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Second generation of BACE-1 inhibitors part 2: Optimisation of the non-prime side substituent

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ABSTRACT

Our first generation of hydroxyethylamine transition-state mimetic BACE-1 inhibitors allowed us to validate BACE-1 as a key target for Alzheimer's disease by demonstrating amyloid lowering in an animal model, albeit at rather high doses. Finding a molecule from this series which was active at lower oral doses proved elusive and demonstrated the need to find a novel series of inhibitors with improved pharmacokinetics. This Letter describes the discovery of such inhibitors.

In the preceding paper,[1] we demonstrated that the chemical space occupied by our first generation of inhibitors was unlikely to contain a BACE-1 inhibitor with the combination of in vitro potency, solubility, permeability and metabolic stability that would provide efficacy in an animal model at low oral dose (≈10–20 mg/kg). We needed to find a novel series with similar in vitro efficacy but which occupied an area of physicochemical space which we reasoned would lead to improved in vivo pharmacokinetics.

From X-ray crystallography studies the hydroxyethylamine core of inhibitors such as GSK188909 (compound **1**, Fig. 1) binds strongly in the active site to the catalytic aspartyl residues therefore we did not want to modify this part of the molecule at this stage.[2] The prime side meta substituted benzylic residue was one

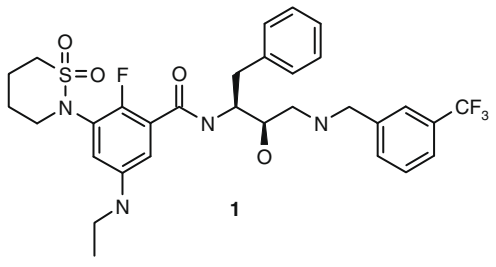

Figure 1. Structure of GSK188909.

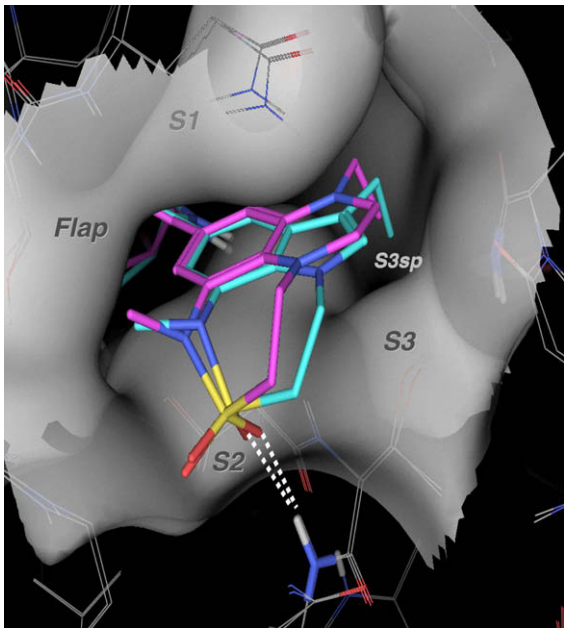

Figure 2. Non-prime side of inhibitor **9** (blue) and **10** (magenta) bound to BACE-1.

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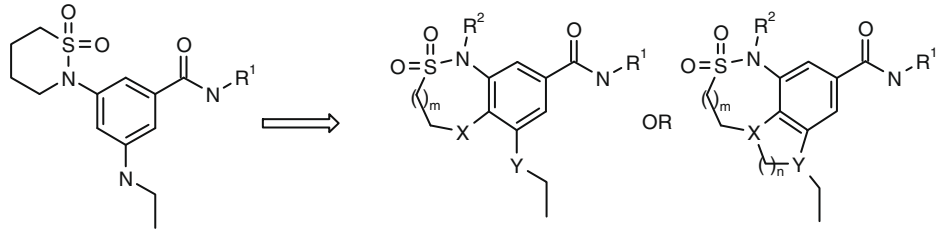
of the only active substituents identified from a large library and was unlikely to be easily replaced.[3] On the other end, key interactions made by the non-prime side residue of our inhibitors are (1) a hydrogen bond between the sulfonamide and Asn294; (2) a lipophilic interaction between the other meta-substituent (NHEt in this case) and the S3 pocket of the enzyme. Abolishing either of these interactions led to roughly a 100-fold loss of potency.[4]

