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Discovery of novel 2-(alkylmorpholin-4-yl)-6-(3-fluoropyridin-4-yl)-pyrimidin-4(3*H*)-ones as orally-active GSK-3β inhibitors for Alzheimer's disease

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ABSTRACT

We herein describe the results of further evolution of GSK-3β inhibitors for Alzheimer's disease from our promising compounds with in vivo tau phosphorylation inhibitory activity by oral administration. Introduction of a low alkyl group instead of the phenyl group at the 3-position of the morpholine moiety aiming to improve pharmacokinetic profiles resulted in potent low molecular weight GSK-3β inhibitors with good in vitro pharmacokinetic profiles, which also showed in vivo tau phosphorylation inhibitory activity by oral administration. Effect of the stereochemistry of the alkyl moiety is also discussed using docking models.

Alzheimer's disease (AD) is a neurodegenerative disorder reported by Dr. A. Alzheimer in 1907. Patients of AD show progressive and irreversible memory loss and cognitive impairment with psychiatric symptoms such as psychosis, hallucinations, depression, anxiety, sleep disorder and aberrant behavior disturbance. By the progression of the disease, patients are gradually impaired cognitively and functionally and it is difficult for them to live alone, and finally caregivers will make all basic activity of daily living of the patients. These facts make tremendous burdens physically, economically and mentally to the patients' family, and also afford a great economic loss in worldwide. Pathology of AD shows three major characteristics: (i) massive neuronal loss in hippocampus as well as frontal and temporal cortices, (ii) neurofibrillary tangles (NFTs), and (iii) senile plaques (SP). NFTs are mainly composed of abnormally hyperphosphorylated microtubule-associated protein tau, the hyperphosphorylation of which GSK-3β mainly contributes to. GSK-3β is a promising therapeutic target against AD due to the increase in activity in AD brains and the involvement in the acceleration of tau pathology.[1–4]

Recently we reported the discovery of a GSK-3β inhibitor for AD, UDA-680 which exhibited potent GSK-3β inhibitory activity with high kinase selectivity and in vivo tau phosphorylation inhibitory activity by oral administration in mice. Pharmacokinetic studies indicated that UDA-680 had high brain/plasma ratio with moderate bioavailability (37%, rat).[5]

In order to identify further promising compounds for clinical trials, evolution of UDA-680 was planned. UDA-680 had an acceptable level of bioavailability and water solubility, which, however, remained to be improved. It is already known that reducing the number of carbon atoms of a phenyl group according to a so-called 'carbon-cutting approach' may improve bioavailability,[6] and we tried to transform the phenyl group into a small alkyl group to evolve a new chemical series of GSK-3β inhibitors. A 3-fluoropyridin-4-yl group was selected as the substituent at the 6-position of the pyrimidone moiety in order to compensate the loss of a cation–π interaction between the phenyl group and Arg141 in the hydrophobic site of GSK-3β (Fig. 1).[5] We envisioned that this modification would afford increased hydrophilicity, reduced molecular weight and resulting improvement of in vivo pharmacological profiles.

Preliminary results of the transformation of the phenyl group into alkyl groups are shown in Table 1.[7] Both a 2-alkyl group and

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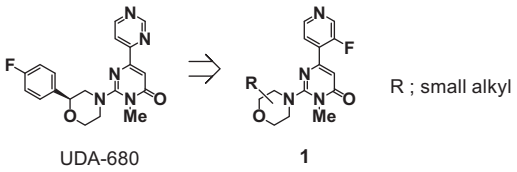


Figure 1. Structure of UDA-680 and its transformation.

a 3-alkyl group increased in vitro activity compared to unsubstituted morpholine **2**. Compounds with 2-alkyl groups showed the same level of in vitro activity as UDA-680 accompanying potent CYP1A2 inhibition and poor metabolic stability. In the case of compounds with a 3-alkyl group, a 3-(*R*)-isomer also showed the same level of potency as UDA-680, and in vitro activity of the (*S*)-isomer was lower than that of the (*R*)-isomer. Compound **6** with a 3-(*R*)-Me group was particularly interesting as it had the most potent activity and the smallest molecular weight coupled with better metabolic stability and moderate CYP inhibition comparable to UDA-680.

