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Rapid P1 SAR of brain penetrant tertiary carbinamine derived BACE inhibitors

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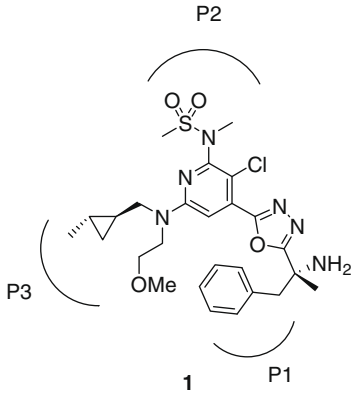
ABSTRACT

This Letter describes the one pot synthesis of tertiary carbinamine **3** and related analogs of brain penetrant BACE-1 inhibitors via the alkylation of the Schiff base intermediate **2**. The methodology developed for this study provided a convenient and rapid means to explore the P1 region of these types of inhibitors, where the P1 group is installed in the final step using a one-pot two-step protocol. Further SAR studies led to the identification of **10** which is twofold more potent in vitro as compared to the lead compound. This inhibitor was characterized in a cisterna magna ported rhesus monkey model, where significant lowering of CSF Aβ40 was observed.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder initially manifested by memory loss. Progression of the disease leads to behavior and personality changes and ultimately death as no true disease modifying therapy currently exists. The implications of AD in terms of the financial and emotional burden of caring for affected patients is immense.[1] According to the amyloid cascade hypothesis, the deposition of amyloid β-peptide (Aβ) in the brain is one of the characteristics of AD pathogenesis.[2] Aβ is formed by sequential processing of amyloid precursor protein (APP) by two aspartyl proteases, β-secretase (BACE-1, β-site APP Cleaving Enzyme) followed by γ-secretase. BACE-1 knockout mice show a complete absence of Aβ production but are otherwise similar to wild type animals. BACE-1 is therefore hypothesized to be an attractive therapeutic target for the treatment of AD.[3]

The development of brain penetrant BACE-1 inhibitors has been extremely challenging.[4] Recently, the discovery of a tertiary carbinamine derived BACE-1 inhibitor was disclosed.[5] This compound, represented by structure **1** (Fig. 1), is a brain penetrant BACE-1 inhibitor with excellent in vitro (IP[6] = 5 nM at pH 6.5)[7] and cell-based potencies (IC_{50} = 40 nM). Significant pharmacokinetic liabilities were associated with **1**, such as high clearance and poor oral bioavailability in multiple species due to extensive first pass



1

BACE-1 IP (pH 4.5) = 0.4 nM
BACE-1 IP (pH 6.5) = 5 nM
sAPP_NF IC_{50} = 46 ± 22 nM
PGP ratio: (h) = 1.9, (m) = 2.3, Papp = 22
Brain/plasma ratio (i.p. 30 mpk): 9%
Log P > 3.2
PB > 98%

Figure 1. Profile of BACE-1 inhibitor **1**.

Abbreviations: IP, inflection point; sAPP_NF, cell based assay utilizing NF mutation for sAPP. See Ref. 15; PGP, P-glycoprotein; Papp, apparent permeability value; PB, protein binding; IP, dosing: intraperitoneal dosing; Mpk, milligrams of compound per kilogram of animal; BLQ, below limit of quantitation.

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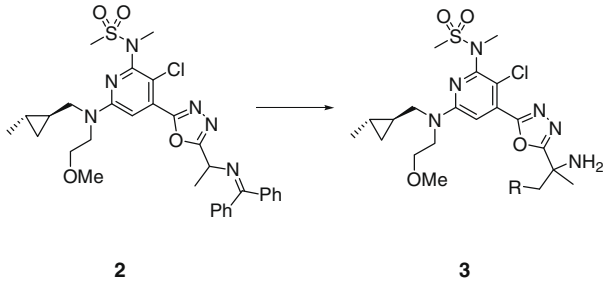
metabolism. Nevertheless, upon IP dosing in transgenic mice at high doses, reduction of brain Aβ levels was observed. Upon co-dosing with ritonavir, a CYP3A4 inhibitor widely used to improve the pharmacokinetics of HIV protease inhibitors,[8] twice daily dosing with **1** (15 mpk) reduced CSF Aβ levels by ~40% in cisterna magna ported (CMP) rhesus monkeys. This data provided, to the best of our knowledge, the first example of Aβ lowering upon oral dosing of a BACE-1 inhibitor in non-human primates.

