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Design, synthesis, and SAR of macrocyclic tertiary carbinamine BACE-1 inhibitors

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Abstract—This Letter describes the design and synthesis of tertiary carbinamine macrocyclic inhibitors of the β-secretase (BACE-1) enzyme. These macrocyclic inhibitors, some of which incorporate novel P2 substituents, display a 2- to 100-fold increase in potency relative to the previously described acyclic analogs while affording greater stability.
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Alzheimer's disease (AD) is a neurodegenerative disease of the brain that leads to a progressive decline in cognitive function and ultimately incapacitation and death.[1] AD is characterized by the presence of insoluble amyloid plaques and fibrillary tangles, key pathological features of this devastating disease. Years of research have established that the major component of the amyloid plaques is $A\beta_{40-42}$, a neurotoxic peptide fragment of β-amyloid precursor protein (APP), generated by the proteolytic action of the β- and γ-secretase enzymes. Of these two enzymes, β-secretase (β-site APP Cleaving Enzyme or BACE-1), an aspartyl protease, is considered rate-limiting in this proteolytic cleavage process.[2] Based on the key role of BACE-1 in the β-amyloid cascade, inhibition of BACE-1 is widely recognized as one of the most promising therapeutic approaches for the treatment and prevention of AD.[3]

However, the development of small molecule, brain penetrant BACE-1 inhibitors has been challenging. Most efforts so far have relied on the use of transition state isosteres (hydroxyethylamines, reduced amides, statines, and aminostatines) to interact with the catalytic aspartates, often with a negative impact on pharmacokinetics and permeation of the blood–brain barrier (BBB).

Recently, we disclosed the discovery of non-transition state isostere derived BACE-1 inhibitors which utilize a single tertiary carbinamine to bind to the catalytic aspartates.[4] Inhibitors derived from this novel scaffold, exemplified by **1a** (BACE-1 IC_{50} = 326 nM) and **1b** (BACE-1 IC_{50} = 27 nM), displayed comparable potency to classical hydroxyethylene amine (HEA) derived inhibitors (Fig. 1). However, stability, pharmacokinetics, and brain penetration remain an issue with this new series. Examination of X-ray crystallography data for ester **1b** bound to BACE-1 revealed the close spatial proximity between the P1 aryl group and the P3 methyl (Fig. 2).

Keywords: BACE; AD; Aspartate protease; Macrocycle; P-gp.
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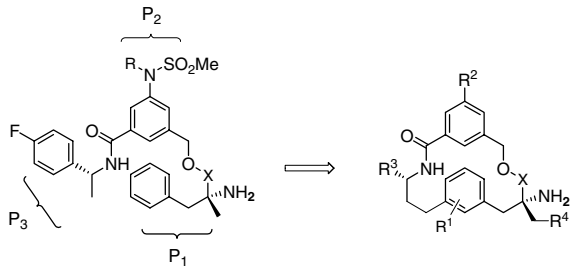


1a: X = CH_2, R = *n*-Pr
1b: X = CO, R = Me

2, X = CH_2, CO

Figure 1. Tertiary carbinamine BACE inhibitor **1** and envisioned P1–P3 macrocyclization strategy.

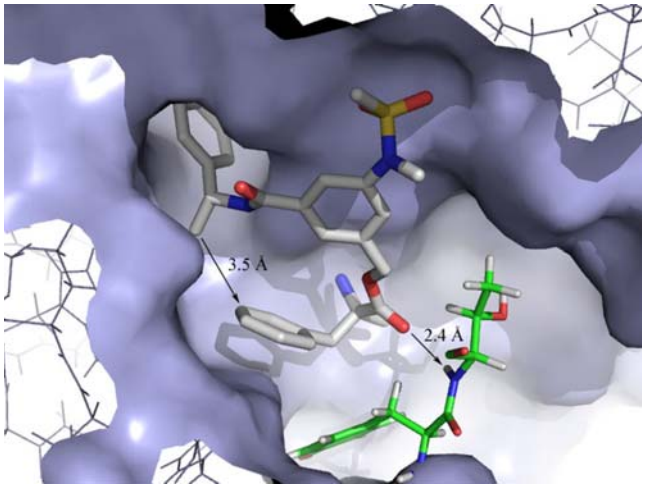


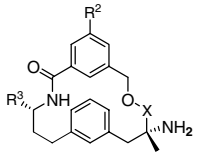
Figure 2. X-ray of inhibitor **1b** complexed with BACE-1. RCSB ID code: rcsb042372; PDB ID code: 2PH6.

