LATVIJAS UNIVERSITĀTE

ĶĪMIJAS FAKULTĀTE

Linda Kinēna

ASPARTILPROTEĀŽU INHIBITORU IZSTRĀDE MALĀRIJAS ĀRSTĒŠANAI

Promocijas darbs

Darba zinātniskā vadītāja:

Dr. chem. vad. pētn. Vita Ozola

RĪGA, 2020

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Darbs sastāv no ievada, 3 nodaļām, secinājumiem, literatūras saraksta, 4 pielikumiem.

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ANOTĀCIJA

Aspartilproteāžu inhibitoru izstrāde malārijas ārstēšanai. Kinēna L., zinātniskā vadītāja *Dr. chem.* Ozola V. Promocijas darbs, 235 lappuses, 85 attēli, 18 tabulas, 115 literatūras avoti, 4 pielikumi. Latviešu valodā.

Pētījuma ietvaros ir izstrādāti nepeptidomimētiski un peptidomimētiski plazmepsīna II un plazmepsīna IV inhibitori. Nepeptidomimētisku inhibitoru izstrāde ietver tetrahidroizohinolīna un azolu atvasinājumu sintēzi. Noskaidrots, ka azolu atvasinājumu rindā augstāko inhibitoro aktivitāti uz plazmepsīnu II uzrāda 1,2,3-triazola atvasinājumi. Veikta 1,2,3-triazola atvasinājumu struktūras-aktivitātes likumsakarību izpēte ar dažādiem bifenilfunkcijas, fenilgredzena un aminofunkcijas aizvietotājiem. Sintezētie nepeptidomimētiskie inhibitori uzrāda inhibitoro aktivitāti uz Plm II un Plm IV mikromolārā līmenī. Sintezēta rinda peptidomimētisko inhibitoru, kuru struktūras pamatā ir hidroksietilamīna fragments. Hidroksietilamīna atvasinājumi tika optimizēti, variējot fenilgredzena un amīda grupas aizvietotājus. Savienojumiem, kuri uzrādīja visaugstākās inhibitorās aktivitātes vērtības uz Plm IV, tika noteikta to inhibēšanas spēja inficētu asins šūnu testā un spēja inhibēt Plm X. Sintezētie hidroksietilamīna atvasinājumi ir selektīvi attiecībā pret cilvēka aspartilproteāzi katepsīnu D.

Atslēgvārdi: MALĀRIJA, PLAZMEPSĪNI, KATEPSĪNS D, 1,2,3-TRIAZOLS, TETRAHIDROIZOHINOLĪNI, HIDROKSIETILAMĪNA ATVASINĀJUMI.

ABSTRACT

Design and synthesis of aspartic protease inhibitors as antimalarial agents. Kinēna L., supervisor Dr. chem. Ozola V. Doctoral thesis, 235 pages, 85 figures, 18 tables, 115 literature references, 4 appendices. In Latvian.

A series of non-peptidomimetic and peptidomimetic inhibitors of plasmepsin II and plasmepsin IV were designed and synthesized. Development of non-peptidomimetic inhibitors have been focused on the synthesis of tetrahydroisoquinoline and azole derivatives. 1,2,3-Triazole based inhibitors showed the highest plasmepsin II inhibition activity in the azole series. Research of structure-activity relationship of 1,2,3-triazole derivatives with various substituents in biaryl-unit, phenyl ring and amino-function were made. The designed non-peptidomimetic inhibitors show inhibitory activity toward plasmepsin II and plasmepsin IV at micromolar level. Hydroxyethylamine based peptidomimetic inhibitors were synthesized. Optimization of hydroxyethylamine based inhibitors was performed by inducing substituents at phenyl ring and amide function. Inhibition capacity of parasite growth in vitro and plasmepsin X inhibition activity was determined for the most active compounds. Hydroxyethylamine based inhibitors display selectivity against human aspartic protease cathepsin D.

Keywords: MALARIA, PLASMEPSINS, CATHEPSIN D, 1,2,3-TRIAZOLE, TETRAHYDROISOQUINOLINE, HYDROXYETHYLAMINE DERIVATIVES.

SATURA RĀDĪTĀJS

APZĪMĒJUMU SARAKSTS

3D	–	Trīsdimensionāls
3D7	–	*P. falciparum* celms, klonēts no NF54, raksturīgs Āfrikas reģiona parazītiem
Ac	–	Acetil-
AD	–	Asimetriskā dihidroksilēšana
AD-mix-α	–	Reaģentu maisījums, kas satur $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3
AEŠH	–	Augsti efektīvā šķidruma hromatogrāfija
AIMS	–	Augstas izšķirtspējas masspektrometrija
Asp	–	Asparagīnskābe
BACE	–	*β*-Sekretāze
BINAP	–	(2,2′-Bis(difenilfosfino)-1,1′-binaftils)
Bn	–	Benzil-
Boc	–	*terc*-Butoksikarbonil-
Cat	–	Katepsīns
COD	–	1,5-Ciklooktadiēns
Cp	–	Ciklopentadienil-
Dba	–	Dibenzilidēnacetons
DCC	–	*N*,*N*'-Dicikloheksilkarbodiimīds
DCE	–	Dihloretāns
DEAD	–	Dietilazodikarboksilāts
DIPEA	–	Diizopropiletilamīns
DMAP	–	4-Dimetilaminopiridīns
DMF	–	*N*,*N*-Dimetilformamīds
DPAP 1	–	Dipeptidilaminopeptidāze 1
Dppf	–	1,1'-Bis(difenilfosfino)ferrocēns
EC_{50}	–	Efektīvā koncentrācija, kurā tiek sasniegti 50 % no maksimālā bioloģiskā efekta noteiktā laika posmā
EDC	–	1-Etil-3-(3-dimetilaminopropil)karmodiimīds
FRET	–	Fluorescences rezonanses enerģijas pārnese
Gln	–	Glutamīns
Gly	–	Glicīns
HAP	–	Histoaspartilproteāze

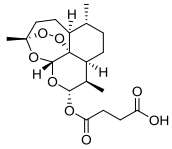
HBTU	–	(2-(1*H*-Benztriazol-1-il)-1,1,3,3-tetrametiluronija heksafluorfosfāts
HIV-1	–	Cilvēka imūndeficīta vīrusa 1. tips
HMBC	–	Kodolu magnētiskās rezonanses eksperiments, kurā tiek noteikta heteroatomu un ūdeņraža atomu sadarbība caur vairākām saitēm
HOBt	–	1-Hidroksibenztriazols
HTS	–	Augstas caurlaidības skrīnings
IC_{50}	–	Antagonista koncentrācija, kas attiecīgo bioloģisko efektu inhibē līdz 50 % no maksimālā efekta līmeņa
Ile	–	Izoleicīns
i-Pr	–	Izopropil-
K_i	–	Inhibēšanas konstante
KMR	–	Kodolu magnētiskā rezonanse
Leu	–	Leicīns
LiHMDS	–	Litija heksametildisilazīds
Lys	–	Lizīns
m.d.	–	Miljonās daļas
Met	–	Metionīns
MsCl	–	Metānsulfonskābes hlorīds
MSP1	–	Merozoītu virsmas proteīns 1
NBS	–	*N*-Bromsukcīnimīds
n-Bu	–	*n*-Butil-
NCS	–	*N*-Hlorsukcīnimīds
NF54	–	*P. falciparum* celms, raksturīgs Āfrikas reģiona parazītiem
NMP	–	*N*-Metil-2-pirolidons
nn	–	Nav noteikts
NOESY	–	Kodola Overhauzera efekta spektroskopija
PEXEL	–	(*Plasmodium* export element) – *Plasmodium* eksporta elements
PĒ	–	Petrolēteris
Pf	–	*Plasmodium falciparum*
Ph	–	Fenil-
Phe	–	Fenilalanīns
Pin	–	Bis(pinakol)dibors
Plm	–	Plazmepsīns

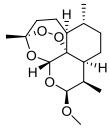
Pro	–	Prolīns
RBC	–	Sarkanās asins šūnas
Ser	–	Serīns
SERA5	–	Serīniem bagātais antigēns 5
SUB1	–	Subtilizīns 1
THF	–	Tetrahidrofurāns
Thr	–	Treonīns
TMP	–	2,2,6,6-Tetrametilpiperidīns
TMS	–	Trimetilsilil-
Tol	–	Toluols
Ts	–	*p*-Toluolsulfonil-
Tyr	–	Tirozīns
UEŠH	–	Ultra efektīvā šķidruma hromatogrāfija
Val	–	Valīns
W2	–	*P. falciparum* celms, kas ir rezistents pret hlorohīnu
X-Phos	–	2-Dicikloheksilfosfīno-2′,4′,6′-triizopropilbifenils

IEVADS

Malārija ir dzīvībai bīstama infekcijas slimība, kuru izraisa *Anopheles* moskītu pārnēsātie *Plasmodium knowlesi*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* un *Plasmodium falciparum* (*P. falciparum*) parazītiskie vienšūņi. 2017. gadā *P. falciparum* parazīts izraisīja 99.7 % malārijas gadījumu Āfrikas reģionā, un tas ir atzīts par visbīstamāko no iepriekš minētajām parazītu sugām.[1] 2017. gadā tika reģistrēti 219 miljoni malārijas inficēšanās gadījumu, no kuriem 435 tūkstoši bija letāli. Tai skaitā 266 000 (61 %) nāves gadījumu attiecināmi uz bērniem, kuri ir jaunāki par 5 gadiem. Pasaules Veselības organizācija stratēģijas "*Global technical strategy for malaria 2016-2030*" ietvaros ir izvirzījusi mērķi līdz 2030. gadam samazināt malārijas izraisīto nāves gadījumu skaitu par 90 % salīdzinājumā ar 2015. gadu.[1]

Malārijas infekcijas riskam ir pakļauta aptuveni puse no pasaules populācijas – Āfrikas, Vidējo Austrumu, Dienvidaustrumāzijas, Dienvidamerikas, Centrālamerikas, Karību salu un Okeānijas iedzīvotāji.[1] Aprēķināts, ka valstīs ar augstu saslimšanas līmeni malārijas infekcijas rezultātā ekonomiskā izaugsme samazinās par 1.3 % gadā. Saslimšana ar malāriju daudzās mazattīstītajās valstīs ir atzīta par vienu no nabadzības cēloņiem un sekām.[2], [3] Infekcijas riskam ir pakļauti ne tikai minēto reģionu iedzīvotāji, bet arī tūristi no pārējām pasaules valstīm. Diemžēl praktiski pret visām pašlaik izmantotajām medikamentu grupām ir izveidojušies rezistentie malārijas celmi.[4] Artemisinīnu grupas preparāti **1**-**4** pašlaik ir vienīgie efektīvie rezistentās malārijas ārstniecības līdzekļi, un tos sekmīgi izmanto kombinācijā ar citām pretmalārijas zāļvielām, tomēr ir zināmi rezistences veidošanās gadījumi arī pret šīs grupas medikamentiem.[5]


Artesunāts **1**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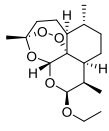
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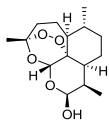
Dihidroartemisinīns **4**

Lai novērstu draudus, ka malārija nākotnē varētu kļūt par masveida neārstējamu saslimšanu lielai daļai planētas iedzīvotāju, ir akūti nepieciešams radīt jaunus rezistences brīvus pretmalārijas ārstniecības līdzekļus.[6]

Gan cilvēka organismā, gan *P. falciparum* parazītā atrodas aspartil-proteāzes – farmakoloģiski nozīmīga endoproteāžu klase, kuras vieno kopīgs katalītiskās darbības mehānisms peptīdu substrātu amīdsaites šķelšanā, iesaistot divas enzīma katalītiskā centra aspartātu sānu ķēdes.[7] Līdz šim klīniskajā praksē veiksmīgi ir ieviesti vairāki aspartilproteāžu inhibitori, piemēram, HIV-1 proteāzes inhibitori HIV ārstēšanai un renīna inhibitors hipertensijas ārstēšanai.[8] Šie piemēri rāda, ka aspartilproteāzes var tikt veiksmīgi izmantotas kā zāļvielu mērķi jaunu preparātu radīšanai. Tomēr *P. falciparum* parazīta aspartilproteāžu – plazmepsīnu (I, II, IV, HAP, V, IX, X) terapeitiskais potenciāls malārijas ārstēšanai joprojām nav izmantots.

Promocijas darba mērķis ir plazmepsīna II un plazmepsīna IV inhibitoru izstrāde malārijas ārstēšanai.

Darba mērķa īstenošanai izvirzīti šādi **uzdevumi**:

1) sintezēt tetrahidroizohinolīnu un azolu saturošu savienojumu rindas struktūras-aktivitātes likumsakarību pētījumiem;
2) noteikt iegūto savienojumu inhibitorās aktivitātes vērtības uz Plm II. Noskaidrot optimālos farmakoforus inhibitoru molekulā, kas nepieciešami aktivitātes nodrošināšanai;
3) uzlabot peptidomimētisku hidroksietilamīna fragmentu saturošu inhibitoru selektivitāti attiecībā pret cilvēka aspartilproteāzi Cat D un veikt struktūras-aktivitātes likumsakarību analīzi;
4) aktīvākajiem savienojumiem noteikt *P. falciparum* parazīta augšanas inhibēšanas spēju inficētu asins šūnu testā un to spēju inhibēt plazmepsīnu X.

Promocijas darba **zinātniskā novitāte** un **praktiskā nozīme**. Pētījuma ietvaros ir dizainēti un sintezēti jauni nepeptidomimētiski azolu rindas (izoksazola, pirola, triazola, imidazola) un tetrahidroizohinolīna ciklu saturoši plazmepsīna II inhibitori. Tika sintezēti peptidomimētiski hidroksietilamīna atvasinājumi ar dažādiem mono- un di- aizvietotiem amīdiem. Struktūras-aktivitātes likumsakarību analīze ļāva noskaidrot Plm IV un Cat D S3 sub-kabatu telpiskos izmērus un tādējādi uzlabot Plm IV / Cat D selektivitāti. Sintezētie hidroksietilamīna atvasinājumi uzrāda inhibitorās aktivitātes vērtības pret Plm IV nanomolārā līmenī, Plm IV / Cat D selektivitātes faktora vērtībai sasniedzot 50. Aktīvākie savienojumi inhibē *P. falciparum* parazīta augšanu inficētās sarkanajās asins šūnās nanomolārā līmenī. Ir noskaidrots, ka

hidroksietilamīna atvasinājumi spēj inhibēt arī Plm X. Tā kā pašlaik nav zināma Plm IX un Plm X struktūra, Plm IV var kalpot kā modeļproteīns jaunu inhibitoru izstrādē.

Promocijas darba rezultātu aprobācija:

Zinātniskās publikācijas:

1. Kinena, L.; Ozola, V. Tetrahydroisoquinoline-based non-peptidomimetic plasmepsin inhibitors. *Chem. Heterocycl. Comp.* **2020**, *56*, 60-66.
   L. Kinēna izstrādāja 100 % no eksperimentālā darba apjoma, noformēja pētījuma rezultātus atbilstoši žurnāla prasībām, kā arī sniedza ieguldījumu publikācijas rakstīšanā.
2. Zogota, R.; Kinena, L.; Withers-Martinez, C.; Blackman, M. J.; Bobrovs, R.; Pantelejevs, T.; Kanepe-Lapsa, I.; Ozola, V.; Jaudzems, K.; Suna, E.; Jirgensons, A. Peptidomimetic plasmepsin inhibitors with potent anti-malarial activity and selectivity against cathepsin D. *Eur. J. Med. Chem.* **2019**, *163*, 344-352.
   L. Kinēna izstrādāja 80 % no eksperimentālā darba apjoma, sniedza ieguldījumu pētījumu rezultātu noformēšanā un publikācijas rakstīšanā.
3. Kinena, L.; Leitis, G.; Kanepe-Lapsa, I.; Bobrovs, R.; Jaudzems, K.; Ozola, V.; Suna, E.; Jirgensons, A. Azole-based non-peptidomimetic plasmepsin inhibitors. *Arch. Pharm. Life Sci*. **2018**, *351*, 1800151.
   L. Kinēna izstrādāja 80 % no eksperimentālā darba apjoma, noformēja pētījuma rezultātus atbilstoši žurnāla prasībām, kā arī sniedza ieguldījumu publikācijas rakstīšanā.

Patents:

Kinena, L.; Ozola, V.; Suna, E.; Leitis, G.; Jirgensons, A.; Jaudzems, K.; Kanepe-Lapsa, I.; Domracheva, I. 2. Substituted aminoalkylazoles as malarial aspartic protease inhibitors. WO 2017069601 A1, 27 Apr, 2017.

Konferenču tēzes:

1. Linda Kinena, Rimants Zogota, Vita Ozola, Aigars Jirgensons, Edgars Suna. Peptidomimetic Plasmepsin Inhibitors with Potent Anti-malarial Activity and Selectivity Against Cathepsin D. VIII EFMC International Symposium on Advances in Synthetic and Medicinal Chemistry, 1.–5. septembris, **2019**, Atēnas, Grieķija, stenda referāts.

2. Rimants Zogota, Linda Kinena. The Development of Malaria Aspartic-Protease Inhibitors with Improved Selectivity. Balticum Organicum Syntheticum, 1.–4. jūlijs, **2018**, Tallina, Igaunija, stenda referāts.
3. Rimants Zogota, Linda Kinena. The Development of Aspartic-Protease Inhibitors for Malaria Treatment. 10th Paul Walden Symposium, 15.–16. jūnijs, **2017**, Rīga, Latvija, stenda referāts.

Pateicība

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Dr. Sergejam Beļakovam par rentgenstruktūranalīzes veikšanu.

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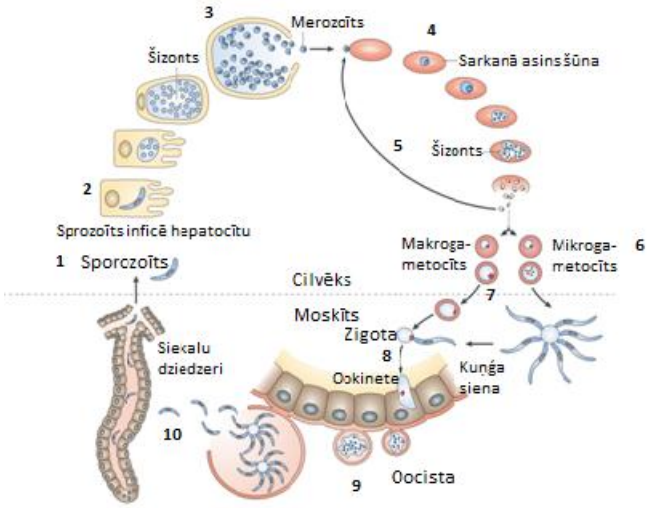
1. LITERATŪRAS APSKATS

1.1. Malārijas parazīta *P. falciparum* dzīves cikls

Malārijas parazīta dzīves cikls ir sarežģīts un sastāv no daudzām atsevišķām fāzēm (1.1. att.). Ir divi tā galvenie cikli – cikls, kas noris cilvēka organismā, un cikls, kas noris *Anopheles* moskītā. Cilvēka organismā notiekošais dzīves cikla posms tiek iedalīts aknu jeb preeritrocītiskajā posmā un eritrocītiskajā posmā.[9], [10]

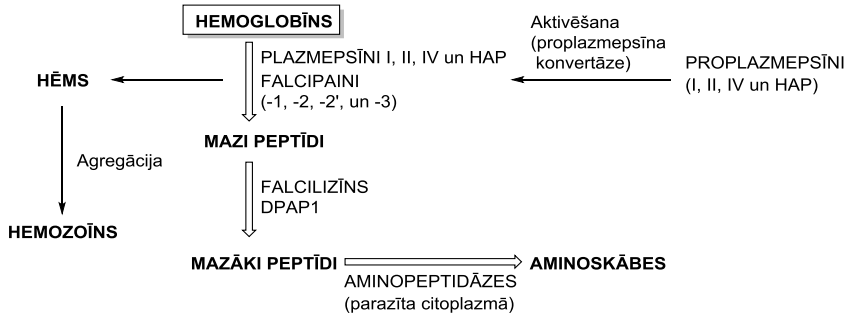


1.1. att. **Malārijas parazīta dzīves cikls [2]**

Pēc cilvēka inficēšanās sporozoīti (**1**) nonāk cilvēka asinsritē un tiek nogādāti aknās, kur tie inficē hepatocītus (**2**). Šo parazīta *P. falciparum* dzīves cikla posmu sauc par "aknu posmu". Aknās veidojušies merozoīti (**3**) inficē sarkanos asins ķermenīšus (**4**), kur tie iziet apļa posmu, trofozītu posmu un asins šizonta posmu (**5**) [9], kā rezultātā notiek merozoītu pavairošanās, kuri tiek atbrīvoti asinsrites sistēmā un inficē veselās asins šūnas (eritrocītiskais posms). Pēc šī posma cilvēkam parādās klīniskie slimības simptomi (drudzis). Tas noved pie inficētā organisma parazitēmijas palielināšanās.[11] Asins šūnās daži merozoīti attīstās par makro- un mikro- gametocītiem (**6**). Gametocīti koncentrējas ādas kapilāros, un reizē ar cilvēka asinīm tie tiek uzņemti *Anopheles* moskītā (**7**). Moskītā vīrišķie gametocīti veido gametas, kas saplūst ar sievišķajām gametām,

veidojot zigotas, kas pagarinās par ookineti (**8**). Šīs ookinetes migrē uz moskīta kuņģa vidusdaļu, šķērso kuņģa sienu un veido oocistas (**9**). Mejotiskās dalīšanās rezultātā veidojas sporozoīti, kuri migrē uz moskīta siekalu dziedzeriem (**10**), un moskīts var tālāk izplatīt *P. falciparum* parazītus.[2]

Slimības simptomi visbiežāk ir asins posma rezultāts, tādēļ svarīga ir medikamenta izstrāde, kas inhibētu šo parazīta dzīves posmu.[11], [12] Asins posma laikā notiek merozoītu pavairošanās asins šūnās. Šajā laikā tiek patērēts liels barības vielu daudzums, taču parazītam ir ierobežotas iespējas sintezēt aminoskābes *de novo*. Hemoglobīna katabolisma rezultātā iegūtās aminoskābes parazīts izmanto kā barību. Hemoglobīna proteolīze notiek parazīta vakuolās dažādu proteolītisko enzīmu – plazmepsīnu, falcipainu – vai metaloproteāzes falcilizīna klātienē (1.2. att.).[9], [12]



1.2. att. **Hemoglobīna proteolīze [9]**

Hemoglobīna noārdīšanas laikā rodas hēms [Fe(II)], kas tālāk tiek oksidēts par hematīnu [Fe(III)], kā blakusproduktu izdalot ūdeņraža peroksīdu, kas ir kaitīgs parazītam. *P. falciparum* parazītam ir izstrādājies tā neitralizēšanas mehānisms. Hemoglobīna noārdīšana ir parazīta specifisks katabolisks process, un daži šī procesa soļi jau tiek izmantoti kā zāļu mērķi.[12]

1.2. *P. falciparum* parazīta aspartilproteāzes – plazmepsīni

Plazmepsīni ir aspartilproteāzes – farmakoloģiski nozīmīga endoproteāžu klase, kuru vieno kopīgs katalītiskās darbības mehānisms peptīdu substrātu amīdsaites šķelšanā, iesaistot divas enzīma katalītiskā centra aspartātu sānu ķēdes.[7] Ir zināmi 10 gēni, kas kodē aspartilproteāzes *P. falciparum* parazīta genomā (Plm I, II, IV, V, VI, VII, VIII, IX, X un strukturāli līdzīgā HAP (histoaspartilproteāze, viena katalītiskā aspartilatlikuma vietā satur histidīnu)). Līdz šim laikam nav precīzi noskaidrota katra plazmepsīna loma parazīta

metabolismā, taču ir zināms, ka Plm I, II, IV, V, IX, X un HAP piedalās eritrocītiskajā stadijā, savukārt Plm VI, VII un VIII piedalās eksoeritrocītiskajā stadijā, un to funkcijas parazīta organismā nav zināmas.[9]

Visvairāk pētītie ir Plm I, II un IV. Tie piedalās hemoglobīna metabolismā parazīta vakuolās. Plm I, II, IV un HAP uzrāda 60–70 % aminoskābju secības līdzību, tādējādi inhibitoram jāspēj inhibēt vairākus plazmepsīnus vienlaikus. Aktīvajā centrā Plm II uzrāda 84 % sekvences līdzību ar Plm I, 68 % ar Plm IV un 39 % ar HAP. Arī cilvēka organismā atrodas aspartilproteāzes – katepsīns D (Cat D), katepsīns E, renīns un pepsīns A. Plazmepsīns II un katepsīns D uzrāda 35 % aminoskābju secības līdzību, aktīvajā centrā tā ir vēl augstāka, tādēļ, izstrādājot inhibitorus plazmepsīnu inhibēšanai, ir jāņem vērā selektivitātes problēma.[9]

Gremošanas vakuolas plazmepsīni I, II un IV ir plaši pētīti, un ir noskaidrota to loma *P. falciparum* parazīta dzīves ciklā. Jaunu zāļu mērķu definēšanai ir svarīgi noskaidrot arī plazmepsīnu V, IX un X struktūru un nozīmi parazīta dzīves ciklā. Zināms, ka šie plazmepsīni neatrodas parazīta gremošanas vakuolās un tādējādi nepiedalās hemoglobīna proteolīzē.[13]

Plm V ir endoplazmatiskā tīkla proteāze, kas sastāv no 590 aminoskābju atlikumiem [14] un pēc sekvences vairāk līdzinās cilvēka aspartilproteāzei BACE (β-sekretāze).[15] Pēc inficēšanās parazīta izdzīvošanai eritrocītā ir nepieciešams izvadīt proteīnus caur parazīta vakuolas membrānu uz eritrocīta citoplazmu, izmantojot proteīna eksporta mehānismus. Šie proteīni tālāk remodelē inficēto eritrocītu un tā virsmu. Lielākā daļa proteīnu, kas paredzēti izvadīšanai no parazīta vakuolas, satur aminoterminālu motīvu PEXEL (*Plasmodium* EXport Element, piecu aminoskābju fragments R × L × Q / E / D – arginīns × leicīns × glutamīns / glutamīnskābe / asparagīnskābe). Plm V atpazīst šos proteīnus ar PEXEL motīvu un proteolītiski sagatavo tos eksportēšanai no parazīta vakuolas uz eritrocītu.[16]–[18] Plm V ir svarīgs parazīta attīstībai un izdzīvošanai, tādēļ var kalpot kā labs zāļu mērķis.[14]

Malārijas parazīta *P. falciparum* dzīves cikla asins posms ietver vairākus ciklus ar merozoītu pavairošanos, to atbrīvošanu asinsrites sistēmā un veselo asins šūnu inficēšanu. Lai parazīts spētu izdzīvot, ir svarīgs tā egress no eritrocīta un atkārtota asins šūnu inficēšana. Sekojošu proteolītisku procesu norise ļauj merozoītam izkļūt no eritrocīta, kas tiek iniciēta ar serīna proteāzes subtilizīna 1 (SUB1) eksocitozi.[19] Plm X galvenā bioloģiskā funkcija ir SUB1 proteolītiska aktivēšana. Plm IX neietekmē parazīta egresu no eritrocīta, bet tam

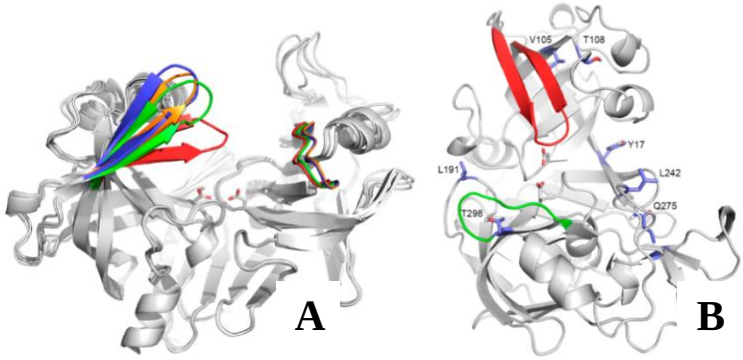
ir būtiska nozīme asins šūnu inficēšanā. Pētījumi rāda, ka, inhibējot plazmepsīnus IX un X (strukturāli līdzīgi gremošanas plazmepsīniem Plm I, Plm II un Plm IV), iespējams, tiek bloķēta parazīta replikācija, tādēļ Plm IX un Plm X ir svarīgi parazīta izdzīvošanai un var kalpot kā zāļu mērķi.[20]–[22]

Plazmepsīni (I, II, IV, V un HAP), kuriem ir pieejami kristālu rentgendifraktometrijas dati, uzrāda augstu strukturālo līdzību (1.3. att. (**A**)). Vienīgā būtiskā atšķirība starp tiem ir plazmepsīna V papildu spirāļu kopums *C*-terminālajā domēnā un 19 aminoskābju atlikumus saturoša cilpa *N*-terminālajā domēnā (ciānkrāsa 1.3. att. (**C**)).[23]

1.3. att. **Plazmepsīnu lentveida 3D struktūru attēlojums. A – viens virs otra salikti Plm I, Plm II un Plm IV 3D struktūru attēli; B – Plm III 3D struktūra; C – Plm V 3D struktūra [23]**

Plazmepsīnu (I, II, IV, V un HAP) struktūra sastāv no 3 atšķirīgiem reģioniem: diviem topoloģiski līdzīgiem *N*- un *C*- terminālajiem domēniem (zils un rozā 1.3. att.) un 6-*β*-lokšņu kopuma, kas savieno šīs divas daļas (oranžs 1.3. att.). Enzīma aktīvā kabata atrodas *N*- un *C*-terminālo domēnu saskares vietā un satur divus aspartilatlikumus (Asp34 un Asp214 Plm II), protonu donoru un akceptoru, kuri veido katalītisko cilpu peptīdsaites šķelšanas laikā. Viens no katalītiskajiem aspartilatlikumiem plazmepsīnā III (HAP) ir aizvietots ar histidīnu (1.3. att. (**B**)). Līdzīgi kā citām aspartilproteāzēm [24], plazmepsīnu (I, II, IV, V un HAP) *N*-terminālais domēns satur vienu garu *β-hairpin* struktūru, ko sauc par vārsta cilpu (Lys72-Phe85 plazmepsīnā II). Tā novietojas perpendikulāri katalītiskajai cilpai (sarkans 1.3. att.). Vārsta cilpas pretējā pusē (*C*-terminālajā domēnā) atrodas vēl viena kustīga vārsta-tipa cilpas struktūra (Gly291-Pro297 plazmepsīnā II) (zaļš 1.3. att.). Šāds reģions ir raksturīgs visu gremošanas vakuolu plazmepsīniem, un, iespējams, tas sadarbojas ar hemoglobīna virsmas hidrofobajiem atlikumiem, un tam ir būtiska loma hemoglobīna noārdīšanas pirmajos soļos.[25] Lielākajā daļā Plm II kristālu

rentgenstruktūru ir redzama aizvērtā vārsta cilpas konformācija (1.4. att. (**A**)).[23]



1.4. att. **Plazmepsīna II struktūras 3D attēlojums. A – dažādas Plm II vārsta un fleksiblās cilpas konformācijas (sarkans – aizvērtā konformācija, zils, zaļš, oranžs – atvērtā konformācija); B – plazmepsīna II struktūras lentīšveida attēls, ar lillā krāsojumu apzīmētas 7 aminoskābes, kas sastopamas parazīta aspartilproteāzēs [23]**

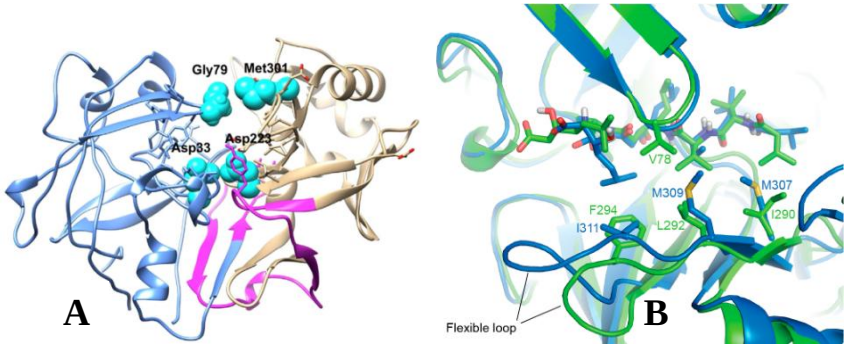
Ir publicēti plasmepsīna II kristāla rentgendifrakrometrijas dati [26], [27], kuros redzams, ka inhibitors ir saistījies ar atvērtu enzīma konformāciju (1.4. att. (**A**)). Tas nozīmē, ka vārsta cilpa ir samērā kustīgs reģions. Lai šo reģiona īpašību izmantotu pēc iespējas efektīvāk jaunu, selektīvu inhibitoru dizainēšanā un izstrādē, ir identificēti aktīvā centra aminoskābju atlikumi, kuri ir sastopami visās plazmepsīnu izoformās (Plm I, II, IV), bet nav sastopami cilvēka organisma aspartilproteāzēs (Cat D, E) (1.4. att. (**B**)). Pētījuma rezultātā tika identificētas septiņas aminoskābes – Tyr17, Val105, Thr108, Leu191, Leu242, Gln275 un Thr298 (numerācija saskaņā ar Plm II). Divas no šīm aminoskābēm – Val105 un Thr108 – atrodas vārsta kabatas dziļumā, kur tās sadarbojas ar to nepeptidomimētisko inhibitoru funkcionālajām grupām, kas piesaistījušies plazmepsīnam atvērtajā vārsta konformācijā.[23], [28]

1.3. Cilvēka aspartilproteāze katepsīns D

Aspartilproteāzes atrodas arī cilvēka organismā. Cilvēka genomā ir zināmi 8 gēni, kas kodē aspartilproteāzes – katepsīns D, katepsīns E, napsīns A, BACE, BACE 2, pepsīns, gastricsīns, renīns. Katepsīns D ir 346 aminoskābju atlikumu [29] garš lizosomāls enzīms, kas sastopams lielākajā daļā cilvēka šūnu.[15] Salīdzinot Plm II struktūru ar cilvēka organisma aspartilproteāžu struktūrām, ir

noskaidrots, ka Cat D strukturāli ir visvairāk līdzīgs Plm II.[30] Izstrādājot selektīvus plazmepsīnu inhibitorus, svarīgi ir nodrošināt selektivitāti attiecībā pret katepsīnu D.[15]

Salīdzinot Plm II un Cat D kristālu rentgendifraktometrijas datus [31] (1.5. att. **A**, **B**), ir noskaidrots, ka Plm II un Cat D struktūras līdzīgie elementi ir vārsta cilpa, aktīvais centrs un β-lokšņu kopums.

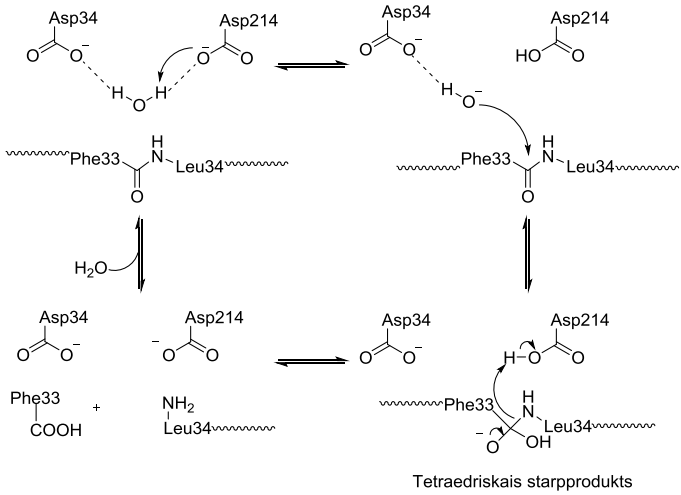


1.5. att. **A – Cat D 3D struktūra [29]; B – Plm II un Cat D 3D struktūru salīdzinājums (zaļš – Plm II, zils – Cat D) [23]**

Tomēr ir novērojamas samērā būtiskas atšķirības kustīgās vārsta cilpas reģionā, kas ir eikariotisko aspartilproteāžu raksturīgs struktūras elements.[32] Cat D un renīna fleksiblā cilpa satur trīs blakus esošus prolīna atlikumus, bet Plm II struktūrā viens prolīna atlikums ir aizvietots ar valīnu, kā arī cilpa ir daudz īsāka, un tās konformācija ir tāda, kas veido atvērtāku saistīšanās vietu. Cat D vārsta cilpas gala atlikums ir Gly79, bet Plm II tas ir Val78. Piesaistes vietas pretējā pusē katepsīnā D ir Met309, bet plazmepsīnā II – Leu292. Plazmepsīna II Val78 un Leu292 pāris ir mazliet lielāks un hidrofobāks nekā attiecīgā Cat D daļa, tādējādi hidrofobi inhibitori varētu labāk inhibēt Plm II.[23] Cat D inhibēšana cilvēka organismā var izraisīt nevēlamas blakusparādības (attīstības traucējumi, redzes zudums, epilepsija) [33] un ārstēšanās laikā samazināt inhibitora efektīvo koncentrāciju asinīs.[15]

1.4. Aspartilproteāžu katalītiskais darbības mehānisms

Aspartilproteāžu katalītiskais mehānisms tiek pētīts, izmantojot izotopu iezīmēšanas, teorētisko aprēķinu un rentgenstruktūras analīzes metodes. Kaut gan daži aspekti vēl nav noskaidroti, tiek uzskatīts, ka pamatā ir skābes–bāzes mehānisms. Shematisks aspartilproteāžu katalītiskais darbības mehānisms ir parādīts 1.6. attēlā. Visās pepsīnu un retrovīrusu aspartilproteāžu struktūrās ir katalītiska ūdens molekula, kas ar ūdeņraža saišu palīdzību ir piesaistīta diviem aktīvās kabatas aspartilatlikumiem – Asp34 un Asp214.



1.6. att. **Aspartilproteāžu katalītiskais darbības mehānisms [34]**

Aktivētā ūdens molekula veic nukleofīlu uzbrukumu substrāta Phe33–Leu34 amīda karbonilgrupai, izveidojot tetraedrisko starpproduktu, kurš sabrūk pēc amīna protonēšanās, veidojot divus peptīdus un reģenerējot aspartilatlikumus katalītiskajā ciklā.[9], [34]

1.5. Mūsdienās izmantotie medikamenti malārijas ārstēšanai

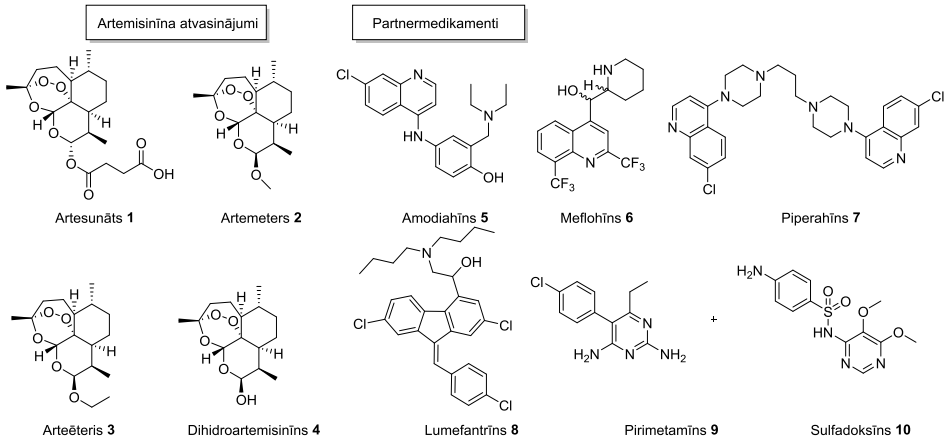
Pasaules Veselības organizācija šobrīd ir atzinusi 14 medikamentus malārijas ārstēšanai un 4 medikamentus profilaktiskai ārstēšanai. Medikamentus malārijas ārstēšanai iedala 4 kategorijās:

1) hinolīna atvasinājumi – hlorohīns (*Chloroquine*), amodiahīns (*Amodiaquine*), hinīns (*Quinine*), meflohīns (*Mefloquine*), primahīns (*Primaquine*), piperahīns (*Piperaquine*). Šie medikamenti akumulējas

parazīta gremošanas vakuolās un veido kompleksus ar hēmu, tādējādi novēršot tā izkristalizēšanos parazīta gremošanas vakuolā. Tiek inhibēta hēma polimerizēšanās, kas izraisa citotoksiskā hēma uzkrāšanos parazītā;

2) antifolāti – pirimetamīns (*Pyrimethamine*) / sulfadoksīns (*Sulfadoxine*), atovakvons (*Atovaquone*) / proguanils (*Proguanil*). Folātu antagonisti iedarbojas uz parazīta dihidrofolāta reduktāzi;
3) artemisīna atvasinājumi – artesunāts (*Artesunate*), artemeters (*Artemether*), dihidroartemisinīns (*Dihydroartemisinin*) – molekulas endoperoksīda tiltiņš sadarbojas ar parazīta hēma dzelzs joniem un izveido skābekļa radikāļus. Šie radikāļi selektīvi saistās ar membrānu proteīniem, izraisa lipīdu peroksidāciju, bojā endoplazmatisko tīklu un inhibē proteīnu sintēzi, kā rezultātā parazīts aiziet bojā;
4) antibiotikas – tetraciklīns, doksiciklīns, klindamicīns, azitromicīns – iedarbojas uz parazīta mitohondrija 70S ribosomām.[35]

Šobrīd visefektīvākā ārstniecības metode ir divu medikamentu kombināciju lietošana (1.7. att.), kas visbiežāk ietver artemisīna atvasinātus medikamentus **1**, **2**, **3**, **4** un lumefantrīnu (*Lumefantrine*) **8**, meflohīnu **6**, piperahīnu **7**, amodiahīnu **5**, sulfadoksīnu **10** un pirimetamīnu **9** kā partnermedikamentu.[36], [37]



1.7. att. **Pašlaik izmantotās medikamentu kombinācijas malārijas ārstēšanai [37]**

Medikamentu kombināciju lietošana malārijas ārstēšanai ļauj izvairīties no rezistences veidošanās. Artemisīna atvasinājumi ir efektīvi pret visām *P. falciparum* rezistences formām. Visplašāk izmanto tādus atvasinājumus kā artemeteru **2**, artesunātu **1** un arteēteri **3** (1.7. att.).[2] Šie daļēji sintētiskie atvasinājumi parazītā tiek pārvērsti aktīvajā metabolītā – dihidroartemisinīnā **4**.

Medikamentu kombināciju lietošanai ir būtiska priekšrocība attiecībā pret viena medikamenta ārstēšanu – artemisīns ātri un efektīvi iznīcina lielāko daļu parazītu (tā darbības puslaiks ir < 1 h), bet pēc tam sāk iedarboties otrs medikaments, kas iznīcina atlikušos parazītus ilgākā laika posmā.[37] Tā kā artemisinīna atvasinājumu darbības puslaiks ir mazāks par 1 stundu, tad rezistence pret šo medikamentu veidojas lēnāk. Tomēr 2008. gadā Kambodžā tika novērots pirmais rezistences gadījums pret šīs grupas medikamentiem. 2018. gadā tika fiksēti jau 30 rezistences gadījumi [37], tādēļ ir svarīgi definēt jaunus zāļu mērķus un izstrādāt rezistences brīvus medikamentus malārijas ārstēšanai. Ir vairākas pieejas, kas tiek lietotas jaunu pretmalārijas līdzekļu izstrādē:

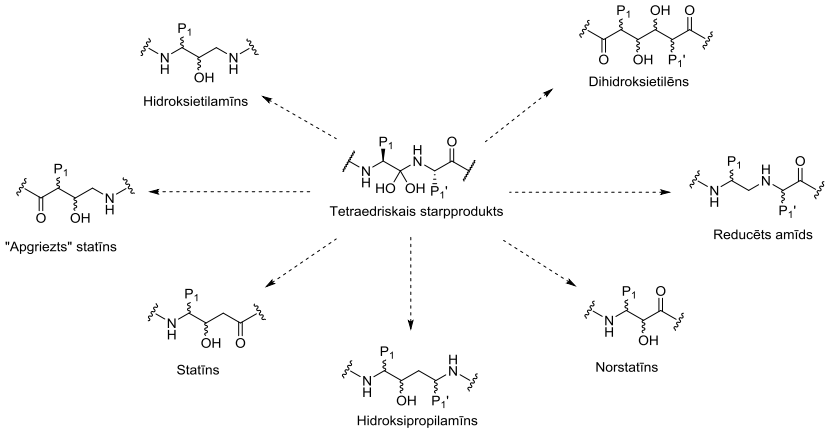
1) visplašāk lietotā pieeja ir jau esošo medikamentu analogu sintēze [38];
2) plašu savienojumu bibliotēku un dabasvielu skrīnings uz potenciālo zāļu mērķi;
3) medikamentu izmantošana, kas jau ir aktīvi pret citām saslimšanām;
4) kovalentā biterapija – ārstēšanā izmanto savienojumus, kuri satur divus kovalenti saistītus farmakoforus, kas katrs darbojas uz citu / to pašu zāļu mērķi ar atšķirīgiem darbības mehānismiem [35];
5) jaunu zāļu mērķu atrašana un to inhibitoru sintēze.

P. falciparum parazīta genoma izpēte ir ļāvusi identificēt jaunus zāļu mērķus antimalārijas medikamentu un vakcīnu izstrādei.[39] Malārijas parazīta gadījumā jaunos potenciālos zāļu mērķus var iedalīt trīs kategorijās: 1) hemoglobīna metabolisma process (proteāzes falcipains un plazmepsīni); 2) mērķi, kas ietekmē makromolekulu un metabolītu sintēzi (1-dezoksi-D-ksilozes 5-fosfāta reduktoizomerāze); 3) membrānu transporta sistēma (holīna transportieri un proteīnu kināzes).[9]

Līdz šim zinātniskajā literatūrā nav aprakstīti pretmalārijas medikamenti, kuru darbības mehānisms ietvertu plazmepsīnu inhibēšanu. Taču plazmepsīni var kalpot kā labs zāļu mērķis vairāku iemeslu dēļ – ar vienu savienojumu ir iespējams inhibēt vairākus plazmepsīnus, tādējādi paaugstinot inhibitora efektivitāti, un to inhibēšanas rezultātā *P. falciparum* parazīts ietu bojā. Kā arī ir iegūti Plm I [40], II [41], [42], IV [43], V (*Plasmodium vivax*) [44] un strukturāli līdzīgās HAP [45] kristālu rentgendifraktometrijas dati, kas ļauj mērķtiecīgi izstrādāt jaunus plazmepsīnu inhibitorus.

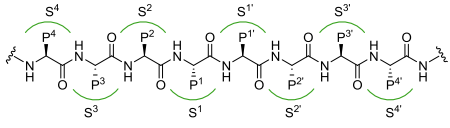
1.6. Plazmepsīnu inhibitori

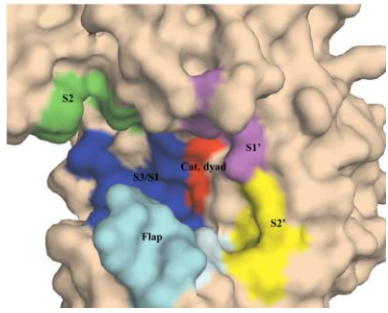
P. falciparum parazīta plazmepsīnu inhibitori tiek iedalīti divās galvenajās grupās – peptidomimētiski un nepeptidomimētiski inhibitori. Peptidomimētiskie inhibitori bieži tiek dēvēti par pārejas stāvokļa analogiem, jo tie imitē tetraedrisko starpproduktu, bet ir noturīgi pret enzimātisko šķelšanu modificētās struktūras dēļ (1.8. att.).[9]



1.8. att. **Tetraedriskā starpprodukta analogi [9]**

Tetraedriskā pārejas stāvokļa analogu nomenklatūra tiek veidota no peptīdtipa savienojuma aminoskābju sānu ķēdes atrašanās vietas molekulā – P1, P1’, P2, P2’, P3, P3’, kas atbilst to saistīšanās vietai proteīnā – S1, S2, S3, S4, S1’, S2’, S3’, S4’ (1.9. att.).[12]





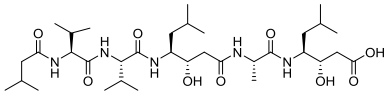
1.9. att. **Peptidomimētisko inhibitoru nomenklatūra [12] un Plm II saistīšanās vietu attēlojums [46]**

Ne visi tetraedriskā starpprodukta analogi ir pētīti vai sintezēti to atvasinājumi (1.8. att.), tādēļ tālāk tiks aplūkoti vairāk izpētītie pārejas stāvokļa

analogi: statīna, norstatīna, 1,2-dihidroksietilēna un hidroksietilamīna atvasinājumi.

1.6.1. Plazmepsīnu peptidomimētiskie inhibitori

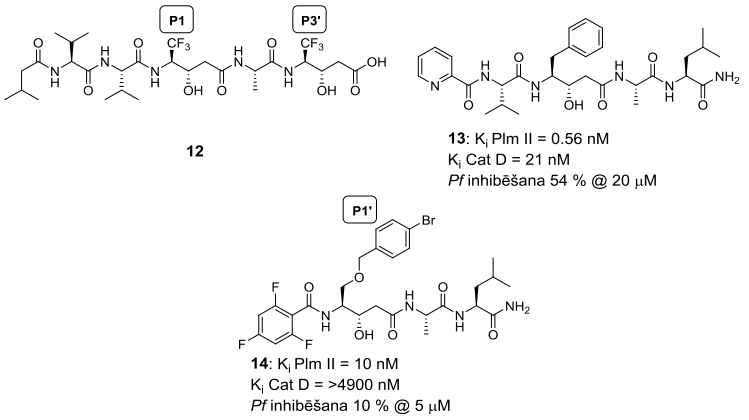
Statīna atvasinājumi. Statīna atvasinājumu raksturīgs struktūrelements ir statīna grupa ((3*S*,4*S*)-4-amino-3-hidroksi-6-metilheptānskābe). No aktinomicetām izdalītais aspartilproteāžu inhibitors pepstatīns A **11** satur divas statīna grupas.[47] Pepstatīns A **11** uzrāda nanomolāras IC_{50} vērtības uz Plm I, Plm II (K_i = 0.025 nM), Plm III un Plm IV, bet tas uzrāda vēl augstāku inhibitoro aktivitāti uz cilvēka aspartilproteāzi Cat D (K_i = 0.0038 nM). Turklāt pepstatīns A uzrāda pazeminātu inhibitoro aktivitāti inficētu asins šūnu testā (IC_{50} RBC = 4 μM) (1.10. att.).[48]



Pepstatīns A 11: K_i Plm II = 0.025 nM
K_i Cat D = 0.0038 nM
IC_{50} RBC[a] = 4 μM
[a]- IC_{50} RBC - parazīta *P. falciparum* augšanas inhibēšana inficētās sarkanajās asins šūnās

1.10. att. **Pepstatīna A struktūra [48]**

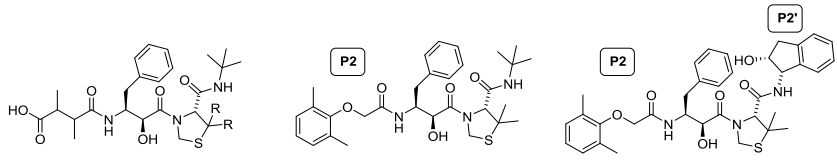
Lai uzlabotu savienojumu selektivitāti attiecībā pret Cat D, ir sintezēta rinda statīna atvasinājumu (1.11. att.). Tā, piemēram, ievadot molekulas P1 un P3' pozīcijās CF_3 grupas, selektivitāte būtiski uzlabojas, tomēr inhibitorā aktivitāte samazinās (IC_{50} Plm II = 1.3 nM).[49] Inhibitoru šķīdības un šūnu caurlaidības uzlabošanai ir sintezēti mazāki savienojumi **13**, **14**, kuri uzrāda zemāku inhibitoro aktivitāti, bet labāku selektivitāti attiecībā pret Cat D.[9], [50] Pepstatīna atvasinājuma **13** K_i pret parazīta aspartilproteāzi Plm II ir 0.56 nM, bet attiecībā pret Cat D K_i ir 38 reizes zemāka.[50]



1.11. att. **Sintezētie statīna atvasinājumi 12-14**

Ievadot molekulas P1' pozīcijā *para*-brombenziloksiaizvietotāju, selektivitāte būtiski uzlabojas (inhibitora **14** K_i Plm II = 10 nM, K_i Cat D = >4900 nM), tomēr inhibitorā aktivitāte inficētu asins šūnu testā ir zema. Datormodelēšanas rezultāti liecina, ka šajā gadījumā metil-*O*-metil tiltiņš nodrošina pietiekamu P1' pozīcijas sānu ķēdes kustīgumu, lai ieņemtu S1 / S3 kabatu. Pētījumos noskaidrots, ka inhibitoro aktivitāti ietekmē P1 aizvietotājs, savukārt selektivitāte atkarīga no P2 aizvietotāja.[51]

Norstatīna atvasinājumi. Allofenilnorstatīna-dimetilprolīna atvasinājumi iepriekš ir veiksmīgi izmantoti kā HIV-1 proteāžu inhibitori [52], [53], tāpēc *A. Nezami* izmantoja tos plazmepsīnu inhibitoru dizainam.[54] Zināms, ka šie savienojumi uzrāda zemu toksicitāti un labu biopieejamību.[54] Pētījumā noskaidroja, ka inhibitorās aktivitātes nodrošināšanā pret Plm II būtiska nozīme ir tioprolīna cikla dimetilgrupām. Nomainot dimetilgrupas pret ūdeņraža atomiem, inhibitorā aktivitāte būtiski samazinās (**16** K_i Plm II = 30.7 μM), un savienojums **16** inhibē arī cilvēka aspartilproteāzi Cat D (K_i = 18.2 μM) (1.12. att.).[54]



15: R = Me, K_i Plm II = 8.6 μM
K_i Cat D = 37.7 μM
16: R = H, K_i Plm II = 30.7 μM
K_i Cat D = 18.2 μM

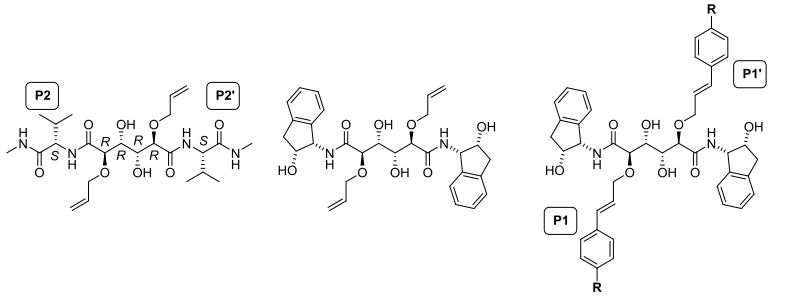
17: K_i Plm II = 70 nM
K_i Cat D = 1600 nM
IC_{50} RBC = 10 μM

18: K_i Plm II = 0.5 nM
K_i Cat D = 2 nM
IC_{50} RBC = 6.8 μM

1.12. att. **Norstatīna atvasinājumi**

Inhibitorā aktivitāte uzlabojas, ja molekulas P2 daļā ievada dimetilfeniloksiacetilgrupu (inhibitora **17** K_i = 70 nM). Šajā gadījumā uzlabojas arī selektivitāte (K_i Cat D = 1600 nM).[54] Visaugstākā inhibitorā aktivitāte (savienojuma **18** K_i Plm II = 0.5 nM) ir iegūta, ievadot molekulas **18** P2 daļā dimetilfeniloksiacetilgrupu, bet P2' daļā (1*S*,2*R*)-1-amino-2-indanola grupu. Indāna sastāvā esošā hidrofobā benzola gredzena daļa un hidroksilgrupas kombinācija nodrošina optimālo sadarbību veidošanos ar S2' kabatu, tomēr šis savienojums **18** uzrāda inhibitoro aktivitāti arī uz Cat D (K_i = 2 nM). Inhibitors **18** uzrāda būtiski zemāku inhibitoro aktivitāti inficētu asins šūnu testā (IC_{50} RBC = 6.8 μM), kas skaidrojams ar to, ka plazmepsīnu inhibitoriem ir jāizkļūst caur 4 membrānām – eritrocīta, parazīta vakuolas, plazmas un gremošanas vakuolas (1.12. att.).[55]

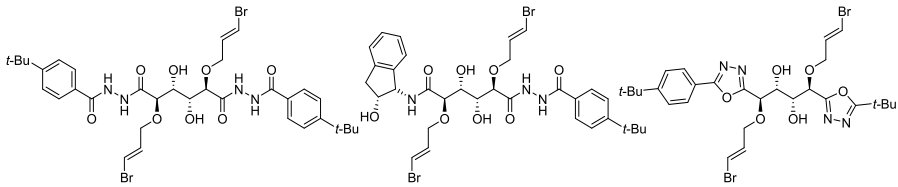
1,2-Dihidroksietilēna atvasinājumi. Molekulārās dinamikas pētījumos ir noskaidrots, ka 1,2-dihidroksietilēna atvasinājumu stereoķīmiskajai konfigurācijai ir būtiska nozīme inhibitorās aktivitātes nodrošināšanā. Piemēram, tikai viens savienojuma **19** stereoizomērs (*SRRRRS*) uzrāda inhibitoro aktivitāti uz Plm II (IC_{50} = 4100 nM).[56] Aizvietojot divas P2 / P2' valīna metilamīda grupas C_2-simetriskā 1,2-dihidroksietilēna atvasinājumā **19** ar (1*S*,2*R*)-1-amino-2-indanola fragmentu, inhibitorā aktivitāte palielinājās 40 reizes, salīdzinot ar savienojumu **19**, un inhibitors **20** uzrāda selektivitāti attiecībā pret Cat D (1.13. att.).[57] Noskaidrots, ka 2-indanola hidroksilgrupas ir iesaistītas iekšmolekulāro ūdeņraža saišu veidošanā.[56]



1.13. att. **1,2-Dihidroksietilēna atvasinājumi 19-23**

Inhibitorā aktivitāte uz Plm II ir augstāka, ja molekulas P1 / P1' pozīcijā ir *para*-acetilfenilgrupa (savienojums **21** uzrāda visaugstāko inhibitoro aktivitāti K_i

Plm II = 6 nM). Būtiski uzlabojas arī 1,2-dihidroksietilēna atvasinājuma **21** inhibitorā aktivitāte inficētu asins šūnu testā (*Pf* inhibēšana 78 % @ 5 μM), salīdzinot ar savienojuma **20** inhibēšanas spēju. Aizvietotāji fenilgredzena *para*-pozīcijā būtiski ietekmē inhibitoro aktivitāti. Neaizvietota fenilgredzena gadījumā **22** K_i Plm II = 29 nM, bet *para*-metilgrupu saturošs inhibitors **23** uzrāda 20 reizes zemāku inhibitoro aktivitāti uz Plm II kā savienojums **21** (1.13. att.).[57] Pētījuma ietvaros ir sintezēti arī savienojumi **24** un **26** ar atšķirīgiem amīda bioizostēriem: brīvi rotējošu diacilhidrazīna elementu (var iesaistīties ūdeņraža saišu veidošanā) un 1,3,4-oksadiazolu (var darboties kā ūdeņraža saišu akceptors) (1.14. att.).[58]

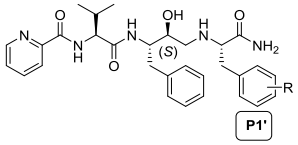


1.14. att. **1,2-Dihidroksietilēna atvasinājumi ar amīda bioizostēriem – diacilhidrazīna fragmentu 24, 25 un 1,3,4-oksadiazolu 26**

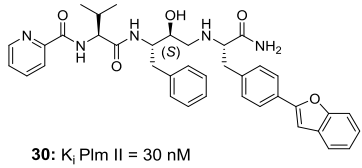
Rezultāti liecina, ka inhibitoro aktivitāti uz Plm II uzrāda tikai hidrazīna fragmentu saturoši savienojumi **24** un **25**. Augstāka inhibitorā aktivitāte ir tajos gadījumos, ja molekula satur diacilhidrazīna fragmentu un aizvietotāji nav simetriski (**25** K_i Plm II = 142 nM, K_i Cat D = >6000 nM). 1,2-Dihidroksietilēna atvasinājumi, kas satur 1,3,4-oksadiazola fragmentu, inhibitoro aktivitāti uz Plm II un Cat D neuzrāda (**26** K_i Plm II = >5000 nM, K_i Cat D = >6000 nM) (1.14. att.).[58]

Hidroksietilamīna atvasinājumi. Vairākas zinātnieku grupas ir strādājušas pie hidroksietilamīna atvasinājumu kā pretmalārijas līdzekļu izveides un to efektivitātes paaugstināšanas.[59], [60] Hidroksietilamīna analogā ir ieviesta bāziska otrējā amīna grupa, kas veidotu sadarbības ar aktīvajā centrā esošajiem Asp34 un Asp214 atlikumiem Plm II struktūrā un koncentrētu inhibitoru parazīta vakuolās (1.15. att.). Ir noskaidrots, ka inhibitorās aktivitātes nodrošināšanā ir svarīgi lielas *meta*- vai *para*-aizvietotas benzilgrupas inhibitora P1' pozīcijā.[9] Piemēram, ja molekula šajā pozīcijā satur neaizvietotu fenilgredzenu, inhibitorā aktivitāte pret Plm II ir zema un nav selektivitātes attiecībā pret Cat D, savienojuma **27** K_i Plm II = 3200 nM, K_i Cat D = >2800 [60], bet

bifenilaizvietotāja gadījumā inhibitora **29** K_i Plm II = 120 nM (1.15. att.). Ir noskaidrots, ka, mainot P1' aizvietotājus, iespējams ietekmēt Plm I / Plm II selektivitāti, piemēram, hidroksietilamīna atvasinājums **28**, kas satur *meta*-fenilaizvietotu benzilgrupu, uzrāda zemu inhibitoro aktivitāti uz Plm I (K_i = 1000 nM).[60], [61]



27: R = *p*-Ph, K_i Plm II = 120 nM
K_i Plm I = 68 nM
K_i Cat D = >2000
28: R = *m*-Ph, K_i Plm II = 579 nM
K_i Plm I = 1000 nM
29: R = H, K_i Plm II = 3200 nM
K_i Cat D = >2800



30: K_i Plm II = 30 nM
K_i Cat D = 1400 nM
Pf inhibēšana 72 % @ 2 μM

1.15. att. **Hidroksietilamīna atvasinājumi 27-30**

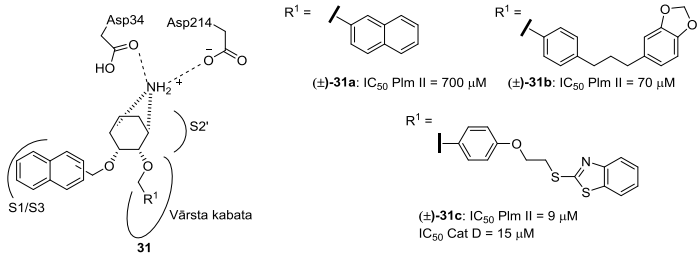
Lai noskaidrotu P3 un P1' pozīciju aizvietotājus, kuri ļauj iegūt augstāku inhibitoro aktivitāti pret Plm II, ir veikts savienojumu bibliotēkas skrīnings. Pētījuma rezultātā ir noskaidrots, ka, ieviešot molekulas P1' pozīcijā lielu, skābekli saturošu biciklu, savienojumu inhibitorā aktivitāte pret Plm II būtiski pieaug (savienojuma **30** K_i Plm II = 30 nM) un inhibitors uzrāda arī aktivitāti inficētu asins šūnu testā.[61] Inhibitora-plazmepsīna II afinitātes nodrošināšanā centrālajai hidroksilgrupai ir jābūt (*S*) konfigurācijā, un terminālais slāpekļa atoms nedrīkst būt aizvietots.[9]

1.6.2. Plazmepsīnu nepeptidomimētiskie inhibitori

Nepeptidomimētisku inhibitoru izstrāde varētu ļaut iegūt savienojumus ar augstāku Plm II / Cat D selektivitāti, jo ir zināms, ka nepeptidomimētiski inhibitori spēj saistīties ar Plm II atvērto vārsta konformāciju, bet katepsīni šādu konformāciju ieņem grūtāk vai neieņem.[62] Vairākas zinātnieku grupas ir identificējušas jaunas nepeptidomimētiskas inhibitoru struktūras, izmantojot savienojumu datubāzu augstas caurlaidības skrīningu (HTS), molekulāro modelēšanu, enzimātiskos testus uz savienojumu bibliotēkām, kā arī savienojumu datubāzu virtuālo skrīningu. Sākotnēji nepeptidomimētisko Plm II inhibitoru identificēšanā, izmantojot augstas caurlaidības skrīningu, tika izmantota Plm II un renīna (cilvēka aspartilproteāze, EC 3.4.23.15) kristālu rentgendifraktometrijas datu līdzība [9], taču šobrīd ir pieejami Plm I [40], II [41], [42], IV [43], V (*Plasmodium vivax*) [44] un strukturāli līdzīgās HAP [45]

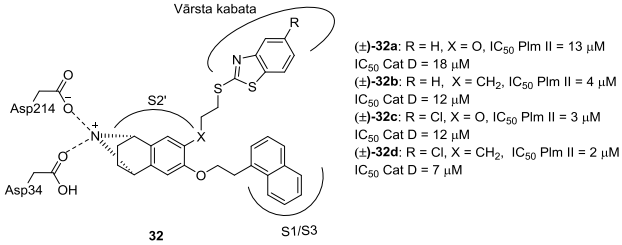
kristālu rentgendifraktometrijas dati, kas ļauj izstrādāt jaunus nepeptidomimētiskus inhibitorus.

Azacikliski inhibitori. Pirmā Plm II un pepstatīna A kompleksa rentgendifraktometrijas dati tika publicēti 1996. gadā.[63] Profesora *F. Diederich* grupa, balstoties uz aizvērtās Plm II konformācijas un renīna atvērtās konformācijas rentgendifraktometrijas datiem, ir izstrādājusi nepeptidomimētiskus, azacikliskus Plm II inhibitorus.[64], [65] Pirmās paaudzes inhibitori **31** satur protonētu centrālo aminogrupu, kura sadarbojas ar aktīvajā centrā esošajiem aspartilatlikumiem, naftilaizvietotāju (R^1), kas ievietojas S1 / S3 kabatā, un heteroaromātisku grupu, kura atrodas vārsta kabatā (1.16. att.).[46] Šie azanorbornāna atvasinājumi **31** uzrāda samērā zemas IC_{50} vērtības uz Plm II ((±)-**31a** IC_{50} Plm II = 700 μM; (±)-**31b** IC_{50} Plm II = 70 μM). Visaugstāko inhibitoro aktivitāti uz Plm II uzrāda savienojums (±)-**31c** (IC_{50} Plm II = 9 μM), bet šis savienojums (±)-**31c** nav selektīvs attiecībā pret cilvēka aspartilproteāzi Cat D (1.16. att.).[65]



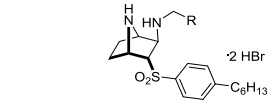
1.16. att. **Azaciklisku kodolu saturoši inhibitori** (±)**-31a-c un shematisks to attēlojums Plm II aktīvajā centrā [46]**

Azacikliski atvasinājumi **32** ir otrās paaudzes azaciklu saturoši inhibitori. Arī šie inhibitori satur protonētu aminogrupu, kas sadarbojas ar aktīvā centra aspartilatlikumiem, naftilaizvietotāju, kas ietilpst S1 / S3 kabatā, un benztiazola ciklu, kas aizņem vārsta kabatu (1.17. att.). Savienojums (±)-**32a** (IC_{50} Plm II = 13 μM) uzrāda līdzīgu inhibitoro aktivitāti uz Plm II kā pirmās paaudzes azanorbornāna atvasinājums (±)-**31c** (IC_{50} Plm II = 9 μM). Augstāka inhibitorā aktivitāte novērojama savienojumiem, kuros X = CH_2 ((±)-**32b** IC_{50} Plm II = 4 μM) vai satur hlora atomu benztiazola ciklā ((±)-**32c** IC_{50} Plm II = 3 μM, (±)-**32d** IC_{50} Plm II = 2 μM). Minētie savienojumi nav selektīvi, jo tiem ir salīdzinoši augsta katepsīna D inhibitorā aktivitāte (IC_{50} Cat D = 7–18 μM) (1.17. att.).[64]



1.17. att. **Azaciklisku kodolu saturoši inhibitori (±)-32a-d [64] un shematisks to attēlojums Plm II aktīvajā centrā [46]**

Šī inhibitoru klase ir attīstīta tālāk, ievadot aminogrupu bicikla 2. pozīcijā, kas veido papildu sadarbību ar aktīvajā centrā esošajiem aspartilatlikumiem. Sulfonilgrupas ģeometrijas dēļ fenilfgrupas alkilķēde tiek ievirzīta vārsta kabatā, tādējādi nodrošinot hidrofobo saistību veidošanos starp inhibitoru un proteīnu. Iegūtais savienojums (±)-**33** (racemāts) uzrāda zemākas IC_{50} vērtības uz plazmepsīnu II (IC_{50} Plm II = 210 nM) nekā otrās paaudzes azacikliskie atvasinājumi **32a-d** (IC_{50} Plm II = 2–13 μM), kā arī ir uzlabojusies selektivitāte attiecībā pret cilvēka aspartilproteāzi Cat D ((±)-**33** IC_{50} Cat D = 2800 nM). Enantiotīrs azanorbornāna atvasinājums (-)-**33a** uzrāda vēl augstāku inhibitoro aktivitāti (IC_{50} Plm II = 45 nM) (1.18. att.).[66], [67]



(±)-**33**, R = *m*-Bromfenil-
IC_{50} Plm II = 210 nM
IC_{50} Cat D = 2800 nM
(±)-**34**, R = Naftil-
IC_{50} Plm II = 130 nM
IC_{50} Cat D = 2030 nM

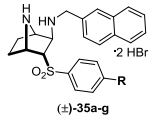
(-)-33a
IC_{50} Plm II = 45 nM

1.18. att. **Azanorbornāna atvasinājumi (±)-33, (-)-33a un (±)-34**

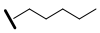
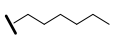
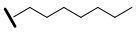
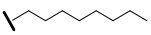
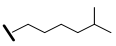
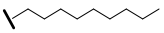
Tā kā savienojums (±)-**28** (IC_{50} Plm II = 130 nM) uzrāda augstāku inhibitoro aktivitāti uz Plm II nekā *meta*-bromfenilgrupu saturošs azanorbornāns (±)-**33** (IC_{50} Plm II = 210 nM), turpmākie pētījumi ir veikti ar naftilaizvietotiem atvasinājumiem. Lai noskaidrotu piemērotāko fenilgrupas aizvietotāja R alkilķēdes garumu un aizvietotāju ietekmi uz inhibitoro aktivitāti, ir sintezēta azanorbornānu (±)-**35a-g** rinda.[68]

1.1. tabula

Savienojumu (±)-35a-f inhibitorās aktivitātes vērtības uz Plm II un Plm IV



(±)-35a-g

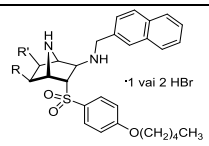
			IC_{50}, nM	
Nr. p. k.	**Sav. nr.**	**R**	**Plm II**	**Plm IV**
1.	(±)-**35a**		4190	1480
2.	(±)-**35b**		680	240
3.	(±)-**35c**		130	50
4.	(±)-**35d**		50	210
5.	(±)-**35e**		70	430
6.	(±)-**35f**		30	90
7.	(±)-**35g**		150	1360

Rezultāti rāda (1.1. tabula), ka plazmepsīna II gadījumā piemērotākais alkilķēdes garums ir no C_6 līdz C_8 (1.1. tabula, 3.–5. rinda). Savukārt plazmepsīna IV gadījumā piemērotākais alkilķēdes garums ir C_6 (1.1. tabula, 3. rinda). Īsāku alkilķēžu gadījumā inhibitorās aktivitātes vērtības pazeminās (1.1. tabula, 1., 2. rinda). Redzams, ka, ievadot molekulā garākas alkilķēdes (1.1. tabula, 7. rinda), būtiskāks inhibitorās aktivitātes kritums novērojams Plm IV gadījumā. Sazarotu alkilaizvietotāju gadījumā (1.1. tabula, 6. rinda) IC_{50} vērtības pazeminājās Plm II gadījumā, bet paaugstinājās attiecībā pret Plm IV. Tas nozīmē, ka atšķiras plazmepsīna II un plazmepsīna IV vārsta kabatas izmērs un forma. Ir noskaidrots, ka svarīgs ir ne tikai alkilķēdes garums, bet arī tas, cik daudz tā aizpilda vārsta kabatu. Rezultāti rāda, ka zemākas IC_{50} vērtības ir tad, ja šis aizpildījums ir 55 ± 9 % no pieejamās telpas.[68] Datormodelēšanas pētījumos noskaidrots, ka *n*-heptilķēde aizņem ~50 % no Plm II vārsta kabatas. Garāku aizvietotāju gadījumā (*n*-oktil-, *n*-nonil-) notiek alkilķēdes salocīšanās, kā rezultātā neveidojas alkilķēdes un Plm II vārsta kabatas sadarbības, un inhibitorā aktivitāte pret Plm II pazeminās. Dažādu funkcionālo grupu (ētera, alkēna, alkinola vai spirta) ievadīšana arilgredzena *para*-pozīcijā inhibitoro aktivitāti uz Plm II un Plm IV neuzlaboja.[46]

Balstoties uz pieejamajiem Plm II kristāla rentgendifraktometrijas datiem, profesora *F. Diederich* grupa ir attīstījusi 7-azanorbornāna atvasinājumus (±)-**36a-f**, kas satur *exo*-aizvietotājus 7-azanorbornāna 5. vai 6. pozīcijā. Šie aizvietotāji spētu aizpildīt plazmepsīna II S1' sub-kabatu.[69]

1.2. tabula

7-Azanorbornāna atvasinājumu (±)-36a-f IC_{50} vērtības uz Plm II, Plm IV un Cat D



(±)-**36a-f**

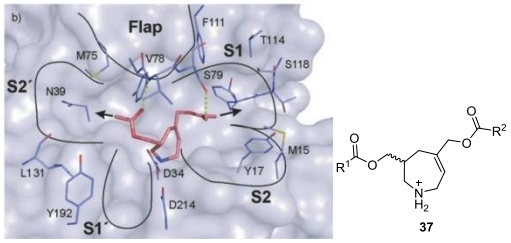
Nr. p. k.	Sav. nr.	R	R'	IC_{50}, nM			IC_{50} (nM)
				Plm II	Plm IV	Cat D	*Pf* NF54
1.	(±)-**36a**		H	72	18	3250	2554
2.	(±)-**36b**		H	107	11	1997	829
3.	(±)-**36c**	H		198	139	6800	nn[a]
4.	(±)-**36d**		H	127	6	2100	1050
5.	(±)-**36e**		H	218	185	2690	nn[a]
6.	(±)-**36f** [b]	H	H	494	223	4900	nn[a]

[a] nn – nav noteikts
[b] references savienojums

6-*exo*-aizvietotāja ievadīšana 7-azanorbornāna molekulā ((±)-**36b**) uzlaboja inhibitoro aktivitāti uz Plm II, Plm IV un Plm II / Cat D selektivitāti, salīdzinot ar 6. pozīcijā neaizvietotu inhibitoru (±)-**36f** (1.2. tabula, 2., 6. rinda). Savukārt 5-*exo*-aizvietotāju gadījumā ((±)-**36c**) inhibitorā aktivitāte uz Plm II bija zemāka nekā 6-*exo*-aizvietotiem 7-azanorbornāna atvasinājumiem ((±)-**36b**) (1.2. tabula, 2., 3. rinda). Plm IV gadījumā inhibitorās aktivitātes kritums ir vēl ievērojamāks (1.2. tabula, 2., 3. rinda). Tas liecina, ka savienojums (±)-**36c** neveido produktīvas sadarbības starp inhibitoru un proteīna S1' sub-kabatu. Cikloalkilamīnu atvasinājumi (±)-**36a**, (±)-**36b** un (±)-**36d** uzrāda visaugstāko inhibitoro aktivitāti gan uz Plm II (IC_{50} = 72–127 nM), gan uz Plm IV (IC_{50} = 6–18 nM) (1.2. tabula, 1., 2., 4. rinda). Vēl ir svarīgi minēt, ka savienojumi (±)-**36a**, (±)-**36b** un (±)-**36d** ir arī selektīvi attiecībā pret cilvēka aspartilproteāzi Cat D, piemēram, inhibitors (±)-**36d** ir 350 reizes aktīvāks pret Plm IV nekā uz

Cat D. Heteroaromātisku aizvietotāju gadījumā 6-*exo* pozīcijā inhibitorā aktivitāte pazeminās (1.2. tabula, 5. rinda). Savienojumi (±)-**36a**, (±)-**36b** un (±)-**36d** uzrāda zemāku *P. falciparum* parazīta augšanas inhibēšanas spēju inficētu asins šūnu testā nekā izolēta enzīma gadījumā (1.2. tabula, 1., 2., 4. rinda).[69]

Tetrahidroazepīna atvasinājumi. Literatūrā ir aprakstīti tetrahidroazepīna ciklu saturoši *P. falciparum* parazīta aspartilproteāžu inhibitori. To struktūra tika modelēta un optimizēta, izmantojot *in silico* aprēķinus.[70] Iespējamais tetrahidroazepīna **37** novietojums proteīnā ir parādīts 1.19. attēlā, kurā redzams, ka protonētā amino grupa veido sadarbības ar aktīvajā centrā esošajiem aspartilatlikumiem, bet 3. un 5. pozīcijas aizvietotāji aizņem S2'un S1 sub-kabatas.

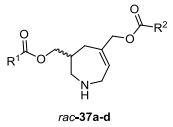


1.19. att. **Tetrahidroazepīna atvasinājuma 37 novietojums proteīnā (dokinga rezultāti) [70]**

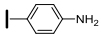
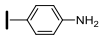
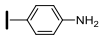
Aprēķini liecina, ka tetrahidroazepīna atvasinājumi **37** saistās ar proteīnu atvērtajā vārsta konformācijā, līdz ar to ir svarīgi atrast piemērotākos aizvietotājus S2' un S1 kabatā. Šim nolūkam pētījuma ietvaros ir sintezēti savienojumi *rac*-**37a-d** ar dažādiem arilaizvietotājiem R^1 un R^2 pozīcijā.[70]

1.3. tabula

Tetrahidroazepīna atvasinājumu *rac*-37a-d K_i vērtības



rac-37a-d

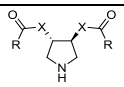
Nr. p. k.	Sav. nr.	R^1	R^2	K_i (μM)		
				Plm II	Plm IV	Cat D
1.	*rac*-**37a**			303	211	>320
2.	*rac*-**37b**			4.7	7.2	260
3.	*rac*-**37c**			1.0	1.5	262
4.	*rac*-**37d**			0.4	5.7	165

No rezultātiem redzams, ka inhibitorās aktivitātes nodrošināšanā būtiska nozīme ir R^1 aizvietotāja arilfunkcijas *para*-pozīcijas aizvietotājiem (1.3. tabula). Neaizvietotu fenilgredzenu gadījumā inhibitorā aktivitāte uz Plm II ir 303 μM (*rac*-**37a**, 1.3. tabula, 1. rinda), bet, ieviešot pozīcijā R^1 *para*-aminofenilgrupu, inhibitorā aktivitāte uz Plm II un Plm IV uzlabojas – K_i Plm II = 4.7 μM, K_i Plm IV = 7.2 μM (*rac*-**37b**, 1.3. tabula, 2. rinda). Tas liecina, ka šajā pozīcijā ir nepieciešama ūdeņraža saišu donora grupa. Savukārt, ieviešot R^2 pozīcijā 3-indolilaizvietotāju, inhibitorā aktivitāte uzlabojas – K_i Plm II = 1.0 μM, kā arī savienojums *rac*-**37c** uzrāda samērā augstu inhibitoro aktivitāti uz Plm IV K_i = 1.5 μM (*rac*-**37c**, 1.3. tabula, 3. rinda). Savienojums *rac*-**37d** ar broma atomu molekulā uzrāda vislabāko rezultātu un sasniedz nanomolāru līmeni – K_i Plm II = 400 nM (1.3. tabula, 4. rinda).[70]

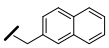
Pirolidīna atvasinājumi. Šīs sērijas savienojumi dizainēti, balstoties uz HIV-1 proteāžu inhibitoriem.[71] Pirolidīna atvasinājumu sadarbības ar plazmepsīnu II ir līdzīgas kā tetrahidroazepīna atvasinājumiem – protonētais pirolidīna slāpekļa atoms veido ūdeņraža saites ar aktīvajā centrā esošajiem aspartilatlikumiem, un estera vai amīda karbonilgrupas skābekļa atoms ar ūdeņraža saitēm sadarbojas ar vārsta aminoskābju atlikumiem Ser79 un Val78. Pētījuma ietvaros ir sintezētas trīs savienojumu klases – simetriski esteri, simetriski amīdi un nesimetriski amīdi.[72]

1.4. tabula

Pirolidīna atvasinājumu 38a-d un 39 K_i vērtības



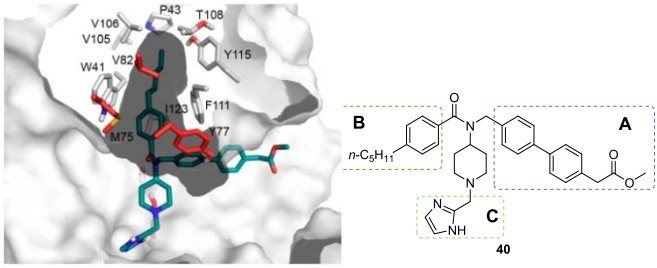
38a-d, 39

Nr. p. k.	Sav. nr.	X	R	K_i (μM)		
				Plm II	**Plm IV**	**Cat D**
1.	**38a**	O		51.2	168	>1000
2.	**38b**	O		11.4	7.4	53.4
3.	**38c**	O		1.0	0.8	0.5
4.	**38d**	O		0.10	0.09	0.37
5.	**39**	NR^1 R^1 =		0.43	1.5	11.7

Savienojumu inhibitorā aktivitāte uz Plm II ir zemāka, ja pirolidīna atvasinājumi **38a-d** satur alkilaizvietotus esterus (**38a**, 1.4. tabula, 1. rinda), taču inhibitorā aktivitāte uzlabojas benzil- un 2-naftilmetil- aizvietotu esteru gadījumā – K_i vērtības attiecīgi ir 11.4 un 1.0 μM (**38b**, **38c**, 1.4. tabula, 2., 3. rinda). Visaugstāko inhibitoro aktivitāti uz Plm II uzrāda 1-naftilmetilgrupu saturošs pirolidīna atvasinājums **38d** (K_i Plm II = 0.10 μM, K_i Plm IV = 0.09 μM) (1.4. tabula, 4. rinda). Ir redzama tendence, ka, palielinoties aizvietotāju stēriskajiem izmēriem, inhibitorā aktivitāte pret Plm II un Plm IV paaugstinās. Acīmredzot stēriski lielāki aizvietotāji var veidot ciešākas hidrofobās sadarbības ar S2 un S2' sub-kabatām. Uzlabojoties savienojumu inhibitorajai aktivitātei uz Plm II, pasliktinās selektivitāte attiecībā pret Cat D.[72] Arī simetriskas amīda grupas saturošs pirolīdina atvasinājums **39** uzrāda inhibitoro aktivitāti pret Plm II (K_i Plm II = 0.43 μM) un Plm IV (K_i Plm IV = 1.5 μM). Sintezētie nesimetriskie amīdi neuzrādīja Plm II / Cat D selektivitāti. Šis pētījumu virziens netika attīstīts tālāk, jo, modificējot pirolidīna atvasinājumus, neizdevās uzlabot Plm II / Cat D selektivitāti.

Aminopiperidīna atvasinājumi. Izmantojot augstas caurlaidības FRET testu, kā plazmepsīna II inhibitori ir identificēti 4-aminopiperidīna atvasinājumi.[12], [23], [42], [73] Šo savienojumu sintēze ir vienkārša un ļauj

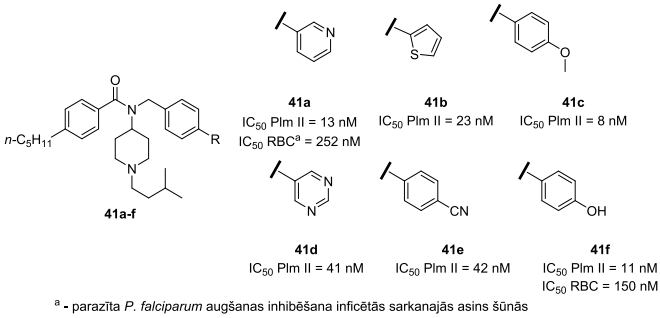
veikt dažādas molekulas modifikācijas. 4-Aminopiperidīna atvasinājuma **40** un Plm II kompleksa rentgenstruktūranalīze atklāj liganda novietojumu proteīnā, un redzams, ka šis inhibitors ir saistījies ar Plm II atvērtajā vārsta konformācijā, kurā vārsta cilpa ir nobīdījusies no aktīvajā centrā esošajiem aspartilatlikumiem, tādējādi ļaujot piperidīna slāpeklim izveidot spēcīgas ūdeņraža saites caur katalītisko ūdens molekulu ar aspartilatlikumiem (1.20. att.).[26], [42]



1.20. att. **4-Aminopiperidīna atvasinājuma 40 un Plm II kompleksa rentgendifraktometrijas dati [23]**

Pētījumā noskaidrots, ka inhibitorās aktivitātes nodrošināšanā svarīgs ir molekulas **B** daļas benzoilaizvietotājs ar *n*-pentilķēdi *para*-pozīcijā (1.20. att.). 4-Metilfenilaizvietotāja gadījumā inhibitorā aktivitāte samazinās gandrīz 2000 reižu.[42] Tāpat šajā molekulas daļā nav vēlams ievadīt nepiesātinātas saites (alkenil-, alkinil-) vai heteroatomus, bet inhibitorā aktivitāte nemainās, ja fenilgrupu aizstāj ar 4-*n*-pentilcikloheksilgrupu.[12], [42]

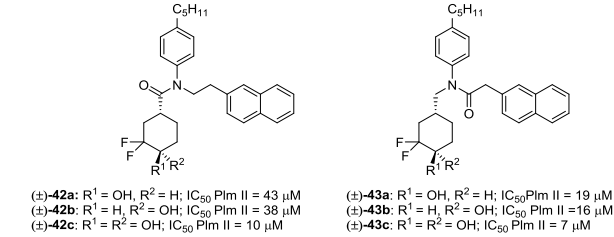
Savukārt molekulas **A** daļā piemērotākais S1 / S3 kabatā ietilpstošais aizvietotājs ir *para*-pozīcijā aizvietota bifenilgrupa. Pētījumā izmantotie savienojumi **41a-f** ar dažādiem fenilgrupas aizvietotājiem *para*-pozīcijā redzami 1.21. attēlā. Ievadot molekulā heteroaromātiskus ciklus – piridīnu (**41a**), pirimidīnu (**41d**), tiofēnu (**41b**), inhibitorā aktivitāte būtiski nemainās (IC_{50} Plm II = 13–41 nM) (1.21. att.). Zemāku inhibitoro aktivitāti uzrāda 4-aminopiperidīna atvasinājums **41e**, kas fenilgredzenā satur elektronus atvelkošu cianogrupu, savukārt elektronu donoru grupu gadījumā (**41c**, **41f**) inhibitorā aktivitāte ir augstāka (IC_{50} Plm II = 8–11 nM). Noskaidrots, ka molekulas **C** daļā ir vēlams 3-metilbutilaizvietotājs (1.21. att.). Zināms, ka piperidīna ciklā esošais slāpekļa atoms veido stipru ūdeņraža saiti ar katalītiskajā centrā esošo ūdens molekulu, tādējādi nodrošinot sadarbību ar Plm II aspartilatlikumiem. Biarilaizvietotājs veido hidrofobas sadarbības ar plazmepsīnu II, bet benzoilaizvietotājs nodrošina ideālu novietojumu, lai *n*-pentilķēde ieņemtu vārsta kabatu.[23], [73]



1.21. att. **4-Aminopiperidīna atvasinājumi 41a-f ar dažādiem fenilgrupas aizvietotājiem**

Būtiski, ka savienojumi **41a** un **41f** uzrāda zemākas inhibitorās aktivitātes vērtības inficētu asins šūnu testā, kas var liecināt par to, ka zāļu mērķis nav Plm I, Plm II vai Plm IV, bet varētu būt Plm IX, Plm X.[12]

***α,α*-Difluorcikloheksanona atvasinājumi.** Veicot 4-aminopiperidīna atvasinājumu bioizostēro aizvietošanu, profesora *F. Diederich* grupa sintezējusi *α,α*-difluorcikloheksanona atvasinājumus (±)-**42c** un (±)-**43c** (1.22. att.). Izmantojot ^{19}F-kodolu magnētiskās rezonanses spektroskopiju, noskaidrots, ka ūdens šķīdumā šie savienojumi pilnībā hidratējas, tādēļ IC_{50} vērtības ir noteiktas to hidrātiem.[74] Lai noskaidrotu hidroksigrupu nozīmi inhibitorās aktivitātes nodrošināšanā pret Plm II, ir sintezēti arī monohidroksiatvasinājumi (±)-**42a,b** un (±)-**43a,b** (1.22. att.).

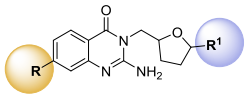


1.22. att. ***α,α*-Difluorcikloheksanona atvasinājumu (±)-42a-c, (±)-43a-c IC_{50} vērtības**

Pētījuma rezultātā noskaidrots, ka inhibitori (±)-**43a-c**, kuros amīda funkcijas karbonilgrupa ir orientēta S1 / S3 kabatas virzienā, ir aktīvāki nekā inhibitori (±)-**42a-c**, kuros *N* atoms ir orientēts S1 / S3 kabatas virzienā. Datormodelēšanas rezultāti liecina, ka inhibitoru (±)-**42a-c** amīda grupas karbonilgrupa neiesaistās ūdeņraža saišu veidošanā, savukārt savienojumu (±)-**43a-c** gadījumā amīda grupas karbonilgrupa veido ūdeņraža saites ar plazmepsīna II Tyr77 atlikumu.

No iegūtajiem rezultātiem redzams, ka salīdzināmas inhibitorās aktivitātes pret Plm II uzrāda monohidroksiatvasinājumi (±)-**42a**, (±)-**42b** (IC_{50} Plm II = ~40 μM) un (±)-**43a**, (±)-**43b** (IC_{50} Plm II = ~20 μM). Augstāku inhibitoro aktivitāti uzrāda pilnībā hidratēti *α*,*α*-difluorcikloheksanona atvasinājumi (±)-**42c** (IC_{50} Plm II = 10 μM) un (±)-**43c** (IC_{50} Plm II = 7 μM) (1.22. att.).[74] Pētījuma ietvaros ir sintezēti arī citi *α*,*α*-difluorcikloheksanona atvasinājumi, taču tie neuzrāda labāku inhibitoro aktivitāti uz Plm II.[75]

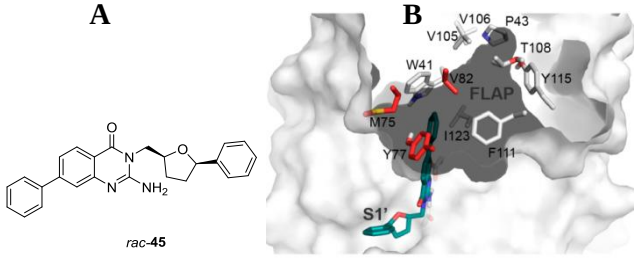
2-Aminohinazolīn-4-(3*H*)-ona atvasinājumi. 2-Aminohinazolīn-4-(3*H*)-ona atvasinājumi *rac*-**44** kā plazmepsīnu inhibitori ir identificēti, veicot *ChemBridge* fragmentu bibliotēkas skrīningu, izmantojot KMR metodi. Pētījuma laikā tika noskaidrots, ka 2-aminohinazolīn-4-(3*H*)-ona 7. pozīcijā un THF grupas 5. pozīcijā nepieciešams ievadīt fenilgrupu (1.23. att.).[41]



rac-**44**: R, R^1 = H, IC_{50} Plm II = 24.3 μM
rac-**45**: R = Ph, R^1 = Ph, IC_{50} Plm II = 0.57 μM

1.23. att. **2-Aminohinazolīn-4-(3*H*)-ona atvasinājumi *rac*-44, *rac*-45 kā plazmepsīnu inhibitori**

Savienojums *rac*-**45** uzrāda 42 reizes augstāku inhibitoro aktivitāti uz Plm II (IC_{50} = 0.57 μM) salīdzinājumā ar neaizvietotu hinazolinonu *rac*-**44** un ir aktīvs arī uz Plm IV (IC_{50} = 0.60 μM) (1.24. att. (**A**)). Ir iegūti inhibitora *rac*-**45** un Plm II kompleksa rentgendifraktometrijas dati (1.24. att. (**B**)).[41]



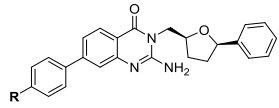
1.24. att. **A – Inhibitora *rac*-45 struktūra; B – 2-Aminohinazolīn-4-(3*H*)-ona atvasinājuma *rac*-45 un Plm II kompleksa rentgendifraktometrijas dati [41]**

Analizējot inhibitora *rac*-**45** un Plm II kompleksa rentgendifraktometrijas datus, ir noskaidrots, ka Plm II satur plaši atvērtu, neaizpildītu vārsta kabatu, kuru ir iespējams aizpildīt, ievadot lineāru, alifātisku aizvietotāju fenilgrupas

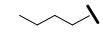
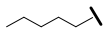
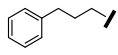
para-pozīcijā (1.24. att. (**B)**). Tālākā inhibitora struktūras optimizācija ir veikta, ievadot dažādus aizvietotājus 2-aminohinazolīn-4-(3*H*)-ona fenilgrupas *para*-pozīcijā.[41]

1.5. tabula

2-Aminohinazolīn-4-(3*H*)-ona atvasinājumu *rac*-45, *rac*-46a-d struktūras-aktivitātes likumsakarības



rac-**46a-d**

Nr. p. k.	Sav. nr.	R	IC_{50}, μM			EC_{50} *Pf* 3D7, μM
			Plm II	Plm IV	Cat D	
1.	*rac*-**45**	H	0.57	0.60	13.8	nn*
2.	*rac*-**46a**		0.23	0.2	2.2	0.9
3.	*rac*-**46b**		0.15	0.10	5.0	1.1
4.	*rac*-**46c**		0.40	0.30	3.2	nn*
5.	*rac*-**46d**		10.0	0.13	6.0	1.2

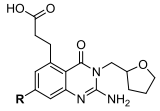
*nn – nav noteikts

Ievadot 2-aminohinazolīn-4-(3*H*)-ona fenilgredzena *para*-pozīcijā *n*-butil- vai *n*-pentil- grupu (*rac*-**46a**,**b**), inhibitorā aktivitāte uz Plm II uzlabojas divas (*rac*-**46a**, 1.5. tabula, 2. rinda) un trīs reizes (*rac*-**46b**, 1.5. tabula, 3. rinda) salīdzinājumā ar *para*-pozīcijā neaizvietotu fenilgredzenu (*rac*-**45**, 1.5. tabula, 1. rinda). Redzams, ka ciklisku alkilaizvietotāju (sav. *rac*-**46c**) gadījumā inhibitorā aktivitāte būtiski nemainās (1.5. tabula, 4. rinda). Inhibitors *rac*-**46d** ar 3-fenilpropilgrupu fenilgredzena *para*-pozīcijā uzrāda samērā augstu inhibitoro aktivitāti pret Plm IV (IC_{50} = 0.13 μM), taču zemāku aktivitāti pret Plm II (IC_{50} = 10.0 μM) (1.5. tabula, 5. rinda). Molekulārās modelēšanas pētījumi rāda, ka Plm IV vārsta kabata ir mazliet lielāka kabatas dziļumā, tādējādi stēriski lieli aizvietotāji spēj piesaistīties daudz efektīvāk. Savienojumi *rac*-**46a**,**b**,**d** inficētu asins šūnu testā uzrāda parazīta augšanas inhibēšanas spēju mikromolārā līmenī (1.5. tabula, 2., 3., 5. rinda). Interesanti, ka inhibitora *rac*-**46d** uzrādītā aktivitāte inficētu asins šūnu testā ir augstāka nekā izolēta enzīma gadījumā (Plm II). Tas nozīmē, ka īstais zāļu mērķis varētu būt plazmepsīnam IV strukturāli līdzīgie Plm IX un Plm X.[23], [41]

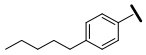
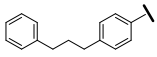
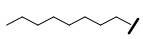
Lai samazinātu inhibitora lipofilitāti un uzlabotu selektivitāti attiecībā pret Cat D, ir veikta 2-aminohinazolīn-4-(3*H*)-ona atvasinājumu struktūras optimizācija. Rezultātā ir iegūts inhibitors *rac*-**47a**, kas satur neaizvietotu THF grupu un karboksietilgrupu 2-aminohinazolīn-4-(3*H*)-ona 5. pozīcijā. Savienojums *rac*-**47a** uzrāda salīdzināmu inhibitoro aktivitāti ar savienojumu *rac*-**45** pret Plm II un Plm IV, bet ir daudz selektīvāks attiecībā pret cilvēka aspartilproteāzi Cat D (1.5. tabula, 1. rinda un 1.6. tabula, 1. rinda).

1.6. tabula

2-Aminohinazolīn-4-(3*H*)-ona atvasinājumu *rac*-47a-d struktūras-aktivitātes likumsakarības



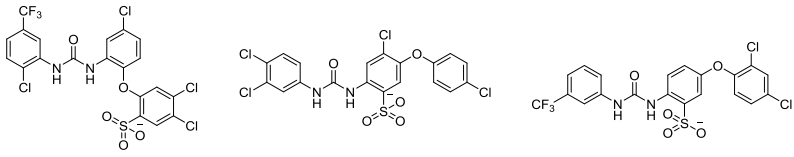
rac-**47a-d**

Nr. p. k	Sav. nr.	R	IC_{50}, μM		
			Plm II	**Plm IV**	**Cat D**
1.	*rac*-**47a**		0.50	6.0	>100
2.	*rac*-**47b**		0.027	0.042	21.0
3.	*rac*-**47c**		0.080	0.030	13.0
4.	*rac*-**47d**		0.022	0.110	0.43

Ievadot 2-aminohinazolīn-4-(3*H*)-ona molekulā dažādus fenilgrupas aizvietotājus – *n*-pentil- (*rac*-**47b**), fenilpropil- (*rac*-**47c**) un *n*-oktil- grupu (*rac*-**47d**) (1.6. tabula, 2.–4. rinda) –, ir būtiski paaugstinājusies inhibitorā aktivitāte uz Plm II (IC_{50} = 0.022–0.080 μM) un Plm IV (IC_{50} = 0.030–0.110 μM).[23], [76] Jāpiebilst, ka savienojums *rac*-**47b** uzrāda augstu Plm II / Cat D selektivitāti (IC_{50} Plm II = 0.027 μM, IC_{50} Cat D = 21.0 μM).

