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Diastereoselective synthesis of fused cyclopropyl-3-amino-2,4-oxazine β-amyloid cleaving enzyme (BACE) inhibitors and their biological evaluation

Jonathan D. Low [a,*], Michael D. Bartberger [b], Yuan Cheng [a], Doug Whittington [c,e], Quifen Xue [a], Stephen Wood [d], Jennifer R. Allen [a], Ana E. Minatti [a,*]

[a] Department of Medicinal Chemistry, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[b] Department of Molecular Engineering, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[c] Department of Molecular Engineering, Amgen Inc., 360 Binney Street, Cambridge, MA 02142, USA
[d] Department of Neuroscience, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA

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ABSTRACT

The diastereoselective synthesis and structure activity relationship (SAR) of a series of fused cyclopropyl-3-amino-2,4-oxazine (2-oxa-4-azabicyclo[4.1.0]hept-3-en-3-amine)-containing BACE inhibitors is described. Through these efforts compound **2** was identified as a potent (cell IC_{50} = 15 nM) BACE inhibitor with acceptable ADME properties. When tested in vivo, compound **2** demonstrated a significant reduction of brain and cerebral spinal fluid (CSF) $A\beta_{40}$ levels (46% and 66%, respectively) in a rat pharmacodynamic study and thus represents a suitable starting point for the further development of in vivo efficacious compounds for the treatment of Alzheimer's disease.

Alzheimer's disease (AD), the most common form of age-related dementia, is a slowly progressive neurodegenerative disease, characterized clinically by a gradual decline in memory and other cognitive functions, depression, and apathy. It is estimated that roughly 5.5 million Americans and as many as 44 million individuals world-wide are currently living with AD. It is the sixth leading cause of death in the US, fifth among individuals 65 and older. Cost of care in the US alone was estimated to be $259 billion in 2017 and is expected to rise to $1.1 trillion by 2050.[1]

AD is pathologically defined by extensive neuronal loss along with the accumulation of intracellular neurofibrillary tangles composed of a microtubule-associated protein known as tau, as well as extracellular amyloid plaques composed of an aggregated form of a peptide called Amyloid-β (Aβ) in the brain. The amyloid cascade hypothesis postulates that Aβ aggregation plays a key role in initiating the pathogenic cascade and is supported by genetic, biochemical, and neuropathological evidence.[2–4] Aβ is produced by the sequential, endoproteolytic cleavage of the amyloid precursor protein (APP) by β-site APP cleaving enzyme-1 (BACE1) and γ-secretase. Discovered in 1999, the aspartyl protease BACE1 is the enzyme responsible for the rate-limiting step in the production of Aβ and therefore inhibition of BACE1 represents an attractive disease-modifying strategy for the treatment of AD.[5,6]

At Amgen, significant effort has resulted in the development of amino-oxazoline xanthene[7–10] (AOX)-derived BACE1 inhibitors with superb in vivo central nervous system (CNS) penetration, excellent oral efficacy in rats and non-human primates, and most recently, improved selectivity against Cathepsin D (CatD).[11] As part of our on-going effort to develop new BACE1 inhibitor scaffolds, we turned our attention to a class of lower-molecular-weight BACE inhibitors bearing a six-membered amidine warhead and an amide-substituted S1 phenyl ring, a motif initially described by Shionogi and heavily utilized in numerous disclosed BACE1 inhibitors.[12–15]

Herein we disclose the synthesis and pharmacodynamic (PD) response of a new series of BACE1 inhibitors employing 2-oxa-4-azabicyclo[4.1.0]hept-3-en-3-amine as the amino heterocyclic warhead, colloquially referred to as the fused cyclopropyl oxazine.[16]

* Corresponding authors.
E-mail addresses: low@amgen.com (J.D. Low), aminatti@amgen.com (A.E. Minatti).
[e] Present address: Tango Therapeutics, 11 Hurley Street, Cambridge, MA 02141, USA.

