Bioorganic & Medicinal Chemistry Letters 23 (2013) 2181–2186

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure-based design of novel dihydroisoquinoline BACE-1 inhibitors that do not engage the catalytic aspartates

Simeon Bowers [a,*], Ying-zi Xu [a], Shendong Yuan [a], Gary D. Probst [a], Roy K. Hom [a], Wayman Chan [a], Andrei W. Konradi [a], Hing L. Sham [a], Yong L. Zhu [b], Paul Beroza [b], Hu Pan [b], Eric Brecht [b], Nanhua Yao [b], Julie Lougheed [b], Danny Tam [b], Zhao Ren [b], Lany Ruslim [b], Michael P. Bova [b], Dean R. Artis [b]

[a] Department of Chemical Sciences, Elan Pharmaceuticals, 180 Oyster Point Boulevard, South San Francisco, CA 94080, USA
[b] Department of Molecular Design, Elan Pharmaceuticals, 180 Oyster Point Boulevard, South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 16 November 2012
Revised 16 January 2013
Accepted 22 January 2013
Available online 4 February 2013

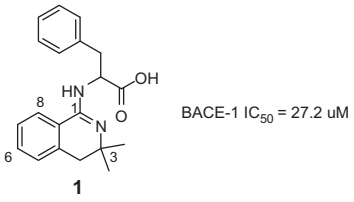
Keywords:
Alzheimer's disease
Beta-secretase
BACE-1 inhibitor

ABSTRACT

The structure–activity relationship of a series of dihydroisoquinoline BACE-1 inhibitors is described. Application of structure-based design to screening hit **1** yielded sub-micromolar inhibitors. Replacement of the carboxylic acid of **1** was guided by X-ray crystallography, which allowed the replacement of a key water-mediated hydrogen bond. This work culminated in compounds such as **31**, which possess good BACE-1 potency, excellent permeability and a low P-gp efflux ratio.

Alzheimer's disease (AD) is the leading cause of dementia and is characterized by a slow progressive loss of memory and cognitive ability.[1] Pathologically, AD manifests itself by the formation of extracellular insoluble amyloid plaques composed of -amyloid (A) peptide.[2] Aβ-peptides are generated by the proteolytic cleavage of the β-amyloid precursor protein (β-APP) by two aspartic acid proteases, referred to as β-secretase (BACE-1) and γ-secretase, respectively.[3] BACE-1 knockout mice are healthy, and fertile,[4] but because they lack compensatory activity, are unable to generate A in the brain,[5] which suggests that BACE-1 would be an attractive therapeutic target for the development of disease modifying treatments for AD.[6] Consequently, BACE-1 inhibitors have been intensely investigated by many laboratories as potential AD modifying therapeutics.[7,8]

As part of a new high throughput screen/high throughput crystallization campaign (HTS/HTX), we sought to develop an assay for compound binding that would be more sensitive than our fluorescence polarization functional screen and be better suited to a high-concentration screening approach. We reasoned that an Alpha-Screen format with an avidity component, utilizing a probe that bound to the active site and did not displace the central water between the two aspartic acids, would provide the needed characteristics.[8j] A satisfactory probe was developed from a member of the hydantoin class of BACE-1 inhibitors, which, when functionalized with a biotinylated small PEG linker, gave an approximately 10-fold boost in assay sensitivity.[9] Approximately 2000 hits from a 500 μM HTS screen of 500,000 compounds were crystallographically screened to identify a BACE-1 inhibitor complex with a novel structure. Of the compounds identified in this screen, dihydroisoquinoline **1** (Fig. 1) was intriguing.



1

Figure 1. Screening hit.

Although it had modest potency against BACE-1 (IC_{50} = 27.2 μM), the crystal structure revealed an interesting binding mode (Fig. 2). The (*S*)-enantiomer of compound **1** occupies the non-prime region of BACE-1 and interestingly, does not interact with the catalytic aspartates or the catalytic water molecule. The carboxylic acid of compound **1** engages in one direct hydrogen bond with BACE-1 to the backbone NH of Thr293 and also engages in a water-mediated interaction with the backbone NH of Asn294. Furthermore, the carboxylic acid also engages in an intramolecular hydrogen bond with the amine at the 1-position of the dihydroisoquinoline. The phenyl ring points towards the S3 pocket and could

* Corresponding author.
E-mail address: simeongbowers@gmail.com (S. Bowers).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.01.103