Difenilurīnvielas atvasinājumi. Izmantojot *Walter Reed* savienojumu datubāzes skrīningu, kā Plm II inhibitori ir identificēti difenilurīnvielas atvasinājumi **48**-**50**. Būtiski, ka šie savienojumi uzrāda inhibitoro aktivitāti uz Plm II nanomolārā līmenī (K_i Plm II = 50–680 nM) un ir selektīvi attiecībā pret cilvēka aspartilproteāzi Cat D – savienojumu **48**-**50** inhibitorā aktivitāte uz Plm II ir vairāk nekā 1000 reižu augstāka nekā uz Cat D (1.25. att.). Difenilurīnvielas atvasinājums **48** ir šīs savienojumu sērijas aktīvākais savienojums. Lai gan savienojumi **48**-**50** uzrāda spēju inhibēt Plm II nanomolārā līmenī, parazīta

P. falciparum parazīta augšanu tie inhibē vāji (IC_{50} vērtības ir augstākas par 6 μg / mL).[77]



48: K_i Plm II = 0.05 μM
K_i Cat D = 83 μM
IC_{50} *Pf* W2 = 15 μg/mL

49: K_i Plm II = 0.19 μM
K_i Cat D = 191 μM
IC_{50} *Pf* W2 = 20 μg/mL

50: K_i Plm II = 0.11 μM
K_i Cat D = 157 μM
IC_{50} *Pf* W2 = 21 μg/mL

1.25. att. **Difenilurīnvielas atvasinājumi 48-50 kā Plm II inhibitori**

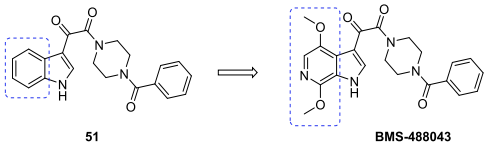
Molekulārās modelēšanas pētījumā ir noskaidrots, ka urīnvielas funkcionālās grupas slāpekļa atomi sadarbojas ar Plm II aktīvajā centrā esošajiem aspartilatlikumiem Asp34 un Asp214 un feniloksi- fragments aizņem S2' kabatu.[77]

Plazmepsīnu (I, II, HAP, IV, V (*Plasmodium vivax*)) rentgendifraktometrijas datu publicēšana ir ļāvusi dizainēt un identificēt jaunus plazmepsīnu inhibitorus. Ir sintezēti gan peptidomimētiski, gan nepeptidomimētiski inhibitori. Plazmepsīnu inhibitoru izstrāde ir apgrūtināta vairāku iemeslu dēļ – inhibitoram ir jāspēj inhibēt vairākus plazmepsīnu subtipus un inhibitoram ir jābūt selektīvam attiecībā pret cilvēka aspartilproteāzi katepsīnu D. Kā arī bieži nav novērojama rezultātu korelācija starp savienojumu inhibitoro aktivitāti uz Plm II un parazīta augšanas inhibēšanas spēju inficētu asins šūnu testā. Tas nozīmē, ka īstais zāļu mērķis varētu būt negremošanas plazmepsīni IX un X, kuri strukturāli visvairāk ir līdzīgi Plm IV.

2. REZULTĀTI UN TO IZVĒRTĒJUMS

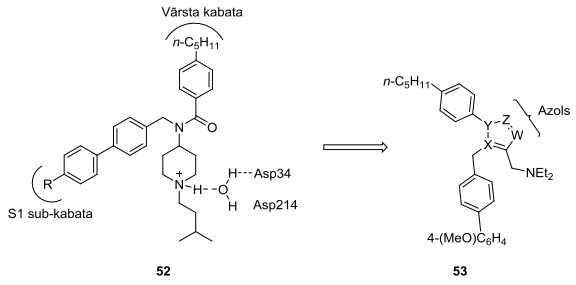
2.1. Nepeptidomimētisku Plm II inhibitoru izstrāde

Viena no pieejām jaunu zāļvielu izveidē ir jau zināma bioloģiski aktīva savienojuma bioizostēru sintēze – molekulā esošo atomu, funkcionālo grupu vai ciklu aizvietošana ar citiem atomiem vai atomu grupām, saglabājot līdzīgas ķīmiskās vai fizikālķīmiskās īpašības un bioloģisko efektu.[78] Šī pieeja ir veiksmīgi izmantota HIV-1 inhibitoru izstrādē (2.1. att.) – piridīna cikla ieviešana savienojuma **51** molekulā ļāva saglabāt tā inhibitoro aktivitāti pret HIV-1, uzlabojot savienojuma farmakokinētiskās īpašības.[79]



2.1. att. **Fenilgrupas bioizostērā aizvietošana ar piridīna ciklu**

Bioizostērās aizvietošanas pieeju izmantojām jaunu nepeptidomimētisku Plm II inhibitoru izstrādē, par pamatu ņemot *Actelion* zinātnieku izstrādātos nepeptidomimētiskos Plm II inhibitorus **52**, kuru pamatstruktūru veido 4-aminopiperidīna grupa.[12], [26], [42] Ir noskaidrots, ka aminopiperidīna tipa inhibitori saistās ar enzīma atvērto vārsta konformāciju.[26] Inhibitora-enzīma kompleksa rentgendifraktometrijas datu analīze atklāja, ka piperidīna slāpekļa atoms veido jonu tipa sadarbību ar aktīvajā centrā esošo Asp214 atlikumu un ūdens molekulas mediētu ūdeņraža saiti ar katalītisko atlikumu Asp34. Molekula satur arī citus svarīgus farmakoforos elementus – bifenilgrupu (atrodas S1 sub-kabatā) un *n*-pentilķēdi (atrodas vārsta kabatā) (2.2. att.).[26]

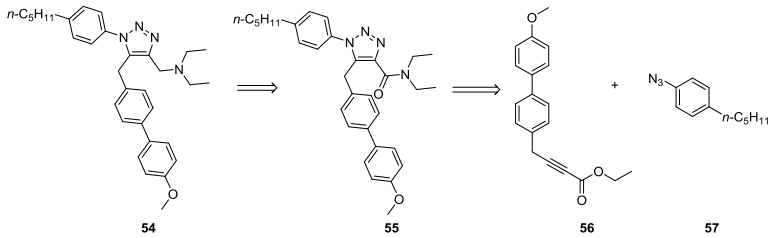


2.2. att. **Nepeptidomimētisko inhibitoru dizainēšana**

4-Aminopiperidīna inhibitoriem piemīt trūkumi – tie satur enzimātiski nestabilu amīdsaiti, kas neļauj šos savienojumus izmantot kā orāli pieejamus medikamentus.[80] Minētie inhibitori **52** uzrāda nepietiekoši labas fizikālķīmiskās īpašības – augstas clogP vērtības un zemu šķīdību, kā arī savienojumu **52** aktivitāte inficētu sarkano asins šūnu testā ir 2.5 līdz 20 reizes zemāka nekā izolēta enzīma gadījumā.[12] Lai izvairītos no minētajiem trūkumiem, izstrādājām jaunu nepeptidomimētisku inhibitoru dizainu, kas balstīts uz amīda funkcijas aizvietošanu savienojumā **52** ar tā bioizostēriem – azoliem. Nolēmām sintezēt amīda grupas bioizostērus – 1,2,3-triazolu, izoksazolu, imidazolu un pirolu saturošus Plm II inhibitorus, saglabājot jau esošos farmakoforos elementus – *n*-pentilķēdi, aminofunkciju un bifenilaizvietotāju (2.2. att.).

2.1.1. Azolu atvasinājumu sintēze un to struktūras-aktivitātes likumsakarības

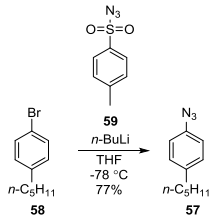
1,2,3-Triazolu sintēzē visbiežāk tiek izmantota *Huisgen* 1,3-dipolārā ciklopievienošanās reakcija starp azīdiem un alkīniem.[81]–[83] Reakcijas norise iespējama termiskos apstākļos, bet bieži tiek izmantoti varu (I) (veicina 1,4- aizvietotu triazolu veidošanos) vai rutēniju (II) saturoši katalizatori (veicina 1,5- un 1,2,3- aizvietotu triazolu veidošanos).[83] Triazola atvasinājuma **55** sintēzē nolēmām izmantot 1,3-dipolāru ciklopievienošanās reakciju starp alkīna fragmentu **56** un 4-*n*-pentilfenilazīdu (**57**) (2.3. att.).



2.3. att. **1,2,3-Triazola 54 retrosintēzes shēma**

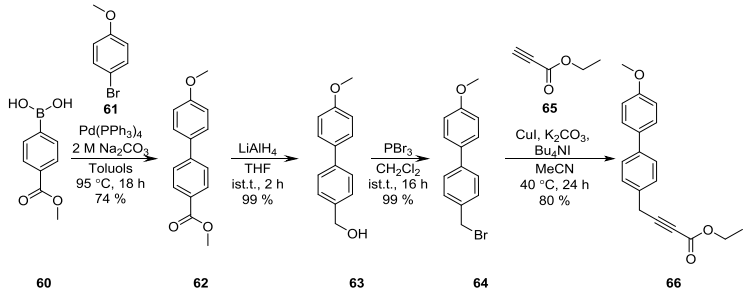
Fenilazīda **57** iegūšanai sākotnēji mēģinājām izmantot literatūrā [84] aprakstītu metodi fenilazīdu iegūšanai no fenilhalogenīdiem, izmantojot vara (I) katalizētu reakciju ar NaN_3 nātrija askorbāta un *N*,*N*-dimetiletilēndiamīna klātienē. Substrāta **58** gadījumā, izmantojot aprakstītos reakciju apstākļus, vēlamais azidoatvasinājums **57** neveidojās. 4-Pentilfenilazīda (**57**) sintēzē izmantojām azīda pārneses reaģentu – tozilazīdu **59**, kas ir viegli iegūstams no

tozilhlorīda un NaN_3.[85] Tozilazīdu **59** tālāk izmantojām fenilazīda **57** sintēzē no attiecīgā bromīda **58** litijēšanas reakcijā.[86] Produktu **57** ieguvām ar 77 % iznākumu (2.4. att.).



2.4. att. **4-Pentilfenilazīda (57) iegūšana**

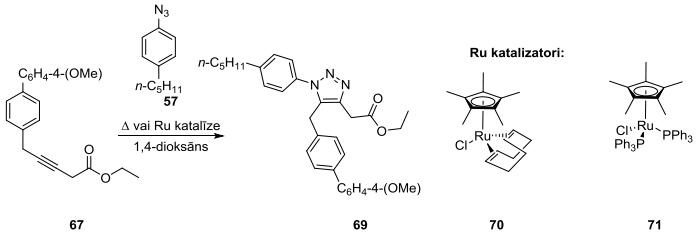
Alkīna fragmenta iegūšanai kā izejvielu izmantojām bifenilatvasinājumu **64**, kuru sintezējām 3 stadiju procesā (2.5. att.). Sākumā *Suzuki-Miyaura* šķērssametināšanas reakcijā no 4-bromanisola (**61**) un 4-metoksikarbonilborskābes (**60**) $Pd(PPh_3)_4$ un Na_2CO_3 klātienē ieguvām savienojumu **62**. Tālāk ar $LiAlH_4$ reducējām estera grupu un ieguvām benzilspirtu **63**.[87] Benzilspirtam **63** reaģējot ar fosfora tribromīdu (metilēnhlorīds, istabas temperatūra, 16 stundas), ieguvām 4'-brommetil-4-metoksibifenilu (**64**), kuru tālāk izmantojām alkīna **66** iegūšanai. Trīskāršās saites ievadīšanai sākotnēji izmantojām trimetilsililacetilēnu vara (I) bromīda un *i*-PrMgCl klātienē [88], taču nebija novērojama produkta **66** veidošanās. Turpretim bromīda **64** reakcija ar etilpropiolātu (**65**) vara (I) jodīda, K_2CO_3 un tetrabutilamonija jodīda klātienē (acetonitrils, 40 °C, 24 stundas) [89] ļāva iegūt produktu **66** ar teicamu (80 %) iznākumu (2.5. att.).



2.5. att. **4'-Brommetil-4-metoksibifenila (66) sintēze**

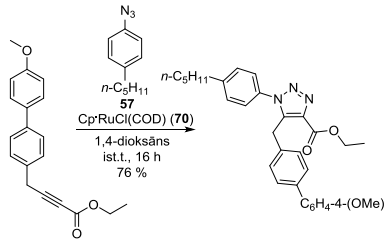
Lai noskaidrotu piemērotākos apstākļus *Huisgen* 1,3-dipolārai ciklopievienošanās reakcijai, izmantojām modeļsavienojumus alkīnu **67** un azīdu

57 (2.6. att.). Sākotnējie mēģinājumi iegūt 1,2,3-triazola atvasinājumu **69** termiskos apstākļos bija neveiksmīgi, ciklopievienošanās nenotika, un reakcijas maisījumā bija neizreaģējušas izejvielas **67** un **57**. Reakciju veicām toluolā 90 °C temperatūrā 16 stundas, pēc tam temperatūru paaugstinājām līdz 145 °C, taču vēlamais produkts **69** neveidojās. Arī nomainot šķīdinātāju uz 1,4-dioksānu un sildot 100 °C, produkta **69** veidošanos ar AEŠH analīzi nenovērojām. Tādēļ nolēmām pētījumus turpināt, izmantojot rutēnija (II) katalizētu ciklopievienošanos (2.6. att.). Šajā reakcijā izmantojām katalizatorus – Cp·RuCl(COD) (**70**) un Cp·RuCl$(PPh_3)_2$ (**71**).[83] Reakciju veicām ar 2 mol% katalizatora 1,4-dioksānā, 60 °C, 16 stundas. Cp·RuCl(COD) (**70**) gadījumā reakcijas maisījumā saskaņā ar AEŠH analīzi bija novērojama 1,2,3-triazola veidošanās (konversija 2 %), bet, izmantojot Cp·RuCl$(PPh_3)_2$ (**71**), triazols **69** neveidojās. Turpmāko reakcijas optimizēšanu veicām ar katalizatoru Cp·RuCl(COD) (**70**). Paaugstinot temperatūru līdz 110 °C, triazola **69** iznākums neuzlabojās, jo notiek azīda **57** sadalīšanās. Tādēļ palielinājām katalizatora **70** daudzumu līdz 10 mol% un, veicot reakciju istabas temperatūrā 16 stundas, saskaņā ar AEŠH analīzi konversija sasniedza 60 % (2.6. att.).

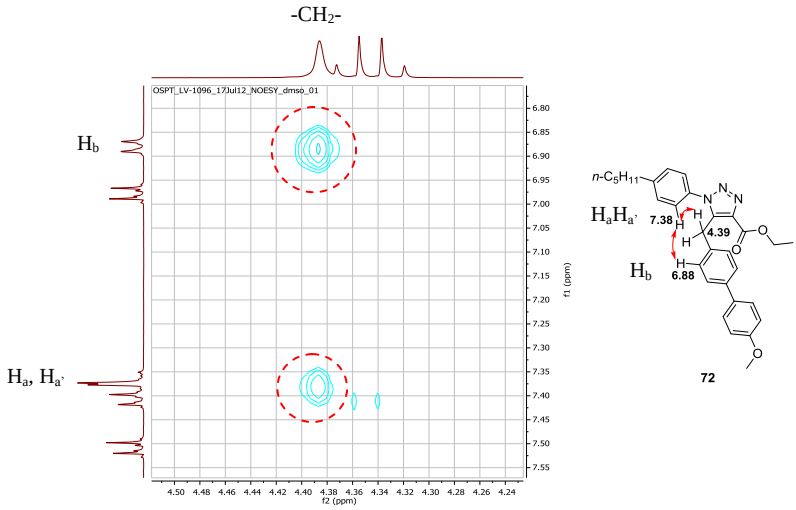


2.6. att. **1,2,3-Triazola 69 sintēze termiskos apstākļos un izmantojot Ru katalizatorus 70 un 71**

Alkīnu **66** izmantojām triazola atvasinājuma **72** sintēzē. Triazola **72** iegūšanai no alkīna **66** un 4-pentilfenilazīda (**57**) izmantojām uz modeļsavienojuma **67** piemeklētos reakciju apstākļus rutēnija (II) katalizētai 1,3-dipolārai ciklopievienošanās reakcijai. Alkīna **66** gadījumā reakcijā veidojās viens produkta reģioizomērs – 1,2,3-triazolu **72** ieguvām ar 76 % iznākumu (2.7. att.). Tā struktūras pierādīšanai izmantojām divdimensionālo HMBC kodolu magnētiskās rezonanses spektru – NOESY un HMBC – analīzi (2.8. att.).



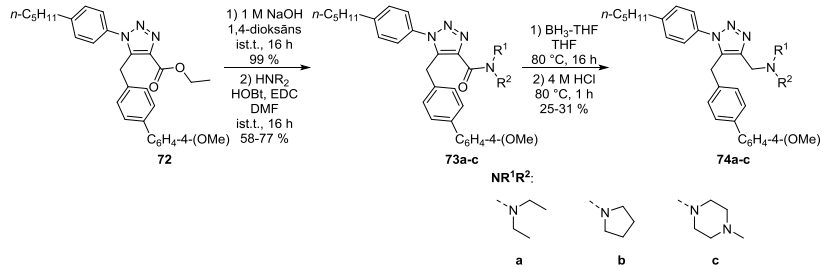
2.7. att. **1,2,3-Triazola atvasinājuma 72 iegūšana**



2.8. att. **1,2,3-Triazola atvasinājuma 72 NOESY spektra fragments**

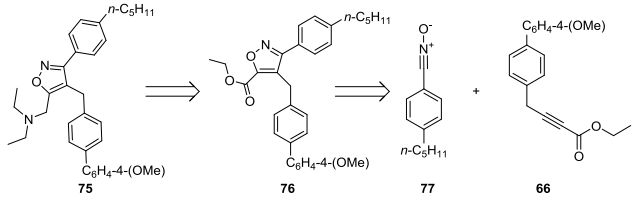
Triazola **72** NOESY spektrā redzama sadarbība starp metilēngrupas protoniem (δ = 4.39 ppm) un aromātiskā gredzena protoniem Ha, Ha' (δ = 7.38 ppm), kā arī starp aromātisko gredzenu protoniem Ha, Ha' un Hb (δ = 6.88 ppm un δ = 7.38 ppm). Redzamās sadarbības apstiprina savienojuma **72** struktūru (2.8. att.).

Tālāk mērķsavienojumu **74a-c** iegūšanai hidrolizējām estera grupu savienojumā **72** ar 1 M NaOH, iegūstot attiecīgo karbonskābi. Tad, izmantojot HOBt / EDC kā kondensējošos aģentus, sintezējām amīdus **73a-c**. Izmantojot amīda **73a** reducēšanai $LiAlH_4$ (THF, istabas temperatūra), veidojās sarežģīts produktu maisījums, tādēļ tālākajā darbā amīdu **73a-c** reducēšanai lietojām BH_3-THF kompleksu. Reakcijas rezultātā radās amīna-borāna komplekss, kuru apstrādājot ar 4 M HCl ūdens šķīdumu (80 °C, 1 stunda), ieguvām amīnus **74a-c** (2.9. att.).



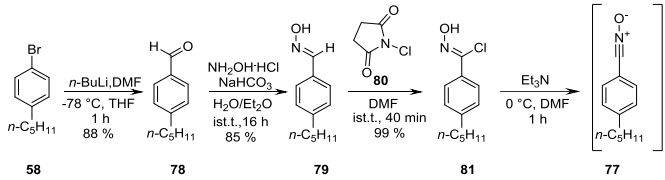
2.9. att. **Mērķsavienojumu 74a-c sintēze**

Izoksazola cikla sintēzē visbiežāk izmanto 1,3-dipolāru ciklopievienošanās reakciju starp alkīnu un nitrila *N*-oksīdu.[90] Izoksazola atvasinājumu **76** plānojām sintezēt no benzonitrila *N*-oksīda **77** un alkīna **66** saskaņā ar 2.10. attēlā parādīto retrosintēzes shēmu.



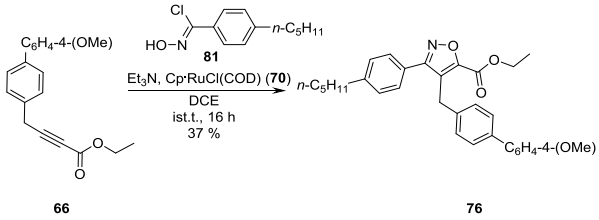
2.10. att. **Izoksazola atvasinājuma 75 retrosintēzes shēma**

Benzonitrila *N*-oksīdu **77** ieguvām 4 stadiju sintēzē no komerciāli pieejamā bromīda **58**. Sākotnēji savienojumā **58** veicām broma-litija apmaiņu ar *n*-BuLi un tai sekojošu DMF pievienošanu, iegūstot aldehīdu **78** (2.11. att.).[91] Sintezēto aldehīdu **78** tālāk izmantojām kondensācijas reakcijā ar hidroksilamīna hidrohlorīdu ($NaHCO_3$, H_2O / Et_2O maisījums, 16 stundas), iegūstot oksīmu **79** ar 85 % iznākumu. *N*-Hidroksibenzimidoilhlorīdu **81** ieguvām, oksīmam **79** lēni piepilinot *N*-hlorsukcīnimīda (**80**) šķīdumu dimetilformamīdā (0 ºC, pēc tam istabas temperatūra 40 minūtes).[92] No literatūras [93] ir zināms, ka oksīma hlorīda atvasinājumi mēdz būt nestabili, tādēļ savienojumu **81** sintezējām tieši pirms tālākās reakcijas veikšanas un izmantojām bez papildu attīrīšanas. *N*-Hidroksibenzimidoilhlorīdu **81** ieguvām ar kvantitatīvu iznākumu. Tā kā sintēzes rezultātā noskaidrojām, ka nitrila *N*-oksīds **77** ir nestabils un sadalās, arī uzglabājot pazeminātā temperatūrā, tad nolēmām to ģenerēt *in situ* ciklopievienošanās reakcijas laikā (2.11. att.).



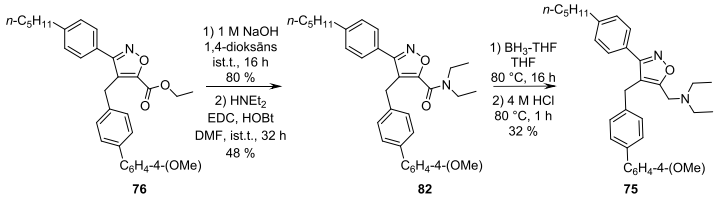
2.11. att. **Benzonitrila *N*-oksīda 77 sintēze**

Izoksazola **76** sintēzē izmantojām iepriekš optimizētos reakcijas apstākļus – alkīna **66** un *N*-hidroksibenzimidoilhlorīda **81** šķīdumam dihloretānā pievienojām Cp·RuCl(COD) (**70**) un lēni piepilinājām trietilamīnu. Iegūto šķīdumu maisījām istabas temperatūrā 16 stundas. Šajos reakcijas apstākļos rodas viens izoksazola **76** izomērs (2.12. att.). Izoksazola esteri **76** ieguvām ar 37 % iznākumu un savienojuma **76** struktūru pierādījām, izmantojot divdimensionālos NOESY un HMBC kodolu magnētiskās rezonanses spektrus.



2.12. att. **Izoksazola 76 iegūšana**

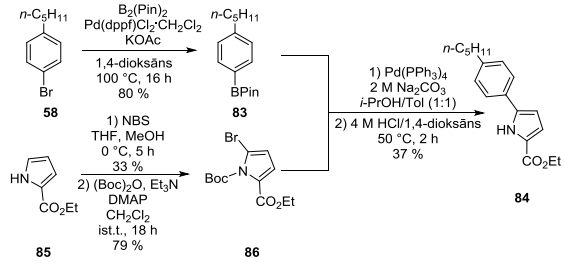
Mērķsavienojuma **75** sintēzē izmantojām iepriekš triazolu **74a-c** gadījumā aprakstīto sintēzes soļu secību. Pēc estera grupas hidrolīzes savienojumā **76** ieguvām attiecīgo karbonskābi, kuru tālāk izmantojām amīda **82** sintēzē, izmantojot HOBt / EDC kā kondensējošos reaģentus. Tālāk ar BH_3-THF kompleksu reducējām amīdu **82**, iegūstot amīnu **75** (2.13. att.).



2.13. att. **Mērķsavienojuma 75 sintēze**

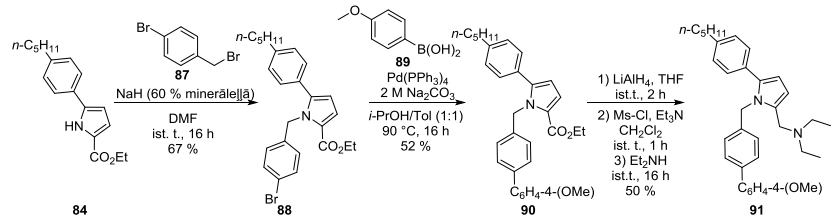
Inhibitora **84** sintēzē izmantojām komerciāli pieejamo pirol-2-karboksilātu (**85**). Sākumā bromējām pirolu **85** 5. pozīcijā, izmantojot *N*-bromsukcīnimīdu

tetrahidrofurāna un metanola maisījumā. Vēlamo produktu **86** ieguvām ar 33 % iznākumu, kas skaidrojams ar dibromaizvietota blakusprodukta veidošanos. Pirola **85** slāpekļa atomu aizsargājām ar Boc aizsarggrupu, izmantojot Boc_2O trietilamīna un DMAP klātienē. Sintezēto bromīdu **86** tālāk izmantojām Pd-katalizētā šķērssametināšanas reakcijā ar boronātu **83**. Iegūtajam produktam pievienojām 4 M HCl 1,4-dioksānā un šķīdumu sildījām 50 °C 2 stundas, lai nošķeltu Boc aizsarggrupu. Pirolu **84** ieguvām ar 37 % iznākumu (2 stadijās) (2.14. att.).



2.14. att. **Pirola atvasinājuma 84 sintēze**

Tālāk pirolu **84** alkilējām ar 4-brombenzilbromīdu (**87**). Sekojoša iegūtā arilbromīda **88** *Suzuki-Miyaura* šķērssametināšanas reakcija ar 4-metoksifenilborskābi (**89**) deva savienojumu **90**. Pirolā **90** estera grupu reducējām līdz spirtam ($LiAlH_4$, THF, istabas temperatūra, 2 stundas), to pārvērtām par attiecīgo mezilatvasinājumu (Ms-Cl, Et_3N, istabas temperatūra, 1 stunda), kurš reakcijā ar dietilamīnu deva mērķsavienojumu **91** (50 % iznākums 3 stadijās) (2.15. att.).

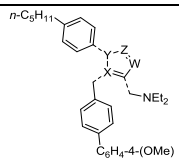


2.15. att. **Mērķsavienojuma 91 sintēze**

Sintezētajiem 4-aminopiperidīna heterocikliskajiem analogiem – triazolam **74a**, imidazolam **92**, pirolam **91** un izoksazolam **75** – tika noteiktas IC_{50} vērtības pret Plm II.

2.1. tabula

Azolu 74a, 75, 92 un 91 inhibitorās aktivitātes vērtības pret Plm II (IC_{50}, μM)



74a, **75**, **92**, **91**

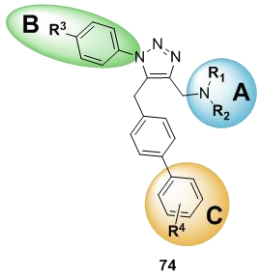
Nr. p. k.	Sav. nr.	Azols	IC_{50} Plm II, μM
1.	**74a**		4.3 ± 0.2
2.	**75**		10.0 ± 0.5
3.	**92**[a]		5.3 ± 0.2
4.	**91**		10.0 ± 0.4

[a] Savienojums iegūts sadarbībā ar G. Leiti

Iegūtie azoli **74a**, **75**, **92**, **91** uzrāda līdzīgu inhibitoro aktivitāti pret Plm II mikromolārā līmenī (2.1. tabula). Triazola **74a** un imidazola atvasinājumi **92** uzrādīja nedaudz augstāku inhibitoro aktivitāti (attiecīgi IC_{50} Plm II = 4.3 μM un 5.3 μM) (2.1. tabula, 1., 3. rinda), tādēļ turpmākajos pētījumos izmantojām 1,2,3-triazola atvasinājumus.

2.1.2. 1,2,3-Triazola atvasinājumi kā Plm II inhibitori

1,2,3-Triazola **74** molekula satur 3 fragmentus **A**, **B**, **C**, kurus iespējams modificēt, lai uzlabotu inhibitoro aktivitāti uz Plm II (2.16. att.). Molekulas **74 A** fragmenta daļā nolēmām mainīt alkilķēdes garumu starp heterociklisko kodolu un aminofunkciju un izpētīt aminogrupas aizvietotāju ietekmi uz inhibitoro aktivitāti. Molekulas **B** daļā nolēmām mainīt alkilķēdes garumu un ieviest aizvietotājus fenilgredzenā. Savukārt benzilaizvietotāja ievadīšana triazola pirmajā pozīcijā ļauj novietot fenilgredzenu leņķī pret centrālo triazola gredzenu un noskaidrot fenilgredzena pozīcijas ietekmi uz inhibitoro aktivitāti. Mainot fenilgredzena aizvietotājus molekulas **C** daļā, iespējams noskaidrot to ietekmi uz inhibitoro aktivitāti (2.16. att.).

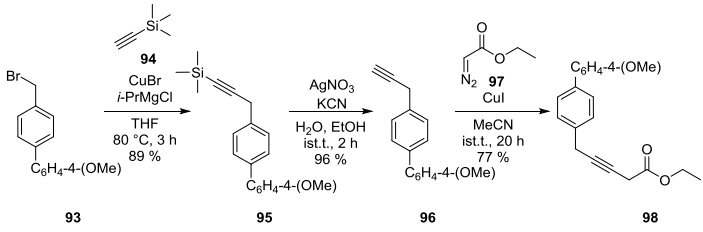


74

2.16. att. **1,2,3-Triazola molekulas modificējamie fragmenti**

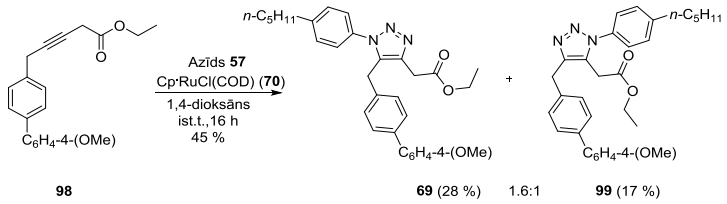
2.1.2.1. 1,2,3-Triazola inhibitoru fragmenta A modificēšana un struktūras aktivitātes likumsakarības

Mērķsavienojumu sintēzē izmantojām būvblokus – alkīna fragmentu saturošu biarilatvasinājumu **98** un iepriekš iegūto fenilazīdu **57**. Alkīnu **95** sintezējām no iepriekš iegūtā benzilbromīda **93**. Alkīna fragmentu molekulā **93** ievadījām, izmantojot trimetilsililacetilēnu (**94**) vara (I) bromīda un *i*-PrMgCl klātienē (THF, 80 °C, 3 stundas). Produktu **95** ieguvām ar 89 % iznākumu. Trimetilsililaizsarggrupas nošķelšanai savienojumā **95** izmantojām $AgNO_3$ un KCN (etanola / ūdens maisījums, istabas temperatūra, 2 stundas) [94] un ieguvām produktu **96**. Mērķsavienojumu **101a-g** iegūšanai bija nepieciešams savienojumā **96** ievadīt funkcionalizējamu grupu – etilkarboksimetilgrupu, kas ļautu iegūt aminogrupu saturošus atvasinājumus 2 oglekļa atomu attālumā no triazola cikla. Būvbloku **98** sintezējām ar 77 % iznākumu no alkīna atvasinājuma **96**, izmantojot etildiazoacetātu (**97**) vara (I) jodīda klātienē (MeCN, 20 stundas) (2.17. att.).[95]



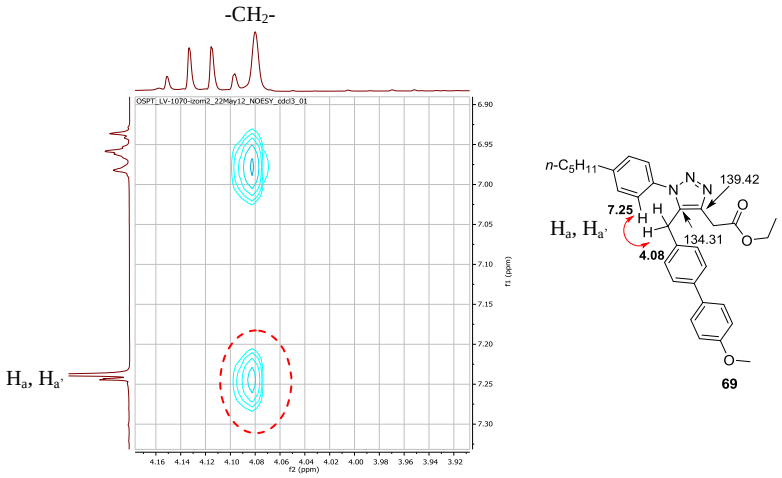
2.17. att. **Alkīna grupu saturoša biarilsavienojuma 98 sintēze**

Iegūto alkīna fragmentu saturošo būvbloku **98** un azīdu **57** tālāk izmantojām rutēnija (II) katalizētā ciklopievienošanās reakcijā. Saskaņā ar AEŠH analīzi reakcijā radās divu reģioizomēru **69** un **99** maisījums attiecībā 1.6:1 (2.18. att.). Izomērus **69** un **99** atdalījām ar kolonnas hromatogrāfiju.



2.18. att. **1,2,3-Triazola atvasinājumu 69 un 99 iegūšana**

Izmantojot protonu kodolmagnētiskās rezonanses spektrus, nebija iespējams iegūt viennozīmīgu apstiprinājumu produktu struktūrām, tādēļ savienojumu **69** un **99** struktūru pierādīšanai izmantojām divdimensionālo kodolu magnētiskās rezonanses spektru – HMBC un NOESY – analīzes.

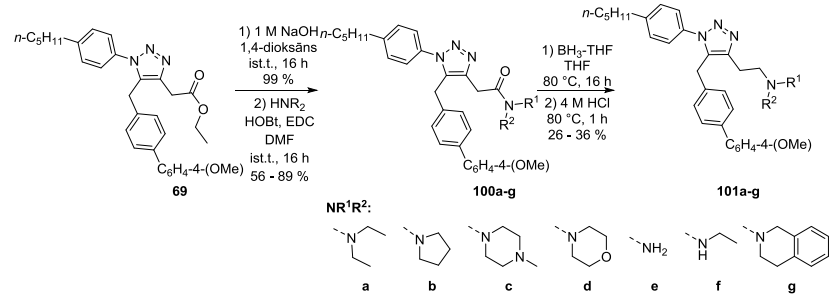


2.19. att. **1,2,3-Triazola 69 NOESY spektra fragments**

Savienojuma **69** NOESY spektrā redzama sadarbība starp metilēngrupas protoniem (δ = 4.08 ppm) un fenilgrupas protoniem Ha, Ha' (δ = 7.25 ppm) (2.19. att.). Savukārt savienojuma HMBC spektrā novērojamas sadarbības starp aromātiskā gredzena protoniem Ha, Ha' (δ = 7.25 ppm) un triazola cikla oglekļa atomu (δ = 134.31 ppm). Noteiktās sadarbības apstiprina aizvietotāju novietojumu 1,2,3-triazolā **69**.

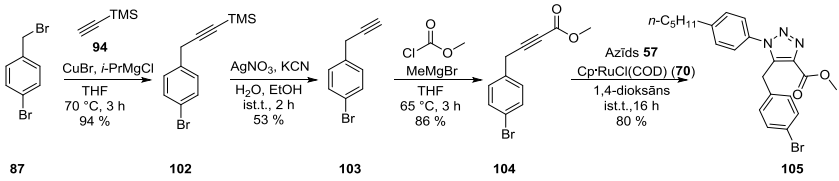
Nākamajā solī pēc savienojuma **69** estera grupas hidrolīzes ieguvām karbonskābi, kuru tālāk izmantojām bez papildu attīrīšanas. Lai noskaidrotu inhibitoru aminogrupas aizvietotāju ietekmi uz Plm II inhibēšanas aktivitāti, izvēlējāmies amīnus ar atšķirīgiem stēriskajiem izmēriem. Amīdus **100a-g** ieguvām no karbonskābes, kā kondensējošos reaģentus izmantojot HOBt / EDC.

Mērķsavienojumus **101a-g** ieguvām pēc amīda grupas reducēšanas ar BH_3-THF kompleksu (80 °C, 16 stundas) (2.20. att.).



2.20. att. **Mērķsavienojumu 101a-g sintēze**

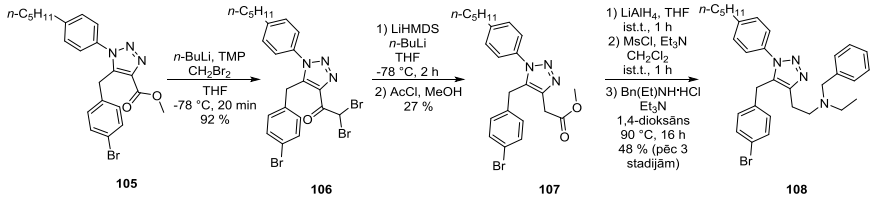
Mērķsavienojuma **108** iegūšanai izvēlējāmies alternatīvu sintēzes ceļu, kas ļauj izvairīties no reģioizomēru veidošanās rutēnija (II) katalizētā ciklopievienošanās reakcijā (2.21. att., 2.22. att.). Alkīnu **104** ieguvām 3 stadiju sintēzē no 4-brombenzilbromīda (**87**). Alkīna fragmentu benzilbromīda **87** molekulā ievadījām nukleofīlās aizvietošanas ceļā, izmantojot trimetilsililacetilēnu (**94**) vara (I) bromīda un *i*-PrMgCl klātienē (70 °C, 3 stundas). Produktu **102** ieguvām ar 94 % iznākumu. Kā desililēšanas reaģentus izmantojot $AgNO_3$ un KCN (etanola / ūdens maisījums, istabas temperatūra, 2 stundas), ieguvām alkīnu **103** (2.21. att.). Estera grupu alkīna **103** molekulā ievadījām reakcijā ar metilhloroformātu metilmagnija bromīda klātienē (THF, 65 °C, 3 stundas). Iegūto diaizvietoto alkīnu **104** izmantojām rutēnija (II) katalizētā ciklopievienošanās reakcijā ar azīdu **57**. Reakcijas rezultātā veidojās viens triazola reģioizomērs **105** ar 80 % iznākumu (2.21. att.).



2.21. att. **1,2,3-Triazola 105 sintēze**

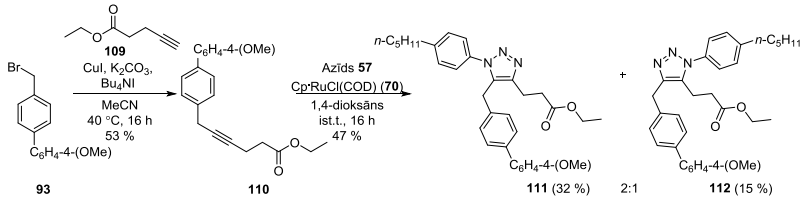
Estera **107** iegūšanai izmantojām *Kowalski* estera homologācijas reakciju [96], kas ļauj iegūt produktu **108** ar vajadzīgo alkilķēdes (2 oglekļa atomu) garumu starp triazola ciklu un aminofunkciju. *Kowalski* estera homologācijas reakcijā kā starpprodukts rodas *α*,*α*-dibromketons **106**, no kura tālāk ieguvām

homologizēto analogu **107**. Mērķsavienojumu **108** ieguvām 3 soļu sintēzē, kas ietver – estera grupas reducēšanu ar $LiAlH_4$, iegūstot attiecīgo spirtu, spirta grupas pārvēršanu par mezilatvasinājumu, kā arī sekojošu nukleofīlās aizvietošanas reakciju ar benziletilamīnu, veidojot mērķsavienojumu **108**.



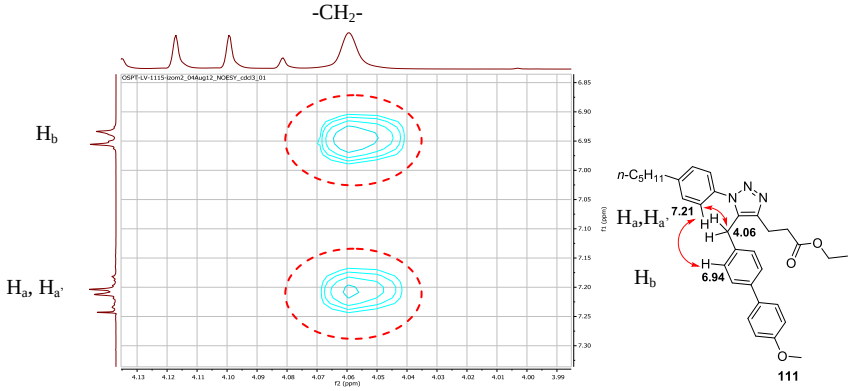
2.22. att. **Mērķsavienojuma 108 sintēze**

Struktūras-aktivitātes likumsakarību noskaidrošanai bija nepieciešams sintezēt triazola inhibitorus **114a-c**, kas satur 3 oglekļa atomus starp centrālo gredzenu un aminofunkciju. Alkīna **110** iegūšanai izmantojām iepriekš sintezēto benzilbromīdu **93**. Būvbloku **110** sintezējām, izmantojot alkīnu **109** vara (I) jodīda, K_2CO_3 un tetrabutilamonija jodīda klātienē (MeCN, 40 °C, 24 stundas) (2.23. att.).[89]



2.23. att. **1,2,3-Triazola atvasinājumu 111 un 112 iegūšana**

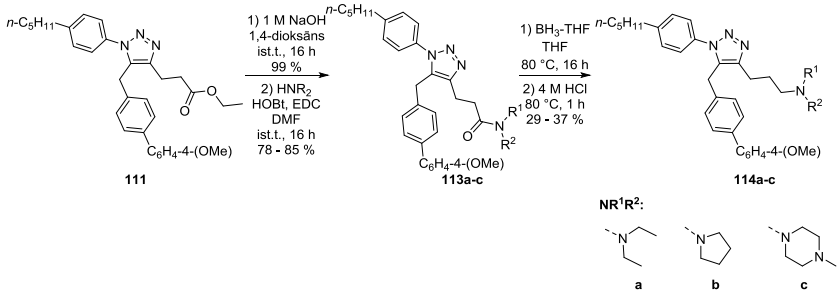
Iegūto alkīnu **110** izmantojām Ru katalizētā triazola estera **111** sintēzē. Alkīna **110** gadījumā ciklopievienošanās reakcijas rezultātā veidojās reģioizomēru **111** un **112** maisījums attiecībā 2:1 (2.23. att.). Pilnīgai izomēru **111** un **112** atdalīšanai izmantojām preparatīvo apgrieztās fāzes hromatogrāfiju. Pēc attīrīšanas vajadzīgo reģioizomēru **111** ieguvām ar 32 % iznākumu un tā struktūru pierādījām ar divdimensionālajiem kodolu magnētiskās rezonanses HMBC un NOESY spektriem (2.24. att.).



2.24. att. **1,2,3-Triazola 111 NOESY spektra fragments**

Savienojuma **111** NOESY spektrā redzamas sadarbības starp metilēngrupas protoniem (δ = 4.06 ppm) un aromātiskā gredzena protoniem Ha, Ha' (δ = 7.21 ppm), kā arī starp aromātisko ciklu protoniem Ha, Ha' (δ = 7.21 ppm) un Hb (δ = 6.94 ppm) (2.24. att.). Redzamās sadarbības apstiprina triazola **111** struktūru.

Mērķsavienojumus **114a-c** sintezējām analoģiski kā triazola **74a-c** atvasinājumus. Estera grupas hidrolīze savienojumā **111** ar 1 M NaOH šķīdumu deva karbonskābi, no kuras tālāk sintezējām nepieciešamos amīdus **113a-c** (2.25. att.). Amīdu reducēšanai kā reducētāju izmantojām BH_3-THF kompleksu. Reakcijas gaitā radušos amīna-borāna kompleksu apstrādājām ar 4 M HCl ūdens šķīdumu (80 °C, 1 stunda) un ieguvām amīnus **114a-c**.



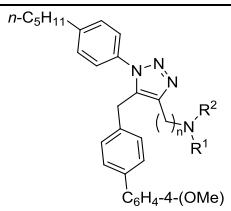
2.25. att. **Mērķsavienojumu 114a-c sintēze**

Pētījuma ietvaros sintezējām 1,2,3-triazola atvasinājumus, kas satur vienu, divus un trīs oglekļa atomus garu alkilķēdi starp centrālo gredzenu un aminofunkciju. Kā arī tika iegūti inhibitori ar atšķirīgiem aminofunkcijas

aizvietotāju stēriskajiem izmēriem. Triazola atvasinājumu **74a-c**, **101a-g**, **114a-c**, **108** inhibitorās aktivitātes vērtības uz Plm II parādītas 2.2. tabulā.

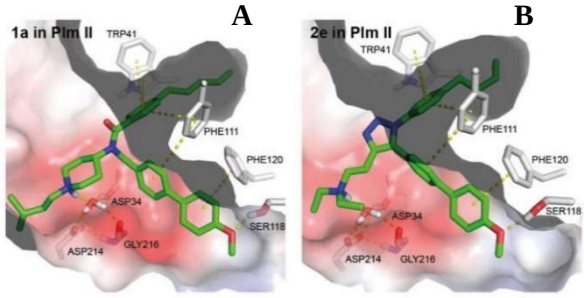
2.2. tabula

1,2,3-Triazola atvasinājumu inhibitorās aktivitātes vērtības pret Plm II (IC_{50}, μM)



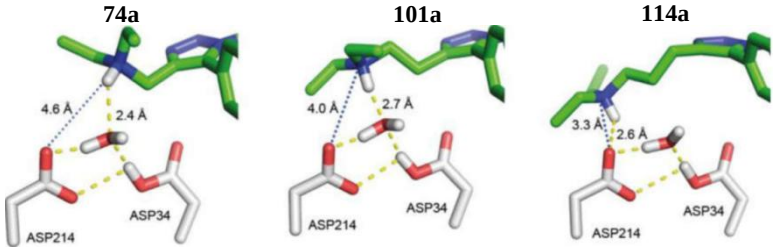
Nr. p. k.	Sav. nr.	-NR^1R^2	n	IC_{50} Plm II, μM
1.	**74a**		1	4.3 ± 0.2
2.	**101a**		2	0.60 ± 0.03
3.	**114a**		3	2.7 ± 0.2
4.	**74b**		1	4.7 ± 0.2
5.	**101b**		2	0.60 ± 0.03
6.	**114b**		3	2.9 ± 0.2
7.	**74c**		1	7.5 ± 0.3
8.	**101c**		2	1.8 ± 0.1
9.	**114c**		3	5.9 ± 0.3
10.	**101d**		2	2.9 ± 0.2
11.	**108**		2	5.0 ± 0.2
12.	**101e**		2	6.5 ± 0.3
13.	**101f**		2	3.0 ± 0.2
14.	**101g**		2	2.2 ± 0.2

Noskaidrojām, ka divu oglekļa atomu garš linkeris starp centrālo ciklu un aminofunkciju ir piemērotākais inhibitorās aktivitātes nodrošināšanai (2.2. tabula, 2., 5., 8. rinda). Ja linkeris satur 1 oglekļa atomu (2.2. tabula, 1., 4., 7. rinda) vai 3 oglekļa atomus (2.2. tabula, 3., 6., 9. rinda), tad inhibitorā aktivitāte uz Plm II ir krietni zemāka. Iegūtie rezultāti sakrīt ar datormodelēšanas pētījuma datiem, kuri parāda, ka triazola **101a** gadījumā centrālais heterocikls ir uz šķīdinātāju vērsts, un pārējie farmakoforie elementi veido analoģiskas sadarbības kā 4-aminopiperidīna atvasinājums **A** (2.26. att.).



2.26. att. **A – 4-Aminopiperidīna atvasinājuma 52; B – triazola 101a molekulārais modelis Plm II aktīvajā kabatā**

Molekulārajā modelī redzams, ka divu oglekļa atomus garš linkeris savienojumā **101a** nodrošina protonētā slāpekļa atoma optimālu pozīciju virs negatīvi lādētā aspartilatlikuma Asp214 un vienlaicīgu ūdeņraža saišu veidošanu caur ūdens tiltiņu ar aspartilatlikumiem Asp214 un Asp34 (2.27. att.).



2.27. att. **Molekulārais modelis, kas parāda alkilķēdes garuma ietekmi uz sadarbības veidošanos starp *N,N*-dialkilamino grupu savienojumos 74a, 101a, 114a un katalītiskajiem aspartilatlikumiem**

Īsākas (1 oglekļa atoms) vai garākas (3 oglekļa atomi) alkilķēdes gadījumā aminofunkcija tiek virzīta suboptimālā pozīcijā, kurā sadarbība tiek veidota ar vienu no aspartilatlikumiem, bet ne abiem reizē (2.27. att.). Tā kā visaugstāko inhibitoro aktivitāti uzrādīja triazola atvasinājumi, kas satur 2 oglekļa atomus starp centrālo triazola gredzenu un aminofunkciju, tad aminogrupas aizvietotāju ietekmes uz aktivitāti noskaidrošanai izmantojām tikai šos atvasinājumus.

Lai novērtētu aminogrupas aizvietotāju veida un izmēra ietekmi uz inhibitoro aktivitāti, sintezējām nelielu triazola atvasinājumu sēriju (2.2. tabula, 2., 5., 8., 10.–14. rinda). Visaugstākās IC_{50} vērtības uzrādīja triazola atvasinājumi, kas satur dietilamino- un pirolidīna aizvietotājus (2.2. tabula, 2., 5. rinda). Saskaņā ar Plm II un inhibitoru molekulārās modelēšanas rezultātiem, *N*-alkilgrupas sadarbojas ar S1’ sub-kabatas Tyr192, Ile212, Phe294 un Ile300 atlikumiem, un

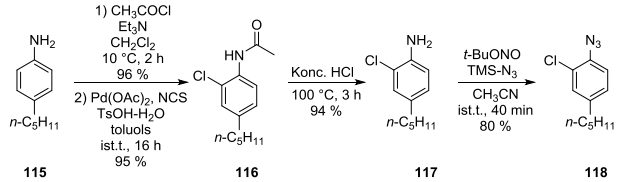
liganda saistīšanos galvenokārt ietekmē šo grupu forma un izmērs. Inhibitori, kas satur stēriski mazākas (amino- un etilamino-) grupas (2.2. tabula, 12., 13. rinda), uzrāda zemāku inhibitoro aktivitāti nekā dietilamino- atvasinājums. Acīmredzot stēriski mazas grupas neaizpilda enzīma hidrofobo S1' sub-kabatu. Savukārt stēriski lielu grupu (benzil-, *N*-metilpiperazīn-, morfolīn-, tetrahidroizohinolīn-) (2.2. tabula, 8. 10., 11., 13. rinda) gadījumā inhibitorā aktivitāte ir zemāka, jo aizvietotājs neietilpst S1' sub-kabatā.

2.1.2.2. 1,2,3-Triazola inhibitoru B fragmenta modificēšana un struktūras-aktivitātes likumsakarības

Lai noskaidrotu **B** fragmenta ietekmi uz inhibitoro aktivitāti, sintezējām 1,2,3-triazola atvasinājumus, kas **B** fragmentā satur:

1) hlora atomu fenilgredzena *orto*-pozīcijā. Šī modifikācija veicinātu fenilgredzena izgriešanos no molekulas plaknes, kas ļautu izveidot papildu sadarbības ar enzīmu;
2) 4-pentilbenzilaizvietotāju. Modifikācijas rezultātā tiktu palielināts fenilgredzena kustīgums;
3) 4-metilfenilaizvietotāju. Šī modifikācija apstiprinātu hipotēzi, ka inhibitorās aktivitātes nodrošināšanā uz Plm II būtiska nozīme ir sadarbību veidošanai ar vārsta kabatu.

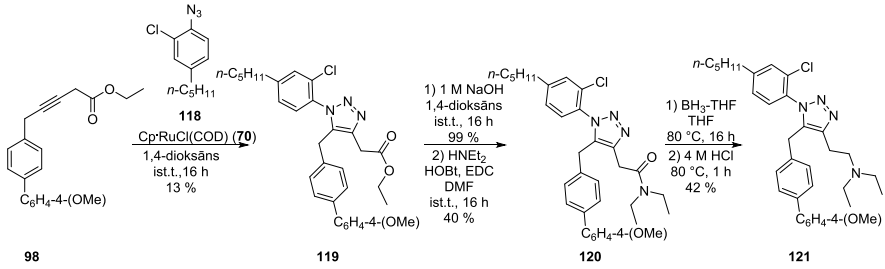
Mērķsavienojuma, kurš **B** fragmentā satur fenilfgredzenu ar hlora atomu *orto*-pozīcijā, iegūšanai bija nepieciešams sintezēt azīdu **118**. Azīda **118** sintēzē galvenā stadija bija *N*-acetil-anilīna Pd-katalizēta C-H *orto*-hlorēšana.[97] Produktu **116** ieguvām ar 95 % iznākumu un tālāk izmantojām bez papildu attīrīšanas. Pēc acetilgrupas nošķelšanas ieguvām anilīnu **117**, kuru pārvērtām par azīdu **118**, izmantojot *terc*-butilnitrītu un azidotrimetilsilānu (2.28. att.).[98]



2.28. att. **Azīda 118 iegūšana**

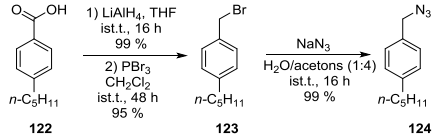
Iegūto azīdu **118** tālāk izmantojām rutēnija (II) katalizētā ciklopievienošanās reakcijā ar alkīnu **98**, veidojot triazola atvasinājumu **119.** Arī šajā gadījumā ciklopievienošanas reakcijas rezultātā radās triazola reģioizomēru maisījums

attiecībā 1:1, kurus atdalījām, izmantojot kolonnas hromatogrāfiju. Triazola atvasinājuma **119** struktūra tika pierādīta, izmantojot divdimensionālo NOESY un HMBC kodolu magnētiskās rezonanses spektru analīzi. Tālāk hidrolizējām estera grupu ar tai sekojošu amīda **120** iegūšanu. Mērķsavienojuma **121** iegūšanai reducējām amīdsaiti, izmantojot BH_3-THF kompleksu (2.29. att.).



2.29. att. **1,2,3-Triazola atvasinājuma 121 sintēze**

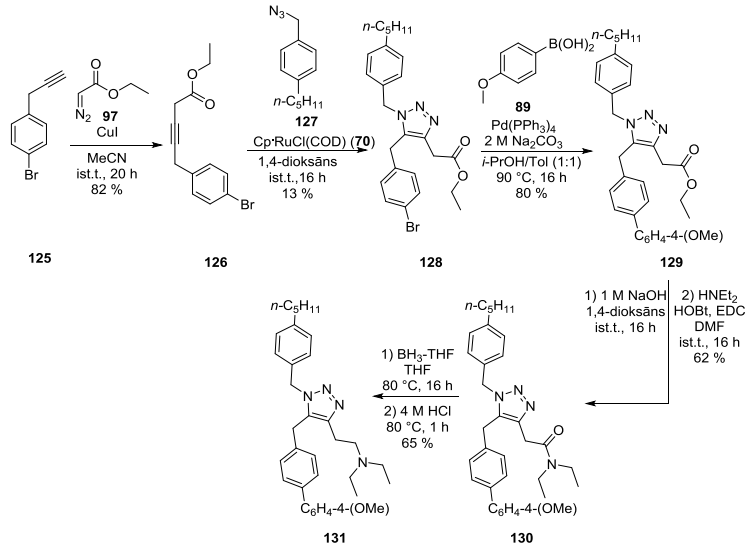
Benzilazīdu **124** sintezējām saskaņā ar 2.30. attēlā redzamo shēmu un kā izejvielu izmantojām komerciāli pieejamo 4-*n*-pentilbenzoskābi (**122**). Pēc karbonskābes grupas reducēšanas savienojumā **122** ar $LiAlH_4$ ieguvām attiecīgo benzilspirtu **123**. Tālāk veicām hidroksilgrupas nomaiņu pret bromu, izmantojot fosfora tribromīdu (metilēnhlorīds, istabas temperatūra, 16 stundas). Tai sekojošas nukleofīlās aizvietošanas reakcijas rezultātā ar azīda anjonu, izmantojot nātrija azīdu (H_2O / acetona maisījums, istabas temperatūra, 16 stundas), ieguvām benzilazīdu **124** (2.30. att.).[99]



2.30. att. **Azīda 124 sintēze**

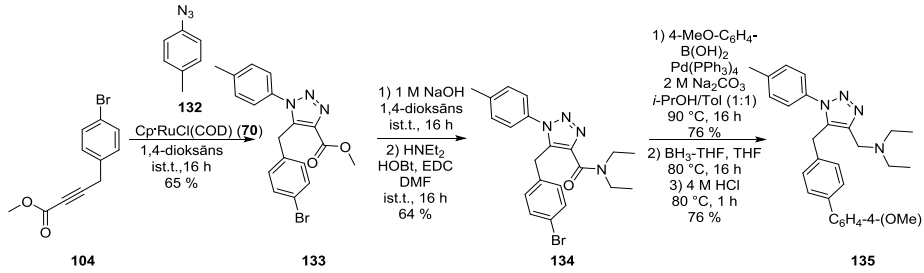
Rutēnija (II) katalizētā 1,3-dipolārā ciklopievienošanas reakcijā izmantojām benzilazīdu **124** un acetilēnu **126**. Reakcijas rezultātā veidojās triazola reģioizomēru maisījums attiecībā 3:1, kurus sadalījām, izmantojot kolonnas hromatogrāfiju. Vajadzīgais triazola reģioziomērs **128** tika iegūts ar 13 % iznākumu. Zemais iznākums skaidrojams ar reģioizomēru veidošanos un nepilnīgu izejvielas **126** konversiju. Tālāk *Suzuki-Miyaura* šķērssametināšanas reakcijā no bromīda **128** un 4-metoksikarbonilborskābes (**89**) palādija katalizatora $Pd(PPh_3)_4$ un Na_2CO_3 klātienē ieguvām bifenilatvasinājumu **129**.

Pēc estera grupas hidrolīzes savienojumā **128** ieguvām karbonskābi, kuru izmantojām amīda **130** sintēzē. Pēc amīdsaites reducēšanas ar BH_3-THF kompleksu un apstrādes ar 4 M HCl šķīdumu ieguvām amīnu **131** (2.31. att.).



2.31. att. **1,2,3-Triazola atvasinājuma 131 iegūšana**

Pēc analoģiskas sintēzes shēmas ieguvām arī triazola atvasinājumu **135**. Acetilēna **104** un azīda **132**, kuru savukārt ieguvām litijēšanas ceļā no attiecīgā bromīda, 1,3-dipolārās ciklopievienošanas reakcijas rezultātā radās tikai viens triazola **133** reģioizomērs ar 65 % iznākumu. Tālāk, izmantojot iepriekš aprakstīto reakciju secību – karbonskābes estera hidrolīzi, amīdsaites veidošanu, *Suzuki-Miyaura* šķērssametināšanas reakciju un amīdsaites reducēšanu, ieguvām mērķsavienojumu **135** (2.32. att.).

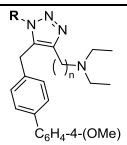


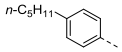
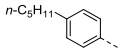
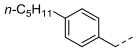
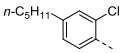
2.32. att. **1,2,3-Triazola atvasinājuma 135 sintēze**

No literatūras ir zināms, ka 4-aminopiperidīna **52** 4-*n*-pentilfenilgrupa ir virzīta Plm II vārsta kabatā.[26] Saskaņā ar dokinga pētījumiem arī triazola pirmās pozīcijas aizvietotājs ir virzīts Plm II vārsta kabatā, kas nodrošina alkilķēdes un vārsta kabatas sadarbību veidošanos. Alkilķēdes garuma saīsināšana no *n*-pentilgrupas uz metilgrupu izraisīja būtisku aktivitātes samazinājumu no 4.7 μM līdz 200 μM, bet, salīdzinot ar aktīvāko savienojumu **101a**, inhibitorās aktivitātes kritums pārsniedz 300 reizes (2.3. tabula, 1., 2., 3. rinda). Līdzīgi rezultāti tika novēroti, pētot 2-aminohinazolīn-4-(3*H*)-ona atvasinājumus **46a-d** kā Plm II inhibitorus. Pētījumā tika noskaidrots, ka vārsta kabatā ietilpstoša gara alkilķēde stabilizē atvērto vārsta konformāciju un notur liganda kodolu stingri virs aspartātu atlikumiem.[41] Var secināt, ka, saīsinot alkilķēdes garumu, šāda stabilizācija nenotiek.

2.3. tabula

Triazola atvasinājumu 74a, 101a, 121, 131, 135 inhibitorā aktivitāte uz Plm II (IC_{50}, μM)



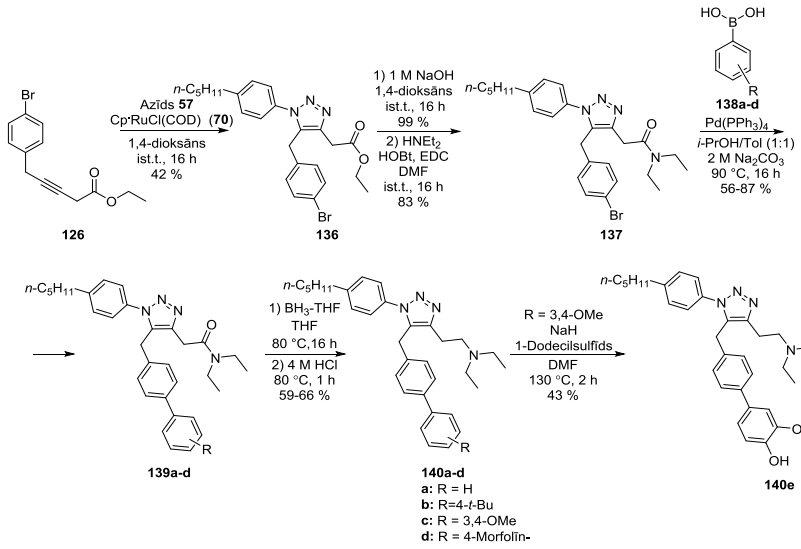
Nr. p. k.	Sav. nr.	R	n	IC_{50} Plm II, μM
1.	**101a**		2	0.60 ± 0.03
2.	**74a**		1	4.7 ± 0.2
3.	**135**		1	>200
4.	**131**		2	5.2 ± 0.2
5.	**121**		2	0.60 ± 0.03

Ievadot benzilgrupu triazola cikla 1. pozīcijā, inhibitorā aktivitāte uz Plm II samazinājās desmitkārtīgi (2.3. tabula, 4. rinda), tādējādi apstiprinot to, ka fenilgrupa ir piemērotākais aizvietotājs šajā pozīcijā. Molekulārās modelēšanas dati liecina, ka *para*-*n*-pentilbenzilķēde atrodas leņķī pret centrālo gredzenu, tādējādi samazinot molekulas sadarbības ar aspartilatlikumiem un S1' sub-kabatu. Hlora atoma ievadīšana fenilgredzena *orto*-pozīcijā inhibitoro aktivitāti neietekmēja (2.3. tabula, 5. rinda).

2.1.2.3. 1,2,3-Triazola inhibitoru C fragmenta modificēšana un struktūras-aktivitātes likumsakarības

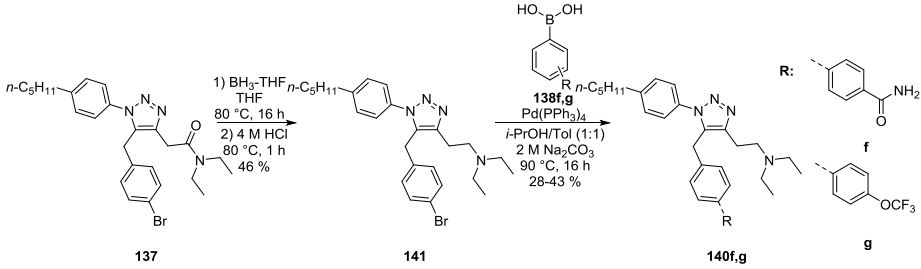
Lai noskaidrotu S1 sub-kabatā ietilpstošo **C** fragmenta aizvietotāju ietekmi uz Plm II inhibitoro aktivitāti, sintezējām triazola atvasinājumus ar dažādiem fenilgrupas aizvietotājiem – savienojumus, kuri satur stēriski lielas grupas (*t*-Bu, morfolīn-), neaizvietotu fenilgredzenu, ūdeņraža saišu akceptoras un donoras grupas.

Mērķsavienojumu sintēzē **140a-e** izmantojām iepriekš sintezēto alkīna būvbloku **126**. Rutēnija (II) katalizētā ciklopievienošanās reakcijā ar azīdu **57** un alkīnu **126** ieguvām 2 reģioizomēru maisījumu attiecībā 2:1, kurus atdalījām, izmantojot kolonnas hromatogrāfiju (2.33. att.). Nepieciešamo triazola reģioizomēru **136** ieguvām ar 42 % iznākumu un tā struktūru pierādījām, izmantojot divdimensionālo kodolu magnētiskās rezonanses HMBC un NOESY spektru analīzi. Pēc estera grupas hidrolīzes savienojumā **136** ieguvām karbonskābi, no kuras tālāk sintezējām dietilamidoatvasinājumu **137**. Savienojumu **137** modificējām, izmantojot *Suzuki-Miyaura* šķērssametināšanas reakciju, broma atomu nomainot pret dažādi aizvietotu fenilgrupu. Produktus **139a-d** ieguvām ar vidēji augstiem iznākumiem. Pēc amīda grupas reducēšanas ar BH_3-THF kompleksu un apstrādes ar 4 M HCl šķīdumu ieguvām atbilstošos aminoatvasinājumus **140a-d**. Hidroksiaizvietotu triazola atvasinājumu **140e** ieguvām pēc metilgrupu nošķelšanas savienojumā **140c**, izmantojot NaH un 1-dodecilsulfīdu (2.33. att.).[100]



2.33. att. **Mērķsavienojumu 140a-e sintēze**

Inhibitorus **140f**,**g** sintezējām pēc 2.34. attēlā redzamās shēmas. Amīnu **141** ieguvām pēc amīda grupas reducēšanas ar BH_3-THF kompleksu savienojumā **137**. Mērķsavienojumus **140f**,**g** ieguvām *Suzuki-Miyaura* sametināšanas reakcijā, palādija katalizatora $Pd(PPh_3)_4$ un Na_2CO_3 klātienē.

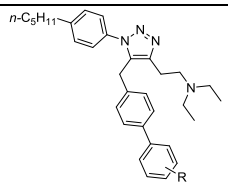


2.34. att. **Mērķsavienojumu 140f,g sintēze**

Saglabājot nemainīgus iepriekš noskaidrotos molekulas farmakoforus, noskaidrojām, kā bifenilfunkcijas aizvietotāji triazola molekulas **C** daļā ietekmē inhibitoro aktivitāti pret Plm II (2.4. tabula).

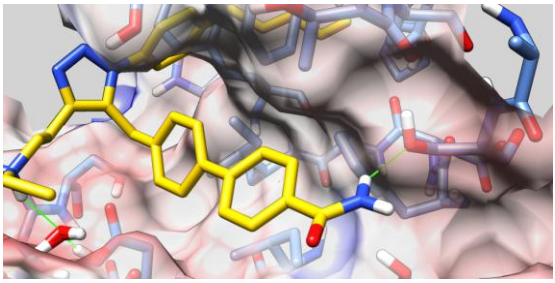
2.4. tabula

1,2,3-Triazola atvasinājumi ar dažādiem bifenilfunkcijas aizvietotājiem un to IC_{50}, μM



Nr. p. k.	Sav. nr.	R	IC_{50} Plm II, μM
1.	**140a**	H	1.8
2.	**140b**	4-*t*-Bu	2.8
3.	**140c**	3,4-OMe	1.9
4.	**140d**	4-Morfolino	1.2
5.	**140e**	3,4-OH	1.5
6.	**140f**	4-$CONH_2$	0.78
7.	**140g**	4-OCF_3	3.3
8.	**101a**	4-OMe	0.6

No iegūtajiem rezultātiem redzams, ka triazola inhibitoru bifenilfunkcijas aizvietotāji molekulas **C** daļā maz ietekmē inhibitoro aktivitāti. Labākie rezultāti iegūti, ja bifenilfunkcijas *para*-pozīcijā atrodas metoksigrupa (**101a**, IC_{50} Plm II = 0.6 μM) (2.4. tabula, 8. rinda). Saskaņā ar datormodelēšanas rezultātiem, metoksigrupas gadījumā veidojas hidrofoba sadarbība starp metilgrupu un proteīna S1 sub-kabatas Ile14 un Met15 aminoskābju atlikumiem, bet skābekļa atoms atrodas pārāk tālu no citiem heteroatomiem, lai veidotu ūdeņraža saites. Savukārt, ievadot fenilgredzena *para*-pozīcijā trifluormetoksigrupu, inhibitorā aktivitāte uz Plm II samazinājās 5 reizes (**140g**, IC_{50} Plm II = 3.3 μM) (2.4. tabula, 7. rinda). Ja fenilgredzens *para*-pozīcijā satur neaizvietotu amīda grupu, inhibitorā aktivitāte (**140f**, IC_{50} = 0.78 μM, 2.4. tabula, 6. rinda) uz Plm II ir līdzīga kā *para*-metoksiaizvietota triazola **101a** gadījumā (2.4. tabula, 8. rinda). Tas skaidrojams ar stabilizējošas ūdeņraža saites veidošanos starp amīdgrupas slāpekļa atomu un aminoskābju atlikuma Ser118 skābekļa atomu (2.35. att.). Savukārt, ievadot molekulā *meta*-pozīcijā papildu metoksigrupu, inhibitorā aktivitāte pret Plm II pazeminās 3 reizes IC_{50} = 1.9 μM (**140c**, 2.4. tabula, 3. rinda).



2.35. att. **Triazola 140f amīdfunkcijas ūdeņraža saites veidošanās ar proteīna aminoskābes atlikumu (dokinga rezultāts)**

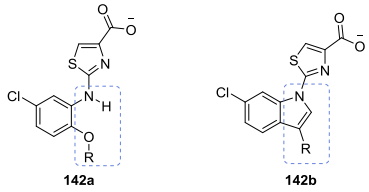
3,4-Hidroksiaizvietota (**140e**, 2.4. tabula, 5. rinda) un neaizvietota fenilgredzena (**140a**, 2.4. tabula, 1. rinda) gadījumā IC_{50} vērtības uz Plm II ir zemākas, jo neveidojas ūdeņraža saites starp inhibitoru un proteīnu. Inhibitoro aktivitāti ietekmē arī fenilgredzena aizvietotāju stēriskie izmēri. Telpiski lielu aizvietotāju gadījumā – 4-*t*-Bu (**140b**, IC_{50} Plm II = 2.8 μM), 4-morfolino- (**140d**, IC_{50} Plm II = 1.2 μM) (2.4. tabula, 2., 4. rinda) – inhibitorā aktivitāte nedaudz samazinās. Šajā gadījumā stēriski lieli aizvietotāji neietilpst S1 sub-kabatā, līdz ar to starp proteīnu un ligandu neveidojas sadarbība.

No sintezētajiem azolu rindas (izoksazola, pirola, triazola, imidazola) atvasinājumiem triazola atvasinājumi uzrādīja visaugstāko inhibitoro aktivitāti uz Plm II. Savienojumi, kas satur divus oglekļa atomus garu alkilķēdi starp triazola ciklu un aminofunkciju, uzrādīja visaugstāko inhibitoro aktivitāti pret Plm II. Variējot dažādus aminogrupas aizvietotājus, noskaidrojām, ka augstākās IC_{50} vērtības uzrāda dietilamino- un pirolidīna grupu saturoši triazola atvasinājumi. Svarīgi ir triazola molekulā saglabāt *n*-pentilķēdi, kas veido hidrofobās sadarbības ar vārsta kabatu. **C** fragmenta fenilgredzena aizvietotāji inhibitoro aktivitāti ietekmē maz.

2.1.3. Tetrahidroizohinolīna atvasinājumi kā Plm II inhibitori un struktūras-aktivitātes likumsakarības

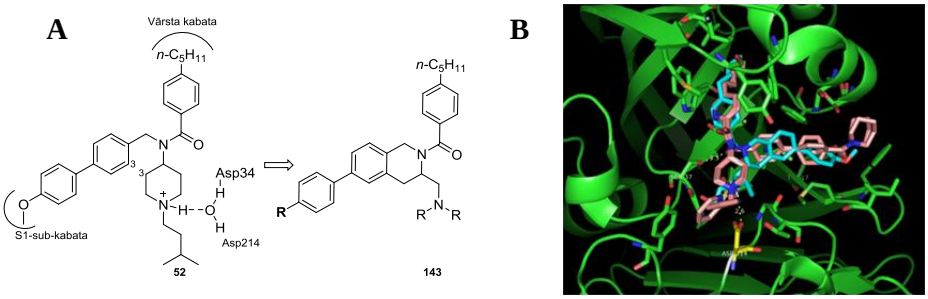
Jaunu nepeptidomimētisku inhibitoru dizainēšanā iespējams izmantot pieeju, kurā zināmi savienojumi ar vismaz vienu ciklu tiek modificēti, atverot šo ciklu vai saslēdzot jaunu ciklu, bet saglabājot esošos farmakoforos elementus. Izmantojot šo pieeju jaunu molekulu izstrādē, ir iespējams uzlabot savienojuma fizikālķīmiskās īpašības, kā arī iegūt jaunas struktūras. Šis molekulu modificēšanas veids ir veiksmīgi izmantots prostaglandīnu EP1 receptoru antagonistu izstrādē. Pēc cikla saslēgšanas savienojumā **142a** molekula tika

fiksēta bioaktīvajā konformācijā, saglabājot nanomolāru aktivitāti saistīšanās un funkcionālajos EP1 antagonistu testos (2.36. att.).[101]



2.36. att. **Prostaglandīna EP1 receptora antagonisti**

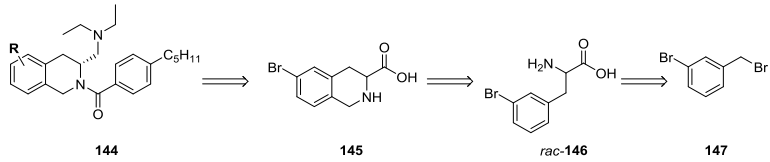
Par pamatstruktūru jaunu Plm II nepeptidomimētisku inhibitoru dizainā izmantojām iepriekš aprakstīto 4-aminopiperidīnu **52.**[12], [26], [42] Modificējot molekulu **52**, izveidojot saiti starp fenilgredzena 3. pozīciju un piperidīna cikla 3. pozīciju un atverot piperidīna ciklu, tika dizainēts tetrahidroizohinolīna atvasinājums **143**. Savienojums **143** saglabā esošos farmakoforos elementus – aminofunkciju (veido jonu tipa sadarbības ar aspartilatlikumiem un ūdens molekulas mediētu ūdeņraža saiti ar katalītisko Asp34 atlikumu), bifenilaizvietotāju (aizņem S1 sub-kabatu) un *n*-pentilķēdi (ietilpst vārsta kabatā) (2.37. att.).



2.37. att. **A – Plazmepsīnu II inhibitoru dizainēšana; B –Tetrahidroizohinolīna atvasinājumu 143 dokings**

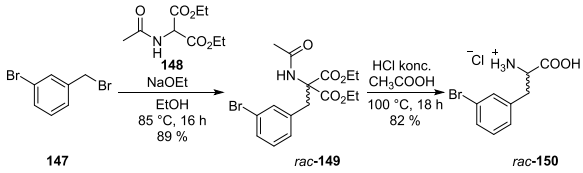
Izmantojot dokinga pētījumus, pārliecinājāmies, ka mūsu dizainētais tetrahidroizohinolīna atvasinājums **143** saistās ar Plm II līdzīgi kā aminopiperidīna atvasinājums (2.37. att.). Nolēmām sintezēt nelielu tetrahidroizohinolīna atvasinājumu sēriju ar dažādiem fenilgredzena aizvietotājiem un noteikt to inhibitorās aktivitātes vērtības pret Plm I, Plm II un Plm IV.

Analizējot literatūru [102], secinājām, ka ērtākā metode tetrahidroizohinolīna atvasinājumu **144** sintēzei ir *Pictet-Spengler* tetrahidroizohinolīnu sintēzes metode, kuras pamatā ir feniletilamīnu kondensācija ar karbonilsavienojumiem protonu vai Lūisa skābes klātienē (2.38. att.).[103]



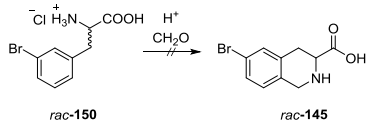
2.38. att. **Tetrahidroizohinolīna atvasinājumu 144 retrosintēzes shēma**

Aminoskābes atvasinājumu *rac*-**150** nolēmām sintezēt 2 stadiju procesā no komerciāli pieejamā bromīda **147**. Savienojumu *rac*-**149** ieguvām, *C*-alkilējot 3-brombenzilbromīdu (**147**) ar dietilacetoamidomalonātu (**148**) nātrija etoksīda klātienē, vārot etanolā (2.39. att.).[104] Sildot savienojumu *rac*-**149** koncentrētas sālsskābes un etiķskābes maisījumā, notiek acetilgrupas nošķelšanās un dekarboksilēšanās, kā rezultātā ieguvām aminoskābes atvasinājumu *rac*-**150** (2.39. att.).



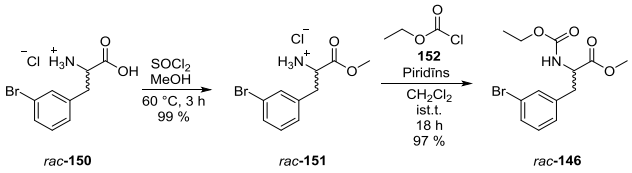
2.39. att. **Aminoskābes *rac*-150 sintēze**

Iegūt tetrahidroizohinolīna atvasinājumu **145** no neaizsargātas aminoskābes *rac*-**150**, izmantojot nelielu paraformaldehīda pārākumu (1.1 ekv.) etiķskābes un sērskābes klātienē, neizdevās.[105] Tetrahidroizohinolīna *rac*-**145** iegūšanai izmēģinājām arī formaldehīda šķīdumu ūdenī (37 %) 6 M HCl šķīduma [105] vai trifluoretiķskābes un HBr (33 % etiķskābē) [106] klātienē, taču arī šajos apstākļos aminoskābe *rac*-**150** nereaģēja (2.40. att.).



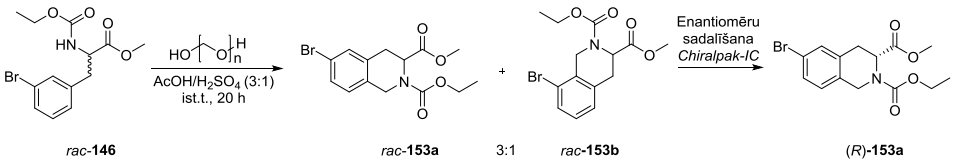
2.40. att. **Tetrahidroizohinolīna atvasinājuma *rac*-145 sintēzes mēģinājums**

Tādēļ nolēmām aizsargāt skābes un amīna funkcionālās grupas aminoskābes *rac*-**150** molekulā. Skābes funkciju savienojumā *rac*-**150** aizsargājām ar metilgrupu esterificēšanas reakcijas rezultātā ar tionilhlorīdu un metanolu. Savukārt aminogrupas aizsargāšanai izmantojām etilhloroformātu **152** piridīna klātienē, kas ļāva iegūt karbamātu *rac*-**146** (2.41. att.).



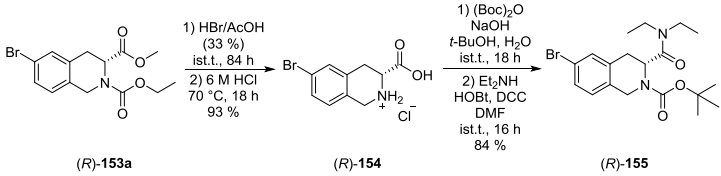
2.41. att. **Aizsargātas aminoskābes *rac*-146 sintēze**

Iegūto karbamātu *rac*-**146** izmantojām tetrahidroizohinolīna **153a** iegūšanai iepriekš aprakstītajos reakcijas apstākļos ar paraformaldehīdu etiķskābes / sērskābes (3:1) maisījumā. Reakcijas rezultātā radās 2 reģioizomēri **153a** un **153b** attiecībā 3:1, kurus atdalījām, izmantojot preparatīvo kolonnu hromatogrāfiju (2.42. att.). Savukārt enantiotīrus savienojumus **153a** ieguvām ar 17 % un 18 % iznākumu pēc enantiomēru atdalīšanas ar kolonnu hromatogrāfiju, izmantojot hirālu stacionāro fāzi.



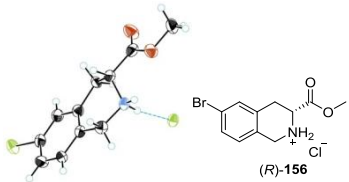
2.42. att. **Tetrahidroizohinolīna atvasinājumu 153a un 153b sintēze**

Lai iegūtu tetrahidroizohinolīnu (*R*)-**154**, kas nepieciešams turpmāko pārvērtību realizēšanai, vajadzēja nošķelt aizsarggrupas savienojumā (*R*)-**153a** (2.43. att.). Savienojumā (*R*)-**153a** *N*-karboksietilaizsarggrupu nošķēlām ar 33 % HBr / etiķskābes šķīdumu, maisot istabas temperatūrā 16 stundas. Tālāk karbonskābes atvasinājumu (*R*)-**154** ieguvām pēc estera grupas hidrolīzes ar 6 M HCl šķīdumu, sildot 70 °C.[105]



2.43. att. **Tetrahidroizohinolīna atvasinājuma (*R*)-155 sintēze**

Savienojuma **154** stereocentra absolūtās konfigurācijas noskaidrošanai nolēmām izmantot kristālu rentgendifraktometrijas datu analīzi. Diemžēl skābes atvasinājumam **154** nebija iespējams noteikt absolūto konfigurāciju, tādēļ sintezējām atbilstošo skābes metilesteri. Šajā gadījumā, izmantojot rentgendifraktometrijas datu analīzi, noskaidrojām, ka tetrahidroizohinolīna **154** stereocentra konfigurācija ir (*R*) (2.44. att.).

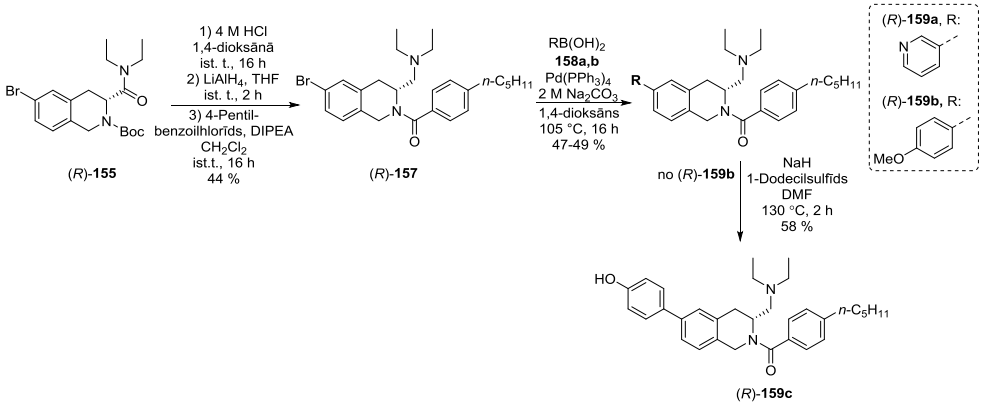


2.44. att. **Tetrahidroizohinolīna (*R*)-156 kristāla rentgendifraktometrijas dati**

Tālākajā sintēzes gaitā bija nepieciešams aizsargāt aminogrupu savienojumā (*R*)-**154**, kas ļautu izvairīties no blakusproduktu veidošanās turpmākajās stadijās. Kā aizsarggrupu izvēlējāmies *terc*-butoksikarbonilgrupu.[107] Reakciju veicām *t*-BuOH / ūdens maisījumā, NaOH un Boc_2O klātienē. Boc aizsargātu tetrahidroizohinolīna atvasinājumu tālāk izmantojām amīda (*R*)-**155** sintēzē, kā kondensējošo reaģentu izmantojām HOBt / DCC un produktu (*R*)-**155** ieguvām ar 84 % iznākumu pēc 2 stadijām (2.43. att.).

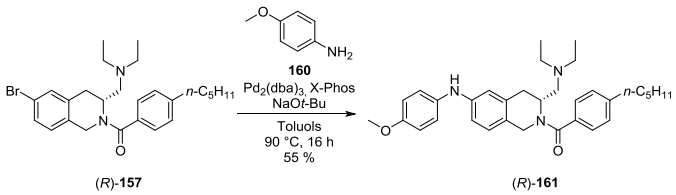
Iegūtajam amīdam (*R*)-**155** Boc-aizsarggrupu nošķēlām, izmantojot 4 M HCl 1,4-dioksānā (50 °C, 2 stundas) (2.45. att.). Iegūto produktu tālākajās reakcijās izmantojām bez papildu attīrīšanas. Pēc amīda grupas reducēšanas ar $LiAlH_4$ tetrahidrofurānā ieguvām attiecīgo aminoatvasinājumu. Lai molekulā ievadītu *n*-pentilķēdi saturošu farmakoforu, iepriekš sintezēto amīnu acilējām ar 4-*n*-pentilbenzoilhlorīdu DIPEA klātienē (metilēnhlorīds, istabas temperatūra, 18 stundas). Produktu (*R*)-**157** ieguvām ar 44 % iznākumu pēc 3 stadijām. Mērķsavienojumus (*R*)-**159a**,**b** ieguvām *Suzuki-Miyaura* sametināšanas reakcijā palādija katalizatora $Pd(PPh_3)_4$ un Na_2CO_3 klātienē. Hidroksiatvasinājumu

(*R*)-**159c** ieguvām no iepriekš sintezētā produkta (*R*)-**159b**, nošķeļot metilgrupu ar 1-dodeciltiolu NaH klātienē (2.45. att.).[100]



2.45. att. **Mērķsavienojumu (*R*)-159a-c sintēze**

Lai noskaidrotu, vai fenilgrupas novietojums tetrahidroizohinolīna molekulā ietekmē inhibitoro aktivitāti uz plazmepsīnu II, sintezējām aminotiltiņu saturošu savienojumu (*R*)-**161** (2.46. att.). Mērķsavienojumu (*R*)-**161** ieguvām *Buchwald-Hartwig* reakcijas ceļā no 4-metoksianilīna (**160**) un bromīda (*R*)-**157** katalizatora $Pd_2(dba)_3$, fosfīna liganda X-Phos un NaO*t*-Bu klātienē (90 °C, 16 stundas) (2.46. att.).[108]



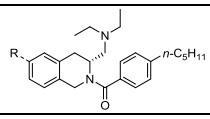
2.46. att. **Aminotiltiņu saturoša inhibitora (*R*)-161 iegūšana**

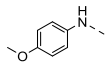
Sintezētajiem mērķsavienojumiem (*R*)-**159a-c** un (*R*)-**161** tika noteikta inhibitorā aktivitāte pret plazmepsīnu I, II un IV. Iegūtie rezultāti ir apkopoti 2.5. tabulā. Tetrahidroizohinolīna atvasinājums (*R*)-**159a** uzrāda inhibitoro aktivitāti pret Plm I un Plm IV mikromolārā līmenī, bet ir neaktīvs uz Plm II. Visaugstāko Plm II inhibēšanas spēju uzrādīja metoksifenilgrupu saturošs inhibitors (*R*)-**159b**, savukārt hidroksifenilgrupu un aminotiltiņu saturoši inhibitori (*R*)-**159c** un (*R*)-**161** uzrādīja 2 līdz 3 reizes zemāku inhibitoro

aktivitāti uz Plm I, Plm II un Plm IV, salīdzinot ar aktīvāko savienojumu (*R*)-**159b** (2.5. tabula, 2.–4. rinda).

2.5. tabula

Tetrahidroizohinolīna atvasinājumu (*R*)-159a-c, (*R*)-161 IC_{50} vērtības



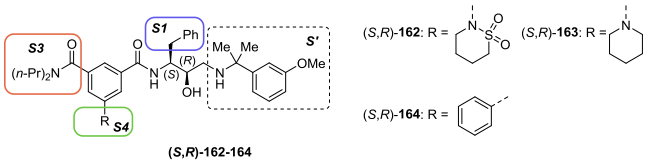
Nr. p. k.	Sav. nr.	R	IC_{50}, μM		
			Plm I	Plm II	Plm IV
1.	(*R*)-**159a**		22 ± 1	-[a]	~100
2.	(*R*)-**159b**		1.9 ± 0.1	16.2 ± 0.8	46.6 ± 2.0
3.	(*R*)-**159c**		7.3 ± 0.3	40 ± 2	~100
4.	(*R*)-**161**		7.0 ± 0.3	45.5 ± 2.0	73.6 ± 3.0

[a] Koncentrāciju reģionā (0.01–100 μM) inhibitors neuzrāda efektu

Pētījuma ietvaros tika sintezēti jauni nepeptidomimētiski tetrahidroizohinolīna ciklu saturoši Plm II inhibitori. Inhibitoru sintēzē kā atslēgas stadija tika izmantota *Pictet-Spengler* reakcija. Labākie tetrahidroizohinolīna atvasinājumi uzrādīja inhibitorās aktivitātes vērtības pret Plm II mikromolārā līmenī.

2.2. Hidroksietilamīna atvasinājumi kā plazmepsīnu inhibitori

2010. gadā kompānija *GlaxoSmithKline* publicēja liela apjoma šūnu testa HTS rezultātus, lai veicinātu jaunu antimalārijas medikamentu izstrādi. Pētījuma rezultātā tika identificēti 13 533 savienojumi, kas inhibēja *P. falciparum* parazīta augšanu inficētās asins šūnās vismaz 80 % 2 μM koncentrācijā. Identificētās struktūras tika sadalītas 47 grupās, izmantojot kemoinformātikas analīzi. Viena no šīm grupām saturēja 74 hidroksietilamīna fragmentu saturošus savienojumus.[109] No publicētajiem savienojumiem tālākajai attīstīšanai mēs izvēlējāmies aktīvāko hidroksietilamīna fragmentu saturošu atvasinājumu (*S*,*R*)-**162** (2.47. att.).



2.47. att. **Hidroksietilamīna atvasinājumi (*S*,*R*)-162-164**

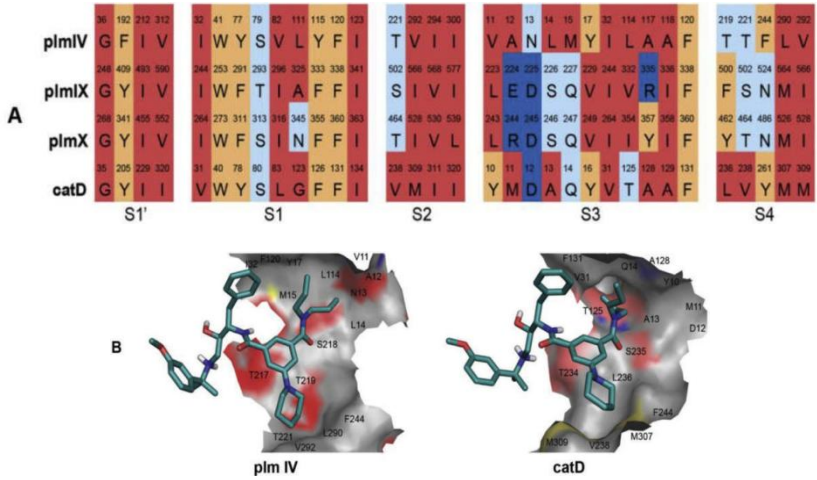
Iepriekšējos pētījumos [110] tika noskaidrots, ka šis savienojums uzrāda inhibitoro aktivitāti uz *P. falciparum* aspartilproteāzēm – plazmepsīnu subtipiem Plm I, Plm II un Plm IV. Arī strukturāli vienkāršotie savienojuma (*S*,*R*)-**162** analogi (*S*,*R*)-**163**, (*S*,*R*)-**164** saglabāja augstu inhibēšanas spēju *P. falciparum* inficētu asins šūnu testā (EC_{50} = 0.002–0.006 μM), tomēr šie inhibitori uzrāda arī augstu Cat D inhibēšanas spēju (2.6. tabula, 1.–3. rinda).[110]

2.6. tabula

Literatūrā [110] aprakstītie Plm inhibitori un to inhibitorā aktivitāte

Nr. p. k.	Sav. nr.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	EC_{50} *Pf* 3D7, μM
1.	(*S*,*R*)-**162**		0.029	0.043	0.002
2.	(*S*,*R*)-**163**		0.024	0.042	0.006
3.	(*S*,*R*)-**164**	Ph	0.006	0.054	0.002

Tādēļ kā galveno uzdevumu izvirzījām – hidroksietilamīna atvasinājumu selektivitātes uzlabošanu attiecībā pret cilvēka aspartilproteāzi Cat D, izmantojot informāciju par plazmepsīnu un Cat D strukturālajām atšķirībām. Iepriekšējos SAR pētījumos [110] tika noskaidrots, ka savienojuma (*S*,*R*)-**162** izopropil-2-(3-metoksifenil)- aizvietotājs, kas aizņem galveno sub-kabatu (S'daļa) (2.47. att.), ir optimāls plazmepsīnu inhibēšanai. Tādēļ turpmākajos pētījumos nolēmām modificēt aizvietotājus, kas aizņem S3 un S4 sub-kabatas. Lai novērtētu inhibitora atpazīšanas atšķirības starp Plm IV, IX, X un Cat D, izveidojām šo proteīnu aminoskābju sekvences salīdzinājumu un, izmantojot dokingu, salīdzinājām proteīnu sadarbības ar inhibitoru (*S*,*R*)-**163** (2.48. att. **A**, **B**).

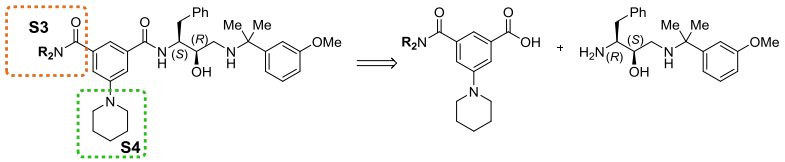


2.48. att. **A – Plm IV, Plm IX un Plm X S1' un S1-S4 kabatu aminoskābju secības salīdzinājums; B – Plm IV un Cat D S3 un S4 kabatu virsmas attēlojums (dokinga modelis ar inhibitoru (*S*,*R*)-163)**

Tā kā nav pieejamas eksperimentāli noteiktas Plm IX un Plm X struktūras, lai izvairītos no iespējamām neprecizitātēm, kas saistītas ar homoloģijas modeļu izmantošanu, Plm IX un Plm X dokinga pētījumi tika veikti, balstoties uz Plm IV kristāla rentgendifraktometrijas datiem, kas ir to tuvākais analogs ar pieejamiem kristāla rentgendifraktometrijas datiem.[111] 2.48. attēlā **A** redzams, ka S3 sub-kabata uzrāda vislielākās aminoskābju rindas atšķirības starp plazmepsīniem un Cat D. Savukārt 2.48. attēlā **B** redzams, ka Plm IV S3 sub-kabata ir platāka, daudz seklāka un hidrofobāka nekā Cat D sub-kabata. Minēto atšķirību dēļ inhibitoru selektivitātes uzlabošanu uzsākām, modificējot *N*,*N*-dipropilamīda funkciju savienojumā (*S*,*R*)-**163**, kas aizņem S3 sub-kabatu.

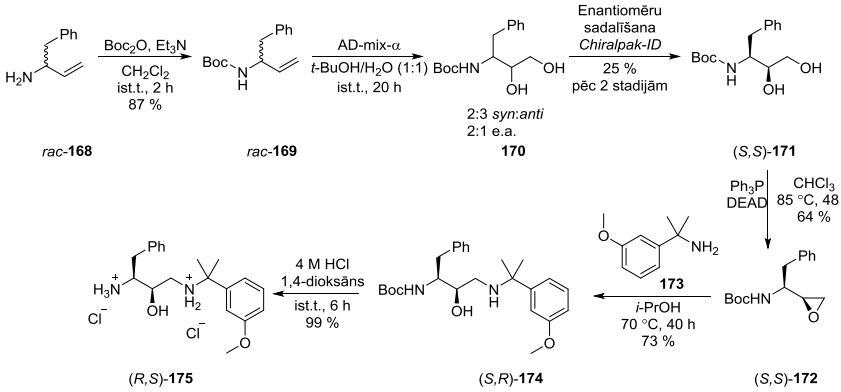
2.2.1. Diaizvietotu amīdu saturošu hidroksietilamīna atvasinājumu sintēze un struktūras-aktivitātes likumsakarības

Pirmais uzdevums bija sintezēt inhibitora (*S*,*R*)-**163** *N*,*N*-diaizvietotu amīdu analogu rindu ar dažādām hidrofobām grupām, kuras varētu aizņemt Plm IV hidrofobo S3 sub-kabatu (2.49. att.). Mērķsavienojumus (*S*,*R*)-**165** plānojām sintezēt pēc 2.49. attēlā redzamās retrosintēzes shēmas, kā galvenos būvblokus izmantojot benzoskābes atvasinājumus **166** un aminospirtu (*R*,*S*)-**167**.



