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Design and synthesis of potent hydroxyethylamine (HEA) BACE-1 inhibitors carrying prime side 4,5,6,7-tetrahydrobenzazole and 4,5,6,7-tetrahydropyridinoazole templates

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ABSTRACT

A set of low molecular weight compounds containing a hydroxyethylamine (HEA) core structure with different prime side alkyl substituted 4,5,6,7-tetrahydrobenzazoles and one 4,5,6,7-tetrahydropyridinoazole was synthesized. Striking differences were observed on potencies in the BACE-1 enzymatic and cellular assays depending on the nature of the heteroatoms in the bicyclic ring, from the low active compound **4** to inhibitor **6**, displaying BACE-1 IC_{50} values of 44 nM (enzyme assay) and 65 nM (cell-based assay).

For more than a decade, research teams have been developing small and selective high affinity molecules for the inhibition of β-site amyloid precursor protein cleaving enzyme-1 (BACE-1) with the aim that these inhibitors would slow down the progression of the neurodegenerative disorders in Alzheimer's disease (AD).[1,2] This drug hunting approach has been guided by the 'Amyloid Cascade Hypothesis',[3] which suggests a chronic imbalance between production and clearance of amyloid-beta peptides (Aβ) resulting in gradual accumulation of aggregated Aβ deposits in the brain tissue, one of the hallmarks associated with AD.[3] Thus partial inhibition of BACE-1 activity to restore the balance is a particularly attractive concept since it catalyzes the first step in the amyloid production. The aspartyl proteases BACE-1 and γ-secretase are sequentially involved in proteolytic processing of the membrane bound amyloid precursor protein (APP)[4] leading to the generation of Aβ isoforms ranging from 37 to 42 amino acid residues. $Aβ_{40}$ is the most abundant isoform, whilst the deposits primarily consist of aggregated $Aβ_{42}$.[5] Data from animal studies have further helped to consider BACE-1 as potentially suitable target for the treatment of AD.[6]

An abundant number of BACE-1 inhibitors with the hydroxyl ethylamine (HEA) core template have been reported in literature.[7] This core has been used for extension into the non-prime and/or prime side of the BACE-1 active site, often generating potent inhibitors. Besides achieving satisfactory compromises between potency, DMPK properties and selectivity, primarily against Cathepsin D (Cat-D), the ability of the BACE-1 inhibitors to penetrate the blood brain barrier (BBB) presents an additional challenge.[8]

As one part of our BACE-1 program, we have investigated a class of low molecular weight compounds as shown in Figure 1. It has been shown that the pK_a of the HEA NH-functionality plays a significant role for the inhibitory activity of HEA-compounds as the protonated form of the NH-functionality makes hydrogen bonds with the aspartic acid 289[9a] and the glycine 95[9a] (Asp 228 and Gly 34)[9b] upon binding into the active site.[9a,9c] This implies that the pK_a value should be >6.0 in order to exert inhibition of amyloid secretion in cell based assays.[9a] Conversely a too high basicity may render problems with both intestinal and BBB permeability. We have therefore designed and synthesized different alkyl substituted 4,5,6,7-tetrahydrobenzazole and 4,5,6,7-tetrahydropyridinoazole derivatives to fine tune the basicity of the inhibitors and thereby modulate the overall potency-permeability-metabolic stability properties.[7d]

We initiated the program with the synthesis of the 2,2-dimethylchroman analogue **1**,[10a–c] which showed high BACE-1 potency (IC_{50} = 12 nM, enzyme assay) and high cellular activity (11 nM,

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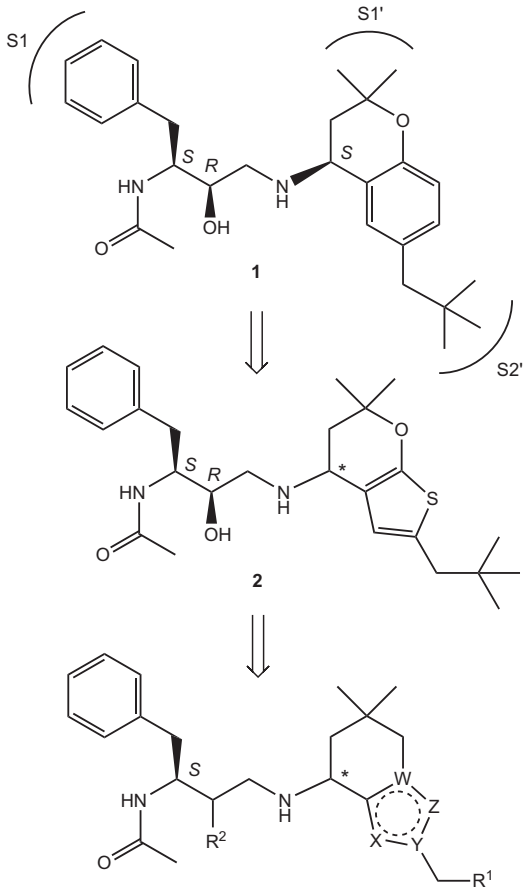


