Bioorganic & Medicinal Chemistry Letters 23 (2013) 466–471

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The discovery of fused oxadiazepines as gamma secretase modulators for treatment of Alzheimer's disease

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ARTICLE INFO

Article history:
Received 30 September 2012
Revised 10 November 2012
Accepted 14 November 2012
Available online 29 November 2012

Keywords:
Alzheimer's disease
Oxadiazepine
Amyloid precursor protein
Gamma secretase modulator
hERG

ABSTRACT

In an attempt to further improve overall profiles of the oxadiazine series of GSMs, in particular the hERG activity, conformational modifications of the core structure resulted in the identification of fused oxadiazepines such as **7i** which had an improved hERG inhibition profile and was a highly efficacious GSM in vitro and in vivo in rats. These SAR explorations offer opportunities to identify potential drugs to treat Alzheimer's disease.

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that affects millions of older people in the United States. AD is characterized by neuronal loss, neurofibrillary tangle (NFT) formation, and extracellular deposition of amyloid-β (Aβ) peptide plaques. Patients normally show symptoms of cognitive impairment, as well as disturbance in language, memory, movement, attention, and orientation. It is estimated that more than 35 million people currently suffer from AD worldwide, with an annual cost of over $600 billion and a potential population increase to more than 115 million patients by 2050.[1] Despite enormous efforts AD, there is no solution to this medical problem.[2] Due to this urgent unmet medical need, academic and industrial laboratories are working very aggressively to develop therapies to halt or even reverse AD. It is believed that the accumulation of amyloid-beta (Aβ) peptide and hyperphosphorylated protein tau contribute to AD progression.[3] Aβ peptide is formed from a larger amyloid precursor protein (APP) via sequential proteolytic cleavage by β- and γ-secretases. γ-Secretase cleaves the APP C-terminal fragment at multiple sites leading to Aβ peptides of 37–42 amino acids. Of these peptides $Aβ_{42}$, the more hydrophobic form, is the most amyloidogenic and neurotoxic. While development of γ-secretase inhibitors (GSIs) holds promise for the treatment of AD,[4] recent preclinical experiments demonstrated that inhibition of γ-secretase resulted in mechanism-based GI toxicity such as thymus atrophy and intestinal goblet cell hyperplasia. This toxicity is due to disruption of the processing of other γ-secretase substrates such as Notch, which is important for cellular gene transcription,[5] and ERB4. Recent clinical results of the GSI semagacestat showed that it not only failed to slow disease progression but also increased the incidence of skin cancer of patients in the treatment group compared to the placebo group.[6] Although the molecular mechanism of action remains largely unknown, γ-secretase modulators (GSMs) are believed to act at an allosteric site to shift the predominant site of γ-secretase cleavage toward shorter, non-amyloidogenic peptides (e.g., Aβ38), without blocking overall γ-secretase function.[7] This potentially offers a better selectivity window over GSIs versus for example notch processing. From an in vitro point of view, $Aβ_{total}/Aβ_{42}$ ratio can be used to distinguish GSMs from GSIs with a ratio of >10 for GSMs and <3 for GSIs. There are two major classes of GSMs in clinical trials: the non-steroidal anti-inflammatory acids (NSAIDs),[8] and the non-NSAID class such as the lactam **E-2012** from Eisai.[9] Among our efforts in the AD area,[10] we have recently identified cyclic hydroxyamidines such as oxadiazolines (**1**) and oxadiazines (**2**) as highly efficacious GSMs in both in vitro studies and in vivo animal models. They were found to not only be

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http://dx.doi.org/10.1016/j.bmcl.2012.11.055

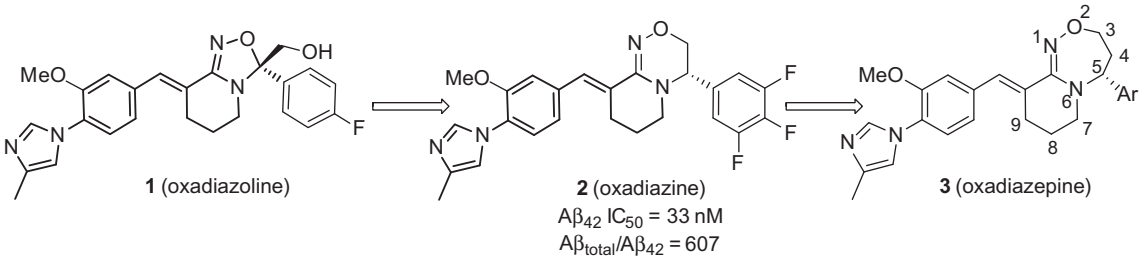


Figure 1. Design rationale of oxadiazepine GSMs.

