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BACE-1 inhibition by a series of ψ[CH_2NH] reduced amide isosteres

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Abstract—A series of β-site amyloid precursor protein cleaving enzyme (BACE-1) inhibitors containing a ψ(CH_2NH) reduced amide bond were synthesized. Incorporation of this reduced amide isostere as a non-cleavable peptide surrogate afforded inhibitors possessing low nanomolar potencies in both an enzymatic and cell-based assay.

Alzheimer's disease (AD) is a devastating neurodegenerative disease that accounts for the majority of dementia diagnosed in the elderly. AD is characterized clinically by a slow and progressive decline in cognitive function that inevitably leads to incapacitation and death.

Current AD therapy relies primarily on palliative treatment that can temporarily alleviate the symptoms but fails to provide disease modifying amelioration. The predominant hypothesis that guides current research into disease modifying therapies is based on the amyloid cascade.[1] This hypothesis states that the gradual and chronic imbalance between the production and clearance of Aβ peptides results in their accumulation in the brain. These secreted peptides, known as $A\beta_{40}$ and $A\beta_{42}$, are the result of the proteolytic processing of the amyloid precursor protein (APP) by two enzymes, β- and γ-secretase. Once formed, the Aβ monomers can aggregate and form neurotoxic oligomers that disrupt neuronal function and lead to cell death and memory loss that is the phenotype of AD.[2]

β-Secretase (BACE-1) is an aspartyl protease that functions as the rate limiting step in the generation of these Aβ peptide fragments. Given the central role of Aβ in AD pathology, as well as the rich history in the field of aspartic acid protease inhibition, there has been much support for the pursuit of BACE-1 as a target for AD therapy.[3]

Most of the inhibitors of aspartyl proteases (e.g., HIV protease, renin, plasmepsin, and cathepsin D) that have been reported in the literature contain a transition-state isostere as the key binding element. In a similar fashion, the majority of the BACE-1 inhibitors that have appeared thus far are peptide-based analogs of the enzyme substrate that replace the scissile amide bond with a non-cleavable isostere. Typically the aspartate-bound water molecule responsible for peptide cleavage is mimicked by an alcohol or amine and is contained within the framework of an hydroxyethylamine (HEA), hydroxyethylene (HE), statine, or norstatine pseudopeptide backbone[4] (Fig. 1).

The incorporation of a ψ[CH_2NH] peptide bond isostere into various peptide substrates is well documented and has produced many important compounds for structure activity investigations. In the context of aspartyl

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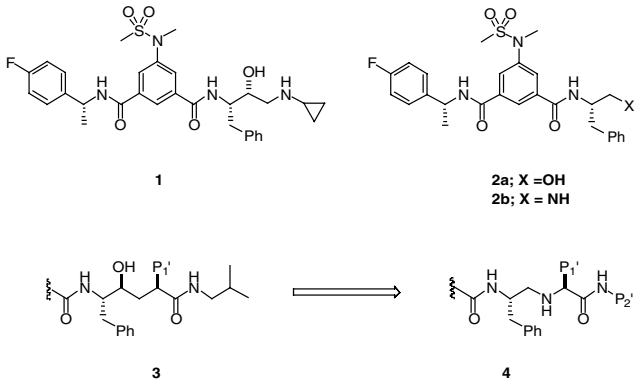


Figure 1. Structures of BACE inhibitors.

protease inhibition, the concept of the reduced amide isostere was first exploited by Szelke et al. to generate potent inhibitors of human renin.[5] X-ray crystallographic analysis showed that these inhibitors interacted with the enzyme in a manner similar to their statine and HE analogs.[6] Since then, this isostere has been incorporated into the skeleton of inhibitors of other aspartyl proteases, such as rhizopuspepsin,[7] Sap2[8] and HIV protease.[9]

In the course of our research to develop inhibitors of β-secretase, we investigated utilizing the reduced amide isostere as a key binding element. Previously we reported that HEA inhibitors such as **1**, when incorporated into an N-terminal isophthalamide scaffold, were potent inhibitors of β-secretase.[10] The 1,3,5-trisubstituted aromatic scaffold used in this paradigm was designed to function as a P_3–P_1 Val-Asn-Phe peptidomimetic of an optimized β-secretase cleavage site of APP.[11] In those examples there was a two pronged engagement of the catalytic dyad upon binding with the hydroxyl and secondary amino groups and further binding energy was gained as the cyclopropyl group filled the S_1' binding pocket.

To understand the limits of engaging the catalytic dyad, compound **2a**, which was devoid of any prime-side binding elements, was synthesized. While a potency loss of 60-fold (IC_{50} = 630 nM) was observed from truncation to the terminal primary alcohol, it was noteworthy that this small molecule inhibitor displayed even moderate activity since it occupied only three sub-sites of the BACE-1 enzyme. More discouraging was that an additional 10-fold loss in potency was observed when the hydroxyl group was substituted with a primary amine (**2b**; IC_{50} = 5.9 μM). A similar loss in potency was observed when amino statine isosteres were compared to the corresponding statine-derived inhibitors of BACE-1.[12] In an effort to regain this precipitous loss in potency, we targeted the alkylation of the amine for extension toward the prime-side of the enzyme.