Figure 1. The development of lead HEA compound **1** to the final target HEAs.

cell-based assay). However, this analogue was found to be metabolically unstable in human liver microsomes (HLM) (Table 1). We then synthesized and tested the 2,2-dimethyl-3,4-dihydro-2*H*-thieno[2,3-*b*]pyran analogue **2**[10a–c,11], which still delivered high potency (IC_{50} = 8.5 nM, enzyme assay) and high cellular activity (18 nM, cell-based assay), and with improved metabolic stability. We then envisioned that replacing the oxygen in the dihydropyran ring in **2** with a methylene, we should maintain the potency of **2** and further improve on the DMPK and selectivity properties. Our endeavour led to quite simple and robust syntheses of compounds **4–10** (Fig. 1 and Scheme 1).

Our strategy for the synthesis of compounds **4–10** is summarized in Scheme 1. The 4-amino derivatives **36**, **37**, **40**, **47–50** were synthesized as depicted in Schemes 6–8, followed by reaction with the commercially available epoxide **3**, to give the *t*-butoxycarbonyl (Boc) protected intermediates (including the bis-Boc derivative **5a**). These intermediates were treated with HCl in dioxane/MeOH, followed by acetylation of the free amine with acetyl imidazole[12] to give the final products **4**, **5b**, **6–9**. Compound **7** was converted to the 3-oxo derivative **10** in three steps in an overall isolated yield of 48%, by first protecting the HEA NH function with the Boc-group and Dess-Martin oxidation of the 3-hydroxy group, followed by deprotection of the Boc-group (Scheme 1).

The 4-keto derivatives **11, 13, 19, 20, 31** were prepared as the key intermediates for the subsequent synthesis of the 4-amino derivatives **36, 37, 40, 47–50**. Depicted in Scheme 2 is the reaction of the oxazole **11** to provide in one step the imidazole **12**,[13–17] followed by the NH protection with Boc, to give **13**. The oxazole **11** was prepared in four steps from 5,5-dimethylcyclohexane-1,3-dione (dimedone) and neopentoyl chloride analogously to published procedures, which employ two convenient rearrangement reactions.[17,13]

Scheme 3 shows the synthesis of the indazole analogues **19** and **20** with 2-*N*-neopentyl and 2-*N*-isobutyl substituents respectively. Compound **14**[19,18] was refluxed with the *t*-butyl *N*-alkyl hydrazinecarboxylates[20] **15** and **16** to generate in high yields the adducts **17** and **18**, which were readily ring closed by treatment with TFA to give **19** (74%) and **20** (70%). This sequence allows the full regiocontrol in contrast to earlier reported one pot procedure[18] which gave the mixtures of 1-, and 2-substituted indazole-4-ones.

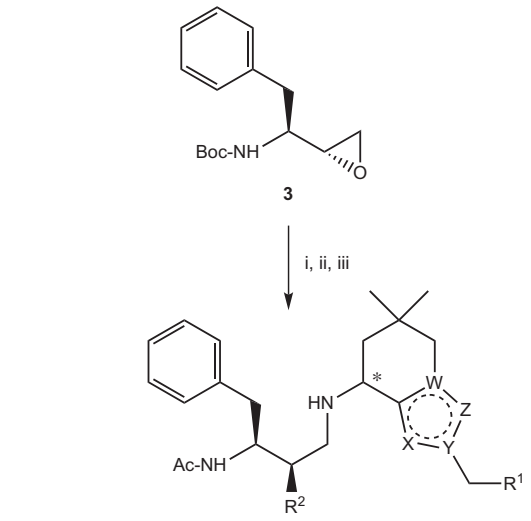
A novel synthesis of the 3,3-dimethylbutyrolactone (**25**)[21] is shown in Scheme 4 which was a crucial component for the preparation of the pyrazolo[1,5-*a*]pyridin-4(5*H*)-one derivative **31** (Scheme 5). 2,2-Dimethyl-1,3-propanediol (**21**) was treated with thionyl chloride to furnish the cyclic sulphite **22**.[22] A ruthenium catalyzed periodate oxidation of compound **22** provided corresponding sulphate diester **23**.[22] This oxidation facilitated the S_N2 reaction with sodium cyanide to generate a sodium salt of sulfuric acid mono-(3-cyano-2,2-dimethyl-propyl) ester. Without separation, this salt was subjected to a mild acidic hydrolysis to give 2,2-dimethyl-4-hydroxy butyronitrile (**24**). Subsequently nitrile **24** was hydrolyzed and cyclised under harsh acidic conditions to the lactone **25** which was isolated by extraction and distillation as a colourless oil in 51% overall yield from cyclic sulphate **23**. The oil solidified upon standing in the freezer.[23] In a second experiment, the lactone **25** was also obtained from cyclic sulphate **23** in one pot without separation of butyronitrile **24** with improved yield of 78% (step vi, Scheme 4).

