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BACE-1 inhibitors part 2: Identification of hydroxy ethylamines (HEAs) with reduced peptidic character

Brian Clarke, Emmanuel Demont,* Colin Dingwall, Rachel Dunsdon, Andrew Faller, Julie Hawkins, Ishrut Hussain, David MacPherson, Graham Maile, Rosalie Matico, Peter Milner, Julie Mosley, Alan Naylor, Alistair O'Brien, Sally Redshaw, David Riddell, Paul Rowland, Virginie Soleil, Kathrine J. Smith, Steven Stanway, Geoffrey Stemp, 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, United Kingdom

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Abstract—This paper describes the discovery of non-peptidic, potent, and selective hydroxy ethylamine (HEA) inhibitors of BACE-1 by replacement of the prime side of a lead di-amide **2**. Inhibitors with nanosmolar potency and high selectivity were identified. Depending on the nature of the P'_1 and P'_2 substituents, two different binding modes were observed in X-ray co-crystal structures.

The preceding paper in this series[1] described how a focused library of potential BACE-1 inhibitors delivered a hit (compound **1**, Table 1), which was then optimised to give compounds such as **2**, a sub-micromolar inhibitor of amyloid-β (Aβ40 and Aβ42) production in cells expressing amyloid precursor protein (APP) Wild-Type. A precise combination of *meta*-substituents on the phenyl ring of the non-prime side benzamide was found to be important for good potency and selectivity.

The next round of optimisation focused on modifications to the prime side. The strategy was to replace the amide bond-containing $P'_1 - P'_2$ fragment by groups with lower molecular weight, fewer heteroatoms and a lower polar surface area, in order to obtain inhibitors with improved potential for oral absorption and brain penetration.

The routes used to generate a library of such compounds are depicted in Figure 1 and involved either opening of commercially available epoxide **3** or reductive aminations using amine **4**.[3]

In total, 150 potential inhibitors were prepared and four were found to have IC_{50}s of less than 200 nM (Fig. 2).

The first of these, compound **6**, which incorporates a cyclohexylamine at the prime side, was especially interesting since it is only 10-fold less potent than lead compound **2**, despite significant truncation (Table 2). Analogues containing cyclopentylamine or cycloheptylamine were ~ 10-fold less potent (compounds **7** and **8**). Introduction of a heteroatom into the ring (compound **9**) or replacing the cyclohexyl with cyclohexylmethyl (compound **10**) was also detrimental to activity. Compounds in which the cyclohexyl ring was replaced with smaller alkyl fragments were also more than 10-fold less potent than **6** (data not shown).

The co-crystal structure of inhibitor **6** with the enzyme[4] showed that the cyclohexyl moiety was binding into the S'_1 pocket which contrasts with the observation that the peptidic prime side of inhibitor **2** binds into the S'_2 pocket (Fig. 3). It was difficult to pinpoint additional potential interactions within the pocket and consequently further derivatization of the cyclohexyl ring was not explored.

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

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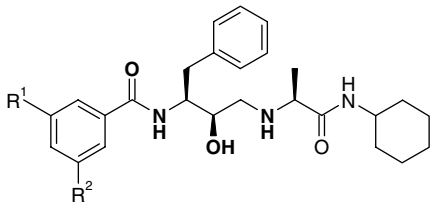
Table 1. Activity and selectivity of inhibitors **1** and **2**



Compound	R^1	R^2	BACE-1[a,b] IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	Aβ40[b,c] IC_{50} (μM)	Aβ42[b,c] IC_{50} (μM)
1	CH_3SO_2-	H	5400 (3)	41770	7700	—	—
2		-NHEt	13 (2)	1810	2695	0.31	0.36

[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. 2 for protocol.
[c] IC_{50} values are means of at least two separate experiments. Each IC_{50} is within 3-fold of the mean value.

