Published in final edited form as:

Bioorg Med Chem Lett. 2018 August 15; 28(15): 2605–2610. doi:10.1016/j.bmcl.2018.06.045.

Design, synthesis, X-ray studies, and biological evaluation of novel BACE1 inhibitors with bicyclic isoxazoline carboxamides as the P3 ligand

Arun K. Ghosh^{*,a,b}, Koena Ghosh^a, Margherita Brindisi^a, Emma K. Lendy^c, Yu-Chen Yen^d, Nagaswamy Kumaragurubaran^a, Xiangping Huang^e, Jordan Tang^{e,f}, and Andrew D. Mesecar^{a,c,d}

^aDepartment of Chemistry, Purdue University, West Lafayette, IN 47907

^bDepartment of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907

^cDepartment of Biochemistry, Purdue University, West Lafayette, IN 4790

^dDepartment of Biological Sciences, Purdue University, West Lafayette, IN 47907

^eProtein Studies Program, Oklahoma Medical Research Foundation

^fDepartment of Biochemistry and Molecular Biology, University of Oklahoma Health Science Center, Oklahoma City, OK 73104

Abstract

We describe design, synthesis, X-ray studies, and biological evaluation of novel BACE1 inhibitors containing bicyclic isoxazoline carboxamides as the P3 ligand in combination with methyl cysteine, methylsulfonylalanine and Boc-amino alanine as P2 ligands. Inhibitor **3a** displayed a BACE1 K_i value of 10.9 nM and EC_{50} of 343 nM. The X-ray structure of **3a** bound to the active site of BACE1 was determined at 2.85 Å resolution. The structure revealed that the major molecular interactions between BACE1 and the bicyclic tetrahydrofuranyl isoxazoline heterocycle are van der Waals in nature.

Graphical Abstract

A series of BACE1 inhibitors incorporating bicyclic isoxazoline carboxamides as the P3 ligands is described. An X-ray crystal structure of **3a**-bound BACE1 provided insight into the ligand-binding site interactions.

*Corresponding author. Tel.: +1-765-494-5323; fax: +1-765-496-1612; akghosh@purdue.edu (AKG).

Supplementary data

Supplementary data associated with this article can be found in the online version.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

β -secretase; Alzheimer's disease; Memapsin 2; BACE-1; Isoxazoline

Alzheimer's disease (AD) is a progressive degenerative disorder that leads to considerable neuronal cell death and ultimately destroys brain function critical to cognition and memory.^{1,2} The World Health Organization estimates that there were 44 million people suffering from AD in 2017 and the number is expected to be tripled by 2050.³ There exists no effective treatment that could prevent or slow down the progression of AD. The histopathological hallmark of AD has been the formation of β -amyloid ($A\beta$) peptides which results in the accumulation of amyloid plaques and neurofibrillary tangles ultimately causing neuronal cell death, cognitive decline, and AD.^{4,5}

The amyloid cascade hypothesis continues to play a major role in today's AD drug discovery efforts. $A\beta$ peptide is generated through a sequential cleavage of amyloid precursor protein (APP), first by β -secretase (memapsin 2, BACE1) and then by γ -secretase.^{6,7} BACE1 activity is the rate limiting step for APP processing and it is responsible for the generation of 40/42 residue peptides, the key constituent of the brain amyloid plaques. Due to its central role in $A\beta$ peptides, therapeutic inhibition of BACE1 has become a major focus in AD drug therapy.^{8,9} Since the discovery of β -secretase in 1999, many BACE1 inhibitors have been reported.^{10,11} A number of BACE1 inhibitors have entered in clinical trials in recent years.^{12,13}

Early on, on the basis of initial kinetics and substrate specificity data, we designed pseudopeptide inhibitor **1** (Figure 1) by incorporating a nonhydrolyzable Leu-Ala hydroxyethylene dipeptide isostere at the cleavage site.^{14,15} This inhibitor showed potent BACE1 inhibitory activity; however, it did not show selectivity over BACE2 or Cathepsin D. We subsequently determined the X-ray structure of inhibitor **1**-bound BACE1 which provided key molecular insight into the ligand-binding site interactions.¹⁶ On the basis of this X-ray structure, we designed highly selective peptidomimetic inhibitor **2** with a pyrazolemethyl urethane as the P2–P3 ligand.^{17,18} The X-ray structure of inhibitor **2**-bound BACE1 was determined and based upon this structural information, we explored a number of isoxazoline-based bicyclic derivatives as P2–P3 ligands. Herein, we report the results of our studies in which we synthesized a number of tetrahydrofuro- and tetrahydropyrano[3,2-*d*]isoxazole-3-carboxamides with hydrogen bond donor and acceptor groups to interact with the residues in S2–S3 subsites of BACE1.

