Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 418–422
Bioorganic & Medicinal Chemistry Letters

Discovery of an orally efficaceous 4-phenoxypyrrolidine-based BACE-1 inhibitor

U. Iserloh,[a,*] J. Pan,[a] A. W. Stamford,[a] M. E. Kennedy,[b] Q. Zhang,[b] L. Zhang,[b] E. M. Parker,[b] N. A. McHugh,[b] L. Favreau,[c] 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*
[c]*Department of Drug Metabolism and Pharmacokinetics, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*

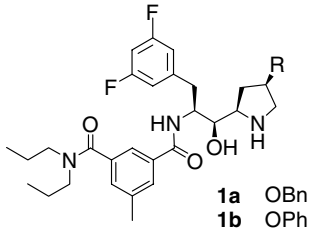
Received 23 July 2007; revised 2 October 2007; accepted 5 October 2007
Available online 18 October 2007

Abstract—Based on a lead compound identified from the patent literature, we developed patentably novel BACE-1 inhibitors by introducing a cyclic amine scaffold as embodied by **1a** and **1b**. Extensive SAR studies assessed a variety of isophthalamide replacements including substituted pyrrolidinones and ultimately led to the identification of **11**. Due to its favorable overall profile, **11** has been extensively profiled in various in vivo settings.

Alzheimer's disease (AD)[1] is a progressive and ultimately fatal neurodegenerative disorder, for which no effective treatment is currently available. One of the major pathological hallmarks of AD is the abnormal deposition of amyloid plaques comprised of $A\beta_{40,42}$ peptides in the brain of AD patients. These peptides arise from sequential cleavage of the amyloid precursor protein (APP) by β-amyloid cleaving enzyme-1 (BACE-1)[2] and γ-secretase.[3] As BACE-1 catalyzes the first committed step in the synthesis of β-amyloid, inhibitors of BACE-1 are expected to be useful treatments for AD.

In the preceding paper in this issue,[4] we described the discovery of conformationally constrained versions of the hydroxyethylamine (HEA) motif found in many peptidomimetic inhibitors.[5] Based on a lead structure identified from the patent literature,[6] we developed 4-benzyloxypyrrolidine and 4-phenoxypyrrolidine containing inhibitors **1a** and **1b** with good in vitro potency (5 nM and 3 nM, respectively) although with low cellular activity and modest selectivity against other human aspartyl proteases (Fig. 1). In addition, both inhibitors exhibited insufficient pharmacokinetic properties in rats as evidenced by low plasma levels following oral dosing.

We now report on studies of various isophthalamide replacements to improve the bioactivity, pharmacokinetic parameters as well as ancillary profile of our cyclic amine BACE-1 inhibitors, ultimately resulting in the discovery of **11**.



Assay[a]	**1a** (OBn)	**1b** (OPh)
BACE-1 IC_{50}	5 nM	3 nM
BACE-2 IC_{50}	45 nM	54 nM
Cell IC_{50} (HEK 293)	150 nM	165 nM
Cathepsin D K_i	36 nM	291 nM
Cathepsin E K_i	5 nM	24 nM
Pepsin K_i	158 nM	232 nM
$AUC_{0\text{-}6h}$ (rat, 10 mpk po)	162 nM*hr	227 nM*hr

Figure 1. Profile of pyrrolidine-containing BACE-1 inhibitors **1a** and **1b**. [a]See Ref. 7 for details of in vitro and cell assays.

Keywords: Alzheimer's disease; β-Secretase inhibitors; Aspartyl proteases.
* 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.2007.10.053

Inspection of the X-ray structure for 4-benzyloxypyrrolidine-based inhibitor **1a** bound to BACE-1 revealed two hydrogen-bonds from the isophthalamide carbonyl-groups to the peptide backbone NH-groups of Thr232 (S3 subsite) and Thr72 (flap), along with hydrophobic contacts within the S3 and S2 enzyme subsites (Fig. 2).[8] As part of our plan to identify isophthalamide replacements, we decided to retain the internal amide with its key interaction to the flap Thr72 while broadly screening for more appropriate S2–S3 moieties. Synthetically, this would entail amide formation from our key intermediate, aminoalcohol **2**,[4] followed by Boc-deprotection (Scheme 1).

