Discovery of Isonicotinamide Derived β-Secretase Inhibitors: In Vivo Reduction of β-Amyloid

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Abstract: β-Secretase inhibition offers an exciting opportunity for therapeutic intervention in the progression of Alzheimer's disease. A series of isonicotinamides derived from traditional aspartyl protease transition state isostere inhibitors has been optimized to yield low nanomolar inhibitors with sufficient penetration across the blood−brain barrier to demonstrate β-amyloid lowering in a murine model.

Alzheimer's disease (AD) represents a major unmet medical need. Despite substantial effort, there exists no disease modifying treatment for AD.[1] It has been proposed that the biological pathway leading to the observed disease pathology is reliant on the processing of amyloid precursor protein (APP).[2] Proteolysis of APP by the secretase enzymes (α, β, and γ) generates peptide fragments, with the most relevant to AD being $A\beta_{40}$ and $A\beta_{42}$. These fragments are derived from the action of both γ- and β-secretase and are the primary components of the insoluble amyloid plaques in AD patients. Disruption of this cascade via γ- and more recently β-secretase inhibition has and continues to be a central focus of drug discovery efforts.[3]

Inhibition of the β-secretase (β-site APP cleaving enzyme or BACE-1) pathway by small molecule interference has been a goal of the pharmaceutical industry since the identification and characterization of BACE-1 in 1999.[4] Particularly encouraging for this strategy was the discovery in 2001 that BACE-1 knockout mice were devoid of β-secretase activity, did not generate Aβ, and displayed a relatively normal phenotype.[5]

Previous work in our laboratories revealed **1**, a potent BACE-1 inhibitor derived from a 1,3,5-trisubstituted aromatic core and containing a traditional aspartyl protease inhibitor motif, the hydroxyethylamine (HEA) transition state isostere (Figure 1).[6] Interaction of the HEA with the aspartic acid residues in the catalytic region of the enzyme is critical for activity. Despite the excellent cellular activity of **1** (sAPPβ = 20 nM), brain penetration of this class of compounds following i.v. administration in mice is negligible. The alleged culprits for this lack of CNS penetration are poor permeability and Pgp-mediated efflux, presumably due to the compound's multiple hydrogen bond donors and acceptors.[7] With this in mind, the HEA isostere was truncated to a simple primary alcohol (**2**). This led to a substantial loss in BACE-1 inhibitory activity. Investigations focusing on replacement of the *P*3 amide and optimization of the 1,3,5-trisubstituted aromatic core have been the subject of previous communications.[8a,b] It was found that small cyclopropylmethylamine *P*3 groups in combination with the isonicotinamide core (**3**) could provide improved potency relative to the larger α-methylbenzamide (**2**). This communication describes further refinement of the *P*2 sulfonamide and optimization of the primary amine aspartyl binding region leading to **4**, a molecule suitable for in vivo evaluation in a murine model.

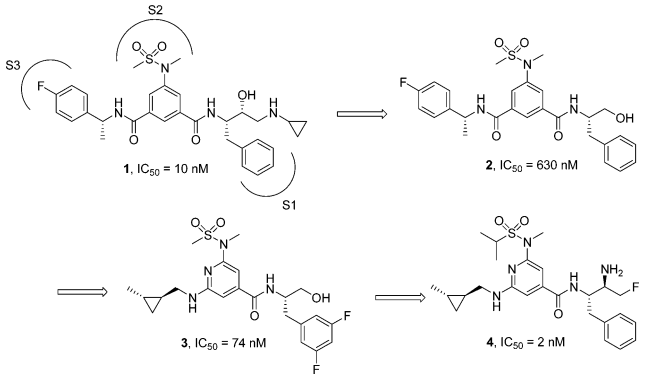
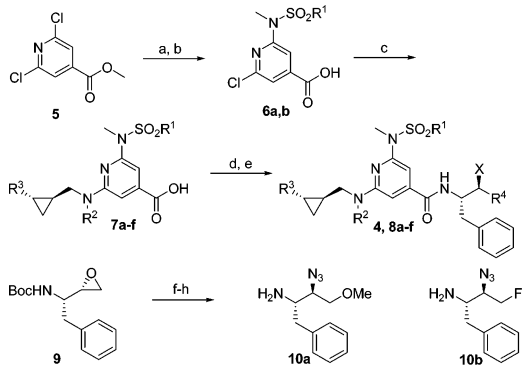