The synthesis of the pyrazolo[1,5-*a*]pyridin-4(5*H*)-one derivative **31** is shown in Scheme 5 and was carried out analogously to

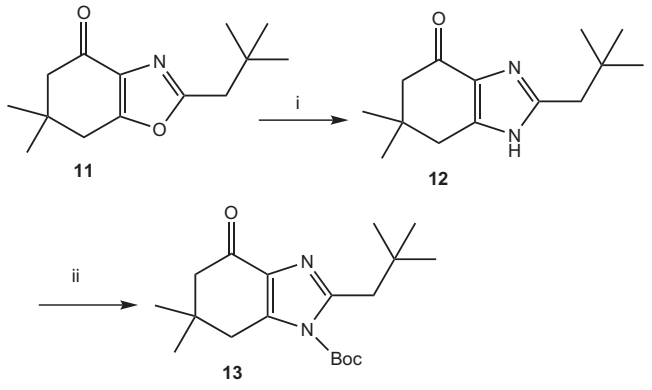
Table 1
In vitro inhibition and DMPK data of **1**, **2** and **4–10** (Fig. 1 and Scheme 1)

Compd[a]	BACE-1[30] IC_{50} (nM)	$A\beta_{40}$[b] IC_{50} (nM)	BACE-2 IC_{50} (nM)	Cathepsin D[30] K_i (nM)	Caco-2 (cm/s × 10^{-6})	HLM Clint (μl/min/mg)
1	12	11	4.5	120	21	200
2	8.5	18	3.9	9.9	5.6	82
4	>10,000	>10,000	>10,000	>5000	—	—
5b	1900	230	390	750	—	15
6[c,d]	44	65	88	37	1.5	25
7	150	19	22	240	1.6	16
8	5600	—	1900	5000	—	—
9	99	440	—	36	5	70
10	290	>10,000	—	—	—	—

[a] Ratios of (*S*):(*R*)-configurations (diastereomeric mixture) at the C4 of the bicyclic ring: **1** (fully *S*), **2** (75:25), **4** (fully racemic), **5b** (fully racemic), **6** (79:21), **7** (fully *S*), **8** (fully *R*), **9** (fully *S*).
[b] Cell-based assays: Human Embryonic Kidney (HEK) cells transfected with APP Swedish Mutant where the levels of $A\beta_{40}$ were monitored by ELISA.
[c] MDCK MDR1 ratio = 150; MDCK wt ratio = 18.
[d] Protein binding in mouse serum, free unbound = 10%. Cell toxicity assays (HEK) gave F-CC_{50} >50 μM for all compounds except for **2**, which had F-CC_{50} = 7.4 μM.



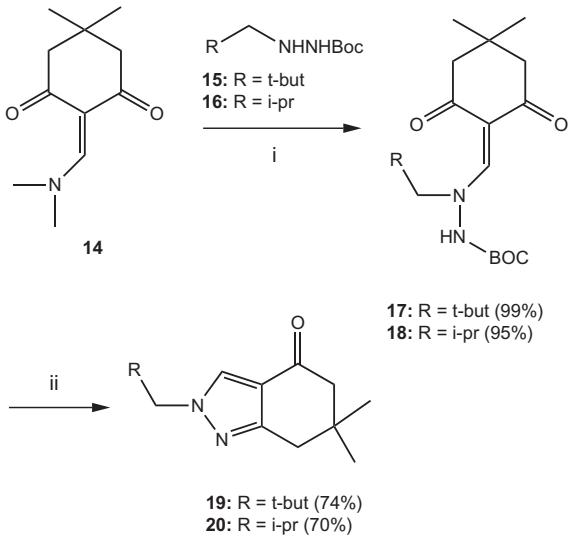
Scheme 1. Reagents and conditions: (i) amines **36**, **37**, **40** or **47**–**49**, EtOH, 60 °C; (ii) TFA, DCM, Et_3SiH, rt or HCl, dioxane, MeOH, rt; (iii) Ac-imidazole, DCM, rt, yields in 3 steps from **3**:**4** (31%), **5b** (22%), **6** (38%), **7** (24%), **8** (17%), **9** (13%) (iv) (a) Boc_2O, DCM, rt, 86%, (b) Dess-Martin periodane, DCM, rt, 80%, (c) 4 M HCl, dioxane, rt, 70%.



