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Bioorganic & Medicinal Chemistry Letters 17 (2007) 73–77
Bioorganic & Medicinal Chemistry Letters

Design of potent inhibitors of human β-secretase. Part 1

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Received 11 July 2006; revised 23 September 2006; accepted 29 September 2006
Available online 4 October 2006

Abstract—We describe a novel series of potent inhibitors of human β-secretase. These compounds possess the hydroxyethyl amine transition state isostere. A 2.5 Å crystal structure of inhibitor **32** bound to BACE is provided.

β-Secretase or β-site APP-cleaving enzyme (BACE) has been identified as an aspartyl protease responsible for the first mandatory proteolytic step to generate amyloidogenic β-peptides from the naturally occurring amyloid precursor protein (APP).[1–4] Site specific cleavage of APP (both wild-type and Swedish mutation) by β-secretase yields the 99 amino acid C-terminal fragment which is further processed by the γ-secretase/presenilin(s) complex at residue 711 or 713 to liberate a peptide of 40 residues ($A\beta_{40}$) or of 42 residues ($A\beta_{42}$) Generation of Aβ is thought to be causal for the pathology and cognitive decline in Alzheimer's disease AD.[5,6]

Our goal was to discover and develop potent, safe, and efficacious agents to block formation of Aβ plaque via the β-secretase pathway and thus block the onset and/or progression of AD. Recently, we disclosed a potent series of BACE inhibitors utilizing a hydroxyethyl amine (HEA) transition state isostere (TSIs)[7a–7d] see Figure 1.

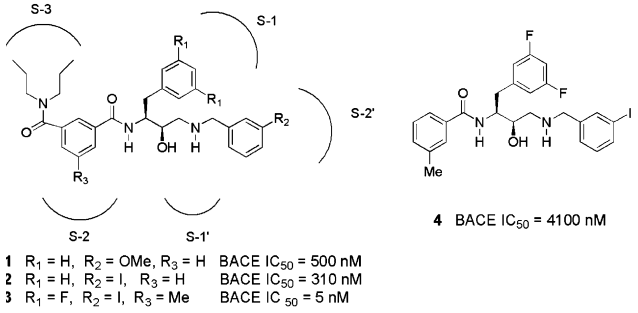

Figure 1. Evolution of the isophthalate hydroxyethyl amine series.

Interestingly in this series phenyl (Phe) at P_1 is more potent than isobutyl (Leu), which is found at P_1 in the Swedish mutant that helped in the initial identification of BACE, and 3,5-difluorophenyl was found to be optimum. It typically gives ~10 to 100× boost in potency over phenyl while the *m*-iodo for *m*-methoxy is ~2× (**1** vs **2**). It is also noteworthy how important it is to occupy S-3 in order to maintain potency (**3** vs **4**). After the initial SAR of the isophthalide series was described we found that others had initiated similar work on the HEA series of inhibitors.[8] A recent review on synthesis of BACE inhibitors has also appeared.[9]

In the present letter, we describe our attempts to replace the potent isophthalate N-terminus with novel acyclic

Keywords: β-Secretase; Hydroxyethylamine (HEA) isostere.
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doi:10.1016/j.bmcl.2006.09.092

scaffolds that maintain good enzyme and cellular potency for BACE and selectivity for other aspartyl proteases in particular the ubiquitous protease Cathepsin-D (Cat-D).[7d] Also we will outline the initial SAR exploration, which was done in the phenylalanine series at P_1, while a following paper will discuss utilization of the more potent 3,5-difluorophenylalanine at P_1 to increase potency for BACE as well as optimization of the S_2 and S_3 side chains to improve selectivity against Cat-D while maintaining potency for BACE.

