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Second generation of BACE-1 inhibitors part 3: Towards non hydroxyethylamine transition state mimetics

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ABSTRACT

Our first generation of hydroxyethylamine BACE-1 inhibitors proved unlikely to provide molecules that would lower amyloid in an animal model at low oral doses. This observation led us to the discovery of a second generation of inhibitors having nanomolar activity in a cell-based assay and with the potential for improved pharmacokinetic profiles. In this Letter, we describe our successful strategy for the optimization of oral bioavailability and also give insights into the design of compounds with the potential for improved brain penetration.

In the two preceding papers,[1] we have shown that our first generation of BACE-1 inhibitors was unlikely to deliver a compound capable of lowering amyloid in an animal model at low (≈10–20 mg/kg) oral doses. We then described the unique properties (in terms of in vitro potency and selectivity) of a series of compounds bearing a 7,6,5 indole-derived tricyclic non prime side substituent. Notably, exemplars of these hydroxyethylamine transition-state mimetics can achieve nanomolar potency in a cell-based assay without interacting with the prime side of the enzyme.

A potential disadvantage of such compounds was, however, suggested by the synthetic route used to access them. The preparation of the tricyclic moiety involves Michael addition of the indole N–H to a vinyl sulfonamide and we were concerned that a reverse elimination reaction could take place in vivo, generating a transient Michael acceptor such as **2** that could be irreversibly trapped by endogenous thiol-containing nucleophiles (Fig. 1) and potentially lead to a sub-optimal toxicological profile.

In order to assess this possibity further, we examined the stability of the compounds using phenyl methane thiol as a surrogate nucleophile. The tricyclic indoles[2] were stable in the presence of 3 equiv of phenyl methane thiol in ethanol at 80 °C and no decomposition (in particular, no adduct such as **3**) was observed by LC–MS after 4 h. It was only when phenyl methane thiol was used as a solvent that, after 6 h at 40 °C, 90% of the tricyclic inhibitor was converted to the opened adduct **3** (ratio determined by LC–MS). The conditions needed to generate and trap the transient Michael acceptor **2** were thus significantly harsher than physiological conditions and the potential for toxicity via this mechanism was considered to be extremely low.

However, this line of thinking led us to assess the potential of inhibitors incorporating the analogous 'reverse' tricycle non prime side substituent **4** (Scheme 1). These were expected to have a similar binding efficiency, but without the potential to generate a Michael acceptor in vivo. The synthesis of this novel ring system used a high yielding (93%) intramolecular condensation of an alpha-sulfonamide ester to a Vilsmeier–Haack adduct, followed by decarboxylation and hydrogenation. These reactions could be performed on a 50 g scale. Because of previously developed SAR,[3] we chose to focus only on compounds in which the sulfonamide nitrogen was methylated and the indole nitrogen was ethylated.

A comparison of the activity and selectivity of the two tricyclic series is shown below (Table 1). Surprisingly, the small structural modification inherent to the new tricycle system led to an increase in activity in both enzyme and cell-based assays, whilst inhibition of BACE-2 and Cat-D remained similar (providing an increase in selectivity). Interestingly, compounds incorporating the new ring system also appeared to be less lipophilic in general. Overall, nanomolar potency could be achieved in a cell-based assay with compounds of relatively low (for protease inhibitors)[4] molecular

