Structure-Based Design of Potent and Selective Cell-Permeable Inhibitors of Human β-Secretase (BACE-1)

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Abstract: We describe the development of cell-permeable β-secretase inhibitors that demonstratively inhibit the production of the secreted amino terminal fragment of an artificial amyloid precursor protein in cell culture. In addition to potent inhibition in a cell-based assay (IC_{50} < 100 nM), these inhibitors display impressive selectivity against other biologically relevant aspartyl proteases.

Alzheimer's disease (AD) is a neurodegenerative disease of the brain that is characterized by the progressive formation of insoluble amyloid plaques and fibrillary tangles.[1] Plaques are extracellular constructs consisting primarily of aggregated Aβ_{42}, a peptide fragment formed by the sequential proteolytic processing of β-amyloid precursor protein (APP) by two enzymes, β- and γ-secretase.[2] Although there is debate as to whether insoluble plaques or their oligomeric progenitors are culpable for the pathogenic cascade that ultimately leads to neuronal loss and dementia, inhibition of APP proteolysis has emerged as a central concept in therapeutic approaches to AD.

β-Secretase (β-site APP cleaving enzyme or BACE-1), a novel type I transmembrane aspartyl protease whose identity remained elusive until 1999,[3] is believed to be the key enzyme that commits APP catabolism to the amyloidogenic pathway. Mice genetically deficient in BACE-1 show no Aβ production.[4] More importantly the mice are viable and display a minimally altered phenotype.[5] As such, β-secretase inhibition is considered an attractive therapeutic target for the treatment and prevention of AD.

In this report we describe the discovery of a series of isophthalamides that are potent and selective BACE-1 inhibitors. Among these, **3** (Figure 1) displays excellent activity as an inhibitor of BACE-1 in assays with purified enzyme and in a cell-based assay optimized for monitoring BACE-1 cleavage.

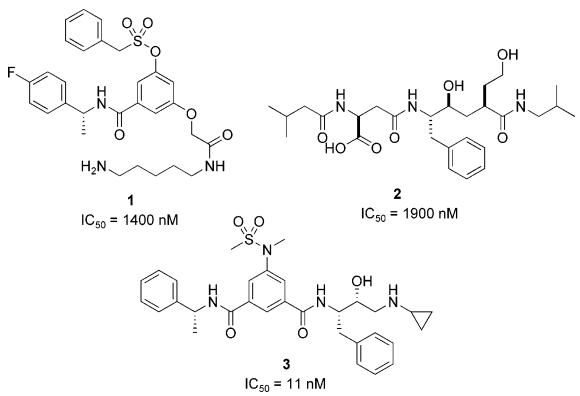

Figure 1. Nontraditional, hydroxyethylene (HE) and hydroxyethylamine (HEA) BACE inhibitors.

To date, the majority of the BACE-1 inhibitors in the literature are peptide-based analogues that replace the scissile amide bond with a noncleavable isostere.[6] While these statine or hydroxyethylene (HE) based dipeptide isosteres (Figure 1, **2**) result in potent enzymatic inhibition, their peptidic character precludes their use as therapeutic agents.

We recently reported the structure of an inhibitor that displayed a novel mode of binding to aspartyl proteases.[7] Resorcyclic compound **1** (Figure 1) was shown to inhibit BACE-1 (IC_{50} = 1.4 μM) in our enzymatic assay. The discovery of **1** was the result of the cumulative effort of high-throughput screening and subsequent lead optimization. While **1** was not a high-affinity ligand for BACE-1, we were successful in obtaining a crystal structure of the inhibitor bound in the BACE-1 active site. The crystal structure revealed two important pieces of structural information that influenced future compound design. First, it was apparent that **1** was bound in the S_1−S_4 region of the enzyme, confirming our competition study experiments and allowing a more rational design approach to emerge. However, the most notable feature was the lack of a direct interaction between the inhibitor and the catalytic aspartate dyad. Instead, **1** interacts with the aspartates indirectly through a water-mediated hydrogen bond. Previous research in the field of aspartyl protease inhibition has shown that direct interaction with the catalytic aspartates is usually paramount for activity. We surmised that by modifying **1** to interact directly with the catalytic dyad we could dramatically enhance the potency of this series.

