Discovery of a Tetrahydrobenzisoxazole Series of γ -Secretase ModulatorsZhiqiang Zhao,^{*,†} Dmitri A. Pissarnitski,[†] Xianhai Huang,[†] Anandan Palani,[†] Zhaoning Zhu,[†] William J. Greenlee,[†] Lynn A. Hyde,[‡] Lixin Song,[‡] Giuseppe Terracina,[‡] Lili Zhang,[‡] and Eric M. Parker[‡][†]Department of Medicinal Chemistry and [‡]Department of Neurobiology, Merck Research Laboratories, 2000 Galloping Hill Road, Kenilworth, New Jersey 07033, United States

Supporting Information

ABSTRACT: The design and synthesis of a new series of tetrahydrobenzisoxazoles as modulators of γ -secretase activity and their structure–activity relationship (SAR) 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 nontransgenic 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 3 h after an acute oral dosing at 30 mg/kg).

KEYWORDS: Alzheimer's disease, γ -secretase modulators, tetrahydrobenzisoxazole

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.

$A\beta$ 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 $A\beta$ peptides; however, deposited $A\beta$ 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 $A\beta$ 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 $A\beta$ 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 research-

ers.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 Eisai¹⁷ and **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

Figure 1. Design of GSMs from known structures.

bond present in structures **1–3** mitigate 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**.

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

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Scheme 1. Synthesis of Intermediates 12 and 13

From previously known SAR, the left side of 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 R² is *N*-methylamino, the A β ₄₂ inhibition activity is only 12 μ M with no selectivity (**14h**, clogD = 1.07, pK_a = 8.10).

The reaction scheme illustrates the synthesis of 14a-d and 17a-b from compound 12. Compound 12, 2-(4-methoxyphenyl)-2-(4-methyl-1H-imidazol-2-yl)-2-nitroso-1,2,3,4-tetrahydro-1H-benzocyclohepta[b]pyridine, reacts with R-MgBr in THF at 0 °C to form 15a-b, 2-(4-methoxyphenyl)-2-(4-methyl-1H-imidazol-2-yl)-2-nitroso-1,2,3,4-tetrahydro-1H-benzocyclohepta[b]pyridine-1-ol. Compound 15a-b is then treated with TMSCl and NaI in CH₃CN at 60 °C to form 16, 2-(4-methoxyphenyl)-2-(4-methyl-1H-imidazol-2-yl)-2-nitroso-1,2,3,4-tetrahydro-1H-benzocyclohepta[b]pyridine-1-yl 4,4'-difluorobiphenyl ether. Compound 16 is then treated with Ar-CH₂-Br and NaH/DMF at 0 °C to form 18, 2-(4-methoxyphenyl)-2-(4-methyl-1H-imidazol-2-yl)-2-nitroso-1,2,3,4-tetrahydro-1H-benzocyclohepta[b]pyridine-1-yl 4-(4-fluorophenyl)methyl ether. Compound 12 also reacts with 1. R-NH₂, THF, Ti(OPr-i)₄, 60 °C to form 14a-j, 2-(4-methoxyphenyl)-2-(4-methyl-1H-imidazol-2-yl)-2-nitroso-1,2,3,4-tetrahydro-1H-benzocyclohepta[b]pyridine-1-yl R-amine. Compound 14a-j is then treated with 2. NaBH₄, EtOH, 0 °C and 3. Chiral OD column separation to form 14a-d. Compound 12 also reacts with R-OH, DEAD, Ph₃P, THF, 50 °C to form 17a-b, 2-(4-methoxyphenyl)-2-(4-methyl-1H-imidazol-2-yl)-2-nitroso-1,2,3,4-tetrahydro-1H-benzocyclohepta[b]pyridine-1-yl R-ether.

Mass spectrometric analysis of A β peptides secreted by HEK293 cells carrying the London–Swedish double mutation after treatment with vehicle (DMSO) or 10 μ M solutions of compounds **14a**, **14c**, and **14e** is shown in Figure 2. As

Table 1. Cellular IC₅₀ of Tetrahydrobenzisoxazole Analogues

Compound	R ¹	R ²	Aβ ₄₂ IC ₅₀ , (μM)*	Aβ _{tot} /Aβ 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

Compound	R ¹	R ²	Aβ ₄₂ IC ₅₀ , (μM)*	Aβ _{tot} /Aβ ₄₂
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.

expected for γ -secretase modulators, compounds **14a**, **14c**, and **14e** blocked the production of Aβ₄₂ and Aβ₄₀, while the relative proportion of Aβ₃₈ and Aβ₃₇ is increased in comparison to Aβ₄₀ (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

Compounds **14a**, **14c**, **14d**, and **15a** were then tested using an *in vivo* rat model. Compound **14a** significantly reduced cerebrospinal fluid (CSF) Aβ₄₂ by 58% (*p* = 0.0066, *n* = 5) 3 h after a single oral dose at 30 mg/kg in rats compared to the vehicle-treated animals (Figure 3).

