Bioorganic & Medicinal Chemistry Letters 20 (2010) 4639–4644

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

BACE-1 hydroxyethylamine inhibitors using novel edge-to-face interaction with Arg-296

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ARTICLE INFO

Article history:
Received 3 May 2010
Revised 26 May 2010
Accepted 29 May 2010
Available online 8 June 2010

Keywords:
Alzheimer
BACE-1
Aspartic protease
Hydroxyethylamine
Edge-to-face

ABSTRACT

Inhibition of the aspartyl protease BACE-1 has the potential to deliver a disease-modifying therapy for Alzheimer's disease. Herein, is described a series of potent inhibitors based on an hydroxyethylamine (HEA) transition state mimetic template. These inhibitors interact with the non prime side of the enzyme using a novel edge-to-face interaction with Arg-296.

Alzheimer's disease is a devastating neurodegenerative disorder for which no disease-modifying therapy is currently available.[1] An increasing body of evidence suggests this disease is triggered by the formation of amyloid plaques in the brain, mainly constituted of amyloid-beta peptides (Aβ-40, Aβ-42).[2] The aspartyl protease BACE-1[3] (for β-site APP cleaving enzyme, also known as β-secretase, memapsin-2 or Asp-2) plays a key role in the formation of these peptides and has therefore been the subject of intensive medicinal chemistry efforts.[4] Indeed, it has been shown that BACE-1 inhibitors can reduce amyloid-beta formation in pre-clinical species.[5]

We have recently disclosed our medicinal chemistry effort starting from the hydroxyethylamine transition state mimetic[6] **1** (Fig. 1) which led to GSK188909 **2**-[7] the first BACE-1 inhibitor shown to reduce brain amyloid levels in transgenic mice following oral administration.[8] Further optimization led to inhibitors such as **3** with improved pharmacokinetic profiles.[9]

In parallel with this Lead Optimization effort, and bearing in mind the inherent difficulties associated with identifying an orally efficacious and brain penetrant BACE-1 inhibitor derived from a transition state mimetic, we were keen to identify alternative starting points that might address some of these issues.

The compounds highlighted above all make an important hydrogen bonding interaction with Asn-294 on the non prime side of the enzyme, either via a lactam (compound **1**) or a sulfonamide (compounds **2**–**3**) oxygen atom. Removing this interaction generally led to very significant (>100-fold) losses of potency. Therefore we prepared a series of analogs varying the side chain and incorporating groups with the capacity to interact through an hydrogen bond with Asn-294, using the available GSK proprietary acid collection (Fig. 2).

This effort lead to the identification of compound **4**, markedly more active (350-fold) than our first hit **1** (Table 1) and showing some selectivity against BACE-2 and cathepsin D (Cat-D), the two aspartic proteases most closely related to BACE-1 and which comprised our primary selectivity panel.

The first round of SAR around this hit demonstrated the importance of the presence and nature of the H-bond acceptor (compare activity of **5** and **6** vs **4**) and of the distal aromatic (compares activity of **7** and **8** with **4**). All other substitution patterns tested led to significant decrease in potency (see representative examples **9**–**14**).

Intensive efforts were directed towards obtaining a co-crystal of inhibitor **4** with a BACE-1 construct in order to aid the prioritization of our chemistry efforts around this hit. Interestingly, the X-ray co-crystal structure succeeded in demonstrating that inhibitor **4** interacts with the non prime side of the enzyme not only via

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doi:10.1016/j.bmcl.2010.05.111

