Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1022–1026
Bioorganic & Medicinal Chemistry Letters

BACE-1 inhibitors part 3: Identification of hydroxy ethylamines (HEAs) with nanomolar potency in cells

Paul Beswick, Nicolas Charrier, Brian Clarke, Emmanuel Demont,* Colin Dingwall, Rachel Dunsdon, Andrew Faller, Robert Gleave, Julie Hawkins, Ishrut Hussain, Christopher N. Johnson, David MacPherson, Graham Maile, Rosalie Matico, Peter Milner, Julie Mosley, Alan Naylor, Alistair O'Brien, Sally Redshaw, David Riddell, Paul Rowland, John Skidmore, Virginie Soleil, Kathrine J. Smith, Steven Stanway, Geoffrey Stemp, Alistair Stuart, Sharon Sweitzer, Pam Theobald, David Vesey, Daryl S. Walter, John Ward and Gareth Wayne

Neurology and Gastrointestinal Centre of Excellence for Drug Discovery, GlaxoSmithKline R&D, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

Received 30 October 2007; revised 7 December 2007; accepted 11 December 2007
Available online 15 December 2007

Abstract—This article is focusing on further optimization of previously described hydroxy ethylamine (HEA) BACE-1 inhibitors obtained from a focused library with the support of X-ray crystallography. Optimization of the non-prime side of our inhibitors and introduction of a 6-membered sultam substituent binding to Asn-294 as well as a fluorine in the C-2 position led to derivatives with nanomolar potency in cell-based assays.

In two previous papers,[1] we outlined how BACE-1 inhibitors with sub-micromolar potency in cells expressing APP wild-type (WT) were obtained from a focused library with the help of X-ray crystallography. To maximize the chance of achieving efficacy in vivo, we wanted to achieve low nanomolar potency in a cell based assay. We therefore further investigated the SAR of our inhibitors.

As the SAR on the prime side of our inhibitors and at the S_3 pocket had already been extensively explored and the substitution pattern optimized accordingly, it was felt that the best chance of achieving significant increase in potency lay in further optimization of the substituents which had been shown to form a H-bond to Asn-294, or which would bind more tightly in the S_1 pocket. The H-bonding interaction was examined first and initial efforts focused on replacing the pyrrolidinone substituent present in compound **1**.[2]

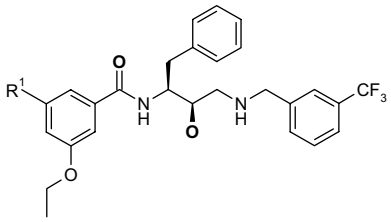
It proved possible to replace the lactam with other H-bond acceptors (HBA) such as ketones **2** and **3** or nitrile **4** with some improvements in potency, albeit with lower selectivity (Table 1). Much more impressive increases in potency were seen when sultams were introduced: Inhibitors **5** and particularly **6** were significantly more potent (at least 25-fold) and selective (up to 1000-fold against Cat-D) than all the previously prepared analogues, with a non-peptidic prime side, and further work focused on the 6-membered sultam as HBA to Asn-294.

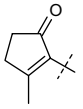
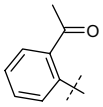
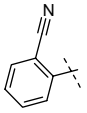
This increase in potency in the enzyme assay also translated to increased cellular activity. In fact, the level of potency achieved in the enzyme assay at this stage led to a second cell-based assay being introduced to help in ranking of relative potencies of the compounds. This additional cell assay was similar to the one already in use, but used cells expressing the Swedish (SWE) APP substrate[3] in place of the wild-type (WT) substrate.

Keywords: Alzheimer; BACE-1; Aspartic protease; Hydroxy ethylamine.
* Corresponding author. Tel.: +44 (0) 1279643468; fax: +44 (0) 1279622727; e-mail: emmanuel.h.demont@gsk.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.12.020

Table 1. Substitution of the pyrrolidinone as hydrogen bond acceptor (HBA) interacting with Asn-294



Compound	R^1	BACE-1[a,b] IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
1		380 (2)	26,840	34,325
2		250 (1)	4130	1750
3		280 (1)	3080	2940
4		160 (1)	1050	2020
5		50 (1)	4680	8510
6		6 (1)	1020	5890

[a] In all tables, IC_{50}s reported are means of the values of n different experiments, n being reported in bracket and identical for BACE-1, BACE-2 and Cat-D. Each IC_{50} is within 3-fold of the mean value.
[b] See Ref. 3 for protocol. FRET-based assay.