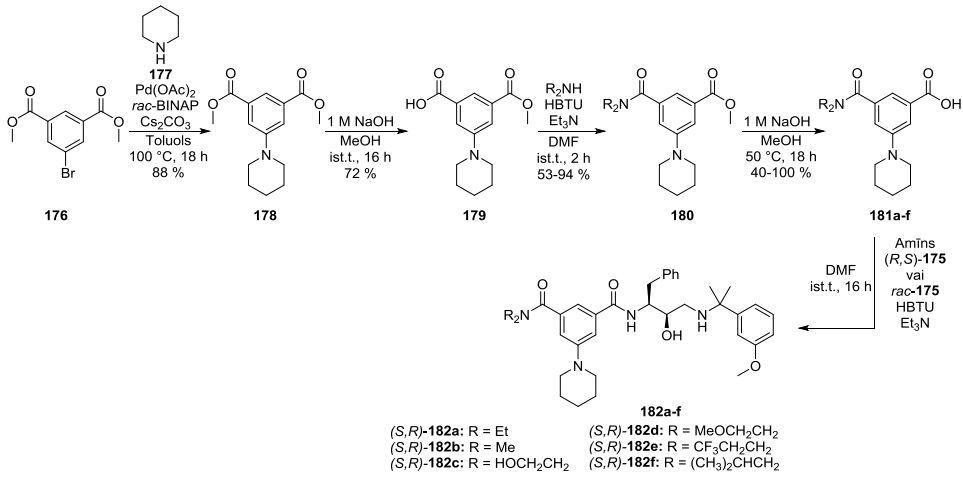
2.49. att. **Hidroksietilamīna fragmentu saturošu inhibitoru (*S*,*R*)-165 retrosintēzes shēma**

Aminospirta (*R*,*S*)-**175** sintēzē izmantojām racēmisku alilamīnu *rac*-**168**. Aminofunkciju savienojumā *rac*-**168** aizsargājām ar Boc-grupu (Boc_2O, metilēnhlorīds, istabas temperatūra, 2 stundas), iegūstot karbamātu *rac*-**169**. Alkēna *rac*-**169** dihidroksilēšanai izmantojām AD-mix-α (*t*-BuOH / ūdens = 1:1 maisījums, istabas temperatūra, 20 stundas). Diolu **170** ieguvām kā *syn* / *anti* diastereomēru maisījumu attiecībā 2:3 un kā enantiomēru maisījumu attiecībā 2:1. Enantiotīru diolu (*S*,*S*)-**171** ieguvām pēc *syn* / *anti* diastereomēru atdalīšanas ar kolonnu hromatogrāfiju, kam sekoja *syn* **170** sadalīšana enantiomēros, izmantojot kolonnas hromatogrāfiju ar hirālu sorbentu. Katram no iegūtajiem enantiotīrajiem dioliem noteicām polarizētas gaismas griešanas leņķa (α) vērtības un iegūtos rezultātus salīdzinājām ar literatūras datiem.[112] No iegūtajiem rezultātiem secinājām, ka pārākumā rodas (*S*,*S*)-**171** – tas ir mums nepieciešamais produkts. Izmantojot *Mitsunobu* reakcijas apstākļus (Ph_3P, DEAD, 85 °C, 48 stundas), no (*S*,*S*)-**171** diola ieguvām epoksīdu (*S*,*S*)-**172**. Veicot epoksīda (*S*,*S*)-**172** aminolīzi ar 2-(3-metoksifenil)propān-2-amīnu (**173**) (izopropanols, 70 °C, 40 stundas) [110], ieguvām *N*-Boc aizsargātu aminospirtu (*S*,*R*)-**174**. Tālāk, nošķeļot Boc aizsarggrupu savienojumā (*S*,*R*)-**174** ar 4 M HCl 1,4-dioksānā, ieguvām aminospirtu (*R*,*S*)-**175**, ko izmantojām plazmepsīnu inhibitoru sintēzē (2.50. att.).



2.50. att. **Hidroksietilamīna fragmentu saturoša būvbloka (*R*,*S*)-175 sintēze**

Benzoskābju **181a-f** sintēzē kā izejvielu izmantojām dimetil-5-bromizoftalātu (**176**). Bromīda **176** Pd katalizētā aminēšanas reakcijā ar piperidīnu **177**, izmantojot $Pd(OAc)_2$ kā katalizatoru un racēmisku BINAP kā ligandu (toluols, 100 °C, 18 stundas), ieguvām izoftalāta atvasinājumu **178**. Pēc hidrolīzes ar 1 M NaOH šķīdumu metanolā (istabas temperatūra, 16 stundas) ieguvām izoftalskābes monoesteri **179**. Iegūto monoesteri **179** tālāk izmantojām HBTU mediētā kondensācijas reakcijā ar amīniem, veidojot amīdus **180a-f**. Esterus **180a-f** hidrolizējot ar 1 M NaOH šķīdumu, ieguvām benzoskābes atvasinājumus **181a-f**. Izoftalskābes atvasinājumu **181b-e** kondensācijā ar amīnu (*R*,*S*)-**175** ieguvām mērķsavienojumus (*S*,*R*)-**182b-e**. Inhibitorus **182a** un **182f** sintezējām no racēmiska amīna *rac*-**175**. Iegūtos produktus attīrījām, izmantojot kolonnas ar hirāliem sorbentiem *Chiralpak-IC* un *Chiralpak-ID*, iegūstot mērķsavienojumus (*S*,*R*)-**182a** un (*S*,*R*)-**182f** ar 18 % un 33 % iznākumu (2.51. att.).

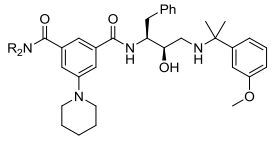


2.51. att. **Mērķsavienojumu 182a-f sintēze**

Sintezētajai *N*,*N*-diaizvietotu amīda analogu rindai (*S*,*R*)-**182a-f** (2.7. tabula) ar dažādām hidrofobām grupām, kuras varētu aizņemt Plm IV hidrofobo S3 sub-kabatu, tika noteiktas IC_{50} vērtības uz Plm IV un Cat D.

2.7. tabula

Sintezētie savienojumi (*S,R*)-182a-f un to inhibitorā aktivitāte



(*S*,*R*)-**163**, (*S*,*R*)-**182a-f**

Nr. p. k.	Sav. nr.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
1.	(*S*,*R*)-**163**	*n*-Pr	0.024[b]	0.042[b]	1.8
2.	**(*S*,*R*)-182a**	**Et**	**0.014**	**0.25**	**17.9**
3.	(*S*,*R*)-**182b**	Me	0.087	0.5	5.7
4.	(*S*,*R*)-**182c**	$HOCH_2CH_2$	0.068	0.27	4.0
5.	(*S*,*R*)-**182d**	$MeOCH_2CH_2$	0.037	0.10	2.7
6.	(*S*,*R*)-**182e**	$CF_3CH_2CH_2$	0.21	0.12	0.57
7.	(*S*,*R*)-**182f**	$(CH_3)_2CHCH_2$	0.5	1.3	2.6

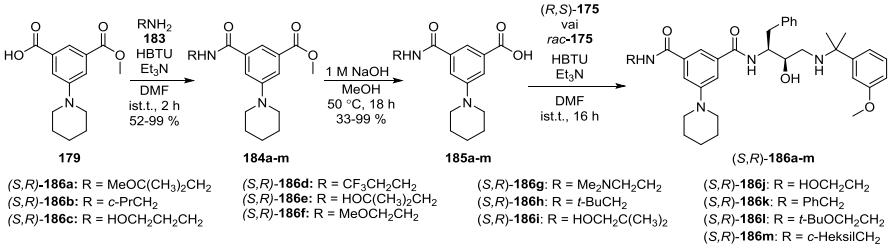
[a] Plm IV / Cat D inhibēšanas selektivitātes faktors
[b] Literatūras dati [110]

Interesanti, ka *N*,*N*-dietil- un *N*,*N*-dimetilaizvietotie savienojumi (*S*,*R*)-**182a** un (*S*,*R*)-**182b** uzrādīja visaugstāko Plm IV / Cat D inhibēšanas selektivitātes faktora vērtību (2.7. tabula, 2., 3. rinda), lai gan dokinga pētījumi uzrādīja, ka

platajā Plm IV S3 sub-kabatā ietilpst lielāki aizvietotāji. Savienojums (*S*,*R*)-**182c** ar divām *N*-hidroksietilgrupām uzrādīja 4 reizes zemāku inhibitoro aktivitāti uz Plm IV (IC_{50} Plm IV = 0.068 μM), salīdzinot ar amīdu (*S*,*R*)-**182a** (IC_{50} Plm IV = 0.014 μM) (2.7. tabula, 2., 4. rinda). Lielāku lineāru aizvietotāju gadījumā – *N*,*N*-di(metoksietil)- ((*S*,*R*)-**182d**) un *N*,*N*-di(3,3,3-trifluorpropil)- ((*S*,*R*)-**182e**) uzlabojās inhibitorā aktivitāte pret Cat D, kas tādējādi pazemināja selektivitātes faktora vērtību (2.7. tabula, 5., 6. rinda). Inhibitorās aktivitātes pieaugums pret Cat D liecina par to, ka minētās lineārās grupas labi ietilpst dziļajā Cat D S3 sub-kabatā. Savienojumam (*S*,*R*)-**182f** inhibitorā aktivitāte pret Plm IV samazinās (IC_{50} Plm IV = 0.5 μM) (2.7. tabula, 7. rinda). Tas nozīmē, ka diizobutilaizvietotājs ir pārāk liels, lai ietilptu Plm IV un Cat D S3 sub-kabatās. No iegūtajiem rezultātiem redzams, ka inhibitorās aktivitātes nodrošināšanai pret Plm IV S3 kabatā vēlami stēriski nelieli aizvietotāji, turpretī stēriski lielu aizvietotāju gadījumā novērojams inhibitorās aktivitātes pret Plm IV kritums un selektivitātes faktora vērtības samazināšanās.

2.2.2. Monoaizvietotu amīdu saturošu hidroksietilamīna atvasinājumu sintēze un struktūras aktivitātes likumsakarības

Lai iegūtu informāciju par Plm IV un Cat D S3 sub-kabatas izmēriem, turpmākajā darbā sintezējām hidroksietilamīna atvasinājumus ar monoaizvietotiem amīdiem. Savienojumus (*S*,*R*)-**186a-m** sintezējām pēc analoģiskas shēmas kā iepriekš (2.51. att., 2.52. att.).



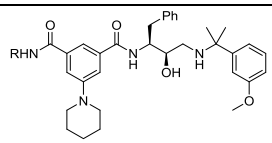
2.52. att. **Plazmepsīnu inhibitoru (*S*,*R*)-186a-m sintēze**

Amīdu **184a-m** sintēzē izmantojām iepriekš iegūto izoftalskābes monoesteri **179**. Pēc estera grupas hidrolīzes iegūtās skābes **185a-m** izmantojām amīdu (*S*,*R*)-**186a-m** sintēzē, kā kondensējošo reaģentu izmantojot HBTU (2.52. att.). Mērķsavienojumus (*S*,*R*)-**186a**,**c**,**e-g**,**i**,**j**,**l**,**m** sintezējām no (*R*,*S*)-**175** un produktus ieguvām ar 57–77 % iznākumu. Savukārt amīdus (*S*,*R*)-**186b**,**d**,**h**,**k**

sintezējām no racēmiska amīna *rac*-**175** un enantiotīrus produktus (*S*,*R*)-**186b**,**d**,**h**,**k** ieguvām ar 22–33 % iznākumu pēc attīrīšanas, izmantojot kolonnas ar hirālu sorbentu *Chiralpak – IC* un *Chiralpak – ID*. Sintezētajiem mērķsavienojumiem (*S*,*R*)-**186a-m** tika noteiktas IC_{50} vērtības uz Plm IV un Cat D (2.8. tabula).

2.8. tabula

Sintezētie savienojumi (*S,R*)-186a-m un to inhibitorā aktivitāte



(*S*,*R*)-**187**, (*S*,*R*)-**186a-m**

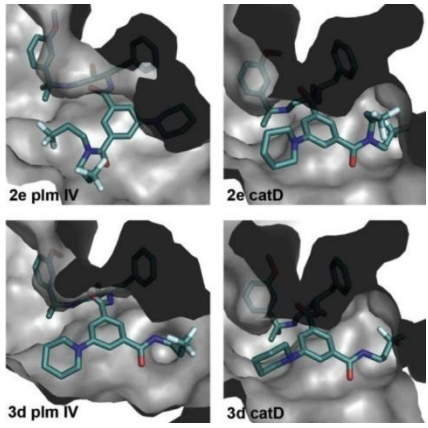
Nr. p. k.	Sav. nr.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
1.	(*S*,*R*)-**187**	*n*-Pr	0.038[b]	0.11[b]	2.9
2.	**(*S*,*R*)-186a**	**$MeOC(CH_3)_2CH_2$**	**0.048**	**2.1**	**43.8**
3.	(*S*,*R*)-**186b**	*c*-$PrCH_2$	0.030	0.76	25.3
4.	(*S*,*R*)-**186c**	$HOCH_2CH_2CH_2$	0.093	2.25	24.2
5.	(*S*,*R*)-**186d**	$CF_3CH_2CH_2$	0.024	0.58	24.2
6.	(*S*,*R*)-**186e**	$HOC(CH_3)_2CH_2$	0.10	1.66	16.6
7.	(*S*,*R*)-**186f**	$MeOCH_2CH_2$	0.05	0.75	15.0
8.	(*S*,*R*)-**186g**	$Me_2NCH_2CH_2$	0.36	4.8	13.3
9.	(*S*,*R*)-**186h**	*t*-$BuCH_2$	0.027	0.40	14.8
10.	(*S*,*R*)-**186i**	$HOCH_2C(CH_3)_2$	0.12	1.46	12.2
11.	(*S*,*R*)-**186j**	$HOCH_2CH_2$	0.21	1.42	6.8
12.	(*S*,*R*)-**186k**	$PhCH_2$	0.038	0.22	5.8
13.	(*S*,*R*)-**186l**	*t*-$BuOCH_2CH_2$	0.031	0.15	4.8
14.	(*S*,*R*)-**186m**	*c*-$HeksilCH_2$	0.09	0.15	1.7

[a] Plm IV / Cat D inhibēšanas selektivitātes faktors
[b] Literatūras dati [110]

Monoaizvietoti amīdi uzrādīja zemāku inhibitoro aktivitāti uz Cat D nekā to atbilstošie *N*,*N*-diaizvietotie amīdu analogi ((*S*,*R*)-**182d** (IC_{50} Cat D = 0.10 μM), (*S*,*R*)-**186f** (IC_{50} Cat D = 0.75 μM) un (*S*,*R*)-**182e** (IC_{50} Cat D = 0.12 μM), (*S*,*R*)-**186d** (IC_{50} Cat D = 0.58 μM)) (2.8. tabula, 5., 6. rinda, 2.8. tabula, 5., 7. rinda), kā rezultātā selektivitātes faktora vērtība palielinājās pat 40 reizes. Stēriski lielāku aizvietotāju gadījumā ((*S*,*R*)-**186**,**h**,**k**,**l**,**m**) inhibitorā aktivitāte pret Cat D uzlabojās un līdz ar to samazinājās selektivitātes faktora vērtība

(S = 1.7–14.8) (2.8. tabula, 9., 12., 13., 14. rinda). Stēriski mazākas grupas (R = *c*-PrCH$_2$, (*S*,*R*)-**186b**) gadījumā savienojuma inhibēšanas spēja pret Cat D samazinājās un selektivitātes faktora vērtība palielinājās (S = 25.3) (2.8. tabula, 3. rinda).

Savienojumu (*S*,*R*)-**182e** un (*S*,*R*)-**186d** dokinga modeļi Plm IV un Cat D kristālu struktūrās ir redzami 2.53. attēlā, kas parāda šo savienojumu stēriskās prasības. Dokinga pētījumos noskaidrots, ka ūdeņraža saites donoru vai ūdeņraža saites akceptoru ievadīšana varētu veicināt elektrostatisko sadarbību veidošanos ar elektronbagātajām funkcionālajām grupām S3 sub-kabatā. Ūdeņraža saites donoras grupas var sadarboties ar Plm IV aminoskābju atlikumu Asn13 un Leu14 karbonilgrupām un Cat D Asp323, Tyr15, Gln14 un Ala13 atlikumiem. Savukārt ūdeņraža saišu akceptoras grupas var sadarboties ar Plm IV Asn13 atlikumu un Cat D Gln14 atlikumu.

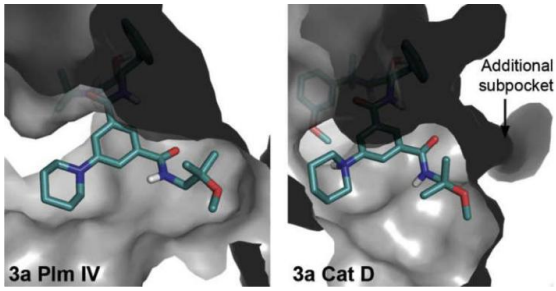


2.53. att. **Savienojumu (*S*,*R*)-182e un (*S*,*R*)-186d dokinga modeļi Plm IV un Cat D kristālu struktūrās**

Ūdeņraža saišu donoru ((*S*,*R*)-**186c**, (*S*,*R*)-**186e**, (*S*,*R*)-**186i**, (*S*,*R*)-**186j**) vai ūdeņraža saišu akceptoru ((*S*,*R*)-**186a**, (*S*,*R*)-**186f**) grupu ievadīšana S3 sub-kabatā ietilpstošo aizvietotāju pozīcijā samazināja inhibitoro aktivitāti pret Cat D (2.8. tabula, 2., 4., 6., 7., 10., 11. rinda). Salīdzinot savienojumu (*S*,*R*)-**163** (R = di-*n*-Pr) (IC_{50} Plm IV = 0.024 μM) (2.6. tabula, 2. rinda) un (*S*,*R*)-**186a** (IC_{50} Plm IV = 0.048 μM) (2.8. tabula, 2. rinda) IC_{50} rezultātus, redzams, ka inhibitorā aktivitāte samazinājusies 2 reizes. Tomēr ir būtiski samazinājusies inhibitora (*S*,*R*)-**186a** aktivitāte uz Cat D (IC_{50} Cat D = 2.1 μM), tādējādi

savienojums (*S*,*R*)-**186a** ir šīs sērijas selektīvākais savienojums (S = 43.8) (2.8. tabula, 1., 2. rinda).

Izmantojot savienojuma (*S*,*R*)-**186a** dokinga modeli ar Plm IV un Cat D, ir iespējams izskaidrot būtisko inhibitorās aktivitātes kritumu pret Cat D savienojumiem ar ūdeņraža saišu donorām grupām. Tas skaidrojams ar neaizpildītu hidrofobu sub-kabatu, kuras solvatācija ir entropiski neizdevīga. Tas nozīmē, ka, pateicoties iepriekš minētajām sadarbībām ar aminoskābju atlikumiem, amīda pozīcija abos enzīmos ir nemainīga (2.54. att.), kā rezultātā notiek entropiski neizdevīga Cat D hidrofobās sub-kabatas aizpildīšana ar ūdeni un samazinās inhibitorā aktivitāte pret Cat D.



2.54. att. **Savienojuma (*S*,*R*)-186a un Plm IV, Cat D kompleksa dokinga modelis**

Viszemāko inhibitoro aktivitāti pret Plm IV šajā sērijā uzrādīja savienojums (*S*,*R*)-**186g** (IC_{50} = 0.36 μM). Acīmredzot protonētā amino grupa veido ūdeņraža saites un jonu tipa sadarbības ar aminoskābju atlikumiem, kas atrodas ārpus S3 sub-kabatas.

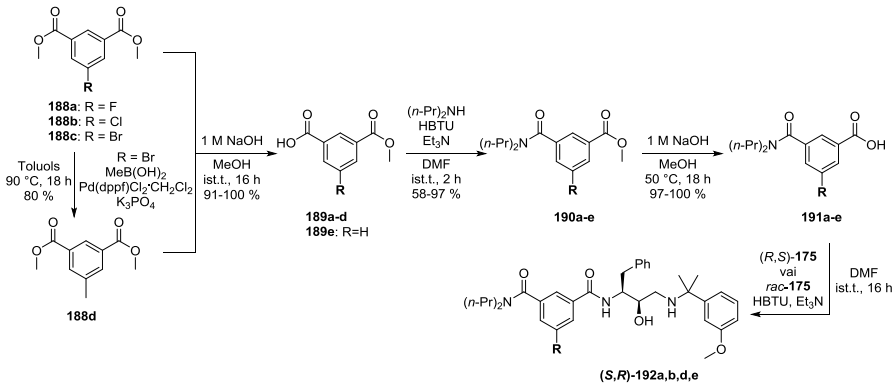
Aktīvākie šīs sērijas savienojumi uzrāda līdzīgu inhibitoro aktivitāti kā labākie *N*,*N*-diaizvietoto amīdu sērijas (*S*,*R*)-**182** savienojumi. Būtiski, ka šie savienojumi uzrāda zemāku Cat D inhibitoro aktivitāti, tādējādi uzlabojot selektivitāti. Neskatoties uz to, ka tika variētas S3 sub-kabatā ietilpstošās ūdeņraža saišu akceptoras un ūdeņraža saišu donoras grupas, dokinga pētījumi rāda, ka galvenokārt inhibitoro aktivitāti uz Plm IV un Cat D ietekmē aizvietotāju izmērs un forma. Sazaroti un gari aizvietotāji neietilpst Cat D šaurajā S3 padziļinājumā, savukārt atvērtā Plm IV S3 sub-kabatā šādi aizvietotāji ietilpst. Tas nozīmē, ka selektivitāti pār Cat D iespējams paaugstināt, aizpildot S3 sub-kabatu ar monoaizvietotu amīda funkciju, kas satur lineāras vai sazarotas hidrofobas grupas.

Enzīmu S4 sub-kabata (2.48. att. **A**) ir vēl viens inhibitoru saistīšanās apgabals, kurš būtiski atšķiras Plm IV un Cat D. Lai gan šī sub-kabata ir

hidrofoba abos enzīmos, Plm IV S4 sub-kabata ir plakanāka un vairāk vērsta uz šķīdinātāju, tādēļ turpmākajā darbā pētījām to aizvietotāju ietekmi uz inhibitoro aktivitāti, kas ietilpst S4 sub-kabatā.

2.2.3. Hidroksietilamīna atvasinājumu sintēze, variējot fenilgredzena aizvietotājus un struktūras-aktivitātes likumsakarības

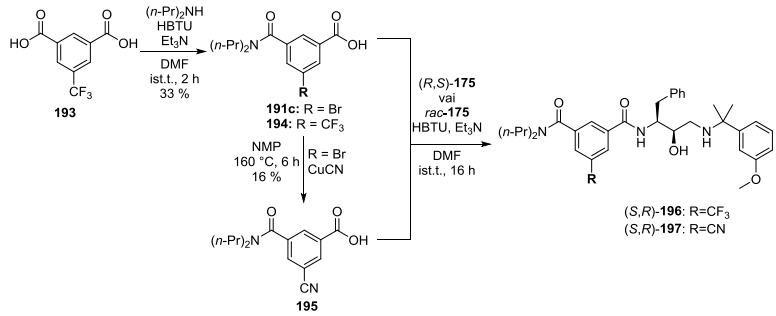
Fenilgredzena piektajā pozīcijā aizvietotus savienojumus sintezējām līdzīgi kā iepriekšējās sērijas inhibitorus. Monoesteru **189a-d** sintēzē izmantojām komerciāli pieejamos dimetilizoftalāta atvasinājumus **188a-c**. Dimetil-5-metilizoftalātu (**188d**) ieguvām no bromīda **188c** Pd-katalizētā šķērssametināšanā ar metilborskābi kālija fosfāta klātienē (toluols, 90 °C, 18 stundas). Pēc estera grupas hidrolīzes ieguvām produktus **189a-d** ar augstu iznākumu, kurus tālākajās stadijās izmantojām bez papildu attīrīšanas. Skābes **189a-d** tālāk izmantojām amīdu **190a-e** sintēzē, kurus ieguvām ar 58–97 % iznākumu. Pēc estera grupas hidrolīzes iegūtās skābes **191a-e** izmantojām inhibitoru (*S*,*R*)-**192a**,**b**,**d**,**e** sintēzē. Mērķsavienojumu (*S*,*R*)-**192a** sintezējām no enantiotīra amīna (*R*,*S*)-**175** un produktu ieguvām ar 62 % iznākumu. Savukārt inhibitorus (*S*,*R*)-**192b**,**d**,**e** sintezējām no racēmiska amīna *rac*-**175** un enantiotīrus produktus (*S*,*R*)-**192b**,**d**,**e** ieguvām ar 16–24 % iznākumu pēc attīrīšanas, izmantojot kolonnas ar hirālu sorbentu *Chiralpak – IC* un *Chiralpak – ID* (2.55. att.).



2.55. att. **Inhibitoru (*S*,*R*)-192a,b,d,e sintēze**

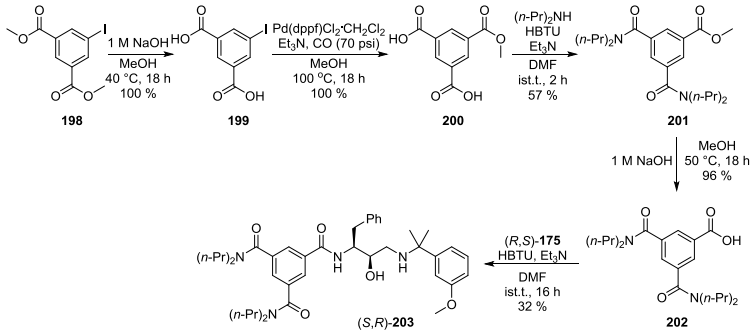
Pēc analoģiskas shēmas sintezējām arī būvblokus **194** un **195**. Amīdu **194** ieguvām komerciāli pieejamās 5-(trifluormetil)izoftalskābes (**193**). Cianīdu **195** sintezējām no iepriekš iegūtā bromīda **191c**, izmantojot vara (I) katalizētu broma

aizvietošanu ar ciano grupu (NMP, 160 °C, 6 stundas).[113] Vēlamo produktu **195** ieguvām ar 16 % iznākumu, kas skaidrojams ar nepilnīgu izejvielas **191c** konversiju. Iegūtās skābes **194** un **195** izmantojām savienojumu (*S*,*R*)-**196** un (*S*,*R*)-**197** sintēzē. Mērķsavienojumu (*S*,*R*)-**197** sintezējām no enantiotīra amīna (*R*,*S*)-**175**. Savukārt hidroksietilamīna atvasinājumu (*S*,*R*)-**196** sintezējām no racēmiska amīna *rac*-**175** un enantiotīru produktu (*S*,*R*)-**196** ieguvām ar 19 % iznākumu pēc attīrīšanas, izmantojot kolonnu ar hirālu sorbentu *Chiralpak – ID* (2.56. att.).



2.56. att. **Inhibitoru (*S*,*R*)-196 un (*S*,*R*)-197 sintēze**

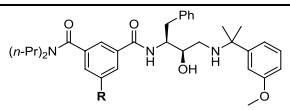
Karbonskābes **202** sintēzei izmantojām komerciāli pieejamo dimetil-jodizoftalātu **198**. Estera grupas ievadīšanai izoftalskābes **199** molekulā izmantojām Pd-katalizētu metoksikarbonilēšanas reakciju ar oglekļa monoksīdu [114], par katalizatoru lietojot $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (Et_3N, metanols, 100 ^oC, 18 stundas). Esteri **200** ieguvām ar kvantitatīvu iznākumu un tālākajās stadijās izmantojām bez papildu attīrīšanas. No iegūtās dikarbonskābes **200** tālāk sintezējām diamīda atvasinājumu **201**. Pēc estera grupas hidrolīzes ieguvām skābi **202**, kuru izmantojām mērķsavienojuma (*S*,*R*)-**203** sintēzē iepriekš minētos amīdu iegūšanas apstākļos, un enantiotīru produktu (*S*,*R*)-**203** ieguvām ar 32 % iznākumu (2.57. att.).



2.57. att. **Hidroksietilamīna fragmentu saturoša inhibitora (*S*,*R*)-203 sintēze**

Sintezētajiem mērķsavienojumiem (*S*,*R*)-**192a**,**b**,**d**,**e**, (*S*,*R*)-**196**, (*S*,*R*)-**197**, (*S*,*R*)-**203** tika noteiktas IC_{50} vērtības uz Plm IV un Cat D (2.9. tabula). *N*,*N*-Dipropilamīda grupas ievadīšana molekulā (*S*,*R*)-**203** būtiski neuzlaboja inhibitoro aktivitāti uz Plm IV (IC_{50} Plm IV = 0.018 μM), salīdzinot ar piperidīna ciklu saturošo inhibitoru (*S*,*R*)-**163** (IC_{50} Plm IV = 0.024 μM), taču inhibitors (*S*,*R*)-**203** uzrādīja 15 reizes zemāku aktivitāti uz Cat D (IC_{50} Cat D = 0.7 μM), kā rezultātā būtiski pieauga selektivitātes faktora vērtība (S = 38.9) (2.9. tabula, 1., 9. rinda). Savukārt stēriski nelielu aizvietotāju gadījumā (*S*,*R*)-**192a**, (*S*,*R*)-**192e** inhibitorā aktivitāte uz Plm IV nedaudz samazinājās, salīdzinot ar piperidinil- (*S*,*R*)-**163** un fenil- aizvietotiem (*S*,*R*)-**164** inhibitoriem. Tomēr šo savienojumu aktivitāte uz Cat D samazinājās būtiski, ļaujot paaugstināt Plm IV / Cat D inhibēšanas selektivitātes faktora vērtību līdz 20 (2.9. tabula, 1., 2., 3., 6. rinda).

Sintezētie inhibitori (*S*,*R*)-192a,b,d,e, (*S*,*R*)-196, (*S*,*R*)-197, (*S*,*R*)-203 un to inhibitorā aktivitāte



(*S*,*R*)-**163**, (*S*,*R*)-**164**
(*S*,*R*)-**192a**,**b**,**d**,**e**
(*S*,*R*)-**196**, (*S*,*R*)-**197**, (*S*,*R*)-**203**

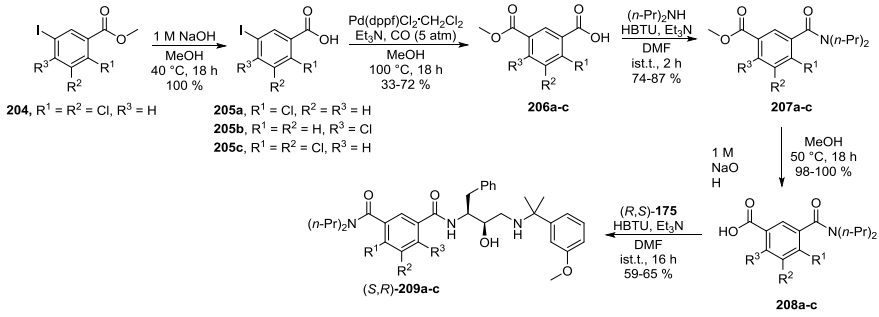
Nr. p. k.	Sav. nr.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
1.	(*S*,*R*)-**163**	1-Piperidinil	0.024[b]	0.042[b]	1.8
2.	(*S*,*R*)-**164**	Ph	0.006[b]	0.054[b]	9.0
3.	(*S*,*R*)-**192a**	F	0.050	1.0	20.0
4.	(*S*,*R*)-**192b**	Cl	0.008	0.096	12.0
5.	(*S*,*R*)-**192d**	Me	0.023	0.21	9.1
6.	(*S*,*R*)-**192e**	H	0.058	1.15	19.8
7.	(*S*,*R*)-**196**	CF_3	0.015	0.067	4.5
8.	(*S*,*R*)-**197**	CN	0.059	0.56	9.5
9.	(*S*,*R*)-**203**	(*n*-Pr)$_2$NC(=O)	0.018	0.7	38.9

[a] Plm IV / Cat D inhibēšanas selektivitātes faktors
[b] Literatūras dati [110]

Interesanti, ka inhibitors (*S*,*R*)-**192b**, kas satur hlora atomu fenilgredzenā, uzrādīja visaugstāko inhibitoro aktivitāti uz Plm IV un samērā augstu inhibitoro aktivitāti uz Cat D, kā rezultātā Plm IV / Cat D inhibēšanas selektivitātes faktors nepieauga (2.9. tabula, 4. rinda). Citu aizvietotāju gadījumā – metil- (*S*,*R*)-**192d**, ciano- (*S*,*R*)-**197**, trifluormetil- (*S*,*R*)-**196** – savienojumu inhibitorā aktivitāte uz Plm IV netika būtiski ietekmēta (2.9. tabula, 5., 7., 8. rinda). No iegūtajiem rezultātiem (2.9. tabula) redzams, ka nav novērojamas skaidras struktūras-aktivitātes likumsakarības. Visticamāk, ka Plm IV un Cat D S4 sub-kabatas aizvietotāju uzrādītās inhibitorās aktivitātes vērtības ir saistītas ar hidrofobām mijiedarbībām un polārām nekovalentām sadarbībām.

Lai iegūtu plašāku informāciju par struktūras-aktivitātes likumsakarībām, sintezējām inhibitora (*S*,*R*)-**192b** analogus **209a-c**, kuri satur hlora atomu *orto*- un *para*- pozīcijā, kā arī dihloraizvietotu analogu, amīda daļā saglabājot di-*n*-propilfragmentu. Benzoskābes būvblokus **208a-c** sintezējām saskaņā ar 2.58. attēlā redzamo sintēzes shēmu. Būvblokus **208a-c** sintezējām no komerciāli pieejamām izejvielām **204** un **205a**,**b**, kuras molekulā satur gan hlora, gan joda atomus. Estera funkcijas ievadīšanai molekulā izmantojām

Pd-katalizētu alkoksikarbonilēšanas reakciju. Ir zināms, ka šajā reakcijā hlora atoms reaģē daudz lēnāk nekā joda atoms [115], kas ļauj selektīvi iegūt vēlamos produktus un saglabāt molekulā hlora atomus. Jodīdu **205a-c** alkoksikarbonilēšanas reakcijā ar oglekļa monoksīdu un metanolu, par katalizatoru izmantojot $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, esterus **206a**,**b** ieguvām ar vidēji augstiem iznākumiem (attiecīgi 72 % un 66 %), savukārt savienojuma **206c** gadījumā iznākums bija zemāks – 33 %. Tālāk sekoja iepriekš aprakstītā sintēzes secība – amīdu iegūšana un tai sekojoša hidrolīzes reakcija, kas ļāva iegūt benzoskābes **208a-c**. Mērķsavienojumu (*S*,*R*)-**209a-c** sintēzē izmantojām iepriekš aprakstītos reakciju apstākļus kondensācijas reakcijai starp skābes skābēm **208a-c** un amīnu (*R*,*S*)-**175**. Amīdus (*S*,*R*)-**209a-c** ieguvām ar 59–65 % iznākumiem (2.58. att.).

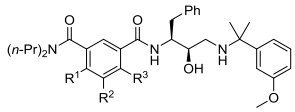


2.58. att. **Mērķsavienojumu (*S*,*R*)-209a-c sintēze**

Iegūtajiem savienojumiem (*S*,*R*)-**209a-c** noteicām inhibitoro aktivitāti uz Plm IV un Cat D, lai pārbaudītu, vai hlora atomu pozīcijas maiņa molekulā dod ieguldījumu selektivitātes uzlabošanā (2.10. tabula).

2.10. tabula

Sintezētie inhibitori (*S*,*R*)-192b (*S*,*R*)-209a-c un to inhibitorā aktivitāte



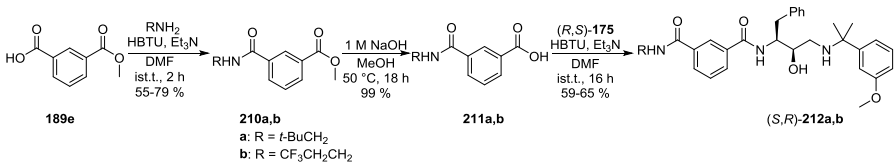
(*S*,*R*)-**192b**
(*S*,*R*)-**209a-c**

Nr. p. k.	Sav. nr.	Cl-	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
1.	(*S*,*R*)-**192b**	R^1, R^3 = H, R^2 = Cl	0.008	0.096	12.0
2.	(*S*,*R*)-**209a**	R^1 = Cl, R^2, R^3 = H	0.16	0.57	3.6
3.	(*S*,*R*)-**209b**	R^1, R^2 = H, R^3 = Cl	0.75	0.51	0.68
4.	(*S*,*R*)-**209c**	R^1, R^2 = Cl, R^2 = H	0.050	0.080	1.6

[a] Plm IV / Cat D inhibēšanas selektivitātes faktors

No rezultātiem redzams, ka hlora atoma pozīcijas maiņa būtiski ietekmē inhibitoro aktivitāti uz Plm IV. Hlora atoma novietojuma maiņas rezultātā no R^2 pozīcijas ((*S*,*R*)-**192b**, IC_{50} Plm IV = 0.008 μM) uz R^1 pozīciju ((*S*,*R*)-**209a**, IC_{50} Plm IV = 0.16 μM) inhibitorā aktivitāte uz Plm IV samazinājās 20 reizes, savukārt R^3 pozīcijā aizvietota inhibitora (*S*,*R*)-**209b** gadījumā aktivitāte samazinājās pat 90 reizes (IC_{50} Plm IV = 0.75 μM), bet R^1, R^2 pozīcijās aizvietota inhibitora (*S*,*R*)-**209c** gadījumā IC_{50} vērtība uz Plm IV samazinājās 6 reizes (2.10. tabula, 1.–4. rinda). Interesanti, ka R^3 pozīcijā ar hlora atomu aizvietots inhibitors (*S*,*R*)-**209b** uzrāda augstāku aktivitāti uz Cat D nekā uz Plm IV (IC_{50} Plm IV = 0.75 μM, IC_{50} Cat D = 0.51 μM, 2.10. tabula, 3. rinda). Kopumā redzams, ka hlora atoma pozīcijas maiņas rezultātā inhibitorā aktivitāte uz Plm IV ir pazeminājusies un nav izdevies uzlabot inhibēšanas selektivitātes faktora vērtību (2.10. tabula).

Tā kā savienojums (*S*,*R*)-**192e**, kurš nesatur aizvietotāju fenilgredzena piektajā pozīcijā, uzrādīja visaugstāko selektivitātes faktora vērtību (S = 19.8) (2.9. tabula, 4. rinda), nolēmām apvienot abu sēriju aktīvākos savienojumus un sintezēt *N*-mono-aizvietoto amīdu inhibitoru (*S*,*R*)-**186d** un (*S*,*R*)-**186h** analogus ar neaizvietotu fenilgredzenu (2.59. att.).

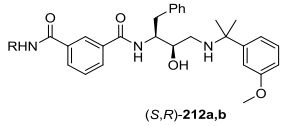


2.59. att. **Hidroksietilamīna fragmentu saturošu inhibitoru (*S*,*R*)-212a,b sintēze**

Savienojumus (*S*,*R*)-**212a**,**b** sintezējām saskaņā ar 2.59. attēlā redzamo sintēzes shēmu. Amīdus **210a**,**b** ieguvām no komerciāli pieejamās benzoskābes **189**, kā kondensējošo reaģentu izmantojot HBTU. Pēc hidrolīzes ar 1 M NaOH šķīdumu metanolā iegūtās skābes **211a**,**b** izmantojām mērķsavienojumu (*S*,*R*)-**212a**,**b** sintēzē. Iegūtajiem inhibitoriem (*S*,*R*)-**212a**,**b** noteicām IC_{50} vērtības uz Plm IV un Cat D (2.11. tabula).

2.11. tabula

Sintezētie inhibitori (*S*,*R*)-212a,b un to inhibitorā aktivitāte



Nr. p. k.	Sav. nr.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
1.	(*S*,*R*)-**212a**	*t*-$BuCH_2$	0.076	3.8	50.0
2.	(*S*,*R*)-**212b**	$CF_3CH_2CH_2$	0.15	4.9	32.6

[a] Plm IV / Cat D inhibēšanas selektivitātes faktors

Iegūtie rezultāti rāda, ka amīdu (*S*,*R*)-**212a**,**b** inhibitorā aktivitāte uz Plm IV ir nedaudz pazeminājusies, tomēr ir būtiski samazinājušās IC_{50} vērtības uz Cat D (2.11. tabula, 1., 2. rinda). Ieviesto modifikāciju rezultātā savienojums (*S*,*R*)-**212a** uzrāda visaugstāko Plm IV / Cat D inhibēšanas selektivitātes faktora vērtību (S = 50.0) (2.11. tabula, 1. rinda).

2.2.4. Selektīvāko savienojumu IC_{50}, EC_{50} vērtības un Plm X inhibēšana

Savienojumiem, kuri uzrādīja visaugstākās Plm IV / Cat D inhibēšanas selektivitātes faktora vērtības, noteicām *P. falciparum* augšanas inhibēšanas vērtības (EC_{50}) un inhibitorās aktivitātes vērtības uz Plm I, Plm II un Plm IV (2.12. tabula).

2.12. tabula

Selektīvāko savienojumu IC_{50} vērtības uz Plm I, Plm II, Plm IV un EC_{50}

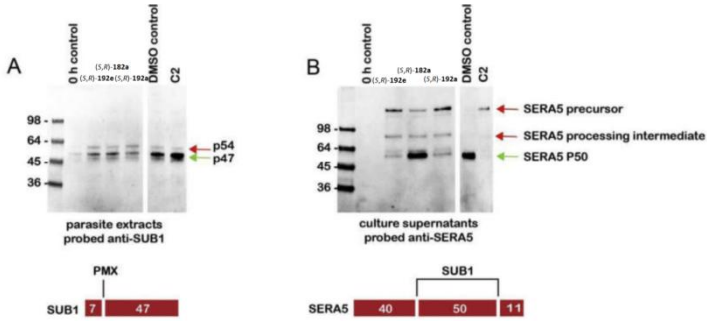
Nr. p. k.	Sav. nr.	IC_{50} Plm I, μM	IC_{50} Plm II, μM	IC_{50} Plm IV, μM	S[a]	EC_{50}[b] *Pf* 3D7, nM
1.	(*S*,*R*)-**182a**	0.8	0.16	0.014	17.9	1.5
2.	(*S*,*R*)-**186a**	7.4	5.4	0.048	43.8	2.0
3.	(*S*,*R*)-**186b**	1.8	0.5	0.030	25.3	1.8
4.	(*S*,*R*)-**186d**	2.5	2.2	0.024	24.2	2.0
5.	(*S*,*R*)-**186h**	2.0	0.85	0.027	14.8	6.0
6.	**(*S*,*R*)-192e**	**3.1**	**1.7**	**0.058**	**19.8**	**0.3**
7.	(*S*,*R*)-**192a**	1.1	1.1	0.050	20.0	1.5
8.	(*S*,*R*)-**203**	0.78	0.27	0.018	38.9	6.0
9.	(*S*,*R*)-**212a**	5.6	7.1	0.076	50.0	2.0
10.	(*S*,*R*)-**212b**	10.3	10.4	0.15	32.6	6.0

[a] Plm IV / Cat D inhibēšanas selektivitātes faktors
[b] EC_{50} vērtības tika noteiktas, izmantojot SYBR *Green* testu ar 96 stundu inkubācijas laiku

No rezultātiem redzams, ka visi testētie savienojumi ir samērā vāji Plm I un Plm II inhibitori (2.12. tabula). Augstākās IC_{50} vērtības uz Plm II uzrādīja savienojumi ar hidrofobiem un stēriski lieliem aizvietotājiem molekulas amīda daļā ((*S*,*R*)-**186b**, (*S*,*R*)-**186h**, (*S*,*R*)-**203**, 2.12. tabula, 3., 5., 8. rinda). Visi 2.12. tabulā minētie savienojumi uzrādīja EC_{50} vērtības nanomolārā līmenī, kas liecina par labu *P. falciparum* parazīta augšanas inhibēšanas spēju inficētu asins šūnu testā. Šie rezultāti liecina, ka nav novērojama sakarība starp inhibitorās aktivitātes vērtībām uz Plm I, Plm II un EC_{50} vērtībām. Bet inhibitorās aktivitātes vērtības uz Plm IV korelē ar rezultātiem inficētu asins šūnu testā (EC_{50}). Interesanti, ka savienojums (*S*,*R*)-**192e**, kurš uzrādīja 3 reizes zemāku IC_{50} vērtību uz Plm IV nekā savienojums (*S*,*R*)-**203**, uzrādīja 20 reizes labāku *P. falciparum* augšanas inhibēšanas vērtību (2.12. tabula, 6., 8. rinda). Savienojums (*S*,*R*)-**192e** uzrādīja visaugstāko *P. falciparum* augšanas inhibēšanas spēju inficētu asins šūnu testā EC_{50} = 0.3 nM (2.12. tabula, 6. rinda).

Savienojumiem (*S*,*R*)-**182a**, (*S*,*R*)-**192e**, (*S*,*R*)-**192a**, kuri uzrādīja augstāko inhibitoro aktivitāti inficētu asins šūnu testā, noteicām arī spēju inhibēt arī Plm X. Plazmepsīna X galvenā bioloģiskā funkcija ir SUB1 proteolītiska aktivēšana.[19] SUB1 aktīvās formas veidošana ietver 2 soļus. Pirmajā solī tiek nošķelts ~82 kDa pre-proenzīms, veidojot 54 kDa proteīnu (p54), no kura otrajā solī tiek atšķelts 47kDa terminālais produkts (p47), kurš akumulējas intraeritrocītiskās parazīta attīstības pēdējo 12 stundu laikā.[21], [22] Pirmais solis ir autokatalītisks, bet otro soli no p54 uz p47 veicina Plm X.[21] Izmantojot *Western blot* testu, savienojumiem (*S*,*R*)-**182a**, (*S*,*R*)-**192e**, (*S*,*R*)-**192a** noteicām to ietekmi uz SUB1 aktivēšanu un parazīta egresu (2.60. att.).

SUB1 aktīvā forma šķeļ serīniem bagātu antigēnu 5 (SERA5; merozoītu egresa negatīvais regulators) un citus efektorus, ieskaitot merozoītu virsmas proteīnu 1 (MSP1), kas atver eritrocītu un ļauj parazītam iebrukt jaunā šūnā. Tādējādi SUB1 ir atbildīgs par merozoītu egresu no eritrocīta.[19], [21], [22] Šis egress tika kvantificēts, izmērot šķīstošā parazīta proteīna SERA5 daudzumu parazīta kultūras supernatantā. SERA5 arī ir SUB1 substrāts, kas tiek atbrīvots P50 formā, kas rodas no SUB1-mediētas lielāka prekursora šķelšanas. SERA5 P50 formas esamība liecina par SUB1 aktivitāti un efektīvu parazīta egresu no eritrocīta. Pakļaujot attīstības stadijā esošus parazītus inhibitoru (*S*,*R*)-**182a**, (*S*,*R*)-**192e**, (*S*,*R*)-**192a** iedarbībai, notiek SUB1 p54 formas uzkrāšanās. Tas liecina, ka tiek inhibēta p54 formas pārvēršanās par p47 formu (2.60. att. (**A)**).



2.60. att. **Savienojumu (*S*,*R*)-182a, (*S*,*R*)-192e, (*S*,*R*)-192a *P. falciparum* SUB1 aktivēšana un parazīta egresa inhibēšana**

Inhibitoru (*S*,*R*)-**182a**, (*S*,*R*)-**192e**, (*S*,*R*)-**192a** iedarbībai pakļautajiem parazītiem atļāva sasniegt egresa stadiju. Šo kultūru supernatantu analīze (2.60. att. (**B**)) parādīja, ka savienojumu (*S*,*R*)-**192e** un (*S*,*R*)-**192a** klātienē notiek SERA5 P50 formas daudzuma samazināšanās. Tomēr savienojumu

(*S*,*R*)-**182a**, (*S*,*R*)-**192e**, (*S*,*R*)-**192a** klātienē palielinājās SERA5 prekursora vai procesa starpproduktu daudzums. Tas liecina par defektiem parazīta egresā un SERA5 šķelšanā. Šie rezultāti liecina, ka savienojumu (*S*,*R*)-**192e** un (*S*,*R*)-**192a** parazīta augšanas inhibēšanas mehānisms ietver Plm X inhibēšanu.

Pētījuma ietvaros tika sintezēti hidroksietilamīna atvasinājumi ar dažādiem mono- un diaizvietotiem amīdiem. Tika noskaidrots, ka selektivitāti attiecībā pret Cat D iespējams paaugstināt, aizpildot S3 sub-kabatu ar monoaizvietotu amīda funkciju, kas satur lineāras vai sazarotas hidrofobas grupas. Sazaroti un gari aizvietotāji neietilpst Cat D šaurajā S3 padziļinājumā, savukārt atvērtajā Plm IV S3 sub-kabatā šādi aizvietotāji ietilpst. Variējot S4 sub-kabatā ietilpstošos fenilgredzena aizvietotājus, nav novērojamas skaidras struktūras-aktivitātes likumsakarības. Visticamāk, ka Plm IV un Cat D S4 sub-kabatas aizvietotāju uzrādītās inhibitorās aktivitātes vērtības var skaidrot ar hidrofobām mijiedarbībām un polārām nekovalentām sadarbībām. Sintezētie hidroksietilamīna atvasinājumi uzrāda inhibitorās aktivitātes vērtības uz Plm IV nanomolārā līmenī, selektivitātes faktora vērtībai pret Cat D sasniedzot 50. Aktīvākie savienojumi uzrāda *P. falciparum* šūnu augšanas inhibēšanas spēju nanomolārā līmenī. Izmantojot *Western blot* testu, ir noskaidrots, ka šie savienojumi spēj inhibēt arī Plm X. Tā kā pašlaik nav zināmas Plm IX un Plm X struktūras, Plm IV var kalpot kā modeļproteīns jaunu inhibitoru izstrādē, jo pastāv strukturāla līdzība starp šiem proteīniem.

3. EKSPERIMENTĀLĀ DAĻA

Sintēzēm, kurām bija nepieciešama sausa vide, traukus žāvēja žāvskapī 120 °C temperatūrā un atdzesēja argona plūsmā. Reakcijām inertā vide tika nodrošināta ar argona atmosfēru. THF žāvēja *M-BRAUN MB SPS-800* šķīdinātāju iekārtā.

Reaģenti un šķīdinātāji tika iegādāti no *Acros Organics*, *Sigma Aldrich*, *Strem Chemicals* un *Alfa Aesar* izplatītājiem un izmantoti bez papildu attīrīšanas.

Reakcijas gaitu kontrolēja un vielu tīrību noteica ar UEŠH *Waters Acquity*, detektoru *Acquity UPLC PDA eλ*, datu apstrādes sistēmu *MassLynx*, apgrieztās fāzes kolonnu *Acquity UPLC® BEH C18*, 50 × 2.1 mm, 1.7 μm, ar kustīgo fāzi: acetonitrils ar 0.01 % trifluoretiķskābes šķīdumu ūdenī, eluenta sastāvam 6 minūšu laikā, mainoties no 10 % acetonitrila ūdens šķīduma līdz 95 % acetonitrila ūdens šķīduma, plūsmas ātrums 0.5 mL / min, UV absorbcija no 180 nm līdz 800 nm). Masspektrometrs *SQ Detector 2* ar elektroizsmidzināšanas jonizāciju un kvadrupola analizatoru.

AIMS analīzes veiktas ar *Waters Acquity UPLC H-Class* UEŠH, kas apvienots ar *Waters Synapt G2 Si TOF MS* masspektrometru.

Tiešās fāzes kolonnu hromatogrāfija veikta ar *Armen Spot Flash* iekārtu. Par eluentiem izmantoja petrolētera (frakcijas ar $T_{virš.}$ = 40–60 °C) / etilacetāta, etilacetāta / MeOH vai CH_2Cl_2 / MeOH maisījumus. Detektēšanai izmantoja UV gaismas absorbciju pie 254 nm un 210 nm.

Iegūto savienojumu ^{1}H-KMR un ^{13}C-KMR spektri uzņemti $CDCl_3$ un CD_3OD šķīdumos, izmantojot *Varian Mercury-400* vai *Bruker fourier-300* spektrometrus. Kā standarti tika izmantoti šķīdinātāju signāli ^{1}H: $CDCl_3$, δ = 7.260 m.d., CD_3OD, δ = 4.780, 3.310 m.d. ^{13}C: $CDCl_3$, δ = 77.16 m.d., CD_3OD, δ = 49.1 m.d.

Optiskā griešana noteikta ar *Perkin-Elmer 141* un *Kruess P3000* polarimetru.

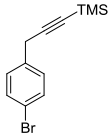
Kolonnu hromatogrāfijai izmantots *Acros* silikagels (0.060-0.200 nm). Vielu svēršanai izmantoti analītiskie svari *Sartorius BP 211D* (līdz 80 mg d = 0.01 mg, līdz 210 mg d = 0.1 mg) un *Boeco Germany* svari (d = 0.01 g).

Mērķsavienojumu **74a-c**, **75**, **91**, **101a-g**, **108**, **114a-c**, **121**, **131**, **135**, **140a**,**b**,**d**,**e**, **140f**,**g** sintēžu apraksti un eksperimentālie dati publicēti *Arch. Pharm. Life Sci*. **2018**, *351*, 1800151.

Mērķsavienojumu (*R*)-**159a-c**, (*R*)-**161** sintēžu apraksti un eksperimentālie dati publicēti *Chem. Heterocycl. Comp.* **2020**, *56*, 60-66.

Mērķsavienojumu (*S*,*R*)-**182a-f**, (*S*,*R*)-**186a-m**, (*S*,*R*)-**192a**,**b**,**d**,**e**, (*S*,*R*)-**196**, (*S*,*R*)-**197**, (*S*,*R*)-**203**, (*S*,*R*)-**209a-c**, (*S*,*R*)-**212a**,**b** sintēžu apraksti un eksperimentālie dati publicēti *Eur. J. Med. Chem*. **2019**, *163*, 344-352.

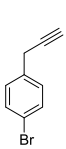
(3-(4-Bromfenil)prop-1-īn-1-il)trimetilsilāns (102)



Izkarsētā un argona plūsmā atdzesētā augstspiediena ampulā ielēja 15 mL THF, tad trimetilsililacetilēnu (**92**) (3.14 g, 4.5 mL, 32.0 mmol). Šķīdumu atdzesēja ledus vannā līdz 0 ^{o}C un pilinot pievienoja *i*-PrMgCl šķīdumu THF (2.0 M šķīdums THF, 12.0 mL, 24.0 mmol). Maisīja 0 ^{o}C 30 minūtes, tad istabas temperatūrā 1 stundu. Pievienoja CuBr (0.69 g, 4.80 mmol), maisīja istabas temperatūrā 30 minūtes, tad pievienoja 4-brombenzilbromīda (**87**) (2.00 g, 8.00 mmol) šķīdumu 5 mL THF. Vārīja 3 stundas. Reakcijas maisījumu ietvaicēja pazeminātā spiedienā. Pārpalikumam pievienoja 20 mL destilētu H_2O un 20 mL EtOAc, izveidojās oranžas nogulsnes. Tās nofiltrēja caur celīta slāni. Celīta slāni mazgāja ar destilētu H_2O un EtOAc. Filtrātu pārnesa dalāmajā piltuvē un nodalīja EtOAc slāni, H_2O slāni ekstrahēja ar EtOAc (2 × 40 mL). Organiskos slāņus apvienoja, mazgāja ar destilētu ūdeni (50 mL), ar piesātinātu NaCl šķīdumu (50 mL), žāvēja virs b/ū Na_2SO_4, nofiltrēja, ietvaicēja pazeminātā spiedienā. Atlikumu attīrīja ar ARMEN tiešās fāzes hromatogrāfu. Eluents PĒ / EtOAc no 90:10 līdz 40:60, 100 g silikagela kolonna, plūsmas ātrums 30 mL / min. Ieguva 2.02 g (94 %) produkta **102** kā gaiši dzeltenu eļļu.

^{1}H-KMR ($CDCl_3$) δ: 7.28–7.23 (m, 2H), 7.05–7.01 (m, 2H), 3.41 (s, 2H), 0.00 (s, 9H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 135.4, 131.5, 129.6, 120.4, 103.5, 87.4, 25.7, 0.02 m.d. R_f = 0.72 (PĒ / EtOAc, 9:1).

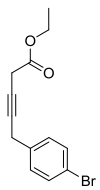
1-Brom-4-(prop-2-īn-1-il)benzols (103)



[3-(4-Bromfenil)-prop-1-īnil]-trimetilsilānu (**102**) (2.02 g, 7.56 mmol) izšķīdināja 10 mL etanola, tad pilinot pievienoja $AgNO_3$ (1.93 g, 9.77 mmol) šķīdumu 12 mL H_2O / EtOH maisījumā attiecībā 1:3. Maisīja istabas temperatūrā 30 minūtes, tad pilinot pievienoja KCN

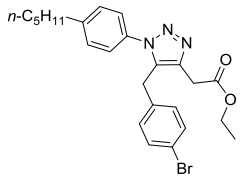
(4.87 g, 74.84 mmol) šķīdumu H_2O (15 mL). Maisīja istabas temperatūrā 2 stundas. Reakcijas maisījumam pievienoja EtOAc un H_2O. Organisko slāni mazgāja ar destilētu H_2O (2 × 20 mL), tad ar piesātinātu NaCl šķīdumu (20 mL), žāvēja virs b/ū Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Ieguva 0.78 g (53 %) produkta **103**, ko tālākajās reakcijās izmantoja bez papildu attīrīšanas.
^{1}H-KMR ($CDCl_3$) δ: 7.50–7.41 (m, 2H), 7.26–7.21 (m, 2H), 3.56 (d, *J* = 2.7 Hz, 2H), 2.20 (t, *J* = 2.7 Hz, 1H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 135.2, 131.7, 129.7, 120.7, 81.4, 70.9, 24.5 m.d. R_f = 0.72 (PĒ / EtOAc, 9:1).

5-(4'-Metoksibifenil-4-il)-pent-3-īnskābes etilesteris (126)



Apaļkolbā ar izejvielu **103** (0.78 g, 4.03 mmol) pievienoja 5 ml sausa acetonitrila, tad CuI (38 mg, 0.20 mmol) un etildiazoacetātu (**97**) (446 μL, 4.43 mmol). Maisīja istabas temperatūrā 20 stundas. Reakcijas maisījumam pievienoja piesātinātu NH_4Cl ūdens šķīdumu (10 mL) un ekstrahēja ar EtOAc (3 × 15 mL). Organiskos slāņus apvienoja, žāvēja virs b/ū Na_2SO_4, nofiltrēja un ietvaicēja pazeminātā spiedienā. Atlikumu attīrīja ar ARMEN tiešās fāzes hromatogrāfu. Eluents PĒ / EtOAc no 95:5 līdz 90:10, 30 g silikagela kolonna, plūsmas ātrums 20 mL / min. Ieguva 0.93 g (82 %) produkta **126** kā gaiši dzeltenu eļļu.
^{1}H-KMR ($CDCl_3$) δ: 7.45–7.41 (m, 2H), 7.25–7.22 (m, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.57 (t, *J* = 2.5 Hz, 2H), 3.31 (t, *J* = 2.5 Hz, 2H), 1.29 (t, *J* = 7.1 Hz, 3H) m.d.

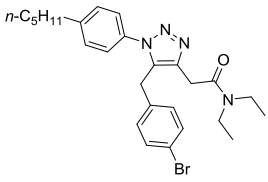
Etil 2-(5-(4-brombenzil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)acetāts (136)



Izkarsētā un argona plūsmā atdzesētā apaļkolbā iesvēra 4-pentilfenilazīdu (**57**) (689 mg, 3.64 mmol), alkīnu (**126**) (930 mg, 3.31 mmol), Cp·RuCl(COD) (**70**) (126 mg, 0.33 mmol) un izšķīdināja 15 mL sausa 1,4-dioksāna. Maisīja istabas temperatūrā 16 stundas. Reakcijas maisījumu ietvaicēja pazeminātā spiedienā un attīrīja, izmantojot kolonnas hromatogrāfiju. Produktu eluēja ar sistēmu PĒ / EtOAc no 9:1 līdz 1:1. Ieguva 660 mg (42 %) produkta **136** kā gaiši dzeltenu eļļu.
^{1}H-KMR ($CDCl_3$) δ: 7.35–7.31 (m, 2H), 7.26–7.22 (m, 2H), 7.21–7.16 (m, 2H), 6.83–6.78 (m, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 4.01 (s, 2H), 3.67 (s, 2H), 2.65 (t, *J* = 7.7 Hz, 2H), 1.62 (t, *J* = 7.5 Hz, 2H), 1.39–1.29 (m, 4H), 1.25 (t, *J* = 7.2 Hz, 3H), 0.90 (t, *J* = 7.0 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 170.0, 144.9, 138.9,

135.2, 133.8, 133.6, 131.7, 129.8, 129.3, 125.3, 120.8, 61.2, 35.5, 31.6, 31.3, 30.9, 28.4, 22.4, 14.1, 14.0 m.d. AIMS (*m/z*): $[M+H]^+$ aprēķināts $C_{24}H_{29}N_3O_2Br$: 470.1443. Noteikts: 470.1444.

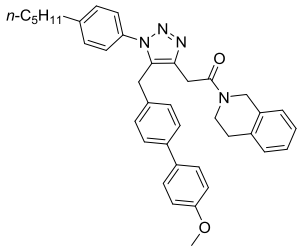
2-(5-(4-Brombenzil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietilacetamīds (137)



Esteri **136** (665 mg, 1.41 mmol) izšķīdināja 1,4-dioksānā (15 mL) un pievienoja 1 M NaOH ūdens šķīdumu (9 mL). Šķīdumu maisīja istabas temperatūrā 16 stundas. Reakcijas maisījumu paskābināja ar 1 M HCl šķīdumu līdz pH = 1 (universālais indikatorpapīrs) un ekstrahēja EtOAc (3 × 15 mL). Organiskos slāņus apvienoja, mazgāja ar piesātinātu NaCl šķīdumu (30 mL), žāvēja virs Na_2SO_4 (b/ū), filtrēja un ietvaicēja pazeminātā spiedienā. Ieguva 623 mg (100 %) produkta, kuru bez papildu attīrīšanas izmantoja tālākajās stadijās. Izkarsētā un argona plūsmā atdzesētā apaļkolbā iesvēra iepriekš iegūto skābi (623 mg, 1.41 mmol), HOBt (247 mg, 1.83 mmol) un EDC (351 mg, 1.83 mmol) un izšķīdināja sausā DMF (10 mL). Šķīdumu maisīja 0 °C 1 stundu, tad pievienoja dietilamīnu (154 mg, 219 μL, 2.11 mmol) un turpināja maisīt istabas temperatūrā 18 stundas. Reakcijas maisījumam pievienoja destilētu H_2O (15 mL) un ekstrahēja ar CH_2Cl_2 (3 × 15 mL). Organiskos slāņus apvienoja, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Atlikumu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents PĒ / EtOAc no 9:1 līdz 1:1. Iegūtais produkts **137** saturēja DMF, tādēļ to izšķīdināja *t*-butilmetilēterī (15 mL) un mazgāja ar H_2O (3 × 10 mL). Organisko slāni žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Ieguva 581 mg (83 %) amīda **137** kā brūnu, cietu vielu.

^{1}H-KMR ($CDCl_3$) δ: 7.34–7.28 (m, 2H), 7.27–7.22 (m, pārklājas ar $CDCl_3$, 2H), 7.21–7.16 (m, 2H), 6.84–6.79 (m, 2H), 4.12 (s, 2H), 3.69 (s, 2H), 3.50 (q, *J* = 7.1 Hz, 2H), 3.36 (q, *J* = 7.1 Hz, 2H), 2.65 (t, *J* = 7.7 Hz, 2H), 1.63 (kvintets, *J* = 7.5 Hz, 2H), 1.40–1.27 (m, 4H), 1.19 (t, *J* = 7.1 Hz, 3H), 1.12 (t, *J* = 7.1 Hz, 3H), 0.90 (t, *J* = 7.0 Hz, 3H) m.d.

1-(3,4-Dihidroizohinolīn-2(1*H*)-il)-2-(5-((4'-metoksi-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)etān-1-ons (100g)



Esteri **69** (90 mg, 0.18 mmol) izšķīdināja 1,4-dioksānā (2 mL) un pievienoja 1 M NaOH ūdens šķīdumu (1 mL). Šķīdumu maisīja istabas temperatūrā 16 stundas. Reakcijas maisījumu paskābināja ar 1 M HCl šķīdumu līdz pH = 1 (universālais indikatorpapīrs) un ekstrahēja EtOAc (3 × 15 mL). Organiskos slāņus apvienoja, mazgāja ar piesātinātu NaCl šķīdumu (30 mL), žāvēja virs Na_2SO_4 (b/ū), filtrēja un ietvaicēja pazeminātā spiedienā. Ieguva 89 mg (100 %) produkta, kuru bez papildu attīrīšanas izmantoja tālākajās stadijās. Izkarsētā un argona plūsmā atdzesētā apaļkolbā iesvēra iepriekš iegūto skābi (89 mg, 0.18 mmol), HOBt (31 mg, 0.23 mmol) un EDC (44 mg, 0.23 mmol) un izšķīdināja sausā DMF (1 mL). Šķīdumu maisīja 0 °C 1 stundu, tad pievienoja 1,2,3,4-tetrahidroizohinolīnu (36 mg, 34 μL, 0.27 mmol) un turpināja maisīt istabas temperatūrā 18 stundas. Reakcijas maisījumam pievienoja destilētu H_2O (5 mL) un ekstrahēja ar CH_2Cl_2 (3 × 5 mL). Organiskos slāņus apvienoja, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Atlikumu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents PĒ / EtOAc no 9:1 līdz 1:1. Ieguva 76 mg (72 %) amīda **100g**.

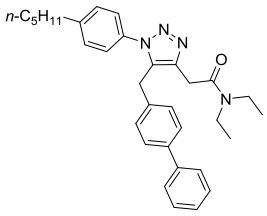
^{1}H-KMR ($CDCl_3$) δ: 7.44–7.37 (m, 2H), 7.35–7.29 (m, 1H), 7.26 (s, 9H, pārklājas ar $CDCl_3$), 6.99–6.90 (m, 4H), 4.81–4.66 (m, 2H), 4.21–4.08 (m, 2H), 3.93–3.75 (m, 7H), 2.85 (d, *J* = 5.9 Hz, 1H), 2.80 (t, *J* = 5.9 Hz, 1H), 2.65 (t, *J* = 7.7 Hz, 2H), 1.63 (kvintets, *J* = 7.3 Hz, 2H), 1.40–1.28 (m, 4H), 0.89 (t, *J* = 6.7 Hz, 3H) m.d. ^{13}C-KMR (101 MHz, $CDCl_3$) δ: 168.3, 159.29, 144.9, 139.9, 139.3, 135.1, 134.7, 134.2, 133.3, 133.0, 129.4, 128.9, 128.0, 127.0, 126.8, 126.7, 125.5, 114.3, 55.5, 47.9, 44.6, 44.0, 40.4, 35.7, 32.3, 31.5, 31.1, 29.5, 28.6, 22.6, 14.1.

Vispārīgā *Suzuki-Miyaura* šķērssametināšanas reakcijas procedūra A

Izkarsētā un Ar plūsmā atdzesētā augstspiediena ampulā iesvēra bromīdu (1.0 ekviv.), borskābi (1.05 ekviv.) un $Pd(PPh_3)_4$ (3 mol %), tad pievienoja *i*-PrOH (5 mL / 1 mmol bromīda), toluolu (5 mL / 1 mmol bromīda) un 2 M Na_2CO_3 ūdens šķīdumu (3.0 ekviv.). Iegūto divslāņu šķīdumu sildīja 90 °C 16 stundas. Reakcijas maisījumu atdzesēja un dalāmajā piltuvē pievienoja H_2O un

ekstrahēja ar EtOAc. Organiskos slāņus apvienoja, mazgāja ar piesātinātu NaCl šķīdumu, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Atlikumu attīrīja ar kolonnas hromatogrāfiju.

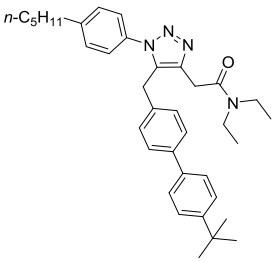
2-(5-([1,1'-Bifenil]-4-ilmetil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietilacetamīds (139a)



Amīdu **139a** 83 mg (87 %) ieguva pēc vispārīgās *Suzuki-Miyaura* šķērssametināšanas reakcijas procedūras **A** no bromīda **137** (100 mg, 0.20 mmol), borskābes **138a** (26 mg, 0.21 mmol), $Pd(PPh_3)_4$ (7 mg, 0.006 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents PĒ / EtOAc no 95:5 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.56–7.50 (m, 2H), 7.46–7.39 (m, 4H), 7.36–7.30 (m, 1H), 7.28–7.23 (m, pārklājas ar $CDCl_3$, 4H), 7.05–6.99 (m, 2H), 4.21 (s, 2H), 3.72 (s, 2H), 3.48 (q, *J* = 7.1 Hz, 2H), 3.37 (q, *J* = 7.1 Hz, 2H), 2.65 (t, *J* = 7.7 Hz, 2H), 1.63 (kvintets, *J* = 7.5 Hz, 2H), 1.39–1.28 (m, 4H), 1.16 (t, *J* = 7.1 Hz, 3H), 1.13 (t, *J* = 7.1 Hz, 3H), 0.89 (t, *J* = 7.0 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 168.4, 144.7, 140.5, 140.2, 139.5, 135.8, 134.6, 134.1, 129.2, 128.7, 128.7, 127.3, 127.2, 126.9, 125.4, 42.5, 40.6, 35.5, 31.3, 31.3, 30.9, 28.5, 22.4, 14.3, 14.0, 13.0 m.d.

2-(5-((4'-(*terc*-Butil)-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietilacetamīds (139b)

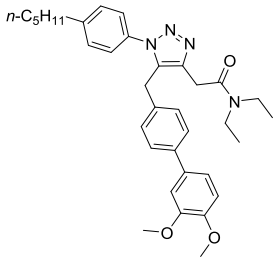


Amīdu **139b** 87 mg (83 %) ieguva pēc vispārīgās *Suzuki-Miyaura* šķērssametināšanas reakcijas procedūras **A** no bromīda **137** (95 mg, 0.19 mmol), borskābes **138b** (36 mg, 0.20 mmol), $Pd(PPh_3)_4$ (7 mg, 0.006 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents PĒ / EtOAc no 95:5 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.50–7.40 (m, 6H), 7.25-7.24 (m, 4H), 7.02–6.97 (m, 2H), 4.20 (s, 2H), 3.71 (s, 2H), 3.47 (q, *J* = 7.2 Hz, 2H), 3.37 (q, *J* = 7.2 Hz, 2H), 2.65 (t, *J* = 7.7 Hz, 2H), 1.67–1.58 (m, 2H), 1.35 (s, 9H), 1.34–1.29 (m, 4H), 1.16 (t, *J* = 7.2 Hz, 3H), 1.13 (t, *J* = 7.2 Hz, 3H), 0.89 (t, *J* = 7.0 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 168.4, 150.3, 144.6, 140.2, 139.3, 137.6,

135.5, 134.6, 134.1, 129.2, 128.6, 127.0, 126.5, 125.7, 125.4, 42.5, 40.6, 35.5, 34.5, 31.3, 31.3, 30.9, 28.5, 22.5, 14.3, 14.0, 13.0 m.d.

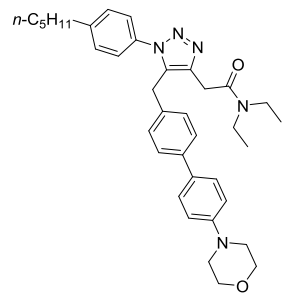
2-(5-((3',4'-Dimetoksi-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietilacetamīds (139c)



Amīdu **139c** 83 mg (79 %) ieguva pēc vispārīgās *Suzuki-Miyaura* šķērssametināšanas reakcijas procedūras **A** no bromīda **137** (95 mg, 0.19 mmol), borskābes **138c** (36 mg, 0.20 mmol), $Pd(PPh_3)_4$ (7 mg, 0.006 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents PĒ / EtOAc no 95:5 līdz 1:1, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.42–7.38 (m, 2H), 7.26–7.25 (m, 4H), 7.09 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.05 (d, *J* = 2.1 Hz, 1H), 7.03–6.98 (m, 2H), 6.93 (d, *J* = 8.3 Hz, 1H), 4.20 (s, 2H), 3.93 (s, 3H), 3.92 (s, 3H), 3.72 (s, 2H), 3.49 (q, *J* = 7.2 Hz, 2H), 3.37 (d, *J* = 7.2 Hz, 2H), 2.68–2.62 (m, 2H), 1.63 (p, *J* = 7.7 Hz, 2H), 1.39–1.28 (m, 4H), 1.17 (t, *J* = 7.2 Hz, 3H), 1.13 (t, *J* = 7.2 Hz, 3H), 0.92–0.87 (m, 3H) m.d. ^{13}C-KMR (101 MHz, $CDCl_3$) δ: 168.5, 149.0, 144.8, 140.4, 139.5, 137.0, 135.6, 134.8, 134.2, 133.7, 132.2, 129.4, 128.7, 128.4, 127.0, 126.8, 125.5, 119.3, 111.0, 56.1, 42.7, 40.7, 35.7, 31.4, 31.1, 28.8, 22.6, 14.5, 14.1, 13.2.

***N*,*N*-Dietil-2-(5-((4'-morfolīn-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)acetamīds (139d)**



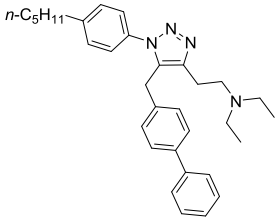
Amīdu **139d** 73 mg (62 %) ieguva pēc vispārīgās *Suzuki-Miyaura* šķērssametināšanas reakcijas procedūras **A** no bromīda **137** (100 mg, 0.20 mmol), borskābes **138d** (43 mg, 0.21 mmol), $Pd(PPh_3)_4$ (7 mg, 0.006 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents PĒ / EtOAc no 9:1 līdz 0:100, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.49–7.44 (m, 2H), 7.42–7.37 (m, 2H), 7.26 (m, 4H), 7.01–6.94 (m, 4H), 4.19 (s, 2H), 3.91–3.85 (m, 4H), 3.71 (s, 2H), 3.47 (q, *J* = 7.1 Hz, 2H), 3.37 (q, *J* = 7.1 Hz, 2H), 3.24–3.17 (m, 4H), 2.68–2.61 (m, 2H), 1.63 (p, *J* = 7.5 Hz, 2H), 1.39–1.28 (m, 4H), 1.19–1.09 (m, 6H), 0.93–0.86 (m, 3H) m.d.

Vispārīgā amīdu reducēšanas procedūra B

Izkarsētā un Ar plūsmā atdzesētā ampulā iesvēra amīdu (1.0 ekviv.), tad pievienoja sausu THF (7 mL / 1 mmol amīda) un BH_3-THF kompleksu (1 M šķīdums THF, 2.5–3.6 ekviv.). Šķīdumu sildīja 80 °C 16 stundas. Gaiši dzeltenajam šķīdumam pievienoja 4 M HCl ūdens šķīdumu (7 mL / 1 mmol amīda) un sildīja 80 °C 1 stundu. Reakcijas maisījumu atdzesēja, neitralizēja ar piesātinātu $NaHCO_3$ šķīdumu līdz pH = 8 un ekstrahēja ar CH_2Cl_2. Organiskos slāņus apvienoja, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Atlikumu attīrīja ar kolonnas hromatogrāfiju.

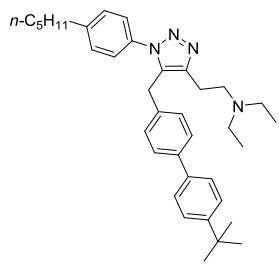
2-(5-([1,1'-Bifenil]-4-ilmetil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietiletān-1-amīns (140a)



Amīnu **140a** 49 mg (73 %) ieguva pēc vispārīgās amīdu reducēšanas procedūras **B** no amīda **139a** (70 mg, 0.14 mmol) un BH_3-THF kompleksa (1 M šķīdums THF, 0.5 mL, 0.5 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.56–7.50 (m, 2H), 7.48–7.39 (m, 4H), 7.37–7.30 (m, 1H), 7.26–7.20 (m, 4H), 7.04–6.98 (m, 2H), 4.08 (s, 2H), 2.94–2.86 (m, 4H), 2.72–2.57 (m, 6H), 1.67–1.58 (m, 2H), 1.40–1.23 (m, 4H), 1.06 (t, *J* = 7.1 Hz, 6H), 0.94–0.84 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 144.9, 140.6, 139.9, 136.2, 134.2, 132.9, 129.5, 128.9, 128.6, 127.5, 127.5, 127.1, 125.4, 52.2, 47.0, 35.7, 31.5, 31.1, 28.5, 22.6, 14.1 m.d. AIMS (*m/z*): $[M+H]^+$ aprēķināts $C_{32}H_{41}N_4$: 481.3331. Noteikts: 481.3331.

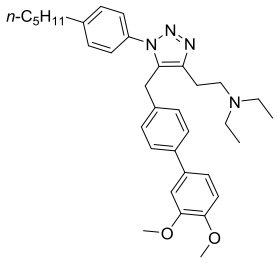
2-(5-((4'-(*terc*-Butil)-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietiletān-1-amīns (140b)



Amīnu **140b** 55 mg (66 %) ieguva pēc vispārīgās amīdu reducēšanas procedūras **B** no amīda **139b** (85 mg, 0.15 mmol) un BH_3-THF kompleksa (1 M šķīdums THF, 0.5 mL, 0.5 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.43–7.35 (m, 5H), 7.21–7.14 (m, 5H), 6.95–6.90 (m, 2H), 4.02 (s, 2H), 2.97–2.85 (m, 4H), 2.73–2.62 (m, 4H), 2.59 (t, *J* = 7.7 Hz, 2H), 1.59–1.51 (m, 2H), 1.28 (s, 9H), 1.28–1.20 (m, 4H), 1.05 (t, *J* = 7.2 Hz, 6H), 0.86–0.79 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 150.6, 144.9, 139.8, 137.6, 135.8, 134.2, 133.3, 129.5, 128.6, 127.4, 126.7, 125.9, 125.5, 62.9, 51.9, 46.9, 35.7, 34.7, 31.5, 31.1, 30.0, 28.5, 22.6, 14.1 m.d.

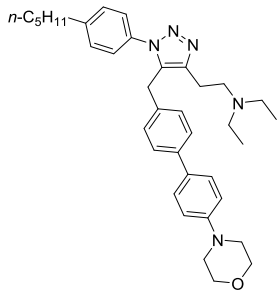
2-(5-((3',4'-Dimetoksi-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietiletān-1-amīns (140c)



Amīnu **140c** 100 mg (60 %) ieguva pēc vispārīgās amīdu reducēšanas procedūras **B** no amīda **139c** (172 mg, 0.31 mmol) un BH_3-THF kompleksa (1 M šķīdums THF, 1.1 mL, 1.1 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.45–7.40 (m, 2H), 7.29-7.22 (m, pārklājas ar $CDCl_3$, 4H), 7.09 (d, *J* = 8.3, 2.1 Hz, 1H), 7.05 (d, *J* = 2.1 Hz, 1H), 7.02–6.98 (m, 2H), 6.92 (d, *J* = 8.3 Hz, 1H), 4.09 (s, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.07-2.95 (m, 4H), 2.87-2.70 (m, 4H), 2.66 (t, *J* = 7.7 Hz, 2H), 1.63 (kvintets, *J* = 7.5 Hz, 2H), 1.39–1.26 (m, 4H), 1.14 (t, *J* = 7.0 Hz, 6H), 0.89 (t, *J* = 7.0 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 149.3, 148.9, 145.0, 139.8, 135.6, 134.2, 133.6, 129.5, 128.6, 127.2, 125.4, 119.3, 111.6, 110.4, 62.9, 56.1, 56.1, 46.9, 35.7, 31.5, 31.1, 28.4, 22.6, 14.1 m.d.

***N*,*N*-Dietil-2-(5-((4'-morfolīn-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)etān-1-amīns (140d)**

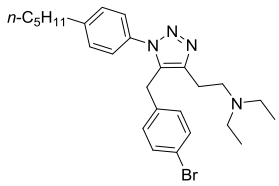


Amīnu **140d** 41 mg (60 %) ieguva pēc vispārīgās amīdu reducēšanas procedūras **B** no amīda **139d** (70 mg, 0.12 mmol) un BH_3-THF kompleksa (1 M šķīdums THF, 0.5 mL, 0.5 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.49–7.45 (m, 2H), 7.44–7.40 (m, 2H), 7.29–7.22 (m, 4H, pārklājas ar $CDCl_3$), 7.01–6.94 (m, 4H), 4.08 (s, 2H), 3.90–3.84 (m, 4H), 3.23–3.17 (m, 4H), 3.08–2.98 (m, 4H), 2.86–2.74 (m,

4H), 2.69–2.62 (m, 2H), 1.67–1.58 (m, 2H), 1.40–1.28 (m, 4H), 1.15 (t, *J* = 7.1 Hz, 6H), 0.93–0.86 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 150.8, 145.0, 139.5, 135.2, 134.1, 133.5, 131.9, 129.5, 128.6, 127.8, 127.7, 126.9, 125.5, 115.9, 67.0, 51.6, 49.2, 46.9, 35.7, 31.5, 31.1, 28.4, 22.6, 14.1 m.d.

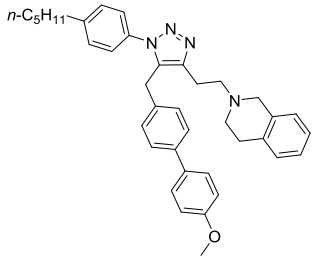
2-(5-(4-Brombenzil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)-*N*,*N*-dietiletān-1-amīns (141)



Amīnu **141** 45 mg (46 %) ieguva pēc vispārīgās amīdu reducēšanas procedūras **B** no amīda **139d** (98 mg, 0.20 mmol) un BH_3-THF kompleksa (1 M šķīdums THF, 0.5 mL, 0.5 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.37–7.31 (m, 2H), 7.27–7.22 (m, 2H), 7.20–7.14 (m, 2H), 6.83–6.78 (m, 2H), 4.02 (s, 2H), 3.09–2.89 (m, 4H), 2.87–2.69 (m, 4H), 2.69–2.62 (m, 2H), 1.67–1.58 (m, 2H), 1.40–1.26 (m, 4H), 1.22–1.04 (m, 6H), 0.90 (d, *J* = 7.0 Hz, 3H) m.d. AIMS (*m/z*): $[M+H]^+$ aprēķināts $C_{26}H_{36}N_4Br$: 483.2123. Noteikts: 483.2105.

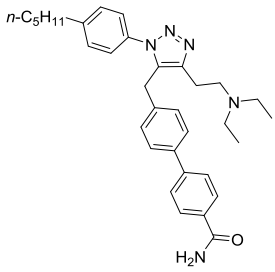
2-(2-(5-((4'-Metoksi-[1,1'-bifenil]-4-il)metil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-4-il)etil)-1,2,3,4-tetrahidroizohinolīns (101g)



Amīnu **101g** 68 mg (47 %) ieguva pēc vispārīgās amīdu reducēšanas procedūras **B** no amīda **100g** (68 mg, 0.12 mmol) un BH_3-THF kompleksa (1 M šķīdums THF, 0.5 mL, 0.5 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 15 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.49–7.44 (m, 2H), 7.40–7.36 (m, 2H), 7.27–7.21 (m, 4H, pārklājas ar $CDCl_3$), 7.13–7.05 (m, 3H), 7.01–6.94 (m, 5H), 4.06 (s, 2H), 3.85 (s, 3H), 3.66 (s, 2H), 3.01–2.85 (m, 6H), 2.77 (t, *J* = 5.9 Hz, 2H), 2.67–2.62 (m, 2H), 1.62 (kvintets, *J* = 7.6 Hz, 2H), 1.38–1.26 (m, 4H), 0.93–0.85 (m, 3H) m.d. ^{13}C-KMR (101 MHz, $CDCl_3$) δ: 159.4, 145.3, 139.6, 135.0, 133.8, 132.9, 129.6, 128.9, 128.1, 127.2, 125.6, 114.4, 55.5, 54.2, 35.7, 31.5, 31.1, 28.6, 23.6, 22.6, 22.2, 14.1

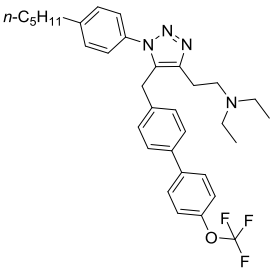
4'-((4-(2-(Dietilamino)etil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-5-il)metil)-[1,1'-bifenil]-4-karboksamīds (140f)



Amīnu **140f** 7 mg (28 %) ieguva pēc vispārīgās *Suzuki-Miyaura* šķērssametināšanas reakcijas procedūras **A** no bromīda **141** (23 mg, 0.05 mmol), borskābes **138f** (8 mg, 0.05 mmol), $Pd(PPh_3)_4$ (1.6 mg, 0.0014 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 12 mL / min.

^{1}H-KMR (CD_3OD) δ: 9.52–9.47 (m, 2H), 9.25–9.19 (m, 2H), 9.13–9.08 (m, 2H), 8.93–8.84 (m, 4H), 8.65–8.59 (m, 2H), 5.72 (s, 2H), 4.45–4.31 (m, 4H), 4.28–4.22 (m, 2H), 4.17 (q, *J* = 7.2 Hz, 4H), 3.27–3.16 (m, 2H), 2.97–2.82 (m, 4H), 2.59 (t, *J* = 7.2 Hz, 6H), 2.50–2.42 (m, 3H) m.d. ^{13}C-KMR (CD_3OD) δ: 172.0, 146.6, 145.1, 144.6, 139.9, 138.3, 135.3, 135.2, 133.7, 130.6, 130.0, 129.3, 128.5, 127.8, 126.7, 52.9, 47.7, 36.4, 32.5, 32.2, 29.1, 23.5, 22.7, 14.4, 11.4 m.d.

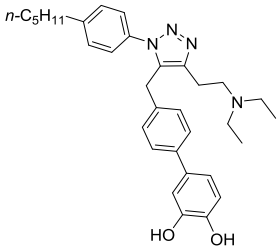
***N*,*N*-Dietil-2-(1-(4-pentilfenil)-5-((4'-(trifluormetoksi)-[1,1'-bifenil]-4-il)metil)-1*H*-1,2,3-triazol-4-il)etān-1-amīns (140g)**



Amīnu **140g** 15 mg (43 %) ieguva pēc vispārīgās *Suzuki-Miyaura* šķērssametināšanas reakcijas procedūras **A** no bromīda **141** (20 mg, 0.062 mmol), borskābes **138g** (14 mg, 0.068 mmol), $Pd(PPh_3)_4$ (2.2 mg, 0.0019 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents CH_2Cl_2 / MeOH no 100:0 līdz 80:20, 10 g silikagela kolonna, plūsmas ātrums 12 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 7.56–7.51 (m, 2H), 7.45–7.39 (m, 2H), 7.29–7.20 (m, 6H), 7.05–6.99 (m, 2H), 4.11 (s, 2H), 3.09–2.94 (m, 4H), 2.86–2.70 (m, 4H), 2.69–2.61 (m, 2H), 1.63 (p, *J* = 7.4 Hz, 2H), 1.39–1.24 (m, 4H), 1.14 (t, *J* = 7.2 Hz, 6H), 0.95–0.84 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 148.9, 145.0, 139.3, 138.6, 136.6, 134.1, 129.5, 128.8, 128.4, 127.5, 125.5, 121.4, 51.9, 47.0, 35.7, 31.5, 31.1, 28.5, 22.6, 14.1 m.d. AIMS (*m/z*): $[M+H]^+$ aprēķināts $C_{33}H_{40}N_4OF_3$: 565.3154. Noteikts: 565.3154.

4'-((4-(2-(Dietilamino)etil)-1-(4-pentilfenil)-1*H*-1,2,3-triazol-5-il)metil)-[1,1'-bifenil]-3,4-diols (140e)



Izkarsētā un Ar plūsmā atdzesētā ampulā iesvēra NaH (60 % minerāleļļā, 60 mg, 1.48 mmol), to mazgāja ar sausu Et_2O (3 × 2 mL). Pievienoja sausu DMF (1 mL), suspensiju atdzesēja līdz 0 °C un pilinot pievienoja 1-dodeciltiolu (300 mg, 352 μL, 1.48 mmol). Pelēkbalto suspensiju maisīja istabas temperatūrā 1 stundu, tad pievienoja amīna **140c** (100 mg, 0.18 mmol) šķīdumu sausā DMF (1 mL). Reakcijas maisījumu sildīja 130 °C 2 stundas. Šķīdumu atdzesēja līdz istabas temperatūrai, tad pievienoja piesātinātu NH_4Cl ūdens šķīdumu (2 mL) un H_2O (3 mL), ekstrahēja ar EtOAc (3 × 10 mL). Organiskos slāņus apvienoja, mazgāja ar H_2O (10 mL), piesātinātu NaCl šķīdumu (10 mL), žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Atlikumu attīrīja ar kolonnas hromatogrāfiju, izmantojot eluentu sitēmu CH_2Cl_2 / MeOH no 100:0 līdz 80:20. Ieguva 40 mg (43 %) amīna **140g**.
^{1}H-KMR (CD_3OD) δ: 7.43–7.27 (m, 6H), 7.01–6.94 (m, 3H), 6.89 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 4.10 (s, 2H), 2.93–2.81 (m, 4H), 2.76–2.65 (m, 6H), 1.71–1.59 (m, 2H), 1.42–1.26 (m, 4H), 1.08 (t, *J* = 7.2 Hz, 6H), 0.94–0.85 (m, 3H) m.d. ^{13}C-KMR (CD_3OD) δ: 146.7, 146.6, 143.9, 141.3, 136.2, 135.6, 135.2, 133.6, 130.6, 129.6, 127.8, 126.7, 119.3, 116.7, 114.8, 52.6, 47.9, 36.4, 32.5, 32.2, 23.5, 22.3, 14.4, 10.9 m.d.

Vispārīgā Pd – katalizētas alkoksikarbonilēšanas reakcijas procedūra C

Izkarsētā un Ar plūsmā atdzesētā stikla mēģenē iesvēra jodbenzoskābi (1.0 ekviv.), tad pievienoja sausu MeOH (5.6 mL / 1 mmol benzoskābes). Šķīdumam pievienoja Et_3N (2.2 ekviv.) un $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (0.1 ekviv.). Mēģeni ar šķīdumu ievietoja tērauda autoklāvā, to noslēdza un 3 reizes uzpildīja ar CO līdz 5 atmosfēru spiedienam. Reakcijas maisījumu sildīja 100 °C 18 stundas. Pēc sildīšanas autoklāvu atdzesēja un uzmanīgi atbrīvojās no CO pārspiediena. Suspensiju filtrēja caur celītu, mazgāja ar EtOAc un filtrātu ietvaicēja pazeminātā spiedienā. Pārpalikumu izšķīdināja EtOAc un mazgāja ar 1 M HCl šķīdumu un piesātinātu NaCl šķīdumu. Organisko slāni žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Produktu attīrīja ar kolonnas hromatogrāfiju.

Vispārīgā amīdu sintēzes procedūra D

Izkarsētā un Ar plūsmā atdzesētā apaļkolbā iesvēra benzoskābi (1.0 ekviv.) un pievienoja DMF (4.5 mL / 1 mmol benzoskābes). Šķīdumam pievienoja HBTU (1.0 ekviv.) un di-*n*-propilamīnu (1.2 ekviv.). Reakcijas maisījumu atdzesēja līdz 0 °C un piepilināja Et_3N (2.0 ekviv.), maisīja 0 °C 30 minūtes, tad istabas temperatūrā 2 stundas. Iegūtajam šķīdumam pievienoja destilētu H_2O un ekstrahēja ar EtOAc. Organiskos slāņus apvienoja, mazgāja ar destilētu H_2O un piesātinātu NaCl šķīdumu, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Produktu attīrīja ar kolonnas hromatogrāfiju.

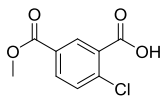
Vispārīgā esteru hidrolīzes procedūra E

Estera (1.0 ekviv.) šķīdumam MeOH (13 mL / 1 mmol estera) pievienoja 1 M NaOH šķīdumu (1.5 ekviv.). Šķīdumu sildīja 50 °C 18 stundas. Reakcijas maisījumu atdzesēja un iekoncentrēja pazeminātā spiedienā. Pārpalikumam pievienoja destilētu H_2O, paskābināja ar 1 M HCl šķīdumu līdz pH = 1 (universālais indikatorpapīrs) un ekstrahēja ar EtOAc. Organiskos slāņus apvienoja, mazgāja ar piesātinātu NaCl šķīdumu, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Iegūto produktu izmantoja tālāk bez papildu attīrīšanas.

Vispārīgā amīdu sintēzes procedūra F

Izkarsētā un Ar plūsmā atdzesētā ampulā iesvēra hidroksietilamīnu (*R*,*S*)-**175** (1.0 ekviv.) un pievienoja DMF (16 mL / 1 mmol amīna). Šķīdumam pievienoja HBTU (1.0 ekviv.) un benzoskābi (1.1 ekviv.). Reakcijas maisījumu atdzesēja līdz 0 °C un piepilināja Et_3N (4.0 ekviv.), maisīja 0 °C 30 minūtes, tad istabas temperatūrā 18 stundas. Iegūtajam šķīdumam pievienoja destilētu H_2O un ekstrahēja ar EtOAc. Organiskos slāņus apvienoja, mazgāja ar destilētu H_2O un piesātinātu NaCl šķīdumu, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Produktu attīrīja ar kolonnas hromatogrāfiju.

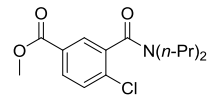
2-Hlor-5-(metoksikarbonil)benzoskābe (206a)



Esteri **206a** 274 mg (72 %) ieguva kā brūnu pulveri pēc vispārīgās Pd – katalizētas alkoksikarbonilēšanas reakcijas procedūras **C** no jodbenzoskābes **205a** (500 mg, 1.77 mmol), CO, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (144 mg, 0.18 mmol), Et_3N (394 mg, 542 μL, 3.89 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluents PĒ / EtOAc no 1:1 līdz 0:1.

^{1}H-KMR (CD_3OD) δ: 8.44 (dd, *J* = 2.2, 0.4 Hz, 1H), 8.08 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.64–7.60 (m, 1H), 3.93 (s, 3H) m.d. ^{13}C-KMR (CD_3OD) δ: 167.9, 166.8, 139.3, 134.0, 133.3, 132.7, 132.5, 130.2, 53.0 m.d. AIMS *(m/z)*: $[M-H]^-$ aprēķināts $C_9H_6O_4Cl$: 212.9955. Noteikts: 212.9946.

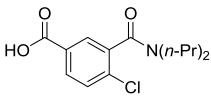
Metil 4-hlor-3-(dipropilkarbamoil)benzoāts (207a)



Amīdu **207a** 145 mg (87 %) ieguva kā bezkrāsainu eļļu pēc vispārīgās amīdu sintēzes procedūras **D** no benzoskābes **206a** (120 mg, 0.56 mmol), di-*n*-propilamīna (68 mg, 92 μL, 0.67 mmol), HBTU (212 mg, 0.56 mmol) un Et_3N (113 mg, 156 μL, 1.12 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluentu sistēma PĒ / EtOAc no 4:1 līdz 1:1.

^{1}H-KMR ($CDCl_3$) δ: 8.00–7.93 (m, 2H), 7.48–7.44 (m, 1H), 3.92 (s, 3H), 3.78–3.64 (m, 1H), 3.35–3.20 (m, 1H), 3.15–2.92 (m, 2H), 1.80–1.67 (m, 2H), 1.58–1.40 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H), 0.73 (t, *J* = 7.4 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 167.4, 165.8, 137.2, 135.5, 130.9, 130.0, 129.3, 129.2, 52.6, 50.3, 46.4, 21.8, 20.8, 11.7, 11.3 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{15}H_{21}NO_3Cl$: 298.1210. Noteikts: 298.1217.

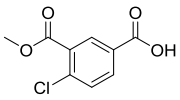
4-Hlor-3-(dipropilkarbamoil)benzoskābe (208a)



Benzoskābi **208a** (128 mg, 100 %) ieguva kā iedzeltenu eļļu pēc vispārīgās esteru hidrolīzes procedūras **E** no estera **207a** (134 mg, 0.45 mmol) un 1 M NaOH (675 μL, 0.67 mmol). Skābi **208a** tālākajās stadijās izmantoja bez papildu attīrīšanas.

^{1}H-KMR ($CDCl_3$) δ: 9.14 (s, 1H), 8.06–7.96 (m, 2H), 7.55–7.47 (m, 1H), 3.81–3.65 (m, 1H), 3.35–3.20 (m, 1H), 3.17–2.94 (m, 2H), 1.80–1.68 (m, 2H), 1.63–1.40 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H), 0.74 (t, *J* = 7.4 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 169.7, 167.5, 137.0, 136.3, 131.5, 130.1, 129.8, 128.6, 50.3, 46.6, 21.8, 20.6, 11.7, 11.3 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{14}H_{19}NO_3Cl$: 284.1053. Noteikts: 284.1053.

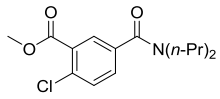
4-Hlor-3-(metoksikarbonil)benzoskābe (206b)



Esteri **206b** 249 mg (65 %) ieguva kā brūnu pulveri pēc vispārīgās Pd–katalizētas alkoksikarbonilēšanas reakcijas procedūra**s C** no jodbenzoskābes **205b** (500 mg, 1.77 mmol), CO, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (144 mg, 0.18 mmol), Et_3N (394 mg, 542 μL,

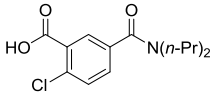
3.89 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluenta sistēma PĒ / EtOAc no 4:1 līdz 0:1, EtOAc / MeOH no 1:0 līdz 9:1.
^{1}H-KMR (CD_3OD) δ: 8.42 (dd, *J* = 2.2, 0.4 Hz, 1H), 8.10 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.64–7.60 (m, 1H), 3.94 (s, 3H) m.d. ^{13}C-KMR (CD_3OD) δ: 167.8, 166.8, 138.9, 134.5, 133.5, 132.4, 131.7, 131.2, 53.1 m.d. AIMS *(m/z)*: $[M-H]^-$ aprēķināts $C_9H_6O_4Cl$: 212.9955. Noteikts: 212.9963.

