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Letters

Aminoethylenes: A Tetrahedral Intermediate Isostere Yielding Potent Inhibitors of the Aspartyl Protease BACE-1

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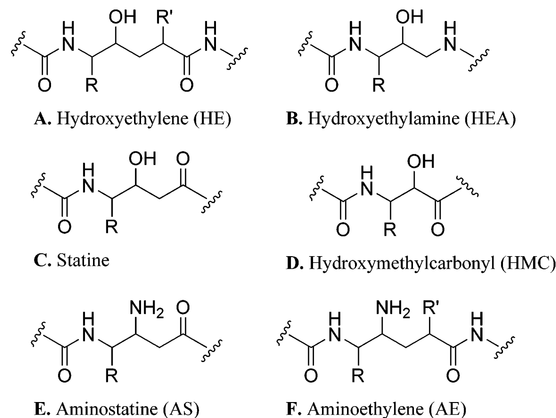
Abstract: A series of novel β-site amyloid precursor protein cleaving enzyme (BACE-1) inhibitors containing an aminoethylene (AE) tetrahedral intermediate isostere were synthesized and evaluated in comparison to corresponding hydroxyethylene (HE) compounds. Enzymatic inhibitory values were similar for both isosteres, as were structure−activity relationships with respect to stereochemical preference and substituent variation (P2/P3, P1, and P2′); however, the AE compounds were markedly more potent in a cell-based assay for reduction of beta-secretase activity. The incorporation of preferred P2/P3, P1, and P2′ substituents into the AE pharmacophore yielded compound **7,** which possessed enzymatic and cell assay IC_{50}s of 26 nM and 180 nM, respectively. A three-dimensional crystal structure of **7** in complex with BACE-1 revealed that the amino group of the inhibitor core engages the catalytic aspartates in a manner analogous to hydroxyl groups in HE inhibitors. The AE isostere class represents a promising advance in the development of BACE-1 inhibitors.

Alzheimer's disease, a condition marked by the deposition of plaques composed of the amyloid beta (Aβ) peptide in the brain, is a debilitating health problem that affects a significant percentage of the elderly population worldwide.[1] Aβ is a 40−42-residue internal peptide segment of the amyloid precursor protein, which is liberated by the action of two proteases, beta secretase and gamma secretase.[2] Evidence gathered in recent years strongly implicates the aspartyl protease, BACE-1 (beta amyloid precursor protein cleaving enzyme), as the predominant beta secretase.[3] BACE-1 is therefore an important Alzheimer's disease therapeutic target, based on the evidence that inhibition of the enzyme should reduce generation of Aβ in the brain.[4]

Chart 1. Aspartyl Protease Inhibitor Core Motifs



Medicinal chemists in industry and academia have accumulated a wealth of knowledge and experience in developing inhibitors of aspartyl proteases, most notably for HIV protease and renin.[5,6] A common strategy in inhibitor design is replacement of the substrate scissile amide bond with a tetrahedral intermediate isostere, typically a secondary alcohol. Four common hydroxyl-containing inhibitor scaffolds include the hydroxyethylene (HE), hydroxyethylamine (HEA), statine, and hydroxymethylcarbonyl (HMC) motifs (Chart 1, **A**−**D**). These pharmacophores, which differ from one another by the functionality alpha and beta to the hydroxyl group, engage one or both of the catalytic aspartates of the enzyme via hydrogen bonds.[6]

For BACE-1, a number of hydroxyl-containing inhibitor series have been described.[7,8] High-resolution crystal structures of HE and HEA inhibitors in complex with BACE-1 have revealed that the active site is similar to other pepsin family aspartyl proteases, and the hydroxyl groups of the cores engage in hydrogen bonds to both Asp32 and Asp228.[9−11]

Given the common hydrogen bond donor/acceptor dual functionality of these hydroxyl core motifs, we sought to