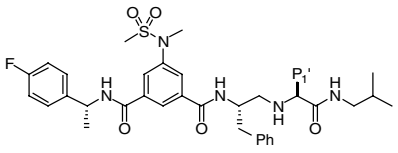
Early work in our laboratory detailed the SAR of a series of micromolar, statine-derived BACE-1 inhibitors **3**.[13] As such, we evaluated the elaboration of amine **2b** with the prime-side binding elements contained in **3** and produced a ψ[CH_2NH] reduced amide inhibitor **4a** (P_1' = H and P_2' = ^{i}Bu), that showed potent activity against BACE-1 in our enzymatic assay (IC_{50} = 117 nM, Table 1). Although quite potent in the enzyme assay, inhibitor **4a** was less effective in the cell culture assay that measured the production of a secreted soluble *N*-terminal fragment of an APP variant from cultured HEK 293T cells (sAPPβ_NF; IC_{50} = 3.7 μM).[14]

In order to optimize activity in both assays, we delineated SAR at the P_1' region of the inhibitor and found that the addition of either small alkyl groups (**4b–d**) or longer hydrophilic substituents (**4e**,**f**) led to inhibitors with enhanced enzymatic potencies. Compounds that incorporated a small alkyl group at P_1' also resulted in a dramatic increase in cellular potency and produced inhibitors with IC_{50} values ranging from 17 to 22 nM. While the more polar substituents maintained enzymatic activity, this modification resulted in diminished cell-based activity, and can most likely be attributed to the lower cell permeability of these compounds. All of the reduced amide derivatives showed some selectivity over the homologous enzyme BACE-2 (5- to 10-fold) and the IC_{50} values versus human renin were greater than 20 μM.

Molecular modeling of compound **4b** docked into the BACE-1 active site revealed that the terminal amide accessed the S_2' binding pocket. As such, we explored the SAR in this region in order to optimize activity (Table 2). We found that small alkyl, cycloalkyl and benzyl groups were tolerated but only at the expense of both cellular and enzymatic potency (**4g–i**). Also apparent was a strict requirement for the presence of a secondary NH group (cf. **4j**) that functioned to properly align the hydrogen bonding network between the enzyme and the inhibitor.

The chemistry employed for the preparation of reduced amide analogs **4** is detailed in Scheme 1. The synthesis relied on the BOP-mediated coupling reaction between carboxylic acid **8**[10] and penultimate diamine **7**.

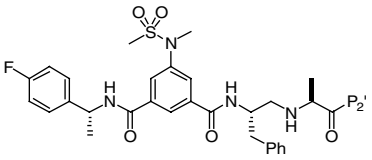
Table 1. BACE-1 enzyme and cell inhibition for P_1' derivatives



Compound	P_1'	BACE-1 IC_{50}[a] (nM)	sAPPβ_NF IC_{50}[a] (nM)
4a	H	117	3710
4b	Me	8	22
4c	Et	7	20
4d	*n*-Pr	4	17
4e	$-CH_2CH_2OH$	13	751
4f	$-CH_2CH_2SO_2Me$	13	17,500

[a] n = 3.

Table 2. BACE-1 enzyme and cell inhibition for P_2' analogs



Compound	P_2'	BACE-1 IC_{50}[a] (nM)	sAPPβ_NF IC_{50}[a] (nM)
4g	NHEt	52	242
4h	NHc-Pr	24	133
4i	NHBn	139	731
4j	Proline	17,200	>20,000

[a] $n = 3$.

Intermediate **7** was synthesized by an amide coupling reaction of isobutylamine to *N*-Boc-L-alanine. Deprotection with HCl gas and reductive amination of the functionalized α-amino amide (**6**) with commercially available *N*-Boc-L-Phe aldehyde afforded a mono-protected diamine precursor. Exposure of this intermediate to HCl in EtOAc afforded the dihydrochloride salt **7**. Coupling to benzoic acid **8** afforded the final compounds in good overall yield.

Owing to our success in obtaining a co-crystal structure of HEA inhibitor **1**, we were able to acquire an X-ray structure of **4b** at 1.8 Å resolution.[15] When compared to compound **1**, both inhibitors were found to occupy the same general space in the active site with a few key differences (Fig. 2).

In both series, the α-methylbenzamide and Phe-derived P_1 groups occupy the S_3 and S_1 binding pockets, respectively and the SAR is conserved between the two structural classes. In addition there are hydrogen bonding interactions between both of the P_1 and P_3 benzamide NH groups and the carbonyl of Gly230 (Fig. 3).

The sulfonamide group present on the aromatic scaffold of inhibitor **4b** occupies the S_2 site in a manner consistent with that of compound **1**. Each sulfonamide oxygen makes important hydrogen bonding interactions with the enzyme; one with the backbone NH of Asn233, and the other with the OH of Ser325.

