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A conformational constraint improves a β-secretase inhibitor but for an unexpected reason

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ABSTRACT

During our ongoing efforts to develop a small molecule inhibitor targeting the β-amyloid cleaving enzyme (BACE-1), we discovered a class of compounds bearing an aminoimidazole motif. Initial optimization led to potent compounds that have high Pgp efflux ratios. Crystal structure-aided design furnished conformationally constrained compounds that are both potent and have relatively low Pgp efflux ratios. Computational studies performed after these optimizations suggest that the introduction of the constraint enhances potency via additional hydrophobic interactions rather than conformational restriction.

Alzheimer's disease (AD) is the most common cause of dementia and is expected to become a more prevalent problem in the aging human population.[1] β-amyloid precursor protein cleaving enzyme (BACE-1) plays an integral role in the formation of β-amyloid (Aβ) peptides, which subsequently form plaques in the brain.[2] Thus, considerable effort has been directed towards developing a small molecule inhibitor of BACE-1, with the hopes of stopping or reversing AD progression.[3] However, progress has been hampered by the requirement that an inhibitor of BACE-1 be both potent and brain penetrant.

Our research laboratories have disclosed inhibitors bearing aminothiazole and aminoimidazole cores.[4,5] These compounds are inhibitors of BACE-1; however, they also have relatively high Pgp efflux ratios (BA/AB) and are not anticipated to be brain penetrant. Herein, we discuss the further development of these aminoheterocyclic BACE-1 inhibitors, focusing on simultaneously enhancing potency and maintaining or lowered Pgp efflux. Of particular interest is the introduction of a conformational constraint inspired by a crystal structure. Introduction of conformational rigidity is a standard strategy in drug discovery and we anticipated this modification would lead to a significant improvement in potency over comparable unconstrained analogs.[6] Interestingly this was only true for certain substitution patterns; furthermore, computational studies suggest that the constraint furnishes more potent compounds by the introduction of additional hydrophobic binding interactions rather than conformational restriction.

Our initial foray into aminoheterocyclic BACE-1 inhibitors has been previously described. Optimization of an aminothiazole HTS hit led to aminoimidazole **1**, a potent compound with a high Pgp efflux ratio (IC_{50} = 0.47 μM; Pgp (BA/AB) = 20). Concurrent with the discovery of inhibitor **1**, we synthesized aminoimidazole **2**, a less potent compound with a lower Pgp efflux ratio (IC_{50} = 1.8 μM; Pgp (BA/AB) = 4.0) (Fig. 1). Of the many derivatives examined, the 2-methoxy-5-nitro substituents on the benzyl subunit were found

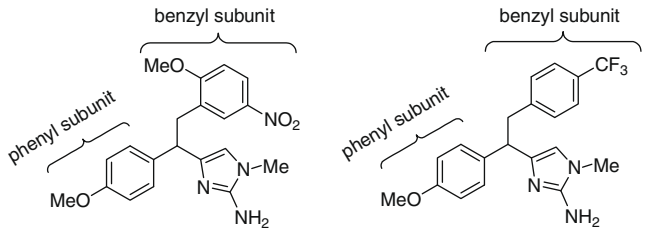

Figure 1. Compound **1** is potent but a substrate for Pgp efflux. Aminoimidazole **2** is less potent but has a lower Pgp efflux ratio.

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to furnish the most potent aminoimidazole inhibitor. However, we also believed that these functional groups were in part responsible for our high Pgp efflux ratio and we sought to prepare potent inhibitors without this substitution pattern.[7] While inhibitor **2** has a significantly attenuated Pgp efflux ratio compared to compound **1**, we believed to move forward the potency of compound **2** would need to be improved. Therefore, we attempted to further optimize this scaffold, hoping to improve potency with substituents that would provide BACE-1 inhibitors with relatively low Pgp efflux.

