Acylguanidines as Small-Molecule β-Secretase Inhibitors

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Abstract: BACE1 is an aspartyl protease responsible for cleaving amyloid precursor protein to liberate Aβ, which aggregates leading to plaque deposits implicated in Alzheimer's disease. We have identified small-molecule acylguanidine inhibitors of BACE1. Crystallographic studies show that these compounds form unique hydrogen-bonding interactions with the catalytic site aspartic acids and stabilize the protein in a flap-open conformation. Structure-based optimization led to the identification of potent analogs, such as **10d** (BACE1 $IC_{50} = 110$ nM).

Alzheimer's (AD) is a progressive, degenerative disease of the brain and the most common form of dementia. A variety of therapeutic strategies for modulating the progression or prevention of AD are currently being investigated. Although the exact pathological mechanism remains unclear, overwhelming evidence implicates amyloid β-peptide (Aβ, 39−43 residues) (most likely in multimeric forms such as oligomers) in the neuronal dysfunction and death that causes the cognitive decline seen in AD.[1] Aβ is produced by the sequential proteolytic cleavage of amyloid precursor protein (APP) via the action of two proteases, β- and γ-secretase. Specifically, β-secretase (also called BACE1, memapsin 2, and Asp2) mediates the primary cleavage of APP,[2] generating the membrane-bound C-terminal APP fragment (βCTF/C99), which in turn is cleaved by γ-secretase liberating the Aβ peptide.[3,4] BACE1 is a membrane-bound aspartyl protease highly expressed in the central nervous system (CNS).[5−9] BACE1 knockout mice have been shown to be healthy with an absence of Aβ production,[10−13] indicating that BACE1 is the key enzyme responsible for Aβ production. Thus, BACE1 is a therapeutic target for inhibitors of Aβ production for the treatment and prevention of AD. The close homologue BACE2 (memapsin1, Asp1) also cleaves APP at the β-site but cleaves more efficiently after Phe19 and Phe20 of Aβ and is poorly expressed in the CNS.[14]

The first crystal structure of BACE1 with the heptapeptide **1** ($K_i = 1.6$ nM) (Figure 1) containing the Leu-Ala hydroxyethylene isostere as the transition state mimic[15] was published by Hong et al.[16] Structure-based optimization of this peptide mimetic led to the identification of potent small molecular weight hydroxyethylene inhibitors such as **2** ($IC_{50} = 30$ nM)[17] as well as a series of constrained analogues, e.g., **3** ($IC_{50} = 10$ nM).[18] Others have replaced the hydroxyethylene isostere with the statin scaffold, leading to inhibitors such as **4** ($IC_{50} = 91$ nM).[19]

Like other aspartic proteases,[20−23] BACE1 consists of two structural domains, the N- and C-terminus, which constitute the active site and a β-hairpin loop which forms the flap region. The flap opens to allow the substrate to enter and then closes down on the substrate during the catalysis step and reopens to release the hydrolyzed products. The design of the transition state based inhibitors described above is based on replacing the cleavage site of the peptide substrate. Not unexpectedly, it has been observed in crystallographic studies that these peptidomimetic inhibitors bind with BACE1 in a closed-flap form.

High-throughput screening (HTS) of the Wyeth Corporate compound library using a FRET assay identified the acylguanidine inhibitor, **7a** (Scheme 1). Acylguanidines have not previously been described as aspartic acid protease inhibitors, and this discovery of a modestly potent, low molecular weight inhibitor represents a new direction in the design of BACE1 inhibitors. Herein, we describe the characterization, cocrystal structure, and optimization of this lead using structure-based design.

Compound **7a** (BACE1 $IC_{50} = 3.7$ μM) was demonstrated to bind to BACE1 by NMR experiments, had a K_d of 2.8 μM by isothermal titration calorimetry, and inhibited $A\beta^{total}$ formation in a cellular assay with an IC_{50} of 8.9 μM. Significantly, in a radiolabeled immunoprecipitation cellular assay (RICA), **7a** caused a dose-dependent reduction of βCTF and Aβ levels without affecting secreted α-secretase amyloid precursor protein (α-sAPP) levels, consistent with a β-secretase mediated inhibition mechanism.