In a continuation of our efforts to discover an improved BACE-1 inhibitor, we sought to improve upon the profile of **1**. The co-crystal structure of **1** with the human BACE-1 enzyme was successfully obtained,[5] revealing that the inhibitor occupied S1-S3 sites as had been observed for other inhibitors derived from this series. The benzyl group occupied the S1 pocket, and we wished to explore the feasibility of improving compound potency via optimization the P1 substituent.

The oxadiazole moiety of **1** was derived from the bridging of two carboxylic acids with hydrazine, followed by dehydration. In particular, the right hand tertiary carbinamine fragment is derived from an α,α-disubstituted amino acid. These types of building blocks are not readily available from commercial sources, necessitating custom synthesis. While accessing α,α-disubstituted amino acids via Schiff base alkylation of α-amino esters is well precedented,[9] the rapid synthesis of P1 variants was precluded due to its multistep nature. Given that oxadiazoles are ester mimetics, we felt that the alkylation of an α-amino oxadiazole Schiff base, though unprecedented, could provide a viable late stage P1 variation strategy (Scheme 1). Given the convenience offered by this approach, we pursued the synthesis of key Schiff base intermediate **2** as shown in Scheme 2.

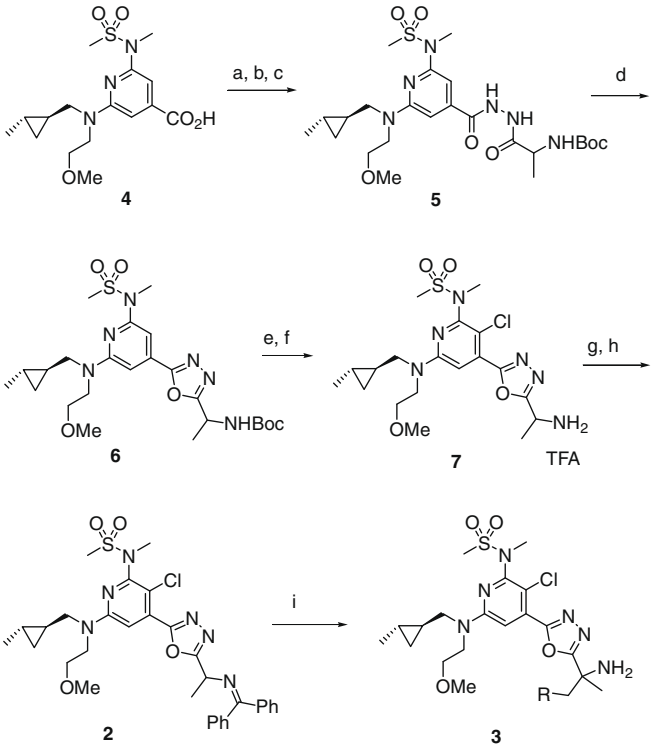
The synthesis was initiated by sequential EDC coupling of isonicotinic acid **4**[5] with Boc-hydrazine, deprotection, then coupling with Boc-DL-Ala-OH to afford the diacylhydrazide **5**. Cyclodehydration with Ph_3P, CBr_4 and imidazole provided desired oxadiazole **6**.[10] Deprotection of the Boc group required mildly acidic TFA conditions to prevent unraveling of the oxadiazole. Chlorination of the amine trifluroacetate salt with NCS gave a 3:1 mixture of regioisomers favoring the desired product which was separable via preparative HPLC. As an interesting aside, we consistently observed that the rate of chlorination was faster when the hydrochloride salt was utilized, at the cost of lower regioselectivity and diminished yield due to oxadiazole hydrolysis. Conversely, the transformation of **7** to the desired Schiff base **2** necessitated the utilization of the HCl salt of **7** to achieve full conversion, presumably due to the insoluble nature of ammonium chloride in dichloromethane driving the equilibrium toward the desired product.