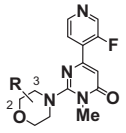
We further sought to increase inhibitory activity by introducing another methyl group at the 2- or 6-position (Table 2). Introduction of a 6-methyl group (**11** and **12**) decreased activity regardless of its configuration, especially for (3*R*,6*R*)-isomer **11**. In the case of 2-methyl group, no increase of activity was observed in both *cis* and *trans* isomers except **16** which slightly increased the activity. 2,2,3-Trimethyl isomer **17** also maintained the activity. These results indicated that the configuration of the 3-methyl group affected the activity and the methyl group at the 2-position made no significant contribution to increase the activity.

Fused cyclic moieties designed by the connection of the two methyl groups of **16** were installed on the morpholine moiety to increase potency by the conformational restriction of the morpholine moiety (Table 3). All fused-morpholine isomers, **18**, **20** and **21**, possessing a cyclopentane, cyclohexane and tetrahydrofuran moiety, respectively, showed increased activity. Compound **19**, the optical isomer of **18** showed decreased activity, again suggesting that the configuration of the carbon atom adjacent to the nitrogen atom of the morpholine ring affected the activity.

In order to analyze the effects of the configuration of the carbon atom of the morpholine moiety, docking studies of **6** and **9** with GSK-3β was performed.[8] Figure 2 shows that the nitrogen atom of the 3-fluoropyridyl group makes hydrogen bonding with the main chain of Val135 and the carbonyl oxygen of the pyrimidone moiety forms a hydrogen bond with the side chain of Lys85. The morpholine moiety of each compound formed a CH–π interaction with the side chain of Phe67,[9] but the binding area of each methyl substituent is different. The methyl group of **6** is buried in the hydrophobic pocket of the ceiling of the active site and that of **9** just looks toward the floor presumably with no interaction with the active site, which resulted in several-fold difference in activity. A nearly 10-fold increase of activity by introducing a methyl group to unsubstituted **2** suggests that this methyl group would confer so-called magic methyl effect by the hydrophobic interaction.[10] Docking studies of **18** and **19** were also performed based on these results. (Fig. 3) The fluoropyridine and pyrimidone moieties make almost the same interactions as the methyl group of **6**, and the fused-morpholine moieties of **18** and **19** are located at different binding positions. The morpholine moiety of **18** is positioned closer to the side chain of Phe67.[11] The position of the morpholine moiety of **18** seems more appropriate to interact with GSK-3β than that of **19**, and therefore **18** shows more potent activity than **19**. Significant difference of activity of **21** and its (+)-isomer was also analyzed by a docking study. The (4a*R*,7a*S*) and (4a*S*,7a*R*) isomers (Fig. 4), which have the same configuration as **18** and **19**, respectively, locate at a similar binding position to **18** and **19**, respectively. In this case, the distance between the CH with Phe67 and the distance between the morpholine moiety with Phe67 are significantly different,[12] which would afford large differences in activity between each stereoisomer.

Table 4 listed the promising compounds by the 'carbon-cutting approach'. All of the compounds had more potent inhibitory activity than UDA-680 and possessed good metabolic stability, CYP inhibition profiles and excellent cell permeability. Ligand efficiency (LE) of these compounds was increased to 0.35 to 0.37 from 0.29 of UDA-680.[13] Lipophilic ligand efficiency (LLE) of these compounds was between 7.05 and 8.92 which are more preferable scores than that of UDA-680 (LLE; 7.21) except **20**.[14] These results showed that the 'carbon-cutting approach' was effective for the evolution of a new subseries with a well-balanced LE-LLE value as oral drugs compared to UDA-680.[15] Furthermore, the number of aromatic rings were decreased from three of UDA-680 to two of the promising compounds in Table 4. This also suggested that the promising

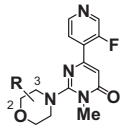
Table 1
Introduction of small alkyl group



Compd	R	GSK-3β IC_{50} (nM)	$CL_{int,\ human}$[a] (mL/min/mg)	CYP inhibition[b] (IC_{50}, μM)		
				1A2	2D6	3A4
2	H	67	—	—	—	—
3	2-(*RS*)-*i*-Pr	17	0.07	3.3	>50.0	>50.0
4	2-(*RS*)-*n*-Bu	12	0.21	7.5	2.3	>50.0
5	2-(*RS*)-cyc-Bu	12	0.20	1.5	37.7	>50.0
6	3-(*R*)-Me	7.0	0.03	18.7	>50.0	>50.0
7	3-(*R*)-Et	19	—	—	—	—
8	3-(*R*)-*i*-Pr	14	—	—	—	—
9	3-(*S*)-Me	23	0.02	5.7	>50.0	>50.0
10	3-(*S*)-Et	30	—	—	—	—
UDA-680		12	0.06	29.4	39.7	>50.0

[a] Human liver microsome assay.
[b] Recombinant human CYP450 isoform inhibition for 1A2, 2D6 and 3A4.