An X-ray crystal structure of **7a** complexed with the BACE1 catalytic domain was solved at 2.4 Å resolution. In this structure the acylguanidine moiety forms four key hydrogen-bonding interactions with the catalytic aspartic acids Asp32 and Asp228 (Figure 2). Important structural changes were observed in BACE1 upon binding of **7a** (Figure 3). In contrast to complexes with peptidomimetic inhibitors in which the flap region closes in over the bound inhibitor,[16] in the **7a** complex the flap adopts an "open conformation" to make room for the diarylpyrrole portion of the inhibitor, with a total movement of 5.5 Å at the tip of the hairpin turn and a movement of 7.5 Å for the Tyr71 hydroxyl moiety. In fact, the diarylpyrrole moiety of **7a** occupies the space occupied by Tyr71 in the peptidomimetic structures. π-Edge stacking between the pyrrole and the phenyl ring of Tyr71 appears to contribute to the stabilization of the flap in this configuration. Stabilization of the flap region in an open position has previously been observed crystallographically for the enzyme/inhibitor complexes of renin[24] and pepsin,[25] and the implications of these conformations for rational drug design have been reviewed by Bursavich and Rich.[26]

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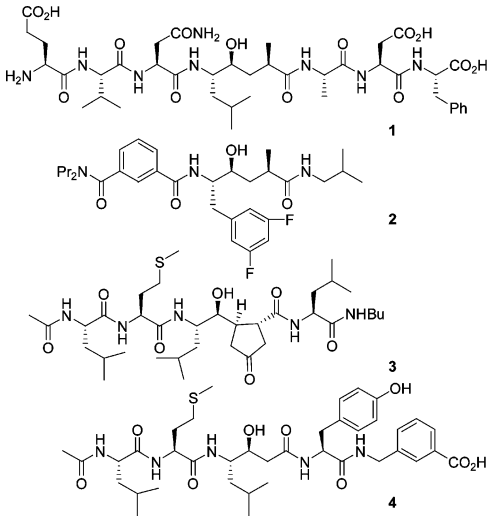


Figure 1. BACE1 peptidomimetic inhibitors: hydroxyethylene based heptapeptide **1**,[16] optimized hydroxyethylene based inhibitor **2**,[17] conformationally constrained analogue **3**,[18] and statine based tetrapeptide **4**.[19]

Further examination of the BACE1/**7a** complex indicates that the two aryl groups extend into the S_1 and S_2' pockets. The large lipophilic S_1 pocket is approximately spherical and appears suboptimally occupied by the flat phenyl ring. In addition, the para position of the P_1 phenyl group projects directly toward the unoccupied S_3 pocket, indicating an opportunity to add substituents to the P_1 phenyl extending into the S_3 pocket and thereby potentially increasing binding affinity. The phenyl occupying S_2' is involved in a π-edge stacking interaction with Trp76. In contrast to the S_1-S_3 pocket, the S_2' pocket provides access to more polar/charged groups (e.g., Trp76, Tyr198, and Arg128) in the immediate vicinity of the ligand and contains several buried water molecules, offering the potential for analogues of **7a** to form additional hydrogen bonds directly with BACE1 or through tightly held water molecules.

While two of the nitrogen groups on the acylguanidine moiety are intimately involved in key hydrogen-bonding interactions with the catalytic aspartic acids, the third nitrogen faces away from the catalytic residues toward the S_1' pocket. Although this nitrogen appears to be involved in an intramolecular hydrogen bond with the adjacent carbonyl group, it does not make any significant interactions with the protein. Thus, we felt substitution on this nitrogen might allow access to the unoccupied S_1' pocket, a pocket that accommodates an Asp residue in the natural substrate and is flanked by Arg235, Lys224, and Thr329, thus presenting potential opportunities to form polar and/or hydrogen-bonding interactions.

