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BACE-1 inhibitors Part 1: Identification of novel hydroxy ethylamines (HEAs)

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Abstract—Inhibition of the aspartyl protease BACE-1 has the potential to deliver a disease-modifying therapy for Alzheimer's disease. Herein, is described the lead generation effort which resulted, with the support of X-ray crystallography, in the discovery of potent inhibitors based on a hydroxy ethylamine (HEA) transition-state mimetic. These inhibitors were capable of lowering amyloid production in a cell-based assay.

Alzheimer's disease is a devastating neurodegenerative disorder characterized by the progressive formation of insoluble amyloid plaques and neurofibrillary tangles in the brain.[1] These plaques are mainly comprised of a small 4 kDa amyloid-β (Aβ) peptide, generated by the proteolytic processing of a larger membrane bound precursor protein, known as the amyloid precursor protein (APP). Cleavage of APP by BACE-1 (for β-site APP cleaving enzyme, also known as β-secretase, Memapsin-2 or Asp-2) releases an extracellular soluble APP fragment. This is concomitant with the generation of a membrane-tethered C-terminal fragment that is subsequently processed by γ-secretase to generate Aβ peptides, predominantly of 40 or 42 amino acids in length (Aβ40, Aβ42).[2] In an alternative non-amyloidogenic pathway, cleavage of APP by α-secretase within the amyloid-β region of APP precludes the release of intact Aβ.

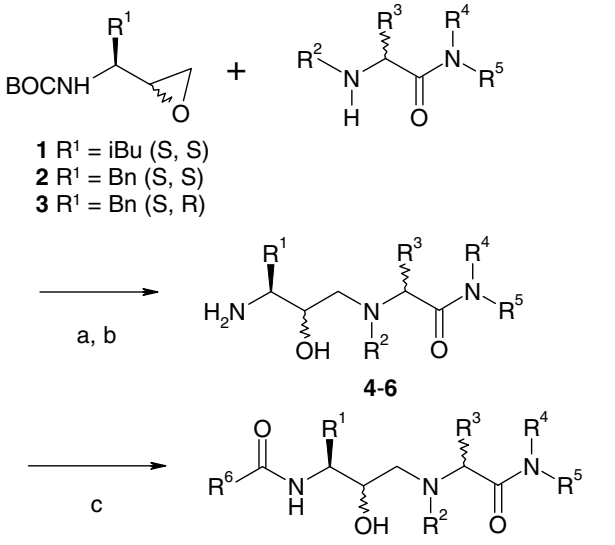

Figure 1. Reagents and conditions: (a) EtOH, reflux; (b) TFA, CH_2Cl_2, 25 °C or HCl, CH_3CN or dioxan, 25 °C; (c) R^6COOH, EDAC·HCl, HOBT, CH_2Cl_2 or DMF, 25 °C.

Keywords: Alzheimer; BACE-1; Aspartic protease; Hydroxy ethylamine.
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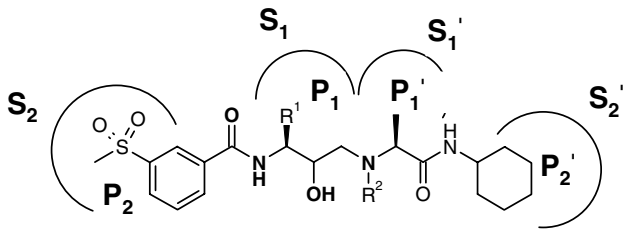
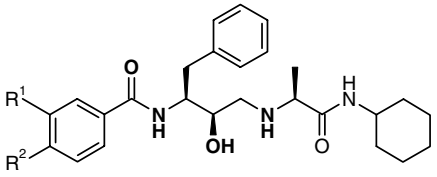
Table 1. BACE-1 inhibition for compounds **7**–**10**



Compound	R^1	CH(OH)	R^2	BACE-1 IC_{50}[a] (μM)
7	CH_2Ph	R	H	5.4
8	$CH_2CH(CH_3)_2$	R	H	>500
9	CH_2Ph	S	H	>500
10	CH_2Ph	R	CH_3	>500

[a] For assay protocol, see Ref. 7. IC_{50} value is the mean of three experiments.