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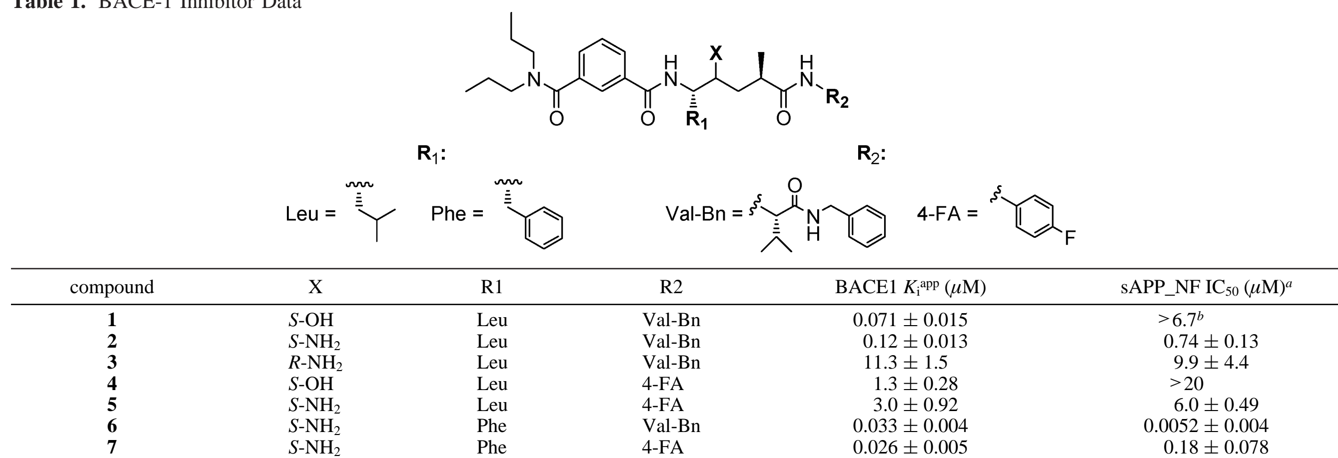
Table 1. BACE-1 Inhibitor Data



compound	X	R1	R2	BACE1 K_i^{app} (μM)	sAPP_NF IC_{50} (μM)[a]
1	*S*-OH	Leu	Val-Bn	0.071 ± 0.015	>6.7[b]
2	*S*-NH_2	Leu	Val-Bn	0.12 ± 0.013	0.74 ± 0.13
3	*R*-NH_2	Leu	Val-Bn	11.3 ± 1.5	9.9 ± 4.4
4	*S*-OH	Leu	4-FA	1.3 ± 0.28	>20
5	*S*-NH_2	Leu	4-FA	3.0 ± 0.92	6.0 ± 0.49
6	*S*-NH_2	Phe	Val-Bn	0.033 ± 0.004	0.0052 ± 0.004
7	*S*-NH_2	Phe	4-FA	0.026 ± 0.005	0.18 ± 0.078

[a] Mean ± SD for two runs in a cell-based assay for the release of the soluble N-terminal fragment of an APP variant from cultured HEK 293T cells.[16] [b] Concentration at which ≥20% cytotoxicity was observed via Trypan Blue assay.

determine if a primary amine group could act as a similar BACE-1 inhibitor pharmacophore. This basic functional group was anticipated to afford distinct physicochemical properties in the resulting compounds, including increased solubility. Primary amines have been used successfully in two classes of renin inhibitors. Compounds containing an aminostatine isostere (Chart 1, **E**) displayed potencies similar to that of statine comparators.[12] A renin inhibitor containing an aminoethylene core (AE, Chart 1, **F**) has also been reported, which possessed potency similar to that of an otherwise identical HE analogue.[13]

In this work, we explored the AE isostere as a central scaffold in a series of novel BACE-1 inhibitors. We noted previous studies on peptidic HE BACE inhibitors that reported preferred P- and P′-segments,[14,15] including an isophthalamide group as a favored P2/P3 peptide replacement,[15] and utilized these components as a starting point. Installation of this isophthalamide on an HE scaffold yielded compound **1**, which showed potent activity in an enzymatic assay (K_i^{app} = 71 nM) but was inactive (IC_{50} > 7 μM) in a cell-based assay for inhibition of sAPPβ_NF secretion[16] (Table 1). Replacement of the HE isostere with the AE core afforded **2**, which possessed slightly reduced enzymatic inhibitory activity but distinctly improved cell potency. Variation of the stereochemistry in the AE isostere indicated that the *S* isomer was strongly preferred for activity (see **3** versus **2**), consistent with what was observed for the statine BACE inhibitors.[17]

To decrease the molecular weight and peptidic character of the compounds, a substitute for the P2′ Val-benzyl amide (Val-Bn) group was sought. An optimization study revealed that 4-fluoroaniline (4-FA) was a particularly good replacement at this position.[18] While this substitution resulted in enzymatic potency decreases of ~20-fold for both the HE (**4**) and the AE (**5**) scaffolds, it eliminated one amide bond and afforded a 95 amu reduction in molecular weight. Compound **5** still retained modest cell potency (Table 1).