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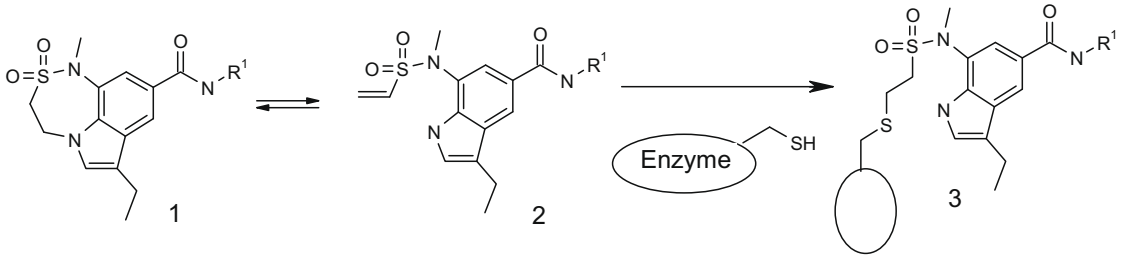
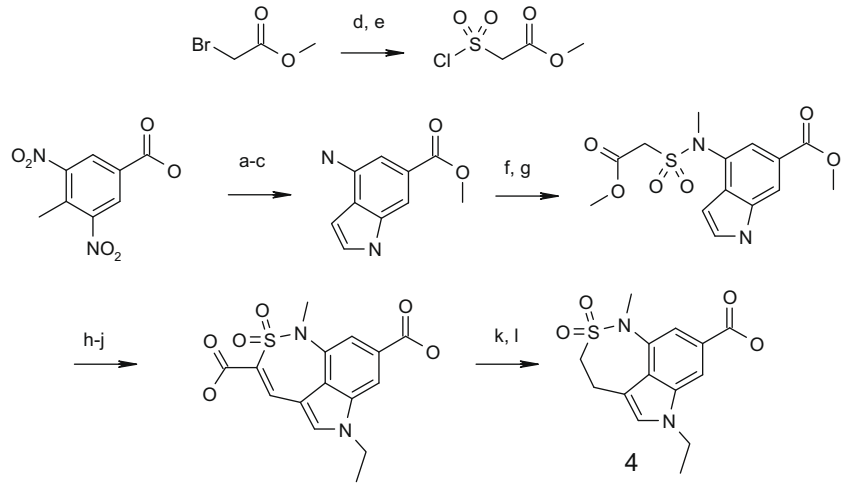
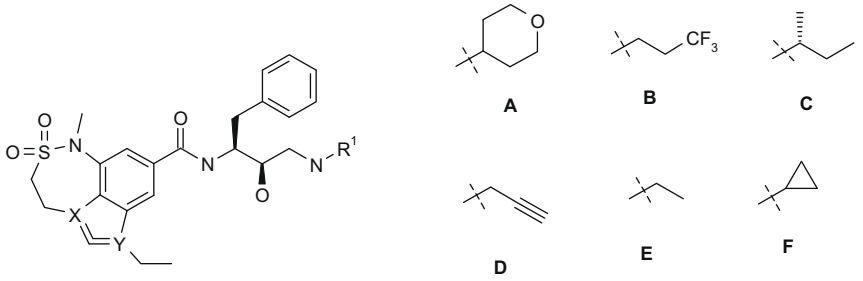


Figure 1. Potential for the formation of Michael acceptor in the tricyclic series of BACE-1 inhibitors.



Scheme 1. Synthesis of novel 7,6,5 indole-like tricyclic non prime side substituent. Reagents and conditions: (a) MeOH, $SOCl_2$, 25 °C, 16 h 95%; (b) DMF, dimethylformamide dimethyl acetal, 40 °C, 24 h, >95%; (c) MeOH, 10% Pd/C, NH_4COOH, 50 °C, 2 h, >90%; (d) Na_2SO_3, H_2O/MeOH, 50 °C, 30 min, >98%; (e) PCl_5, 50 °C to 100 °C, 1 h; (f) $ClSO_2CH_2COOCH_3$, pyridine, DMAP, CH_2Cl_2, $T < 20$ °C, 83%; (g) CH_3I, K_2CO_3, DMF, 16 h, 35–40% (2 steps); (h) $POCl_3$, DMF, 0 °C, 40 min, then 60 °C, 48 h; 93%; (i) NaH, DMF, C_2H_5I, 25 °C, 16 h, 81%; (j) NaOH, MeOH/H_2O, reflux, 2 h, 57%; (k) 2 N HCl, dioxan, reflux, 16 h, 84%; (l) 10% Pd/C, NH_4COOH, EtOH/H_2O, 78 °C, 1 h, 77%.