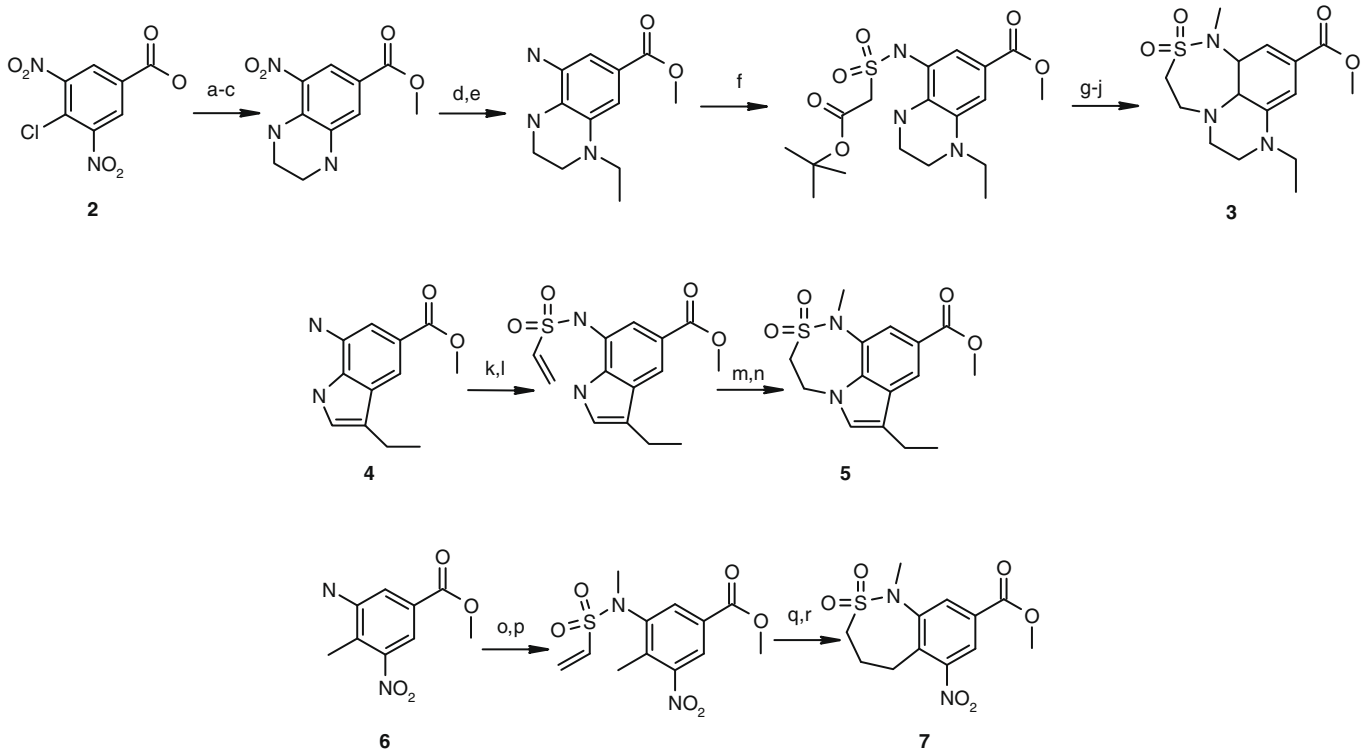
We reasoned that if we could identify a novel template that fulfilled these requirements, but had improved ligand efficiency, this might lead to compounds which would not have to interact with the prime side pockets of the enzyme to maintain good levels of potency. These putative derivatives would therefore have a smaller volume (described by their calculated molar refractivity, CMR), and hence would be more likely to be permeable according to our predictive model (see preceding paper). This would also potentially remove the prime side benzylic position, one of the known metabolic sites in our inhibitors. Moreover, the new non-prime side residues could themselves provide increased metabolic stability compared to the bis meta-substituted benzamide present in GSK188909 and its analogues.