Published data indicated that statine-type inhibitors containing benzyl P1 position substituents (i.e. Phe analogues) had potencies comparable to those containing isobutyl P1 groups (i.e. Leu analogues).[17] Quantitative SAR around the P1 substituent for HE inhibitors is not well documented, although it is clear that benzyl P1 analogues can yield potent inhibitors.[15,19] Upon incorporation of a benzyl P1 substituent in the AE isostere compounds, substantial potency increases were observed relative to the isobutyl analogues (see **6** vs **2**, **7** vs **5**). In the context of a Val-Bn P2′ motif, enzymatic potency was increased at least 4-fold, to an K_i^{app} value approaching the concentration of enzyme in the assay, and cell-based potency was increased approximately 130-fold (**2** vs **6**).[20] Enzymatic and cell potencies were improved approximately 100-fold and 300-fold, respectively, for the 4-FA analogue (**5** vs **7**). Thus, the benzyl P1 substituent afforded dramatically improved affinity in the context of the AE scaffold and appeared to act synergistically with the 4-FA P2′ group.

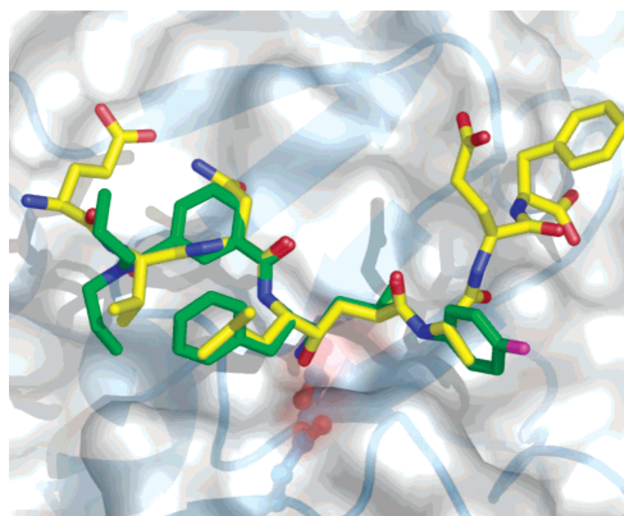


Figure 1. Overlay of the cocrystal structures of the AE inhibitor **7** (green) and the HE octapeptide inhibitor (yellow) from 1FKN[9] in the BACE-1 active site. Enzyme flap residues have been removed to highlight the interactions with the catalytic aspartates (Asp32 and Asp228).

We determined the three-dimensional cocrystal structure of **7** in complex with BACE-1 at 2.4 Å resolution (Figure 1).[21] The overall protein and active site structure is essentially identical (Cα RMSD <0.6 Å) to that of the HE and HEA complexes reported previously.[9−11] The primary amine group of the inhibitor makes hydrogen bond contacts to both catalytic aspartates, Asp32 and Asp228 (2.9 and 2.6 Å, respectively), in a position identical to that for the hydroxyl group of in the HE complexes. In addition, the benzyl P1 element and the isophthalamide P2/P3 substituent overlay precisely with the same groups in an analogous HEA inhibitor structure.[11] On the prime-side, the amide of the 4-FA group of **7** forms a hydrogen bond to Gly34 (2.8 Å), and the aniline ring lies snugly in a trough, surrounded by resides Ser35, Val69, Pro70, Tyr71, Ile126, and Arg128.

The AE motif represents a new class of BACE-1 inhibitor pharmacophore, having an inherent binding affinity for the catalytic site that is similar to the well established HE scaffold. Although the enzymatic inhibition SAR trends and active site binding position of the two isosteres tracked very closely with one another, the cell-based activity was markedly better for the AE compounds. While the source of this potency difference is unclear, an obvious physicochemical difference is the expected protonation, and hence more hydrophilic character, of the AE inhibitors at neutral pH and in the more acidic cellular compartments in which BACE-1 is localized. Recently reported HEA inhibitors, also having an inherently more hydrophilic core than HEs, have also shown high potency in cell-based assays.[8]

In summary, the combination of the AE isostere with optimized replacements for the P2/P3 dipeptide, P2′ dipeptide, and P1 side chain resulted in a compound, **7**, that represents a promising new series of BACE-1 inhibitors that is distinct from others reported to date. Compound **7** displayed good enzymatic and cell-based inhibitory potency, and a molecular weight below 600. Further improvement of these parameters and the incorporation of other drug-like properties into AE isostere inhibitors are the subjects of ongoing efforts.

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Supporting Information Available: Experimental procedures for compound synthesis, analytical measurements, and enzymatic assays. The PDB file for the BACE-1/inhibitor **7** complex has been deposited into the protein data bank PDB (code 2FDP). This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) K_i^{app} values on the order of 10 nM and less may be difficult to determine accurately since 10 nM BACE-1 is used in the assay. The high cell potency of compound **7** may indicate that the true enzymatic K_i may indeed be lower than the measured K_i^{app}.

(21) A self-processing variant of the BACE1 catalytic domain was expressed in *E.coli.* Details of the preparation to be published separately. Coordinates of the structure have been deposited in the PDB (code 2FDP).

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