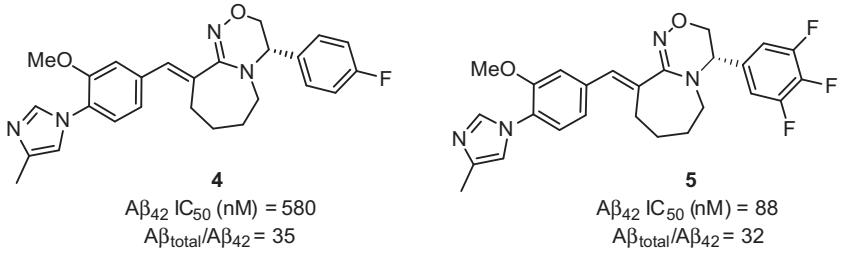
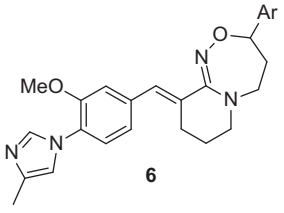
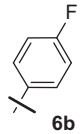
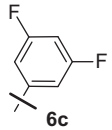
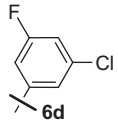
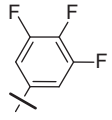


Figure 2. Activity of fused azepane oxadiazine analogs.

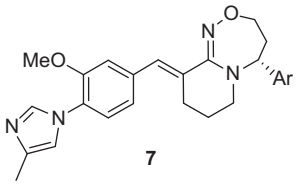
Table 1
SAR studies of C3 mono-substituted oxadiazepines[a]



Ar	$A\beta_{42}$ IC_{50} (nm)	$A\beta_{total}/A\beta_{42}$ ratio	hERG inhibition[b] (%)
 **6a**	41	489	93
 **6b**	43	465	93
 **6c**	41	371	nd
 **6d**	64	262	61
 **6e**	53	364	89

[a] Each IC_{50} value is an average of at least two determinations.
[b] At 10 μM.

Table 2
SAR studies of C5 mono-substituted oxadiazepines[a]



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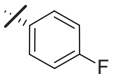
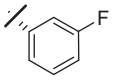
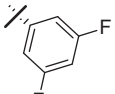
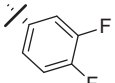
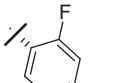
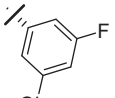
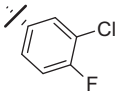
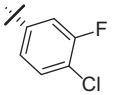
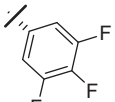
Ar	$A\beta_{42}$ IC_{50} (nm)	$A\beta_{total}/A\beta_{42}$ ratio	CSF $A\beta_{42}$ reduction[b] (%)	hERG inhibition[c] (%)
 **7a**	49	409	−40	81
 **7b**	35	572	nd	86
 **7c**	37	392	−39	49
 **7d**	24	466	−48	56
 **7e**	52	354	−23	nd
 **7f**	16	480	−59	53
 **7g**	40	340	−38	39
 **7h**	95	210	−57	nd
 **7i**	39	510	−61	33

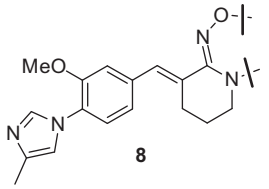
[a] Each IC_{50} value is an average of at least two determinations.
[b] 10 mpk Oral acute dosing at 3 h time point on average 2–3 tests.
[c] At 10 μM.

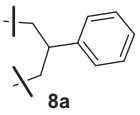
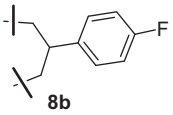
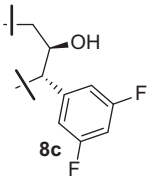
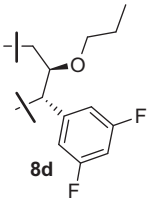
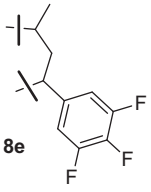
chemically stable but also possess highly desirable pharmacokinetic and toxicological profiles. To further improve the overall profile, especially the hERG activity of compound **2** (76% inhibition of hERG at 10 μM), we continued our search for next generation GSMs. Herein we report the identification of a novel class of fused oxadiazepine GSMs (**3**, Fig. 1).