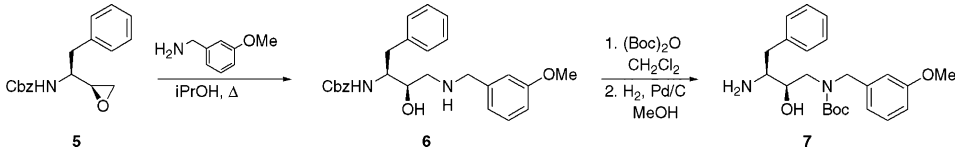
We initially decided to examine acyclic compounds with either a two or three atom spacer between the N-terminal carbonyls. From crystallographic data obtained on **3** we knew that the carbonyl of the dipropyl amide was a key H-bond acceptor with the side-chain hydroxy of Thr-232.[7b,c] Assuming it was critical to maintain this interaction we thus decided to explore acyclic sulfones and sulfonamides with varying spacer atoms between the amide carbonyl and the SO_2 group.

The targeted sulfone and sulfonamides were prepared using a common intermediate **7** (Scheme 1). The reaction of epoxide **5**[10] with excess 3-methoxybenzylamine in refluxing 2-propanol afforded the secondary amine **6** in high yield. Protection with $(Boc)_2O$ followed by removal of the Cbz group by catalytic hydrogenation afforded the desired C-terminal coupling fragment **7**.

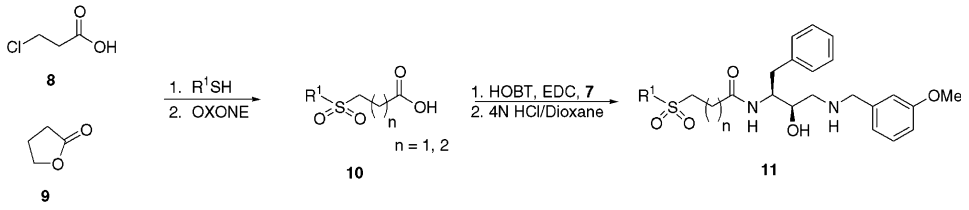
The synthesis of the fully assembled acyclic sulfone inhibitors was straightforward. For the two-carbon spacer the appropriate thiol was reacted with β-chloro propionic acid (**8**) to afford the desired sulfide acid (Scheme 2). Oxidation with OXONE® yielded the desired sulfone acid **10** which was then coupled to the C-terminal fragment **7** using standard peptide coupling (EDC/HOBT) conditions. Removal of the Boc group with 4 N HCl-dioxane afforded the targeted two-carbon sulfone HEA inhibitor **11** ($n = 1$). The three-carbon spacer sulfone was prepared in a similar fashion starting with an S_N2' ring opening of butyrolactone (**9**) with the appropriate thiol. Oxidation to the sulfone followed by coupling and deprotection afforded the targeted three carbon sulfone inhibitor ($n = 2$).

The fully assembled acyclic sulfonamide inhibitors were prepared by reacting potassium thioacetate with either ethyl 3-bromopropionate or ethyl 4-bromobutyrate (**12**) to give the corresponding thioacetate ester (Scheme 3). Chlorine oxidation to the sulfonyl chloride followed by coupling with the appropriate amine and base hydrolysis of the ester gave the desired N-terminal fragment 2 or 3 carbon spacer sulfonamide acid **13**. Coupling to the C-terminal fragment **7** using standard peptide coupling (EDC/HOBT) conditions and removal of the Boc group with 4 N HCl in Dioxane afforded the targeted sulfonamide HEA inhibitor **14**.

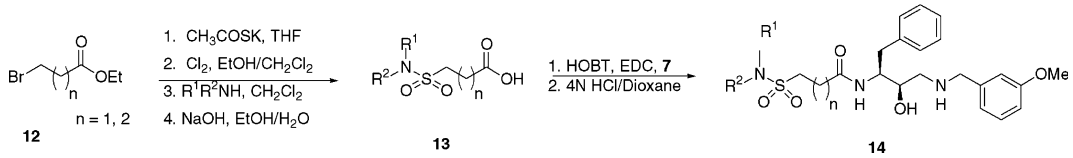
Table 1 summarizes the enzyme inhibition results using a fluorescence polarization assay (FP)[11] to determine the IC_{50} values for inhibition of recombinant human BACE and Cat-D. In contrast to the previous SAR demonstrating that a three-carbon spacer was more potent than two- or four-,[12] both the sulfone and sulfonamide analogues were more potent with a two-carbon spacer. Evidently, the difference in the bond length for



Scheme 1.