The synthetic pathway to the desired analogues is shown in Scheme 1. The 1,4-diarylbutane-1,4-diones **5a** and **5c**,**d** were prepared in one step by coupling enolizable methyl ketones and α-bromomethyl ketones under the action of $ZnCl_2$, *t*-BuOH, and Et_2NH, using the procedure described by Kulinkovich.[27] 1-Aryl-4-adamantylbutane-1,4-dione **5b** was prepared by the reaction of an ethylbenzoyl acetate with 1-adamantyl bromomethyl ketone to give the ethyl 2-aryl-4-oxo-4-alkylbutanoate **8**, which was subjected to ester hydrolysis and decarboxylation to give the 1,4-dione **5b**. The 1,4-diones were condensed with glycine in refluxing acetic acid or with glycine methyl ester in toluene followed by ester hydrolysis to give the different 2-(2,5-disubstituted-1*H*-pyrrol-1-yl)acetic acids **6**. Activation of the acid with 1,1′-carbonyldiimidazole (CDI) and reaction with guanidine hydrochloride gave the unsubstituted acylguanidines **7a**−**c**. Direct alkylation of the acylguanidines **7** proved unsatisfactory, so the desired substituted acylguanidines **10** were prepared by reaction of **6** with 1*H*-pyrazole-1-carboxamidine using CDI activation followed by displacement of the pyrazole leaving group with 3-amino-1-propanol.

Compounds were tested for inhibition of isolated BACE1 in an in vitro peptide cleavage FRET assay. Compound **7b**, in which one of the phenyl groups on **7a** is replaced with the more spherical adamantyl group, inhibited BACE1 activity with an IC_{50} of 0.6 μM, 6-fold more potently than the initial lead. A cocrystal structure of **7b** complexed with BACE1 (not shown) confirmed that the adamantyl group occupies the S_1 pocket as intended. Analogues were also made to project functional groups into the S_3 pocket from the P_1 phenyl. One of the most potent compounds in this initial library was **7c** (BACE1 $IC_{50} = 160$ nM) in which a 4-acetoxyphenoxy group is attached in the para position of the P_1 phenyl substituent. Again, a cocrystal structure (not shown) provided conclusive proof that the 4-(4-acetoxyphenoxy)phenyl group extends through the S_1 pocket into the S_3 pocket as predicted by our models. The 2-chloro group on the P_2' phenyl on this molecule (**7c**) is directed toward the back of the S_2' pocket, but on the basis of the interactions it makes with the protein and our SAR studies, this P_1' substitution does not appear to contribute significantly to potency. As mentioned above, the S_2' pocket presents Tyr198 and Arg128 in the immediate vicinity of the ligand and contains several buried water molecules. Attempts to take advantage of opportunities for improved interactions, and possible displacement, of these buried water molecules through the attachment of polar substituents on the 2-, 3-, and 4-position of P_2' phenyl ring were unsuccessful.

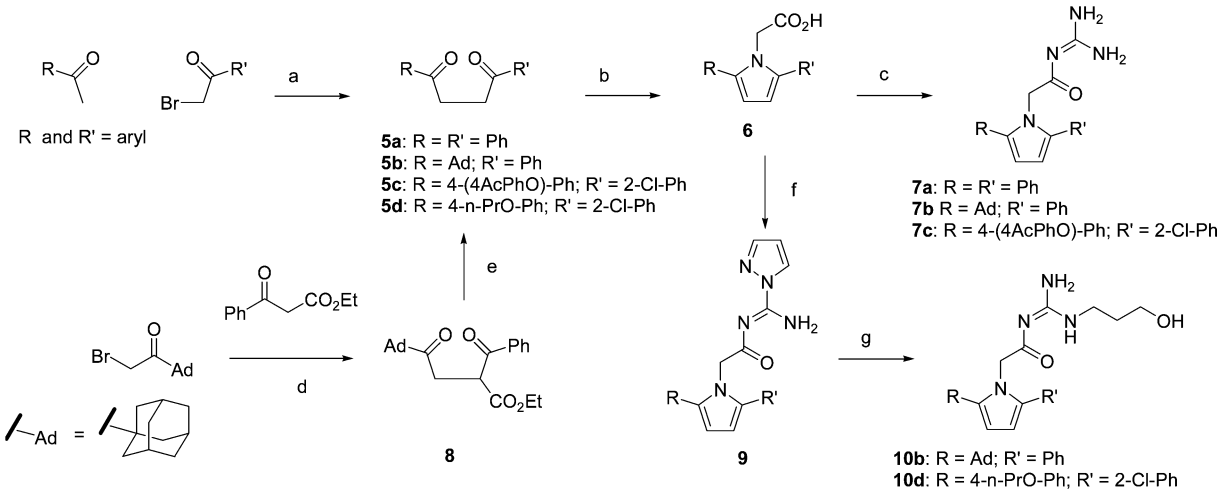
Substitutions of the guanidine nitrogen terminating in polar functionalities were expected to access the S_1' pocket and anticipated to pick up additional hydrogen bonds with, or displace, bound water molecules in this pocket. Substitution on this nitrogen with a 3-propanol group yielded **10b**, which had a BACE1 IC_{50} of 240 nM, approximately 2.5-fold higher potency than the corresponding unsubstituted acylguanidine **7b**.

