Letter

Discovery of a Tetrahydrobenzisoxazole Series of #-Secretase Modulators

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Discovery of a Tetrahydrobenzisoxazole Series of γ-Secretase Modulators

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KEYWORDS: Alzheimer's disease; γ-Secretase modulators; tetrahydrobenzisoxazole.

ABSTRACT: The design and synthesis of a new series of tetrahydrobenzisoxazoles and their structure-activity relationship (SAR) as modulators of γ-secretase activity will be detailed. Several compounds are active γ-secretase modulators (GSMs) with good to excellent selectivity for the reduction of $A\beta_{42}$ in the cellular assay. Compound **14a** was tested *in vivo* in a non-transgenic rat model and was found to significantly reduce $A\beta_{42}$ in the CNS compartment compared to vehicle-treated animals (up to 58% reduction of cerebrospinal fluid $A\beta_{42}$ as measured 3h after an acute oral dosing at 30 mg/kg).

Alzheimer's disease (AD) is the sixth leading cause of death in the United States. An estimated 5.5 million Americans have AD, costing the nation more than $230 billion in 2016.[1] As the elderly population continues to grow, the prevalence of AD will be increasing greatly. Therefore, there is an urgent need to develop an effective therapy to prevent Alzheimer's disease.

Aβ proteins, which are metabolites of the amyloid precursor protein (APP), are considered to be involved in the degeneration and loss of neurons as well as the onset of AD.[2,3] It is known that $A\beta_{40}$ is the most abundant species within the soluble pool of Aβ peptides; however, deposited Aβ plaques primarily consist of $A\beta_{42}$,[4,5] which is the species that is most prone to aggregation, forming aggregates of insoluble fibrils in the brain.[6] The higher aggregation potential of $A\beta_{42}$ may due to the distinct initial folding properties of $A\beta_{40}$ and $A\beta_{42}$.[7] These Aβ fragments are produced when APP is first cleaved by BACE (β-amyloid cleaving enzyme) and subsequently by γ-secretase.[8] Based on these facts, γ-secretase inhibitors (GSIs) and BACE inhibitors have been proposed as treatments for the purpose of reducing the production of Aβ fragments.[9] However, notch-related adverse effects are known as a major obstacle for the development of GSIs for the treatment of AD.[10] Therefore, γ-secretase modulators (GSMs) have attracted much attention in recent years.[11,12]

GSMs selectively inhibit the production of the most amyloidogenic and neurotoxic $A\beta_{42}$ by shifting the position of γ-secretase cleavage toward the formation of shorter, more soluble peptides, without interfering with the overall γ-secretase function, as measured by the formation of total amyloid ($A\beta_{total}$).[13] A larger ratio of $A\beta_{total}/A\beta_{42}$ represents a more selective GSM. More selective GSMs do not shut down the processing of Notch by γ-secretase, and thus offer a potentially improved side effect profile compare to GSIs. Due to this advantage, GSMs have attracted great interest from researchers.[14] There are two major classes of GSMs, the nonsteroidal anti-inflammatory drugs (NSAIDs)[15,16] and the non-NSAIDs class, such as **1** from Eisai,[17] **2** and **3** from Merck.[18,19] In addition, natural product-based GSMs have also been developed.[20,21] Following our efforts[22-27] to develop in the non-NSAID GSMs, we developed a new series, represented by structure **4** (Figure 1),[28] where the replacement of the double bond present in structures **1**– **3** to mitigiate a potential metabolic. Therefore, the double bond and the lactam carbonyl in **1** was replaced by a small heterocyclic fused ring such as tetrahydrobenzisoxazole in **4**.