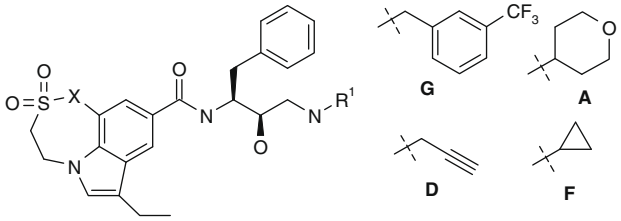
Table 1
Comparison of activity and selectivity of the two tricyclic series of BACE-1 inhibitors



Compd	X	Y	R^1	BACE-1 IC_{50}[a] (μM)	BACE-2 IC_{50} (μM)	Cat-D IC_{50} (μM)	Aβ40 IC_{50}[b] (μM)	Log*D* @ pH 7.4	MW	CMR
5	C	N	A	0.005	0.59	2.11	0.027	0.9	555	15.2
6	N	C		0.015	0.49	6.61	0.090	1.31		
7	C	N	B	0.004	0.075	0.57	0.003	2.59	567	14.4
8	N	C		0.007	0.028	0.89	0.008	2.8		
9	C	N	C	0.010	0.42	1.19	0.064	1.08	527	14.8
10	N	C		0.022	0.43	5.43	0.144	1.52		
11	C	N	D	0.011	1.49	34.59	0.026	—	509	14.1
12	N	C		0.073	3.1	75.5	0.118	—		
13	C	N	E	0.023	2.17	19.54	0.073	0.07	499	13.9
14	N	C		0.048	1.14	36.31	0.262	—		
15	C	N	F	0.009	0.37	6.79	0.014	1.79	511	14.2
16	N	C		0.020	0.263	7.93	0.016	2.17		

[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.
[b] In SHSY5Y wild type cells. See previous paper for details.

Table 2
Comparison of activity and selectivity of sulfone and sulfonamide tricyclic inhibitors



Compd	X	R^1	BACE-1 IC_{50} (μM)	BACE-2 IC_{50} (μM)	Cat-D IC_{50} (μM)
17	N–CH_3	G	0.002	0.059	1.26
18	O		0.008	0.26	2.16
6	N–CH_3	A	0.009	0.21	0.66
19	O		0.086	0.45	0.13
12	N–CH_3	D	0.073	3.1	75.51
20	O		0.36	3.2	15.14
16	N–CH_3	F	0.020	0.263	7.93
21	O		0.25	1.15	2.97

weight (MW) and size (as defined by their calculated molar refractivity, CMR).

The significant effect on activity associated with a shift in the position of a single nitrogen atom in such relatively large inhibitors can be linked with earlier findings in the initial tricyclic series in which the *N*-methyl sulfonamide derivatives were significantly more potent than the sulfonate counterparts[5] (Table 2). The sulfonate derivatives were stable under the experimental conditions used for both enzyme and cell-based assays, ruling out in situ decomposition of the sulfonates as the reason for their weaker activity.

Sulfonamides are known to be weaker hydrogen bond acceptors than sulfones,[6] an observation that can be explained by the absence of resonance between the nitrogen lone pair and the SO_2 group, with only the inductive effect of nitrogen making a contribution.[7] The greater potency of the sulfonamides compared with the sulfonates is therefore unlikely to be related to the strength of the hydrogen bond made with the enzyme.[8] Since the tricycle residues are conformationally very constrained, it is also unlikely that there are significant conformational differences between the sulfonates and sulfonamides and the differences in activity may best be attributed to the impact of these functionalities on the electronics of the bicyclic aromatic ring.

A graphical representation of predictions of oral absorption for the two tricyclic series is shown in Figure 2. Derivatives bearing a benzylic prime side residue are, unsurprisingly, predicted to be less likely to be well absorbed due to their bigger volume compared to inhibitors with a smaller alkyl chain. The chemical space in which compounds with an acceptable lipophilicity profile ($1 < \mathrm{Log}D < 3$) are predicted to be well absorbed is now populated, in contrast to the predictions for our first generation sultams.