Table 2
Introduction of additional methyl group



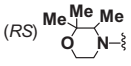
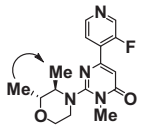
Compd	Morpholine	GSK-3β IC_{50} (nM)	Compd	Morpholine	GSK-3β IC_{50} (nM)
11		453	**15**		19
12		41	**16**		4.9
13	 (+)-isomer	16	**17**		9.7
14	(-)-isomer	32			

Table 3
Fused-morpholine analogues



16

Compd	Morpholine	GSK-3β IC_{50} (nM)	Compd	Morpholine	GSK-3β IC_{50} (nM)
18		4.2	**20**		3.7
19		35	**21**		3.1[a]

[a] We also prepared (+)-isomer, which IC_{50} of in vitro activity was >1000 nM.

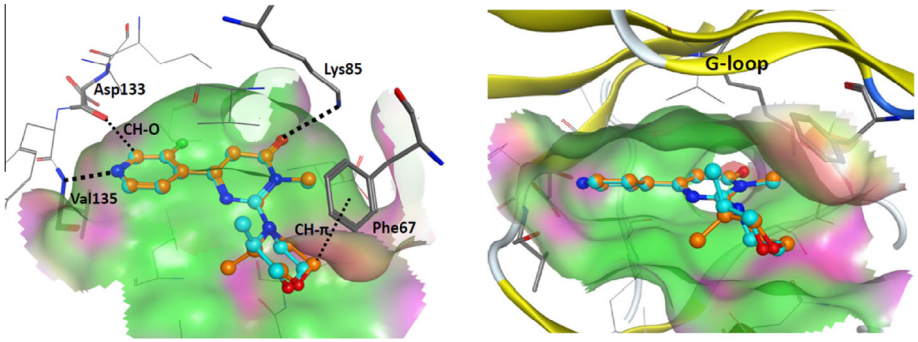


Figure 2. Docking model of **6** (cyan carbon) and **9** (orange carbon) with GSK-3β (PDB code; 3F88, gray carbon). Left figure; interactions between **6**, **9** and GSK-3β. Right figure; binding position of **6** and **9** in GSK-3β seen from the apertural area.

compounds by the 'carbon-cutting approach' possess preferable profiles as oral drug candidates.[16]

Among the compounds in the table, **6** was selected to confirm the effect of the simple methyl group by the 'carbon-cutting approach', and we evaluated its inhibitory effects on in vivo Ser396 tau phosphorylation in mice.[17,18] (Fig. 5) A dose response study showed that **6** significantly decreased tau phosphorylation one hour after administration at a dose of 10 mg/kg or higher doses and that its ED_{50} value was 6.8 mg/kg p.o., 1 h.

2-(Alkyl-substituted morpholin-4-yl)-pyrimidin-4-ones **3**–**21** were synthesized by the condensation of appropriately substituted morpholines and 2-chloropyrimidin-4-one **26** in the presence of triethylamine (Scheme 1). 3-Flurorpyridin-4-carboxylic acid (**23**) was prepared from 3-fluoropyridine (**22**) by *ortho*-lithiation using

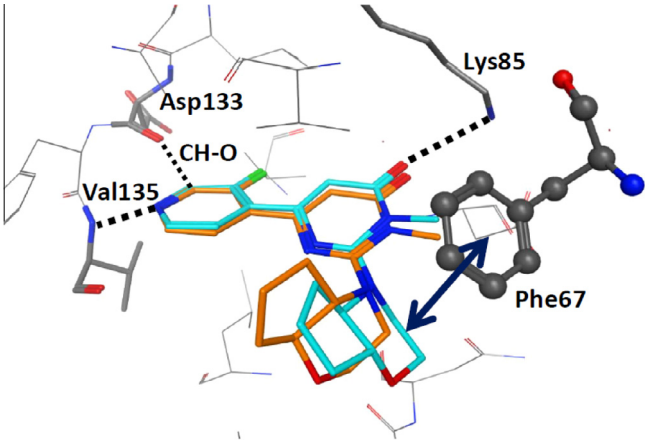


Figure 3. Docking model of **18** (cyan carbon) and **19** (orange carbon) with GSK-3β (gray carbon).