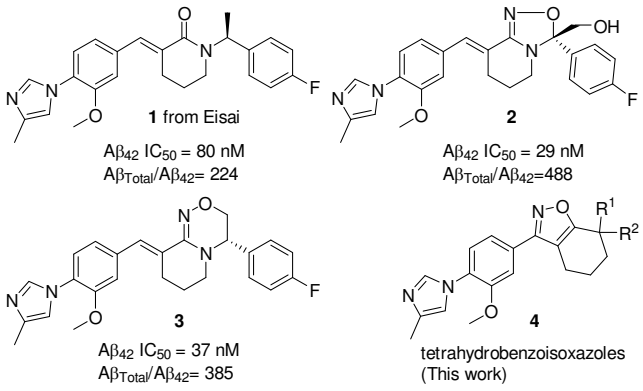
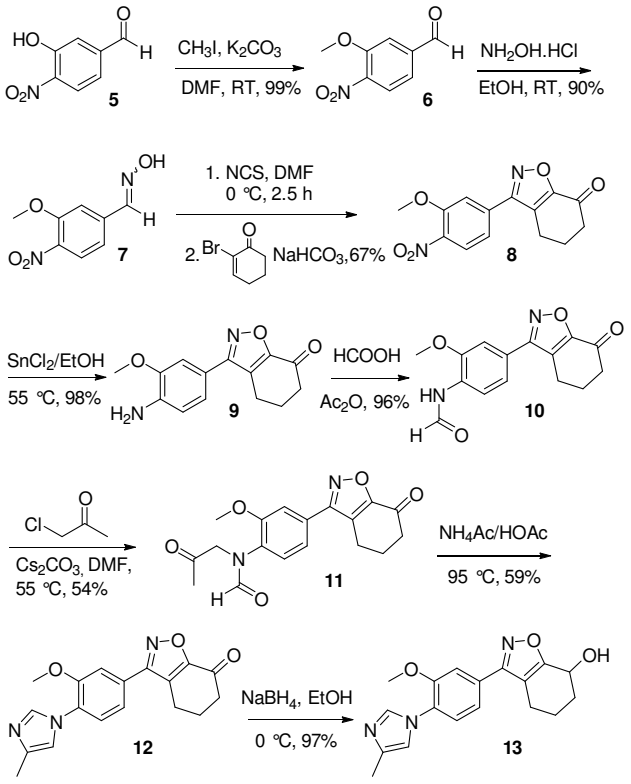


Figure 1. Design of GSMs from known structures

The tetrahydrobenzisoxazole was selected as a linker to lock the side chain in the same conformation as the double bond did in the lead structures **1**– **3**. We synthesized a series of compounds with a core structure of **4**. Our chemical synthesis involves key intermediates **12** and **13** which is summarized in Scheme **1**. The *O*-methylation of **5** afforded **6**, which was converted to the oxime compound **7**. The key step is a [3 + 2]

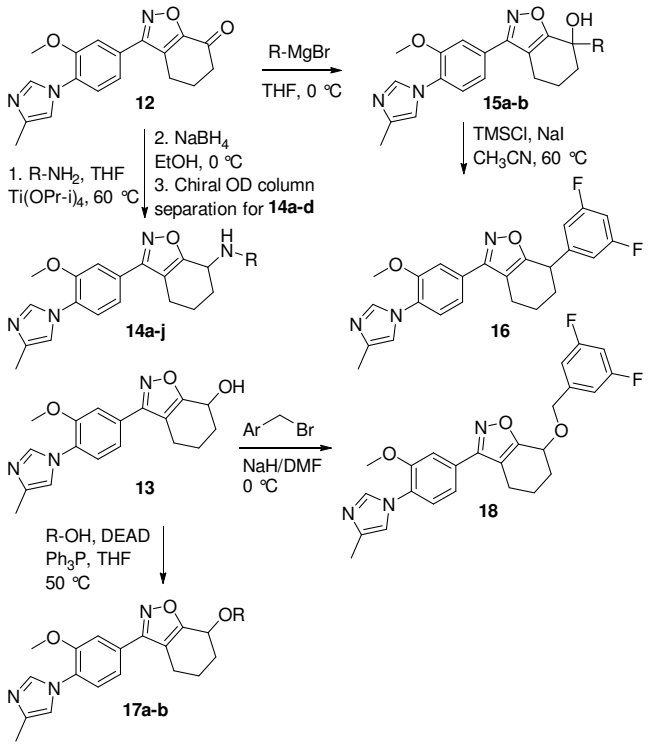
cycloaddition to afford isoxazole **8**. After optimization, we were able to develop a one pot reaction to produce intermediate **8** with a 67% yield (two steps). The nitro group in **8** was reduced to the corresponding amine to afford **9**. The amine group in **9** was converted to formamide **10**. The *N*-alkylation of **10** with 1-chloropropan-2-one, followed by treatment with ammonium acetate afforded **12**, which was reduced by sodium borohydride to deliver **13**.

Scheme 1. Synthesis of intermediates **12** and **13**



From **12** and **13** a variety of final targets were prepared through traditional chemistry: such as reductive amination (**12** to **14a-j**), Grignard reaction (**12** to **15a-b**), Mitsunobu reaction (**13** to **17a-b**), dehydroxylation[29] (**15a** to **16**) and alkylation (**13** to **18**) as illustrated in Scheme 2.

Scheme 2. Synthesis of final GSM compounds

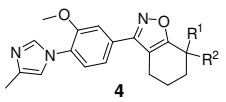


The biological test results are summarized in Table 1. Human embryonic kidney (HEK) 293 cells were treated with a variable concentration of GSM compounds for 5 h. $A\beta_{42}$ and $A\beta_{total}$ in conditioned media were measured using MesoScale Discovery (MSD) sandwich immunoassays.[30] $A\beta_{42}$ was measured using a pair of labeled antibodies, TAG-G2-11 and biotin-4G8; total Aβ was measured using antibodies TAG-W02 and biotin-4G8. The electrochemiluminescence (ECL) signal was measured using an ECLM8 instrument (IGEN International, Inc.) according to the manufacturer's instructions.

