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Evolution of Tertiary Carbinamine BACE-1 Inhibitors: Aβ Reduction in Rhesus CSF upon Oral Dosing

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Alzheimer's disease (AD) is a neurodegenerative disease that leads to progressive decline in cognitive function and ultimately incapacitation and death.[1] One of the most widely accepted theory of disease causation is that perturbations in the rate of formation and/or clearance of the A-beta peptide initiate a cascade of events leading to insoluble amyloid plaques, fibrillary tangles, neuroinflammation, and neuronal cell death.[2a–c] The $A\beta_{40-42}$ peptide is a 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) is a type I membrane associated aspartyl protease and is considered rate-limiting in this proteolytic process.[2d,e] 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 via reduction of $A\beta_{40-42}$ levels in the CNS.[3]

The development of small-molecule BACE-1 inhibitors has been challenging due to the nature of the active site and the CNS localization of the target. Early substrate-based BACE-1inhibitors typically incorporate transition state isosteres such as hydroxyethylamine (HEA) and multiple amide/sulphonamide moieties to ensure efficient binding to the hydrophilic enzyme active site. As a result, these peptidomimetic inhibitors are generally susceptible to P-glycoprotein (P-gp) efflux at the blood–brain–barrier (BBB). This prevents sufficient access to target brain tissue in which the majority of APP processing by BACE-1 takes place.[4] Our efforts to systematically replace amide and sulfonamide components with substituents compatible with CNS penetration, while maintaining acceptable potency, has resulted in several reports describing alternative CNS compatible replacements. The discovery of the oxadiazolyl tertiary carbinamine motif, replacing both the HEA and the P1 amide, was recently described and is illustrated by inhibitor **1**, shown in Figure 1.[5] Alternatively, the HEA could be simplified to a primary amine as exemplified by compound **2**.[6a,b] Furthermore, the benzyl amide-containing P3 group of inhibitor **1** could be replaced with a smaller methylcyclopropylmethylamino P3–P2 scaffold arrangement.[6c] Both inhibitors **1** and **2** retained good intrinsic potency (BACE-1 $IC_{50} = 12$ nM and 2 nM, respectively) and functional activity[7] in cells (sAPPβ_NF $IC_{50} =$ 65 nM and 71 nM, respectively) but remained P-gp substrates.[8] Assuming that the changes incorporated in compounds **1** and **2** may each have had a positive, though not yet measurable, effect on P-gp transport, we prepared hybrid **3**. While intrinsic potency was largely retained at 25 nM, functional activity suffered significantly (sAPPβ_NF $IC_{50} = 2{,}500$ nM). To our satisfaction, however, P-gp efflux susceptibility was greatly diminished (B-A/A-B ratio = 3.6, $P_{app} = 20 \times 10^{-6}$ cm s^{-1}). Previously published studies clearly indicated that both potency and P-gp susceptibility could be further improved with minimal structural modifications. For instance, "capping" of the P3 methylcyclopropylmethylamine NH with a methoxyethyl chain[6] improved both potency (BACE-1 $IC_{50} = 6$ nM) and P-gp susceptibility (B-A/A-B ratio = 1.6, $P_{app} = 25 \times 10^{-6}$ cm s^{-1}). However, functional activity (sAPPβ_NF $IC_{50} = 1{,}300$ nM) was still not considered sufficient to provide efficacy in vivo. In an effort to discover further potency enhancements, we turned to modeling studies to gain insight into the mode of binding of these inhibitors. In the prototypical oxadiazolyl tertiary carbinamine **1**, the oxadiazole is twisted 29° out of the plane of the phenyl scaffold in the bioactive conformation.[5] It was hypothesized that this rotation away from the ground state conformation was induced by a weak electrostatic interaction of the oxadiazole nitrogens with the flap region of the BACE-1 enzyme. Addition of a substituent on the phenyl scaffold, *ortho* to the oxadiazole, should favor a similar out of plane twist in the ground state conformation, therefore mimicking the bioactive conformation. Indeed, installation of a chloro substituent at the 3-position on the pyridine ring of compound **3** yielded inhibitor **5**, which displayed a 25-fold boost in intrinsic potency (BACE-1 $IC_{50} = 1$ nM) and moderate functional activity (sAPPβ_NF $IC_{50} = 146$ nM).[9] P-gp efflux susceptibility was slightly improved compared to com-

