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Design and synthesis of hydroxyethylamine (HEA) BACE-1 inhibitors: Structure–activity relationship of the aryl region

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ABSTRACT

The structure–activity relationship of the prime region of hydroxyethylamine BACE inhibitors is described. Variation in the aryl linker region with 5- and 6-membered heterocycles provided compounds such as **33** with improved permeability and reduced P-gp liability compared to benzyl amine analog **1**.

Alzheimer's disease (AD) is the most prevalent form of dementia and is characterized pathologically by the formation of extracellular insoluble amyloid plaques and intraneuronal fibrillary tangles in the brain.[1] The amyloid plaques are composed of β-amyloid (Aβ)[2] peptide which is generated by cleavage of the β-amyloid precursor protein (β-APP) by two aspartic acid proteases referred to as β-secretase (BACE-1) and γ-secretase. BACE-1 knockout mice are healthy, fertile[3] and are unable to generate Aβ in the brain[4] suggesting that BACE-1 would be an attractive therapeutic target for the development of disease modifying treatments for AD.[5]

During the course of our program to develop BACE-1 inhibitors based on the hydroxyethylamine (HEA) scaffold[6] we discovered that aryl cyclohexyl amines provided excellent potency against BACE-1.[7] The general binding mode of this series of BACE-1 inhibitors is depicted in Figure 1. The hydroxyl and protonated amino group bind to Asp228 and Asp32, respectively, while the difluoroaryl occupies the *S*1 pocket and the cyclohexyl and *tert*-butyl substituents fill the *S*1′ and *S*2′ pockets, respectively. The aryl linker occupies a region between *S*1′ and *S*2′ with Gly34 and Tyr198 on the 'top' side and Thr72, Thr71 and Pro70 at the bottom right side. The lower portion of the benzylamine is exposed to solvent.

During our systematic exploration of the prime region of BACE[7], the SAR of the aryl region of the inhibitors was targeted for study. Our previously reported HEA inhibitors such as **1** suffered from low

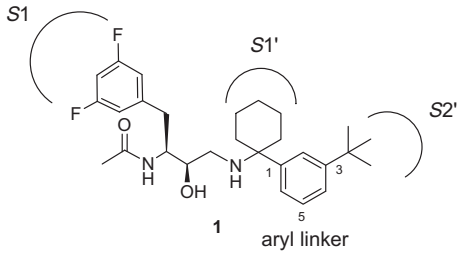

Figure 1. Binding mode of the HEA's.

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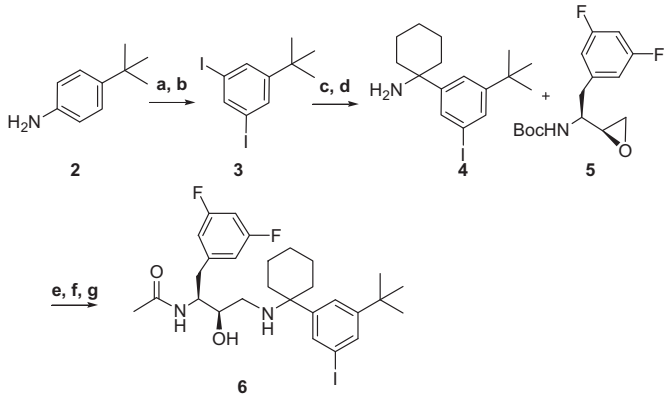


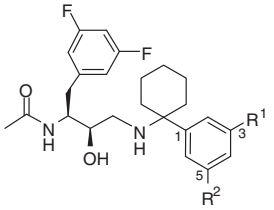
Scheme 1. Reagents and conditions: (a) (i) $BnEt_3NCl$, ICl, H_2O, then (ii) **2**, $CaCO_3$, CH_2Cl_2, MeOH, 18 h, 40 °C; (b) *t*-butyl nitrite, DMF, reflux, 10 min; (c) (i) cyclohexanone, *t*-butyl sulfinamide, $Ti(OEt)_4$, THF, 2 h, room temperature, (ii) *n*-BuLi then **3** $AlMe_3$, toluene, −78 °C; (d) HCl, MeOH, dioxane; (e) **5**, DIPEA, ^{i}PrOH, 18 h, 70 °C; (f) 4 N HCl in dioxane, 0.5 h; (g) Ac_2NOCH_3, CH_2Cl_2, Et_3N, 18 h.