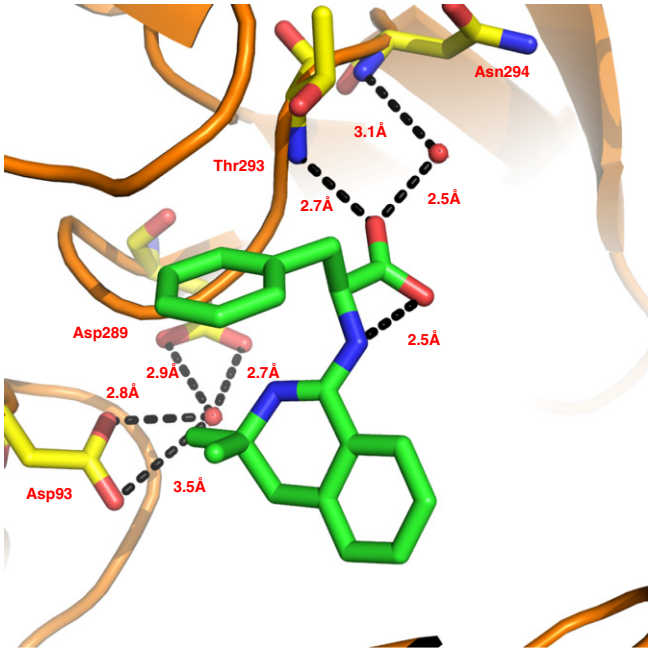


Figure 2. Crystal structure of compound **1** in green bound to BACE-1 (1.9 Å resolution). The catalytic aspartates are shown behind the inhibitor and do not directly interact with it. The catalytic water molecule is also shown. The hydrogen bond network of the inhibitor's carboxylate group, two backbone NH groups, and a water molecule is shown with dotted lines along with the inhibitor's intramolecular hydrogen bond. The PDB deposition code is 4I11. For experimental conditions see Ref. 10.

possibly provide an opportunity to improve the potency of the inhibitors.

Compound design was guided by molecular dynamics simulation. The Molecular Mechanics-Possion Boltzmann Surface Area (MM-PBSA) method was used to estimate binding energies for new analogs.[11] Binding energies were calculated for compounds that were synthesized early in the project, and this yielded a correlation with in vitro potency (Fig. 3). Subsequently, the regression could be used to estimate the potency of designed ligands; those that had more favorable calculated binding energies were prioritized for synthesis.

Molecular modeling suggested that a small pocket in the region of the 6-position of the dihydroisoquinoline ring could accommodate a small hydrophobic substituent such as methyl or chlorine

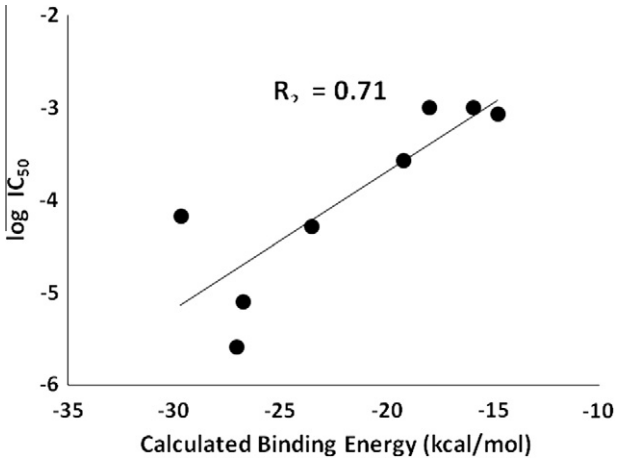


Figure 3. Correlation between calculated binding energy and potency.

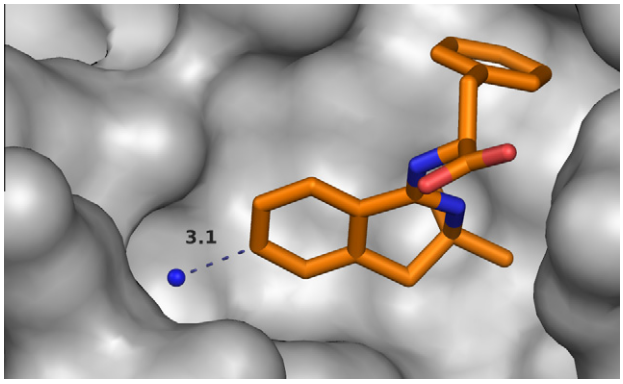
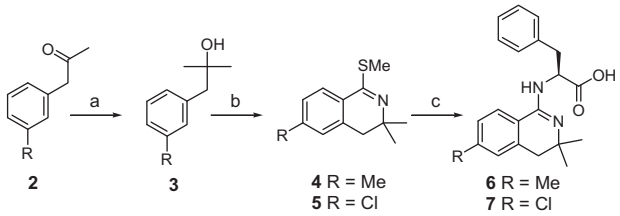


Figure 4. The small hydrophobic pocket at the 6-position of the dihydroisoquinoline ring. The center of the pocket is 3.1 Å from the ring.



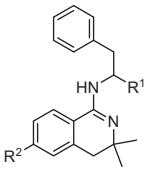
Scheme 1. Reagents and conditions: (a) MeMgBr, THF, −78 °C, 67%; (b) MeSCN, H_2SO_4, rt, 54%; (c) L-Phe, DMSO, 120 °C, 30%.