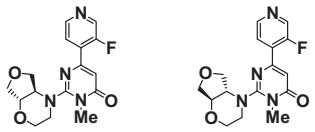


Figure 4. Chemical structure of **21** and its stereoisomer.

lithium diisopropylamide and addition of dry ice. Compound **23** was transformed into the corresponding β-keto ester **24** with potassium monoethylmalonate, carbonyl diimidazole and magnesium chloride.[19] Condensation of **24** and *N*-methylthiourea afforded pyrimidine-4(3*H*)-one **25**, which was converted to chloropyrimidone **26** by using phosphorous oxychloride and *N*,*N*-dimethylformamide.

Preparation of 3-alkylmorpholines **31** is depicted in Scheme 2. N-protected aminoalcohol **28**, prepared by reductive alkylation of optically active aminoalcohol **27**, was acylated by chloroacetyl chloride and successive intramolecular alkylation yielded lactam **29**. Reduction of **29** by diborane generated in situ[20] and deprotection of benzyl group of **30** by 1-chloroethyl chlorformate[21] afforded 3-alkylmorpholine **31** as hydrochloric acid salts.

Preparation of di- and trimethylmorpholines is shown in Schemes 3–5. 2,5-Dimethylmorpholine **35** was prepared according to Scheme 3 using 2-chloropropionyl chloride as the acyl halide. Acylation of aminoalcohol **32** with 2-chloropropionyl chloride followed by intramolecular alkylation afforded lactam **33** as a mixture of diastereomers. Reduction of **33** and separation of each isomer by silica gel column chromatography yielded N-protected morpholine **34**,[22] which was deprotected to give 2,5-dimethylmorpholine **35**.

Preparation of *trans*-2,3-dimethylmorpholine was depicted in Scheme 4. Ring opening of epoxide **36** by (*S*)-1-phenethylamine in the presence of lithium perchlorate afforded aminoalcohol **37** as a mixture of stereoisomers. After cyclization, the resulting lactam was washed with hexane and diethyl ether, and recrystallization from dichloromethane/hexane afforded lactam **38** with (2*R*,3*R*) stereochemistry.[23] Reduction and successive deprotection of 1-phenethyl moiety yielded *trans*-(2*R*,3*R*)-dimethylmorpholine **40**. *Trans*-(2*S*,3*S*)-isomer was also prepared by this route by using (*S*)-1-phenethylamine instead of its (*R*)-isomer.

Scheme 5 shows the preparation of *cis*-2,3-dimethylmorpholine. The sequence began with a ring opening of (*RS*)-epoxide **41** by (*S*)-1-phenethylamine and the resulting aminoalcohol **42** was elaborated to morpholine **44**, which was separated to each isomer[24] by silica gel column chromatography. Deprotection of the 1-methybenzyl group afforded *cis*-2,3-dimethylmorpholine **45**. 2,2,3-Trimethylmorpholine was also synthesized by this sequence using an amino alcohol derived from 2,2,3-trimethyloxirane and benzylamine.

Preparation of a fused-morpholine such as **49** was depicted in Scheme 6. Aminoalcohol **46**[25] prepared from cyclopenteneoxide and (*R*)-1-phenethylamine was acylated, cyclized, reduced and deprotected to yield morpholine **49**. Morpholines fused with cyclohexane and tetrahydrofuran were also prepared from aminoalcohols derived from (*R*)-1-phenethylamine and cyclohexene oxide or dihydrofuran oxide. In the case of tetrahydrofuran-fused morpholine, each stereoisomer was separated after reduction of lactam intermediate.

