Potent and selective isophthalamide S₂ hydroxyethylamine inhibitors of BACE1

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Received 27 February 2007; revised 23 March 2007; accepted 29 March 2007
Available online 3 April 2007

Abstract—The design and synthesis of a novel series of potent BACE1 hydroxyethylamine inhibitors. These inhibitors feature hydrogen bonding substituents at the C-5 position of the isophthalamide ring with improved selectivity over cathepsin D. © 2007 Elsevier Ltd. All rights reserved.

Alzheimer’s disease (AD) is a debilitating and ultimately fatal form of dementia generally affecting people aged 60 and older. The disease progresses from mild cognitive impairment through profound dementia, loss of motor functions and finally death. In addition to the devastating impact AD has on individuals and families there is also a societal impact. The financial cost to society, brought about by the prolonged nature of AD, is estimated at over $100 billion a year in the US alone.2,3

One of the major factors contributing to the onset of AD is the build-up of amyloid plaques in the brain. The primary component of the amyloid plaque is aggregated Aβ-peptide produced from the cleavage of Amyloid Precursor Protein (APP) by beta- and gamma-secretase.4–6 β-Secretase (BACE1) cleaves APP, releasing the soluble portion of APP (sAPP) and leaving behind an APP fragment still anchored to the membrane. Gamma-secretase cleaves the anchored APP fragment a second time producing two forms of peptide, Aβ(1–40) and Aβ(1–42), with Aβ(1–42) being the major component in amyloid plaques.7

Inhibition of BACE1 appears to be a viable therapeutic target for the reduction of Aβ(1–40) and Aβ(1–42). It has been shown that BACE1 knockout mice, transgenic for human APP, do not have amyloid plaque build-up in the brain.6 This data helps validate BACE1 as a therapeutic target for AD.

Hydroxyethylamine (HEA), 1 (Fig. 1) was found to be a potent inhibitor of BACE1 containing the optimized transition state insert (TSI) 3,5-difluoro Phe and C-terminal m-ethyl benzyl.8 Although highly potent, this compound suffered from poor metabolic stability.

Figure 1. Isophthalamide hydroxyethyl amine.
X-ray crystal structures of 1 bound to BACE1, along with modeling, suggested a hydrogen bonding substituent at the C-5 position of the isophthalamide ring (S2 pocket of BACE1) might result in improved binding with BACE1. Subsequent to our work on the isophthalamide HEA series, other groups have described work on related HEA series of inhibitors.9

Although 1 exhibits high affinity for BACE1 (2 nM) and reasonable selectivity over cathepsin D (Cat D) (75-fold), it was rapidly metabolized by rat and human microsomes. The main pathways for metabolism are N-debenzylation at the C-terminus and N-depropylation at the N-terminus (data not shown). Unfortunately, removal of the dipropyl amide results in a significant loss of affinity for BACE1.11 Due to this loss we needed to improve BACE1 affinity before addressing the metabolic stability issues surrounding the HEA template.

The S2 pocket of Cat D is large and contains a lipophilic valine residue whereas the S2 pocket of BACE1 contains an arginine residue.12 Substituting the C-5 position of the isophthalamide ring with hydrogen bonding substituents may create an additional binding interaction with hydrogen bonding substituents in BACE1 and improve selectivity over Cat D. Towards these goals, we have prepared a series of C-5 isophthalamide HEA analogs in order to increase affinity for BACE1 and selectivity over Cat D.

The synthetic route used to prepare the C-3 amide and nitrile isophthalamide rings is outlined in Scheme 1.

Mono methyl 5-nitroisophthalate was converted to isophthalamide 3a–b using standard CDI amide coupling conditions. Nitro 3a–b was reduced to aniline 4a–b under catalytic hydrogenation conditions with palladium on carbon. Diazotization of anilines 4a–b and displacement with either copper (II) bromide or potassium iodide gave halides 5a–d. Under palladium acetate/dppp catalyzed carbonylation conditions halides 5a–d gave amides 6a–d.15 Cyanide displacement of halides 5a–d using copper cyanide gave nitriles 8a–b. The esters of amides 6a–d and 8a–b were hydrolyzed using lithium hydroxide to give acids 7a–f.

