Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 414–417
Bioorganic & Medicinal Chemistry Letters

Potent pyrrolidine- and piperidine-based BACE-1 inhibitors

U. Iserloh,[a,*] Y. Wu,[a] J. N. Cumming,[a] J. Pan,[a] L. Y. Wang,[a] A. W. Stamford,[a] M. E. Kennedy,[b] R. Kuvelkar,[b] X. Chen,[b] E. M. Parker,[b] C. Strickland[a] and J. Voigt[a]

[a]*Department of Chemical Research, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[b]*Department of Neurobiology, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*

Received 23 July 2007; revised 2 October 2007; accepted 5 October 2007
Available online 6 November 2007

Abstract—Based on lead compound **1** identified from the patent literature, we developed novel patentable BACE-1 inhibitors by introducing a cyclic amine scaffold. Extensive SAR studies on both pyrrolidines and piperidines ultimately led to inhibitor **2f**, one of the most potent inhibitors synthesized to date.

Alzheimer's disease (AD)[1] is a neurodegenerative disorder associated with the accumulation of amyloid plaques comprised of $A\beta_{40,42}$ peptides, along with neurofibrillary tangles in the brain. These peptides arise from cleavage of the amyloid precursor protein (APP) by β-amyloid cleaving enzyme-1 (BACE-1),[2] hence inhibitors of this aspartyl protease could have therapeutic benefits for the treatment of AD.

In the course of our program to develop patentable novel inhibitors of BACE-1, we investigated conformationally constrained versions of the hydroxyethylamine motif found in many aspartyl protease inhibitors.[3] Based on our analysis of the binding mode for the prototypical inhibitor **1** complexed to BACE-1,[4,5] we decided to integrate the methylene groups flanking the secondary amine into an azetidine, pyrrolidine, or piperidine ring (**2**, Fig. 1). Computational modeling of these ring-constrained amines suggested a similar binding mode as for **1**, while simultaneously rigidifying the conformational flexibility of the R′-side chain.

From a retrosynthetic perspective, we considered the stereoselective synthesis of key amino alcohol **3** via two complementary routes (Fig. 2): formation of bond A would require an Evans aldol coupling between an enantiopure Boc-protected prolinal (or piperidine-2-aldehyde) and an enolate derived from an appropriate acyloxazolidinone (X_c = auxiliary) to give intermediate **4** with three contiguous stereocenters in their required all-*syn* arrangement.[6,7] Another approach to piperidines through formation of bond B would establish the correct *syn*-stereochemistry of **5** via a Felkin-Anh-controlled addition[8] of substituted 2-lithiopyridines onto (*S*)-*N*,*N*-(dibenzyl)-3,5-difluorophenylalaninal, followed

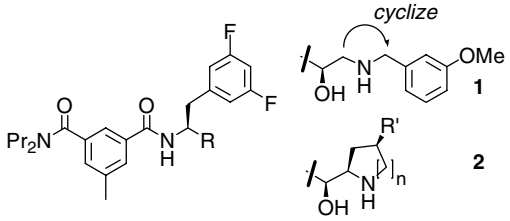


Figure 1. Design of azetidine/pyrrolidine/piperidine-based BACE-1 inhibitors **2** (n = 0–2).

