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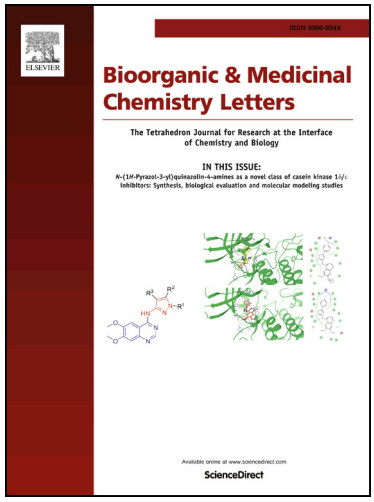

Discovery of novel 2-(4-aryl-2-methylpiperazin-1-yl)-pyrimidin-4-ones as glycogen synthase kinase-3β inhibitors

Toshiyuki Kohara, Kazuki Nakayama, Kazutoshi Watanabe, Shin-ichi Kusaka, Daiki Sakai, Hiroshi Tanaka, Kenji Fukunaga, Shinji Sunada, Mika Nabeno, Ken-Ichi Saito, Jun-ichi Eguchi, Akiko Mori, Shinji Tanaka, Tomoko Bessho, Keiko Takiguchi-Hayashi, Takashi Horikawa

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

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ABSTRACT

We herein describe the results of further evolution of glycogen synthase kinase (GSK)-3β inhibitors from our promising compounds containing a 3-methylmorpholine moiety. Transformation of the morpholine moiety into a piperazine moiety resulted in potent GSK-3β inhibitors. SAR studies focused on the nitrogen atom of the piperazine moiety revealed that a phenyl group afforded potent inhibitory activity toward GSK-3β. Docking studies indicated that the phenyl group on the piperazine nitrogen atom and the methyl group on the piperazine make cation-π and CH-π interactions with GSK-3β respectively. 4-Methoxyphenyl analogue **29** showed most potent inhibitory activity toward GSK-3β with good in vitro and in vivo pharmacokinetic profiles, and **29** demonstrated a significant decrease in tau phosphorylation after oral administration in mice.

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In a previous paper, we described the transformation of the morpholine moiety of previously reported GSK-3β inhibitors with a 2-(2-phenylmorpholin-4-yl)-pyrimidin-4-one skeleton such as UDA-680 into the corresponding piperazine analogues. A docking study indicated that potent inhibitory activity of phenylpiperazine analogues toward GSK-3β was attributed to hydrogen bonding between the nitrogen atom of the piperazine moiety and the oxygen atom of the main chain of Gln185 which was not observed for the phenylmorpholine analogues.[1] We also reported that the alkylmorpholine analogues generally showed potent GSK-3β inhibitory activity together with a certain level of CYP inhibition probably due to the 3-fluoropyridin-4-yl group at the 6-position of the pyrimidone moiety. Based on the information about hydrogen bonding of the piperazine moiety, we hypothesize that transformation of the alkylmorpholine moiety into the corresponding piperazine moiety would introduce new hydrogen bonding toward the enzyme indicated by the docking study of the phenylpiperazine, and that a potential increase in activity by this interaction would enable a transformation of the 3-fluoropyridin-4-yl moiety into a pyridine-4-yl or a pyrimidin-4-yl moiety which would lead to evolution of a new chemical series with decreased CYP inhibition. Namely, we designed 2-methylpiperazine analogues by transforming the morpholine moiety of 3-methylmorpholine analogues such as **1** shown in Fig. 1.[2] In this paper we describe results of the transformation and successive optimization of alkylpiperazine derivatives **2**.

Fig. 1. Transformation of morpholine moiety.

Preliminary results of the transformation of the 3-methylmorpholine moiety into a 2-methylpiperazine moiety are shown in Table 1.[4] Simple transformation of the oxygen atom of the (*R*)-3-methylmorpholine into a nitrogen atom was well tolerated and **3** and **5** showed good inhibitory activity, nearly 2-fold more active for a 4-pyrimidinyl analogue than for a 4-pyridyl analogue. Introduction of a methyl group on the nitrogen atom of the piperazine showed a 2- to 3-fold decrease in activity (**3** vs **4**, **5** vs **6**). The effect of the (*R*)-2-methyl group was clearly observed by comparing it with that of des-methyl piperazine analogue **11**, indicating that introduction of a (*R*)-methyl group afforded a nearly 30-fold increase in activity. The methyl group with (*S*)-configuration generally showed a large decrease in activity. For example, **7** and **9** showed a 6 to 30-fold decrease in activity compared with the corresponding (*R*)-isomers, **3** and **5**, respectively, and *N*-methyl analogues **8** and **10** lost the activity. The (*S*)-2-methyl group seemed detrimental to the inhibitory activity compared with des-methyl analogue **11** which still had weak inhibitory activity.