Metil 2-hlor-5-(dipropilkarbamoil)benzoāts (207b)



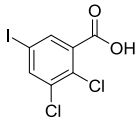
Amīdu **207b** 124 mg (74 %) ieguva kā bezkrāsainu eļļu pēc vispārīgās amīdu sintēzes procedūras **D** no benzoskābes **206b** (120 mg, 0.56 mmol), di-*n*-propilamīna (68 mg, 92 μL, 0.67 mmol), HBTU (212 mg, 0.56 mmol) un Et_3N (113 mg, 156 μL, 1.12 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluentu sistēma PĒ / EtOAc no 4:1 līdz 1:1.
^{1}H-KMR ($CDCl_3$) δ: 7.84 (dd, *J* = 2.1, 0.5 Hz, 1H), 7.49 (dd, *J* = 8.2, 0.5 Hz, 1H), 7.42 (dd, *J* = 8.2, 2.1 Hz, 1H), 3.93 (s, 3H), 3.52–3.37 (m, 2H), 3.24–3.08 (m, 2H), 1.76–1.63 (m, 2H), 1.60–1.48 (m, 2H), 1.06–0.89 (m, 3H), 0.86–0.71 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 169.7, 165.6, 136.0, 134.7, 131.4, 130.8, 130.2, 129.9, 52.7, 50.9, 46.7, 22.1, 20.8, 11.6, 11.2 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{15}H_{21}NO_3Cl$: 298.1210. Noteikts: 298.1199.

2-Hlor-5-(dipropilkarbamoil)benzoskābe (208b)



Benzoskābi **208b** (105 mg, 97 %) ieguva kā iedzeltenu eļļu pēc vispārīgās esteru hidrolīzes procedūras **E** no estera **207b** (113 mg, 0.38 mmol) un 1 M NaOH (570 μL, 0.57 mmol). Skābi **208b** tālākajās stadijās izmantoja bez papildu attīrīšanas.
^{1}H-KMR ($CDCl_3$) δ: 9.25 (s, 1H), 8.02–7.94 (m, 1H), 7.54–7.44 (m, 2H), 3.55–3.41 (m, 2H), 3.24–3.11 (m, 2H), 1.77–1.65 (m, 2H), 1.62–1.50 (m, 2H), 1.04–0.92 (m, 3H), 0.84–0.70 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 170.1, 168.4, 135.6, 135.5, 131.7, 131.4, 130.6, 129.4, 51.1, 47.0, 22.1, 20.8, 11.6, 11.2 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{14}H_{19}NO_3Cl$: 284.1053. Noteikts: 284.1059.

2,3-Dihlor-5-jodbenzoskābe (205c)

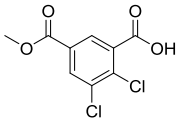


Estera **204** (300 mg, 0.91 mmol) šķīdumam MeOH (10 mL) pievienoja 1 M NaOH šķīdumu (1.36 mL, 1.36 mmol). Šķīdumu

sildīja 40 °C 18 stundas. Reakcijas maisījumu paskābināja ar 5 % $KHSO_4$ šķīdumu līdz pH = 3 (universālais indikatorpapīrs) un ekstrahēja ar EtOAc (3 × 30 mL). Organiskos slāņus apvienoja, mazgāja ar piesātinātu NaCl šķīdumu, žāvēja virs Na_2SO_4, filtrēja un ietvaicēja pazeminātā spiedienā. Ieguva 287 mg (97 %) benzoskābes **205c** kā gaiši brūnu pulveri.

^{1}H-KMR (CD_3OD) δ: 8.04 (d, *J* = 2.1 Hz, 1H), 8.00 (d, *J* = 2.1 Hz, 1H) m.d. ^{13}C-KMR (CD_3OD) δ: 166.8, 142.0, 138.8, 136.6, 136.0, 132.0, 91.5 m.d. AIMS *(m/z)*: $[M-H]^-$ aprēķināts $C_7H_2O_2Cl_2I$: 314.8477. Noteikts: 314.8479.

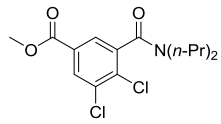
2,3-Dihlor-5-(metoksikarbonil)benzoskābe (206c)



Esteri **206c** 70 mg (33 %) ieguva kā brūnu pulveri pēc vispārīgās Pd – katalizētas alkoksikarbonilēšanas reakcijas procedūra**s C** no jodbenzoskābes **205c** (270 mg, 0.85 mmol), CO, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (70 mg, 0.085 mmol), Et_3N (190 mg, 261 μL, 1.87 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluentu sistēma PĒ / EtOAc no 1:1 līdz 0:100, EtOAc / MeOH no 100:0 līdz 9:1.

^{1}H-KMR (CD_3OD) δ: 8.29 (d, *J* = 2.1 Hz, 1H), 8.23 (d, *J* = 2.1 Hz, 1H), 3.94 (s, 3H) m.d. ^{13}C-KMR (CD_3OD) δ: 165.8, 135.8, 134.1, 131.0, 130.7, 53.3 m.d. AIMS *(m/z)*: $[M-H]^-$ aprēķināts $C_9H_5O_4Cl_2$: 246.9565. Noteikts: 246.9565.

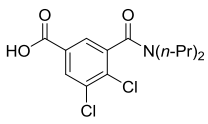
Metil 3,4-dihlor-5-(dipropilkarbamoil)benzoāts (207c)



Amīdu **207c** 67 mg (72 %) ieguva kā baltu cietu vielu pēc vispārīgās amīdu sintēzes procedūras **D** no benzoskābes **206c** (70 mg, 0.28 mmol), di-*n*-propilamīna (34 mg, 46 μL, 0.34 mmol), HBTU (107 mg, 0.28 mmol) un Et_3N (57 mg, 78 μL, 0.56 mmol). Produktu attīrīja ar tiešās fāzes ARMEN hromatogrāfu. Eluents PĒ / EtOAc no 9:1 līdz 3:1, 10 g silikagela kolonna, plūsmas ātrums 12 mL / min.

^{1}H-KMR ($CDCl_3$) δ: 8.12 (d, *J* = 2.0 Hz, 1H), 7.83 (d, *J* = 2.0 Hz, 1H), 3.93 (s, 3H), 3.77–3.64 (m, 1H), 3.31–3.21 (m, 1H), 3.13–3.04 (m, 1H), 3.01–2.92 (m, 1H), 1.79–1.67 (m, 2H), 1.63–1.40 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H), 0.75 (t, *J* = 7.4 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 166.6, 164.8, 139.1, 134.1, 133.8, 131.3, 130.1, 126.9, 52.9, 50.2, 46.5, 21.8, 20.6, 11.7, 11.3 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{15}H_{20}NO_3Cl_2$: 332.0820. Noteikts: 332.0814.

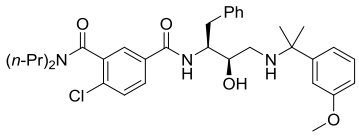
3,4-Dihlor-5-(dipropilkarbamoil)benzoskābe (208c)



Benzoskābi **208c** (62 mg, 97 %) ieguva kā iedzeltenu cietu vielu pēc vispārīgās esteru hidrolīzes procedūras **E** no

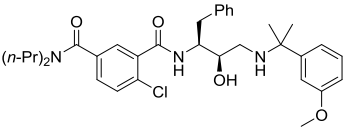
estera **207c** (67 mg, 0.19 mmol) un 1 M NaOH (302 μL, 0.30 mmol). Skābi **208c** tālākajās stadijās izmantoja bez papildu attīrīšanas.
^{1}H-KMR ($CDCl_3$) δ: 8.53 (s, 1H), 8.16 (d, *J* = 2.0 Hz, 1H), 7.88 (d, *J* = 2.0 Hz, 1H), 3.74 (dt, *J* = 14.7, 7.7 Hz, 1H), 3.27 (dt, *J* = 13.4, 7.7 Hz, 1H), 3.16–3.05 (m, 1H), 3.03–2.93 (m, 1H), 1.83–1.67 (m, 2H), 1.64–1.40 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 168.4, 166.8, 138.8, 134.6, 134.3, 131.9, 129.5, 127.4, 50.4, 46.7, 21.8, 20.6, 11.6, 11.3 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{14}H_{18}NO_3Cl_2$: 318.0664. Noteikts: 318.0657.

4-Hlor-*N*^{1}-((2*S*,3*R*)-3-hidroksi-4-((2-(3-metoksifenil)propān-2-il)amino)-1-fenilbutān-2-il)-*N*^{3},*N*^{3}-dipropilizoftalamīds (209a)



Hidroksietilamīnu (*S*,*R*)-**209a** 22 mg (59 %) ieguva kā gaiši brūnu cietu vielu pēc vispārīgās amīdu sintēzes procedūras **F** no amīna (*R*,*S*)-**175** (25 mg, 0.062 mmol), benzoskābes **208a** (19 mg, 0.068 mmol), HBTU (24 mg, 0.062 mmol) un Et_3N (25 mg, 35 μL, 0.25 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluents EtOAc.
^{1}H-KMR ($CDCl_3$) δ: 7.71–7.57 (m, 1H), 7.52–7.35 (m, 2H), 7.23–7.13 (m, 5H), 7.07–6.90 (m, 3H), 6.75 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H), 4.41–4.28 (m, 1H), 3.78 (s, 3H), 3.72–3.62 (m, 1H), 3.56–3.47 (m, 1H), 3.26 (dd, *J* = 13.9, 7.2 Hz, 1H), 3.12–2.88 (m, 4H), 2.82–2.78 (m, 1H), 2.55–2.41 (m, 2H), 1.78–1.65 (m, 2H), 1.47 (d, *J* = 4.8 Hz, 6H), 1.33–1.23 (m, 1H), 0.99 (t, *J* = 7.4 Hz, 4H), 0.72 (t, *J* = 7.4 Hz, 4H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 167.5, 159.8, 133.6, 129.4, 128.7, 126.7, 118.4, 112.6, 70.7, 56.0, 55.3, 50.3, 46.5, 44.5, 38.8, 36.8, 29.8, 21.8, 20.7, 11.7, 11.3 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{34}H_{45}N_3O_4Cl$: 594.3099. Noteikts: 594.3126. $[\alpha]_D^{20}$ -36.3 (*c* 1.50, $CHCl_3$).

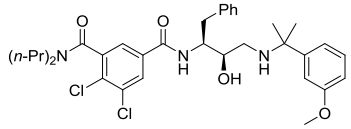
4-Hlor-*N*^{3}-((2*S*,3*R*)-3-hidroksi-4-((2-(3-metoksifenil)propān-2-il)amino)-1-fenilbutān-2-il)-*N*^{1},*N*^{1}-dipropilizoftalamīds (209b)



Hidroksietilamīnu (*S*,*R*)-**209b** 24 mg (65 %) ieguva kā gaiši dzeltenu cietu vielu pēc vispārīgās amīdu sintēzes procedūras **F** no amīna (*R*,*S*)-**175** (25 mg, 0.062 mmol), benzoskābes **208b** (19 mg, 0.068 mmol), HBTU (24 mg, 0.062 mmol) un Et_3N (25 mg, 35 μL, 0.25 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluents EtOAc.

^{1}H-KMR ($CDCl_3$) δ: 7.37–7.31 (m, 1H), 7.30–7.27 (m, 1H), 7.25–7.17 (m, 5H), 7.13 (dd, *J* = 2.1, 0.4 Hz, 1H), 7.03–6.97 (m, 2H), 6.75 (ddd, *J* = 8.2, 2.6, 1.0 Hz, 1H), 6.46 (d, *J* = 9.0 Hz, 1H), 4.41–4.29 (m, 1H), 3.79 (s, 3H), 3.56–3.34 (m, 3H), 3.19–3.00 (m, 3H), 2.88–2.79 (m, 2H), 2.57 (dd, *J* = 12.4, 4.8 Hz, 1H), 2.48 (dd, *J* = 12.4, 4.8 Hz, 1H), 1.77–1.60 (m, 2H), 1.52–1.40 (m, 7H), 1.33–1.24 (m, 1H), 1.05–0.90 (m, 3H), 0.79–0.65 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 169.8, 166.4, 159.7, 149.0, 137.9, 136.3, 135.8, 131.6, 130.4, 129.5, 129.3, 129.2, 128.6, 127.4, 126.6, 118.5, 112.4, 111.4, 71.3, 55.8, 55.3, 53.8, 44.6, 38.7, 36.7, 29.7, 29.5 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{34}H_{45}N_3O_4Cl$: 594.3099. Noteikts: 594.3113. $[\alpha]_D^{20}$ -3.4 (*c* 1.72, $CHCl_3$).

4,5-Dihlor-*N*^{1}-((2*S*,3*R*)-3-hidroksi-4-((2-(3-metoksifenil)propān-2-il)amino)-1-fenilbutān-2-il)-*N*^{3},*N*^{3}-dipropilizoftalamīds (209c)

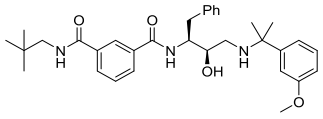


Hidroksietilamīnu (*S*,*R*) **-209c** 20 mg (61 %) ieguva kā gaiši brūnu cietu vielu pēc vispārīgās amīdu sintēzes procedūras **F** no amīna (*R*,*S*)-**175** (21 mg, 0.052 mmol), benzoskābes **208c** (18 mg, 0.057 mmol), HBTU (20 mg, 0.052 mmol) un Et_3N (21 mg, 29 μL, 0.21 mmol). Produktu attīrīja ar kolonnas hromatogrāfiju, eluenta sistēma PĒ / EtOAc no 1:1 līdz 0:100.

^{1}H-KMR ($CDCl_3$) δ: 7.87–7.66 (m, 1H), 7.53–7.29 (m, 2H), 7.28–7.13 (m, 5H), 7.01–6.95 (m, 2H), 6.78–6.72 (m, 1H), 4.38–4.28 (m, 1H), 3.79 (d, *J* = 3.4 Hz, 3H), 3.71–3.61 (m, 1H), 3.55–3.46 (m, 1H), 3.32–3.21 (m, 1H), 3.11–2.84 (m, 5H), 2.55–2.40 (m, 2H), 1.78–1.65 (m, 2H), 1.48 (d, *J* = 4.0 Hz, 6H), 1.44–1.36 (m, 1H), 1.33–1.22 (m, 1H), 0.99 (m, 3H), 0.78–0.69 (m, 3H) m.d. ^{13}C-KMR ($CDCl_3$) δ: 166.7, 164.9, 159.8, 148.6, 138.7, 137.9, 134.7, 134.6, 134.2, 134.0, 129.3, 128.6, 126.7, 124.6, 123.8, 118.4, 112.7, 111.3, 70.5, 55.9, 55.3, 54.7, 50.3, 46.5, 44.4, 36.7, 30.1, 29.1, 21.8, 20.6, 11.7, 11.3 m.d. AIMS *(m/z)*: $[M+H]^+$ aprēķināts $C_{34}H_{44}N_3O_4Cl_2$: 628.2709. Noteikts: 628.2689. $[\alpha]_D^{20}$ -42.5 (*c* 1.59, $CHCl_3$).

GALVENIE REZULTĀTI

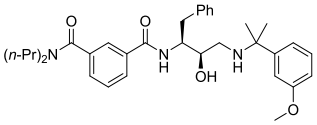
1. Azolu atvasinājumu rindā (izoksazola, pirola, triazola, imidazola) visaugstāko inhibitoro aktivitāti pret Plm II uzrādīja 1,2,3-triazolu saturoši savienojumi.
2. Tetrahidroizohinolīna ciklu saturoši Plm II inhibitori uzrādīja inhibitorās aktivitātes vērtības pret Plm I, Plm II un Plm IV mikromolārā līmenī.
3. Hidroksietilamīna atvasinājums ar neopentilaizvietotu amidogrupu (*S*,*R*)-**212a** uzrāda Plm IV inhibēšanas spēju nanomolārā līmenī un augstu selektivitātes faktora vērtību pret Cat D (S = 50).



IC_{50} (Plm IV) = 0.076 μM
IC_{50} (Cat D) = 3.8 μM
S = 50.0

(*S*,*R*)-**212a**

4. Aktīvākie savienojumi uzrāda *P. falciparum* parazīta augšanas inhibēšanas spēju inficētu asins šūnu testā nanomolārā līmenī. Izmantojot *Western blot* testu, ir noskaidrots, ka šie savienojumi spēj inhibēt arī Plm X.

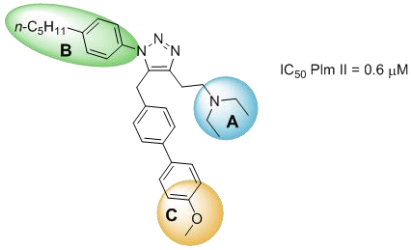


IC_{50} (Plm IV) = 0.058 μM
EC_{50} (*Pf*) = 0.3 nM

(*S*,*R*)-**192e**

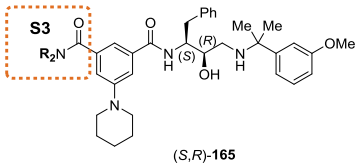
SECINĀJUMI

1. Noskaidrots, ka ir svarīgi saglabāt 1,2,3-triazola molekulas **A** daļā dietilaminogrupu un divus oglekļa atomus garu alkilķēdi, **B** daļā – 4-*n*-pentilfenilgrupu, **C** daļā – *p*-metoksiaizvietotu fenilgredzenu. Savienojums **101a** uzrādīja visaugstāko inhibitoro aktivitāti pret Plm II (IC_{50} = 0.6 μM).



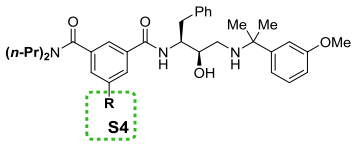
101a

2. Pētījuma ietvaros tika sintezēti peptidomimētiski hidroksietilamīna fragmentu saturoši atvasinājumi ar mono- un di- aizvietotiem amīdiem. Ir noskaidrots, ka Plm IV / Cat D selektivitāti iespējams paaugstināt, aizpildot S3 sub-kabatu ar monoaizvietotu amīda funkciju, kas satur lineāras vai sazarotas hidrofobas grupas.



(*S*,*R*)-**165**

3. Variējot S4 sub-kabatā ietilpstošos fenilgredzena aizvietotājus, ir noskaidrots, ka stēriski mazu aizvietotāju (R = H, R = F) gadījumā uzlabojas Plm IV / Cat D selektivitāte.



(*S*,*R*)-**192a**: R = F
IC_{50} Plm IV = 0.050 μM
IC_{50} Cat D = 1.00 μM
S = 20.0

(*S*,*R*)-**192e**: R = H
IC_{50} Plm IV = 0.058 μM
IC_{50} Cat D = 1.15 μM
S = 19.8

4. Ir noskaidrots, ka hidroksietilamīna atvasinājumu Plm IV inhibēšanas spēja korelē ar aktivitāti inficētu asins šūnu testā. Tas liecina, ka potenciālais zāļu mērķis varētu būt Plm IV, un tas var kalpot kā modeļproteīns Plm IX un Plm X inhibitoru izstrādē.

LITERATŪRAS SARAKSTS

1. *World Malaria report 2018*. World Health Organisation: Geneva, 2018.
2. Wells, T. N. C.; Alonso, P. L.; Gutteridge, W. E. New medicines to improve control and contribute to the eradication of malaria. *Nat. Rev. Drug Discov.* **2009**, *8*, 879–891, DOI: 10.1038/nrd2972.
3. Teklehaimanot, A.; Mejia, P. Malaria and Poverty. *Ann. N. Y. Acad. Sci.* **2008**, *1136*, 32–37, DOI: 10.1196/annals.1425.037.
4. Avery, M.; Choi, S.-R.; Mukherjee, P. The Fight Against Drug-Resistant Malaria: Novel Plasmodial Targets and Antimalarial Drugs. *Curr. Med. Chem.* **2008**, *15*, 161–171, DOI: 10.2174/092986708783330575.
5. Neafsey, D. E. Genome sequencing sheds light on emerging drug resistance in malaria parasites. *Nat. Genet.* **2013**, *45*, 589–590, DOI: 10.1038/ng.2648.
6. Sahu, N. K.; Sahu, S.; Kohli, D. V. Novel Molecular Targets for Antimalarial Drug Development. *Chem. Biol. Drug Des.* **2008**, *71*, 287–297, DOI: 10.1111/j.1747-0285.2008.00640.x.
7. Santos, L.; Garcia-Gomes, A.; Catanho, M.; Sodre, C.; Santos, A.; Branquinha, M.; d'Avila-Levy, C. Aspartic Peptidases of Human Pathogenic Trypanosomatids: Perspectives and Trends for Chemotherapy. *Curr. Med. Chem.* **2013**, *20*, 3116–3133, DOI: 10.2174/0929867311320250007.
8. Abbenante, G.; Fairlie, D. Protease Inhibitors in the Clinic. *Med. Chem.* **2005**, *1*, 71–104, DOI: 10.2174/1573406053402569.
9. Ersmark, K.; Samuelsson, B.; Hallberg, A. Plasmepsins as potential targets for new antimalarial therapy. *Med. Res. Rev.* **2006**, *26*, 626–666, DOI: 10.1002/med.20082.
10. Phillips, M. A.; Burrows, J. N.; Manyando, C.; van Huijsduijnen, R. H.; Van Voorhis, W. C.; Wells, T. N. C. Malaria. *Nat. Rev. Dis. Primer* **2017**, *3*, 17050, DOI: 10.1038/nrdp.2017.50.
11. Biamonte, M. A.; Wanner, J.; Le Roch, K. G. Recent advances in malaria drug discovery. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2829–2843, DOI: 10.1016/j.bmcl.2013.03.067.

12. Boss, C.; Richard-Bildstein, S.; Weller, T.; Fischli, W.; Meyer, S.; Binkert, C. Inhibitors of the Plasmodium falciparum parasite aspartic protease plasmepsin II as potential antimalarial agents. *Curr. Med. Chem.* **2003**, *10*, 883–907, DOI: 10.2174/0929867033457674.
13. Klemba, M.; Goldberg, D. E. Characterization of plasmepsin V, a membrane-bound aspartic protease homolog in the endoplasmic reticulum of Plasmodium falciparum. *Mol. Biochem. Parasitol.* **2005**, *143*, 183–191, DOI: 10.1016/j.molbiopara.2005.05.015.
14. Boonyalai, N.; Collins, C. R.; Hackett, F.; Withers-Martinez, C.; Blackman, M. J. Essentiality of Plasmodium falciparum plasmepsin V. *PLOS ONE* **2018**, *13*, e0207621, DOI: 10.1371/journal.pone.0207621.
15. Coombs, G. H.; Goldberg, D. E.; Klemba, M.; Berry, C.; Kay, J.; Mottram, J. C. Aspartic proteases of Plasmodium falciparum and other parasitic protozoa as drug targets. *Trends Parasitol.* **2001**, *17*, 532–537, DOI: 10.1016/S1471-4922(01)02037-2.
16. Sedwick, C. Plasmepsin V, a Secret Weapon Against Malaria. *PLoS Biol.* **2014**, *12*, e1001898, DOI: 10.1371/journal.pbio.1001898.
17. Marti, M. Targeting Malaria Virulence and Remodeling Proteins to the Host Erythrocyte. *Science* **2004**, *306*, 1930–1933, DOI: 10.1126/science.1102452.
18. Marapana, D. S.; Dagley, L. F.; Sandow, J. J.; Nebl, T.; Triglia, T.; Pasternak, M.; Dickerman, B. K.; Crabb, B. S.; Gilson, P. R.; Webb, A. I.; Boddey, J. A.; Cowman, A. F. Plasmepsin V cleaves malaria effector proteins in a distinct endoplasmic reticulum translocation interactome for export to the erythrocyte. *Nat. Microbiol.* **2018**, *3*, 1010–1022, DOI: 10.1038/s41564-018-0219-2.
19. Boddey, J. A. Plasmepsins on the antimalarial hit list. *Science* **2017**, *358*, 445–446, DOI: 10.1126/science.aaq0002.
20. Munsamy, G.; Ramharack, P.; Soliman, M. E. S. Egress and invasion machinery of malaria: an in-depth look into the structural and functional features of the flap dynamics of plasmepsin IX and X. *RSC Adv.* **2018**, *8*, 21829–21840, DOI: 10.1039/C8RA04360D.

21. Nasamu, A. S.; Glushakova, S.; Russo, I.; Vaupel, B.; Oksman, A.; Kim, A. S.; Fremont, D. H.; Tolia, N.; Beck, J. R.; Meyers, M. J.; Niles, J. C.; Zimmerberg, J.; Goldberg, D. E. Plasmepsins IX and X are essential and druggable mediators of malaria parasite egress and invasion. *Science* **2017**, *358*, 518–522, DOI: 10.1126/science.aan1478.

22. Pino, P.; Caldelari, R.; Mukherjee, B.; Vahokoski, J.; Klages, N.; Maco, B.; Collins, C. R.; Blackman, M. J.; Kursula, I.; Heussler, V.; Brochet, M.; Soldati-Favre, D. A multistage antimalarial targets the plasmepsins IX and X essential for invasion and egress. *Science* **2017**, *358*, 522–528, DOI: 10.1126/science.aaf8675.

23. Bobrovs, R.; Jaudzems, K.; Jirgensons, A. Exploiting Structural Dynamics To Design Open-Flap Inhibitors of Malarial Aspartic Proteases. *J. Med. Chem.* **2019**, *62*, 8931–8950, DOI: 10.1021/acs.jmedchem.9b00184.

24. Eder, J.; Hommel, U.; Cumin, F.; Martoglio, B.; Gerhartz, B. Aspartic Proteases in Drug Discovery. *Curr. Pharm. Des.* **2007**, *13*, 271–285, DOI: 10.2174/138161207779313560.

25. Liu, J.; Istvan, E. S.; Goldberg, D. E. Hemoglobin-degrading Plasmepsin II Is Active as a Monomer. *J. Biol. Chem.* **2006**, *281*, 38682–38688, DOI: 10.1074/jbc.M608535200.

26. Prade, L.; Jones, A. F.; Boss, C.; Richard-Bildstein, S.; Meyer, S.; Binkert, C.; Bur, D. X-ray Structure of Plasmepsin II Complexed with a Potent Achiral Inhibitor. *J. Biol. Chem.* **2005**, *280*, 23837–23843, DOI: 10.1074/jbc.M501519200.

27. Recacha, R.; Leitans, J.; Akopjana, I.; Aprupe, L.; Trapencieris, P.; Jaudzems, K.; Jirgensons, A.; Tars, K. Structures of plasmepsin II from *Plasmodium falciparum* in complex with two hydroxyethylamine-based inhibitors. *Acta Crystallogr. Sect. F Struct. Biol. Commun.* **2015**, *71*, 1531–1539, DOI: 10.1107/S2053230X15022049.

28. Valiente, P. A.; Batista, P. R.; Pupo, A.; Pons, T.; Valencia, A.; Pascutti, P. G. Predicting functional residues in Plasmodium falciparum plasmepsins by combining sequence and structural analysis with molecular dynamics simulations. *Proteins Struct. Funct. Bioinforma.* **2008**, *73*, 440–457, DOI: 10.1002/prot.22068.

29. Arodola, O. A.; Soliman, M. E. S. Molecular Dynamics Simulations of Ligand-Induced Flap Conformational Changes in Cathepsin-D-A Comparative Study: C ONFORMATIONAL C HANGES IN C ATHEPSIN D. *J. Cell. Biochem.* **2016**, *117*, 2643–2657, DOI: 10.1002/jcb.25564.

30. Silva, A. M.; Lee, A. Y.; Erickson, J. W.; Goldberg, D. E. Structural analysis of plasmepsin II. A comparison with human aspartic proteases. *Adv. Exp. Med. Biol.* **1998**, *436*, 363–373, PMID: 9561243.

31. Baldwin, E. T.; Bhat, T. N.; Gulnik, S.; Hosur, M. V.; Sowder, R. C.; Cachau, R. E.; Collins, J.; Silva, A. M.; Erickson, J. W. Crystal structures of native and inhibited forms of human cathepsin D: implications for lysosomal targeting and drug design. *Proc. Natl. Acad. Sci.* **1993**, *90*, 6796–6800, DOI: 10.1073/pnas.90.14.6796.

32. Dunn, B. M. Structure and Mechanism of the Pepsin-Like Family of Aspartic Peptidases. *Chem. Rev.* **2002**, *102*, 4431–4458, DOI: 10.1021/cr010167q.

33. Benes, P.; Vetvicka, V.; Fusek, M. Cathepsin D – Many functions of one aspartic protease. *Crit. Rev. Oncol. Hematol.* **2008**, *68*, 12–28, DOI: 10.1016/j.critrevonc.2008.02.008.

34. Gupta, D.; Yedidi, R. S.; Varghese, S.; Kovari, L. C.; Woster, P. M. Mechanism-Based Inhibitors of the Aspartyl Protease Plasmepsin II as Potential Antimalarial Agents. *J. Med. Chem.* **2010**, *53*, 4234–4247, DOI: 10.1021/jm100233b.

35. Mishra, M.; Mishra, V. K.; Kashaw, V.; Iyer, A. K.; Kashaw, S. K. Comprehensive review on various strategies for antimalarial drug discovery. *Eur. J. Med. Chem.* **2017**, *125*, 1300–1320, DOI: 10.1016/j.ejmech.2016.11.025.

36. Haldar, K.; Bhattacharjee, S.; Safeukui, I. Drug resistance in Plasmodium. *Nat. Rev. Microbiol.* **2018**, *16*, 156–170, DOI: 10.1038/nrmicro.2017.161.

37. Tse, E. G.; Korsik, M.; Todd, M. H. The past, present and future of anti-malarial medicines. *Malar. J.* **2019**, *18*, 93, DOI: 10.1186/s12936-019-2724-z.

38. Dominguez, J. Chemotherapeutic Agents Against Malaria: What Next After Chloroquine? *Curr. Top. Med. Chem.* **2002**, *2*, 1173–1185, DOI: 10.2174/1568026023392986.

39. Hoffman, S. L.; Subramanian, G. M.; Collins, F. H.; Venter, J. C. Plasmodium, human and Anopheles genomics and malaria. *Nature* **2002**, *415*, 702–709, DOI: 10.1038/415702a.

40. Bhaumik, P.; Horimoto, Y.; Xiao, H.; Miura, T.; Hidaka, K.; Kiso, Y.; Wlodawer, A.; Yada, R. Y.; Gustchina, A. Crystal structures of the free and inhibited forms of plasmepsin I (PMI) from Plasmodium falciparum. *J. Struct. Biol.* **2011**, *175*, 73–84, DOI: 10.1016/j.jsb.2011.04.009.

41. Rasina, D.; Otikovs, M.; Leitans, J.; Recacha, R.; Borysov, O. V.; Kanepe-Lapsa, I.; Domraceva, I.; Pantelejevs, T.; Tars, K.; Blackman, M. J.; Jaudzems, K.; Jirgensons, A. Fragment-Based Discovery of 2-Aminoquinazolin-4(3*H*)-ones As Novel Class Nonpeptidomimetic Inhibitors of the Plasmepsins I, II, and IV. *J. Med. Chem.* **2016**, *59*, 374–387, DOI: 10.1021/acs.jmedchem.5b01558.

42. Boss, C.; Corminboeuf, O.; Grisostomi, C.; Meyer, S.; Jones, A. F.; Prade, L.; Binkert, C.; Fischli, W.; Weller, T.; Bur, D. Achiral, Cheap, and Potent Inhibitors of Plasmepsins I, II, and IV. *ChemMedChem* **2006**, *1*, 1341–1345, DOI: 10.1002/cmdc.200600223.

43. Recacha, R.; Jaudzems, K.; Akopjana, I.; Jirgensons, A.; Tars, K. Crystal structure of *Plasmodium falciparum* proplasmepsin IV: the plasticity of proplasmepsins. *Acta Crystallogr. Sect. F Struct. Biol. Commun.* **2016**, *72*, 659–666, DOI: 10.1107/S2053230X16011663.

44. Nguyen, W.; Hodder, A. N.; de Lezongard, R. B.; Czabotar, P. E.; Jarman, K. E.; O'Neill, M. T.; Thompson, J. K.; Jousset Sabroux, H.; Cowman, A. F.; Boddey, J. A.; Sleebs, B. E. Enhanced antimalarial activity of plasmepsin V inhibitors by modification of the P 2 position of PEXEL peptidomimetics. *Eur. J. Med. Chem.* **2018**, *154*, 182–198, DOI: 10.1016/j.ejmech.2018.05.022.

45. Bhaumik, P.; Xiao, H.; Parr, C. L.; Kiso, Y.; Gustchina, A.; Yada, R. Y.; Wlodawer, A. Crystal Structures of the Histo-Aspartic Protease (HAP) from Plasmodium falciparum. *J. Mol. Biol.* **2009**, *388*, 520–540, DOI: 10.1016/j.jmb.2009.03.011.

46. Huizing, A. P.; Mondal, M.; Hirsch, A. K. H. Fighting Malaria: Structure-Guided Discovery of Nonpeptidomimetic Plasmepsin Inhibitors: Miniperspective. *J. Med. Chem.* **2015**, *58*, 5151–5163, DOI: 10.1021/jm5014133.

47. Morishima, H.; Takita, T.; Aoyagi, T.; Takeuchi, T.; Umezawa, H. The structure of Pepstatin. *J. Antibiot. (Tokyo)* **1970**, *23*, 263–265, DOI: 10.7164/antibiotics.23.263.

48. Banerjee, R.; Liu, J.; Beatty, W.; Pelosof, L.; Klemba, M.; Goldberg, D. E. Four plasmepsins are active in the Plasmodium falciparum food vacuole, including a protease with an active-site histidine. *Proc. Natl. Acad. Sci.* **2002**, *99*, 990–995, DOI: 10.1073/pnas.022630099.

49. Molteni, M.; Pesenti, C.; Sani, M.; Volonterio, A.; Zanda, M. Fluorinated peptidomimetics: synthesis, conformational and biological features. *J. Fluor. Chem.* **2004**, *125*, 1735–1743, DOI: 10.1016/j.jfluchem.2004.09.014.

50. Johansson, P.-O.; Chen, Y.; Belfrage, A. K.; Blackman, M. J.; Kvarnström, I.; Jansson, K.; Vrang, L.; Hamelink, E.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. Design and Synthesis of Potent Inhibitors of the Malaria Aspartyl Proteases Plasmepsin I and II. Use of Solid-Phase Synthesis to Explore Novel Statine Motifs. *J. Med. Chem.* **2004**, *47*, 3353–3366, DOI: 10.1021/jm031106i.

51. Johansson, P.-O.; Lindberg, J.; Blackman, M. J.; Kvarnström, I.; Vrang, L.; Hamelink, E.; Hallberg, A.; Rosenquist, Å.; Samuelsson, B. Design and Synthesis of Potent Inhibitors of Plasmepsin I and II: X-ray Crystal Structure of Inhibitor in Complex with Plasmepsin II. *J. Med. Chem.* **2005**, *48*, 4400–4409, DOI: 10.1021/jm040884n.

52. Mimoto, T.; Kato, R.; Takaku, H.; Nojima, S.; Terashima, K.; Misawa, S.; Fukazawa, T.; Ueno, T.; Sato, H.; Shintani, M.; Kiso, Y.; Hayashi, H. Structure−Activity Relationship of Small-Sized HIV Protease Inhibitors Containing Allophenylnorstatine. *J. Med. Chem.* **1999**, *42*, 1789–1802, DOI: 10.1021/jm980637h.

53. Mimoto, T.; Hattori, N.; Takaku, H.; Kisanuki, S.; Fukazawa, T.; Terashima, K.; Kato, R.; Nojima, S.; Misawa, S.; Ueno, T.; Imai, J.; Enomoto, H.; Tanaka, S.; Sakikawa, H.; Shintani, M.; Hayashi, H.; Kiso, Y. Structure-Activity Relationship of Orally Potent Tripeptide-Based HIV Protease Inhibitors Containing Hydroxymethylcarbonyl Isostere. *Chem. Pharm. Bull. (Tokyo)* **2000**, *48*, 1310–1326, DOI: 10.1248/cpb.48.1310.

54. Nezami, A.; Luque, I.; Kimura, T.; Kiso, Y.; Freire, E. Identification and Characterization of Allophenylnorstatine-Based Inhibitors of Plasmepsin II, an Antimalarial Target. *Biochemistry* **2002**, *41*, 2273–2280, DOI: 10.1021/bi0117549.

55. Kiso, A.; Hidaka, K.; Kimura, T.; Hayashi, Y.; Nezami, A.; Freire, E.; Kiso, Y. Search for substrate-based inhibitors fitting the S2' space of malarial aspartic protease plasmepsin II. *J. Pept. Sci.* **2004**, *10*, 641–647, DOI: 10.1002/psc.609.

56. Ersmark, K.; Feierberg, I.; Bjelic, S.; Hultén, J.; Samuelsson, B.; Åqvist, J.; Hallberg, A. C_2-Symmetric inhibitors of Plasmodium falciparum plasmepsin II: synthesis and theoretical predictions. *Bioorg. Med. Chem.* **2003**, *11*, 3723–3733, DOI: 10.1016/S0968-0896(03)00339-0.

57. Ersmark, K.; Feierberg, I.; Bjelic, S.; Hamelink, E.; Hackett, F.; Blackman, M. J.; Hultén, J.; Samuelsson, B.; Åqvist, J.; Hallberg, A. Potent Inhibitors of the *Plasmodium falciparum* Enzymes Plasmepsin I and II Devoid of Cathepsin D Inhibitory Activity. *J. Med. Chem.* **2004**, *47*, 110–122, DOI: 10.1021/jm030933g.

58. Ersmark, K.; Nervall, M.; Hamelink, E.; Janka, L. K.; Clemente, J. C.; Dunn, B. M.; Blackman, M. J.; Samuelsson, B.; Åqvist, J.; Hallberg, A. Synthesis of Malarial Plasmepsin Inhibitors and Prediction of Binding Modes by Molecular Dynamics Simulations. *J. Med. Chem.* **2005**, *48*, 6090–6106, DOI: 10.1021/jm050463l.

59. Haque, T. S.; Skillman, A. G.; Lee, C. E.; Habashita, H.; Gluzman, I. Y.; Ewing, T. J. A.; Goldberg, D. E.; Kuntz, I. D.; Ellman, J. A. Potent, Low-Molecular-Weight Non-Peptide Inhibitors of Malarial Aspartyl Protease Plasmepsin II. *J. Med. Chem.* **1999**, *42*, 1428–1440, DOI: 10.1021/jm980641t.

60. Nöteberg, D.; Hamelink, E.; Hultén, J.; Wahlgren, M.; Vrang, L.; Samuelsson, B.; Hallberg, A. Design and Synthesis of Plasmepsin I and Plasmepsin II Inhibitors with Activity in *Plasmodium falciparum* -Infected Cultured Human Erythrocytes. *J. Med. Chem.* **2003**, *46*, 734–746, DOI: 10.1021/jm020951i.

61. Nöteberg, D.; Schaal, W.; Hamelink, E.; Vrang, L.; Larhed, M. High-Speed Optimization of Inhibitors of the Malarial Proteases Plasmepsin I and II. *J. Comb. Chem.* **2003**, *5*, 456–464, DOI: 10.1021/cc0301014.

62. Zürcher, M.; Diederich, F. Structure-Based Drug Design: Exploring the Proper Filling of Apolar Pockets at Enzyme Active Sites. *J. Org. Chem.* **2008**, *73*, 4345–4361, DOI: 10.1021/jo800527n.

63. Silva, A. M.; Lee, A. Y.; Gulnik, S. V.; Maier, P.; Collins, J.; Bhat, T. N.; Collins, P. J.; Cachau, R. E.; Luker, K. E.; Gluzman, I. Y.; Francis, S. E.; Oksman, A.; Goldberg, D. E.; Erickson, J. W. Structure and inhibition of plasmepsin II, a hemoglobin-degrading enzyme from Plasmodium falciparum. *Proc. Natl. Acad. Sci.* **1996**, *93*, 10034–10039, DOI: 10.1073/pnas.93.19.10034.

64. Carcache, D. A.; Hörtner, S. R.; Bertogg, A.; Binkert, C.; Bur, D.; Märki, H. P.; Dorn, A.; Diederich, F. De Novo Design, Synthesis, and In Vitro Evaluation of a New Class of Nonpeptidic Inhibitors of the Malarial Enzyme Plasmepsin II. *ChemBioChem* **2002**, *3*, 1137–1141, DOI: 10.1002/1439-7633(20021104)3:11<1137::AID-CBIC1137>3.0.CO;2-A.

65. Carcache, D. A.; Hörtner, S. R.; Seiler, P.; Diederich, F.; Dorn, A.; Märki, H. P.; Binkert, C.; Bur, D. Development of a New Class of Inhibitors for the Malarial Aspartic Protease Plasmepsin II Based on a Central 7-Azabicyclo[2.2.1]heptane Scaffold. *Helv. Chim. Acta* **2003**, *86*, 2173–2191, DOI: 10.1002/hlca.200390176.

66. Hof, F.; Schütz, A.; Fäh, C.; Meyer, S.; Bur, D.; Liu, J.; Goldberg, D. E.; Diederich, F. Starving the Malaria Parasite: Inhibitors Active against the Aspartic Proteases Plasmepsins I, II, and IV. *Angew. Chem. Int. Ed.* **2006**, *45*, 2138–2141, DOI: 10.1002/anie.200504119.

67. Zürcher, M.; Hof, F.; Barandun, L.; Schütz, A.; Schweizer, W. B.; Meyer, S.; Bur, D.; Diederich, F. Synthesis of *exo*-3-Amino-7-azabicyclo[2.2.1]heptanes as a Class of Malarial Aspartic Protease Inhibitors: Exploration of Two Binding Pockets. *Eur. J. Org. Chem.* **2009**, *2009*, 1707–1719, DOI: 10.1002/ejoc.200801184.

68. Zürcher, M.; Gottschalk, T.; Meyer, S.; Bur, D.; Diederich, F. Exploring the Flap Pocket of the Antimalarial Target Plasmepsin II: The “55 % Rule” Applied to Enzymes. *ChemMedChem* **2008**, *3*, 237–240, DOI: 10.1002/cmdc.200700236.

69. Aureggi, V.; Ehmke, V.; Wieland, J.; Schweizer, W. B.; Bernet, B.; Bur, D.; Meyer, S.; Rottmann, M.; Freymond, C.; Brun, R.; Breit, B.; Diederich, F. Potent Inhibitors of Malarial Aspartic Proteases, the Plasmepsins, by Hydroformylation of Substituted 7-Azanorbornenes. *Chem. - Eur. J.* **2013**, *19*, 155–164, DOI: 10.1002/chem.201202941.

70. Luksch, T.; Chan, N.-S.; Brass, S.; Sotriffer, C. A.; Klebe, G.; Diederich, W. E. Computer-Aided Design and Synthesis of Nonpeptidic Plasmepsin II and IV Inhibitors. *ChemMedChem* **2008**, *3*, 1323–1336, DOI: 10.1002/cmdc.200700270.

71. Blum, A.; Böttcher, J.; Heine, A.; Klebe, G.; Diederich, W. E. Structure-Guided Design of C_2 -Symmetric HIV-1 Protease Inhibitors Based on a Pyrrolidine Scaffold. *J. Med. Chem.* **2008**, *51*, 2078–2087, DOI: 10.1021/jm701142s.

72. Luksch, T.; Blum, A.; Klee, N.; Diederich, W. E.; Sotriffer, C. A.; Klebe, G. Pyrrolidine Derivatives as Plasmepsin Inhibitors: Binding Mode Analysis Assisted by Molecular Dynamics Simulations of a Highly Flexible Protein. *ChemMedChem* **2010**, *5*, 443–454, DOI: 10.1002/cmdc.200900452.

73. Boss, C.; Corminboeuf, O.; Grisostomi, C.; Weller, T. Inhibitors of aspartic proteases – potential antimalarial agents. *Expert Opin. Ther. Pat.* **2006**, *16*, 295–317, DOI: 10.1517/13543776.16.3.295.

74. Fäh, C.; Hardegger, L. A.; Baitsch, L.; Schweizer, W. B.; Meyer, S.; Bur, D.; Diederich, F. New organofluorine building blocks: inhibition of the malarial aspartic proteases plasmepsin II and IV by alicyclic α,α-difluoroketone hydrates. *Org. Biomol. Chem.* **2009**, *7*, 3947, DOI: 10.1039/b908489d.

75. Fäh, C.; Mathys, R.; Hardegger, L. A.; Meyer, S.; Bur, D.; Diederich, F. Enantiomerically Pure and Highly Substituted Alicyclic α,α-Difluoro Ketones: Potential Inhibitors for Malarial Aspartic Proteases, the Plasmepsins. *Eur. J. Org. Chem.* **2010**, *2010*, 4617–4629, DOI: 10.1002/ejoc.201000712.

76. Rasina, D.; Stakanovs, G.; Borysov, O. V.; Pantelejevs, T.; Bobrovs, R.; Kanepe-Lapsa, I.; Tars, K.; Jaudzems, K.; Jirgensons, A. 2-Aminoquinazolin-4(3*H*)-one based plasmepsin inhibitors with improved hydrophilicity and selectivity. *Bioorg. Med. Chem.* **2018**, *26*, 2488–2500, DOI: 10.1016/j.bmc.2018.04.012.

77. Jiang, S.; Prigge, S. T.; Wei, L.; Gao, Y.-e; Hudson, T. H.; Gerena, L.; Dame, J. B.; Kyle, D. E. New Class of Small Nonpeptidyl Compounds Blocks Plasmodium falciparum Development In Vitro by Inhibiting Plasmepsins. *Antimicrob. Agents Chemother.* **2001**, *45*, 2577–2584, DOI: 10.1128/AAC.45.9.2577-2584.2001.

78. Lima, L.; Barreiro, E. Bioisosterism: A Useful Strategy for Molecular Modification and Drug Design. *Curr. Med. Chem.* **2005**, *12*, 23–49, DOI: 10.2174/0929867053363540.

79. Brown, N. *Bioisosteres in Medicinal Chemistry*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2012; pp 15–29, DOI: 10.1002/9783527654307.ch2.

80. Testa, B.; Mayer, J. M. *Hydrolysis in Drug and Prodrug Metabolism*; Verlag Helvetica Chimica Acta: Zürich, 2006; pp 81–162, DOI: 10.1002/9783906390444.ch4.

81. Wei, F.; Li, H.; Song, C.; Ma, Y.; Zhou, L.; Tung, C.-H.; Xu, Z. Cu/Pd-Catalyzed, Three-Component Click Reaction of Azide, Alkyne, and Aryl Halide: One-Pot Strategy toward Trisubstituted Triazoles. *Org. Lett.* **2015**, *17*, 2860–2863, DOI: 10.1021/acs.orglett.5b01342.

82. Ferrini, S.; Chandanshive, J. Z.; Lena, S.; Comes Franchini, M.; Giannini, G.; Tafi, A.; Taddei, M. Ruthenium-Catalyzed Synthesis of 5-Amino-1,2,3-triazole-4-carboxylates for Triazole-Based Scaffolds: Beyond the Dimroth Rearrangement. *J. Org. Chem.* **2015**, *80*, 2562–2572, DOI: 10.1021/jo502577e.

83. Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. Ruthenium-Catalyzed Azide−Alkyne Cycloaddition: Scope and Mechanism. *J. Am. Chem. Soc.* **2008**, *130*, 8923–8930, DOI: 10.1021/ja0749993.

84. Markiewicz, J. T.; Wiest, O.; Helquist, P. Synthesis of Primary Aryl Amines Through a Copper-Assisted Aromatic Substitution Reaction with Sodium Azide. *J. Org. Chem.* **2010**, *75*, 4887–4890, DOI: 10.1021/jo101002p.

85. Wang, N.; Li, R.; Li, L.; Xu, S.; Song, H.; Wang, B. Rhodium(III)-Catalyzed Intermolecular Amidation with Azides via C(sp^3)–H Functionalization. *J. Org. Chem.* **2014**, *79*, 5379–5385, DOI: 10.1021/jo5008515.

86. Paek, S.; Baik, C.; Kang, M.; Kang, H.; Ko, J. New type of ruthenium sensitizers with a triazole moiety as a bridging group. *J. Organomet. Chem.* **2010**, *695*, 821–826, DOI: 10.1016/j.jorganchem.2009.12.021.

87. Hollinshead, S. P.; Nichols, J. B.; Wilson, J. W. Two Practical Syntheses of Sterically Congested Benzophenones. *J. Org. Chem.* **1994**, *59*, 6703–6709, DOI: 10.1021/jo00101a032.

88. Hameury, T.; Guillemont, J.; Van Hijfte, L.; Bellosta, V.; Cossy, J. Diastereodivergent Addition of Allenylzincs to Aryl Glyoxylates. *Org. Lett.* **2009**, *11*, 2397–2400, DOI: 10.1021/ol900494g.

89. Davies, K. A.; Abel, R. C.; Wulff, J. E. Operationally Simple Copper-Promoted Coupling of Terminal Alkynes with Benzyl Halides. *J. Org. Chem.* **2009**, *74*, 3997–4000, DOI: 10.1021/jo900444x.

90. Gribble, G. W.; Joule, A. J. Progress in Heterocyclic Chemistry. In: *Five-Membered Ring Systems with O & N atoms*; Cordero, F. M.; Giomi, D.; Lascialfari, L.; Elsevier, 2014; Vol. 26, pp 319–348, DOI: 10.1016/B978-0-08-100017-5.00011-X.

91. Zhao, Y.; Wang, Q.; Meng, Q.; Ding, D.; Yang, H.; Gao, G.; Li, D.; Zhu, W.; Zhou, H. Identification of Trypanosoma brucei leucyl-tRNA synthetase inhibitors by pharmacophore- and docking-based virtual screening and synthesis. *Bioorg. Med. Chem.* **2012**, *20*, 1240–1250, DOI: 10.1016/j.bmc.2011.12.035.

92. Pirali, T.; Mossetti, R.; Galli, S.; Tron, G. C. Stereospecific Synthesis of *syn*-α-Oximinoamides by a Three-Component Reaction of Isocyanides, *syn* -Chlorooximes, and Carboxylic Acids. *Org. Lett.* **2011**, *13*, 3734–3737, DOI: 10.1021/ol201397x.

93. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. Copper(I)-Catalyzed Synthesis of Azoles. DFT Study Predicts Unprecedented Reactivity and Intermediates. *J. Am. Chem. Soc.* **2005**, *127*, 210–216, DOI: 10.1021/ja0471525.

94. Masters, K.-S.; Wallesch, M.; Bräse, S. *ortho*-Bromo(propa-1,2-dien-1-yl)arenes: Substrates for Domino Reactions. *J. Org. Chem.* **2011**, *76*, 9060–9067, DOI: 10.1021/jo201679s.

95. Suárez, A.; Fu, G. C. A Straightforward and Mild Synthesis of Functionalized 3-Alkynoates. *Angew. Chem. Int. Ed.* **2004**, *43*, 3580–3582, DOI: 10.1002/anie.200454070.

96. Gray, D.; Concellón, C.; Gallagher, T. Kowalski Ester Homologation. Application to the Synthesis of *β*-Amino Esters. *J. Org. Chem.* **2004**, *69*, 4849–4851, DOI: 10.1021/jo049562h.

97. Bedford, R. B.; Haddow, M. F.; Mitchell, C. J.; Webster, R. L. Mild C-H Halogenation of Anilides and the Isolation of an Unusual Palladium(I)-Palladium(II) Species. *Angew. Chem. Int. Ed.* **2011**, *50*, 5524–5527, DOI: 10.1002/anie.201101606.

98. Barral, K.; Moorhouse, A. D.; Moses, J. E. Efficient Conversion of Aromatic Amines into Azides: A One-Pot Synthesis of Triazole Linkages. *Org. Lett.* **2007**, *9*, 1809–1811, DOI: 10.1021/ol070527h.

99. Berry, M. T.; Castrejon, D.; Hein, J. E. Oxidative Esterification of Aldehydes Using Mesoionic 1,2,3-Triazolyl Carbene Organocatalysts. *Org. Lett.* **2014**, *16*, 3676–3679, DOI: 10.1021/ol501458p.

100. Chae, J. Practical demethylation of aryl methyl ethers using an odorless thiol reagent. *Arch. Pharm. Res.* **2008**, *31*, 305–309, DOI: 10.1007/s12272-001-1156-y.

101. Sun, H.; Tawa, G.; Wallqvist, A. Classification of scaffold-hopping approaches. *Drug Discov. Today* **2012**, *17*, 310–324, DOI: 10.1016/j.drudis.2011.10.024.

102. Chrzanowska, M.; Grajewska, A.; Rozwadowska, M. D. Asymmetric Synthesis of Isoquinoline Alkaloids: 2004–2015. *Chem. Rev.* **2016**, *116*, 12369–12465, DOI: 10.1021/acs.chemrev.6b00315.

103. Kürti, L.; Czakó, B. *Strategic applications of named reactions in organic synthesis: background and detailed mechanisms*; Elsevier Academic Press: Amsterdam; Boston, 2005, pp 348–349.

104. Specker, E.; Böttcher, J.; Heine, A.; Sotriffer, C. A.; Lilie, H.; Schoop, A.; Müller, G.; Griebenow, N.; Klebe, G. Hydroxyethylene Sulfones as a New Scaffold To Address Aspartic Proteases: Design, Synthesis, and Structural Characterization. *J. Med. Chem.* **2005**, *48*, 6607–6619, DOI: 10.1021/jm050224y.

105. Fang, X.; Yin, Y.; Chen, Y. T.; Yao, L.; Wang, B.; Cameron, M. D.; Lin, L.; Khan, S.; Ruiz, C.; Schröter, T.; Grant, W.; Weiser, A.; Pocas, J.; Pachori, A.; Schürer, S.; LoGrasso, P.; Feng, Y. Tetrahydroisoquinoline Derivatives As Highly Selective and Potent Rho Kinase Inhibitors. *J. Med. Chem.* **2010**, *53*, 5727–5737, DOI: 10.1021/jm100579r.

106. Cueva, J. P.; Cai, T. B.; Mascarella, S. W.; Thomas, J. B.; Navarro, H. A.; Carroll, F. I. Synthesis and In Vitro Opioid Receptor Functional Antagonism of Methyl-Substituted Analogues of (3*R*)-7-Hydroxy-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic). *J. Med. Chem.* **2009**, *52*, 7463–7472, DOI: 10.1021/jm900756t.

107. Aggarwal, V. K.; Humphries, P. S.; Fenwick, A. Enantioselective deprotonation of 4-tert-butylcyclohexanone by conformationally constrained chiral lithium amide bases. *J. Chem. Soc. Perkin 1* **1999**, 2883–2889, DOI: 10.1039/a905947d.

108. Hanthorn, J. J.; Valgimigli, L.; Pratt, D. A. Preparation of Highly Reactive Pyridine- and Pyrimidine-Containing Diarylamine Antioxidants. *J. Org. Chem.* **2012**, *77*, 6908–6916, DOI: 10.1021/jo301013c.

109. Gamo, F.-J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J.-L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. Thousands of chemical starting points for antimalarial lead identification. *Nature* **2010**, *465*, 305–310, DOI: 10.1038/nature09107.

110. Jaudzems, K.; Tars, K.; Maurops, G.; Ivdra, N.; Otikovs, M.; Leitans, J.; Kanepe-Lapsa, I.; Domraceva, I.; Mutule, I.; Trapencieris, P.; Blackman, M. J.; Jirgensons, A. Plasmepsin Inhibitory Activity and Structure-Guided Optimization of a Potent Hydroxyethylamine-Based Antimalarial Hit. *ACS Med. Chem. Lett.* **2014**, *5*, 373–377, DOI: 10.1021/ml4004952.

111. Clemente, J. C.; Govindasamy, L.; Madabushi, A.; Fisher, S. Z.; Moose, R. E.; Yowell, C. A.; Hidaka, K.; Kimura, T.; Hayashi, Y.; Kiso, Y.; Agbandje-McKenna, M.; Dame, J. B.; Dunn, B. M.; McKenna, R. Structure of the aspartic protease plasmepsin 4 from the malarial parasite *Plasmodium malariae* bound to an allophenylnorstatine-based inhibitor. *Acta Crystallogr. D Biol. Crystallogr.* **2006**, *62*, 246–252, DOI: 10.1107/S0907444905041260.

112. Ghosh, A. K.; Fidanze, S. Transition-State Mimetics for HIV Protease Inhibitors: Stereocontrolled Synthesis of Hydroxyethylene and Hydroxyethylamine Isosteres by Ester-Derived Titanium Enolate Syn and Anti-Aldol Reactions. *J. Org. Chem.* **1998**, *63*, 6146–6152, DOI: 10.1021/jo980159i.

113. Yang, K. S.; Budin, G.; Tassa, C.; Kister, O.; Weissleder, R. Bioorthogonal Approach to Identify Unsuspected Drug Targets in Live Cells. *Angew. Chem. Int. Ed.* **2013**, *52*, 10593–10597, DOI: 10.1002/anie.201304096.

114. Kaganovsky, L.; Gelman, D.; Rueck-Braun, K. Trans-chelating ligands in palladium-catalyzed carbonylative coupling and methoxycarbonylation of aryl halides. *J. Organomet. Chem.* **2010**, *695*, 260–266, DOI: 10.1016/j.jorganchem.2009.10.001.

115. Brennführer, A.; Neumann, H.; Beller, M. Palladium-Catalyzed Carbonylation Reactions of Aryl Halides and Related Compounds. *Angew. Chem. Int. Ed.* **2009**, *48*, 4114–4133, DOI: 10.1002/anie.200900013.

PIELIKUMI

1. PIELIKUMS – "AZOLE-BASED NON-PEPTIDOMIMETIC PLASMEPSIN INHIBITORS"

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FULL PAPER

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Azole-based non-peptidomimetic plasmepsin inhibitors

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Abstract

The spread of drug-resistant malaria parasites urges the search for new antimalarial drugs. Malarial aspartic proteases – plasmepsins (Plms) – are differentially expressed in multiple stages of the *Plasmodium* parasite's lifecycle and are considered as attractive drug targets. We report the development of novel azole-based non-peptidomimetic plasmepsin inhibitors that have been designed by bioisosteric substitution of the amide moiety in the Actelion amino-piperazine inhibitors. The best triazole-based inhibitors show submicromolar potency toward Plm II, which is comparable to that of the parent Actelion compounds. The new inhibitors can be used as a starting point for the development of a resistance-free antimalarial drug targeting the non-digestive Plm IX or X, which are essential for the malaria parasite life cycle.

KEYWORDS
bioisosteric replacement, inhibitor, malaria, plasmepsins, *Plasmodium falciparum*, triazole

1 | INTRODUCTION

In 2016, an estimated 216 million cases of malaria caused 445000 deaths.[1] Even when non-lethal, malaria is associated with very unpleasant symptoms such as fever, fatigue, vomiting and it can also lead to disability. The treatment and prophylaxis of malaria are encumbered by the spread of drug-resistant parasite strains which motivates to replenish the antimalarial drug arsenal with new chemotherapeutic agents.[2–5] A desirable feature for a resistance free antimalarial drug is interruption of the parasite life cycle by an unexploited mechanism of action. For this reason, we turned our attention to malarial aspartic proteases – plasmepsins, which have been considered as attractive drug targets for decades.[6–8] Digestive plasmepsins (Plm I, II, IV and HAP) are involved in the processing of hemoglobin to amino acids, however, their inhibition may not be sufficient to kill the parasite due to alternative pathways of hemoglobin digestion.[9–11] Recent investigations suggest that the anti-malarial activity in red blood cell (RBC) assays likely can be achieved by targeting the non-digestive Plms such as Plm V,[12,13] Plm IX, and Plm X,[14–17] which are expressed in the blood stage. Of these, Plm V triggers the export protein transfer from parasitic vacuole to the RBC, Plm IX is involved in merozoite invasion of RBC while Plm X is involved in both, the invasion and the egress of merozoites. Unfortunately, the production of recombinant Plm IX and Plm X has been only possible in higher eukaryotic protein expression systems, such as insect or mammalian cells, limiting the availability of these enzymes. In the meantime, Plm IX and Plm X (but not Plm V) share high sequence homology to readily accessible digestive Plms such as Plm I, II, and IV. Therefore, the latter are potentially suited as model proteins to search for novel Plm inhibitors.

Much effort in recent years has been devoted to the discovery of non-peptidomimetic Plm inhibitors.[18–24] These hold a promise to provide metabolically stable drug candidates and also show an improved selectivity profile against human aspartic proteases as compared to peptidomimetic inhibitors. Scientists at Actelion have developed the first non-peptidomimetic Plm II inhibitors **1** (Figure 1) based on the amino-piperidine scaffold.[18–20] The X-ray structure of the inhibitor–enzyme complex demonstrates that the

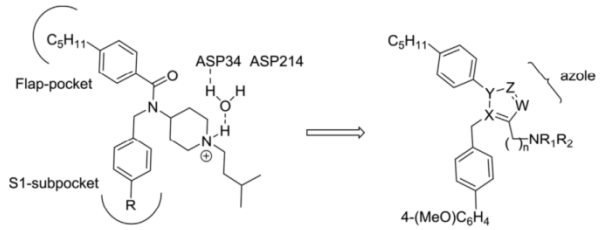


FIGURE 1 Design of azole-based Plm II inhibitors by bioisosteric substitution of amide function

amino-piperidine type inhibitors **1** bind to an open-flap conformation of the enzyme.[20] Analysis of this structure revealed that the piperidine nitrogen forms an ionic interaction with the catalytic residue Asp214 and a water-bridged H-bond interaction with the catalytic Asp34. Other important pharmacophoric elements are the biphenyl group occupying the S1 subpocket and the *n*-pentyl chain, which is placed in the flap-pocket (Figure 1). A drawback of the amino-piperidine inhibitors **1** is that they contain an enzymatically labile amide bond, which could be unattractive for the development of an orally available drug.[25] Moreover, these compounds exhibit unfavorable physicochemical properties such as high clogP values and very low solubility [18]. These drawbacks prompted us to develop a new series of inhibitors **2** (Figure 1). Our approach is based on bioisosteric replacement of the amide moiety by more drug-like scaffolds such as azoles[26,27] (Figure 1).

2 | RESULTS

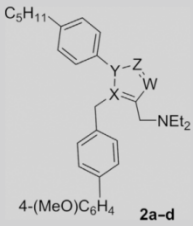
2.1 | Structure–activity relationships

Our study commenced with the re-synthesis of two representative Actelion amino-piperidines **1a**,**b** to measure their Plm II inhibitory potency in our assay conditions. Surprisingly, the determined submicromolar IC_{50} values for both compounds (Figure 1) were considerably higher than those reported in the original publication (**1a**, IC_{50}(Plm II) = 8 nM; **1b**, IC_{50}(Plm II) = 11 nM).[18] Although we were unable to reproduce the published inhibitory activities, we realized that amides **1a**,**b** are well suited as reference compounds for our study.

A series of amino-piperidine heterocyclic analogues **2a–d** was designed by replacing the amide group in the parent inhibitor **1a** with 1,2,3-triazole, isoxazole, imidazole, and pyrrole. In addition, the piperidine moiety was replaced by *N*,*N*-diethylaminomethyl group. All azoles **2a–d** displayed comparable Plm II inhibitory activities at micromolar level (Table 1).

Next, the optimal chain length between the heterocyclic core and the amino function as well as the *N*-substitution pattern was investigated for the triazole analogues **2e–p** (Table 2). The study revealed that a two carbon atom linker between the triazole and the amino function gives the best Plm II inhibitors **2e**,**h**,**k** as compared to one and three carbon analogues. These results are in accordance with docking studies of inhibitor **2e**, which show that the heterocycle is largely solvent exposed and all other pharmacophoric elements are involved in the same interactions that have been observed for inhibitor

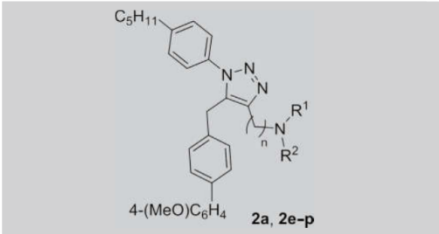
TABLE 1 Plm II inhibitory potency of azole-based inhibitors **2a–d**[a]



Compound	Azole	IC_{50}, μM[b]
2a		4.3 ± 0.2
2b		10.0 ± 0.5
2c		5.3 ± 0.2
2d		10.0 ± 0.4

[a]IC_{50} (Plm II) values for reference compounds: **1a**, 0.42 ± 0.02 μM; **1b**, 0.30 ± 0.015 μM.
[b]Plm II inhibitory activity was determined by enzymatic FRET assay in triplicate experiments.

TABLE 2 SAR of linker length and *N*-substitution pattern in triazole-based inhibitors



2a, **2e–p**

Compound	-NR^{1}R^2	*n*	IC$_{50}$ (Plm II), μM[a]
2a		1	4.3 ± 0.2
2e		2	0.6 ± 0.03
2f		3	2.7 ± 0.15
2g		1	4.7 ± 0.2
2h		2	0.6 ± 0.03
2i		3	2.9 ± 0.15
2j		1	7.5 ± 0.3
2k		2	1.8 ± 0.09
2l		3	5.9 ± 0.3
2m		2	2.9 ± 0.15
2n		2	5.0 ± 0.2
2o		2	6.5 ± 0.3
2p		2	3.0 ± 0.15

[a]Plm II inhibitory activity was determined by enzymatic FRET assay in triplicate experiments.

1a (Figure 2). The two carbon linker in inhibitor **2e** provides proper positioning of the protonated amino nitrogen above the negatively charged Asp214 residue and concomitant hydrogen bonding with both Asp214 and Asp34 through a water bridge (Figure 3). The shorter (one carbon) or longer (three carbon atoms) linkers in **2a**,**f** direct the amino function in a suboptimal position, where it can interact either with Asp34 or Asp214, but not with both residues at the same time.

A small set of compounds with different *N*-alkyl substituents (**2e**,**h**, **k**,**m–p**) was prepared to evaluate the optimal size and type of the alkyl groups. From these, compounds **2e** and **2h** bearing *N*,*N*-diethylamino and pyrrolidine groups, respectively, were found to be the most potent Plm II inhibitors. The activity of these compounds was comparable to that of the parent amino-piperidine derivative **1a**. According to docking studies, the *N*-alkyl groups (R^1 and R^2) interact with Tyr192, Ile212, Phe294, and Ile300 of the S1′ subpocket, and the ligand binding is mostly affected by the shape and size of these groups. Inhibitors with small substituents (**2o** and **2p**) do not fill the hydrophobic S1′ subpocket, whereas inhibitor **2n** bearing a larger R^1 substituent (Bn group) does not fit into the S1′ cavity. These suboptimal inhibitor interactions with the S1′ subpocket residues result in lower activities as compared to inhibitors with medium-sized *N*-substituents.

By analogy with the binding mode of amino-piperidine inhibitor **1b** (2BJU crystal structure), the substituent at the position 1 of the triazole is directed into the flap pocket of Plm II (Figure 1). The shortening of the alkyl-phenyl chain length from the *n*-pentyl to the methyl resulted in a significant drop of the activity (Table 3, compound **2q**). A similar result was observed in our previous studies of 2-aminoquinazolin-4 (3*H*)-one Plm II inhibitors,[24] where we have demonstrated that the long aliphatic chain of the flap pocket substituent allows for stabilization of the open-flap conformation and for locking of the ligand core rigidly above the aspartic dyad. Introduction of a benzylic substituent at the position 1 of the triazole (compound **2r**) resulted in a 10-fold drop of inhibitory potency, thus confirming that the phenyl group is the most optimal substituent at this position. Molecular modeling indicated that the potency drop is due to the bending of the *p*-alkyl-phenyl chain relative to the core, which diminishes interactions with the aspartic dyad and the S1′ subpocket. The introduction of chlorine atom at the *o*-position (compound **2s**) did not affect Plm II inhibition (Table 3).

Recent work by Soldati-Favre and coworkers[16] has shown that the known hydroxylethylamine-based digestive vacuole Plms inhibitors elicit anti-plasmodial activity by targeting the non-digestive Plm IX and X, essential for parasite egress and invasion. To examine if the triazole-based Plm II inhibitors **2** can potentially inhibit Plm IX and X, we docked compound **2e** into homology models which were generated based on the Plm II open-flap crystal structure with PDB ID 2BJU.[20] Comparison of the docked poses of inhibitor **2e** in Plm II, IX, and X (Figure 2) shows that the compound may interact with Plm IX and X in a similar manner as with Plm II, although few differences in the inhibitor recognition are apparent that could be used for selectivity tuning against a particular Plm isoform.

2.2 | Synthesis

1,2,3-Triazole-based inhibitors **2a**, **2e–s** were synthesized using the Huisgen 1,3-dipolar cycloaddition between suitably substituted acetylenes and azides **3**, **4**, **8**, and **11** as the key step. The synthesis of azides **3**, **4** was started with bromine-to-lithium exchange and followed by the reaction of aryllithium intermediate with tosyl azide (Scheme 1).[28] The key step in the synthesis of azide **8** was Pd-catalyzed C—H *ortho* chlorination of *N*-acetyl aniline.[29] Subsequent *N*-deprotection afforded aniline **7**, which was elaborated into azide **8** by treatment with *tert*-butyl nitrite and azidotrimethylsilane.[30] Benzylazide **11** was prepared from acid **9** in a three-step sequence

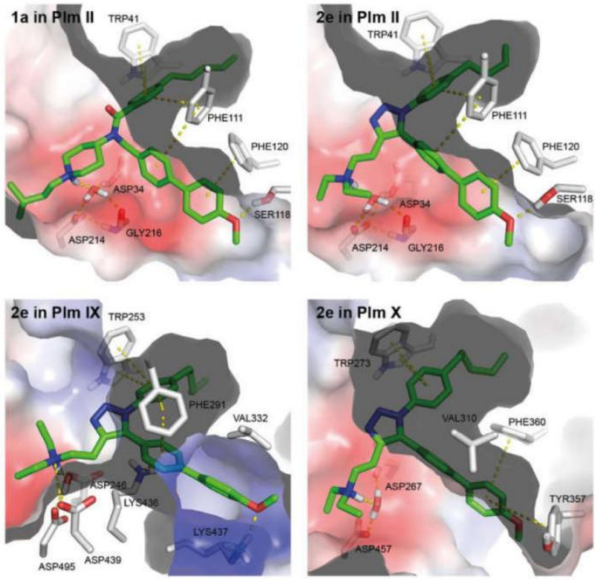


FIGURE 2 Docked poses of amino-piperidine-based inhibitor **1b** and azole-based inhibitor **2e** in the active sites of Plm II, IX, and X. Electrostatic potentials mapped on the Plm II surface were calculated in PyMol [The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC]. Negatively charged regions are shown in red, positively – in blue. Hydrogen bonds and π–π interactions are indicated with yellow-dashed lines. Docking was performed using Schrödinger Glide software on the crystal structure of Plm II solved in complex with an amino-piperidine-based Plm II inhibitor (Ref. [20], PDB ID 2BJU) or homology models of Plm IX and X generated based on the same crystal structure

comprising the initial reduction to benzyl alcohol, followed by conversion to benzyl bromide **10** and alkylation with azide anion (Scheme 1). With prerequisite azides for the 1,3-dipolar cycloaddition in hand, the synthesis of substituted acetylenes **17a–c** and **21**, **22** was addressed. The synthesis of alkynes **17a–c** commenced with Pd-catalyzed Suzuki–Miyaura cross-coupling reaction between boronic acid **12** and 4-bromoanisole to afford biaryl ester **13** (Scheme 2). The reduction of the ester moiety in **13** delivered benzyl alcohol that was

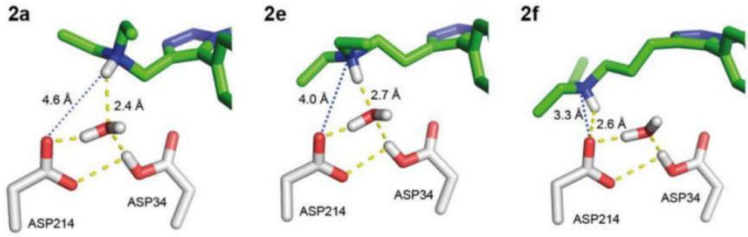
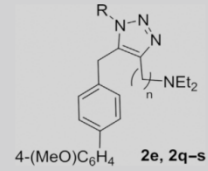
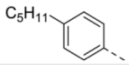
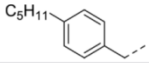
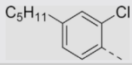


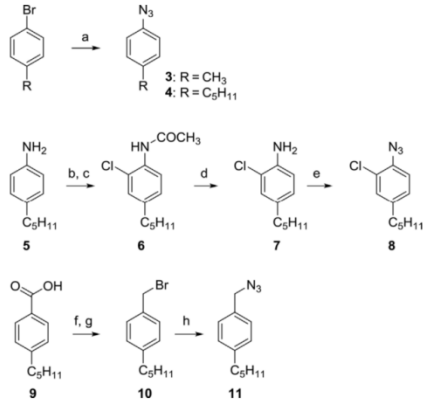
FIGURE 3 Impact of the alkyl chain length on interactions between the *N*,*N*-dialkylamino group and the catalytic aspartates. Hydrogen bonds and ionic interactions are indicated with yellow- and blue-dashed lines, respectively

TABLE 3 SAR of flap pocket substituent



Compound	R	n	IC_{50}, μM[a]
2e		2	0.6 ± 0.03
2q		1	>200
2r		2	5.2 ± 0.25
2s		2	0.6 ± 0.03

[a]Plm II inhibitory activity was determined by enzymatic FRET assay in triplicate experiments.

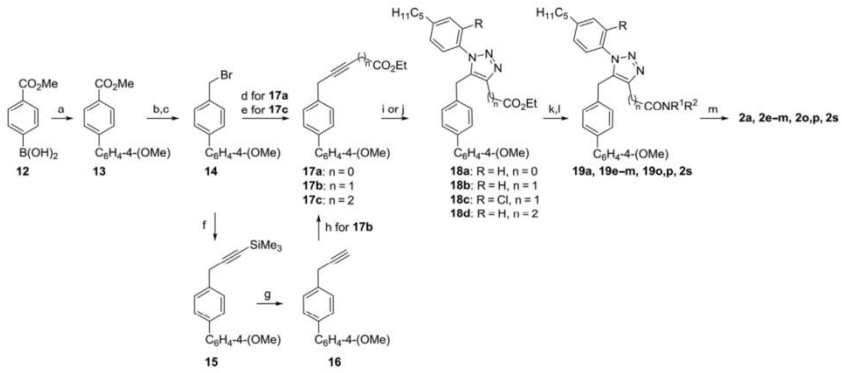


SCHEME 1 Synthesis of azides **3**, **4**, **8**, and **11**. Reagents and conditions: a) *n*-BuLi, THF, −78°C, 1 h, then Tos-N_3, 77%. b) CH_3COCl, NEt_3, CH_2Cl_2, 10°C, 2 h, 96%. c) $Pd(OAc)_2$, NCS, TsOH–H_2O, toluene, r.t., 16 h, 95%. d) Conc. hydrochloric acid, 100°C, 3 h, 94%. e) *t*-BuONO, TMS–N_3, CH_3CN, r.t., 40 min, 80%. f) $LiAlH_4$, THF, r.t., 16 h, 99%. g) PBr_3, CH_2Cl_2, r.t., 48 h, 95%. h) NaN_3, 1:4 H_2O/acetone, r.t., 16 h, 99%

treated with PBr_3 to yield benzyl bromide **14**. The latter was reacted with terminal alkynes in the presence of stoichiometric CuI[31] to form acetylenes **17a**,**c** (Scheme 2). The synthesis of **17b** required an initial alkylation of the *in situ* generated TMS-protected Mg-acetylide with **14** in the presence of sub-stoichiometric amounts of CuBr,[32] followed by cleavage of TMS group[33] in **15** and Cu(I)-catalyzed reaction of the formed acetylene **16** with ethyl diazoacetate (Scheme 2).[34] The latter three-step reaction sequence was also employed for the synthesis of alkyne **22** from *para*-bromobenzyl bromide (Scheme 3). Finally, substituted acetylene **21** was obtained by the acylation of the *in situ* formed Mg-acetylide (from **20**) with methyl chloroformate (Scheme 3). The Huisgen 1,3-dipolar cycloaddition between alkynes **17a–c**, **21**, **22** and azides **3**, **4**, **8**, **11** was accomplished using Cp*RuCl(COD) as the catalyst.[35] Propiolic acid derivatives such as **17a** and **21** ($n = 0$, see Schemes 2 and 3) afforded 1,2,3-triazoles **18a**, **23**, **25** as single regioisomers in the Ru-catalyzed cycloaddition reaction with azides **3** and **4**. In contrast, the cycloaddition involving alkynes **17b**,**c** (Scheme 2) and **22** (Scheme 3) resulted in the formation of the corresponding 1,2,3-triazoles as mixtures of regioisomers. The desired pure 1,2,3-triazoles **18b–d** (Scheme 2) and **24** (Scheme 3) were obtained after column chromatography. Elaboration of triazoles **18a–d** into target inhibitors **2a**,**e–m**,**o**,**p**,**s** was accomplished in three steps, and involved ester hydrolysis under basic conditions to acids **19a–d**, followed by the formation of amides and their reduction with the borane–THF complex (Scheme 2). Similar three-step approach was also employed for the conversion of triazole **28** (obtained from **24** in the Suzuki cross-coupling reaction with 4-methoxyphenylboronic acid) to target **2r** (Scheme 3). Inhibitor **2q** was synthesized using a slightly modified approach (Scheme 3). Accordingly, triazole **25** was converted into amide **26** prior to the Suzuki cross-coupling reaction with 4-methoxyphenylboronic acid. The subsequent reduction of the cross-coupling product **29** with borane–THF complex delivered target **2q**. The six-step synthesis of triazole **2n** from the cycloaddition product **23** started with the Suzuki cross-coupling to afford **27**, and followed with Kowalski ester homologation reaction[36] via the intermediate α,α-dibromoketone **30** to yield ester **31** (Scheme 3). The end game of the synthesis involved the reduction of the ester subunit in **31** to alcohol, its conversion into mesylate and alkylation with *N*-ethyl-*N*-benzylamine.[37]

The key step in the synthesis of isoxazole-derived inhibitor **2b** was Ru(II)-catalyzed cycloaddition between alkyne **21** (see Scheme 3) and *N*-hydroxybenzimidoyl chloride **34** (Scheme 4).[38] The latter was obtained from 1-bromo-4-*n*-pentylbenzene in a reaction sequence comprising metallation with *n*-BuLi and quench of the transient aryllithium with DMF, subsequent condensation of the formed aldehyde **33** with hydroxylamine and chlorination of the intermediate oxime with NCS.[39] Hydrolysis of the ester moiety in **35** and coupling of the intermediate carboxylic acid with the amine furnished amide **36**. The Suzuki cross-coupling reaction of **36** with 4-methoxyphenylboronic acid afforded biaryl **37**, which was reduced with borane–THF complex to yield the target isoxazole **2b**.

The synthesis of imidazole-based inhibitor **2c** was started from nitrile **38** which was subjected to the reaction of with 4-benzylamine in the presence of $AlMe_3$ to give amidine **39** (Scheme 5). The latter was



SCHEME 2 Synthesis of inhibitors **2a**, **2e–m**, **2o,p**, **2s**. Reagents and conditions: a) 4-MeO-C_6H_4-Br, $Pd(PPh_3)_4$ (0.3 mol%), aqueous 2 M Na_2CO_3, 1:1 *i*-PrOH/toluene, 90°C, 9 h, 74%. b) $LiAlH_4$, THF, r.t., 2 h, 99%. c) PBr_3, CH_2Cl_2, r.t., 16 h, 99%. d) HC≡C—CO_2Et, CuI (1.0 equiv.), K_2CO_3, TBA-I, MeCN, 40°C, 16 h, 80%. e) HC≡C—$(CH_2)_2$—CO_2Et, CuI (1.0 equiv.), K_2CO_3, TBA-I, MeCN, 40°C, 16 h, 53%. f) TMS-C≡CH, CuBr (0.6 equiv.), *i*-PrMgCl, THF, reflux, 3 h, 89%. g) $AgNO_3$, KCN, H_2O, EtOH, r.t., 2 h, 88%. h) N_2CHCO_2Et, CuI (5 mol%), MeCN, r.t., 16 h, 77%. i) **4**, Cp*RuCl(COD) (12 mol%), r.t., 16 h, 1,4-dioxane, 76% (**18a**); 28% (**18b**), and 32% (**18d**). j) **8**, Cp*RuCl(COD) (10 mol%), r.t., 16 h, 1,4-dioxane, 13% (**18c**). k) Aqueous 1 N NaOH, 1,4-dioxane, r.t., 16 h. l) Amine, EDC, HOBt, DMF, r.t., 16 h. m) BH_3—THF, THF, 80°C, 16 h, then 4 N HCl, 80°C, 3 h

treated with aldehyde **40** (prepared from 2-bromomalonaldehyde by the acid-catalyzed enol ether formation) to furnish 5-formylimidazole **41**.[40] Biaryl aldehyde **42** was prepared by the Pd-catalyzed Suzuki cross-coupling reaction of bromide **41** and 4-methoxyphenylboronic acid. Finally, aldehyde **42** was subjected to the reductive amination with diethylamine to furnish target **2c** (Scheme 5).

Inhibitor **2d** was obtained from ethyl pyrrole-2-carboxylate (Scheme 6). The synthesis began with the bromination of the heterocycle in position 2 and subsequent protection of the nitrogen with *N*-Boc moiety. The resulting bromide **44** was converted into pyrrole **45** in the Pd-catalyzed cross-coupling with boronate **43**, followed by the cleavage of *N*-Boc protecting group under acidic conditions. *N*-Benzylation of pyrrole anion (from **45** and NaH) was followed by the Suzuki cross-coupling reaction with 4-methoxyphenylboronic acid to afford ester **47**. Finally, the reduction of the ester subunit in **47** to alcohol, its conversion into mesylate and alkylation with diethylamine furnished target **2d**.

3 | CONCLUSION

In summary, we have developed a novel series of triazole-based non-peptidomimetic Plm inhibitors. This has been performed by bioisosteric substitution of amide moiety in the Actelion amino-piperazine inhibitors. The best triazole-based inhibitors show submicromolar potency toward Plm II, an activity, which is comparable to that of the parent Actelion compounds. The new inhibitors found by screening against Plm II as model protein have a potential to be optimized as potent inhibitors of non-digestive Plm IX or Plm X, which are essential for malaria parasite life cycle.

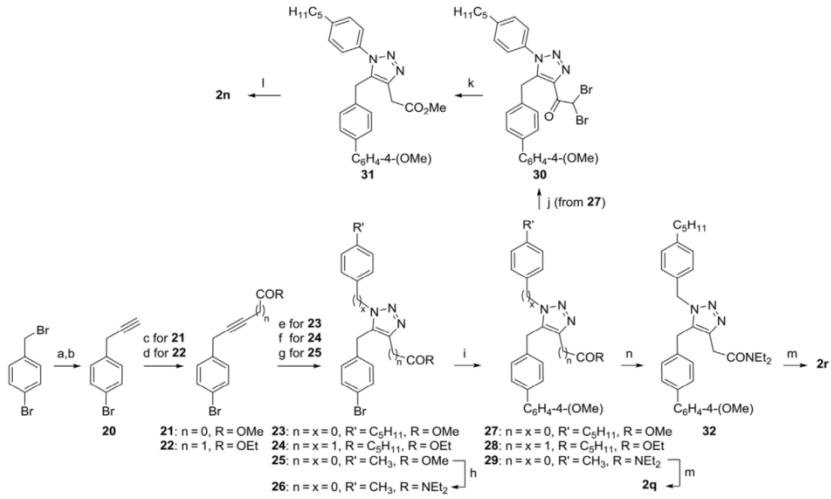
4 | EXPERIMENTAL

4.1 | Molecular docking

Crystal structure of Plm II in complex with an amino-piperidine-based ligand (Ref. [24], PDB ID 2BJU) was used for molecular docking. The protein was prepared using Maestro Protein Preparation Wizard (Schrödinger Release 2017-3: Maestro, Schrödinger, LLC, New York, NY, 2017) by adjusting side chain protonation states at pH 4.6 and by energy minimization allowing a heavy atom convergence up to 0.30 Å. Hydrogen bond network was optimized by choosing optimal orientations of hydroxyl groups, water molecules, and side chain amide groups of Asn and Gln and by selecting appropriate states and orientations of histidine imidazoles. Inhibitors were prepared for docking using the standard protocol implemented in LigPrep at pH 4.6.

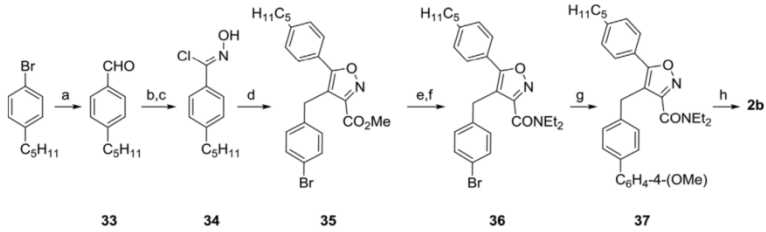
Docking of azole-based Plm II inhibitors was performed using the Induced Fit protocol in Maestro software. Protein and ligand van der Waals scaling was set to 0.50. After initial docking, up to 20 poses were promoted to structure refinement. Protein residues within 7.0 Å from the docked ligand were refined using OPLS3 force field,[41] and the three topmost structures after redocking were examined for each ligand. Results were visualized using PyMOL software (The PyMOL Molecular Graphics System, Version 1.7 Schrödinger, LLC).

The homology models of Plm IX and X were based on the crystal structure of Plm II with PDB ID 2BJU and were generated using

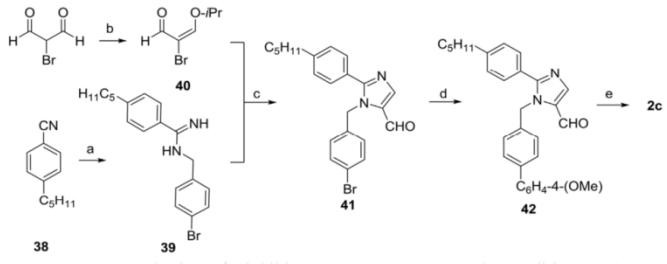


SCHEME 3 Synthesis of inhibitors **2n**, **2q**, and **2r**. Reagents and conditions: a) TMS-C≡CH, CuBr (0.6 equiv.), *i*-PrMgCl, THF, reflux, 3 h, 94%. b) $AgNO_3$, KCN, H_2O, EtOH, r.t., 2 h, 53%. c) MeMgBr, $ClCO_2Me$, THF, 65°C, 3 h, 86%. d) N_2CHCO_2Et, CuI, MeCN, r.t., 16 h, 82%. e) **4**, Cp*RuCl(COD) (10 mol%), r.t., 16 h, 1,4-dioxane, 80%. f) **11**, Cp*RuCl(COD) (10 mol%), 1,4-dioxane, r.t., 16 h, 13%. g) **3**, Cp*RuCl(COD) (10 mol %), 1,4-dioxane, r.t., 16 h, 65%. h) Aqueous 1 N NaOH, 1,4-dioxane, r.t., 16 h, then $HNEt_2$, EDC, HOBt, DMF, r.t., 16 h, then 50°C, 2 h, 64%. i) 4-MeO-C_6H_4-$B(OH)_2$, $Pd(PPh_3)_4$ (3 mol%), aqueous 2 M Na_2CO_3, 1:1 *i*-PrOH/toluene, 90°C, 16 h, 85% for (**27**), 80% (for **28**), 76% (for **29**). j) *n*-BuLi, TMP, CH_2Br_2, THF, −78°C, 20 min, 92%. k) LiHMDS, *n*-BuLi, THF, −78°C, 1 h, then AcCl, MeOH, 27%. l) $LiAlH_4$, THF, then Ms–Cl, NEt_3, CH_2Cl_2, then Bn(Et)NH hydrochloride, NEt_3, 1,4-dioxane, 48%. m) BH_3–THF, THF, 80°C, 16 h, then 4 N HCl, 85°C, 1 h, 76% (for **2q**), 65% (for **2r**). n) Aqueous 1 N NaOH, 1,4-dioxane, r.t., 16 h, then $HNEt_2$, EDC, HOBt, DMF, r.t., 16 h, 62%

SWISS-MODEL homology-modeling server.[42] The obtained homology models were prepared using the previously described protein preparation procedure, and then were subjected to molecular dynamics (MD) simulations to relax the system and to sample several protein conformations. Molecular systems for the MD simulations were prepared using the System builder tool in Maestro. Protein–ligand complexes were solvated in an orthogonal solvent box using SPC solvent model[43] and the system size was set to extend 10 Å beyond the protein in all directions. Na^+ and Cl^- ions were added to maintain physiological salinity (0.15 M) and to obtain a neutral total charge for the system. The complete systems were relaxed and equilibrated using the default Desmond relaxation protocol. All MD



SCHEME 4 Synthesis of inhibitor **2b**. Reagents and conditions: a) *n*-BuLi, DMF, THF, −78°C, 1 h, 99%. b) $NH_2OH^{\bullet}HCl$, $NaHCO_3$, H_2O, Et_2O, r.t., 85%. c) NCS, DMF, r.t., 30 min, 99%. d) **21**, Cp*RuCl(COD) (10 mol%), NEt_3, $(CH_2)_2Cl_2$, r.t., 16 h, 47%. e) Aqueous 1 N NaOH, 1,4-dioxane, r.t., 16 h, 99%. f) $HNEt_2$, EDC, HOBt, DMF, r.t., 16 h, then 50°C, 2 h, 54%. g) 4-MeO-C_6H_4-$B(OH)_2$, $Pd(PPh_3)_4$ (3 mol%), aqueous 2 M Na_2CO_3, 1:1 *i*-PrOH/toluene, 90°C, 16 h, 66%. h) BH_3–THF, THF, 80°C, 16 h, then 4 N HCl, 80°C, 1 h, 55%



SCHEME 5 Synthesis of inhibitor **2c**. Reagents and conditions: a) 4-bromobenzylamine hydrochloride, $AlMe_3$, toluene, 0°C, 0.5 h, then nitrile **38**, 80°C, 4 h, 32%. b) *i*-PrOH, *p*-toluenesulfonic acid, reflux, 3 h. c) K_2CO_3, $CHCl_3$, r.t., 24 h, 63%. d) 4-MeO-C_6H_4-$B(OH)_2$, $Pd(PPh_3)_4$ (10 mol%), aqueous 2 M K_2CO_3 1:1 THF, 90°C, 16 h, 74%. e) Et_2NH, $NaBH(OAc)_3$, AcOH, 4 Å MS, r.t., 24 h, 29%

simulations were performed for 50 ns at constant pressure (1.0 bar) maintained using a Martyna–Tobias–Klein barostat,[44,45] and at constant temperature (300 K) maintained using a Nose–Hoover thermostat.[46,47] Coulombic interaction cutoff was set to 9.0 Å. OPLS3 force field was used for all simulations. The pressure and temperature control used a relaxation time of 5.0 ps. All simulations used a RESPA integrator[48] with a 2.0 fs time step. Ten randomly chosen frames were selected for docking studies. Ligand preparation and docking protocols were identical to those used for docking into Plm II.

4.2 | Enzymatic assay

A fluorescence resonance energy transfer (FRET) assay was performed to evaluate ability of compounds to inhibit Plm II. K_m of the substrate was determined for the enzyme Plm II = 2 ± 0.2 μM. A solution of compounds for testing (concentration 0.01–100 μM) on 96-well plate was added to the enzyme in buffer (0.1 M NaOAc, pH = 4.5, 10% glycerol). The mixture was incubated for 30 min at 37°C. Substrate (DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS, AnaSpec Inc.) was then added to reach a final concentration of 5 μM. Hydrolysis of the substrate was detected as an increase in fluorescence (Em 490 nm, Ex 336 nm) at 37°C. The data points were collected every 1 min within 8–15 min. Compounds were tested in triplicate experiments. IC_{50} values were calculated using software Graph Pad Prism 5.0. Pepstatin A (IC_{50} = 0.42 ± 0.02 nM (Plm II) and compound **1a** were used as positive controls.

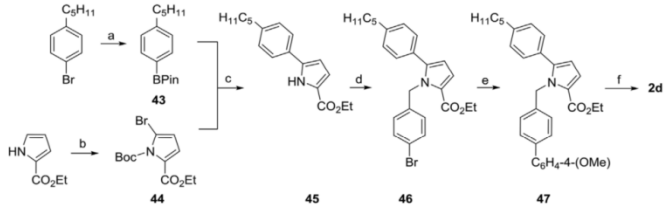
4.3 | Chemistry

4.3.1 | General

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.3.2 | General procedure A for the Suzuki cross-coupling

A pressure tube was charged with a bromide (1.0 equiv), a boronic acid (1.0 equiv), and $Pd(PPh_3)_4$ (0.3 or 3 mol%). A 1:1 (v/v) mixture of anhydrous *i*-PrOH/toluene (1.8 mL/mmol of the bromide) was added,



SCHEME 6 Synthesis of inhibitor **2d**. Reagents and conditions: a) $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (1 mol%), KOAc, 1,4-dioxane, 100°C, 16 h, 80%. b) NBS, THF, MeOH, 0°C, 5 h, 33%, then $(Boc)_2O$, NEt_3, DMAP, CH_2Cl_2, r.t., 18 h, 79%. c) $Pd(PPh_3)_4$ (10 mol%), aqueous 2 M Na_2CO_3, 1:1 *i*-PrOH/toluene, 90°C, 16 h, then 4 M HCl/dioxane, 50°C, 2 h, 37%. d) NaH (60% in mineral oil), 4-Br-C_6H_4-CH_2Br, DMF, r.t., 16 h, 67%. e) 4-MeO-C_6H_4-$B(OH)_2$, $Pd(PPh_3)_4$ (3 mol%), aqueous 2 M Na_2CO_3, 1:1 *i*-PrOH/toluene, 90°C, 16 h, 52%. f) $LiAlH_4$, THF, then Ms–Cl, NEt_3, CH_2Cl_2, then Et_2NH, r.t.; 16 h, 45%

followed by aqueous 2 M Na_2CO_3 solution (4.5 equiv). After stirring at 90°C for 9 h, the orange suspension was cooled to room temperature and the organic layer was decanted. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography.

4.3.3 | General procedure B for the Ru-catalyzed synthesis of azoles

An oven-dried flask was cooled under a stream of argon and charged with an alkyne (1.0 equiv), azide (1.1 or 1.2 equiv), Cp*RuCl(COD) (10 or 12 mol%), and anhydrous 1,4-dioxane (3.7 mL/mmol of the alkyne). The resulting dark brown solution was stirred at room temperature for 16 h. All volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography.

4.3.4 | General procedure C for the synthesis of amides

Corresponding carboxylic acid ester was dissolved in 1,4-dioxane (1 mL/0.1 mmol of the ester) and aqueous 1 N NaOH solution (0.6 mL/0.1 mmol of the ester) was added. After stirring at room temperature for 16 h, the solution was acidified with aqueous 1 N HCl solution to pH 1, diluted with water (10 mL), and extracted with EtOAc (3 × 10 mL). Combined organic extracts were washed with brine (15 mL), dried over Na_2SO_4, and evaporated under reduced pressure to afford carboxylic acid that was used in a subsequent step without purification.

The crude acid from above was dissolved in anhydrous DMF (0.7 mL/0.1 mmol of the ester) and HOBt (1.3 equiv) was added, followed by EDC (1.3 equiv). The resulting colorless solution was stirred at 0°C for 1 h, then amine (1.5 equiv or 3.0 equiv) was added and stirring at room temperature was continued for 16 h. The light yellow solution was diluted with water (10 mL) and extracted with EtOAc (3 × 10 mL). Combined extracts were washed with water (2 × 15 mL), brine (15 mL) and dried over Na_2SO_4. The residue was purified by silica gel column chromatography.

4.3.5 | General procedure D for the reduction of amides

An amide (1.0 equiv) was dissolved in anhydrous THF (1 mL) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol) was added under argon atmosphere. The resulting light yellow solution was stirred at 80°C for 16 h, whereupon aqueous 1 N HCl (1 mL) solution was added and stirring at 80°C was continued for 1 h. After cooling to room temperature, aqueous saturated $NaHCO_3$ solution was added to adjust pH to 8 (Caution! Intense gas evolution). The aqueous layer was extracted with EtOAc (3 × 10 mL), combined organic extracts were washed with brine (20 mL) and dried over Na_2SO_4. Evaporation under reduced pressure afforded residue, which was purified by silica gel column chromatography.

1-Azido-4-methylbenzene (3)

A solution of 1-bromo-4-methylbenzene (650 mg, 3.80 mmol, 1.0 equiv) in anhydrous THF (5 mL) was cooled to −78°C and *n*-BuLi (2.5 M solution in hexanes, 1.7 mL, 4.18 mmol, 1.1 equiv) was added dropwise. The resulting orange solution was stirred at −76°C for 1 h, whereupon a solution of *p*-toluenesulfonyl azide (1.12 g, 5.70 mmol) in anhydrous THF (2 mL) was added dropwise. The dark orange solution was warmed to −45°C, quenched with aqueous saturated NH_4Cl solution (10 mL) and water (10 mL), and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (40 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (24 g silica gel) using 2% EtOAc in petroleum ether to afford azide **3** as a brown oil (342 mg, 68% yield). ^{1}H NMR spectra was identical to that from the literature.[49]

1-Azido-4-pentylbenzene (4)

A solution of 1-bromo-4-pentylbenzene (2.00 g, 8.80 mmol, 1.0 equiv) in anhydrous THF (17 mL) was cooled to −78°C and *n*-BuLi (2.5M in hexanes, 3.9 mL, 9.69 mmol, 1.1 equiv) was added dropwise. The resulting orange solution was stirred at −76°C for 1 h, whereupon a solution of *p*-toluenesulfonyl azide (2.60 g, 13.20 mmol) in anhydrous THF (6 mL) was added dropwise. The dark orange solution was warmed to −45°C and quenched with aqueous saturated NH_4Cl solution (20 mL) and water (15 mL). The reaction mixture was extracted with EtOAc (3 × 35 mL), combined organic layers were washed with brine (70 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (70 g silica gel) using 2% EtOAc in petroleum ether to yield azide **4** as a dark orange oil (1.29 g, 77% yield). ^{1}H NMR spectra were identical to that from the literature.[50]

N-(2-Chloro-4-pentylphenyl)acetamide (6)

To a solution of aniline **5** (500 mg, 3.06 mmol, 1.0 equiv) and triethylamine (510 µL, 3.67 mmol, 1.2 equiv) in anhydrous CH_2Cl_2 (10 mL) was added dropwise acetyl chloride (261 µL, 3.67 mmol, 1.2 equiv). The resulting colorless solution was stirred at 10°C for 2 h and progress of the reaction was monitored by GC-MS analysis. Upon complete conversion of the starting amine **5**, the light yellow solution was washed with aqueous 1 N HCl solution (2 × 15 mL) and aqueous saturated $NaHCO_3$ solution (2 × 15 mL). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to afford crude *N*-acetyl aniline, which was used in the subsequent step without purification.