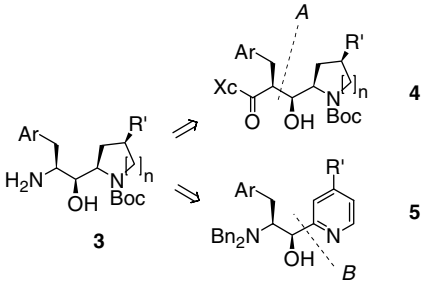
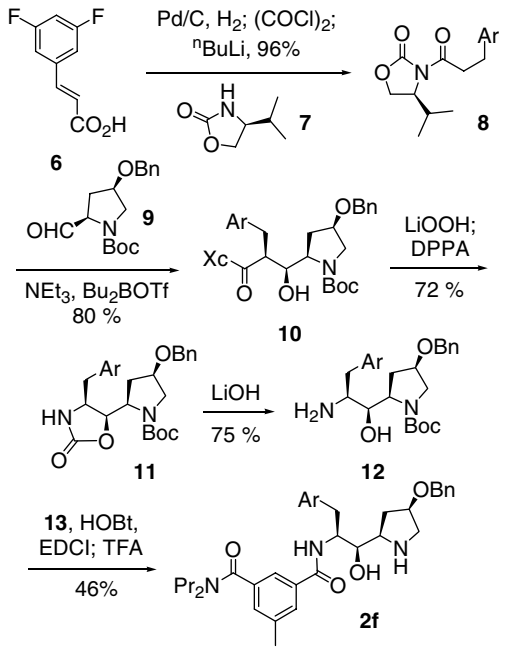


Figure 2. Retrosynthetic options toward amino alcohol **3** (Ar = 3,5-difluorophenyl; X_c = Evans oxazolidinone auxiliary).

Keywords: Alzheimer's disease; β-Secretase; Aspartyl protease inhibitors; Peptidomimetics.
* Corresponding author. Tel.: +1 908 740 7375; fax: +1 908 740 7152; e-mail: ulrich.iserloh@spcorp.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.116



Scheme 1. Synthesis of BACE-1 inhibitor **2f** as a representative example for the Evans aldol route (Ar = 3,5-difluorophenyl; X_c = oxazolidinone auxiliary).

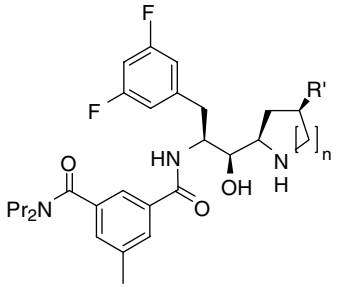
by reduction of the pyridine moiety to the desired piperidine scaffold.

A specific example illustrating the implementation of the Evans aldol route is shown for BACE-1 inhibitor **2f** (Scheme 1), but served well for a host of other cyclic amines described in Table 1. The acyloxazolidinone **8** required for the Aldol coupling was prepared in three steps (96% overall yield) from commercially available 3,5-difluorocinnamic acid **6** based on the literature.[9] *Boc*-protected 4-benzyloxyprolinal **9** was generated in five straightforward steps[10] from *cis*-4-hydroxy-D-proline, and was subsequently coupled with the boron-enolate derived from **8** in 80% isolated yield.

Removal of the oxazolidinone auxiliary from aldol adduct **10**[11] with lithium hydroperoxide provided a β-hydroxy acid, which was exposed to diphenylphosphoryl azide (DPPA). Upon Curtius-rearrangement, the incipient isocyanate was readily captured by the hydroxyl group to furnish oxazolidinone **11** in 72% yield (two steps).[12] Hydrolysis with lithium hydroxide gave the desired amino alcohol **12** in 75% yield, and was subsequently coupled to 5-methyl-*N*,*N*-dipropyl-isophthalic acid **13**[4a] in the presence of HOBt/EDCI (or HATU/HOAt). Final deprotection with trifluoroacetic acid (TFA) furnished BACE-1 inhibitor **2f** in 46% (two steps).

An alternate route to piperidine-based BACE-1 inhibitors is shown in Scheme 2, exemplified by the synthesis of compound **2q**. Addition of (*S*)-*N*,*N*-dibenzyl-3,5-difluorophenylalaninal **14**[13] to 2-lithio-4-chloropyridine (regioselectively formed from 4-chloropyridine **15** and *n*-butyllithium in the presence of 2-(dimethylamino) ethanol)[14] resulted in selective formation of adduct **16**. Treatment with sodium ethoxide afforded intermediate **17**, which was subsequently deprotected to the amino alcohol and converted to amide **18**. Reduction of the pyridine ring with platinum(IV) oxide/hydrogen in acetic acid gave two isomers **2q** and **19**, which were separated by silica gel chromatography.

