Bioorganic & Medicinal Chemistry 17 (2009) 2017–2029

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and structure–activity relationships of 1,3,4-oxadiazole derivatives as novel inhibitors of glycogen synthase kinase-3β

Morihisa Saitoh [a,*], Jun Kunitomo [a], Eiji Kimura [a], Yoji Hayase [b], Hiromi Kobayashi [a], Noriko Uchiyama [c], Tomohiro Kawamoto [c], Toshimasa Tanaka [c], Clifford D. Mol [d], Douglas R. Dougan [d], Garret S. Textor [d], Gyorgy P. Snell [d], Fumio Itoh [a]

[a] Medicinal Chemistry Research Laboratories, Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 17-85, Jusohonmachi 2-Chome, Yodogawa-ku, Osaka 532-8686, Japan
[b] Frontier Research Laboratories, Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 10 Wadai, Tsukuba-shi, Ibaraki 300-4293, Japan
[c] Discovery Research Center, Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd, 17-85, Jusohonmachi 2-Chome, Yodogawa-ku, Osaka 532-8686, Japan
[d] Takeda San Diego, Inc., 10410 Science Center Drive, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 5 December 2008
Revised 8 January 2009
Accepted 9 January 2009
Available online 15 January 2009

Keywords:
GSK-3β
Alzheimer's disease
Oxadiazole
Drug design

ABSTRACT

Glycogen synthase kinase-3β (GSK-3β) is implicated in abnormal hyperphosphorylation of tau protein and its inhibitors are expected to be a promising therapeutic agents for the treatment of Alzheimer's disease. Here we report design, synthesis and structure–activity relationships of a novel series of oxadiazole derivatives as GSK-3β inhibitors. Among these inhibitors, compound **20x** showed highly selective and potent GSK-3β inhibitory activity in vitro and its binding mode was determined by obtaining the X-ray co-crystal structure of **20x** and GSK-3β.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease having pathological features of neurofibrillary tangles (NFTs) and senile plaque. The temporal and spatial distribution of NFTs correlates well with the clinical disease severity. This suggests new strategies to inhibit factor(s) leading to NFT formation and neuronal cell death for the prevention of AD.[1–8] NFTs are composed of abnormally hyperphosphorylated microtubule-associated protein tau. Hyperphosphorylated tau loses its normal function of stabilizing microtubules, leading to a disruption in microtubule assembly and deficits in axonal transport. GSK-3β is a serine/threonine kinase and is thought to be a key factor for aberrant tau phosphorylation.[9] Activated GSK-3β coexists with progression of NFTs and neurodegeneration in the AD brain.[10–12] A conditional GSK-3β overexpressing transgenic mouse exhibits persistent tau hyperphosphorylation, pretangle-like somatodendritic localization of tau, neuronal death in hippocampus and cognitive deficits.[13,14] These studies suggest that GSK-3β is associated with AD progression, and GSK-3β inhibition is expected to be a promising therapeutic approach for AD.

Many GSK-3β inhibitors have been reported and they reviewed in the literature.[15–17] Maleimide derivatives have been reported from many groups.[18,19] In addition, CHIR98023,[20] SB-415286[21] and 2,5-diaminopyrimidines[22] with different chemical structure have been reported. Furthermore, natural product derived GSK-3β inhibitors such as indurubin,[23] paullones,[24] manzamines[25] have been described.

In this paper, we report the design and synthesis of novel small molecule GSK-3β inhibitors based on the 1,3,4-oxadiazole scaffold.

2. Inhibitor design

High-throughput screening of our proprietary compound collection identified hit compound **1** with an IC_{50} value of 65 nM in in vitro assay. The X-ray co-crystal structure of this compound with GSK-3β indicates that this compound binds to the ATP binding site and that the O1 oxygen atom and hydrogen atom on the C2-carbon of the benzodioxole made hydrogen bonds with the amide NH hydrogen and carbonyl oxygen of Val135 in the hinge region, respectively. The 4-methoxy-3-fluorobenzyl group fills the hydrophobic site and both the N3 and N4-nitrogen atoms of the oxadiazole are incorporated into the unique hydrogen bond network between Lys85-Glu97-Asp200 through two water molecules, as shown in Figure 1.