Structural properties such as the conformation of the molecule, basicity and π–π interactions impact the hERG activity of a molecule.[11] We reasoned that it might be possible to improve the hERG profile observed with compound **2** by modifying the oxadiazine core structure (first-degree SAR studies[12]), thereby impacting its interaction with the hERG protein. Thus we prepared the 7,6-member ring fused azepane oxadiazines **4** and **5** (Fig. 2), but found that **4** lost much in vitro activity. Compound **5** had good $A\beta_{42}$ inhibition activity but selectivity decreased by almost 20-fold. This modification suggested that fused piperidinyl ring in **2** was important for both activity and selectivity. Based on this information, we turned our attention to the preparation of fused piperidinyloxadiazepines (**3**) and focused on C3, C4 and C5 modifications.

We first prepared C3 mono-substituted oxadiazepines and focused on introducing aryl substitutions since these were important for potency in the oxadiazine series.[10a] As summarized in Table 1,

Table 3
SAR studies of C4 mono-, C4–C5 and C3–C5 di-substituted oxadiazepines[a]

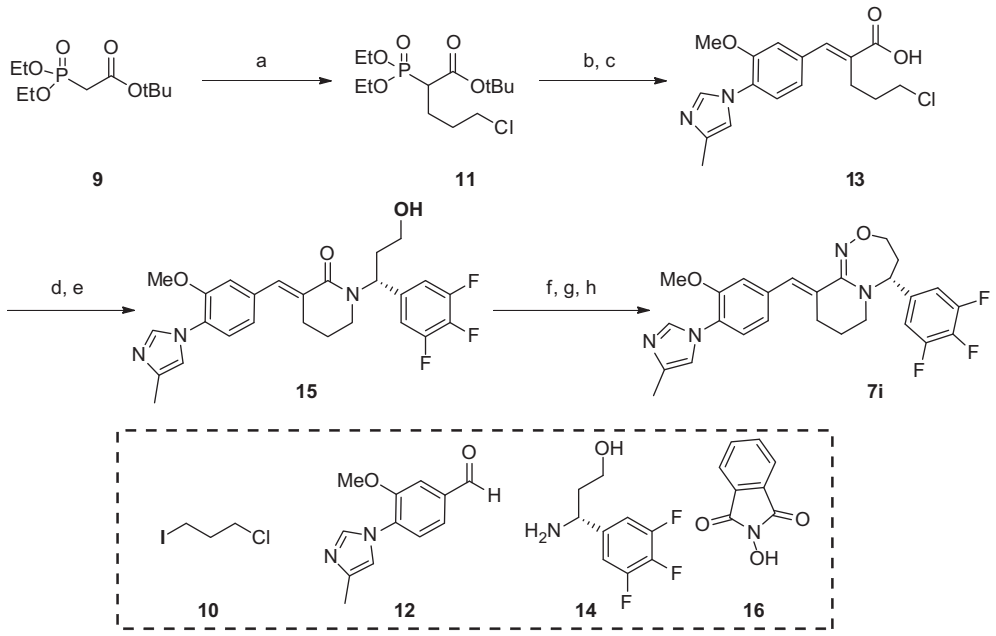


Ar	$A\beta_{42}$ IC_{50} (nm)	$A\beta_{total}/A\beta_{42}$ ratio	hERG inhibition[b] (%)
 **8a**	140	140	nd
 **8b**	130	150	90
 **8c**	240	83	55
 **8d**	1260	16	42
 **8e**	41	480	nd

[a] Each IC_{50} value is an average of at least two determinations.
[b] At 10 μM.