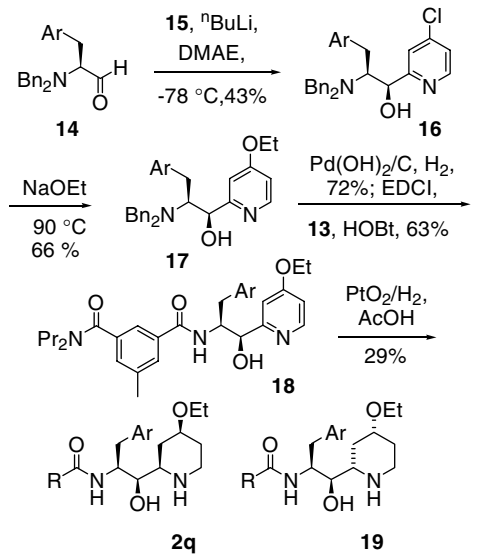
Table 1. BACE-1 binding affinity and cellular activity



Compound	*n* (ring system)	R′	BACE-1 IC_{50}[a] (nM)	Cell IC_{50}[a] (nM)
2a	0 (Azetidine)	H	770	2800
2b	1 (Pyrrolidine)	H	65	330
2c		OH	89	1450
2d		OMe	187	780
2e		OBn	5	150
2f		OPh	3	165
2g	2 (Piperidine)	H	130	240
2h		^{n}Pr	70	1200
2i		Bn	14	522
2j		2-MeOPh CH_2	1350	n/d
2k		3-MeOPh CH_2	330	n/d
2l		4-MeOPh CH_2	440	2000
2m		$Ph(CH_2)_2$	191	1850
2n		Chx	610	4200
2o		$ChxCH_2$	630	2000
2p		OAc	2000	3000
2q		OEt	8	106
2r		OBu	7	160
2s		O^{i}Pr	120	858
2t		$OCH_2{}^c$Pr	6	121
2u		$O(CH_2)_2OMe$	4	110
2v		$O(CH_2)_2NMe_2$	26	482
2w		OCH_2Chx	12	567

[a] See Refs. 15 and 16 for details of in vitro and cell assays.

The bioactivity of these novel BACE-1 inhibitors was assessed in standard in vitro[15] and cellular[16] assays, with select results shown in Table 1. The effect of ring size was probed in a series of unsubstituted analogs **2a**, **2b**, and **2g** (R′ = H, *n* = 0–2). While the azetidine **2a** shows only moderate activity (BACE-1 IC_{50} = 770 nM, cellular IC_{50} = 2800 nM), the increased molecular volume of pyrrolidine **2b** and piperidine **2g** greatly improved in vitro activity (BACE-1 IC_{50} = 65 nM and 130 nM, respectively). This level of potency compares favorably to the lead inhibitor **1** (BACE-1 IC_{50} = 8 nM) given that inhibitors **2b** and **2g** are not garnering binding affinity from the 3-methoxybenzyl substitution.



Scheme 2. Synthesis of BACE-1 inhibitor **2q** via addition to *N*,*N*-dibenzyl-3,5-difluorophenylalaninal (Ar = 3,5-difluorophenyl).