The synthesis of bicyclic tetrahydrofuro[3,2-*d*]isoxazole ligand was carried out using a [3+2] nitrile oxide cycloaddition reaction as shown in Scheme 1. Reaction of dihydrofuran (**4**) with known chlorooxime **5** in diethyl ether in the presence of aqueous Na₂CO₃ at 23 °C for 5 h provided racemic isoxazoline derivative **6** in excellent yield.¹⁹ For assessment of stereochemical preferences, we planned to incorporate this bicyclic ligand with defined stereochemistry. Therefore, racemic ester **6** was saponified with aqueous LiOH to provide acid **7**. Reaction of the racemic acid with optically active methylcysteine methyl ester **8**, with EDCI, HOBt in the presence of diisopropylethylamine (DIPEA) in a mixture of dimethylformamide (DMF) and CH₂Cl₂ at 23 °C for 12 h furnished coupling products **9** as a mixture (1:1) of diastereomers, which could not be separated by silica gel chromatography. However, one of the isomers was separated by crystallization using a 9:1 mixture of chloroform and hexanes. X-ray crystallographic analysis of the crystalline product revealed that the crystallized compound was isomer **9b**. This recrystallized isomer was shown to have optical purity of 96% *ee* by chiral HPLC analysis. The mother liquor containing enriched isomer **9a** was purified by preparative HPLC on a C-18 preparative column using 20% aqueous MeOH. Chiral HPLC analysis showed 99% *ee* for isomer **9a**. Both diastereomers **9a** and **9b** were hydrolyzed with aqueous LiOH to acids **10a** and **10b**, respectively.

Our ¹H-NMR analysis of both diastereomers **9a** and **9b** showed that there are small differences in δ values for these compounds. As shown in Figure 2, the characteristic peaks, assigned by ¹H-NMR COSY experiments are H_D (0.04 ppm difference for H_D'), H_C (0.01 ppm difference for H_C'). All these protons showed more downfield shift for compound **9b**. The most prominent downfield shift was observed for H_D' protons of isomer **9b** in comparison to isomer **9a**. HPLC analysis showed that isomer **9a** has lower retention time compared to isomer **9b**. The absolute configuration of the tetrahydrofuro[3,2-*d*]isoxazole carboxamide was determined to be C-4(*R*), C-8(*S*) and C-11(*R*) as shown in the ORTEP diagram of isomer **9b** in Figure 2.^{20,21}

The synthesis of BACE1 inhibitors with tetrahydrofuro[3,2-*d*]isoxazole ligands is shown in Scheme 2. Dipeptide isostere **11**, reported by us previously,²² was separately coupled with optically active carboxylic acids **10a** and **10b** using EDCI and HOBt in the presence of DIPEA, a mixture of CH₂Cl₂ and DMF at 23 °C for 16 h to provide coupling products **12** and **13**. The cysteine chiral center of **12** was found to be partially epimerized (10–15% by HPLC analysis) under these reaction conditions. However, no epimerization was observed for isomer **13** under these conditions. Treatment of these TBS derivatives with TBAF in THF at 23 °C for 12 h furnished inhibitors **3a** and **3b**.

In an effort to avoid epimerization of methyl cysteine chiral center, we explored an alternative route. As shown in Scheme 3, we first coupled amine **11** with methyl cysteine derivative **14** using EDCI, HOBt in the presence of DIPEA to provide compound **15** in 65% yield. Exposure of compound **15** to trifluoroacetic acid (TFA) in CH₂Cl₂ at 23 °C for 2.5 h removed the Boc-group as well as TBS group under this condition to provide aminoalcohol **16**. The resulting amine was coupled with racemic acid **7** using HATU, HOAt in the presence of collidine at 23 °C for 12 h providing compound **17** as a diastereomeric mixture (1:1 ratio) in 45% yield. These diastereomers were separated using HPLC on chiralpak IC