Attracted by the possibility of increasing potency by stabilizing the bioactive conformation as well as the potential of improving the physiochemical liabilities of the acyclic series, we embarked on the preparation of macroethers and macrolactones of type **2**.[5]

In order to evaluate this strategy, we first synthesized a macrocyclic congener of **1a**. Macroether **2a** was found to inhibit BACE-1 with an IC_{50} of 17 nM in cell-free medium and an IC_{50} of 23 nM in cells,[6] thus affording a 19-fold increase in intrinsic potency relative to **1a** (Fig. 3). Despite making a number of key contacts with the enzyme, **2a** lacks an important interaction with the BACE-1 'flap' region.[4] In acyclic esters of type **1b**, the carbonyl alpha to the tertiary carbinamine accepts a critical H-bond from the 'flap' residue Thr72 (Fig. 2). This provides a significant boost in potency but at the cost of stability, both chemically and toward plasma esterases.[4] We postulated that cyclization into a macrolactone might confer more stability to the ester linkage while improving potency against BACE-1.

Gratifyingly, macrolactone **2b** exhibited improved potency (ca. 10-fold boost over **2a** at 2 nM) and was found to be not only hydrolytically stable at physiological pH

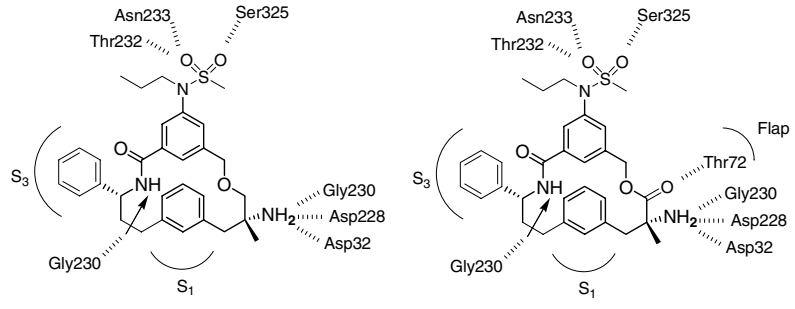
Table 1. P2 sulfonamide and P3 SAR



2

Compound	R^2	R^3	X	BACE-1 IC_{50}[a] (nM)
2a	*n*-Pr-N-SO_2Me	Ph	CH_2	17
2b	*n*-Pr-N-SO_2Me	Ph	CO	2
2c	Me-N-SO_2Me	Ph	CH_2	49
2d	Me-N-SO_2Me	Ph	CO	2
2e	Ph-N-SO_2Me	Ph	CH_2	12
2f	Ph-N-SO_2Me	Ph	CO	1
2g	Me-N-SO_2Et	Ph	CH_2	56
2h	Me-N-SO_2*i*-Pr	Ph	CH_2	196
2i	*n*-Pr-N-SO_2Me	4-F-Ph	CH_2	10
2j	*n*-Pr-N-SO_2Me	Me	CH_2	290[b]
2k	*n*-Pr-N-SO_2Me	Et	CH_2	85
2l	*n*-Pr-N-SO_2Me	*i*-Pr	CH_2	175
2m	*n*-Pr-N-SO_2Me	CCH	CH_2	53
2n	*n*-Pr-N-SO_2Me	*cy*-Pr	CH_2	1100

[a] IC_{50}s are an average of at least three measurements.[6]
[b] Calculated from a mixture of four diastereoisomers.