* Corresponding author. Tel.: +81 6 6300 6120; fax: +81 6 6300 6306.
E-mail address: Saitoh_Morihisa@takeda.co.jp (M. Saitoh).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.01.019

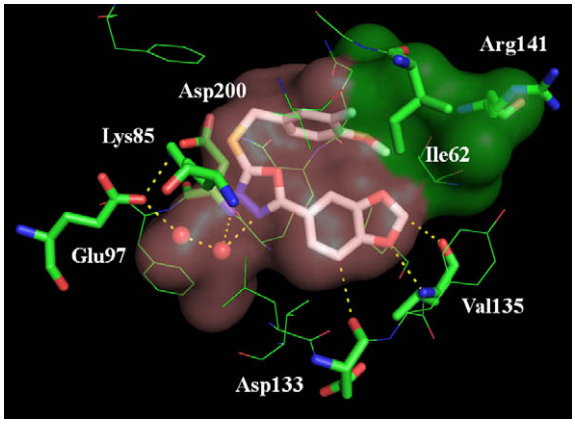

Figure 1. X-ray co-crystal structure of **1** in complex with GSK-3β. The figure was prepared with PyMOL.[26]

The X-ray co-crystal analysis suggested that the 1,3,4-oxadiazole was suitable for interaction with the Lys85-Glu97-Asp200 hydrogen bond network. Thus we fixed the 1,3,4-oxadiazole ring and designed novel compounds having 6,5 or 6,6-fused heterocyclic rings with heteroatom (Z = O, N) and hydrogen atom at appropriate positions for hydrogen bond interaction with the hinge region (Val135). In addition, hydrophobic amino acids were observed near the benzodioxole ring and Arg141 was found adjacent to the gatekeeper. To enhance potency, we also designed compounds incorporating a 4-methoxyphenyl group on the 6,5-fused heterocyclic core, aiming to fill in the hydrophobic site with a phenyl ring and interaction between Arg141 and methoxy group (Fig. 2).

3. Chemistry

The heterobicyclic esters and the hydrazide, the starting materials for oxadiazole derivatives **20a**–**y** were prepared as shown in Scheme 1. Reaction of commercially available aldehyde **4** with iodine and KOH gave ester **5**. Oxidation of dihydrobenzofuran ring of **5** with *N*-bromosuccinimide (NBS) yielded benzofuran **6**. Bromination of benzofuran **6** at the 3-position using bromine gave 3-bromobenzofuran **7** and subsequent coupling with 4-methoxyphenylboronic acid yielded 3-(4-methoxyphenyl)benzofuran **8**. Esterification of carboxylic acid **9** followed by reaction with *p*-anisidine or methylamine gave anilines **11a**,**b**. The nitro groups of **11a**,**b** were reduced with sodium hydrosulfite to provide anilines **12a**,**b**, which were cyclized with formic acid to form benzimidazoles **13a**,**b**. Condensation of the acid **14** with *tert*-butyl carbazate yielded *tert*-butyloxycarbonyl (Boc) hydrazide **15** and removal of the Boc group by exposure to trifluoroacetic acid (TFA) yielded hydrazide **16**.

The synthesis of oxadiazole derivatives **20a**–**y** was performed as outlined in Scheme 2. The heterobicyclic esters **5**,**6**,**8**,**13a**,**b**,**17a**–**c** were treated with hydrazine monohydrate to give hydrazides **18a**–**h**. Treatment of hydrazides **16**,**18a**–**g** in EtOH with carbon disulfide and potassium hydroxide or Et_3N yielded oxadiazolethiols **19a**–**h**. Oxadiazoles **20a**–**y** were prepared by benzylation of oxadiazolethiols **19b**–**d**,**f**–**h** or directly converted from the corresponding hydrazides **18a**,**d**,**h** without isolation of the intermediate oxadiazolethiols.

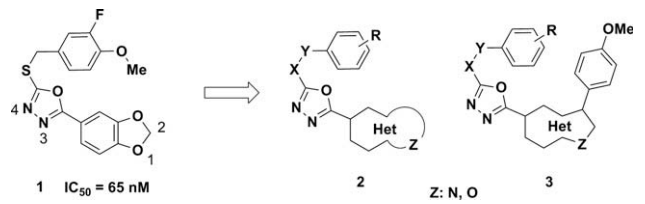

Figure 2. Structure of the hit compound **1** and design of 1,3,4-oxadiazole derivatives **2** and **3**.