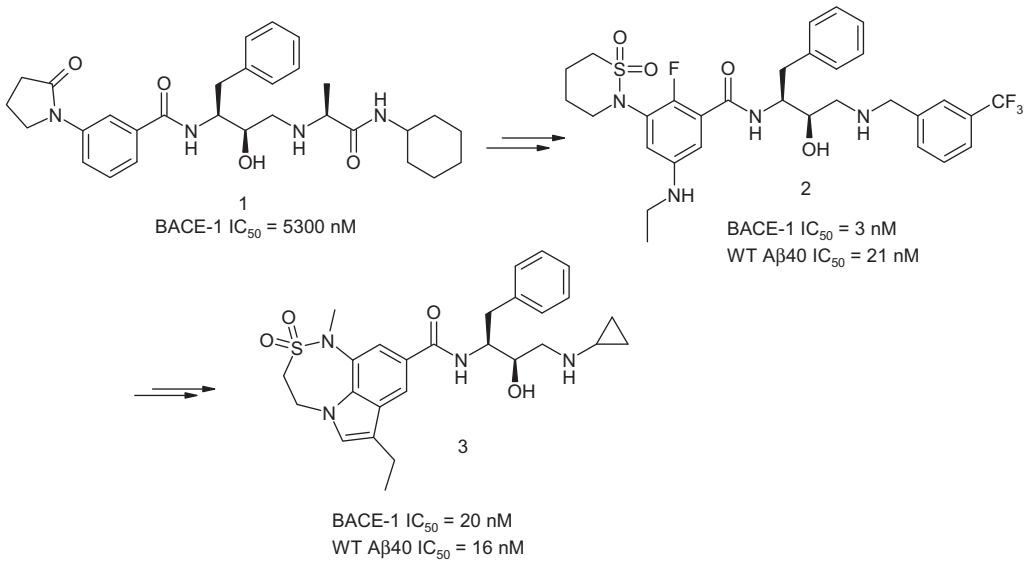


Figure 1. From micromolar hit to nanomolar orally bioavailable BACE-1 hydroxyethylamine inhibitors.

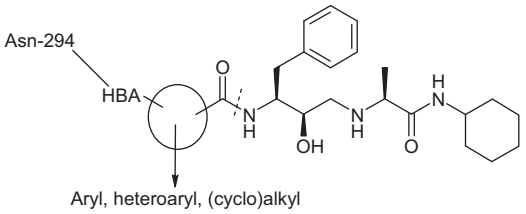
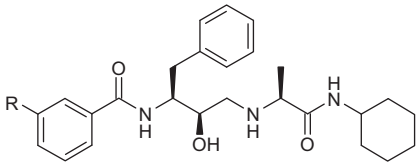


Figure 2. Design of novel HEA inhibitors susceptible of interacting with Asn-294.

Table 1
BACE-1 inhibition for compounds **1**, **4**–**14**



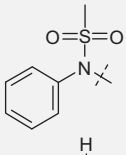
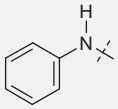
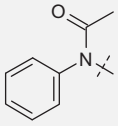
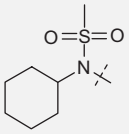
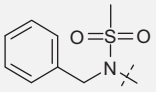
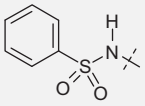
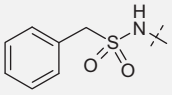
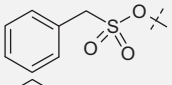
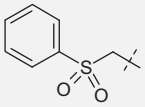
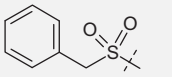
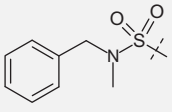
Compd	R^1	BACE-1[a] IC_{50} (μM)	BACE-2[a] IC_{50} (μM)	Cat-D[a] IC_{50} (μM)
1		5.3	79	26
4		0.015	0.655	0.660
5		6.3	0.6	1.7
6		1.3	8.9	6.2

Table 1 (*continued*)

Compd	R^1	BACE-1[a] IC_{50} (μM)	BACE-2[a] IC_{50} (μM)	Cat-D[a] IC_{50} (μM)
7		0.3	7.1	2.4
8		0.18	4.1	0.6
9		7.9	79.4	12.0
10		5.5	132	30
11		13	35	3.5
12		44	46	12
13		50	117	6.6
14		41	200	2.2

[a] In all tables, IC_{50}s reported are means of the values of three different experiments. Each IC_{50} is within threefold of the mean value.

the anticipated H-bond with Asn-294, but also via an unexpected edge-to-face interaction with Arg-296 (Fig. 3).[10,11] The increase of potency against BACE-2 and Cat-D may potentially be explained by a similar interaction but no co-crystallization was attempted in both cases.