Table 2. SAR at the non-prime side of BACE-1 HEA inhibitors



Compound	R^1	R^2	BACE-1[a] IC_{50} (μM)	BACE-2 IC_{50} (μM)	Cat-D IC_{50} (μM)
7	CH_3SO_2–	H	5.4 (3)	42	7.7
11		H	1.9 (4)	>21	10
12		H	5.3 (4)	79	26
13	H	CH_3CONH–	62 (1)	>100	>100
14	*n*-$C_5H_{11}SO_2$–	H	1.8 (1)	3.6	0.6

[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 threefold of the mean value.

Mice genetically deficient in BACE-1 show no Aβ production[3a,b] and are healthy, viable and fertile,[3a–c] suggesting that BACE-1 inhibition is unlikely to be associated with mechanism-based toxicity. For these reasons, inhibition of this enzyme is considered to be an attractive therapeutic target. Following the classification of BACE-1 as an aspartyl protease,[4] extensive efforts have resulted in the discovery of potent and selective inhibitors.[5] In most cases, the strategy for the design of inhibitors has been based on the transition-state mimetic concept, an approach that has been used successfully to design inhibitors of other aspartyl proteases, most notably HIV protease.[6] This approach typically relies on replacement of the scissile amide bond of an appropriate substrate with a stable mimetic of the putative transition state.

GSK188909 was recently reported to be the first BACE-1 inhibitor capable of lowering brain Aβ in APP transgenic mice following oral administration.[7] Herein, and in subsequent letters, the studies which led to the discovery of this orally active inhibitor are described.

Bearing in mind the need to deliver inhibitors with drug-like properties, we preferred not to start with large peptidic compounds based on the EVKM-DAE (APP Wild Type, WT) or EVNL-DAE (APP Swedish Mutation, SWE) sequences of the natural substrates. Our strategy instead focused on the preparation of arrays of hydroxy ethylamines (HEAs) starting from the known[8] Boc epoxides **1**, **2** and **3**, and limiting ourselves to molecules with MW < 700 and containing no more than 2 amide bonds (Fig. 1).

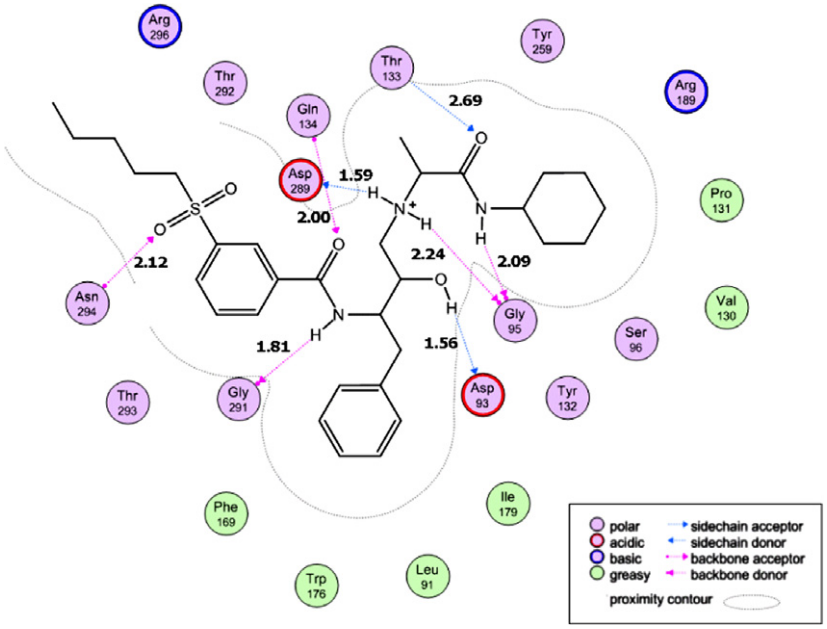


Figure 2. Key interactions between inhibitor **14** and BACE-1.