Table 1
SAR of the 6-position

Compounds	R	BACE-1 IC_{50}[a] (μM)
1[b]	H	27.2
6	Me	5.41
7	Cl	2.84

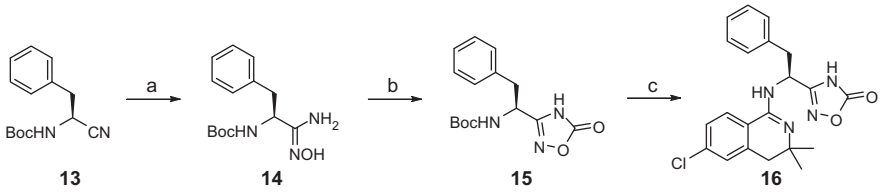
[a] See Ref. 9.
[b] Compound is racemic.

Table 2
SAR of heterocyclic carboxylic acid replacements



Compounds	R^1	R^2	BACE-1 IC_{50}[a] (μM)
1	COOH	H	27.2
8		H	638.6
9		Cl	184.9
10		Cl	339.7
11		Cl	137.1
12		Cl	>250

[a] See Ref. 9.



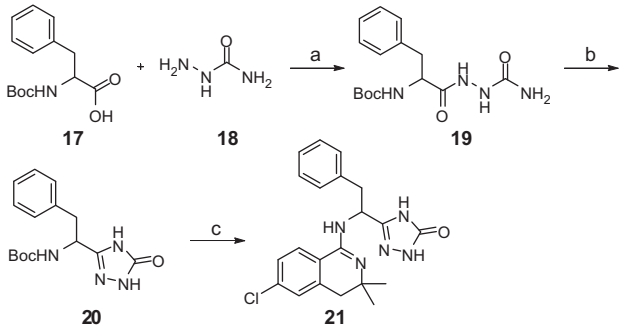
Scheme 2. Reagents and conditions: (a) NH_2OH (aq), EtOH, rt, 86%; (b) CDI, THF, reflux, 81%; (c) (i) TFA, CH_2Cl_2, rt, (ii) **5**, DMSO, 100 °C, 15%.

(Fig. 4). These analogs were readily prepared by the addition of methyl magnesium bromide to an appropriate aryl propanone **2**. Subsequent treatment with methyl thiocyanate and sulfuric acid gave the key intermediates **4** and **5**, which were heated with L-phenylalanine to give compounds **6** and **7** (Scheme 1).

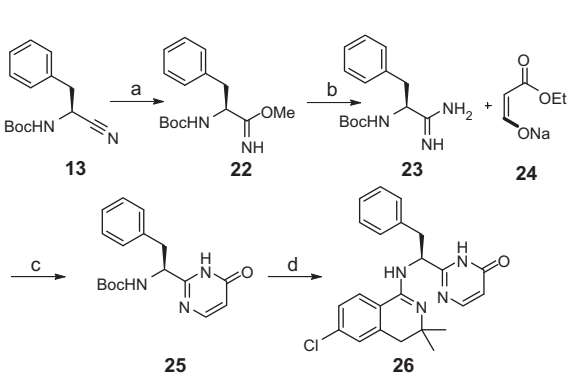
The BACE-1 alpha assay results for these analogs are shown in Table 1. Gratifyingly, both the 6-methyl analog (**6**) and 6-chloro analog (**7**) possessed improved potency against BACE-1 compared to the screening hit **1**.

Although the addition of substituents to the 6-position of the dihydroisoquinoline ring provided significant gains in potency, the major challenge remained the replacement of the carboxylic acid to increase the chances of CNS penetration.[12] As mentioned previously, this moiety interacts with the backbone NH of Thr293 and, via a water molecule, the backbone NH of Asn294. Furthermore, it also engages in an intramolecular hydrogen bond with the amine at the 1-position of the dihydroisoquinoline. Successful replacement of the carboxylic acid would likely require these key interactions. With this in mind, a series of acid replacements was designed. Tetrazoles are well known as carboxylic acid isosteres[12b] and were the first replacements to be prepared. Additionally, a small number of both five-membered and six-membered heterocycles were prepared that could present hydrogen bond acceptors in the same manner as the carboxylic acid. The results of these initial efforts are documented in Table 2.

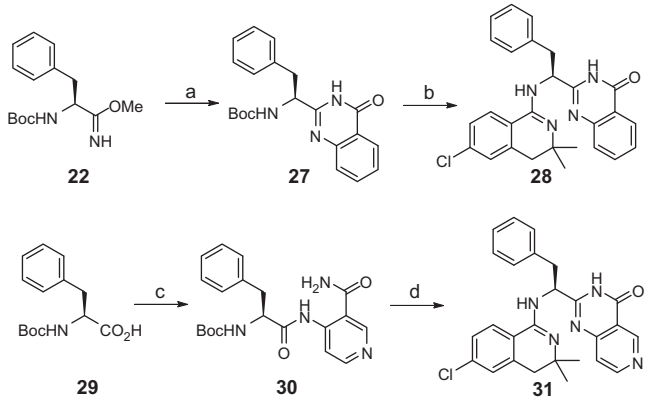
To our disappointment, this initial search for a carboxylic acid replacement was not successful and the most potent analog, pyrimidine **11** was fivefold less potent than the carboxylic acid **1**. At this juncture, we began to reconsider the key water-mediated interaction between the carboxylic acid and the backbone NH of Asn294. As an alternative to interacting with the water molecule, it could be possible to design an acid replacement that would displace the water molecule and allow the inhibitor to interact directly with the protein. To explore this idea, a series of heterocycles bearing exocyclic hydrogen bond acceptors was prepared. Five-membered heterocycles with exocyclic oxygen atoms were prepared as shown in Scheme 2. Treatment of hydroxyamidine **14** with CDI[13] gave the intermediate oxadiazolone **15**, which was deprotected and reacted with **5** to give the final compound **16**.