permeability and high P-gp efflux and our goal was to develop new inhibitors which possessed cellular activity, were highly permeable and were able to penetrate the blood–brain barrier. It was envisioned that the introduction of substituents and heteroatoms into the aryl linker would enable modulation of the pharmacokinetic properties of the inhibitors and enable this goal to be achieved. Initial exploration focused on substituted benzene analogs which could be prepared using a route outlined in Scheme 1.[7] For example, the versatile aryl iodide **6** was prepared by addition of an aryl lithium to the *N*-sulfinyl imine derived from cyclohexanone and *tert*-butyl sulfinamide followed by hydrolysis of the resulting sulfinamide to give cyclohexylamine **4**. Alkylation of epoxide **5** followed by deprotection and acetylation gave the desired compounds. In the case of aryl iodide **6**, further functionalization using cross-coupling methodology allowed expansion of the SAR at the 5-position.

As previously reported[7] the *tert*-butyl analog **1** was identified as one of the more potent inhibitors in this series being significantly more potent than the *neo*-pentyl analog **8** (Table 1). These alkyl chains point into the *S2′* region of BACE-1 and are crucial to providing potency as illustrated with mono-substituted benzyl amine analog **7**. Exploration of the SAR on the 5-position with halogens indicated that larger substituents resulted in a loss of potency in the order F < Br < I. Interestingly the methoxy analog **12** had similar potency to **1** while the methyl analog **11** and phenol **13** were three-fold less potent. Aniline **14** lost seven-fold potency compared to **1** but methylsulfonamide **15** gained back some potency and was only two-fold less active against BACE-1 than **1**. Substitution at the 4-position was not tolerated as shown with **17**. Generally, these compounds were all more potent against Cat-D over BACE-1.

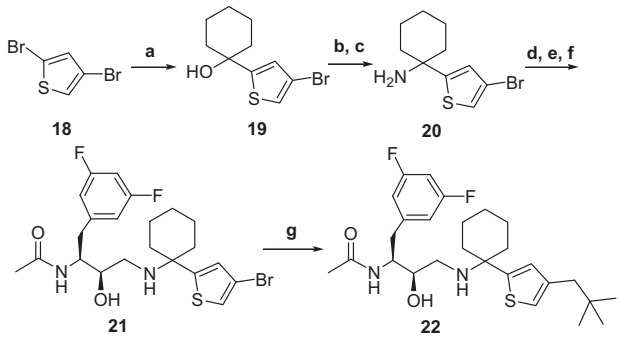
In addition to exploring the SAR of the benzene linker, a series of heterocyclic aryl linkers was also prepared. Thiophenes are well known as phenyl isosteres[8] and a series of thiophene regioisomers was synthesized with various alkyl substituents to fill the *S2′* pocket. One of the general synthetic routes used to access these compounds is outlined in Scheme 2.[9] Metal–halogen exchange of a commercially available di-bromothiophene followed by quenching with cyclohexanone gave the tertiary alcohols which were converted into amines upon treatment with azidotrimethylsilane and BF_3OEt_2. Reduction of the resulting azide with $LiAlH_4$ gave amine **20** which was elaborated into the HEA using the standard protocol to give the key synthetic intermediate **21**. The *P2′* substituents could be conveniently installed using cross-coupling methodology, such as Stille, Negishi, and Sonogashira couplings, to provide the compounds shown in Table 2.

Table 1
BACE inhibition data and cellular activity for compounds **1–17**



Compds	R^1	R^2	BACE IC_{50},[a] (nM)	Cat D IC_{50},[b] (nM)	Cell ED_{50},[c] (nM)
7	H	H	>10,000	na	>1000
1	*tert*-Butyl	H	47	24	17
8	*neo*-Pentyl	H	2100	125	440
9	*tert*-Butyl	F	46	24	9
10	*tert*-Butyl	Br	174	42	435
6	*tert*-Butyl	I	223	55	185
11	*tert*-Butyl	Me	180	67	240
12	*tert*-Butyl	OMe	77	47	68
13	*tert*-Butyl	OH	170	90	na
14	*tert*-Butyl	NH_2	318	105	25
15	*tert*-Butyl	$NHSO_2Me$	93	12	na
16	*tert*-Butyl	C(O)Me	160	45	33
17	*tert*-Butyl	4-Br	6900	1500	2000

[a] See Ref. 10.
[b] See Ref. 11.
[c] See Ref. 12.