column (30% isopropanol in hexanes, flow rate 0.8 mL/min, 25 °C) to provide diastereomerically pure inhibitors **3a** (retention time 6.4 min) and **3b** (retention time 8.4 min). Stereochemical identity of these inhibitors was established by comparison of ¹H-NMR data with established absolute configuration of compound **3b** as mentioned above. Of particular note, ¹H-NMR analysis of compound **3b** showed that H_C' proton shifted downfield compared to H_C proton of compound **3a** (0.03 ppm shift). Similar downfield shift was observed for H_D and H_D' (0.02 ppm shift). As pointed out earlier, H_C proton showed the most prominent downfield shift for compound **3b**. Based upon precedence that sulfone derivatives are often more potent than sulfide derivatives, we oxidized sulfides **3a** and **3b** to the corresponding sulfone derivatives **3c** and **3d** respectively using oxone in a mixture (1:1) of methanol and water at 23 °C for 2 h.

We have also synthesized inhibitors with tetrahydropyrano[3,2-*d*]isoxazole as the P2 ligands as shown in Scheme 4. Cycloaddition of dihydropyran and chlorooxime in diethyl ether as described above provided tetrahydropyrano[3,2-*d*]isoxazole derivative.¹⁹ Saponification of the ester afforded racemic acid **18**. Coupling of amine **16** with racemic acid provided the corresponding diastereomeric mixture (1:1) which was separated by HPLC. Separated diastereomers were oxidized with oxone to provide inhibitors **3e** and **3f** with tetrahydropyrano[3,2-*d*]isoxazoles as the P2 ligands.

We previously observed that Boc-amino- *L*-alanine residue is a suitable P2 ligand.²² We, therefore, prepared inhibitors incorporating Boc-aminoalanine as the P2 ligand. As shown in Scheme 5, coupling of Boc-aminoalanine **19** with amine **11** provided Cbz-derivative **20** in 45% yield. Catalytic hydrogenation of **20** over 10% Pd(OH)₂ in MeOH at 23 °C for 2 h furnished the corresponding TBS-protected alcohols. After coupling with racemic acid **7**, diastereomeric alcohols were then separated by HPLC using a Chiralpak IA column (10% isopropanol in hexanes, flow rate 0.8 mL/min, 25 °C) to provide diastereomerically pure inhibitors **3g** (retention time 10.7 min) and **3h** (retention time 12.1 min). Stereochemical identities of inhibitors **3g** and **3h** were established based upon comparison of ¹H-NMR analysis as well as HPLC retention times.

In order to examine the effect of these bicyclic ligands in combination with hydroxyethylamine isosteres, we prepared inhibitors incorporating GRL-8234 isostere.²³ As shown in Scheme 6, the known amine derivative **22** was reacted with optically pure diastereomer **10a** using EDCI and HOBt in the presence of DIPEA in CH₂Cl₂ to provide the coupling product **23**. Oxidation of the methyl sulfide with oxone in MeOH afforded inhibitor **3i**. Similarly, diastereomeric ligand acid **10b** was coupled with amine **22** to provide the corresponding sulfide **24** which was oxidized with oxone to furnish inhibitor **3j** containing diastereomeric P2–P3 ligand.

The BACE1 inhibitory activity of synthetic compounds was determined using recombinant β-secretase (BACE1) and our previously reported assay protocols.²⁴ The structure and activity of inhibitors are reported in Table 1. As can be seen, ring stereochemistry of the tetrahydrofuro[3,2-*d*]isoxazole plays an important role in inhibitors' binding properties. Inhibitor **3a** with C4(*R*), C8(*S*), and C11(*R*) configuration on the P2–P3 ligand showed K_i value of 10.9 nM. Inhibitor **3b** with C4(*S*), C8(*R*), and C11(*R*) configuration exhibited