The *N*-acetyl aniline from above (578 mg, 2.82 mmol, 1 equiv) was dissolved in anhydrous toluene (10 mL) and *p*-toluenesulfonic acid monohydrate (268 mg, 1.41 mmol, 0.5 equiv) was added, followed by *N*-chlorosuccinimide (392 mg, 2.93 mmol, 1.04 equiv) and $Pd(OAc)_2$ (32 mg, 0.141 mmol, 0.05 equiv). After stirring at room temperature under air for 16 h, the solution was diluted with EtOAc (20 mL) and washed with aqueous 1 N HCl solution (20 mL), then with saturated $NaHCO_3$ solution (20 mL), water (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced

pressure to yield product **6** (643 mg, 95% yield) as a white solid material. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 8.21 (1H, d, *J* = 8.4 Hz), 7.52 (1H, s), 7.18 (1H, d, *J* = 1.9 Hz), 7.07 (1H, dd, *J* = 8.4, 1.9 Hz), 2.54 (2H, t, *J* = 7.7 Hz), 2.22 (3H, s), 1.58 (2H, quintet, *J* = 7.4 Hz), 1.42–1.23 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 168.2, 140.0, 132.3, 128.7, 127.8, 122.6, 121.8, 35.2, 31.4, 31.0, 24.9, 22.6, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{13}H_{19}NOCl$ $[M+H]^+$ 240.1155. Found 240.1160.

2-Chloro-4-pentylaniline (7)

The acetamide **6** (615 mg, 2.57 mmol, 1.0 equiv) was dissolved in concentrated aqueous hydrochloric acid (5 mL) and heated at 100°C for 3 h. The colorless solution was cooled to room temperature and pH was adjusted to 8 by careful addition of aqueous saturated $NaHCO_3$ solution (Caution! Intense gas evolution). The aqueous layer was extracted with EtOAc (3 × 25 mL). Combined organic layers were washed with water (50 mL) and brine (70 mL), dried over Na_2SO_4, and evaporated under reduced pressure to yield product **7** as a light yellow oil (480 mg, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.06 (1H, d, *J* = 2.0 Hz), 6.87 (1H, dd, *J* = 8.1, 2.0 Hz), 6.69 (1H, d, *J* = 8.1 Hz), 3.90 (2H, s), 2.47 (2H, t, *J* = 7.7 Hz), 1.60–1.47 (2H, m), 1.39–1.23 (4H, m), 0.88 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 140.6, 134.2, 129.2, 127.8, 119.3, 116.0, 34.9, 31.5, 31.4, 22.7, 14.2; HRMS-ESI (*m/z*) calcd. for $C_{11}H_{17}ClN$ $[M+H]^+$ 198.1050. Found 198.1048.

1-Azido-2-chloro-4-pentylbenzene (8)

t-BuONO (437 µL, 3.64 mmol, 1.5 equiv) was added dropwise to a cooled solution (0°C) of amine **7** (480 mg, 2.43 mmol, 1.0 equiv) in anhydrous MeCN (5 mL). After stirring at 0°C for 5 min, TMS-N_3 (384 µL, 2.92 mmol, 1.2 equiv) was added dropwise. After stirring for 40 min at room temperature, all volatiles were removed under reduced pressure and pure azide **8** (434 mg, 80% yield) was obtained as a light brown oil after column chromatography on silica gel (20 g silica gel) using 10% EtOAc in petroleum ether. IR (KBr, cm^{-1}) 2121 (N_3); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.21–7.18 (1H, m), 7.12–7.05 (2H, m), 2.55 (2H, t, *J* = 7.7 Hz), 1.59 (2H, quintet, *J* = 7.5 Hz), 1.40–1.24 (4H, m), 0.90 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 141.2, 134.5, 130.6, 128.1, 124.7, 119.6, 35.2, 31.4, 31.0, 22.6, 14.1.

1-(Bromomethyl)-4-pentylbenzene (10)

$LiAlH_4$ (1.0 M solution in THF, 10.1 mL, 10.14 mmol, 1.3 equiv) was added dropwise to a cooled solution (0°C, crushed ice) of benzoic acid **9** (1.50 g, 7.80 mmol, 1.0 equiv) in anhydrous THF (15 mL). After stirring at room temperature for 16 h, the white suspension was cooled to 0°C and quenched by sequential (within intervals of 10 min) addition of water (0.38 mL), aqueous 4 M NaOH solution (0.76 mL) and more water (1.1 mL). Ten minutes after addition of the final amount of water, the white suspension was filtered. The filter cake was washed with EtOAc (80 mL). The filtrate was evaporated to dryness to yield 1.38 g (99% yield) of (4-pentylphenyl)methanol as a white solid material, which was used in subsequent step without purification. To a solution of (4-pentylphenyl)methanol (1.38 g, 7.80 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (10 mL) was dropwise added a solution of PBr_3 (1.4 mL, 14.8 mmol, 1.9 equiv) in anhydrous CH_2Cl_2 (5 mL). After stirring at room temperature for 48 h, water (15 mL) was added to the light yellow solution and stirring was continued for 20 min. The organic layer was washed with water (2 × 40 mL), dried over Na_2SO_4, and evaporated under reduced pressure to yield bromide **10** (1.79 g, 95% yield) as a light yellow solid material. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.33–7.28 (2H, m), 7.18–7.13 (2H, m), 4.50 (2H, s), 2.59 (2H, t, *J* = 7.7 Hz), 1.61 (2H, quintet, *J* = 7.5 Hz), 1.41–1.24 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz).

1-(Azidomethyl)-4-pentylbenzene (11)

Sodium azide (724 mg, 11.1 mmol, 1.5 equiv) was added to a solution of bromide **10** (1.79 g, 7.42 mmol, 1.0 equiv) in a 1:4 (v/v) mixture of H_2O/Me_2CO (15 mL). After 16 h of stirring at room temperature, the light yellow solution was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give azide **11** (1.50 g, 99% yield) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.25–7.17 (4H, m), 4.30 (2H, s), 2.61 (2H, t, *J* = 7.8 Hz), 1.62 (2H, quintet, *J* = 7.4 Hz), 1.40–1.28 (4H, m), 0.89 (3H, t, *J* = 7.0 Hz).

Methyl 4′-methoxy-[1,1′-biphenyl]-4-carboxylate (13)

A pressure tube (200 mL) was charged with boronic acid **12** (2.00 g, 11.1 mmol, 1.0 equiv), 4-bromoanisole (2.08 g, 11.1 mmol, 1.0 equiv), $Pd(PPh_3)_4$ (40 mg, 0.035 mmol, 0.3 mol%), anhydrous *i*-PrOH/toluene = 1:1 (v/v) (20 mL), and aqueous 2 M Na_2CO_3 solution (25 mL). After stirring at 90°C for 9 h, the resulting orange suspension was cooled to room temperature and layers were separated. Aqueous layer was extracted with CH_2Cl_2 (4 × 30 mL), combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (100 g silica gel) using CH_2Cl_2 to yield biphenyl derivative **13** (2.00 g, 74% yield) as a white solid material. ^{1}H NMR spectra was identical to that from the literature.[51]

(Bromomethyl)-4′-methoxy-1,1′-biphenyl (14)

$LiAlH_4$ (2.4 M solution in THF, 4.5 mL, 10.7 mmol, 1.3 equiv) was added dropwise to a cooled solution (0°C, crushed ice) of biphenyl derivative **13** (2.00 g, 8.25 mmol, 1.0 equiv) in anhydrous THF (20 mL). After stirring at room temperature for 2 h, the white suspension was cooled to 0°C and quenched by sequential (within intervals of 10 min) addition of water (0.41 mL), aqueous 4 M NaOH solution (0.82 mL) and more water (1.23 mL). Ten minutes after addition of the final amount of water, the white suspension was filtered. The filter cake was washed with EtOAc (80 mL). The filtrate was evaporated to dryness to yield 1.76 g (99% yield) of (4′-methoxy-[1,1′-biphenyl]-4-yl)methanol as a white solid material, which was used in subsequent step without purification.

To a solution of (4′-methoxy-[1,1′-biphenyl]-4-yl)methanol from above (1.76 g, 8.21 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (25 mL) was added dropwise a solution of PBr_3 (1.5 mL, 15.6 mmol, 1.9 equiv) in anhydrous CH_2Cl_2 (5 mL). After stirring at room temperature for 16 h, water (20 mL) was added to the light yellow solution and stirring was

continued for 15 min. The organic layer was washed with water (2 × 40 mL), dried over Na_2SO_4, and evaporated under reduced pressure to yield benzylbromide **14** (2.26 g, 99% yield) as a light yellow solid material. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.55–7.51 (4H, m), 7.47–7.43 (2H, m), 7.04–6.94 (2H, m), 4.55 (2H, s), 3.86 (3H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.5, 141.1, 136.2, 133.1, 129.6, 128.3, 127.2, 114.4, 55.5, 33.7.

(3-(4′-Methoxy-[1,1′-biphenyl]-4-yl)prop-1-yn-1-yl)-trimethylsilane (15)

To a cooled solution of trimethylsilyl acetylene (2.0 mL, 14.4 mmol, 4.0 equiv) in anhydrous THF (10 mL) was added dropwise *i*-PrMgCl (2.0 M solution in THF, 5.4 mL, 10.8 mmol, 3.0 equiv). The light brown solution was stirred at 0°C for 30 min, and then at room temperature for 1 h, whereupon CuBr (0.31 g, 2.16 mmol, 0.6 equiv) was added to the light brown solution and stirring at ambient temperature was continued for 30 min. A solution of benzylbromide **14** (1.00 g, 3.60 mmol, 1.0 equiv) in anhydrous THF (5 mL) was added to the brown suspension, which was then heated under reflux for 3 h. After cooling to room temperature, all volatiles were removed *in vacuo*. Water (20 mL) and EtOAc (20 mL) were added to the residue, orange precipitate was removed by filtration, and filtrate was extracted with EtOAc (2 × 30 mL). Combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (50 g silica gel) using 5% EtOAc in petroleum ether to give product **15** (0.94 g, 89% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.54–7.49 (4H, m), 7.42–7.37 (2H, m), 7.01–6.95 (2H, m), 3.85 (3H, s), 3.69 (2H, s), 0.20 (9H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.2, 139.3, 134.9, 133.6, 128.4, 128.2, 126.9, 114.3, 104.4, 87.0, 55.5, 26.0, 0.3.

4-Methoxy-4′-(prop-2-yn-1-yl)-1,1′-biphenyl (16)

To a solution of propynylsilane **15** (333 mg, 1.13 mmol, 1.0 equiv) in EtOH (5.9 mL) was added dropwise a solution of $AgNO_3$ (288 mg, 1.70 mmol, 1.5 equiv) in a 1:3 mixture of H_2O and EtOH (6 mL). After stirring at room temperature for 30 min to the light yellow suspension was added a solution of KCN (729 mg, 11.19 mmol, 9.9 equiv) in water (3 mL) and stirring was continued for 2 h. The resulting light yellow solution was partitioned between EtOAc (20 mL) and water (20 mL). Organic layer was washed with water (2 × 20 mL), brine (20 mL), dried over Na_2SO_4, and evaporated under reduced pressure to give product **16** (224 mg, 88% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.55–7.49 (4H, m), 7.43–7.38 (2H, m), 7.00–6.95 (2H, m), 3.85 (3H, s), 3.66–3.63 (2H, m), 2.21 (1H, t, J = 2.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.3, 139.5, 134.6, 133.5, 128.4, 128.2, 127.0, 114.3, 82.1, 70.6, 55.5, 24.6.

Ethyl 4-(4′-methoxy-[1,1′-biphenyl]-4-yl)but-2-ynoate (17a)

An oven-dried pressure tube (200 mL) was cooled under stream of argon and charged with bromide **14** (1.78 g, 6.42 mmol, 1.0 equiv), K_2CO_3 (0.887 g, 6.42 mmol, 1.0 equiv), CuI (1.22 g, 6.42 mmol, 1.0 equiv), tetrabutylammonium iodide (2.37 g, 6.42 mmol, 1.0 equiv), ethyl propiolate (1.3 mL, 12.84 mmol, 2.0 equiv), and anhydrous MeCN (25 mL). After stirring at 40°C for 24 h, the suspension was cooled to room temperature, diluted with aqueous saturated NH_4Cl solution (35 mL), and extracted with diethyl ether (3 × 40 mL). Combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (70 g silica gel) using gradient elution from 12.5% EtOAc in petroleum ether to 50% EtOAc in petroleum ether to give product **17a** (1.52 g, 80% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.57–7.48 (4H, m), 7.42–7.33 (2H, m), 7.02–6.94 (2H, m), 4.24 (2H, q, J = 7.2 Hz), 3.85 (3H, s), 3.77 (2H, s), 1.32 (3H, t, J = 7.2 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.4, 153.9, 140.0, 133.3, 132.6, 128.6, 128.2, 127.2, 114.4, 86.4, 75.0, 62.1, 55.5, 24.8, 14.2.

Ethyl 5-(4′-methoxy-[1,1′-biphenyl]-4-yl)pent-3-ynoate (17b)

Ethyl diazoacetate (286 µL, 2.72 mmol, 1.1 equiv) and CuI (24 mg, 0.12 mmol, 0.05 equiv) were added to a solution of alkyne **16** (550 mg, 2.47 mmol, 1.0 equiv) in anhydrous MeCN (10 mL). After stirring at room temperature for 16 h, all volatiles were removed under reduced pressure and the residue was purified by column chromatography on silica gel (70 g silica gel) using gradient elution from 1% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2. Product **17b** (627 mg, 77% yield) was obtained as a light green oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.55–7.47 (4H, m), 7.44–7.38 (2H, m), 7.00–6.95 (2H, m), 4.24–4.18 (2H, m), 3.85 (3H, s), 3.66 (2H, t, J = 2.6 Hz), 3.33 (2H, t, J = 2.6 Hz), 1.30 (3H, t, J = 7.1 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 168.9, 159.2, 139.3, 135.3, 133.6, 128.4, 128.2, 126.9, 114.3, 81.4, 74.1, 61.7, 55.5, 26.4, 24.9, 14.3.

Ethyl 6-(4′-methoxy-[1,1′-biphenyl]-4-yl)hex-4-ynoate (17c)

An oven-dried pressure tube (100 mL) was cooled under stream of argon and charged with bromide **14** (1.22 g, 4.40 mmol, 1.0 equiv), K_2CO_3 (0.669 g, 4.84 mmol, 1.1 equiv), CuI (0.838 g, 4.40 mmol, 1.0 equiv), tetrabutylammonium iodide (1.63 g, 4.40 mmol, 1 equiv), ethyl pent-4-ynoate (0.61 g, 4.84 mmol, 1.1 equiv), and anhydrous MeCN (15 mL). After stirring at 40°C for 16 h, the suspension was cooled to room temperature, diluted with aqueous saturated NH_4Cl solution (30 mL), and extracted with EtOAc (3 × 30 mL). Combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (70 g silica gel) using gradient elution from 1% EtOAc in petroleum ether to 15% EtOAc in petroleum ether to give product **17c** (0.749 g, 53% yield) as a light yellow solid material. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.53–7.47 (4H, m), 7.40–7.34 (2H, m), 7.00–6.94 (2H, m), 4.16 (2H, q, J = 7.2 Hz), 3.85 (3H, s), 3.59 (2H, s), 2.62–2.50 (4H, m), 1.26 (3H, t, J = 7.2 Hz).

Ethyl 5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazole-4-carboxylate (18a)

The title compound was obtained as a light brown oil (1.00 g, 76% yield) from alkyne **17a** (0.800 g, 2.72 mmol, 1.0 equiv), azide **4** (0.566 g, 2.99 mmol, 1.1 equiv), and Cp*RuCl(COD) (124 mg, 0.326 mmol, 12 mol%) by following general procedure B. Product **18a** was purified by column chromatography on silica gel (100 g silica gel) using gradient

elution from 10% EtOAc in hexanes to 90% EtOAc in hexanes. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 7.54–7.48 (2H, m), 7.44–7.39 (2H, m), 7.39–7.35 (4H, m), 7.00–6.95 (2H, m), 6.90–6.86 (2H, m), 4.39 (2H, s), 4.35 (2H, q, *J* = 7.1 Hz), 3.77 (3H, s), 2.66 (2H, t, *J* = 7.6 Hz), 1.60 (2H, quintet, *J* = 7.3 Hz), 1.39–1.20 (7H, m), 0.85 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ 160.9, 158.9, 145.0, 141.2, 138.1, 136.0, 134.6, 132.8, 131.9, 129.4, 128.3, 127.5, 126.2, 125.7, 114.3, 60.6, 55.2, 34.6, 30.7, 30.4, 28.0, 21.9, 14.1, 13.9.

Ethyl 2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)acetate (18b)

The title compound was obtained as a light brown oil (0.469 g, 13% yield) from alkyne **17b** (1.085 g, 3.52 mmol, 1.0 equiv), azide **4** (0.799 g, 4.22 mmol, 1.2 equiv), and Cp*RuCl(COD) (160 mg, 0.420 mmol, 12 mol%) by following general procedure B. Product **18b** was purified by column chromatography on silica gel (150 g silica gel) using gradient elution from 10% EtOAc in hexanes to 50% EtOAc in hexanes. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.46 (2H, m), 7.44–7.40 (2H, m), 7.27–7.25 (4H, m), 7.03–6.93 (4H, m), 4.14 (2H, q, *J* = 7.1 Hz), 4.10 (2H, s), 3.85 (3H, s), 3.71 (2H, s), 2.66 (2H, t, *J* = 7.7 Hz), 1.63 (2H, quintet, *J* = 7.6 Hz), 1.38–1.29 (4H, m), 1.25 (3H, t, *J* = 7.1 Hz), 0.90 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 170.2, 159.4, 144.9, 139.5, 139.1, 134.8, 134.3, 134.1, 133.1, 129.4, 128.6, 128.1, 127.0, 125.5, 114.4, 61.3, 55.5, 35.7, 31.9, 31.5, 31.1, 28.7, 22.6, 14.3, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{31}H_{36}N_3O_3$ $[M+H]^+$ 498.2757. Found 498.2756.

Ethyl 2-(1-(2-chloro-4-pentylphenyl)-5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)acetate (18c)

The title compound was obtained as a brown oil (100 mg, 13% yield) from alkyne **17b** (433 mg, 1.40 mmol, 1.0 equiv), azide **8** (314 mg, 1.40 mmol, 1.0 equiv), and Cp*RuCl(COD) (53 mg, 0.14 mmol, 10 mol %) by following general procedure B. Product **18c** was purified by column chromatography on silica gel (50 g silica gel) using gradient elution from 10% EtOAc in hexanes to 50% EtOAc in hexanes. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.48–7.43 (2H, m), 7.37–7.32 (3H, m), 7.13–7.05 (2H, m), 7.00–6.91 (4H, m), 4.20–4.12 (2H, m), 3.95 (2H, s), 3.84 (3H, s), 3.72 (2H, s), 2.63 (2H, t, *J* = 7.5 Hz), 1.69–1.56 (2H, m), 1.41–1.28 (4H, m), 1.27–1.22 (3H, m), 0.93–0.87 (3H, m); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 170.0, 159.2, 147.2, 139.3, 138.2, 135.9, 134.1, 133.0, 131.5, 131.4, 130.0, 129.1, 128.8, 127.9, 127.6, 126.6, 114.2, 61.1, 55.3, 35.4, 31.7, 31.2, 30.7, 28.7, 22.4, 14.1, 14.0; HRMS-ESI (*m/z*) calcd. for $C_{31}H_{35}ClN_3O_3$ $[M+H]^+$ 532.2367. Found 532.2366.

Ethyl 3-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)propanoate (18d)

The title compound was obtained as a white solid material (0.374 g, 32% yield) from alkyne **17c** (0.749 g, 2.32 mmol, 1.0 equiv), azide **4** (0.484 g, 2.56 mmol, 1.1 equiv), and Cp*RuCl(COD) (88 mg, 0.232 mmol, 10 mol%) by following general procedure B. Crude **18d** was purified by column chromatography on silica gel (100 g silica gel) using gradient elution from 1% EtOAc in hexanes to 50% EtOAc in hexanes to afford triazole **18d** as a mixture of isomers. The desired pure isomer **18d** was obtained by preparative HPLC (C18-silica) using 85% MeCN in aqueous 0.1% formic acid as a mobile phase. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.45 (2H, m), 7.44–7.39 (2H, m), 7.26–7.19 (4H, m), 6.99–6.93 (4H, m), 4.13 (2H, q, *J* = 7.1 Hz), 4.08 (2H, s), 3.84 (3H, s), 3.03–2.96 (2H, m), 2.87–2.79 (2H, m), 2.65 (2H, t, *J* = 7.7 Hz), 1.62 (2H, quintet, *J* = 7.4 Hz), 1.39–1.27 (4H, m), 1.24 (3H, t, *J* = 7.1 Hz), 0.89 (3H, t, *J* = 7.0 Hz); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 173.1, 159.3, 144.8, 144.3, 139.5, 135.4, 134.2, 133.1, 132.7, 129.4, 128.4, 128.1, 127.1, 125.4, 114.4, 60.6, 55.5, 35.7, 33.4, 31.5, 31.1, 28.3, 22.6, 20.6, 14.4, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{32}H_{38}N_3O_3$ $[M+H]^+$ 512.2913. Found 512.2916.

N,*N*-Diethyl-5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazole-4-carboxamide (19a)

The title compound was obtained from carboxylic acid ester **18a** (100 mg, 0.21 mmol, 1.0 equiv), HOBt (36 mg, 0.27 mmol, 1.3 equiv), EDC (52 mg, 0.27 mmol, 1.3 equiv), and diethylamine (33 µL, 0.31 mmol, 1.5 equiv) by following general procedure C. Purification by column chromatography on silica gel (10 g silica gel) using gradient elution from 10% EtOAc in hexanes to 100% EtOAc afforded the desired **19a** as a light yellow oil (77 mg, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.48–7.42 (2H, m), 7.37–7.32 (2H, m), 7.31–7.27 (2H, m), 7.24–7.19 (2H, m), 7.00–6.96 (2H, m), 6.96–6.92 (2H, m), 4.32 (2H, s), 3.84 (3H, s), 3.77 (2H, q, *J* = 7.0 Hz), 3.55 (2H, q, *J* = 7.0 Hz), 2.68 (2H, t, *J* = 7.7 Hz), 1.66 (2H, quintet, *J* = 7.4 Hz), 1.42–1.25 (7H, m), 1.21 (3H, t, *J* = 7.0 Hz), 0.91 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 162.2, 159.3, 145.4, 140.8, 139.9, 139.2, 135.7, 133.6, 133.4, 129.5, 128.9, 128.1, 126.8, 125.9, 114.3, 55.5, 43.6, 40.7, 31.5, 31.1, 28.9, 22.6, 14.8, 14.2, 13.0; HRMS-ESI (*m/z*) calcd. for $C_{32}H_{39}N_4O_2$ $[M+H]^+$ 511.3073. Found 511.3076.

N,*N*-Diethyl-2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)acetamide (19e)

The title compound was obtained from carboxylic acid ester **18b** (50 mg, 0.10 mmol, 1.0 equiv), HOBt (18 mg, 0.13 mmol, 1.3 equiv), EDC (25 mg, 0.13 mmol, 1.3 equiv), and diethylamine (16 µL, 0.15 mmol, 1.5 equiv) by following general procedure C. Purification by column chromatography on silica gel (10 g silica gel) using gradient elution from 20% EtOAc in hexanes to 100% EtOAc afforded the target **19e** as a light yellow oil (33 mg, 62% yield). ^{1}H NMR (400 MHz, THF-d_8, ppm) δ 7.52–7.44 (2H, m), 7.44–7.36 (2H, m), 7.32–7.27 (4H, m), 7.06–6.98 (2H, m), 6.97–6.90 (2H, m), 4.18 (2H, s), 3.79 (3H, s), 3.70 (2H, s), 3.48 (2H, q, *J* = 7.1 Hz), 3.35 (2H, q, *J* = 7.1 Hz), 2.67 (2H, t, *J* = 7.7 Hz), 1.66 (2H, quintet, *J* = 7.6 Hz), 1.43–1.28 (4H, m), 1.13 (3H, t, *J* = 7.1 Hz), 1.08 (3H, t, *J* = 7.1 Hz), 0.91 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, THF-d_8, ppm) δ 169.2, 160.6, 145.2, 141.3, 140.1, 136.8, 136.0, 135.1, 134.0, 130.1, 128.6, 127.4, 126.4, 115.1, 55.6, 43.3, 41.2, 36.4, 32.5, 32.2, 31.6, 29.1, 23.6, 14.8, 14.7, 14.5, 13.6; HRMS-ESI (*m/z*) calcd. for $C_{33}H_{41}N_4O_2$ $[M+H]^+$ 525.3230. Found 525.3234.

N,*N*-Diethyl-3-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)propanamide (19f)

The title compound was obtained from carboxylic acid ester **18d** (72 mg, 0.14 mmol, 1.0 equiv), HOBt (24 mg, 0.18 mmol, 1.3 equiv), EDC (35 mg, 0.18 mmol, 1.3 equiv), and diethylamine (23 µL, 0.22 mmol, 1.5 equiv) as a light yellow oil (64 mg, 85% yield) by following general procedure C. Product **19f** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 30% EtOAc in hexanes to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.49–7.44 (2H, m), 7.42–7.37 (2H, m), 7.25–7.18 (4H, m), 6.99–6.92 (4H, m), 4.10 (2H, s), 3.84 (3H, s), 3.36 (2H, q, *J* = 7.1 Hz), 3.30 (2H, q, *J* = 7.1 Hz), 3.09–3.02 (2H, m), 2.86–2.80 (2H, m), 2.65 (2H, t, *J* = 7.6 Hz), 1.62 (2H, quintet, *J* = 7.5 Hz), 1.40–1.27 (4H, m), 1.14 (3H, t, *J* = 7.1 Hz), 1.08 (3H, t, *J* = 7.1 Hz), 0.89 (3H, t, *J* = 6.9 Hz).

(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)(pyrrolidin-1-yl)methanone (19g)

The title compound was obtained from carboxylic acid ester **18a** (100 mg, 0.21 mmol, 1.0 equiv), HOBt (36 mg, 0.27 mmol, 1.3 equiv), EDC (52 mg, 0.27 mmol, 1.3 equiv), and pyrrolidine (25 µL, 0.31 mmol, 1.5 equiv) as a light yellow oil (82 mg, 77% yield) by following general procedure C. Product **19g** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 20% EtOAc in hexanes to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.48–7.42 (2H, m), 7.37–7.32 (2H, m), 7.29–7.24 (2H, m), 7.20–7.16 (2H, m), 7.00–6.96 (2H, m), 6.96–6.92 (2H, m), 4.43 (2H, s), 4.11 (2H, t, *J* = 6.9 Hz), 3.84 (3H, s), 3.70 (2H, t, *J* = 6.9 Hz), 2.68 (2H, t, *J* = 7.7 Hz), 2.03–1.90 (4H, m), 1.65 (2H, quintet, *J* = 7.5 Hz), 1.40–1.29 (4H, m), 0.91 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 161.0, 159.2, 145.4, 140.7, 140.3, 139.2, 135.7, 133.6, 133.4, 129.5, 129.0, 128.1, 126.8, 125.9, 114.3, 55.5, 49.2, 46.9, 35.7, 31.5, 31.1, 28.9, 26.8, 24.1, 22.6, 14.2; HRMS-ESI (*m*/*z*) calcd. for $C_{32}H_{37}N_4O_2$ $[M+H]^+$ 509.2917. Found 509.2910.

2-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)-1-(pyrrolidin-1-yl)ethan-1-one (19h)

The title compound was obtained from carboxylic acid ester **18b** (50 mg, 0.10 mmol, 1.0 equiv), HOBt (18 mg, 0.13 mmol, 1.3 equiv), EDC (25 mg, 0.13 mmol, 1.3 equiv), and pyrrolidine (13 µL, 0.15 mmol, 1.5 equiv) as a light yellow oil (40 mg, 75% yield) by following general procedure C. Product **19h** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 10% EtOAc in hexanes to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.44 (2H, m), 7.43–7.37 (2H, m), 7.28–7.22 (4H, m), 7.03–6.98 (2H, m), 6.98–6.94 (2H, m), 4.20 (2H, s), 3.84 (3H, s), 3.68 (2H, s), 3.56 (2H, t, *J* = 6.8 Hz), 3.43 (2H, t, *J* = 6.8 Hz), 2.65 (2H, t, *J* = 7.7 Hz), 1.97–1.88 (2H, m), 1.87–1.79 (2H, m), 1.63 (2H, quintet, *J* = 7.5 Hz), 1.40–1.28 (4H, m), 0.89 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 167.8, 159.3, 144.8, 140.1, 139.3, 135.3, 134.6, 134.2, 133.2, 129.4, 128.8, 128.0, 126.9, 125.5, 114.4, 55.5, 47.2, 46.0, 35.7, 32.8, 31.5, 31.1, 28.6, 26.2, 24.5, 22.6, 14.1; HRMS-ESI (*m*/*z*) calcd. for $C_{33}H_{39}N_4O_2$ $[M+H]^+$ 523.3073. Found 523.3076.

3-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)-1-(pyrrolidin-1-yl)propan-1-one (19i)

The title compound was obtained as a light yellow oil (64 mg, 85% yield) from carboxylic acid ester **18d** (72 mg, 0.14 mmol, 1.0 equiv), HOBt (24 mg, 0.18 mmol, 1.3 equiv), EDC (35 mg, 0.18 mmol, 1.3 equiv), and pyrrolidine (18 µL, 0.22 mmol, 1.5 equiv) by following general procedure C. Product **19i** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 10% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.44 (2H, m), 7.42–7.37 (2H, m), 7.26–7.19 (4H, m), 7.01–6.92 (4H, m), 4.12 (2H, s), 3.84 (3H, s), 3.47–3.36 (4H, m), 3.10–3.00 (2H, m), 2.78 (2H, t, *J* = 7.4 Hz), 2.65 (2H, t, *J* = 7.7 Hz), 1.96–1.87 (2H, m), 1.86–1.78 (2H, m), 1.67–1.58 (2H, m), 1.39–1.26 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz).

(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)(4-methylpiperazin-1-yl)-methanone (19j)

The title compound was obtained as a light yellow oil (65 mg, 58% yield) from carboxylic acid ester **18a** (100 mg, 0.21 mmol, 1.0 equiv), HOBt (36 mg, 0.27 mmol, 1.3 equiv), EDC (52 mg, 0.27 mmol, 1.3 equiv), and 1-methylpiperazine (34 µL, 0.31 mmol, 1.5 equiv) by following general procedure C. Product **19j** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.48–7.43 (2H, m), 7.39–7.33 (2H, m), 7.31–7.27 (2H, m), 7.23–7.18 (2H, m), 7.01–6.92 (4H, m), 4.31 (2H, s), 4.10–4.04 (2H, m), 3.84–3.79 (5H, m), 2.74–2.63 (2H, m), 2.46 (2H, t, *J* = 4.8 Hz), 2.43 (2H, t, *J* = 4.8 Hz), 2.30 (3H, s), 1.65 (2H, quintet, *J* = 7.5 Hz), 1.41–1.29 (4H, m), 0.91 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, CD_3OD-d_4, ppm) δ 163.2, 160.8, 147.1, 141.3, 140.8, 140.7, 136.2, 134.6, 134.1, 130.7, 130.2, 128.8, 127.6, 127.0, 115.3, 56.1, 55.7, 55.4, 45.9, 42.9, 36.5, 32.5, 32.2, 29.5, 23.6, 14.4; HRMS-ESI (*m*/*z*) calcd. for $C_{33}H_{40}N_5O_2$ $[M+H]^+$ 538.3182. Found 538.3185.

2-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)-1-(4-methylpiperazin-1-yl)-ethan-1-one (19k)

The title compound was obtained as a light yellow oil (65 mg, 56% yield) from carboxylic acid ester **18b** (104 mg, 0.21 mmol, 1.0 equiv), HOBt (37 mg, 0.27 mmol, 1.3 equiv), EDC (52 mg, 0.27 mmol, 1.3 equiv), and 1-methylpiperazine (35 µL, 0.32 mmol, 1.5 equiv) by following general procedure C. Product **19k** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.44 (2H, m), 7.43–7.37 (2H, m), 7.27–7.23 (4H, m), 7.02–6.93 (4H, m), 4.16 (2H, s), 3.84 (3H, s), 3.74 (2H, s), 3.66 (2H, t, *J* = 5.2 Hz), 3.61 (2H, t, *J* = 5.2 Hz), 2.65 (2H, t, *J* = 7.7 Hz), 2.36 (4H, q, *J* = 5.2 Hz), 2.28 (3H, s), 1.63 (2H, quintet, *J* = 7.5 Hz), 1.40–1.24 (4H,

m), 0.89 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 167.8, 159.3, 144.9, 140.0, 139.4, 135.1, 134.7, 134.2, 133.1, 129.5, 128.8, 128.1, 126.9, 125.5, 114.4, 55.5, 55.2, 54.7, 46.2, 46.1, 41.9, 35.7, 31.5, 31.5, 31.1, 28.6, 22.6, 14.2; HRMS-ESI (*m/z*) calcd. for $C_{34}H_{42}N_5O_2$ $[M+H]^+$ 552.3339. Found 552.3344.

3-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)-1-(4-methylpiperazin-1-yl)propan-1-one (19l)

The title compound was obtained as a light yellow oil (62 mg, 78% yield) from carboxylic acid ester **18d** (72 mg, 0.14 mmol, 1.0 equiv), HOBt (24 mg, 0.18 mmol, 1.3 equiv), EDC (35 mg, 0.18 mmol, 1.3 equiv), and 1-methylpiperazine (24 µL, 0.22 mmol, 1.5 equiv) by following general procedure C. Product **19l** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.49–7.45 (2H, m), 7.43–7.38 (2H, m), 7.25–7.20 (4H, m), 6.99–6.93 (4H, m), 4.09 (2H, s), 3.84 (3H, s), 3.64 (2H, t, *J* = 4.9 Hz), 3.53–3.47 (2H, m), 3.03 (2H, dd, *J* = 8.5, 6.4 Hz), 2.84 (2H, dd, *J* = 8.5, 6.4 Hz), 2.65 (2H, t, *J* = 7.7 Hz), 2.37 (4H, t, *J* = 4.9 Hz), 2.30 (3H, s), 1.63 (2H, quintet, *J* = 7.6 Hz), 1.39–1.28 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 170.8, 159.4, 145.0, 144.4, 139.4, 135.4, 134.1, 133.0, 133.0, 129.5, 128.5, 128.0, 127.0, 125.4, 114.4, 68.6, 55.5, 53.9, 44.5, 35.7, 31.5, 31.1, 28.4, 27.9, 22.6, 20.7, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{35}H_{44}N_5O_2$ $[M+H]^+$ 566.3495. Found 566.3491.

2-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)-1-morpholinoethan-1-one (19m)

The title compound was obtained as a white solid material (82 mg, 85% yield) from carboxylic acid ester **18b** (90 mg, 0.18 mmol, 1.0 equiv), HOBt (32 mg, 0.23 mmol, 1.3 equiv), EDC (44 mg, 0.23 mmol, 1.3 equiv), and morpholine (23 µL, 0.27 mmol, 1.5 equiv) by following general procedure C. Product **19m** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 15% EtOAc in petroleum ether to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.45 (2H, m), 7.44–7.37 (2H, m), 7.27–7.24 (4H, m), 7.04–6.93 (4H, m), 4.17 (2H, s), 3.84 (3H, s), 3.74 (2H, s), 3.70–3.57 (8H, m), 2.66 (2H, t, *J* = 7.7 Hz), 1.63 (2H, quintet, *J* = 7.6 Hz), 1.40–1.27 (4H, m), 0.90 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 168.1, 159.4, 145.0, 139.8, 139.4, 135.1, 134.6, 134.1, 133.1, 129.5, 128.8, 128.0, 126.9, 125.4, 114.4, 66.9, 66.8, 55.5, 46.8, 42.3, 35.7, 31.5, 31.4, 31.1, 28.6, 22.6, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{33}H_{39}N_4O_3$ $[M+H]^+$ 539.3022. Found 539.3015.

2-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)acetamide (19o)

The title compound was obtained as a white solid material (82 mg, 85% yield) from carboxylic acid ester **18b** (90 mg, 0.18 mmol, 1.0 equiv), HOBt (32 mg, 0.23 mmol, 1.3 equiv), EDC (44 mg, 0.23 mmol, 1.3 equiv), and NH_3 (0.5M in THF, 1.08 mL, 0.54 mmol, 3.0 equiv) by following general procedure C. Product **19o** was purified by column chromatography on silica gel (10 g silica gel) using 2% MeOH in EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.52–7.42 (2H, m), 7.45–7.36 (2H, m), 7.31–7.18 (4H, m), 7.00–6.89 (4H, m), 6.88–6.79 (1H, m), 5.67–5.52 (1H, m), 4.08 (2H, s), 3.84 (3H, s), 3.66 (2H, s), 2.66 (2H, t, *J* = 7.7 Hz), 1.64 (2H, quintet, *J* = 7.7 Hz), 1.44–1.24 (4H, m), 0.90 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 171.4, 159.4, 145.3, 139.9, 139.7, 134.6, 134.3, 133.9, 133.0, 129.6, 128.5, 128.1, 127.2, 125.5, 114.4, 55.5, 35.7, 33.2, 31.5, 28.4, 22.6, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{29}H_{33}N_4O_2$ $[M+H]^+$ 469.2604. Found 469.2601.

N-Ethyl-2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)acetamide (19p)

The title compound was obtained as a white solid material (72 mg, 89% yield) from carboxylic acid ester **18b** (90 mg, 0.18 mmol, 1.0 equiv), HOBt (32 mg, 0.23 mmol, 1.3 equiv), EDC (44 mg, 0.23 mmol, 1.3 equiv), and ethylamine (2.0M in THF, 140 µL, 0.27 mmol, 1.5 equiv) by following general procedure C. Product **19p** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 15% EtOAc in petroleum ether to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.43 (2H, m), 7.42–7.36 (2H, m), 7.31–7.20 (4H, m), 7.01–6.89 (4H, m), 6.75–6.69 (1H, m), 4.08 (2H, s), 3.84 (3H, s), 3.65 (2H, s), 3.30 (2H, qd, *J* = 7.3, 5.6 Hz), 2.67 (2H, t, *J* = 7.7 Hz), 1.64 (2H, quintet, *J* = 7.5 Hz), 1.40–1.28 (4H, m), 1.14 (3H, t, *J* = 7.3 Hz), 0.90 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 168.8, 159.4, 145.2, 140.2, 139.6, 134.8, 134.2, 133.9, 133.1, 129.5, 128.5, 128.1, 127.1, 125.5, 114.4, 55.5, 35.7, 34.8, 33.6, 31.5, 31.1, 28.4, 22.6, 14.9, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{31}H_{37}N_4O_2$ $[M+H]^+$ 497.2917. Found 497.2921.

2-(1-(2-Chloro-4-pentylphenyl)-5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)-*N*,*N*-diethylacetamide (19s)

The title compound was obtained as a white solid material (20 mg, 40% yield) from carboxylic acid ester **18b** (46 mg, 0.089 mmol, 1.0 equiv), HOBt (16 mg, 0.12 mmol, 1.3 equiv), EDC (23 mg, 0.12 mmol, 1.3 equiv), and diethylamine (14 µL, 0.13 mmol, 1.5 equiv) by following general procedure C. Product **19s** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 15% EtOAc in petroleum ether to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.47–7.41 (2H, m), 7.35–7.29 (3H, m), 7.12–7.05 (2H, m), 7.00–6.92 (4H, m), 4.03 (2H, s), 3.84 (3H, s), 3.74 (2H, s), 3.46 (2H, q, *J* = 7.1 Hz), 3.39 (2H, q, *J* = 7.1 Hz), 2.63 (2H, t, *J* = 7.7 Hz), 1.62 (2H, quintet, *J* = 7.5 Hz), 1.40–1.27 (4H, m), 1.14 (3H, t, *J* = 7.1 Hz), 1.13 (3H, t, *J* = 7.1 Hz), 0.90 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 168.5, 159.3, 147.3, 139.6, 139.2, 136.5, 134.8, 133.3, 131.8, 131.6, 130.1, 129.3, 128.0, 127.7, 126.7, 114.3, 55.5, 42.6, 40.6, 35.6, 31.7, 31.4, 30.9, 28.9, 22.6, 14.4, 14.1, 13.2.

N-Ethyl-*N*-((5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamine (2a)

The title compound was obtained as a light yellow oil (14 mg, 25% yield) from amide **19a** (55 mg, 0.11 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 4.5 equiv) by following general procedure D. Product **2a** was purified by column

chromatography on silica gel (10 g silica gel) using gradient elution from 0% EtOAc in CH_2Cl_2 to 30% EtOAc in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.44 (2H, m), 7.40–7.35 (2H, m), 7.24–7.18 (4H, m), 7.02–6.93 (4H, m), 4.15 (2H, s), 3.84 (3H, s), 3.73 (2H, s), 2.68–2.54 (6H, m), 1.67–1.55 (2H, m), 1.39–1.28 (4H, m), 1.04 (6H, t, *J* = 7.1 Hz), 0.89 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (100.6 MHz, CD_3OD-d_4, ppm) δ 160.9, 147.0, 141.1, 139.1, 135.4, 134.9, 130.7, 129.9, 128.8, 128.6, 127.9, 126.8, 115.3, 62.8, 55.8, 36.5, 32.5, 32.2, 30.2, 23.5, 14.4, 9.4; HRMS-ESI (*m/z*) calcd. for $C_{32}H_{41}N_4O$ $[M+H]^+$ 497.3280. Found 497.3280.

N,*N*-Diethyl-2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine hydrochloride (2e)

The title compound was obtained as a white foam (15 mg, 29% yield) from amide **19e** (50 mg, 0.095 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 4.5 equiv) by following general procedure D. Product **2a** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2. The oily residue was converted to hydrochloric salt using 2 M HCl in Et_2O. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 12.35–12.20 (1H, br s), 7.51–7.39 (4H, m), 7.33–7.27 (4H, m), 7.06–6.98 (2H, m), 6.98–6.91 (2H, m), 4.16 (2H, s), 3.84 (3H, s), 3.40–3.29 (4H, m), 3.24–2.99 (4H, m), 2.67 (2H, t, *J* = 7.7 Hz), 1.64 (2H, quintet, *J* = 7.4 Hz), 1.43–1.27 (10H, m), 0.90 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 145.4, 140.4, 139.7, 135.0, 134.5, 133.8, 132.9, 129.7, 128.8, 128.0, 127.2, 125.5, 114.4, 55.5, 50.7, 46.8, 35.7, 31.5, 31.1, 28.4, 22.6, 20.5, 14.1, 8.5; HRMS-ESI (*m/z*) calcd. for $C_{33}H_{43}N_4O$ $[M+H]^+$ 511.3437. Found 511.3441.

N,*N*-Diethyl-3-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)propan-1-amine (2f)

The title compound was obtained as a light yellow oil (7 mg, 37% yield) from amide **19f** (20 mg, 0.037 mmol, 1 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 13.5 equiv) by following general procedure D. Pure product **2f** was obtained after purification by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% EtOH in CH_2Cl_2 to 10% EtOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.46 (2H, m), 7.46–7.42 (2H, m), 7.30–7.27 (4H, m), 7.02–6.93 (4H, m), 4.06 (2H, s), 3.84 (3H, s), 3.06–2.94 (6H, m), 2.71 (2H, t, *J* = 7.0 Hz), 2.66 (2H, t, *J* = 7.7 Hz), 2.28–2.18 (2H, m), 1.64 (2H, quintet, *J* = 7.7 Hz), 1.40–1.26 (10H, m), 0.89 (3H, t, *J* = 7.0 Hz).

5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-4-(pyrrolidin-1-ylmethyl)-1*H*-1,2,3-triazole (2g)

The title compound was obtained as a light yellow oil (11 mg, 25% yield) from amide **19g** (45 mg, 0.088 mmol, 1 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 4.5 equiv) by following general procedure D. Product **2g** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.44 (2H, m), 7.42–7.36 (2H, m), 7.25–7.18 (4H, m), 7.01–6.93 (4H, m), 4.12 (2H, s), 3.84 (3H, s), 3.77 (2H, s), 2.74–2.55 (6H, m), 1.79 (4H, quintet, *J* = 3.2 Hz), 1.73–1.55 (2H, m), 1.41–1.23 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz; HRMS-ESI (*m/z*) calcd. for $C_{32}H_{39}N_4O$ $[M+H]^+$ 495.3124. Found 495.3118.

5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-4-(2-(pyrrolidin-1-yl)ethyl)-1*H*-1,2,3-triazole hydrochloride (2h)

The title compound was obtained as a light yellow foam (12 mg, 36% yield) from amide **19h** (32 mg, 0.061 mmol, 1 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 8.2 equiv) by following general procedure D. Product **2h** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 5% MeOH in CH_2Cl_2. The oily residue was converted to hydrochloric salt using 2 M HCl in Et_2O. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 12.64–12.44 (1H, br s), 7.51–7.45 (2H, m), 7.45–7.38 (2H, m), 7.32–7.22 (4H, m), 7.06–6.97 (2H, m), 6.98–6.90 (2H, m), 4.15 (2H, s), 3.84 (3H, s), 3.81–3.62 (2H, m), 3.56–3.23 (4H, m), 2.89–2.69 (2H, m), 2.67 (2H, t, *J* = 7.7 Hz), 2.29–2.13 (2H, m), 2.12–1.97 (2H, m), 1.64 (2H, quintet, *J* = 7.3 Hz), 1.44–1.15 (4H, m), 0.89 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.4, 145.3, 139.6, 135.0, 133.8, 132.9, 129.6, 128.9, 128.1, 127.2, 125.6, 114.4, 55.5, 54.2, 35.7, 31.5, 31.1, 28.6, 23.6, 22.6, 22.2, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{33}H_{41}N_4O$ $[M+H]^+$ 509.3280. Found 509.3299.

5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-4-(3-(pyrrolidin-1-yl)propyl)-1*H*-1,2,3-triazole (2i)

The title compound was obtained as a light yellow oil (9 mg, 29% yield) from amide **19i** (32 mg, 0.06 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 8.3 equiv) by following general procedure D. Product **2i** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% EtOH in CH_2Cl_2 to 20% EtOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.49–7.45 (2H, m), 7.44–7.40 (2H, m), 7.29–7.23 (4H, m), 6.99–6.92 (4H, m), 4.05 (2H, s), 3.88 (3H, s), 3.10–3.03 (6H, m), 2.73 (2H, t, *J* = 6.9 Hz), 2.65 (2H, t, *J* = 7.7 Hz), 2.35–2.17 (2H, m), 2.12–1.97 (4H, m), 1.62 (2H, quintet, *J* = 7.5 Hz), 1.43–1.22 (4H, m), 0.88 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.4, 145.0, 143.7, 139.6, 135.2, 134.0, 133.2, 132.9, 129.6, 128.6, 128.0, 127.1, 125.4, 114.4, 55.5, 55.1, 53.7, 35.7, 31.5, 31.1, 28.5, 25.0, 23.6, 22.6, 22.4, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{34}H_{43}N_4O$ $[M+H]^+$ 523.3437. Found 523.3455.

1-((5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-4-methylpiperazine (2j)

The title compound was obtained as a light yellow oil (18 mg, 31% yield) from amide **19j** (60 mg, 0.11 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 4.5 equiv) by following general procedure D. Product **2j** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution 0% EtOH in CH_2Cl_2 to 20% EtOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.45 (2H, m), 7.44–7.39 (2H, m), 7.29–7.21 (4H, m), 7.03–6.93 (4H, m), 4.09 (2H, s), 3.84 (3H, s), 3.68 (2H, s), 3.10–3.00 (2H, m),

2.92–2.79 (2H, m), 2.78–2.69 (2H, m), 2.66 (2H, t, *J* = 7.7 Hz), 2.60 (3H, s), 2.58–2.52 (2H, m), 1.63 (2H, quintet, *J* = 7.4 Hz), 1.40–1.27 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz); HRMS-ESI (*m/z*) calcd. for $C_{33}H_{42}N_5O$ $[M+H]^+$ 524.3389. Found 524.3390.

1-(2-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)ethyl)-4-methylpiperazine (2k)

The title compound was obtained as a light yellow oil (14 mg, 26% yield) from amide **19k** (60 mg, 0.10 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 5.0 equiv) by following general procedure D. Product **2k** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% EtOH in CH_2Cl_2 to 20% EtOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.49–7.45 (2H, m), 7.43–7.38 (2H, m), 7.25–7.19 (4H, m), 6.99–6.94 (4H, m), 4.04 (2H, s), 3.84 (3H, s), 2.93–2.83 (2H, m), 2.79–2.71 (2H, m), 2.64 (2H, t, *J* = 7.7 Hz), 2.60–2.33 (8H, m), 2.28 (3H, s), 1.62 (2H, quintet, *J* = 7.5 Hz), 1.41–1.22 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz).

1-(3-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)propyl)-4-methylpiperazine (2l)

The title compound was obtained as a light yellow foam (22 mg, 34% yield) from amide **19l** (62 mg, 0.11 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 4.5 equiv) by following general procedure D. After extraction hydrochloric acid salt of amine **2l** was made using 2 M HCl in Et_2O and washed with diethyl ether and hexane to give pure product. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.54–7.47 (2H, m), 7.46–7.41 (2H, m), 7.34–7.20 (4H, m), 7.02–6.91 (4H, m), 4.02 (2H, s), 3.82 (3H, s), 3.79–3.40 (8H, m), 3.25–3.11 (2H, m), 2.87 (3H, s), 2.77–2.70 (2H, m), 2.64 (2H, t, *J* = 7.7 Hz), 2.33–2.12 (2H, m), 1.61 (2H, quintet, *J* = 7.6 Hz), 1.39–1.25 (4H, m), 0.88 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.4, 145.1, 143.1, 139.4, 135.2, 133.9, 133.3, 132.6, 129.6, 128.7, 128.0, 127.1, 125.5, 114.5, 56.7, 55.5, 50.2, 48.7, 43.3, 35.6, 31.5, 31.0, 28.5, 22.6, 22.2, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{35}H_{46}N_5O$ $[M+H]^+$ 552.3702. Found 552.3707.

4-(2-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)ethyl)morpholine (2m)

The title compound was obtained as a light yellow oil (25 mg, 34% yield) from amide **19m** (69 mg, 0.13 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 3.8 equiv) by following general procedure D. Product **2m** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% EtOH in CH_2Cl_2 to 20% EtOH in CH_2Cl_2. After column chromatography hydrochloric acid salt of amine **2m** was made using 2 M HCl in Et_2O. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.23–11.06 (1H, br s), 7.56–7.49 (2H, m), 7.47–7.42 (2H, m), 7.39–7.28 (4H, m), 7.02–6.97 (2H, m), 6.96–6.93 (2H, m), 4.16 (2H, s), 4.00–3.94 (2H, m), 3.81 (2H, d, *J* = 12.3 Hz), 3.77 (3H, s), 3.50 (2H, d, *J* = 12.3 Hz), 3.46–3.39 (2H, m), 3.23–3.16 (2H, m), 3.16–3.05 (2H, m), 2.69–2.60 (2H, m), 1.59 (2H, quintet, *J* = 7.5 Hz), 1.36–1.21 (4H, m), 0.85 (3H, d, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.4, 145.7, 139.7, 139.6, 135.3, 134.5, 133.4, 132.7, 129.7, 128.9, 128.0, 127.2, 125.5, 114.4, 63.7, 56.0, 55.5, 55.4, 52.2, 35.7, 31.4, 31.0, 28.5, 22.6, 19.7, 14.1.

2-(5-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (2o)

The title compound was obtained as a light yellow oil (15 mg, 30% yield) from amide **19o** (52 mg, 0.11 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 4.5 equiv) by following general procedure D. Product **2o** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 50% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.49–7.44 (2H, m), 7.43–7.37 (2H, m), 7.24–7.21 (4H, m), 6.98–6.90 (4H, m), 4.03 (2H, s), 3.84 (3H, s), 3.34 (2H, t, *J* = 6.3 Hz), 2.99 (2H, t, *J* = 6.3 Hz), 2.64 (2H, t, *J* = 7.7 Hz), 1.62 (2H, quintet, *J* = 7.5 Hz), 1.39–1.28 (4H, m), 0.89 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.3, 145.0, 141.6, 139.4, 134.9, 134.3, 133.9, 133.0, 129.4, 128.6, 128.0, 127.0, 125.6, 114.3, 55.4, 39.4, 35.7, 31.5, 31.1, 29.8, 28.3, 22.6, 14.1; HRMS-ESI (*m/z*) calcd. for $C_{29}H_{35}N_4O$ $[M+H]^+$ 455.2811. Found 455.2806.

N-Ethyl-2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (2p)

The title compound was obtained as a light yellow oil (19 mg, 36% yield) from amide **19p** (56 mg, 0.11 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 4.5 equiv) by following general procedure D. Product **2p** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 50% MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.45 (2H, m), 7.43–7.38 (2H, m), 7.26–7.21 (4H, m), 6.99–6.93 (4H, m), 4.04 (2H, s), 3.84 (3H, s), 3.08 (2H, t, *J* = 6.8 Hz), 2.91 (2H, t, *J* = 6.8 Hz), 2.76 (2H, q, *J* = 7.2 Hz), 2.65 (2H, t, *J* = 7.7 Hz), 1.63 (2H, quintet, *J* = 7.4 Hz), 1.42–1.26 (4H, m), 1.16 (3H, t, *J* = 7.2 Hz), 0.89 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.2, 144.8, 142.8, 139.4, 134.9, 133.89, 133.2, 132.8, 129.3, 128.3, 127.9, 126.9, 125.3, 114.2, 55.3, 47.3, 43.3, 35.5, 31.3, 30.9, 28.2, 23.7, 22.4, 14.0, 13.3; HRMS-ESI (*m/z*) calcd. for $C_{31}H_{39}N_4O$ $[M+H]^+$ 483.3124. Found 483.3119.

2-(1-(2-Chloro-4-pentylphenyl)-5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)-*N*,*N*-diethylethan-1-amine (2s)

The title compound was obtained as a light yellow oil (7 mg, 42% yield) from amide **19s** (16 mg, 0.029 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 17.2 equiv) by following general procedure D. Product **2s** was purified by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2. After column chromatography hydrochloric acid salt of amine **2s** was made using 2 M HCl in Et_2O. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 12.40–12.27 (1H, br s), 7.48–7.42 (2H, m), 7.41–7.36 (3H, m), 7.17–7.13 (2H, m), 7.01–6.97 (2H, m),

6.97–6.93 (2H, m), 4.02 (2H, s), 3.84 (3H, s), 3.39–3.29 (4H, m), 3.24–3.00 (4H, m), 2.66 (2H, t, J = 7.7 Hz), 1.64 (2H, quintet, J = 7.4 Hz), 1.43–1.28 (10H, m), 0.91 (3H, t, J = 7.0 Hz); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 159.4, 147.8, 139.6, 135.9, 134.6, 133.0, 131.5, 131.4, 130.3, 129.3, 129.1, 128.0, 127.9, 127.1, 114.4, 55.5, 50.7, 46.7, 35.6, 31.4, 30.9, 28.6, 22.6, 20.6, 14.1, 8.6.

1-Bromo-4-(prop-2-yn-1-yl)benzene (20)

To a cooled solution of trimethylsilyl acetylene (4.5 mL, 32.0 mmol, 2.0 equiv) in anhydrous THF (10 mL) was added dropwise *i*-PrMgCl (2.0 M solution in THF, 12.0 mL, 24.0 mmol, 3.0 equiv). The light brown solution was stirred at 0°C for 30 min and then 1 h at room temperature. CuBr (0.69 g, 4.80 mmol, 0.6 equiv) was then added to the light brown solution and the stirring was continued for 30 min. A solution of 1-bromo-4-(bromomethyl)benzene (2.00 g, 8.00 mmol, 1.0 equiv) in anhydrous THF (7 mL) was then added and the resulting brown suspension was heated under reflux for 3 h. All volatiles were removed *in vacuo* and water (20 mL) and EtOAc (20 mL) were added to the residue. The orange precipitate was filtered and the filtrate was extracted with EtOAc (2 × 40 mL). Combined organic extracts were washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (100 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 60% EtOAc in petroleum ether to give alkyne (2.02 g, 94% yield) as a light yellow oil. To the solution of alkyne from above (2.02 g, 7.56 mmol, 1.0 equiv) in EtOH (10 mL) was added dropwise a solution of $AgNO_3$ (1.93 g, 11.34 mmol, 1.5 equiv) in 1:3 (v/v) H_2O/EtOH (12 mL). After stirring at room temperature for 30 min, a solution of KCN (4.87 g, 74.84 mmol, 9.9 equiv) in water (15 mL) was added to the light yellow suspension and the stirring was continued for 2 h. The light yellow solution was partitioned between EtOAc and water and organic layer was washed with water (2 × 20 mL), brine (20 mL), dried over Na_2SO_4, and evaporated under reduced pressure to give product **20** (786 mg, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.41 (2H, m), 7.26–7.21 (2H, m), 3.56 (2H, d, J = 2.7 Hz), 2.21 (1H, t, J = 2.7 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 135.2, 131.7, 129.7, 120.7, 81.4, 71, 24.5.

Methyl 4-(4-bromophenyl)but-2-ynoate (21)

An oven-dried pressure tube (100 mL) was cooled under a stream of argon and then charged with alkyne **20** (1.18 g, 6.05 mmol, 1.0 equiv). Anhydrous THF (7 mL) was added, followed by dropwise addition of MeMgBr (1.0 M solution in THF, 7.3 mL, 7.26 mmol, 1.2 equiv). The resulting solution was stirred at 65°C for 3 h, then cooled to room temperature and methyl chloroformate (1.87 mL, 2.04 mmol, 4.0 equiv) was added rapidly with vigorous stirring. The resulting solution was then heated at 65°C for 18 h. After cooling to room temperature, it was diluted with EtOAc (20 mL) and washed with aqueous 1 N HCl solution (15 mL), water (15 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (50 g silica gel) using gradient elution from 2% EtOAc in petroleum ether to 10% EtOAc in petroleum ether to yield product **21** (1.31 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.53–7.41 (2H, m), 7.23–7.16 (2H, m), 3.78 (3H, s), 3.69 (2H, s).

Ethyl 5-(4-bromophenyl)pent-3-ynoate (22)

To a solution of alkyne **20** (786 mg, 4.03 mmol, 1.0 equiv) in anhydrous MeCN (5 mL) were added CuI (38 mg, 0.20 mmol, 0.05 equiv) and ethyl diazoacetate (466 µL, 4.43 mmol, 1.1 equiv). After stirring at room temperature for 20 h, the suspension was diluted with saturated aqueous NH_4Cl solution (10 mL) and extracted with EtOAc (3 × 15 mL). Combined organic extracts were dried over Na_2SO_4, evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel (30 g silica gel) using gradient elution from 5% EtOAc in petroleum ether to 10% EtOAc in petroleum ether to yield product **22** (930 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.45–7.41 (2H, m), 7.25–7.22 (2H, m), 4.21 (2H, q, J = 7.1 Hz), 3.57 (2H, t, J = 2.5 Hz), 3.31 (2H, t, J = 2.5 Hz), 1.29 (3H, t, J = 7.1 Hz).

Methyl 5-(4-bromobenzyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazole-4-carboxylate (23)

The title compound was obtained as a light brown oil (1.42 g, 80% yield) from alkyne **21** (1.02 g, 4.03 mmol, 1.0 equiv), azide **4** (0.841 g, 4.43 mmol, 1.1 equiv), and Cp*RuCl(COD) (0.152 g, 0.40 mmol, 10 mol %) by following general procedure B. Pure material was obtained by column chromatography on silica gel (50 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 33% EtOAc in petroleum ether. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.32–7.27 (4H, m), 7.15–7.12 (2H, m), 6.79–6.75 (2H, m), 4.33 (2H, s), 3.99 (3H, s), 2.68 (2H, t, J = 7.7 Hz), 1.65 (2H, quintet, J = 7.5 Hz), 1.42–1.28 (4H, m), 0.90 (3H, t, J = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 162.2, 146.0, 140.9, 136.7, 135.2, 133.0, 131.9, 130.0, 129.7, 125.8, 121.1, 52.3, 35.7, 31.4, 31.1, 28.6, 22.6, 14.2; HRMS-ESI (*m/z*) calcd. for $C_{22}H_{25}N_3O_2Br$ $[M+H]^+$ 442.1130. Found 442.1106.

Ethyl 2-(5-(4-bromobenzyl)-1-(4-pentylbenzyl)-1*H*-1,2,3-triazol-4-yl)acetate (24)

The title compound was obtained as a light brown oil (88 mg, 13% yield) from alkyne **22** (404 mg, 1.44 mmol, 1.0 equiv), azide **11** (322 mg, 1.58 mmol, 1.1 equiv), and Cp*RuCl(COD) (55 mg, 0.144 mmol, 10 mol %) by following general procedure B. Pure material was obtained by column chromatography on silica gel (40 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.36–7.30 (2H, m), 7.10–7.05 (2H, m), 6.96–6.90 (2H, m), 6.81–6.74 (2H, m), 5.28 (2H, s), 4.10 (2H, q, J = 7.1 Hz), 3.84 (2H, s), 3.64 (2H, s), 2.56 (2H, t, J = 7.7 Hz), 1.63–1.53 (2H, m), 1.41–1.24 (4H, m), 1.21 (3H, t, J = 7.1 Hz), 0.89 (3H, t, J = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 170.2, 143.5, 139.9, 134.8, 132.8, 132.0, 131.8, 130.0, 129.1, 127.2, 121.1, 61.3, 52.3, 35.7, 31.7, 31.6, 31.2, 28.1, 22.6, 14.3, 14.2.

Methyl 5-(4-bromobenzyl)-1-(*p*-tolyl)-1*H*-1,2,3-triazole-4-carboxylate (25)

The title compound was obtained as a light brown oil (282 mg, 65% yield) from alkyne **21** (286 mg, 1.12 mmol, 1.0 equiv), azide **3** (166 mg,

1.24 mmol, 1.1 equiv), and Cp*RuCl(COD) (43 mg, 0.112 mmol, 10 mol %) by following general procedure B. Pure material was obtained by column chromatography on silica gel (30 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.34–7.30 (2H, m), 7.32–7.25 (2H, m), 7.16–7.09 (2H, m), 6.82–6.75 (2H, m), 4.32 (2H, s), 3.99 (3H, s), 2.44 (3H, s).

5-(4-Bromobenzyl)-*N*,*N*-diethyl-1-(*p*-tolyl)-1*H*-1,2,3-triazole-4-carboxamide (26)

The title compound was obtained as a light yellow solid material (200 mg, 64% yield) from carboxylic acid ester **25** (271 mg, 0.70 mmol, 1.0 equiv), HOBt (128 mg, 0.949 mmol, 1.3 equiv), EDC (182 mg, 0.949 mmol, 1.3 equiv), diethylamine (113 µL, 1.096 mmol, 1.5 equiv) by following general procedure C. Pure material was obtained by column chromatography using gradient elution from 20% EtOAc in hexanes to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.30–7.24 (4H, m), 7.16–7.08 (2H, m), 6.85–6.78 (2H, m), 4.23 (2H, s), 3.79 (2H, q, *J* = 7.0 Hz), 3.53 (2H, q, *J* = 7.0 Hz), 2.43 (3H, s), 1.29 (3H, t, *J* = 7.0 Hz), 1.21 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 161.8, 140.6, 140.4, 139.3, 136.1, 133.1, 131.5, 130.1, 130.0, 125.6, 120.6, 43.4, 40.7, 28.6, 21.2, 14.7, 12.8.

Methyl 5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazole-4-carboxylate (27)

The title compound was obtained as a white solid material (900 mg, 85% yield) from boronic acid **12** (378 mg, 2.49 mmol, 1.1 equiv), triazole **23** (1.00 g, 2.26 mmol, 1.0 equiv), and $Pd(PPh_3)_4$ (78 mg, 0.0678 mmol, 3 mol%) by following general procedure A. Pure material was obtained by column chromatography on silica gel (50 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 66% EtOAc in petroleum ether. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.50–7.44 (2H, m), 7.40–7.35 (2H, m), 7.30–7.26 (2H, m), 7.21–7.16 (2H, m), 6.98–6.91 (4H, m), 4.41 (2H, s), 4.00 (3H, s), 3.84 (3H, s), 2.68 (2H, t, *J* = 7.7 Hz), 1.64 (2H, quintet, *J* = 7.4 Hz), 1.41–1.27 (4H, m), 0.89 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 162.2, 159.3, 145.8, 141.5, 139.6, 136.7, 134.6, 133.1, 129.6, 128.7, 128.7, 126.98, 125.8, 114.3, 55.5, 52.3, 35.7, 31.4, 31.1, 28.7, 22.6, 14.1.

Ethyl 2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylbenzyl)-1*H*-1,2,3-triazol-4-yl)acetate (28)

The title compound was obtained as a light yellow solid material (74 mg, 80% yield) from boronic acid **12** (33 mg, 0.22 mmol, 1.2 equiv), triazole **24** (88 mg, 0.18 mmol, 1 equiv), and $Pd(PPh_3)_4$ (6.2 mg, 0.0054 mmol, 3 mol%) by following general procedure A. Pure material was obtained by column chromatography on silica gel (10 g silica gel) using gradient elution from 25% EtOAc in petroleum ether to 50% EtOAc in petroleum ether. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.53–7.45 (2H, m), 7.45–7.39 (2H, m), 7.13–7.07 (2H, m), 7.02–6.92 (6H, m), 5.32 (2H, s), 4.11 (2H, q, *J* = 7.1 Hz), 3.92 (2H, s), 3.85 (3H, s), 3.68 (2H, s), 2.55 (2H, t, *J* = 7.7 Hz), 1.56 (2H, quintet, *J* = 7.4 Hz), 1.39–1.25 (4H, m), 1.21 (3H, t, *J* = 7.1 Hz), 0.88 (3H, t, *J* = 6.9 Hz).

N,*N*-Diethyl-5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(*p*-tolyl)-1*H*-1,2,3-triazole-4-carboxamide (29)

The title compound was obtained as a light yellow solid material (79 mg, 76% yield) from boronic acid (37 mg, 0.26 mmol, 1.05 equiv), triazole **26** (100 mg, 0.23 mmol, 1 equiv), and $Pd(PPh_3)_4$ (8.0 mg, 0.0069 mmol, 3 mol%) by following general procedure A. Pure material was obtained by column chromatography on silica gel (10 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 100% EtOAc. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.48–7.43 (2H, m), 7.38–7.34 (2H, m), 7.29 (2H, d, *J* = 8.2 Hz), 7.23–7.18 (2H, m), 6.99 (2H, d, *J* = 8.2 Hz), 6.97–6.92 (2H, m), 4.31 (2H, s), 3.84 (3H, s), 3.77 (2H, q, *J* = 7.0 Hz), 3.55 (2H, q, *J* = 7.0 Hz), 2.44 (3H, s), 1.28 (3H, t, *J* = 7.0 Hz), 1.21 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 162.2, 159.3, 140.8, 140.4, 139.8, 139.3, 135.7, 133.5, 133.3, 130.2, 128.9, 128.1, 126.8, 125.9, 114.3, 55.5, 43.6, 40.7, 28.9, 21.4, 14.8, 12.9.

N-Ethyl-*N*-((5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(*p*-tolyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamine (2q)

The title compound was obtained as a light yellow oil (51 mg, 76% yield) from amide **29** (70 mg, 0.15 mmol, 1 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 3.3 equiv) by following general procedure D. Pure material was obtained by column chromatography on silica gel (10 g silica gel) using gradient elution from 20% EtOAc in hexanes to 100% EtOAc in hexanes. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.46 (2H, m), 7.39 (2H, d, *J* = 8.2 Hz), 7.25–7.17 (4H, m), 6.99 (2H, d, *J* = 8.3 Hz), 6.97–6.94 (2H, m), 4.14 (2H, s), 3.84 (3H, s), 3.73 (2H, s), 2.60 (4H, q, *J* = 7.1 Hz), 2.40 (3H, s), 1.04 (6H, t, *J* = 7.1 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.3, 139.7, 139.2, 135.7, 134.2, 133.2, 130.0, 128.7, 128.1, 126.9, 125.5, 114.4, 55.5, 46.8, 28.4, 21.4, 11.8.

2,2-Dibromo-1-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-one (30)

To a cooled 0°C (crushed ice) solution of TMP (705 µL, 4.18 mmol, 2.2 equiv) in anhydrous THF (3 mL) was added *n*-BuLi (2.5 M in hexanes, 1.58 mL, 3.80 mmol, 2.0 equiv), and after stirring for 30 min the resulting solution was cooled to −78°C (dry ice bath). In a separate flask, carboxylic acid ester **27** (892 mg, 1.90 mmol, 1.0 equiv) and CH_2Br_2 (267 µL, 3.80 mmol, 2.0 equiv) were dissolved in anhydrous THF (15 mL), cooled to −78°C, and the LTMP solution from above was added to the resulting solution dropwise via cannula. The resulting solution was stirred at −78°C for 20 min, then it was quenched with aqueous 1.2 M HCl solution and extracted with EtOAc (3 × 20 mL). Combined organic extracts were washed with brine (40 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product **30** (1.07 g, 92% yield) was used in subsequent steps without purification. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.48–7.43 (2H, m), 7.41 (1H, s), 7.39–7.35 (2H, m), 7.34–7.30 (2H, m), 7.23–7.19 (2H, m), 6.98–6.90 (4H, m), 4.43 (2H, s), 3.84 (3H, s), 2.70 (2H, t), 1.67 (2H, p, *J* = 7.4 Hz), 1.42–1.29 (4H, m), 0.91 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 181.4, 159.2, 146.0, 142.8, 139.7, 137.9, 133.7, 132.9, 132.5, 129.6, 128.6, 127.9, 126.9, 125.6, 114.2, 55.3,

40.0, 35.6, 31.3, 30.9, 28.7, 22.5, 14.0; HRMS-ESI (*m/z*) calcd. for $C_{29}H_{30}N_3O_2Br_2$ $[M+H]^+$ 610.0705. Found 610.0693.

Methyl 2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)acetate (31)

To a cooled (−78°C) solution of triazole **30** (1.07 g, 1.75 mmol, 1.0 equiv) in anhydrous THF (20 mL) was dropwise added LiHMDS (1.0 M in THF, 1.91 mL, 1.91 mmol, 1.09 equiv). The resulting light yellow solution was stirred at −78°C for 1 h, then *n*-BuLi (2.4 M in hexanes, 1.6 mL, 3.85 mmol, 2.2 equiv) was added dropwise and stirring was continued for 1 h. The solution was warmed to room temperature and added via cannula to a stirred solution of anhydrous HCl in methanol (24 mL; prepared by mixing MeOH with CH_3COCl in a 1:5 (v/v) ratio) at 0°C over a 20 min period. The mixture was partitioned between EtOAc (30 mL) and water (30 mL). The aqueous phase was extracted with EtOAc (3 × 30 mL), combined organic extracts were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (70 g silica gel) using gradient elution from 10% EtOAc in hexanes to 50% EtOAc in hexanes to yield triazole **31** (230 mg, 27% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.44 (2H, m), 7.38–7.32 (2H, m), 7.22–7.18 (2H, m), 7.17–7.13 (2H, m), 6.99–6.93 (4H, m), 4.25 (2H, s), 4.07 (2H, s), 3.84 (3H, s), 3.29 (3H, s), 2.64 (2H, t, *J* = 7.6 Hz), 1.62 (2H, p, *J* = 7.5 Hz), 1.39–1.27 (4H, m), 0.89 (3H, t, *J* = 7.0 Hz).

N-Benzyl-*N*-ethyl-2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)-methyl)-1-(4-pentylphenyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (2n)

$LiAlH_4$ (1.0 M solution in THF, 520 µL, 0.52 mmol, 1.1 equiv) was added dropwise to a cooled solution (0°C, crushed ice) of carboxylic acid ester **31** (230 mg, 0.48 mmol, 1.0 equiv) in anhydrous THF (5 mL). After stirring at room temperature for 1 h, the white suspension was cooled to 0°C and quenched by sequential (within intervals of 10 min) addition of water (20 µL), aqueous 4 M NaOH solution (40 µL), and more water (60 µL). Ten minutes after the addition of the final amount of water, the white suspension was filtered and the filter cake was washed with EtOAc (20 mL). The filtrate was evaporated to dryness to yield 218 mg (99% yield) of alcohol as a white solid material, which was used in a subsequent step without purification. To the solution of alcohol from above (218 mg, 0.48 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (5 mL) was added triethylamine (127 µL, 0.91 mmol, 1.9 equiv). The colorless solution was cooled to 0°C (crushed ice) and methanesulfonyl chloride (56 µL, 0.72 mmol, 1.5 equiv) was added dropwise. After stirring at room temperature for 1 h, water (10 mL) was added to the light yellow solution and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). Combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure to yield mesyl derivative (240 mg, 94% yield), which was used in a subsequent step without purification. To the solution of the mesyl derivative from above (30 mg, 0.056 mmol, 1.0 equiv) in anhydrous 1,4-dioxane (5 mL) were added *N*-benzylethanamine hydrochloride (48 mg, 0.28 mmol, 5.0 equiv) and triethylamine (39 µL, 0.28 mmol, 5.0 equiv). After stirring at 90°C for 16 h, the light yellow solution was diluted with water (5 mL) and extracted with EtOAc (3 × 5 mL). Combined organic extracts were washed with water (10 mL), brine (10 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (20 g silica gel) using gradient elution from using gradient elution from 0% MeOH in CH_2Cl_2 to 20% MeOH in CH_2Cl_2 to yield amine **2n** (15.5 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.49–7.44 (2H, m), 7.41–7.36 (2H, m), 7.31–7.27 (3H, m), 7.25–7.16 (6H, m), 6.99–6.94 (2H, m), 6.92–6.88 (2H, m), 3.95 (2H, s), 3.85 (3H, s), 3.61 (2H, s), 2.84 (4H, s), 2.64 (2H, t), 2.58 (2H, q, *J* = 7.1 Hz), 1.68–1.55 (2H, m), 1.43–1.26 (4H, m), 1.03 (3H, t, *J* = 7.1 Hz), 0.89 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.3, 144.7, 139.4, 135.5, 134.4, 133.2, 132.7, 130.7, 129.4, 128.9, 128.7, 128.5, 128.3, 128.1, 127.0, 126.9, 125.4, 114.4, 58.2, 55.5, 53.0, 47.5, 35.7, 31.5, 31.1, 28.4, 26.0, 23.6, 22.6, 14.2, 12.0; HRMS-ESI (*m/z*) calcd. for $C_{38}H_{45}N_4O$ $[M+H]^+$ 573.3593. Found 573.3601.

N,*N*-Diethyl-2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylbenzyl)-1*H*-1,2,3-triazol-4-yl)acetamide (32)

The title compound was obtained as a light yellow solid material (47 mg, 63% yield) from carboxylic acid ester **28** (74 mg, 0.14 mmol, 1.0 equiv), HOBt (24 mg, 0.18 mmol, 1.3 equiv), EDC (35 mg, 0.18 mmol, 1.3 equiv), diethylamine (22 µL, 0.21 mmol, 1.5 equiv) by following general procedure C. Pure material was obtained by column chromatography on silica gel (10 g silica gel) using gradient elution from 25% EtOAc in hexanes to 4% MeOH in EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.51–7.44 (2H, m), 7.43–7.36 (2H, m), 7.12–7.05 (2H, m), 7.03–6.91 (6H, m), 5.30 (2H, s), 4.04 (2H, s), 3.85 (3H, s), 3.71 (2H, s), 3.48 (2H, q, *J* = 7.1 Hz), 3.34 (2H, q, *J* = 7.1 Hz), 2.54 (2H, t, *J* = 7.7 Hz), 1.56 (2H, quintet, *J* = 7.5 Hz), 1.39–1.22 (4H, m), 1.15 (3H, t, *J* = 7.1 Hz), 1.08 (3H, t, *J* = 7.1 Hz), 0.88 (3H, t, *J* = 6.7 Hz).

N,*N*-Diethyl-2-(5-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-1-(4-pentylbenzyl)-1*H*-1,2,3-triazol-4-yl)ethan-1-amine (2r)

The title compound was obtained as a light yellow oil (30 mg, 65% yield) from amide **32** (47 mg, 0.087 mmol, 1 equiv) and BH_3–THF (1.0 M solution in THF, 0.5 mL, 0.5 mmol, 5.7 equiv) by following general procedure D. Pure material was obtained by column chromatography on silica gel (10 g silica gel) using gradient elution from 0% MeOH in CH_2Cl_2 to 25% MeOH in CH_2Cl_2. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.51–7.45 (2H, m), 7.45–7.39 (2H, m), 7.13–7.06 (2H, m), 7.01–6.93 (6H, m), 5.30 (2H, s), 3.90 (2H, s), 3.85 (3H, s), 3.08–2.72 (4H, m), 2.70–2.39 (6H, m), 1.56 (2H, quintet, *J* = 7.5 Hz), 1.39–1.21 (4H, m), 1.16–0.93 (6H, m), 0.88 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.4, 143.3, 139.7, 134.7, 133.1, 132.2, 132.0, 129.0, 128.5, 128.1, 127.3, 127.2, 114.4, 55.5, 52.4, 52.2, 46.9, 35.7, 31.6, 31.2, 28.0, 22.6, 14.1, 11.2; HRMS-ESI (*m/z*) calcd. for $C_{34}H_{45}N_4O$ $[M+H]^+$ 525.3593. Found 525.3600.

4-Pentylbenzaldehyde (33)

An oven-dried flask was cooled under a stream of argon and then charged with 1-bromo-4-pentylbenzene (2.00 g, 8.8 mmol, 1.0 equiv). Anhydrous THF (10 mL) was added and the colorless solution was

cooled to −78°C (dry ice bath). *n*-BuLi (2.5 M in hexanes, 3.9 mL, 9.7 mmol, 1.1 equiv) was added dropwise and the resulting mixture was stirred at −78°C for 1 h, whereupon anhydrous DMF (1.02 mL, 13.2 mmol, 1.5 equiv) was added dropwise to the colorless solution. The resulting solution was warmed to −45°C and quenched with aqueous saturated NH_4Cl solution (15 mL). The mixture was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, and evaporated under reduced pressure to yield aldehyde **33** (1.54 g, 99% yield) as a colorless oil, which was used in subsequent step without purification. ^{1}H NMR spectra was identical to that from the literature.[52]

N-Hydroxy-4-pentylbenzimidoyl chloride (34)

A mixture of hydroxylamine hydrochloride (1.53 g, 22.1 mmol, 2.5 equiv), $NaHCO_3$ (2.23 g, 26.6 mmol, 3.0 equiv), and aldehyde **33** (1.56 g, 8.85 mmol, 1.0 equiv) in diethyl ether (14 mL) and water (14 mL) was stirred at room temperature for 16 h. Layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography using gradient elution from 2% EtOAc in hexanes to 20% EtOAc in hexanes to yield oxime (1.43 g, 85% yield) as a colorless oil, which was used in a subsequent step without purification.

A solution of NCS (70 mg, 0.52 mmol, 1.0 equiv) in DMF (1 mL) was added dropwise to the solution of oxime from above (100 mg, 0.52 mmol, 1.0 equiv) in DMF (1 mL). The colorless solution was stirred at room temperature for 30 min, then it was diluted with water (5 mL) and extracted with diethyl ether (3 × 10 mL). Combined organic extracts were washed with water (20 mL), brine (15 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product **34** (116 mg, 99% yield) was used further without purification. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 8.33 (1H, s), 7.79–7.70 (2H, m), 7.23–7.18 (2H, m), 2.63 (2H, t, *J* = 7.7 Hz), 1.62 (2H, p, *J* = 7.4 Hz), 1.39–1.25 (4H, m), 0.88 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 146.0, 140.0, 129.9, 128.5, 127.1, 35.7, 31.4, 30.8, 22.5, 14.0.

Methyl 4-(4-bromobenzyl)-5-(4-pentylphenyl)isoxazole-3-carboxylate (35)

An oven-dried flask was cooled under a stream of argon and charged with alkyne **21** (935 mg, 3.69 mmol, 1.0 equiv), benzimidoyl chloride **34** (916 mg, 4.06 mmol, 1.1 equiv), Cp*RuCl(COD) (141 mg, 0.369 mmol, 10 mol%) and DCE (5 mL) was added. Subsequently, a solution of triethylamine (1.03 mL, 7.38 mmol, 2.0 equiv) in DCE (1 mL) was added via syringe pump over a period of 1 h. After stirring at room temperature for 16 h, all volatiles were removed under reduced pressure. Purification of crude product by column chromatography on silica gel (50 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 100% EtOAc afforded isoxazole **35** (768 mg, 47% yield) as a light brown oil. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.41–7.30 (4H, m), 7.24–7.19 (2H, m), 6.95–6.86 (2H, m), 4.17 (2H, s), 3.96 (3H, s), 2.71–2.56 (2H, m), 1.68–1.55 (2H, m), 1.44–1.23 (4H, m), 0.98–0.79 (3H, m); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 164.2, 158.1, 156.5, 145.4, 137.3, 131.8, 129.9, 129.1, 128.4, 125.3, 122.4, 120.5, 52.8, 35.9, 31.5, 31.0, 28.0, 22.6, 14.1.

4-(4-Bromobenzyl)-*N*,*N*-diethyl-5-(4-pentylphenyl)isoxazole-3-carboxamide (36)

The title compound was obtained as a light yellow oil (454 mg, 54% yield) from carboxylic acid ester **35** (768 mg, 1.74 mmol, 1.0 equiv), HOBt (285 mg, 2.11 mmol, 1.3 equiv), EDC (404 mg, 2.11 mmol, 1.3 equiv), and diethylamine (253 µL, 2.44 mmol, 1.5 equiv) by following general procedure C. Full conversion required addition of more HOBt (0.5 equiv), EDC (0.5 equiv), and diethylamine (0.5 equiv) followed by stirring at 50°C for 2 h. Pure material was obtained by column chromatography on silica gel (30 g silica gel) using gradient elution from 10% EtOAc in hexanes to 100% EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.42–7.38 (2H, m), 7.34–7.29 (2H, m), 7.25–7.21 (2H, m), 7.00–6.94 (2H, m), 4.01 (2H, s), 3.49 (2H, q, *J* = 7.1 Hz), 3.36 (2H, q, *J* = 7.1 Hz), 2.64 (2H, t, *J* = 7.7 Hz), 1.72–1.56 (2H, m), 1.40–1.29 (4H, m), 1.26 (3H, t, *J* = 7.1 Hz), 1.17 (3H, t, *J* = 7.1 Hz), 0.90 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 163.2, 161.6, 158.8, 145.2, 138.1, 131.6, 130.3, 129.0, 128.4, 125.7, 120.3, 118.6, 43.2, 40.63, 35.9, 31.6, 31.0, 28.0, 22.6, 14.8, 14.2, 12.7.

N,*N*-Diethyl-4-((4′-methoxy-[1,1′biphenyl]-4-yl)methyl)-5-(4-pentylphenyl)isoxazole-3-carboxamide (37)

The title compound was obtained as a light yellow oil (71 mg, 66% yield) from isoxazole **36** (100 mg, 0.21 mmol, 1.0 equiv), (4-methoxyphenyl)boronic acid (33 mg, 0.22 mmol, 1.05 equiv), and $Pd(PPh_3)_4$ (7.3 mg, 0.0063 mmol, 3 mol%) by following general procedure A. Pure material was obtained by column chromatography on silica gel (20 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 100% EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.49–7.44 (4H, m), 7.42–7.37 (2H, m), 7.26–7.22 (2H, m), 7.15–7.11 (2H, m), 6.98–6.92 (2H, m), 4.08 (2H, s), 3.84 (3H, s), 3.48 (2H, q, *J* = 7.1 Hz), 3.32 (2H, q, *J* = 7.1 Hz), 2.64 (2H, t, *J* = 7.7 Hz), 1.63 (2H, p, *J* = 7.5 Hz), 1.39–1.27 (4H, m), 1.24 (3H, t, *J* = 7.1 Hz), 1.14 (3H, t, *J* = 7.1 Hz), 0.89 (3H, t, *J* = 7.0 Hz).

N-(4-Bromobenzyl)-4-pentylbenzimidamide (39)

4-(Bromophenyl)methanamine hydrochloride (445 mg, 2 mmol) was suspended in toluene (5 mL) and the mixture was cooled in an ice bath. To this, 2 M $AlMe_3$ solution in hexane (1 mL, 2 mmol) was added dropwise. The mixture was warmed to room temperature and stirred for 30 min. Then, a solution of 4-pentylbenzonitrile (**38**) in toluene (12 mL) was added and the mixture was heated to 110°C for 4 h in a sealed reactor. After cooling to room temperature, 1 M aqueous NaOH (20 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine and dried over Na_2SO_4. Solvent was removed *in vacuo* and the residue was treated with Et_2O. The precipitate was collected on a filter and dried to give intermediate **39** (270 mg, 38 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48 (d, *J* = 8.2 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 4.48 (s, 1H), 2.67–2.53 (m, 2H), 1.64–1.54 (m, 1H), 1.38–1.22 (m, 3H), 0.94–0.78 (m, 2H). ^{13}C NMR (400 MHz,

$CDCl_3$) δ: 162.86, 145.44, 138.33, 134.66, 131.62, 129.34, 128.72, 125.91, 120.90, 46.39, 35.64, 31.37, 30.95, 22.47, 13.98.

(*E*)-2-Bromo-3-isopropoxyacrylaldehyde (40)

Bromomalonaldehyde 453 mg (3.00 mmol), 2-propanol 0.92 mL (12.00 mmoL), and *p*-toluenesulfonic acid 9 mg (0.045 mmol) was refluxed for 3 h in 10 mL benzole, with Dean–Stark apparatus. The precipitate was filtered off and the residue was purified by flash chromatography on silica gel, eluent: hexane/EtOAc, 4:1 to give the intermediate **40** as an oil (349 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.13 (s, 1H), 7.63 (s, 1H), 1.45 (d, *J* = 6.2 Hz, 6H).

1-(4-Bromobenzyl)-2-(4-pentylphenyl)-1*H*-imidazole-5-carbaldehyde (41)

A mixture of amidine **39** (270 mg, 0.75 mmol) and (*E*)-2-bromo-3-isopropoxyacrylaldehyde (**40**) (174 mg, 0.90 mmol), 6 M aqueous K_2CO_3 (0.15 mL), and $CHCl_3$ (7 mL) was stirred at room temperature for 48 h. The mixture was diluted with saturated aqueus NaCl and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated. The residue was treated with dichloromethane to give crystalline product **41** (54 mg, 18%) which was collected by filtration. An additional amount of intermediate **41** (130 mg, 42%) was isolated from the mother liquid by radial chromatography on silica gel (eluent, CH_2Cl_2/MeOH = 1:0 to 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.70 (s, 1H), 7.92 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.25–7.21 (m, 2H), 6.84–6.82 (m, 2H), 5.56 (s, 2H), 2.70–2.52 (m, 2H), 1.71–1.50 (m, 2H), 1.41–1.13 (m, 4H), 0.95–0.74 (m, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 186.35, 178.70, 155.08, 145.68, 144.25, 135.98, 132.37, 131.90, 128.84, 127.75, 125.88, 121.58, 48.98, 35.72, 31.35, 30.80, 22.46, 13.97.

1-((4′-Methoxy-[1,1′-biphenyl]-4-yl)methyl)-2-(4-pentylphenyl)-1*H*-imidazole-5-carbaldehyde (42)

Intermediate **41** (135 mg, 0.33 mmol), 4-MeO-phenylboronic acid (75 mg, 0.49 mmol), $Pd(PPh_3)_4$ (38 mg, 10 mol%), 2 M aqueous K_2CO_3 (0.82 mL), and THF (5 mL) was heated at 90°C for 14 h. The mixture was cooled to room temperature and solvents evaporated. The residue was purified by flash chromatography on silica gel (eluent, petroleum ether/EtOAc, 1:1 to 0:1) to give intermediate **42** (106 mg, 74% yield) as colorless solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 9.72 (s, 1H), 7.94 (s, 1H), 7.53–7.42 (m, 6H), 7.23 (d, *J* = 6.4 Hz, 2H), 7.00 (d, *J* = 8.2 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 2H), 5.66 (s, 2H), 3.82 (s, 3H), 2.69–2.55 (m, 2H), 1.67–1.54 (m, 2H), 1.39–1.23 (m, 4H), 0.92–0.81 (m, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 178.71, 159.20, 155.08, 145.51, 144.12, 140.11, 135.30, 132.96, 132.15, 129.07, 128.89, 127.99, 127.01, 126.38, 126.09, 114.19, 55.29, 49.27, 35.73, 31.36, 30.81, 22.47, 13.98.

N-Ethyl-*N*-((1-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-2-(4-pentylphenyl)-1*H*-imidazol-5-yl)methyl)ethanamine (2c)

To the solution of aldehyde **42** (93 mg, 0.21 mmol) and diethylamine (24 µL, 0.23 mmol) in THF (10 mL) 4 (Å) molecular sieves were added and the mixture was cooled in an ice bath. To this, $NaBH(OAc)_4$ (67 mg, 0.225 mmol) and AcOH (48 µL, 0.84 mmol) were added and the mixture was stirred at room temperature for 24 h. Saturated aqueous $NaHCO_3$ was added and the mixture was extracted with EtOAc. The organic phase was separated and dried over Na_2SO_4. The solvent was then removed *in vacuo* and the residue was purified by column chromatography (EtOAc/MeOH = 1:0 to 1:4) to yield product **2c** (30 mg, 28% yield) as colorless solid. ^{1}H NMR (400 MHz, chloroform-*d*) δ: 7.53–7.42 (m, 6H), 7.15 (d, *J* = 8.3 Hz, 2H), 7.05 (s, 1H), 6.99 (d, *J* = 8.3 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 5.51 (s, 2H), 3.83 (s, 3H), 3.34 (s, 2H), 2.60–2.55 (m, 2H), 2.45 (q, *J* = 7.1 Hz, 4H), 1.65–1.51 (m, 2H), 1.37–1.20 (m, 4H), 0.90 (t, *J* = 7.1 Hz, 6H), 0.86 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 159.19, 149.34, 143.51, 139.62, 136.32, 132.98, 129.91, 129.13, 128.53, 128.47, 128.18, 127.94, 126.96, 125.97, 114.22, 55.31, 47.69, 47.40, 46.25, 35.63, 31.38, 30.90, 22.48, 13.99, 11.40.

N-Ethyl-*N*-((4-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-5-(4-pentylphenyl)isoxazol-3-yl)methyl)ethanamine (2b)

The title compound was obtained as a light yellow foam (15 mg, 55% yield) from amide **37** (55 mg, 0.11 mmol, 1.0 equiv) and BH_3–THF complex (1.0 M solution in THF, 0.25 mL, 0.25 mmol, 2.3 equiv) by following general procedure D. Pure material was obtained by column chromatography on silica gel (10 g silica gel) using gradient elution from 10% EtOAc in hexanes to 100% EtOAc in hexanes. The oily residue was converted to hydrochloric acid salt using 2 M HCl in Et_2O. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 13.15–13.00 (1H, br s), 7.53–7.43 (6H, m), 7.29–7.24 (2H, m), 7.17–7.11 (2H, m), 6.99–6.94 (2H, m), 4.18 (4H, s), 3.85 (3H, s), 3.19–3.09 (4H, m), 2.64 (2H, t, *J* = 7.7 Hz), 1.63 (2H, quintet, *J* = 7.5 Hz), 1.47 (6H, t, *J* = 7.2 Hz), 1.39–1.26 (4H, m), 0.89 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 164.1, 159.4, 158.2, 145.6, 139.7, 136.2, 133.0, 129.2, 128.7, 128.4, 128.1, 127.3, 125.2, 119.9, 114.4, 55.5, 48.1, 43.9, 35.9, 31.6, 31.0, 27.8, 22.6, 14.2, 9.5.

4,4,5,5-Tetramethyl-2-(4-pentylphenyl)-1,3,2-dioxaborolane (43)

An oven-dried pressure tube (100 mL) was cooled under a stream of argon and charged with 1-bromo-4-pentylbenzene (500 mg, 2.20 mmol, 1.0 equiv), bis(pinacolato)diborane (670 mg, 2.64 mmol, 1.2 equiv), potassium acetate (1.08 g, 11.0 mmol, 5.0 equiv), Pd(dppf)$Cl_2 \cdot CH_2Cl_2$ (18 mg, 0.022 mmol, 1 mol%), and anhydrous 1,4-dioxane (15 mL). After stirring at 100°C for 18 h, the brown suspension was partitioned between water (15 mL) and EtOAc (15 mL). Layers were separated and organic layer was washed with brine (20 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (50 g silica gel) using gradient elution from 2% EtOAc in hexanes to 20% EtOAc in hexanes to yield product **43** (480 mg, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.75–7.70 (2H, m), 7.21–7.17 (2H, m), 2.61 (2H, t, *J* = 7.7 Hz), 1.62 (2H, quintet, *J* = 7.5 Hz), 1.34 (16H, m), 0.88 (3H, t, *J* = 6.9 Hz).

1-(*tert*-Butyl) 2-ethyl 5-bromo-1*H*-pyrrole-1,2-dicarboxylate (44)

To a cooled (0°C, crushed ice) solution of ethyl 1*H*-pyrrole-2-carboxylate (2.00 g, 14.4 mmol, 1.0 equiv) in a 2:1 (v/v) mixture of

anhydrous THF and MeOH (90 mL) was added NBS (3.30 g, 18.4 mmol, 1.3 equiv) in four portions over a 3 h period. Following the addition of the last portion of NBS, the resulting dark brown solution was stirred at room temperature for 30 min. All volatiles were removed under reduced pressure and the residue was purified initially by column chromatography on silica gel (70 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 15% EtOAc in petroleum ether, and then by the reversed-phase column chromatography (120 g of RP-18 silica gel) using gradient elution from 0% MeCN in water to 100% MeCN in water. 5-Bromopyrrole was obtained as a light yellow solid material (1.04 g, 33% yield), which was used in a subsequent step without purification. Thus, to the solution of the brominated pyrrole from above (1.04 g, 4.8 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (30 mL) were added DMAP (146 mg, 1.2 mmol, 0.25 equiv), di-*tert*-butyl dicarbonate (3.13 g, 14.4 mmol, 3.0 equiv) and triethylamine (3.3 mL, 24.0 mmol, 5.0 equiv). The resulting light yellow solution was stirred at room temperature for 16 h, then pH of the reaction mixture was adjusted to 5 with aqueous 5% $KHSO_4$ solution and product was extracted with CH_2Cl_2 (3 × 30 mL). Combined organic extracts were dried over Na_2SO_4, evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel (50 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 12.5% EtOAc in petroleum ether to yield Boc-protected pyrrole **44** (1.20 g, 79% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 6.82 (1H, d, *J* = 3.9 Hz), 6.22 (1H, d, *J* = 3.9 Hz), 4.30 (2H, q, *J* = 7.1 Hz), 1.63 (9H, s), 1.33 (3H, t, *J* = 7.1 Hz).

Ethyl 5-(4-pentylphenyl)-1*H*-pyrrole-2-carboxylate (45)

A pressure tube (100 mL) was charged with pinacolyl boronate **43** (400 mg, 1.46 mmol, 1.0 equiv), pyrrole **44** (557 mg, 1.75 mmol, 1.2 equiv), $Pd(PPh_3)_4$ (169 mg, 0.146 mmol, 10 mol%), and aqueous 2 M Na_2CO_3 solution (2.9 mL) was added, followed by a 1:1 (v/v) mixture of anhydrous *i*-PrOH and toluene (10 mL). After stirring for 16 h at 90°C, the orange suspension was diluted with water (20 mL) and EtOAc (15 mL). Layers were separated and aqueous layer was extracted with EtOAc (3 × 30 mL). Combined organic extracts were washed with brine (70 mL), dried over Na_2SO_4, and evaporated under reduced pressure. According to LC-MS analysis, the residue contained a mixture of *N*-Boc-protected pyrrole **45**-Boc and the desired product **45**. To achieve complete cleavage of the *N*-Boc protecting group, 4 M HCl solution in 1,4-dioxane (3 mL) was added to the mixture above and the light yellow solution was stirred at room temperature for 4 h. Additional amount (3 mL) of 4 M HCl solution in 1,4-dioxane was added and stirring was continued at 50°C for 2 h. The solution was cooled to room temperature and pH was adjusted to 8 with saturated $NaHCO_3$ solution (Caution! Intense gas evolution). The aqueous layer was extracted with EtOAc (3 × 30 mL). Combined organic extracts were washed with brine (70 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (30 g silica gel) using gradient elution from 10% EtOAc in hexanes to 50% EtOAc in hexanes to yield pyrrole **45** (155 mg, 37% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 9.21 (1H, s), 7.50–7.44 (2H, m), 7.24–7.20 (2H, m), 6.95 (1H, dd, *J* = 3.9, 2.4 Hz), 6.50 (1H, dd, *J* = 3.9, 2.7 Hz), 4.34 (2H, q, *J* = 7.1 Hz), 2.62 (2H, t, *J* = 7.7 Hz), 1.63 (2H, quintet, *J* = 7.6 Hz), 1.41–1.28 (7H, m), 0.90 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 161.4, 143.0, 137.0, 129.2, 128.9, 124.8, 123.2, 116.8, 107.7, 60.5, 35.8, 31.6, 31.2, 22.7, 14.7, 14.2. HRMS-ESI (*m*/*z*) calcd. for $C_{18}H_{24}NO_2$ $[M+H]^+$ 286.1807. Found 286.1813.

Ethyl 1-(4-bromobenzyl)-5-(4-pentylphenyl)-1*H*-pyrrole-2-carboxylate (46)

An oven-dried flask was cooled under a stream of argon and charged with NaH (60% in mineral oil; 33 mg, 0.81 mmol, 1.5 equiv). The mineral oil was removed by washing of NaH with anhydrous diethyl ether (3 × 2 mL). Anhydrous DMF (2 mL) was then added to the dry NaH and the resulting suspension was cooled to 0°C (crushed ice). A solution of pyrrole **45** (155 mg, 0.54 mmol, 1 equiv) in anhydrous DMF (3 mL) was dropwise added to the suspension of NaH in DMF (2 mL). After stirring at 0°C for 30 min, a solution of 1-bromo-4-(bromomethyl)benzene (135 mg, 0.54 mmol, 1.0 equiv) in DMF (3 mL) was added. The resulting light yellow solution was stirred at room temperature for 16 h whereupon it was quenched with H_2O (15 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (30 g silica gel) using gradient elution from 10% EtOAc in hexanes to 50% EtOAc in hexanes to yield pyrrole **46** (165 mg, 67% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.38–7.33 (2H, m), 7.21–7.14 (4H, m), 7.12 (1H, d, *J* = 4.0 Hz), 6.77–6.72 (2H, m), 6.27 (1H, d, *J* = 4.0 Hz), 5.54 (2H, s), 4.20 (2H, q, *J* = 7.1 Hz), 2.61 (2H, t, *J* = 7.8 Hz), 1.62 (2H, quintet, *J* = 7.6 Hz), 1.40–1.25 (7H, m), 0.90 (3H, t, *J* = 6.8 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 161.1, 143.5, 142.3, 138.7, 131.7, 129.3, 128.7, 127.6, 123.0, 120.7, 118.7, 109.8, 60.0, 48.8, 35.8, 31.6, 31.1, 22.7, 14.5, 14.2.