We were aided in this endeavor by the X-ray crystal structure of inhibitor **1** bound to the BACE-1 active site (Fig. 2). Two crucial observations were made: (1) the bioactive conformation of **1** places the hydrogen atom of the central tertiary carbon in close proximity of an *ortho* hydrogen on 4-methoxy phenyl subunit's aromatic ring and; (2) there appears to be sufficient room in the active site to accommodate additional structural modification to the inhibitor.

We hypothesized that the introduction of an appropriate conformational constraint would lead to a more potent inhibitor and we sought to covalently attach an *ortho*-carbon of the phenyl subunit and the central tertiary carbon, consequently leading to a more rigid molecule pre-organized in the bioactive conformation. We immediately added an indane-based constraint to inhibitor **2**, since it was the more potent of the two leading analogs (Fig. 3). Disappointingly, constrained analog **3** was found to be almost *sevenfold less potent* than the unconstrained inhibitor! We were perplexed but not discouraged by this result and simple plastic models led us to hypothesize that perhaps the 2-methoxy-substitutent on the benzyl subunit engages in negative steric interactions with the indane-based constraint. This should in turn lead to a relative destabilization of the putative bioactive conformation.

Therefore, we were cautiously optimistic when we synthesized inhibitor **4**, a constrained variant of aminoimidazole **2**. We were pleased to find that compound **4** is approximately fivefold more potent than **2** (Fig. 4). In fact, constrained compound **4** is even more active than potent non brainpenetrant inhibitor **1**, which bears the 2-methoxy-5-nitro substituted benzyl subunit (IC_{50} = 0.35 μM versus IC_{50} = 0.47 μM). This compound, which lacks the 2-methoxy-5-nitro substitution, led us to believe that it would be possible to synthesize a potent aminoimidazole with a relatively low Pgp efflux ratio. Indeed, additional optimization led to inhibitor **5**, a highly active BACE-1 inhibitor with a relatively low Pgp efflux ratio (IC_{50} = 0.063 μM; Pgp (BA/AB) = 3.6).[8]

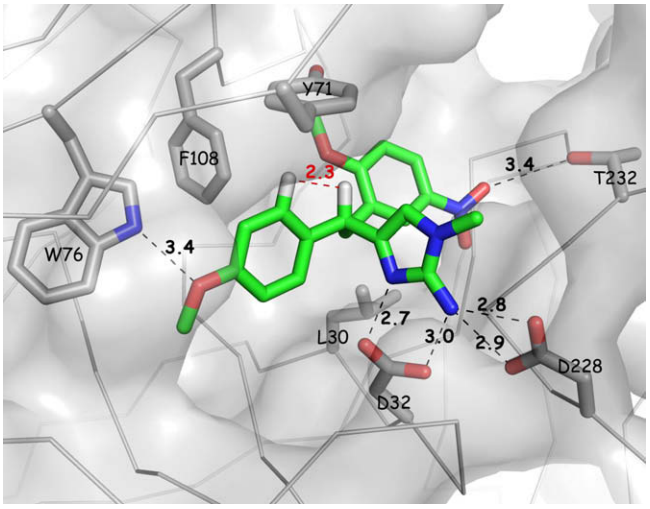


Figure 2. X-ray structure of inhibitor **1** as bound in the BACE-1 active site (PDB ID code: 3H0B). Residues within 3.5 Å of **1** are shown as gray sticks and H-bonding interactions with the inhibitor are labelled. The crucial tertiary carbon hydrogen and the adjacent *ortho* aromatic hydrogen have been added to highlight their alignment and proximity.

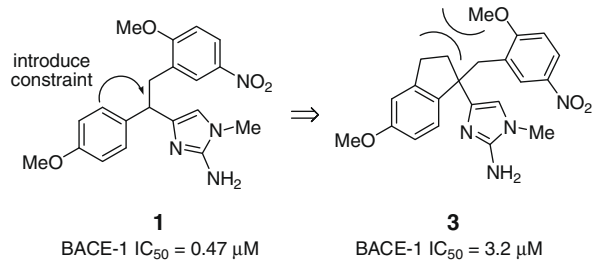


Figure 3. An indane-constraint was introduced to furnish a rigid molecule pre-organized in the bioactive conformation. Initial application of this strategy led to a less potent compound.