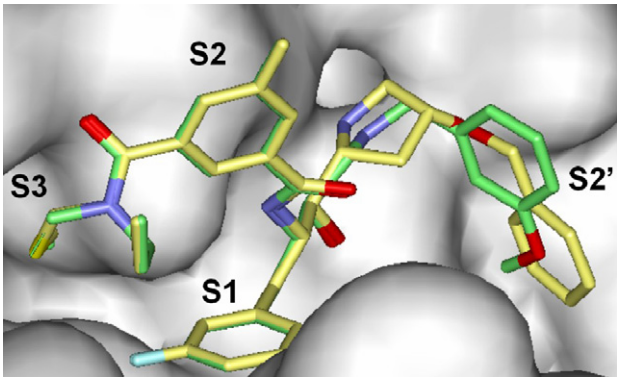


Figure 3. Overlay of BACE-1 X-ray structures for **1** (green) and **2f** (yellow). Excellent overlap is observed in the S1, S2, and S3 enzyme subsites, and a similar space is accessed in the S2′ pocket.

Moreover, additional potency can be regained through appropriate substitution of the pyrrolidine ring as is evident from 4-substituted pyrrolidines **2c**–**2f**. While 4-hydroxy and 4-methoxy analogs **2c** and **2d** exhibit diminished potency relative to its parent, bulkier 4-benzyloxy or 4-phenoxy groups render **2e** (BACE-1 IC_{50} = 5 nM) and **2f** (BACE-1 IC_{50} = 3 nM) equipotent with lead **1**.

An overlay of X-ray structures for **1** and **2f** (Fig. 3, flap removed for clarity) reveals the expected overlap on the nonprime side (S3 through S1 enzyme subsites) along with the conformational constraint imposed by the pyrrolidine ring.[5] As a result, the 4-benzyloxy group accesses a space similar to that occupied by the methoxybenzyl moiety present in inhibitor **1**.

As for the pyrrolidines, substitution of the piperidine ring can also lead to more potent compounds. While **2h** (R = Pr, 70 nM) and **2i** (R = Bn, 14 nM) show improved in vitro activity relative to **2g**, their increased lipophilicity also reduced the cellular activity. In an effort to explore the SAR around 4-benzylpiperidine **2i**, we evaluated methoxybenzyl analogs **2j**–**2l**, phenethyl derivative **2m**, as well as the cyclohexyl (**2n**) and cyclohexylmethyl (**2o**) variants. However, reduced in vitro and cellular activity was observed in each case. Finally, we decided to explore the SAR of 4-alkoxypiperidines **2p**–**2w**. Gratifyingly, small alkoxy groups such as ethoxy, butoxy, cyclopropylmethoxy, and methoxyethoxy showed single-digit nanomolar IC_{50} with good cellular activity (cell IC_{50} = 106–160 nM).

Inspection of the X-ray structure for 4-ethoxypiperidine derivative **2q** reveals a 2.4 Å hydrogen-bond from the Thr72 backbone NH of the enzyme flap to the oxygen atom of the 4-ethoxypiperidine, while the S2′ pocket remains unoccupied (Fig. 4). Energetically, the favorable interaction of the ether oxygen with the flap NH compensates for the absence of hydrophobic interactions that are harnessed by inhibitors such as **2i** with larger substituents in the S2′ pocket, thus enabling improved cellular activity. By comparison, the 4-benzyloxypyrrolidine **2e** cannot establish a similar hydrogen-bonding interaction due to its different trajectory into the S2′ enzyme subsite.

BACE-1 inhibitors **2e** and **2f** were further profiled against a panel of relevant human aspartyl proteases (Table 2). Benzyloxypyrrolidine **2e** exhibited low selectivity against cathepsin D (K_i = 36 nM) and cathepsin E (K_i = 5 nM), while the phenoxypyrrolidine **2f** was slightly more selective (291 nM and 24 nM, respectively).

