

A Potent and Orally Efficacious, Hydroxyethylamine-Based Inhibitor of β -Secretase

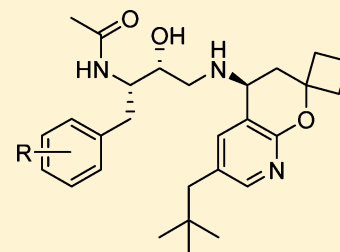
Matthew R. Kaller,^{*,†} Scott S. Harried,^{*,†} Brian Albrecht,^{#,∇} Patricia Amarante,[†] Safura Babu-Khan,[§] Michael D. Bartberger,[‡] James Brown,[†] Ryan Brown,^{#,∞} Kui Chen,^{||} Yuan Cheng,[†] Martin Citron,^{§,¥} Michael D. Croghan,[†] Russell Graceffa,[#] Dean Hickman,[⊥] Ted Judd,[†] Chuck Kriemen,[#] Daniel La,[#] Vivian Li,[‡] Patricia Lopez,[†] Yi Luo,[§] Craig Masse,^{#,○} Holger Monenschein,^{||} Thomas Nguyen,[†] Lewis D. Pennington,[†] Tisha San Miguel,^{||} E. Allen Sickmier,[‡] Robert C. Wahl,^{||} Matthew M. Weiss,[#] Paul H. Wen,[§] Toni Williamson,[§] Stephen Wood,[§] May Xue,[†] Bryant Yang,[†] Jianhua Zhang,[§] Vinod Patel,^{#,+} Wenge Zhong,[†] and Stephen Hitchcock^{†,||}

[†]Chemistry Research and Discovery, [‡]Department of Molecular Structure, [§]Department of Neuroscience, ^{||}Department of HTS and Molecular Pharmacology, and [⊥]Department of Pharmacokinetics and Drug Metabolism, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, United States

[#]Chemistry Research and Discovery, Amgen Inc., One Kendall Square Building 1000, Cambridge, Massachusetts 02139, United States

Supporting Information

ABSTRACT: β -Secretase inhibitors are potentially disease-modifying treatments for Alzheimer's disease. Previous efforts in our laboratory have resulted in hydroxyethylamine-derived inhibitors such as **1** with low nanomolar potency against β -site amyloid precursor protein cleaving enzyme (BACE). When dosed intravenously, compound **1** was also shown to significantly reduce $A\beta_{40}$ levels in plasma, brain, and cerebral spinal fluid. Herein, we report further optimizations that led to the discovery of inhibitor **16** as a novel, potent, and orally efficacious BACE inhibitor.



KEYWORDS: β -site amyloid precursor protein cleaving enzyme (BACE), Alzheimer's disease (AD), hydroxyethylamine (HEA) isostere

The need for a disease-modifying treatment for Alzheimer's disease (AD) continues to grow. In the United States alone, the number of cases exceeds 5 million, and AD is the seventh leading cause of death.¹ The disease is characterized by the progressive loss of cognitive ability, dementia, and eventually death. Extracellular amyloid plaques, largely comprised of $A\beta_{40-42}$ peptides, and intracellular neurofibrillary tangles are the defining pathological characteristics of AD. $A\beta_{40-42}$ is a product of the cleavage of amyloid precursor protein (APP) by β -secretase and γ -secretase. The cloning of the β -site amyloid precursor protein cleaving enzyme (BACE) enzyme and its critical role in the amyloid cascade² have led to the hypothesis that BACE inhibitors are potential disease-modifying treatments for AD.³⁻⁵ During the past decade, numerous pharmaceutical companies have committed considerable resources to develop BACE inhibitors.^{6,7} In this letter, we will describe our effort toward the design and synthesis of a novel, potent, and orally efficacious BACE inhibitor.

Previous work in our laboratories revealed hydroxyethylamine (HEA)-derived compound **1** (Figure 1) to be a potent BACE inhibitor.⁸ On the basis of X-ray analysis, the HEA transition state isostere binds to the two catalytic aspartyl residues in the expected fashion. The *N*-cyclopentyl-3-(2-pyridyl) pyridone amide moiety of **1** provides interactions with

the S2 and S3 binding pockets of BACE, and the 6-ethyl 2,2-spirocyclobutylchromane engages in hydrophobic interactions with the S2' and S1' binding pockets. Compound **1** exhibited potent cellular activity (8.8 nM); however, it also displayed poor metabolic stability (mouse liver microsomes = 568 μ L/min/mg), and its measured *in vivo* clearance was greater than liver blood flow (mouse *iv*, 2 mg/kg, 5.6 L/h/kg). Despite these liabilities, when dosed intravenously in Tg2576 transgenic mice⁹ (30 mg/kg, 4 h time point), compound **1** significantly reduced $A\beta_{40}$ levels by 91, 58, and 57% in plasma, brain, and cerebral spinal fluid (CSF), respectively.