With this information in hand, we were keen to expand the SAR around this diphenylamine sulfonamide template. The chemistry

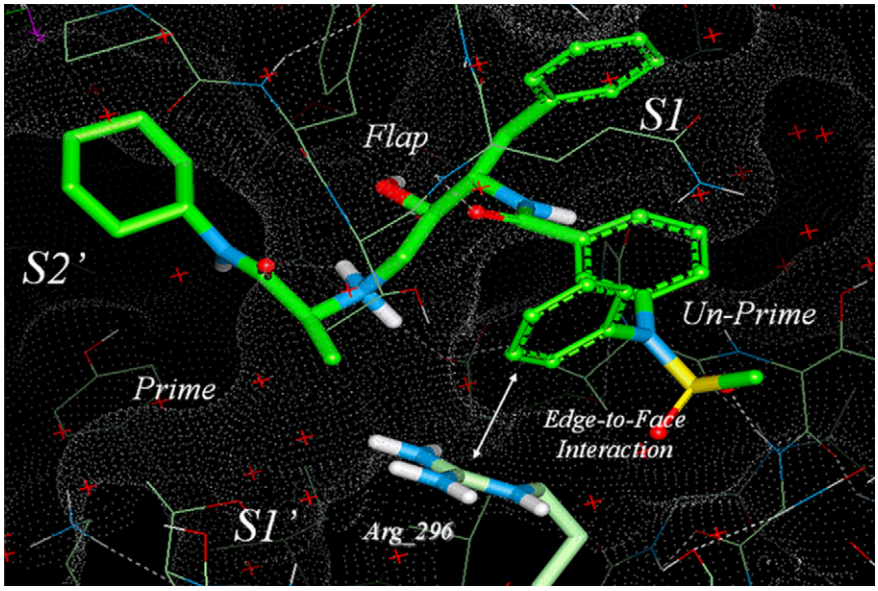
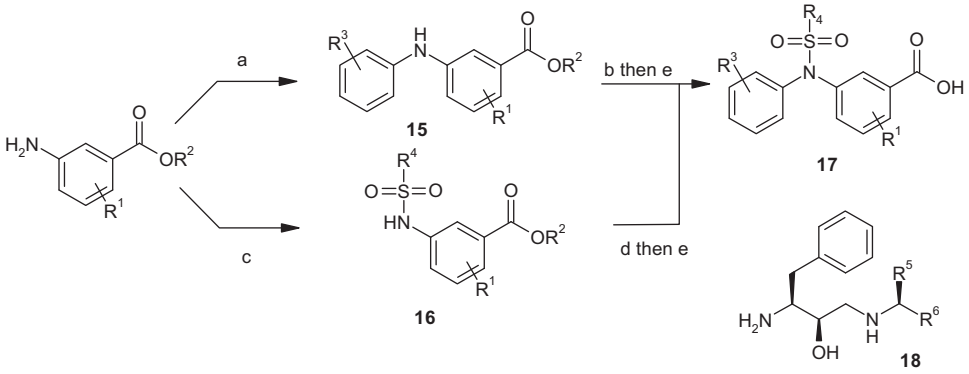
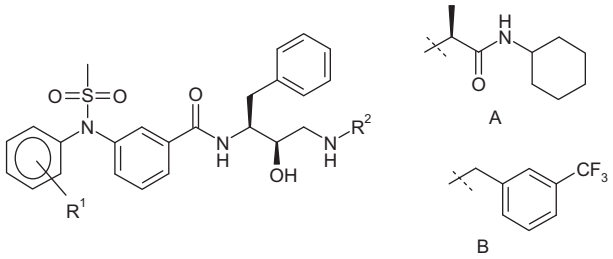


Figure 3. View of the non prime side substituent of inhibitor **4** binding to BACE-1.