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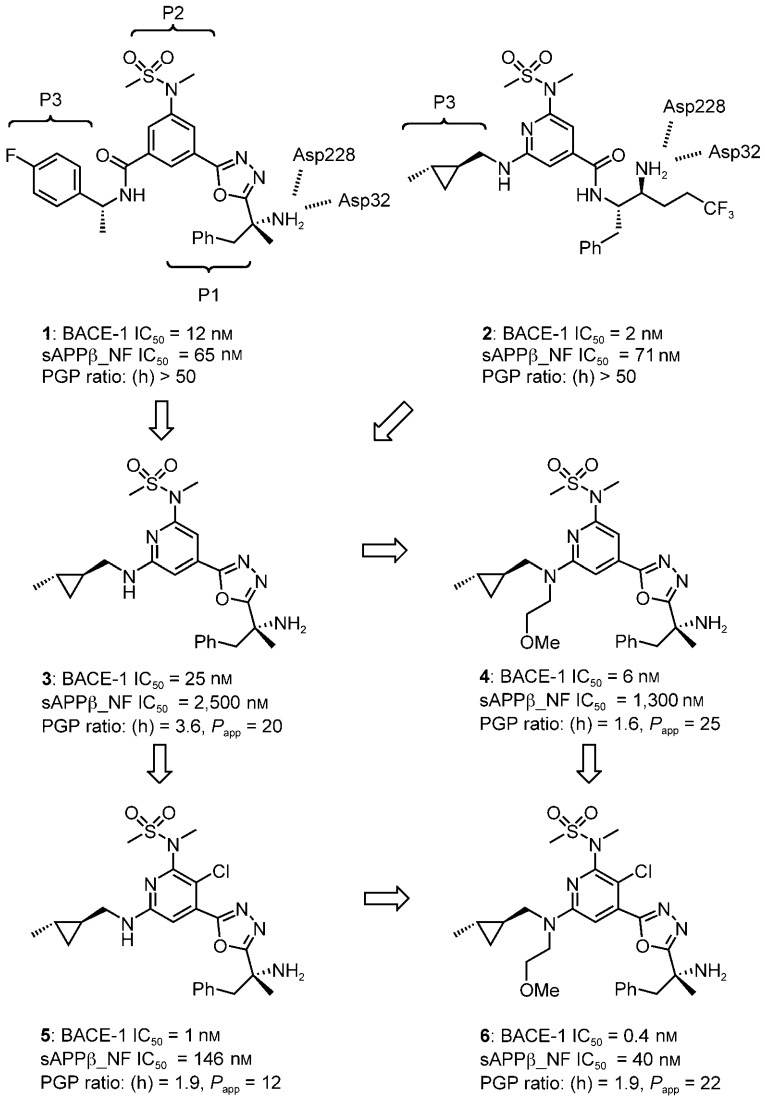


Figure 1. Evolution toward isonicotamide derived tertiary carbinamine inhibitors of BACE-1.

pound **3** (B-A/A-B ratio = 1.9, $P_{app} = 12 \times 10^{-6}$ cm s^{-1}), possibly the result of an inductive effect of the chloro group on the uncapped P3 NH. Combining beneficial modifications observed with compounds **4** and **5** in a single inhibitor led to the discovery of oxadiazolyl tertiary carbinamine **6**, a compound with remarkable intrinsic potency and good functional activity (BACE-1 $IC_{50} = 0.4$ nM; sAPPβ_NF $IC_{50} = 40$ nM), along with minimal P-gp susceptibility suggesting good potential for brain penetration (B-A/A-B ratio = 1.9, $P_{app} = 22 \times 10^{-6}$ cm s^{-1}).