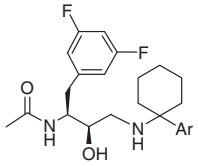


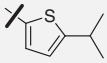
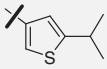
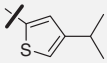
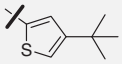
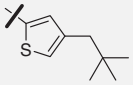
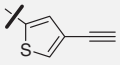
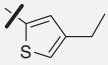
Scheme 2. Reagents and conditions: (a) cyclohexanone, *t*-BuLi, THF 1 h, −78 °C; (b) TMS-N_3, BF_3OEt_2, Et_2O, reflux, 1.5 h; (c) $LiAlH_4$, Et_2O, 2 h; (d) **5**, DIPEA, ^{i}PrOH, 18 h, 70 °C; (e) 4 N HCl in dioxane, 0.5 h; (f) Ac_2NOCH_3, CH_2Cl_2, Et_3N, 18 h; (g) $IZnCH_2C(CH_3)_3$, $Pd_2dba_3{\cdot}CHCl_3$, DTBP, THF, reflux, 20 h.

The 2,5-disubstituted thiophenes proved to be less active than the 2,4-disubstituted analogs. For example, the most potent 2,5-disubstituted thiophene, **24** (BACE IC_{50} = 4.0 μM) was an order of magnitude less potent than the corresponding 2,4-disubstituted thiophenes **25** (BACE IC_{50} = 0.32 μM) and **26** (BACE IC_{50} = 0.35 μM). Further exploration of the SAR of the *S2′* region indicated that *tert*-butyl **27** and *neo*-pentyl **22** were the most potent analogs.

Since it was possible to replace the benzene ring with a thiophene ring and achieve similar BACE-1 inhibition both in the enzyme and cellular assays, the SAR of this region was expanded

Table 2
BACE inhibition data and cellular activity for compounds **22–29**



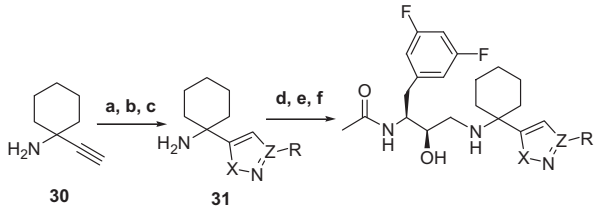
Compds	Ar	BACE IC_{50},[a] (nM)	Cat D IC_{50},[b] (nM)	Cell ED_{50},[c] (nM)
23		>30,000	4420	1330
24		4010	411	1036
25		320	42	<80
26		352	40	<80
27		104	32	10
22		131	9	na
28		4316	276	757
29		1740	228	107

[a] See Ref. 10.
[b] See Ref. 11.
[c] See Ref. 12.

by the preparation of a more diverse series of heterocyclic aryl linkers with particular attention paid to the effect on the pharmacokinetic properties of the compounds.

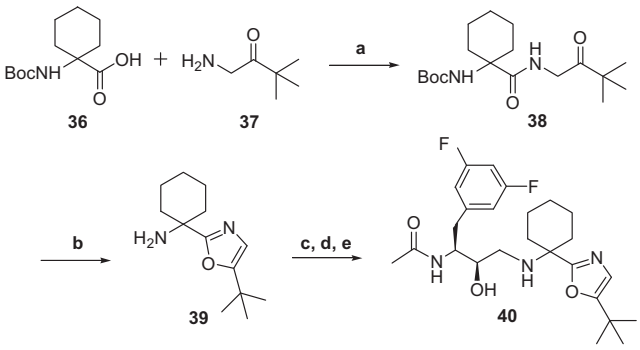
Isoxazoles and 1,2,3-triazoles were prepared by cycloaddition of the commercially available alkyne **30** with aldoximes and alkyl azides, respectively (Scheme 3). Oxazoles were prepared from amino acids using the Robinson–Gabriel oxazole synthesis (Scheme 4) and pyrazoles were prepared by the addition of a metalated-pyrazole into cyclohexanone followed by elaboration into the cyclohexylamines (Scheme 5).