Although compound **1** displays impressive enzymatic inhibition, it is not an efficient inhibitor of BACE (BE = 0.24)¹⁰ and has a relatively high molecular weight of 647 amu. It also has a large total polar surface area (PSA), high clog *P*, and the number of H-bond donors and acceptors are not ideal for a target located in the CNS.¹¹ To optimize the properties of **1**, guided by the well-established binding models, we reasoned

Special Issue: Alzheimer's Disease

Received: January 13, 2012

Accepted: March 29, 2012

Published: March 29, 2012

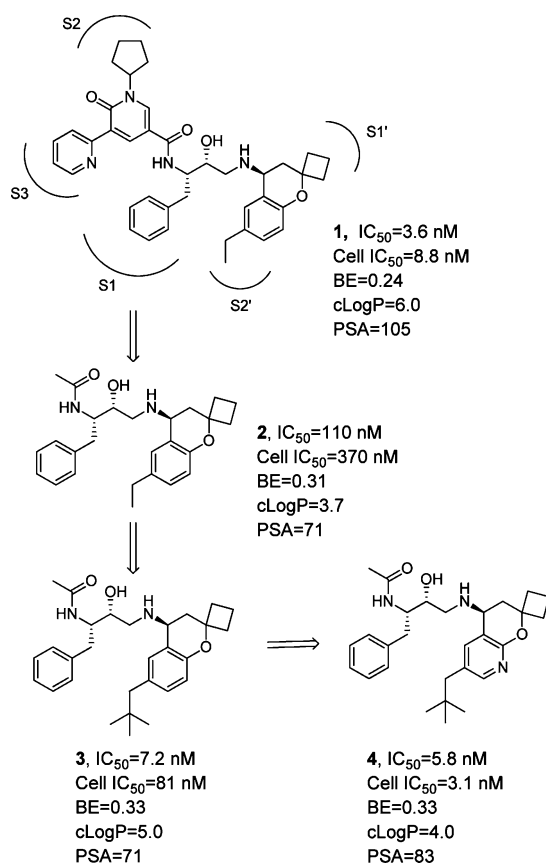


Figure 1. Truncating the amide of **1** and optimizing P2' led to BACE inhibitor **4** (S1–S2' indicate BACE binding domains).

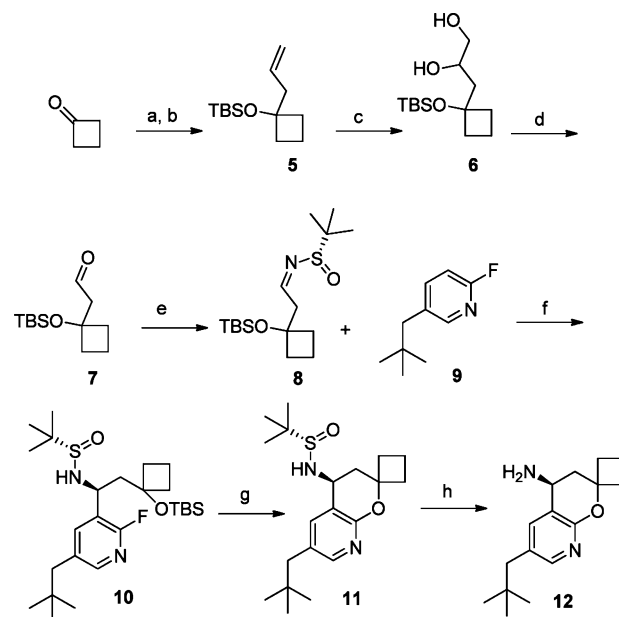
that the HEA core had to be kept intact to properly engage the catalytic aspartic residues of BACE. We decided to target the pyridone and chromane moieties of **1** as the most likely regions to provide meaningful changes to the physicochemical properties of **1**, while maintaining high cellular potency against BACE. A major advancement in our early structure–activity relationship (SAR) was the identification of the highly truncated acetamide analogue **2**.¹² Despite the removal of P2 and P3 interactions with the enzyme, acetamide **2** retained modest activity but with higher binding efficiency (BE = 0.31), thanks to the significant decrease in molecular weight (224 amu). In addition, the apparent permeability of **2** was greatly improved over **1** by 5-fold (14×10^{-6} cm/s, LLC-PK1), which was of particular importance for CNS penetration.