Continued exploration of the S_1-S_3 region of the molecules led to the discovery that the large phenyl is not required for optimal S_3 pocket binding; in fact, the much smaller compound **10d** with the *p*-*n*-propyloxyphenyl group extending from S_1-S_3 (BACE1 $IC_{50} = 110$ nM) achieves similar or greater potency.

A 2.4 Å resolution cocrystal structure of BACE1 with inhibitor **10b** and modeled structure of **10d** (Figure 4) highlight many of the new interactions with these optimized inhibitors. As with **7a**, the acylguanidine moiety forms hydrogen bonds with the two catalytic aspartic acid residues. Substitution of the acylguanidine nitrogen extends into the S_1' pocket, forming hydrogen-bonding interactions with Arg235 and Thr329 via bridging water molecules. The *p*-*n*-propyloxyphenyl group extends into the S_1-S_3 pocket with minimal strain, as was intended.

The acylguanidine inhibitors were tested for their inhibition of the closely related BACE2 enzyme and more distant aspartic proteases cathepsin-D and pepsin (Table 1). In general, these compounds are highly selective for BACE1 over cathepsin (>50-fold, except **7a** which is 16-fold) and pepsin ($IC_{50} > 50$ μM). Compounds with diaryl substituents on the pyrrole generally have lower selectivity for BACE1 over BACE2 (3- to 20-fold), while those with the adamantyl substituent show greater selectivity (14- to 20-fold). Substitution on the guanidine nitrogen also leads to lower BACE1/BACE2 selectivity. Several

Scheme 1[a]



[a] (a) Et_2NH, *t*-BuOH, $ZnCl_2$, toluene, room temp, 2−5 days; (b) glycine, *p*-TSA, EtOH, 80 °C, 3 days; or (i) Gly-OMe, *p*-TSA, toluene, 110 °C, 2 days; (ii) LiOH, THF, room temp, 2 days; (c) (i) CDI, DMF, room temp, 1 h; (ii) guanidine·HCl, Et_3N, room temp, 5 h; (d) NaH, THF, room temp, 4 h; (e) 3 N NaOH, EtOH, 80 °C, 16 h; (f) CDI, DCM, room temp, 5 h, then 1*H*-pyrazole-1-carboximidamide, TEA, DMAP, room temp, 18 h; (g) 3-amino-1-propanol, DIEA, DCM, room temp, 48 h.

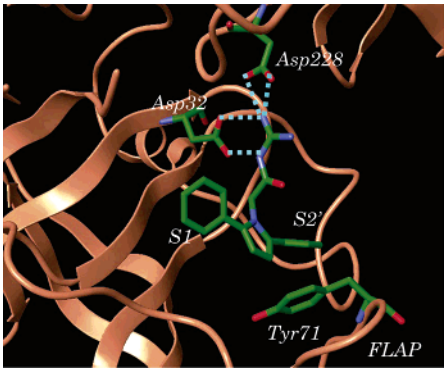


Figure 2. Crystal structure of BACE1 complexed with **7a** highlighting the four key hydrogen-bonding interactions between the catalytic aspartic acids Asp32 and Asp228 and the acylguanidine moiety.

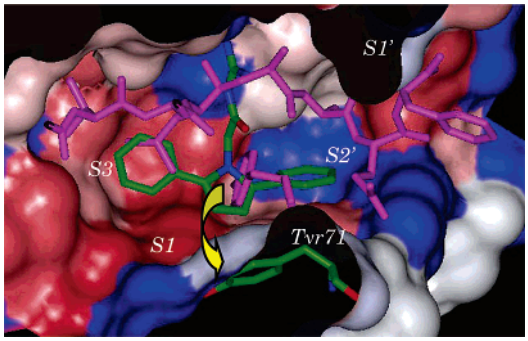


Figure 3. Crystal structure of BACE1 complexed with **7a** (colored by atom type with carbon in green, oxygen in red, nitrogen in blue) overlayed with the published structure of BACE1 complexed with **1**[16] (colored in magenta). The yellow arrow highlights the movement of Tyr71 from the closed position in complex with **1** (Tyr71 shown in magenta) to the open conformation in complex with **7a** (Tyr71 shown in green, blue, and red). A solid Connolly surface of the BACE1/**7a** binding site is shown, with polar/charged regions of the pocket colored blue and lipophilic regions colored red.

compounds had low micromolar activity in the cellular Aβ^{total} lowering ELISA assay.