In this series of compounds bearing heterocyclic aryl linkers, the analogs with a *neo*-pentyl *P2′* substituent had potency comparable to the **1** while the *tert*-butyl analogs were approximately an

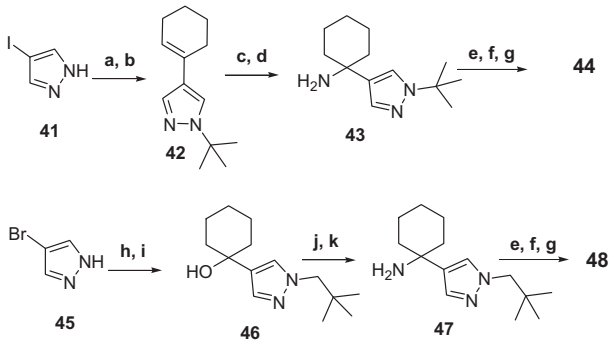


32 X = O Z = C R = ^{t}Bu
33 X = O Z = C R = neopentyl
34 X = N Z = N R = CH_2TMS
35 X = N Z = N R = adamantyl

Scheme 3. Reagents and conditions: (a) $(Boc)_2O$, CH_2Cl_2 X days, rt; (b) for **32** and **33** (i) RCHO, hydroxylamine hydrochloride, pyridine, ethanol, (ii) NCS, Et_3N, CH_2Cl_2; for **34** and **35** RN_3; (c) 4 N HCl in dioxane, 0.5 h; (d) **5**, DIPEA, ^{i}PrOH, 18 h, 70 °C; (e) 4 N HCl in dioxane, 0.5 h; (f) Ac_2NOCH_3, CH_2Cl_2, Et_3N, 18 h.



Scheme 4. Reagents and conditions: (a) EDCI, HOAt, Et_3N, CH_2Cl_2, rt; (b) $POCl_3$, 105 °C; (c) **5**, DIPEA, ^{i}PrOH, 18 h, 70 °C; (d) 4 N HCl in dioxane, 0.5 h; (e) Ac_2NOCH_3, CH_2Cl_2, Et_3N, 18 h.



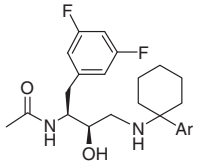
Scheme 5. Reagents and conditions: (a) H_2SO_4, *t*-BuOH, 100 °C; (b) *i*PrMgCl then cyclohexanone, 0 °C; (c) NaN_3, TFA, CH_2Cl_2 rt; (d) Pd-C, H_2, MeOH; (e) **5**, DIPEA, ^{i}PrOH, 18 h, 70 °C; (f) 4 N HCl in dioxane, 0.5 h; (g) Ac_2NOCH_3, CH_2Cl_2, Et_3N, 18 h; (h) *n*-BuLi then cyclohexanone −78 °C to 0 °C; (i) NaH, *neo*-pentyl iodide, DMF, 50 °C; (j) NaN_3, TFA, CH_2Cl_2; (k) $Pd(OH)_2$, H_2, MeOH.

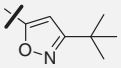
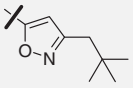
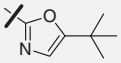
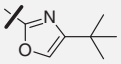
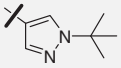
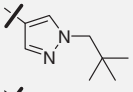
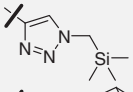
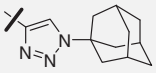
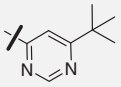
order of magnitude less potent (Table 3). This is in contrast to the phenyl series where the *tert*-butyl analog (**1**) is significantly more potent than *neo*-pentyl analog (**8**). Interestingly, in the thiophene series the *neo*-pentyl (**22**) and *tert*-butyl (**27**) analogs were equipotent suggesting that subtle differences in the bond lengths and bond angles between the five- and six-membered rings places the *P2′* substituent in optimal or sub-optimal positions within the pocket. Furthermore, the *tert*-butyl substituted oxazoles (**40** and **49**) showed dramatic differences in potency with the *tert*-butyl substituent in the 5-position (**49**) being almost three orders of magnitude more potent than the analog with the *P2′* substituent at the 4-position (**40**). Interestingly, the *S2′* pocket proved to be able to accommodate groups as large as adamantyl as demonstrated with triazole **35**. Pyrimidine **59** lost 50-fold potency compared to phenyl analog **1**.