Modelling studies suggested that the non-prime side interactions described above could be realised using sulfonamides cyclised back onto the adjacent phenyl ring (Scheme 1). These studies also suggested that these interactions might also be achieved using the corresponding tricyclic derivatives, which might be synthesized using the chemistry already developed in this area (see synthesis of bicyclics non-prime side residues in the preceding paper).

The synthesis of intermediates **3**, **5** and **7** which were required to test this hypothesis is described in Scheme 2, starting from commercially available materials **2** and **6**, or known intermediate **4**.[5] To facilitate direct comparisons and to simplify the chemistry, the sulfonamide was generally incorporated into a seven-membered ring, which could be readily obtained by Michael addition or amide coupling as shown below. The sulfonamide nitrogen was alkylated in order to remove an additional and likely unnecessary, according



Scheme 1. Potential replacement of the bis meta-substituted non-prime side residue. n = 1,2; m = 0–2; X, Y = C, N.



Scheme 2. Synthesis of key intermediates for the synthesis of novel non-prime side substituent. Reagents and conditions: (a) H_2SO_4, MeOH, reflux, 90%; (b) $Cl(CH_2)_2NH_2{\cdot}HCl$, NEt_3, EtOH, reflux, 86%; (c) Fe(0), AcOH, $T < 30$ °C; (d) CH_3CHO, $NaHB(OAc)_3$, AcOH, $(CH_2Cl)_2$, 25 °C, 71% (two steps); (e) NH_4COOH, Pd/C, MeOH, reflux, 98%; (f) tBuOCOCH$_2$SO$_2$Cl, pyridine, DMAP, CH_2Cl_2, 25 °C then NEt_3, EtOH, reflux, 20%; (g) CH_3I, K_2CO_3, DMF, 25 °C, 100%; (h) 4 N HCl, dioxane, rt, 98%; (i) EDAC, DMF, 25 °C, 60%; (j) BH_3, THF, reflux, 98%; (k) $Cl(CH_2)_2SO_2Cl$, pyridine, DMAP, CH_2Cl_2, 25 °C; (l) NEt_3, CH_2Cl_2, 25 °C; (m) NaH, DMF, 25–100 °C, 85% (three steps); (n) NaH, MeI, DMF, 25 °C, 76%; (o) $Cl(CH_2)_2SO_2Cl$, NEt_3, CH_2Cl_2, 25 °C then K_2CO_3, DMF, 50 °C, 30% (two steps); (p) K_2CO_3, CH_3I, DMF, 25 °C, 92%; (q) K_2CO_3, DMF, 50 °C, 73%; (r) NH_4COOH, Pd/C, MeOH, reflux; 98%.

to modelling based on previous co-crystal structures of inhibitors bound to BACE-1, hydrogen-bond donor.[2]

A comparison of potency and selectivity of a first set of inhibitors, incorporating these novel bicyclic and tricyclic non-prime side substituents is presented in Table 1.[6]

Inhibitors containing the tricyclic residue C or the bicyclic residue D were typically less potent than those containing residues A or B (compare **10**–**11**, **14**, **16**, **19**–**20** with **8**–**9**, **12**–**13**, **15** and **17**–**18**, respectively). Inhibitors containing residue B were more potent against BACE-1 than inhibitors containing residue A, and more selective against Cat-D but less so against BACE-2 (compare **9**, **13** and **18** with **8**, **12** and **17**, respectively). In contrast to what had been observed in the bis meta-substituted series, it was also now possible to achieve excellent BACE-1 inhibition without incorporating large meta-substituted benzyl prime side residues (compounds **15** and **18**). These findings focused our attention on this new series of inhibitors bearing indole-like tricyclic non-prime side residues.

In an attempt to understand the results presented above, a representative of each novel series was co-crystallized with a BACE-1 construct and the structures were elucidated by X-ray crystallography.[7] The non-prime sides of these derivatives bound to BACE-1 are shown below (Figs. 2 and 3).

No significant displacements of enzyme residues or changes to the inhibitor binding motifs were seen and it is difficult to rationalise the observed differences in potency on this basis.