Among potential motifs that might interact with the catalytic aspartates, hydroxyethylamine dipeptide isosteres (HEA) have been applied successfully to a number of aspartyl proteases.[8] We chose to target the HEA motif rather than the related HE because the former was lower in molecular weight and contained one less amide bond. These are important considerations with respect to obtaining compounds with good pharmacokinetics. Recently, Tamamura reported the use of a HEA dipeptide isostere as a BACE inhibitor, although this series was still predominantly peptidic in design and possessed *S* stereochemistry at the hydroxyl center that interacts with the catalytic aspartates.[9] Studies on HIV protease

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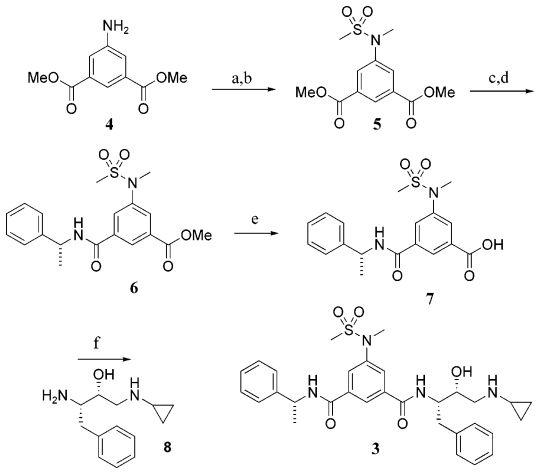
Scheme 1. Synthesis of Designed HEA Hybrid[a]



[a] (a) MsCl, pyridine, 75%; (b) NaH, MeI, DMF, 98%; (c) 1 N NaOH, 82%; (d) BOP, (*R*)-α-methylbenzylamine, 95%; (e) 1 N NaOH, 99%; (f) BOP, DIPEA, 84%.

inhibitors based on this motif have shown that the (*S*)-hydroxyl stereochemistry is preferred for a statine or HE type isostere (**2**, Figure 1) but the opposite *R* stereochemistry is preferred in the HEA motif.[10,11]

We set out to synthesize a hybrid structure between inhibitor **1** and a hydroxyethylamine isostere. In addition, we chose to replace the sulfonate ester present in **1** with a more chemically robust sulfonamide. To synthesize the hybrid compound, we began by mesylating 5-amino isophthalic ester **4** followed by N-alkylation with methyl iodide (Scheme 1). Monohydrolysis of the bis-ester **5** followed by BOP mediated amide coupling with (*R*)-α-methylbenzlyamine and hydrolysis of the remaining ester **6** produced the requisite benzoic acid **7** in high yield. Finally, amide coupling of **7** with **8** resulted in the formation of the desired hydroxyethylamine containing **3**. It should be noted that coupling of the unprotected diamine **8** took place exclusively at the primary amine as verified by ^{1}H NMR. We were gratified when **3** was shown to exhibit enhanced potency toward BACE-1 compared with **1**. In fact, the hybrid construct **3** had an IC_{50} of 15 nM toward the purified enzyme, which represents approximately a 100-fold increase in potency.

Owing to our success in obtaining a cocrystal of **1** in the BACE-1 active site, we were able to obtain an X-ray structure of **3** via a soaking exchange. Figure 2 displays an overlay of **1** with hydroxyethylamine construct **3**, showing that they occupy the same general space in the active site with a few key differences. In both series, the α-methylbenzamide occupies the S_3 subpocket in the enzyme and its SAR is conserved between the two structural classes;[7] i.e., the *R* stereochemistry at the methyl is preferred and the addition of a *p*-fluoro group on the aryl ring adds slightly to potency, as in **10**. Like the sulfonate ester of **1**, the sulfonamide of **3** occupies the S_2 site but is lacking the phenyl appendage, which is involved in a cation−π interaction with Lys321 in the S_4 site. However, in both series, each sulfonamide oxygen makes important hydrogen-bonding interactions with the enzyme, one with the backbone NHs of Thr 232 and Asn233 and the other with ND2 of Asn233 and NE of Arg235. These interactions appear to contribute significantly to binding. The R_2 substituent of the

