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Structure–activity relationships of 3,5-disubstituted benzamides as glucokinase activators with potent in vivo efficacy

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ABSTRACT

The optimization of our lead GK activator **2a** to 3-[(1*S*)-2-hydroxy-1-methylethoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (**6g**), a potent GK activator with good oral availability, is described, including to uncouple the relationship between potency and hydrophobicity. Following oral administration, this compound exhibited robust glucose lowering in diabetic model rodents.

1. Introduction

Glucokinase (GK), a member of the hexokinase family,[1] catalyzes the first step in glycolysis involving the phosphorylation of glucose to glucose-6-phosphate. GK plays an important role as a glucose sensor for maintaining plasma glucose homeostasis by enhancing insulin secretion from pancreatic β-cells and glucose metabolism in the liver.[2–4] Therefore, activation of GK is expected to be a novel therapeutic strategy for the treatment of Type 2 diabetes.[5–8]

We previously reported discovery of orally active 3-alkoxy-5-phenoxy-*N*-thiazolylbenzamide GK activators (**2a** and **2b**),[9] which was discovered by optimization of our original lead GK activator, 2-aminobenzamide (**1**)[10,11] (Fig. 1). As shown in the report, compound **2a** demonstrated good glucose lowering effects in normal mice at 30 mg/kg oral dosing and in oral glucose tolerance test (OGTT) studies in rats at 10 and 30 mg/kg dosing. On the other hand, the solubility in water (pH 7.4) was very low: <0.1 μg/mL. The hydrophobicity was also high, log *D* (pH 7.4): >5 (Table 1). In pre-clinical or clinical studies, the poor solubility of candidates may cause trouble,[12] such as absorption saturation.[13] Therefore, development of more soluble and hydrophilic GK activators is required.

Here, we describe the optimization of our lead GK activators **2a** and **2b**, and the identification of a aqueous soluble and hydrophilic derivative (**6g**).[14,15] We also describe the in vivo profile of **6g**, pharmacokinetic profiles in animals and glucose lowering effects in diabetic rodent models.

2. Chemistry

The preparation of lead compounds is summarized in Schemes 1–4. Alkylation of **3**[9] was conducted by substitution reaction or

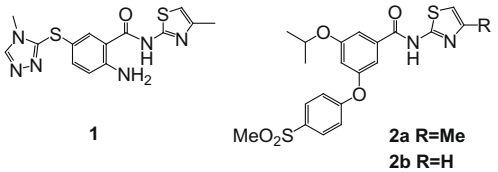

Figure 1. Previously reported our GK activators.

Table 1
Profiles of the lead compounds **2a** and **2b**

Compound	2.5 mM Glc EC_{50} (μM)	10 mM Glc EC_{50} (μM)	Solubility in water (pH 7.4, μg/mL)	Log *D* (pH 7.4)
2a	0.33	0.13	<0.1	>5
2b	0.17	0.06	NT	>5

NT: not tested.

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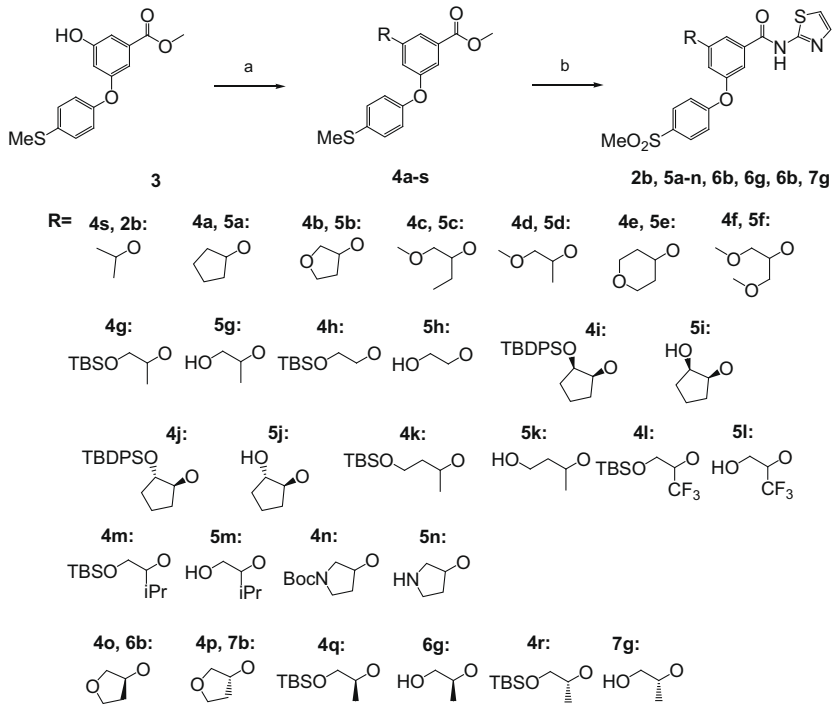
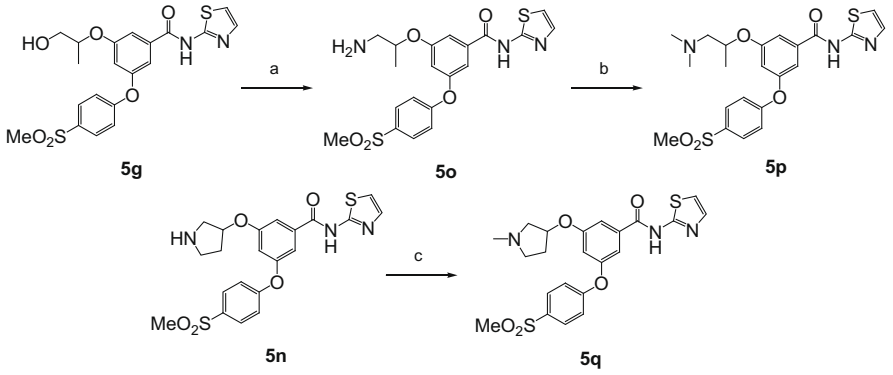
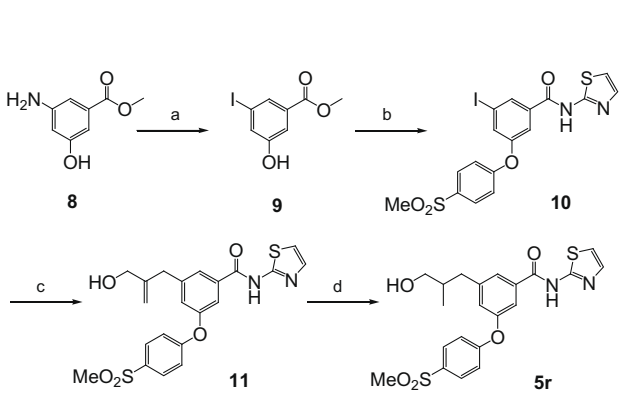