Looking in more detail at the compounds with an alkyl non prime side substituent, three comments can be made. (1) Increasing the size of the alkyl substituent (whilst maintaining the basicity of the nitrogen bound to the catalytic aspartates) increases lipophilicity and CMR in parallel so that the absorption prediction remains similar (see representative examples Fig. 3). (2) It is possible to increase the lipophilicity of adducts with similar CMR by lowering the basicity of the nitrogen bound to the aspartate residues. This can be achieved either by using a cyclopropyl amine or by introducing electron withdrawing fluorine atoms into the prime side residue (Fig. 4), both of these strategies lead to lower basicity and hence higher log D @ pH 7.4;[9] (3) For compounds with the same CMR, it is possible to modify lipophilicity by the choice of tricycle non prime side substituent (see $\Delta\mathrm{Log}D$, Table 1). Increased lipophilicity can also be achieved by substituting the S1 phenyl

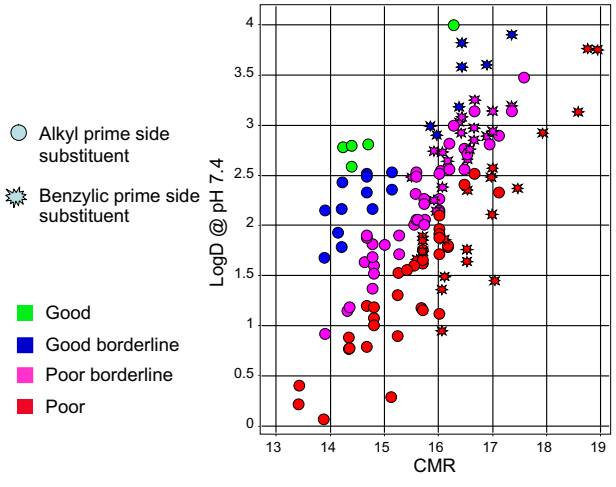


Figure 2. Prediction of absorption for the tricyclic series of inhibitors.

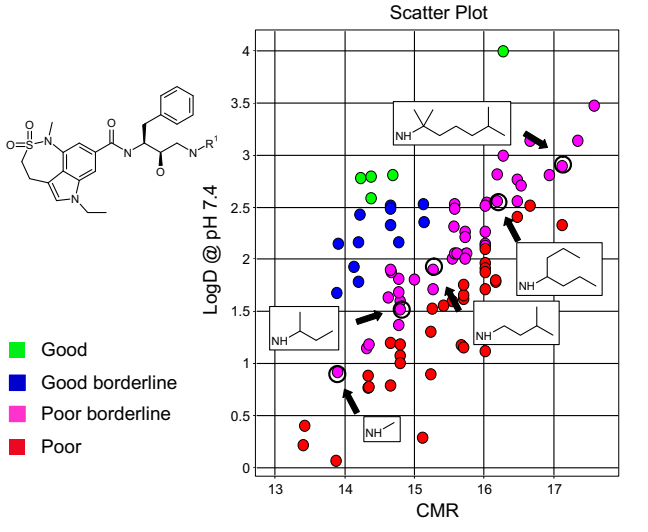


Figure 3. Prediction of absorption for inhibitors with increase alkyl prime side substituent.

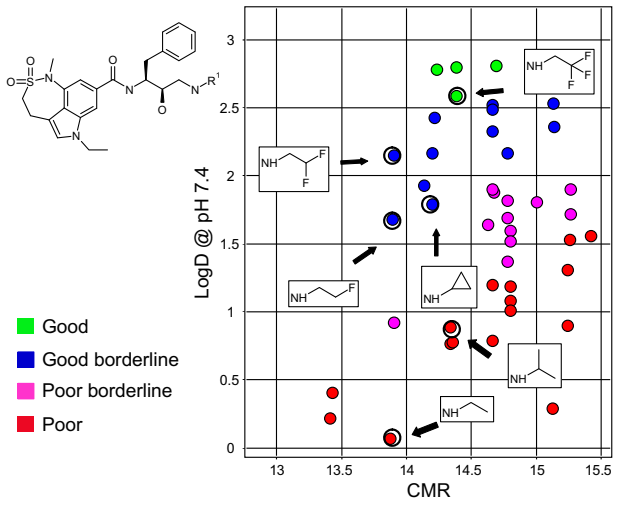


Figure 4. Prediction of absorption for inhibitors bearing cyclopropyl or polyfluorinated alkyl prime side substituents.