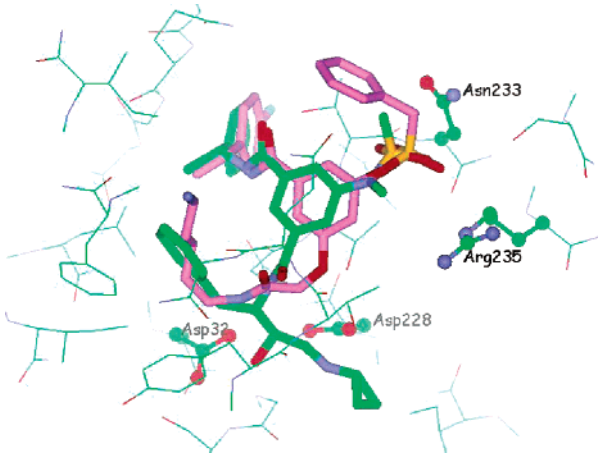


Figure 2. Overlay of cocrystal structures of **1** (magenta) and **3** (green) in the BACE-1 active site.

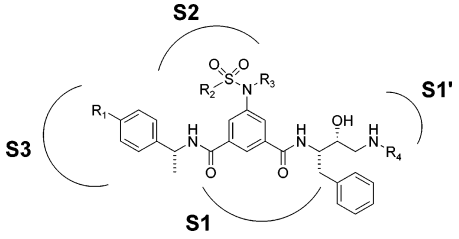
sulfonamide is somewhat indiscriminate, and a variety of modifications are tolerated without significantly reducing potency. In contrast, we believe the alkyl group, R_3, on the nitrogen of the sulfonamide serves mainly to orient the binding conformation. Whereas there is a slight loss in activity between the methylated **3** and nonmethylated **9** analogues, there is little difference in activity when alkylated with larger substituents presumable because of its location near the solvent interface.

The most notable differences between **1** and **3** relate to the filling of the S_1 pocket and the interactions of the HEA fragment with the catalytic aspartates. In structure **1** the S_1 pocket is occupied by the pentylamine ligand, whereas in structure **3** the phenylalanyl group occupies this hydrophobic space in a manner similar to that of known peptidic inhibitors. Another important difference is that, as mentioned previously, **1** interacts indirectly with the catalytic aspartates through a water-mediated hydrogen-bonding network. In contrast, the hydroxylamine motif is involved in direct interactions with the catalytic dyad as seen in Figure 2. There is a two-pronged interaction wherein the hydroxyl functionality of the inhibitor engages Asp32, while the protonated α-amino group simultaneously engages Asp228. This 2-fold interaction may also contribute to additional binding by assisting in the complex proton shuttling that occurs at the dyad upon inhibitor binding. Furthermore, the cyclopropyl group on the amine in **3** is oriented toward the S_1' site of the enzyme, which is somewhat promiscuous in nature. A variety of R_4 groups are tolerated with a slight difference in activity. Here, the P_1' SAR seems to have a more dramatic effect on cellular potency than on enzymatic activity. This may be a result of differences in cell permeability.

As mentioned previously, there are several reports of low nanomolar BACE-1 inhibitors in the literature.[12] However, with the exception of an HE-based inhibitor that displayed an IC_{50} of 400 nM,[13] very few of these are reported to have activity in whole cells. This is likely attributed to poor membrane permeability and low aqueous solubility.

To evaluate the cellular activity of our inhibitors, an optimized cell-based assay was developed. HEK293 cells were stably transfected with a mutant form of APP containing the synthetic BACE-1 cleavage sequence

Table 1. SAR of BACE-1 Inhibitor



compd	R_1	R_2	R_3	R_4	BACE-1 IC_{50} (nM)	sAPP_NF IC_{50} (nM)	BACE-2 IC_{50} (nM)	renin IC_{50} (nM)
3	H	Me	Me	cyclopropyl	15 ± 0.4	29 ± 4.1	230 ± 15	>50 000
9	F	Me	H	cyclopropyl	63 ± 4.5	1180[a]	210[a]	>50 000
10	F	Me	Me	cyclopropyl	10 ± 2.0	19[a]	69 ± 6.5	>10 000
11	F	Me	Me	H	23 ± 1.0	5389[a]	443 ± 4.5	>50 000
12	F	Me	Me	ethyl	15[a]	396[a]	260 ± 25	>50 000
13	F	Me	Me	*tert*-butyl	15 ± 9.9	2122[a]	NA	>50 000
14	H	^{i}Pr	Me	cyclopropyl	41 ± 0.5	25 ± 7.1	360 ± 35	>50 000
15	H	$N(Me)_2$	Me	cyclopropyl	14[a]	9 ± 3.5	110 ± 2.5	>50 000

[a] Indicates single experiment.