Somewhat unexpectedly, a significant decrease in potency was observed when comparing the lowering of amyloid production in the cells expressing SWE APP to that seen in the cells expressing WT APP (Table 2). The reason for this reduction in the level of potency was not entirely clear as the FRET enzyme assay also used the SWE substrate. Nonetheless, to progress compounds further, we resolved to maximize potency in both cell lines by further rounds of optimization.

Due to the similar binding modes, the SAR observed in the S_3 pocket in both the lactam and sultam series was similar: O- and N-linked derivatives were more potent than their carbon analogues (compare **6** and **7** with **8**), and a linear three-atom substituent gave the best compromise between activity and selectivity (compare **7** and **9**). The N-linked S_3 substituents were also generally more potent than their oxygen analogues, particularly in cells expressing SWE APP (compare **10** and **11** for example) and consequently the non-prime side of inhibitor **7** was selected as the basis for further optimization. Co-crystallisation confirmed that these derivatives had a similar binding mode to that seen with the corresponding 5-lactam derivatives (Fig. 1).[4]

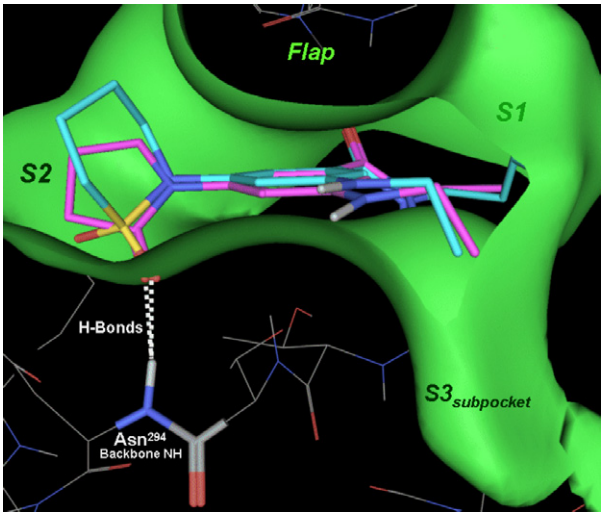
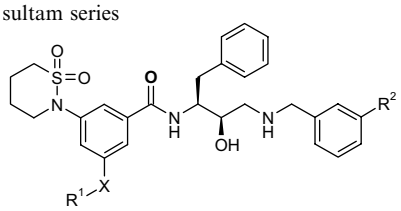


Figure 1. Superimposition of 6-sultam (light-blue) and 5-lactam (magenta) derivatives bound to BACE-1.

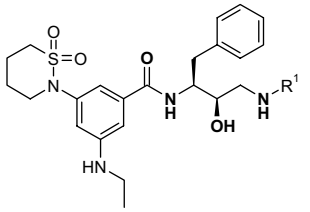
Table 2. SAR at the S_3 pocket in the 6-membered sultam series

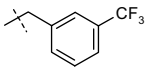
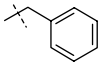
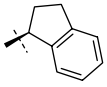
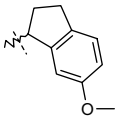
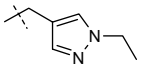


Compound	X	R^1	R^2	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	WT Aβ40[a] IC_{50} (nM)	WT Aβ42[a] IC_{50} (nM)	SWE Aβ40[a] IC_{50} (nM)	SWE Aβ42[a] IC_{50} (nM)
6	O	C_2H_5	CF_3	6 (1)	1020	5890	27	21	715	205
7	N	C_2H_5	CF_3	3 (2)	1430	3900	21	30	529	175
8	C	C_2H_5	CF_3	5 (1)	1450	4790	40	40	844	366
9	N	$CH(CH_3)_2$	CF_3	11 (1)	1660	25,120	58	62	910	414
10	O	C_2H_5	OCF_3	5 (1)	760	6170	25	18	711	216
11	N	C_2H_5	OCF_3	5 (1)	600	2570	47	34	102	39

[a] See Ref. 3 for protocol. IC_{50} values are means of at least two separate experiments.

