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Discovery and SAR of isonicotinamide BACE-1 inhibitors that bind β-secretase in a N-terminal 10s-loop down conformation

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Abstract—A series of low-molecular weight 2,6-diamino-isonicotinamide BACE-1 inhibitors containing an amine transition-state isostere were synthesized and shown to be highly potent in both enzymatic and cell-based assays. These inhibitors contain a *trans-S,S*-methyl cyclopropane P_3 which bind BACE-1 in a 10s-loop down conformation giving rise to highly potent compounds with favorable molecular weight and moderate to high susceptibility to P-glycoprotein (P-gp) efflux.

Beta-site amyloid precursor protein cleaving enzyme (BACE-1) is a single-membrane associated aspartyl protease belonging to the pepsin family of proteolytic enzymes responsible for the processing of the amyloid precursor protein (APP).[1] Cleavage of APP by BACE-1 is the rate-limiting step in the amyloid cascade leading to the production of $A\beta_{40}$ and $A\beta_{42}$ peptide fragments. In particular, $A\beta_{42}$ is the primary species that is thought to be responsible for the neurotoxicity and amyloid plaque formation that lead to memory and cognitive defects in Alzheimer's disease (AD).[2] Initial BACE-1 KO-mouse studies,[3a–c] which showed no apparent adverse phenotype or detectable levels of Aβ, continue to support the rationale for the pursuit of BACE-1 inhibitors[4a,b] as a potential means for therapeutic intervention for AD.

Previously, **1**, which contains a reduced amide transition-state isostere as an aspartate interacting element, in conjunction with a novel olefin P_3-amide replacement, was described as a potent BACE-1 inhibitor with good cell permeability.[5a,b] However, despite the successful substitution of the P_3-amide within the isophthalate series, inhibitors in this class remained susceptible to significant P-gp efflux, thereby limiting their ability to penetrate the CNS. In an effort to develop BACE-1

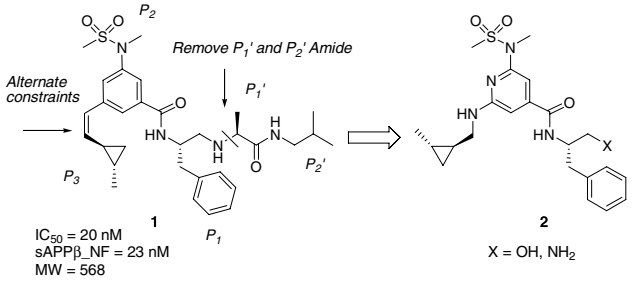

Figure 1. Reduced amide and isonicotinamide BACE-1 inhibitors.