To further improve enzyme potency, insights from X-ray cocrystal structures and molecular modeling suggested that an ethyl group in the highly hydrophobic S2' binding pocket was probably not optimal. Modifying **2** to better fill the S2' pocket with larger hydrophobic substituents led to the neopentyl chromane derivative **3** as a potent BACE inhibitor (IC_{50} = 7.2 nM). Upon examination of modeled structures, we realized that the 8-position of the chromane moiety is open to a solvent-exposed area, suggesting that introduction of some polarity would not be detrimental to enzyme potency but could modulate physicochemical properties. We thus incorporated a nitrogen atom into the chromane at the 8-position. Gratifyingly, we found that such a subtle structural variation resulted in compound **4**, which not only was equipotent as compound **3** in the enzyme assay but also displayed potent cellular activity (cell IC_{50} , 3.1 nM). We next turned our attention to improving

compound intrinsic stability in liver microsomes as a means to improve in vivo clearance in rodents. This communication will describe our efforts in detail on further optimization of **4**, particularly modifications to the P1 substituent, leading ultimately to the discovery of **16** as a novel, potent, and orally bioavailable efficacious BACE inhibitor.

The synthesis of **4** and analogues **16**–**33** began with commercially available cyclobutanone (Scheme 1). The

Scheme 1. Synthesis of Azachromane Intermediate **12**^a



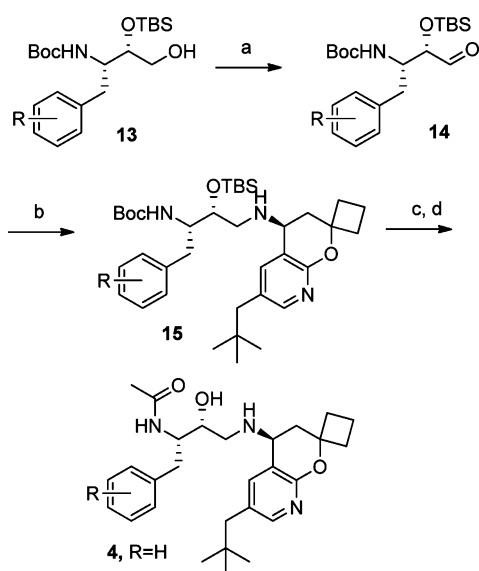
^aReagents and conditions: (a) Allylmagnesium bromide, THF, -78 °C. (b) TBSOTf, TEA, DCM, 0 °C. (c) OsO₄, NMO, H₂O:*t*-BuOH:THF (1:1:0.03). (d) NaIO₄, THF:*t*-BuOH:H₂O (1:1:2). (e) CuSO₄, (*R*)-2-methylpropane-2-sulfinamide, DCM. (f) *n*BuLi, 2,2,6,6-tetramethylpiperidine, THF, -78 °C. (g) CsF, DMSO, 120 °C. (h) 4 M HCl, DCM.

addition of allylmagnesium bromide to cyclobutanone, followed by protection of the secondary alcohol with *tert*-butyldimethylsilyl triflate, gave the silyl ether **5**. Dihydroxylation was effected with osmium tetroxide and *N*-methylmorpholine-*N*-oxide to give diol **6**, which was then treated with sodium periodate to afford aldehyde **7**. The sulfinylimine **8** was formed by condensation with Ellman's chiral *tert*-butylsulfinamide auxiliary with copper sulfate.¹³ The fluoropyridine **9** was produced via a palladium-catalyzed zinc cross-coupling between commercially available reagents 2-fluoro-5-bromopyridine and neopentylzinc chloride. Fluoropyridine **9** was regioselectively deprotonated with lithium tetramethylpiperidide at the 3-position and then treated with sulfinylimine **8** to give the sulfinylamide adduct **10** in good yield with moderate diastereoselectivity (2:1) in favor of the desired diastereomer. Silyl ether deprotection of **10** with concurrent cyclization in the presence of cesium fluoride in DMSO at 120 °C furnished azachromane **11**, which was readily separated from the minor diastereomer via column chromatography. The chiral auxiliary was then smoothly cleaved under acidic conditions to give enantiomerically pure azachromane amine **12**.