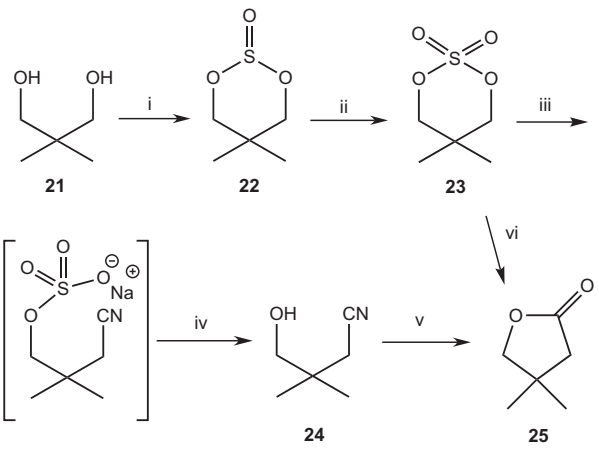
Scheme 2. Reagents and conditions: (i) $NH_4^+TFA^-$, toluene, DMF, 115 °C, 66%; (ii) Boc_2O, THF, 50 °C, 84%.

published procedures.[24–27] The N-alkylation of the 3-neopentylpyrazole **29** with lactone **25** in presence of strong base (KH) generated two regioisomers **30a** and **30b** in 87:13 ratio respectively. This mixture was treated with *t*-butyl lithium whereby only the main isomer **30a** cyclised to provide **31** in 50% isolated yield calculated from the estimated amount of **30a** in the mixture.[27]

After completing the synthesis of the bicyclic ketones **11**, **13**, **19**, **20** and **31**, three synthetic routes were explored to convert these ketones into the corresponding amines **36**, **37**, **40**, **47–50**. In the case of the 4-ketones **11** and **13**, a racemic conversion to the 4-amines **36** and **37** was carried out (Scheme 6). The ketones were reduced with $NaBH_4$ to the *racemic* alcohols **32** and **33**, which were each converted by treatment with DPPA and DBU in toluene to the *racemic* azides **34**, **35** and subsequently reduced with hydrazine



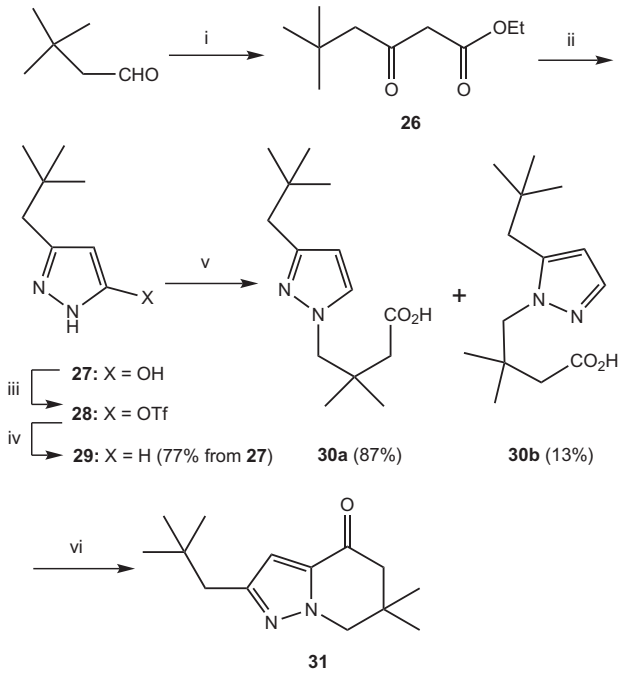
Scheme 3. Reagents and conditions: (i) **15** or **16**, toluene, reflux; (ii) TFA, DCM, rt.



