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SAR of tertiary carbinamine derived BACE1 inhibitors: Role of aspartate ligand amine pK_a in enzyme inhibition

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ABSTRACT

The optimization of tertiary carbinamine derived inhibitors of BACE1 from its discovery as an unstable lead to low nanomolar cell active compounds is described. Five-membered heterocycles are reported as stable and potency enhancing linkers. In the course of this work, we have discovered a clear trend where the activity of inhibitors at a given assay pH is dependent on pK_a of the amino group that interacts directly with the catalytic aspartates. The potency of compounds as inhibitors of Aβ production in a cell culture assay correlated much better with BACE1 enzyme potency measured at pH 7.5 than at pH 4.5.

Alzheimer's disease (AD) is a progressive neurodegenerative condition for which only palliative treatments exist at present. Given the complexity of caring for AD patients as well as the aging demographics of the world population, an urgent need for true disease modifying therapies exist. β-Site of APP Cleaving Enzyme (BACE1) is an attractive and widely pursued target in this respect.[1] Over the past years many reports highlighting the creative efforts at solving the problem of inhibiting an aspartyl protease in the brain with small molecule inhibitors have appeared in the literature.[2] We previously reported on the discovery of a novel tertiary carbinamine derived aspartate ligand[3] for BACE1 inhibition and further optimized these leads toward molecules compatible with brain penetration.[4] In this Letter, we would like to further describe the SAR around the tertiary carbinamine warhead, and how these efforts lead to a better understanding of the correlation between enzyme and cell-based activities for our program.

The serendipitous discovery of the novel tertiary carbinamine derived inhibitor **1** was followed by efforts to chemically stabilize the molecule as well as identify its binding mode to BACE1 (Table 1). The typical workflow for the project involved measuring enzyme potency for newly synthesized inhibitors in an ECL format BACE1 assay at the optimal pH for enzyme activity of 4.5, then following up selected compounds in a cell-based assay.[5] Molecular modeling, confirmed by the X-ray crystal structure of **1** bound to the BACE1 enzyme, led to the hypothesis that the central amine was interacting with the two catalytic aspartates, Asp32 and Asp228, as well as backbone Gly230. Based on proximity, we also assumed that the hydroxyl group formed an important interaction with Asp32. As anticipated, N-methylation of the amine gave compound **2**, which was inactive in our primary assay. Interchange of the unstable amino ester to the more stable amide **3** also led to a large loss in potency. Given that hydroxyls are the prevalent aspartate ligands for aspartyl protease inhibitors,[6] we attempted to replace the central amine with a hydroxyl group as with compounds **4** and **5**, which were far less active.

Efforts to stabilize the amino ester led to the ether linked compound **6** (Table 2). The deletion of the ester carbonyl mediated flap interaction lead to a significant intrinsic potency loss, but afforded a stable inhibitor with activity in the BACE1 cell-based assay. Inter-

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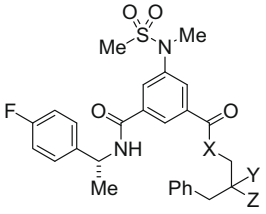
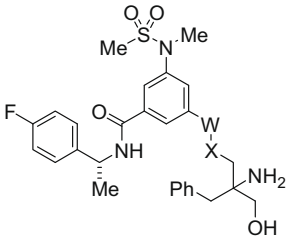
Table 1
Initial SAR of tertiary carbinamine lead



Entry	X	Y	Z	BACE1 IC_{50} (μM)
1	O	NH_2	CH_2OH	0.193
2	O	NHMe	CH_2OH	>100
3	NH	NH_2	CH_2OH	10.8
4	O	OH	CH_2NH_2	22.2
5	NH	OH	CH_2OH	24.9

Table 2
Linker SAR



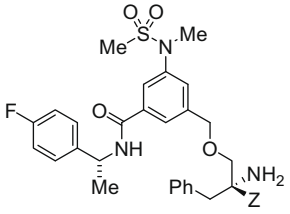
Entry	W	X	BACE1 IC_{50} (μM)	sAPPβ_NF IC_{50} (μM)
6	CH_2[a]	O	1.37	0.580
7	CH_2[a]	NH	10.1	3.47
8	CH_2[a]	CH_2	22.4	—
9	O	CH_2	70.5	—
10	N	CH_2	>100	—
11	S	CH_2	>100	—
12	SO_2	CH_2	>100	—

[a] Data reported for enantiopure ***R*** configuration at tertiary carbinamine stereocenter.

estingly, we noted that the cell-based potency of this compound was superior to the intrinsic BACE1 activity, an observation that we could not account for at the time. Co-crystallization of **6** with the target enzyme revealed that the ether oxygen atom was in close proximity to the Gly230 carbonyl. Attempts to establish an interaction with this residue utilizing benzylamine linker **7** or replacement of the oxygen with the methylene linked compound **8**, which presumably avoids unfavorable dipole interactions with the Gly230 carbonyl again resulted in less active inhibitors. Other variations of the linker, including phenol, aniline, sulfide and sulfonamide linked compounds **9**, **10**, **11**, and **12** all led to complete loss of intrinsic activity, reinforcing the notion that the benzyl ether or ester linker motif was unique.