Scheme 1. Reagents and conditions: (a) ArX (X = Br, I), Cs_2CO_3, XantPhos™, $Pd_2(dba)_3$, toluene, 100 °C; (b) LDA, THF, −78 °C then RSO_2Cl, −78 °C to room temperature; (c) RSO_2Cl, CH_2Cl_2, pyridine, DMAP, room temperature; (d) $ArB(OH)_2$, $Cu(OAc)_2$, CH_2Cl_2, NEt_3, room temperature; (e) NaOH, MeOH/H_2O, room temperature.

Table 2
SAR at the distal aryl ring of diphenylamine sulfonamide non prime side substituted BACE-1 inhibitors



Compd	R^1	Ar	R^2	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
4	H	Ph	A	15	665 (44)	660 (44)
19	H	Ph	B	23	870	450
20	2-MeO	Ph	A	126	3615	405
21	2-CN	Ph	A	310	10,110	1090
22	3-MeO	Ph	A	18	765	640
23	3-CN	Ph	A	8	510	600
24	4-MeO	Ph	A	11	435	350
25	4-CF3	Ph	A	20	690	580

(*continued on next page*)

Table 2 (*continued*)

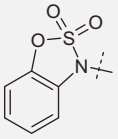
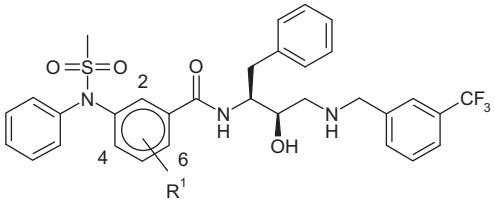
Compd	R^1	Ar	R^2	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
26	4-Cl	Ph	A	10	420	350
27	4-CN	Ph	A	190	4215	5145
28	3,5-Cl	Ph	A	11	430	170
29	—		B	910	1260	1860
30	H	2-Pyridyl	B	290	8320	1020
31	H	3-Pyridyl	B	25	480	470
32	H	4-Pyridyl	B	210	10,230	25,700

Table 3
SAR at the proximal aryl ring of diphenylamine sulfonamide non prime side substituted BACE-1 inhibitors



Compd	R^1	Ar	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
4	H	Ph	15	665	660
33	2-CH_3	Ph	26	620	110
34	4-CH_3	Ph	930	6920	2140
35	6-CH_3	Ph	3470	24,550	980
36	H	2-Pyridyl	3630	11,220	2090
37	H	4-Pyridyl	15	580	280
38	H	5-Pyridyl	21	2290	400
39	H	6-Pyridyl	4570	28,180	5890

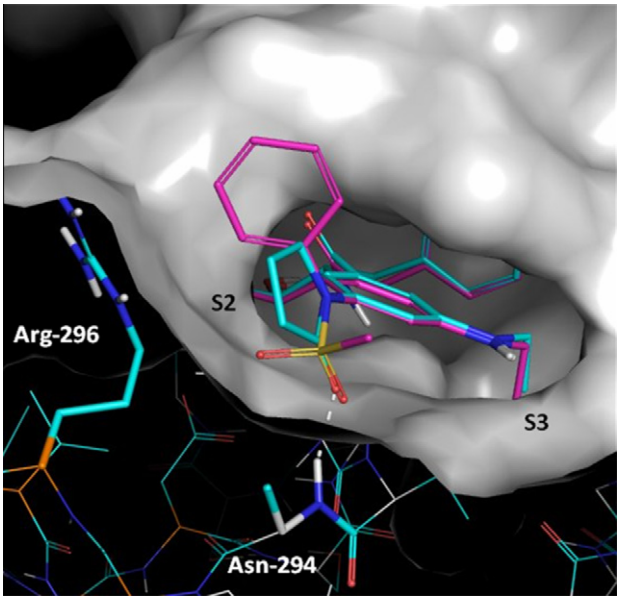
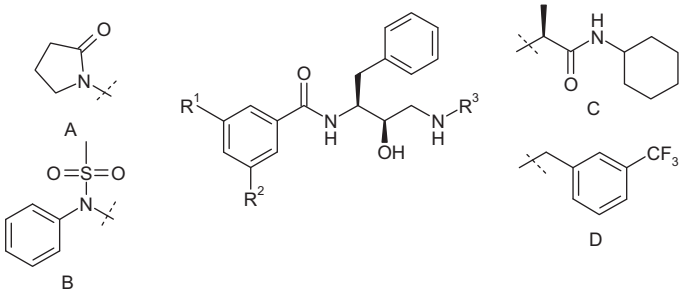