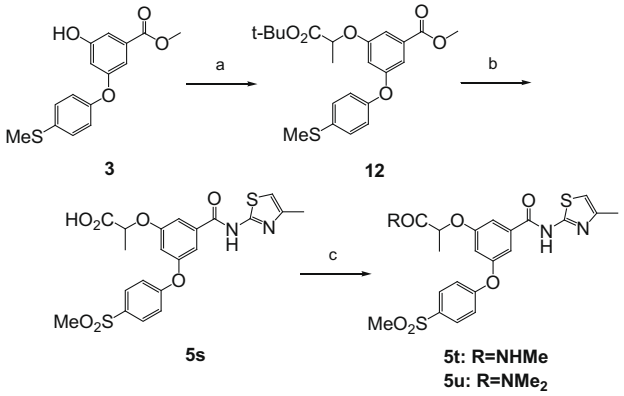
Scheme 1. Reagents and conditions: (a) (i) RX, K_2CO_3, DMF, rt −60 °C or ROH, Ph_3P, DEAD, THF (R: including protecting groups; TBS or TBDPS for OH group, Boc for NH group); (b) (i) *m*CPBA, THF, 0 °C; (ii) NaOHaq, MeOH; (iii) 2-aminothiazole, WSC, HOBT, CH_2Cl_2 or $POCl_3$, pyridine then 2-aminothiazole; (iv) HClaq, dioxane or TBAF, THF (for hydroxy derivatives); HClaq dioxane (**5n**).



Scheme 2. Reagents and conditions: (a) (i) MsCl, Et_3N, 0 °C; (ii) NaN_3, DMF, 60 °C; (iii) Ph_3P, H_2O, THF, 60 °C; (b) HCHO, $NaBH_4$–$ZnCl_2$, MeOH; (c) HCHO, $NaBH_4$–$ZnCl_2$, MeOH.

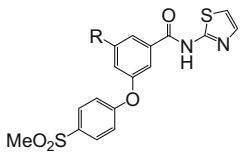


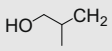
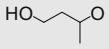
Scheme 3. Reagents and conditions: (a) (i) $NaNO_2$, HClaq, dioxane then KI, H_2O, Et_2O; (b) (i) 4-$MeSO_2C_6H_4B(OH)_2$, $Cu(OAc)_2$, Et_3N, CH_2Cl_2; (ii) NaOHaq, MeOH; (iii) 2-aminothiazole, WSC, HOBT, CH_2Cl_2; (c) 2-methyl-2-propen-1-ol, $PdCl_2(PPh_3)_2$, $NaHCO_3$, DMF, 110 °C; (d) H_2, Pd–C, MeOH.



Scheme 4. Reagents and conditions: (a) (i) *tert*-butyl 2-bromopropanoate, NaH, DMF, rt; (b) (i) *m*CPBA, THF, 0 °C; (ii) NaOHaq, MeOH; (iii) 2-amino-4-methylthiazole, WSC, HOBT, CH_2Cl_2; (iv) HClaq dioxane; (c) $MeNH_2$–HCl or Me_2NH–HCl, HOBT, Et_3N, WSC, DMF, CH_2Cl_2.

Table 2
GK activity and log *D* (pH 7.4) of GK activators around isopropyl moiety of **2b**



Compound	R	2.5 mM Glc EC_{50}[a] (μM)	10 mM Glc EC_{50}[a] (μM)	Log *D* (pH 7.4)
2b		0.17	0.06	>5
5a		0.49	0.24	>5
5b[b]		0.43	0.11	4.2
5c[b]		0.19	0.06	>5
5d[b]		0.19	0.07	4.0
5e		0.46	0.12	4.1
5f		0.61	0.12	>4
5g[b]		0.15	0.07	2.7
5h		0.78	0.20	2.4
5i[b]		0.46	0.12	3.4
5j[b]		0.28	0.17	NT
5r[b]		0.75	0.25	3.4
5k[b]		0.15	0.06	3.4
5l[b]		0.79	0.24	>4
5m[b]		0.36	0.20	>4
5o[b]		1.5	0.21	1.1
5p[b]		12	2.6	NT
5n[b]		18	4.2	NT
5q[b]		24	7.3	NT

NT: not tested.
[a] Values are the means of two or more independent assays. Compound **1** is the internal standard (EC_{50}: 0.42 ± 0.09 and 0.14 ± 0.04).
[b] Racemate.