Keywords: BACE-1 inhibitors; Alzheimer's disease; 2,6-Diamino-isonicotinamide.
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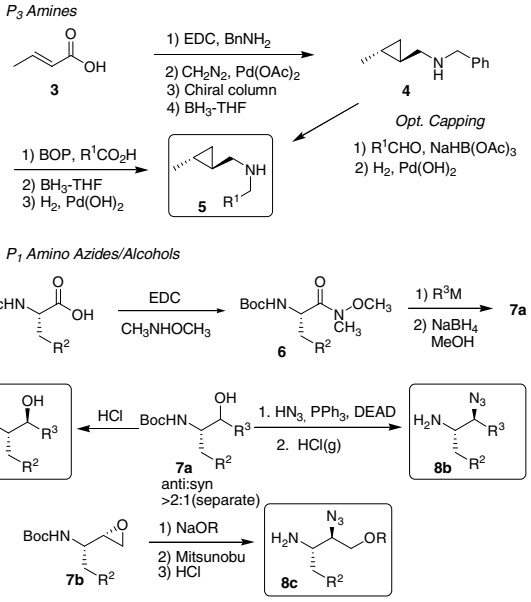
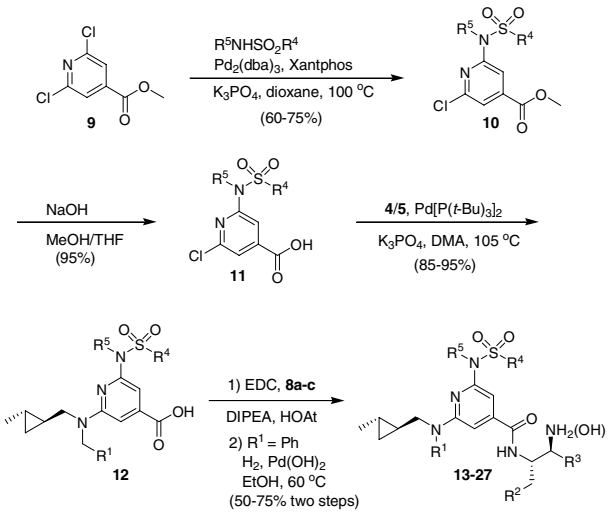
inhibitors with improved CNS penetration and pharmacokinetic stability, we have investigated a series of truncated amine and hydroxyl isosteres of type **2** (Fig. 1). Our strategy was to remove prime-side elements from inhibitors such as **1**, in particular the $P_{1'}$ and $P_{2'}$ amide, with the goal of further mitigating P-gp efflux and improving CNS penetration. For this study, we chose an N-terminal 2,6-diamino-isonicotinamide core with a cyclopropylmethyl amine as an isosteric and non-olefinic P_3-amide replacement. Although a potency loss was initially anticipated using a truncated prime-side design,[5a] the reduction in both the overall MW and the number of amide bonds was expected to favorably impact P-gp efflux and pharmacokinetic stability.

Schemes 1 and 2 summarize the routes used to prepare isonicotinamides **13**–**27**. The intermediates required for installation of the P_3 cyclopropanes and right-hand P_1 amino-azides are described in Scheme 1. The requisite *trans*-methyl cyclopropyl methyl amines, **5**, were prepared from all *trans*-crotonic acid **3** beginning with EDC coupling using benzyl amine followed by cyclopropanation via $Pd(OAc)_2$-catalyzed decomposition of diazomethane. Subsequent chiral column chromatography gave the preferred *S*,*S*-*trans* enantiomer,[6] which upon further functional group manipulation provided the chiral amine **5**. Amino alcohol and amino azide intermediates (Scheme 1, **8a**–**c**) were prepared in good yield and selectivity (2*S*,3*R* configuration preferred) using standard methods from either the appropriate BOC-protected amino acid or amino epoxides.[7] Intermediate **7a** (*anti*:*syn* >2:1), prepared in two steps via Weinreb amide **6**, was separated into its *syn* (1*S*,2*S*) and *anti* (1*S*,2*R*) diastereomers and utilized to prepare amino alcohols **8a** (from *anti* isomer) or amino azides **8b** where R^3 = alkyl (from *syn* isomer). Epoxide **7b**, upon nucleophilic opening, was similarly utilized to prepare intermediates where $R^3 = CH_2OR$.



Scheme 1. Synthesis of P_3 and P_1 intermediates **5** and **8a**–**c**.



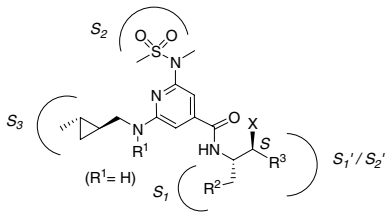
Scheme 2. Synthesis of trisubstituted isonicotinamides **13**–**27**.

Scheme 2 summarizes the convergent three-step coupling and reduction sequence chosen to prepare target compounds **13**–**27**. Starting with methyl-2,6 dichloroisonicotinate (**9**) and various alkyl sulfonamides, dilute Pd-catalyzed N-arylation proceeded overnight to give the monoarylated products of type **10**. Saponification provided the key P_2-containing isonicotinic acids **11**. A modified Pd-catalyzed amination reaction (**4/5** + **11**) using DMA as solvent generated in 85–95% yield the 2-amino-6-sulfonamido isonicotinic acids **12**.[8] The amination products were subsequently coupled to amino alcohol or amino azides **8a**–**c** using EDC, which after reduction and concomitant deprotection (R^1 = Ph) provided the target isonicotinamides **13**–**27**.