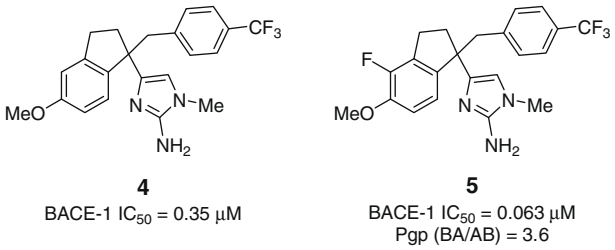


Figure 4. Indane-constrained aminoimidazoles bearing a 4-trifluoromethyl substituted benzyl subunit are potent and have relatively low Pgp efflux ratios.

With these results in hand, we were interested to see if computations would support our conjecture that the indane constraint leads to more potent inhibitors by reducing conformational flexibility. We also wanted to further understand why constrained inhibitor **3** is less potent than inhibitor **1**. Gratifyingly, modeling of **1** and **3**,[9] both bound and unbound to BACE-1, clearly shows that introduction of the indane constraint reduces the percentage of conformers that resemble the bound conformer of compound **3**. Modeling shows that 18.5% of the conformers of compound **1** adopt a bound-like geometry. However, the bound-like conformation of **3** is disfavored by 1.2 kcal/mol and only 8.9% of the conformers of **3** closely resemble the conformation required for effective binding to the BACE-1 active site. This is consistent with the observed decrease in potency and is presumably due to negative steric interactions. As illustrated in Figure 5, the lowest energy conformer for compound **3** (**A**, green) is quite dissimilar from the conformer believed to be required for binding to BACE-1 (**A**, white).

We next examined inhibitors **2** and **4**. When we studied unconstrained analog **2**, we found that 52% of the conformers adopt a

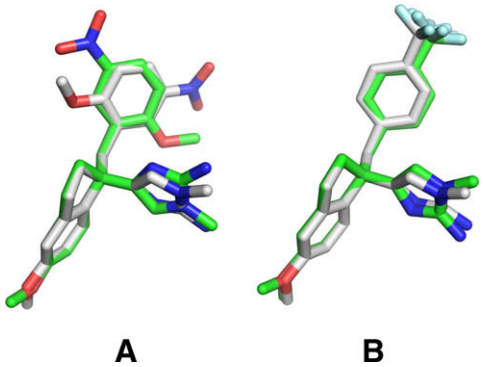
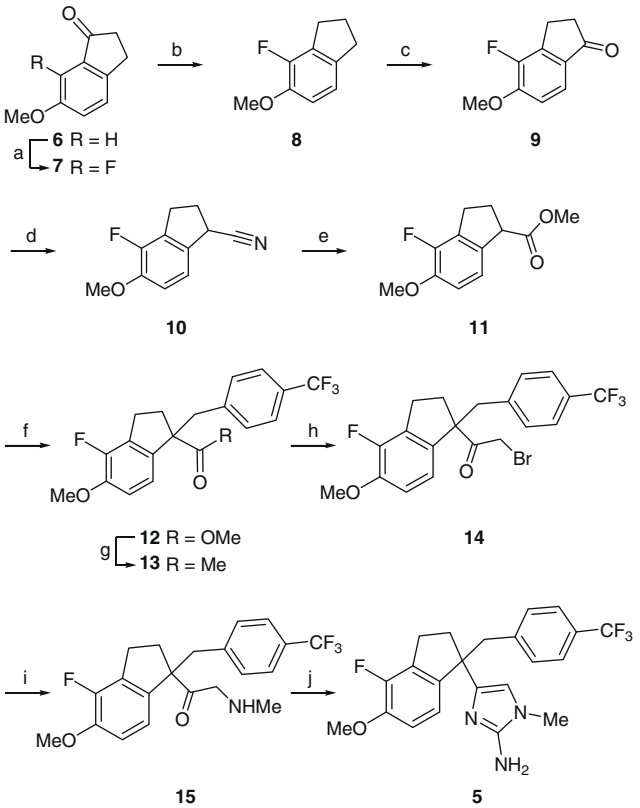