Mitsunobu reaction to afford intermediates **4a–s** (Scheme 1). Intermediates **4b–d**, **4g**, **4i–m** are racemates, while **4o–r** are chiral. Oxidation of the methanesulfonyl group using *m*CPBA, hydrolysis of the methyl ester by NaOH and amidation with 2-aminothiazole or 2-amino-4-methylthiazole gave racemic **5a–n** and chiral derivatives **6b**, **7b**, **6g**, and **7g** after removing the hydroxy, amine or carboxylic acid protecting groups.

Racemate **5c** was optically resolved using chiral HPLC to give enantiomers **6c** and **7c**. In the same manner, **6i** and **7i** were prepared from **5i,** and **6j** and **7j** from **5j**.

Primary amine **5o** was prepared from hydroxy compound **5g** by converting the alcohol to the azide followed by reduction using the Ph_3P–H_2O system. Dimethylamino derivative **5p** was prepared by reductive amination of **5o** with formaldehyde. *N*-Methyl pyrrolidine derivative **5q** was also obtained by reductive amination of **5n** with formaldehyde (Scheme 2).

Iodophenol compound **9** was prepared from aminophenol **8** by diazonium reaction followed by addition of KI (Scheme 3). Coupling of **9** with 4-$MeSO_2C_6H_4B(OH)_2$ and amidation with 2-aminothiazole afforded the key intermediate **10.**[16] Heck reaction of **10** with 2-methyl-2-propen-1-ol gave the olefin compound **11**.[17] The carbon-linked derivative **5r** was obtained after hydrogenation of **11**.

Condensation of **5s** with methylamine or dimethylamine afforded **5t** or **5u**, respectively (Scheme 4).

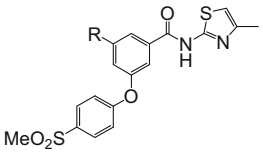
3. Biological results and discussion

An in vitro GK assay was conducted at two different glucose concentrations, 2.5 mM and 10 mM, which simulated low and high (post prandial) blood glucose conditions, respectively. SARs around the isopropoxy moiety of **2a** and **2b** were explored from the point of view of GK potency and hydrophilicity (log *D* value) (Table 2).

The ring derivatives, cyclopentyl (**5a**) and tetrahydrofuryl (**5b**) were less potent than **2b**, however, the log *D* value of **5b** improved to 4.2 (Table 2). We focused on the tetrahydrofuran group and modified **5b** to prepare **5c–f**. Ring-opened derivatives (**5c–d**) were found comparable to **2b** in GK potency, but their hydrophilicities were not significantly improved. Compounds with no chiral center (**5e–f**) showed weaker GK potency. To enhance the hydrophilicity of these derivatives, hydroxyl derivatives were prepared. 2-Hydroxy-1-methylethoxy derivative (**5g**) was strongly potent (equipotent to **2b** as racemate) and more hydrophilic than **2b** (log *D* value: 2.7). A drop in GK potency of 2-hydroxyethoxy derivative **5h** indicated the importance of the branched methyl group of **5g**. Cyclic analogs of **5g** (**5i** and **5j**), conversion to a carbon linker (**5r**), and conversion of the branched methyl group of **5g** to trifluoromethyl (**5l**) or isopropyl (**5m**) groups resulted in a decrease in GK potency compared to that of **5g**. Elongation of **5g** (**5k**) did not affect GK potency but led to less hydrophilicity (log *D*: 3.4). Amine derivatives (**5o–q**) and carboxyl derivatives (**5s–u**) exhibited weak potency (Table 3).

To confirm the effect of the chiral center on GK activity, chiral isomers were assessed by in vitro assay (Table 4). The *S*-tetrahydrofuran compound (**6b**) was twice as potent as racemate **5b**, while the *R*-tetrahydrofuranyl isomer (**7b**) was not effective in GK. Ring-opened isomers **6c** and **7c** showed the same trend, though their absolute structures are not yet confirmed. These results suggested that the chiral center affected the GK potency. We also studied the effect of chirality in the alcohol derivatives. In the same manner of **6b**, *S*-isomer (**6g**) showed excellent potency. Enantiomers of **5i** and **5j** were prepared to study the effect of the alcohol of **6g** in GK. Chiral diastereomers **6i** and **6j** showed good potency but their enantiomers (**7i** and **7j**) were less potent. However, stereoconfiguration such as *trans* and *cis* did not confer considerably different potencies. *trans* Derivative **6j** was only slightly more potent than the corresponding *cis* isomer **6i**.

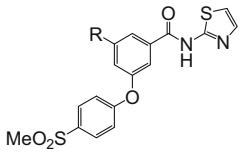
Table 3
GK activity and log *D* (pH 7.4) of carboxylic acid derivatives of **2a**



Compound	R	2.5 mM Glc EC_{50}[a] (μM)	10 mM Glc EC_{50}[a] (μM)	Log *D* (pH 7.4)
2a		0.33	0.13	>5
5s[b]		30	7.4	NT
5t[b]		>30	>30	2.9
5u[b]		>30	>30	2.6

NT: not tested.
[a] Values are the means of two or more independent assays. Compound **1** is the internal standard (EC_{50}: 0.42 ± 0.09 and 0.14 ± 0.04).
[b] Racemate.