Table 1
Preliminary structure-activity relationship of 2 and/or 4-substituted piperazine derivatives.

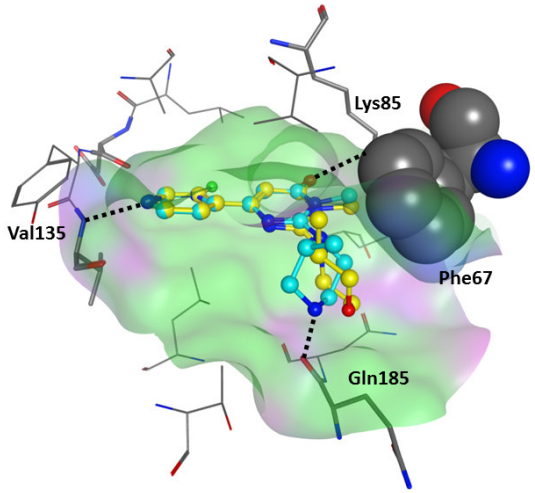


Fig. 2. Docking model of **1** (yellow) and **5** (cyan) with GSK-3β.

In order to analyze the effects of the piperazine moiety, an induced fit docking simulation of **1** and **5** with GSK-3β was performed.[4] Fig. 2 shows that the pyrimidone moiety of both compounds makes interactions which are common to our pyrimidine-type GSK-3β inhibitors. The nitrogen atom at the 1-position of the pyrimidin-4-yl group makes hydrogen bonding with the main chain of Val135 in the hinge region, and the carbonyl oxygen atom of the pyrimidone moiety forms hydrogen bond with the side chain of Lys85.[2,5] The methyl group of 3-(*R*)-methylmorpholine of **1** was buried in a hydrophobic pocket of the active site in the enzyme.[2] The amino moiety of the piperazine of **5** makes hydrogen bonding with the oxygen atom of the main chain of Gln185. This hydrogen bonding is the same interaction as that of phenylpiperazine.[1] And the methyl group with (*R*)-configuration on the piperazine makes a CH-π interaction with Phe67. We consider that the interaction between the piperazine and Gln185 would rotate the piperazine moiety, which would result in making a CH-π interaction between the methyl group of the piperazine and Phe67.

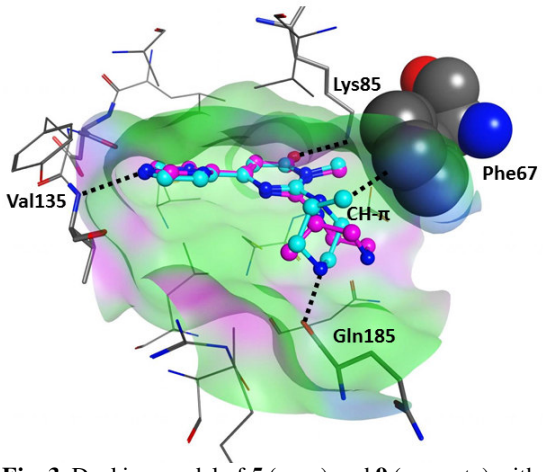


Fig. 3. Docking model of **5** (cyan) and **9** (magenta) with GSK-3β.

The effect of the configuration of the methyl group was also analyzed using docking simulation (Fig. 3). The (*R*)-enantiomer, **5** makes a CH-π interaction with Phe67 and hydrogen bonding with Gln185. The docking study suggests that there are no effective interactions between the enzyme and the piperazine moiety of **9** which have (*S*)-configuration at the methyl group. We think lack of effective interactions between the piperazine moiety of **9** and the enzyme leads to decreased inhibitory activity of **9**.

Table 2
Effect of the substituent on the nitrogen atom of the piperazine moiety (1).