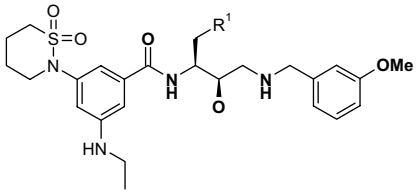
Table 3. SAR at prime side in the sultam series

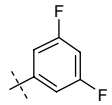


Compound	R^1	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	WT Aβ40 IC_{50} (nM)	WT Aβ42 IC_{50} (nM)	SWE Aβ40 IC_{50} (nM)	SWE Aβ42 IC_{50} (nM)
7		3 (2)	1430	3900	21	30	529	175
12		120 (1)	32,360	26,300	573	565	—	—
13		34 (1)	2290	3090	229	154	—	—
14[a]		18 (1)	2950	1070	36	26	—	—
15		12 (2)	6920	56,230	78	72	3633	1113

[a] Obtained as a 1:1 mixture of isomers.

Table 4. SAR at the P_1 position



Compound	R^1	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	WT Aβ40 IC_{50} (nM)	WT Aβ42 IC_{50} (nM)	SWE Aβ40 IC_{50} (nM)	SWE Aβ42 IC_{50} (nM)
16		8 (2)	4270	6760	19	41	928	299
17		5 (1)	630	2400	26	30	450	203
18		40 (1)	13,215	15,140	129	108	—	—
19		2690 (1)	$>10^5$	$>10^5$	—	—	—	—
20		5750 (1)	$>10^5$	$>10^5$	—	—	—	—

The SAR of the benzylic prime side was similar to that previously observed in the lactam series:[1] *meta*-substitution was important for activity (compare **7** and **12**, Table 3); Constraining the benzylic group appeared beneficial for activity (compare **13** and **12**) but *meta*-substituted constrained benzylic derivatives were disappointingly not as potent as expected (compare the increase in activity from **13** to **14** versus the difference in activity between **12** and **7**). It was possible to replace the prime side phenyl ring by heteroaryls (compound **15**) but overall, a compound with nanomolar potency across all assays was not observed.

At this stage it was felt that compounds with increased activity might be obtained by further optimization of the P_1 substituent[5] and an array of compounds with saturated and unsaturated P_1 substituents was prepared.

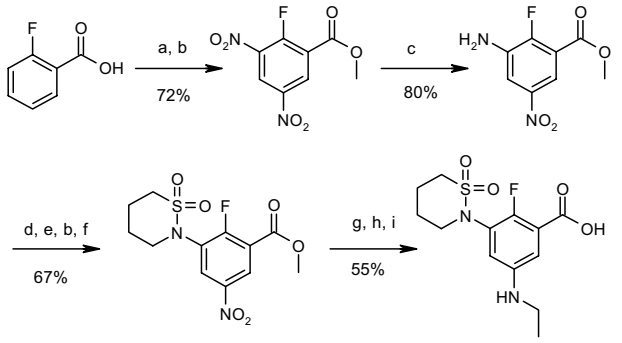


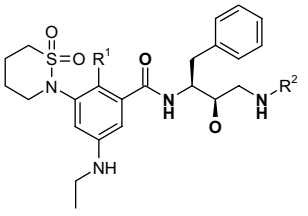
Figure 2. Reagents and conditions: (a) HNO_3, H_2SO_4 10 °C to 95 °C; (b) MeOH, H_2SO_4, reflux; (c) Fe, AcOH, T < 35 °C (1 isomer); (d) $Cl(CH_2)_4SO_2Cl$, NEt_3, CH_2Cl_2, 25 °C; (e) NaOH, MeOH/H_2O, 25 °C; (f) NEt_3, EtOH, reflux; g) Pd/C, NH_4COOH, EtOH/H_2O, reflux; (h) CH_3CHO, $NaHB(OAc)_3$, AcOH, $(CH_2Cl)_2$, 25 °C; (i) NaOH, THF/H_2O, 25 °C.

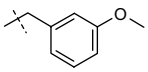
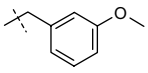
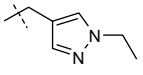
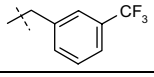
As shown in Table 4, compounds with substituted aryls (compound **17**) or electron rich heteroaryls (compound **18**) were more potent and selective than electron poor heteroaryls (compound **19**) or alkyl substituents (compound **20**). However, these compounds offered no advantage compared to the lead **16**. These results, and the fact that the core of compound **16** could be obtained from readily available phenylalanine, led us to explore other ways of improving potency.