The inhibitory data of a select set of 'non-capped' isonicotinamides (R^1 = H) are shown in Table 1. Compound **13**, derived from 2*S*-3,5-difluorophenylalaninol, represents the simplest transition-state analog wherein X is a primary alcohol. This early example, with an IC_{50} of 74 nM and MW of 482 amu, encouraged us to evaluate additional prime-side analogs devoid of amide functionality which would limit CNS penetration. Extension of **13** with a simple butyl branch to give **14** resulted in a 5-fold improvement in potency for the diastereomeric mixture.

The 3*S*-OH stereochemistry shown for **14** was preferred and is similar to that found in other known hydroxyethylene (HE) and aminoethylene (AE) transition-state analogs for BACE-1;[9a,b,c] however, in contrast to these transition-state isosteres there are no additional amides present toward the prime-side. Unfortunately, despite the good inhibitory activity in our enzymatic activity, neither **13** nor **14** displayed significant functional activity in a sAPP_NF cellular assay.[10] Replacing the hydroxyl group with an amine however, such as that found in **15**, resulted in an inhibitor with good inhibitory potency in the enzymatic assay and in the cellular assay. The incorporation of a basic amine to engender cellular activity against BACE-1 has been noted by others[9c] and is thought to function by permitting the inhibitor

Table 1. SAR of P_3 'non-capped' inhibitors **13**–**20**



Compound	X	R^2	R^3	BACE-1[a] (IC_{50} nM)	sAPP_NF (IC_{50} nM)
13	OH		H	74	>20,000
14[b]	OH		*n*-Bu	13	>6700
15[b]	NH_2		*n*-Bu	9	110
16[b]	NH_2	Ph	H	34	279
17[b]	NH_2	Ph	*n*-Bu	20	110
18[b]	NH_2	Ph	$-CH_2CH_2CF_3$	2	71
19[b]	NH_2		*n*-Bu	11	38
20[b]	NH_2		CH_3	32	54

[a] IC_{50} determinations were performed using an ECL-based assay[10] and were run $n > 2$ with a CV < 0.3.
[b] Mixt. of *trans* methyl(cyclopropylmethyl)diastereomers.

to target acidic organelles wherein BACE-1 and sAPP are co-localized. Truncation of the R^3 group back to hydrogen and substituting phenyl in P_1 afforded **16**, resulting in a marginal loss in inhibition relative to **15** and **17**. Alternatively, replacement of the R^3 *n*-butyl group with a trifluoropropyl chain (**18**) gave a ~10-fold improvement in inhibitory activity relative to **17**; however, increased lipophilicity for this compound appears to have limited its inhibitory impact in cells (measured HPLC $\log P_{\text{octanol/water}}$: $\log P > 3.5$ for **18** vs measured $\log P = 2.51$ for **17**).

Structure–activity relationships in P_1 revealed a 4-fold preference for a 2-thienyl substituent (**19**), thus permitting truncation of the prime-side alkyl to **20**, which in cells proved to be nearly equipotent to **19**. As a BACE-1 inhibitor occupying the S_1–S_3 pockets (vide infra) the potency of **20** is impressive considering its moderate MW (465 amu).[12] With respect to activity against other aspartyl proteases, the series generally showed moderate selectivity versus BACE-2 (>15-fold) and excellent selectivity versus Cathepsin D (>5000-fold).

To gain an understanding of the binding mode for this class of inhibitors, and in particular the interaction of the R^3 sidechain within the enzyme active-site, a co-crystal structure of **19** with a human BACE-1 variant was successfully obtained.[13a,b] The **19**/BACE-1 complex (Fig. 2) revealed the inhibitor occupied the S_3–S_1 sites as predicted from modeling and previously obtained structures containing similar hydrophilic P_2 groups.[13a] In addition, the primary amine was found to engage both catalytic aspartates, Asp_{32} and Asp_{228}, with hydrogen bonding contacts. With respect to S_3, these inhibi-