In conclusion, transformation of the phenyl group of morpholine moiety of UDA-680 into a small alkyl group was well tolerated and led to the discovery of a novel alkylmorpholine series of potent GSK-3β inhibitors. Extending these findings identified a conformationally restrained fused-morpholine series. Several

Table 4
Promising compounds

Compd	Morpholine	GSK-3β IC_{50} (nM)	$CL_{int,\ human}$[a] (mL/min/mg)	CYP inhibition[b] (IC_{50}, μM)			Caco-2 (10^{-7} cm/s)	*c*log*P*	LE	LLE
				1A2	2D6	3A4				
6		7.0	0.03	19	>50	>50	546	0.47	0.37	7.68
16		4.9	n.t.	16	>50	>50	485	0.99	0.36	7.32
18		4.2	0.04	21	>50	>50	445	0.83	0.35	7.55
20		3.7	0.20	14	>50	>50	418	1.38	0.34	7.05
21		3.1	0.02	>50	>50	>50	545	−0.41	0.35	8.92

n.t.; not tested.
LE; ligand efficiency.
LLE; lipophilic ligand efficiency.
[a] Human liver microsome assay.
[b] Recombinant human CYP450 isoform inhibition for 1A2, 2D6 and 3A4.

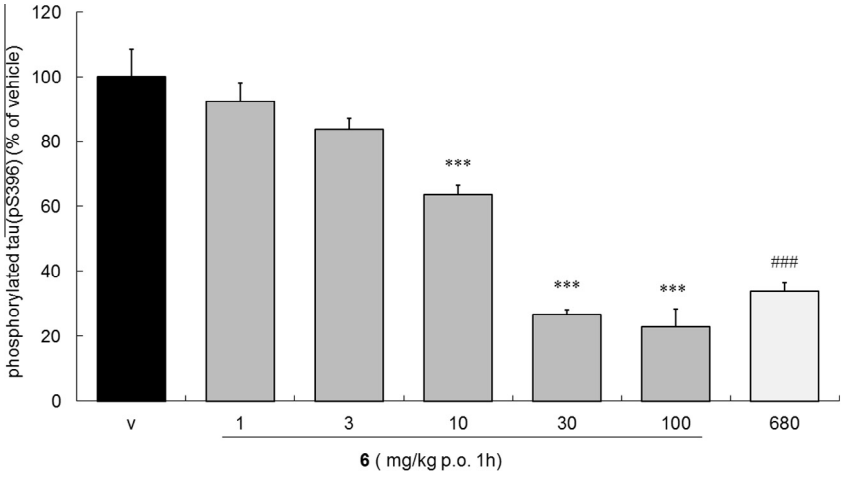
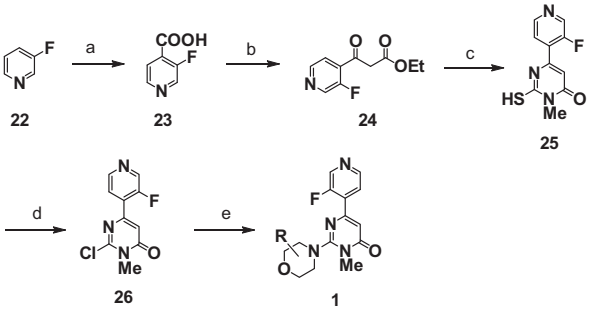
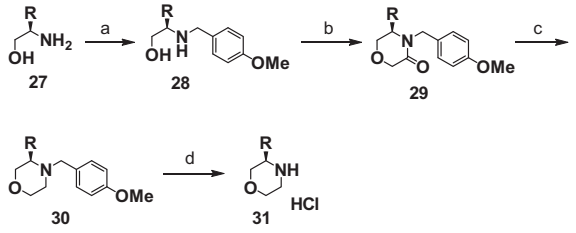


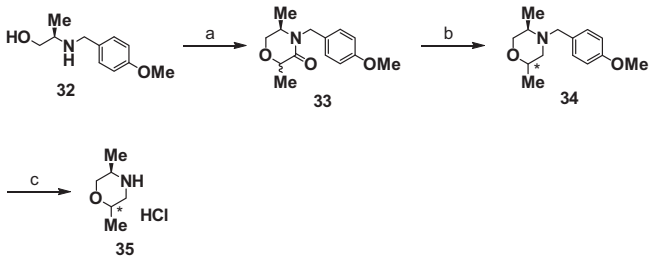
Figure 5. Dose–response of **6** for decrease of phosphorylated tau protein 1 h after administration. Data represents mean ± SE ($N = 6$). v: vehicle. 680: UDA-680 100 mg/kg p.o. treated as a positive control. $^{###}p$ <0.001 versus vehicle treated group by Student's t-test. $^{***}p$ <0.001 versus vehicle treated group by Dunnett's test.