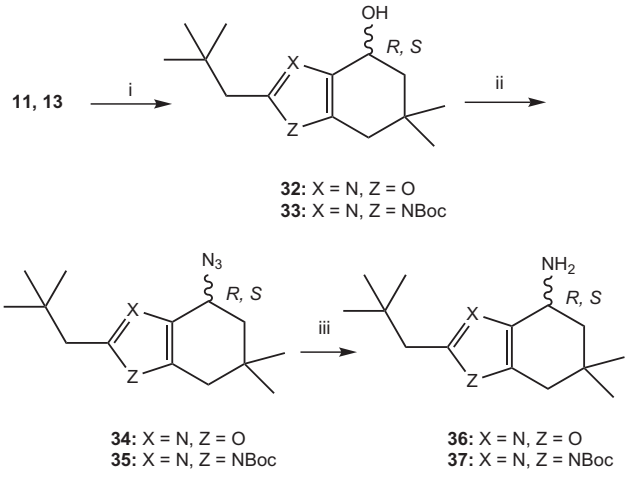
Scheme 4. Reagents and conditions: (i) $SOCl_2$, DCM, 0 °C to rt, 91%; (ii) $NaIO_4$, $RuCl_3$, H_2O, DCM, MeCN, rt, crude, 97%; (iii) NaCN, DMF, rt; (iv) 20% aq H_2SO_4, 61% from **23**; (v) 20% aq HCl, 80 °C, 83%; (vi) (a) NaCN, DMF, rt, (b) 20% aq HCl, 80 °C, 78% from **23**.

hydrate and Raney-nickel to the *racemic* amines **36** and **37**. These racemic mixtures were coupled to **3** (Scheme 1) to generate at the end the final products **4** and **5b**, as inseparable diastereomeric mixtures (50:50 and 55:45 ratios respectively).

Due to the failure to separate the diastereomers of the final compounds a chiral synthesis of the amines was required. It was tried to adopt the highly effective racemic synthetic sequence shown in Scheme 6 to a chiral variant (Scheme 7). Thus, chiral reduction of **19** using the (*S*)-(−)-2-Me-Cbs-oxazaborolidine—BH_3SMe_2 procedure[28] rendered the 4-(*R*) alcohol **38** in high yield and excellent enantiomeric purity (ee >99, $[\alpha]_D^{20}$ −85.5, c 1, $CHCl_3$). However the subsequent treatment with DPPA and DBU in toluene at ambient temperature to produce the inverted 4-(*S*) azide **39** resulted in substantial racemisation. The destabilization of benzylic type of the carbocation should decrease the S_N1 pass of the reaction and therefore the rate of racemisation. The same reaction repeated in heptane at 0 °C resulted in improved stereocontrol albeit with compromised chemical yield of 4-(*S*) azide **39** (ee 58, yield 52%). Reduction of the azide with hydrazine hydrate and Raney-nickel gave the enantiomerically enriched 4-(*S*) amine **40**. This



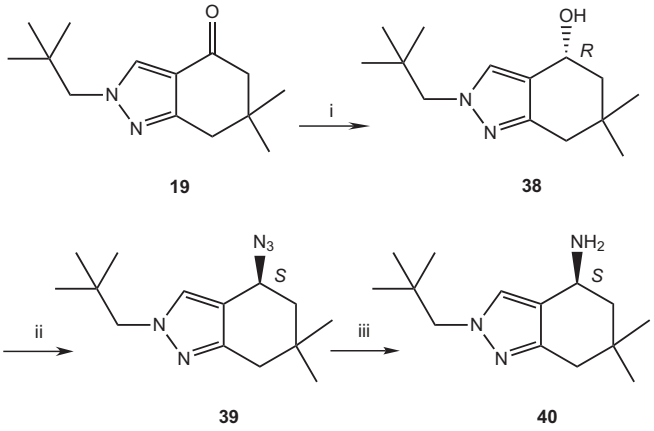
Scheme 5. Reagents and conditions: (i) diazaethylacetate, $SnCl_2$, DCM, rt, 90%; (ii) NH_2NH_2 H_2O, EtOH, 70 °C, 70%; (iii) $PhN(Tf)_2$, Et_3N, THF, 0 °C to rt, 65%; (iv) $Pd(OH)_2/C$, H_2 (1 atm.), EtOAc, AcOH, >95%; (v) **25**, 8 M KH in mineral oil, DMF, 160 °C; (vi) *t*-butyl lithium, pentane, THF, −75 °C to rt, 50%.