In addition to exploring the SAR of the aryl region of the cyclohexylamine based inhibitors, a range of cyclopropyl amines bearing various aryl and heteroaryl linkers were prepared. In general, the synthesis of these analogs relied upon the conversion of a suitably substituted heterocyclic nitrile to a cyclopropyl amine [13] followed by elaboration into the HEA as depicted in Scheme 6.[14]

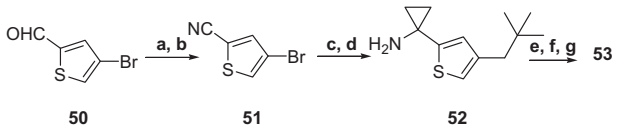
As with the cyclohexyl amine series, the phenyl linker combined with a *tert*-butyl *P2′* substituent proved to be the most potent combination (**54**, IC_{50} = 170 nM) (Table 4) although it was three-fold less active than its cyclohexyl counterpart **1**. This difference may be attributed to the less effective filling of the *S1′* pocket and/or due to differences in the conformation of the rings. Replacement of the benzene ring with pyridine gave a moderate loss in potency.

Table 3
BACE inhibition data and cellular activity for compounds **32–49**



Compds	Ar	BACE IC_{50},[a] (nM)	Cat D IC_{50},[b] (nM)	Cell ED_{50},[c] (nM)
32		4457	263	3711
33		139	14	88
49		1580	310	760
40		13,7800	12,170	>10,000
44		1840	140	235
48		210	29	23
34		154	12	6
35		392	8	na
59		2430	245	348

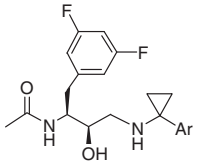
[a] See Ref. 10.
[b] See Ref. 11.
[c] See Ref. 12.

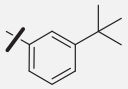
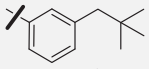
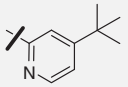
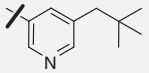
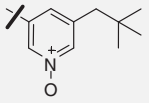


Scheme 6. Reagents and conditions: (a) hydroxylamine hydrochloride, EtOH, (b) Ac_2O; (c) *neo*-pentylzinc iodide, $PdCl_2[(o\text{-}Tol_3P)]_2$, THF; (d) EtMgBr, $Ti(OiPr)_4$, BF_3OEt_2, Et_2O; (e) **5**, DIPEA, ^{i}PrOH, 18 h, 70 °C; (f) 4 N HCl in dioxane, 0.5 h; (g) Ac_2NOCH_3, CH_2Cl_2, Et_3N, 18 h.

As observed with the cyclohexylamine series, the position of the sulfur atom in the thiophenes proved to be important in determining the BACE-1 activity of the inhibitors (Table 5). 2,4-Disubstituted thiophenes were 3–6 fold more potent that their 2,5-disubstituted analogs. A screen of oxazole (prepared as in Scheme 4, using amino cyclopropyl carboxylic acid in place of amino cyclohexyl carboxylic acid) and thiazole aryl linkers was performed but this did not provide any compounds with sub-micromolar potency (Table 5). As with the cyclohexylamine series, placing the nitrogen atom of the heterocycle in between the cycloalkyl and the *P2′* substituent resulted in a dramatic loss in potency with nitrogen having a more deleterious effect than oxygen. Pyrazole **67** and triazole **69** were the most potent analogs in this series and had activity similar to that of thiophene **53** indicating that a

Table 4
BACE inhibition data and cellular activity for compounds **54–58**



Compds	Ar	BACE IC_{50},[a] (nM)	Cat D IC_{50},[b] (nM)	Cell ED_{50},[c] (nM)
54		140	290	51
55		341	379	720
56		640	850	229
57		780	530	310
58		720	1050	8700

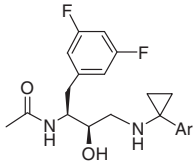
[a] See Ref. 10.
[b] See Ref. 11.
[c] See Ref. 12.