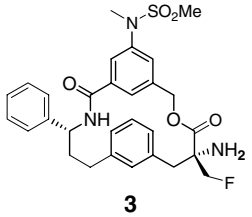
2a
BACE-1 IC_{50} = 17 nM
sAPP_NF IC_{50} = 23 nM

2b
BACE-1 IC_{50} = 2 nM
sAPP_NF IC_{50} = 5 nM

Figure 3. Macrocyclic tertiary carbinamine BACE inhibitors **2a** and **2b**. For both **2a** and **2b**, the sulfonamide imparts potency due to interactions with Thr232, Asn233, and Ser325; the lactam NH interacts with Gly230; the two phenyl rings partially fill S1 and S3, and the tertiary carbinamine makes critical interactions with both Asp32 and Asp228 as well as Gly230. In **2b**, the ester carbonyl makes additional interactions with Thr72 of the Flap.

but also stable in rat and human plasma and in microsomal preparations in the absence of NADPH (Fig. 3). Together, these data validated our initial proposal and warranted the synthesis of additional macrocyclic analogs.

Table 1 lists macroethers and macrolactones of type **2** bearing a variety of P2 sulfonamides and P3 hydrophobic groups. As observed with the prototypical compounds, macrolactones **2b**, **2d**, and **2f** are >8-fold more potent than the corresponding macroethers **2a**, **2c**, and **2e**. SAR of the *N*-alkyl portion of the P2 group is rather flat, especially in the macrolactone mode (**2a**–**f**). The alkyl-sulfone portion appears somewhat more responsive (**2g**,**h**), clearly favoring the smaller methyl-sulfone substituent as in compound **2c**.



3

BACE-1 IC_{50} = 23 nM

hu P-gp: P_{App} = 22, B-A/A-B = 56

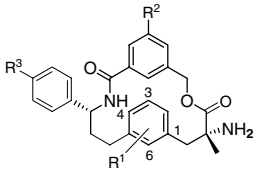
Figure 4. Attenuation of the tertiary carbinamine basicity by β-fluorination.

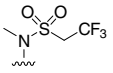
In contrast, the R^3 position is highly sensitive to modification. While fluoro substitution at the *para* position of the P3 phenyl provides a modest improvement in potency (**2i**), any attempt to reduce the size of the P3 group (**2j**–**n**) results in diminished potency.

Macrolactone **2d** represents the best combination of potency and molecular weight, with a 2 nM IC_{50} toward BACE-1 and a MW of 535. Unfortunately, like many other analogs described in Table 1, inhibitor **2d** suffers from poor brain penetration ($[\mathbf{2d}]_{brain}/[\mathbf{2d}]_{plasma} < 5\%$).[7] This could be the result of low apparent permeability ($P_{App} = 9$) and high P-gp efflux (B–A/A–B = 16).[8] Macrolactone **2d** also displayed poor pharmacokinetics (iv clearances approaching hepatic blood flow in rats, dogs, and rhesus) likely due to extensive oxidative metabolism at various positions of the P1–3 aromatic groups, as observed from incubation with microsomes.

In order to increase the likelihood of brain penetration, we proposed to modulate the Lewis acidity/basicity of the polar functional groups. Initially, we evaluated the effect of reducing the basicity of the tertiary carbinamine

Table 2. Macrolactone optimization



Compound	R^1	R^2	R^3	BACE-1 IC_{50}[a] (nM)	hu P-gp	
					P_{App}[b]	B–A/A–B[c]
2d	H	Me-N-SO_2Me	H	2	9	16
4a	H		H	16	12	19
4b	H		H	38	13	13
4c	H		H	4	15	32
5	H	Br	H	270	9	5
6a	H		H	780	19	1
6b	H	2-CN-Ph	H	27	9	1
6c	3-F	2-CN-Ph	F	130	ND	ND
6d	4-F	2-CN-Ph	F	46	9	3
6e	6-F	2-CN-Ph	F	78	6	2
6f	H	2-CN-3-F-Ph	F	22	7	1
6g	4-F	2-CN-3-F-Ph	F	46	6	5

[a] IC_{50}s are an average of at least three measurements.[6]