Several dozen inhibitors including aliphatic amides (**3**), benzamides (**4**), arylacetamides (**5**), and cinnamides (**6**) were synthesized yet elicited uniformly poor bioactivity (BACE-1 IC_{50}: 10–100 μM) and were not further profiled (Scheme 1). Amides (**7**) and sulfonamides (**8**) incorporating azetidine-3-carboxylic acid exhibited reasonable potency (BACE-1 IC_{50} = 1–10 μM) yet demonstrated a fairly flat SAR most likely due to reduced hydrophobic contacts in the S2 subsite and suboptimal interactions with Thr232.

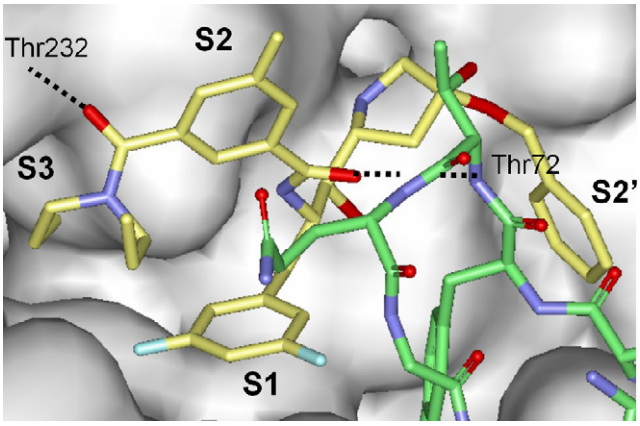
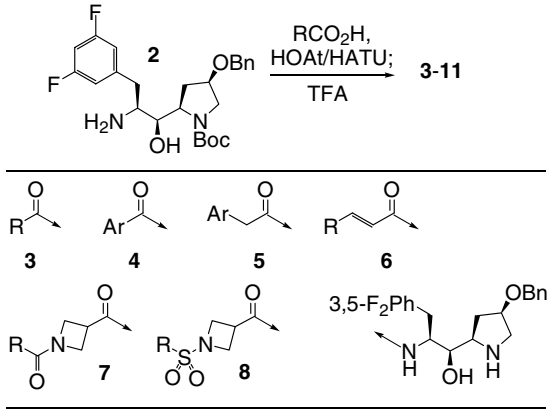


Figure 2. X-ray structure of **1a** bound to BACE-1, with enzyme flap shown in green. Key hydrogen-bonds from **1a** to Thr232 and flap Thr72 are indicated by dotted lines.



Scheme 1. Synthesis of BACE-1 inhibitors **3**–**11**; IC_{50} (BACE-1) >10 μM (**3**–**6**), >1 μM (**7**–**8**).

Considering that pyrrolidinones have a larger molecular volume than azetidinones and display the pyrrolidine *N*-substituent differently with respect to Thr232 and the S3-enzyme subsite, we decided to investigate BACE-1 inhibitors containing *N*-substituted pyrrolidinones **9**[9] (Tables 1 and 2).

The modest potency of the prototypical inhibitor **9a** with its small *N*-ethyl group (BACE-1 IC_{50} = 7 μM) underlines the importance of hydrophobic interactions in the S3 enzyme subsite (Table 1). With increasing chain length (**9b**, **9d**–**9f**), in vitro potencies greatly improved reaching a maximum of 45 nM for **9e**. While further branching was tolerated (**9c**, **9g**, and **9h**), incorporation of a less hydrophobic oxygen atom resulted in diminished activity (**9i**–**9l**).