Figure 5. Comparison of modeled bound poses (white) relative to the lowest energy conformers (green) for inhibitors **3** and **4**. **A.** In the lowest energy conformer computed for **3**, both the 2-methoxy-5-nitro phenyl and the aminoimidazole rings are flipped 180° relative to the modeled bound pose. **B.** By contrast, there are 3 low energy conformers of **4** that are almost identical to the modeled bound pose.

conformation very similar to the bound geometry. Interestingly, as can be seen in Figure 5, the lowest energy conformer for compound **4** (**B**, green) is closely related to the conformer modeled in the BACE-1 active site (**B**, white). However, to our surprise, there is a modest decrease in the population of conformers that naturally resemble the bound conformation for constrained compound **4** (only 42% of the conformers are similar to the bound pose). This is likely due to a 0.09 kcal/mol energy penalty between the bound-like pose and the lowest energy conformer. This observation alone would lead us to conclude that a small erosion of potency should occur when a constraint is installed to furnish **4**. However, force-field based and empirical scoring methods[11] suggest that the observed enhanced potency may be due to a favorable change in the binding energy, as a result of additional van der Waals contacts between residues on the BACE flap (e.g., Tyr71) and the indane constraint. Thus, the addition of a constraint does lead to a more potent inhibitor. However, computations suggest this strategy is *not successful due to conformational restriction*; rather it is due to gaining *additional hydrophobic interactions*.

This class of constrained aminoimidazole BACE-1 inhibitors is synthesized in a straightforward manner (Scheme 1). The synthesis of compound **5** begins with the fluorination of 6-methoxy-1-indanone to furnish **7**. The carbonyl of **7** is readily removed via hydrogenation and subsequent oxidation by CrO_3 yields isomeric indanone **9**. Treatment of **9** with TosMIC and KO*t*-Bu allows for the isolation of nitrile **10**. Standard acidic methanolysis leads to methyl ester **11**, which is then alkylated using NaHMDS and (trifluoromethyl)benzyl chloride in THF to give **12**. The methyl ester is then converted to a methyl ketone through the addition of (trimethylsilyl)methyllithium to provide intermediate **13**. Formation of a putative TMS-enolate via NaHMDS and TMSCl, followed by addition of trimethylphenylammonium tribromide leads to alpha-bromo ketone **14**. Simple substitution of the bromide by methylamine furnishes amino ketone **15**. Finally treatment of the amino ketone with 2-ethyl-2-thiopseudourea under basic conditions allows the isolation of indane-constrained aminoimidazole **5**.



Scheme 1. Reagents and conditions: (a) Accuflour™ NFTh (1 equiv), MeCN, reflux, 4 h; (b) Pd/C, H_2, H_2SO_4, EtOAc, rt, overnight; (c) CrO_3, AcOH/H_2O, 0 °C, 5 h; (d) TosMIC, KO*t*-Bu, DME, 0–50 °C, overnight; (e) HCl, MeOH, 60 °C, overnight; (f) NaHMDS, 4-(trifluoromethyl)benzyl chloride, THF, −78 °C, 30 min; (g) (trimethylsilyl)methyllithium, THF, rt, 1 h; (h) (1) NaHMDS, TMSCl, THF, −78 °C, 30 min; (2) trimethylphenylammonium tribromide, THF, rt, overnight; (i) Methylamine, THF, rt, 2 h; (j) 2-ethyl-2-thiopseudourea hydrobromide, NaOH, THF/H_2O, rt, overnight.

In summary, we have been able to achieve our initial goal of producing an inhibitor of BACE-1 with improved potency *and a relatively low Pgp efflux ratio*. This was accomplished by introducing an indane-based constraint that was effective for certain analogs (**2** vs **4**) but not others (**1** vs **3**). Computational studies unexpectedly suggest that the constraint enhances potency via additional favorable hydrophobic interactions, rather than conformational restriction.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.071.

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