An X-ray crystal structure of inhibitor **6** bound to the BACE-1 active site was obtained via the soaking exchange of a weak ligand (Figure 2).[10] The central tertiary carbinamine interacts with catalytic aspartates Asp 32 and Asp 228, as well as the Gly 230 residue, and the oxadiazole nitrogens appear to be within electrostatic interaction distance to the flap region Thr 72 and Gln 73, as observed earlier with inhibitor **1**.[5] The oxadiazole ring is now twisted out of the plane defined by the central aryl scaffold with a dihedral angle of approximately 40° (compared with 29° dihedral angle for inhibitor **1**), and the sulfonamide lies 70° out of the plane. Interestingly, the methoxy group terminating the P3 amine cap is within H-bonding distance of residue Gln 73; this may be responsible for the additional boost in potency (**3** vs **4** and **5** vs **6**). Although not depicted in Figure 2 for clarity reasons, the 10 s loop adopts the down conformation induced by the permitted Thr 232–Ser 10 interaction, as expected.[6c] Also not shown is the proximity of the 3-chloro group to Thr 72 and Arg 235.[9,10]

Tertiary carbinamine **6** was assessed in an acute brain penetration model to be only marginally able to penetrate the BBB (brain/plasma ratio = 9% in rats, 2 h post-20 mpk i.p. dosing in 25%DMSO/75% PEG300), despite its promising P-gp profile.[11] High plasma protein binding in rats, as well as dogs, monkeys and humans (free fraction < 3%), was assumed to be a strong limiting influence on acute brain penetration.[12] The question remained, however, as to whether sustained plasma exposure would allow for more efficient brain permeation over hours or days. Unfortunately, inhibitor **6** suffers from high clearance and poor oral bioavailability in multiple species ($Cl_{rat} = 58$ mL min^{-1} kg^{-1}, $Cl_{dog} = 26$ mL min^{-1} kg^{-1}, $Cl_{rhesus} = 24$ mL min^{-1} kg^{-1}; < 2% F in all three species) as a result of extensive first pass metabolism, implying that sustained exposure would require continuous i.v. infusion. Metabolism studies indicated that inhibitor **6** was extensively oxidized by CYP 3A4. Following a strategy originally developed in the HIV pro-

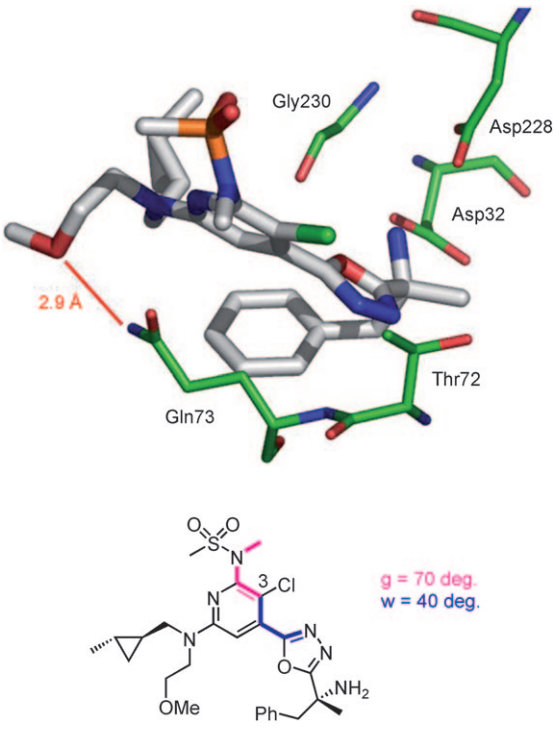


Figure 2. X-ray structure of the BACE-1–inhibitor **6** complex (PDB code: 2NTR), and the chemical structure of inhibitor **6** showing the dihedral angles.

tease field,[13] compound **6** was co-dosed with the CYP 3A4 inhibitor ritonavir (10 mpk p.o., for both ritonavir and compound **6**) resulting in 83% oral bioavailability in rhesus and sustained plasma exposure upon p. o. dosing. The effect was less pronounced in dogs or rats.