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Amide-containing 2-amino-4H-5,6-dihydro-[1,3]-oxazines (general structure, Fig. 1) have been described as BACE1 and BACE2 inhibitors with high enzymatic inhibitory activity but moderate brain penetration, resulting in low CNS pharmacodynamic activity in mice.[17–20] Following the trend observed with other amidine-warhead containing BACE1 inhibitors, the high basicity of the 1,3-oxazine warhead (e.g., pK_a = 9.8 for **1,** Fig. 1) was identified as an undesired liability giving rise to low permeability across the blood brain barrier[19,21] and potential risks of endosomal accumulation and polypharmacology.[21–25] As a result, there has been a significant effort among research groups to modulate the pK_a of the amidine, for example, through incorporation of fluorine and other electron-withdrawing groups on the warhead.[21,26]

We envisioned that fusion of a cyclopropyl moiety onto the (*S*)-2-amino-1,3-oxazine warhead would reduce the pK_a due to the inductive effect of the cyclopropyl group[27] and potentially overcome the aforementioned liabilities. In addition to the electronic impact, the fused cyclopropyl group was also anticipated to impart conformational rigidity, possibly reducing the conformational entropy of the unbound state, and potentially improving binding.[28] These hypothesized improvements resulting from the installation of the fused cyclopropyl group were especially attractive considering the minimal increase in molecular weight (MW) by a single carbon. Depending on the orientation of the cyclopropyl ring, two diastereomers are possible, resulting in either a (*S*)-*trans* (**2**) or a (*S*)-*cis*-isomer (**3**) with respect to the cyclopropyl and aryl groups (Fig. 1). Molecular modeling of **2** and **3** with the BACE1 protein based on a co-crystal structure of BACE1 with a closely related analog of **1** suggested that the cyclopropyl group would be well accommodated within the protein and no preference for either the *trans*- or the *cis*-isomer was revealed. Therefore access to both isomers was desired.

Compounds **2** and **3** provided some unique synthetic challenges. Two different retrosynthetic routes were considered (Fig. 2). Route A featured a late-stage cyclopropanation of the unsaturated oxazine **4**. Although multi-step syntheses of analogous unsaturated oxazines is well precedented,[12,20] cyclopropanation via Simmons-Smith Reaction or Johnson-Corey-Chaykovsky sulfur ylide could be burdened by the electron rich nature of the oxazine and would most likely require protection of the amidine nitrogen. Another challenge associated with this route was that the cyclopropanation reaction would be substrate-controled, rendering this strategy even more unfavorable as both *trans*- and *cis*-isomers needed to be accessed.

As an alternative, Route B featured an early installation of the cyclopropyl group, with the key step consisting of a nucleophilic addition to the C=N bond of the respective racemic isoxazolines **2c** and **3c**. Based on work by Suzuki[29,30] and Mapp[31] this type of addition was anticipated to be sterically controlled (>90% de) with preferential nucleophilic attack occurring anti to the cyclopropyl group. We therefore anticipated the need of two complementary syntheses in order to access both diastereomers **2** and **3**: the addition of an aryl nucleophile into methyl isoxazoline ***rac*-2c** (Fig. 2, Route B, top) and a methyl nucleophile into aryl isoxazoline ***rac*-3c** (Fig. 2, Route B, bottom), respectively. Subsequent reduction would provide diastereomeric aminocyclopropanols ***rac*-2e** and ***rac*-3e**, which would undergo cyclization followed by further derivatization and chiral separation to furnish the desired (*S*)-*trans* and (*S*)-*cis* analogs **2** and **3,** respectively.