With intermediate **2** in hand, we wished to examine the feasibility of the key alkylation reaction. The optimal conditions that were discovered involved the deprotonation of **2** in DMF with LiHMDS, followed by addition of the alkylating agent. Once the reaction was complete, the addition of a few drops of 6 N HCl achieved the removal of the Schiff base in the same pot. Purification of the reaction mixture via preparative HPLC gave the desired product as a mixture of diastereomers at the α-amino acid center.[11] The diastereomeric mixtures could be readily resolved via chiral chromatography for analogs of interest.[12] The abovementioned alkylation reaction proved to be robust, where desired analogs were obtained with various substituted benzyl halides (Table 1) as well as heteroaryl substituted halo methane derivatives and alkyl halides.



2 **3**

Scheme 1. P1 scan strategy.



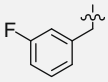
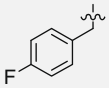
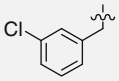
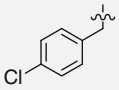
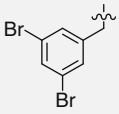
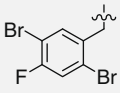
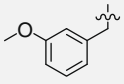
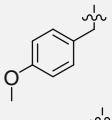
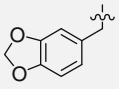
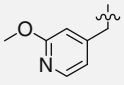
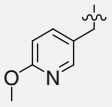
4 **5** **6** **7** **2** **3**

Scheme 2. Synthesis of tertiary carbinamine derived BACE inhibitors. Reagents: (a) Boc-hydrazine, EDC, HOAt, DMF, 72%; (b) HCl, EtOAc, 100%; (c) Boc-DL-Ala-OH, EDC, HOAt, Hunig's base, DMF, 81%; (d) Ph_3P, CBr_4, imidazole, DCM, 74%; (e) TFA, DCM, 100%; (f) NCS, DCM; (g) HCl, DCM, 58% (2 steps); (h) benzophenone imine, DCM, 84%; (i) LiHMDS, RCH_2Br or RCH_2Cl, THF, then aq 6 N HCl.

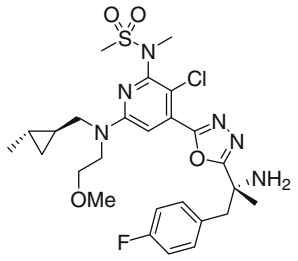
In general, SAR trends revealed that substitution at the para position was better tolerated than at either the ortho or meta positions. In fact, the incorporation of a 4-fluoro substituent gave compound **10**,[13] with a twofold improvement in both our in vitro and cell based assays as compared to **1**. However, a substituent larger than fluorine at the para position lead to a large potency loss compared to reference compound **1**. A variety of heteroaryl groups and cyclopropyl derived phenyl replacements were also screened, but these transformations universally led to large losses of potency. Unfortunately no improvement in the intrinsic metabolic clearance was observed for the fluorinated compound **10** (Fig. 2).

As compound **10** represented a twofold improvement for in vitro potency over our lead compound **1**, we wondered how this comparison would translate in vivo. In particular, we hoped that in the established CMP rhesus monkey model,[14] we would observe enhanced reduction of CSF Aβ levels at the same dose, or similar reduction of the biomarker at a lower dose. The rhesus pharmacokinetics of compound **10** when co-administered with ritonavir were determined in a satellite experiment ($n = 3$ rhesus). Even though the profile for compound **1** was determined previously, the experiment was repeated, and the plasma levels at 4 h and 24 h are tabulated below (Table 2). Given the small sampling size, we were faced with large standard deviations for the averages of plasma levels at a given timepoint.

Table 1
Summary of in vitro data for P1 analogs

Compound	R	BACE-1 IP (nM)	sAPP_NF[15] IP (nM)
1		5	46 ± 22
8		15*	195*
9		17*	304*
10		*2**	*15 ± 6**
11		340*	>6700*
12		65*	4315*
13		4*	320*
14		>600,000*	
15		29,000	
16		3500	
17		1500	
18		180	4300
19		119	1100
20		9900	
21		1400	
22		375	3186
23		142	1934
24		49,000	
25		1300	>2200

* Racemate was separated by chiral HPLC. Data showed is of the active enantiomer.



10

BACE-1 IP = 2 nM
sAPP_NF IC_{50} = 15 ± 6 nM
PGP ratio: (h) = 1.1, (m) = 1.9, Papp = 20
Prot. Bind 97.8%

Figure 2. Profile of BACE-1 inhibitor **10**.