Preliminary in vitro pharmacokinetic studies indicated that **5** had no CYP450 inhibition (>50 μM, recombinant human CYP1A2, 2D6 and 3A4). From these results, 2-((*R*)-2-methylpiperazin-1-yl)-pyrimidin-4-one analogues with a 4-pyridyl or pyrimidin-4-yl group at the 6-position seemed to have preferable profiles for GSK-3β inhibitory activity and CYP inhibition, and we selected **6** as a lead compound for further optimization because **6** had acceptable inhibitory activity and no hydrogen bonding donor which was preferable structural profile as a CNS-acting drug.

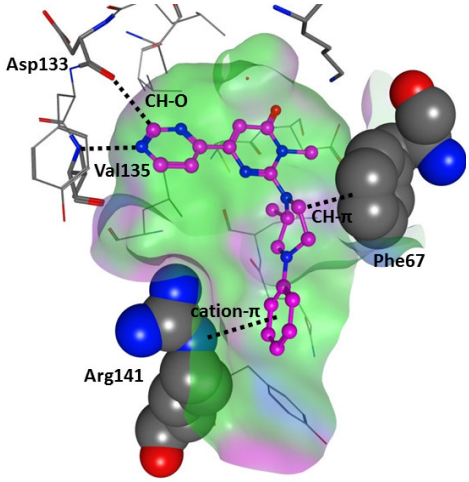


Fig. 4. Docking model of **14** with GSK-3β.

Table 2 shows the results of the optimization of the substituent on the nitrogen atom of the piperazine. Introduction of alkyl groups such as isopropyl (**12**) and benzyl (**13**) showed a moderate increase in activity. The phenyl analogue **14** was over 10-fold more active than **6** and showed moderate metabolic stability and CYP2D6 inhibition. In the case of acyl, alkoxycarbonyl and sulfonyl groups, the substituents containing a phenyl group such as benzoyl (**16**) and 4-toluenesulfonyl (**19**) were tolerant and those without a phenyl group showed decreased activity (**15**, **18**). Among the compounds possessing a phenyl group, **16** which had moderate in vitro activity and **19** which showed poor metabolic stability were not attractive for further optimization toward drug candidates, and **14** which had potent inhibitory activity was chosen for further optimization.

Table 3
Effect of the alkyl group on the piperazine moiety.

The result of a docking study of **14** with GSK-3β is shown in Fig. 4. The phenyl group on the piperazine moiety makes a cation-π interaction with Arg141 and the ethylene moiety of the piperazine makes a CH-π interaction with Phe67. The methyl substituent on the piperazine is bound to the hydrophobic pocket of the enzyme composed of the side chains of Cys199 and Asn186. In this case, no hydrogen bonding interaction between the piperazine and Gln185 is observed, and the cation-π and CH-π interactions observed above would afford potent inhibitory activity toward GSK-3β.

Effect of the position of the methyl group was studied using *N*-benzyl analogues (Table 3). A methyl group at the 3-position was not tolerant regardless of its stereochemistry (**20**, **21**). Effect of a larger alkyl group was also studied. An ethyl group (**22**) afforded a 5-fold decrease in activity. From these results, we concluded that the 2-(*R*)-methyl group was preferable as the substituent of the ethylene moiety of the piperazine.

Table 4
Effect of the substituent on the nitrogen atom of the piperazine moiety (2).

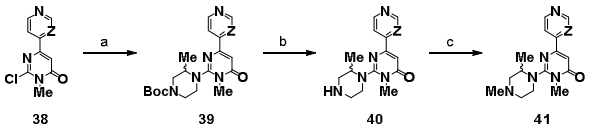
Results of the optimization of the substituent on the phenyl group of **14** are shown in Table 4. Halogen atoms such as a fluorine atom and a chlorine atom, a methoxy and a cyano group were introduced as the substituents. Except the cyano group, the substituents on the phenyl group generally increased the activity, especially the fluorine and methoxy groups afforded potent inhibitory activity regardless their position. No clear relationship between the activity and the position of the substituent was observed. For example, the compound with a substituent at the 2-position showed weaker activity than that of the 3- and 4-position in the case of methoxy (0.8 nM of **27** vs 0.2, 0.1 nM of **28**, **29**, respectively) and cyano (4.2 nM of **30** vs 2.2, 2.7 nM of **31**, **32**, respectively) groups, and slightly potent activity in the case of a fluorine atom (0.2 nM of **23** vs 0.5, 0.4 nM of **24**, **25**, respectively). Among the compounds, 4-methoxy analogue **29** showed most potent inhibitory activity. Most of the compounds showed poor metabolic stability regardless of their electronic nature and the position of the substituent, and many compounds showed moderate one or two CYP isoforms inhibition. No clear relationship between those profiles and structural profiles of the