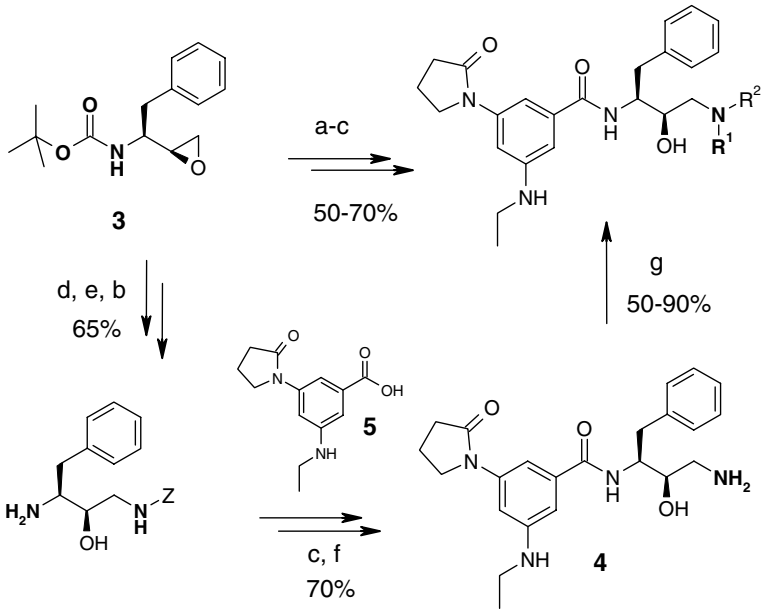


Figure 1. Conditions and reagents: (a) NHR^1R^2, EtOH, reflux; (b) TFA, CH_2Cl_2, 0 °C or HCl, CH_3CN or Dioxan, 25 °C; (c) **5**, EDAC.HCl, HOBT, CH_2Cl_2 or DMF, 25 °C; (d) NH_3, $MeOH/H_2O$, 25 °C; (e) Z-Cl, NEt_3, DMF, 25 °C; (f) NH_4COOH, Pd/C, $EtOH/H_2O$, 60 °C; (g) aldehyde or ketone, AcOH, $(CHCl_2)_2$, $NaHB(OAc)_3$, 0 °C.

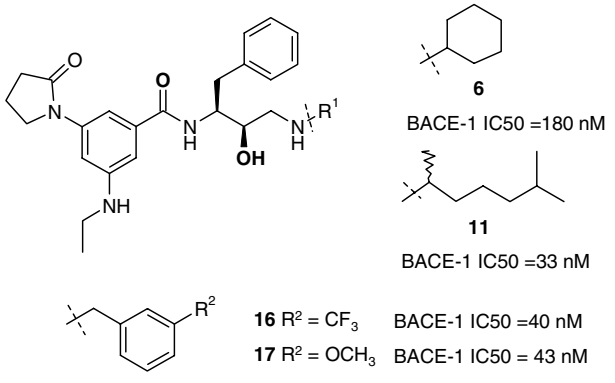
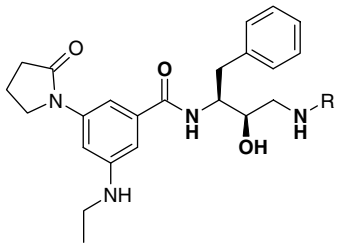


Figure 2. Activity of the four hits from the prime side replacement library.

The second hit (compound **11**, Table 3) was tested as a mixture of diastereoisomers.[5] However, the analogous dimethyl derivative **12** proved to be equipotent and the co-crystal structure of this inhibitor with the enzyme showed that the prime side adopted an extended conformation when interacting with the enzyme and could be superimposed on the peptidic prime side of compound **2** (Fig. 4).

The SAR around inhibitor **12** was investigated. Removal of both methyl groups from the carbon adjacent to the nitrogen led to a 10-fold decrease in potency (compound **13**). Removal of one of the terminal methyl substituents (compound **14**) also resulted in a significant reduction in potency. Incorporating a heteroatom in the

Table 2. Prime side SAR from inhibitor **6**



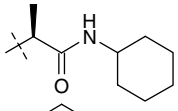
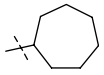
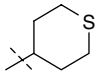
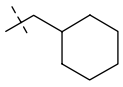
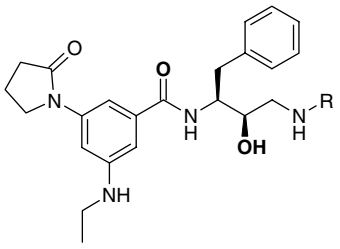
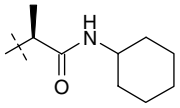
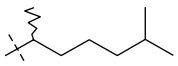
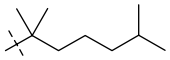
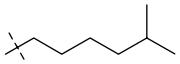
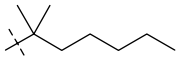
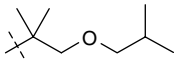
Compound	R	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
2		13 (2)	1810	2695
6		180 (1)	5010	780
7		1120 (1)	12,020	2400
8		2450 (1)	41,690	10,960
9		650 (1)	5890	1550
10		5890 (1)	57,540	12,020