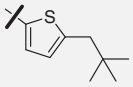
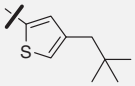
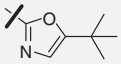
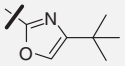
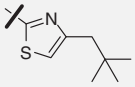
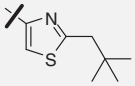
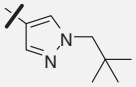
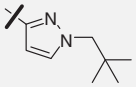
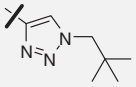
CH in this position is crucial for activity. Examination of the crystal structure of **53** (Fig. 2) clearly shows the CH in the 3-position of the thiophene engaging in a CH···O[15] non-classical hydrogen bond with the carbonyl of Gly34 at a distance of 3.2 Å. This interaction explains the observed SAR in this region, the addition of heteroatoms such as N or O causes a repulsive interaction with Gly34 C=O resulting in reduced BACE-1 potency.

The in vitro derived pharmacokinetic properties for a selected number of inhibitors are shown in Table 6. In the cyclohexylamine series, the permeability was poor with benzene as the aryl linker but replacement with both five-membered and six-membered heterocyclic aromatic rings gave compounds with generally good permeability. For example, isoxazole **33** had much higher permeability (180 nm/s) and lower P-gp liability compared to benzylamine **1** (4 nm/s). In general, the cyclopropylamine analogs have increased permeability and reduced P-gp liability compared to the cyclohexylamines as exemplified by comparing analog **54** to compound **1**. Interestingly, thiazole **64** proved not to be a substrate for P-gp.

The pK_a of a subset of these compounds was measured (Table 6) revealing a good correlation between the basicity of the cycloalkyl amine and the permeability of the compounds. Moving from a cyclohexyl amine (**1**) to a cyclopropyl amine (**54**) reduces the pK_a by 1.6 units resulting in a neutral molecule in the assay medium and allowing greater permeability through the lipid bi-layer.[7b] Similar effects were observed by replacing the phenyl group with heterocycles, allowing the pyrimidine **59** (pK_a = 6.7) to have much higher permeability (P_{app} = 265 nm/s) compared to **1** (pK_a = 8.2, P_{app} = 4 nm/s) but unfortunately this improvement in permeability did not have any effect on the P-gp efflux ratio of these compounds.[16] The log *P* of the inhibitors was also measured and it indicates that a high rate of permeability is only obtained when the log *P* falls in the range of 3.0–4.3 and it declines dramatically when the log *P* of the compound is greater than 4.6.

Table 5
BACE inhibition data and cellular activity for compounds **53–64**



Compds	Ar	BACE IC_{50},[a] (nM)	Cat D IC_{50},[b] (nM)	Cell ED_{50},[c] (nM)
60		1620	1720	1475
53		470	395	346
62		4900	3470	>10,000
63		42,880	>200,000	>10,000
64		9360	3110	>10,000
65		3050	1950	3850
67		346	720	68
68		870	3080	430
69		450	200	725

[a] See Ref. 10.
[b] See Ref. 11.
[c] See Ref. 12.

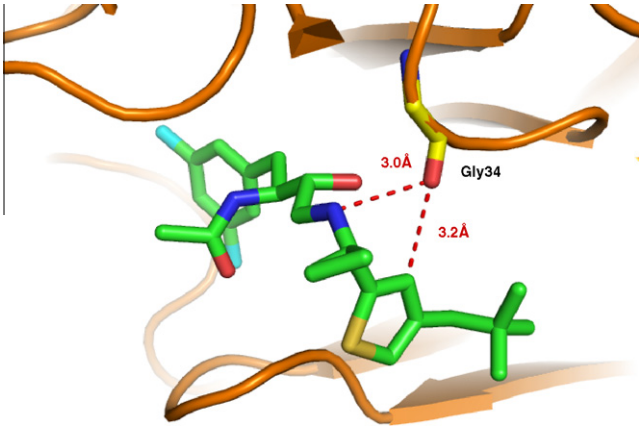
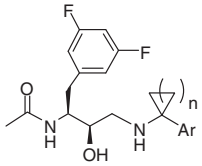
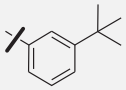
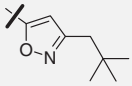
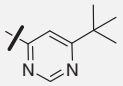
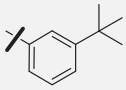
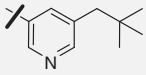
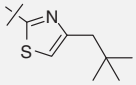
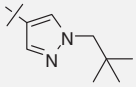


Figure 2. Crystal structure of truncated (57–453) human BACE-1 bound to **53** in green (2.2 Å resolution). The PBD deposition code is 3NSH. See supplemental material for experimental details.