In conclusion, we have successfully demonstrated the potential of novel pyrrolidine- and piperidine-based BACE-1 inhibitors. As conformationally constrained versions of hydroxyethylamine-type peptidomimetics, their cyclic amine core allows for targeted substitution into the S2′ subsite. In addition, a hydrogen-bonding interaction has been discovered that significantly improved potency for 4-alkoxypiperidines that do not

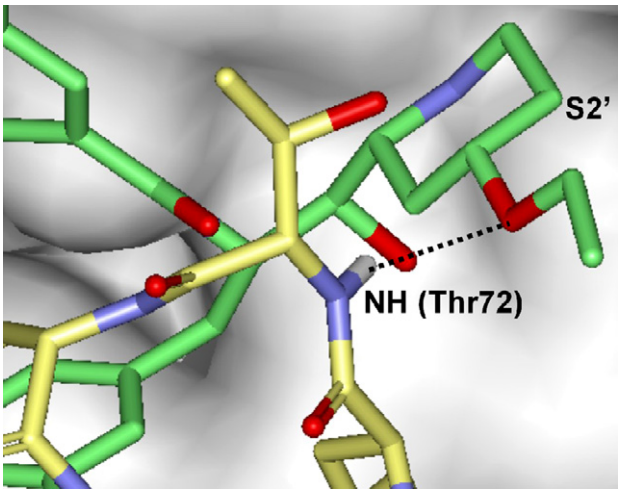
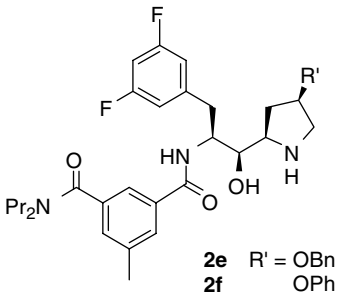


Figure 4. Close-up view of BACE-1 X-ray structure for **2q** (green) and flap (yellow) in the S2′ enzyme subsite. A hydrogen-bond is formed from the flap Thr72 NH to the oxygen atom of the 4-ethoxypiperidine.

Table 2. Detailed profile for novel BACE-1 inhibitors **2e** and **2f**



Assay, IC_{50}	**2e** (R = OBn)	**2f** (R = OPh)
BACE-1 IC_{50} (nM)	5	3
BACE-2 IC_{50} (nM)	45	54
Cell IC_{50} (HEK 293) (nM)	150	165
Cathepsin D K_i (nM)	36	291
Cathepsin E K_i (nM)	5	24
Pepsin K_i (nM)	158	232

exploit binding in the S2′ pocket. Further SAR studies will be reported in the subsequent paper in this issue.[17]

Acknowledgments

The authors are thankful to Drs. A. Buevich and T.-M. Chan for NMR analyses, and Dr. William Greenlee for continued support. Use of the IMCA-CAT beamline 17-ID at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with the Center for Advanced Radiation Sources at the University of Chicago. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

