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Macrocyclic peptidomimetic inhibitors of β-secretase (BACE): First X-ray structure of a macrocyclic peptidomimetic-BACE complex

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Abstract—The synthesis of novel macrocyclic peptidomimetic inhibitors of the enzyme BACE1 is described. These macrocycles are derived from a hydroxyethylene core structure. Compound **7** was co-crystallized with BACE1 and the X-ray structure of the complex elucidated at 1.6 Å resolution. This molecule inhibits the production of the Aβ peptide in HEK293 cells overexpressing APP751sw.

Alzheimer's disease (AD) is a major neurodegenerative disorder that affects 10% of the population over 65% and 40% over 80 years.[1,2] Symptoms of the disease are behavioral disturbances and loss of cognitive function. AD represents an unmet medical need that adds approximately 100 billion dollars to healthcare costs in the United States alone. In addition, AD also causes great deal of suffering to the patient and places a burden on their families.

Treatments currently available for AD are cholinesterase inhibitors and NMDA-receptor antagonists. The rationale behind the former approach is that these agents increase cholinergic transmission by interfering with degradation of the Ach neurotransmitter. Unfortunately, this approach does not stop the progressive loss of cholinergic neurons, and eventually the treatment becomes ineffective. Treatment with NMDA-receptor antagonists appears to be no more effective in terms of progression of the disease. A better approach would be to develop agents that interfere with the mechanism that leads to neurodegeneration.

AD is characterized by the presence of amyloid plaques that are believed to produce neuronal toxicity and cell death.[3] These plaques are constituted mainly from an insoluble form of Aβ amyloid, a 40–42 amino acid peptide produced in the brain from the amyloid precursor protein (APP). APP is processed by at least three secretases. α-Secretase cleaves APP to form a carboxy terminal 83 amino acid fragment (C83), which is processed by γ-secretase to produce p3, a non amyloidogenic form of Aβ–peptide. Alternatively, APP can be processed initially by β-secretase (BACE1) and the resulting C99 membrane bound C-terminal peptide can be hydrolyzed by γ-secretase to form the 40 amino acid Aβ-peptide ($A\beta_{40}$). Shorter and longer forms of the peptide also are produced, in particular $A\beta_{42}$, which has a high propensity to aggregate and is the principal Aβ species found in amyloid plaques. Notably, the BACE1 enzyme is only found in ER/Golgi or endosome compartments of neurons, whereas several α-secretase candidates and the γ-secretase complex have broad tissue distribution.

Conceptually, the inhibition of either β- or γ-secretase by a small molecule would preclude the formation of Aβ and therefore plaque formation. γ-Secretase inhibitors have been reported to attenuate the formation of Aβ and plaques in animal models. However, the therapeutic index of these inhibitors requires careful monitoring. This is due to the implication of γ-secretase in

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essential functions, including facilitating Notch signaling, which is important for specifying cell fates during development and for regulating differentiation of self-renewing cell types in adults.[4] In contrast, β-secretase KO mice are viable and free of the gross phenotypic changes that are observed, for example with presenilin knock-outs, except for the complete absence of Aβ in their brains.[5]

Beta amyloid cleaving enzyme (BACE1) has recently been identified as the principal β-secretase in neurons.[6] It is a trans-membrane aspartyl protease expressed in neuronal cells that cleaves APP inside vesicles associated with ER/Golgi apparatus and endosomes. Tang et al. reported in 2000 that OM99-2 (Fig. 1), a peptidomimetic based on the hydroxyethylene transition state isostere, was a nanomolar inhibitor of BACE. The Oklahoma group subsequently reported the X-ray structure of the OM99-2-BACE complex showing the main features of the enzyme–inhibitor interactions.[7]

With the goal of designing low molecular weight BACE1 inhibitors,[8] we envisioned that linking two residues of our small peptidomimetic inhibitors[9] would provide a macrocyclic structure that would be more potent, and would have improved absorption properties, relative to the corresponding open chain analog. The linkage of distant residues should produce a decrease in the number of possible low energy conformations, and therefore, if one of the remaining conformations is complementary to the enzyme active site, the binding affinity should be increased. In addition, it is known that cyclic peptides are more resistant to gastric tract degradation adding an additional attractive feature to this class of inhibitors.[10]

Examination of Tang's,[7] and our X-ray data[9] suggested P1–P3 and P1–N2 as suitable points of union due to their proximity, orientation, and localization into the large P1–P3 lipophillic pocket (Fig. 2).