A screen of benzyl groups (**9m**–**9s**) revealed the incompatibility of para-substitution—even a small fluoro-substituent (**9n**) led to a 10-fold loss of activity (vs. *N*-benzyl **9m**). *Meta*- and *ortho*-substitution was tolerated with BACE-1 IC_{50}s similar to the parent compound, though cellular activity was diminished. To moderate the cellular potency, we also screened a variety of heteroaryl groups (**9t**–**9y**). Despite maintaining in vitro potency for all but **9y** (BACE-1 IC_{50}: 219–312 nM), cellular activities were largely unimproved.

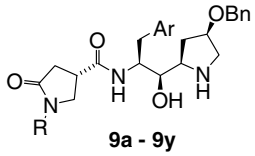
Since X-ray crystallography of **9d** and **9e** suggested that stereodefined 2-substituted pyrrolidinones may yield additional binding affinity, we evaluated inhibitors **10a**–**10h** (Table 2). Although substituting the C2-position of **9d** with alkyl or benzyl groups (**10a**–**10d**) moderately improved the in vitro and cellular activity, we did not observe a similar improvement for the *N*-pentyl series (**10e**–**10h**).

Taken together, these studies revealed that the pyrrolidinone scaffold appeared inferior to the isophthalamide moiety, prompting us to revisit further modifications of **1a** and **1b**.

Another isophthalamide moiety widely adopted throughout the patent literature[10] forms the basis for BACE-1 inhibitor **11** and its benzyloxypyrrolidine congener (Fig. 3).[8] Replacement of the *N,N*-dipropylamide present in **1b** with a 2-(*R*)-methoxymethylpyrrolidine amide resulted in a marked increase in cellular potency (BACE-1 K_i = 0.7 nM, cell IC_{50} = 21 nM), while maintaining good selectivity over related human aspartyl proteases such as cathepsin D, cathepsin E, and renin.

When tested in rats (10 mpk po dose), **11** exhibited improved pharmacokinetic properties with reasonable AUC (841 nM·h) though a low brain/plasma ratio of less then 0.1. In a study with a 5 mpk iv dose, a rat plasma half-life of 3.2 h was determined, with a high volume of distribution (V_d = 15 L/kg) and clearance (Fig. 3). BACE-1 inhibitor **11** has a good ancillary profile and did not inhibit the tested CYP enzymes (>30 μM). The compound has high human and mouse plasma protein binding, reasonable kinetic solubility and no significant inhibition in a rubidium-efflux hERG assay. Although

Table 1. SAR for *N*-substituted pyrrolidinone BACE-1 inhibitors



9a - 9y

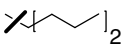
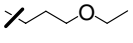
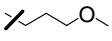
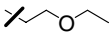
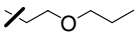
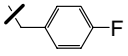
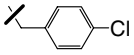
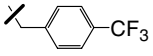
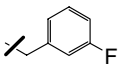
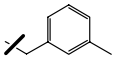
Compound	R	BACE-1 IC_{50}[a] (nM)	Cell IC_{50}[a] (nM)
9a	Ethyl	7000	
9b	Propyl	900	5000
9c	Isopropyl	3000	
9d	Butyl	370	2300
9e	Pentyl	45	610
9f	Hexyl	100	582
9g		71	1200
9h		155	795
9i		2000	2000
9j		2950	
9k		270	1200
9l		850	1500
9m		290	1700
9n		2800	5150
9o		18800	
9p		46000	
9q		338	2130
9r		350	2150
9s		365	1400
9t		312	3600
9u		219	5600

Table 1 (*continued*)

Compound	R	BACE-1 IC_{50}[a] (nM)	Cell IC_{50}[a] (nM)
9v		225	1070
9x		303	1350
9y		1200	6200

Ar = 3,5-difluorophenyl.
[a] See Ref. 7 for details of in vitro and cell assays.

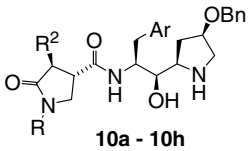
moderate permeability was observed in the Caco-2 P-gp assay, the efflux ratio was high (B–A/A–P = 174) indicating that **11** is most likely a substrate for the P-gp transporter. This may account for the low brain/plasma ratio observed in the rat PK studies.