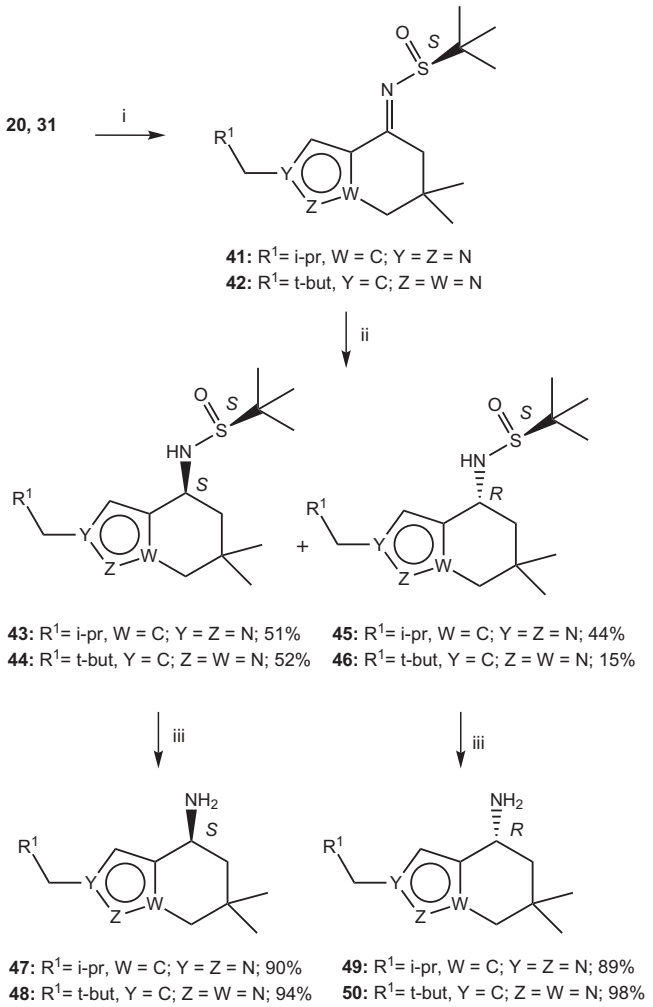
Scheme 6. Reagents and conditions: (i) $NaBH_4$, EtOH, rt, 84–90%; (ii) DPPA, DBU, toluene, rt, 74–81%; (iii) $NH_2NH_2xH_2O$, Raney-Ni, H_2O, EtOH, rt, >95%.

stereoisomeric mixture of **40** was coupled to **3** to give compound **6** (Scheme 1), with basically the same ratio ((*S*) 79:(*R*) 21) of inseparable diastereomers.

With a limited success of S_N2 inversion methodology to produce chiral tetrahydroindazol amine **40** as depicted in Scheme 7, we then explored the Ellman procedure[29a–g] for the synthesis of enantiomerically pure amines **47–50** (Scheme 8). The ketones **20** and **31** were smoothly converted to the corresponding sulfinimines **41** and **42** by reaction with (*S*) isomer of *t*-butylsulfinamide and titanium tetraethoxide.[29c] These sulfinimines were reduced with $NaBH_4$ at 0 °C to give predominantly (*S,4S*) diastereomers **43**, **44** which in both cases were easily separated by silica gel column chromatography from the minor (*S, 4R*) diastereomers **45**, **46**. The stereoselectivity in the reduction of **41** was only 7% *de* in favour of the 4-(*S*) isomer, while 37% *de* was achieved for **42**. Each of the diastereomers was separately deprotected with HCl in dioxane to furnish the pure enantiomers of amines **47–50** respectively. In case of the isobutyl-tetrahydroindazol amine, both enantiomers



Scheme 7. Reagents and conditions: (i) (*S*)-(−)-2-Me-CBS-oxazaborolidine, BH_3SMe_2, toluene, 0 °C to rt, 86%; (ii) DPPA, DBU, heptane, 0 °C, 52%; (iii) $NH_2NH_2xH_2O$, Ra-Ni, H_2O, EtOH, rt, 92%.



Scheme 8. Reagents and conditions: (i) (*S*)-*t*-butS(*O*)NH_2, $Ti(OEt)_4$, THF, 60 °C, 71–79%; (ii) $NaBH_4$, THF, H_2O, 0 °C; (iii) 4 M HCl, dioxane, MeOH, rt.

47 and **49** were used in the coupling with **3** (Scheme 1) to provide the final products **7** and **8**. For the neopentyl-tetrahydropyrazolopyridine amine only the biologically more interesting 4-(*S*) isomer **48** was coupled to epoxide **3** to give **9**.

Table 1 shows the in vitro activities[30] and the in vitro DMPK results. As it turned out, the tetrahydrobenzazole and tetrahydropyridinoazole derived compounds **4**–**10** displayed lower potencies than the dihydropyran analogues **1** and **2**. No major improvements were apparent on selectivity versus Cat-D and BACE-2, or on Caco-2 permeability. However, it was possible to significantly improve on the metabolic stability for several of the inhibitors.

Figure 2 illustrates the co-crystal structures[30] of human BACE-1 with inhibitors **2** and **6** (with the (*S*)-configuration at C4 of the bicyclic ring) superimposed on each other (with the flap removed for convenience) and showing their positions relative to the Asp228 and 32 and Gly34. Analysis of co-crystals of human BACE-1 with **1** and **2** suggest that the dihydropyran oxygen has hydrogen bonding to the hydroxyl group of Thr72 of the flap. This hydrogen bonding is prevented in the case of the methylene analogue **6** as can be visualized in Figure 3. This could contribute to the higher potencies seen for inhibitors **1** and **2**. In addition, the lipophilic benzo ring of **1** and similarly the large sulphur atom in **2** are better accommodated into the rather lipophilic space between S1′ and S2′ cavities than the more polar nitrogen atoms in the representative inhibitor **6**.