Table 3. Prime side SAR from inhibitor **11**



Compound	R	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
2		13 (2)	1810	2695
11		33 (2)	3090	1095
12		33 (1)	5890	2510
13		305 (2)	3610	970
14		550 (1)	30,200	10,720
15		790 (2)	69,010	22,325

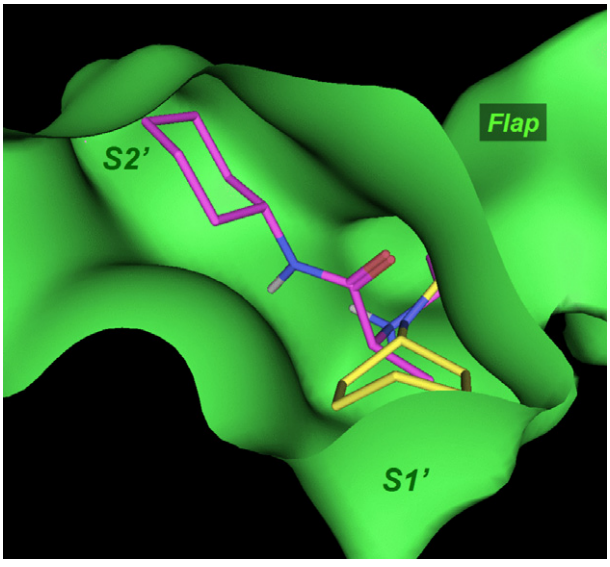


Figure 3. Superimposition of inhibitors **2** (red) and **6** (yellow) prime sides bound to BACE-1.

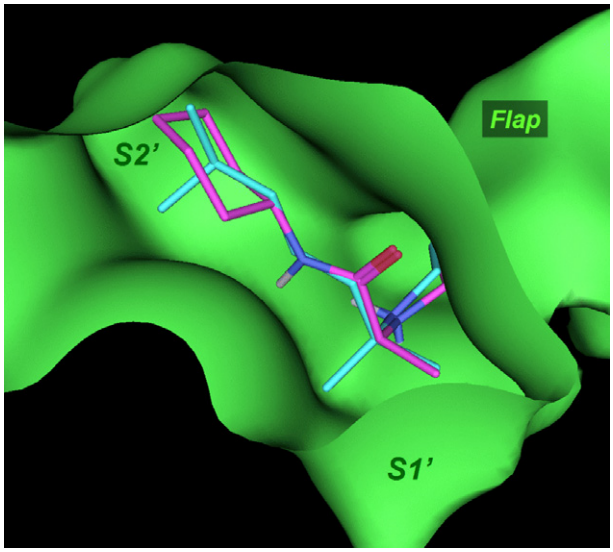
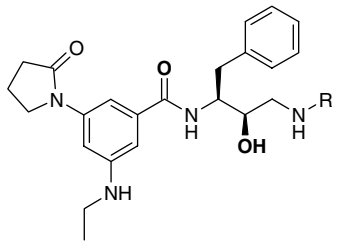


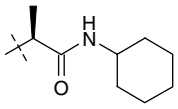
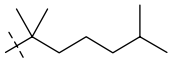
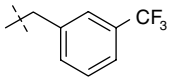
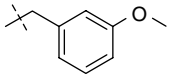
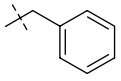
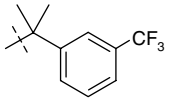
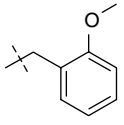
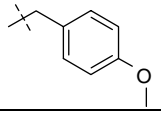
Figure 4. Superimposition of compounds **2** (red) and **12** (blue) prime sides bound to BACE-1.

alkyl chain also led to at least a 10-fold reduction in potency (see compound **15** for a representative example) and replacement of the alkyl prime side of compound **12** by shorter branched alkyl chains always resulted in at least an order of magnitude loss of potency (data not shown).