Table 4
GK activity of chiral derivatives of **5g**



Racemate Compound	Chiral Compound	R	2.5 mM Glc EC_{50}[a] (μM)	10 mM Glc EC_{50}[a] (μM)
2b	**2b**		0.17	0.06
5b	**6b**		0.19	0.05
	7b		1.8	0.3
5c	**6c**[b]		0.13	0.06
	7c[b]		1.2	0.36
5g	**6g**		0.08	0.05
	7g		2.2	0.91
5i	**6i**[b]		0.26	0.11
	7i[b]		3.4	1.2
5j	**6j**[b]		0.16	0.09
	6j[b]		3.7	1.6

[a] Values are the means of two or more independent assays. Compound **1** is the internal standard (EC_{50}: 0.42 ± 0.09 and 0.14 ± 0.04).
[b] Absolute stereostructure speculated.

4. Pharmacological results and discussion

To compare the efficacy of the lead derivative **2a** (log *D*: >5) and the alcohol **6g** (log *D*: 2.7) that showed excellent GK activity and hydrophilicity, in vivo studies in mice were conducted. In normal fed mice, at 3 and 10 mpk oral dosing, **2a** did not show any glucose lowering effects, while **6g** was sufficiently efficacious (Fig. 2). We supposed that this difference of efficacy in vivo was due to the GK potency and solubility of **6g** (5.2 μg/mL in water (pH 7.4)).

Evaluation of glucose lowering effects of **6g** in HFD mice and KKAy mice was conducted (Figs. 3 and 4). **6g** was significantly efficacious at 3 and 10 mg/kg by oral dosing in HFD mice and 10 and 30 mpk in KKAy mice.

The PK study of **6g** in SD rats and beagle dogs indicated that oral bioavailability was good, 33% and 57%, and half lives were 1.3 h and 5.4 h, respectively (Table 5).

5. Conclusion

In conclusion, exploration of SAR around the lead compounds **2a** and **2b** with the aim to find a more soluble orally active GK activator led to the identification of compound **6g**. Aqueous solubility of this compound was improved to 5.2 μg/mL and **6g** demonstrated significant glucose lowering efficacy in HFD mice at 3 and 10 mg/kg dosing and in KKAy mice at 10 and 30 mg/kg dosing. Further development of this GK activator is currently underway.

6. Experimental

6.1. Chemistry

In general, reagents and solvents were used as purchased without further purification. The ^{1}H NMR spectra were obtained at 300 MHz on a Gemini-300, 400 MHz on a Mercury-400 (Varian) or 400 MHz on a JMN-AL400 (JEOL) spectrometer, with chemical shifts (δ, ppm) expressed relative to TMS as an internal standard. Mass spectra were recorded with electron-spray ionization (ESI) or atmospheric pressure chemical ionization (APCI) on a Waters micromass ZQ, micromass Quattro II or micromass Q-TOF-2 instrument. Flash chromatography was carried out with prepacked silica gel columns (KP-Sil silica) from Biotage or (Purif-Pack) from Moritex. Preparative thin-layer chromatography (TLC) was performed with TLC Silica Gel 60 F (Merck KGaA). Preparative HPLC purification was carried out on a YMC-Pack *Pro* C18 (YMC, 50 mm × 30 mm i.d.), eluting with a gradient of CH_3CN:aqueous CF_3CO_2H (0.1%) 10:90 to 50:50 over 8 min at a flow rate of 40 mL/min. High-resolution mass spectra were recorded with electron-spray ionization on a micromass Q-TOF-2 instrument. HPLC analysis was performed on a SUPELCO Ascentis Express (4.6 × 150 mm i.d.), eluting with a gradient of (a) 5:95–90:10 CH_3CN/aqueous H_3PO_4 (0.1%), linear gradient over 7 min followed by 90:10 isocratic over 1 min and (b) 5:95–80:20 CH_3CN/potassium phosphate

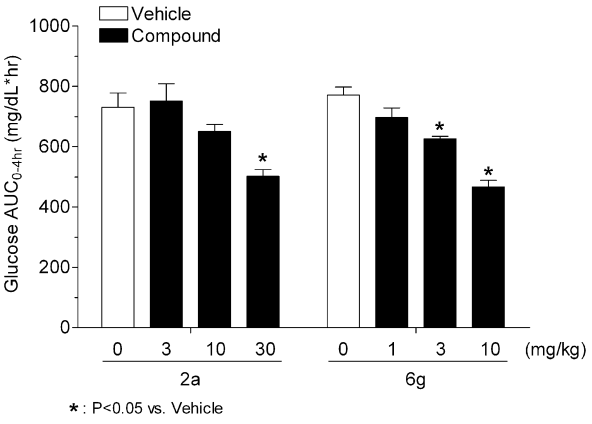


Figure 2. Glucose lowering effects of **2a** and **6g** in normal fed mice.

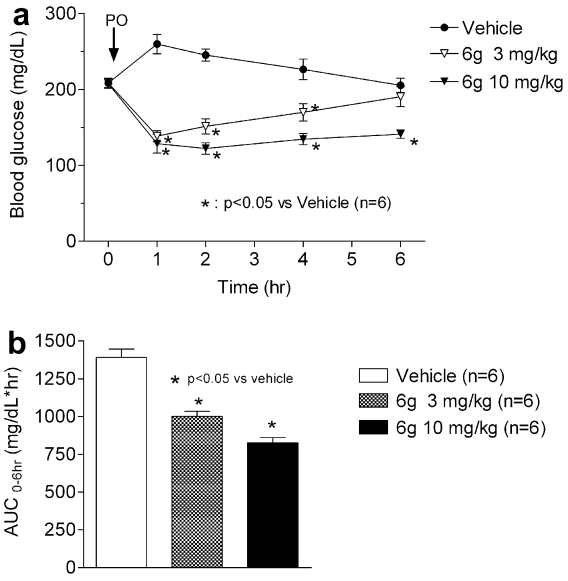


Figure 3. (a) Blood glucose levels of **6g** in HFD mice. (b) Glucose AUC of **6g** in HFD mice.