ring with halogen atoms which has no effect on enzyme or cellular activity (data not shown).[10]

Within the set of compounds predicted to be well absorbed, compounds **7** and **16** proved to have good oral exposure in multiple species (Table 3).[11]

Not unexpectedly for aspartyl protease inhibitors, CNS penetration studies comparing MDR knockout mice and wild-type mice showed that the compounds are Pgp substrates.[12] The ratio of brain to blood concentrations was markedly higher in the knockout mice, suggesting a high degree of active efflux in the wild-type mice in the case of inhibitors **7** and **16**. (Table 4).

Since these tricyclic inhibitors incorporate extensively optimized non prime side residues and need to form minimal interactions with the prime side of the enzyme, one of the few remaining options likely to reduce Pgp interactions and increase the likelihood of brain penetration is to modify the transition state mimetic itself and reduce the number of hydrogen bond donors and acceptors present.[13] This approach has been used successfully in another series of inhibitors.[14]

Compounds in the tricyclic series appear to need a basic nitrogen in order to see a good correlation of activities in both the

Table 3
In vivo pharmacokinetics profile of inhibitors **7**and **16** in rat and dog

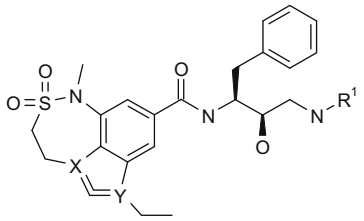
Species	Compd	Cl_{blood}[a] (mL/min/kg)	Vd[a] (L/kg)	$T_{1/2}$[a] (h)	C_{max}[b] (μM)	%F
Rat $n = 2–3$	**16**	72,62	11,8	3, 2.8	0.184 ± 0.082	17, 22
	7	57 ± 12	11.0 ± 3.6	3.3 ± 1.5	0.088 ± 0.03	13 ± 3
Dog $n = 3$	**16**	23 ± 5	4.9 ± 0.9	4.2 ± 0.2	2.969 ± 0.787	79 ± 50 [c]
	7	14 ± 4	4.8 ± 1.6	6.8 ± 0.2	3.586 ± 2.343	52 ± 9

[a] 1 mg free base (fb)/kg/h i.v. dose (solution of mesylate salt in 0.9% w/v saline containing 10% w/v Kleptose).
[b] 10 mg fb/kg (solution of mesylate salt in 1% v/v Tween 80 and 1% w/v methylcellulose aqueous).
[c] Two dogs had Fpo ≈50% whilst the third dog had Fpo markedly higher (>100%) than would have been predicted from the blood clearance.

Table 4
CNS penetration study data for inhibitors **7** and **16**

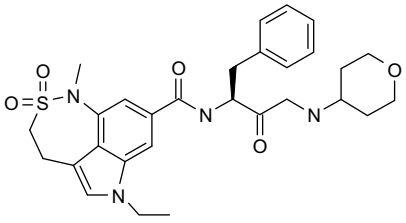
Compd	*Mouse Strain*	Css blood (μM)	Css brain (μM)	CLb (mL/min/kg)	Brain:blood ratio	Ratio (−/−):(+/+)
16	*mdr*1a/b (−/−)	0.397, 0.544	0.989, 1.310	89, 62	2.49, 2.41	19.4
	*mdr*1a/b (+/+)	0.490 ± 0.095	0.061 ± 0.009	72 ± 14	0.13 ± 0.01: 1	
7	*mdr*1a/b (−/−)	0.339 ± 0.037	0.335 ± 0.045	94 ± 11	0.99 ± 0.11:1	9.5
	mdr1a/b (+/+)	0.381 ± 0.032	0.040 ± 0.008	85 ± 6	0.10 ± 0.02:1	