With this in mind, a number of α-amino amides were selected to add to epoxides **1**–**3**. Removal of the Boc protecting group provided amines **4**–**6** which were then coupled to a small set of carboxylic acids.

From an initial array, **7** was one of the first compounds to show micromolar activity against BACE-1 (Table 1). The analogue **8**, with Leu in the P_1 position, proved much less active. This was somewhat surprising since the Swedish-mutant substrate has Leu in the P_1 position and is turned over more rapidly than the wild-type substrate. The requirement for *R*-stereochemistry at the hydroxyl group was confirmed by compound **9** derived from the *S*,*R*-epoxide **3**. N-Methylation of the hydroxy ethylamine isostere was not tolerated (compound **10**). All subsequent SAR was therefore developed using the central core of compound **7**.

Initially, the effect of modifications of the P_1' and the P_2' substituents on BACE-1 inhibition was explored, but despite extensive effort, no improvement of potency could be found compared to the hit **7**. The contribution of the *meta*-substituted benzamide non-prime side was then investigated (Table 2), choosing BACE-2 and Cat-D,[9] both structurally related to BACE-1, as representative aspartyl protease for selectivity screens. A large range of hydrogen bond acceptors (HBAs) were tolerated in this position (see compounds **11** and **12** as representative examples) whilst inhibitors with other substitution patterns were less potent (see compound **13** for example).[10] More lipophilic *meta*-HBAs led to a modest increase in potency (compound **14**) which was sufficient to obtain a co-crystal structure of the inhibitor with a human BACE-1 construct.[11]

The key H-bond interactions between inhibitor **14** and BACE-1 are depicted below (Fig. 2) and help with understanding some of the results presented above.

Interestingly, the co-crystal structure showed that the sulfone on the non-prime side of the inhibitor makes a hydrogen bond with the enzyme, allowing access to the S_3 pocket from the other *meta*-position (Fig. 3). It seemed reasonable that filling this narrow pocket with an appropriate substituent might increase the potency of our inhibitors.

This idea was explored further after first replacing the sulfone H-bond acceptor with a pyrrolidinone. Incorporation of this group gave inhibitors with similar potency (compare **7** and **12**, Table 2), but it was felt that the lower polar surface area would increase the chance of obtaining compounds with a degree of brain penetration. The substituted benzoic acids required to complete this study

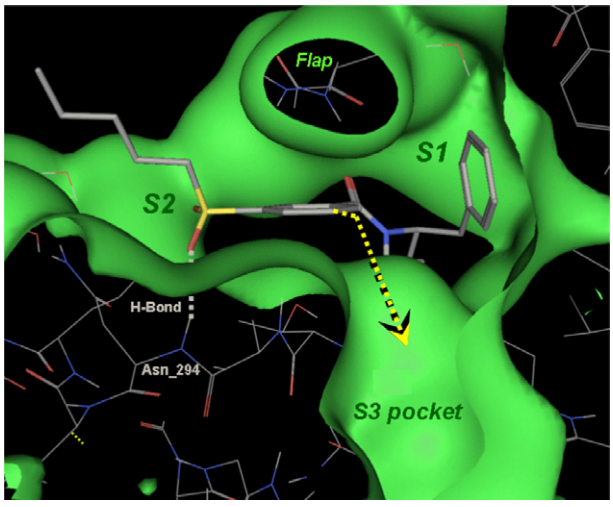


Figure 3. Non-prime side of inhibitor **14** bound to BACE-1 and potential access to the S_3 pocket.