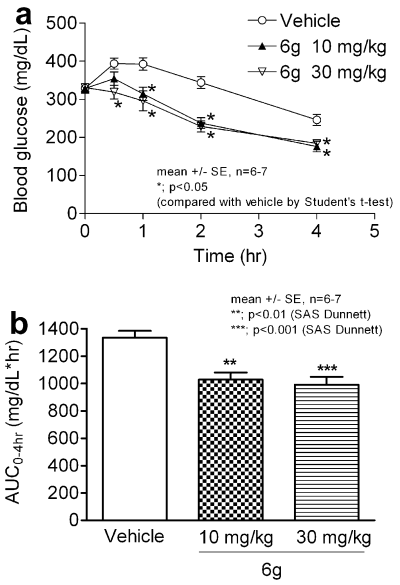
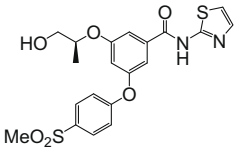


Figure 4. (a) Blood glucose levels of **6g** in KKAy mice. (b) Glucose AUC of **6g** in KKAy mice.

Table 5
Pharmacokinetic parameters of compound **6g** in SD rats and Beagle dogs



	$AUC_{0-\infty}$ (μM * h)	CLp (mL/min/kg)	$T_{1/2}$ (h)	V_{dss} (L/kg)	C_{max} (μM)	T_{max} (h)	F (%)
Rat							
IV (1 mg/kg, 40% PEG)	10	3.9	1.3	0.19			
PO (3 mg/kg, 0.5% MC)	12				3.4	0.3	33
Dog							
IV (0.3 mg/kg, 40% PEG)	2.4	4.6	5.4	1.56			
PO (1 mg/kg, 0.5% MC)	4.6				0.8	1.2	57

F: oral bioavailability; PEG: polyethylene glycol 400; MC: methylcellulose.

buffer (10 mM), linear gradient over 7 min followed by 80:20 isocratic over 1 min (detection at 210 nm).

6.1.1. 3-Isopropoxy-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (2b)

To a solution of **4s** (41.0 mg, 0.12 mmol) in $CHCl_3$ (5 mL) was added *m*CPBA (64.0 mg, 0.37 mmol) at 0 °C and the mixture was stirred for 20 min. The reaction was quenched with saturated $Na_2S_2O_3$ solution and the resulting mixture was extracted with EtOAc. The organic phase was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC on silica gel (50% EtOAc/hexane) to provide the sulfone product (43.9 mg, 98%) as a colorless foam.

To a solution of the above-obtained ester (14.0 mg, 0.035 mmol) in MeOH (0.5 mL) was added 2 N NaOH solution (0.18 mL, 0.36 mmol), and the mixture was stirred at room temperature overnight. The mixture was neutralized with 2 N HCl solution and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. To a solution of the above–obtained carboxylic acid in $CHCl_3$ (0.5 mL) were added 2-amino-1,3-thiazole (5.1 mg, 0.051 mmol), HOBt-hydrate (9.3 mg, 0.068 mmol) and EDCI (13.0 mg, 0.068 mmol), and the mixture was stirred at room temperature overnight. The mixture was evaporated and the residue was purified by silica gel column chromatography (70% EtOAc/hexane) to give **2b** (7.2 mg, 49%) as a colorless foam. ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (6H, d, J = 6.0 Hz), 3.07 (3H, s), 4.57–4.61 (1H, m), 6.83 (1H, t, J = 2.0 Hz), 6.99 (1H, d, J = 3.4 Hz), 7.14 (2H, d, J = 8.8 Hz), 7.20 (1H, d, J = 2.0 Hz), 7.28 (1H, d, J = 3.4 Hz), 7.34 (1H, d, J = 2.0 Hz), 7.92 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 433 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{21}N_2O_5S_2$ 433.0892; found 433.0896 $[M+H]^+$.

6.1.2. 3-(Cyclopentyloxy)-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5a)

To a solution of **3** (30.0 mg, 0.10 mmol) in DMF (1 mL) were added K_2CO_3 (71.0 mg, 0.52 mmol) and bromocyclopentane (0.033 mL, 0.31 mmol), and the mixture was stirred at 80 °C overnight. After cooling, the mixture was partitioned between water and EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (25% EtOAc/hexane) to give **4a** (31.9 mg, 86%) as a colorless foam.

Compound **5a** was prepared as a colorless foam from **4a** in a similar manner as described for compound **2b**. Yield: 74%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.61–1.93 (8H, m), 3.07 (3H, s), 4.75–4.79 (1H, m), 6.81 (1H, d, J = 2.0 Hz), 6.97 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.6 Hz), 7.20 (1H, s), 7.21 (1H, d, J = 3.6 Hz), 7.33 (1H, d, J = 2.0 Hz), 7.92 (2H, d, J = 8.6 Hz); MS (ESI) m/z = 459 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}N_2O_5S_2$ 459.1048; found 459.1054 $[M+H]^+$.

6.1.3. 3-[4-(Methylsulfonyl)phenoxy]-5-[tetrahydrofuran-3-yloxy]-*N*-1,3-thiazol-2-ylbenzamide (5b)

To a solution of **3** (40.0 mg, 0.14 mmol) in THF (1.0 mL) were added tetrahydrofuran-3-ol (0.022 mL, 0.28 mmol), PPh_3 (72.0 mg, 0.28 mmol) and DIAD (0.055 mL, 0.39 mmol), and the mixture was stirred at room temperature overnight. The mixture was evaporated and the residue was purified by preparative TLC on silica gel (33% EtOAc/hexane) to give **4b** (37.6 mg, 76%) as a colorless foam.