We synthesized compounds **4** and **5b** to investigate the possibility of improving potency by creating additional hydrogen bonding between the inhibitor prime side hetero atoms and amino acid residues in the active site, including the flap amino acid residues. Modelling studies based on co-crystal data on this series indicate that one imidazole NH-tautomer of **5b** could form a hydrogen bond (for the desired diastereomer, (*S*)-configuration at the C4 of the bicyclic ring), although non-optimally, to the carbonyl of Gly34 (data not shown). However, the activity data for **5b** did not corroborate this prediction, showing it being a very weak binder. The oxazole ring in **4** completely abolished the affinity to BACE-1. Inhibitor **6** was prepared and tested as a diastereomeric mixture (79(*S*):21(*R*) at C4 of the bicyclic ring) and was found to be the most potent inhibitor in this series. As expected, compound **8** lost most of its BACE-1 affinity because of the less favourable (*R*)-configuration at the C4 of the bicyclic ring.[10a–c] Following the report that oxidation of the HEA-scaffold to an alpha-amino ketone gave in some cases inhibitors which bound to BACE-1 in a hydrated form and show an excellent potency in cell-based assay,[9a] we oxidized the 3-(*R*)-OH in **7** to the 3-oxo derivative **10**. However, this resulted in partial loss of potency in the enzyme based assay and abolished the activity in the cell-based assay. This activity loss could in this case be due to the insufficient hydration of the carbonyl or protonation of the vicinal HEA NH-bridge, which would disrupt hydrogen bonding to Asp228, Asp32 dyad and Gly34. Finally, compound **9** maintained a modestly good BACE-1 potency, but had greater affinity to Cat-D, and was metabolically less stable.

The stability in HLM was improved from the chroman analogue **1** to **5b**, **6** and **7** (Table 1). The Caco-2 p_{app} values ranged from 1.5 to 5.0, and for **6** the MDCK MDR-1 ratio over the wild type ratio was measured and proved to be high (footnote of Table 1) indicating that **6** is a P-glycoprotein (Pgp) -substrate and may therefore have suboptimal BBB penetration.[31]

Nevertheless, inhibitor **6** was administered iv to C57/B1 male mice at a dose of 20 μmol/kg. A brain exposure of 0.20 μM and a plasma exposure of 4.75 μM was observed 0.5 h after dose. Brain exposure was measurable up to 6 h after dose (0.068 μM). Inhibitor **6** was also administered subcutaneously via mini pumps at a dose of 2.9 μmol/kg/h for 24 h. A mean steady-state plasma concentration of 0.45 ± 0.09 μM was measured with a mean brain/plasma ratio of 0.69 ± 0.10 μM ($n = 3$). These results show that we could achieve appreciable brain exposure with the potent inhibitor **6** in spite of indications of it being a Pgp-substrate in the MDCK assay.

$A\beta_{40}$ levels in brain and plasma were also measured in the mice which were treated intravenously with inhibitor **6**. Plasma $A\beta_{40}$ levels were reduced by 24% 3 h post-dose, whereas the brain expo-