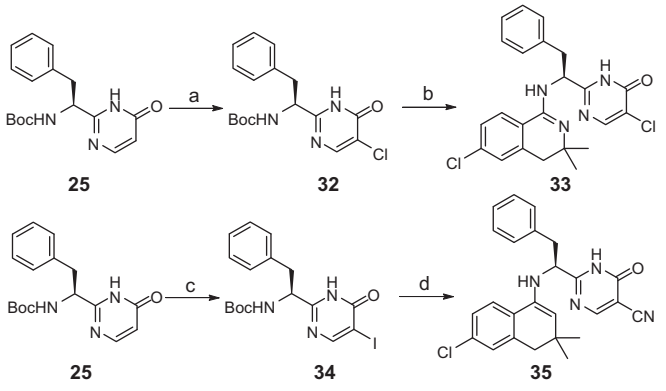
Scheme 3. Reagents and conditions: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, rt, 75%; (b) NaOH, H_2O, reflux, 51%; (c) (i) TFA, CH_2Cl_2, rt, (ii) **5**, DMSO, 120 °C, 10%.



Scheme 4. Reagents and conditions: (a) NaOMe, MeOH, 50 °C; (b) NH_4Cl, MeOH, 50 °C; (c) EtOH, H_2O, 91%, (3-steps); (d) (i) TFA, CH_2Cl_2, rt, (ii) **5**, DMSO, 100 °C, 18%.



Scheme 5. Reagents and conditions: (a) 2-aminobenzoic acid, MeOH, reflux, 62%; (b) (i) TFA, CH_2Cl_2, rt, (ii) **5**, DMSO, 100 °C, 19%; (c) 4-aminonicotinamide, EDCI, HOBt, Et_3N, CH_2Cl_2, 79%; (d) (i) NaOH (aq), EtOH, 60 °C, 96%, (ii) TFA, CH_2Cl_2, rt, (iii) **5**, DMSO, 100 °C, 23%.



Scheme 6. Reagents and conditions: (a) NCS, AcOH, rt, 90%; (b) **5**, DMSO, 100 °C, 12%; (c) NIS, $CHCl_3$, rt, 67%; (d) (i) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 110 °C, microwave, 40 min, 56%, (ii) TFA, CH_2Cl_2, rt, (iii) **5**, DMSO, 100 °C, 15%.

Triazolones were prepared by cyclization of semicarbazide **19**,[14] which was elaborated to the final analog using standard methods (Scheme 3).

A number of six-membered and bicyclic heterocycles were also prepared using methods shown in Schemes 4 and 5. Pyrimidinones were prepared via cyclo-condensation of amidine **23** with sodium 2-ethoxycarbonyl ethanolate (**24**)[15] to provide compound **26**.

Quinazolinone **28** was readily prepared by condensation of imidate **22** with 2-aminobenzoic acid, while pyridopyrimidone **31** was prepared by cyclization of amide **30** (Scheme 5).

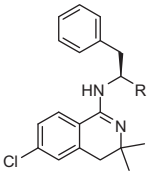
Further modification of pyrimidone **25** was achieved by halogenation to give intermediates **32** and **34** (Scheme 6). Aryl iodide **34** was converted into nitrile **35** via palladium catalyzed cross coupling.

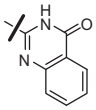
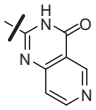
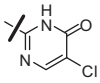
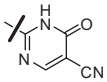
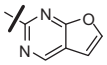
In an attempt to remove the protic nature of these heterocyclic acid replacements, the exocyclic oxygen was incorporated into a bicyclic ring system. Sonogashira coupling of ethynyltrimethylsilane with iodide **34**, followed by subsequent removal of the TMS group and heat-mediated cyclization, gave furopyrimidine analog **39** (Scheme 7).