substituent was observed. In the case of electron-withdrawing substituents, a cyano group afforded increased metabolic stability for 3- and 4- substituted analogues **31** and **32** and no improvement of metabolic stability was observed for 2-substituted analogue **30** compared with unsubstituted analogue **14**. The compounds with the fluorine group at the 3- and 4-positions, **24** and **25** respectively, afforded nearly the same level of metabolic stability as the unsubstituted analogue **14**, and decreased metabolic stability was observed for 2-substituted analogue **23**. In the case of electron donating methoxy group, 3-substituted analogue **28** showed poor metabolic stability and good metabolic stability was observed for 4-substituted analogue **29**. Cell permeability of the compounds we tested was excellent.

Pyridyl and pyrimidinyl groups were also introduced as the aromatic substituent on the nitrogen atom of the piperazine moiety. Compounds possessing pyridyl groups showed nearly 3-fold weaker activity than those with a phenyl group. A pyrimidinyl groups largely decreased the activity regardless of its substituted position. All pyridyl and pyrimidinyl compounds showed weak CYP inhibition and some compounds had preferable metabolic stability (**33**, **37**).

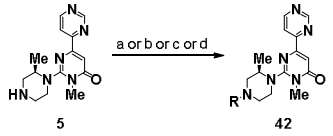
Compound **29**, which was the most potent compound, possessed good metabolic stability and cell permeability together with acceptable CYP inhibition profiles, and we checked its mouse in vivo pharmacokinetic profiles. C_{max} of **29** was 248, 528 ng/ml in plasma and brain, respectively one hour after oral administration at a dose of 10 mg/kg, and AUC (0-4 hr) was 770, 1614 ngh/mL in plasma and brain, respectively. Kp brain for both C_{max} and AUC was 2.1. These data indicated that **29** had higher brain concentration than plasma concentration and preferable pharmacokinetic profiles as a CNS-acting drug. In vivo Ser396 tau phosphorylation inhibition of **29** in mice was also evaluated.[6] Compound **29** significantly demonstrated 60% of decrease of tau phosphorylation one hour after oral administration at a dose of 10 mg/kg. The positive control UDA-680[7] at 100 mg/kg one hour after oral administration showed 73% of inhibition of tau phospholylation.

2-(2-(*R*)- or (*S*)-methylpiperazin-1-yl)-pyrimidin-4-ones **3–10** were synthesized by condensation of commercially available 2-(*R*)- or (*S*)-methyl-4-(*tert*-butoxycarbonyl)-piperazine and the corresponding 2-chloropyrimidin-4-one **38** with triethylamine in tetrahydrofuran as a solvent (Scheme 1).[2,5,8] After deprotection of the *tert*-butoxycarbonyl group with hydrogen chloride in ethyl acetate, the methyl group was introduced by reductive alkylation with formaldehyde for **4**, **6**, **8** and **10**.



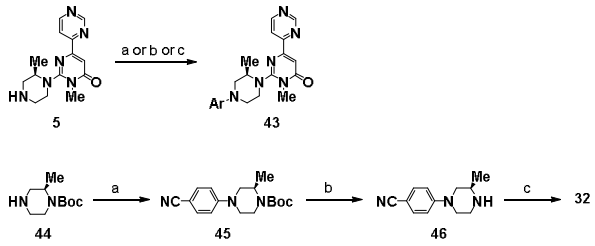
Scheme 1. Reagents and conditions: a) (*R*)- or (*S*)-2-methyl-4-*tert*-butoxycarbonylpiperazine, Et_3N, THF, 65 ºC, 48% ((*R*)-isomer, Z = C-H), 52% ((*R*)-isomer, Z = N), 52% ((*S*)-isomer, Z = C-H), 28% ((*S*)-isomer, Z = N); b) 4N HCl/AcOEt, CH_2Cl_2 or AcOEt, r.t. then 1N NaOH, 68% ((*R*)-isomer, Z = C-H), 49% ((*R*)-isomer, Z = N), 77% ((*S*)-isomer, Z = C-H), 73% ((*S*)-isomer, Z = N); c) HCHO, AcOH, $NaBH(OAc)_3$, MeOH, CH_2Cl_2, r.t., 57% ((*R*)-isomer, Z = C-H), 47% ((*R*)-isomer, Z = N), 44% ((*S*)-isomer, Z = C-H), 44% ((*S*)-isomer, Z = N).