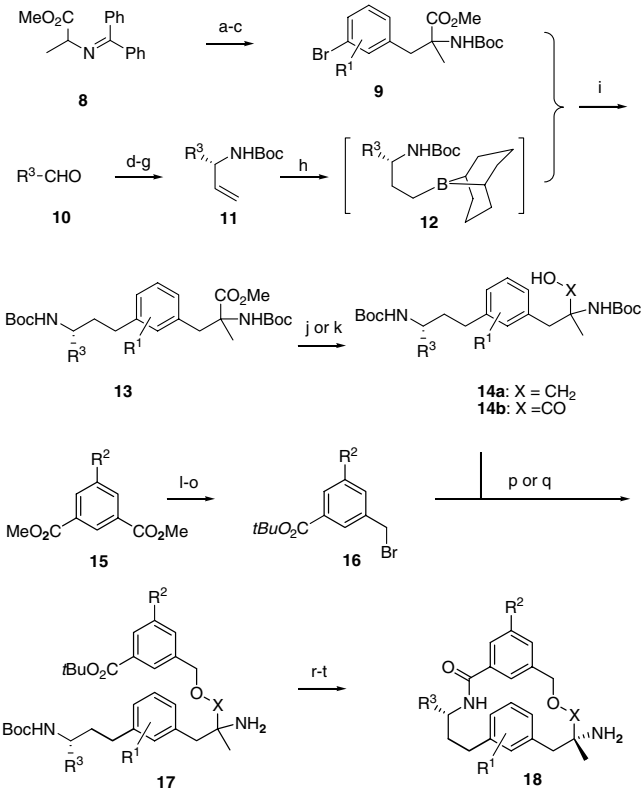
[b] Apparent permeability (10^{-6} cm/s).

[c] Efflux ratio.

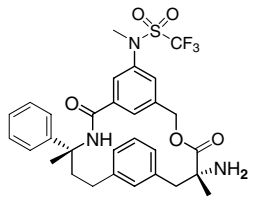
by β-fluorination. The resulting methyl-fluoro macrolactone **3** (Fig. 4) displayed a 10-fold loss in potency versus **2d** while improving apparent permeability (P_{App} = 22) and increasing P-gp efflux (B–A/A–B = 56). Similarly, attempts at modulating the electronics of the P2 sulfonamide via inductive or resonance effects (**4a**–**c**, Table 2) resulted in improved P_{App} but did not affect P-gp efflux. Despite the critical BACE-1 binding contributions made by the P2 sulfonamide (Fig. 3), it was also perceived as a major P-gp liability. Following unsuccessful attempts to attenuate P-gp efflux via modulation of the sulfonamide moiety (**4a**–**c**, Table 2), efforts centered on finding alternative P2 groups. Replacement of the P2 sulfonamide moiety with a bromo substituent (**5**) resulted in a 135-fold loss in potency; however, P-gp efflux[9] was improved. Guided by earlier findings[10] and encouraged by this result, we focused on P2 hydrophobic replacements. Although it displayed reduced potency, P2 oxazole[11] derivative **6a** was the first macrolactone to display high intrinsic permeability (P_{App} = 19) without P-gp efflux susceptibility (B–A/A–B = 1). Installation of a 2-cyano-phenyl P2 afforded biaryl inhibitor **6b** (BACE-1 IC_{50} = 27 nM, sAPP_NF IC_{50} = 68 nM, 10-fold loss in intrinsic potency relative to **2d**)[6] which was also devoid of P-gp efflux. Unfortunately, this came at the cost of permeability. A modest 16% brain penetration was achieved by **6b** when administered ip at 30 mpk.[7] In an attempt to increase P_{App} while maintaining low P-gp efflux, fluorine atoms[12] were sequentially installed around the P1–3 phenyl groups (**6c**–**g**); unfortunately, this strategy did not improve permeability (P_{App} = 6–9).

