Bioorganic & Medicinal Chemistry Letters 27 (2017) 3726–3732

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel 2-(3-phenylpiperazin-1-yl)-pyrimidin-4-ones as glycogen synthase kinase-3β inhibitors

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ARTICLE INFO

Article history:
Received 29 March 2017
Revised 16 June 2017
Accepted 29 June 2017
Available online 5 July 2017

Keywords:
glycogen synthase kinase-3
Alzheimer's disease
phenylpiperazine

ABSTRACT

We herein describe the results of further evolution of glycogen synthase kinase (GSK)-3β inhibitors from our promising compounds containing a 2-phenylmorpholine moiety. Transformation of the morpholine moiety into a piperazine moiety resulted in potent GSK-3β inhibitors. SAR studies focused on the phenyl moiety revealed that a 4-fluoro-2-methoxy group afforded potent inhibitory activity toward GSK-3β. Based on docking studies, new hydrogen bonding between the nitrogen atom of the piperazine moiety and the oxygen atom of the main chain of Gln185 has been indicated, which may contribute to increased activity compared with that of the corresponding phenylmorpholine analogues. Effect of the stereochemistry of the phenylpiperazine moiety is also discussed.

Glycogen synthase kinase-3 (GSK-3) is a proline-directed serine-threonine kinase which was initially identified as an enzyme that was able to deactivate glycogen synthase through phosphorylation.[1] GSK-3 exists as two isoforms, GSK-3α (51 kDa) and GSK-3β (47 kDa) with high homology in their kinase domains. Both isoforms are ubiquitously expressed, with particularly high levels observed in the brain and testes.[2]

It has been reported that GSK-3β is involved in several physiological processes such as cell proliferation, neural function, embryonic development, apoptosis, immune response,[2d,3] and GSK-3β would be an attractive drug target for the treatment of Alzheimer's disease, Parkinson's disease, stroke, Huntington's disease, amyotrophic lateral sclerosis, type 2 diabetes, cancer and inflammation.[4]

We previously reported that 6-(4-pyridinyl or pyrimidin-4-yl)-3-methylpyrimidones with a 2-phenylmorpholine moiety at the 2-position were found to be potent GSK-3β inhibitors as useful therapeutic drugs for the treatment of Alzheimer's disease, and that UDA-680 was discovered as a promising compounds.[5] UDA-680 exhibited potent GSK-3β inhibitory activity and in vivo tau phosphorylation inhibitory activity by oral administration in mice. UDA-680 showed excellent cell permeability, and pharmacokinetic studies indicated that UDA-680 had high brain/plasma ratio with moderate bioavailability (37%, rat). In order to identify further promising compounds for clinical studies, we transformed the morpholine moiety of UDA-680 into a piperazine moiety in order to improve water solubility of UDA-680 and to evolve a new chemical series (Fig. 1). In this communication, we report synthesis and results of structure–activity relationship of 3-(substituted phenyl) piperazine analogues as well as computational docking studies.

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http://dx.doi.org/10.1016/j.bmcl.2017.06.078
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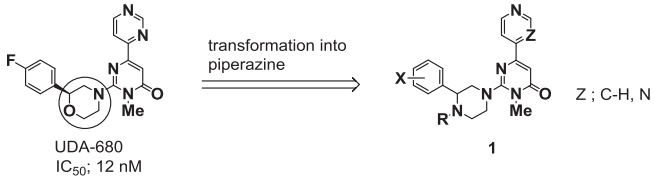


Fig. 1. Transformation of morpholine moiety.

First we examined the transformation of the morpholine moiety into the corresponding piperazine with no substitution on the nitrogen atom. 4-Pyridinyl and pyrimidin-4-yl groups were selected as a substituent at the 6-position of the pyrimidone moiety because our previous studies indicated that a 3-fluoropyridin-4-yl group which was also an effective substituent for the 6-position of the pyrimidone generally had potent CYP inhibition activity together with good GSK-3β inhibitory activity.[6] Racemic phenylpiperazine analogues with various substituents on the phenyl moiety were prepared and their effect toward GSK-3β inhibitory activity was examined (Table 1). This result indicated that the transformation into phenylpiperazine was preferable compared with IC_{50} of corresponding phenylmorpholine analogue (51 nM).[5,7] Introduction of a substituent on the phenyl moiety generally increased the inhibitory activity in the case of compounds with a 4-pyridinyl group. Methoxy and chlorine groups increased activity in all substituted positions (**3**, **5**, **7** and **17**, **19**, **21** respectively) and a fluorine group in 2- and 3-positions (**11** and **13**) and a bromine atom at the 2-position (**23**) also increased inhibitory activity. A 2-ethoxy group (**9**) showed no effects toward the activity despite 10-fold increase of activity by introduction of a 2-methoxy group (**3**) and further investigation of alkoxy substituents were not pursued. A cyano group which was generally known as a halogen mimic did not show clear effects toward the inhibitory activity (**28**). Phenylpiperazine analogues with a pyrimidin-4-yl moiety showed comparable or more potent inhibitory activity compared with those possessing a 4-pyridinyl moiety except 3-fluorophenyl analogue **14**. Pyrimidin-4-yl analogues with chlorine and bromine groups on the phenyl moiety showed 3 to 5-fold more potent activity than corresponding 4-pyridinyl analogues (**17** versus **18**, **19** versus **20** and **21** versus **22** for a chlorine atom and **23** versus **24** for a bromine atom).