4-Substituted-2-(*R*)-methyl-piperazine analogues were prepared by alkylation or acylation of unsubstituted 2-(*R*)-methyl-piperazine **5** (Scheme 2). The benzyl group was introduced by reductive alkylation with benzaldehyde. Alkylation with isopropylbromide afforded *N*-isopropyl analogue **12**. The acyl and sulfonyl groups were introduced by acylation or sulfonylation with the corresponding acyl or sulfonyl halide.



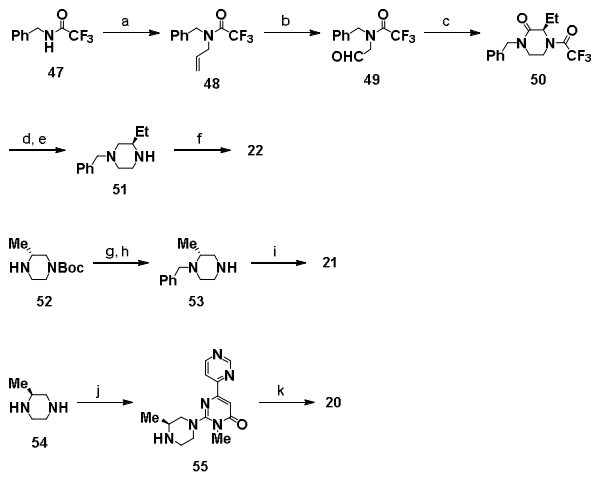
Scheme 2. Reagents and conditions: a) PhCHO, $NaBH(OAc)_3$, cat. AcOH, r.t., 29% (R = $PhCH_2$); b) *i*-PrBr, K_2CO_3, DMF, 80 ºC (R = *i*-Pr); c) acyl or sulfonyl chloride, Et_3N, THF, r.t., 66% (R = PhCO), 56% (R = MeCO), 64% (R = $MeSO_2$), 60% (R = 4-Me-$PhSO_2$); d) ClCOOMe, Et_3N, 60% (R = MeOCO).

Scheme 3 shows the preparation of 4-aryl derivatives. 4-Aryl-2-(*R*)-methyl-piperazine analogues were prepared by arylation of unsubstituted 2-(*R*)-methyl-piperazine **5** with the corresponding aryl bromide. Tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct was used as a catalyst, and following two sets of a ligand and a base were used; BINAP (2,2'-bis(diphenylphosphino)-1,1'-binaphthalene) and sodium *tert*-butoxide, or Xphos (2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl) and potassium phosphate. Introduction of the 2-pyrimidinyl group was performed by nucleophilic substitution of **5** with 2-chloropyrimidine. 4-(4-Cyanophenyl)-2-methylpiperazine (**46**) was prepared by nucleophilic substitution with 4-fluorobenzonitrile and 1-*tert*-butoxycarbonyl-2-(*R*)-methyl-piperazine (**44**) followed by deprotection of the *tert*-butoxycarbonyl group, and **46** was reacted with 2-chloro-3-methyl-6-(pyrimidin-4-yl)-pyrimidine-4-one (**38**) (Z = N) to afford **32**.