Table 5
Influence of pK_a on cellular activity of BACE-1 inhibitors



Compd	X	Y	R^1	BACE-1 IC_{50} (μM)	Aβ40 IC_{50} (μM)	IC_{50}s ratio[a]	pK_a[b]
22	C	N	$(CH_2)_2CH_3$	0.008	0.029	3.7	10.58
23	N	C	CH_2CH_2F	0.050	0.209	4.2	9.19
24	C	N	CH_2CH_2F	0.040	0.245	6.2	9.19
16	N	C	c-C_3H_5	0.011	0.030	2.7	8.76
7	C	N	$(CH_2)_2CF_3$	0.003	0.003	1.0	8.6
25	C	N	CH_2CHF_2	0.020	0.051	2.6	7.45
26	C	N	$CH_2CF_2CF_3$	0.032	4.427	140.0	5.7
27	C	N	CH_2CF_3	0.079	2.109	26.6	5.4
28	C	N	Phenyl	0.005	0.739	147.5	4.6

[a] Calculated as Aβ40 IC_{50}/BACE-1 IC_{50}.
[b] Reported value of pK_a for R^1NH_2. See Ref. 9 for details.



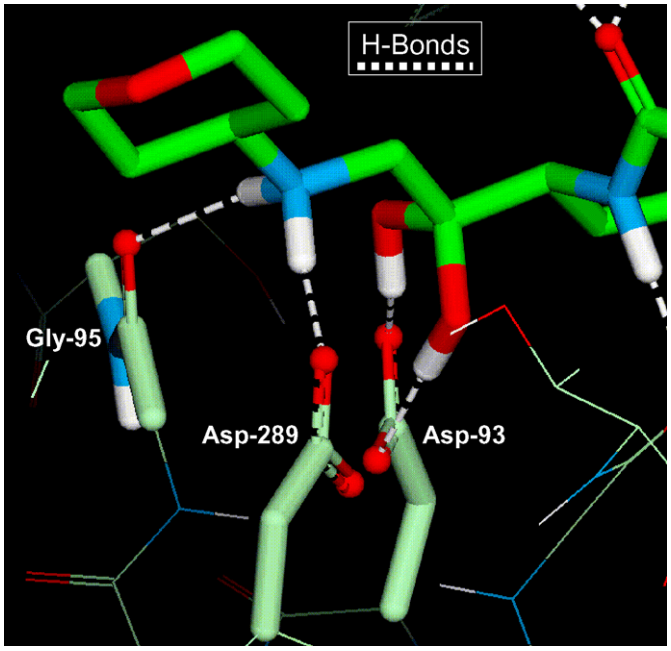


Figure 5. Structure and binding mode to the BACE-1active site of inhibitor **29**.

enzyme and cell-based assays (Table 5); compounds with amines of low basicity ($pK_a < 6$) do not generally inhibit amyloid secretion in cell-based assays.

The alpha-amino ketone **29** (Fig. 5) is a first example of a compound demonstrating that a tricyclic BACE-1 inhibitor without a hydroxyethylamine transition state mimetic - and with fewer hydrogen bond donors and acceptors - can achieve excellent potency in cell-based assays (BACE-1 $IC_{50} = 13$ nM; Aβ40 $IC_{50} = 48$ nM). Interestingly, this derivative binds to the enzyme's aspartyl residues as a hydrate, whilst is only present as the ketone in solution (based on NMR studies).[15]

In summary, in this and the two preceding papers, we have presented the results and reasoning that led us to discover a second generation of stable, potent and orally bioavailable BACE-1 inhibitors. These derivatives are Pgp-substrates and have sub-optimal brain penetration but early findings suggest that non hydroxyethylamine inhibitors with better likelihood of crossing the blood brain barrier can be generated from this series. Further results in this area will be reported in due course.

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9. For measured pK_a of cyclopropyl amine or poly fluoro alkylamines, see: (a) Kluger, R.; Hunt, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 566; (b) Willberg, K. B.; Ross, B. S.; Isbell, J. J.; McMurdie, N. *J. Org. Chem.* **1993**, *58*, 1372; (c) Sakai, T.; Korenaga, T.; Washio, N.; Nishio, Y.; Minami, S.; Ema, T. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1001.
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