An inhibitor with nanomolar potency in all of the key assays appeared elusive but, in another series of BACE-1 inhibitors we were developing,[6] the introduction of a fluorine in the 2-position of the benzamide non-prime side led to an increase in cell activity. It was hoped that this effect might also be observed in the sultam series. The synthetic route used to access the key building blocks that were required to test this idea is depicted in Figure 2.[7]

Satisfyingly, it was found that the introduction of this fluoro substituent did indeed prove to be beneficial (compare **16**–**21**, Table 5)[8] and excellent selectivity could be achieved, particularly against Cat-D (compound **22**). From a set of derivatives bearing a *meta*-substituted benzyl amine prime side, GSK188909 (compound **23**) was identified as one of the first selective, nanomolar inhibitors in the cells expressing SWE APP assay. GSK188909 also had a favourable pharmacokinetic profile which allowed us to determine whether a BACE-1 inhibitor would lower amyloid production in an animal model of Alzheimer's disease. The positive read-out of this study has been reported previously:[3] following oral administration (250 mg/kg twice daily for 5 days), decrease of brain Aβ40 (18%) and Aβ42 (23%) was observed in TASTPM mice. This lowering can be significantly enhanced (up to 68% and 55% for Aβ40

Table 5. Activity and selectivity of inhibitors bearing a 2-F benzamide non-prime side



Compound	R^1	R^2	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	WT Aβ40 IC_{50} (nM)	WT Aβ42 IC_{50} (nM)	SWE Aβ40 IC_{50} (nM)	SWE Aβ42 IC_{50} (nM)
16	H		8 (2)	4270	6760	19	41	928	299
21	F		4 (4)	785	6545	14	12	283	92
22	F		6 (2)	2465	87,100	14	12	361	176
23	F		4 (81)	177	2653	5	5	40	18

and Aβ42, respectively) after a single 250 mg/kg oral dose when GSK188909 is co-administed with a Pgp inhibitor.

To conclude, we have shown that further exploration of the SAR in this HEA series allowed us to achieve the level of potency in both of our key cellular assays. In particular, optimization of the HBA binding to Asn-294 and introduction of a fluorine atom in the C-2 position of our benzamide non-prime side led to a 100-fold increase in potency. These inhibitors represent useful tools for the further study, in animal models, of the possibilities for treating Alzheimer's disease via BACE-1 inhibition. Further optimization of these derivatives towards more drug-like inhibitors will be reported in due course.[6]

References and notes

1. See the two preceding papers.
2. An $-OC_2H_5$ S_3 substituent was used rather than an $-NHC_2H_5$ substituent in order to simplify the synthesis of analogues.
3. Hussain, I.; Hawkins, J.; Harisson, D.; Hille, C.; Wayne, G.; Cutler, L.; Buck, T.; Walter, D.; Demont, E.; Howes, C.; Naylor, A.; Jeffrey, P.; Gonzalez, M. I.; Dingwall, C.; Michel, A.; Redshaw, S.; Davis, J. B. *J. Neurochem.* **2007**, *100*, 802.
4. See preceding paper for references on BACE-1 construct used. The X-ray data were collected at ESRF beamline ID14-4. The PDB deposition code for the BACE-1 complex crystal structure is 2vij. The structure was refined to 1.6 Å resolution ($R = 0.187$, $R_{free} = 0.215$). It is difficult to explain why the sultams appeared more potent than the lactam as sulfonamides are in general less powerful HBAs than amides, see: Abraham, M. H.; Duce, P. P.; Prior, D. V.; Barratt, D. G.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc. Perkin Trans. II.* **1989**, *10*, 1355, This increase in potency could be explained by a better orientation of the S═O bond compared to the C═O bond or by a higher similarity between low energy conformation and enzyme-bound conformation for the sultam compared to the lactam.
5. For the synthesis of the corresponding epoxides, see: Dalla Croce, P.; La Rosa, C.; Pizzatti, E. *Tetrahedron: Asymmetry* **2000**, *11*, 2635; US Patent App. 2003/0004360(A1).
6. Manuscript in preparation.
7. For experimental procedures, see: Demont, E.; Faller, A.; Macpherson, D.; Milner, P.; Naylor, A.; Redshaw, S.; Stanway, S.; Vesey, D. Walter, D. PCT Int. Appl. (2004), WO 2004050619.
8. This trend is observed regardless of the nature of the group forming a H-bond with Asn-294 or the group binding into the S_3 pocket. The 2-MeO analogue has similar potency and selectivity to the 2-H derivative. X-ray structures of inhibitors bound to BACE-1 having 2-H or 2-F substituents can be superimposed, hence it is difficult to rationalise why 2-F derivatives are more potent. An inductive effect may be part of the explanation.