Compound **5b** was prepared as a colorless foam from **4b** in a similar manner as described for compound **2b**. Yield: 77%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.14–2.27 (2H, m), 3.08 (3H, s), 3.91–3.99 (4H, m), 4.96–4.97 (1H, m), 6.82 (1H, d, J = 1.7 Hz), 6.99 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.9 Hz), 7.18 (1H, d, J = 3.6 Hz), 7.25 (1H, s), 7.30 (1H, d, J = 1.7 Hz), 7.93 (2H, d, J = 8.9 Hz); MS (ESI) m/z = 461 $[M+H]^+$.

6.1.4. 3-[4-(Methylsulfonyl)phenoxy]-5-[(3*S*)-tetrahydrofuran-3-yloxy]-*N*-1,3-thiazol-2-ylbenzamide (6b)

To a solution of **3** (40.0 mg, 0.14 mmol) in THF (1.5 mL) were added (3*R*)-tetrahydrofuran-3-ol (0.022 mL, 0.28 mmol), PPh_3 (72.3 mg, 0.28 mmol) and DIAD (0.076 mL, 0.39 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with saturated NH_4Cl solution and the resulting mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (50% $CHCl_3$/hexane) to give **4o** (12.1 mg, 24%) as a colorless foam. HPLC (Chiralpak AD-H, 0.5 × 25 cm, hexane.EtOH = 75:25, 0.4 mL/min) t_R = 27.7 min, >98% ee.

Compound **6b** was prepared as a colorless foam from **4o** in a similar manner as described for compound **2b**. Yield: 57%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.14–2.27 (2H, m), 3.08 (3H, s), 3.91–3.99 (4H, m), 4.96–4.97 (1H, m), 6.82 (1H, d, J = 1.7 Hz), 6.99 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.9 Hz), 7.18 (1H, d, J = 3.6 Hz), 7.25 (1H, s), 7.30 (1H, d, J = 1.7 Hz), 7.93 (2H, d, J = 8.9 Hz); MS (ESI) m/z = 461 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{21}N_2O_6S_2$ 461.0841; found 461.0840 $[M+H]^+$; HPLC (a) 96.7%; (b) 97.4%.

6.1.5. 3-[4-(Methylsulfonyl)phenoxy]-5-[(3*R*)-tetrahydrofuran-3-yloxy]-*N*-1,3-thiazol-2-ylbenzamide (7b)

To a solution of **3** (40.0 mg, 0.14 mmol) in THF (1.5 mL) were added (3*S*)-tetrahydrofuran-3-ol (0.022 mL, 0.28 mmol), PPh_3 (72.3 mg, 0.28 mmol) and DIAD (0.076 mL, 0.39 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with saturated NH_4Cl solution and the resulting mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (50% $CHCl_3$/hexane) to give **4p** (14.9 mg, 30%) as a colorless foam. HPLC (Chiralpak AD-H, 0.5 × 25 cm, hexane/EtOH = 75:25, 0.4 mL/min) t_R = 37.2 min, 97% ee.

Compound **7b** was prepared as a colorless foam from **4p** in a similar manner as described for compound **2b**. Yield: 49%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.14–2.27 (2H, m), 3.08 (3H, s), 3.91–3.99 (4H, m), 4.96–4.97 (1H, m), 6.82 (1H, d, J = 1.7 Hz), 6.99 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.9 Hz), 7.18 (1H, d, J = 3.6 Hz), 7.25 (1H, s), 7.30 (1H, d, J = 1.7 Hz), 7.93 (2H, d, J = 8.9 Hz); MS (ESI) m/z = 461 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{21}N_2O_6S_2$ 461.0841; found 461.0840 $[M+H]^+$; HPLC (a) 97.9%; (b) 98.5%.

6.1.6. 3-{[1-(Methoxymethyl)propyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5c)

Compound **5c** was prepared as a colorless oil from **3** with 1-methoxybutan-2-ol in a similar manner as described for compound **2b**. Yield: 16%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.4 Hz), 1.70–1.76 (2H, m), 3.08 (3H, s), 3.37 (3H, s), 3.53–3.61 (2H, m), 4.34–4.40 (1H, m), 6.89–6.95 (1H, m), 6.97–7.01 (1H, m), 7.15 (2H, d, J = 8.8 Hz), 7.22–7.24 (1H, m), 7.25–7.29 (1H, m), 7.42–7.43 (1H, m), 7.93 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 477 $[M+H]^+$.

6.1.7. 3-{[(1*S* or 1*R*)-1-(Methoxymethyl)propyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (6c) and 3-{[(1*R* or 1*S*)-1-(Methoxymethyl)propyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (7c)

Optical resolution of **5c** by Chiralpak AD column (2 × 25 cm, hexane/EtOH = 1:2, 10 mL/min) afforded **6c** (slower, 2.2 mg) as a colorless oil and **7c** (faster, 2.5 mg) as a colorless oil.

Compound ***6c***: HPLC (Chiralpak AD, 0.5 × 25 cm, hexane/EtOH = 1:2, 0.4 mL/min) t_R = 36.0 min, 96% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.4 Hz), 1.70–1.76 (2H, m), 3.08 (3H, s), 3.37 (3H, s), 3.53–3.61 (2H, m), 4.34–4.40 (1H, m), 6.89–6.95 (1H, m), 6.97–7.01 (1H, m), 7.15 (2H, d, J = 8.8 Hz), 7.22–7.24 (1H, m), 7.25–7.29 (1H, m), 7.42–7.43 (1H, m), 7.93 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 477 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{25}N_2O_6S_2$ 477.1154; found 477.1156 $[M+H]^+$; HPLC (a) 99.3%; (b) 99.5%.