For the carboxylic acid replacement to be successful, it must not only provide equal or greater potency than the acid but must also allow the inhibitor to reach its target. This requires the compound to be highly permeable and possess a low P-gp efflux in order to cross the blood brain barrier.[17] The SAR of the carboxylic acid replacements, along with their permeability and P-gp efflux ratios derived from MDR-MDCK cells, are shown in Table 3. Gratifyingly, oxadiazolone **16** proved to be an effective replacement for the carboxylic acid (IC_{50} = 0.75 μM) providing a fourfold improvement in potency compared to acid **7**. Similarly, triazolone **21** also had good potency (IC_{50} = 3.37 μM), albeit slightly attenuated compared to **16**. The crystal structure of BACE-1 in complex with compound **16** was determined to 1.8 Å resolution as shown in Figure 5. This structure revealed that the exocyclic oxygen effectively displaces the water molecule found in the structure of **1** and interacts directly with the backbone NH of Asn294 at 3.1 Å distance. Furthermore, the contact with Thr293 is maintained.

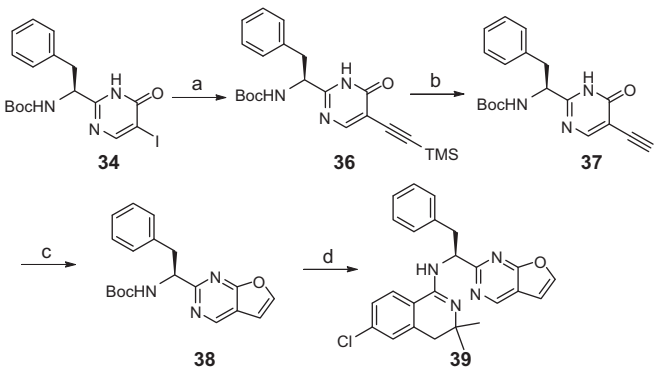
Unfortunately, oxadiazolone **16** had a low pK_a (4.0) indicating that the compound would exist as a zwitterion at physiological pH and may have a lower chance of CNS penetration compared to a basic molecule.[12a] Compound **16** had low passive permeability (26 nm/s) and a high P-gp efflux ratio (47), thereby limiting its potential as an acid replacement for a CNS target. Although triazolone **21** possesses a more reasonable pK_a (7.1), it still suffers from low permeability (23 nm/s) and a very high P-gp efflux ratio (60), which also limits its usefulness as an acid replacement.

Table 3
SAR of acid replacements



#	R	BACE-1 IC_{50}[a] μM	pK_a[b]	Papp nm/s %recovery[c]	P-gp efflux[c]
7	COOH	2.84	2.1	nd	nd
16		0.75	4.0	26 nm/s 75%	47
21[d]		3.37	7.1	23 nm/s 72%	60
26		2.71	6.3	231 nm/s 60%	2.6
28		7.23	7.2	Low recovery	—
31		0.17	nd	196 nm/s 51%	1.9
33		0.56	nd	213 nm/s 65%	5.5
35		0.47	nd	73 nm/s 75%	39
39		73.7	nd	nd	nd
40		0.59	nd	nd	nd

[a] See Ref. 9.
[b] Determined by capillary electrophoresis
[c] See Ref. 16.
[d] Compound is racemic; nd; not determined.



Scheme 7. Reagents and conditions: (a) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, CuI, Et_3N, microwave, 120 °C, 20 min; (b) K_2CO_3, MeOH; (c) MeOH, 120 °C, 45% (3-steps); (d) (i) TFA, CH_2Cl_2, rt, (ii) **5**, DMSO, 100 °C, 12%.

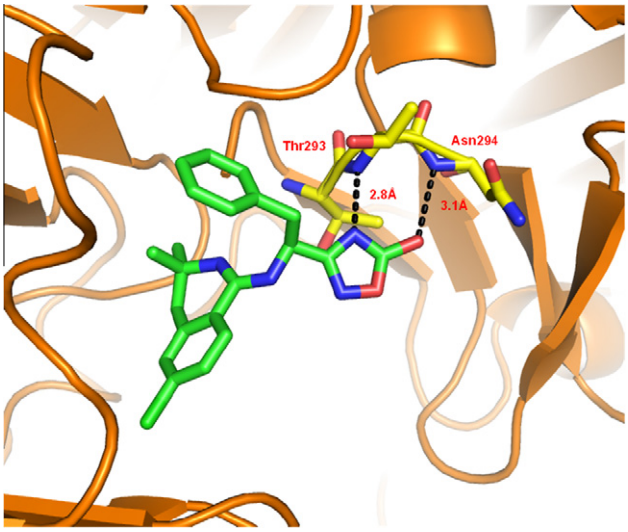


Figure 5. Crystal structure of compound **16** in green bound to BACE-1 (1.8 Å resolution). The PDB deposition code is 4HZT. For experimental conditions see Ref. 10.

For the six-membered heterocyclic acid replacement, pyrimidinone **26** had similar potency (BACE-1 IC_{50} = 2.71 μM), compared to acid **7**, but interestingly, it also possessed a significantly lower P-gp efflux ratio and higher passive permeability compared to **16** and **21**. Molecular modeling suggested that there would be sufficient space on the 5-position of the pyrimidine ring to place a substituent. Indeed, chlorine, iodine and cyano pyrimidinones **33**, **40** and **35** all had improved potency compared to **26**, with the cyanopyrimidone **35** being sixfold more potent than the carboxylic acid. The crystal structure of nitrile **35** with BACE-1 was determined to 1.8 Å resolution (Fig. 6). This structure revealed that the inhibitor's pyrimidinone ring interacts with Thr293 and Asn294 in a similar manner as oxadiazolone **16**, but its nitrile group forms a new interaction with the side-chain of Ser-386 at 3.0 Å distance.