Scheme 3. Reagents and conditions: a) ArBr, *tert*-BuONa, $Pd_2(dba)_3$•$CHCl_3$, BINAP, Tol, dioxane, 90 ºC, 36% (Ar = Ph), 28% (Ar = 2-F-Ph), 28% (Ar = 3-F-Ph), 19% (Ar = 4-F-Ph), 25% (Ar = 2-MeO-Ph), 11% (Ar = 4-MeO-Ph); b) ArBr, $Pd_2(dba)_3$• $CHCl_3$, Xphos, K_3PO_4, DME, reflux, 21% (Ar = 4-Cl-Ph), 58% (Ar = 2-CN-Ph), 21% (Ar = 3-CN-Ph), 26% (Ar = 2-Py), 60% (Ar = 3-Py), 56% (Ar = 4-Py), 21% (Ar = 5-pyrimidinyl); c) 2-chloropyrimidine, Et_3N, THF, 85 ºC (sealed tube), 71%; d) 4-fluorobenzonitrile, K_2CO_3, DMSO, 120 ºC, 83%; e) 4N HCl/AcOEt then basification; f) 2-chloro-3-methyl-6-(pyrimidin-4-yl)-pyrimidine-4-one, Et_3N, THF, 85 ºC (sealed tube), 55%.

Preparation of 2-(*R*)-ethyl analogue **22** and 3-methyl analogues, **21** and **20**, are depicted in Scheme 4. 2-(*R*)-Ethyl-4-benzylpiperazine (**51**) was prepared by one-pot tandem reductive amination – transamidation – cyclization reported by Beshore and Dinsmore.[9] *N*-Benzyl-*N*-(2-oxoethyl)trifluoeoacetamide (**49**), prepared by allylation of *N*-benzyltrifluoroacetamide (**47**) followed by oxidative cleavage of the allyl moiety, and methyl 2-(*R*)-aminobutylate hydrochloride was reacted with triacetoxyborohydride. After deprotection of the trifluoroacetyl group, reduction of the resulting piperazinone with lithium aluminum hydride afforded **51**. 1-Benzyl-2-(*R*)-methylpiperazine (**53**) for 3-(*R*)-methyl analogue **21** was prepared by benzylation of 4-*tert*-butoxy-2-(*R*)-methylpiperazine (**52**) followed by deprotection of the *tert*-butoxycarbonyl group. Resulting piperazines were reacted with 2-chloro-3-methyl-6-(pyrimidin-4-yl)-pyrimidine-4-one (**38**) (Z = N) to afford **21**. In the case of 3-(*S*)-methyl analogue **20**, commercially available (*S*)-2-methylpiperazine was reacted with 2-chloro-3-methyl-6-(pyrimidin-4-yl)-pyrimidine-4-one (**38**) (Z = N) which was reacted at less-hindered nitrogen atom at the 4-position to afford des-benzyl analogue **55**, and successive *N*-benzylation afforded **20**.



Scheme 4. Reagents and conditions: a) allyl bromide, K_2CO_3, acetone, reflux, 68%; b) H_5IO_6, K_2OsO_4•2 H_2O, THF, H_2O, 0 ºC to r.t.; c) methyl 2-(*R*)-aminobutylate hydrochloride, $NaBH(OAc)_3$, MeCN, AcOH, molecular sieves 4A, 0 ºC to r.t., 42%, 2 steps; d) K_2CO_3, MeOH, H_2O, 0 ºC; e) $LiAlH_4$, cyclopentyl methyl ether, reflux, 75%, 2 steps; f) 2-chloro-3-methyl-6-(pyrimidin-4-yl)-pyrimidine-4-one, Et_3N, THF, 100 ºC, sealed tube, 13%; g) benzyl bromide, K_2CO_3, DMF, 70 ºC; h) 4N HCl/AcOEt, AcOEt, r.t., 61%, 2 steps; i) 2-chloro-3-methyl-6-(pyrimidin-4-yl)-pyrimidine-4-one, Et_3N, THF, r.t., 25%; j) 2-chloro-3-methyl-6-(pyrimidin-4-yl)-pyrimidine-4-one, Et_3N, THF, r.t., 56%; k) benzyl bromide, K_2CO_3, DMF, 70 ºC, 60%.

In conclusion, the transformation of the 3-(*R*)-methylmorpholine moiety of **1** into the corresponding 2-(*R*)-methylpiperazine moiety was well tolerated and the optimization of the substituent on the nitrogen atom of the piperazine led to the discovery of a novel *N*-aryl-2-(*R*)-methylpiperazine series of potent GSK-3β inhibitors. Among several potent compounds **29** exhibited the most potent GSK-3β inhibitory activity with good in vitro and in vivo pharmacokinetic profiles, especially high cell permeability and brain/plasma concentration ratio. **29** also demonstrated a significant decrease of tau phosphorylation in mice by oral administration at a dose of 10 mg/kg. Further optimization of this series will be published elsewhere.