Due to its favorable overall profile, inhibitor **11** was extensively profiled in the transgenic CRND8 mouse model (Fig. 4).[11] Upon oral dosing to 6-week old pre-plaque CRND8 mice at 10, 30, and 100 mpk, plasma $A\beta_{40}$ was reduced by 4%, 25%, and 70% relative to the vehicle group.

Subcutaneous dosing further improved exposure levels resulting in even greater reduction of plasma $A\beta_{40}$ (10 mpk: −58%; 30 mpk: −77%; 100 mpk: −88%). Despite whole brain concentrations in excess of 50-fold its cellular IC_{50}, **11** had no effect on cortical $A\beta_{40}$ levels.

In conclusion, replacement of the isophthalamide portion of BACE-1 inhibitor **1a** with various truncated amides and *N*-substituted pyrrolidinones did not improve in vitro or cellular potency. However, incorpora-

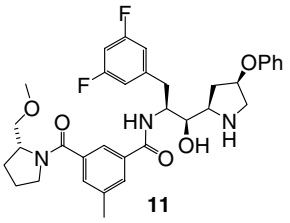
Table 2. SAR for enantiomer 2-substituted pyrrolidinone BACE-1 inhibitors



10a - 10h

Compound	R	R^2	BACE-1 IC_{50} (nM)	Cell IC_{50} (nM)
9d	Butyl	H	370	2300
10a		Ethyl	142	400
10b		Propyl	110	330
10c		Isobutyl	57	260
10d		Benzyl	78	1200
9e	Pentyl	H	45	610
10e		Cyclopropyl	570	2400
10f		Cyclopentyl	295	4250
10g		Isobutyl	365	2600
10h		Benzyl	293	4000

Ar = 3,5-difluorophenyl.



Assay		Assay	
BACE-1 Ki	0.7 nM	CYP 2D6	>30 uM
BACE-2 Ki	20 nM	CYP 3A4	>30 uM
Cell IC50 (HEK)	21 nM	CYP 2C9	>30 uM
Cathepsin D	2525 nM		
Cathepsin E	170 nM	PPB[c] (human)	96.0%
Renin	500 nM	PPB[c] (mouse)	97.7%
		Solubility	175 uM
Rat $AUC_{0\text{-}6h}$[a]	841 nM*hr	hERG Rb Efflux	12%
C_{6h}[a]	101 ng/mL	(Inh. at 5 ug/mL)	
Brain/plasma[a]	<0.1	Caco-2 Efflux ratio	x 174
$t_{1/2}$[b]	3.2 hr	(B-A/A-B)	
Clearance[b]	147 mL/min/kg		
V_d[b]	15 L/kg		

Figure 3. Detailed profile for novel BACE-1 inhibitor **11.** [a]10 mpk po dose (rat). [b]5 mpk iv dose (rat). [c]Protein plasma binding.

tion of a methoxymethylpyrrolidine isophthalamide achieved a significant improvement in cellular activity, prompting us to broadly profile **11** in the CRND8 mouse model. Here, dose-dependent reduction of plasma $A\beta_{40}$ was observed both upon oral and subcutaneous dosing, demonstrating the utility of **11** as a tool compound suitable for in vivo studies. Further SAR studies will be reported in due course.