In conclusion, optimization of the aryl linker region of a series of HEA BACE-1 inhibitors resulted in cell potent compounds with improved permeability and reduced P-gp liability compared to our previously reported compounds. The addition of heterocycles into the aryl linker region of the inhibitors lowered the pK_a and log *P* of the compounds into a range that is amenable to high rates of permeability. Subsequent efforts to address the remaining issues with these compounds will be reported in due course.

Table 6
Pharmacokinetic properties of selected inhibitors



Compds	*n*	Ar	log *P*	pK_a[c]	P_{app}[a] (nm/s) % recovery	P-gp efflux[b]
1	4		4.8	8.2	4 nm/s 7%	19
33	4		4.2	6.4	180 nm/s 100%	3.5
59	4		3.7	6.7	265 nm/s 112%	20
54	1		4.6	6.6	50 nm/s 27%	1.3
57	1		4.2	6.3	135 nm/s 94%	na
64	1		4.3	na	113 nm/s 54%	1
67	1		3.0	7.0	106 nm/s 67%	11

[a] See Ref. 17.
[b] See Ref. 18.
[c] See Ref. 19.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.070.

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10. Compounds serially diluted in DMSO were added to recombinant BACE purified from *E. coli* in 100 mM sodium acetate buffer containing 0.001% Tween-20 at pH 4.5 and allowed to incubate for 20 min at room temperature. A biotinylated peptide substrate, based on the Swedish mutant APP sequence, containing an Oregon Green moiety at the C-terminus, was added to initiate the reaction which was allowed to proceed for 3 h at 37 °C. The reaction was quenched by the addition of a five-fold volume excess of 100 mM sodium phosphate buffer pH = 7.4 containing a 1.5 μM final concentration of streptavidin. The extent of fluorescence polarization was measured using a LJL Analyst.
11. The Cathepsin-D was obtained from Sigma (cat. #C8696). Cathepsin-D was first dissolved with water to a 2 μM concentration and then subsequently with 100 mM sodium acetate buffer pH 4.5 to a 1.8 nM working concentration. A biotinylated peptide Cathepsin-D substrate was labeled at the C-terminus of the peptide with an Oregon Green fluorophore. Compounds were serially diluted three-fold in DMSO at a 100× concentration and then subsequently diluted 33× with 100 mM sodium acetate buffer pH 4.5. Compound was added to Cathepsin-D (0.6 nM final concentration) for 30 min before the addition of peptide substrate to initiate the reaction. The reaction was allowed to proceed for 110 min at 37 °C and then quenched by the addition of streptavidin in 200 mM sodium phosphate buffer pH 7.5. The amount of fluorescence polarization in the well was measured using a LJL Analyst (Perkin Elmer). If Cathepsin-D cleaves the peptide substrate then polarization will be reduced.
12. (a) Dovey, H. F. et al *J. Neurochem.* **2001**, *76*, 173. in which the HEK 293 cells were modified to express the Swedish mutation of APP FAD isoform; (b) Johnson-Wood, K. et al *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1550; (c) Seubert, P. et al *Nature* **1992**, *359*, 325.
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17. Compounds (5 μM) in mHBSS (pH 7.4) were incubated with MDCK II cell monolayers for 120 min at 37 °C. Samples were taken from apical and basolateral chambers, and analyzed using LC/MS/MS.
18. Compounds (5 μM) in mHBSS (pH 7.4) were incubated with MDR1-MDCK cell monolayers for 120 min at 37 °C with and without a P-gp inhibitor. Samples were taken from apical (A) and basolateral (B) chambers, and analyzed using LC/MS/MS. The efflux ratio was determined by dividing the rate of the A to B direction with and without a P-gp inhibitor.
19. Determined by capillary electrophoresis.