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 ~six-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 four-fold higher than **14a** (*P*_{a-b} = 376 nm/s, *P*_{b-a} = 600 nm/s, ER = 1.6). The higher efflux ratio of **14c** could be the cause for its low brain penetration and poor *in vivo* efficacy.

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 that are potent GSMs with good to excellent selectivity. Specifically, **14a** and **14c** are two-fold

Figure 2. Mass spectrometric analysis of A β profile.

Figure 3. CSF A β_{42} reduction in rat, measured 3 h after an acute oral dosing at 30 mg/kg compared to the vehicle.

Table 2. Drug Levels in Plasma and Brain

compd	plasma concentration (μM)*	brain concentration (μM)*
14a	10.4	14.3
14c	6.01	2.19

*Measured 3 h after the dosing ($n = 3$).

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 β_{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 at DOI: 10.1021/acsmchemlett.7b00178.

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

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AD, Alzheimer's disease; A β , 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

■ REFERENCES

- (1) Alzheimer's Association. 2017 Alzheimer's Disease Facts and Figures. *Alzheimer's Dementia* **2017**, 13, 325–373.
- (2) Esparza, T. J.; Zhao, H.; Cirrito, J. R.; Cairns, N. J.; Bateman, R. J.; Holtzman, D. M.; Brody, D. L. Amyloid-beta Oligomerization in Alzheimer Dementia vs. High Pathology Controls. *Ann. Neurol.* **2013**, 73 (1), 104–119.
- (3) Sadigh-Eteghad, S.; Sabermarouf, B.; Majdi, A.; Talebi, M.; Farhoudi, M.; Mahmoudi, J. Amyloid-Beta: A Crucial Factor in Alzheimer's Disease. *Med. Princ. Pract.* **2015**, 24 (1), 1–10.
- (4) Kreft, A. F.; Martone, R.; Porte, A. Recent Advances in the Identification of γ -Secretase Inhibitors to Clinically Test the A β Oligomer Hypothesis of Alzheimer's Disease. *J. Med. Chem.* **2009**, 52, 6169–6188.
- (5) Irvine, G. B.; El-Agnaf, O. M.; Shankar, G. M.; Walsh, D. M. Protein Aggregation in the Brain: The Molecular Basis for Alzheimer's and Parkinson's Diseases. *Mol. Med.* **2008**, 14 (7–8), 451–464.
- (6) Gu, L.; Guo, Z. Alzheimer's A β_{42} and A β_{40} Peptides form Interlaced Amyloid Fibrils. *J. Neurochem.* **2013**, 126 (3), 305–311.
- (7) Chen, Y.; Glabe, C. G. Distinct Early Folding and Aggregation Properties of Alzheimer Amyloid- β Peptides A β_{40} and A β_{42} . *J. Biol. Chem.* **2006**, 281 (34), 24414–24422.
- (8) Jonsson, T.; Atwal, J. K.; Steinberg, S.; Snaedal, J.; Jonsson, P. V.; Bjornsson, S.; Stefansson, H.; Sulem, P.; Gudbjartsson, D.; Maloney, J.; Hoyte, K.; Gustafson, A.; Liu, Y.; Lu, Y.; Bhangale, T.; Graham, R. R.; Huttenlocher, J.; Bjornsdottir, G.; Andreassen, O. A.; Joensson, E. G.; Palotie, A.; Behrens, T. W.; Magnusson, O. T.; Kong, A.; Thorsteinsdottir, U.; Watts, R. J.; Stefansson, K. A Mutation in APP Protects Against Alzheimer's Disease and Age-Related Cognitive Decline. *Nature* **2012**, 488, 96–99.
- (9) Yan, R.; Vassar, R. Targeting the β -Secretase BACE1 for Alzheimer's Disease Therapy. *Lancet Neurol.* **2014**, 13, 319–329.
- (10) Choi, S. H.; Norstrom, E. Moderate Reduction of γ -Secretase: Is There a Therapeutic Sweet Spot? *J. Neurosci.* **2007**, 27, 13579–13580.
- (11) Bursavich, M. G.; Harrison, B. A.; Blain, J.-F. Gamma Secretase Modulators: New Alzheimer's Drugs on the Horizon? *J. Med. Chem.* **2016**, 59, 7389–7409.
- (12) Xia, W.; Wong, S. T.; Hanlon, E.; Morin, P. γ -Secretase Modulator in Alzheimer's Disease: Shifting the End. *J. Alzheimer's Dis* **2012**, 31, 685–696.
- (13) Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrzik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Bulter, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. A Subset of NSAIDs Lower Amyloidogenic A β_{42} Independently of Cyclooxygenase Potency. *Nature* **2011**, 414, 212–216.