Linking positions P1–P3 has recently been described in BACE1 inhibitors for structures derived from an ethanolamine core;[11] also, other macrocycles derived from a hydroxyethylene core structure have been synthesized based on the linkage of positions P2–P3.[12]

The strategy developed to build the macrocyclic structures was to modify our lead inhibitor **1** by Grubbs cyclization of alkene moieties at P1 and P3 or between P1 and the nitrogen of the P2 amino acid. It was determined by iterative docking studies[13] that the best P1–P3 linkage was a six-atom long saturated chain that forms a 13-membered ring (including the inhibitor backbone). For the P1–N2 connection, the five-carbon saturated linker was predicted to be optimal, leading to a 10-membered ring.

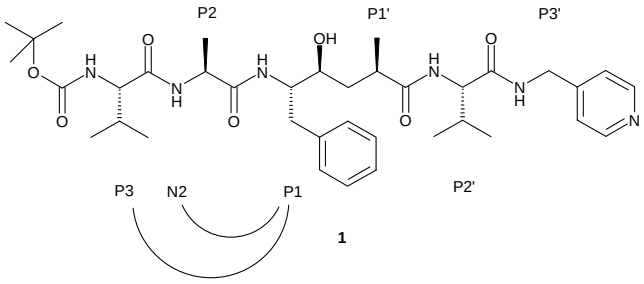

Figure 2.

The synthesis of the macrocyclic peptidomimetics was performed as depicted in Scheme 1. *N*-Boc serine methyl ester was converted into the iodoserine derivative by treatment with iodine and imidazole. Conversion of the alkyl iodide into the corresponding alkylzinc reagent followed by alkylation with allyl chloride[14] afforded, after hydrolysis, intermediate **8**.

Compound **8** was transformed into the pivotal lactone **9** by reduction to *N*-Boc homoallylglycinal,[15] alkylation with the Zn–Cu reagent obtained from 3-iodopropionic acid ethyl ester,[16] thermal lactonization and, for P1′ = Me, stereoselective methylation with LHDMS and methyl iodide.

The C-terminal fragment **10** was prepared by standard EDCI coupling of *N*-Boc alanine and 4-aminomethyl pyridine or benzylamine followed by cleavage of the Boc group under acidic conditions. The N-terminal fragment **11**, used to prepare compounds **2**–**5**, was prepared by EDCI coupling of **8** and alanine methyl ester, followed by ester hydrolysis.

The N-terminal fragment **12** used to build macrocyles **6**–**7** was prepared starting with L-alanine. The *p*-nitrobenzenesulfonyl group was introduced as an amino protecting group to facilitate the N–H alkylation with allyl bromide in the presence of potassium carbonate. Deprotection of the *N*-allylsulfonamide by treatment with thiophenol and potassium carbonate provided *N*-allyl-Ala-(OMe).[17] The coupling of this amino acid with

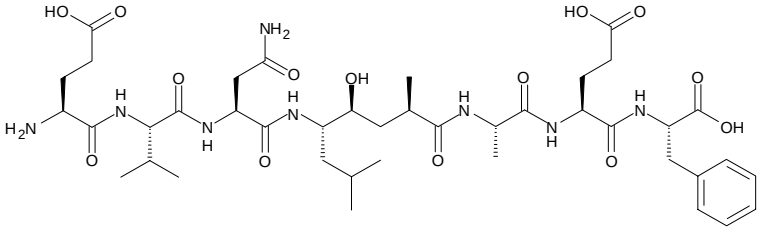

Figure 1. OM-99-2.