Figure 4. Superimposition of non prime side of inhibitors **40** (blue) and **41** (pink) bound to BACE-1.

Table 4
Effect of substituent interacting with the S3 pocket in lactam and phenyl sulfonamide series



Compd	R^1	R^2	R^3	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)	Aβ-40 IC_{50} (nM)	Aβ-42 IC_{50} (nM)
1	A	H	C	5270	78,530	25,630		
40	A	–NHC_2H_5	C	13	1810	2695	307	363
4	B	H	C	15	655	660	570	510
41	B	–NHC_2H_5	C	3	170	630	21	53
42	B	OC_2H_5	D	3	160	680	50	41
43	B	–$CH(CH_3)_2$	D	5	580	520	180	126
44	B	Phenyl	D	13	68	250	—	—
45	B		D	6	145	350	29	35
46	B	$SO_2C_2H_5$	D	14	1510	550	210	220

to access these types of inhibitors is described below (Scheme 1) and involves either Buchwald coupling followed by sulfonylation of the intermediate amine **15**, or arylation of an intermediate N–H aryl sulfonamide **16**. Saponification of the ester obtained leads to acid **17** which can then be coupled with known hydroxyethylamine fragments **18** in order to obtain new inhibitors.[12]

The key SAR data regarding the distal aromatic ring are presented in Table 2 and was generated either in comparison with inhibitor **4** or with its analogue **19** bearing one of our favored non-peptidic prime side residues.[13] Overall, electron donating or withdrawing substituents were tolerated in all positions but *ortho* to the sulfonamide moiety (compare activity of **20** and **21** with **4**, **22**–**26**), with some exceptions (such as compound **27**). Di-substituted derivatives were only of similar activity to the parent (compare **28** and **4**) whilst all attempts to conformationally constrain this fragment of the inhibitor led to decreases in potency (see inhibitor **29** as a representative example). The most potent inhibitors were at least 30-fold selective against BACE-2 and Cat-D. Replacement of the phenyl ring with pyridyl was only well tolerated in the case of the 3-pyridyl group (compare **19** with **30**–**32**). Overall, no significant gains in activity or selectivity were obtained relative to the lead molecule.

We next turned our attention to the proximal aromatic ring. A methyl scan showed that substitution was tolerated at C-2 but was detrimental to activity at the C-4 and C-6 positions (compare **33**–**35** with **4**, Table 3). Only two phenyl-to-pyridyl replacements were tolerated (compare **36**–**39** vs **4**) in this ring.

However, the most surprising results were observed at the C-5 position which when substituted in previous series led to significantly increased BACE-1 potency by virtue of filling the S3 pocket of the enzyme (compare activity of **1** and **40**, Table 4).[6] In this novel series, substitution in this position led only to a modest increase (5- and 10-fold in the enzyme and cell based assays, respectively) in potency (compare **41** and **4**).

Co-crystallization of inhibitors **40** and **41** with a BACE-1 construct showed however that in both cases the NHC_2H_5 meta-substituent fills the S3 pocket of the enzyme in a similar way (Fig. 4). These data possibly suggest differences in binding kinetic between the two series although this hypothesis was not investigated rigorously.