We synthesized HEAs **2** and **3** (Figure 1) following well-established literature procedures that utilize a nucleophilic opening of an amino acid-derived epoxide with the appropriate

chromane amine.⁸ We discovered that this methodology was sluggish and inefficient when an 8-azachromane amine was used as the nucleophile in this alkylation reaction. Several attempts to improve this epoxide opening with azachromane **12**, including using Lewis acid additives, did not provide synthetically useful yields. Therefore, a reductive amination approach, one that circumvents the poor nucleophilic reactivity of **12**, was developed to allow efficient coupling between the azachromane amine **12** with a suitable aldehyde **14** (Scheme 2).¹⁴ To

Scheme 2. Synthesis of BACE Inhibitors 16–32^a



^aReagents and conditions: (a) Dess–Martin periodinane, Na₂CO₃, DCM. (b) Compound **12**, NaBH(OAc)₃, trimethylorthoformate, DCM. (c) 4 M HCl, MeOH, 50 °C. (d) 1-(1*H*-imidazol-1-yl)ethanone, DIPEA, DMF.

complete the synthesis of inhibitors **4** and **16–32**, the alcohol **13**¹⁵ was oxidized with Dess–Martin periodinane to give aldehyde **14**. Sodium triacetoxyborohydride-promoted¹⁶ reductive amination gave a very smooth and highly efficient coupling reaction to provide adduct **15** in excellent yields. It is noteworthy that no epimerization of aldehyde **14** was observed in either the oxidation or the reductive amination sequence. Global deprotection of **15** was effected with HCl in methanol to give the penultimate hydroxyl diamine, which was then selectively acylated with acyl imidazole to give acetamides **4** and **16–32**.

Metabolic identification studies of **4** in the presence of rat liver microsomes revealed that oxidation on the P1 ring and P2' side chain was the major sites of oxidative metabolism. We surmised that the poor metabolic stability was one of the sources of high in vivo clearance (rat iv, 2 mg/kg, 5.3 L/h/kg). We explored modifications on the P1 aryl ring to address this metabolic soft spot of inhibitor **4**. The results are summarized in Table 1.

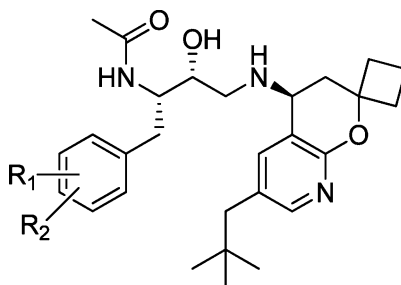
In general, we found that a wide range of substituents on the P1 aryl ring were well tolerated in terms of potency against the BACE enzyme. The first modification was to substitute a hydrogen atom with a single fluorine atom at different positions around the aryl ring. Placing a fluorine atom at the 4-position of the aryl ring (**16**) provided good potency and permeability. Moving the fluorine to the meta position (**17**) improved the potency 5-fold but was detrimental to stability in human liver

microsomes (621 μL/mL/min). Moving the fluorine to the 2-position (**18**) provided good potency, but this compound was also not stable in human liver microsomes (342 μL/mL/min). Several alternative substitutions were tried at the 4-position of the aryl ring, such as chlorine and trifluoromethoxy (**19** and **20**), but both were inferior to fluorine in terms of potency and metabolic stability. Substituting at both the 3- and the 5-positions with fluorine atoms (**21**) was consistent with single substitution with good potency but poor metabolic stability. Substituting trifluoromethyl for fluorine (**22**) in the 3-position of the P1 phenyl group provided improved potency over **16** but suffered from poor human microsomal stability and reduced permeability. Placing fluorine atoms at both the 2- and the 4-positions (**23**) resulted in modest enzyme potency, but the increased lipophilicity led to a larger enzyme to cell shift. Realizing that fluorine at the 4-position provided some stability and that the 3-position provided some improved potency, various substituents were tried at the 3-position in combination with the 4-fluoro substituent (**24–29**). The compounds were all comparable to **16** in potency, but almost all demonstrated poor stability in human liver microsomes. Other substituents at the 4-position (**30–32**) with fluorine at the 3-position generally provided good potency but failed to improve the metabolic stability of the molecules. These substituted P1 analogues have good potency and good permeability but generally suffer from poor in vitro microsomal stability (rat and human).