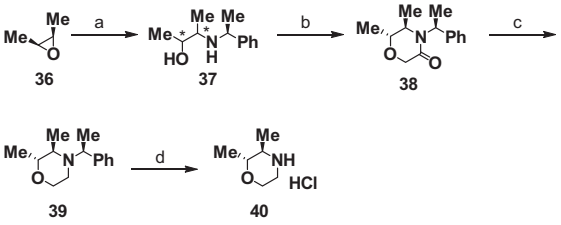
Scheme 1. Reagents and conditions: (a) THF, LDA, −78 °C, 1 h then add s.CO_2, −78 °C to rt, 1 h, 70%; (b) carbonyldiimidazole, THF, reflux, 1 h then add $EtOOCCH_2COOK$, $MgCl_2$, 60 °C, 66%; (c) *N*-methylthiourea, DBU, toluene, 110 °C, 5 h, 68%; (d) $POCl_3$, DMF, 60 °C, 2 h, 73%; (e) morpholines, Et_3N, THF, 37–75%.



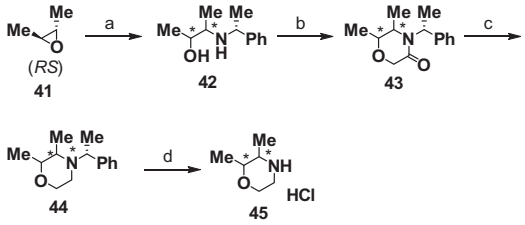
Scheme 2. Reagents and conditions: (a) 4-methoxybenzaldehyde, $NaBH_4$, MeOH, 0 °C to rt, 1 h, 100% (R = Me); (b) $ClCH_2COCl$, Et_3N, THF, 1 h then add NaH, 0 °C, 63% (R = Me), 82% (R = Et, 2 steps), 59% (R = *i*-Pr, 2 steps); (c) $LiBH_4$, Me_3SiCl, THF, rt, 1 h, 96% (R = Me), 69% (R = Et), 75% (R = *i*-Pr); (d) (i) $ClCOOCH(Cl)CH_3$, $ClCH_2CH_2Cl$, 80 °C, 6 h, (ii) MeOH, reflux, 1 h, 74% (R = Me), 89% (R = Et), 58% (R = *i*-Pr).



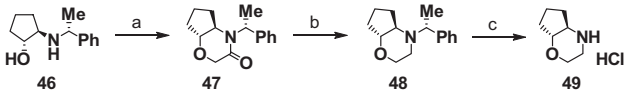
Scheme 3. Reagents and conditions: (a) $MeCH(Cl)COCl$, Et_3N, THF then add NaOMe in MeOH, 0 °C to rt, overnight, 93%; (b) $LiBH_4$, Me_3SiCl, THF, 0 °C to rt, 2 h, separation of isomer, 13% (*cis*), 29% (*trans*); (c) (i) $ClCOOCH(Cl)CH_3$, $ClCH_2CH_2Cl$, reflux, 2 h, (ii) MeOH, reflux, 2 h, 52% (*cis*), 21% (*trans*).



Scheme 4. Reagents and conditions: (a) (*S*)-1-phenethylamine, $LiClO_4$, MeCN, reflux, 12 h; (b) (i) $ClCH_2COCl$, Et_3N, CH_2Cl_2, 0 °C, 1 h, (ii) KOH, *i*-PrOH, 0 °C to rt, 3 h, (iii) wash with hexane and Et_2O then recryst. from CH_2Cl_2/hexane; 19%, 4 steps (c) $LiBH_4$, Me_3SiCl, THF, 0 °C to rt, 3 h, 62%; (d) (i) $ClCOOCH(Cl)CH_3$, $ClCH_2CH_2Cl$, reflux, 10 h, (ii) MeOH, reflux, 7 h, 69%.