Compound ***7c***: HPLC (Chiralpak AD, 0.5 × 25 cm, hexane/EtOH = 1:2, 0.4 mL/min) t_R = 31.0 min, >98% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (3H, t, J = 7.4 Hz), 1.70–1.76 (2H, m), 3.08 (3H, s), 3.37 (3H, s), 3.53–3.61 (2H, m), 4.34–4.40 (1H, m), 6.89–6.95 (1H, m), 6.97–7.01 (1H, m), 7.15 (2H, d, J = 8.8 Hz), 7.22–7.24 (1H, m), 7.25–7.29 (1H, m), 7.42–7.43 (1H, m), 7.93 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 477 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{25}N_2O_6S_2$ 477.1154; found 477.1147 $[M+H]^+$; HPLC (a) 99.4%; (b) 99.6%.

6.1.8. 3-(2-Methoxy-1-methylethoxy)-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5d)

Compound **5d** was prepared as a colorless oil from **3** with 1-methoxypropan-2-ol in a similar manner as described for compound **2b**. Yield: 14%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (3H, d, J = 6.3 Hz), 3.07 (3H, s), 3.38 (3H, s), 3.54–3.56 (2H, m), 4.58–4.59 (1H, m), 6.89–6.90 (1H, m), 6.98 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.21–7.22 (1H, m), 7.25 (1H, d, J = 3.6 Hz), 7.37–7.39 (1H, m), 7.92 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 463 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{23}N_2O_6S_2$ 463.0998; found 463.1004 $[M+H]^+$.

6.1.9. 3-[4-(Methylsulfonyl)phenoxy]-5-(tetrahydro-2*H*-pyran-4-yloxy)-*N*-1,3-thiazol-2-ylbenzamide (5e)

Compound **5e** was prepared as a colorless foam from **3** with tetrahydro-2*H*-pyran-4-ol in a similar manner as described for compound **2b**. Yield: 28%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.76–1.84 (2H, m), 2.03–2.08 (2H, m), 3.07 (3H, s), 3.55–3.61 (2H, m), 3.94–3.99 (2H, m), 4.54–4.58 (1H, m), 6.84 (1H, t, J = 2.2 Hz), 6.99 (1H, d, J = 2.1 Hz), 7.12 (2H, d, J = 7.7 Hz), 7.22 (1H, s), 7.30 (1H, br s), 7.38 (1H, s), 7.90 (2H, d, J = 7.7 Hz); MS (ESI) m/z = 475 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}N_2O_6S_2$ 475.0998; found 475.0995 $[M+H]^+$.

6.1.10. 3-[2-Methoxy-1-(methoxymethyl)ethoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5f)

Compound **5f** was prepared as a colorless oil from **3** with 2-bromo-1,3-dimethoxypropane in a similar manner as described for compound **2b** and **5a**. Yield: 14%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (3H, s), 3.39 (6H, s), 3.63 (4H, d, J = 4.7 Hz), 4.56–4.58 (1H, m), 6.97–6.99 (2H, m), 7.15 (2H, d, J = 8.9 Hz), 7.25–7.28 (2H, m),

7.44–7.45 (1H, m), 7.93 (2H, d, J = 8.9 Hz); MS (ESI) m/z = 493 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{25}N_2O_7S_2$ 493.1103; found 493.1102 $[M+H]^+$.

6.1.11. 3-(2-Hydroxy-1-methylethoxy)-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5g)

To a solution of **3** (700 mg, 2.4 mmol) in THF (10 mL) were added 1-(*t*-butyldimethylsiloxy)-2-hydroxypropane (1.14 g, 6.0 mmol), PPh_3 (1.50 g, 6.0 mmol) and DEAD (2.6 mL, 6.0 mmol) at 0 °C, and the mixture was stirred at room temperature overnight. The reaction was quenched with water and the resulting mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (5% EtOAc/hexane) to give racemate **4g** (1.04 g, 95%) as a colorless oil.

To a solution of **4g** (800 mg, 1.73 mmol) in $CHCl_3$ (17 mL) was added *m*CPBA (896 mg, 5.19 mmol) at 0 °C and the mixture was stirred for 30 min. The reaction was quenched with saturated $Na_2S_2O_3$ solution and the resulting mixture was extracted with EtOAc. The organic phase was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated to give the crude sulfone.

To a solution of the above-obtained sulfone in MeOH (17 mL) was added 2 N NaOH solution (4.3 mL, 8.65 mmol), and the mixture was stirred at room temperature overnight. The mixture was neutralized with 10% citric acid solution and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (2% MeOH/$CHCl_3$) to give the carboxylic acid (402 mg, 49%) as a colorless oil.

To a solution of the above-obtained carboxylic acid (402 mg, 0.84 mmol) in $CHCl_3$ (8.4 mL) were added 2-amino-1,3-thiazole (252 mg, 2.52 mmol), HOBt-hydrate (341 mg, 2.52 mmol) and EDCI (321 mg, 1.68 mmol), and the mixture was stirred at room temperature overnight. The mixture was quenched with water and extracted with EtOAc. The organic phase was washed with 10% citric acid solution and brine, dried over $MgSO_4$, and evaporated to give the crude amide.