However, we were able to prioritise work on one of the novel series and to develop this series further. We turned our attention to developing the SAR of the sulfonamide ring. A comparison of activity between the six- and seven-membered rings is highlighted below (Table 2). Inhibitors bearing a 6,6,5 tricycle non-prime side residue demonstrated nanomolar potency against BACE-1, particularly when interacting with the S2′ pocket (compound **21**). However, truncation of the prime side residue results in a 10-fold drop in activity relative to the 7,6,5 tricyclic inhibitors

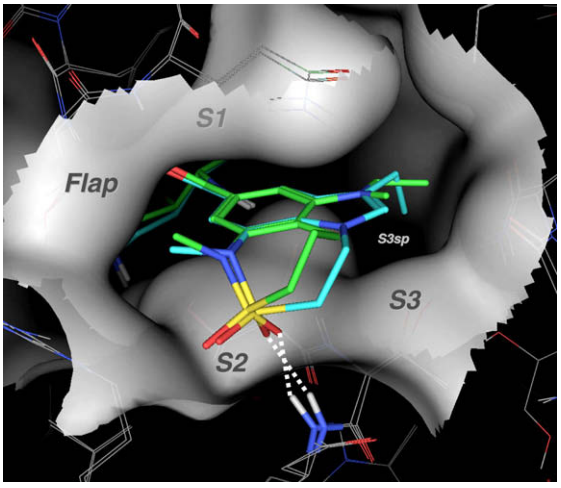
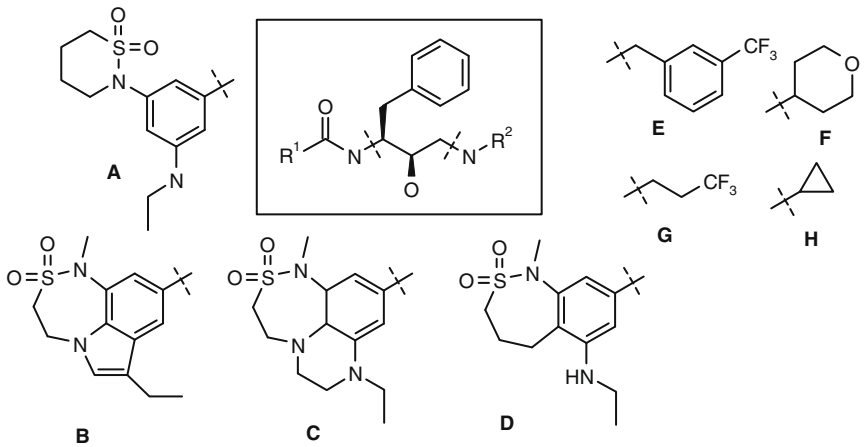


Figure 3. Non-prime side of inhibitor **9** (blue) and **11** (green) bound to BACE-1.

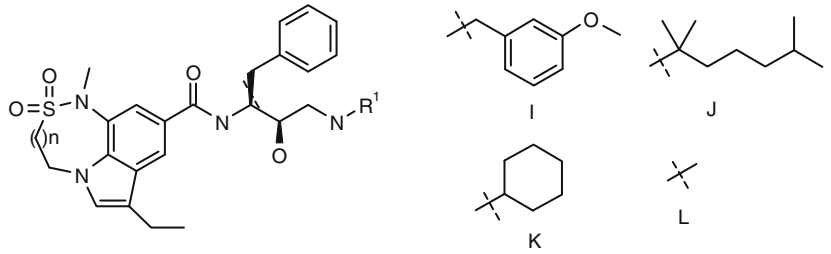
Table 1
Activity and selectivity of selected BACE-1 inhibitors



Compd	R^1	R^2	BACE-1 IC_{50}[a] (μM)	BACE-2 IC_{50}[b] (μM)	Cat-D IC_{50}[b] (μM)	Aβ40 IC_{50}[c] (μM)
8	A	E	0.007	4.26 (589)	6.76 (933)	0.019
9	B		0.002	0.059 (24)	1.259 (525)	0.006
10	C		0.026	3.8 (146)	2.24 (86)	0.084
11	D		0.019	3.59 (189)	1.21 (64)	0.056
12	A	F	0.091	11.19 (123)	19.95 (219)	–
13	B		0.015	0.49 (33)	6.61 (441)	0.090
14	D		0.19	18.71 (98)	4.1 (22)	1.185
15	B	G	0.007	0.028 (4)	0.89 (127)	0.008
16	D		0.026	1.23 (47)	1.07 (41)	0.180
17	A	H	0.12	19.5 (163)	30.2 (252)	–
18	B		0.020	0.263 (13)	7.93 (397)	0.016
19	C		0.55	16.22 (12)	26.3 (48)	–
20	D		0.15	13.55 (90)	8.15 (54)	1.574

