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Hydroxyethylamine-based inhibitors of BACE1: P_1–P_3 macrocyclization can improve potency, selectivity, and cell activity

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ABSTRACT

We describe a systematic study of how macrocyclization in the P_1–P_3 region of hydroxyethylamine-based inhibitors of β-site amyloid precursor protein (APP)-cleaving enzyme (BACE1) modulates in vitro activity. This study reveals that in a number of instances macrocyclization of bis-terminal dienes leads to improved potency toward BACE1 and selectivity against cathepsin D (CatD), as well as greater amyloid β-peptide (Aβ)-lowering activity in HEK293T cells stably expressing APP_{SW}. However, for several closely related analogs the benefits of macrocyclization are attenuated by the effects of other structural features in different regions of the molecules. X-ray crystal structures of three of these novel macrocyclic inhibitors bound to BACE1 revealed their binding conformations and interactions with the enzyme.

Aloysius Alzheimer reported in 1907 a previously unrecognized neurodegenerative disease clinically presented as a presenile and progressive loss of cognitive function and dementia, and characterized by the presence of neurofibrillary tangles and amyloid plaques in the brains of deceased patients.[1,2] These biochemical hallmarks of Alzheimer's disease (AD) and their involvement in its etiology have been studied extensively over the last 30 years.[3] The amyloid cascade hypothesis posits that the accumulation and aggregation of amyloid β-peptide (Aβ) is neurotoxic, triggering a variety of pathogenic processes leading to cognitive impairment and neuronal death.[3,4] Since Alzheimer's initial description of this eponymous disease, aged populations have increased globally and a disease-modifying therapy has remained a critical unmet medical need for this grievous and costly illness.[5]

The rate-limiting first step of the proteolysis cascade converting β-amyloid precursor protein (APP) to the pathogenic peptides $Aβ_{40}$ and $Aβ_{42}$ was revealed in 1999 to be mediated by β-site APP-cleaving enzyme (BACE1).[6] Subsequently, gene-knockout studies in mice[7] and genome-wide association studies in humans[8] have lent further credence to the promise of this enzyme as a therapeutic target for AD. Despite tremendous effort by the biomedical research community, however, no inhibitor of BACE1 has yet been approved for the treatment of AD.[9] That BACE1 is an aspartyl protease situated within neurons of the central nervous system (CNS) and shielded by the blood–brain barrier (BBB) presents a significant obstacle to this endeavor.[9,10] Another challenge is achieving selectivity against cathepsin D (CatD), a related aspartyl protease with high sequence homology to BACE1 at the active site, the inhibition of which may give rise to toxic side-effects.[11,12]

The first X-ray crystal structure of BACE1 was reported in 2000 as a complex with OM99-2, a potent peptide-based inhibitor incorporating a hydroxyethylene transition state isostere (BACE1 K_i = 0.0016 μM).[13,14] Over the last 13 years, myriad chemotypes have been disclosed as BACE1 inhibitors, including peptides and a wide variety of peptidomimetic and heterocyclic compounds.[9] In patent applications published in December 2002, Pulley et al.

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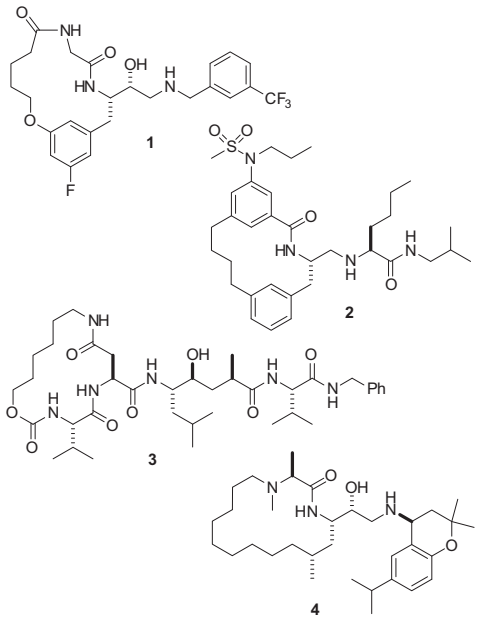

Figure 1. A selection of early macrocyclic inhibitors of BACE1.