Table 2
Rhesus pharmacokinetics for compounds **1** and **10** when co-administered with 10 mpk ritonavir (RTV)

Dose (+10 mpk RTV)	$(Plasma)_{4\ h}$ (μM)	$(Plasma)_{24\ h}$ (μM)
15 mpk **1**	3.36 ± 1.95	0.10 ± 0.09
5 mpk **10**	1.31 ± 0.88	BLQ
15 mpk **10**	2.34 ± 1.96	0.03 ± 0.02s

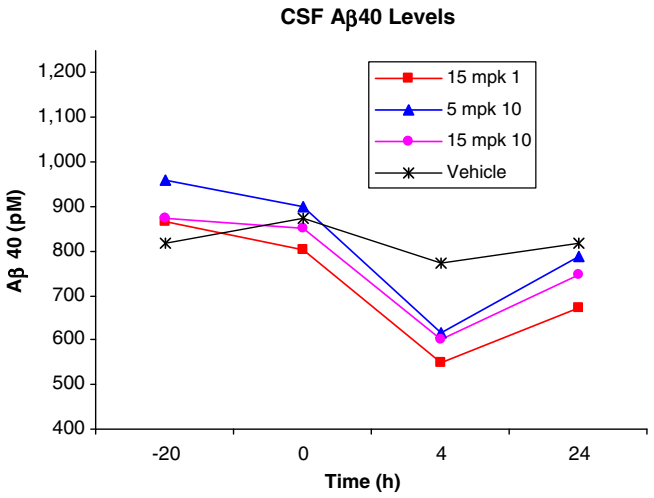


Figure 3. CSF Aβ40 reduction in rhesus monkeys.

In order to compare the pharmacodynamics of the two analogs, a four-way single dose crossover experiment was designed. Both plasma and CSF were sampled at time points −20 (predose), 0, 4 and 24 h and analyzed for peptides related to the amyloid cascade (Fig. 3), Gratifyingly, significant reductions of Aβ40 (~30%) were observed in the CSF at 4 h in all three treated groups. Surprisingly, the reduction of this biomarker at a 5 mpk dose of **10** was comparable to the 15 mpk dose **1**. However, increasing the dose of **10** to 15 mpk did not result in enhanced Aβ40 at the 4 h timepoint. Also observed were decreases in Aβ42 and sAPPβ concentrations, which are consistent with the inhibition of BACE-1.[14c] At the second and final timepoint of 24 h, more robust Aβ40 reduction was observed when treated with 15 mpk of compound **1**, which could be attributed to its superior pharmacokinetics when co-administered with ritonavir. Overall, the pharmacodynamics of compound **10** were not meaningfully different from **1** despite the in vitro potency improvement.

In summary, we developed a one pot procedure for the facile synthesis of P1 analogs of active BACE-1 inhibitor **1** via late stage Schiff base alkylation. A more potent inhibitor of the enzyme was identified from this effort and its efficacy in the rhesus CSF Aβ lowering was evaluated. Unfortunately, the modest improvement of in vitro potency did not result in superior pharmacodynamic response for compound **10**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.005.

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6. See abbreviations.
7. It is more predictive of the measured cellular potency if the enzymatic BACE-1 assay is conducted at PH 6.5 rather than pH 4.5. The data from our study suggests that APP cleavage is occurring in less acidic cellular components than previously theorized. For additional information see: Stachel, S. J.; Coburn, C. A.; Rush, D.; Jones, K. L. G.; Zhu, H.; Rajapakse, H. A.; Graham, S. L.; Simon, A.; Holloway, M. K.; Allison, T. J.; Munshi, S. K.; Espeseth, A. S.; Zuck, P.; Colussi, D.; Wolfe, A.; Pietrak, B. L.; Lai, M.-T.; Vacca, J. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2977.
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11. The purities of the target compounds and the enantiomeric excess for compounds that were separated by chiral chromatography were greater than 97%. For experimental details, see Supplementary data.
12. The diastereomers resulting from this alkylation could be separated on a ChiralPack AD, gradient from 90/10 to 60/40 hexane/*i*PrOH (hexane modified w/0.1% DEA).
13. The stereochemistry of the active diastereomer is known to always be R at the tertiary carbinamine center based on earlier X-ray crystallographic analysis of tertiary carbinamine inhibitors Refs. 5,9
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