Figure 1. Isonicotinamide BACE inhibitor (**4**) derived from hydroxyethylamine (HEA) **1** (IC_{50} values from the in vitro ECL assay).

Scheme 1. Synthesis of Isonicotinamide β-Secretase Inhibitors **4** and **8a**−**f**[a]



[a] Reagents and conditions: (a) $CH_3NHSO_2R^1$, $Pd_2(dba)_3$, Xantphos, K_3PO_4, toluene, 100 °C; (b) LiOH, MeOH, THF; (c) $R^3[CH(CH_2)CH]CH_2$-NHR^2, Pd(*t*-$Bu_3P)_2$, K_3PO_4, DMF, 120 °C; (d) amine ((2*S*)-2-amino-3-phenylpropan-1-ol, (2*S*)-1-azido-3-phenylpropan-2-amine, **10a**,**b**), BOP, TEA, DCM; (e) H_2, $Pd(OH)_2$, EtOH, TFA; (f) NaOMe (**10a**) or KF·HF, Bu_4NF·2HF (**10b**); (g) HN_3, PPh_3, DEAD, THF; (h) EtOAc, HCl.

Synthesis of isonicotinamide inhibitors **4** and **8a**−**f** began with methyl 2,6-dichloroisonicotinate (**5**; Scheme 1). Monosulfonamide incorporation according to the procedure described by Buchwald, followed by saponification, gave isonicotinic acids **6a**,**b**.[9] Amination under modified Hartwig conditions generated the 2-amino-6-sulfonamidoisonicotinic acids **7a**−**f**.[10] Coupling of these acids to either the amino alcohol or the amino azides derived from phenylalanine, followed by reduction of the azide and/or removal of the benzyl protecting group (R^2 = benzyl) gave compounds **4** and **8a**−**f**. Azides **10a**,**b** were prepared by epoxide opening with the appropriate nucleophile (MeO^-, F^-) followed by a Mitsunobu reaction with hydrazoic acid and deprotection of the amine.

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Table 1. Binding Affinity, Pgp Efflux, and Brain Penetration for Test Compounds[a]

cmpd[b]	R^1	R^2	R^3	R^4	X	IC_{50}[c]	sAPPβ[d]	MDR1[e]	[brain][f]	[brain]/sAPPβ
8a	CH_3	H	H	H	OH	1100	n.d.	n.d.	n.d.	
8b	$CH(CH_3)_2$	$CH_2CH_2CH_3$	H	H	OH	350	>20 000	3.6	1400 ± 1350	<0.07
8c	$CH(CH_3)_2$	H	CH_3	H	OH	14	>20 000	n.d.	n.d.	
8d	CH_3	H	CH_3[g]	H	NH_2	34	280 ± 49	36.5	420 ± 157	1.5
8e	CH_3	CH_3	CH_3	CH_2OCH_3	NH_2	12 ± 14	173 ± 12	n.d.	1100 ± 492	6.4
8f	$CH(CH_3)_2$	CH_3	CH_3	CH_2F	NH_2	18	910	4.8	3080 ± 2400[h]	3.4
4	$CH(CH_3)_2$	H	CH_3	CH_2F	NH_2	2 ± 0.2	49 ± 18	>50	560 ± 128[h]	11.4

[a] All values lacking standard deviation were measured as single data points. [b] All compounds were >95% pure by HPLC and characterized by ^{1}H NMR and HRMS. [c] Biochemical IC_{50}. Values are reported in nM and were determined via electrochemiluminescence assay. [d] Cell-based assay. Values are reported as IC_{50}'s values in nM and were determined via Alpha Screen assay. [e] B/A−A/B ratio. [f] Concentrations determined 30 min post 20 mg/kg i.v. dose (*n* of 3) and are reported in nM. [g] Compound exists as a mixture of *trans*-methyl(cyclopropylmethyl) diastereomers. [h] Represents concentrations from dosing of mixture of *trans*-methyl(cyclopropylmethyl) diastereomers.