Additionally, pharmacokinetic properties remained poor across this series despite blocking multiple oxidative metabolism locations by fluorination. The only molecular subunit not yet addressed in these macrocyclic tertiary carbinamine BACE inhibitors was the P2–P3 amide. Although the amide appeared critical for potency (H-bond to Gly230),[4] it also represented a classical recognition element for the P-gp efflux pump. Indeed, P3-α-methylation (**7**, Fig. 5) as a means to increase steric hindrance around the P2 to P3 amide did improve P-gp efflux susceptibility when compared to non-methylated analog **4b**, but it also resulted in a 10-fold loss in potency.

A general synthetic route to macrocyclic tertiary carbinamine BACE inhibitors is illustrated in Scheme 1.[13] Installation of the P1 group is achieved via alkylation of methyl alanine benzophenone imine **8**, which after hydrolysis and Boc protection of the tertiary carbinamine provides intermediate **9**. The P3 building block is elaborated from aldehydes of type **10** via Ellman's methodology[14] to provide vinylic intermediate **11**. Hydroboration, followed by Pd-mediated coupling of aryl bromide **9**, affords the P1/P3 subunit **13**. Reduction or hydrolysis leads to alcohol **14a** or acid **14b**. Alkylation with benzylic bromide **16** leads to the acyclic P1–3 full assembly **17** which is deprotected and macrocyclized to **18**. If the R^2 group is carried intact from the original diester **15**, separation of the diastereoisomers (at the tertiary carbinamine center) is performed by preparative chiral HPLC. Alternatively, the R^2 group can be installed postmacrocyclization via Pd-mediated coupling, starting originally from dimethyl 3-bromoisophthalate.



Scheme 1. Reagents and conditions: (a) base, $ArCH_2Br$; (b) HCl, MeOH; (c) Boc_2O, DIEA; (d) Ellman sulfinimine; (e) vinyl Grignard, THF; (f) HCl, MeOH; (g) Boc_2O, Hunig's base; (h) 9-BBN, THF; (i) $Pd(PPh_3)_4$, 3 N NaOH, toluene, 85 °C; (j) for **14a**: $LiBH_4$,THF; (k) for **14b**: 1 N LiOH, THF; (l) 1 equiv NaOH, MeOH; (m) CDI, *t*-BuOH; (n) $LiBH_4$,THF; (o) CBr_4, PPh_3, DCM; (p) for **14a**: AgOTf, 2,6-di-*t*-Bu-pyr resin, DCE; (q) for **14b**: Cs_2CO_3, DMF; (r) HCl, DCM; (s) BOP, DIEA, DMF; (t) chiral preparative HPLC; for R^2 = Br: Boc_2O; flash chromatography; R^2–M, Pd(0); HCl, DCM.



7
BACE-1 IC_{50} = 380 nM
hu P-gp: P_{App} = 18, B-A/A-B = 1

Figure 5. P3 amide modulation.

In conclusion, macrocyclization of tertiary carbinamine BACE-1 inhibitors provided 2- to 100-fold improvement in potency and maintained greater than 1000-fold levels of selectivity against Renin and Cathepsin D.[15] Modification of the P2 group did afford minor improvements in brain penetration but often at the cost of potency. Initial results also indicated that modifications of the P2–P3 amide moiety could be more fruitful; this will be the subject of future work, to be described in due time.

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15. IC_{50} against Renin for **2a**, **2b**, **6b**, respectively: 144, 169, 275 μM. IC_{50} against Cathepsin D for **2a**, **2b**, **6b**, respectively: 275, 97, 48 μM. IC_{50} against BACE-2 for **2a**, **2b**, **6b**, respectively: 1.6 μM, 54 nM, 41 nM. It is rationalized that this selectivity is built into the isophthalimide scaffold, resulting from the hydrophilic nature of the BACE-1 S2 pocket as compared to BACE-2, Renin, and Cathepsin D.[4] For additional reference, see: Brady, S. F.; Singh, S.; Crouthamel, M.-C.; Holloway, M. K.; Coburn, C. A.; Garsky, V. M.; Bogusky, M.; Pennington, M. W.; Vacca, J. P.; Hazuda, D.; Lai, M.-T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 601.