The similar potencies of compounds **11** and **12** were difficult to rationalize until the co-crystal structures of these compounds were superimposed onto those of the remaining hits from the initial array (compounds **16** and **17**, Table 4). These inhibitors were as potent and selective as compound **12** but, interestingly, removal of

Table 4. Prime side SAR from inhibitors **16** and **17**



Compound	R	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
2		13 (2)	1810	2695
12		33 (1)	5890	2510
16		40 (1)	4070	5750
17		43 (1)	7590	5010
18		760 (1)	63,100	19,050
19		17 (1)	600	930
20		1350 (1)	44,670	9770
21		2090	$>10^6$	18,620

the *meta*-substituent from the phenyl ring gave a 50-fold reduction in potency (compound **18**). Di-methylation of the benzylic position of compound **16** gave compound **19** with similar potency but slightly lower selectivity.

Figure 5 shows the enzyme-bound conformations of the prime side of inhibitors **12** and **16** superimposed. The *meta*-CF_3 group of compound **16** fills the same sub-pocket as the terminal methyl substituent of compound **12** which may explain the similar loss of potency when these substituents are removed. The 10-fold difference in potency between dimethyl compound **12** and the unsubstituted analogue **13** is not replicated in compounds with a benzylic prime side. Here, compound **16** and the dimethyl analogue **19** show similar potency. It may be hypothesized that in the former case, the substituents are necessary to orientate the alkyl chain towards the crucial S_2' pocket: filling this pocket is essential for good activity as shown by the significantly reduced

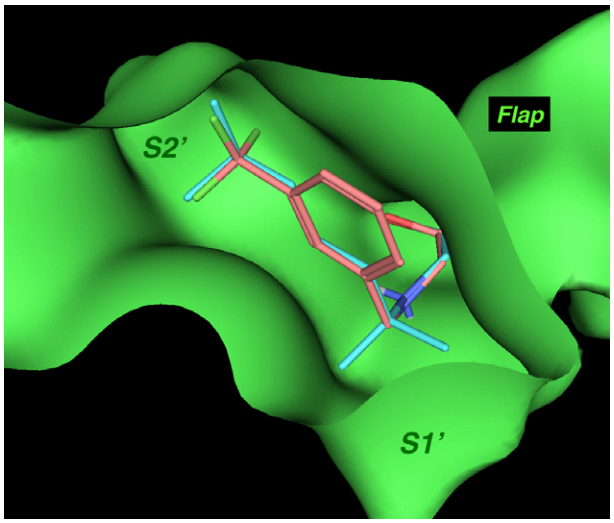
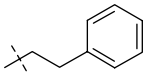
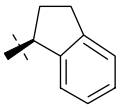
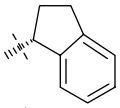
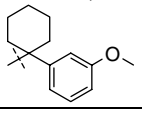


Figure 5. Superimposition of compounds **12** (blue) and **16** (red) prime sides bound to BACE-1.

potency of compounds lacking the *meta*- substituent or those in which the substituent is moved to the *ortho*- or *para*-position (compare activities of **20** and **21** versus **17**).

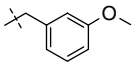
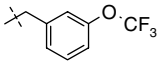
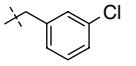
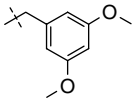
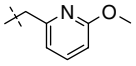
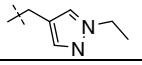
Having identified benzylamines and, specifically, meta-substituted benzylamines as suitable prime side replacements, variations in the linker between the amine and the aromatic ring were explored. As shown in Table 5, compound **22**, incorporating a phenyl prime side, was somewhat less potent than compound **18** whilst compound **23,** with a phenethyl residue, had similar potency to **18**. However, the potency of compound **23** was not significantly increased by substitution in the *ortho- meta*- or *para*-positions (data not shown) and further efforts focused on the benzylamine series. Modelling based on co-crystal structures suggested that conformational restriction of the benzylic residue should be possible

Table 5. Chain length modification and constrained derivatives

Compound	R[a]	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
22		1700 (1)	21380	4680
23		600 (1)	3390	1780
24		160 (1)	3720	5250
25		34,670 (1)	93,330	10,230
26		14 (2)	370	380

[a] See structure above in Table 4 for definition of R.