From previously known SAR, the left side 3-methoxyphenyl-4-methyl-1*H*-imidazole is required for GSM activity. Therefore, we focused our efforts on the SAR development of the right side of the molecule. The SAR is summarized in Table 1. When R^2 is *N*-methylamino, the $A\beta_{42}$ inhibition activity is only 12 μM with no selectivity (**14h,** clogD = 1.07, pKa = 8.10). It is known that increasing lipophilicity often results in improved *in vitro* activity and improved blood brain barrier permeability.[31] Controlling the pKa was a successful approach to improve the CNS penetration.[32] We then introduced fluorine to modulate both lipophilicity and basicity of the target molecules. Substitution with *N*-trifluoroethylamino led to improved $A\beta_{42}$ inhibition activity and selectivity (464 nM and 43-fold for **14i**, clogD = 2.77, pKa = 5.42). When the *N*-trifluoroethylamino (**14i**) was replaced by an *N*-para-fluorobenzylamino (**14e**, clogD = 3.71, pKa = 7.08), the $A\beta_{42}$ activity of **14e** increased more than 3 fold. This result suggests that the phenyl ring can increase activity. When the *N*-cyclohexanamino (**14j**, clogD = 2.86, pKa = 8.42) was replaced by an *N*-(4-fluorophenyl) amino, the $A\beta_{42}$ inhibition activity and selectivity are greatly improved (39 nM and 499-fold for **14a**, clogD = 4.20, pKa = 5.33). This result is probably due to the strong electron withdrawing effect and the lipophilic effect of the fluorine introduced on the phenyl ring. When a tertiary amino (**14f**) or *N*-alpha-methyl para-fluorobenzylamino was introduced (**14g**), the compounds lost some activity when compared to **14e**. When nitrogen was replaced by oxygen (**17a, b** and **18**) the compound also lost

some activity relative to **14e**. Nevertheless, we found that the enantiomerically pure compounds **14a** and **14c** are two compounds in the series with both good activity and high selectivity. To this point, the double bond and the lactam carbonyl present in **1** was successfully replaced by a tetrahydrobenzisoxazole ring. The new GSM compounds, **14a** and **14c**, are two-fold more active than GSM **1**, and have similar activity to **2** and **3**. Additionally, **14a** and **14c** are more selective than **1** and **3**, and have similar selectivity to **2**.

Table 1. Cellular IC_{50} of Tetrahydrobenzisoxazole Analogs



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Compound	R^1	R^2	$A\beta_{42}$ IC_{50}, (μM)*	$A\beta_{tot}/A\beta_{42}$
14a, b	H		3.9×10^{-2} (**14a**)	499
			0.23 (**14b**)	86
14c, d	H		2.9×10^{-2} (**14c**)	575
			0.64 (**14d**)	28
14e	H		0.13	32
14f	H		0.25	79
14g	H		0.26	77
14h	H		1.2×10	2
14i	H		0.46	43
14j	H		0.31	64
15a	OH		0.19	105
15b	OH		0.15	102
16	H		0.11	178
17a	H		0.29	68
17b	H		0.20	98
18	H		0.51	39

* n = 3

Mass spectrometric analysis of Aβ peptides secreted by HEK293 cells carrying the London-Swedish double mutation after treatment with vehicle (DMSO) or 10 μM solutions of compounds **14a**, **14c**, **14e** is shown in Figure 2. As expected for γ-secretase modulators, compounds **14a**, **14c**, and **14e** blocked the production of $A\beta_{42}$ and $A\beta_{40}$, while the relative proportion of $A\beta_{38}$ and $A\beta_{37}$ is increased in comparison to $A\beta_{40}$ (numbers in spectra are amyloid-β numbering). The Aβ profile in conditioned medium was analyzed using surface enhanced laser desorption/ionization (SELDI) mass spectrometry as described previously.[33]