References and notes

1. General reviews of AD: (a) Nguyen, J.; Yamani, A.; Kiso, Y. *Curr. Pharm. Des.* **2006**, *12*, 4295; (b) Zimmermann, M.; Gardoni, F.; Di Luca, M. *Drugs Aging* **2005**, *22*, 27; (c) Citron, M. *Nat. Rev. Neurosci.* **2004**, *5*, 677.
2. BACE-1 is also known as β-secretase. Recent efforts toward BACE-1 inhibitors have been amply reviewed: (a) Durham, T. B.; Shepherd, T. A. *Curr. Opin. Drug Discovery Dev.* **2006**, *9*, 776; (b) Guo, T.; Hobbs, D. W. *Curr. Med. Chem.* **2006**, *13*, 1811; (c) Thompson, L. A.; Bronson, J. J.; Zusi, F. C. *Curr. Pharm. Des.* **2005**, *11*, 3383; (d) Baxter, E. W.; Reitz, A. B. *Ann. Rep. Med. Chem.* **2005**, *40*, 35; (e) Cumming, J. N.; Iserloh, U.; Kennedy, M. E. *Curr. Opin. Drug Discovery Dev.* **2004**, *7*, 536.
3. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
4. (a) Maillaird, M.; Hom, R.; Gailunas, A.; Jagodzinska, B.; Fang, L. Y.; John, V.; Freskos, J. N.; Pulley, S. R.; Beck, J. P.; Tenbrink, R. E. PCT Int. Appl., WO 2002002512, 2002; (b) Freskos, J. N.; Fobian, Y. M.; Benson, T. E.; Bienkowski, M. J.; Brown, D. L.; Emmons, T. L.; Heintz, R.; Laborde, A.; McDonald, J. J.; Mischke, B. V.; Molyneaux, J. M.; Moon, J. B.; Mullins, P. B.; Prince, D. B.; Paddock, D. J.; Tomasselli, A. G.; Winterrowd, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 73.
5. X-ray coordinates for the complex of BACE-1 with inhibitors **1**, **2e**, **2f**, and **2q** have been deposited in the Protein Data Bank (http://www.rcsb.org), and can be accessed under PDB ID 2QK5, 2QMD, 2QMF, and 2QP8.
6. (a) Shioiri, T.; Hayashi, K.; Hamada, Y. *Tetrahedron* **1993**, *49*, 1913; (b) Hayashi, K.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1991**, *49*, 7287.
7. Pettit, G. R.; Burkett, D. D.; Barkoczy, J.; Breneman, G. L.; Pettit, W. E. *Synthesis* **1996**, 719.
8. Reetz, M. T.; Reif, W.; Holdgrun, X. *Heterocycles* **1989**, *28*, 707.
9. (a) Kruse, L. I.; Kaiser, C.; DeWolf, W. E.; Frazee, J. S.; Ross, S. T.; Wawro, J.; Wise, M.; Flaim, K. E.; Sawyer, J. L.; Erickson, R. W.; Ezekiel, M.; Ohlstein, E. H.; Berkowitz, B. A. *J. Med. Chem.* **1987**, *30*, 486; (b) Gage, J. R.; Evans, D. A. *Org. Synth.* **1989**, *68*, 83.
10. Synthesis of **9**: BnOH, TsOH; Boc_2O (70%, two steps); BnBr, NaH (71%); $LiBH_4$ (81%); Swern oxidation (98%); see: Russell, M. G. N.; Beer, M. S.; Stanton, J. A.; Sohal, B.; Mortishire-Smith, R. J.; Castron, J. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2491.
11. Stereochemistry for **10** was confirmed by its cyclization to the dihydropyrrolizine, followed by extensive NMR analysis (see Ref. 7).
12. Takacs, J. M.; Jaber, M. R.; Vellekoop, A. S. *J. Org. Chem.* **1998**, *63*, 2742.
13. Prepared from commercially available Boc-3,5-difluorophenylalanine: Reetz, M. T.; Drewes, M. W.; Schwickardi, R. *Org. Synth.* **1999**, *76*, 110.
14. Choppin, S.; Gros, P.; Fort, Y. *Eur. J. Org. Chem.* **2001**, 603.
15. BACE-1 enzymatic inhibition was determined using an APP-derived peptide containing the Swedish mutant: Kennedy, M. E.; Wang, W.; Song, L.; Lee, J.; Zhang, L.; Wong, G.; Wang, L.; Parker, E. *Anal. Biochem.* **2003**, *319*, 49.
16. Compound IC_{50}s for inhibition of $A\beta_{40}$ production were determined by incubating HEK-293 cells, stably transfected with the human APP cDNA containing both Swedish and London FAD mutations, with increasing concentrations of BACE-1 inhibitors. $A\beta_{40}$ levels were measured in the cell culture media using an $A\beta_{1-40}$ specific ELISA assay: Zhang, L.; Song, L.; Terracina, G.; Liu, Y.; Pramanik, B.; Parker, E. *Biochemistry* **2001**, *40*, 5049.
17. Iserloh, U.; Pan, J.; Stamford, A. W.; Kennedy, M. E.; Zhang, Q.; Zhang, L.; Parker, E. M.; McHugh, N. A.; Favreau, L.; Strickland, C.; Voigt, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 418.