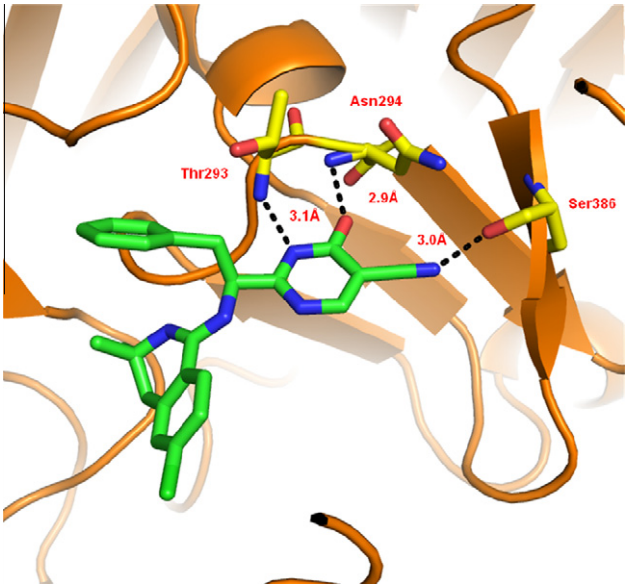


Figure 6. Crystal structure of compound **35** in green bound to BACE-1 (1.8 Å resolution). The PDB deposition code is 4I0Z. For experimental conditions see Ref. 10.

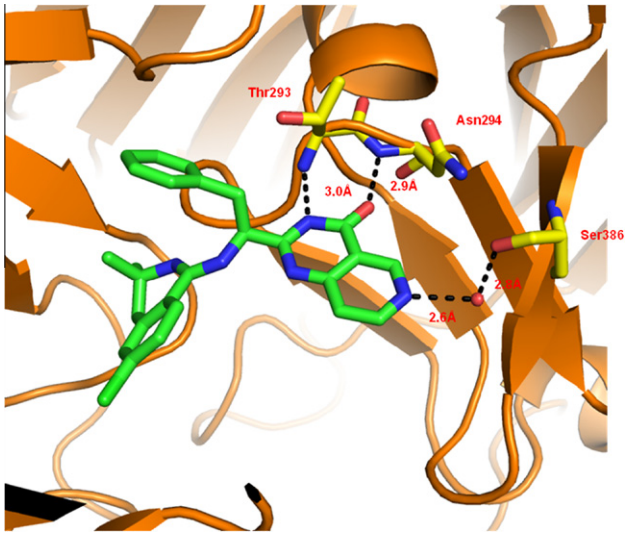


Figure 7. Crystal structure of compound **31** in green bound to BACE-1 (2.1 Å resolution). The PDB deposition code is 4I10. For experimental conditions see Ref. 10.

Molecular modeling also indicated that larger, bicyclic rings could be accommodated and, indeed, quinazoline **28** proved that this was the case (IC_{50} = 7.2 μM). Since **28** had poor permeability and low solubility (20 μM),[16] it was envisioned that the addition of heteroatoms to the benzo ring would improve the pharmacokinetic properties of the inhibitor. Molecular modeling indicated that nitrogen, placed at the 6-position of the ring, could form further interactions with BACE-1. This was confirmed with the synthesis of pyrido[4,3-*d*]pyrimidin-4(3*H*)-one **31**. Compound **31** gained significant potency compared to **35** and, furthermore, had improved solubility (57 μM), high permeability (196 nm/s) and low P-gp efflux (efflux ratio = 1.9). The crystal structure of **31** with BACE-1 was also solved (Fig. 7), and, as observed in the structure of pyrimidine **35**, the interactions between the acid replacement and Thr293 and Asn294 are maintained. Additionally, the nitrogen in the 6-position of the pyridine ring forms a water-mediated hydrogen bond with the side-chain of Ser-186.

Finally, we attempted to remove the protic nature of the heterocyclic acid replacements with furanopyrimidine **39**. To our disappointment, this endeavor was not successful, and compound **39** lost an order of magnitude in potency compared to acid **7**.

In conclusion, guided by structure-based design, we were able to rapidly modify the initial screening hit **1** to gain significant improvements in potency against BACE-1. Key to the continued development of this series of analogs was the replacement of the carboxylic acid. Molecular modeling aided in the design of heterocycles bearing exocyclic hydrogen bond acceptors that effectively replaced a water mediated hydrogen bond. Compound **31** proved to be the most promising analog in this series with good potency, low P-gp efflux and high permeability, all hallmarks of a CNS penetrant small molecule. The continued development of this series of compounds will be reported in due course.