Table 6. Optimisation of the prime side substituent

Compound	R[a]	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
17		42 (1)	7590	5010
27		20 (1)	2000	3550
28		83 (1)	15,140	5620
29		100 (1)	6920	5890
30		330 (1)	33,100	26,300
31		230 (1)	33,110	46,770

[a] See structure above in Table 4 for definition of R.

Table 7. Cellular activity of representative inhibitors

Compound	BACE-1 IC_{50} (nM)	Aβ40[a,b] IC_{50} (μM)	Aβ42[a,b] IC_{50} (μM)
2	13 (2)	0.31	0.36
6	180 (1)	—	—
12	33 (1)	0.23	0.21
16	40 (1)	0.18	0.20

[a] See Ref. 2 for protocol.
[b] IC_{50} values are means of at least two separate experiments. Each IC_{50} is within 3-fold of the mean value.

and that the *S*-indane should be more potent than the corresponding *R*-isomer. This was confirmed experimentally (compounds **24** and **25**) with the *S*-indane **24** showing a 5-fold improvement in potency compared with **18**.[6,7]

Combining some of the observations described above, it was hoped that filling both the S_1' and S_2' pockets simultaneously might lead to increased potency but compound **26**, incorporating both the S_1' cyclohexyl group and the S_2' *meta*-methoxy benzyl group, was not significantly more potent than inhibitor **17** (3-fold) which is able to interact with only one of the prime-side pockets. Compound **26** was also less selective versus BACE-2 and Cat-D than compound **17**.

Further exploration of different *meta*-substituents on the phenyl ring did not lead to more potent inhibitors. A large range of lipophilic substituents was tolerated but no significant improvement in potency or selectivity was obtained compared with the initial hit **17** (Table 6, see representative examples **27** and **28**). Moreover, incorporation of additional substituents in the aromatic ring resulted in no significant benefit in terms of potency or selectivity (compare for example **29** versus **17**). Replacement of the *meta*-substituted phenyl ring by *meta*-substituted heteroaromatic rings led to compounds such as **30** and **31** with decreased potency and selectivity.

In summary, it was shown that reduction in the peptidic nature of the prime side of compound **2** led to a range of nanomolar inhibitors of BACE-1 with two different binding modes and with improved cell potency when compared to **2** (Table 7).

The absorption and brain penetrance of these compounds were predicted to be sub-optimal (high PSA , CMR and MW, large number of HBAs and HBDs).[8] We postulated that, with modest levels of CNS penetration, amyloid lowering in an animal model would most likely be achieved by a compound with low nanomolar potency in a cell-based assay.[9] The further optimisation of this series of inhibitors towards highly potent, orally active inhibitors will form the subject of a subsequent publication.

References and notes

1. See the preceding paper.
2. 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.
3. The synthesis of acid **5** is described in the preceding paper.
4. See preceding paper for references on BACE-1 construct. The X-ray data were collected at ESRF beamline ID14-4. The PDB deposition codes and refinement details for the BACE-1 complex crystal structures are: compound **2** (2vj6, 1.8Å resolution, $R = 0.187$, $R_{free} = 0.212$), compound **6** (2vj9, 1.6Å resolution, $R = 0.203$, $R_{free} = 0.232$), compound **12** (2vie, 1.9Å resolution, $R = 0.191$, $R_{free} = 0.216$) and compound **16** (2vj7, 1.6Å resolution, $R = 0.209$, $R_{free} = 0.231$).
5. The stereoisomers of compound **11** were not separated.
6. Similar conformational constraint of the phenethyl prime side did not give an improvement in potency (data not shown).
7. In a further optimised series of compounds, introduction of a *meta*-substituent into the *S*-indane did not lead to a significant improvement in potency. See following paper.
8. For discussion on absorption and brain-penetration, see for examples: (a) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3; (b) Clark, D. E. *J. Pharm. Sci.* **1999**, *88*, 807; (c) Seelig, A. *Eur. J. Biochem.* **1998**, *251*, 252.
9. It was felt that $IC_{50} \approx 10$ nM could be achieved in a cell-based assay, as it has been in the HIV protease field. See for example: Ami, E.; Nakahara, K.; Sato, A.; Nguyen, J.-T.; Hidaka, K.; Hamada, Y.; Nakatani, S.; Kimura, T.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett* **2007**, *17*, 4213.