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References and notes

1. Usui, Y.; Tanaka, H.; Watanabe, K.; Shouda, A.; Uehara, F.; Hiki, S.; Sunada, S.; Aritomo, K.; Adachi, T.; Yokoshima, S.; Mabeno, M.; Saito, K,; Eguchi, J.; Yamagami, K.; Asano, S.; Tanaka, S.; Yuki, S.; Yoshii, N.; Fujimura, M.; Horikawa, T. *Bioorg. Med. Chem. Lett*., in press.
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3. Human GSK-3β cell free enzyme inhibition assay; 7.5 μM of prephosphorylated GS1 peptide and 10 μM [γ-^{32}P]ATP were incubated in 50 mM *N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulfonic acid) (HEPES)-sodium hydroxide (pH 7.2), 1 mM dithiothreitol (DTT), 1 mM magnesium chloride, 0.02% Tween-20 buffer for 1 hr at room temperature in the presence of human recombinant GSK-3β. The reaction was stopped with 0.1 volume of 21% perchloric acid. An aliquot of the reaction mixture was then transferred onto Whatman P81 cation exchange filters and the filters were washed three times with 75 mM phosphoric acid solution, once with water and once with acetone. Incorporated ^{32}P radioactivity was determined by liquid scintillation spectrometry. The prephosphorylated GS1 peptide had the following sequence; NH_2-YRRAAVPPSPSLSRHSSPHQS(P)EDEE-COOH. IC_{50} values are the mean of at least two experiments.
4. Method of docking study; The X-ray crystal structure of GSK-3β (PDB code: 3F88) was utilized in the docking calculations. The compounds were docked into GSK-3β using Glide, version 6.7 (*). Induced fit docking simulation was performed using Glide, version 6.7 and Prime, version 4.0 (*).
*Schrodinger, LLC, New York, NY. 2015.
http://www.schrodinger.com/.
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6. Inhibitory activity on tau phosphorylation in vivo; Test compound was administrated to male CD-1 mice of 5–6 weeks weighing 25–35 g (Charles River Japan, Inc.) at 10 mg/kg p.o. (0.5% Tween/H_2O suspension) and after 1 hr, mice were decapitated and cortex was promptly removed, followed by being frozen in liquid N_2. Cortex was directly homogenized with 2.3% SDS homogenization buffer (62.5 mM Tris–HCl, 2.3% SDS, 1 mM each of EDTA, EGTA and DTT, protease inhibitor cocktail (sigma P2714) containing 0.2 μM 4-(2-aminoethyl)benzenesulfonylfluoride (AEBSF), 13 μM bestatin, 1.4 μM E-64, 0.1 mM leupeptin, 30 nM aprotinin, pH 6.8) and centrifuged at 15000g for 15 min at 4 ºC. Protein concentrations were determined using DC protein assay kit (BIO-RAD). Supernatants were diluted with sample buffer (62.5 mM Tris–HCl, 25% glycerol, 2% SDS, 0.01% bromophenol blue, pH 6.8) to adjust the protein concentrations around 0.5–2 mg/mg and then boiled for 5 min. 10 μg of samples were applied on 10% SDS–PAGE mini slab gels and transferred onto PVDF membranes. Membranes were incubated with PBS containing 5% non-fat milk for 1 hr at room temperature and then probed with pS396 anti-body (BIOSOURCE) overnight at 4 ºC. Anti-rabbit IgG HRP-conjugated anti-body (Promega) was used as secondary anti-body. Membranes were visualized by ECL kit (Amersham Bioscience)

and detected by LAS 1000 (Fuji Photo Film). We have already checked that the amount of total tau was not changed by the compounds which were analogue of UDA-680 assayed in this screening. Therefore, we did not measure the amount of total tau but measured pS396 phosphorylated tau normalized with total protein concentration for compound screening in vivo.