NFEV at the site of proteolysis.[14] Compared to the wild-type sequence, KMDA, or the Swedish sequence, NLDA, the secretion of the BACE-1 dependent N-terminal fragment of APP_NFEV ("sAPP_NF") was increased dramatically, thus providing a robust and direct measure of BACE-1 activity in cells. As shown in Table 1, all of the isophthalamide-derived inhibitors quantifiably reduced sAPP_NF formation in this assay, with several of the inhibitors exhibiting low nanomolar potency. Potency was impaired when R_4 was removed or sterically demanding (Table 1, **11**, **13**). A notable example is sulfamide **15**, which displayed an IC_{50} of 9 nM.[15]

The catalytic domain of BACE-1 is similar to that of other known aspartyl proteases such as renin and cathepsin D. Here, the sequence identities in the active site (i.e., within 6 Å of **3**) are 29% and 39%, respectively. Selectivity versus these enzymes will be needed for clinical development of a BACE-1 inhibitor. However, a particular challenge is selectivity versus BACE-2, a close relative of BACE-1 with 79% sequence identity in the active site.[16] While BACE-1 is localized in neurons, BACE-2 is ubiquitous. The biological functions of BACE-2 have yet to be delineated, but it is known to also cleave APP within the Aβ domain. Although BACE-1/BACE-2 double knockout mice have been reported and appear to be viable,[17] enzyme selectivity is still desirable to avoid unanticipated side effects. BACE-1 inhibitors reported to date have displayed poor selectivity versus other aspartyl proteases such as cathepsin D and little if any selectivity toward BACE-2.[18] In fact, some structural modifications on BACE-1 inhibitors that have resulted in increased selectivity versus cathepsin D and renin actually reduced selectivity between BACE-2 and BACE-1.[19]

Despite similarities with other aspartyl proteases, there are also some key differences in the BACE-1 active site; e.g., it is more open and less hydrophobic than other aspartyl proteases, suggesting that enzyme selectivity is indeed possible with a small-molecule inhibitor. In fact, all of the isophthalamides reported in Table 1 exhibited remarkable enzyme selectivity *and showed little to no inhibition of renin up to 500 μM!* Compound **3** was also counterscreened against cathepsin D and demonstrated approximately 500-fold selectivity (IC_{50} = 7620 nM). All of the compounds tested also displayed moderate selectivity for BACE-1 over BACE-2, ranging from 8- to 20-fold. The prominent factors suspected for imparting the observed selectivity are the P_2 sulfonamide and the P_1' group. As noted previously, the sulfonamide oxygens residing in the S_2 region of the enzyme are engaged in two key hydrogen-bonding interactions, namely, Asn233 and Arg235. In contrast, Asn233 is a leucine in BACE-2 and cathepsin D and is a tyrosine in renin. Additionally, while Arg235 is conserved between BACE-1 and BACE-2, it is a valine in cathepsin D and a serine residue in renin. The second contributor we suspect for the observed selectivity resides within the S_1' site. Here, the more hydrophilic S_1' site of BACE-1 better accommodates a small hydrophobic group, whereas cathepsin D and renin have longer, primarily hydrophobic S_1' regions that should prefer to interact with large hydrophobic groups.[19] In contrast to the S_1' topology of cathepsin D and renin, which have an extended β-hairpin loop in this region, the difference between BACE-1 and BACE-2 is more subtle consisting of only two amino acid differences, Gln326 to Pro and Thr329 to Asn. Thus, this region may primarily affect selectivity over cathepsin D and renin rather than differentiate between BACE-1 and BACE-2 activity. These key residue differences could explain the enhanced selectivity against related aspartyl proteases, making these compounds the most selective BACE-1 inhibitors reported to date.

In conclusion, on the basis of the cocrystal structure of the novel resorcyclic inhibitor **1** in the BACE-1 active site, we succeeded in identifying nonpeptidic low molecular weight β-secretase inhibitors. Compound **3** was shown to exhibit potent inhibition of sAPP_NF production in a cell-based assay. In addition, **3** displayed greater than 500-fold selectivity versus cathepsin D and renin, two structurally related aspartyl proteases. Further studies to evaluate **3** in vivo are ongoing and will be reported in due course.

Supporting Information Available: Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Selkoe, D. J. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* **1999**, *399A*, 23.