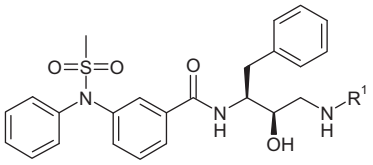
It is worth mentioning that a large range of substituents were tolerated in this position (compounds **42**–**46,** Table 4), even if again no increase in potency or selectivity was observed versus the lead **4**.

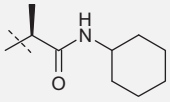
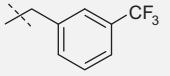
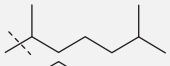
With these results in hand, we turned our attention to the SAR on the prime side of the enzyme, using our favoured set of substituents at S1′ and S2′ pockets.[13,14] As seen in Table 5, truncation of the substituent[15] in this position led to a significant drop in potency (compare activities of **47**–**49** vs **4** and **19**, Table 5).

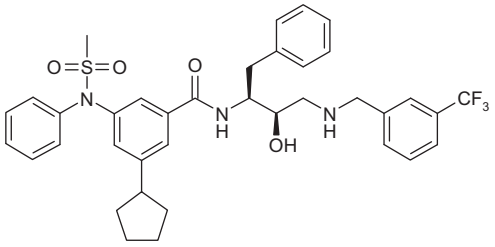
Moreover, in vitro and in vivo pharmacokinetic data were suboptimal in this series (see profile of inhibitor **50**, Fig. 5) and none of the inhibitors tested showed oral bioavailability in rats. Overall, this series did not present significant advantages compared to the ones already described[7,9] and was therefore not progressed further.

In conclusion, we have described a series of inhibitors which bind to BACE-1 using an edge-to-face interaction with Arg-296 which has not previously been described in the literature. This new binding-mode appears to impact significantly the SAR observed in previous series, particularly whilst filling the S3 pocket

Table 5
SAR at the prime side of diphenylamine BACE-1 inhibitors



Compd	R^1	BACE-1 IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cat-D IC_{50} (nM)
4		15	665	660
19		23	870	450
47		32	780	207
48		243	3243	193
49		440	12,020	9550



IC_{50} (BACE-1, BACE-2, Cat-D; nM)		4, 42, 430
IC_{50} (Aβ-40, Aβ-42; nM)		275, 205
MW, PSA, CHI LogD @ pH 7.4		680, 99, 3.90
CYP IC_{50}s (1A2, 2C19, 2C9, 2D6, 3A4 DEF, 3A4 PPR; μM)		>100, 14.0, 6.9, 3.7, 0.3, 0.6
Rat PK*	CLb (mL/min/kg)	76
	T½ (h)	0.7
	Vss (L/kg)	9.3

* 1 mg/kg i.v. in 2% (v/v) DMSO added to 5% (w/v) glucose containing 10% (w/v) Kleptose

Figure 5. In vitro profile and in vivo pharmacokinetics in rat of inhibitor **50**.

of the enzyme. Current efforts are directed towards a better understanding of the binding kinetics (k_{on} and k_{off}) for this series. Results in this area will be reported in due course.

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11. The PDB deposition codes and refinement details for the BACE-1 complex crystal structures are: **4** (2xfi, 1.7 Å resolution, $R = 0.171$, $R_{free} = 0.198$); **40** (2xfj, 1.8 Å resolution, $R = 0.166$, $R_{free} = 0.191$); **41** (2xfk, 1.8 Å resolution, $R = 0.163$, $R_{free} = 0.195$). For crystallographic experimental details see Ref. 6.
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14. Increasing the size of the alkyl chain linked to the SO_2 group did not increase potency or selectivity (data not shown).
15. Truncation of the prime side substituent of our inhibitors was expected to improve their permeability, hence their absorption, as well as remove a potential site of metabolism. See Ref. 9.