all these modifications retained good in vitro activity and selectivity (**6a**–**6e**) compared to the oxadiazine series. However, little reduction of hERG activity was observed. While this was disappointing, we nonetheless turned our attention to C5 modifications to complete the SAR (Table 2). Gratifyingly, C5-aryl substituted oxadiazepines again showed very good in vitro $A\beta_{42}$ activity and selectivity. With respect to hERG inhibition, mono fluorinated compounds **7a** and **7b** were not improved, however dihalogenation (**7c**–**7h**) moderately reduced hERG activity. The trifluorinated compound **7i** had a hERG inhibition of 33% at 10 μM. In order to differentiate these compounds for further follow up, they were tested in in vivo rat CSF $A\beta_{42}$ reduction studies (10 mpk oral acute dosing at 3 h time point). Monofluorinated compound **7a** showed 40% $A\beta_{42}$ reduction in vivo, while the 3,5- (**7c**) and 3,4-di-fluorinated (**7d**) compounds showed similar in vivo activity whereas 2,4-di-fluorination resulted in loss of activity (**7e**). On the other hand, fluorochlorination and trifluorination improved in vivo activity with compounds **7f**, **7h** and **7i** having similar $A\beta_{42}$ CSF reduction. With an improved hERG profile and good in vivo activity, compound **7i** was further characterized in rising dose in vivo studies, PK and ancillary profile evaluations. Acute oral administration of **7i** to rats dose dependently reduced $A\beta_{42}$ levels in CSF at the 3 h time point (−78% at 30 mpk, −61% at 10 mpk, and −43% at 3 mpk). The oral 10 mpk dose showed very good exposure with a plasma AUC of 15,800 nM h, a high brain concentration (1803 ng/g) and a brain/plasma ratio of 3.2 at 6 h post-dose. The compound was clean in CYP P450 inhibition (CYP3A4, 2D6, 2C9 IC_{50} >30 μM) and PXR enzyme induction studies (relative signal strength to control, CYP1A1: 2%, 2B1/2: 0%, 3A1: 0%), when tested at concentrations up to 30 μM.

We also prepared C4 mono-, C4–C5 and C3–C5 di-substituted oxadiazepines to determine whether further improvement of hERG activity could be achieved. As shown in Table 3, C4 mono-substitution didn't help to improve either activity (**8a** and **8b**) or the hERG profile (**8b**). The introduction of a polar hydroxy group at C4 of the C4–C5 di-substituted compound **8c** led to a small decrease in activity and resulted in comparable hERG activity to **7c** (without OH at C4). Alkylation on C4 hydroxy (**8d**) resulted in further loss of activity and selectivity. On the other hand, C3–C5 di-substitution (**8e**, mixture of isomers) showed good $A\beta_{42}$ inhibition and selectivity which provided opportunity for further SAR studies.



Scheme 1. The Synthesis of fused oxadiazepine GSMs. Reagents and conditions: (a) **10**, NaH, THF, 95%; (b) **12**, LiOH, THF; (c) TFA, 90% over two steps; (d) **14**, EDCI/HOBt, DMF; (e) NaOMe, MeOH, 75% over two steps; (f) **16**, 1,1′-(azodicarbonyl)dipiperidine, PBu_3, THF, 80 °C, 65%; (g) $NH_2NH_2{\cdot}xH_2O$, CH_3CN, 75%; (h) P_2O_5, EtOH, 80 °C, 35%.

The synthesis of the fused oxadiazepine analogs was similar to that of the fused oxadiazines[10a] (Scheme 1). Commercially available ester **9** was alkylated with iodide **10** under basic conditions to give chloride **11**. A Wittig reaction between **11** and aldehyde **12** proceeded smoothly under mild basic conditions to give an *E*-alkene, which was deprotected with TFA to provide acid **13**. Compound **13** was then coupled with the appropriate aminoalcohol **14** using EDCI/HOBt, and subsequently cyclized in the presence of NaOMe to give primary alcohol **15**. Compound **15** was reacted with N-hydroxyphthalimide **16** under Mitsunobu reaction conditions, followed by deprotection of the nitrogen with hydrazine hydrate to give an alkoxyamine intermediate which was cyclized to the desired final product **7i** under dehydrative conditions.

In summary, in an attempt to improve the hERG profile of lead oxadiazine GSM **2**, conformational modification of the core structure resulted in the identification of fused oxadiazepines as potent GSMs with compound **7i** showing comparable in vitro and in vivo activity to **2** and an improved hERG profile. This compound was highly efficacious in vitro and in vivo in rats with highly desirable physical and pharmacological properties. Further exploration of these compounds as potential drugs to treat Alzheimer's disease is underway and will be the subject of future communications.

Acknowledgments

We thank Drs. Andrew Stamford, Christopher Boyce and Chad Bennett for comments on the preparation of the manuscript. We thank Dr. Eric Parker for his strong support of the program. We thank Drs. Alexei Buevich and Li-Kang Zhang for technical support.

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