Hydroxyl methylation of ether **6** led to inactive compound **13** (Table 3), which was not surprising given that this group appeared to interact with Asp32. Deletion of the hydroxymethyl moiety afforded compound **14**, which surprisingly maintained some inhibitory activity despite the excision of what we believed to be a key interaction with the catalytic Asp32 of the BACE enzyme. Even more surprisingly, the α-methyl-Phe 'warhead' motif-containing compound **15** proved to be more active than analog **6** and was within the potency range of lead compound **1**. The proximity of the hydroxyl group to Asp32 did not lead to an overall gain in

Table 3
Tertiary carbinamine methyl substituent SAR



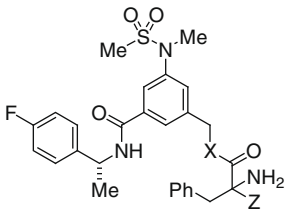
Entry	Z	BACE1 IC_{50} (μM)	sAPPβ_NF IC_{50} (μM)
13	CH_2OCH_3[a]	>100	—
14	H	10.1	1.71
15	CH_3	0.344	0.138
16	Et[a]	22.0	—
17	CH_2F[a]	0.997	0.432
18	CHF_2[a]	0.925	>20

[a] Data reported for diastereomeric mixture at tertiary carbinamine stereocenter.

binding energy, possible due to unfavorable interactions with the 'hydrophobic wall' of the BACE1 enzyme defined by Gly34, Ser35, and Tyr71, and the simple methyl substituent appears ideally fill this pocket. Again, we noted that the cell-based potency for inhibitor **15** was superior to enzyme activity at pH 4.5. Attempts to incorporate larger substituents such as ethyl substituted derivative **16** lead to a steady decline in activity. We then explored attenuating the basicity of the amine via fluorination of the α-methyl substituent to afford **17** and **18**. The enzyme and cell potency of monofluorinated compound **17** proved to be similar to that of parent **15**. Difluorination afforded analog **18** which maintained similar enzyme potency but featured much reduced cellular activity, giving us an important first indication that the pK_a of the amine derived aspartate ligand was critical for inhibiting BACE1 in a living cell.

Compound **15** provided a simplified aspartate ligand comprising of only a tertiary carbinamine interacting with the BACE1 catalytic machinery, and appeared to hold more promise for CNS exposure compared to other more traditional aspartyl protease ligands.[7] In terms of further potency enhancement proximal to the warhead group, structural information obtained during the course of our studies led us to believe that interaction with the Thr72 and Gln73 enzyme flap residues could be established from the methylene group proximal to the amine. Synthesis of the amino ester analog **19** afforded an active BACE1 inhibitor (Table 4). Utilizing SAR developed previously, we found that deletion of the hydroxyl group again proved to be potency enhancing, affording **20** with

Table 4
Alternate vector to reestablish flap interaction



Entry	X	Z	BACE1 IC_{50} (μM)	sAPPβ_NF IC_{50} (μM)
19	O	CH_2OH	0.310	0.684
20	O	CH_3	0.019	0.163
21	NH	CH_2OH	18.1	—
22	NH	CH_3	5.72	—

excellent enzyme and cell-based activity. Despite the considerable steric hindrance surrounding amino esters **19** and **20**, these compounds also proved to be very prone to hydrolysis, even at physiological pH.[8] Similar to previously observed SAR, incorporation of an amide linker to improve stability led to large losses in potency, as exemplified by compounds **21** and **22**.

Given the inability to establish a flap interaction via a carbonyl group from the linker, we next explored the possibility of utilizing a heterocycle derived ester mimetic to this end. Examination of overlays of compounds **1** and **20** co-crystallized with BACE1 supported this hypothesis (Fig. 1). Incorporation of a 1,3,4-oxadiazole into the linker provided compound **23**, with excellent potency against the target and stability in the pH 4–10 range (Table 5). A co-crystal of **23** bound to BACE1 was obtained, which when compared to bound inhibitors **1** and **20**, revealed that the oxazadiazole nitrogen atoms overlaid closely with the respective carbonyl oxygen atoms of ester linked inhibitors, implying hydrogen bonding with the flap residue.