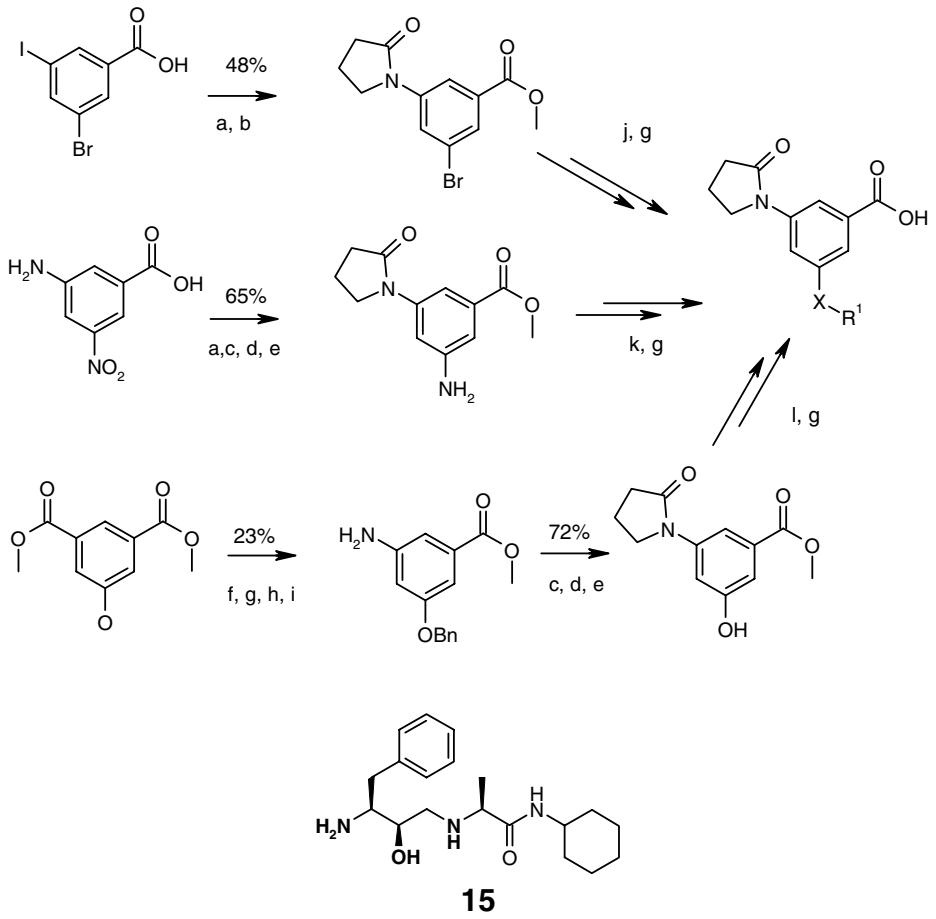
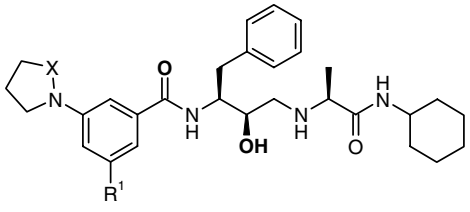


Figure 4. Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) pyrrolidinone, $Pd_2(dba)_3$, xantphos, Cs_2CO_3, dioxane, 55 °C; (c) $Cl(CH_2)_3COCl$, NEt_3, CH_2Cl_2, 25 °C; (d) NaH, THF, 25 °C; (e) H_2, Pd/C, MeOH, 25 °C; (f) BnBr, K_2CO_3, acetone, reflux; (g) NaOH, THF/H_2O, 25 °C; (h) DPPA, toluene, 80 °C then $(CH_3)_3Si(CH_2)_2OH$; (i) TBAF, THF, 25 °C; (j) BINAP, alkyl amine, toluene, K_2CO_3, $Pd(OAc)_2$, 100 °C; (k) aldehyde or ketone, $(CH_2Cl)_2$, $NaHB(OAc)_3$, 25 °C; (l) R^1Br or R^1I, acetone, K_2CO_3, reflux.