The synthesis[32] of (*S*)-*trans* (**2**) and (*S*)-*cis* (**3**) and their corresponding enantiomers (*R*)-*trans* (***ent*-2**) and (*R*)-*cis* (***ent*-3**) is shown in Scheme 1. In situ generation of the corresponding nitrile oxides from imidoyl chlorides **2a** and **3a,** followed by a 1,3-dipolar cycloaddition with allyl chloride yielded racemic 5-chloromethyl isoxazolines ***rac*-2b** and ***rac*-3b**, respectively. KO*t*Bu–mediated

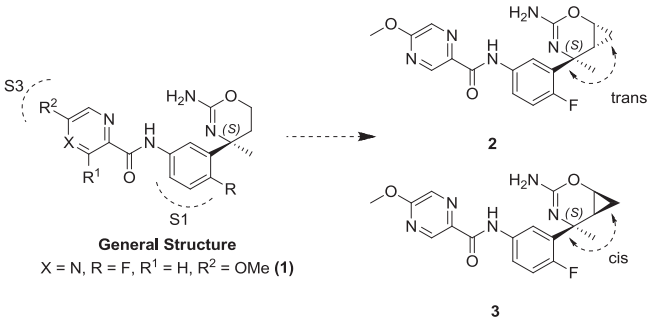

Fig. 1. Work described in this publication.

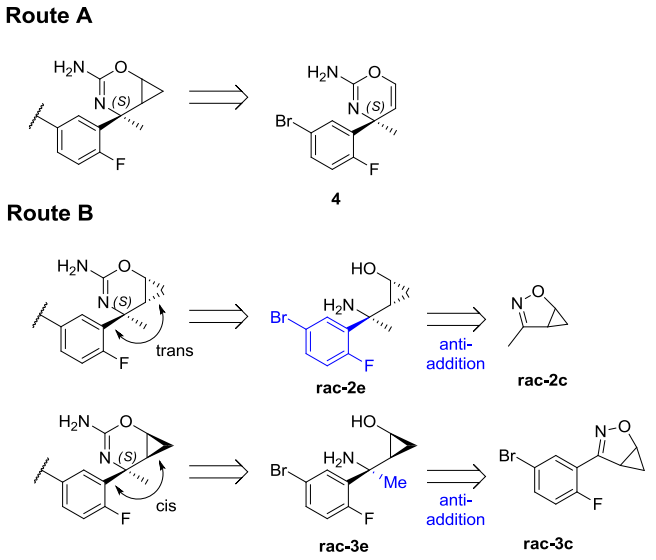

Fig. 2. Possible retrosynthetic key steps to access *trans*- and *cis*-isomers **2** and **3.**

cyclization afforded racemic methyl (***rac*-2c**) and phenyl (***rac*-3c**) cyclopropyl isoxazolines, respectively.

With the cyclopropyl isoxazolines in hand, we set out to investigate the critical nucleophilic addition reaction. Employing the conditions described by Suzuki[29,30] and Mapp,[31] racemic isoxazoline ***rac*-2c** was activated by $BF_3.OEt_2$ and then treated with a solution of 4-bromo-1-fluoro-2-lithiobenzene in THF at −78 °C, providing ***rac*-2d** in 64% yield and > 99% de as determined by ^1H NMR. Toluene was identified as the preferred solvent, while reactions performed entirely in THF or in DCM provided significantly diminished yields (30 and <1%, respectively). The relative stereochemistry of ***rac*-2d** was confirmed to be *trans* based on a strong cross peak between the methyl and cyclopropyl methylene protons in its NOESY spectra.

When applied to the synthesis of isoxazolidine ***rac*-3d,** the aforementioned addition reaction required optimization. Addition of a solution of MeLi in THF to pre-complexed isoxazoline ***rac*-3c** in toluene resulted only in recovery of the starting material. A solvent screen revealed dichloromethane as the preferred solvent, affording isoxazolidine ***rac*-3d** in 16% yield along with 77% recovered starting material. Switching the nucleophilic species to methyl lithium complexed with lithium bromide (MeLi·LiBr) improved the conversion and ***rac*-3d** was isolated in 52% yield. Similar to ***rac*-2d**, ***rac*-3d** was obtained as a single diastereomer and was confirmed to have the expected *cis* relative stereochemical configuration with respect to the phenyl and cyclopropyl groups, implying nucleophilic attack occurred in both cases from the face opposite the cyclopropyl group.