The C-3 acetonitrile was prepared using the synthesis outlined in Scheme 2. Acid 9 was reduced to alcohol 10 using borane dimethyl sulfide complex. Selective hydrolysis of one ester of alcohol 10 was accomplished using 0.9 equiv of lithium hydroxide to give acid 11. Acid 11 was coupled with dipropylamine using EDC/HOBt to give amide 12.
The alcohol of amide 12 was converted to bromide 13 using PBr₃ at 50 °C. Bromide 13 was displaced with sodium cyanide to give nitrile 14. Hydrolysis of the ester of nitrile 14 gave acid 7g.

The P₁ – P₂ fragments synthesis and final coupling are outlined in Scheme 3.

Epoxides 15a–c were refluxed with benzyl amines 16a–b to form P₁ – P₂ fragments 17a–c. BOC deprotection of P₁ – P₂ fragments 17a–c using TFA gave amines 18a–c. HATU coupling of amines 18a–c to acids 7a–g gave HEA analogs 19a–k and 19m–n. The lower yields associated with this coupling reaction was due to unwanted coupling of the acid with the benzyl amine portion of 18a–c.

The ester and acid HEA analogs 19l and 19n, respectively were made from acid 9 utilizing standard EDC/HOBt coupling to form the desired amide and selective hydrolysis as described above gave the desired isophthalamide. The acid of 19l was formed by saponification of ester 19n using lithium hydroxide.

BACE1 and Cat D IC₅₀ values are contained in Table 1. Compounds 19a and 19b are included for comparison.

Direct comparison of the parent compound 19b with the corresponding carboxamide substituted analog 19c showed comparable values for BACE1 but a 6-fold decrease in Cat D affinity. The dimethyl amide 19m exhibited an 8-fold decrease in Cat D affinity when compared with the parent compound. Increasing the alkyl chain length of the C-5 amide to propyl as in 19i resulted in little change in Cat D affinity. BACE1 affinity was reduced by a factor of 2.5 as compared with the parent compound. Acid 19l was 3-fold more potent and 4-fold more selective than amide 19c while ester 19n lost selectivity 4-fold compared to 19c.

Compounds 19d and 19j illustrate the contributions the difluoro Phe and m-ethyl benzyl amine moieties make toward BACE1 affinity compared to the amide 19c. Replacing the P₁ Phe with 3,5 difluoro Phe increased BACE1 affinity 10-fold whereas Cat D affinity was unchanged compared to 19c. Replacing the P₂ m-methoxy 19d with m-ethyl 19j results in a two-fold increase in BACE1 affinity.

The X-ray crystal structure of 19d in the BACE1 enzyme shows a stacking interaction between the amide carbonyl group and Arg 235 as shown by Figure 2. The distance between the amide carbonyl group and Arg 235 is between 2.8 and 3.1 Å. In Cat D the amide carbonyl likely interacts with a more lipophilic Val 238, resulting in a less desirable interaction which is reflected in the affinities for Cat D. This same interaction may be responsible for the results obtained for 19e.

In an effort to increase the interaction between the amide carbonyl and Arg 235, a methylene spacer was
Scheme 3. Synthesis of (2R,3S)-3-amino-1-(benzylamino)-4-phenylbutan-2-ols 18a-c and final coupling. Reagents and conditions: (a) isopropanol/reflux/5 h (70–80%); (b) TFA/CH₂Cl₂/rt/1 h/sodium bicarbonate (90%); (c) HATU/TEA/CH₂Cl₂/rt/18 h (30–50%).

Table 1. Table of BACE1 and Cat D IC₅₀ values

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<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>BACE IC₅₀ (nM)</th>
<th>Cat D IC₅₀ (nM)</th>
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placed between the isophthalamide ring and the nitrile substituent to give 19f. Unfortunately this brought about a decrease in BACE1 affinity with a slight increase in the Cat D affinity. Attempts to convert 19f to the primary carboxamide were unsuccessful.

Since a major metabolite of compounds such as 19a is depropylation of the dipropyl amide on the N-terminus, compounds such as 19g and 19h were prepared to eliminate this mode of metabolism and improve overall metabolic stability. Unfortunately the added interaction at the C-5 position of the isophthalamide ring did not compensate for the loss in affinity resulting from the removal of one of the propyl groups in the dipropyl amide. BACE1 affinity decreased ~9 to 19-fold for the mono propyl amide analogs.