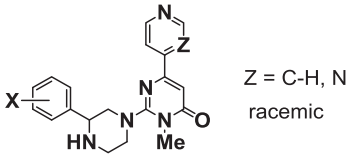
No clear tendency of the effect relating the substituent position was observed. For example, the 2-position was the most preferable position for a methoxy group with both 4-pyridyl and pyrimidin-4-yl groups (**3**, **4**), and the 3-position for a 4-pyridyl (**13**) and both 2- and 4-positions for a pyrimidin-4-yl (**12**, **16**) was the most preferable position toward GSK-3β inhibition for a fluorine atom. A chlorine atom at the 3- and 4-positions showed comparatively potent activity for both 4-pyridyl and pyrimidin-4-yl groups (**19** and **21**, **20** and **22**, respectively). Substituents except a fluorine atom showed a similar substituent effect for the phenyl group between the 4-pyridyl and the pyrimidin-4-yl analogues.

In vitro pharmacokinetic profiles were examined for methoxy-substituted phenylpiperazine analogues which showed potent inhibitory activity against GSK-3β (Table 2). 2- and 3-substituted analogues **3**, **5** showed potent CYP2D6 inhibition and poor metabolic stability, and 4-substituted analogue **7** showed weak CYP inhibition profile including CYP2D6 and good metabolic stability. These results indicate that a substituent at the 4-position may block the possible metabolism of the phenyl moiety and confer weak CYP inhibition activity, which resulted in preferable in vitro pharmacokinetic profiles.

Based on the information above, we further introduced substituents at the 4-position of the phenyl moiety to improve in vitro pharmacokinetic profiles and at the 2- or 3-positions to increase GSK-3β inhibitory activity (Table 3). 4-Fluoro-2-methoxy analogues, **30** and **31** showed potent GSK-3β inhibitory activity, and further substitution by a fluorine atom at the 6-position (**32** and **33**) resulted in two-fold potency decrease. On the other hand, other combinations of substituents did not increase the activity; thus, 4-fluoro-2-methyl analogue **34** was more than 20-fold less active compared to the corresponding 4-fluoro-2-methoxy analogue **30**, and 3, 4-disubstituted analogues showed no significant increase of activity compared to 3- or 4-monosubstitued analogues (**35**, **36** for methoxy groups and **37**, **38** for chlorine groups).

As we successively increased the inhibitory activity by introducing substituents at the 4- and 2-positions, we resolved **30** and **31** to investigate in vitro pharmacokinetic profiles of both enantiomers (Table 4). As we expected, **30** and **31** which possessed a substituent at the 4-position showed good metabolic stability,

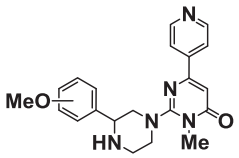
Table 1
GSK-3β inhibitory activity of substituted phenylpiperazines.



X	Z	GSK-3β inhibition (IC_{50}, nM)[1]					
		Position of the substituent X					
		2-		3-		4-	
H	C—H	**2**	18				
MeO	C—H	**3**	1.8	**5**	11	**7**	4.7
	N	**4**	1.8	6	6.7	**8**	3.5
EtO	C—H	**9**	25	–		–	
	N	**10**	20	–		–	
F	C—H	**11**	11	**13**	22	**15**	18
	N	**12**	8.2	**14**	12	**16**	8.6
Cl	C—H	**17**	9.6	**19**	5.4	**21**	6.6
	N	**18**	2.6	20	1.1	**22**	1.7
Br	C—H	**23**	13	–		**26**	22
	N	**24**	4.3	25	2.0	**27**	8.1
CN	C—H	–		–		28	15
	N	–		–		**29**	7.4

[1] Compounds were assayed as corresponding hydrochlorides except **15**, **16**, **17**, **18**, **19**, **20**, **23** and **24**.

Table 2
In vitro pharmacokinetic profiles of methoxy-substituted phenylpiperazines.



Compound	Position	GSK-3β inhibition (IC_{50}, nM)[1]	$CL_{int,\ mouse}$[2] (ml/min/mg protein)	CYP inhibition (IC_{50}, μM)		
				1A2[3]	2D6[3]	3A4[3]
3	2	1.8	0.158	>50	<0.2	>50
5	3	11	0.123	>50	0.4	>50
7	4	4.7	0.078	>53[4]	45[4]	>53[4]

[1] All compounds were assayed as corresponding hydrochlorides.
[2] Mouse liver microsome.
[3] Recombinant human CYP450.
[4] Human liver microsome.