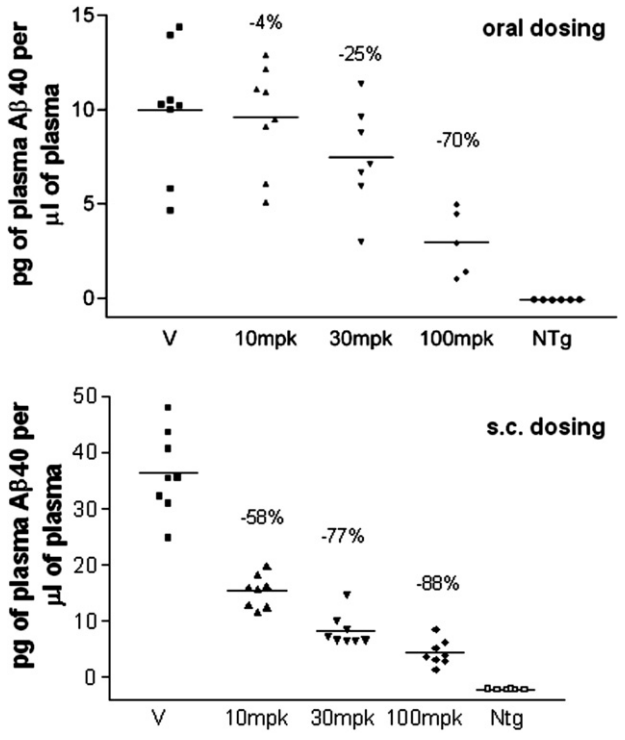


Figure 4. Dose-dependent plasma $A\beta_{40}$ lowering in 6-week-old CRND8 mice at 3 h post-dosing relative to vehicle group. Top panel shows results from oral dosing, bottom panel highlights efficacy upon s.c. dosing. NTg: non-transgenic mice devoid of APP mutations.

Acknowledgments

The authors thank Drs. A. Buevich and T.-M. Chan for NMR analyses, R. Kuvelkar and X. Chen for K_i and IC_{50} determinations, 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 US 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 Discov. Devel.* **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. Reports Med. Chem.* **2005**, *40*, 35; (e) Cumming, J. N.; Iserloh, U.; Kennedy, M. E. *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 536.
3. Kimberly, W. T.; Wolfe, M. S. *J. Neurosci. Res.* **2003**, *74*, 353.
4. Iserloh, U.; Wu, Y.; Cumming, J. N.; Pan, J.; Wang, L. Y.; Stamford, A. W.; Kennedy, M. E.; Kuvelkar, R.; Chen, X.; Parker, E. M.; Strickland, C.; Voigt, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 414.
5. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305.
6. (a) HEA-based peptidomimetic BACE-1 inhibitor disclosed by Elan/Pharmacia/Pfizer: 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.
7. BACE-1 enzymatic inhibition was determined using an APP-derived peptide containing the Swedish mutant: (a) Kennedy, M. E.; Wang, W.; Song, L.; Lee, J.; Zhang, L.; Wong, G.; Wang, L.; Parker, E. *Anal. Biochem.* **2003**, *319*, 49; 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 inhibitors. $A\beta_{40}$ levels were measured in the cell culture media using an $A\beta_{1-40}$ specific ELISA: (b) Zhang, L.; Song, L.; Terracina, G.;

Liu, Y.; Pramanik, B.; Parker, E. *Biochemistry* **2001**, *40*, 5049.
8. X-ray coordinates for the complex of BACE-1 with inhibitors **1a**, **1b**, and **11** have been deposited in the Protein Data Bank (www.rcsb.org), and can be accessed under PDB ID 2QMD, 2QMF, and 2QMG.
9. Racemic pyrrolidinone-3-carboxylic acids were obtained by condensation of primary amines with dimethyl itaconate; diastereomers were separated after coupling to **2**: (a) Felluga, F.; Pitacco, G.; Prodan, M.; Pricl, S.; Visintin, M.; Valentin, E. *Tetrahedron Asymmetr.* **2001**, *12*, 3241; (b) Beltaief, I.; Besbes, R.; Ben Amor, F.; Amri, H.; Villieras, M.; Villieras, J. *Tetrahedron* **1999**, *55*, 3949.
10. First reported by Elan Pharmaceuticals and Pharmacia & Upjohn: Varghese, J.; Maillard, M.; Jagodzinska, B.; Beck, J.P.; Gailunas, A.; Fang, L.; Sealy, J.; Tenbrink, R.; Freskos, J.; Mickelson, J.; Samala, L.; Hom, R. PCT Int. Appl., WO 2003040096, 2003.
11. CRND8-APP mice are an aggressive model of amyloid pathology that utilize a prion promoter to drive expression of human APP containing the Swedish and Indiana mutations which enhance β-secretase cleavage and $A\beta_{42}$ production, respectively.