On the basis of favorable permeability and moderate in vitro microsomal stability, **16** was selected for further profiling. Pharmacokinetic analysis of **16** showed that in vivo clearance in rat was 1.2 L/h/kg, Vd_{ss} was 1.6 L/kg, and T_{1/2} was 7.2 h following an iv dose (2 mg/kg). Oral bioavailability was measured at 40% based on a 10 mg/kg dose.

Compound **16** was evaluated in a rat pharmacodynamic (PD) model. The model allowed for the measurement of acute reductions in soluble Aβ₄₀ in plasma, brain, and CSF. The compound was administered at 100 mg/kg orally as a suspension in 2% HPMC, 1% Tween-80 at pH 2.0 to wild-type male Sprague–Dawley rats. Samples were collected from plasma, brain, and CSF 4 h after dosing.¹⁷ Compound **16** showed Aβ₄₀ level reductions of 71, 32, and 35% in plasma, brain, and CSF, respectively (Figures 2–4). Concentrations of **16** at this time point were measured at 4.3, 0.66, and 0.006 μM, respectively, for the plasma, brain, and CSF. On the basis of the rat plasma protein binding (98.5%) of **16**, the estimated unbound concentration was 64 nM in plasma and 10 nM in brain. The estimated unbound brain concentration is similar to the measured CSF concentration. The reduction in Aβ₄₀ levels was consistent with the exposure levels and the in vitro cellular IC₅₀.

The binding of mode of compound **16** was determined by X-ray crystallography and is depicted in Figure 5.¹⁹ The hydroxyl group and basic amine of the HEA core engage in the expected contacts with the two catalytic aspartyl residues Asp32 and Asp228, respectively. The latter amine–Asp interaction is augmented by a hydrogen bond between the amine and the carbonyl oxygen of Gly34 (not shown.) Hydrogen-bonding interactions involving the acetamide moiety of the inhibitor and both the underside C=O of Gly230 and the backbone NH of flap Thr72, along with a CH...O type contact (ca. 3.4 Å) between C5 of the azachromane group and the C=O of Gly34, round out the additional, anchoring polar contacts between the inhibitor and the active site of BACE. The 4-fluoro substituent on the P1 aryl ring, although likely experiencing a somewhat

Table 1. SAR of Phenyl Ring Substituents^a

compd ^b	R ¹	R ²	BACE IC ₅₀ (nM) ^c	cell IC ₅₀ (nM) ^a	μL/min/mg ^d		
					RLM	HLM	LLC-PK1 P _{app} (×10 ⁻⁶ cm/s)
4	H	H	5.8 ± 6.3	3.1 ± 4.2	241	465	22
16	4-F	H	5.0 ± 3.0	12 ± 6.1	180	107	21
17	3-F	H	1.0 ± 0.47	1.8 ± 1.2	227	621	16
18	2-F	H	3.6 ± 0.86	5.1 ± 4.3	206	342	13
19	4-Cl	H	8.3 ± 2.7	13 ± 9.1	160	443	21
20	4-OCF ₃	H	14 ± 1.3	65 ± 4.3	101	181	
21	3-F	5-F	6.9 ± 8.7	6.6 ± 7.5	186	921	15
22	3-CF ₃	H	2.4 ± 0.97	4.0 ± 1.6	179	308	12
23	4-F	2-F	6.5 ± 5.3	18 ± 0.41	173	71	
24	4-F	3-F	2.0	2.4 ± 1.1			16
25	4-F	3-CH ₃	4.0 ± 3.0	4.6 ± 2.6	>399	829	15
26	4-F	3-CF ₃	11 ± 14	18 ± 8.8	116	134	12
27	4-F	3-OCF ₃	2.1	2.6 ± 1.8	220	360	8.6
28	4-F	3-CN	2.4 ± 0.06	4.5 ± 1.1	185	188	
29	4-F	3-OCH ₃	4.5 ± 0.99	7.6 ± 3.4	165	628	20
30	4-CH ₃	3-F	0.99 ± 0.37	2.2 ± 0.82	155	>399	18
31	4-OCH ₃	3-F	3.6 ± 0.73	8.5 ± 6.4	258	162	28
32 ^c	4-CF ₃	3-F	44 ± 11	160	121	443	16

^aAll values lacking standard deviations were measured as single data points. ^bAll compounds were >95% pure by HPLC and characterized by ¹H NMR and HRMS. ^cCompound is mixture of diastereomers (1:1, 2*S*,3*R* and 2*R*,3*S*). ^dCompounds are incubated with microsomes for 30 min at a concentration of 1 μM.