The implantation of a permanent port in the cisterna magna of rhesus monkeys[14a,b] provides a convenient access to the CSF compartment, allowing the assessment of both inhibitor and biomarker concentrations in the CNS.[14c] In a multiple dose oral cross-over study, tertiary carbinamine **6** was administered twice daily (p.o., 15 mpk), two hours after ritonavir (p.o., 10 mpk), for 3.5 days in rhesus monkeys ($n=8$).[15] The peak concentration of inhibitor **6** averaged over the course of the experiment was 4.7 μM in plasma and 35 nM in CSF, ~35% of the calculated unbound fraction in plasma.[16] A significant and sustained reduction of CSF $A\beta_{42}$ was observed throughout the treatment period (Figure 3), averaging 47% at peak and 42% for all time points ($p<0.001$, pairwise t-test, $n=8$ animals). The reduction persisted at trough concentrations, which ranged from 0.7 to 1.9 μM in plasma and averaged 25 nM in CSF.

The synthesis[17] of tertiary carbinamine **6** is centered around the coupling of the sulfonamide and cyclopropylmethylamine moieties to a central isonicotinate core followed by elaboration of the oxadiazole ring from the carboxylic acid functionality (Scheme 1). Amine **7** was prepared from *trans*-2-methylcyclopropane carboxylic acid via benzylamine coupling, subsequent reduction and chiral separation.[18] The methoxyethyl cap was added using the same methodology to give amine **8**. Mesylmethylamine was coupled to methyl-2,6-dichloroisonicotinate using Pd_2dba_3 and Xantphos to afford acid **9** after hydrolysis of the corresponding methyl ester. At this point a second Pd^0 mediated coupling of amine **8** to aryl chloride **9** provided intermediate **10**. Sequential coupling of Boc-hydrazine (followed by deprotection) and *N*-Boc-α-methyl-phenylalanine yielded the corresponding bis-amide. Cyclodehydration to construct the oxadiazole moiety, followed by Boc removal and chlorination[19] of the aryl ring completed the synthesis of tertiary carbinamine **6**.

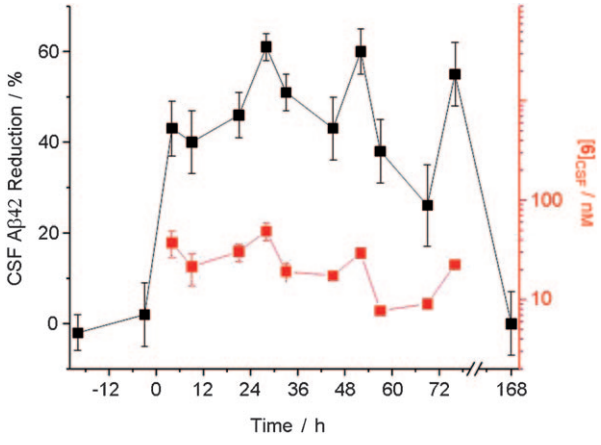
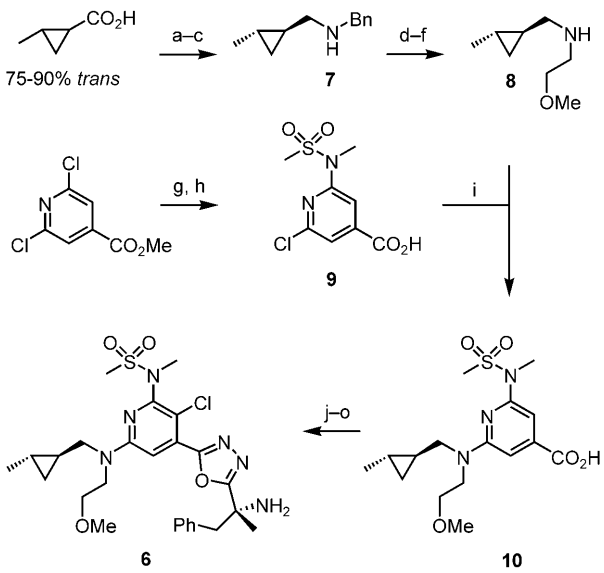