significant reduction in inhibitory activity. Oxidation of methyleysteine side chain in compound **3a** to sulfone derivative **3c** resulted in a 10-fold reduction of potency compared to sulfide derivative **3a** (entries 1 and 3). Inhibitor **3d** with diastereomeric P2-P3 ligand with a P2-sulfone side chain showed BACE1 K_i of 280 nM (entry 4). Inhibitors **3e** and **3f** containing tetrahydropyrano[3,2-*d*]isoxazole exhibited similar activity trends as the tetrahydrofuro[3,2-*d*]isoxazole ligands (entries 4, 5). Incorporation of Boc-amino-*L*-alanine as P2 ligand in combination with tetrahydrofuro[3,2-*d*]isoxazole resulted in significant reduction in BACE1 inhibitory potency. Inhibitor **3g** with C4(*R*), C8(*S*) configuration for P3 ligand, showed (K_i = 808 nM) better inhibitory activity over inhibitor **3h** (K_i = 4124 nM) with C4(*S*), C8(*R*) configuration (entries 7, 8). Inhibitors (**3i** and **3j**) with tetrahydrofuro[3,2-*d*]isoxazole and methyl sulfonyl alanine as the P2 and P3 ligands in combination with hydroxyethylamine isostere showed only marginal inhibitory activity (entries 9 and 10). We also measured the potency of inhibitor **3a** in a cell-based assay. It displayed an EC_{50} value of 343 nM. Furthermore, we evaluated inhibitory activity of compound **3a** against BACE2 and it showed only marginal selectivity against BACE2 with a K_i value of 46 nM.

To gain molecular insight into the BACE1-inhibitor interactions, we determined the X-ray crystal structure of inhibitor **3a** bound to BACE1 at 2.85 Å resolution (R_{free} = 21.9%, R_{work} = 17.2%).²⁵ Methods for BACE1 co-crystallization with **3a** and X-ray data collection are provided in Supplementary Data along with the final X-ray data collection and refinement statistics. A wall-eye stereoview of the active site of BACE1 with its interactions with inhibitor **3a** is shown in Figure 3. The transition-state hydroxyl group at the Leu-Ala isostere forms two hydrogen bonds (2.4 Å and 2.8 Å bond distances) with active site aspartic acid residues Asp32 and Asp228 respectively.¹⁶ The P2-methylcysteine side chain appears to be involved in van der Waals interactions with Thr72, Gln73, Thr231 and Arg235 in the S2 subsite. The other inhibitor-BACE1 interactions due to P1, P1', and P2' ligands are very similar to Leu-Ala hydroxyethylene isostere-derived BACE1 inhibitors reported by us previously.^{17,26}

The P3 bicyclic tetrahydrofuranyl isoxazoline occupies the S3 subsite and it is involved in a number of key van der Waals (VDW) interactions with BACE1 including the backbone carbonyl oxygens of Gly11 and Gly230; the alpha-carbon hydrogen of Gln12; and the side chains of Leu30, Ile110, Trp115 and Thr232 (Figure 3). The shape of the BACE1 pocket surrounding the bicyclic tetrahydrofuranyl isoxazoline is shown in Figure 4. The bicyclic group is partially solvent exposed, and the surrounding pocket is electrostatically almost neutral. No hydrogen bonds are observed between BACE1 and the oxygens or nitrogen of the bicyclic tetrahydrofuranyl isoxazoline heterocycle. The ring junction stereochemistry (3a*R*, 6a*S*) of the bicyclic ligand allows for more optimal VDW interactions within the active site. In contrast, the corresponding (3a*S*, 6a*R*) stereochemistry in inhibitor **3b** appears to disrupt these favorable VDW interactions and this may explain nearly 50-fold differences in BACE1 inhibitory activity for inhibitor **3a**.

The carboxamide carbonyl group forms a hydrogen bond with the Thr232 backbone NH. It is also involved in a water-mediated hydrogen bond with the Asn233 backbone NH (Figures 3 and 4). The carboxamide nitrogen group forms a water-mediated hydrogen bond with the backbone carbonyl oxygen and the side chain of Gln73 (Figure 3).

In summary, we have designed and synthesized a series of BACE1 inhibitors containing Leu-Ala hydroxyethylene isosteres incorporating bicyclic isoxazolines as the P3 ligand. In particular, we have designed tetrahydrofuro[3,2-*d*]isoxazole and tetrahydropyrano[3,2-*d*]isoxazole-derived P3 ligands for specific interactions in the S3 subsite. We have synthesized these heterocyclic ligands using [3+2] nitrile oxide cycloaddition reactions of dihydrofuran and dihydropyran with a readily prepared chlorooxime. Racemic compounds were coupled with optically active P2 ligands and diastereomers were separated by HPLC. Absolute configuration was established by X-ray crystallographic analysis. We investigated methylcysteine, methylsulfonyl alanine and Boc-amino-*L*-alanine as the P2-ligands. The methylcysteine ligand provided an inhibitor with enhanced activity compared to inhibitors with a P2-methylsulfonyl or Boc-amino alanine derivatives. Inhibitor **3a** displayed the most potent BACE1 inhibitory activity. We determined the X-ray structure of inhibitor **3a**-BACE1 complex to 2.85 Å resolution. The structure provided important molecular insight into the ligand-binding site interactions in the S2 and S3 subsites of β -secretase. Further design and improvement of inhibitor properties are currently in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support by the National Institutes of Health (AG 18933) and the Purdue University Office of the Executive Vice President for Research is gratefully acknowledged. NMR, Mass Spectrometry and BACE1 crystallization were all performed using shared resources which are partially supported by the Purdue Center for Cancer Research through NIH grant (P30CA023168). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357. Use of the Lilly Research Laboratories Collaborative Access Team (LRL-CAT) beamline at Sector 31 of the Advanced Photon Source was provided by Eli Lilly Company, which operates the facility.