References and notes

1. Ferri, C.; Prince, M.; Brayne, C.; Brodaty, H.; Fratiglioni, L.; Ganguli, M.; Hall, K.; Hasegawa, K.; Hendrie, H.; Huang, Y. *Lancet* **2005**, *366*, 2112.
2. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
3. Sinha, S.; Liberburg, I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11049.
4. (a) 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.; Citron, M.; Vassar, R. *Nat. Neurosci.* **2001**, *4*, 231; (b) Cai, H.; Wang, Y.; McCarthy, D.; Wen, H.; Borchelt, D. R.; Price, D. L.; Wong, P. C. *Nat. Neurosci.* **2001**, *4*, 233; (c) Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M.; 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.; Keuhn, 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. *Hum. Mol. Genet.* **2001**, *10*, 1317.
5. Luo, Y.; Bolon, B.; Damore, M. A.; Fitzpatrick, D.; Liu, H.; Zhang, J.; Yan, Q.; Vassar, R.; Citron, M. *Neurobiol. Dis.* **2003**, *14*, 81.
6. Schmidt, B.; Baumann, S.; Braun, H. A.; Larbig, G. *Curr. Top. Med. Chem.* **2006**, *6*, 377.
7. Probst, G. D.; Xu, Y. Z. *Expert Opin. Ther. Pat.* **2012**, *5*, 511.
8. (a) Maillard, M. C.; 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.; Tucker, J. A.; Dappen, M. S.; Brogley, L.; Thorsett, E. D.; Jewett, N.; Sinha, S.; John, V. *J. Med. Chem.* **2007**, *50*, 776; (b) Sealy, J. M.; Truong, A. P.; Tso, L.; Probst, G. D.; Aquino, J.; Hom, R. K.; Jagodzinska, B. M.; Dressen, D.; Wone, D. W. G.; Brogley, L.; John, V.; Tung, J. S.; Pleiss, M. A.; Tucker, J. A.; Konradi, A. W.; Dappen, M. S.; Tóth, G.; Pan, H.; Ruslim, L.; Miller, J.; Bova, M. P.; Sinha, S.; Quinn, K. P.; Sauer, J.-M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6386; (c) Truong, A. P.; Toth, G.; Probst, G. D.; Sealy, J. M.; Bowers, S.; Wone, D. W. G.; Dressen, D.; Hom, R. K.; Konradi, A. W.; Sham, H. L.; Wu, J.; Peterson, B. T.; Ruslim, L.; Bova, M. P.; Kholodenko, D.; Motter, Ruth N.; Bard, F.; Santiago, P.; Ni, H.; Chian, D.; Soriano, F.; Cole, T.; Brigham, E. F.; Wong, K.; Zmolek, W.; Goldbach, E.; Samant, B.; Chen, L.; Zhang, H.; Nakamura, D. F.; Quinn, K. P.; Yednock, T. A.; Sauer, J.-M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6231; (d) Probst, G. D.; Bowers, S.; Sealy, J. M.; Stupi, B.; Dressen, D.; Jagodzinska, B. M.; Aquino, J.; Gailunas, A.; Truong, A. P.; Tso, L.; Xu, Y.-Z.; Hom, R. K.; John, V.; Tung, J. S.; Pleiss, M. A.; Tucker, J. A.; Konradi, A. W.; Sham, H. L.; Jagodzinski, J.; Toth, G.; Brecht, E.; Yao, N.; Pan, H.; Lin, M.; Artis, D. R.; Ruslim, L.; Bova, M. P.; Sinha, S.; Yednock, T. A.; Gauby, S.; Zmolek, W.; Quinn, K. P.; Sauer, J.-M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6034; (e) Truong, A. P.; Probst, G. D.; Aquino, J.; Fang, L.; Brogley, L.; Sealy, J. M.; Hom, R. K.; Tucker, J. A.; John, V.; Tung, J. S.; Pleiss, M. A.; Konradi, A. W.; Sham, H. L.;