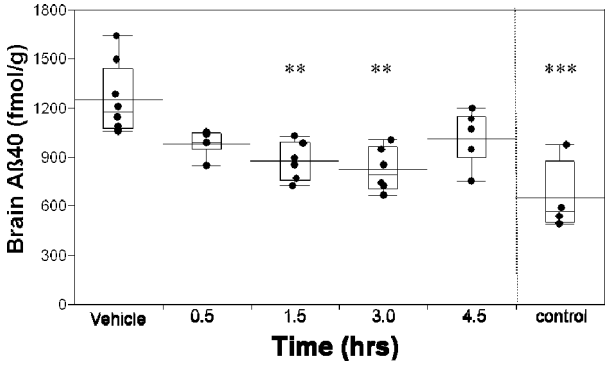

Figure 2. Time course study of Aβ reduction in APP-YAC mice upon treatment with **4** (50 mg/kg i.v.).

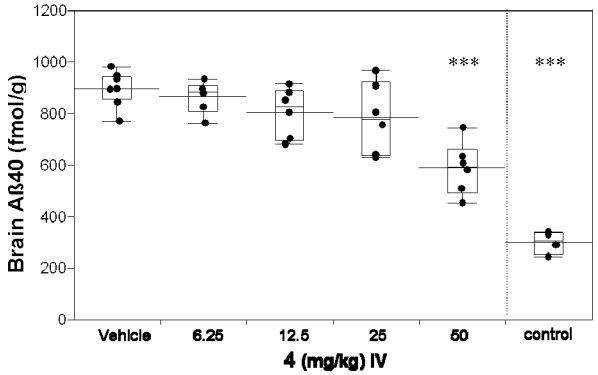

Figure 3. Dose response study of Aβ reduction in APP-YAC mice upon treatment with **4** (3.0 h post i.v. bolus).

Initial efforts in the isonicotinamide series of BACE-1 inhibitors revealed that truncation of the HEA to a simple primary alcohol gave reasonable activity for such a simple, low molecular weight (mw = 432) compound (**8a**, Table 1). Optimization of the *P*2 sulfonamide (Me to *i*-Pr) and alkylation of the *P*3 amine (NH to $NCH_2CH_2CH_3$; **8b**) gave both improved biochemical potency and, more importantly, greater passive permeability and decreased Pgp efflux. These improved properties were reflected in the in vivo model for brain penetration, with **8b** achieving brain concentrations of 1.4 μM after i.v. administration (30 min post-dose, 20 mg/kg). Encouraged by this result, the *P*3 region of the molecule was further optimized to the [*S*,*S*]-*trans*-methylcyclopropyl group, imparting further benefits with respect to potency (**8c**, 14 nM).[8b] Despite the low nanomolar activity of this series, activity in the functional cell-based assay was not observed (sAPPβ > 20 μM).

Incorporation of an amino residue in place of the hydroxyl group (alternative aspartyl ligand)[8b,c] provided molecules with activity in cell culture (**4**, **8d**−**f**). Compound **8d** achieved CNS exposure (20 mg/kg i.v. dose) in excess of the molecules cellular IC_{50}. Optimization of this series was focused on the transition state isostere. Incorporation of branched alkyl substituents increased the biochemical potency (**4**, **8e**−**f**). Attenuation of the pK_a of the amino functionality by incorporation of a fluoromethyl substituent gave improved potency and, in the case of **8f**, reduced Pgp efflux. Analysis of the brain concentrations relative to the cellular IC_{50} values revealed compound **4** as a leading candidate for in vivo studies.