Conversion of the linker (S–CH_2) between the oxadiazole and the phenyl ring to ethylene (CH_2CH_2), aminomethylene (NH–CH_2), oxymethylene (O–CH_2) and methylenethio (CH_2–S) is shown in Scheme 3. Condensation of hydrazide **18a** with 3-(3-fluorophenyl)propionic acid in phosphorus oxychloride provided phenethyl oxadiazole **21**. Reaction of **18a** with 3-fluorobenzyl isocyanate and subsequent cyclization using polystyrene-bound triphenylphosphine resin (PS–PPh_3) gave the benzylamino oxadiazole **23**. Methylation of thiol **19a** and subsequent oxidation of the sulfide yielded sulfone **25** followed by substitution of sulfone with 3-fluorobenzyl alcohol to give the benzyloxy oxadiazole **26**. Treatment of **18a** with 2-chloro-1,1,1-triethoxyethane yielded chloromethyl oxadiazole **27** and substitution of chloride with 3-fluorobenzenethiol provided **28**.

4. Results and discussion

The compounds were evaluated for GSK-3β inhibitory activity in a non-RI kinase assay using Kinase-Glo reagent (Promega, U.S.A.) and the results are shown in Table 1. Initially, we replaced the benzodioxole core as the hinge binding unit of compound **1** to dihydrobenzofuran. The dihydrobenzofuran derivative **20j** showed improved activity with an IC_{50} value of 44 nM compared to compound **1**.

Next, we examined the effect of substituents on the S-benzyl group of dihydrobenzofuran derivatives. Removal of the methoxy group and fluorine atom of **20j** led to unsubstituted phenyl derivative **20a**, which resulted in a 5-fold decrease in activity. The 3-fluorobenzyl derivative **20b** retained activity, however the 4-methoxybenzyl derivative **20c** showed less activity than **20j**. Introduction of a chlorine atom at the 3-position of the phenyl ring showed a 2-fold enhancement of activity compared to **20a**, while introduction at the 2- or 4-position showed no improvement of activity (**20d**,**f**). Compound **20g**, with an electron-withdrawing cyano group at the 3-position, showed similar activity to 3-chloro derivative **20e**, and introduction of a 3-trifluoromethyl group (**20i**) slightly increased activity compared to 3-chlorobenzyl derivative **20e**. However 3-methoxycarbonylbenzyl derivative **20h** was less potent than 3-chlorobenzyl derivative **20e**. It is speculated that introduction of an electron-withdrawing group of the appropriate size into the 3-position increased activity. Furthermore, introduction of a methoxy group at the 4-position of the phenyl ring in 3-chloro, 3-cyano and 3-trifluoromethyl benzyl derivatives (**20k**–**m**) resulted in high potency compared to compound **1** or benzyl derivatives monosubstituted at the 3-position (**20e**,**g**,**i**). Among these derivatives, 4-methoxy-3-(trifluoromethyl)benzyl derivative **20m** showed the most potent inhibitory activity with an IC_{50} of 5.7 nM. These results suggested that combination of an electron-withdrawing group at the 3-position and an electron-donating methoxy group at 4-position on the phenyl ring enhanced activity.

Next, we modified the linker between the oxadiazole and the phenyl ring. Replacement of the sulfur atom to carbon or nitrogen (**21**,**23**) resulted in a 6-fold less potency compared to **20b** and replacement of the sulfur atom with oxygen (**26**) markedly reduced potency. Phenylthiomethyl derivative **28** showed about 10-fold less potency than **20b**.

On the basis of these results, we further examined the effect of other heterocycles as potential hinge binders. Benzofuran derivative **20n** showed a slight increase in activity and benzothiazole derivative **20o** showed a 2-fold increase in activity compared to dihydrobenzofuran derivative **20m**. Indazole, imidazo[1,2-*a*]pyridine and quinoline derivatives **20p**–**r** showed decreased activity.