- (14) Crump, C. J.; Johnson, D. S.; Li, Y.-M. Development and Mechanism of γ -Secretase Modulators for Alzheimer's Disease. *Biochemistry* **2013**, *52*, 3197–3216.
- (15) Joo, Y.; Kim, H.-S.; Woo, R.-S.; Park, C. H.; Shin, K.-Y.; Lee, J.-P.; Chang, K.-A.; Kim, S.; Suh, Y.-H. Mefenamic Acid Shows Neuroprotective Effects and Improves Cognitive Impairment in in vitro and in vivo Alzheimer's Disease Models. *Mol. Pharmacol.* **2006**, *69*, 76–84.
- (16) Davis, K. L. NSAID and Alzheimer's Disease; Possible Answers and New Questions. *Mol. Psychiatry* **2002**, *7*, 925–926.
- (17) Kimura, T.; Kawano, K.; Doi, E.; Kitazawa, N.; Shin, K.; Miyagawa, T.; Kaneko, T.; Ito, K.; Takaishi, M.; Sasaki, T.; Hagiwara, H. Preparation of Cinnamide, 3-Benzylidenepiperidin-2-one, Phenylpropynamide Compounds as Amyloid β Production Inhibitors. PCT Int. Appl. 2005, WO 2005115990 A1 20051208.
- (18) Sun, Z.-Y.; Asberom, T.; Bara, T.; Bennett, C.; Burnett, D.; Chu, I.; Clader, J.; Cohen-Williams, M.; Cole, D.; Czarniecki, M.; Durkin, J.; Gallo, G.; Greenlee, W.; Josien, H.; Huang, X.; Hyde, L.; Jones, N.; Kazakevich, I.; Li, H.; Liu, X.; Lee, J.; MacCoss, M.; Mandal, M. B.; McCracken, T.; Nomeir, A.; Mazzola, R.; Palani, A.; Parker, E. M.; Pissarnitski, D. A.; Qin, J.; Song, L.; Terracina, G.; Vicarel, M.; Voigt, J.; Xu, R.; Zhang, L.; Zhang, Q.; Zhao, Z.; Zhu, X.; Zhu, Z. Cyclic Hydroxyamidines as Amide Isosteres: Discovery of Oxadiazolines and Oxadiazines as Potent and Highly Efficacious γ -Secretase Modulators in vivo. *J. Med. Chem.* **2012**, *55*, 489–502.
- (19) Huang, X.; Zhou, W.; Liu, X.; Li, H.; Sun, G.; Mandal, M.; Vicarel, M.; Zhu, X.; Bennett, C.; McCracken, T.; Pissarnitski, D.; Zhao, Z.; Cole, D.; Gallo, G.; Zhu, Z.; Palani, A.; Aslanian, R.; Clader, J.; Czarniecki, M.; Greenlee, W.; Burnett, D.; Cohen-Williams, M.; Hyde, L.; Song, L.; Zhang, L.; Chu, I.; Buevich, A. Synthesis and SAR Studies of Fused Oxadiazines as γ -Secretase Modulators for Treatment of Alzheimer's Disease. *ACS Med. Chem. Lett.* **2012**, *3*, 931–935.
- (20) Haugabook, S. J.; Yager, D. M.; Eckman, E. A.; Golde, T. E.; Younkin, S. G.; Eckman, C. B. High Throughput Screens for the Identification of Compounds That Alter the Accumulation of The Alzheimer's Amyloid Beta Peptide (A β). *J. Neurosci. Methods* **2001**, *108*, 171–179.
- (21) Findeis, M. A.; Schroeder, F. C.; Creaser, S. P.; McKee, T. D.; Xia, W. Natural Product and Natural Product-Derived Gamma Secretase Modulators from *Actaea Racemosa* Extracts. *Medicines* **2015**, *2*, 127–140.
- (22) Huang, X.; Aslanian, R.; Zhou, W.; Zhu, X.; Qin, J.; Greenlee, W.; Zhu, Z.; Zhang, L.; Hyde, L.; Chu, I.; Cohen-Williams, M.; Palani, A. The Discovery of Pyridone and Pyridazone Heterocycles as γ -Secretase Modulators. *ACS Med. Chem. Lett.* **2010**, *1*, 184–187.