Further SAR revealed that incorporating regioisomeric oxadiazoles **24** and **25**, as well as thiadiazole **26** as the linker led to large potency losses. One regioisomeric oxazole, exemplified by compound **27**, was synthesized and proved to be equipotent to **23**. Surprisingly, furan **28** proved to be active against BACE1, with superior potency in the cell-based assay. The conclusions that we drew from this data were that while oxadiazole **23** and oxazole **27** were capable of hydrogen bonding with the flap region, the heterocycle embedded in the linker served more as a conformational constraint via π-stacking with the P1 phenyl substituent.[9] The reduced affinity of the two regioisomeric triazoles **29** and **30**, which presumably could interact with the flap residue similar to oxadiazole **23** reinforces this point.

An interesting trend identified during the tertiary carbinamine optimization effort warranted further thought: the role of the central amine pK_a and why certain compounds displayed greater cell-based potency compared to enzyme activity at pH 4.5. Previously reported work from these laboratories have illustrated the importance of a basic amine aspartate ligand for activity in the cellular assay.[10,11] BACE1 has been demonstrated to have the highest catalytic activity for processing sAPPβ at pH 4.5, and our program utilized a primary enzyme assay at this pH to conserve enzyme while obtaining the best possible S/N ratio. However, in general we observed poor potency correlation between the enzyme- and cell-based assays (Fig. 2). Given the range of central amine basicity accessed during the course of this optimization, we could investigate the role of amine pK_a at varying assay pH to better understand this issue.

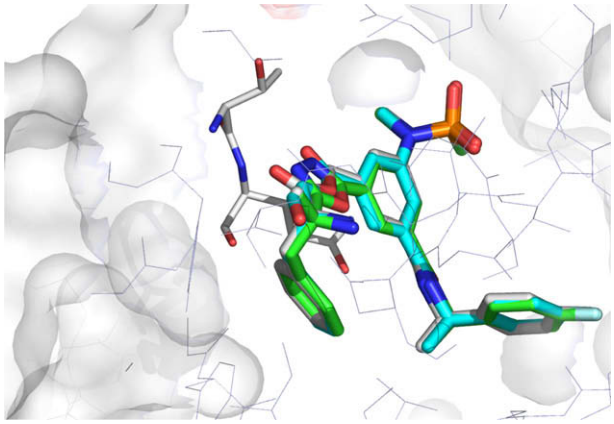
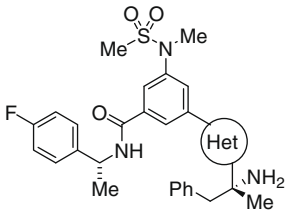


Figure 1. Overlay of inhibitors **1** (gray), **20** (blue) and **23** (green). Flap residues Thr72 and Gln73 are illustrated in stick format for clarity.

Table 5
Linker heterocycle SAR



Entry	Het	BACE1 IC_{50} (μM)	sAPPβ_NF IC_{50} (μM)
23		0.012	0.060
24		1.83	–
25		2.06	–
26		29.9	–
27		0.007	0.005
28		0.020[a]	0.004
29		0.822	4.74
30		16.3	–

[a] Inhibitor synthesized as a mixture of diastereomers at tertiary carbinamine stereocenter.

Ether linked compound **6**, which displayed superior potency in the cell-based assay compared to enzyme potency at pH 4.5 was calculated to have an average pK_a of 9.2 (Table 6). This compound displayed progressively improving BACE1 enzyme IC_{50} values as the pH of the assay was increased. Two other inhibitors with calculated pK_a around 8, the ether linked fluoromethyl derivative **17** and furan linked inhibitor **27** also proved to be increasingly potent at higher pH. Oxadiazole linker inhibitor **23**, with an averaged calculated amine pK_a of 7.2 did not exhibit much pH dependent variation for BACE1 enzyme inhibition. On the other hand, ether linked difluoromethyl compound **18**, with an average calculated pK_a of 5.7 proved to be similarly active against BACE1 at pH 4.5 and 6.5. However, at assay pH $\geqslant$7.2, large loss of activity in the purified enzyme and cellular assays was measured. Overall, the correlation between the BACE1 enzyme assay at pH 7.5 and the cell-based assay was significantly better than that at pH 4.5 for this limited set of compounds. This observation may be extended to a larger and more diverse set of compounds (Fig. 2).[12] This data highlights the fact that significant amine protonation is critical for inhibiting the BACE1 enzyme at any given pH, and could be attributed to the nature of the electrostatic interactions with the catalytic aspartates.[13] The potency improvement of compounds **6**, **17**, and **27**, all with calculated central amine pK_a >8, with increasing assay pH is not fully understood, and may be a consequence of protonation state changes of the BACE1 catalytic aspartates.