As a result of the ratio of brain concentration to cellular IC_{50}, compound **4** was chosen as a proof of concept molecule for studying the effect of inhibition of BACE-1 on brain levels of Aβ_{40} (Figure 2). Transgenic mice expressing human WT APP under the control of a yeast artificial chromosome vector[11] were administered 50 mg/kg of **4** as an i.v. bolus and brain concentrations of Aβ_{40} were measured at 0.5, 1.5, 3, and 4.5 h post-dose. The results of this study are depicted in Figure 2 and show a maximal reduction of Aβ_{40} (34%) at 3 h ($p < 0.01$, Tukey−Kramer HSD) compared with that of vehicle-treated animals, while a positive control γ-secretase inhibitor lowered brain Aβ_{40} ~50% ($p < 0.001$, Tukey−Kramer HSD). Determination of concentrations of **4** in the brain at these time points indicated a maximal concentration of 5.8 μM at 0.5 h, with 0.7 μM remaining at the 4.5 h time point (data not shown).

Table 2. Pharmacokinetic Parameters of Isonicotinamide BACE Inhibitors in Rat

cmpd	Cl[a] (mg/min/kg)	Vd[a] (l/kg)	$t_{1/2}$[b] (hr)	C_{max}[b] (μM)	%*F*
8e	42.6	5.3	2.7	2.7	69
8f	59.1	4.2	1.6	0.2	8
4	45.8	3.9	1.6	0.3	13

[a] 2 mg/kg i.v. dose (solution in 25%DMSO/75%H_2O). [b] 10 mg/kg oral dose (solution in 1% methylcellulose).

Having determined a maximal Aβ_{40} reduction at 3 h post-dose, a full dose−response study was performed (Figure 3). The Aβ_{40} reduction measured in the time-course study was confirmed in this study with ~34% reduction of Aβ_{40} at 50 mg/kg at 3 h after dosing relative to vehicle ($p < 0.001$, Tukey−Kramer HSD).[12] The Aβ_{40} reduction was dose proportional, with doses <25 mg/kg not showing statistical significance. The concentration of drug in the brain was 1.9 μM and 0.7 μM for the 50 and 25 mg/kg dose groups, respectively, and below the limit of quantification in the 12.5 and 6.25 mg/kg groups (data not shown).

The pharmacokinetic parameters of selected isonicotinamide BACE-1 inhibitors were investigated in rat (Table 2). In general, compounds from this series displayed high clearance and high volumes of distribution. The i.v. half-lives were moderate and the oral bioavailability was poor. The notable exception with respect to bioavailability is compound **8e**, which is 69% bioavailable, with a good oral maximum concentration of 2.7

μM. The challenge remains to find molecules that combine the reasonable pk parameters of **8e**, with the potency/efficacy of **4**.

AD remains one of the more substantial unmet medical needs. Small molecule interference in the amyloid cascade represents an attractive therapeutic option. β-Secretase is a particularly appealing target with knock-out mice demonstrating Aβ reduction and a relatively normal phenotype.[5] The challenges surrounding β-secretase inhibitor design are substantial and are highlighted by the difficulty in uncovering small molecules that maintain potency while demonstrating desirable penetration across the blood−brain barrier. Starting from compounds containing a known aspartyl protease transition state isostere, isonicotinamide-based inhibitors were discovered that allowed for truncation of the HEA isostere to a simple amine. Optimization for potency and brain penetration led to **4**, a low nanomolar BACE-1 inhibitor that was effective in reducing Aβ levels in a murine model in a dose-dependent manner. Issues that remain to be resolved are Pgp-mediated efflux and poor pharmacokinetics. Efforts are currently under way to address these liabilities and will be the subject of future communications.

Supporting Information Available: Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Three out of 33 mice did not survive at the highest dose (50 mg/kg) of **4** for the two studies in Figures 2 and 3 (compared to 0 deaths in 16 control animals). No deaths occurred in the lower doses of **4**.

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