Table 3. SAR at the S_3 pocket using a pyrrolidinone as HBA



Compound	X	R^1	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	Aβ40[a,b] IC_{50} (μM)	Aβ42[a,b] IC_{50} (μM)
12	CO	H	5270 (4)	78,530	25,630	—	—
16	CO	$-OCH_3$	745 (2)	28,600	30,015	—	—
17	CO	$-OC_2H_5$	59 (1)	5130	7940	0.97	1.42
18	CO	$-OCH(CH_3)_2$	120 (1)	4790	12,300	1.13	1.18
19	CO	$-OC_5H_{11}$	32 (1)	400	430	—	—
20	CH_2	$-OC_2H_5$	1450 (1)	12,880	6170	—	—
21	CO	$-OC_3H_7$	605 (2)	8235	47,080	1.69	1.38

[a] See Ref. 7 for protocol.

[b] In Tables 3 and 4, IC_{50} values are means of at least two separate experiments. Each IC_{50} is within threefold of the mean value.

were prepared as depicted in Figure 4. The acids were coupled to amine **15** to complete the synthesis of the inhibitors. Access to the S_3 pocket could be achieved via a nitrogen, oxygen, or carbon linker.

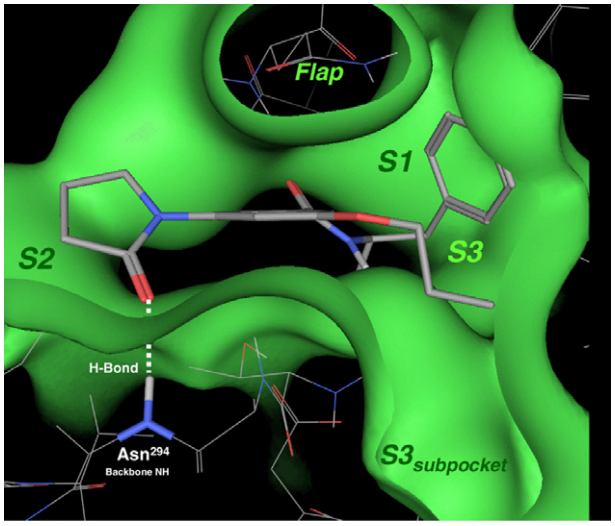


Figure 5. Key interactions between the non-prime side of inhibitor **21** and the enzyme.

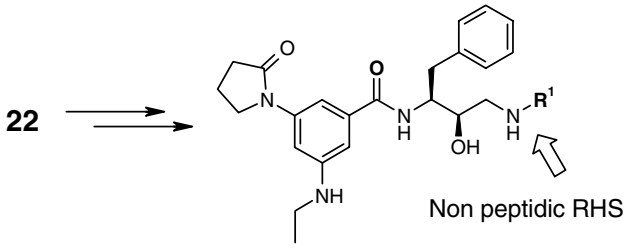


Figure 6. Towards drug-like inhibitors.

An additional substituent of sufficient bulk did appear to improve binding and inhibitors **17**–**19** (Table 3), for example, were more potent than the unsubstituted analogue **12** or derivative **16** with the smaller methoxy substituent. On examining the length of the chain in the S_3 pocket, it was found that a three-atom chain length led to a more substantial increase in potency (100-fold, compare **17**, **18** and **12**, **16**), whilst compounds with a longer chain in this position proved no more active and were generally less selective versus BACE-2 and Cat-D (compound **19**). The inhibition of BACE-1 by these compounds was also demonstrated to result in a reduction in amyloid-β (Aβ40 and Aβ42) production in cells expressing APP WT.

Replacement of the pyrrolidinone by a pyrrolidine ring (compound **20**) resulted in a 25-fold reduction in potency, suggesting that a hydrogen bonding interaction with the enzyme in this position was needed in order to lock the benzamide non-prime side in a conformation that allows access and additive binding in the S_3 pocket by a group in the other *meta*-position.