Dappen, M. S.; Toth, G.; Yao, N.; Brecht, E.; Pan, H.; Artis, D. R.; Ruslim, L.; Bova, M. P.; Sinha, S.; Yednock, T. A.; Zmolek, W.; Quinn, K. P.; Sauer, J.-M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4789; (f) Mandal, M.; Zhu, Z.; Cumming, J. N.; Liu, X.; Strickland, C.; Mazzola, R. D.; Caldwell, J. P.; Leach, P.; Grzelak, M.; Hyde, L.; Zhang, Q.; Terracina, G.; Zhang, L.; Chen, X.; Kuvelkar, R.; Kennedy, M. E.; Favreau, L.; Cox, K.; Orth, P.; Buevich, A.; Voigt, J.; Wang, H.; Kazakevich, I.; McKittrick, B. A.; Greenlee, W.; Parker, E. M.; Stamford, A. W. *J. Med. Chem.* **2012**, *55*, 9331; (g) Malamas, M. S.; Erdei, J.; Gunawan, I.; Turner, J.; Hu, Y.; Wagner, E.; Fan, K.; Chopra, R.; Olland, A.; Bard, J.; Jacobsen, S.; Magolda, R. L.; Pangalos, M.; Robichaud, A. J. *J. Med. Chem.* **2010**, *53*, 1146; (h) Zhu, Z.; Sun, Z.-Y.; Ye, Y.; Voigt, J.; Strickland, C.; Smith, E. M.; Cumming, J.; Wang, L.; Wong, J.; Wang, Y.-S.; Wyss, D. F.; Chen, X.; Kuvelkar, R.; Kennedy, M. E.; Favreau, L.; Parker, E.; McKittrick, B. A.; Stamford, A.; Czarniecki, M.; Greenlee, W.; Hunter, J. C. *J. Med. Chem.* **2010**, *53*, 951; (i) May, P. C.; Dean, R. A.; Lowe, S. L.; Martenyi, F.; Sheehan, S. M.; Boggs, L. N.; Monk, S. A.; Mathes, B. M.; Mergott, D. J.; Watson, B. M.; Stout, S. L.; Timm, D. E.; LaBell, E. S.; Gonzales, C. R.; Nakano, M.; Jhee, S. S.; Yen, M.; Ereshefsky, L.; Lindstrom, T. D.; Calligaro, D. O.; Cocke, P. J.; Hall, D. G.; Friedrich, S.; Citron, M.; Audia, J. E. *J. Neurosci.* **2011**, *31*, 6507; (j) Brodney, M. A.; Barreiro, G.; Ogilvie, K.; Hajos-Korcsok, E.; Murray, J.; Vajdos, F.; Ambroise, C.; Christoffersen, C.; Fisher, K.; Lanyon, L.; Liu, J. H.; Nolan, C. E.; Withka, J. M.; Borzilleri, K. A.; Efremov, I.; Oborski, C. E.; Varghese, A.; O'Neill, B. R. *J. Med. Chem.* **2012**, *55*, 9224.

9. Ren, Z.; Tam, D.; Xu, Y.-Z.; Wone, D.; Yuan, S.; Jobling, M.; Cheung, H.; Chen, X.; Rudolph, D.; Sham, H.; Artis, D. R.; Bova, M. P. *J. Biomol. Screen.* submitted for publication.
10. *Crystallography conditions*: BACE-1 protein from construct pQE70[8e] was concentrated to 10 mg/ml in 100 mM borate pH 8.5. Apo crystals were grown at 4 °C in 1 μL sitting drops with a 1:1 (v/v) ratio of protein to reservoir, a solution of 9% PEG 8000, 100 mM sodium acetate pH 5.3 and 10 mM $ZnCl_2$. Compounds at 10 mM DMSO solution were added to a soaking solution consisting of 100 mM sodium acetate pH 6.6, 10% PEG 8000, 5% ethylene glycol, 5 mM zinc chloride and 10%DMSO. Apo crystals were transferred to soaking solution and incubated overnight at room temperature. 80 Individual compounds were soaked with apo BACE-1 crystals in one batch. 80 Soaked crystals in one batch were frozen directly into liquid nitrogen, data sets were collected and processed automatically by the Rigaku X-ray Homelab Highflex system with an ACTOR auto sampler. Electron density was analyzed and interpreted manually.
11. Kollman, P. A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; Donini, O.; Cieplak, P.; Srinivasan, J.; Case, D. A.; Cheatham, T. E., 3rd *Acc. Chem. Res.* **2000**, *33*, 889.
12. (a) Gleeson, P. M. *J. Med. Chem.* **2008**, *51*, 817; (b) Meanwell, N. A. *J. Med. Chem.* **2011**, *54*, 2529.
13. Mangette, J. E.; Johnson, M. R.; Le, V.-D.; Shenoy, R. A.; Roark, H.; Stier, M.; Belliotti, T.; Capiris, T.; Guzzo, P. R. *Tetrahedron* **2009**, *65*, 9536.
14. Kandeel, K. A.; Youssef, A. S. A.; Abou-Elmagd, W. S. I.; Hashem, A. I. *J. Heterocycl. Chem.* **2006**, *43*, 957.
15. Mylari, B. L.; Oates, P. J.; Beebe, D. A.; Brackett, N. S.; Coutcher, J. B.; Dina, M. S.; Zembrowski, W. J. *J. Med. Chem.* **2001**, *44*, 2695.
16. For details on in vitro pharmacokinetic assays, see: Truong, A. P.; Aubele, D. A.; Probst, G. D.; Neitzel, M. L.; Semko, C. M.; Bowers, S.; Dressen, D.; Hom, R. K.; Konradi, A. W.; Sham, H. L.; Garofalo, A. W.; Keim, P. S.; Wu, J.; Dappen, M. S.; Wong, K.; Goldbach, E.; Quinn, K. P.; Sauer, J.-M.; Brigham, E. F.; Wallace, W.; Nguyen, L.; Hemphill, S. S.; Bova, M. P.; Basi, G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4920.
17. Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. *ACS Chem. Neurosci.* **2010**, *1*, 420.