In conclusion, we have discovered acylguanidines as a novel template for inhibitors of aspartyl proteases. These small-molecule inhibitors form four hydrogen-bonding interactions with the two catalytic aspartic acids and stabilize the protein in a flap-open conformation. Structure-based design was used to optimize the series, leading to potent analogues such as **10d** (BACE1 IC_{50} = 110 nM).

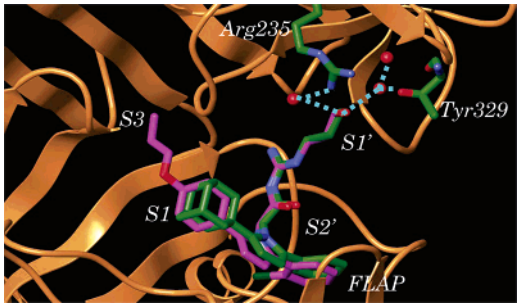


Figure 4. X-ray structure of BACE1 complexed with **10b** (colored by atom type with carbon in green, oxygen in red, nitrogen in blue), overlaid with a modeled structure of **10d** (colored in magenta). Interactions between the hydroxypropyl group and bound water molecules in the S1′ pocket are indicated as light-blue dashed lines.

Table 1. IC_{50} Values[a] of Inhibition of BACE1 and Related Aspartyl Proteases by Compounds **7** and **10**

	BACE1 IC_{50}, μM	BACE2 IC_{50}, μM	cathD IC_{50}, μM	pepsin IC_{50}, μM	cellular Aβ^{total} ED_{50}, μM
7a	3.7 ± 1.0	>50	60.0 ± 11.4	>50	8.9 ± 1.6
7b	0.6 ± 0.2	12.9 ± 2.8	30.2 ± 6.8	>50	3.1 ± 1.9
7c	0.16 ± 0.01	3.2 ± 0.2	8.2 ± 1.0	>50	8.6 ± 3.8
10b	0.24 ± 0.05	3.5 ±0.6	19.4 ± 0.9	>50	1.8 ± 0.6
10d	0.11 ± 0.01	0.34 ± 0.03	5.9 ± 0.3	>50	ND

[a] IC_{50} values are reported as the median and standard deviation of at least three individual determinations.

Supporting Information Available: Experimental details and characterization data for **5**−**10**, BACE1, BACE2, cathepsin-D, and pepsin enzyme; RICA and Aβ cell assay conditions. 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**, *399* (6738), A23−A31.
(2) Sinha, S.; Lieberburg, I. Cellular mechanisms of beta-amyloid production and secretion. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96* (20), 11049−11053.
(3) Wolfe, M. S.; Xia, W.; Ostaszewski, B. L.; Diehl, T. S.; Kimberly, W. T.; Selkoe, D. J. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature* **1999**, *398* (6727), 513−517.

(4) Li, Y. M.; Xu, M.; Lai, M. T.; Huang, Q.; Castro, J. L.; DiMuzio-Mower, J.; Harrison, T.; Lellis, C.; Nadin, A.; Neduvelil, J. G.; Register, R. B.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* **2000**, *405* (6787), 689−694.

(5) Lin, X.; Koelsch, G.; Wu, S.; Downs, D.; Dashti, A.; Tang, J. Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97* (4), 1456−1460.

(6) Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, 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. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **1999**, *286* (5440), 735−741.

(7) 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. E. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* **1999**, *402* (6761), 533−537.

(8) 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 beta-secretase. *Mol. Cell. Neurosci.* **1999**, *14* (6), 419−427.

(9) 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.; Suomensaari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* **1999**, *402* (6761), 537−540.

(10) Ohno, M.; Chang, L.; Tseng, W.; Oakley, H.; Citron, M.; Klein, W. L.; Vassar, R.; Disterhoft, J. F. Temporal memory deficits in Alzheimer's mouse models: rescue by genetic deletion of BACE1. *Eur. J. Neurosci.* **2006**, *23* (1), 251−260.