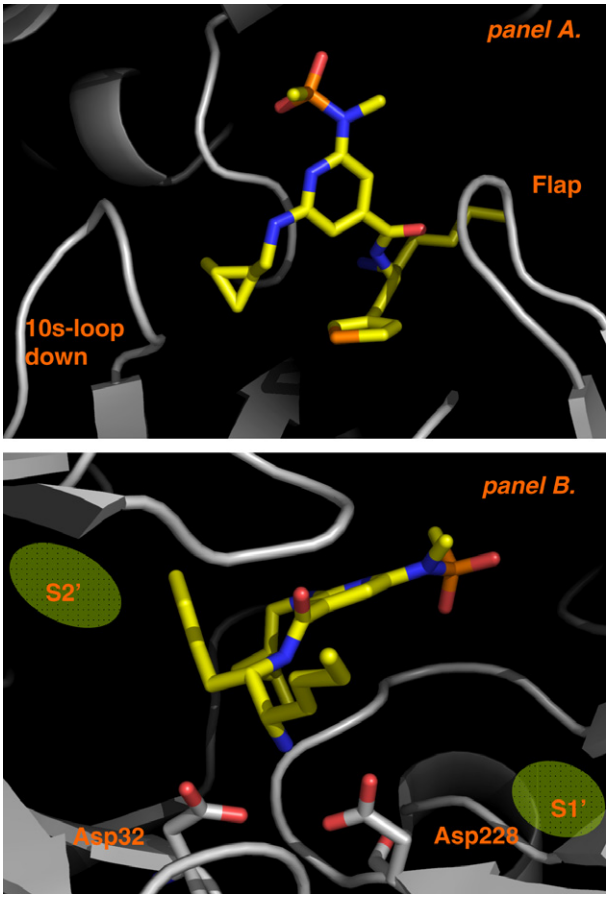


Figure 2. BACE-1/**19** view of S_3/S_1 (panel a) and view of prime-side aspartic acid residue (32, 228) interactions (panel b).[11]

tors bind to and stabilize a 10s-loop down conformation which is also found in the peptidic Val-P_3 containing OM99-2/BACE-1 crystal structure.[14] The 10s loop, which is known to be quite flexible, defines the size of the S_3 pocket and appears to vary depending on the P_3 presentation.[15] Relative to a similar 1,3,5-isophthalate inhibitor[5a] bearing a (*R*)-α-methylbenzyl-carboxamide P_3 residue in the $S_{3^{sp}}$ (sp = subpocket 10s-loop up), the conformational change observed for this complex energetically constitutes a >20-fold enhancement for the inhibitor–enzyme complex, while fortuitously reducing inhibitor MW by ~80 amu and eliminating one amide bond. Control inhibitors lacking the terminal *trans*-3*S*-methyl group of the cyclopropane result in a potency loss comparable in magnitude to the above comparison and suggest that the presence of the terminal S_3 methyl group is responsible for most of the binding enhancement. Relative to the OM99-2/BACE-1[14] crystal structure, the *trans*-methyl cyclopropane group occupies the S_3 pocket more deeply than the OM99-2 Val-P_3 and appears to garner a number of additional key van der Waals contacts in this region of the enzyme, including the alpha carbons of Gly_{230}, Thr_{231} and in particular the methyl sidechain of Ala_{335}. In addition, an alternate rotamer for the sidechain of Leu_{30} is observed and appears to allow for optimal van der Waals contact with the methylene portion of the cyclopropane ring.

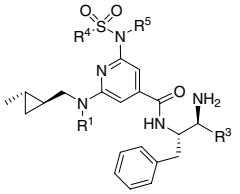
An additional somewhat unexpected feature for the BACE-1/**19** complex was that the R^3 butyl sidechain did not have a direct prime-side interaction in either the $S_{1'}$ or $S_{2'}$ sites (Fig. 2, panel b). In an attempt to conformationally lock R^3 into a favorable gauche conformation which would poise the prime-side group toward the more proximal S'_1 pocket, a β-alkoxyl substituent was incorporated.[16] This modification is exemplified within a series of 'capped' ($R_1 \neq H$) compounds shown in Table 2 (**21**–**27**). Gratifyingly, introduction of a methoxy methyl branch (**24** vs **21**) had a modest impact on potency while reducing the overall MW. Extension of the hypothesized $P_{1'}$ group from methoxy (**25**) to ethoxy (**26**) proved invariable in terms of BACE-1 inhibition; an observation which was similarly noted in $S_{1'}$ by others utilizing HE isosteres.[9b]