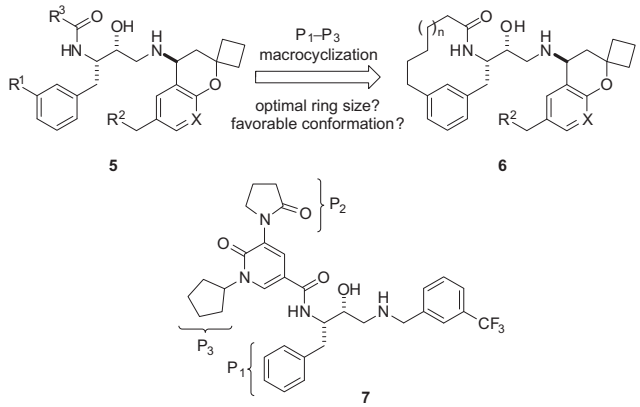

Figure 2. Strategy for pursuing macrocyclization in the P_1–P_3 region of hydroxyethylamine-based inhibitors of BACE1.

revealed the first macrocyclic inhibitors of BACE1, including hydroxyethylamine-based macro-bis-lactam **1** (Fig. 1; BACE1 IC_{50} <50 μM).[15] Subsequent early reports from the laboratories of Stachel,[16] Ghosh,[17] and Machauer[18] unveiled BACE1-inhibiting macrocycles including **2** (BACE1 IC_{50} = 0.0040 μM), **3** (BACE1 K_i = 0.025 μM), and **4** (BACE1 IC_{50} = 0.0020 μM), respectively. Since these seminal reports, the appeal of macrocycles[19] as conformationally constrained congeners of existing acyclic chemotypes with potentially improved potency, selectivity, and other drug-like properties has led to several other disclosures of macrocyclic inhibitors of BACE1.[20]

Our laboratory recently disclosed hydroxyethylamine-based inhibitors of BACE1 represented by generic structure **5** (Fig. 2; e.g., R^1 = H, R^2 = Me, R^3 = Me, X = CH; BACE1 IC_{50} = 0.11 μM; CatD IC_{50} = 0.70 μM; Cell $A\beta_{40}$ IC_{50} = 0.37 μM).[21] Inspired by the initial reports of macrocycles **1**–**4** in Figure 1 and the analysis of several X-ray crystal structures of Amgen inhibitors bound to BACE1,[21] we began our exploration of macrocyclization in late 2004. We desired first to examine simple methylene-linked macrocycles closely related to **5** to allow rapid entry into this arena (e.g., **5** → **6**; Fig. 2). A divergent synthesis hinging upon a late-stage ring-closing methathesis (RCM)–reduction sequence of simple bis-terminal diene substrates was envisioned to allow ready comparison of both the different chain-length dienes and their cognate methylene-linked macrocycles (e.g., **5**: R^1 = $-CH_2CHCH_2$, R^2 = Me, R^3 = $-[CH_2]_nCHCH_2$, X = CH; **6**: n = 1–3, R^2 = Me, X = CH).[22] Though unclear at the outset of these studies what ring size and conformation would be optimal, preliminary computational studies suggested that 13- and 14-membered rings would be favored (i.e., **6**: n = 2 or 3).[23] Our group has also explored hydroxyethylamine-based inhibitors containing a pyridone ring with a variety of substituents penetrating into the S_2 and S_3 pockets of BACE1 (e.g., **7**; BACE1 IC_{50} = 0.076 μM; CatD IC_{50} >10 μM; Cell $A\beta_{40}$ IC_{50} = 0.21 μM; Fig. 2).[24] Anticipating that modification of the linker region of the simple methylene-linked macrocycles depicted in Figure 2 might be necessary to improve BACE1 potency and CatD selectivity, as well as to modulate physicochemical properties, evaluation of more complex macrocycles based on inhibitor **7** was also planned.