Ethyl 1-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-5-(4-pentylphenyl)-1*H*-pyrrole-2-carboxylate (47)

The title compound was obtained as a white solid material (50 mg, 52% yield) from (4-methoxyphenyl)boronic acid (32 mg, 0.21 mmol, 1.05 equiv), pyrrole **46** (89 mg, 0.20 mmol, 1 equiv), and $Pd(PPh_3)_4$ (6.9 mg, 0.006 mmol, 3 mol%) by following general procedure A. Pure material was obtained by column chromatography on silica gel (20 g silica gel) using gradient elution from 10% EtOAc in petroleum ether to 50% EtOAc in petroleum ether. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.51–7.45 (2H, m), 7.45–7.40 (2H, m), 7.28–7.23 (2H, m), 7.19–7.15 (2H, m), 7.15 (1H, d, *J* = 3.9 Hz), 6.97–6.94 (2H, m), 6.94–6.90 (2H, m), 6.29 (1H, d, *J* = 3.9 Hz), 5.64 (2H, s), 4.21 (2H, q, *J* = 7.1 Hz), 3.84 (3H, s), 2.61 (2H, t, *J* = 7.7 Hz), 1.62 (2H, quintet, *J* = 7.3 Hz), 1.40–1.30 (4H, m), 1.28 (3H, t, *J* = 7.1 Hz), 0.90 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 161.0, 159.0, 143.1, 142.2, 139.1, 138.0, 133.4, 129.4, 129.2, 128.5, 127.9, 126.7, 126.0, 123.0, 118.5, 114.1, 109.5, 59.8, 55.3, 48.9, 35.6, 31.5, 31, 22.5, 14.3, 14.0. HRMS-ESI (*m*/*z*) calcd. for $C_{32}H_{36}NO_3$ $[M+H]^+$ 482.2695. Found 482.2673.

N-Ethyl-*N*-((1-((4′-methoxy-[1,1′-biphenyl]-4-yl)methyl)-5-(4-pentylphenyl)-1*H*-pyrrol-2-yl)methyl)ethanamine (2d)

$LiAlH_4$ (1.0 M solution in THF, 100 µL, 0.1 mmol, 1.0 equiv) was added dropwise to a cooled solution (0°C, crushed ice) of carboxylic acid ester **47** (48 mg, 0.10 mmol, 1 equiv) in anhydrous THF (2 mL). After stirring at room temperature for 2 h, an additional amount of $LiAlH_4$ (1.0 M solution in THF, 100 µL, 0.1 mmol, 1.0 equiv) was added. After stirring at room temperature for 1 h, the white suspension was cooled to 0°C and quenched by sequential (within intervals of 10 min) addition of water (8 µL), aqueous 4 M NaOH solution (16 µL), and more water (24 µL). Ten minutes after the addition of the final amount of water, the white suspension was filtered. The filter cake was washed with EtOAc (10 mL). The filtrate was evaporated to dryness to yield 40 mg (91% yield) of alcohol as a white solid material, which was used in a subsequent step without purification. Thus to the solution of alcohol from above (34 mg, 0.08 mmol, 1 equiv) in anhydrous CH_2Cl_2 (2 mL) was added triethylamine (20 µL, 0.15 mmol, 1.9 equiv). The resulting colorless solution was cooled to 0°C and methanesulfonyl chloride (9 µL, 0.12 mmol, 1.5 equiv) was added dropwise. After stirring at 0°C for 1 h, diethylamine (41 µg, 0.40 mmol, 5.0 equiv) was added to the light yellow solution. The stirring was continued at room temperature for 16 h, then the yellow solution was diluted with water (5 mL) and extracted with EtOAc (3 × 5 mL). Combined extracts were washed with brine (10 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (20 g silica gel) using gradient elution from 15% EtOAc in hexanes to 100% EtOAc to yield amine **2d** (20 mg, 50% yield) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.55–7.49 (2H, m), 7.48–7.44 (2H, m), 7.30–7.23 (2H, m), 7.15–7.10 (2H, m), 6.99–6.96 (2H, m), 6.96–6.92 (2H, m), 6.22 (1H, d, *J* = 3.4 Hz), 6.16 (1H, d, *J* = 3.4 Hz), 5.46 (2H, s), 3.85 (3H, s), 3.37 (2H, s), 2.58 (2H, t, *J* = 7.7 Hz), 2.49 (4H, q, *J* = 7.1 Hz), 1.61 (2H, quintet, *J* = 7.6 Hz), 1.39–1.27 (4H, m), 0.94 (6H, t, *J* = 7.1 Hz), 0.90 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ 159.2, 141.6, 139.2, 138.6, 136, 133.5, 131.3, 131.2, 128.8, 128.5, 128.1, 126.9, 126.2, 114.3, 109.9, 107.4, 55.5, 50.4, 47.3, 46.6, 35.7, 31.7, 31.2, 22.7, 14.2, 11.6; HRMS-ESI (*m/z*) calcd. for $C_{34}H_{43}N_2O$ $[M+H]^+$ 495.3375. Found 495.3376.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

[1] WHO, World Malar. Rep. 2017 World Health Organ. Geneva **2017** http://www.who.int/malaria/publications/world-malaria-report-2017/report/en/.
[2] T. N. C. Wells, R. H. van Huijsduijnen, W. C. Van Voorhis, *Nat. Rev. Drug Discov.* **2015**, *14*, 424.
[3] T. N. C. Wells, P. L. Alonso, W. E. Gutteridge, *Nat. Rev. Drug Discov.* **2009**, *8*, 879.
[4] J. E. Hyde, *FEBS J.* **2007**, *274*, 4688.
[5] S. Martin, *Arch. Pharm.* **2008**, *341*, 149.
[6] K. Ersmark, B. Samuelsson, A. Hallberg, *Med. Res. Rev.* **2006**, *26*, 626.
[7] M. J. Meyer, D. E. Goldberg, *Curr. Top. Med. Chem.* **2012**, *12*, 445.
[8] D. Edgar, *FEBS J.* **2017**, *284*, 2604.
[9] J. A. Bonilla, T. D. Bonilla, C. A. Yowell, H. Fujioka, J. B. Dame, *Mol. Microbiol.* **2007**, *65*, 64.
[10] J. A. Bonilla, P. A. Moura, T. D. Bonilla, C. A. Yowell, D. A. Fidock, J. B. Dame, *Int. J. Parasitol.* **2007**, *37*, 317.
[11] P. A. Moura, J. B. Dame, D. A. Fidock, *Antimicrob. Agents Chemother.* **2009**, *53*, 4968.
[12] A. N. Hodder, B. E. Sleebs, P. E. Czabotar, M. Gazdik, Y. Xu, M. T. O'Neill, S. Lopaticki, T. Nebl, T. Triglia, B. J. Smith, K. Lowes, J. A. Boddey, A. F. Cowman, *Nat. Struct. Mol. Biol.* **2015**, *22*, 590.
[13] B. E. Sleebs, M. Gazdik, M. T. O'Neill, P. Rajasekaran, S. Lopaticki, K. Lackovic, K. Lowes, B. J. Smith, A. F. Cowman, J. A. Boddey, *J. Med. Chem.* **2014**, *57*, 7644.
[14] C.-L. Ciana, R. Siegrist, H. Aissaoui, L. Marx, S. Racine, S. Meyer, C. Binkert, R. de Kanter, C. Fischli, S. Wittlin, C. Boss, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 658.
[15] K. Jaudzems, K. Tars, G. Maurops, N. Ivdra, M. Otikovs, J. Leitans, I. Kanepe-Lapsa, I. Domraceva, I. Mutule, P. Trapencieris, M. J. Blackman, A. Jirgensons, *ACS Med. Chem. Lett.* **2014**, *5*, 373.
[16] P. Pino, R. Caldelari, B. Mukherjee, J. Vahokoski, N. Klages, B. Maco, C. R. Collins, M. J. Blackman, I. Kursula, V. Heussler, M. Brochet, D. Soldati-Favre, *Science* **2017**, *358*, 522.
[17] A. S. Nasamu, S. Glushakova, I. Russo, B. Vaupel, A. Oksman, A. S. Kim, D. H. Fremont, N. Tolia, J. R. Beck, M. J. Meyers, J. C. Niles, J. Zimmerberg, D. E. Goldberg, *Science* **2017**, *358*, 518.
[18] C. Boss, S. Richard-Bildstein, T. Weller, W. Fischli, S. Meyer, C. Binkert, *Curr. Med. Chem.* **2003**, *10*, 883.
[19] C. Boss, O. Corminboeuf, C. Grisostomi, S. Meyer, A. F. Jones, L. Prade, C. Binkert, W. Fischli, T. Weller, D. Bur, *ChemMedChem* **2006**, *1*, 1341.
[20] L. Prade, A. F. Jones, C. Boss, S. Richard-Bildstein, S. Meyer, C. Binkert, D. Bur, *J. Biol. Chem.* **2005**, *280*, 23837.
[21] A. P. Huizing, M. Mondal, A. K. H. Hirsch, *J. Med. Chem.* **2015**, *58*, 5151.
[22] F. Hof, A. Schütz, C. Fäh, S. Meyer, D. Bur, J. Liu, D. E. Goldberg, F. Diederich, *Angew. Chem. Int. Ed.* **2006**, *45*, 2138.
[23] M. Zürcher, T. Gottschalk, S. Meyer, D. Bur, F. Diederich, *ChemMedChem* **2008**, *3*, 237.
[24] D. Rasina, M. Otikovs, J. Leitans, R. Recacha, O. V. Borysov, I. Kanepe-Lapsa, I. Domraceva, T. Pantelejevs, K. Tars, M. J. Blackman, K. Jaudzems, A. Jirgensons, *J. Med. Chem.* **2016**, *59*, 374.
[25] B. Testa, J. M. Maye, *Hydrolysis in Drug and Prodrug Metabolism*. Verlag Helvetica Chimica Acta AG, Zürich **2006**, Ch. 4.
[26] I. Mohammed, I. R. Kummetha, G. Singh, N. Sharova, G. Lichinchi, J. Dang, M. Stevenson, T. M. Rana, *J. Med. Chem.* **2016**, *59*, 7677.
[27] A. Proteau-Gagné, K. Rochon, M. Roy, P.-J. Albert, B. Guérin, L. Gendron, Y. L. Dory, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5267.
[28] S. Paek, C. Baik, M.-S. Kang, H. Kang, *J. Organomet. Chem.* **2010**, *695*, 821.
[29] R. B. Bedford, M. F. Haddow, C. J. Mitchell, R. L. Webster, *Angew. Chem. Int. Ed.* **2011**, *50*, 5524.
[30] K. Barral, A. D. Moorhouse, J. E. Moses, *Org. Lett.* **2007**, *9*, 1809.
[31] K. A. Davies, R. C. Abel, J. E. Wulff, *J. Org. Chem.* **2009**, *74*, 3997.
[32] T. Hameury, J. Guillemont, L. Van Hijfte, V. Bellosta, J. Cossy, *Org. Lett.* **2009**, *11*, 2397.
[33] M. E. Jung, J. A. Hagenah, *J. Org. Chem.* **1987**, *52*, 1889.
[34] A. Suárez, G. C. Fu, *Angew. Chem. Int. Ed.* **2004**, *43*, 3580.

[35] B. C. Boren, S. Narayan, L. K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia, V. V. Fokin, *J. Am. Chem. Soc.* **2008**, *130*, 8923.
[36] D. Gray, C. Concellón, T. Gallagher, *J. Org. Chem.* **2004**, *69*, 4849.
[37] H. J. Finlay, J. Lloyd, M. Nyman, M. L. Conder, T. West, P. Levesque, K. Atwal, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2714.
[38] T. Pirali, R. Mossetti, S. Galli, G. C. Tron, *Org. Lett.* **2011**, *13*, 3734.
[39] S. Grecian, V. V. Fokin, *Angew. Chem. Int. Ed.* **2008**, *47*, 8285.
[40] S. C. Shilcrat, M. K. Mokhallalati, J. M. D. Fortunak, L. N. Pridgen, *J. Org. Chem.* **1997**, *62*, 8449.
[41] E. Harder, W. Damm, J. Maple, C. Wu, M. Reboul, J. Y. Xiang, L. Wang, D. Lupyan, M. K. Dahlgren, J. L. Knight, J. W. Kaus, D. S. Cerutti, G. Krilov, W. L. Jorgensen, R. Abel, R. A. Friesner, *J. Chem. Theory Comput.* **2016**, *12*, 281.
[42] A. Waterhouse, M. Bertoni, S. Bienert, G. Studer, G. Tauriello, R. Gumienny, F. T. Heer, T. A. P. de Beer, C. Rempfer, L. Bordoli, R. Lepore, T. Schwede, *Nucleic Acids Res.* **2018**, *46*, W296.
[43] C. D. Berweger, W. F. van Gunsteren, F. Müller-Plathe, *Chem. Phys. Lett.* **1995**, *232*, 429.
[44] H. Kamberaj, R. J. Low, M. P. Neal, *J. Chem. Phys.* **2005**, *122*, 224114.
[45] G. J. Martyna, M. E. Tuckerman, D. J. Tobias, M. L. Klein, *Mol. Phys.* **1996**, *87*, 1117.
[46] S. Nosé, *J. Chem. Phys.* **1984**, *81*, 511.
[47] W. G. Hoover, *Phys. Rev. A* **1985**, *31*, 1695.
[48] M. Tuckerman, B. J. Berne, G. J. Martyna, *J. Chem. Phys.* **1992**, *97*, 1990.
[49] H. Yang, Y. Li, M. Jiang, J. Wang, H. Fu, *Chem. Eur. J.* **2011**, *17*, 5652.
[50] M. Hu, J. Li, S. Q. Yao, *Org. Lett.* **2008**, *10*, 5529.
[51] V. Papoian, T. Minehan, *J. Org. Chem.* **2008**, *73*, 7376.
[52] L. P. Hernández-Eguía, R. J. Brea, L. Castedo, P. Ballester, J. R. Granja, *Chem. Eur. J.* **2011**, *17*, 1220.

SUPPORTING INFORMATION

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2. PIELIKUMS – "PEPTIDOMIMETIC PLASMEPSIN INHIBITORS WITH POTENT ANTI-MALARIAL ACTIVITY AND SELECTIVITY AGAINST CATHEPSIN D"

Zogota, R.; Kinena, L.; Withers-Martinez, C.; Blackman, M. J.; Bobrovs, R.; Pantelejevs, T.; Kanepe-Lapsa, I.; Ozola, V.; Jaudzems, K.; Suna, E.; Jirgensons, A.

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Research paper

Peptidomimetic plasmepsin inhibitors with potent anti-malarial activity and selectivity against cathepsin D

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ABSTRACT

Following up the open initiative of anti-malarial drug discovery, a GlaxoSmithKline (GSK) phenotypic screening hit was developed to generate hydroxyethylamine based plasmepsin (Plm) inhibitors exhibiting growth inhibition of the malaria parasite *Plasmodium falciparum* at nanomolar concentrations. Lead optimization studies were performed with the aim of improving Plm inhibition selectivity *versus* the related human aspartic protease cathepsin D (Cat D). Optimization studies were performed using Plm IV as a readily accessible model protein, the inhibition of which correlates with anti-malarial activity. Guided by sequence alignment of Plms and Cat D, selectivity-inducing structural motifs were modified in the S3 and S4 sub-pocket occupying substituents of the hydroxyethylamine inhibitors. This resulted in potent anti-malarials with an up to 50-fold Plm IV/Cat D selectivity factor. More detailed investigation of the mechanism of action of the selected compounds revealed that they inhibit maturation of the *P. falciparum* subtilisin-like protease SUB1, and also inhibit parasite egress from erythrocytes. Our results indicate that the anti-malarial activity of the compounds is linked to inhibition of the SUB1 maturase plasmepsin subtype Plm X.

1. Introduction

Malaria is a life-threatening disease caused by *Plasmodium* parasites which are transmitted by mosquitoes [1]. More than half of the earth's population lives in malaria endemic areas, rendering the disease a global health problem. Extensive eradication campaigns have been implemented, leading to considerably reduced malaria morbidity [2]. A key future goal, according to the Global Technical Strategy for Malaria 2016–2030, is a 90% reduction in clinical cases and deaths by 2030 as compared with 2015 [3]. However, these efforts are impeded by widespread resistance of the parasite to all currently used drugs, including artemisinins, the current front line drug class [4–6]. Consequently, new antimalarial drugs with new modes of action are urgently needed. Their development faces notable hurdles, one of which is a low expected profit after market approval. This has prompted several open innovation initiatives by private and academic organizations, including the disclosure of preclinical research data to the scientific community [7–10]. To support one such initiative, GlaxoSmithKline (GSK) recently published the results of a large-scale cell-based (phenotypic) HTS screening campaign that provided a number of starting points for anti-malarial drug discovery [7]. From the pool of parasite growth inhibitory compounds we selected hydroxyethylamine derivative **1a** for further development (Table 1) [11]. In our previous studies we showed that compound **1a** is an inhibitor of the *Plasmodium falciparum* aspartic proteases - plasmepsin subtypes Plm I, Plm II and Plm IV with particularly high potency against Plm IV. Structurally simplified potent Plm IV inhibitors **1b,c** were developed as compound **1a** analogues, retaining high potency in *P. falciparum* growth assays (see Table 1).

It is important to note that despite more than two decades of research on plasmepsin inhibitor discovery, only a few compounds (including inhibitors **1a-c**) exhibiting parasite growth inhibition at

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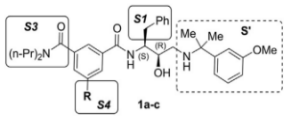
Table 1
Representative Plm inhibitors **1a-c** from previous studies [11]



Comp.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	EC_{50} *Pf* growth, μM
(*S,R*)-**1a**		0.029	0.043	0.002
(*S,R*)-**1b**		0.024	0.042	0.006
(*S,R*)-**1c**	Ph	0.006	0.054	0.002

low nanomolar concentration have been identified [11,12]. This is likely attributable to the fact that most of the efforts so far have been devoted to inhibiting plasmepsin subtypes involved in hemoglobin digestion (Plms I-IV) [13–22]. Gene disruption studies have revealed that none of these hemoglobinase Plms are essential in the parasite asexual blood stage lifecycle, indicating a high degree of redundancy in the hemoglobin catabolic pathway [23–25]. In contrast, the three other plasmepsin subtypes expressed in the *P. falciparum* blood stages, Plms V [26–28], IX, and X [29–32], all appear to be essential for parasite viability. The hemoglobinase plasmepsins (Plm I, II, IV) share high sequence homology with Plms IX and X, but not Plm V. It might therefore be expected that inhibitors developed to target the hemoglobinase Plms would exhibit activity in cell-based assays only if they additionally target Plms IX and/or Plm X. Recombinant expression of both Plms IX and X has been recently reported [29,30], but this could be achieved only in higher eukaryotic protein expression systems, such as insect or mammalian cells. For our further work to develop the hydroxyethylamine based inhibitors (*S,R*)-**1a-c** as anti-malarials we therefore used Plm IV as a readily accessible model plasmepsin, an approach also supported by the previously observed good correlation between inhibition of this enzyme and potency in parasite growth assays in erythrocytes [11,20].

The successful development of protease inhibitors as drugs requires optimization of on-target potency and minimization of undesirable off-target activity, particularly against related host proteases. The human lysosomal aspartic protease cathepsin D (Cat D) plays critical roles in protein catabolism and retinal function [33]. Recent work focused on development of inhibitors of the human aspartic protease β-secretase (BACE1) revealed the importance of ensuring selectivity against Cat D in order to avoid off-target ocular toxicity [34,35]. In view of this, we decided that the next step for optimization of the hydroxyethylamine based Plm inhibitors **1** should aim to improve their selectivity for their malarial target(s) over human Cat D.

2. Results and discussion

2.1. Structural factors determining the selectivity of inhibitor binding to Plms vs Cat D

Our previous SAR investigations revealed that the substituents of the inhibitor (*S,R*)-**1** occupying the prime sub-pockets (part S', Table 1) are optimal for Plm inhibition [11]. Therefore, we focused our efforts on optimisation of selectivity inducing motifs in the substituents occupying the non-prime sub-pockets (Table 1). To assess the differences in inhibitor recognition between Plms IV, IX, X and Cat D, we generated a structure-based sequence alignment of these proteins and compared their interactions with inhibitor **1b** in docking models (Fig. 1). Since Plm IX and X lack experimentally determined structures and in order to avoid possible inaccuracies associated with the use of homology models, the docking studies were performed on the crystal structure of Plm IV (PDB ID 2ANL), which is the closest homologue with an available crystal structure [36]. As can be seen from Fig. 1A, the S3 sub-pocket shows the largest differences in amino acid composition between the Plms and Cat D. Additionally, this revealed that the S3 sub-pocket of Plm IV is wider, more shallow and more hydrophobic than that of Cat D (Fig. 1B). For these reasons, selectivity improvement was first attempted by modifying the *N,N*-dipropylamide moiety in inhibitors (*S,R*)-**1** which occupies the S3 sub-pocket.

Several *N,N*-disubstituted amide analogues (*S,R*)-**2a,b,d-f** were synthesized (see Section 2.3) bearing hydrophobic groups that would prefer the more hydrophobic S3 sub-pocket of Plm IV. Unexpectedly the *N,N*-diethyl and *N,N*-dimethyl substituted analogues (*S,R*)-**2a** and (*S,R*)-**2b** showed the highest selectivity factor for Plm IV inhibition over Cat D, even though our docking studies indicated that the wider S3 sub-pocket of Plm IV could accommodate bulkier groups. Analogue (*S,R*)-**2c** bearing *N*-hydroxyethyl groups showed 3-fold weaker Plm IV inhibition potency than compound (*S,R*)-**2a**. Introduction of larger linear substituents such as *N,N*-dipropyl (compound (*S,R*)-**1b**), *N,N*-di(methoxyethyl) (compound (*S,R*)-**2d**) and *N,N*-di(3,3,3-trifluoropropyl) (compound (*S,R*)-**2e**) resulted in improved Cat D inhibition and correspondingly lower selectivity factors, suggesting that these groups fit well in the deep S3 sub-pocket of Cat D. Analogue (*S,R*)-**2f** bearing a *N,N*-diisobutyl amide showed poor inhibition of both enzymes, indicating that this group is too large to fit into the S3 sub-pocket of both Plm IV and Cat D.

We further explored *N*-monosubstituted amide analogues (*S,R*)-**3a-m** (see Section 2.3 for synthesis). The best compounds in this series showed similar Plm IV inhibition potency compared to the most potent *N,N*-disubstituted amides (*S,R*)-**2**. Gratifyingly, these appeared to be less potent Cat D inhibitors, leading to improved selectivity factors. The removal of one *N*-substituent was more beneficial for compounds with larger or branched substituents (e.g. (*S,R*)-**2e** and (*S,R*)-**2d** compared to (*S,R*)-**3d** and (*S,R*)-**3f**) while for compounds bearing smaller substituents a slight decrease in Plm IV inhibitory activity was observed e.g. (*S,R*)-**1b** (Table 2) compared to (*S,R*)-**1d** (Table 3) (see Fig. 2 for docking of compounds **2e** and **3d** into Plm IV and Cat D representing the difference of steric requirements). The docking studies suggested that the introduction of a hydrogen bond donor (inhibitors (*S,R*)-**3c,e,i,j**) or hydrogen bond acceptor ((*S,R*)-**3a,l,f**) group in the S3 sub-pocket substituents could potentially enable additional electrostatic interactions with electron-rich functional groups in this pocket. Hydrogen bond donor groups could interact with Asn13 and Leu14 backbone carbonyl in Plm IV; and Asp323, Tyr15, Gln14 and Ala13 in CatD, whereas for the hydrogen bond acceptors the most likely interactions are with Asn13 in Plm IV and Gln14 in Cat D (Fig. S1, see supporting information). Although the introduction of hydrogen bond donor or acceptor groups in the S3 sub-pocket occupying substituent reduced Plm IV inhibition activity (up to 2 times if comparing **1b** and **3a**), it also produced the most selective ligand in this series - (*S,R*)-**3a**, as the activity decrease for Cat D is even higher (up to 19 times if comparing (*S,R*)-**1b** and (*S,R*)-**3a**). The docking studies suggest that the relatively higher drop in activity against Cat D for the compounds bearing the hydrogen bond donor or acceptor groups is due to an unfilled hydrophobic sub-pocket (resulting in an entropic penalty of solvating the non-polar sub-pocket). That is, the position of the amide substituent remains the same in both

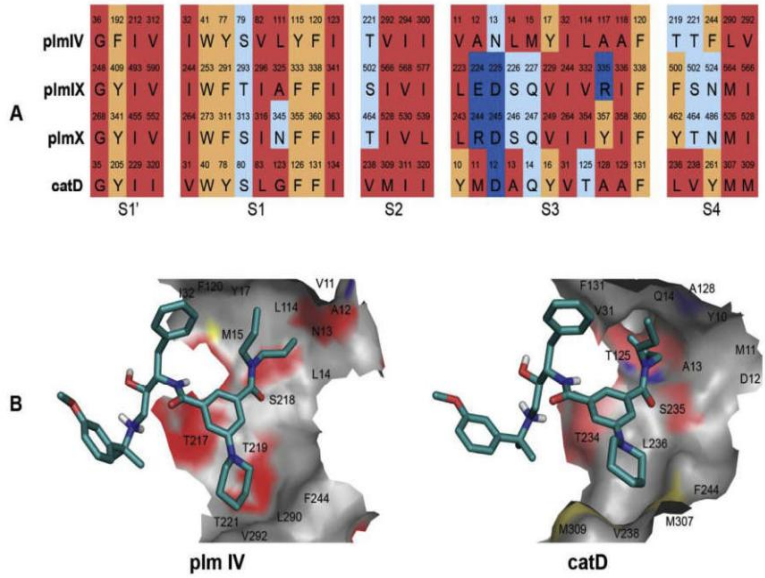
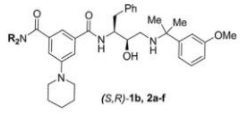


Fig. 1. (A) Structure-based sequence alignment of the amino acid residues making up the S1′ and S1–S4 pockets of Plm IV, Plm IX, Plm X and Cat D. (B) Surface representation of the S3 and S4 pockets in Plm IV and Cat D docking models with the hydroxyethylamine based inhibitor (*S,R*)-**1b**. The figure was prepared in PyMol [37]. Surface oxygen atoms are colored in red, nitrogens in blue, sulfurs in yellow and hydrogens and carbons in grey. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

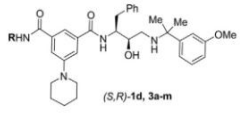
Table 2
SAR of *N,N*-disubstituted amide analogues (*S,R*)-**1b**, **2a-f**



Comp.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
(*S,R*)-**1b**	*n*-Pr	0.024[b]	0.042[b]	1.8
(*S,R*)-**2a**	Et	0.014	0.25	17.9
(*S,R*)-**2b**	Me	0.087	0.5	5.7
(*S,R*)-**2c**	$HOCH_2CH_2$	0.068	0.27	4.0
(*S,R*)-**2d**	$MeOCH_2CH_2$	0.037	0.10	2.7
(*S,R*)-**2e**	$CF_3CH_2CH_2$	0.21	0.12	0.57
(*S,R*)-**2f**	$(CH_3)_2CHCH_2$	0.5	1.3	2.6

[a] Selectivity factor of Plm IV/Cat D inhibition.
[b] Data from literature [11].

Table 3
SAR of *N*-mono-substituted amide analogues (*S,R*)-**1d**, **3a-m**



Comp.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
(*S,R*)-**1d**	n-Pr	0.038[b]	0.11[b]	2.9
(*S,R*)-**3a**	$MeOC(CH_3)_2CH_2$	0.048	2.1	43.8
(*S,R*)-**3b**	c-$PrCH_2$	0.030	0.76	25.3
(*S,R*)-**3c**	$HOCH_2CH_2CH_2$	0.093	2.25	24.2
(*S,R*)-**3d**	$CF_3CH_2CH_2$	0.024	0.58	24.2
(*S,R*)-**3e**	$HOC(CH_3)_2CH_2$	0.10	1.66	16.6
(*S,R*)-**3f**	$MeOCH_2CH_2$	0.05	0.75	15.0
(*S,R*)-**3g**	$Me_2NCH_2CH_2$	0.36	4.8	13.3
(*S,R*)-**3h**	t-$BuCH_2$	0.027	0.40	14.8
(*S,R*)-**3i**	$HOCH_2C(CH_3)_2$	0.12	1.46	12.2
(*S,R*)-**3j**	$HOCH_2CH_2$	0.21	1.42	6.8
(*S,R*)-**3k**	$PhCH_2$	0.038	0.22	5.8
(*S,R*)-**3l**	t-$BuOCH_2CH_2$	0.031	0.15	4.8
(*S,R*)-**3m**	c-$HexCH_2$	0.09	0.15	1.7

[a] Selectivity factor of Plm IV/Cat D inhibition.
[b] Data from literature [11].

enzymes due to additional interactions with aforementioned residues (Fig. 3), but by doing so it creates a situation where the Cat D hydrophobic sub-pocket is filled with water resulting in an entropic penalty and reduced Cat D inhibition activity.

Despite varying hydrogen bond donor and acceptor groups in the S3 sub-pocket filling substituents, docking studies also suggest that the main factor affecting Plm IV and Cat D inhibition potency is the size and shape of the substituent; branched and long substituents cannot fit into the narrow S3 recess of Cat D whereas the open S3 sub-pocket of Plm IV can accommodate such

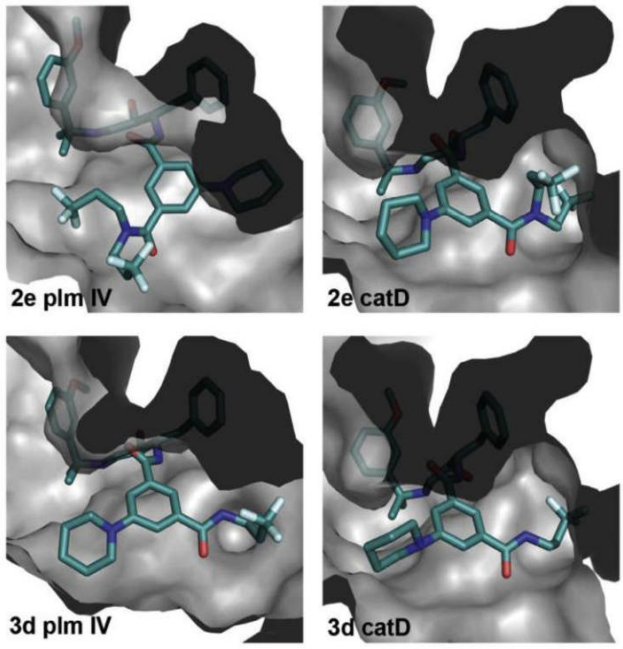


Fig. 2. Docking models of compounds (*S,R*)-**2e** and (*S,R*)-**3d** in crystal structures of Plm IV and Cat D.

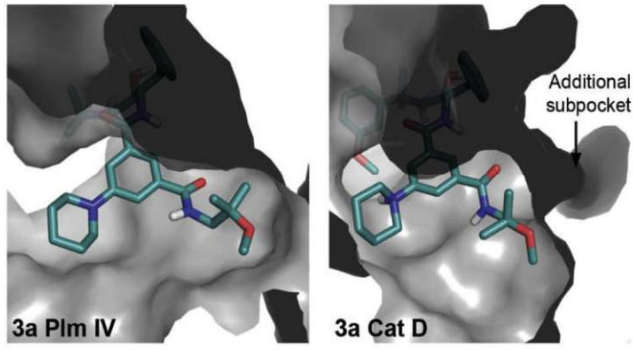


Fig. 3. Docking models of compound **3a** in complex with Plm IV and Cat D.

substituents. The weakest inhibitor of the *N*-mono-substituted amide analogues was compound (*S,R*)-**3g**. This could be explained by the protonated amine group as the amide substituent which, according to the docking studies, tends to form hydrogen bonds and ionic interactions with residues outside of the S3 sub-pocket. Altogether these results indicate that the selectivity against Cat D can be improved by targeting the S3 sub-pocket with mono-substituted amide moieties containing linear or branched hydrophobic groups.

The S4 sub-pocket is another inhibitor binding region which is

notably distinct between Plm IV and Cat D (Fig. 1A). Although predominantly hydrophobic in both enzymes, the S4 sub-pocket of Plm IV is flatter and more solvent exposed. Therefore, we investigated SAR for substituents occupying the S4 sub-pocket in the series of compounds (*S,R*)-**4a-g** (Table 4, see Section 2.3. for synthesis). Installation of an *N,N*-dipropylamide group (compound (*S,R*)-**4g**) resulted in practically unchanged Plm IV inhibitory activity compared to parent compound **1b**, however this was paralleled by a more than 15-fold drop in activity against Cat D resulting in improvement of the selectivity factor. Installation of fluorine or removal of the S4 filling substituent provided compounds **4b,c** with slightly decreased Plm IV inhibitory potency, whereas inhibition of Cat D was considerably lower which again improved the selectivity factor. Installation of chlorine in the benzene ring (compound **4d**) improved the activity for both Plm IV and Cat D. Cyano, methyl and trifluoromethyl groups in the S4 sub-pocket (compounds **4a,e-f**) made less difference for inhibitor binding to Plm IV and Cat D. Collectively, these effects of S4 occupying substituents are difficult to explain from our molecular modelling data, and presumably arise from an interplay of hydrophobic and polar non-covalent interactions.

Considering that the lack of an S4 occupying substituent considerably improves selectivity against Cat D, analogues (*S,R*)-**5a,b** of the most potent *N*-mono-substituted amide inhibitors (*S,R*)-**3d**,**h** were prepared and tested (Table 5, see Section 2.3. for synthesis). As expected, a slight drop in Plm IV inhibitory potency was observed; however the modifications appeared to be additive for a considerable improvement in the selectivity factor.

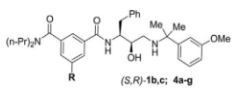
2.2. Relation of Plm subtype inhibition with *P. falciparum* growth inhibition

The capacity to inhibit growth in vitro of asexual blood stage *P. falciparum* was determined for selected compounds (*S,R*)-**2a**,**3a**,**b**,**d**,**h**,**4b**,**c**,**g**,**5a**,**b** (Table 6). In all cases, the compounds showed strong growth inhibitory potency with EC_{50} values in the low nanomolar range. Inhibitory activity against Plm I, Plm II and Plm IV was determined for the same compounds. This revealed no correlation between inhibitory potency in the parasite growth assay and inhibition of Plm I and II enzyme activity. In contrast, there was a much better correlation between parasite growth inhibition potency and Plm IV inhibition potency, although even here there were some notable exceptions; for instance, the most potent growth inhibitory compound, (*S,R*)-**4b,** was a 3-fold weaker inhibitor of Plm IV than compound (*S,R*)-**4g**, yet showed a 20-fold better inhibition of parasite growth than (*S,R*)-**4g**.

These results implied that the important parasite target(s) engaged by the growth inhibitory compounds are not the hemoglobinase plasmepsins. Recent reports have shown that inhibitors of the non-hemoglobinase plasmepsins Plm IX and Plm X (which are structurally similar to Plm I, II and IV) can potently block parasite replication [29,30]. A key biological function of Plm X is the proteolytic maturation of SUB1, a parasite subtilisin-like serine protease that plays an essential role in regulating parasite release (egress) from the infected host erythrocyte [38]. SUB1 maturation comprises 2 steps in which the initial ~82 kDa pre-proenzyme is cleaved to form first a 54 kDa protein (p54) then a 47 kDa terminal product (p47) which accumulates during the latter ~12 h of intra-erythrocytic parasite development. Whilst the first SUB1 processing step is autocatalytic, the second p54-to-p47 step is believed to be mediated by Plm X [29,30]. We used a Western blot-based assay to examine the effects of selected compounds (*S,R*)-**2a**, (*S,R*)-**4b** and (*S,R*)-**4c** on both SUB1 maturation and parasite egress (Fig. 4).

Egress was quantified by measuring the release of a soluble parasite protein called SERA5 into parasite culture supernatants. SERA5 is also a SUB1 substrate, and is generally released in a P50 form that results from SUB1-mediated cleavage of a larger precursor. Release of correctly processed SERA5 P50 is therefore an indicator of both SUB1 activity and efficiency of egress. As shown in Fig. 4A, treatment of developing intracellular parasites with the three Plm inhibitors (*S,R*)-**2a**, (*S,R*)-**4b** and (*S,R*)-**4c** resulted in a relative enrichment of the p54 form of SUB1, indicating inhibition of conversion of p54 to the terminal p47 form. Analysis of culture

Table 4
SAR of phenylgroup substitution in analogues **1b**,**c**, **4a-g**

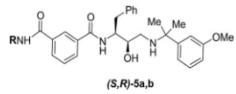


(*S,R*)-**1b,c; 4a-g**

Comp.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
(*S,R*)-**1b**	1-piperidinyl	0.024[b]	0.042[b]	1.8
(*S,R*)-**1c**	Ph	0.006[b]	0.054[b]	9.0
(*S,R*)-**4a**	Me	0.023	0.21	9.1
(*S,R*)-**4b**	H	0.058	1.15	19.8
(*S,R*)-**4c**	F	0.050	1.0	20
(*S,R*)-**4d**	Cl	0.008	0.096	12.0
(*S,R*)-**4e**	CF_3	0.015	0.067	4.5
(*S,R*)-**4f**	CN	0.059	0.56	9.5
(*S,R*)-**4g**	$(n\text{-}Pr)_2NC(=O)$	0.018	0.7	38.9

[a] Selectivity factor of Plm IV/Cat D inhibition.
[b] Data from literature [11].

Table 5
Combining selectivity inducing structural motives in analogues (*S,R*)-**5a,b**



(*S,R*)-**5a,b**

Comp.	R	IC_{50} Plm IV, μM	IC_{50} Cat D, μM	S[a]
(*S,R*)-**5a**	t-$BuCH_2$	0.076	3.8	50.0
(*S,R*)-**5b**	$CF_3CH_2CH_2$	0.15	4.9	32.6

[a] Selectivity factor of Plm IV/Cat D inhibition.

Table 6
Plm I, II, IV inhibition and *P. falciparum* growth inhibition activity of selected compounds.

Comp.	IC_{50} Plm I, μM	IC_{50} Plm II, μM	IC_{50} Plm IV, μM	S[a]	EC_{50}[b] *Pf* Growth, nM
(*S,R*)-**2a**	0.8	0.16	0.014	17.9	1.5
(*S,R*)-**3a**	7.4	5.4	0.048	43.8	2.0
(*S,R*)-**3b**	1.8	0.5	0.030	25.3	1.8
(*S,R*)-**3d**	2.5	2.2	0.024	24.2	2.0
(*S,R*)-**3h**	2.0	0.85	0.027	14.8	6.0
(*S,R*)-**4b**	3.1	1.7	0.058	19.8	0.3
(*S,R*)-**4c**	1.1	1.1	0.050	20	1.5
(*S,R*)-**4g**	0.78	0.27	0.018	38.9	6.0
(*S,R*)-**5a**	5.6	7.1	0.076	50.0	2.0
(*S,R*)-**5b**	10.3	10.4	0.15	32.6	6.0

[a] Selectivity factor of Plm IV/Cat D inhibition.
[b] The EC_{50} values for *P. falciparum* growth were determined using a SYBR Green-based assay with an incubation time of 96 h (2 erythrocytic cycles). Samples were each measured in triplicate, in 2 separate biological assays. Compound TCMDC-134674[11,20] was used as a positive control (see Supporting Information).

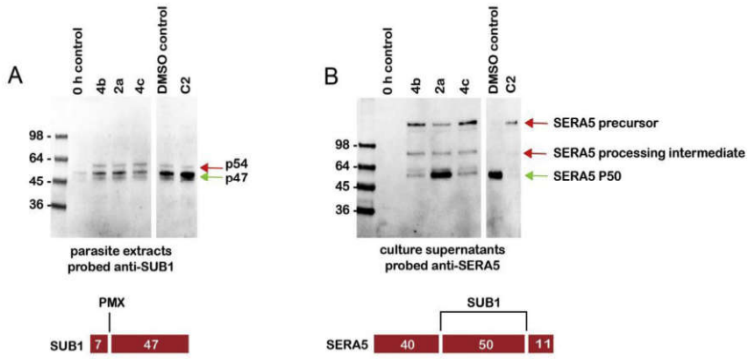


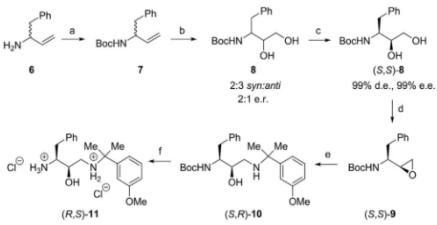
Fig. 4. Inhibition of *P. falciparum* SUB1 maturation and egress by selected compounds indicates that they target Plm X. (A) Synchronous cultures of immature intracellular parasites were treated for ~8 h with compounds (*S,R*)-**2a** or (*S,R*)-**4b,c** (10 nM), or vehicle only (DMSO, 1% v/v), or the cGMP-dependent protein kinase inhibitor (4-[7-[(dimethylamino) methyl]-2-(4-fluorphenyl)imidazo[1,2-α]pyridine-3-yl]pyrimidin-2-amine (compound 2; C2, 2 μM) which inhibits egress but not SUB1 maturation. Extracts of the parasites were then analyzed by Western blot, probing with an antibody to SUB1. The positions of migration of the SUB1 p54 and p47 forms (green arrow) are indicated. The schematic below indicates the mode by which Plm X converts SUB1 p54 to the terminal p47 form. (B) Parasites treated for ~24 h as in (A) were transferred to fresh medium containing the various compounds and allowed to undergo egress for 4 h before the culture supernatants were analysed by Western blot, probing with antibodies to the parasite protein SERA5. The positions of migration of the SERA5 precursor, a processing intermediate and the terminal P50 form (green arrow) are indicated. The schematic below indicates the mode by which SUB1 converts the SERA5 precursor to the P50 form. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

supernatants from treated parasites allowed to proceed to egress (Fig. 4B) showed that compounds (*S,R*)-**4b** and (*S,R*)-**4c** both produced a clear reduction in SERA5 P50 release, whilst all three compounds produced an increase in the release of SERA5 precursor or processing intermediates, indicating defects in egress and SERA5 processing. These results strongly suggest that the mechanism of parasite growth inhibition by compounds (*S,R*)-**4b** and (*S,R*)-**4c** (as well as possibly (*S,R*)-**2a**) involves Plm X inhibition, since the effects on egress were linked to inhibition of SUB1 maturation and its enzymatic activity against an endogenous substrate. Given their structural similarity, it is likely that the other compounds **3a,b,d,h**, **4g** and **5a,b** exerting potency in the parasite growth assay (Table 6) also target Plm X.

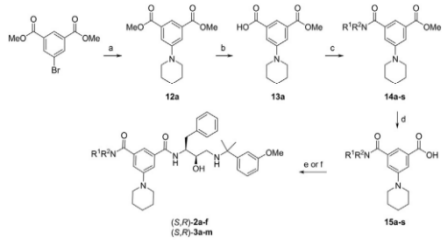
2.3. Synthesis of inhibitors (*S,R*)–**2-5**

Plm inhibitors (*S,R*)–**2-5** were synthesized from substituted benzoic acids **15**, **17** and **19** and either enantiomerically pure amino alcohol (*R,S*)–**11** or the corresponding racemate *rac*-**11** (Schemes 1–5). Inhibitors (*S,R*)–**2b-e**, (*S,R*)–**3a,c,e-g,i,j,l,m**, (*S,R*)–**4c,f,g** and (*S,R*)–**5a,b** were obtained from enantiomerically pure amino alcohol (*R,S*)–**11**, whereas targets **2a,f**, **3b,d,h,k** and **4a,b,d,e** were obtained as mixtures of stereoisomers from racemic alcohol *rac*-**11**. Enantiomerically pure inhibitors (*S,R*)–**2a,f**, (*S,R*)–**3b,d,h,k** and (*S,R*)–**4a,b,d,e** were obtained by separation of diastereomers using chromatography on silica gel, followed by separation of enantiomers using the chromatography on a chiral stationary phase.

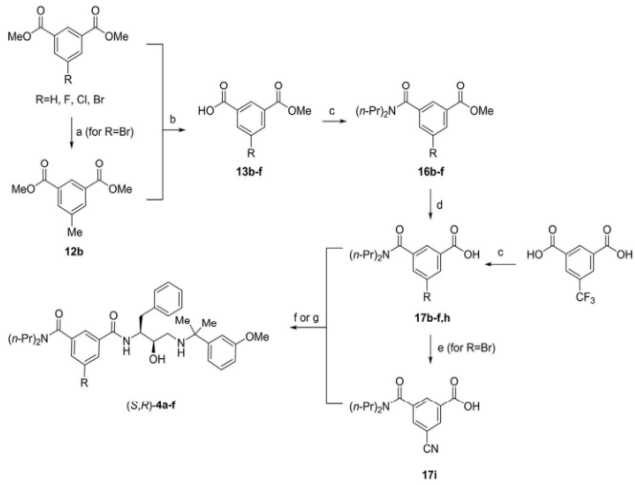
Synthesis of aminoalcohol **11** commenced with *N*-Boc protection of *rac*-**6** (Scheme 1). Dihydroxylation of the resulting carbamate *rac*–**7** with AD-mix-α afforded diol **8** as a 2:3 mixture of *syn:anti* diastereomers and a 2:1 mixture of enantiomers. Enantiomerically pure diol (*S,S*)–**8** was obtained by an initial separation



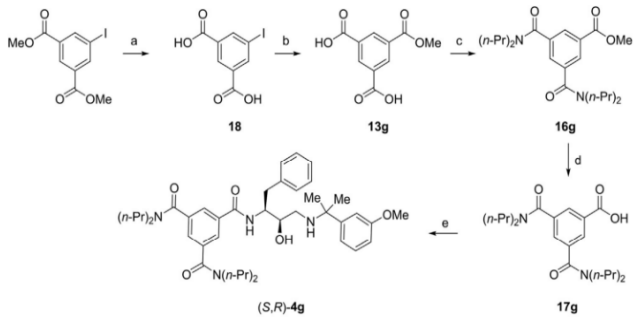
Scheme 1. Synthesis of amino alcohol intermediate (*R,S*)–**11**. Reagents and conditions: a) Boc_2O (1.25 equiv), NEt_3 (2 equiv), DCM, rt, 2 h, 87%. b) AD-mix-α (1 equiv), 1:1 (v/v) *t*-BuOH:water, rt, 20 h c) preparative HPLC on chiral stationary phase (*Chiralpak-ID*), 25% in two steps. d) Ph_3P (1.1 equiv), DEAD (1.1 equiv), $CHCl_3$, 85 °C, 48 h, 64%. e) 2-(3-Methoxyphenyl)propan-2-amine [11] (1.05 equiv), *i*-PrOH, 70 °C, 40 h, 73%. f) 4 M HCl in dioxane, rt, 6 h, 99%.



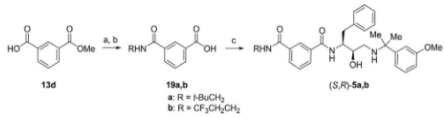
Scheme 2. Synthesis of Plm inhibitors (*S,R*)–**2a-f** and (*S,R*)–**3a-m**. Reagents and conditions: a) piperidine (1 equiv), $Pd(OAc)_2$ (5 mol%), *rac*-BINAP (5 mol%), Cs_2CO_3 (1.5 equiv), toluene, 100 °C, 18 h, 88%. b) General procedure A: aqueous 1 M NaOH (1 equiv), MeOH, rt, 16 h c) General procedure B: R^1R^2NH (1.2 equiv), HBTU (1 equiv), NEt_3 (2 equiv), DMF, rt, 2 h d) General procedure C: aqueous 1 M NaOH (1.5 equiv), MeOH, 50 °C, 18 h e) General procedure D: amino alcohol (*R,S*)–**11** (1.0 equiv), HBTU (1.0 equiv), NEt_3 (4 equiv), DMF, rt, 16 h f) amino alcohol *rac*–**11** (1.0 equiv), HBTU (1.0 equiv), NEt_3 (4 equiv), DMF, rt, 16 h; then separation of enantiomers by HPLC on chiral stationary phase (*Chiralpak-ID*).



Scheme 3. Synthesis of Plm inhibitors (*S,R*)–**4a-f**. Reagents and conditions: a) $MeB(OH)_2$ (1.2 equiv), $Pd(dppf)Cl_2xCH_2Cl_2$ (5 mol%), K_3PO_4 (3 equiv), toluene, 90 °C, 18 h b) General procedure A: aqueous 1 M NaOH (1 equiv), MeOH, rt, 16 h c) General procedure B: $(n\text{-}Pr)_2NH$ (1.2 equiv), HBTU (1 equiv), NEt_3 (2 equiv), DMF, rt, 2 h d) General procedure C: aqueous 1 M NaOH (1.5 equiv), MeOH, 50 °C, 18 h e) CuCN (2 equiv), NMP, 160 °C, 6 h f) General procedure D: amino alcohol (*R,S*)–**11** (1.0 equiv), HBTU (1.0 equiv), NEt_3 (4 equiv), DMF, rt, 16 h g) amino alcohol *rac*–**11** (1.0 equiv), HBTU (1.0 equiv), NEt_3 (4 equiv), DMF, rt, 16 h; then separation of enantiomers by HPLC on chiral stationary phase (*Chiralpak-ID*).



Scheme 4. Synthesis of Plm inhibitor (*S,R*)–**4g**. Reagents and conditions: a) aqueous 1 N NaOH (3 equiv), MeOH, 40 °C, 18 h b) $Pd(dppf)Cl_2xCH_2Cl_2$ (10 mol%), NEt_3 (2.2 equiv), CO (70 psi), MeOH, 100 °C, 18 h c) General procedure B: $(n\text{-}Pr)_2NH$ (2.2 equiv), HBTU (2 equiv), NEt_3 (4 equiv), DMF, rt, 2 h d) General procedure C: aqueous 1 M NaOH (1.5 equiv), MeOH, 50 °C, 18 h e) General procedure D: amino alcohol (*R,S*)–**11** (1.0 equiv), HBTU (1.0 equiv), NEt_3 (4 equiv), DMF, rt, 16 h.



Scheme 5. Synthesis of Plm inhibitors **5a**,**b**. Reagents and conditions: a) General procedure B: $(n\text{-}Pr)_2NH$ (1.2 equiv), HBTU (1 equiv), NEt_3 (2 equiv), DMF, rt, 2 h b) General procedure C: aqueous 1 M NaOH (1.5 equiv), MeOH, 50 °C, 18 h c) General procedure D: amino alcohol (*R,S*)–**11** (1.0 equiv), HBTU (1.0 equiv), NEt_3 (4 equiv), DMF, rt, 16 h.

of *syn/anti* diastereomers using chromatography on silica gel, followed by separation of *syn*–**8** enantiomers by chromatography on a chiral stationary phase. The major enantiomer turned out to be the desired diol (*S,S*)–**8** as evidenced by comparison of optical rotation data with that from the literature [39]. Conversion of (*S,S*)–**8** into epoxide (*S,S*)–**9** under Mitsunobu conditions was followed by aminolysis with 2-(3-methoxyphenyl)propan-2-amine [11] to afford *N*-Boc protected amino alcohol (*S,R*)–**10**. Finally, the cleavage of *N*-Boc protecting group yielded amino alcohol (*R,S*)–**11**.

Benzoic acids **15a-s** were prepared from dimethyl 5-bromoisophthalate (Scheme 2). Pd-catalyzed amination with piperidine afforded **12a**, which was hydrolysed to isophthalic monoester **13a** under basic conditions. Subsequent HBTU-mediated condensation with amines afforded amides **14a-s**, which were

Table 7
Substitution pattern of compounds in Scheme 2.

R^1	R^2	No.	No.	No.	Comments
Et	Et	**14a**	**15a**	(*S*,*R*)-**2a**	from *rac*–**11** (step f)
Me	Me	**14b**	**15b**	(*S*,*R*)-**2b**	
$HOCH_2CH_2$	$HOCH_2CH_2$	**14c**	**15c**	(*S*,*R*)-**2c**	
$MeOCH_2CH_2$	$MeOCH_2CH_2$	**14d**	**15d**	(*S*,*R*)-**2d**	
$CF_3CH_2CH_2$	$CF_3CH_2CH_2$	**14e**	**15e**	(*S*,*R*)-**2e**	
$(CH_3)_2CHCH_2$	$(CH_3)_2CHCH_2$	**14f**	**15f**	(*S*,*R*)-**2f**	from *rac*–**11** (step f)
H	$MeOC(CH_3)_2CH_2$	**14g**	**15g**	(*S*,*R*)-**3a**	
H	c-$PrCH_2$	**14h**	**15h**	(*S*,*R*)-**3b**	from *rac*–**11** (step f)
H	$HOCH_2CH_2CH_2$	**14i**	**15i**	(*S*,*R*)-**3c**	
H	$CF_3CH_2CH_2$	**14j**	**15j**	(*S*,*R*)-**3d**	from *rac*–**11** (step f)
H	$HOC(CH_3)_2CH_2$	**14k**	**15k**	(*S*,*R*)-**3e**	
H	$MeOCH_2CH_2$	**14l**	**15l**	(*S*,*R*)-**3f**	
H	$Me_2NCH_2CH_2$	**14m**	**15m**	(*S*,*R*)-**3g**	
H	t-$BuCH_2$	**14n**	**15n**	(*S*,*R*)-**3h**	from *rac*–**11** (step f)
H	$HOCH_2C(CH_3)_2$	**14o**	**15o**	(*S*,*R*)-**3i**	
H	$HOCH_2CH_2$	**14p**	**15p**	(*S*,*R*)-**3j**	
H	$PhCH_2$	**14q**	**15q**	(*S*,*R*)-**3k**	from *rac*–**11** (step f)
H	t-$BuOCH_2CH_2$	**14r**	**15r**	(*S*,*R*)-**3l**	
H	c-$HexCH_2$	**14s**	**15s**	(*S*,*R*)-**3m**	

Table 8
Substitution pattern of compounds in Scheme 3.

R	No.	No.	No.	No.	No.	Comments
Me	**12b**	**13b**	**16b**	**17b**	(*S*,*R*)–**4a**	from *rac*–**11** (step g)
Br	-	**13c**	**16c**	**17c**	–	
H	-	**13d**	**16d**	**17d**	(*S*,*R*)–**4b**	from *rac*–**11** (step g)
F	-	**13e**	**16e**	**17e**	(*S*,*R*)–**4c**	
Cl	-	**13f**	**16f**	**17f**	(*S*,*R*)–**4d**	from *rac*–**11** (step g)
CF_3	-	-	-	**17h**	(*S*,*R*)–**4e**	from *rac*–**11** (step g)
CN	-	-	-	**17i**	(*S*,*R*)–**4f**	

hydrolysed to benzoic acids **15a-s**. (see Table 7)

Similar synthetic approach was also used for the preparation of acids **17b-i** (Scheme 3). Accordingly, monoesters **13c-f** were obtained from commercially available dimethyl isophthates. The synthesis of methyl ester **13b** required an initial Pd-catalyzed alkylation of dimethyl 5-bromoisophthalate with methylboronic acid, followed by hydrolysis of one of the two ester moieties. Benzoic acids **13b-f** were converted into amides **16b-f** and ester moieties were hydrolysed to afford acids **17b-f**. Benzoic acid **17h** was obtained directly from trifluoromethyl isophthalic acid and *n*-Pr_2NH in the presence of HBTU, whereas the synthesis of **17i** was accomplished by Cu(I)-catalyzed substitution of bromide in benzoic acid **17c** for cyano group (Scheme 3). (see Table 8)

Synthesis of benzoic acid **17g** (Scheme 4) commenced with hydrolysis of commercially available dimethyl iodo-isophthalate to the corresponding isophthalic acid **18**, followed by Pd-catalyzed methoxycarbonylation [40] to afford ester **13g**. Subsequent conversion to diamide **16g** in the presence of HBTU was followed by ester hydrolysis to form benzoic acid **17g** (Scheme 4). The amide bond formation-hydrolysis sequence was also used for the preparation of benzoic acids **19a,b** (Scheme 5).

3. Summary

The optimization of hydroxyethylamine based Plm inhibitors was performed with the aim of improving selectivity against the related human aspartic protease Cat D. The studies were performed using Plm IV as a readily accessible model protein, the inhibition of which was previously found to correlate with *Plasmodium falciparum* growth inhibition. Based on sequence alignment of Plm IV and Cat D, putative selectivity inducing structural motifs were sought in S3 and S4 sub-pocket-occupying substituents of the inhibitors. Installation of an S3 sub-pocket targeting mono-substituted amide moiety in compounds (*S*,*R*)-**3** containing linear or branched hydrophobic groups resulted in up to 40-fold selectivity (compound (*S*,*R*)-**3a**) against Cat D. Plm IV inhibitors (*S*,*R*)-**4b,c** with no substituents or fluorine targeting the S4 sub-pocket led to 20-fold selectivity against Cat D, though with some loss of Plm IV inhibition potency. Surprisingly, installation of amide as the S4 sub-pocket filling group in compound (*S*,*R*)-**4g** resulted in potent Plm IV inhibition with almost 40-fold selectivity against Cat D. Selectivity-inducing factors in S3 and S4 positions were additive as evidenced by compound (*S*,*R*)-**5a** (50-fold selectivity). Determination of *P. falciparum* growth inhibition potency for ten of the new Plm inhibitors showed them to display activities in the low nanomolar range. The potent anti-malarial activity did not correlate with the relatively weak inhibition of Plm I and II, whilst in contrast there was a much better correlation with Plm IV inhibition. More detailed investigation of the mechanism of action of the selected compounds showed that they interfered with parasite egress and maturation of the parasite serine protease SUB1, indicating a strong link between anti-malarial activity and inhibition of the non-hemoglobinase plasmepsin and SUB1 maturase Plm X. Future studies should clarify whether cooperative Plm IV and Plm X inhibition or only Plm X inhibition is necessary to achieve optimal anti-malarial activity.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2018.11.068.

References

[1] WHO, World Malar. Rep. 2016, World Health Organ, Geneva, 2016. http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/.
[2] T.N.C. Wells, R.H. van Huijsduijnen, W.C. Van Voorhis, Malaria medicines: a glass half full? Nat. Rev. Drug Discov. 14 (6) (2015) 424–442.
[3] WHO, http://www.Who.Int/Malaria/Publications/Atoz/9789241564991/En/.
[4] T.N.C. Wells, P.L. Alonso, W.E. Gutteridge, New medicines to improve control and contribute to the eradication of malaria, Nat. Rev. Drug Discov. 8 (11) (2009) 879–891.
[5] J.E. Hyde, Drug-resistant malaria – an insight, FEBS J. 274 (18) (2007) 4688–4698.
[6] A.M. Dondorp, F. Nosten, P. Yi, D. Das, A.P. Phyo, J. Tarning, K.M. Lwin, F. Ariey, W. Hanpithakpong, S.J. Lee, et al., Artemisinin resistance in Plasmodium falciparum malaria, N. Engl. J. Med. 361 (5) (2009) 455–467.
[7] F.-J. Gamo, L.M. Sanz, J. Vidal, C. de Cozar, E. Alvarez, J.-L. Lavandera, D.E. Vanderwall, D.V.S. Green, V. Kumar, S. Hasan, et al., Thousands of chemical starting points for antimalarial lead identification, Nature 465 (7296) (2010) 305–310.
[8] F. Calderón, D. Barros, J.M. Bueno, J.M. Coterón, E. Fernández, F.J. Gamo, J.L. Lavandera, M.L. León, S.J.F. Macdonald, A. Mallo, et al., An invitation to open innovation in malaria drug discovery: 47 quality starting points from the TCAMS, ACS Med. Chem. Lett. 2 (10) (2011) 741–746.
[9] T.N.C. Wells, P. Willis, J.N. Burrows, R.H. van Huijsduijnen, Open data in drug discovery and development: lessons from malaria, Nat. Rev. Drug Discov. 15 (2016) 661.
[10] M. Pieroni, E. Azzali, N. Basilico, S. Parapini, M. Zolkiewski, C. Beato, G. Annunziato, A. Bruno, F. Vacondio, G. Costantino, Accepting the invitation to open innovation in malaria drug discovery: synthesis, biological evaluation, and investigation on the structure–activity relationships of benzo[b]thiophene-2-carboxamides as antimalarial agents, J. Med. Chem. 60 (5) (2017) 1959–1970.
[11] K. Jaudzems, K. Tars, G. Maurops, N. Ivdra, M. Otikovs, J. Leitans, I. Kanepe-Lapsa, I. Domraceva, I. Mutule, P. Trapencieris, et al., Plasmepsin inhibitory activity and structure-guided optimization of a potent hydroxyethylamine-based antimalarial hit, ACS Med. Chem. Lett. 5 (4) (2014) 373–377.
[12] C.-L. Ciana, R. Siegrist, H. Aissaoui, L. Marx, S. Racine, S. Meyer, C. Binkert, R. de Kanter, C. Fischli, S. Wittlin, et al., Novel in vivo active anti-malarials based on

a hydroxy-ethyl-amine scaffold, Bioorg. Med. Chem. Lett 23 (3) (2013) 658–662.
[13] T.S. Haque, A.G. Skillman, C.E. Lee, H. Habashita, I.Y. Gluzman, T.J.A. Ewing, D.E. Goldberg, I.D. Kuntz, J.A. Ellman, Potent, low-molecular-weight non-peptide inhibitors of malarial aspartyl protease plasmepsin II, J. Med. Chem. 42 (8) (1999) 1428–1440.
[14] D. Nöteberg, E. Hamelink, J. Hultén, M. Wahlgren, L. Vrang, B. Samuelsson, A. Hallberg, Design and synthesis of plasmepsin I and plasmepsin II inhibitors with activity in Plasmodium falciparum-infected cultured human erythrocytes, J. Med. Chem. 46 (5) (2003) 734–746.
[15] K. Ersmark, B. Samuelsson, A. Hallberg, Plasmepsins as potential targets for new antimalarial therapy, Med. Res. Rev. 26 (5) (2006) 626–666.
[16] K. Ersmark, I. Feierberg, S. Bjelic, E. Hamelink, F. Hackett, M.J. Blackman, J. Hultén, B. Samuelsson, J. Åqvist, A. Hallberg, Potent inhibitors of the Plasmodium falciparum enzymes plasmepsin I and II devoid of cathepsin D inhibitory activity, J. Med. Chem. 47 (1) (2004) 110–122.
[17] C. Boss, O. Corminboeuf, C. Grisostomi, S. Meyer, A.F. Jones, L. Prade, C. Binkert, W. Fischli, T. Weller, D. Bur, Achiral, cheap, and potent inhibitors of plasmepsins I, II, and IV, ChemMedChem 1 (12) (2006) 1341–1345.
[18] M.J. Meyer, D.E. Goldberg, Recent advances in plasmepsin medicinal chemistry and implications for future antimalarial drug discovery efforts -, Curr. Top. Med. Chem. 12 (5) (2012) 445–455.
[19] A. Kumar Singh, V. Rajendran, S. Singh, P. Kumar, Y. Kumar, A. Singh, W. Miller, V. Potemkin, Poonam, M. Grishina, et al., Antiplasmodial activity of hydroxyethylamine analogs: synthesis, biological activity and structure activity relationship of plasmepsin inhibitors, Bioorg. Med. Chem. 26 (13) (2018) 3837–3844.
[20] D. Rasina, M. Otikovs, J. Leitans, R. Recacha, O.V. Borysov, I. Kanepe-Lapsa, I. Domraceva, T. Pantelejevs, K. Tars, M.J. Blackman, et al., Fragment-based discovery of 2-aminoquinazolin-4(3H)-Ones as novel class non-peptidomimetic inhibitors of the plasmepsins I, II, and IV, J. Med. Chem. 59 (1) (2016) 374–387.
[21] D. Rasina, G. Stakanovs, O.V. Borysov, T. Pantelejevs, R. Bobrovs, I. Kanepe-Lapsa, K. Tars, K. Jaudzems, A. Jirgensons, 2-Aminoquinazolin-4(3H)-One based plasmepsin inhibitors with improved hydrophilicity and selectivity, Bioorg. Med. Chem. 26 (9) (2018) 2488–2500.
[22] L. Kinena, G. Leitis, I. Kanepe-Lapsa, R. Bobrovs, K. Jaudzems, V. Ozola, E. Suna, A. Jirgensons, Azole-based non-peptidomimetic plasmepsin inhibitors, Arch. Pharm. 351 (9) (2018), 1800151.
[23] J.A. Bonilla, T.D. Bonilla, C.A. Yowell, H. Fujioka, J.B. Dame, Critical roles for the digestive vacuole plasmepsins of Plasmodium falciparum in vacuolar function, Mol. Microbiol. 65 (1) (2007) 64–75.
[24] J.A. Bonilla, P.A. Moura, T.D. Bonilla, C.A. Yowell, D.A. Fidock, J.B. Dame, Effects on growth, hemoglobin metabolism and paralogous Gene expression resulting from disruption of genes encoding the digestive vacuole plasmepsins of Plasmodium falciparum, Int. J. Parasitol. 37 (3–4) (2007) 317–327.
[25] P.A. Moura, J.B. Dame, D.A. Fidock, Role of Plasmodium falciparum digestive vacuole plasmepsins in the specificity and antimalarial mode of action of cysteine and aspartic protease inhibitors, Antimicrob. Agents Chemother. 53 (12) (2009) 4968–4978.
[26] A.N. Hodder, B.E. Sleebs, P.E. Czabotar, M. Gazdik, Y. Xu, M.T. O'Neill, S. Lopaticki, T. Nebl, T. Triglia, B.J. Smith, et al., Structural basis for plasmepsin V inhibition that blocks export of malaria proteins to human erythrocytes, Nat. Struct. Mol. Biol. 22 (8) (2015) 590–596.
[27] B.E. Sleebs, M. Gazdik, M.T. O'Neill, P. Rajasekaran, S. Lopaticki, K. Lackovic, K. Lowes, B.J. Smith, A.F. Cowman, J.A. Boddey, Transition state mimetics of the Plasmodium export element are potent inhibitors of plasmepsin V from P. Falciparum and P, Vivax. J. Med. Chem. 57 (18) (2014) 7644–7662.
[28] W. Nguyen, A.N. Hodder, R.B. de Lezongard, P.E. Czabotar, K.E. Jarman, M.T. O'Neill, J.K. Thompson, H. Jousset Sabroux, A.F. Cowman, J.A. Boddey, et al., Enhanced antimalarial activity of plasmepsin V inhibitors by modification of the P2 position of PEXEL peptidomimetics, Eur. J. Med. Chem. 154 (2018) 182–198.
[29] P. Pino, R. Caldelari, B. Mukherjee, J. Vahokoski, N. Klages, B. Maco, C.R. Collins, M.J. Blackman, I. Kursula, V. Heussler, et al., A multistage antimalarial targets the plasmepsins IX and X essential for invasion and egress, Science 358 (6362) (2017) 522.
[30] A.S. Nasamu, S. Glushakova, I. Russo, B. Vaupel, A. Oksman, A.S. Kim, D.H. Fremont, N. Tolia, J.R. Beck, M.J. Meyers, et al., Plasmepsins IX and X are essential and druggable mediators of malaria parasite egress and invasion, Science 358 (6362) (2017) 518.
[31] J.A. Boddey, Plasmepsins on the antimalarial hit list, Science 358 (6362) (2017) 445.
[32] S. Crunkhorn, Blocking malaria parasite invasion and egress, Nat. Rev. Drug Discov. 17 (2017) 17.
[33] R. Steinfeld, K. Reinhardt, K. Schreiber, M. Hillebrand, R. Kraetzner, W. Brück, P. Saftig, J. Gärtner, Cathepsin D deficiency is associated with a human neurodegenerative disorder, Am. J. Hum. Genet. 78 (6) (2006) 988–998.
[34] A.M. Zuhl, C.E. Nolan, M.A. Brodney, S. Niessen, K. Atchison, C. Houle, D.A. Karanian, C. Ambroise, J.W. Brulet, E.M. Beck, et al., Chemoproteomic profiling reveals that cathepsin D off-target activity drives ocular toxicity of β-secretase inhibitors, Nat. Commun. 7 (2016), 13042.
[35] A.K. Ghosh, B.S. Reddy, Y.-C. Yen, E.L. Cárdenas, K.V. Rao, D. Downs, X. Huang, J. Tang, A.D. Mesecar, Design of potent and highly selective inhibitors for human β-secretase 2 (memapsin 1), a target for type 2 diabetes, Chem. Sci. 7 (5) (2016) 3117–3122.
[36] J.C. Clemente, L. Govindasamy, A. Madabushi, S.Z. Fisher, R.E. Moose, C.A. Yowell, K. Hidaka, T. Kimura, Y. Hayashi, Y. Kiso, M. Agbandje-McKenna, J.B. Dame, B.M. Dunn, R. McKenna, Structure of the aspartic protease plasmepsin 4 from the malarial parasite Plasmodium malariae bound to anallophenylnorstatine-based inhibitor, Acta Crystallogr. D 62 (2006) 246–252.
[37] The PyMOL Molecular Graphics System, Version 1.7.4; Schrödinger, LLC.
[38] J.A. Thomas, M.S.Y. Tan, C. Bisson, A. Borg, T.R. Umrekar, F. Hackett, V.L. Hale, G. Vizcay-Barrena, R.A. Fleck, A.P. Snijders, H.R. Saibil, M.J. Blackman, A protease cascade regulates release of the human malaria parasite Plasmodium falciparum from host red blood cells, Nat Microbiol 3 (4) (2018) 447–455.
[39] A.K. Ghosh, S. Fidanze, Transition-state mimetics for HIV protease Inhibitors: stereocontrolled synthesis of hydroxyethylene and hydroxyethylamine isosteres by ester-derived titanium enolate syn and anti-aldol reactions, J. Org. Chem. 63 (18) (1998) 6146–6152.
[40] L. Kaganovsky, D. Gelman, K. Rueck-Braun, Trans-chelating ligands in palladium-catalyzed carbonylative coupling and methoxycarbonylation of aryl halides, J. Organomet. Chem. 695 (2) (2010) 260–266.

Supporting information

Peptidomimetic Plasmepsin Inhibitors with Potent anti-Malarial activity and Selectivity Against Cathepsin D

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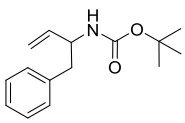
1. Synthesis

General procedure A for hydrolysis of diesters No.13a-f. An aqueous 1 M NaOH solution (1.0 eq) was added to a solution of diester **No.12a-b** (1.0 eq) in MeOH (1 mL/0.1 mmol of the diester). After stirring at room temperature for 16 h, the solution was acidified with aqueous 5% $KHSO_4$ solution to pH 3, diluted with water (20 mL) and extracted with EtOAc (3 x 20 mL). Combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford monoester.

General procedure B for the synthesis of amides No.14a-s; 16b-g. An oven-dried 20 mL pressure vial was charged with monoester **No.13a-f** (1.0 eq) and dissolved in anhydrous DMF (0.5 mL/0.1 mmol of the monoester). The corresponding amine (1.2 eq) was added, followed by HBTU (1.0 eq). The resulting solution was stirred at 0 °C for 5 min, then TEA (2.0 eq) was added and stirring at 0 °C was continued for 0.5 h. The resulting mixture was warmed to room temperature and stirring was continued for 2 h. The brownish solution was diluted with water (20 mL) and extracted with EtOAc (3 x 15 mL). Combined organic extracts were washed with water (15 mL), brine (15 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography.

General procedure C for hydrolysis of benzoates No.15a-s; 17b-g; 19a-b. An aqueous 1 M NaOH solution (1.5 eq) was added to a solution of benzoate **No.14a-s; 16b-g** (1.0 eq) in MeOH (1 mL/0.1 mmol of the benzoate). The resulting solution was stirred at 50 °C for 18 h. After cooling to room temperature, all volatiles were removed under reduced pressure. The white solid was diluted with water (20 mL) and acidified to pH 1 with aqueous 1M HCl solution. The aqueous layer was extracted with EtOAc (3 x 20 mL). Combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford benzoic acid.

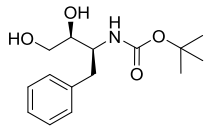
General procedure D for the synthesis of inhibitor No.2a-f; 3a-m; 4a-g; 5a-b. An oven-dried 10 mL pressure vial was cooled under a stream of argon and charged with hydroxyethylamine hydrochloride (1.0 eq), benzoic acid (1.0 eq), HBTU (1.0 eq), and anhydrous DMF (1.2 mL/0.1 mmol of the hydroxyethylamine hydrochloride). The resulting solution was stirred at 0 °C for 5 min, then TEA (4.0 eq) was added and stirring at 0 °C was continued for 0.5 h. After warming to room temperature and stirring for 16 h, the brownish solution was diluted with water (20 mL) and extracted with EtOAc (3 x 15 mL). Combined organic extracts were washed with water (15 mL), brine (15 mL), dried over anhydrous Na_2SO_4. Evaporation under reduced pressure afforded residue, which was purified by silica gel column chromatography.



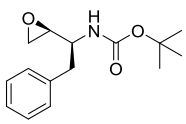
***tert*-Butyl (1-phenylbut-3-en-2-yl)carbamate (7)**. To a stirred solution of amine **6** (2.70 g, 18.4 mmol, 1.0 eq) in anhydrous DCM (50 mL) was added TEA (5.12 mL, 36.7 mmol, 2.0 eq), followed by di-*tert*-butyl dicarbonate (5.01 g, 23.0 mmol, 1.25 eq). After stirring at room temperature for 2 h the yellowish solution was extracted with DCM (3 x 30 mL), combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using gradient elution from 2% EtOAc in petroleum ether to 10% EtOAc in petroleum ether. Fraction spots on TLC were visualized using $KMnO_4$. The fractions containing the product **7** were evaporated to dryness under reduced pressure to give 3.95 g (87% yield) of **7** as a white solid. ^{1}H-NMR spectrum was identical to that from the literature.[1]

[1] Jaudzems, K.; Tars, K.; Maurops, G.; Ivdra, N.; Otikovs, M.; Leitans, J.; Kanepe-Lapsa, I.; Domraceva, I.; Mutule, I.; Trapencieris, P.; Blackman, M. J.; Jirgensons, A.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.32-7.27 (m, 2H, Ar-H), 7.25-7.16 (m, 3H, Ar-H), 5.85-5.75 (m, 1H, CH), 5.14-5.05 (m, 2H, CH_2), 4.54-4.33 (m, 2H, CH_2), 2.84 (d, *J* = 6.0 Hz, 2H, CH_2), 1.41 (s, 9H, CH_3×3) ppm. HRMS-ESI *(m/z)*: $[M+Na]^+$ Calcd for $C_{15}H_{21}NO_2Na$ 270.1470; Found 270.1473.

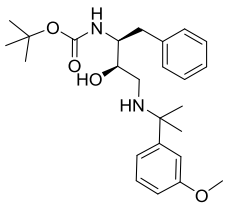


***tert*-Butyl ((2*S*,3*S*)-3,4-dihydroxy-1-phenylbutan-2-yl)carbamate ((*S*,*S*)-8)**. To a mixture of alkene **7** (3.94 g, 16.0 mmol, 1.0 eq) and 1:1 (v:v) *t*-BuOH/water (120 mL) was added AD-mix-α (12.42 g, 16.0 mmol, 1.0 eq). After stirring at room temperature for 20 h the orange solution was cooled to 0 °C (crushed ice) and quenched with solid Na_2SO_3 (12.42 g). The stirring was continued for 0.5 h whereupon the orange color of the solution turned brown. The suspension was extracted with EtOAc (3 x 40 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (eluent 10% *i*-PrOH in hexane). Fraction spots were visualized on thin layer chromatography silica gel using $KMnO_4$. The fractions containing the product were evaporated to dryness under reduced pressure. Enantiomerically pure product was obtained by preparative HPLC on chiral stationary phase (*Chiralpak-ID*) using 5% *i*-PrOH in hexanes as a mobile phase (flow rate 40 mL/min, detector UV 220 nm), to give 1.11 g (25% yield) of (*S*,*S*)-**8** as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.35-7.30 (m, 2H, Ar-H), 7.26-7.22 (m, 3H, Ar-H), 4.54 (d, *J* = 8.7 Hz, 1H, NH), 3.89-3.79 (m, 1H, CH), 3.71-3.57 (m, 2H, CH, OH), 3.41-3.27 (m, 2H, OCH_2, OH), 3.10 (dd, *J* = 14.3, 4.3 Hz, 1H, OCH_2), 2.91 (dd, *J* = 14.3, 7.8 Hz, 1H, CH_2), 2.76 (d, *J* = 8.7 Hz, 1H, CH_2), 1.38 (s, 9H, CH_3×3) ppm. HRMS-ESI *(m/z)*: $[M+Na]^+$ Calcd for $C_{15}H_{23}NO_4Na$ 304.1525; Found 304.1521. Optical rotation $[\alpha]^{20}_D$ +10.1 (*c* 1.01, $CHCl_3$); Lit.[2] $[\alpha]^{23}_D$ +8.06 (*c* 0.62, $CHCl_3$)

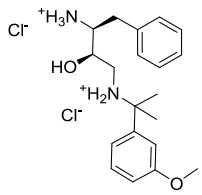


***tert*-Butyl ((*S*)-1-((*S*)-oxiran-2-yl)-2-phenylethyl)carbamate ((*S*,*S*)-9)**. Ph_3P (1.13 g, 4.30 mmol, 1.1 eq) was added to a solution of diol (*S*,*S*)-**8** (1.10 g, 3.91 mmol, 1.0 eq) in anhydrous $CHCl_3$ (14 mL). Diethyl azodicarboxylate (0.68 mL, 4.30 mmol, 1.1 eq) was then added dropwise over a period of 10 min. Upon completion of the addition the light brown solution was stirred at 85 °C for 48 h. After cooling to room temperature water (20 mL) was added and the solution was extracted with DCM (3 x 20 mL), dried over Na_2SO_4 and evaporated under

reduced pressure. The crude product was purified by column chromatography on silica gel using gradient elution from 5% EtOAc in petroleum ether to 15% EtOAc in petroleum ether to give product (*S*,*S*)-**9** as a white solid (655 mg, 64% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.34-7.29 (m, 2H, Ar-H), 7.26-7.20 (m, 3H, Ar-H), 4.43 (br s, 1H, NH), 3.69 (br s, 1H, CH), 2.97 (dd, *J* = 14.0, 5.1 Hz, 1H, CH), 2.94-2.83 (m, 2H, CH_2), 2.80 (t, *J* = 4.9 Hz, 1H, CH_2), 2.77-2.73 (m, 1H, CH_2), 1.38 (s, 9H, CH_3×3) ppm. HRMS-ESI *(m/z)*: $[M+Na]^+$ Calcd for $C_{15}H_{21}NO_3Na$ 286.1419; Found 286.1419. Optical rotation $[\alpha]^{20}_D$ +3.0 (*c* 0.34, $CHCl_3$). Lit.[2] $[\alpha]^{23}_D$ +6.45 (*c* 1.86, $CHCl_3$)



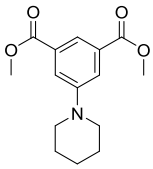
***tert*-Butyl ((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)carbamate ((*S*,*R*)-10)**. To a stirred solution of epoxide (*S*,*S*)-**9** (653 mg, 2.48 mmol, 1.0 eq) in anhydrous *i*-PrOH (7 mL) was added 2-(3-methoxyphenyl)propan-2-amine (430 mg, 2.60 mmol, 1.05 eq). After stirring at 70 °C for 40 h the yellowish solution was cooled to room temperature and then concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using gradient elution from 25% EtOAc in petroleum ether to 100% EtOAc to give product (*S*,*R*)-**10** as a colorless oil (775 mg, 73% yield). ^{1}H-NMR spectrum was identical to that from the literature.[3]
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.30-7.16 (m, 6H, Ar-H), 7.04-6.91 (m, 2H, Ar-H), 6.75 (dd, *J* = 8.2, 2.5 Hz, 1H, Ar-H), 4.54 (d, *J* = 9.3 Hz, 1H, NH), 3.80 (s, 3H, OCH_3), 3.79-3.74 (m, 1H, CH), 3.30 (dt, *J* = 8.0, 4.6 Hz, 1H, CH), 2.96 (dd, *J* = 14.1, 4.6 Hz, 1H, CH_2), 2.81 (dd, *J* = 14.1, 8.0 Hz, 1H, CH_2), 2.46 (dd, *J* = 12.4, 4.6 Hz, 1H, CH_2), 2.38 (dd, *J* = 12.4, 3.2 Hz, 1H, CH_2), 1.45 (s, 6H, CH_3×2), 1.36 (s, 9H, CH_3×3) ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{25}H_{37}N_2O_4$ 429.2753; Found 429.2754. Optical rotation $[\alpha]^{20}_D$ –12.4 (*c* 0.43, $CHCl_3$).



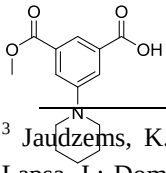
(2*R*,3*S*)-2-Hydroxy-*N*^{1}-(2-(3-methoxyphenyl)propan-2-yl)-4-phenylbutane-1,3-diaminium chloride ((*R*,*S*)-11). HCl (4M solution in 1,4-dioxane, 8.2 mL, 33.0 mmol, 19.0 eq) was added to the amino alcohol (*S*,*R*)-**10** (744 mg, 1.74 mmol, 1.0 eq). After stirring at room temperature for 6 h the

[2] Ghosh, A. K.; Fidanze, S. *J. Org. Chem*., **1998**, *63*, 6146-6152.

colorless solution was concentrated under reduced pressure to obtain hydroxyethylamine hydrochloride (*R*,*S*)-**11** (697 mg, 100% yield) as a light yellow solid, which was used in subsequent steps without purification.
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.75 (t, *J* = 10.4 Hz, 1H, NH), 9.28 (t, *J* = 11.0 Hz, 1H, NH), 8.18 (d, *J* = 5.4 Hz, 2H, NH_2), 7.39-7.33 (m, 1H, Ar-H), 7.28-7.20 (m, 4H, Ar-H), 7.17-7.13 (m, 2H, Ar-H), 7.12-7.08 (m, 1H, Ar-H), 6.99-6.95 (m, 1H, Ar-H), 6.22-6.14 (m, 1H, NH), 4.11-4.02 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.55-3.49 (m, 2H, CH, OH), 2.86-2.61 (m, 3H, CH_2, CH_2), 2.47-2.36 (m, 1H, CH_2), 1.67 (s, 3H, CH_3), 1.66 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, DMSO-d_6) δ: 159.6, 140.9, 136.0, 129.9, 129.3, 128.6, 126.9, 118.3, 114.1, 112.2, 72.2, 70.6, 66.2, 61.4, 55.4, 54.6, 45.0, 33.2, 25.6, 25.1 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{20}H_{29}N_2O_2$ 329.2229; Found 329.2239.



Dimethyl 5-(piperidin-1-yl)isophthalate (12a). An oven-dried pressure tube (200 mL) was cooled under stream of argon and charged with dimethyl 5-bromoisophthalate (2.00 g, 7.32 mmol, 1.0 eq), palladium(II) acetate (82 mg, 0.37 mmol, 0.05 eq), *rac*-BINAP (228 mg, 0.37 mmol, 0.05 eq), Cs_2CO_3 (3.58 g, 11.0 mmol, 1.5 eq) and piperidine (0.72 mL, 7.32 mmol, 1.0 eq). The substances were degassed and anhydrous toluene (15 mL) was added. After heating at 100 ^{o}C for 18 h, the brownish suspension was cooled to room temperature and filtered through Celite. The filter plug was washed with EtOAc and filtrate was dried over anhydrous Na_2SO_4. Volatiles were evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using 10% EtOAc in petroleum ether to give product **12a** as a yellow solid (1.78 g, 88% yield). ^{1}H-NMR spectrum was identical to that from the literature.[3]
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.85 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.66 (d, *J* = 1.4 Hz, 2H, Ar-H), 3.86 (s, 6H, OCH_3×2), 3.24 (t, *J* = 4.9 Hz, 4H, NCH_2×2), 1.68-1.50 (m, 6H, CH_2×3) ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{20}NO_4$ 278.1392; Found 278.1404.

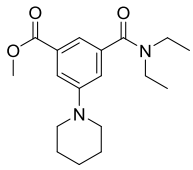


3-(Methoxycarbonyl)-5-(piperidin-1-yl)benzoic acid (13a). The title compound was obtained as a light yellow solid material

[3] Jaudzems, K.; Tars, K.; Maurops, G.; Ivdra, N.; Otikovs, M.; Leitans, J.; Kanepe-Lapsa, I.; Domraceva, I.; Mutule, I.; Trapencieris, P.; Blackman, M. J.; Jirgensons, A. *ACS Med. Chem. Lett.* **2014**, 5, 373–377.

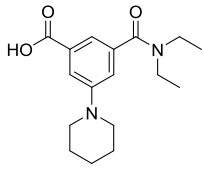
(1.03 g, 72% yield) from diester **12a** (1.52 g, 5.50 mmol, 1.0 eq) and aqueous 1 M NaOH solution (5.50 mL, 5.50 mmol, 1.0 eq) by following general procedure A. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 100% EtOAc. ^{1}H-NMR spectrum was identical to that from the literature.[4]

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.16 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.81 (d, *J* = 1.4 Hz, 2H, Ar-H), 3.94 (s, 3H, OCH_3), 3.31-3.26 (m, 4H, NCH_2×2), 1.77-1.69 (m, 4H, CH_2×2), 1.66-1.59 (m, 2H, CH_2) ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{14}H_{18}NO_4$ 264.1236; Found 264.1240.



Methyl 3-(diethylcarbamoyl)-5-(piperidin-1-yl)benzoate (14a). The title compound was obtained as a colorless oil (241 mg, 91% yield) from monoester **13a** (220 mg, 0.84 mmol, 1.0 eq), diethylamine (0.10 mL, 1.00 mmol, 1.2 eq), HBTU (317 mg, 0.84 mmol, 1.0 eq) and TEA (0,23 mL, 1.70 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc.

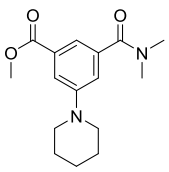
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.42 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.07 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.90 (s, 3H, OCH_3), 3.58-3.50 (m, 2H, NCH_2), 3.29-3.20 (m, 6H, NCH_2×3), 1.73-1.66 (m, 4H, CH_2×2), 1.64-1.56 (m, 2H, CH_2), 1.29-1.20 (m, 3H, CH_3), 1.16-1.07 (m, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.0, 167.1, 152.2, 138.4, 131.0, 118.1, 117.6, 117.3, 52.3, 50.1, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{27}N_2O_3$ 319.2022; Found 319.2028.



3-(Diethylcarbamoyl)-5-(piperidin-1-yl)benzoic acid (15a). The title compound was obtained as a pink solid (134 mg, 60% yield) from benzoate **14a** (235 mg, 0.74 mmol, 1.0 eq) and aqueous 1 M NaOH solution (1.11 mL, 1.11 mmol, 1.5 eq) by following general procedure C. The crude product **15a** was used further without purification.

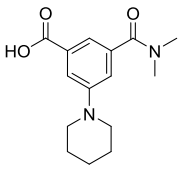
[4] Jaudzems, K.; Tars, K.; Maurops, G.; Ivdra, N.; Otikovs, M.; Leitans, J.; Kanepe-Lapsa, I.; Domraceva, I.; Mutule, I.; Trapencieris, P.; Blackman, M. J.; Jirgensons, A. *ACS Med. Chem. Lett.* **2014**, 5, 373–377.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.46 (dd, *J* = 2.7, 1.4 Hz, 1H, Ar-H), 7.19-7.17 (m, 1H, Ar-H), 7.05 (dd, *J* = 2.7, 1.4 Hz, 1H, Ar-H), 3.50-3.26 (m, 2H, overlapped with DMSO water, NCH_2), 3.25-3.12 (m, 6H, NCH_2×3), 1.65-1.51 (m, 6H, CH_2×3), 1.19-0.99 (m, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, DMSO-d_6) δ: 169.6, 167.2, 151.3, 138.4, 131.7, 116.8, 116.1, 115.9, 49.0, 25.0, 23.8 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{25}N_2O_3$ 305.1865; Found 305.1869.



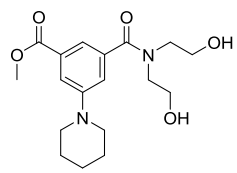
Methyl 3-(dimethylcarbamoyl)-5-(piperidin-1-yl)benzoate (14b). The title compound was obtained as a colorless oil (130 mg, 91% yield) from monoester **13a** (130 mg, 0.49 mmol, 1.0 eq), dimethylamine (2M solution in THF, 0.40 mL, 0.79 mmol, 1.6 eq), HBTU (187 mg, 0.49 mmol, 1.0 eq) and TEA (0.14 mL, 0.99 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 20% EtOAc in petroleum ether to 50% EtOAc in petroleum ether.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60 (dd, *J* = 2.6, 1.5 Hz, 1H. Ar-H), 7.45-7.42 (m, 1H, Ar-H), 7.12 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 3.89 (s, 3H, OCH_3), 3.26-3.20 (m, 4H, NCH_2×2), 3.09 (s, 3H, NCH_3), 2.97 (s, 3H, NCH_3), 1.73-1.65 (m, 4H, CH_2×2), 1.62-1.56 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.4, 167.1, 152.2, 137.5, 131.0, 118.7, 117.9, 52.3, 50.0, 39.7, 35.4, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{16}H_{23}N_2O_3$ 291.1709; Found 291.1706.



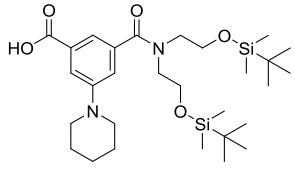
3-(Dimethylcarbamoyl)-5-(piperidin-1-yl)benzoic acid (15b). The title compound was obtained as a yellow solid (44 mg, 40% yield) from benzoate **14b** (115 mg, 0.40 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.59 mL, 0.59 mmol, 1.5 eq) by following general procedure C. The crude product **15b** was used further without purification.

^{1}H-NMR (400 MHz, CD_3OD) δ: 7.65 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.43-7.40 (m, 1H, Ar-H), 7.17 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.28-3.23 (m, 4H, NCH_2×2), 3.10 (s, 3H, NCH_3), 3.00 (s, 3H, NCH_3), 1.76-1.68 (m, 4H, CH_2×2), 1.67-1.59 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 173.4, 169.5, 153.6, 138.4, 133.1, 119.5, 119.1, 118.9, 51.2, 40.0, 35.6, 26.6, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{21}N_2O_3$ 277.1552; Found 277.1558.



Methyl 3-(bis(2-hydroxyethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14c). The title compound was obtained as a yellowish oil (174 mg, 64% yield) from monoester **13a** (205 mg, 0.78 mmol, 1.0 eq), diethanolamine (90 µL, 0.93 mmol, 1.2 eq), HBTU (295 mg, 0.78 mmol, 1.0 eq) and TEA (0.22 mL, 1.56 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 100% EtOAc to 10% MeOH in EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61-7.59 (m, 1H, Ar-H), 7.50-7.48 (m, 1H, Ar-H), 7.20-7.17 (m, 1H, Ar-H), 4.03-3.94 (m, 2H, NCH_2), 3.89 (s, 3H, OCH_3), 3.76-3.67 (m, 4H, NCH_2, OCH_2), 3.50-3.41 (m, 2H, OCH_2), 3.26-3.20 (m, 4H, NCH_2×2), 1.72-1.65 (m, 4H, CH_2×2), 1.63-1.55 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 173.5, 167.1, 152.1, 137.4, 131.2, 118.7, 117.9, 117.8, 61.4, 53.6, 52.4, 50.0, 25.6, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{27}N_2O_5$ 351.1920; Found 351.1929.



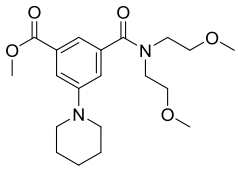
3-(Bis(2-((*tert*-butyldimethylsilyl)oxy)ethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15c). To a stirred solution of benzoate **14c** (173 mg, 0.49 mmol, 1.0 eq) in anhydrous DMF (6 mL) was added imidazole (202 mg, 2.96 mmol, 6.0 eq), followed by *tert*-butyldimethylsilyl chloride (223 mg, 1.48 mmol, 3.0 eq). After stirring at room temperature for 18 h water (20 mL) was added and the colorless solution was extracted with DCM (3 x 20 mL), combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using 30% EtOAc in petroleum ether to give a protected intermediate as a yellow oil (257 mg, 90% yield) that was used in the next step.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.66-7.57 (m, 1H, Ar-H), 7.49-7.41 (m, 1H, Ar-H), 7.13-7.03 (m, 1H, Ar-H), 3.92-3.86 (m, 5H, NCH_2, OCH_3), 3.74-3.61 (m, 4H, NCH_2, OCH_2), 3.53-3.45 (m, 2H, OCH_2), 3.26-3.19 (m, 4H, NCH_2×2), 1.76-1.65 (m, 4H, CH_2×2), 1.63-1.55 (m, 2H, CH_2), 0.91 (s, 9H, CH_3×3), 0.84 (s, 9H, CH_3×3), 0.09 (s, 6H, $SiCH_3$×2), -0.01 (s, 6H, $SiCH_3$×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.9, 167.1, 152.2, 138.1, 131.1, 118.4, 117.7, 117.6, 61.3,

52.3, 50.1, 48.4, 26.0, 25.8, 25.7, 24.3, 18.4, -3.4, -5.2, -5.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{30}H_{55}N_2O_5Si_2$ 579.3650; Found 579.3636.

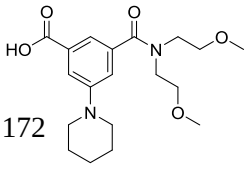
The title compound was obtained as a colorless oil (50 mg, 20% yield) from protected intermediate (253 mg, 0.44 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.66 mL, 0.66 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 100% EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.64 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.51 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.13 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.96-3.87 (m, 2H, NCH_2), 3.70 (t, *J* = 5.5 Hz, 2H, NCH_2), 3.65 (t, *J* = 6.1 Hz, 2H, OCH_2), 3.56-3.46 (m, 2H, OCH_2), 3.28-3.20 (m, 4H, NCH_2×2), 1.75-1.65 (m, 4H, CH_2×2), 1.64-1.56 (m, 2H, CH_2), 0.92 (s, 9H, CH_3×3), 0.85 (s, 9H, CH_3×3), 0.10 (s, 6H, $SiCH_3$×2), -0.01 (s, 6H, $SiCH_3$×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.8, 171.1, 152.2, 138.1, 130.3, 119.2, 118.3, 118.1, 61.2, 52.5, 50.1, 48.5, 29.8, 26.0, 25.7, 24.3, 18.4, -5.2, -5.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{29}H_{53}N_2O_5Si_2$ 565.3493; Found 565.3477.



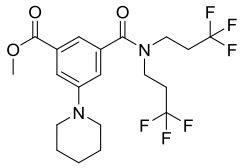
Methyl 3-(bis(2-methoxyethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14d). The title compound was obtained as a yellowish oil (176 mg, 94% yield) from monoester **13a** (130 mg, 0.49 mmol, 1.0 eq), bis(2-methoxyethyl)amine (72 µL, 0.49 mmol, 1.0 eq), HBTU (187 mg, 0.49 mmol, 1.0 eq) and TEA (0.14 mL, 0.99 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 100% EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.59 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.47 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.15 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.89 (s, 3H, OCH_3), 3.78-3.71 (m, 2H, NCH_2), 3.69-3.63 (m, 2H, NCH_2), 3.55-3.48 (m, 2H, OCH_2), 3.47-3.41 (m, 2H, OCH_2), 3.38 (s, 3H, OCH_3), 3.31-3.19 (m, 7H, NCH_2×2, OCH_3), 1.74-1.66 (m, 4H, CH_2×2), 1.63-1.56 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.9, 166.8, 152.4, 136.4, 131.4, 118.3, 117.6, 116.6, 110.2, 52.5, 49.9, 25.6, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{20}H_{31}N_2O_5$ 379.2223; Found 379.2233.



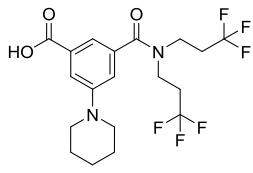
3-(Bis(2-methoxyethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15d). The title compound was obtained as a reddish oil (151 mg, 96% yield) from benzoate **14d** (163 mg, 0.43 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.65 mL, 0.65 mmol, 1.5 eq) by following general procedure C. The crude product **15d** was used further without purification.

^{1}H-NMR (400 MHz, CD_3OD) δ: 7.63 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.43 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.22 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.78-3.72 (m, 2H, NCH_2), 3.70-3.63 (m, 2H, NCH_2), 3.57-3.45 (m, 4H, OCH_2×2), 3.40 (s, 3H, OCH_3), 3.29-3.23 (m, 7H, NCH_2×2, OCH_3), 1.77-1.68 (m, 4H, CH_2×2), 1.67-1.60 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 174.3, 169.4, 153.3, 138.6, 133.0, 119.8, 119.1, 118.8, 71.3, 59.1, 51.2, 46.2, 26.6, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{19}H_{29}N_2O_5$ 365.2076; Found 365.2086.



Methyl 3-(bis(3,3,3-trifluoropropyl)carbamoyl)-5-(piperidin-1 yl)benzoate (14e). The title compound was obtained as a colorless oil (119 mg, 53% yield) from monoester **13a** (130 mg, 0.49 mmol, 1.0 eq), bis(3,3,3-trifluoropropyl)amine (103 mg, 0.49 mmol, 1.0 eq), HBTU (187 mg, 0.49 mmol, 1.0 eq) and TEA (0.14 mL, 0.99 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 10% EtOAc in petroleum ether to 50% EtOAc in petroleum ether.

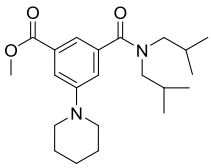
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.65 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.35 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.02 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.91 (s, 3H, OCH_3), 3.72-3.51 (m, 4H, NCH_2×2), 3.29-3.21 (m, 4H, NCH_2×2), 2.70-2.22 (m, 4H, CH_2×2), 1.73-1.67 (m, 4H, CH_2×2), 1.65-1.57 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 172.0, 167.1, 151.9, 137.8, 131.0, 118.9, 118.0, 117.7, 110.2, 59.0, 52.3, 50.1, 25.7, 24.3 ppm. ^{19}F-NMR (376 MHz, $CDCl_3$) δ: -70.9, -72.8 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{20}H_{25}N_2O_3F_6$ 455.1769; Found 455.1775.