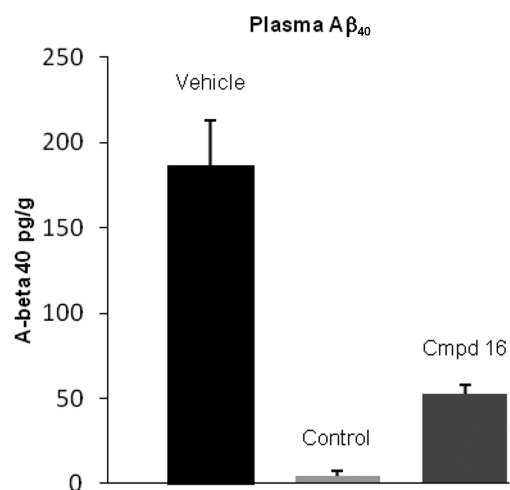


Figure 2. PD study of 16 in rat, plasma Aβ₄₀ concentration (100 mg/kg po, 4 h postdose, *P* < 0.05). A literature γ-secretase inhibitor¹⁸ was used as a positive control (10 mg/kg, po).

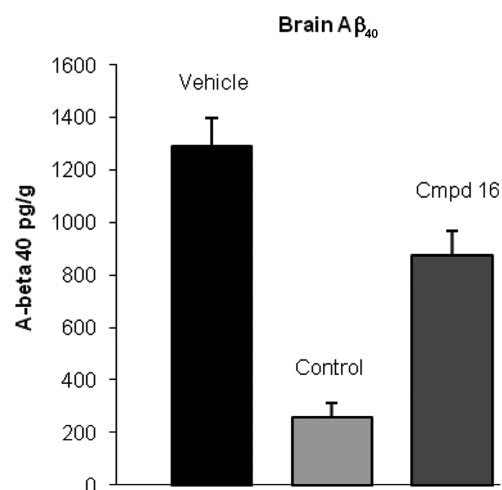


Figure 3. PD study of 16 in rat, brain Aβ₄₀ concentration (100 mg/kg po, 4 h postdose, *P* < 0.05). A literature γ-secretase inhibitor¹⁸ was used as a positive control (10 mg/kg, po).

repulsive interaction with the backbone carbonyl of Phe108, interacts with the edge of Trp115 and also likely helps to augment the attractive, edge-to-face interaction between the fluorophenyl substituent and the electron-rich π face of flap Tyr71. Interactions between the azachroman with Tyr198 (ca. 4.1 Å; not marked), occupation of S1' by the spirocyclobutyl

moiety, and occupation of the large S2' cavity by the hydrophobic neopentyl group provide additional contacts contributing to the high binding affinity of 16.

In summary, starting from 1, we have improved several key parameters to help increase the likelihood of achieving CNS penetration. Compound 16 has a lower molecular weight (163

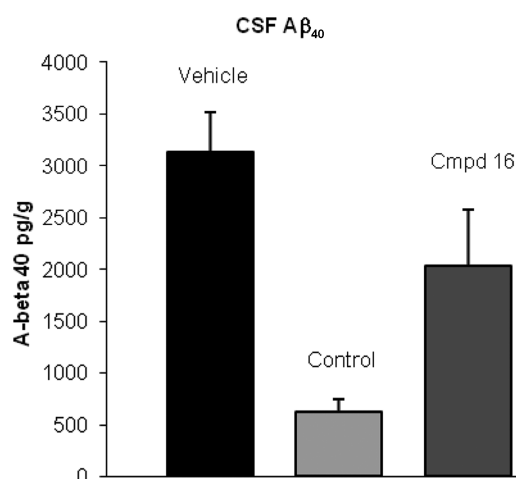


Figure 4. PD study of 16 in rat, CSF Aβ₄₀ concentration (100 mg/kg po, 4 h postdose, $P < 0.05$). A literature γ -secretase inhibitor¹⁸ was used as a positive control (10 mg/kg, po).