7. Fukunaga, K.; Uehara, F.; Aritomo, K.; Shoda, A.; Hiki, S.; Okuyama, M.; Usui, Y.; Watanabe, K.; Yamakoshi, K.; Kohara, T.; Hanano, T.; Tanaka, H.; Tsuchiya, S.; Sunada, S.; Saito, K.; Eguchi, J.; Yuki, S.; Asano, S.; Tanaka, S.; Mori, A.; Yamagami, K.; Baba, H.; Horikawa, T.; Fujimura, M. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6933.
8. Uehara, F.; Shoda, A.; Aritomo, K.; Fukunaga, K.; Watanabe, K.; Ando, R.; Shinoda, M.; Ueno, H.; Kubodera, H.; Sunada, S.; Saito, K.; Kaji, T.; Asano, S.; Eguchi, J.; Yuki, S.; Tanaka, S.; Yoneyama, Y.; Niwa, Y. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6928.
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Table 1

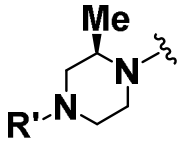
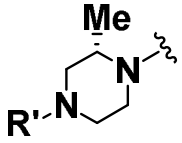
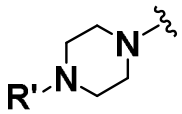
piperazine	compound	Z	R'	GSK-3β inhibition (IC_{50}, nM)
	**3**	C-H	H	29
	4	C-H	Me	80
	5	N	H	13
	6	N	Me	22
	**7**	C-H	H	920
	8	C-H	Me	>1000
	9	N	H	76
	10	N	Me	>1000
	**11**	N	Me	630

Table 2

compound	R'	GSK-3β inhibition (IC_{50}, nM)	$CL_{int, human}$ [a] (mL/min/mg)	CYP inhibition (IC_{50}, μM) [b]			Caco-2 ($x10^{-7}$ cm/s)
				1A2	2D6	3A4	
6	Me	22	-----	-----	-----	-----	-----
12	i-Pr	13	N.D.	>50	>50	>50	N.D.
13	$PhCH_2$	8.3	0.18	>50	21	>50	-----
14	Ph	1.4	0.23	>50	23	>50	-----
15	MeCO	49	-----	-----	-----	-----	-----
16	PhCO	21	0.05	>50	>50	N.D.	333
17	MeOCO	70	-----	-----	-----	-----	-----
18	$MeSO_2$	46	-----	-----	-----	-----	-----
19	4-$MePhSO_2$	19	0.37	>50	34	1.4	566

N.D.; not determined

a) human liver microsome.

b) Recombinant human CYP450.

Table 3

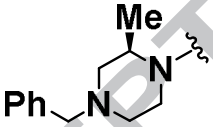
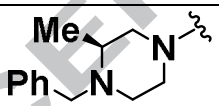
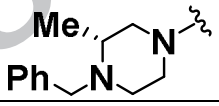
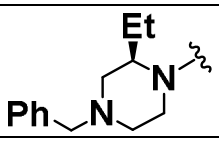
compound	piperazine	GSK-3β inhibition (IC_{50}, nM)
13		8.3
20		780
21		>1000
22		43

Table 4

compound	Ar	GSK-3β inhibition (IC_{50}, nM)	$CL_{int, human}$ [a] (mL/min/mg)	CYP inhibition (IC_{50}, μM) [b]			Caco-2 ($x10^{-7}$cm/s)
				1A2	2D6	3A4	
14	Ph	1.4	0.23	>50	23	>50	-----
23	2-F-Ph	0.2	0.43	42	19	N.D.	645
24	3-F-Ph	0.5	0.24	41	10	>50	511
25	4-F-Ph	0.4	0.26	43	10	>50	620
26	4-Cl-Ph	1.2	0.14	30	7.6	>50	-----
27	2-MeO-Ph	0.8	N.D.	41	10	>50	450
28	3-MeO-Ph	0.2	0.38	28	19	9.6	630
29	4-MeO-Ph	0.1	0.09	18	>25	25	610
30	2-CN-Ph	4.2	0.27	>50	27	N.D.	-----
31	3-CN-Ph	2.2	0.02	8.3	15	>50	N.D.
32	4-CN-Ph	2.7	0.11	>50	24	N.D.	-----
33	4-Py	3.8	0.01	>50	>50	>50	-----
34	3-Py	3.9	0.13	>50	>50	N.D.	-----
35	2-Py	4.1	0.40	>50	40	>50	-----
36	2-pyrimidinyl	24	-----	-----	-----	-----	-----
37	5-pyrimidinyl	14	0.04	>50	>50	N.D.	-----

N.D.; not determined

a) human liver microsome.

b) Recombinant human CYP450.

Graphical Abstract

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

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