Scheme 5. Reagents and conditions: (a) (*S*)-1-phenethylamine, $LiClO_4$, MeCN, reflux, 10 h; (b) (i) $ClCH_2COCl$, Et_3N, THF, 0 °C, 1 h, (ii) KOH, *i*-PrOH, 0 °C to rt, 4 h, 77% (3 steps, mixture of isomer); (c) $LiBH_4$, Me_3SiCl, THF, 0 °C to rt, 6 h, then separation of isomer, 18% (*cis*, upper), 11% (*cis*, lower); (d) (i) $ClCOOCH(Cl)CH_3$, 130 °C (neat), (ii) MeOH, reflux, 7 h.



Scheme 6. Reagents and conditions: (a) (i) $ClCH_2COCl$, Et_3N, THF, 0 °C, 30 min then add NaOMe in MeOH, 0 °C to rt, overnight, 88%; (b) $LiBH_4$, Me_3SiCl, THF, 0 °C to rt, 2 h, 91%; (c) (i) $ClCOOCH(Cl)CH_3$, $ClCH_2CH_2Cl$, reflux, 10 h, (ii) MeOH, reflux, 1 h, 58%.

potent compounds exhibited good in vitro pharmacokinetic profiles, and **6** inhibited in vivo tau phosphorylation in mice by oral administration. LE and LLE score of the promising compounds are better than those of UDA-680, which indicated that this novel new chemical series would bind to GSK-3β more effectively than

UDA-680. Further optimization of this series will be published elsewhere.

Acknowledgments

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7. 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 h 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 3 times with 75 mM phosphoric acid solution, once in water and once in 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.
8. The X-ray crystal structure of GSK-3β (PDB code: 3F88) was utilized in the docking calculations after restrained minimization by applying the constraint to converge the non-hydrogen atoms to an RMSD of 0.3 Å using OPLS 2005 force field. The compounds were docked into GSK-3β using Glide 5.8 (XP) which was available from Schrodinger, LLC, New York, NY. 2012 (http://www.schrodinger.com/). After above docking study, the atomic charges of each compound with the docked conformation were calculated. The charges were obtained by electrostatic potential (ESP) fitting at the single point energy, using Jaguar 7.9 at the B3LYP/6-31G** level. Jaguar was available from Schrodinger, LLC, New York, NY. 2011 (http://www.schrodinger.com/).
9. The distance between the CH with Phe67 is 4.0 Å for **6** and 3.6 Å for **9**, and the distance between centers of the morpholine moiety and the benzene ring of Phe67 is 4.7 Å for **6** and 4.6 Å for **9**.
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11. The distances between centers of the morpholine moiety and the benzene ring of Phe67 of **18** and **19** are 4.7 Å and 5.1 Å, respectively. In this case the distance between the CH–π and Phe67 is the same between **18** and **19** (4.2 Å for **18** and 4.2 Å for **19**).
12. The distances between the CH with Phe67 of the (4a*R*,7a*S*) and (4a*S*,7a*R*) isomers are 3.7 Å and 4.3 Å, respectively, and the distances between centers of the morpholine moiety and the benzene ring of Phe67 of the (4a*R*,7a*S*) and (4a*S*,7a*R*) isomers are 4.5 Å and 5.1 Å, respectively.
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17. 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 1, 3, 10, 30 mg/kg po (0.5% Tween/H_2O suspension) and after 1 h, 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 15000*g* for 15 min at 4 °C. 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 h at room temperature and then probed with pS396 anti-body (BIOSOURCE) overnight at 4 °C. 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).
18. Analytical data of **6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (t, J = 7.2 Hz, 3H), 3.10–3.17 (m, 1H), 3.40–3.46 (m, 1H), 3.55 (s, 3H), 3.60–3.63 (m, 1H), 3.68–3.72 (m, 1H), 3.83–3.91 (m, 3H), 6.90 (s, 1H), 7.97 (dd, J = 1.2 Hz, 4.2 Hz, 1H), 8.52 (d, J = 4.2 Hz, 1H), 8.54 (d, J = 1.2 Hz, 1H); MS m/z 305 ($M+H^+$).
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22. Relative stereochemistry of the isomer was determined by ^{1}H NMR.
23. No stereoisomer was observed by ^{1}H NMR spectroscopy (400 MHz). Absolute stereochemistry was determined by X-ray crystallography using lactam derived from (*S*)-1-phenethylamine (optical isomer of **38**).
24. The absolute configuration of each isomer was not determined.
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