Due to the loss in affinity for BACE1 in compounds such as 19g and 19h we needed to further improve upon the affinity for BACE1. Based on the X-ray crystal structure of 19d in the BACE1 enzyme, Phe-108, which resides in the bottom of the S1 pocket of BACE1, may offer an additional hydrogen bonding opportunity. In an attempt to gain hydrogen bonding between the BACE1 inhibitor and the carbonyl oxygen of Phe-108, we replaced the difluoro phenyl oxygen of 19d with a para-hydroxy group; however 19k showed an approximately 30-fold decrease in BACE1 affinity. As a result, further analogs with the tyrosine HEA insert were not pursued.

A carboxamide HEA with optimized P1 and P2 groups 19j resulted in a 5 nM BACE1 inhibitor with 320-fold selectivity over Cat D compared with 75-fold selectivity for 19a. The added interaction with BACE1 at S2 was not enough to overcome the affinity loss associated with the removal of one of the propyl substituents of the N-terminal dipropyl amide. Several other HEAs with various substituents in the C-5 position of the isophthalamide ring were prepared but did not improve upon 19d.

Acknowledgments

We would like to thank Benjamin Amore and Richard Steenwick for their work in metabolic stability. We would also like to acknowledge the use of the IMCA-CAT beamline 17-1D at the Advanced Photon Source which is supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with the Center for Advanced Radiation Sources at the University of Chicago. Use of the Advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

References and notes

11. Freskos, J. N.; Fobian, Yvette M.; Benson, Timothy E.; Bienkowski, Micheal K.; Brown, David L.; Emmons, Thomas L.; Heintz, Robert; Labrude, Alice; McDonald, Joseph J.; Mischke, Brent V.; Molyneaux, John M.; Moon, Joseph B.; Mullins, Patrick B.; Prince, D. Bryan; Paddock,

13. Coordinates for the complex of BACE with inhibitor 36d have been deposited in the Protein Data Bank (www.rcsb.org) under PDB ID 2P83.

14. The BACE1 and cathepsin D assays were used to measure enzymatic activities in a fluorescence polarization format and in an endpoint mode. Both assays were performed in non-binding 384-well plates and in a volume of 30 µL/well. Enzyme, substrate, and stop solutions were added to the assay plates with a LabSystems Multidrop 384. The reactions in the BACE1 assay were run at 37 °C for 3 h under the following conditions: 100 µM sodium acetate (pH 4.5), 150 nM substrate 1, 0.1 nM BACE1, 2% DMSO, and 0.001% (v/v) Tween-20. The cathepsin D assay reactions were run at 37 °C for 90 min under the following conditions: 100 µM sodium acetate (pH 4.5), 150 nM substrate 2, 0.5 nM cathepsin D, 2% DMSO, and 0.001% (v/v) Tween-20. Reactions were terminated by the addition of stop solution (30 µL/well). Following a 15-min incubation at room temperature, sample fluorescence polarization was measured on a LJL Biosystems Acquest (Sunnyvale, CA) using an excitation 485 nm filter, a 530 nm emission, and G factor settings of 0.872 and 0.864 for the BACE1 and the cathepsin D assays, respectively.

15. Carbonylation procedure for compound 6d: To a mixture of methyl 3-[(dipropylamino)carbonyl]-5-iodobenzoate (0.42 g, 1.08 mmol) in dry NMP (6.0 mL) bubbled with carbon monoxide for 10 min was added diisopropyl ethylamine (0.14 g, 1.08 mmol), palladium (II) acetate (0.025 g, 0.108 mmol), 2 M dimethylamine in THF (1.0 mL, 2.0 mmol) and diphenylphosphino propane (0.067 g, 0.162 mmol). The mixture was stirred at room temperature under a carbon monoxide atmosphere overnight. The mixture was partitioned between water and ethyl acetate. The layers were separated and the organic layer washed twice with water, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified via flash chromatography on silica gel (100 mL) using 3% CH₃OH in CH₂Cl₂ to give 0.249 g (69%) of the title compound: 1H NMR (CDCl₃, 400 MHz) δ 0.745–0.765 (m, 3 H), 0.972–0.998 (m, 3H), 1.51–1.59 (m, 2H), 1.65–1.73 (m, 2H), 2.99 (s, 3H), 3.09–3.17 (m, 5H), 3.41–3.53 (m, 2H), 3.94 (s, 3H), 7.62 (d, J = 1.2 Hz, 1H), 8.07 (d, J = 1.2 Hz, 1H), 8.12 (d, J = 1.2 Hz, 1H).