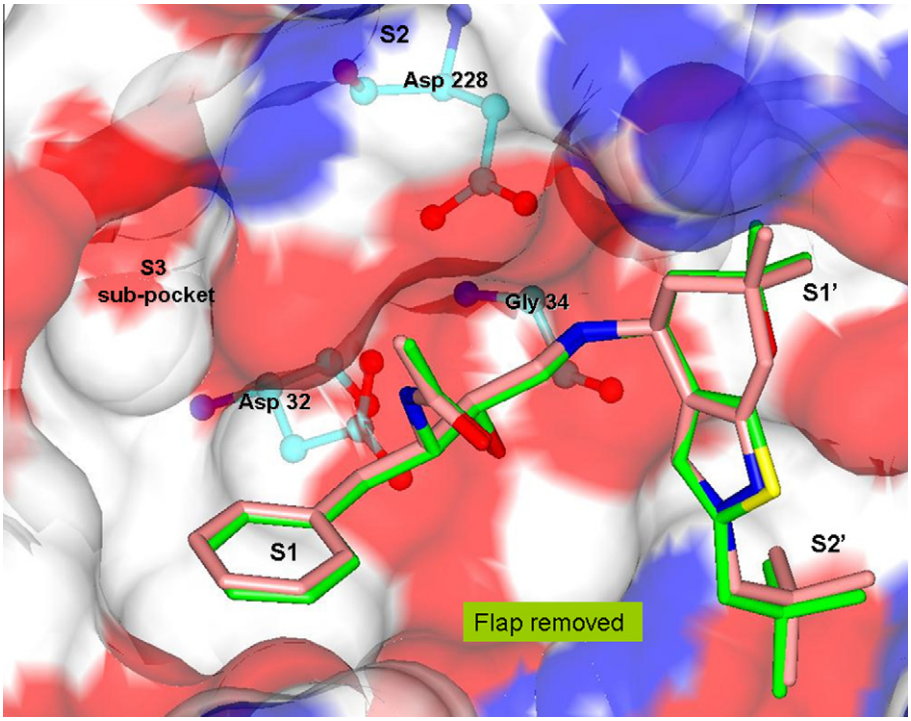


Figure 2. Superimposed X-ray structures of **2** (green, PDB 4EXG) and **6** (brown, PDB 4EWO) bound to human BACE-1 (the flap removed for clarity). 1.8 Å resolution.

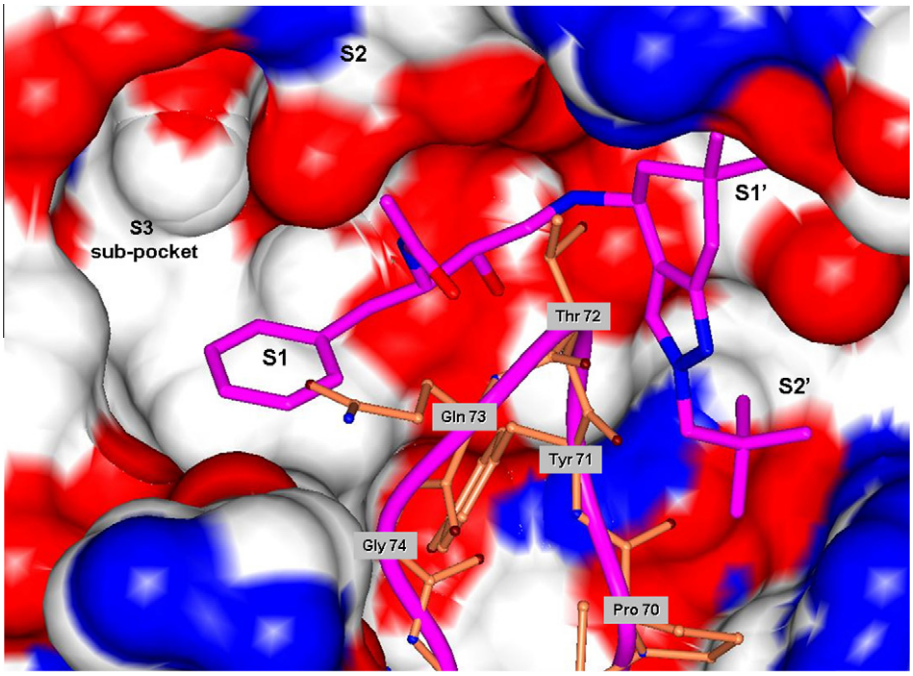


Figure 3. X-ray structure of **6** (PDB 4EWO) bound to human BACE-1 (the flap outlined for clarity). 1.8 Å resolution.

sures achieved with inhibitor **6** were not sufficient to affect $A\beta_{40}$ levels in the brain. The relatively weak efficacy in vivo is probably not due to excessive protein binding (free unbound in mouse serum = 10%).

In summary, guided by the lead compounds **1** and **2**, a series of low molecular weight and in some cases potent BACE-1 inhibitors (**4**–**10**) were synthesized. Different heteroatom combinations on the prime side core template, consisting of either 4,5,6,7-tetrahydrobenzazoles or a 4,5,6,7-tetrahydropyridinoazole, were explored with the aim of fine tuning the basicity of the molecules and thereby influence their inhibitory activities and DMPK properties. Less polar heteroaromatics as in the pyrazolo analogues **6**, **7** and **9** seemed to be preferred for good BACE-1 inhibitory activity, whereas the more polar oxazole **4** and imidazole **5b** analogues were less preferred. Oxidizing the 3-hydroxyl group of **7** to the 3-oxo function to give **10** led to a weakened potency in the enzyme based assay and to abolition of potency in the cell based assay. Selectivity over Cathepsin D and BACE-2 was poor for this series of compounds. The stability of the compounds in HLM could be improved compared to the lead compounds **1** and **2** whereas permeability was lower probably due to Pgp affinity. Compound **6** was dosed in vivo in mice and although the compound reached the brain, the exposure achieved did not elicit any change in $A\beta_{40}$ levels.

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