The results of the study are detailed in Table 1. Dienes **8**, **9**, and **10** exhibit similar potency toward BACE1 (BACE1 IC_{50} = 0.11, 0.045, and 0.092 μM, respectively)[25,26] and similarly increased potency against CatD (CatD IC_{50} = 0.029, 0.0063, and 0.036 μM, respectively).[27] Interestingly, whereas dienes **8** and **9** display comparable decreases in cell potency versus biochemical potency (Cell $A\beta_{40}$ IC_{50} = 1.5 and 0.81 μM, respectively; Cell $A\beta_{40}$ IC_{50}/BACE1 IC_{50} = 14 and 18, respectively), the more lipophilic diene **10** shows a much greater cell shift (Cell $A\beta_{40}$ IC_{50} >10 μM; Cell $A\beta_{40}$ IC_{50}/BACE1 IC_{50} >110).[28] Macrocycles **12**, **13**, and **14** display some analogous, and several notably different, bioassay trends from their respective parental dienes **8**, **9**, and **10**. Whereas cyclization of diene **8** to 12-membered macrocycle **12** results in nearly identical potency toward BACE1 (BACE1 IC_{50} = 0.12 μM), cyclization of **9** and **10** to 13- and 14-membered macrocycles **13** and **14**, respectively, leads to improved BACE1 potency (BACE1 IC_{50} = 0.017 and 0.036 μM, respectively). In contrast to dienes **8**, **9**, and **10**, macrocycles **12**, **13**, and **14** exhibit decreased potency against CatD (CatD IC_{50} = 0.23, 0.37, and 0.89 μM, respectively). The CatD selectivity is especially favorable for macrocycles **13** and **14** (CatD IC_{50}/BACE1 IC_{50} = 22 and 25, respectively). Compared to parental dienes **8** and **9**, macrocycles **12** and **13** also display a much smaller cell shift (Cell $A\beta_{40}$ IC_{50} = 0.25 and 0.058 μM, respectively; Cell $A\beta_{40}$ IC_{50}/BACE1 IC_{50} = 2.1 and 3.4, respectively). Although a much greater cell shift was observed for the more lipophilic macrocycle **14** (Cell $A\beta_{40}$ IC_{50} = 0.96 μM; Cell $A\beta_{40}$ IC_{50}/BACE1 IC_{50} = 27), the magnitude is still less than that observed for its diene precursor **10**. As previous studies in our laboratory revealed that a neopentyl group at C6 of the chroman moiety increased BACE1 potency and N atom substitution at C8 of this ring system mitigated cell shifts in potency, albeit with lower CatD selectivity (Fig. 2; e.g., **5**: R^1 = H, R^2 = *t*-Bu, R^3 = Me, X = N; BACE1 IC_{50} = 0.0058 μM; CatD IC_{50} = 0.0045 μM; Cell $A\beta_{40}$ IC_{50} = 0.0031 μM),[21] we desired to incorporate these structural motifs into the best diene/macrocycle pair (**9**/**13**). Although the resultant diene **11** exhibits nearly 10-fold better potency toward BACE1 (BACE1 IC_{50} = 0.0047 μM), it also displays increased potency against CatD and a large cell shift (CatD IC_{50} = 0.00084 μM; Cell $A\beta_{40}$ IC_{50} = 0.066 μM; Cell $A\beta_{40}$ IC_{50}/BACE1 IC_{50} = 14). Compared to macrocycle **13**, macrocycle **15** shows a ~3-fold increase in potency toward BACE1 (BACE1 IC_{50} = 0.0061 μM) and negated selectivity against CatD (CatD IC_{50} = 0.0043 μM); pleasingly, **15** displays a negligible cell shift (Cell $A\beta_{40}$ IC_{50} = 0.0059 μM).

An overlay of the X-ray crystal structures of **7**, **13**, and **14** bound to BACE1 is depicted in Figure 3.[29] As predicted,[23] **13** and **14** display similar binding conformations and key contacts with the enzyme, with only minor differences in the linker region. The conformational constraints afforded upon macrocyclization of **9** and **10** may help to explain the increased BACE1 potency and improved CatD selectivity of **13** and **14**.[30] The large number of