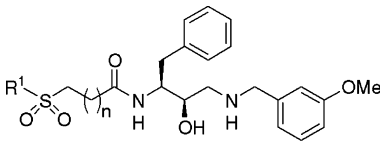


Scheme 2.



Scheme 3.

Table 1. Enzyme inhibition values for acyclic sulfones and sulfonamides



Compound	*n*	R^1	BACE-FP IC_{50} (nM)	CatD-FP IC_{50} (nM)
Sulfones				
15	3	Phe	>20,000	11,202
16	2	Phe	4131	>20,000
17	1	Phe	5214	6042
18	2	Cyclohexyl	>20,000	>20,000
19	1	Cyclohexyl	3670	6946
20	1	Methyl	>20,000	7593
21	2	Isopentyl	>20,000	>20,000
22	1	Isopentyl	5614	10,986
23	1	4-Heptyl	596	9669
Sufonamides				
24	2	Piperidinyl	6461	>20,000
25	1	Piperidinyl	3785	>20,000
26	1	Isopentylamino	5015	2116
27	1	Dipropylamino	1090	4900
28	2	Dipropylamino	7950	11,700
29	1	Dibutylamino	484	4880

a C—S bond versus a C—C bond required one less carbon spacer to appropriately position one of the oxygens in the sulfonyl group for the desired interaction with Thr-232. Within the more potent two carbon series, potency against BACE increased for analogues with alkyl substitution at the N-terminus over either aryl or cyclic substituted analogues. Furthermore, potency for BACE increased when the substitution more closely mimicked the diisopropylamide N-terminus of the isophthalate series.

Within both series it was possible to achieve equipotent BACE inhibitors by replacing the isophthalate N-terminus of **1** with an appropriate acyclic sulfone (**23**) or sulfonamide (**29**). Additionally each of these analogues maintained excellent potency in the HEK-293 Swe amyloid inhibition cell assay[13] with 314 and 130 nM values, respectively.

In an effort to further improve potency for BACE by accessing the S_2 pocket as shown for the isophthalate HEA inhibitor **3**, the α-Cbz-amine **32** was proposed (Fig. 2). As shown in Scheme 4, the Michael acceptor methyl-2-acetamidoacrylate (**30**) was reacted with 4-mercaptoheptane and then oxidized with OXONE® to give the β-sulfone amino acid. Hydrolysis of the methyl ester and *N*-acyl group with concentrated hydrochloric acid in glacial acetic acid afforded the free amino acid which was protected using Cbz-succinimide to yield **31**. Coupling of **31** with the C-terminal fragment **7** followed by removal of the Boc group then afforded the desired inhibitor, **32**. When tested in the fluorescence polarization assay, **32** inhibited BACE with an IC_{50} = 112 nM. This compound was even more potent for Cat-D with an IC_{50} = 67 nM. This represented approximately a 5-fold increase in BACE activity for

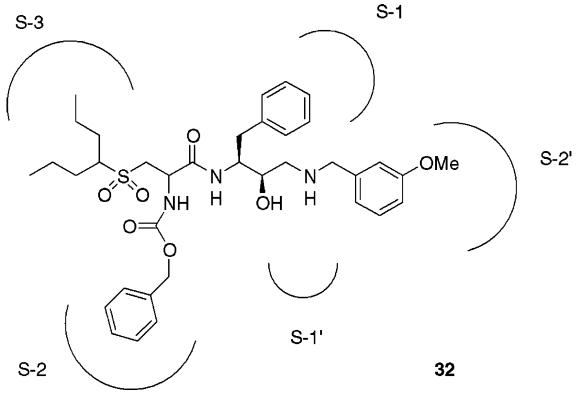
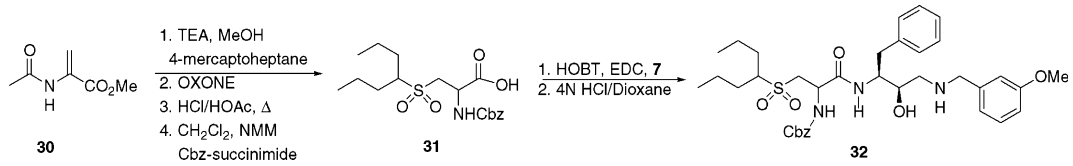