- (23) Qin, J.; Zhou, W.; Huang, X.; Dhondi, P.; Palani, A.; Aslanian, R.; Zhu, Z.; Greenlee, W.; Cohen-Williams, M.; Jones, N.; Hyde, L.; Zhang, L. Discovery of a Potent Pyrazolopyridine Series of γ -Secretase Modulators. *ACS Med. Chem. Lett.* **2011**, *2*, 471–476.
- (24) Qin, J.; Dhondi, P.; Huang, X.; Mandal, M.; Zhao, Z.; Pissarnitski, D.; Zhou, W.; Aslanian, R.; Zhu, Z.; Greenlee, W.; Clader, J.; Zhang, L.; Cohen-Williams, M.; Jones, N.; Hyde, L.; Palani, A. Discovery of Fused 5,6-Bicyclic Heterocycles as γ -Secretase Modulators. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 664–669.
- (25) Caldwell, J. P.; Bennett, C. E.; McCracken, T. M.; Mazzola, R. D.; Bara, T.; Buevich, A. V.; Burnett, D. A.; Chu, I.; Cohen-Williams, M.; Jones, N. T.; Josien, H.; Hyde, L. A.; Lee, J.; McKittrick, B.; Song, L.; Terracina, G.; Voigt, J. H.; Zhang, L.; Zhu, Z. Iminoheterocycles as Gamma-Secretase Modulators. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5380–5384.
- (26) Zhu, Z.; Greenlee, W. J.; Li, H.; Vicarel, M. L.; Qin, J.; Dhondi, P. K.; Huang, X.; Palani, A.; Liu, X.; Sun, Z.-Y.; Josien, H. B.; Xu, R.; Cole, D. J.; Burnett, D. A.; Bennett, C. E.; McCracken, C. M.; Maccoss, M. Heterocyclic compounds as gamma-secretase modulators and their preparation and use in the treatment of CNS diseases. PCT Int. Appl. 2010, WO 2010056722 A1 20100520.
- (27) Zhu, Z.; Greenlee, W. J.; Cole, D. J.; Pissarnitski, D. A.; Gallo, G. V.; Li, H.; Josien, H. B.; Qin, J.; Knutson, C. E.; Mandal, M.; Vicarel, M. L.; Rajagopalan, M.; Dhondi, P. K.; Xu, R.; Sun, Z.-Y.; Bara, T. A.; Huang, X.; Zhu, X.; Zhao, Z.; Clader, J. W.; Palani, A.; Asberom, T.; McCracken, T.; Bennett, C. E. Gamma Secretase Modulators. PCT Int. Appl. 2010, WO 2010054078 A1 20100514.
- (28) Greenlee, W. J.; Pissarnitski, D. A.; Zhao, Z.; Zhu, Z. Gamma Secretase Modulators. PCT Int. Appl. 2013, WO 2013066740 A1 20130510.
- (29) Sakai, T.; Miyata, K.; Utaka, M.; Takeda, A. Me₃SiCl-NaI-CH₃CN as an Efficient and Practical Reducing Agent for Benzylic Alcohols. *Tetrahedron Lett.* **1987**, *28* (33), 3817–3818.
- (30) Please see the [Supporting Information](#) for details.
- (31) Rankovic, Z. CNS Drug Design: Balancing Physicochemical Properties for Optimal Brain Exposure. *J. Med. Chem.* **2015**, *58*, 2584–2608.
- (32) Charifson, S. P.; Walters, W. P. Acidic and Basic Drugs in Medicinal Chemistry. *J. Med. Chem.* **2014**, *57*, 9701–9717.
- (33) Lee, J.; Song, L.; Terracina, G.; Bara, T.; Josien, H.; Asberom, T.; Sasikumar, T. K.; Burnett, D. A.; Clader, J.; Parker, E. M.; Zhang, L. Identification of Presenilin 1-Selective γ -Secretase Inhibitors With Reconstituted γ -Secretase Complexes. *Biochemistry* **2011**, *50*, 4973–4980.