References and notes

1. Rutten BP, Steinbusch HW. *Mol Neurodegener.* 2013; 8:33. [PubMed: 24148188]
2. Lansdall CJ. *Biosci Horizons.* 2014; 7:1–11.
3. *Alzheimers.net.* [Accessed 15 May 2018] 2016 Alzheimer's statistics. 2016. <http://www.alzheimers.net/resources/alzheimers-statistics/>
4. Alzheimer's Association. *Alzheimers Dement.* 2012; 8:131–168. [PubMed: 22404854]
5. Selkoe DJ. *Physiol Rev.* 2001; 81:741–766. [PubMed: 11274343]
6. Selkoe DJ. *Nature.* 1999; 399:A23–A31. [PubMed: 10392577]
7. Citron M. *Nat Rev Drug Discov.* 2010; 9:387–398. [PubMed: 20431570]
8. Vassar R. *Alzheimer's Res Ther.* 2014; 6:89. [PubMed: 25621019]
9. Sinha S. BACE: a (almost) perfect target for staving off Alzheimer's disease. In: Ghosh AK, editor *Aspartic acid proteases as therapeutic targets*, vol 45, *Methods and principles in medicinal chemistry*. Wiley-VCH; Weinheim: 2010. 393–412.
10. Ghosh AK, Cardénas EL, Osswald HL. *The Design, Development, and Evaluation of BACE1 Inhibitors for the Treatment of Alzheimer's Disease.* *Top Med Chem.* 2016:1–59.
11. Ghosh AK, Osswald HL. *Chem Soc Rev.* 2014; 43:6765–6813. [PubMed: 24691405]
12. Schneider LS, Mangialasche F, Andreasen N, Feldmen H, Giacobini E, Jones R, Mantua V, Mecocci P, Pani L, Winblad B, Kivipelto M. *J Intern Med.* 2014; 275:251–283. [PubMed: 24605808]

13. Cummings JL, Morstorf T, Zhong K. *Alzheimers Res Ther.* 2014; 6:37. [PubMed: 25024750]
14. Ghosh AK, Shin D, Downs D, Koelsch G, Lin X, Ermolieff J, Tang J. *J Am Chem Soc.* 2000; 122:3522–3523.
15. Ghosh AK, Bilcer G, Harwood C, Kawahama R, Shin D, Hussain KA, Hong L, Loy JA, Nguyen C, Koelsch G, Ermolieff J, Tang J. *J Med Chem.* 2001; 44:2865–2868. [PubMed: 11520194]
16. Hong L, Koelsch G, Lin X, Terzyan S, Ghosh AK, Zhang XC, Tang J. *Science.* 2000; 290:150–153. [PubMed: 11021803]
17. Ghosh AK, Kumaragurubaran N, Hong L, Lei H, Hussain KA, Liu CF, Devasamudram T, Weerasena V, Turner R, Koelsch G, Bilcer G, Tang J. *J Am Chem Soc.* 2006; 128:5310–5311. [PubMed: 16620080]
18. Ghosh AK, Rao KV, Yadav ND, Anderson DD, Gavande N, Huang X, Terzyan S, Tang J. *J Med Chem.* 2012; 55:9195–9207. [PubMed: 22954357]
19. Kozikowski AP, Adamcz M. *J Org Chem.* 1983; 48:366–372.
20. Single-crystal X-ray analysis was performed in-house: Dr. Phil Fanwick, X-ray Crystallography Laboratory, Department of Chemistry, Purdue University, West Lafayette, IN 47907.
21. CCDC 1565312 contains supplementary crystallographic data for compound 9b. This data can be obtained free of charge via www.ccdc.cam.ac.uk/datarequest/cif.
22. Ghosh AK, Brindisi M, Yen YC, Xu X, Huang T, Devasamudram T, Bilcer G, Lei H, Koelsch G, Mesecar AD, Tang J. *Bioorg Med Chem Lett.* 2015; 25:668–672. [PubMed: 25537272]
23. Ghosh AK, Kumaragurubaran N, Hong L, Kulkarni S, Xu X, Miller HB, Reddy DS, Weerasena V, Turner R, Chang W, Koelsch G, Tang J. *Bioorg Med Chem Lett.* 2008; 18:1031–1036. [PubMed: 18180160]
24. Ermolieff J, Loy JA, Koelsch G, Tang J. *Biochem.* 2000; 39:12450–12456. [PubMed: 11015226]
25. PDB Code: 6DHC. For details of X-ray studies, please see supporting information.
26. Ghosh AK, Kumaragurubaran N, Hong L, Kulkarni S, Weerasena V, Turner R, Koelsch G, Bilcer G, Tang J. *J Med Chem.* 2007; 50:2399–2407. [PubMed: 17432843]