Figure 2.

the racemic Cbz-analogue **32** versus the sulfone analogue **23** with no P_2 substituent (Fig. 3).

Modeling of **32** in the active site of BACE predicted the preferred stereochemistry for substitution at the α position to be *S*. A subsequent 2.5 Å X-ray crystal structure of recombinant human BACE expressed and purified from *Escherichia coli*[14] and co-crystallized in the presence **32** (a racemic mixture) resulted in binding of a single isomer confirming the preferred stereochemistry was indeed *S*. One of the sulfone oxygens provides a hydrogen bond acceptor for the Thr232 NH (3.0 Å). The Cbz group projects toward P2 but reaches past Arg235 into the solvent. The carbonyl of the Cbz is involved in a long water mediated interaction to the main chain NH of Asn233 (3.7 Å), and the benzyl moiety is nestled adjacent to Gln73. The absence of direct interactions with Arg235 most likely accounts for the lack of specificity with respect to Cat-D. Other interactions between **32**



Scheme 4.

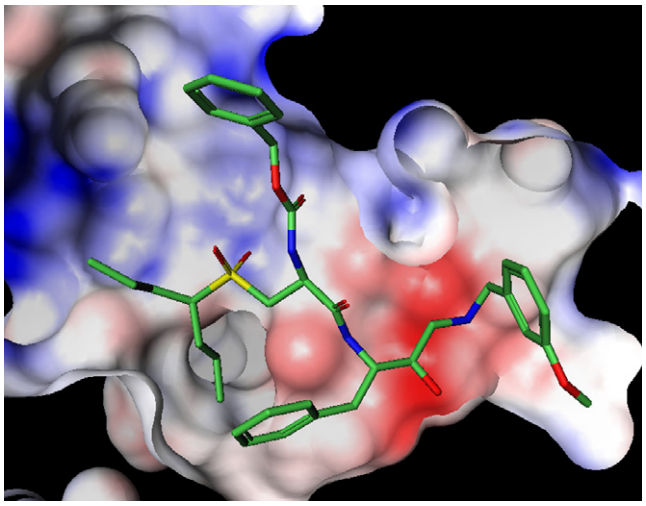


Figure 3. Compound **32** bound to human recombinant BACE.

are similar to those previously observed for other BACE-HEA co-crystal structures.[7b,c,15]

In summary, we have described a series of potent BACE inhibitors utilizing several novel acyclic scaffolds to replace the isophthalate N-termini of existing hydroxyethylamine inhibitors. Both access the critical S_3 subsite of BACE. Equipotent inhibition for BACE was achieved through the proper choice of carbon linker and appropriate N-termini substitution. Additional potency was achieved by occupying the S_2 pocket of the enzyme through incorporation of an α-side chain on the N-terminus of the acyclic scaffold. Finally, a preferred stereochemistry for this substitution was predicted by modeling and subsequently confirmed by X-ray crystallography.