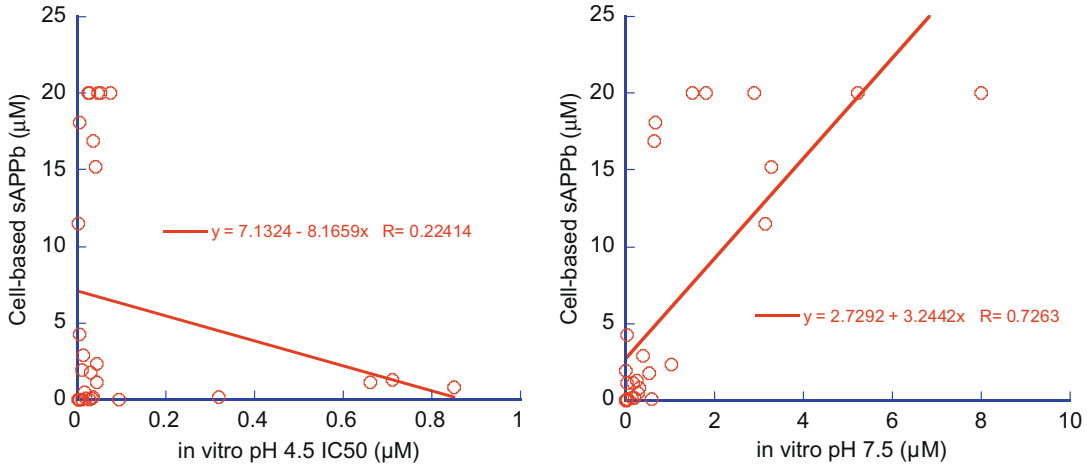


Figure 2. Correlation between cell-based assay and enzyme assay at pH 4.5 and 7.5 for a diverse set of compounds.

Table 6
pH dependent BACE1 enzyme inhibition for selected tertiary carbinamines

Entry	ACD pK_a[a] (calcd)	Jaguar pK_a[b] (calcd)	Avg pK_a[c] (calcd)	BACE1 IP[d] (μM) (pH 4.5)	BACE1 IP[d] (μM) (pH 6.5)	BACE1 IP[d] (μM) (pH 7.5)	sAPPβ_NF IC_{50} (μM) (pH 7.2)
6	8.1	10.3	9.2	6.50	0.719	0.290	0.506
17	7.1	8.7	7.9	4.01	0.608	0.522	0.432
18	6.5	4.9	5.7	10.8	6.94	49.7	>20
23	6.1	8.3	7.2	0.042	0.020	0.060	0.157
27	8.1	8.5	8.3	0.073	0.012	0.016	0.005

[a] Calculated using ChemDraw ACD Labs v11.0 plugin.
[b] Calculated using Jaguar v4.2 distributed by Scrodinger.
[c] Average of two calculated values.
[d] MESO assay format.

In summary, a fortuitously discovered tertiary carbinamine derived inhibitor of BACE1 was optimized to a 'warhead' motif compatible with brain penetration. The optimization of these compounds toward fully CNS penetrant BACE1 inhibitors capable of lowering non-human primate CSF Aβ levels has already been reported in the literature.[4,14] During this process, we discovered that not only did cell active BACE1 inhibitors require an amine derived aspartate ligand, but also that the pK_a of this group should be at the very least close to that of the assay medium pH. This data might also imply that contrary to some reports in the literature,[15] BACE1 action may not be functionally important in acidic cellular compartments. Instead, it could operate in cleaving APP in neutral compartments on or near the cell surface in the cell assay that we employed.[14]

Supplementary material

The synthesis of compounds **1** (WO2005004803), **6**, **10**, **15** (WO2005032471), **23**–**30** (WO2005103020) have been described in the patent literature. The BACE1/inhibitor complexes for compounds **1**, **20** and **23** have been deposited with the Protein Data Bank with identifiers 2ISO, 2PH6, and 2IRZ, respectively.

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12. Hydroxyethylamine and tertiary carbinamine derived BACE1 inhibitors, as well as inhibitors described in Refs. 10 and 11 were included in the data described in Figure 2.
13. By definition, an amine with a pK_a of 7.2 is 50% protonated at pH 7.2, while one with a pK_a of 8.2 is over 90% protonated at the same pH.
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