Figure 1.
Structures of BACE1 inhibitors **1**, **2**, and **3a**.

Author Manuscript

Figure 3.

Stereoview (wall-eye) of the X-ray structure of inhibitor **3a** (green carbon chain) bound to BACE1 (grey carbon chain). Chain B in the asymmetric unit is shown. Potential hydrogen bonds between the inhibitor and BACE1 are shown as black dotted lines. The PDB code for this structure is 6DHC.

Figure 4.

Stereoview (wall-eye) of inhibitor **3a** (green carbon chain) bound to BACE1 (surface representation). The solvent exposed surface of BACE1 is shown and is colored according to Coulombic electrostatic potential (negative = red, zero = white, positive = blue). Values are shaded from -20 to 20 in kcal/mol*e and are shown in the color key. The intense red in the active site is the result of the catalytic aspartates (Asp32 and Asp228). Potential hydrogen bonds between the inhibitor and BACE1 are shown as yellow lines.

Scheme 1.

Reagents and conditions: (a) aq Na₂CO₃, Et₂O, 23 °C (85%); (b) aq LiOH, THF, 23 °C (90–95%); (c) **8**, EDCI, HOBT, DIPEA, CH₂Cl₂, DMF, 23 °C (55%); (d) HPLC separation.

Scheme 2.

Reagents and conditions: (a) EDCI, HOBT, DIPEA, 90%) CH₂Cl₂, DMF, 23 °C (63%); (b) TBAF, THF, 23 °C (90–92%).

Scheme 3.

Reagents and conditions: (a) EDCI, HOBT, DIPEA, CH₂Cl₂, DMF, 23 °C, (65%); (b) TFA, CH₂Cl₂, 23 °C (99%); (c) **7**, HATU, HOAt, collidine, DMF, 23 °C (45%); (d) HPLC separation on Chiralpak IC; (e) oxone, MeOH, 23 °C (99%).

Scheme 4.

Reagents and conditions: (a) HATU, HOAt, collidine, DMF, 23 °C (45%); (b) HPLC separation on Chiralpak IC; (c) oxone, MeOH, 23 °C (99%).

Scheme 5.

Reagents and conditions: (a) **11**, EDCI, HOBt, DIPEA, CH₂Cl₂, DMF, 23 °C (45%); (b) H₂, Pd(OH)₂, MeOH, 23 °C (99%); (c) **7**, HATU, HOAt, collidine, CH₂Cl₂, DMF, 23 °C (55%); (d) HPLC separation.

Scheme 6.

Reagents and conditions: (a) **10a** or **10b**, EDCI, HOBt, DIPEA, CH₂Cl₂, 23 °C (33%); (b) oxone, MeOH, 23 °C.

Table 1Enzyme inhibitory activity of inhibitors **3a–j**.

Entry	Inhibitor	K_i (nM)
1.		10.9
	3a	
2.		553.7
	3b	
3.		110.5
	3c	
4.		279.9
	3d	
5.		28.1
	3e	

Entry	Inhibitor	K_i (nM)
6.	3f	630
7.	3g	808.6
8.	3h	4124
9.	3i	41,126
10.	3j	28,758