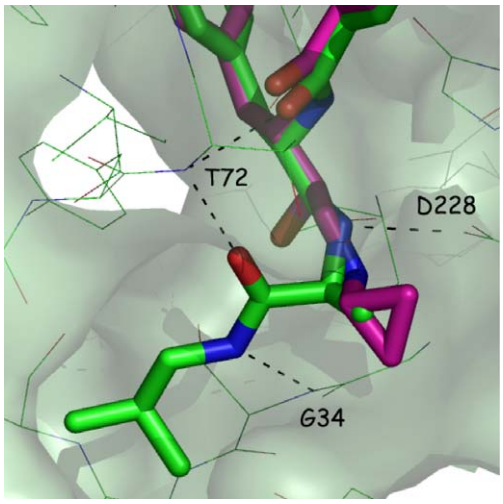


Figure 2. Overlay of HEA inhibitor **1** and reduced amide **4b**.

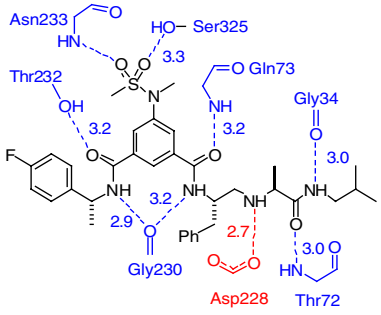
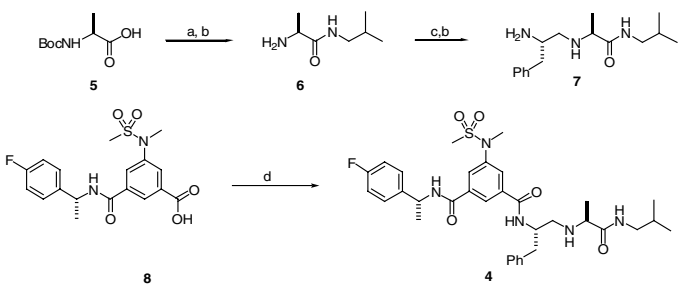


Figure 3. Hydrogen bonding network of inhibitor **4b**.

The amide bond of the reduced amide subunit is involved in two key interactions with the flap of the enzyme that provided added potency. Namely, there is one interaction between the Gln73 NH (3.2 Å) and the P_1 carbonyl and a second between the P2′ isobutyl amide carbonyl and Thr72 NH (3.0 Å) of BACE-1. The carbonyl of Gly34 is in close proximity to the isobutyl amide NH (3.0 Å).

Further analysis of the crystal structure of inhibitor **4b** in complex with BACE-1 revealed that unlike the mode of binding of typical transition state isosteres, where the hydroxyl and/or amino group is in close contact with both catalytic aspartates, the nitrogen atom at the scissile bond of reduced amide **4b** shared a hydrogen bond



Scheme 1. Reagents: (a) isobutylamine, BOP reagent, DIPEA, DCM, 88%; (b) $HCl_{(g)}$, EtOAc, 99%; (c) *N*-Boc-L-Phe-CHO, $NaBH_3CN$, MeOH, 74%; (d) BOP reagent, amine **7**, DIPEA, DMF, 78%.

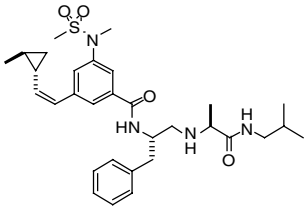


Figure 4. Reduced amide inhibitor **9**.

with only half of the dyad, specifically Asp228. This observation is consistent with the known binding properties of ψ[CH_2NH]pseudopeptides to aspartic acid proeteases.[7–9]

Cognizant of the fact that this motif introduced another amide bond into the framework of the inhibitor, we incorporated the reduced amide binding element into one of our previously described non-amide P_3 analogs.[16] Gratifyingly, we substituted the P_3 benzamide ligand of inhibitor **4b** with the isosteric *cis* vinylcyclopropane moiety that also contained a *trans* methyl substituent (Fig. 4). As such, we produced inhibitor **9** that was equipotent in both the enzyme and cell-based assays (BACE-1; IC_{50} = 20 nM; sAPPβ_NF; IC_{50} = 23 nM). This modification also improved the cell permeability of the series ($P_{app} = 24 \times 10^{-6}$ cm/s) when compared to either HEA inhibitor **1** ($P_{app} = 0.6 \times 10^{-6}$ cm/s) or reduced amide **4b** ($P_{app} = 10 \times 10^{-6}$ cm/s). However, both compounds were susceptible to P-glycoprotein transport.

The reduced amide isostere represents a new application of an established pharmacophore in the field of aspartyl protease inhibition and an alternative structural class of BACE-1 inhibitors. The incorporation of the 5-substituted isophthalamide scaffold at the N-terminal site of this isostere resulted in potent compounds that display impressive cellular IC_{50} values. Further modification of this general template has paved the way toward more potent and efficacious inhibitors with improved physical properties. Additional results from these modifications will be forthcoming.

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