Acknowledgments

The authors would like to acknowledge Dr. Daniel Rich for his advice and support of this work. Dr. T.E.B. would like to acknowledge the use of the IMCA-CAT beamline 17-ID 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 U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

References and notes

1. Yan, R.; Bienkowski, M. J.; Shuck, M. E.; Miao, H.; Tory, M. C.; Pauley, A. M.; Brashier, J. R.; Stratman, N. C.; Mathews, W. R.; Buhl, A. E.; Carter, D. B.; Tomasselli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. *Nature* **1999**, *402*, 533.
2. Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. *Nature* **1999**, *402*, 537.
3. Vasser, R.; Bennett, B. D.; Babu-Khan, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*, 735.
4. Lin, X.; Koelsch, G.; Wu, S.; Downs, D.; Dashti, A.; Tang, J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1456.
5. Selkoe, D. J. *Nature* **1999**, *399*(6738 Suppl), A23.
6. Sinha, S.; Lieberburg, I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11049; Citron, M. *Science* **1999**, *286*, 735.
7. (a) Maillard, M.; Hom, R.; Gailunas, A.; Jagodzinska, B.; Fang, L. Y.; John, V.; Freskos, J. N.; Pulley, S. R.; Beck, J. P.; Tenbrink, R. E. 2002, WO Patent 0202512.; (b) Hom, R. K; Maillard, M; Mamo, S.; Dappen, M. A.; Brogley, L.; John, V.; Thorsett, E. D.; Benson, T. E.; *Abstracts of Papers*, 232nd National Meeting of the American Chemical Society, San Francisco, CA. September 10–14, 2006.; (c) Maillard, M; Hom, R. K.; Benson, T. E,; Moon, J. B.; Mamo, S.; Bienkowski, M.; Tomasselli, A. G.; Woods, D. D.; Prince, D. B.; Paddock, D. J.; Emmons, T. L.; Galiunas, A.; Tucker, J.; Dappen, M. A.; Brogley, L.; Thorsett, G.; Jewett, N.; Sinha, S. John, V., *J. Med. Chem.*, in preparation.; (d) Freskos, J. N.; Aquino, J.; Brown, D. L.; Fang, L.; Fobian, Y. M.; Gailunas, A. F.; Gunn, A.; John, V.; Romereo, A. G.; Tucker, J.; Tung, J.; Walker, D. 2002 WO Patent 020988849.
8. Stachel, Shawn J.; Coburn, Craig A.; Steele, Thomas G.; Crouthamel, Min-Chi; Pietrak, Beth L.; Lai, Ming-Tain; Holloway, M. Katharine; Munshi, Sanjeev K.; Graham, Samuel L.; Vacca, Joseph P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 641; Stachel, Shawn J.; Coburn, Craig A.; Steele, Thomas G.; Jones, Kristen G.; Loutzenhiser, Elizabeth F.; Gregro, Alison R.; Rajapakse, Hemaka A.; Lai, Ming-Tain; Crouthamel, Ming-Chih; Xu, Min; Tugusheva, Katherine; Lineberger, Janet E.; Pietrak, Beth L.; Espeseth, Amy S.; Shi, Xiao-Ping; Chen-Dodson, Elizabeth; Holloway, M. Katharine; Munshi, Sanjeev; Simon, Adam J.; Kuo, Lawrence; Vacca, Joseph P. *J. Med. Chem.* **2004**, *47*, 6447.
9. Ghosh, A. K.; Kumaragubaran, N.; Tang, J. *Curr. Topics Med. Chem.* **2005**, *5*, 1609; Baxter, E. W.; Reitz, A. B. *Annu. Reports Med. Chem.* **2005**, *1*, 35.

10. Getman, D. P.; DeCrescenzo, G. A.; Heintz, R. M.; Reed, K. L.; Talley, J. J.; Bryant, M. L.; Clare, M.; Houseman, K. A.; Marr, J. J.; Mueller, R. A.; Vazquez, M. L.; Shieh, H-S.; Stallings, W. C.; Stegeman, R. A. *J. Med. Chem.* **1993**, *36*, 288.
11. Unpublished work of Dr. Mike Bienkowski and Dr. Alice Laborde.
12. Unpublished results of Dr. John Tucker.
13. Dovey, H. F. et al. *J. Neurochem.* **2001**, *76*, 173.
14. Tomasselli, A. G.; Benson, T. E. et al., in preparation.
15. Coordinates for the complex of BACE with inhibitor **32** have been deposited in the Protein data bank (www.rcsb.org) under PDB ID 2HIZ.