3-(Bis(3,3,3-trifluoropropyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15e). The title compound was obtained as a light yellow solid (107 mg, 99% yield) from benzoate **14e** (111 mg, 0.24 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.37 mL, 0.37 mmol, 1.5

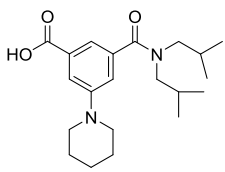
eq) by following general procedure C. The crude product **15e** was used further without purification.

^{1}H-NMR (400 MHz, CD_3OD) δ: 7.68 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.39 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.14 (dd, *J* = 2.6, 1.4 Hz, 1H. Ar-H), 3.81-3.70 (m, 2H, NCH_2), 3.65-3.54 (m, 2H, NCH_2), 3.29-3.25 (m, 4H, NCH_2×2), 2.72-2.60 (m, 2H, CH_2), 2.58-2.47 (m, 2H, CH_2), 1.76-1.68 (m, 4H, CH_2×2), 1.67-1.60 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 174.0, 169.3, 153.6, 137.8, 133.4, 119.2, 118.7, 118.1, 51.0, 44.3, 40.2, 26.6, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{19}H_{23}N_2O_3F_6$ 441.1613; Found 441.1617.



Methyl 3-(diisobutylcarbamoyl)-5-(piperidin-1-yl)benzoate (14f). The title compound was obtained as a colorless oil (193 mg, 78% yield) from monoester **13a** (173 mg, 0.66 mmol, 1.0 eq), diisobutylamine (0.14 mL, 0.79 mmol, 1.2 eq), HBTU (249 mg, 0.66 mmol, 1.0 eq) and TEA (0.18 mL, 1.31 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 10% EtOAc in petroleum ether to 50% EtOAc in petroleum ether.

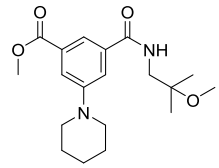
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.59 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.37 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.05 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.90 (s, 3H, OCH_3), 3.35 (d, *J* = 7.5 Hz, 2H, NCH_2), 3.26-3.18 (m, 4H, NCH_2×2), 3.09 (d, *J* = 7.5 Hz, 2H, NCH_2), 2.20-2.04 (m, 1H, CH), 1.96-1.77 (m, 1H, CH), 1.75-1.64 (m, 4H, CH_2×2), 1.64-1.55 (m, 2H, CH_2), 0.99 (d, *J* = 6.6 Hz, 6H, CH_3×2), 0.75 (d, *J* = 6.6 Hz, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 172.2, 167.2, 152.3, 138.6, 130.9, 119.0, 118.0, 117.6, 56.6, 52.3, 51.2, 50.2, 26.9, 26.3, 25.7, 24.3, 20.4, 20.0 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{22}H_{35}N_2O_3$ 375.2648; Found 375.2663.



3-(Diisobutylcarbamoyl)-5-(piperidin-1-yl)benzoic acid (15f). The title compound was obtained as a yellowish solid (176 mg, 100% yield) from benzoate **14f** (183 mg, 0.49 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.73 mL, 0.73 mmol, 1.5 eq) by following general procedure C. The crude product **15f** was used further without purification.

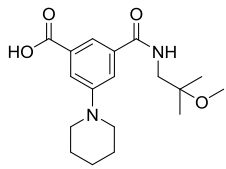
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.64-7.61 (m, 1H, Ar-H), 7.45-7.41 (m, 1H, Ar-H), 7.10-7.05 (m, 1H, Ar-H), 3.35 (d, *J* = 7.5 Hz, 2H, NCH_2), 3.23 – 3.17 (m,

4H, NCH_2×2), 3.09 (d, *J* = 7.5 Hz, 2H, NCH_2), 2.16-2.04 (m, 1H, CH), 1.90-1.78 (m, 1H, CH), 1.71-1.64 (m, 4H, CH_2×2), 1.61-1.53 (m, 2H, CH_2), 0.97 (d, *J* = 6.6 Hz, 6H, CH_3×2), 0.73 (d, *J* = 6.6 Hz, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 172.4, 152.2, 138.3, 119.1, 118.5, 118.2, 56.7, 51.3, 50.2, 26.9, 26.3, 25.7, 24.3, 20.4, 20.0 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{21}H_{33}N_2O_3$ 361.2491; Found 361.2509.



Methyl 3-((2-methoxy-2-methylpropyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14g). The title compound was obtained as a colorless oil (169 mg, 85% yield) from monoester **13a** (150 mg, 0.57 mmol, 1.0 eq), 2-methoxy-2-methylpropan-1-amine (70 mg, 0.68 mmol, 1.2 eq), HBTU (216 mg, 0.57 mmol, 1.0 eq) and TEA (0.16 mL, 1.14 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 100% EtOAc.

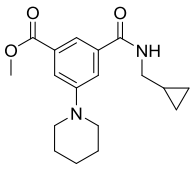
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.74-7.67 (m, 2H, Ar-H), 7.64-7.59 (m, 1H, Ar-H), 6.53-6.46 (m, 1H, NH), 3.92 (s, 3H, OCH_3), 3.49 (d, *J* = 5.6 Hz, 2H, NCH_2), 3.29-3.25 (m, 4H, NCH_2×2), 3.23 (s, 3H, OCH_3), 1.74-1.67 (m, 4H, CH_2×2), 1.64-1.56 (m, 2H, CH_2), 1.22 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.4, 167.0, 136.0, 131.2, 119.5, 116.9, 74.5, 52.4, 50.1, 49.6, 48.2, 25.7, 24.3, 22.7 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{19}H_{29}N_2O_4$ 349.2127; Found 349.2118.



3-((2-Methoxy-2-methylpropyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15g). The title compound was obtained as a brownish solid (49 mg, 33% yield) from benzoate **14g** (154 mg, 0.44 mmol, 1.0 eq) and aqueous 1M NaOH solution (0.66 mL, 0.66 mmol, 1.5 eq) by following general procedure C. The crude product **15g** was used further without purification.

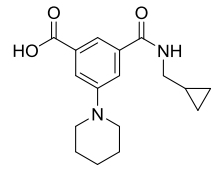
^{1}H-NMR (400 MHz, CD_3OD) δ: 8.02-7.98 (m, 1H, Ar-H), 7.89-7.84 (m, 1H, Ar-H), 7.75-7.72 (m, 1H, Ar-H), 3.47 (s, 2H, CH_2), 3.39-3.34 (m, 4H, NCH_2×2), 3.28 (s, 3H, OCH_3), 1.85-1.76 (m, 4H, CH_2×2), 1.70-1.63 (m, 2H, CH_2), 1.22 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 169.7, 169.0, 137.4, 133.4,

121.8, 121.3, 76.5, 52.6, 49.9, 48.3, 26.4, 24.7, 23.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{27}N_2O_4$ 335.1971; Found 335.1961.



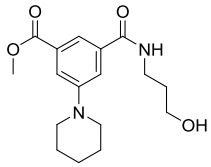
Methyl 3-((cyclopropylmethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14h). The title compound was obtained as a colorless oil (225 mg, 85% yield) from monoester **13a** (220 mg, 0.83 mmol, 1.0 eq), 1-cyclopropylmethanamine (87 μL, 1.00 mmol, 1.2 eq), HBTU (317 mg, 0.83 mmol, 1.0 eq) and TEA (0.23 mL, 1.67 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using 50% EtOAc in petroleum ether.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.69-7.67 (m, 2H, Ar-H), 7.63 (dd, *J* = 2.7, 1.5 Hz, 1H, Ar-H), 6.33-6.25 (m, 1H, NH), 3.93 (s, 3H, OCH_3), 3.31 (dd, *J* = 7.2, 5.4 Hz, 2H, NCH_2), 3.29-3.25 (m, 4H, NCH_2×2), 1.74-1.66 (m, 4H, CH_2×2), 1.64-1.57 (m, 2H, CH_2), 1.13-1.01 (m, 1H, CH, cyclopropyl), 0.62-0.53 (m, 2H, CH_2, cyclopropyl), 0.33-0.24 (m, 2H, CH_2, cyclopropyl) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.2, 167.1, 152.4, 135.9, 131.1, 119.5, 119.4, 116.7, 52.4, 50.0, 45.2, 25.7, 24.3, 10.9, 3.7 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{25}N_2O_3$ 317.1865; Found 317.1873.



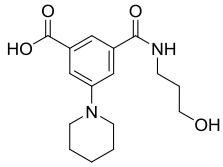
3-((Cyclopropylmethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15h). The title compound was obtained as a white solid (177 mg, 84% yield) from benzoate **14h** (220 mg, 0.70 mmol, 1.0 eq) and aqueous 1 M NaOH solution (1.04 mL, 1.04 mmol, 1.5 eq) by following general procedure C. The crude product **15h** was used further without purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.66 (t, *J* = 5.7 Hz, 1H, NH), 7.83-7.80 (m, 1H, Ar-H), 7.61-7.58 (m, 1H, Ar-H), 7.54 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 3.25-3.20 (m, 4H, NCH_2×2), 3.16-3.09 (m, 2H, NCH_2), 1.67-1.60 (m, 4H, CH_2×2), 1.59-1.53 (m, 2H, CH_2), 1.08-0.98 (m, 1H, CH, cyclopropyl), 0.47-0.38 (m, 2H, CH_2, cyclopropyl), 0.27-0.19 (m, 2H, CH_2, cyclopropyl) ppm. ^{13}C-NMR (101 MHz, DMSO-d_6) δ: 167.4, 165.7, 165.6, 151.5, 135.7, 131.7, 118.4, 118.0, 49.3, 43.6, 25.1, 23.8, 11.0, 3.4 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{23}N_2O_3$ 303.1709; Found 303.1720.



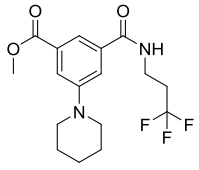
Methyl 3-((3-hydroxypropyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14i). The title compound was obtained as a light yellow oil (102 mg, 60% yield) from monoester **13a** (140 mg, 0.53 mmol, 1.0 eq), 3-amino-1-propanol (48 mg, 0.64 mmol, 1.2 eq), HBTU (202 mg, 0.53 mmol, 1.0 eq) and TEA (0.15 mL, 1.06 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 100% EtOAc to 2% MeOH in EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.69-7.66 (m, 2H, Ar-H), 7.63-7.60 (m, 1H, Ar-H), 6.75-6.66 (m, 1H, NH), 3.91 (s, 3H, OCH_3), 3.70 (t, J = 5.7 Hz, 2H, NCH_2), 3.63 (q, J = 6.2 Hz, 2H, OCH_2), 3.29-3.24 (m, 4H, NCH_2×2), 3.21 (s, 1H, OH), 1.80 (quintet, J = 5.7 Hz, 2H, CH_2), 1.74-1.65 (m, 4H, CH_2×2), 1.64-1.56 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.4, 167.0, 152.4, 135.3, 131.2, 119.6, 119.3, 116.7, 59.6, 52.4, 50.0, 37.1, 32.4, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{25}N_2O_4$ 321.1814; Found 321.1824.



3-((3-Hydroxypropyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15i). The title compound was obtained as a yellow solid (84 mg, 94% yield) from benzoate **14i** (93 mg, 0.29 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.44 mL, 0.44 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 15 mL/min) using gradient elution from 100% water to 100% MeCN.

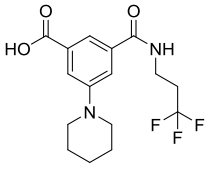
^{1}H-NMR (400 MHz, CD_3OD) δ: 8.61-8.59 (m, 1H, Ar-H), 8.49 (dd, J = 2.4, 1.5 Hz, 1H, Ar-H), 8.42 (dd, J = 2.4, 1.5 Hz, 1H, Ar-H), 3.78-3.71 (m, 4H, NCH_2×2), 3.67 (t, J = 6.2 Hz, 2H, NCH_2), 3.52 (t, J = 7.0 Hz, 2H, OCH_2), 2.15-2.06 (m, 4H, CH_2×2), 1.92-1.79 (m, 4H, CH_2×2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 167.2, 166.8, 144.2, 138.8, 135.1, 130.5, 126.2, 125.7, 60.5, 58.3, 38.4, 33.1, 24.9, 22.0 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{16}H_{23}N_2O_4$ 307.1658; Found 307.1656.



Methyl 3-(piperidin-1-yl)-5-((3,3,3-trifluoropropyl)carbamoyl) benzoate (14j). The title compound was obtained as a colorless oil (183 mg, 61% yield) from monoester **13a** (220 mg, 0.83 mmol, 1.0 eq), 3,3,3-trifluoropropylamine (113 mg, 1.00 mmol, 1.2 eq),

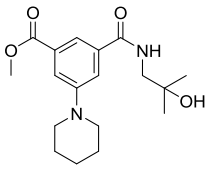
HBTU (317 mg, 0.83 mmol, 1.0 eq) and TEA (0.23 mL, 1.67 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 50% EtOAc in petroleum ether.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.70 (dd, *J* = 2.7, 1.4 Hz, 1H, Ar-H), 7.64 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.58 (dd, *J* = 2.7, 1.4 Hz, 1H, Ar-H), 6.47-6.40 (m, 1H, NH), 3.92 (s, 3H, OCH_3), 3.75-3.68 (m, 2H, NCH_2), 3.29-3.25 (m, 4H, NCH_2×2), 2.55-2.41 (m, 2H, CH_2), 1.74-1.66 (m, 4H, CH_2×2), 1.64-1.56 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.5, 166.9, 152.4, 135.2, 131.3, 119.8, 119.1, 116.6, 52.5, 50.0, 34.0, 33.8, 33.7, 25.7, 24.3 ppm. ^{19}F-NMR (376 MHz, $CDCl_3$) δ: -65.1 (t, *J* = 10.9 Hz) ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{22}N_2O_3F_3$ 359.1583; Found 359.1586.



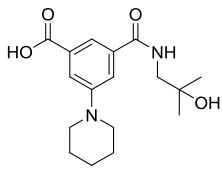
3-(Piperidin-1-yl)-5-((3,3,3-trifluoropropyl)carbamoyl)benzoic acid (15j). The title compound was obtained as a white solid (142 mg, 85% yield) from benzoate **14j** (173 mg, 0.48 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.72 mL, 0.72 mmol, 1.5 eq) by following general procedure C. The crude product **15j** was used further without purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.77 (t, *J* = 5.6 Hz, 1H, NH), 7.79 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.58-7.54 (m, 2H, Ar-H), 3.52-3.45 (m, 2H, NCH_2), 3.25-3.20 (m, 4H, NCH_2×2), 2.62-2.51 (m, 2H, CH_2), 1.66-1.60 (m, 4H, CH_2×2), 1.59-1.52 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, DMSO-d_6) δ: 167.3, 166.0, 151.5, 135.1, 128.2, 125.5, 118.2, 117.7, 49.2, 32.8, 32.6, 32.3, 25.0, 23.8 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{16}H_{20}N_2O_3F_3$ 345.1426; Found 345.1440.



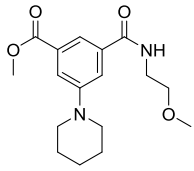
Methyl 3-((2-hydroxy-2-methylpropyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14k). The title compound was obtained as a light yellow oil (194 mg, 80% yield) from monoester **13a** (190 mg, 0.72 mmol, 1.0 eq), 1-amino-2-methyl-2-propanol (77 mg, 0.87 mmol, 1.2 eq), HBTU (274 mg, 0.72 mmol, 1.0 eq) and TEA (0.20 mL, 1.44 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 100% EtOAc, followed by 2% MeOH in EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.72-7.70 (m, 1H, Ar-H), 7.69-7.67 (m, 1H, Ar-H), 7.64-7.60 (m, 1H, Ar-H), 6.74-6.66 (m, 1H, NH), 3.91 (s, 3H, OCH_3), 3.30-3.23 (m, 4H, NCH_2×2), 2.79 (d, *J* = 0.8 Hz, 2H, NCH_2), 2.52 (s, 1H, OH), 1.74-1.65 (m, 4H, CH_2×2), 1.63-1.56 (m, 2H, CH_2), 1.29 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.3, 167.0, 152.4, 135.5, 131.2, 119.6, 119.4, 116.8, 71.3, 52.4, 51.0, 50.0, 38.8, 27.6, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{27}N_2O_4$ 335.1971; Found 335.1978.



3-((2-Hydroxy-2-methylpropyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15k). The title compound was obtained as a light yellow solid (139 mg, 77% yield) from benzoate **14k** (188 mg, 0.56 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.84 mL, 0.84 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 15 mL/min) using gradient elution from 100% water to 50% MeCN in water.

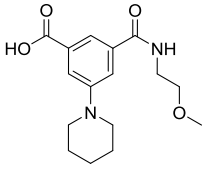
^{1}H-NMR (400 MHz, CD_3OD) δ: 7.90 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.73 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 7.62 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 3.41 (s, 2H, NCH_2), 3.29-3.25 (m, 4H, NCH_2×2), 1.78-1.69 (m, 4H, CH_2×2), 1.67-1.59 (m, 2H, CH_2), 1.24 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 170.4, 169.5, 153.6, 136.8, 120.8, 120.3, 119.6, 72.0, 51.6, 51.3, 27.3, 26.7, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{25}N_2O_4$ 321.1814; Found 321.1825.



Methyl 3-((2-methoxyethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14l). The title compound was obtained as a colorless oil (156 mg, 99% yield) from monoester **13a** (130 mg, 0.49 mmol, 1.0 eq), 2-methoxyethylamine (47 µL, 0.54 mmol, 1.1 eq), HBTU (187 mg, 0.49 mmol, 1.0 eq) and TEA (0.14 mL, 0.99 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 100% EtOAc.

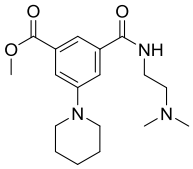
^{1}H-NMR (400 MHz, CD_3OD) δ: 7.86-7.84 (m, 1H, Ar-H), 7.70-7.68 (m, 1H, Ar-H), 7.62-7.59 (m, 1H, Ar-H), 3.91 (s, 3H, OCH_3), 3.58-3.55 (m, 4H, NCH_2, OCH_2), 3.38 (s, 3H, OCH_3), 3.29-3.24 (m, 4H, NCH_2×2), 1.77-1.69 (m, 4H, CH_2×2), 1.67-1.59 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 169.8, 168.3, 153.6, 136.8, 132.4, 120.4, 120.3, 119.2, 71.9, 58.9, 52.8, 51.2, 40.8,

26.7, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{25}N_2O_4$ 321.1814; Found 321,1822.



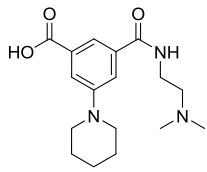
3-((2-Methoxyethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15l). The title compound was obtained as a dark red solid (85 mg, 57% yield) from benzoate **14l** (156 mg, 0.49 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.73 mL, 0.73 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 14 mL/min) using gradient elution from 100% water to 50% MeCN in water.

^{1}H-NMR (400 MHz, CD_3OD) δ: 7.87 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.72 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 7.60 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 3.58-3.55 (m, 4H, NCH_2, OCH_2), 3.38 (s, 3H, OCH_3), 3.29-3.24 (m, 4H, NCH_2×2), 1.77-1.69 (m, 4H, CH_2×2), 1.67-1.59 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 170.0, 169.6, 153.6, 136.7, 133.1, 120.8, 120.2, 119.6, 71.9, 58.9, 51.3, 40.8, 26.7, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{16}H_{23}N_2O_4$ 307.1658; Found 307.1668.



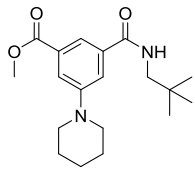
Methyl 3-((2-(dimethylamino)ethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14m). The title compound was obtained as a colorless oil (90 mg, 47% yield) from monoester **13a** (150 mg, 0.57 mmol, 1.0 eq), *N*,*N*-dimethylethylene-diamine (69 µL, 0.63 mmol, 1.1 eq), HBTU (216 mg, 0.57 mmol, 1.0 eq) and TEA (0.16 mL, 1.14 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 100% DCM to 6% MeOH in DCM.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.72-7.70 (m, 1H, Ar-H), 7.68-7.66 (m, 1H, Ar-H), 7.63-7.60 (m, 1H, Ar-H), 6.88-6.82 (m, 1H, NH), 3.91 (s, 3H, OCH_3), 3.56-3.50 (m, 2H, NCH_2), 3.29-3.23 (m, 4H, NCH_2×2), 2.56 (t, *J* = 5.9 Hz, 2H, NCH_2), 2.30 (s, 6H, CH_3×2), 1.74-1.66 (m, 4H, CH_2×2), 1.63-1.56 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.5, 167.1, 152.3, 135.8, 131.1, 119.5, 117.1, 58.0, 52.4, 50.1, 45.3, 37.5, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{28}N_3O_3$ 334.2131; Found 334.2132.



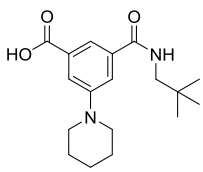
3-((2-(Dimethylamino)ethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15m). The title compound was obtained as a reddish solid (46 mg, 54% yield) from benzoate **14m** (89 mg, 0.27 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.40 mL, 0.40 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 25 mL/min) using gradient elution from 100% water to 50% MeCN in water.

^{1}H-NMR (400 MHz, CD_3OD) δ: 7.86-7.83 (m, 1H, Ar-H), 7.50-7.47 (m, 1H, Ar-H), 7.46 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 3.85-3.79 (m, 2H, NCH_2), 3.39-3.33 (m, 2H, NCH_2), 3.18-3.12 (m, 4H, NCH_2×2), 2.89 (s, 6H, CH_3×2), 1.72-1.64 (m, 4H, CH_2×2), 1.62-1.56 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 170.9, 153.1, 135.2, 121.5, 119.9, 118.8, 58.0, 51.6, 43.4, 36.3, 26.8, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{26}N_3O_3$ 320.1974; Found 320.1962.



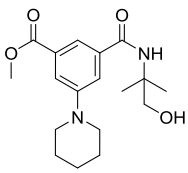
Methyl 3-(neopentylcarbamoyl)-5-(piperidin-1-yl)benzoate (14n). The title compound was obtained as a colorless oil (148 mg, 78% yield) from monoester **13a** (150 mg, 0.57 mmol, 1.0 eq), 2,2-dimethylpropan-1-amine (74 μL, 0.63 mmol, 1.1 eq), HBTU (216 mg, 0.57 mmol, 1.0 eq) and TEA (0.16 mL, 1.14 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 10% EtOAc in petroleum ether to 20% EtOAc in petroleum ether.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.68 (dd, *J* = 2.7, 1.5 Hz, 1H, Ar-H), 7.65 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.62 (dd, *J* = 2.7, 1.5 Hz, 1H, Ar-H), 6.25-6.18 (m, 1H, NH), 3.92 (s, 3H, OCH_3), 3.31-3.24 (m, 6H, NCH_2, NCH_2×2), 1.75-1.66 (m, 4H, CH_2×2), 1.64-1.57 (m, 2H, CH_2), 0.98 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.4, 167.1, 152.4, 136.2, 131.1, 119.5, 119.4, 116.4, 52.4, 51.2, 50.0, 32.4, 27.5, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{19}H_{29}N_2O_3$ 333.2178; Found 333.2177.

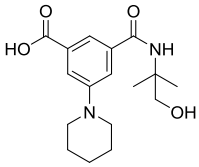


3-(Neopentylcarbamoyl)-5-(piperidin-1-yl)benzoic acid (15n). The title compound was obtained as a white solid (133 mg, 99% yield) from benzoate **14n** (140 mg, 0.42 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.84 mL, 0.84 mmol, 2.0 eq) by following general procedure C. The crude product **15n**

was used further without purification.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.75-7.71 (m, 2H, Ar-H), 7.68-7.65 (m, 1H, Ar-H), 6.44-6.35 (m, 1H, NH), 3.31-3.23 (m, 6H, NCH_2, NCH_2×2), 1.73-1.65 (m, 4H, CH_2×2), 1.63-1.56 (m, 2H, CH_2), 0.98 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 170.9, 167.6, 152.3, 136.2, 130.7, 120.2, 119.9, 117.1, 51.3, 50.0, 32.4, 27.5, 25.6, 24.3 ppm.
HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{27}N_2O_3$ 319.2022; Found 319.2031.

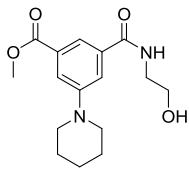


Methyl 3-((1-hydroxy-2-methylpropan-2-yl)carbamoyl)-5-(piperidin-1-yl)benzoate (14o). The title compound was obtained as a white solid (157 mg, 82% yield) from monoester **13a** (150 mg, 0.57 mmol, 1.0 eq), 2-amino-2-methyl-1-propanol (61 mg, 0.68 mmol, 1.2 eq), HBTU (216 mg, 0.57 mmol, 1.0 eq) and TEA (0.16 mL, 1.14 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 30% EtOAc in petroleum ether to 100% EtOAc.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.67 (dd, *J* = 2.7, 1.5 Hz, 1H, Ar-H), 7.60 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.57 (dd, *J* = 2.7, 1.5 Hz, 1H, Ar-H), 6.25 (s, 1H, NH), 4.65 (s, 1H, OH), 3.92 (s, 3H, OCH_3), 3.70 (s, 2H, OCH_2), 3.30-3.23 (m, 4H, NCH_2×2), 1.74-1.66 (m, 4H, CH_2×2), 1.64-1.57 (m, 2H, CH_2), 1.42 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.2, 166.9, 152.3, 135.9, 131.1, 119.6, 119.3, 116.6, 70.8, 56.8, 52.5, 50.0, 25.6, 24.9, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{18}H_{27}N_2O_4$ 335.1971; Found 335.1974.



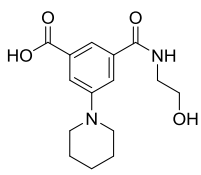
3-((1-Hydroxy-2-methylpropan-2-yl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15o). The title compound was obtained as an orange solid (94 mg, 66% yield) from benzoate **14o** (148 mg, 0.44 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.66 mL, 0.66 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 15 mL/min) using gradient elution from 100% water to 100% MeCN.
^{1}H-NMR (400 MHz, CD_3OD) δ: 7.78 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.70 (dd, *J* = 2.7, 1.4 Hz, 1H, Ar-H), 7.52 (dd, *J* = 2.7, 1.4 Hz, 1H, Ar-H), 3.70 (s, 2H, CH_2), 3.28-3.23 (m, 4H, NCH_2×2), 1.77-1.68 (m, 4H, CH_2×2), 1.67-1.59 (m, 2H, CH_2), 1.41 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 170.5, 153.5,

138.1, 120.6, 120.2, 119.7, 69.2, 56.8, 51.4, 26.7, 25.3, 24.0, 23.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{17}H_{25}N_2O_4$ 321.1814; Found 321.1814.



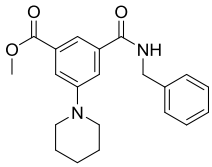
Methyl 3-((2-hydroxyethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14p). The title compound was obtained as a yellowish oil (97 mg, 52% yield) from monoester **13a** (160 mg, 0.61 mmol, 1.0 eq), ethanolamine (42 µL, 0.70 mmol, 1.2 eq), HBTU (230 mg, 0.61 mmol, 1.0 eq) and TEA (0.17 mL, 1.22 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 100% EtOAc to 5% MeOH in EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.71-7.69 (m, 1H, Ar-H), 7.68-7.66 (m, 1H, Ar-H), 7.62-7.60 (m, 1H, Ar-H), 6.84-6.77 (m, 1H, NH), 3.90 (s, 3H, OCH_3), 3.84 (t, *J* = 5.4 Hz, 2H, OCH_2), 3.63 (quartet, *J* = 5.4 Hz, 2H, NCH_2), 3.28-3.23 (m, 4H, NCH_2×2), 1.74-1.66 (m, 4H, CH_2×2), 1.64-1.55 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.4, 167.0, 152.3, 135.3, 131.1, 119.6, 119.4, 117.0, 62.6, 52.4, 50.0, 43.1, 25.6, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{16}H_{23}N_2O_4$ 307.1658; Found 307.1672.



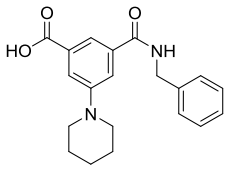
3-((2-Hydroxyethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15p). The title compound was obtained as a pink solid (67 mg, 85% yield) from benzoate **14p** (83 mg, 0.27 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.41 mL, 0.41 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 15 mL/min) using gradient elution from 100% water to 100% MeCN.

^{1}H-NMR (400 MHz, CD_3OD) δ: 7.89 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.71 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 7.62 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 3.72 (t, *J* = 5.8 Hz, 2H, OCH_2), 3.51 (t, *J* = 5.8 Hz, 2H, NCH_2), 3.29-3.24 (m, 4H, NCH_2×2), 1.77-1.68 (m, 4H, CH_2×2), 1.67-1.59 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 170.2, 169.6, 153.6, 136.8, 133.1, 120.8, 120.2, 119.7, 61.6, 51.3, 43.6, 26.7, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{21}N_2O_4$ 293.1501; Found 293.1510.



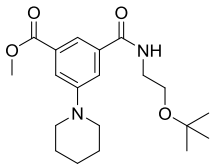
Methyl 3-(benzylcarbamoyl)-5-(piperidin-1-yl)benzoate (14q). The title compound was obtained as a colorless oil (233 mg, 79% yield) from monoester **13a** (220 mg, 0.84 mmol, 1.0 eq), benzylamine (0.11 mL, 1.00 mmol, 1.2 eq), HBTU (317 mg, 0.84 mmol, 1.0 eq) and TEA (0.23 mL, 1.67 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc.

^{1}H-NMR (400 MHz, CD_3OD) δ: 7.89 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.70 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 7.63 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 7.37-7.30 (m, 4H, Ar-H), 7.27-7.22 (m, 1H, Ar-H), 4.57 (s, 2H, Ar-CH_2), 3.91 (s, 3H, OCH_3), 3.28-3.24 (m, 4H, NCH_2×2), 1.75-1.68 (m, 4H, CH_2×2), 1.66-1.59 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 169.6, 168.3, 153.7, 140.2, 136.8, 132.4, 129.5, 128.6, 128.2, 120.5, 120.3, 119.2, 52.8, 51.2, 44.6, 26.7, 25.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{21}H_{25}N_2O_3$ 353.1865; Found 353.1867.



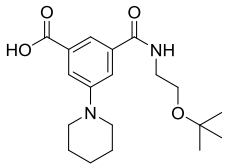
3-(Benzylcarbamoyl)-5-(piperidin-1-yl)benzoic acid (15q). The title compound was obtained as a white solid (175 mg, 78% yield) from benzoate **14q** (233 mg, 0.66 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.99 mL, 0.99 mmol, 1.5 eq) by following general procedure C. The crude product **15q** was used further without purification.

^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.14 (t, *J* = 6.0 Hz, 1H, NH), 7.86 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.65 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.56 (dd, *J* = 2.6, 1.4 Hz, 1H, Ar-H), 7.36-7.29 (m, 4H, Ar-H), 7.27-7.21 (m, 1H, Ar-H), 4.47 (d, *J* = 6.0 Hz, 2H, Ar-CH_2), 3.25-3.20 (m, 4H, NCH_2×2), 1.66-1.60 (m, 4H, CH_2×2), 1.58-1.52 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, DMSO-d_6) δ: 167.3, 165.8, 151.5, 139.6, 135.3, 131.7, 128.3, 127.3, 126.8, 118.4, 118.1, 118.0, 49.2, 42.7, 25.0, 23.8 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{20}H_{23}N_2O_3$ 339.1709; Found 339.1715.

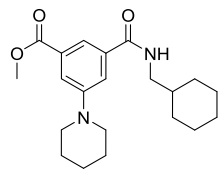


Methyl 3-((2-(*tert*-butoxy)ethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14r). The title compound was obtained as a colorless oil (153 mg, 85% yield) from monoester **13a** (130 mg, 0.49 mmol, 1.0 eq), 2-*tert*-butoxyethan-1-amine (64 µL, 0.54 mmol, 1.1 eq), HBTU (187 mg, 0.49 mmol, 1.0 eq) and TEA (0.14 mL, 0.99 mmol, 2.0 eq) by following general procedure B. Pure

material was obtained by column chromatography using gradient elution from 15% EtOAc in petroleum ether to 50% EtOAc in petroleum ether.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.70-7.67 (m, 2H, Ar-H), 7.60 (dd, *J* = 2.7, 1.6 Hz, 1H, Ar-H), 6.64-6.55 (m, 1H, Ar-H), 3.91 (s, 3H, OCH_3), 3.63-3.57 (m, 2H, OCH_2), 3.56-3.51 (m, 2H, NCH_2), 3.30-3.24 (m, 4H, NCH_2×2), 1.74-1.66 (m, 4H, CH_2×2), 1.64-1.57 (m, 2H, CH_2), 1.21 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.2, 167.0, 152.3, 136.0, 131.2, 119.4, 119.3, 116.9, 73.4, 60.6, 52.4, 50.0, 40.6, 27.7, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{20}H_{31}N_2O_4$ 363.2284; Found 363.2292.

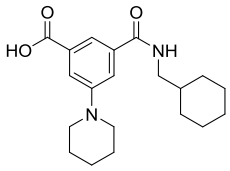


3-((2-(*tert*-Butoxy)ethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15r). The title compound was obtained as a pink solid (107 mg, 84% yield) from benzoate **14r** (133 mg, 0.37 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.55 mL, 0.55 mmol, 1.5 eq) by following general procedure C. Pure material was obtained by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 14 mL/min) using gradient elution from 100% water to 50% MeCN in water.
^{1}H-NMR (400 MHz, CD_3OD) δ: 8.48-8.43 (m, 1H, Ar-H), 8.39-8.33 (m, 1H, Ar-H), 8.30-8.24 (m, 1H, Ar-H), 3.67-3.62 (m, 4H, NCH_2×2), 3.61-3.57 (m, 2H, OCH_2), 3.55-3.50 (m, 2H, NCH_2), 2.10-2.00 (m, 4H, CH_2×2), 1.84-1.74 (m, 2H, CH_2), 1.21 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 138.4, 134.7, 74.5, 61.3, 42.1, 27.8, 25.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{19}H_{29}N_2O_4$ 349.2127; Found 349.2128.



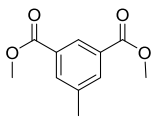
Methyl 3-((cyclohexylmethyl)carbamoyl)-5-(piperidin-1-yl)benzoate (14s). The title compound was obtained as a colorless oil (156 mg, 76% yield) from monoester **13a** (150 mg, 0.57 mmol, 1.0 eq), cyclohexanemethylamine (77 mg, 0.68 mmol, 1.2 eq), HBTU (216 mg, 0.57 mmol, 1.0 eq) and TEA (0.16 mL, 1.14 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 20% EtOAc in petroleum ether to 35% EtOAc in petroleum ether.
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.67 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 7.65 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.62 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 6.28-6.21 (m, 1H, NH), 3.92 (s, 3H, OCH_3), 3.33-3.24 (m, 6H, NCH_2×2, Cy- CH_2), 1.82-1.52

(m, 12H, CH_2×6), 1.32-1.12 (m, 3H, CH, CH_2), 1.06-0.92 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.3, 167.1, 152.4, 136.1, 131.1, 119.5, 119.4, 116.6, 52.4, 50.0, 46.5, 38.2, 31.1, 26.5, 26.0, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{21}H_{31}N_2O_3$ 359.2335; Found 359.2349.



3-((Cyclohexylmethyl)carbamoyl)-5-(piperidin-1-yl)benzoic acid (15s). The title compound was obtained as a light yellow solid (137 mg, 98% yield) from benzoate **14s** (146 mg, 0.41 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.61 mL, 0.61 mmol, 1.5 eq) by following general procedure C. The crude product **15s** was used further without purification.

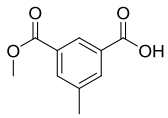
^{1}H-NMR (400 MHz, CD_3OD) δ: 7.87 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.71 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 7.59 (dd, *J* = 2.6, 1.5 Hz, 1H, Ar-H), 3.29-3.24 (m, 4H, NCH_2×2), 3.21 (d, *J* = 7.0 Hz, 2H, Cy- CH_2), 1.83-1.58 (m, 12H, CH_2×6), 1.37-1.15 (m, 3H, CH, CH_2), 1.07-0.94 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 170.0, 169.6, 153.6, 137.1, 133.0, 120.7, 120.2, 119.6, 51.3, 47.4, 39.2, 32.1, 27.6, 27.0, 26.7, 25.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{20}H_{29}N_2O_3$ 345.2178; Found 345.2178.



Dimethyl 5-methylisophthalate (12b). An oven-dried 20 mL pressure vial was cooled under stream of argon and charged with dimethyl 5-bromoisophthalate (500 mg, 1.83 mmol, 1.0 eq), methylboronic acid (132 mg, 2.20 mmol, 1.2 eq), potassium phosphate (1.17 g, 5.49 mmol, 3.0 eq), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (75 mg, 0.092 mmol, 0.05 eq) and anhydrous toluene (7 mL). After strirring at 90 °C for 18 h, the brown suspension was cooled to room temperature and filtered through Celite. The filter plug was washed with EtOAc and filtrate was dried over anhydrous Na_2SO_4. Volatiles were evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using 5% EtOAc in hexanes to give product **12b** as a white solid (305 mg, 80% yield). ^{1}H-NMR spectrum was identical to that from the literature.[5]

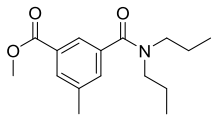
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.51-8.46 (m, 1H, Ar-H), 8.07-8.01 (m, 2H, Ar-H), 3.93 (s, 6H, OCH_3×2), 2.45 (s, 3H, Ar-CH_3) ppm.

[5] Sivakumar, C.; Sultan, N. Journal of Polymer Science: Part A: Polymer Chemistry. 2009, 47, 3337-3351.



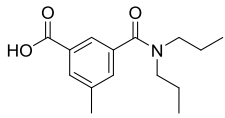
3-(Methoxycarbonyl)-5-methylbenzoic acid (13b). The title compound was obtained as a white solid material (266 mg, 97% yield) from diester **12b** (295 mg, 1.42 mmol, 1.0 eq) and aqueous 1M NaOH solution (1.42 mL, 1.42 mmol, 1.0 eq) by following general procedure A. The crude product **13b** was used in subsequent step without purification. ^{1}H-NMR spectrum was identical to that from the literature.[6]

^{1}H-NMR (400 MHz, CD_3OD) δ: 8.46-8.41 (m, 1H, Ar-H), 8.07-8.03 (m, 2H, Ar-H), 3.93 (s, 3H, OCH_3), 2.46 (s, 3H, Ar-CH_3) ppm. HRMS-ESI *(m/z)*: $[M-H]^-$ Calcd for $C_{10}H_9O_4$ 193.0501; Found 193.0502.



Methyl 3-(dipropylcarbamoyl)-5-methylbenzoate (16b). The title compound was obtained as a colorless oil (246 mg, 66% yield) from monoester **13b** (261 mg, 1.34 mmol, 1.0 eq), dipropylamine (0.22 mL, 1.61 mmol, 1.2 eq), HBTU (510 mg, 1.34 mmol, 1.0 eq) and TEA (0.37 mL, 2.69 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using 20% EtOAc in petroleum ether.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.88-7.86 (m, 1H, Ar-H), 7.81-7.79 (m, 1H, Ar-H), 7.38-7.35 (m, 1H, Ar-H), 3.91 (s, 3H, OCH_3), 3.49-3.40 (m, 2H, NCH_2), 3.19-3.10 (m, 2H, NCH_2), 2.41 (s, 3H, Ar-CH_3), 1.76-1.62 (m, 2H, CH_2), 1.58-1.46 (m, 2H, CH_2), 0.97 (t, J = 7.6 Hz, 3H, CH_3), 0.74 (t, J = 7.6 Hz, 3H, CH_3) ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{16}H_{24}NO_3$ 278.1756; Found 278.1758.

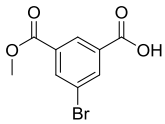


3-(Dipropylcarbamoyl)-5-methylbenzoic acid (17b). The title compound was obtained as a white solid (227 mg, 100% yield) from benzoate **16b** (240 mg, 0.86 mmol, 1.0 eq) and aqueous 1 M NaOH solution (1.30 mL, 1.30 mmol, 1.5 eq) by following general procedure C. The crude product **17b** was used further without purification.

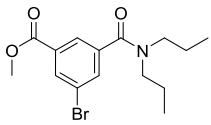
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.95-7.92 (m, 1H, Ar-H), 7.89-7.86 (m, 1H, Ar-H), 7.44-7.42 (m, 1H, Ar-H), 6.48-6.07 (br s, 1H, COOH), 3.55-3.39 (m, 2H,

[6] Gosh, A. K.; Takayama, J.; Kassekert, L. A.; Ella-Menye, J. R.; Yashchuk, S.; Agniswamy, J.; Wang, Y. F.; Aoki, M.; Amano, M.; Weber, I. T.; Mitsuya, H. Bioorg. Med. Chem. Lett. 2015, 25, 4903-4909.

NCH_2), 3.24-3.10 (m, 2H, NCH_2), 2.43 (s, 3H, Ar-CH_3), 1.77-1.65 (m, 2H, CH_2), 1.62-1.48 (m, 2H, CH_2), 0.99 (t, J = 7.6 Hz, 3H, CH_3), 0.76 (t, J = 7.6 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.0, 170.7, 139.1, 137.7, 132.7, 131.5, 129.6, 125.4, 51.0, 46.7, 22.1, 21.4, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{22}NO_3$ 264.1600; Found 264.1606.

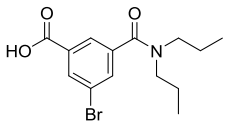


3-Bromo-5-(methoxycarbonyl)benzoic acid (13c). The title compound was obtained as a white solid material (568 mg, 100% yield) from dimethyl 5-bromoisophthalate (600 mg, 2.20 mmol, 1.0 eq) and aqueous 1 M NaOH solution (2.20 mL, 2.20 mmol, 1.0 eq) by following general procedure A. The crude product **13c** was used in subsequent step without purification. ^{1}H-NMR spectra was identical to that from the literature.[7]



Methyl 3-bromo-5-(dipropylcarbamoyl)benzoate (16c). The title compound was obtained as a colorless oil (484 mg, 64% yield) from monoester **13c** (568 mg, 2.19 mmol, 1.0 eq), dipropylamine (0.36 mL, 2.63 mmol, 1.2 eq), HBTU (831 mg, 2.19 mmol, 1.0 eq) and TEA (0.61 mL, 4.38 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using 10% EtOAc in petroleum ether.

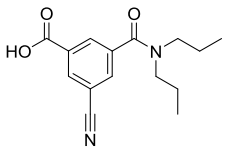
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.20-8.18 (m, 1H, Ar-H), 7.95-7.93 (m, 1H, Ar-H), 7.70-7.67 (m, 1H, Ar-H), 3.93 (s, 3H, OCH_3), 3.45 (t, J = 7.5 Hz, 2H, NCH_2), 3.14 (t, J = 7.5 Hz, 2H, NCH_2), 1.75-1.62 (m, 2H, CH_2), 1.60-1.49 (m, 2H, CH_2), 0.98 (t, J = 7.5 Hz, 3H, CH_3), 0.77 (t, J = 7.5 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.1, 165.3, 139.5, 134.0, 133.2, 132.2, 126.3, 122.8, 52.8, 50.9, 46.7, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{21}NO_3Br$ 342.0705; Found 342.0691.



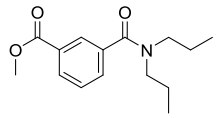
3-Bromo-5-(dipropylcarbamoyl)benzoic acid (17c). The title compound was obtained as a colorless oil (454 mg, 100% yield) from benzoate **16c** (473 mg, 1.38 mmol, 1.0 eq) and aqueous 1 M NaOH solution (2.07 mL, 2.07 mmol, 1.5 eq) by following general procedure C. The crude product **17c** was used further without purification.

[7] Choi, K.; Hamilton, A. D. *J. Am. Chem. Soc.* **2003**, *125*, 10241–10249.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.83 (s, 1H, COOH), 8.26-8.23 (m, 1H, Ar-H), 8.02-7.99 (m, 1H, Ar-H), 7.76-7.73 (m, 1H, Ar-H), 3.47 (t, *J* = 7.7 Hz, 2H, NCH_2), 3.16 (t, *J* = 7.7 Hz, 2H, NCH_2), 1.77-1.64 (m, 2H, CH_2), 1.62-1.49 (m, 2H, CH_2), 0.99 (t, *J* = 7.4 Hz, 3H, CH_3), 0.78 (t, *J* = 7.4 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.3, 168.9, 139.3, 134.7, 133.8, 131.7, 126.8, 122.9, 51.0, 46.9, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{14}H_{19}NO_3Br$ 328.0548; Found 328.0557.

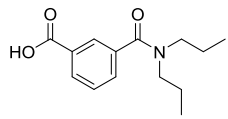


3-Cyano-5-(dipropylcarbamoyl)benzoic acid (17i). Copper(I) cyanide (82 mg, 0.92 mmol, 2.0 equiv) was added to a solution of benzoic acid **17c** (150 mg, 0.46 mmol, 1.0 equiv) in *N*-methyl-2-pyrrolidone (1 mL). After 6 h of stirring at 160 °C, the colorless solution was cooled to room temperature and aqueous 2N HCl solution (10 mL) and EtOAc (15 mL) were added. The organic layer was washed with an aqueous 2N HCl solution (2 x 10 mL), then brine (2 x 10 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by the reversed-phase column chromatography (30 g KP-C18-HS column, flow rate 18 mL/min) using gradient elution from 100% water to 100% MeCN to give product **17i** as a brown oil (20 mg, 16% yield).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.62 (s, 1H, COOH), 8.39-8.34 (m, 1H, Ar-H), 8.29-8.25 (m, 1H, Ar-H), 7.89-7.84 (m, 1H, Ar-H), 3.49 (t, *J* = 7.7 Hz, 2H, NCH_2), 3.14 (t, *J* = 7.7 Hz, 2H, NCH_2), 1.72 (sextet, *J* = 7.4 Hz, 2H, CH_2), 1.56 (sextet, *J* = 7.4 Hz, 2H, CH_2), 0.99 (t, *J* = 7.4 Hz, 3H, CH_3), 0.77 (t, *J* = 7.4 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.8, 167.4, 138.7, 134.5, 134.3, 132.1, 117.2, 113.7, 51.1, 47.1, 22.1, 20.8, 11.6, 11.2 ppm. IR (KBr, cm^1) 2236 (C≡N), 1724 (C=O). HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{19}N_2O_3$ 275.1396; Found 275.1406.



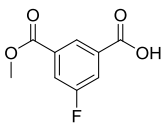
Methyl 3-(dipropylcarbamoyl)benzoate (16d). The title compound was obtained as a colorless oil (426 mg, 97% yield) from 3-methoxycarbonylbenzoic acid (300 mg, 1.66 mmol, 1.0 eq) (**13d**), dipropylamine (0.27 mL, 2.00 mmol, 1.2 eq), HBTU (632 mg, 1.66 mmol, 1.0 eq) and TEA (0.46 mL, 3.33 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 10% EtOAc in petroleum ether to 25% EtOAc in petroleum ether.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.08-8.01 (m, 2H, Ar-H), 7.56 (ddd, *J* = 7.6, 1.8, 1.3 Hz, 1H, Ar-H), 7.50-7.45 (m, 1H, Ar-H), 3.93 (s, 3H, OCH_3), 3.50-3.40 (m, 2H, NCH_2), 3.21-3.08 (m, 2H, NCH_2), 1.76-1.64 (m, 2H, CH_2), 1.58-1.46 (m, 2H, CH_2), 1.04-0.93 (m, 3H, CH_3), 0.82-0.67 (m, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 170.8, 166.6, 137.8, 131.2, 130.4, 130.3, 128.8, 127.8, 52.4, 50.9, 46.6, 22.1, 20.9, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{22}NO_3$ 264.1600; Found 264.1598.



3-(Dipropylcarbamoyl)benzoic acid (17d). The title compound was obtained as a white solid material (382 mg, 97% yield) from benzoate **16d** (416 mg, 1.58 mmol, 1.0 eq) and aqueous 1 M NaOH solution (2.37 mL, 2.37 mmol, 1.5 eq) by following general procedure C. The crude product **17d** was used further without purification. ^{1}H-NMR spectra was identical to that from the literature.[8]

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 10.29 (br s, 1H, COOH), 8.23-8.01 (m, 2H, Ar-H), 7.66-7.58 (m, 1H, Ar-H), 7.55-7.46 (m, 1H, Ar-H), 3.59-3.37 (m, 2H, NCH_2), 3.24-3.06 (m, 2H, NCH_2), 1.82-1.63 (m, 2H, CH_2), 1.61-1.46 (m, 2H, CH_2), 0.99 (t, *J* = 7.4 Hz, 3H, CH_3), 0.76 (t, *J* = 7.4 Hz, 3H, CH_3) ppm.

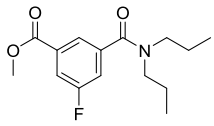


3-Fluoro-5-(methoxycarbonyl)benzoic acid (13e). The title compound was obtained as a white solid material (213 mg, 91% yield) from dimethyl 5-fluoroisophthalate (250 mg, 1.18 mmol, 1.0 eq) and aqueous 1 M NaOH solution (1.18 mL, 1.18 mmol,

[8] Bjoerklund, C.; Oscarson, S.; Benkestock, K.; Borkakoti, N.; Jansson, K.; Lindberg, J.; Vrang, L.; Hallberg, A.; Rosenquist, A.; Samuelsson, B.. *J. Med. Chem.* **2010**, *53*, 1458–1464.

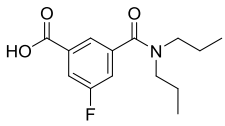
1.0 eq) by following general procedure A. The crude product **13e** was used in subsequent step without purification.

^{1}H-NMR (400 MHz, CD_3OD) δ: 8.44 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.95-7.89 (m, 2H, Ar-H), 3.95 (s, 3H, OCH_3) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 167.3 (d, *J* = 3.0 Hz), 166.4 (d, *J* = 3.0 Hz), 165.0, 162.6, 134.1 (d, *J* = 7.5 Hz), 127.4 (d, *J* = 3.0 Hz), 121.6 (d, *J* = 23.4 Hz), 121.2 (d, *J* = 23.7 Hz), 53.2 ppm. HRMS-ESI *(m/z)*: $[M-H]^-$ Calcd for $C_9H_6O_4F$ 197.0250; Found 197.0247.



Methyl 3-(dipropylcarbamoyl)-5-fluorobenzoate (16e). The title compound was obtained as a colorless oil (163 mg, 58% yield) from monoester **13e** (199 mg, 1.00 mmol, 1.0 eq), dipropylamine (0.16 mL, 1.20 mmol, 1.2 eq), HBTU (381 mg, 1.00 mmol, 1.0 eq) and TEA (0.28 mL, 2.01 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 25% EtOAc in petroleum ether to 50% EtOAc in petroleum ether.

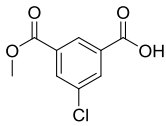
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.81 (dd, *J* = 1.4, 1.4 Hz, 1H, Ar-H), 7.74 (ddd, *J* = 8.9, 2.6, 1.4 Hz, 1H, Ar-H), 7.29-7.26 (m, 1H, Ar-H), 3.94 (s, 3H, OCH_3), 3.45 (t, *J* = 7.7 Hz, 2H, NCH_2), 3.14 (t, *J* = 7.7 Hz, 2H, NCH_2), 1.74-1.63 (m, 2H, CH_2), 1.60-1.47 (m, 2H, CH_2), 0.99 (t, *J* = 7.3 Hz, 3H, CH_3), 0.76 (t, *J* = 7.3 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.3 (d, *J* = 2.3 Hz), 165.4 (d, *J* = 3.0 Hz), 163.7, 161.2, 139.8 (d, *J* = 6.8 Hz), 123.5 (d, *J* = 3.3 Hz), 118.5 (d, *J* = 22.9 Hz), 117.3 (d, *J* = 23.1 Hz), 52.7, 50.9, 46.7, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{21}NO_3F$ 282.1505; Found 282.1518.



3-(Dipropylcarbamoyl)-5-fluorobenzoic acid (17e). The title compound was obtained as a colorless sticky oil (146 mg, 100% yield) from benzoate **16e** (154 mg, 0.55 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.82 mL, 0.82 mmol, 1.5 eq) by following general procedure C. The crude product **17e** was used further without purification.

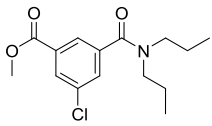
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.46 (br s, 1H, COOH), 7.89-7.85 (m, 1H, Ar-H), 7.78 (ddd, *J* = 8.7, 2.7, 1.3 Hz, 1H, Ar-H), 7.32 (ddd, *J* = 8.1, 2.7, 1.3 Hz, 1H, Ar-H), 3.47 (t, *J* = 7.8 Hz, 2H, NCH_2), 3.15 (t, *J* = 7.8 Hz, 2H, NCH_2), 1.75-1.64 (m, 2H, CH_2), 1.61-1.49 (m, 2H, CH_2), 0.98 (t, *J* = 7.3 Hz, 3H, CH_3), 0.77

(t, J = 7.3 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.5 (d, J = 2.2 Hz), 169.2 (d, J = 2.3 Hz), 163.7, 161.2, 139.5 (d, J = 6.7 Hz), 124.0 (d, J = 3.1 Hz), 119.2 (d, J = 22.9 Hz), 117.9 (d, J = 23.0 Hz), 51.0, 46.9, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{14}H_{19}NO_3F$ 268.1349; Found 268.1356.



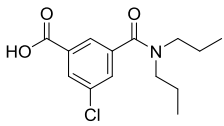
3-Chloro-5-(methoxycarbonyl)benzoic acid (13f). The title compound was obtained as a white solid material (282 mg, 100% yield) from dimethyl 5-chloroisophthalate (300 mg, 1.31 mmol, 1.0 eq) and aqueous 1 M NaOH solution (1.31 mL, 1.31 mmol, 1.0 eq) by following general procedure A. The crude product **13f** was used in subsequent step without purification.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.64 (dd, J = 1.5, 1.5 Hz, 1H, Ar-H), 8.26 (dd, J = 1.5, 1.5 Hz, 2H, Ar-H), 3.98 (s, 3H, OCH_3) ppm.



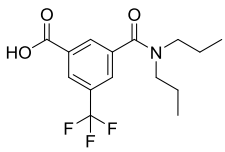
Methyl 3-chloro-5-(dipropylcarbamoyl)benzoate (16f). The title compound was obtained as a colorless oil (300 mg, 77% yield) from monoester **13f** (282 mg, 1.31 mmol, 1.0 eq), dipropylamine (0.22 mL, 1.58 mmol, 1.2 eq), HBTU (498 mg, 1.31 mmol, 1.0 eq) and TEA (0.37 mL, 2.63 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using 15% EtOAc in petroleum ether.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.06-8.00 (m, 1H, Ar-H), 7.93-7.87 (m, 1H, Ar-H), 7.56-7.50 (m, 1H, Ar-H), 3.94 (s, 3H, OCH_3), 3.49-3.41 (m, 2H, NCH_2), 3.18-3.09 (m, 2H, NCH_2), 1.75-1.64 (m, 2H, CH_2), 1.60-1.49 (m, 2H, CH_2), 0.98 (t, J = 7.4 Hz, 3H, CH_3), 0.77 (t, J = 7.4 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.2, 165.4, 139.3, 135.0, 132.1, 131.2, 130.3, 125.9, 52.8, 50.9, 46.7, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{21}NO_3Cl$ 298.1210; Found 298.1223.



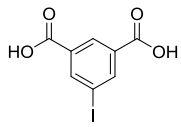
3-Chloro-5-(dipropylcarbamoyl)benzoic acid (17f). The title compound was obtained as a white solid material (275 mg, 98% yield) from benzoate **16f** (293 mg, 0.98 mmol, 1.0 eq) and aqueous 1 M NaOH solution (1.48 mL, 1.48 mmol, 1.5 eq) by following general procedure C. The crude product **17f** was used further without purification.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.09 (dd, *J* = 1.8, 1.8 Hz, 1H, Ar-H), 7.96 (dd, *J* = 1.8, 1.8 Hz, 1H, Ar-H), 7.59 (dd, *J* = 1.8, 1.8 Hz, 1H, Ar-H), 3.51-3.43 (m, 2H, NCH_2), 3.16 (t, *J* = 7.4 Hz, 2H, NCH_2), 1.77-1.65 (m, 2H, CH_2), 1.62-1.50 (m, 2H, CH_2), 0.99 (t, *J* = 7.4 Hz, 3H, CH_3), 0.78 (t, *J* = 7.4 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.4, 168.9, 139.2, 135.2, 131.9, 131.5, 130.9, 126.4, 51.0, 46.8, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{14}H_{19}NO_3Cl$ 284.1053; Found 284.1064.



3-(Dipropylcarbamoyl)-5-(trifluoromethyl)benzoic acid (17h). The title compound was obtained as a brownish oil (90 mg, 33% yield) from 5-(trifluoromethyl)isophthalic acid (200 mg, 0.85 mmol, 1.0 eq), dipropylamine (0.12 mL, 0.85 mmol, 1.0 eq), HBTU (324 mg, 0.85 mmol, 1.0 eq) and TEA (0.24 mL, 1.71 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using 25% EtOAc in petroleum ether.

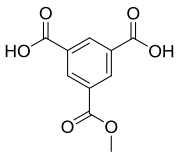
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.38-8.35 (m, 1H, Ar-H), 8.27-8.24 (m, 1H, Ar-H), 7.87-7.84 (m, 1H, Ar-H), 3.55-3.43 (m, 2H, NCH_2), 3.20-3.09 (m, 2H, NCH_2), 1.80-1.66 (m, 2H, CH_2), 1.63-1.51 (m, 2H, CH_2), 1.01 (t, *J* = 7.6 Hz, 3H, CH_3), 0.78 (t, *J* = 7.6 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.5, 168.6, 138.5, 132.0, 131.4, 128.4, 127.8, 124.7, 122.0, 51.1, 47.0, 22.1, 20.8, 11.6, 11.1 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{15}H_{19}NO_3F_3$ 318.1317; Found 318.1321.



5-Iodoisophthalic acid (18). To a solution of dimethyl 5-iodoisophthalate (500 mg, 1.56 mmol, 1.0 eq) in MeOH (20 mL) was added an aqueous 1 M NaOH solution (4.69 mL, 4.69 mmol, 3.0 eq). After stirring at 40 ^oC temperature for 18 h, the brown suspension was diluted with water (10 mL) and acidified to pH 3 with aqueous 1N HCl solution. The aqueous layer was extracted with EtOAc (3 x 20 mL). Combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield product **18** as a yellowish solid (455 mg, 100% yield), which was used in subsequent step without purification. ^{1}H-NMR spectrum was identical to that from the literature.[9]

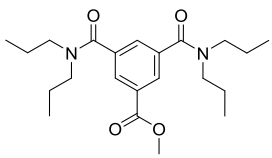
[9] Zhang, S.; Liu, Q.; Shen, M.; Hu, B.; Chen, Q.; Li, H.; Ammoureux, J. P. *Dalton Trans.* **2012**, 41, 4692-4698.

^{1}H-NMR (400 MHz, CD_3OD) δ: 8.59 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 8.52 (d, *J* = 1.5 Hz, 2H, Ar-H) ppm. HRMS-ESI *(m/z)*: $[M-H]^-$ Calcd for $C_8H_4O_4I$ 290.9154; Found 290.9165.



5-(Methoxycarbonyl)isophthalic acid (13g). Isophthalic acid **18** (451 mg, 1.54 mmol, 1.0 eq), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (126 mg, 0.15 mmol, 0.10 eq), TEA (0.47 mL, 3.40 mmol, 2.2 eq) and dry MeOH (10 mL) were combined in a 100 mL glass liner, which was then placed in stainless steel autoclave with a pressure gauge. The autoclave was sealed and purged three times with carbon monoxide and then pressurized to 70 psi with carbon monoxide. The mixture was heated at 100 ^oC. After 18 h the autoclave was cooled down to room temperature and the excess of carbon monoxide was carefully released under fume hood. The dark brown suspension was filtered through Celite. The filter plug was washed with EtOAc and filtrate was dried over anhydrous Na_2SO_4. Volatiles were evaporated under reduced pressure. An aqueous 1N HCl solution was added to pH 1 and then suspension was extracted with EtOAc (3 x 20 mL). Combined organic extracts were washed with brine (15 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, to give 346 mg (100% yield) of **13g** as a dark brown solid, which was used in the subsequent step without purification.

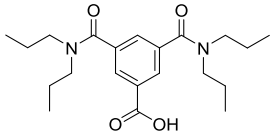
^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.68-8.65 (m, 1H, Ar-H), 8.63 (d, *J* = 1.6 Hz, 2H, Ar-H), 3.92 (s, 3H, OCH_3) ppm. ^{13}C-NMR (101 MHz, DMSO-d_6) δ: 165.8, 164.9, 133.9, 133.2, 132.5, 130.6, 52.7 ppm. HRMS-ESI *(m/z)*: $[M-H]^-$ Calcd for $C_{10}H_7O_6$ 223.0243; Found 223.0250.



Methyl 3,5-bis(dipropylcarbamoyl)benzoate (16g). The title compound was obtained as a colorless oil (337 mg, 57% yield) from monoester **13g** (340 mg, 1.52 mmol, 1.0 eq), dipropylamine (0.46 mL, 3.34 mmol, 2.2 eq), HBTU (1.15 g, 3.03 mmol, 2.0 eq) and TEA (0.85 mL, 6.07 mmol, 4.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 25% EtOAc in petroleum ether to 100% EtOAc.

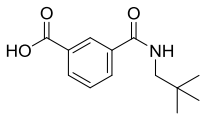
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.05 (d, *J* = 1.6 Hz, 2H, Ar-H), 7.55 (dd, *J* = 1.6, 1.6 Hz, 1H, Ar-H), 3.93 (s, 3H, OCH_3), 3.45 (t, *J* = 7.5 Hz, 4H, NCH_2×2), 3.14 (t, *J* = 7.5 Hz, 4H, NCH_2×2), 1.74-1.63 (m, 4H, CH_2×2), 1.58-1.48 (m, 4H,

CH_2×2), 0.98 (t, *J* = 7.3 Hz, 6H, CH_3×2), 0.75 (t, *J* = 7.3 Hz, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 170.0, 165.9, 138.2, 130.7, 129.4, 128.3, 52.6, 50.9, 46.6, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{22}H_{35}N_2O_4$ 391.2597; Found 391.2600.



3,5-Bis(dipropylcarbamoyl)benzoic acid (17g). The title compound was obtained as a colorless sticky oil (301 mg, 96% yield) from benzoate **13g** (324 mg, 0.83 mmol, 1.0 eq) and aqueous 1 M NaOH solution (1.24 mL, 1.24 mmol, 1.5 eq) by following general procedure C. The crude product **17g** was used further without purification.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.08 (br s, 1H, COOH), 8.08 (d, *J* = 1.6 Hz, 2H, Ar-H), 7.59 (dd, *J* = 1.6, 1.6 Hz, 1H, Ar-H), 3.46 (t, *J* = 7.7 Hz, 4H, NCH_2×2), 3.15 (t, *J* = 7.7 Hz, 4H, NCH_2×2), 1.75-1.64 (m, 4H, CH_2×2), 1.58-1.48 (m, 4H, CH_2×2), 0.98 (t, *J* = 7.3 Hz, 6H, CH_3×2), 0.74 (t, *J* = 7.3 Hz, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 170.2, 168.9, 138.0, 130.5, 129.8, 128.8, 51.0, 46.7, 22.0, 20.8, 11.6, 11.1 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{21}H_{33}N_2O_4$ 377.2440; Found 377.2450.



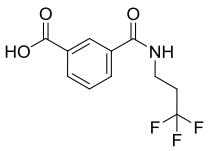
3-(Neopentylcarbamoyl)benzoic acid (19a). The benzoate intermediate was obtained as a yellow oil (164 mg, 79% yield) from 3-methoxycarbonylbenzoic acid (150 mg, 0.83 mmol, 1.0 eq) (**13d**), 2,2-dimethylpropan-1-amine (87 mg, 1.00 mmol, 1.2 eq), HBTU (316 mg, 0.83 mmol, 1.0 eq) and TEA (0.23 mL, 1.66 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 25% EtOAc in petroleum ether to 100% EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.38-8.35 (m, 1H, Ar-H), 8.18-8.13 (m, 1H, Ar-H), 8.05-8.00 (m, 1H, Ar-H), 7.56-7.49 (m, 1H, Ar-H), 6.31-6.18 (m, 1H, NH), 3.94 (s, 3H, OCH_3), 3.30 (d, *J* = 6.4 Hz, 2H, NCH_2), 0.99 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 166.8, 166.5, 135.5, 132.4, 131.9, 130.6, 129.0, 127.5, 52.5, 51.3, 32.4, 27.5 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{14}H_{20}NO_3$ 250.1443; Found 250.1449.

The title compound was obtained as a white solid material (144 mg, 99% yield) from benzoate intermediate (154 mg, 0.62 mmol, 1.0 eq) and aqueous 1 M

NaOH solution (0.93 mL, 0.93 mmol, 1.5 eq) by following general procedure C. The crude product **19a** was used further without purification.

^{1}H-NMR (400 MHz, CD_3OD) δ: 8.59-8.50 (m, 1H, NH), 8.48-8.45 (m, 1H, Ar-H), 8.20-8.14 (m, 1H, Ar-H), 8.03 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H, Ar-H), 7.61-7.54 (m, 1H, Ar-H), 3.25-3.21 (m, 2H, NCH_2), 0.98 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 169.8, 169.0, 136.7, 133.4, 132.6, 132.4, 129.8, 129.6, 51.9, 33.8, 27.9 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{13}H_{18}NO_3$ 236.1287; Found 236.1287.

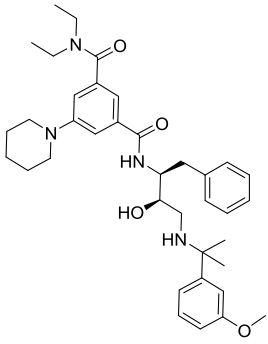


3-((3,3,3-Trifluoropropyl)carbamoyl)benzoic acid (19b). The benzoate intermediate was obtained as a yellow oil (127 mg, 55% yield) from 3-methoxycarbonylbenzoic acid (150 mg, 0.83 mmol, 1.0 eq) (**13d**), 3,3,3-trifluoropropylamine (94 mg, 0.83 mmol, 1.0 eq), HBTU (316 mg, 0.83 mmol, 1.0 eq) and TEA (0.23 mL, 1.66 mmol, 2.0 eq) by following general procedure B. Pure material was obtained by column chromatography using gradient elution from 25% EtOAc in petroleum ether to 100% EtOAc.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.37-8.34 (m, 1H, Ar-H), 8.20-8.15 (m, 1H, Ar-H), 8.03-7.98 (m, 1H, Ar-H), 7.54 (td, *J* = 7.8, 0.7 Hz, 1H, Ar-H), 6.58-6.49 (m, 1H, NH), 3.94 (s, 3H, OCH_3), 3.77-3.70 (m, 2H, NCH_2), 2.57-2.42 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 166.8, 166.4, 134.4, 132.8, 131.8, 130.8, 129.2, 127.7, 52.6, 33.9, 33.8, 33.7 ppm. ^{19}F-NMR (376 MHz, $CDCl_3$) δ: -65.0 (t, *J* = 10.6 Hz) ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{12}H_{13}NO_3F_3$ 276.0848; Found 276.0848.

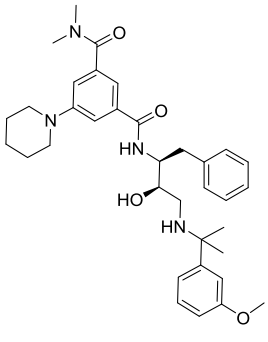
The title compound was obtained as a white solid material (114 mg, 99% yield) from benzoate intermediate (121 mg, 0.44 mmol, 1.0 eq) and aqueous 1 M NaOH solution (0.66 mL, 0.66 mmol, 1.5 eq) by following general procedure C. The crude product **19b** was used further without purification.

^{1}H-NMR (400 MHz, CD_3OD) δ: 8.48 (td, *J* = 1.8, 0.5 Hz, 1H, Ar-H), 8.21-8.16 (m, 1H, Ar-H), 8.03 (ddd, *J* = 7.8, 1.8, 1.2 Hz, 1H, Ar-H), 7.58 (td, *J* = 7.8, 0.5 Hz, 1H, Ar-H), 3.64 (t, *J* = 7.0 Hz, 2H, NCH_2), 2.61-2.46 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, CD_3OD) δ: 169.3, 168.8, 135.8, 133.7, 132.6, 129.9, 129.5, 126.6, 34.5, 34.2, 33.9 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{11}H_{11}NO_3F_3$ 262.0691; Found 262.0692.



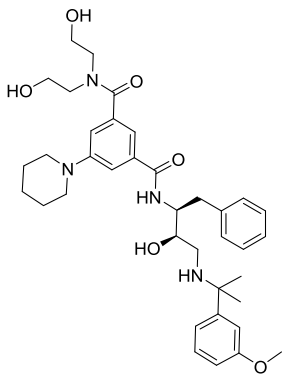
***N*[1],*N*[1]-Diethyl-*N*[3]-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-2a)**. The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **15a** (38 mg, 0.12 mmol, 1.0 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc, followed by 2% MeOH in EtOAc afforded *rac*-**2a** as a white solid (41 mg, 54% yield). Enantiomerically pure material (*S*,*R*)-**2a** (14 mg, 18% yield) was obtained by preparative HPLC on chiral stationary phase (*Chiralpak-IC*), using 50% EtOAc/50% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 15 mL/min, detector UV 230 nm).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.26-7.15 (m, 7H, Ar-H, NH), 7.05-6.99 (m, 2H, Ar-H), 6.93 (dd, *J* = 2.5, 1.2 Hz, 1H, Ar-H), 6.89-6.85 (m, 1H, Ar-H), 6.78-6.73 (m, 1H, Ar-H), 6.58 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.42-4.33 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.56-3.48 (m, 2H, CH_2), 3.27-3.12 (m, 5H, NCH_2×2, CH), 3.08-2.87 (m, 2H, CH_2), 2.54-2.44 (m, 2H, CH_2), 1.71-1.65 (m, 4H, CH_2×2), 1.63-1.58 (m, 2H, CH_2), 1.54 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 1.36-1.17 (m, 6H, CH_3, CH_2, OH), 1.14-1.00 (m, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.0, 168.1, 159.9, 152.2, 138.5, 137.8, 129.6, 129.5, 128.7, 126.7, 118.4, 116.2, 115.3, 113.9, 112.4, 70.7, 55.4, 53.5, 50.0, 36.7, 25.7 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{37}H_{51}N_4O_4$ 615.3910; Found 615.3904. Optical rotation $[\alpha]^{20}_D$ –35.7 (*c* 0.62, $CHCl_3$).



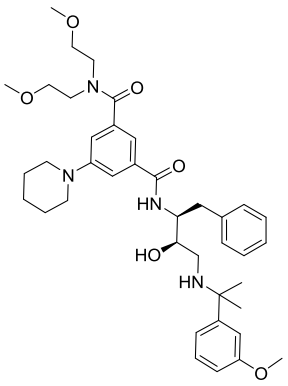
***N*[1]-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl) amino)-1-phenylbutan-2-yl)-*N*[3],*N*[3]-dimethyl-5-(piperidin-1-yl) isophthalamide ((*S*,*R*)-2b)**. The title compound was obtained from (*R*,*S*)-**11**(25 mg, 0.062 mmol, 1.0 eq), benzoic acid **15b** (17 mg, 0.062 mmol, 1.0 eq), HBTU (24 mg, 0.062 mmol, 1.0 eq) and TEA (35 µL, 0.25 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel

using gradient elution from 25% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**2b** as a colorless sticky oil (17 mg, 47% yield).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.25-7.14 (m, 7H, Ar-H, NH), 7.02-6.97 (m, 3H, Ar-H), 6.94-6.89 (m, 1H, Ar-H), 6.76-6.64 (m, 2H, Ar-H), 4.39-4.29 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.53-3.46 (m, 1H, CH), 3.23-3.15 (m, 4H, NCH_2×2), 3.08 (s, 3H, NCH_3), 3.01-2.75 (m, 5H, NCH_3, CH_2), 2.46 (d, *J* = 4.3 Hz, 2H, CH_2), 1.71-1.63 (m, 4H, CH_2×2), 1.62-1.56 (m, 2H, CH_2), 1.48 (s, 3H, CH_3), 1.47 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.4, 167.9, 159.8, 152.2, 148.6, 137.9, 137.6, 135.7, 129.5, 129.4, 128.6, 126.6, 118.5, 116.9, 115.6, 114.6, 112.4, 111.6, 70.8, 56.1, 55.3, 53.8, 50.0, 44.6, 36.7, 29.9, 29.0, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{35}H_{47}N_4O_4$ 587.3597; Found 587.3580. Optical rotation $[\alpha]^{20}_D$ –26.7 (*c* 1.24, $CHCl_3$).



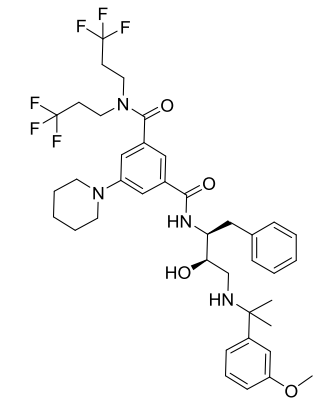
N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3,N^3-bis(2-hydroxyethyl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-2c). The protected intermediate was obtained from (*R*,*S*)-**11** (35 mg, 0.087 mmol, 1.0 eq), benzoic acid **15c** (49 mg, 0.087 mmol, 1.0 eq), HBTU (33 mg, 0.087 mmol, 1.0 eq) and TEA (49 µL, 0.35 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 25% EtOAc in petroleum ether to 50% EtOAc in petroleum ether afforded protected intermediate as a colorless oil (60 mg, 79% yield), which was used in subsequent step without purification. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{49}H_{79}N_4O_6Si_2$ 875.5538; Found 875.5530.
To a solution of protected intermediate from above (53 mg, 0.060 mmol, 1.0 eq) in anhydrous THF (4 mL) was dropwise added TBAF (1M solution in THF, 0.24 mL, 0.24 mmol, 4.0 eq). After stirring for 1 h at room temperature all the volatiles were evaporated under reduced pressure. The residue was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). Combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and evaporated under reduced pressure. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 100% EtOAc to 5% MeOH in EtOAc afforded (*S*,*R*)-**2c** as a white solid (16 mg, 41%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.39 (d, *J* = 8.7 Hz, 1H, NH), 7.24-7.11 (m, 8H, Ar-H), 7.05 (dd, *J* = 2.4, 1.2 Hz, 1H, Ar-H), 7.01-6.94 (m, 2H, Ar-H), 6.76-6.71 (m, 1H, Ar-H), 4.36-4.27 (m, 1H, CH), 3.93-3.83 (m, 2H, OCH_2), 3.76 (s, 3H, OCH_3), 3.69-3.53 (m, 5H, OCH_2, CH_2, CH), 3.43-3.29 (m, 2H, CH_2), 3.22-3.11 (m, 4H, NCH_2×2), 2.94-2.79 (m, 2H, NCH_2), 2.50-2.39 (m, 2H, NCH_2), 1.67-1.60 (m, 4H, CH_2×2), 1.60-1.53 (m, 2H, CH_2), 1.44 (s, 3H, CH_3), 1.43 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 173.6, 168.0, 159.7, 152.2, 148.7, 138.3, 137.1, 135.6, 129.4, 128.5, 126.4, 118.5, 117.0, 115.9, 115.2, 112.5, 111.4, 71.2, 60.8, 55.9, 55.3, 54.4, 53.5, 49.9, 44.8, 36.5, 30.1, 28.8, 25.6, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{37}H_{51}N_4O_6$ 647.3809; Found 647.3796. Optical rotation $[\alpha]^{20}_D$ –38.7 (*c* 1.16, $CHCl_3$).



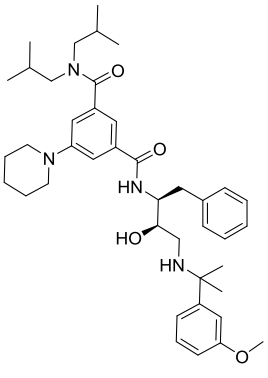
***N*^{1}-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl) amino)-1-phenylbutan-2-yl)-*N*^{3},*N*^{3}-bis(2-methoxyethyl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-2d)**. The title compound was obtained from (*R*,*S*)-**11** (35 mg, 0.087 mmol, 1.0 eq), benzoic acid **15d** (32 mg, 0.087 mmol, 1.0 eq), HBTU (33 mg, 0.087 mmol, 1.0 eq) and TEA (49 µL, 0.35 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 10% MeOH in EtOAc afforded (*S*,*R*)-**2d** as a colorless sticky oil (18 mg, 31% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.25-7.15 (m, 7H, Ar-H, NH), 7.05 (dd, *J* = 2.5, 1.2 Hz, 1H, Ar-H), 7.02-6.96 (m, 3H, Ar-H), 6.73 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H, Ar-H), 6.54 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.39-4.29 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.75-3.62 (m, 4H, OCH_2×2), 3.53-3.32 (m, 8H, OCH_3, NCH_2×2, CH), 3.27-3.17 (m, 7H, OCH_3, NCH_2×2), 3.05-2.91 (m, 2H, CH_2), 2.50-2.40 (m, 2H, CH_2), 1.72-1.64 (m, 4H, CH_2×2), 1.63-1.56 (m, 2H, CH_2), 1.47 (s, 3H, CH_3), 1.46 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 172.0, 167.8, 159.7, 152.1, 148.9, 137.9, 137.7, 135.5, 129.5, 129.3, 128.7, 126.6, 118.5, 117.2, 115.4, 114.6, 112.4, 111.5, 71.0, 59.0, 55.9, 55.3, 53.6, 49.9, 44.5, 36.7, 30.0, 29.2, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{39}H_{55}N_4O_6$ 675.4122; Found 675.4120. Optical rotation $[\alpha]^{20}_D$ –35.1 (*c* 1.28, $CHCl_3$).



***N*^{1}-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)-*N*^{3},*N*^{3}-bis(3,3,3-trifluoropropyl)isophthalamide (**(*S*,*R*)-2e)**. The title compound was obtained from (*R*,*S*)-**11** (35 mg, 0.087 mmol, 1.0 eq), benzoic acid **15e** (38 mg, 0.087 mmol, 1.0 eq), HBTU (33 mg, 0.087 mmol, 1.0 eq) and TEA (49 µL, 0.35 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**2e** as a white solid (39 mg, 60% yield).

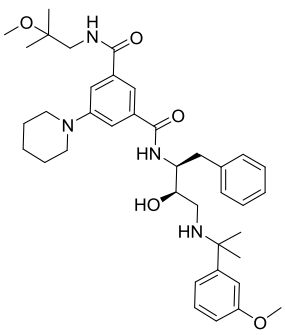
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.25-7.17 (m, 7H, Ar-H, NH), 7.04-6.97 (m, 2H, Ar-H), 6.89 (dd, *J* = 2.6, 1.3 Hz, 1H, Ar-H), 6.84-6.82 (m, 1H, Ar-H), 6.75 (dd, *J* = 8.1, 2.6 Hz, 1H, Ar-H), 6.48 (d, *J* = 8.7 Hz, 1H, Ar-H), 4.38-4.29 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.69-3.45 (m, 5H, NCH_2×2, CH), 3.22-3.17 (m, 4H, NCH_2×2), 3.04-2.93 (m, 2H, CH_2), 2.62-2.49 (m, 2H, CH_2), 2.48 (d, *J* = 4.3 Hz, 2H, CH_2), 2.35-2.22 (m, 2H, CH_2), 1.73-1.65 (m, 4H, CH_2×2), 1.64-1.58 (m, 2H, CH_2), 1.49 (s, 3H, CH_3), 1.48 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.9, 167.7, 159.8, 152.3, 137.8, 136.6, 136.1, 129.5, 129.4, 128.7, 126.7, 118.4, 115.9, 115.8, 113.6, 112.4, 111.7, 70.7, 56.4, 55.3, 53.7, 49.8, 44.6, 38.8, 36.6, 29.8, 29.6, 29.0, 25.6, 24.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{39}H_{49}N_4O_4F_6$ 751.3658; Found 751.3660. Optical rotation $[\alpha]^{20}_D$ –22.4 (*c* 3.12, $CHCl_3$).



***N*^{1}-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl) amino)-1-phenylbutan-2-yl)-*N*^{3},*N*^{3}-diisobutyl-5-(piperidin-1-yl)isophthalamide (**(*S*,*R*)-2f)**. The title compound was obtained from *rac*-**11** (51 mg, 0.13 mmol, 1.0 eq), benzoic acid **15f** (46 mg, 0.13 mmol, 1.0 eq), HBTU (48 mg, 0.13 mmol, 1.0 eq) and TEA (71 µL, 0.51 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using 1% MeOH in EtOAc afforded *rac*-**2f** as a white solid (58 mg, 68% yield). Enantiomerically pure material (*S*,*R*)-**2f** (28 mg, 33% yield) was obtained

by preparative HPLC on chiral stationary phase (*Chiralpak-ID*), using 85% EtOAc/15% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 30 mL/min, detector UV 254 nm).

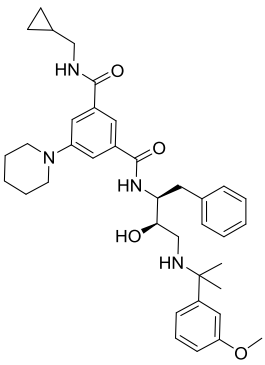
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.25-7.13 (m, 7H, Ar-H, NH), 7.03-6.99 (m, 2H, Ar-H), 6.93-6.88 (m, 1H, Ar-H), 6.85-6.80 (m, 1H, Ar-H), 6.75 (dd, *J* = 8.2, 2.4 Hz, 1H, Ar-H), 6.51 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.90-4.74 (m, 2H, NH, OH), 4.41-4.29 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.60-3.52 (m, 1H, CH), 3.41-3.28 (m, 2H, NCH_2), 3.22-3.13 (m, 4H, NCH_2×2), 3.07-2.92 (m, 4H, NCH_2, CH_2), 2.52-2.45 (m, 2H, CH_2), 2.15-2.04 (m, 1H, CH), 1.91-1.78 (m, 1H, CH), 1.72-1.65 (m, 4H, CH_2×2), 1.63-1.57 (m, 2H, CH_2), 1.47 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 0.98 (d, *J* = 6.6 Hz, 6H, CH_3×2), 0.73 (d, *J* = 6.6 Hz, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 172.2, 168.0, 159.9, 152.2, 138.7, 137.7, 135.5, 129.6, 128.7, 126.7, 118.4, 117.2, 115.4, 114.6, 112.4, 112.2, 70.5, 57.2, 55.4, 53.3, 50.1, 45.2, 36.5, 28.9, 28.4, 25.7, 24.3, 20.4, 20.0 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{41}H_{59}N_4O_4$ 671.4536; Found 671.4534. Optical rotation $[\alpha]^{20}_D$ –21.6 (*c* 2.11, $CHCl_3$).



N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3-(2-methoxy-2-methylpropyl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-**3a**. The title compound was obtained from (*R*,*S*)-**11** (30 mg, 0.075 mmol, 1.0 eq), benzoic acid **15g** (25 mg, 0.075 mmol, 1.0 eq), HBTU (28 mg, 0.075 mmol, 1.0 eq) and TEA (42 μL, 0.30 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**3a** as a white solid (30 mg, 62% yield).

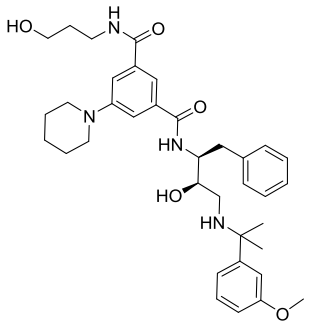
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.47 (dd, *J* = 2.6, 1.4 Hz, 1H, NH), 7.34-7.27 (m, 4H, Ar-H, NH), 7.25-7.19 (m, 4H, Ar-H), 7.06-6.99 (m, 2H, Ar-H), 6.76 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H, Ar-H), 6.72-6.65 (m, 1H, Ar-H), 6.55-6.49 (m, 1H, Ar-H), 4.43-4.34 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.57-3.47 (m, 3H, CH, NCH_2), 3.25 (s, 3H, OCH_3), 3.25-3.21 (m, 4H, NCH_2×2), 3.02 (d, *J* = 6.6 Hz, 2H, CH_2), 2.49 (d, *J* = 4.3 Hz, 2H, CH_2), 1.75-1.67 (m, 4H, CH_2×2), 1.66-1.60 (m, 2H, CH_2), 1.50 (s, 6H, CH_3×2), 1.24 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101

MHz, $CDCl_3$) δ: 167.8, 167.6, 159.7, 152.4, 149.0, 137.9, 136.1, 135.8, 129.5, 129.3, 128.7, 126.7, 118.5, 117.6, 117.1, 114.4, 112.4, 111.4, 74.6, 70.9, 55.8, 55.3, 53.8, 50.1, 49.6, 48.4, 44.5, 36.7, 30.1, 29.1, 25.7, 24.3, 22.6 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{38}H_{53}N_4O_5$ 645.4016; Found 645.4012. Optical rotation $[\alpha]^{20}_D$ –39.3 (*c* 2.03, $CHCl_3$).



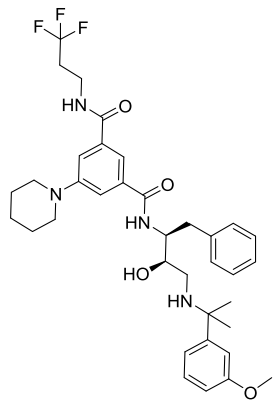
***N*^{1}-(Cyclopropylmethyl)-*N*^{3}-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3b)**. The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **15h** (38 mg, 0.12 mmol, 1.0 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc, followed by 5% MeOH in EtOAc afforded *rac*-**3b** as a white solid (48 mg, 63% yield). Enantiomerically pure material (*S*,*R*)-**3b** (17 mg, 22% yield) was obtained by semi-preparative HPLC on chiral stationary phase (*Chiralpak-IC*), using 40% EtOAc/60% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 2.0 mL/min, detector UV 254 nm).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46 (dd, *J* = 2.6, 1.4 Hz, 1H, NH), 7.29-7.26 (m, 1H, NH), 7.25-7.15 (m, 7H, Ar-H), 7.03-6.96 (m, 2H, Ar-H), 6.80-6.70 (m, 2H, Ar-H), 6.49-6.40 (m, 1H, Ar-H), 4.40-4.31 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.54-3.50 (m, 1H, CH), 3.28 (ddd, *J* = 7.2, 5.4, 1.9 Hz, 2H, NCH_2), 3.22-3.16 (m, 4H, NCH_2×2), 2.97 (d, *J* = 6.7 Hz, 2H, CH_2), 2.49-2.40 (m, 2H, CH_2), 1.71-1.64 (m, 4H, CH_2×2), 1.63-1.55 (m, 2H, CH_2), 1.48 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 0.93-0.80 (m, 1H, CH), 0.60-0.50 (m, 2H, CH_2), 0.31-0.21 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.0, 167.3, 159.8, 152.4, 137.9, 136.1, 135.6, 129.6, 129.4, 128.7, 126.7, 118.5, 117.9, 116.9, 114.4, 112.4, 111.9, 70.7, 55.4, 53.8, 50.0, 45.2, 44.7, 36.7, 29.8, 28.5, 25.7, 24.3, 10.9, 3.8 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{37}H_{49}N_4O_4$ 613.3754; Found 613.3769. Optical rotation $[\alpha]^{20}_D$ –40.3 (*c* 1.32, $CHCl_3$).



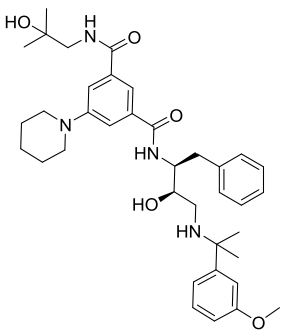
N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3-(3-hydroxypropyl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3c). The title compound was obtained from (*R*,*S*)-**11** (32 mg, 0.080 mmol, 1.0 eq), benzoic acid **15i** (24 mg, 0.080 mmol, 1.0 eq), HBTU (30 mg, 0.080 mmol, 1.0 eq) and TEA (44 µL, 0.32 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 100% EtOAc to 2% MeOH in EtOAc afforded (*S*,*R*)-**3c** as a white solid (37 mg, 75% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46 (dd, *J* = 2.6, 1.4 Hz, 1H, NH), 7.28-7.26 (m, 1H, NH), 7.25-7.14 (m, 7H, Ar-H), 7.05-6.88 (m, 4H, Ar-H), 6.77-6.71 (m, 1H, Ar-H), 4.40-4.30 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.68 (t, *J* = 5.5 Hz, 2H, OCH_2), 3.62-3.49 (m, 3H, NCH_2, CH), 3.22-3.15 (m, 4H, NCH_2×2), 2.93 (dd, *J* = 6.8, 3.9 Hz, 2H, CH_2), 2.54-2.41 (m, 2H, CH_2), 1.76 (quintet, *J* = 5.7 Hz, 2H, CH_2), 1.71-1.63 (m, 4H, CH_2×2), 1.62-1.56 (m, 2H, CH_2), 1.47 (s, 3H, CH_3), 1.45 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.3, 168.1, 159.7, 152.4, 148.8, 138.0, 135.9, 135.4, 129.5, 129.4, 128.7, 126.7, 118.5, 117.6, 117.0, 114.2, 112.5, 111.6, 70.7, 59.8, 55.9, 55.4, 54.2, 49.9, 44.5, 37.3, 36.8, 32.2, 30.4, 28.7, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{36}H_{49}N_4O_5$ 617.3703; Found 617.3705. Optical rotation $[\alpha]^{20}_D$ –45.9 (*c* 2.51, $CHCl_3$).



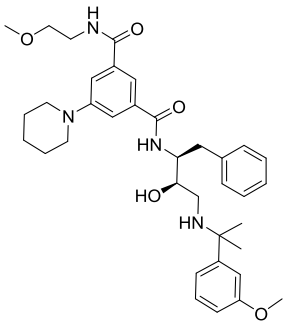
N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)-N^3-(3,3,3-trifluoropropyl)isophthalamide ((*S*,*R*)-3d). The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **15j** (42 mg, 0.12 mmol, 1.0 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 2% MeOH in EtOAc afforded *rac*-**3d** as a colorless sticky oil (60 mg, 73% yield). Enantiomerically pure material (*S*,*R*)-**3d** (21 mg,

26% yield) was obtained by semi-preparative HPLC on chiral stationary phase (*Chiralpak-IC*), using 40% EtOAc/60% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 2.5 mL/min, detector UV 254 nm).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.47-7.42 (m, 1H, NH), 7.30-7.26 (m, 1H, NH), 7.25-7.13 (m, 7H, Ar-H), 7.03-6.96 (m, 1H, Ar-H), 6.92-6.82 (m, 1H, Ar-H), 6.73 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H, Ar-H), 6.70-6.54 (m, 2H, Ar-H), 4.40-4.30 (m, 1H, CH), 3.80-3.77 (m, 3H, OCH_3), 3.70-3.63 (m, 2H, NCH_2), 3.58-3.48 (m, 1H, CH), 3.25-3.17 (m, 4H, NCH_2×2), 2.98-2.92 (m, 2H, CH_2), 2.52-2.39 (m, 4H, CH_2×2), 1.72-1.64 (m, 4H, CH_2×2), 1.63-1.57 (m, 2H, CH_2), 1.49-1.45 (m, 4H, CH_3), 1.39 (d, *J* = 5.5 Hz, 2H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.0, 167.5, 159.7, 152.4, 137.8, 135.9, 135.3, 129.5, 129.4, 128.7, 126.8, 118.5, 117.5, 117.1, 114.2, 112.5, 111.9, 70.7, 56.3, 55.4, 53.8, 49.9, 44.6, 36.7, 33.9, 33.6, 30.1, 28.5, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{36}H_{46}N_4O_4F_3$ 655.3471; Found 655.3477. Optical rotation $[\alpha]^{20}_D$ –31.8 (*c* 0.33, $CHCl_3$).



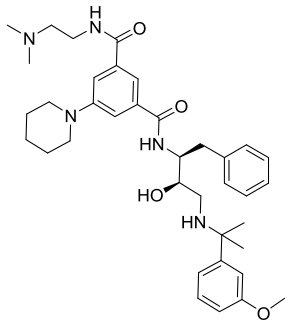
***N*^{1}-(2-Hydroxy-2-methylpropyl)-*N*^{3}-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3e)**. The title compound was obtained from (*R*,*S*)-**11** (32 mg, 0.080 mmol, 1.0 eq), benzoic acid **15k** (26 mg, 0.080 mmol, 1.0 eq), HBTU (30 mg, 0.080 mmol, 1.0 eq) and TEA (44 µL, 0.32 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**3e** as a white solid (30 mg, 60% yield).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.43 (dd, *J* = 2.6, 1.4 Hz, 1H, NH), 7.31-7.28 (m, 1H, NH), 7.24-7.12 (m, 8H, Ar-H), 7.00-6.91 (m, 3H, Ar-H), 6.73 (ddd, *J* = 8.2, 2.5, 1.0 Hz, 1H, Ar-H), 4.39-4.30 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 3.57-3.50 (m, 1H, CH), 3.49-3.38 (m, 2H, NCH_2), 3.19-3.12 (m, 4H, NCH_2×2), 2.99-2.83 (m, 2H, CH_2), 2.54-2.40 (m, 2H, CH_2), 1.69-1.61 (m, 4H, CH_2×2), 1.61-1.54 (m, 2H, CH_2), 1.46 (s, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.26 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.5, 168.1, 159.7, 152.3, 148.8, 138.0, 135.9, 135.6, 129.4, 129.3, 128.7, 126.6, 118.5, 117.7, 117.1, 114.4, 112.5, 111.6, 71.2, 70.6, 55.9, 55.4, 54.4, 51.1, 49.9, 44.5, 36.7, 30.3, 28.8, 27.6, 27.5,

25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{37}H_{51}N_4O_5$ 631.3859; Found 631.3856. Optical rotation $[\alpha]^{20}_D$ –46.9 (*c* 2.20, $CHCl_3$).



N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3-(2-methoxyethyl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3f). The title compound was obtained from (*R*,*S*)-**11** (35 mg, 0.087 mmol, 1.0 eq), benzoic acid **15l** (27 mg, 0.087 mmol, 1.0 eq), HBTU (33 mg, 0.087 mmol, 1.0 eq) and TEA (49 µL, 0.35 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 25% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**3f** as a colorless sticky oil (36 mg, 67% yield).

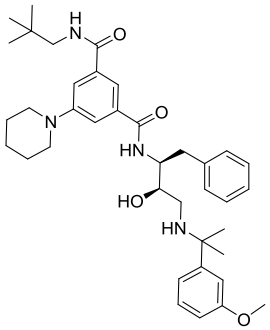
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.46-7.43 (m, 1H, NH), 7.30-7.27 (m, 1H, NH), 7.25-7.16 (m, 7H, Ar-H), 7.02-6.97 (m, 2H, Ar-H), 6.77-6.71 (m, 1H, Ar-H), 6.67-6.56 (m, 2H, Ar-H), 4.40-4.30 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.67-3.60 (m, 2H, OCH_2), 3.58-3.48 (m, 3H, NH_2, CH), 3.37 (s, 3H, OCH_3), 3.22-3.17 (m, 4H, NCH_2×2), 3.06-2.79 (m, 4H, CH_2, NH, OH), 2.46 (d, *J* = 4.2 Hz, 2H, CH_2), 1.72-1.64 (m, 4H, CH_2×2), 1.63-1.56 (m, 2H, CH_2), 1.48 (s, 3H, CH_3), 1.48 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.9, 167.4, 159.7, 152.4, 137.9, 135.9, 135.8, 129.6, 129.3, 128.7, 126.7, 118.5, 117.7, 117.1, 114.4, 112.4, 111.6, 71.3, 70.8, 59.0, 56.0, 55.3, 53.8, 50.0, 44.6, 40.0, 36.7, 30.2, 28.9, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{36}H_{49}N_4O_5$ 617.3703; Found 617.3708. Optical rotation $[\alpha]^{20}_D$ –36.3 (*c* 2.39, $CHCl_3$).



N^1-(2-(Dimethylamino)ethyl)-N^3-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3g). The title compound was obtained from (*R*,*S*)-**11** (30 mg, 0.075 mmol, 1.0 eq), benzoic acid **15m** (24 mg, 0.075 mmol, 1.0 eq), HBTU (28 mg, 0.075 mmol, 1.0 eq) and TEA (42 µL, 0.30 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica

gel using gradient elution from 100% EtOAc to 10% MeOH in EtOAc afforded (*S*,*R*)-**3g** as a yellowish sticky oil (27 mg, 57% yield).

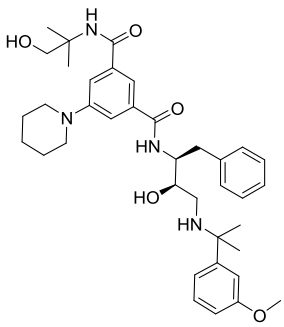
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.51-7.44 (m, 1H, NH), 7.38-7.30 (m, 1H, NH), 7.29-7.26 (m, 1H, Ar-H), 7.25-7.13 (m, 6H, Ar-H), 7.02-6.95 (m, 2H, Ar-H) 6.89-6.83 (m, 1H, Ar-H), 6.81-6.76 (m, 1H, Ar-H), 6.75-6.70 (m, 1H, Ar-H), 4.40-4.31 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.55-3.47 (m, 3H, NCH_2, CH), 3.26-3.17 (m, 4H NCH_2×2), 3.08-2.67 (m, 4H, CH_2, NH, OH), 2.51 (t, *J* = 6.0 Hz, 2H, NCH_2), 2.46 (d, *J* = 4.3 Hz, 2H, CH_2), 2.26 (s, 6H, NCH_3×2), 1.71-1.64 (m, 4H, CH_2×2), 1.62-1.55 (m, 2H, CH_2), 1.49-1.43 (m, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.8, 167.5, 159.7, 152.3, 149.0, 138.0, 136.0, 135.7, 129.5, 129.3, 128.7, 126.7, 118.5, 117.8, 117.1, 114.5, 112.4, 111.5, 70.9, 58.0, 55.8, 55.3, 53.9, 50.1, 45.3, 44.6, 37.5, 36.7, 30.2, 29.0, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{37}H_{52}N_5O_4$ 630.4019; Found 630.4048. Optical rotation $[\alpha]^{20}_D$ –39.2 (*c* 1.97, $CHCl_3$).