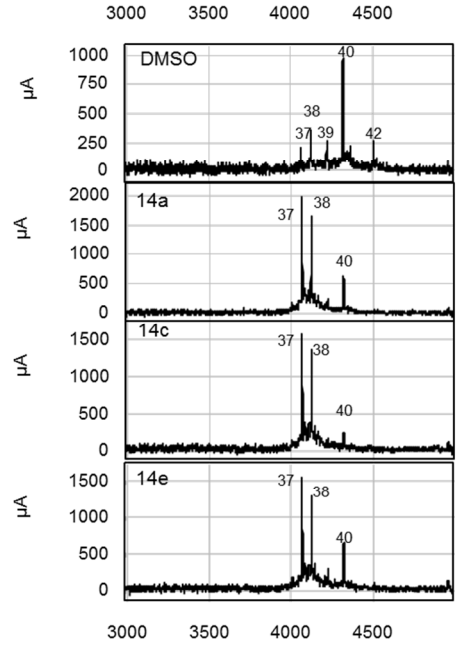

Figure 2. Mass spectrometric analysis of Aβ profile

Compounds **14a, 14c, 14d** and **15a** were then tested *in vivo* rat model. Compound **14a** significantly reduced cerebrospinal fluid (CSF) $A\beta_{42}$ by 58% (p = 0.0066, n = 5) 3h after a single oral dose at 30 mg/kg in rats compared to the vehicle-treated animals (Figure 3).

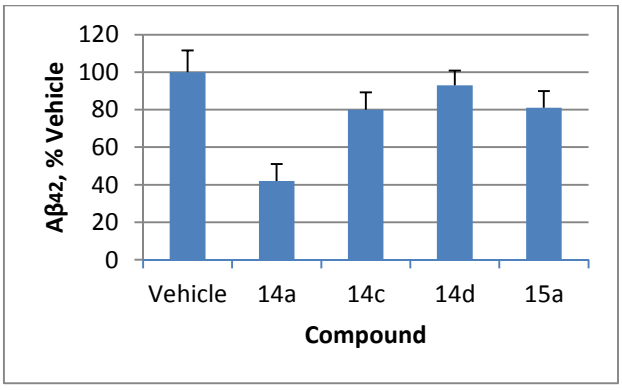

Figure 3. CSF $A\beta_{42}$ reduction in rat*

* Measured 3 h after an acute oral dosing at 30 mg/kg compared to the vehicle

Compound **14a** was the most efficacious among compounds tested, albeit it was not as potent as **14c** *in vitro*. To get an insight into this discrepancy, plasma and brain drug levels were assessed in the rat tissues collected after dosing. We concluded that the efficacy of **14a** was driven by its excellent penetration into the CNS compartment, which provided a ~6-fold higher exposure compared to **14c** (Table 2). This conclusion was supported by the fact that **14c** is a PGP efflux substrate (P_{a-b} = 68 nm/s, P_{b-a} = 462 nm/s, ER = 6.8). The efflux ratio of **14c** is 4-fold higher than **14a** (P_{a-b} = 376 nm/s, P_{b-a} = 600 nm/s, and ER= 1.6). The higher efflux ratio of **14c** could be the cause for its low brain penetration and poor *in vivo* efficacy.

Table 2. Drug levels in plasma and brain

Compound	Plasma Concentration (μM)*	Brain Concentration (μM)*
14a	10.4	14.3
14c	6.01	2.19

*measured 3 h after the dosing (n = 3)

In summary, we designed and synthesized a series of GSM compounds containing a unique tetrahydrobenzisoxazole ring. The unique tetrahydrobenzisoxazole ring successfully replaced the double bond in previously published GSMs. This series contained several compounds which are potent GSMs with good to excellent selectivity. Specifically, **14a** and **14c**, are two-fold more active than **1** and have similar activity to GSM **2** and **3**. In addition, **14a** and **14c** are more selective than GSM **1** and **3**, and have similar selectivity to **2**. Selected compounds were tested *in vivo*. Among them, compound **14a** significantly reduced CSF $A\beta_{42}$ by 58% after 3 h, dosed orally at 30 mg/kg in rats compared to vehicle-treated animals.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Biological assay protocols, general experimental descriptions, procedures and characterization of final compounds. (File type, PDF)

AUTHOR INFORMATION

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ABBREVIATIONS

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; ; AE, adverse effects; APP, amyloid precursor protein; BACE, β-amyloid cleaving enzyme; CSF, cerebrospinal fluid; DEAD, diethyl azodicarboxylate; GSM, γ-secretase modulators; GSI, γ-secretase inhibitor; NCS, N-chlorosuccinimide; TMS, trimethylsilyl.

Notes

The authors are or were employees of Merck.

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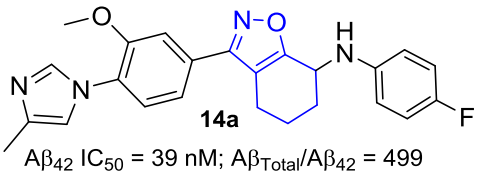

14a

$A\beta_{42}$ IC_{50} = 39 nM; $A\beta_{Total}/A\beta_{42}$ = 499
58% reduction of rat CSF $A\beta_{42}$ at 30 mpk oral dosing