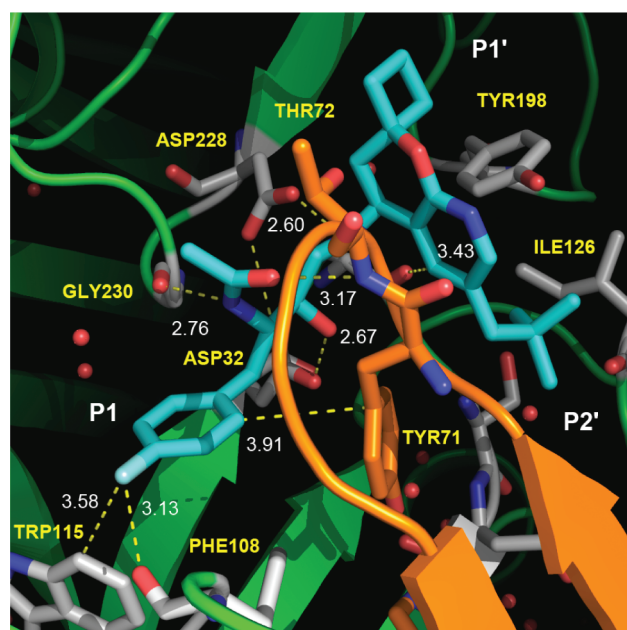


Figure 5. Crystal structure of inhibitor 16 bound to the active site of BACE.

amu), fewer hydrogen bond donors and acceptors, and a lower PSA (from 105 to 83 Å²) as compared to inhibitor 1. We have explored the S1 pocket of BACE with several analogues, with compound 16 having the best profile. Oral administration of inhibitor 16 in a rat PD model demonstrated statistically significant lowering of Aβ_{40–42} peptide.

■ ASSOCIATED CONTENT

Supporting Information

Procedures and characterization for all newly prepared compounds, coordinates for the cocrystal structure of inhibitor 16 with BACE, and experimental procedures for the enzyme and cellular assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 805-447-8004. E-mail: mkaller@amgen.com (M.R.K.) and harried@gmail.com (S.S.H.).

Present Addresses

[∇]Constellation Pharmaceuticals, 215 First Street, Suite 200, Cambridge, Massachusetts 02142, United States.

[○]Nimbus Discovery, 25 First Street, Suite 303, Cambridge, Massachusetts 02141, United States.

[¶]Envoy Therapeutics, 555 Heritage Drive, Jupiter, Florida 33458, United States.

⁺Sanofi-Aventis, 270 Albany Street, Cambridge, Massachusetts 02139, United States.

[‡]Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, United States.

Notes

The authors declare no competing financial interest.

[∞]Deceased.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the Advanced Light Source. The Advanced Light Source is supported by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.

■ REFERENCES

- http://www.alz.org/documents_custom/report_alzfactsfigures2010.pdf.
- Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiáz, 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. β -Secretase Cleavage of Alzheimer's Amyloid Precursor Protein by the Transmembrane Aspartic Protease BACE. *Science* **1999**, *286*, 735–741.
- Hardy, J.; Selkoe, D. J. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science* **2002**, *297*, 353–356.
- Tanzi, R. E.; Bertram, L. Twenty Years of the Alzheimer's Disease Amyloid Hypothesis: A Genetic Perspective. *Cell* **2005**, *120*, 545–555.
- De Strooper, B.; Vasar, R.; Golde, T. The secretases: Enzymes with therapeutic potential in Alzheimer's disease. *Nature Rev. Neurol.* **2010**, *6*, 99–107.
- Silvestri, R. Boom in the development of non-peptidic beta-secretase (BACE1) inhibitors for the treatment of Alzheimer's disease. *Med. Res. Rev.* **2008**, *29*, 295–338.
- Stachel, S. J. Progress toward the development of a viable BACE-1 inhibitor. *Drug Dev. Res.* **2009**, *70*, 101–110.
- Lopez, P.; Zhong, W.; Albrecht, B.; Amarante, P.; Andersen, D.; Babu-Khan, S.; Bartberger, M. D.; Brown, J.; Chen, K.; Cheng, Y.; Citron, M.; Croghan, M. D.; Graceffa, R.; Harried, S. S.; Hickman, D.; Hungate, R.; Jordan, S.; Kaller, M. R.; Kriemen, C.; La, D.; Luo, Y.; San Miguel, T.; Monenschein, H.; Nguyen, T.; Nixey, T.; Pennington, L. D.; Wahl, R. C.; Weiss, M. M.; Wood, S.; Xue, M.; Yang, B.; Yan, Q.; Zhang, J.; Zhang, J.; Patel, V.; Hitchcock, S. Optimization of P1' Interactions in a Series of Hydroxyethylamine Derived Small Molecule BACE1 Inhibitors. Manuscript in preparation.
- Lanz, T. A.; Himes, C. S.; Pallante, G.; Adams, L.; Yamazaki, S.; Amore, B.; Merchant, K. M. The γ -secretase inhibitor *N*-[*N*-(3,5-difluorophenacetyl)-*L*-alanyl]-*S*-phenylglycine *t*-butyl ester reduces Aβ₄₂ levels in vivo in plasma and cerebrospinal fluid in young (plaque-free) and aged (plaque-bearing) Tg2576 mice. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 864–871.
- Reynolds, C. H.; Tounge, B. A.; Bembek, S. D. Ligand Binding Efficiency: Trends, Physical Basis, and Implications. *J. Med. Chem.* **2008**, *51*, 2432–2438 and references therein.