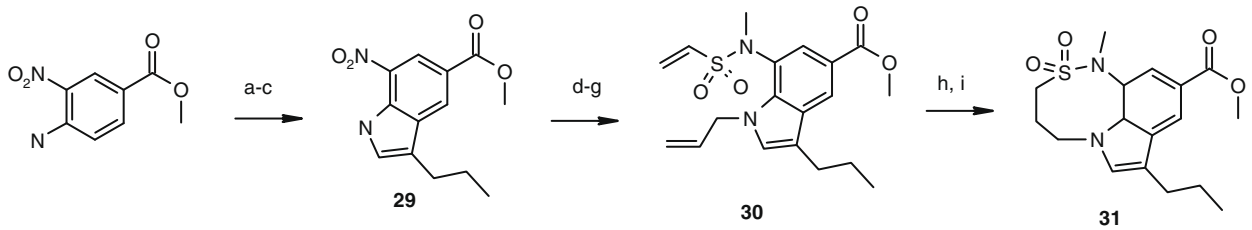
[a] In all tables, IC_{50}S reported are means of the values of 3 different experiments. Each IC_{50} is within threefold of the mean value.
[b] Data in parentheses indicates compound selectivity as ratio of enzyme and BACE-1 IC_{50}S.
[c] In SHSY5Y wild type cells. See previous paper for details.

Table 2
Comparison of BACE-1 activity for 6,6,5 and 7,6,5 tricyclic non-prime side



Compd	N	R^1	BACE-1 IC_{50} (μM)	BACE-2 IC_{50}[a] (μM)	Cat-D IC_{50}[a] (μM)	Aβ40 IC_{50} (μM)
21	0	I	0.008	0.35 (44)	1.00 (125)	–
22	1		0.002	0.096 (48)	2.95 (1476)	0.012
23	0	J	0.023	0.39 (17)	0.78 (34)	0.129
24	1		0.005	0.11 (23)	1.00 (200)	0.072
25	0	K	0.060	0.62 (10)	0.30 (5)	–
26	1		0.009	0.21 (23)	0.66 (73)	0.093
27	0	L	0.091	1.17 (13)	6.61 (73)	0.868
28	1		0.015	1.26 (84)	58.88 (3925)	0.106

[a] Data in parentheses indicates compound selectivity as ratio of enzyme and BACE-1 IC_{50}S.



Scheme 3. Synthesis of key intermediate for the synthesis of 8,6,5 tricyclic non-prime side residue. Reagents and conditions: (a) I_2, $AgSO_4$, MeOH, 25 °C, 95%; (b) $nC_3H_7CCSi(CH_3)_3$, $Pd(OAc)_2$, LiCl, K_2CO_3, DMF, 100 °C, 53%; (c) TBAF, THF, 0 °C, 89%; (d) Allyl bromide, NaH, DMF, 25 °C, 95%; (e) Fe, AcOH, 60 °C, 96%; (f) $Cl(CH_2)_2SO_2Cl$, pyridine, DMAP, CH_2Cl_2, 25 °C, 75%; (g) CH_3I, K_2CO_3, DMF, 25 °C, 90%; (h) Grubbs II, toluene, reflux, 52%; (i) NH_4COOH, Pd/C, MeOH, reflux; 73%.

(compare **23**, **25** and **27** with **24**, **26** and **28**, respectively). The 6,6,5 derivatives also appeared to be less selective against BACE-2 and Cat-D.