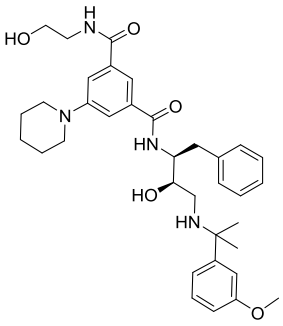
***N*^{1}-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-*N*^{3}-neopentyl-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3h)**. The title compound was obtained from *rac*-**11** (60 mg, 0.15 mmol, 1.0 eq), benzoic acid **15n** (52 mg, 0.16 mmol, 1.1 eq), HBTU (57 mg, 0.15 mmol, 1.0 eq) and TEA (83 µL, 0.60 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 10% MeOH in EtOAc afforded *rac*-**3h** as a light yellowish solid (66 mg, 70% yield). Enantiomerically pure material (*S*,*R*)-**3h** (31 mg, 33% yield) was obtained by preparative HPLC on chiral stationary phase (*Chiralpak-ID*), using 85% EtOAc/15% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 20 mL/min, detector UV 254 nm).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.47 (dd, *J* = 2.6, 1.4 Hz, 1H, NH), 7.32-7.30 (m, 1H, NH), 7.24-7.16 (m, 7H, Ar-H), 7.04-6.98 (m, 2H, Ar-H), 6.84 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.79-6.73 (m, 1H, Ar-H), 6.52-6.46 (m, 1H, Ar-H), 5.26-5.04 (m, 2H, NH, OH), 4.39-4.30 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 3.73-3.67 (m, 1H, CH), 3.24 (d, *J* = 6.4 Hz, 2H, NCH_2), 3.21-3.17 (m, 4H, NCH_2×2), 3.06-2.92 (m, 2H, CH_2), 2.61-2.46 (m, 2H, CH_2), 1.71-1.63 (m, 4H, CH_2×2), 1.62-1.53 (m, 8H, CH_2, CH_3×2), 0.96 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz,

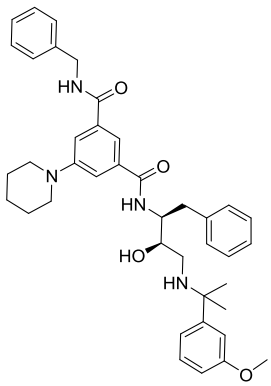
$CDCl_3$) δ: 168.0, 167.7, 159.9, 152.4, 137.8, 136.3, 135.4, 129.7, 129.6, 128.7, 126.7, 118.3, 117.9, 117.0, 114.3, 112.4, 70.2, 57.8, 55.4, 53.6, 51.2, 50.0, 45.4, 36.3, 32.5, 28.7, 27.7, 27.5, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{38}H_{53}N_4O_4$ 629.4067; Found 629.4067. Optical rotation $[\alpha]^{20}_D$ –40.6 (*c* 0.59, $CHCl_3$).



N^1-(1-Hydroxy-2-methylpropan-2-yl)-N^3-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3i). The title compound was obtained from (*R*,*S*)-**11** (30 mg, 0.075 mmol, 1.0 eq), benzoic acid **15o** (24 mg, 0.075 mmol, 1.0 eq), HBTU (28 mg, 0.075 mmol, 1.0 eq) and TEA (42 μL, 0.30 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**3i** as a white solid (27 mg, 57% yield).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37 (dd, *J* = 2.7, 1.4 Hz, 1H, NH), 7.28-7.26 (m, 1H, NH), 7.25-7.10 (m, 7H, Ar-H), 7.09-7.01 (m, 1H, Ar-H), 7.00-6.94 (m, 2H, Ar-H), 6.73 (ddt, *J* = 8.1, 2.3, 1.1 Hz, 1H, Ar-H), 6.42-6.35 (m, 1H, Ar-H), 4.39-4.30 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 3.67 (d, *J* = 1.2 Hz, 2H, OCH_2), 3.57-3.52 (m, 1H, CH), 3.23-3.13 (m, 4H, NCH_2×2), 3.02-2.85 (m, 2H, CH_2), 2.54-2.41 (m, 2H, CH_2), 1.70-1.63 (m, 4H, CH_2×2), 1.62-1.53 (m, 2H, CH_2), 1.46 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.40 (s, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.4, 167.9, 159.7, 152.3, 148.8, 137.9, 136.2, 135.8, 129.5, 129.4, 128.7, 126.7, 118.5, 117.5, 117.0, 114.4, 112.5, 111.4, 70.7, 70.6, 56.7, 55.9, 55.3, 54.3, 50.0, 44.5, 36.7, 30.3, 28.9, 25.7, 24.7, 24.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{37}H_{51}N_4O_5$ 631.3859; Found 631.3839. Optical rotation $[\alpha]^{20}_D$ –41.2 (*c* 2.00, $CHCl_3$).

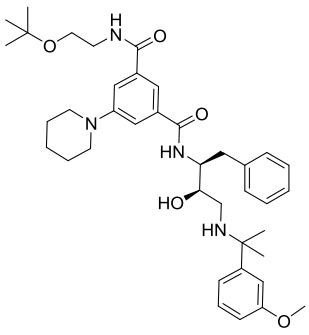


N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3-(2-hydroxyethyl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3j). The title compound was obtained from (*R*,*S*)-**11** (32 mg, 0.080 mmol, 1.0 eq), benzoic acid **15p** (23 mg, 0.080 mmol, 1.0 eq), HBTU (30 mg, 0.080 mmol, 1.0 eq) and TEA (44 µL, 0.32 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 100% EtOAc to 2% MeOH in EtOAc afforded (*S*,*R*)-**3j** as a white solid (37 mg, 77% yield).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.40-7.31 (m, 2H, NH×2), 7.25-7.08 (m, 9H, Ar-H), 7.02-6.95 (m, 2H, Ar-H), 6.74 (ddd, *J* = 8.2, 2.5, 1.1 Hz, 1H, Ar-H), 4.39-4.30 (m, 1H, CH), 3.80 (t, *J* = 4.9 Hz, 2H, OCH_2), 3.76 (s, 3H, OCH_3), 3.62-3.53 (m, 3H, CH, NCH_2), 3.22-3.10 (m, 4H, NCH_2×2), 3.00-2.81 (m, 2H, CH_2), 2.56-2.41 (m, 2H, CH_2), 1.68-1.61 (m, 4H, CH_2×2), 1.60-1.53 (m, 2H, CH_2), 1.46 (s, 3H, CH_3), 1.43 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.5, 168.3, 159.7, 152.2, 148.7, 138.0, 135.8, 135.4, 129.4, 128.7, 126.6, 118.5, 117.6, 117.0, 114.4, 112.5, 111.6, 70.7, 62.2, 55.9, 55.4, 54.6, 49.9, 44.6, 43.2, 36.6, 30.3, 28.6, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{35}H_{47}N_4O_5$ 603.3546; Found 603.3544. Optical rotation $[\alpha]^{20}_D$ –49.7 (*c* 2.85, $CHCl_3$).



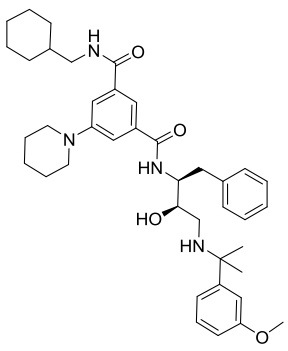
N^1-Benzyl-N^3-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl) propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3k). The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **15q** (42 mg, 0.12 mmol, 1.0 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 3% MeOH in EtOAc afforded *rac*-**3k** as a yellowish sticky oil (60 mg, 74% yield). Enantiomerically pure material (*S*,*R*)-**3k** (18 mg, 22% yield) was obtained by semi-preparative HPLC on chiral stationary phase

(*Chiralpak-IC*), using 40% EtOAc/60% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 3.0 mL/min, detector UV 254 nm).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.51 (dd, *J* = 1.9 Hz, 1H, NH), 7.37-7.27 (m, 6H, NH, Ar-H), 7.23-7.10 (m, 7H, Ar-H), 7.02-6.95 (m, 2H, Ar-H), 6.86-6.75 (m, 2H, Ar-H), 6.73-6.68 (m, 1H, Ar-H), 4.61 (d, *J* = 5.8 Hz, 2H, NCH_2), 4.41-4.27 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 3.59-3.53 (m, 1H, CH), 3.21-3.16 (m, 4H, NCH_2×2), 2.99-2.89 (m, 2H, CH_2), 2.55-2.40 (m, 2H, CH_2), 1.70-1.62 (m, 4H, CH_2×2), 1.62-1.56 (m, 2H, CH_2), 1.50 (s, 3H, CH_3), 1.49 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.2, 167.2, 159.8, 152.3, 138.5, 137.8, 135.6, 131.0, 129.6, 129.5, 128.8, 128.7, 128.1, 127.6, 126.7, 118.4, 118.0, 117.2, 114.3, 112.5, 55.4, 53.8, 49.9, 44.2, 38.9, 36.4, 29.8, 29.1, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{40}H_{49}N_4O_4$ 649.3754; Found 649.3755. Optical rotation $[\alpha]^{20}_D$ –35.3 (*c* 1.20, $CHCl_3$).



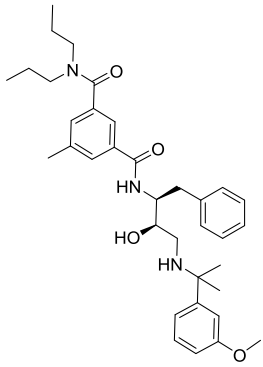
N^1-(2-(*tert*-Butoxy)ethyl)-N^3-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3l). The title compound was obtained from (*R*,*S*)-**11** (35 mg, 0.087 mmol, 1.0 eq), benzoic acid **15r** (30 mg, 0.087 mmol, 1.0 eq), HBTU (33 mg, 0.087 mmol, 1.0 eq) and TEA (49 µL, 0.35 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 15% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**3l** as a colorless sticky oil (33 mg, 57% yield).
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.44-7.40 (m, 1H, NH), 7.30-7.27 (m, 1H, NH), 7.26-7.17 (m, 7H, Ar-H), 7.02-6.96 (m, 2H, Ar-H), 6.76-6.70 (m, 1H, Ar-H), 6.68-6.57 (m, 2H, Ar-H), 4.39-4.31 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.62-3.45 (m, 6H, OCH_2, NCH_2, CH, NH), 3.25-3.15 (m, 4H, NCH_2×2), 3.06-2.92 (m, 3H, CH_2, OH), 2.49-2.43 (m, 2H, CH_2), 1.71-1.64 (m, 4H, CH_2×2), 1.63-1.57 (m, 2H, CH_2), 1.48 (s, 6H, CH_3×2), 1.21-1.18 (m, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 167.8, 167.4, 159.7, 152.3, 148.8, 137.9, 136.1, 135.8, 129.6, 129.3, 128.7, 126.7, 118.5, 117.4, 117.0, 114.4, 112.4, 111.6, 73.4, 70.8, 60.6, 55.9, 55.3, 53.7, 50.0, 44.5, 40.8, 36.7, 30.1, 29.0, 27.7, 25.7, 24.3 ppm. HRMS-

ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{39}H_{55}N_4O_5$ 659.4172; Found 659.4191. Optical rotation $[\alpha]^{20}_D$ –34.6 (*c* 2.31, $CHCl_3$).



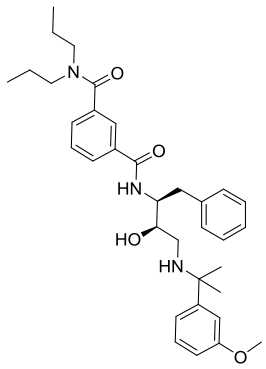
N^1-(Cyclohexylmethyl)-N^3-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-(piperidin-1-yl)isophthalamide ((*S*,*R*)-3m). The title compound was obtained from (*R*,*S*)-**11** (25 mg, 0.062 mmol, 1.0 eq), benzoic acid **15s** (24 mg, 0.069 mmol, 1.1 eq), HBTU (24 mg, 0.062 mmol, 1.0 eq) and TEA (35 µL, 0.25 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using 1% MeOH in EtOAc afforded (*S*,*R*)-**3m** as a white solid (28 mg, 69% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.45 (dd, *J* = 2.5, 1.4 Hz, 1H, NH), 7.29-7.26 (m, 1H, NH), 7.25-7.15 (m, 7H, Ar-H), 7.03-6.97 (m, 2H, Ar-H), 6.76-6.68 (m, 2H, Ar-H), 6.32 (d, *J* = 6.3 Hz, 1H, Ar-H), 4.40-4.30 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.54-3.48 (m, 1H, CH), 3.30-3.23 (m, 2H, NCH_2), 3.22-3.16 (m, 4H, NCH_2×2), 3.03-2.91 (m, 2H, CH_2), 2.46 (d, *J* = 4.3 Hz, 2H, CH_2), 1.80-1.71 (m, 4H, CH_2×2), 1.70-1.63 (m, 5H, CH_2×2, CH), 1.62-1.54 (m, 3H, CH_2, OH), 1.47 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.32-1.08 (m, 4H, CH_2×2), 1.04-0.91 (m, 2H, CH_2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.0, 167.4, 159.7, 152.4, 137.9, 136.2, 135.8, 129.6, 129.3, 128.7, 126.7, 118.5, 117.7, 116.8, 114.2, 112.4, 111.6, 70.8, 55.9, 55.4, 53.9, 50.0, 46.5, 44.5, 38.8, 38.2, 36.7, 31.1, 30.3, 28.8, 26.5, 26.0, 25.7, 24.3 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{40}H_{55}N_4O_4$ 655.4223; Found 655.4229. Optical rotation $[\alpha]^{20}_D$ –33.2 (*c* 2.00, $CHCl_3$).



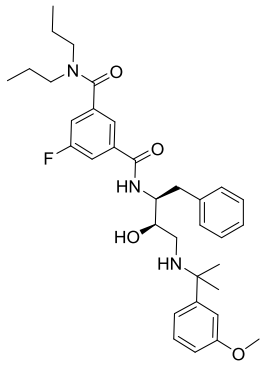
N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-5-methyl-N^3,N^3-dipropylisophthalamide ((*S*,*R*)-**4a)**. The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **17b** (33 mg, 0.12 mmol, 1.0 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 2% MeOH in EtOAc afforded *rac*-**4a** as a colorless sticky oil (50 mg, 70% yield). Enantiomerically pure material (*S*,*R*)-**4a** (17 mg, 24% yield) was obtained by semi-preparative HPLC on chiral stationary phase (*Chiralpak-IC*), using 60% EtOAc/40% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 2.5 mL/min, detector UV 270 nm).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.43-7.37 (m, 2H, NH, Ar-H), 7.29-7.26 (m, 5H, Ar-H), 7.24-7.15 (m, 2H, Ar-H), 6.95-6.88 (m, 2H, Ar-H), 6.70 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H, Ar-H), 6.52 (d, *J* = 9.0 Hz, 1H, Ar-H), 4.24-4.15 (m, 1H, CH), 3.75 (s, 3H, OCH_3), 3.56 (dd, *J* = 9.9, 3.9 Hz, 1H, CH), 3.51-3.40 (m, 2H, NCH_2), 3.19-3.08 (m, 2H, NCH_2), 3.06-2.91 (m, 2H, CH_2), 2.45 (dd, *J* = 12.3, 4.0 Hz, 1H, CH (CH_2)), 2.40 (s, 3H, Ar-CH_3), 2.27 (dd, *J* = 12.2, 9.7 Hz, 1H, CH (CH_2)), 1.75-1.65 (m, 2H, CH_2), 1.57-1.47 (m, 2H, CH_2), 1.42 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.04-0.95 (m, 3H, CH_3), 0.79-0.68 (m, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 171.1, 167.4, 159.8, 148.7, 139.0, 137.9, 137.8, 134.9, 130.1, 129.5, 129.4, 128.7, 128.4, 126.7, 122.0, 118.5, 112.5, 111.5, 70.8, 56.0, 55.3, 53.9, 46.5, 44.5, 36.9, 30.0, 29.1, 22.0, 21.4, 20.9, 11.6, 11.2. ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{35}H_{48}N_3O_4$ 574.3645; Found 574.3670. Optical rotation $[\alpha]^{20}_D$ –32.4 (*c* 1.02, $CHCl_3$).



N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3,N^3-dipropylisophthalamide ((*S*,*R*)-4b). The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **17d** (31 mg, 0.12 mmol, 1.0 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 2% MeOH in EtOAc afforded *rac*-**4b** as a colorless sticky oil (45 mg, 64% yield). Enantiomerically pure material (*S*,*R*)-**4b** (16 mg, 23% yield) was obtained by semi-preparative HPLC on chiral stationary phase (*Chiralpak-IC*), using 40% EtOAc/60% $CHCl_3$/0.1% DEA as a mobile phase (flow rate 2.5 mL/min, detector UV 254 nm).

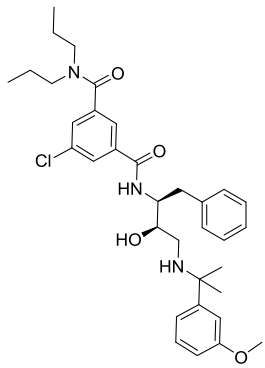
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.67-7.62 (m, 1H, NH), 7.60-7.57 (m, 1H, Ar-H), 7.46-7.37 (m, 2H, Ar-H), 7.26-7.15 (m, 6H, Ar-H), 7.05-6.99 (m, 2H, Ar-H), 6.85 (d, *J* = 8.6 Hz, 1H, Ar-H), 6.77 (ddd, *J* = 8.1, 2.3, 1.0 Hz, 1H, Ar-H), 4.46-4.35 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.60-3.54 (m, 1H, CH), 3.50-3.40 (m, 2H, NCH_2), 3.16-3.07 (m, 2H, NCH_2), 3.03-2.95 (m, 2H, CH_2), 2.59-2.45 (m, 2H, CH_2), 1.75-1.63 (m, 2H, CH_2), 1.59-1.44 (m, 8H, CH_2, CH_3×2), 1.03-0.93 (m, 3H, CH_3), 0.77-0.69 (m, 3H, CH_3) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.8, 167.4, 159.9, 137.9, 137.6, 134.6, 129.6, 129.5, 128.9, 128.8, 127.7, 126.8, 125.2, 118.4, 112.5, 70.5, 55.4, 53.6, 50.9, 46.6, 45.0, 36.7, 29.9, 28.5, 22.1, 20.9, 14.3, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{34}H_{46}N_3O_4$ 560.3488; Found 560.3507. Optical rotation $[\alpha]^{20}_D$ –24.1 (*c* 0.37, $CHCl_3$).



5-Fluoro-N^1-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl) propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3,N^3-dipropyl isophthalamide ((*S*,*R*)-4c). The title compound was obtained from (*R*,*S*)-**11** (19 mg, 0.047 mmol, 1.0 eq), benzoic acid **17e** (15 mg, 0.057 mmol, 1.2 eq), HBTU (18 mg, 0.047 mmol, 1.0 eq) and TEA (26 µL, 0.19 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 50% EtOAc in

petroleum ether to 100% EtOAc afforded (*S*,*R*)-**4c** as a brown solid (17 mg, 62% yield).

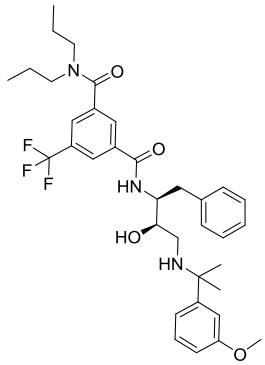
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.40 (ddd, *J* = 9.0, 2.5, 1.5 Hz, 1H, NH), 7.33 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.25-7.10 (m, 8H, Ar-H), 7.01-6.97 (m, 2H, Ar-H), 6.77-6.73 (m, 1H, Ar-H), 4.40-4.30 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.50 (dt, *J* = 6.4, 4.1 Hz, 1H, CH), 3.47-3.39 (m, 2H, NCH_2), 3.14-3.05 (m, 2H, NCH_2), 2.95 (d, *J* = 6.8 Hz, 2H, CH_2), 2.53-2.42 (m, 2H, CH_2), 1.72-1.62 (m, 2H, CH_2), 1.55-1.44 (m, 8H, CH_2, CH_3×2), 0.98 (t, *J* = 7.1 Hz, 3H, CH_3), 0.74 (t, *J* = 7.1 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.4 (d, *J* = 2.2 Hz), 165.8 (d, *J* = 2.3 Hz), 163.8, 161.3, 159.8, 148.7, 139.7 (d, *J* = 6.8 Hz), 137.8, 137.5 (d, *J* = 7.1 Hz), 129.4, 128.7, 126.7, 120.5, 118.4, 116.6 (d, *J* = 23.0 Hz), 115.2 (d, *J* = 22.7 Hz), 112.7, 111.2, 70.6, 55.9, 55.3, 54.4, 50.8, 46.7, 44.4, 36.9, 30.1, 29.1, 22.0, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{34}H_{45}N_3O_4F$ 578.3394; Found 578.3397. Optical rotation $[\alpha]^{20}_D$ –32.8 (*c* 1.10, $CHCl_3$).



5-Chloro-*N*^{1}-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl) propan-2-yl)amino)-1-phenylbutan-2-yl)-*N*^{3},*N*^{3}-dipropyl isophthalamide ((*S*,*R*)-4d). The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **17f** (35 mg, 0.12 mmol, 1.0 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 3% MeOH in EtOAc afforded *rac*-**4d** as a colorless sticky oil (34 mg, 46% yield). Enantiomerically pure material (*S*,*R*)-**4d** (12 mg, 16% yield) was obtained by preparative HPLC on chiral stationary phase (*Chiralpak-ID*), using 80% EtOAc/20% hexanes/0.1% DEA as a mobile phase (flow rate 15 mL/min, detector UV 270 nm).

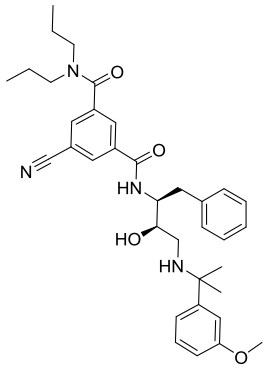
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.66-7.63 (m, 1H, NH), 7.46-7.41 (m, 1H, Ar-H), 7.40-7.35 (m, 1H, Ar-H), 7.28-7.26 (m, 1H, Ar-H), 7.24-7.15 (m, 6H, Ar-H), 7.01-6.96 (m, 2H, Ar-H), 6.78-6.73 (m, 1H, Ar-H), 4.42-4.31 (m, 1H, CH), 3.79 (s, 3H, OCH_3), 3.52 (d, *J* = 5.6 Hz, 1H, CH), 3.48-3.38 (m, 2H, NCH_2), 3.15-3.04 (m, 2H, NCH_2), 2.95 (d, *J* = 6.7 Hz, 2H, CH_2), 2.71 (br s, 2H, NH, OH), 2.56-2.42 (m, 2H, CH_2), 1.74-1.63 (m, 2H, CH_2), 1.54-1.45 (m, 8H, CH_2,

CH_3×2), 1.03-0.94 (m, 3H, CH_3), 0.79-0.70 (m, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.4, 165.8, 159.8, 139.3, 137.8, 136.8, 135.1, 129.4, 128.7, 128.0, 126.8, 123.2, 118.4, 112.7, 111.4, 110.2, 70.6, 56.1, 55.3, 54.4, 50.9, 46.7, 44.5, 36.9, 30.0, 29.0, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{34}H_{45}N_3O_4Cl$ 594,3099; Found 594,3101. Optical rotation $[\alpha]^{20}_D$ –42.1 (*c* 0.82, $CHCl_3$).



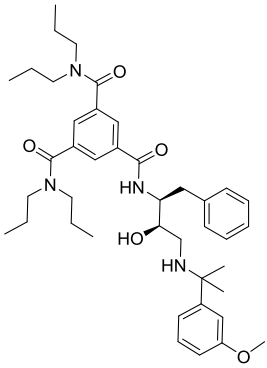
N^1-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-N^3,N^3-dipropyl-5-(trifluoromethyl)isophthalamide ((*S*,*R*)-4e). The title compound was obtained from *rac*-**11** (50 mg, 0.12 mmol, 1.0 eq), benzoic acid **17h** (47 mg, 0.15 mmol, 1.2 eq), HBTU (47 mg, 0.12 mmol, 1.0 eq) and TEA (70 µL, 0.50 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 100% EtOAc to 2% MeOH in EtOAc afforded *rac*-**4e** as a colorless sticky oil (30 mg, 38% yield). Enantiomerically pure material (*S*,*R*)-**4e** (15 mg, 19% yield) was obtained by preparative HPLC on chiral stationary phase (*Chiralpak-ID*), using 80% EtOAc/20% hexanes/0.1% DEA as a mobile phase (flow rate 15 mL/min, detector UV 270 nm).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.91-7.87 (m, 1H, Ar-H), 7.79-7.74 (m, 1H, Ar-H), 7.69-7.65 (m, 1H, Ar-H), 7.33-7.15 (m, 7H, NH, Ar-H), 7.03-6.95 (m, 2H, Ar-H), 6.75 (dd, *J* = 8.2, 2.6, Hz, 1H, Ar-H), 4.44-4.34 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.58-3.52 (m, 1H, CH), 3.50-3.41 (m, 2H, NCH_2), 3.13-3.04 (m, 2H, NCH_2), 2.95 (dd, *J* = 6.9, 2.4 Hz, 2H, CH_2), 2.90-2.70 (m, 2H, NH, OH), 2.50 (qd, *J* = 12.3, 4.2 Hz, 2H, CH_2), 1.80-1.61 (m, 2H, CH_2), 1.59-1.45 (m, 8H, CH_2, CH_3×2), 1.00 (t, *J* = 7.5 Hz, 3H, CH_3), 0.74 (t, *J* = 7.5 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 169.4, 165.7, 159.8, 138.6, 137.7, 136.1, 131.7, 131.3, 129.5, 129.4, 128.8, 128.5, 126.8, 126.2, 124.7, 122.0, 118.4, 112.7, 111.6, 70.5, 56.3, 55.3, 54.5, 50.9, 46.8, 44.7, 36.8, 29.8, 28.9, 22.1, 20.8, 11.6, 11.1 ppm. ^{19}F-NMR (376 MHz, $CDCl_3$) δ: -62.8 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{35}H_{45}N_3O_4F_3$ 628.3362; Found 628.3393. Optical rotation $[\alpha]^{20}_D$ –33.7 (*c* 1.10, $CHCl_3$).



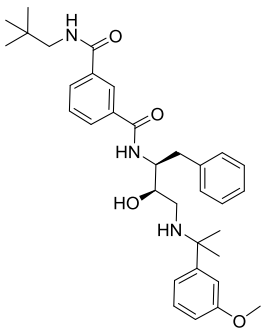
5-Cyano-*N*^{1}-((2*S*,3*R*)-3-hydroxy-4-((2-(3-methoxyphenyl) propan-2-yl)amino)-1-phenylbutan-2-yl)-*N*^{3},*N*^{3}-dipropyl isophthalamide ((*S*,*R*)-4f). The title compound was obtained from (*R*,*S*)-**11** (24 mg, 0.060 mmol, 1.0 eq), benzoic acid **17i** (16 mg, 0.060 mmol, 1.0 eq), HBTU (23 mg, 0.060 mmol, 1.0 eq) and TEA (33 µL, 0.24 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 25% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**4f** as a colorless sticky oil (14 mg, 40% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.95-7.92 (m, 1H, Ar-H), 7.83-7.79 (m, 1H, Ar-H), 7.70-7.66 (m, 1H, Ar-H), 7.56-7.49 (m, 1H, NH), 7.24-7.13 (m, 6H, Ar-H), 7.02-6.96 (m, 2H, Ar-H), 6.80-6.74 (m, 1H, Ar-H), 4.37 (quintet, *J* = 7.0 Hz, 1H, CH), 3.79 (s, 3H, OCH_3), 3.62-3.55 (m, 1H, CH), 3.48-3.41 (m, 2H, NCH_2), 3.12-3.04 (m, 2H, NCH_2), 2.97-2.72 (m, 4H, CH_2, NH, OH), 2.58-2.44 (m, 2H, CH_2), 1.74-1.62 (m, 2H, CH_2), 1.56-1.44 (m, 8H, CH_2, CH_3×2), 0.98 (t, *J* = 7.3 Hz, 3H, CH_3), 0.74 (t, *J* = 7.3 Hz, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 168.6, 164.9, 159.8, 148.1, 139.0, 137.8, 136.5, 132.4, 131.3, 129.5, 129.3, 128.7, 126.8, 118.4, 117.4, 113.4, 112.8, 111.4, 70.4, 56.2, 55.4, 54.8, 51.0, 44.6, 36.8, 28.9, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{35}H_{45}N_4O_4$ 585.3441; Found 585.3419. Optical rotation $[\alpha]^{20}_D$ –31.3 (*c* 1.15, $CHCl_3$).



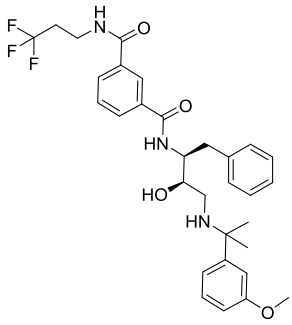
***N*^{1}-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-*N*^{3},*N*^{3},*N*^{5},*N*^{5}-tetrapropylbenzene-1,3,5-tricarboxamide ((*S*,*R*)-4g).** The title compound was obtained from (*R*,*S*)-**11** (18 mg, 0.045 mmol, 1.0 eq), benzoic acid **17g** (20 mg, 0.054 mmol, 1.2 eq), HBTU (17 mg, 0.045 mmol, 1.0 eq) and TEA (25 µL, 0.18 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on silica gel using gradient elution from 50% EtOAc in petroleum ether to 100% EtOAc afforded (*S*,*R*)-**4g** as a brownish solid (10 mg, 32% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 1.5 Hz, 2H, NH, Ar-H), 7.44 (dd, *J* = 1.5, 1.5 Hz, 1H, Ar-H), 7.25-7.13 (m, 6H, Ar-H), 7.02-6.90 (m, 3H, Ar-H), 6.74 (ddd, *J* = 8.2, 2.5, 0.9 Hz, 1H, Ar-H), 4.41-4.32 (m, 1H, CH), 3.78 (s, 3H, OCH_3), 3.52-3.37 (m, 5H, CH, NCH_2×2), 3.18-3.07 (m, 4H, NCH_2×2), 3.00-2.93 (m, 2H, CH_2), 2.47 (dd, *J* = 4.3, 2.9 Hz, 2H, CH_2), 1.74-1.61 (m, 4H, CH_2×2), 1.55-1.42 (m, 10H, CH_2×2, CH_3×2), 1.02-0.93 (m, 6H, CH_3×2), 0.79-0.67 (m, 6H, CH_3×2) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 170.1, 166.2, 159.8, 138.2, 137.8, 135.2, 129.4, 128.7, 127.7, 126.7, 125.8, 118.4, 112.5, 111.4, 70.7, 55.3, 54.1, 50.9, 46.6, 44.6, 36.7, 29.8, 29.3, 22.1, 20.8, 11.6, 11.2 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{41}H_{59}N_4O_5$ 687.4485; Found 687.4495. Optical rotation $[\alpha]^{20}_D$ –30.6 (*c* 0.79, $CHCl_3$).



***N*^{1}-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-*N*^{3}-neopentylisophthalamide ((*S*,*R*)-5a)**. The title compound was obtained from (*R*,*S*)-**11** (30 mg, 0.075 mmol, 1.0 eq), benzoic acid **19a** (18 mg, 0.075 mmol, 1.0 eq), HBTU (28 mg, 0.075 mmol, 1.0 eq) and TEA (42 µL, 0.30 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 25% EtOAc in petroleum ether to 60% EtOAc in petroleum ether afforded (*S*,*R*)-**5a** as a white solid (23 mg, 56% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.05-8.01 (m, 1H, Ar-H), 7.90-7.85 (m, 1H, NH), 7.75-7.69 (m, 1H, NH), 7.47-7.39 (m, 1H, Ar-H), 7.29-7.26 (m, 1H, Ar-H), 7.24-7.15 (m, 6H, Ar-H), 7.02-6.95 (m, 2H, Ar-H), 6.77-6.71 (m, 1H, Ar-H), 6.38-6.29 (m, 1H, Ar-H), 4.46-4.35 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 3.58-3.51 (m, 1H, CH), 3.27 (d, *J* = 6.5 Hz, 2H, NCH_2), 3.03-2.87 (m, 2H, CH_2), 2.55-2.41 (m, 2H, CH_2), 1.47 (s, 6H, CH_3×2), 0.98 (s, 9H, CH_3×3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 166.9, 159.7, 148.8, 137.8, 135.5, 134.9, 130.2, 129.5, 129.4, 129.4, 129.0, 128.7, 126.7, 125.3, 118.5, 112.6, 111.4, 70.5, 55.9, 55.3, 54.4, 51.3, 44.4, 36.9, 32.4, 30.1, 29.0, 27.5 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{33}H_{44}N_3O_4$ 546.3332; Found 546.3341. Optical rotation $[\alpha]^{20}_D$ –49.0 (*c* 1.73, $CHCl_3$).



***N*[1]-((2*S*,3*R*)-3-Hydroxy-4-((2-(3-methoxyphenyl)propan-2-yl)amino)-1-phenylbutan-2-yl)-*N*[3]-(3,3,3-trifluoropropyl) isophthalamide ((*S*,*R*)-5b)**. The title compound was obtained from (*R*,*S*)-**11** (30 mg, 0.075 mmol, 1.0 eq), benzoic acid **19b** (20 mg, 0.075 mmol, 1.0 eq), HBTU (28 mg, 0.075 mmol, 1.0 eq) and TEA (42 µL, 0.30 mmol, 4.0 eq) by following general procedure D. Purification by column chromatography on amino-functionalized silica gel using gradient elution from 25% EtOAc in petroleum ether to 60% EtOAc in petroleum ether afforded (*S*,*R*)-**5b** as a white solid (27 mg, 63% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.01-7.97 (m, 1H, Ar-H), 7.90-7.86 (m, 1H, NH), 7.73-7.67 (m, 1H, NH), 7.45-7.39 (m, 1H, Ar-H), 7.33 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.28-7.26 (m, 1H, Ar-H), 7.24-7.16 (m, 5H, Ar-H), 7.02-6.96 (m, 2H, Ar-H), 6.78-6.71 (m, 2H, Ar-H), 4.46-4.35 (m, 1H, CH), 3.77 (s, 3H, OCH_3), 3.74-3.66 (m, 2H, NCH_2), 3.59-3.53 (m, 1H, CH), 3.02-2.84 (m, 2H, CH_2), 2.59-2.40 (m, 4H, CH_2×2), 1.48 (s, 3H, CH_3), 1.47 (s, 3H, CH_3) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ: 166.9, 166.9, 159.7, 148.8, 137.8, 135.1, 134.5, 130.3, 129.8, 129.4, 129.1, 128.7, 127.9, 126.7, 125.4, 118.5, 112.6, 111.5, 70.4, 55.9, 55.4, 54.6, 44.4, 37.0, 33.9, 33.6, 30.2, 28.8 ppm. HRMS-ESI *(m/z)*: $[M+H]^+$ Calcd for $C_{31}H_{37}N_3O_4F_3$ 572.2736; Found 572.2737. Optical rotation $[\alpha]^{20}_D$ –45.2 (*c* 2.04, $CHCl_3$).

2. Molecular modeling

Docking was performed using the standard precision protocol in Glide. Docked complexes were further refined with Prime MM-GBSA (Molecular Mechanics/Generalized Born Surface Area) method using VSGB solvation model and OPLS3 force field. For MM-GBSA calculations, a 7-Å active region around the ligands for full molecular mechanics minimization was used. Results were visualized using UCSF Chimera v1.12 and Maestro.

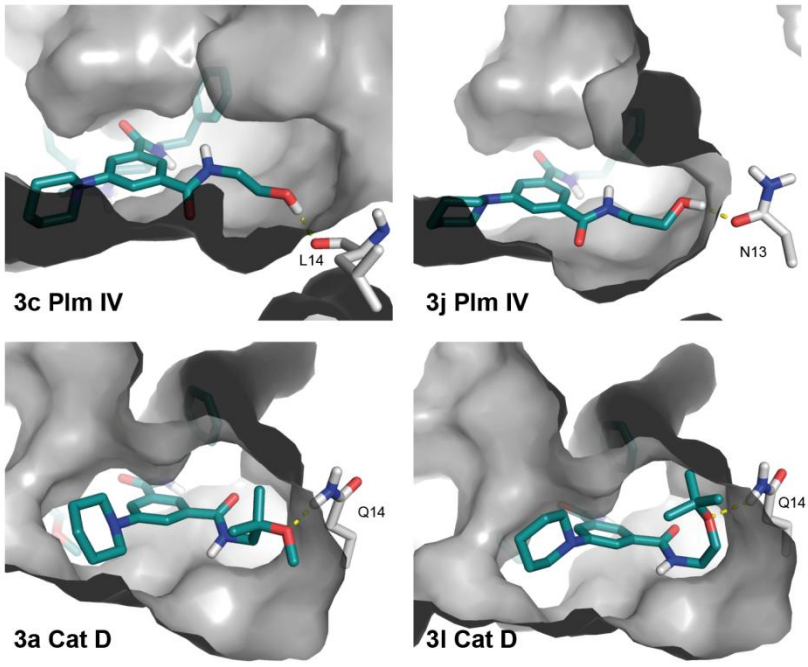


Fig. S1. Docking models of compounds containing hydrogen bond donor in S3 substituent (**3c,j** in complex with Plm IV); and compounds containing hydrogen bond acceptor group in S3 substituents (**3a,l** in complex with Cat D). Hydrogen bonds are indicated with yellow dashed line.

3. Biological assays

3.1. Enzymatic assay

A fluorescence resonance energy transfer (FRET) assay was performed to evaluate ability of compounds to inhibit PlmI, II, IV, and Cat D. K_m of the substrate was determined for each enzyme: PlmII = 2 ± 0.2 μM; PlmI = 2.7 ± 0.3μM; PlmIV = 2.8 ± 0.2μM; Cat D = 1.8 ± 0.2 μM. A solution of compounds for testing (concentration 0.01−100 μM) on 96-well plate was added to the enzyme (Plm I, II, IV or Cat D) in buffer (0.1 M NaOAc, pH = 4.5, 10% glycerol). The mixture was incubated for 30 min at 37 °C. Substrate (DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS, AnaSpec Inc.) was then added to reach a final concentration of 5 μM. Hydrolysis of the substrate was detected as an increase in fluorescence (Em 490 nm, Ex 336 nm) at 37 °C. The data points were collected every 1 min within 8−15 min. For the rate calculation, only the linear interval was used, which was slightly different for each enzyme.

Compounds were tested in triplicate experiments. IC_{50} values were calculated using software Graph Pad Prism 5.0. Pepstatin A (IC_{50} = 0.42 ± 0.02 nM (Plm II); IC_{50} = 0.9 ± 0.02 nM (Plm I); IC_{50} = 0.3 ± 0.04 nM (Plm IV)) and resveratrol (IC_{50} = 138 μM) were used as positive controls.

3.2. Red blood cell assay

The effects of compounds on growth of blood-stage *plasmodium falciparum* (clone 3D7) was assessed using a SYBR Green I assay, essentially as described previously.[1] Test compounds (dissolved in DMSO at concentrations ranging from 1 mM–0.1 μM) were added in triplicate to wells of flat bottomed, 96 well microtitre plates (1 μL per well). Wells were then supplemented with 100 μL per well of a synchronous *P. falciparum* parasite culture at 0.1% parasitaemia, 1% haematocrit. Each assay plate also included DMSO only control wells, as well as additional control wells containing uninfected erythrocytes only. Plates were incubated in sealed humidified gassed chambers at 37 ^{o}C for 96 h to allow the parasites to undergo two entire cycles of erythrocytic growth. Wells were then supplemented with 100 μL of a 1:5,000 dilution of stock SYBR Green I (Life Technologies, catalogue #S7563) diluted in 20 mM Tris-HCl pH 7.5, 5 mM EDTA, 0.008 % (w/v) saponin, 0.08% (v/v) Triton X100. Plates were agitated to mix, incubated for a further 1 h in the dark at room temperature, then transferred to a Cary Eclipse fluorescence spectrophotometer (Varian) equipped with a 96-well microplate reader accessory for fluorescence readings (Ex 485 nm, Em 530 nm). EC_{50} values were determined from dose-response curves obtained after subtracting background fluorescence values (obtained from the erythrocyte only wells) from all experimental readings.

3. PIELIKUMS – "TETRAHYDROISOQUINOLINE-BASED NON-PEPTIDOMIMETIC PLASMEPSIN INHIBITORS"

Kinena, L.; Ozola, V.

Chem. Heterocycl. Comp. **2020**, *56*, 60-66.

Латвийский институт органического синтеза

Химия гетероциклических соединений **2020**, *56*(1), 60–66

Химия гетероциклических соединений

Tetrahydroisoquinoline-based non-peptidomimetic plasmepsin inhibitors

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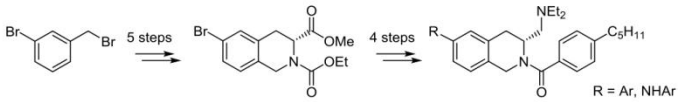


A series of tetrahydroisoquinoline derivatives containing different aryl substituents were designed and synthesized using Pictet–Spengler reaction as the key step. The synthesized tetrahydroisoquinoline derivatives displayed micromolar inhibitory activity against plasmepsins I and II.

Keywords: plasmepsins, tetrahydroisoquinoline, malaria, Pictet–Spengler reaction, X-ray analysis.

Malaria is a potentially life-threatening disease caused by *Plasmodium falciparum* parasite. It is transmitted through the bite of an infected mosquito. The increasing resistance of the malaria parasite to currently available drugs requires urgent development of new antimalarial agents.[1–3] Malarial aspartic proteases – plasmepsins (Plm I, Plm II, Plm IV) are involved in hemoglobin degradation and are potential drug targets.[4,5] There are two kinds of plasmepsin inhibitors: peptidomimetic and non-peptidomimetic inhibitors. Peptidomimetic plasmepsin inhibitors usually show high inhibitory activity, but low selectivity against human aspartic proteases.[6] Several studies have been performed to discover new non-peptidomimetic plasmepsin inhibitors in order to overcome selectivity issues.[7–9] Scientists from Actelion have published first non-peptidomimetic Plm II inhibitor **A** based on the aminopiperidine scaffold (Fig. 1). Aminopiperidine-based inhibitors have several drawbacks – they display adverse physicochemical properties such as high ClogP and low solubility.[10,11] Therefore, we decided to design a new series of inhibitors by rescaffolding Actelion aminopiperidine-type inhibitor **A** (Fig. 1).

A series of tetrahydroisoquinoline derivatives **B** were designed by introducing bond between C-3 carbon atom of aromatic ring and C-3 carbon atom of piperidine and opening piperidine cycle. According to the docking studies, the newly designed tetrahydroisoquinoline inhibitor **B** binds to the enzyme similarly to aminopiperidine derivative **A**. Our design retains the key pharmacophoric elements needed for inhibitory activity: amino moiety (makes ionic interaction with Asp 214 residue and water-bridged H-bonding interaction with the catalytic Asp 34), biphenyl substituent (occupies S1 subpocket), and *n*-pentyl chain (placed in the flap pocket) (Fig. 2).[12]

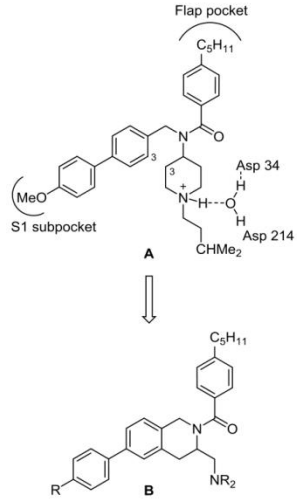


Figure 1. Design strategy of tetrahydroisoquinoline-based inhibitors **B** by rescaffolding Actelion aminopiperidine-type inhibitors **A**.

Figure 2. Binding mode of tetrahydroisoquinoline-based inhibitor **B** to the active site of Plm II. Docking was performed using the AutoDock Vina software package[18] on the crystal structure of Plm II solved in complex with an aminopiperidine-based Plm II inhibitor (Protein Data Bank ID 2IGX[11]).

A small series of tetrahydroisoquinoline derivatives (*R*)-**9a**–**c**, (*R*)-**10** with different aryl substituents was prepared to evaluate the inhibitory activity against Plm I, Plm II, and Plm IV isoforms. Tetrahydroisoquinoline-based inhibitors (*R*)-**9a**–**c**, (*R*)-**10** were synthesized from ester **4** and paraformaldehyde using Pictet–Spengler reaction as the key step. The synthesis was started by C-alkylation of diethyl 2-acetamidomalonate with *meta*-bromobenzyl bromide (**1**) in presence of NaOEt.[13] Subsequent hydrolysis of ester and deprotection of the amino group in compound **2** was followed by decarboxylation to afford amino acid **3**.[13] The Pictet–Spengler reaction involving protected amino acid **4** and paraformaldehyde[14] resulted in the formation of tetrahydroisoquinoline derivative as a mixture of regioisomers **5a** and **5b**. Enantiomerically pure tetrahydroisoquinoline (*R*)-**5a** was obtained by initial separation of regioisomers by preparative column chromatography, followed by separation of enantiomers on a chiral stationary phase (Scheme 1).

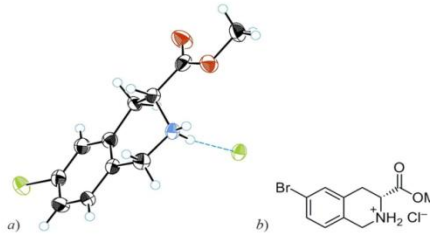
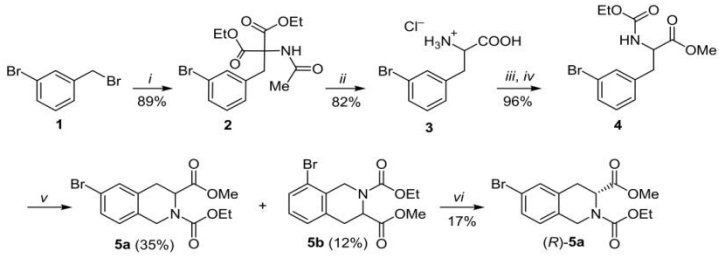


Figure 3. The molecular (*a*) and chemical (*b*) structure of met ester (*R*)-**6a** with atoms represented by thermal vibration el soids of 50% probability.

After hydrolysis and deprotection of amino function compound **5a**, acid **6** was obtained (Scheme 2). determine the absolute configuration of carboxylic a (*R*)-**6** it was converted to methyl ester (*R*)-**6a**. Single crys X-ray analysis of methyl ester (*R*)-**6a** confirm (*R*) absolute configuration of stereogenic center (Fig. 3).

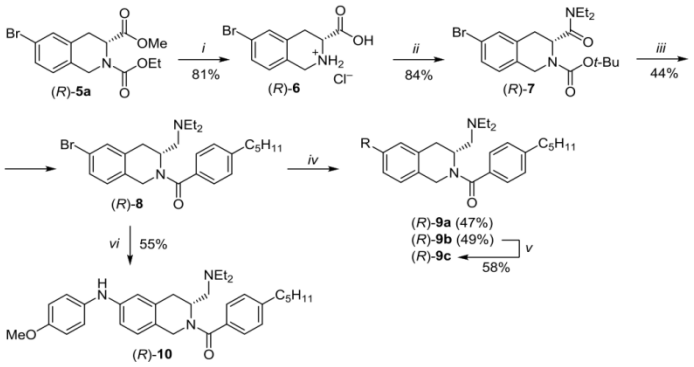
For further modifications, only acid (*R*)-**6** was us Next, amino function was protected with Boc moiety yielding tetrahydroisoquinoline which was reacted w diethylamine in the presence of HOBt leading tetrahydroisoquinoline derivative (*R*)-**7** (Scheme 2). Af cleavage of the *N*-Boc protecting group, the amide moi was reduced with $LiAlH_4$ to give amine. The latter v acylated with 4-pentylbenzoyl chloride in the presence DIPEA yielding tetrahydroisoquinoline (*R*)-**8**. This v used as the key building block. Suzuki–Miyaura cro coupling reaction between bromide (*R*)-**8** and boronic ac in the presence of $Pd(PPh_3)_4$ was used for the synthesis diaryl derivatives (*R*)-**9a**,**b**. Demethylation of tetrahyd isoquinoline derivative (*R*)-**9b** was accomplished w NaH/1-dodecanethiol in DMF[16] to yield hydroxy grou containing derivative (*R*)-**9c**. Buchwald–Hartwig aminati reaction[17] was used for the introduction of 4-metho phenylamino substituent yielding tetrahydroisoquinoli derivative (*R*)-**10** (Scheme 2).

Scheme 1



i: $AcNHCH(CO_2Et)_2$, NaOEt, EtOH, 85°C, 16 h; *ii*: HCl, AcOH, 100°C, 18 h
iii: $SOCl_2$, MeOH, 60°C, 3 h; *iv*: $ClCO_2Et$, Py, CH_2Cl_2, rt, 18 h
v: paraformaldehyde, AcOH, H_2SO_4, rt, 20 h; *vi*: preparative HPLC on chiral stationary phase (Chiralpak-IC)

Scheme 2



i: 33% HBr in AcOH, rt, 84 h, then aq 6 M HCl, 70°C, 18 h
ii: $(Boc)_2O$, NaOH, *t*-BuOH–H_2O, 1:1, rt, 18 h, then Et_2NH, DCC, HOBt·H_2O, DMF, rt, 16 h
iii: 4 M HCl in dioxane, rt, 16 h, then $LiAlH_4$, THF, rt, 2 h, then 4-pentylbenzoyl chloride, DIPEA, CH_2Cl_2, rt, 16 h
iv: $RB(OH)_2$, $Pd(PPh_3)_4$ (3 mol %), aq 2 M Na_2CO_3, 1,4-dioxane, 105°C, 16 h
v: 1-dodecanethiol, NaH, DMF, 0°C, then 130°C, 2 h
vi: 4-methoxyaniline, $Pd_2(dba)_3$ (2 mol %), X-Phos (8 mol %), NaO*t*-Bu, PhMe, 90°C, 16 h

The synthesized tetrahydroisoquinoline derivatives (*R*)-**9a**–**c**, (*R*)-**10** were tested for their Plm I, Plm II, and Plm IV inhibiting properties (Table 1). Tetrahydroisoquinoline **9a** displayed micromolar inhibitory activity against Plm I, but hardly any activity against Plm II and Plm IV was observed. Inhibitor **9b** with *p*-OMe substituent at the phenyl ring showed the highest activity against Plm I, Plm II, and Plm IV. In contrast, *p*-OH-substituted and amino-linker-containing inhibitors **9c** and **10** showed 2–3 times lower inhibitory activity against Plm I, Plm II, Plm IV (Table 1).

Table 1. Plm I, Plm II, and Plm IV inhibitory activity of compounds **9a**–**c**, **10**, **A**

Compound	R	IC_{50}*, μM		
		Plm I	Plm II	Plm IV
9a		22	–**	~100
9b		1.9 ± 0.09	16.2 ± 0.8	46.6 ± 2
9c		7.3 ± 0.3	40 ± 2	~100
10		7 ± 0.3	45.5 ± 2	73.6 ± 3
A**		0.4	0.42	0.92

* Plm I, Plm II, and Plm IV inhibitory activity was determined by enzymatic FRET assay in triplicate experiments.
** The assay was performed at five concentrations of the inhibitor (0.01–100 μM). At this range of concentrations inhibitor **9a** did not show any effect.
*** Reference compound, see Figure 1.

In summary, we have developed a new series of tetrahydroisoquinoline-based non-peptidomimetic Plm inhibitors. Synthesis of the inhibitors was performed using Pictet–Spengler reaction as the key step. Plm I, Plm II, and Plm IV inhibiting properties of the synthesized tetrahydroisoquinoline derivatives were tested. The best tetrahydroisoquinoline derivatives show inhibitory activity toward plasmepsins I and II at micromolar level. However, inhibition potency of tetrahydroisoquinoline derivatives is lower than Actelion aminopiperidine-type inhibitor. Hence, the performed rescaffolding does not allow the molecule to adopt the bioactive conformation in the active site of enzyme.

Experimental

^1H and ^{13}C NMR spectra (400 and 100 MHz, respectively) were recorded on a Bruker 400 spectrometer in $CDCl_3$, DMSO-d_6, or CD_3OD; TMS was used as internal standard. HRMS (ESI) spectra were recorded on a Waters Acquity UPLC H-Class apparatus with a time-of-flight (TOF) mass analyzer Waters Synapt G2 Si TOF MS. Analytical thin-layer chromatography (TLC) was performed on precoated Merck silica gel F-254 plates.

Unless otherwise noted, all chemicals were used as obtained from commercial sources and all reactions were performed under nitrogen or argon atmosphere in glassware dried in an oven (120°C) and cooled under a stream of argon. Dry PhMe, THF, CH_2Cl_2, and Et_2O were obtained by passing commercially available anhydrous solvents through activated alumina columns.

Diethyl (acetylamino)(3-bromobenzyl)propanedioate (2). A pressure tube was charged with diethyl 2-acetamidomalonate (1.74 g, 8.00 mmol) and NaOEt (2.59 g, 8.00 mmol), then anhydrous EtOH (10 ml) was added and light-yellow suspension formed. A solution of *m*-bromo-

benzyl bromide (**1**) (2.00 g, 8.00 mmol) in anhydrous EtOH (10 ml) was added, and the resulting orange solution was heated at 85°C for 16 h. After cooling to room temperature, orange suspension was concentrated under reduced pressure and the residue was partitioned between EtOAc (50 ml) and H_2O (50 ml). Water phase was extracted with EtOAc (3 × 50 ml), combined extracts were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (100 g), eluent hexane–EtOAc, gradient from 10 to 100% EtOAc. Yield 2.76 g (89%), light-yellow solid. Analytical data of the compound were identical to the literature data.[13]

2-Amino-3-(3-bromophenyl)propanoic acid hydrochloride (3). Diethyl (acetylamino)(3-bromobenzyl)propanedioate (**2**) (2.95 g, 7.66 mmol) was dissolved in a mixture of concentrated AcOH (7.6 ml) and concentrated aqueous HCl (23 ml). The light-yellow solution was heated at 100°C for 18 h. The light-brown suspension was concentrated under reduced pressure, the precipitate was filtered off and washed with petroleum ether, dried over P_2O_5 at 60°C for 18 h. The product was used in a subsequent step without purification. Yield 1.75 g (82%), light-brown solid. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 14.22–13.52 (1H, br. s, COOH); 8.78–8.32 (3H, m, NH_3^+); 7.56–7.51 (1H, m, H Ar); 7.51–7.45 (1H, m, H Ar); 7.35–7.27 (2H, m, H Ar); 4.25–4.13 (1H, m, CH_2CH); 3.16 (2H, d, J = 6.3, CH_2CH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 170.1; 137.9; 132.3; 130.6; 130.1; 128.7; 121.8; 52.9; 35.1. Found, *m/z*: 243.9971 $[M+H]^+$. $C_9H_{11}BrNO_2$. Calculated, *m/z*: 243.9973.

Methyl 3-(3-bromophenyl)-2-[(ethoxycarbonyl)amino]-propanoate (4). $SOCl_2$ (1.13 ml, 15.66 mmol) was added to cooled (0°C) anhydrous MeOH (20 ml), and the resulting solution was stirred for 10 min. Hydrochloride **3** (1.75 g, 6.27 mmol) was then added in portions, and the light-brown solution was stirred at 60°C for 3 h. The volatiles were removed under reduced pressure, and anhydrous PhMe (2 × 20 ml) was added and evaporated under reduced pressure. The precipitate was dried over P_2O_5 at 40°C and used in subsequent step without purification. Yield 1.82 g (98%), light-brown powder. Pyridine (1.50 ml, 18.54 mmol) was added dropwise to a solution of methyl 2-amino-3-(3-bromophenyl)propanoate hydrochloride from the previous step (1.82 g, 6.18 mmol) in anhydrous CH_2Cl_2 (20 ml). The light-brown solution was cooled to 0°C, and ethyl chloroformate (737 mg, 649 µl, 6.80 mmol) was added dropwise. After stirring at room temperature for 18 h, the light-brown suspension was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 ml), washed with aqueous 1 M HCl solution (30 ml), saturated aqueous $NaHCO_3$ solution (50 ml), and brine (30 ml). The organic layer was evaporated under reduced pressure, and product **4** was used in a subsequent step without purification. Yield 1.98 g (96%), light-brown oil. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.38 (1H, ddd, J = 7.9, J = 2.0, J = 1.1, H Ar); 7.30–7.26 (1H, m, H Ar); 7.16 (1H, dd, J = 7.9, J = 7.9, H Ar); 7.08–7.04 (1H, m, H Ar); 5.15 (1H, d, J = 8.2, NH); 4.68–4.56 (1H, m, CH_2CH); 4.11 (2H, q, J = 7.1, CH_2CH_3); 3.72 (3H, s, CH_3); 3.15–2.98 (2H, m, CH_2CH); 1.23 (3H, t, J = 7.1, CH_2CH_3). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 171.9; 155.9; 138.4; 132.5; 130.4; 130.2; 128.0; 122.7; 61.4; 54.7; 52.5; 38.1; 14.7. Found, *m/z*: 330.0331 $[M+H]^+$. $C_{13}H_{17}BrNO_4$. Calculated, *m/z*: 330.0341.

2-Ethyl 3-methyl (*R*)-6-bromo-3,4-dihydroisoquinoline-2,3(1*H*)-dicarboxylate (5a) and 2-ethyl 3-methyl 8-bromo-3,4-dihydroisoquinoline-2,3(1*H*)-dicarboxylate (5b). AcOH (7.4 ml), H_2SO_4 (2.5 ml), and paraformaldehyde (198 mg, 6.60 mmol) were added to methyl 3-(3-bromophenyl)-2-[(ethoxycarbonyl)amino]propanoate (**4**) (1.98 g, 6.00 mmol), and the resulting light-brown suspension was stirred at room temperature for 20 h, then the second portion of paraformaldehyde (198 mg, 6.60 mmol) was added and stirring was continued for 20 h more. The light-brown solution was poured into ice water (25 ml). After 20 min, the suspension was extracted with EtOAc (3 × 50 ml). The combined extracts were washed with saturated $NaHCO_3$ solution (2 × 50 ml), brine (50 ml), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (100 g), eluent hexane–EtOAc, gradient from 25 to 100% EtOAc. The crude product was obtained as a mixture of regioisomers **5a**:**5b** in ratio 2.9:1. The regioisomers **5a** and **5b** were separated by preparative chromatography (Sunfire™ Prep Silica OBD™ 5µm, 30 × 100 column), eluent hexane–EtOAc, 9:1, flow rate 40 ml/min, detector UV 230 nm, 260 nm. Yield of compound **5a** 717 mg (35%), yield of compound **5b** 247 mg (12%). Enantiomerically pure material (*R*)-**5a** was obtained by preparative HPLC on chiral stationary phase (DAICEL, Chiralpak-IC), eluent hexane–EtOAc, 5:1, flow rate 36 ml/min, detector UV 230 nm, 260 nm. Yield of compound (*R*)-**5a** 351 mg (17%), light-yellow oil, mixture of rotamers, $[\alpha]_D^{20}$ –45.7 (*c* 1.03, $CHCl_3$).

Compound 5a. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.36–7.28 (2H, m, H Ar); 7.02 (0.45H, d, J = 8.0, H Ar); 6.98 (0.55H, d, J = 8.0, H Ar); 5.18 (0.55H, dd, J = 6.1, J = 2.9, CH); 4.96 (0.45H, dd, J = 6.1, J = 4.2, CH); 4.77–4.67 (1H, m, CH_2N); 4.56–4.42 (1H, m, CH_2N); 4.32–4.13 (2H, m, CH_2CH_3); 3.63 (3H, s, OCH_3); 3.33–3.06 (2H, m, CH_2CH); 1.33 (1.65H, t, J = 7.1, CH_2CH_3); 1.26 (1.35H, t, J = 7.1, CH_2CH_3). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 171.7; 171.5; 156.3; 155.7; 134.0; 132.2; 131.6; 131.5; 131.1; 130.2; 130.1; 128.2; 128.0; 120.5; 62.2; 62.1; 53.3; 52.6; 44.1; 44.0; 31.3; 31.0; 14.8; 14.7. Found, *m/z*: 342.0327 $[M+H]^+$. $C_{14}H_{17}BrNO_4$. Calculated, *m/z*: 342.0341.

Compound 5b. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.46–7.40 (1H, m, H Ar); 7.14–7.08 (1H, m, H Ar); 7.08–7.02 (1H, m, H Ar); 5.25 (0.6H, dd, J = 6.3, J = 2.5, CH); 5.06 (0.4H, dd, J = 6.3, J = 3.2, CH); 4.86 (0.4H, d, J = 17.6, CH_2N); 4.79 (0.6H, d, J = 17.6, CH_2N); 4.45 (0.6H, d, J = 17.6, CH_2N); 4.43 (0.4H, d, J = 17.6, CH_2N); 4.31–4.16 (2H, m, CH_2CH_3); 3.64 (3H, s, OCH_3); 3.33–3.13 (2H, m, CH_2CH); 1.35 (1.8H, t, J = 7.1, CH_2CH_3); 1.28 (1.2H, t, J = 7.1, CH_2CH_3). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 171.5; 171.4; 156.4; 134.1; 133.9; 132.3; 131.8; 131.2; 131.1; 128.1; 128.0; 127.6; 122.6; 122.5; 62.3; 62.1; 53.1; 52.6; 52.4; 45.2; 31.6; 31.2; 14.8; 14.7.

(*R*)-6-Bromo-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (6). 2-Ethyl 3-methyl (*R*)-6-bromo-3,4-dihydroisoquinoline-2,3(1*H*)-dicarboxylate (**5a**) (351 mg, 1.03 mmol) was dissolved in 33% HBr in AcOH (4.1 ml, 24.6 mmol) and the orange solution was stirred at room temperature for 84 h. Volatiles were removed under reduced pressure, and the residue was treated with aqueous 6 M HCl solution (10 ml). The light-brown suspension was stirred at 70°C for 18 h. The formed precipitate was filtered off, dried over P_2O_5 at 50°C. Yield 244 mg (81%), light-brown solid, $[\alpha]_D^{20}$ 66.5 (*c* 1.05, MeOH). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 7.55–7.50 (1H, m, H Ar); 7.46 (1H, dd, *J* = 8.3, *J* = 2.1, H Ar); 7.20 (1H, d, *J* = 8.3, H Ar); 4.50–4.34 (3H, m, CH_2N, CH_2CH); 3.48 (1H, dd, *J* = 17.4, *J* = 5.3, CH_2CH); 3.23 (1H, dd, *J* = 17.4, *J* = 11.6, CH_2CH). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 170.7; 134.2; 132.9; 131.7; 129.6; 128.1; 122.9; 55.0; 45.2; 29.3. Found, *m*/*z*: 255.9970 $[M+H]^+$. $C_{10}H_{11}BrNO_2$. Calculated, *m*/*z*: 255.9973.

Methyl (*R*)-6-bromo-1,2,3,4-tetrahydroisoquinoline-3-carboxylate hydrochloride (6a). $SOCl_2$ (12 ml, 0.17 mmol) was added to the cooled (0°C) anhydrous MeOH (1 ml), and resulting solution was stirred for 10 min. Hydrochloride **6** (20 mg, 0.068 mmol) was then added, and the light-brown solution was stirred at 60°C for 3 h. The volatiles were removed under reduced pressure, and anhydrous PhMe (2 × 1 ml) was added and evaporated under reduced pressure. The precipitate was dried over P_2O_5 at 40°C. Yield 20 mg (95%), light-brown powder, $[\alpha]_D^{20}$ 55.2 (*c* 0.55, MeOH). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 7.55–7.49 (1H, m, H Ar); 7.47 (1H, dd, *J* = 8.3, *J* = 2.0, H Ar); 7.20 (1H, d, *J* = 8.3, H Ar); 4.56–4.34 (3H, m, CH_2N, CH_2CH); 3.91 (3H, s, OCH_3); 3.47 (1H, dd, *J* = 17.4, *J* = 5.2, CH_2CH); 3.28–3.17 (1H, m, CH_2CH). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 169.9; 133.8; 132.9; 131.7; 129.6; 128.0; 122.9; 55.1; 54.0; 45.3; 29.1. Found, *m*/*z*: 270.0141 $[M+H]^+$. $C_{11}H_{13}BrNO_2$. Calculated, *m*/*z*: 270.0130.

***tert*-Butyl (*R*)-6-bromo-3-(diethylcarbamoyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (7)**. NaOH (70 mg, 1.76 mmol) was added to a suspension of hydrochloride **6** (215 mg, 0.84 mmol) in *t*-BuOH–H_2O, 1:1 (6 ml). The reaction mixture was stirred till clear solution was formed, then Boc_2O (183 mg, 0.84 mmol) was added. The light-yellow solution was stirred at room temperature for 16 h. The solution was evaporated under reduced pressure to 1/3 of volume, then acidified with aqueous 5% $KHSO_4$ solution to pH 3 and extracted with EtOAc (3 × 15 ml). The combined extracts were washed with brine (20 ml), dried over Na_2SO_4, and evaporated under reduced pressure. The crude product (258 mg, 0.72 mmol) was dissolved in anhydrous DMF (4 ml) and HOBt·H_2O (144 mg, 0.94 mmol) was added, followed by DCC (194 mg, 0.94 mmol). The resulting light-yellow solution was stirred at 0°C for 1 h, then diethylamine (79 mg, 112 µl, 1.09 mmol) was added and stirring was continued at room temperature for 16 h. The light-yellow solution was diluted with H_2O (10 ml) and extracted with EtOAc (3 × 10 ml). The combined extracts were washed with H_2O (15 ml), brine (15 ml), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (25 g), eluent hexane–EtOAc, gradient from 15 to 85% EtOAc. Yield 250 mg (84%), light-yellow oil, mixture of rotamers, $[\alpha]_D^{20}$ 27.9 (*c* 0.86, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.33–7.26 (2H, m, H Ar); 7.04–6.91 (1H, m, H Ar); 5.33–5.24 (0.6H, m, CH_2CH); 5.00–4.84 (0.8H, m, CH_2N, CH_2CH); 4.78 (0.6H, d, *J* = 16.6, CH_2N); 4.35 (0.6H, d, *J* = 16.6, CH_2N); 4.25 (0.4H, d, *J* = 16.2, CH_2); 3.56–3.16 (4H, m, $(CH_2CH_3)_2$); 3.14–2.92 (2H, m, CH_2CH); 1.47 (9H, s, $C(CH_3)_3$); 1.31–1.19 (3H, m, CH_2CH_3); 1.14–1.01 (3H, m, CH_2CH_3). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 170.3; 154.7; 135.5; 135.3; 132.6; 131.2; 130.6; 129.6, 129.3; 127.7; 127.4; 120.4; 120.2; 80.9; 51.5; 49.5; 44.3; 43.7; 42.0; 40.6; 31.4; 30.8; 28.6; 14.6; 13.0. Found, *m*/*z*: 433.1086 $[M+Na]^+$. $C_{19}H_{27}BrNaN_2O_3$. Calculated, *m*/*z*: 433.1103.

(*R*)-{6-Bromo-3-[(diethylamino)methyl]-3,4-dihydroisoquinolin-2(1*H*)-yl}(4-pentylphenyl)methanone (8). 4 M HCl in 1,4-dioxane (1.52 ml, 6.10 mmol) was added dropwise to a solution of amide (*R*)-**7** (251 mg, 0.61 mmol) in anhydrous 1,4-dioxane (4 ml). The light-yellow solution was stirred at room temperature for 16 h, then solvent was evaporated under reduced pressure to give unprotected tetrahydroisoquinoline (207 mg, 98%) which was used in the next step without purification. $LiAlH_4$ (2.4 M solution in THF, 496 µl, 1.19 mmol) was added dropwise to a cooled solution (0°C) of crude product from previous step (207 mg, 0.59 mmol) in anhydrous THF (6 ml). After stirring at room temperature for 2 h, the light-yellow solution was cooled to 0°C and quenched by sequential (within intervals of 10 min) addition of H_2O (45 µl), aqueous 4 M NaOH solution (90 µl), and more H_2O (135 µl). Ten minutes after addition of the final amount of H_2O, the white suspension was filtered. The filter cake was washed with EtOAc (20 ml). The filtrate was evaporated to dryness to yield 162 mg (92%) of (*R*)-*N*-[(6-bromo-1,2,3,4-tetrahydroisoquinolin-3-yl)methyl]-*N*-ethylethanamine as a light-yellow oil, which was used in subsequent step without purification. DIPEA (283 µl, 211 mg, 1.64 mmol) was added to a cooled (0°C) solution of amine from previous step (162 mg, 0.54 mmol) in anhydrous CH_2Cl_2 (7 ml) followed by 4-pentylbenzoyl chloride (122 µl, 126 mg, 0.60 mmol). The light-yellow solution was stirred at room temperature for 16 h, then evaporated under reduced pressure. The residue was partitioned between EtOAc (10 ml) and H_2O (10 ml) and extracted with EtOAc (3 × 10 ml). The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (15 g), eluent hexane–EtOAc, gradient from 10 to 100% EtOAc. Yield 113 mg (44% in three steps), light-yellow oil, mixture of rotamers, $[\alpha]_D^{20}$ 10.2 (*c* 0.98, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.38–7.28 (4H, m, H Ar); 7.24–7.17 (2H, m, H Ar); 7.11–7.03 (1H, m, H Ar); 5.23 (1H, d, *J* = 17.9, CH_2N); 4.57–4.12 (2H, m, CH_2N, CH_2CH); 3.20–2.97 (1H, m, CH_2CH); 2.89–2.75 (1H, m, CH_2CH); 2.69–2.45 (4H, m, 1-CH_2, CH_2CH_3); 2.44–2.10 (4H, m, $CHCH_2NEt_2$, CH_2CH_3); 1.62 (2H, quint, *J* = 7.3, 2-CH_2); 1.42–1.20 (4H, m, 3,4-CH_2); 1.01–0.93 (3H, m, CH_2CH_3); 0.89 (3H, t, *J* = 6.9, 5-CH_3);

0.82–0.63 (3H, m, $CH_2C\underline{H}_3$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 172.3; 144.9; 134.6; 133.9; 132.3; 131.4; 129.7; 128.6; 128.3; 126.9; 120.3; 54.3; 51.7; 47.3; 45.1; 41.7; 35.9; 31.5; 31.4; 31.1; 22.7; 14.2; 11.8. Found, *m*/*z*: 471.2007 $[M+H]^+$. $C_{26}H_{36}BrN_2O$. Calculated, *m*/*z*: 471.2011.

(*R*)-{3-[(Diethylamino)methyl]-6-(pyridin-3-yl)-3,4-dihydroisoquinolin-2(1*H*)-yl}(4-pentylphenyl)methanone (9a). A vial was charged with bromide (*R*)-**8** (20 mg, 0.042 mmol), pyridin-3-ylboronic acid (7.8 mg, 0.064 mmol), and $Pd(PPh_3)_4$ (1.5 mg, 0.0013 mmol), then 1,4-dioxane (0.5 ml) was added, followed by aqueous 2 M Na_2CO_3 solution (42 μl, 0.084 mmol). After stirring at 105°C for 16 h, the orange suspension was cooled to room temperature and diluted with H_2O (5 ml) and EtOAc (5 ml). The organic layer was decanted, and the aqueous layer was extracted with EtOAc (3 × 5 ml). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (15 g), eluent hexane–EtOAc, gradient from 50 to 100% EtOAc, then EtOAc–MeOH, 9:1. Yield 9 mg (47%), light-yellow oil, mixture of rotamers, $[\alpha]_D^{20}$ 7.0 (*c* 0.62, $CHCl_3$). ^{1}H NMR spectrum (CD_3OD), δ, ppm: 8.84–8.76 (1H, m, H Ar); 8.55–8.47 (1H, m, H Ar); 8.15–8.04 (1H, m, H Ar); 7.59–7.48 (3H, m, H Ar); 7.46–7.36 (3H, m, H Ar); 7.36–7.28 (2H, m, H Ar); 5.24 (1H, d, *J* = 18.5, CH_2N); 4.65–4.24 (2H, m, CH_2N, $CH_2C\underline{H}$); 3.30–3.17 (1H, m, $C\underline{H}_2CH$); 3.07–2.94 (1H, m, $C\underline{H}_2CH$); 2.76–2.53 (4H, m, 1-CH_2, $C\underline{H}_2CH_3$); 2.52–2.13 (4H, m, $CHC\underline{H}_2NEt_2$, $C\underline{H}_2CH_3$); 1.66 (2H, quintet, *J* = 7.5, 2-CH_2); 1.45–1.23 (4H, m, 3,4-CH_2); 1.03 (3H, t, *J* = 7.1, $CH_2C\underline{H}_3$); 0.96–0.84 (3H, m, 5-CH_3); 0.77 (3H, t, *J* = 7.1, $CH_2C\underline{H}_3$). ^{13}C NMR spectrum (CD_3OD), δ, ppm: 174.4; 148.8; 148.3; 138.2; 137.2; 136.4; 134.8; 133.5; 133.1; 133.0; 130.0; 129.9; 129.8; 129.2; 128.5; 128.1; 126.4; 125.5; 55.2; 53.6; 49.3; 48.5; 43.1; 36.7; 32.4; 32.3; 23.6; 14.4; 12.1. Found, *m*/*z*: 470.3164 $[M+H]^+$. $C_{31}H_{40}N_3O$. Calculated, *m*/*z*: 470.3171.

(*R*)-{3-[(Diethylamino)methyl]-6-(4-methoxyphenyl)-3,4-dihydroisoquinolin-2(1*H*)-yl}(4-pentylphenyl)-methanone (9b). A vial was charged with bromide (*R*)-**8** (50 mg, 0.106 mmol), (4-methoxyphenyl)boronic acid (24 mg, 0.159 mmol), and $Pd(PPh_3)_4$ (3.7 mg, 0.0032 mmol), then 1,4-dioxane (1 ml) was added, followed by aqueous 2 M Na_2CO_3 solution (106 μl, 0.212 mmol). After stirring at 105°C for 16 h, the orange suspension was cooled to room temperature and diluted with H_2O (5 ml) and EtOAc (5 ml). The organic layer was decanted, and the aqueous layer was extracted with EtOAc (3 × 5 ml). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (15 g), eluent hexane–EtOAc, gradient from 10 to 100% EtOAc. Yield 26 mg (49%), light-yellow oil, $[\alpha]_D^{20}$ 13.3 (*c* 1.03, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.54–7.46 (2H, m, H Ar); 7.44–7.28 (4H, m, H Ar); 7.25–7.19 (3H, m, H Ar); 7.01–6.93 (2H, m, H Ar); 5.31 (1H, d, *J* = 18.9, CH_2N); 4.61–4.16 (2H, m, CH_2N, $CH_2C\underline{H}$); 3.85 (3H, s, OCH_3); 3.29–3.07 (1H, m, $C\underline{H}_2CH$); 2.95–2.83 (1H, m, $C\underline{H}_2CH$); 2.72–2.49 (4H, m, 1-CH_2, $C\underline{H}_2CH_3$); 2.47–2.37 (1H, m, $CHC\underline{H}_2NEt_2$); 2.34–2.13 (3H, m, $CHC\underline{H}_2NEt_2$, $C\underline{H}_2CH_3$); 1.63 (2H, quint, *J* = 7.4, 2-CH_2); 1.41–1.23 (4H, m, 3,4-CH_2); 1.08–0.94 (3H, m, $CH_2C\underline{H}_3$); 0.90 (3H, t, *J* = 6.8, 5-CH_3); 0.76 (3H, t, *J* = 7.1, $CH_2C\underline{H}_3$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 172.3; 159.3; 144.7; 139.3; 134.2; 133.5; 132.6; 130.8; 128.6; 128.1; 127.7; 127.0; 126.2; 125.0; 114.3; 55.5; 54.5; 52.1; 47.4; 47.2; 41.9; 35.9; 31.5; 31.1; 22.7; 14.2; 11.8. Found, *m*/*z*: 499.3322 $[M+H]^+$. $C_{33}H_{43}N_2O_2$. Calculated, *m*/*z*: 499.3325.

(*R*)-{3-[(Diethylamino)methyl]-6-(4-hydroxyphenyl)-3,4-dihydroisoquinolin-2(1*H*)-yl)}(4-pentylphenyl)-methanone (9c). A vial was charged with NaH (60% suspension in mineral oil) (7.4 mg, 0.184 mmol) and washed with anhydrous Et_2O (3 × 1 ml), then anhydrous DMF (1 ml) was added. The suspension was cooled to 0°C, and 1-dodecanethiol (44 μl, 0.184 mmol) was added dropwise (*Caution! Gas evolution!*). After stirring at room temperature for 10 min, solution of isoquinoline derivative (*R*)-(**9b**) (23 mg, 0.046 mmol) in anhydrous DMF (0.5 ml) was added to the white suspension. The yellow solution was stirred at 130°C for 2 h, then evaporated, and H_2O (3 ml) was added to the residue and extracted with EtOAc (3 × 3 ml). The combined extracts were washed with H_2O (7 ml), brine (7 ml), dried, and evaporated. The mixture was purified by column chromatography on silica gel (10 g), eluent CH_2Cl_2, gradient to CH_2Cl_2–MeOH, 96:4. Yield 13 mg (58%), light-yellow oil, $[\alpha]_D^{20}$ 9.8 (*c* 1.16, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.46–7.34 (4H, m, H Ar); 7.34–7.17 (5H, m, H Ar); 7.00–6.82 (2H, m, H Ar); 5.32 (1H, d, *J* = 18.0, CH_2N); 4.66–4.17 (2H, m, CH_2N, $CH_2C\underline{H}$); 3.31–3.06 (1H, m, $C\underline{H}_2CH$); 2.96–2.84 (1H, m, $C\underline{H}_2CH$); 2.76–2.54 (4H, m, 1-CH_2, $C\underline{H}_2CH_3$); 2.50–2.37 (1H, m, $CHC\underline{H}_2NEt_2$); 2.35–2.15 (3H, m, $CHC\underline{H}_2NEt_2$, $C\underline{H}_2CH_3$); 1.71–1.54 (2H, m, 2-CH_2); 1.44–1.23 (4H, m, 3,4-CH_2); 1.12–0.96 (3H, m, $CH_2C\underline{H}_3$); 0.90 (3H, t, *J* = 6.7, 5-CH_3); 0.76 (3H, t, *J* = 6.9, $CH_2C\underline{H}_3$). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 172.6; 156.0; 144.9; 139.5; 133.8; 133.0; 132.4; 130.5; 128.6; 128.2; 127.6; 127.3; 127.0; 126.2; 125.0; 116.0; 54.5; 52.2; 47.3; 42.0; 35.9; 31.5; 31.1; 22.7; 14.2; 11.7. Found, *m*/*z*: 514.3428 $[M+H]^+$. $C_{33}H_{44}N_3O_2$. Calculated, *m*/*z*: 514.3434.

(*R*)-{3-[(Diethylamino)methyl]-6-[(4-methoxyphenyl)-amino]-3,4-dihydroisoquinolin-2(1*H*)-yl}(4-pentylphenyl)-methanone (10). A vial was charged with $Pd_2(dba)_3$ (0.78 mg, 0.0009 mmol), X-Phos (0.81 mg, 0.0017 mmol), then anhydrous PhMe (1 ml) was added and the solution was heated to 60°C. After 10 min, bromide (*R*)-**8** (20 mg, 0.042 mmol), 4-methoxyaniline (6.3 mg, 0.051 mmol), and NaO*t*-Bu (5.7 mg, 0.059 mmol) were added. After stirring at 90°C for 16 h, the yellow suspension was cooled to room temperature and diluted with H_2O (5 ml) and EtOAc (5 ml). The organic layer was decanted, and the aqueous layer was extracted with EtOAc (3 × 5 ml). The combined organic layers were washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (15 g), eluent hexane–EtOAc, gradient from 50 to 100% EtOAc. Yield 12 mg (55%), light-yellow oil, $[\alpha]_D^{20}$ 11.5 (*c* 0.81, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 7.40–7.33

(2H, m, H Ar); 7.25–7.18 (2H, m, H Ar); 7.10–6.98 (3H, m, H Ar); 6.90–6.83 (2H, m, H Ar); 6.82–6.66 (2H, m, Ar); 5.43 (1H, s, NH); 5.19 (1H, d, J = 17.4, CH_2N); 4.47–4.06 (2H, m, CH_2N, CH_2CH); 3.80 (3H, s, OCH_3); 3.17–2.96 (1H, m, CH_2CH); 2.72 (1H, d, J = 16.2, CH_2CH); 2.67–2.49 (4H, m, 1-CH_2, CH_2CH_3); 2.44–2.33 (1H, m, $CHCH_2NEt_2$); 2.31–2.12 (3H, m, $CHCH_2NEt_2$, CH_2CH_3); 1.71–1.54 (2H, m, 2-CH_2); 1.44–1.19 (4H, m, 3,4-CH_2); 1.03–0.94 (3H, m, CH_2CH_3); 0.93–0.86 (3H, m, 5-CH_3); 0.75 (3H, t, J = 7.1, CH_2CH_3). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 172.2; 155.4; 144.6; 143.8; 136.1; 134.3; 133.2; 128.5; 127.6; 127.2; 127.0; 122.4; 122.1; 116.2; 114.8; 55.7; 54.6; 52.1; 47.4; 41.7; 35.9; 31.6; 31.2; 27.7; 22.7; 14.2; 11.9. Found, m/z: 514.3428 $[M+H]^+$. $C_{33}H_{44}N_3O_2$. Calculated, m/z: 514.3434.

Inhibitory activity assays of compounds 9a–c, 10. A fluorescence resonance energy transfer (FRET) assay was performed to evaluate the ability of compounds to inhibit Plm I, Plm II, and Plm IV. K_m of the substrate was determined for each enzyme: Plm I – 2.7 ± 0.3 μM, Plm II – 2 ± 0.2 μM, Plm IV – 2.8 ± 0.2 μM. A solution of each compound for testing (concentration 0.01–100 μM) was added to the enzyme (Plm I, Plm II, or Plm IV) in buffer (0.1 M NaOAc, pH 4.5, 10% glycerol) on 96-well plate. The mixture was incubated for 30 min at 37°C. Substrate (DABCYL-Glu-Arg-Nle-Leu-Ser-Phe-Pro-EDANS, AnaSpec Inc.) was then added to reach the final concentration of 5 μM. Hydrolysis of the substrate was detected as an increase in fluorescence (Em 490 nm, Ex 336 nm) at 37°C. The data points were collected every 1 min within 8–15 min. For the rate calculation, only the linear interval was used, which was slightly different for each enzyme. Compounds were tested in triplicate experiments. IC_{50} values were calculated using the software Graph Pad Prism 5.0. Pepstatin A (IC_{50}, nM: 0.42 ± 0.02 (Plm II), 0.9 ± 0.02 (Plm I), 0.3 ± 0.04 (Plm IV)) and compound **A** were used as positive control.

X-ray structural analysis of compound (*R*)-6a. Single crystals of $C_{11}H_{13}BrClNO_2$ were investigated on a Rigaku, XtaLAB Synergy, Dualflex, HyPix diffractometer. The crystal was kept at 150.0(1) K during data collection. Using Olex2,[19] the structure was solved with the ShelXT[20] structure solution program using Intrinsic Phasing and refined with the olex2.refine[21] refinement package using Levenberg–Marquardt minimization. Crystal data for $C_{11}H_{13}BrClNO_2$ (M 306.59 g/mol): triclinic, space group $P1$; a 7.0731(4), b 7.9997(4), c 12.2609(4) Å; α 77.412(4), β 74.682(4), γ 68.708(5)°; V 617.68(6) Å^3; Z 1; μ(Cu$K\alpha$) 6.417 mm^{-1}; d_{calc} 1.6483g/cm^3. 16651 reflections measured ($2\Theta \leq 155.0°$), 4805 unique (R_{int} 0.0475, R_{sigma} 0.0349) which were used in all calculations. The final R_1 was 0.0438 ($I > 2\sigma(I)$) and wR_2 was 0.1318 (all data). The complete crystallographic dataset was deposited at the Cambridge Crystallographic Data Center (deposit CCDC 1953912).

Docking studies. Docking studies were performed using the AutoDock Vina software package.[18]

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References

1. Wells, T. N. C.; Hooft van Huijsduijnen, R.; Van Voorhis, W. C. *Nat. Rev. Drug Discovery* **2015**, *14*, 424.
2. Wells, T. N.; Alonso, P. L.; Gutteridge, W. E. *Nat. Rev. Drug Discovery* **2009**, *8*, 879.
3. Schlitzer, M. *Arch. Pharm.* **2008**, *341*, 149.
4. Ersmark, K.; Samuelsson, B.; Hallberg, A. *Med. Res. Rev.* **2006**, *26*, 626.
5. Miller, W. A., III; Teye, J.; Achieng, A. O.; Mogire, R. M.; Akala, H.; Ong'echa, J. M.; Rathi, B.; Durvasula, R.; Kempaiah, P.; Kwofie, S. K. *Curr. Top. Med. Chem.* **2018**, *18*, 2022.
6. Bobrovs, R.; Jaudzems, K.; Jirgensons, A. *J. Med. Chem.* **2019**, *62*, 8931.
7. Kinena, L.; Leitis, G.; Kanepe-Lapsa, I.; Bobrovs, R.; Jaudzems, K.; Ozola, V.; Suna, E.; Jirgensons, A. *Arch. Pharm.* **2018**, *351*, 1800151.
8. Huizing, A. P.; Mondal, M.; Hirsch, A. K. H. *J. Med. Chem.* **2015**, *58*, 5151.
9. Rasina, D.; Otikovs, M.; Leitans, J.; Recacha, R.; Borysov, O. V.; Kanepe-Lapsa, I.; Domraceva, I.; Pantelejevs, T.; Tars, K.; Blackman, M. J.; Jaudzems, K., Jirgensons, A. *J. Med. Chem.* **2016**, *59*, 374.
10. Boss, C.; Richard-Bildstein, S.; Weller, T.; Fischli, W.; Meyer, S.; Binkert, C. *Curr. Med. Chem.* **2003**, *10*, 883.
11. Boss, C.; Corminboeuf, O.; Grisostomi, C.; Meyer, S.; Jones, A. F.; Prade, L.; Binkert, C.; Fischli, W.; Weller, T.; Bur, D. *ChemMedChem* **2006**, *1*, 1341.
12. Prade, L.; Jones, A. F.; Boss, C.; Richard-Bildstein, S.; Meyer, S.; Binkert, C.; Bur, D. *J. Biol. Chem.* **2005**, *280*, 23837.
13. Specker, E.; Böttcher, J.; Heine, A.; Sotriffer, C. A.; Lilie, H.; Schoop, A.; Müller, G.; Griebenow, N.; Klebe, G. *J. Med. Chem.* **2005**, *48*, 6607.
14. Fang, X.; Yin, Y.; Chen, Y. T.; Yao, L.; Wang, B.; Cameron, M. D.; Lin, L.; Khan, S.; Ruiz, C.; Schröter, T.; Grant, W.; Weiser, A.; Pocas, J.; Pachori, A.; Schürer, S.; LoGrasso, P.; Feng, Y. *J. Med. Chem.* **2010**, *53*, 5727.
15. Aggarwal, V. K.; Humphries, P. S.; Fenwick, A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2883.
16. Chae, J. *Arch. Pharmacal Res.* **2008**, *31*, 305.
17. Hanthorn, J. J.; Valgimigli, L.; Pratt, D. A. *J. Org. Chem.* **2012**, *77*, 6908.
18. Trott, O.; Olson, A. J. *J. Comput. Chem.* **2010**, *31*, 455.
19. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschman, H. *J. Appl. Crystallogr.* **2009**, *42*, 339.
20. Sheldrick, G. M. *Acta Crystallogr., Sect. A: Found. Adv.* **2015**, *A71*, 3.
21. Bourhis, L. J.; Dolomanov, O. V.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *Acta Crystallogr., Sect. A.: Found. Adv.* **2015**, *A71*, 59.

4. PIELIKUMS – KONFERENČU TĒZES

Linda Kinena, Rimants Zogota, Vita Ozola, Aigars Jirgensons, Edgars Suna. Peptidomimetic Plasmepsin Inhibitors with Potent Anti-malarial Activity and Selectivity Against Cathepsin D. VIII EFMC International Symposium on Advances in Synthetic and Medicinal Chemistry.

Rimants Zogota, Linda Kinena. The Development of Malaria Aspartic-Protease Inhibitors with Improved Selectivity. Balticum Organicum Syntheticum.

Rimants Zogota, Linda Kinena. The Development of Aspartic-Protease Inhibitors for Malaria Treatment. 10th Paul Walden Symposium.

PEPTIDOMIMETIC PLASMEPSIN INHIBITORS WITH POTENT ANTI-MALARIAL ACTIVITY AND SELECTIVITY AGAINST CATHEPSIN D

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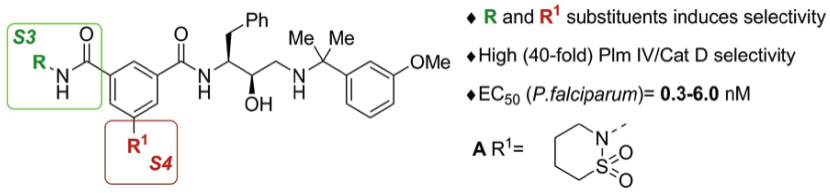
Malaria is a life-threatening disease caused by *Plasmodium* parasites which are transmitted by mosquites.[1] The spread of drug-resistant malaria parasites urges the development of new antimalarial drugs. Malarial aspartic proteases – plasmepsins (Plm I, Plm II, Plm IV, HAP) – are found in the food vacuole of the parasite. The plasmepsins are involved in processing of hemoglobin to amino acids and are considered as attractive drug targets.[2]

Hydroxyethylamine derivative **A** was recently published by *GlaxoSmithKline* as potent antimalarial which shows high inhibitory activity against Plm IV but no selectivity *versus* human aspartic protease Cathepsin D (CatD).[3] For the lead optimization Plm IV was used as a readily accessible model protein, the inhibition of which was previously found to correlate with results of parasite growth assay. Based on sequence alignment of Plm IV and Cat D, the selectivity factor (S) Plm IV/Cat D of antimalarial hit **A** was optimizied by changing substituents occupying *S3* and *S4* sub-pockets.[4]



Introduction of an *S3* sub-pocket targeting mono-substituted amide moiety containing linear or branched hydrophobic groups resulted in up to 40-fold Plm IV/Cat D selectivity factor. Plm IV inhibitors with no substituents or fluorine targeting the *S4* sub-pocket led to 20-fold selectivity against Cat D. Determination of parasite growth inhibition potency for selected Plm inhibitors showed activities in the low nano-molar range (0.3-0.6 nM).[4]

References

1) WHO, World Malar. Rep. 2016, World Health Organ, Geneva, 2016. http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/.
2) A. Hallberg, J. Åqvist, B. Samuelsson, M. J. Blackman, B. M. Dunn, J. C. Clemente, L. K. Janka, E. Hamelink, M. Nervall, K. J. Ersmark, J. Med. Chem. 2005, 48, 6090.
3) F.J. Gamo, L.M. Sanz, J. Vidal, C. de Cozar, E. Alvarez, J.-L. Lavandera, D.E. Vanderwall, D.V. Green, V. Kumar, S. Hasan, et al., Nature 2010, 465, 305.
4) R. Zogota, L. Kinena, C. Withers-Martinez, M. Blackman, R. Bobrovs, T. Pantelejevs, I. Kanepe-Lapsa, V. Ozola, K. Jaudzems, E. Suna, A. Jirgensons, Eur. J. Med. Chem. 2019, 163, 344.

The Development of Malaria Aspartic-Protease Inhibitors with Improved Selectivity

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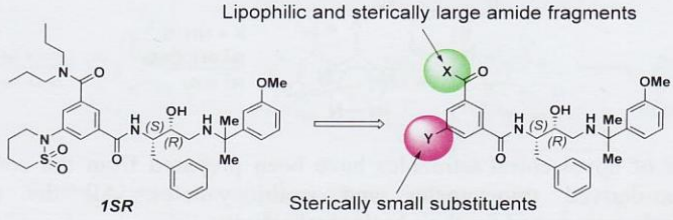
Malaria is one of the most life-threatening infectious disease spread by mosquitoes. The rapidly growing resistance of parasite has affected most of the anti-malarial drugs. The potential anti-malarial drug targets could be aspartic proteases - Plm I, Plm II, Plm IV that are found in the food vacuole of the parasite and degrade 75% of host hemoglobin.

Antimalarial hit ***ISR*** was identified to be potent Plm II and Plm IV inhibitor with no selectivity over Cathepsin D (the human aspartic protease). CatD shares ~35% sequence identity with the plasmepsins of the malaria parasite so anti-malarial drugs targeting the plasmepsins must have reduced activity on CatD to avoid adverse side-effects such as neuronal ceroid lipofuscinosis (NCL), developmental regression, visual loss and epilepsy [1]. Majority of the ***ISR*** analogues which has the best potency against Plm IV possess poor selectivity over CatD [2]. To improve the selectivity (selectivity factor of CatD/Plm IV inhibition), we attempted to optimize this molecule by structural variations in positions **X** and **Y**.



Among various fragments tested at the position **X** and **Y** we can confirm that high IC_{50} values and good CatD/PlmIV selectivity factor can be achieved if inhibitor contains secondary amide with lipophilic substituent at position **X** and sterically small substituent at position **Y**.

References: 1. Benes, P.; Vetvicka, V.; Fusek, M. *Critical Reviews in Oncology/Hematology*, **2008**, *68*, 12.
2. Jaudzems, K.; Tars, K.; Maurops, G.; Ivdra, N.; Otikovs, M.; Leitans, J.; Kanepe-Lapsa, I.; Domraceva, I.; Mutule, I.; Trapencieris, P.; Blackman, M. J.; Jirgensons, A. *ACS Med. Chem. Lett.* **2014**, *5*, 373.

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The Development of Aspartic-Protease Inhibitors for Malaria Treatment

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Malaria is one of the most life-threatening infectious disease spread by mosquitoes. *Plasmodium falciparum* is one of the four species of *Plasmodium* that cause malaria in humans. The rapidly growing resistance of parasite has affected most of the anti-malarial drugs. The potential anti-malarial drug targets could be aspartic proteases - Plm I, Plm II, Plm IV that are found in the food vacuole of the parasite and degrade 75% of host hemoglobin [1].

Antimalarial hit ***ISR*** (Figure 1) was identified by *GlaxoSmithKline* to be potent Plm II and Plm IV inhibitor with no selectivity over Cathepsin D (the human aspartic protease) [2]. CatD shares a ~35% sequence identity with the plasmepsins of the malaria parasite [3]. Anti-malarial drugs targeting the plasmepsins must have reduced activity on CatD to avoid adverse side-effects such as neuronal ceroid lipofuscinosis (NCL), developmental regression, visual loss and epilepsy [4]. Majority of the ***ISR*** analogues, which has the best potency against Plm IV, possess poor selectivity over CatD. To improve the selectivity (inhibitory activity on CatD against inhibitory activity on Plm IV), we attempted to optimize this molecule by structural variations in positions **X** and **Y**.

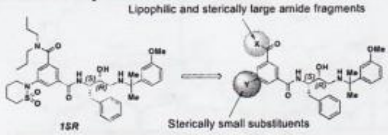

Figure 1. The optimization of the ***ISR*** molecule.

Among various secondary and tertiary amides tested at the position **X**, the best CatD/PlmIV selectivity was obtained with secondary amides possessing lipophilic and sterically large substituents, such as 3,3,3-trifluoropropyl **1**. Significant decrease in selectivity was observed using tertiary amides, such as *bis*-3,3,3-trifluoropropyl **2** (Figure 2).

Position **Y** required sterically small substituents such as hydrogen and chlorine (Figure 3) to achieve high IC_{50} values and CatD/PlmIV selectivity. Molecule **3** lacking substituent at position **Y** shows the highest selectivity in a series.

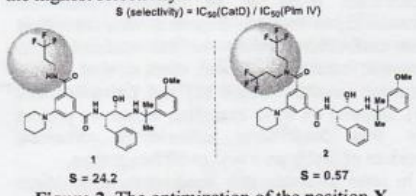

Figure 2. The optimization of the position **X**.

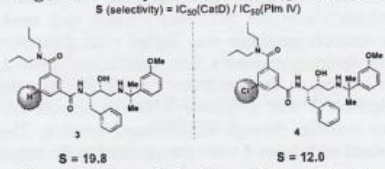

Figure 3. The optimization of the position **Y**.

In summary, high IC_{50} values and good CatD/PlmIV selectivity can be achieved if inhibitor contains secondary amide with lipophilic nitrogen substituent at position **X** and sterically small substituent at position **Y**.

Supervisor: Prof. E. Suna.

REFERENCES

[1] Hallberg, A.; Åqvist, J.; Samuelsson, B.; Blackman, M. J; Dunn, B. M; Clemente, J. C; Janka, L. K; Hamelink, E; Nervall, M; Ersmark, K. *J. Med. Chem.* **2005**, *48*, 6090. [2] Jaudzems, K.; Tars, K.; Maurops, G.; Ivdra, N.; Otikovs, M.; Leitans, J.; Kanepe-Lapsa, I.; Domraceva, I.; Mutule, I.; Trapencieris, P.; Blackman, M. J.; Jirgensons, A. *ACS Med. Chem. Lett.* **2014**, *5*, 373. [3] Liu, P.; Marcahn, M. R.; Robbins, A. H.; Gutiérrez-de-Ter án, H.; Rodriguez, D.; McClung, S. H.; Stevens, S. M.; Yowell, C. A.; Dame, J. B.; McKenna, R., Dunn, B. M. *Biochemistry* **2009**, *48(19)*, 4086. [4] Benes, P.; Vetvicka, V.; Fusek, M. *Critical Reviews in Oncology/Hematology* **2008**, *68*, 12.

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Zinātniskā vadītāja: *Dr. chem.* Vita Ozola

Paraksts:_____/ Vita Ozola /

Darbs iesniegts
LU Ķīmijas nozares Promocijas padomē 2020. gada 9. martā

Promocijas padomes sekretāre: Vita Rudoviča_____
(*paraksts*)

Darbs aizstāvēts Promocijas padomes sēdē

2020. gada 18. maijā, protokols Nr._____
(*protokola Nr.*)

Promocijas padomes sekretāre: Vita Rudoviča_____
(*paraksts*)