(11) Hitchcock, S. A.; Pennington, L. D. Structure-Brain Exposure Relationships. *J. Med. Chem.* **2006**, *49*, 7559–7583.

(12) Maillard, M.; Baldwin, E. T.; Beck, J. T.; Hughes, R. T.; Varghese, J.; Pulley, S. R.; Tenbrink, R. Acetyl 2-Hydroxy-1,3-diaminoalkanes. PCT Int. Appl. WO 2004024081, 2004.

(13) Ellman, J. A.; Owens, T. D.; Tang, T. P. *N*-tert-Butanesulfinyl Imines: Versatile Intermediates for the Asymmetric Synthesis of Amines. *Acc. Chem. Res.* **2002**, *35*, 984–995.

(14) Parkes, K. E. B.; Bushnell, D. J.; Crackett, P. H.; Dunsdon, S. J.; Freeman, A. C.; Gunn, M. P.; Hopkins, R. A.; Lambert, R. W.; Martin, J. A.; Merret, J. H.; Redshaw, S.; Spurden, W. C.; Thomas, G. J. Studies toward the large-scale synthesis of the HIV proteinase inhibitor Ro 31-8959. *J. Org. Chem.* **1994**, *59*, 3656–3664.

(15) Harried, S. S.; Croghan, M. D.; Kaller, M. R.; Lopez, P.; Zhong, W.; Hungate, R.; Reider, P. J. Stereoselective Synthesis of anti-*N*-Protected 3-Amino-1,2-epoxides by Nucleophilic Addition to *N*-tert-Butanesulfinyl Imine of a Glycerinaldehyde Synthone. *J. Org. Chem.* **2009**, *74*, 5975–5982.

(16) Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. Reductive Amination of Aldehydes and Ketones with Sodium Triacetoxyborohydride. Studies on Direct and Indirect Reductive Amination Procedures. *J. Org. Chem.* **1996**, *61*, 3849–3862.

(17) Drug levels were quantified using the standard LC/MS/MS methods. A β 40 levels were quantified using ELISA-based Meso Scale Discovery (MSD) technology.

(18) Example 7-B [(*S*)-2-hydroxy-3-methyl-*N*-((*S*)-1-(((*S*)-5-methyl-6-oxo-6,7-dihydro-5*H*-dibenzo[*b,d*]azepin-7-yl)amino)-1-oxopropan-2-yl)butanamide]. Wu, J.; Tung, J. S.; Thorsett, E. D.; Pleiss, M. A.; Nissen, J. S.; Neitz, J.; Latimer, L. H.; Varghese, J.; Freedman, S.; Britton, T. C.; Audia, J. E.; Reel, J. K.; Mabry, T. E.; Dressman, B. A.; Cwi, C. L.; Droste, J. J.; Henry, S. S.; McDaniel, S. L.; Scott, W. L.; Stucky, R. D.; Porter, W. J. Cycloalkyl, lactam, lactone and related compounds, pharmaceutical compositions comprising same, and methods for inhibiting β -amyloid peptide release and/or its synthesis by use of such compounds. International Patent Application WO 98/28268, 1998.

(19) Coordinates for the complex of BACE with inhibitor **16** have been deposited in the Protein Data Bank under PDB ID code 4DUS.