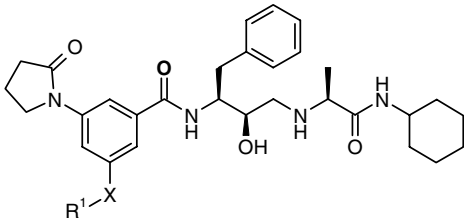
The latter hypothesis appeared to be corroborated by a co-crystal structure of compound **21** with the enzyme (Fig. 5) in which an appropriate H-bond was observed. The structure did not, however, make it entirely clear as to why an *n*-propyl chain led to a 5- to 20-fold loss in potency when compared with shorter or longer alkyl chains in the same position.[12]

Extensive exploration of the SAR at the S_3 pocket was initiated, with the aim of increasing activity and selectivity whilst keeping the polar surface area as low as possible in order to improve the likelihood of obtaining brain penetrant inhibitors. It appeared that a linear three-atom chain gave the best compromise between size, potency and selectivity (Table 4, compare **22** and **23**–**25**), with the NH-linked derivatives being the most potent and selective (compare **22** and **17**, **26**).

At this stage, efforts were shifted towards further simplification of the prime-side by replacement of the P_1'–P_2' amide, as it was felt that this strategy was more likely to lead to the identification of drug-like inhibitors of BACE-1 with the potential for good oral bioavailability and CNS penetration (Fig. 6).

In summary, in this first round of optimisation of hydroxy ethylamine BACE-1 inhibitors, P_2 and P_3 substituents which gave good potency and selectivity over

Table 4. SAR at the S_3 pocket: influence of linker and nature of the substituent



Compound	X	R^1	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	Aβ40 IC_{50} (μM)	Aβ42 IC_{50} (μM)
22	NH	C_2H_5	13 (2)	1810	2695	0.31	0.36
23	NH	$CH(CH_3)_2$	39 (6)	830	5413	0.33	0.28
24	NH	$CH_2CH(CH_3)_2$	180 (1)	650	3090		
25	Pyrrolidine		860 (3)	8793	9277		
26	CH_2	C_2H_5	72 (1)	1580	4570	0.99	0.69

other aspartyl proteases were identified. Efforts to reduce the peptidic nature of these compounds and increase the drug-like properties of this series will form the subject of a subsequent publication.

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10. Substitution *ortho* to the amide functionality was in general poorly tolerated (data not shown).
11. Asp2N153, 172, 223, 354Q/Fc was expressed in CHO secretion system with thrombin cleavage sites engineered after residues 45 and 460 (pro/thr/Asp2QQQQ/thr/Fc). Pro/thr/Asp2QQQQ/thr/Fc was captured by ProSep-A High Capacity resin (Bioprocessing Limited) and neutralized eluate dialysed into 25 mM Hepes, 0.25 M NaCl, pH 7.4. Soluble Asp2QQQQ was released by cleavage with bovine alpha thrombin (Haematologic Technologies Inc.). ProSep A unbound of the thrombin digest was further truncated by pepsin at pH 4.6. V61-E452 form of Asp2QQQQ was purified from the neutralized pepsin digest by MonoQ (GE Healthcare) at pH 7.4. Crystals of apo Asp2 were grown at 20 °C using the hanging drop vapour diffusion method combined with streak seeding. Protein at 10 mg/mL was mixed in a 1:1 ratio with the reservoir solution consisting of 10% PEG8000 and 0.1 M glycine, pH 3.2. Crystals were soaked overnight in the mother liquor to which approximately 0.1 mg of solid compound was added. The crystals were cryoprotected by serial transfer into a solution of mother liquor with 30% glycerol and then flash-frozen in liquid nitrogen prior to data collection.
    The X-ray was collected at ESRF. The PDB deposition codes and refinement details for the BACE-1 complex crystal structures are: **14** (2viy, 1.8 Å resolution, $R = 0.181$, $R_{free} = 0.216$); **21** (2viz, 1.6 Å resolution, $R = 0.198$, $R_{free} = 0.227$).
12. This effect was also observed with HBAs other than pyrrolidinone.