(2) Sinha, S.; Lieberburg, I. Cellular mechanisms of β-amyloid production and secretion. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11049−11053.

(3) (a) Vassar, R.; Bennett, B. D.; Babu-Kahn, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edendon, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. β-Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science* **1999**, *286*, 735. (b) 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.; Leiberburg, I.; Powern M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; John, V. Purification and cloning of amyloid precursor protein β-secretase from human brain. *Nature* **1999**, *402*, 537−540. (c) 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.; Tomasseelli, A. G.; Parodi, L. A.; Heinrikson, R. L.; Gurney, M. E. Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature* **1999**, *402*, 533−537. (d) Hussain, I.; Powell, D.; Howlett, D. R.; Tew, D. G.; Meek, T. D.; Chapman, C.; Gloger, I. S.; Murphy, K. E.; Southan, C. D.; Ryan, D. M.; Smith, T. S.; Simmons, D. L.; Walsh, F. S.; Dingwall, C.; Christie, G. Identification of a Novel Aspartic Protease (Asp 2) as β-Secretase *Mol. Cell. Neurosci*. **1999**, *14*, 419−427.

(4) Cai, H.; Wang, Y.; McCarthy, D.; Wen, H.; Borchelt, D. R.; Price, D. L.; Wong, P. C. BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. *Nat. Neurosci*. **2001**, *4*, 233−234.

(5) Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sihna, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. BACE knockout mice are healthy despite lacking the primary β-secretase activity in the brain. *Hum. Mol. Genet*. **2001**, *10*, 1317−1324.

(6) (a) Hom, R. K.; Gailunas, A. F.; Mamo, S.; Fang, L. Y.; Tung, J. S.; Walker, D. E.; Davis, D.; Thorsett, E. D.; Jewett, N. E.; Moon, J. B.; John, V. Design and Synthesis of Hydroxyethylene-Based Peptidomimetic Inhibitors of Human β-Secretase. *J. Med. Chem*. **2004**, *47*, 158−164. (b) Tung, J. S.; Davis, D. L.; Anderson, J. P.; Walker, D. E.; Mamo, S.; Jewett, N.; Hom, R. K.; Sinha, S.; Thorsett, E. D.; John, V. Design of Substrate-Based Inhibitors of Human β-Secretase. *J. Med. Chem.* **2002**, *45*, 259−262. (c) Ghosh, A. K.; Shin, D.; Downs, D.; Koelsch, G.; Lin, X.; Ermolieff, J.; Tang, J. *J. Am. Chem. Soc.* **2000**, *122*, 3522−3523. (d) Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*, 150−153. (e) Ghosh, A. K.; Bilcer, G.; Harwood, C.; Kawahama, R.; Shin, D.; Hussain, K. A.; Hong, L.; Loy, J. A.; Nguyen, C.; Koelsch, G.; Ermolieff, J.; Tang, J. *J. Med. Chem.* **2001**, *44*, 2865−2868. (f) Hong, L.; Turner, R. T.; Koelsch, G.; Shin, D.; Ghosh, A. K.; Tang, J. *Biochemistry* **2002**, *41*, 10963−10967. (g) Chen, S.; Lamar, J.; Guo, D.; Kohn, T.; Yang, H.; McGee, J.; Timm, D.; Erickson, J.; Yip, Y.; May, P.; McCarthy, J. P3 cap modified Phe*-Ala series BACE inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 245−250

(7) Coburn, C. A.; Stachel, S. J.; Rush, D. M.; Steele, T. S.; Holloway, M. K.; Li, M.; Lai, M.-T..; DiMuzio, J.; Crouthamel, M.; Sardona, V.; Chen, Z.; Munshi, S.; Kuo, L.; Makara, G.; Vacca, J. P. Identification of a Small Molecule Non-Peptide Active Site BACE-1 Inhibitor That Displays a Novel Binding Mode for Aspartyl Proteases. *J. Med Chem.*, in press.

(8) Leung, D.; Abbenante, G.; Fairlie, D. P. Protease Inhibitors: Current Status and Future Prospects. *J. Med. Chem.* **2000**, *43*, 305−341.

(9) Tamamura, H.; Kato, T.; Otaka, A.; Fujii. Synthesis of potent β-secretase inhibitors containing a hydroxyethylamine dipeptide isostere and their structure−activity relationship studies. *Org. Biomol. Chem*. **2003**, *1*, 2468−2473.