(11) 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* (3), 233−234.

(12) Luo, Y.; Bolon, B.; Kahn, S.; Bennett, B. D.; Babu-Khan, S.; Denis, P.; Fan, W.; Kha, H.; Zhang, J.; Gong, Y.; Martin, L.; Louis, J. C.; Yan, Q.; Richards, W. G.; Citron, M.; Vassar, R. Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation. *Nat. Neurosci.* **2001** *4* (3), 231−232.

(13) Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S. B.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T. 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.; Sinha, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics. *Hum. Mol. Genet.* **2001**, *10* (12), 1317−1324.

(14) Farzan, M.; Schnitzler, C. E.; Vasilieva, N.; Leung, D.; Choe, H. BACE2, a beta-secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97* (17), 9712−9717.

(15) Rich, D. H. Peptidase Inhibitors. In *Comprehensive Medicinal Chemistry: The Rational Design, Mechanistic Study and Therapeutic Application of Chemical Compounds*; Hansh, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: New York; 1990, pp 391−441.

(16) Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. *Science* **2000**, *290* (5489), 150−153.

(17) 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 beta-secretase. *J. Med. Chem.* **2004**, *47* (1), 158−164.

(18) Hanessian, S.; Yun, H.; Hou, Y.; Yang, G.; Bayrakdarian, M.; Therrien, E.; Moitessier, N.; Roggo, S.; Veenstra, S.; Tintelnot-Blomley, M.; Rondeau, J. M.; Ostermeier, C.; Strauss, A.; Ramage, P.; Paganetti, P.; Neumann, U.; Betschart, C. Structure-based design, synthesis, and memapsin 2 (BACE) inhibitory activity of carbocyclic and heterocyclic peptidomimetics. *J. Med. Chem.* **2005**, *48* (16), 5175−5190.

(19) Hu, J.; Cwi, C. L.; Smiley, D. L.; Timm, D.; Erickson, J. A.; McGee, J. E.; Yang, H. C.; Mendel, D.; May, P. C.; Shapiro, M.; McCarthy, J. R. Design and synthesis of statine-containing BACE inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13* (24), 4335−4339.

(20) Fruton, J. S. The mechanism of the catalytic action of pepsin and related acid proteinases. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1976**, *44*, 1−36.

(21) James, M. N.; Sielecki, A. R.; Hayakawa, K.; Gelb, M. H. Crystallographic analysis of transition state mimics bound to penicillopepsin: difluorostatine- and difluorostatone-containing peptides. *Biochemistry* **1992**, *31* (15), 3872−3886.

(22) Pearl, L.; Blundell, T. The active site of aspartic proteinases. *FEBS. Lett.* **1984**, *174* (1), 96−101.

(23) Suguna, K.; Padlan, E. A.; Smith, C. W.; Carlson, W. D.; Davies, D. R. Binding of a reduced peptide inhibitor to the aspartic proteinase from Rhizopus chinensis: implications for a mechanism of action. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84* (20), 7009−7013.

(24) Guller, R.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Hirth, G.; Jenny, C.; Kansy, M.; Montavon, F.; Muller, M.; Oefner, C.; Stadler, H.; Vieira, E.; Wilhelm, M.; Wostl, W.; Marki, H. P. Piperidine-renin inhibitors compounds with improved physicochemical properties. *Bioorg. Med. Chem. Lett.* **1999**, *9* (10), 1403−1408.

(25) Bursavich, M. G.; West, C. W.; Rich, D. H. From peptides to non-peptide peptidomimetics: design and synthesis of new piperidine inhibitors of aspartic peptidases. *Org. Lett.* **2001**, *3* (15), 2317−2320.

(26) Bursavich, M. G.; Rich, D. H. Designing non-peptide peptidomimetics in the 21st century: inhibitors targeting conformational ensembles. *J. Med. Chem.* **2002**, *45* (3), 541−558.

(27) Nevar, N. M.; Kel'in, A. V.; Kulinkovich, O. G. One step preparation of 1,4-diketones from methyl ketones and a-bromomethyl ketones in the presence of $ZnCl_2$•*t*-BuOH. Et_2NR as a condensation agent. *Synthesis* **2000**, *9*, 1259−1262.

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