Figure 3. CSF $A\beta_{42}$ reduction (baseline normalized, left Y-axis, ■) and $[\mathbf{6}]_{CSF}$ (right Y-axis, ■) in rhesus monkeys, after twice daily treatment with compound **6** (p.o., 15 mpk), two hours following ritonavir treatment (p.o., 10 mpk), for three and a half days in a full crossover study design. Samples were collected at baseline (−20, −1 h) prior to first dose, at peak time point (+4 h) after morning dosing, and finally at recovery, 96 h after the last dose.



Scheme 1. Synthesis of tertiary carbinamine inhibitors. *Reagents and conditions*: a) benzylamine, EDC, DCM, recrystallization from EtOAc/hexane, 50%; b) chiral separation: Chiralpak OD; c) BH_3-THF, THF, 100%; d) methoxyacetic acid, EDC, HOAt, Hunig's base, DMF, 91%; e) BH_3·THF, THF, 50 °C; f) H_2, $Pd(OH)_2$/C, EtOH, 93% (2 steps); g) $MeNHSO_2Me$, Pd_2dba_3, Xantphos, K_3PO_4, dioxane, 73%; h) 1N NaOH, THF, MeOH 99%; i) $Pd(PtBu_3)_2$, K_3PO_4, DMA; j) (Boc-hydrazine, EDC, HOAt, DCM, 77% (2 steps); k) $HCl_{(g)}$, DCM, 100%; l) *N*-Boc-D-α-Me-Phe, EDC, HOAt, Hunig's base, DMF, 90%; m) Ph_3P, CBr_4, imidazole, DCM, 94%; n) TFA, DCM; o) NCS, DCM, 3–3.5:1 regioisomeric mix, preparative HPLC, 50–60% (2 steps).

In summary, incorporation of an isonicotinic core containing a P3 methylcyclopropyl group into our previously reported oxadiazolyl tertiary carbinamine based BACE-1 inhibitors has led to the identification of the exquisitely potent inhibitor **6**, which displays good permeability and low susceptibility to the P-gp efflux pump. Upon co-dosing with CYP 3A4 inhibitor ritonavir, tertiary carbinamine 6 was found to be orally bioavailable. Following twice daily oral administration to monkeys, it was shown to penetrate the CNS and lower $A\beta_{42}$ levels in the CSF by >40% over the course of a 3 day experiment. Further structural improvements aimed at optimization of pharmacokinetics and brain penetration will be the subject of future work, as will studies of the impact of central BACE-1 inhibitor-mediated $A\beta_{42}$ lowering on cognition.

Experimental Section

The Supporting Information contains full experimental details for the synthesis of the BACE-1 inhibitors outlined in Scheme 1. Details of the animal studies conducted can also be found in the Supporting Information.

All animal procedures were done in accordance with guidelines from the Institutional Animal Care and Use Committee at Merck.

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Keywords: alzheimer's disease · aβ reduction · inhibitors · BACE-1 · β-secretase

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[19] Conditions were carefully optimized to yield full conversion and a 3–3.5:1 ratio of chlorination between sulphonamide and oxadiazole moieties (as desired) as opposed to between sulphonamide and cyclopropylmethylamine moieties. The isomers were separated by reverse phase HPLC (Waters Sunfire C18 OBD 5 μm, 30×150 mm, multiple 0.7 mL injections at 320 mg mL^{-1} in DMF; CH_3CN/water 35 mL min^{-1} gradient (with 0.1% TFA modifier): 5–30% over 2 min, 30–45% over 8 min, ramp to 100% over 1 min and wash with 100% over 3 min).

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