With respect to P_2 sidechain SAR, a propyl group at R^5 (i.e., **22**–**23**) resulted in a dramatic loss of inhibition in whole cells, suggesting these compounds had exceeded a lipophilic threshold disfavoring cell-penetration. However, in contrast to homologation at R^5, increasing steric bulk at R^4 with an *i*-propyl group (i.e., **25**) proved more successful in retaining favorable cell permeability properties.

The presence of a methyl capping group ($R^1 = CH_3$ vs $R^1 = H$, Tables 1 and 2) generally resulted in a reduction in intrinsic and cellular inhibition (i.e., 5-fold **17** vs **21**). In contrast, capping R^1 had a positive affect in reducing susceptibility to P-gp efflux. For example, in a cell-line expressing human P-gp, **25** was a moderate substrate for P-gp efflux (B/A–A/B: MDR1 = 13), while a closely related non-capped analog, **17**, exhibited significant efflux (B/A–A/B: MDR1 > 50).[18] An alternative capping group that was highly beneficial for potency, although somewhat deleterious in terms of susceptibility to P-gp efflux within the β-alkoxy series, was a methoxyethyl group as illustrated by compound **27**. In direct comparison with compound **25**, **27** was 4- and 12-fold more potent in terms of enzymatic and cell-inhibition, respectively, and represents in the cell-based assay the only sub-100 nM BACE-1 inhibitor within the 'capped' series.

In summary, we have shown that a series of isonicotinamides occupying the S_1–S_3 pockets in conjunction with either a primary amine or alcohol are both highly potent and selective against BACE-1. Although potency optimization was readily achieved, susceptibility to in vitro P-gp efflux remained a challenge when maintaining good BACE-1 inhibition in whole cells. The incorporation of a *trans*-methylcyclopropane P_3 provides a significant improvement in both potency and overall MW relative to preceding isophthalate series investigated to date and appears to originate from enhanced van der Waals contacts within the context of a 10s-loop down BACE-1 conformation. Further efforts incorporating and retaining the favorable structural elements within the isonicotinamide series into new inhibitor designs are ongoing.[17] These efforts and additional in vivo data are forthcoming.

Table 2. SAR of P_3 capped inhibitors **21**–**27**



Compound	R^1	R^3	R^4/R^5	BACE-1[a] (IC_{50} nM)	sAPP-NF (IC_{50} nM)
21	CH_3	*n*-Bu	CH_3/CH_3	31	589
22	CH_3	*n*-Bu	CH_3/*n*-Pr	39	>6700
23	CH_3	CH_3	CH_3/*n*-Pr	67	>6700
24	CH_3	CH_2OMe	CH_3/CH_3	12	173
25	CH_3	CH_2OMe	*i*-Pr/CH_3	24	845
26	CH_3	CH_2OEt	*i*-Pr/CH_3	24	402
27	MeO	CH_2OMe	*i*-Pr/CH_3	6	66

[a] IC_{50} determinations were performed using an ECL-based assay ($n > 2$, CV < 0.3).[9]

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6. The all *trans* cyclopropane amide (preparation of **4**, step 3) was resolved using a 50×5 cm 20 μ ChiralPak OD column with 95% hexanes (modified with 0.1% TFA) and 5% EtOH at 70 mL/min. The individual enantiomers were carried through subsequent transformations with **11** to give diastereomeric compounds using *S*-phenylalaninol in P_1. The more 'active' diastereomer (data not shown) was found to have originated from the second eluting peak from the requisite amide intermediate. A binary complex of BACE-1/**19** confirmed the *S*,*S*-*trans* configuration.
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