Comparison between the 8,6,5 and 7,6,5 tricyclic derivatives proved less straightforward due to the chemical inaccessibility of the former system (Scheme 3): Indole **29** was obtained by Larock cyclisation.[5] Sulfonamide **30** could be engaged in a ring-closing metathesis reaction and reduction of the vinyl sulfonamide formed led to intermediate **31**. The chemistry to synthesise the desired hydroxyethylamine BACE-1 inhibitors from **31** has already been described.[2]

A comparison of activity between inhibitors bearing 8,6,5 and 7,6,5 tricyclic non-prime side residues is shown in Table 3. Again, nanomolar potency could be achieved against BACE-1 with the extended sulfonamide (compound **32**). However, inhibitors of this type are less potent and less selective that their 7,6,5 counterparts (compare **32** and **33** with **22** and **18**, respectively). Overall, the 7,6,5 ring system consistently provided the most potent inhibitors.

The effect of the sulfonamide nitrogen substituent was also explored (Table 4). The presence of an acidic hydrogen is somewhat detrimental to activity (compare **22** and **34**). Methyl and ethyl substitution afforded a similar profile (Compare **13**, **26** and **18** with **35**, **38** and **39**, respectively) whilst branched or more lipophilic alkyl chains did not seem to provide any advantage in terms of potency (compounds **36** and **37**).

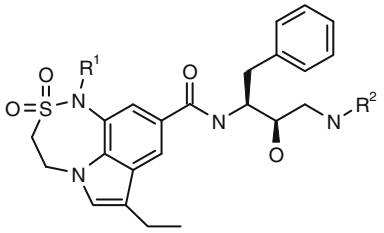
Overall, the data presented highlight the unique profile of this novel series of hydroxyethylamine transition-state mimetics bearing indolic 7,6,5 tricyclic non-prime side substituents, both in terms of potency and selectivity, particularly against Cat-D. A de-

Table 3
Comparison of BACE-1 activity for 8,6,5 and 7,6,5 tricyclic non-prime side

Compd	N[a]	R^1 [a]	BACE-1 IC_{50} (μM)	BACE-2 IC_{50}[b] (μM)	Cat-D IC_{50}[b] (μM)	Aβ40 IC_{50} (μM)
22	1	I	0.002	0.096 (48)	2.95 (1476)	0.012
32	2		0.028	1.86 (66)	1.57 (56)	0.339
18	1	H	0.020	0.263 (13)	7.93 (397)	0.016
33	2		0.49	5.46 (11)	2.9 (6)	–

[a] See Table 2 for definition of n and R^1.
[b] Data in parentheses indicates compound selectivity as ratio of enzyme and BACE-1 IC_{50}S.

Table 4
Comparison of BACE-1 activity for N-substituted 7,6,5 tricyclic sulfonamides BACE-1 inhibitors



Compd	R^1	R^2	BACE-1 IC_{50} (μM)	BACE-2 IC_{50}[a] (μM)	Cat-D IC_{50}[a] (μM)	Aβ40 IC_{50} (μM)
22	CH_3	I	0.002	0.096 (48)	2.95 (1476)	0.012
34	H		0.014	1.047 (76)	14.12 (1023)	0.260
13	CH_3	F	0.015	0.49 (33)	6.61 (441)	0.090
35	C_2H_5		0.005	0.15 (30)	1.62 (324)	0.048
36	$CH(CH_3)_2$		0.023	0.94 (41)	2.96 (129)	0.227
37	CH_2CF_3		0.029	0.68 (23)	5.01 (173)	0.248
26	CH_3	K	0.009	0.21 (23)	0.66 (73)	0.093
38	C_2H_5		0.007	0.089 (13)	0.15 (21)	0.030
18	CH_3	H	0.020	0.263 (13)	7.93 (397)	0.016
39	C_2H_5		0.02	0.331 (17)	12.30 (617)	0.017

[a] Data in parentheses indicates compound selectivity as ratio of enzyme and BACE-1 IC_{50}S.

tailed account of our findings in this series will be the subject of the following publication.

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6. For IC_{50} < 10 nM, the FRET assay was considered as not reliable for SAR, which was then driven by cellular activity; All compounds tested had purity > 95% by LC–MS and/or NMR.
7. The PDB deposition codes and refinement details for the BACE-1 complex crystal structures are **9** (2wf1, 1.6 Å resolution, $R = 0.184$, $R_{free} = 0.207$); **10** (2wf2, 1.8 Å resolution, $R = 0.208$, $R_{free} = 0.247$); **11** (2wf3, 2.1 Å resolution, $R = 0.164$, $R_{free} = 0.217$).