(10) Rich, D. H.; Sun, C.; Vara Prasad, J. V. N.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M.; Mueller, R. A.; Houseman, K. Effect of Hydroxyl Group Configuration in Hydroxyethylamine Dipeptide Isosteres on HIV Protease Inhibition. Evidence of Multiple Binding Modes. *J. Med. Chem.* **1991**, *34*, 1222−1225.

(11) During the course of this work it became apparent in the patent literature that groups at Elan and Pharmacia had embarked on a similar strategy. (a) Maillaird, M.; Hom, C.; Gailunas, A.; Jagodzinska, B.; Fang, L. Y.; John, V.; Freskos, J. N.; Pulley, S. R.; Beck, J. P.; Tenbrink, R. E. Preparation of substituted amines to treat Alzheimer's disease. PCT Int. Appl. WO 02002512, CAN 136:102190, 2002. (b) Gailunas, A.; Hom, R.; John, V.; Maillard, M.; Chrusciel, R. A.; Fisher, J.; Jacobs, J.; Freskos, J. N.; Brown, D. L.; Fobian, Y. M. Preparation of *N*-(3-amino-2-hydroxy-propyl) substituted alkanamides as inhibitors of the beta secretase enzyme for treating Alzheimer's disease. PCT Int. Appl. WO 2003006423, CAN 138:136938, 2003.

(12) Hom, R. K.; Fang, L. Y.; Mamo, S.; Tung, J. S.; Guinn, A. C.; Walker, D. E.; Davis, D. L.; Gailunas, A. F.; Thorsett, E. D.; Sinha, S.; Knops, J. E.; Jewett, N. E.; Anderson, J. P.; John, V. Design and Synthesis of Statine-Based Cell-Permeable Peptidomimetic Inhibitors of Human β-Secretase. *J. Med. Chem.* **2003**, *46*, 1799−1802.

(13) Lamar, J.; Hu, J.; Bueno, A. B.; Yang, H.; Guo, D.; Copp, J. D.; McGee, J.; Gitter, B.; Timm, D.; May, P.; McCarthy, J.; Chen, S. Phe*-Ala-based pentapeptide mimetics are BACE inhibitors: P2 and P3 SAR. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 239−243.

(14) Shi, X.-P.; Tugusheva, K.; Brice, J. E.; Lucka, A.; Chen-Dodson, E.; Hu, B.; Wu, G.-X.; Price, E.; Register, R. B.; Lineberger, J.; Gates, A.; Miller, R.; Tang, M.-J.; Espeseth, A.; Kahana, J.; Wolfe, A.; Crouthamel, M.-C.; Sankaranarayanan, S.; Simon, A.; Chen, L.; Lai, M.-T.; Pietrak, B.; DiMuzio, J.; Li, Y.; Xu, M.; Huang, Q.; Garsky, V.; Sardana, M.; Hazuda, D. Novel mutations introduced at the beta-site of amyloid precursor protein enhance beta-secretase cleavage in vitro and in cells. *J. Neurosci*., submitted.

(15) All compounds reported in Table 1 were noncytotoxic at 10 μM in cells.

(16) (a) Farzan, M.; Schnitzler, C. E.; Vasillieva, N.; Leung, D.; Choe, H. BACE2, a β-secretase homolog, cleaves at the β site and within the amyloid-β region of the amyloid-β-precursor protein. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 9712−9717. (b) Reference 6b.

(17) Harper, A. J.; Harrison, S. M.; Morrison, A. D.; Austin, A. A.; Dingwell, C. *BACE 1 and 2 double knowckout mice are indistinguishable from wild type littermate controls*; Program No. 842.12.2003; Society for Neuroscience: Washington, DC, 2003.

(18) Ghosh, A. K.; Shin, D.; Downs, D.; Koelsch, G.; Lin, X.; Ermolieff, J.; Tang, J. Design of Potent Inhibitors for Human Brain Memapsin 2 (β-Secretase). *J. Am. Chem. Soc.* **2000**, *122*, 3522−3523.

(19) Brady, S. F.; Singh, S.; Crouthamel, M.; Holloway, M. K.; Coburn, C. A.; Garsky, V. M.; Bogusky, M.; Pennington, M. W.; Vacca, J. P.; Hazuda, D.; Lai, M. Rational design and synthesis of selective BACE-1 inhibitors. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 601−604. This reference also details sequence differences in the S_1' region.

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