To a solution of the above-mentioned amide in 1,4-dioxane (6.0 mL) was added 6 N HCl solution (2.0 mL, 12 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was evaporated, and then excess Et_3N was added to the residue and the reaction was evaporated again. The residue was purified by silica gel column chromatography (80% EtOAc/hexane) to give the racemate **5g** (290 mg, 77%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (3H, d, J = 6.2 Hz), 3.10 (3H, s), 3.79–3.81 (2H, m), 4.54–4.57 (1H, m), 6.87–6.89 (1H, m), 7.03 (1H, d, J = 3.6 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.20–7.23 (1H, m), 7.35–7.39 (2H, m), 7.96 (2H, d, J = 8.8 Hz), 10.8 (1H, br s); MS (ESI) m/z = 449 $[M+H]^+$.

6.1.12. 3-[(1*S*)-2-Hydroxy-1-methylethoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (6g) and 3-[(1*R*)-2-hydroxy-1-methylethoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (7g)

Optical resolution of **5g** by Chiralpak AS column (2 × 25 cm, hexane/EtOH = 50:50) afforded **6g** (faster, 91.3 mg) as a colorless solid and **7g** (slower, 83.5 mg) as a colorless solid.

Using (2*R*)-1-(*t*-butyldimethylsiloxy)-2-hydroxypropane instead of 1-(*t*-butyldimethylsiloxy)-2-hydroxypropane, **6g** was prepared from **3** in a similar manner as described for compound **5g**. On the other hand, **7g** was obtained from **3** with (2*S*)-1-(*t*-butyldimethylsiloxy)-2-hydroxypropane.

Compound ***6g***: HPLC (Chiralpak AS, 0.5 × 25 cm, hexane/EtOH = 50:50, 0.4 mL/min) t_R = 17.9 min, 98% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (3H, d, J = 6.2 Hz), 3.10 (3H, s), 3.79–3.81 (2H, m), 4.54–4.57 (1H, m), 6.87–6.89 (1H, m), 7.03 (1H, d, J = 3.6 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.20–7.23 (1H, m), 7.35–7.39 (2H, m), 7.96 (2H, d, J = 8.8 Hz), 10.8 (1H, br s); MS (ESI) m/z = 449 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{21}N_2O_6S_2$ 449.0841; found 449.0840 $[M+H]^+$; HPLC (a) 99.8%; (b) 99.8%.

Compound ***7g***: HPLC (Chiralpak AS, 0.5 × 25 cm, hexane:EtOH = 50:50, 0.4 mL/min) t_R = 23.3 min, 98% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (3H, d, J = 6.2 Hz), 3.10 (3H, s), 3.79–3.81 (2H, m), 4.54–4.57 (1H, m), 6.87–6.89 (1H, m), 7.03 (1H, d, J = 3.6 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.20–7.23 (1H, m), 7.35–7.39 (2H, m), 7.96 (2H, d, J = 8.8 Hz), 10.8 (1H, br s); MS (ESI) m/z = 449 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{21}N_2O_6S_2$ 449.0841; found 449.0849 $[M+H]^+$; HPLC (a) 98.7%; (b) 99.4%.

6.1.13. 3-(2-Hydroxyethoxy)-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5h)

Compound **5h** was prepared as a colorless foam from **3** with 2-{[*tert*-butyl(dimethyl)silyl]oxy}ethanol in a similar manner as described for compound **5g**. Yield: 12%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.10 (3H, s), 4.01 (2H, t, J = 4.5 Hz), 4.14 (2H, t, J = 4.5 Hz), 6.85–6.88 (1H, m), 7.02 (1H, d, J = 3.0 Hz), 7.16 (2H, d, J = 8.4 Hz), 7.29–7.32 (2H, m), 7.36–7.38 (1H, m), 7.95 (2H, d, J = 8.4 Hz), 11.3 (1H, br s); MS (ESI) m/z = 435 $[M+H]^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_2O_6S_2$ 435.0685; found 435.0686 $[M+H]^+$.

6.1.14. 3-{[(*cis*)-2-Hydroxycyclopentyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5i)

Compound **5i** was prepared as a colorless foam from **3** with *trans*-2-{[*tert*-butyl(diphenyl)silyl]oxy}cyclopentanol in a similar manner as described for compound **5g**. Yield: 10%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62–2.08 (6H, m), 3.08 (3H, s), 4.24–4.30 (1H, m), 4.55–4.60 (1H, m), 6.87 (1H, t, J = 2.0 Hz), 7.00 (1H, d, J = 3.6 Hz), 7.14 (2H, d, J = 8.8 Hz), 7.25 (1H, t, J = 2.0 Hz), 7.25 (1H, d, J = 3.6 Hz), 7.40 (1H, t, J = 2.0 Hz), 7.93 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 475 $[M+H]^+$.

6.1.15. 3-{[(1*S*,2*R* or 1*R*,2*S*)-2-Hydroxycyclopentyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (6i) and 3-{[(1*R*,2*S* or 1*S*,2*R*)-2-Hydroxycyclopentyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (7i)

Optical resolution of **5i** by Chiralpak AS column (2 × 25 cm, hexane/EtOH = 1:2) afforded **6i** (faster, 3.8 mg) as a colorless solid and the antipode **7i** (slower, 4.2 mg) as a colorless solid.

Compound ***6i***: HPLC (Chiralpak AS, 0.5 × 25 cm, hexane/EtOH = 1:2, 0.4 mL/min) t_R = 16.3 min, >99% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62–2.08 (6H, m), 3.08 (3H, s), 4.24–4.30 (1H, m), 4.55–4.60 (1H, m), 6.87 (1H, t, J = 2.0 Hz), 7.00 (1H, d, J = 3.6 Hz), 7.14 (2H, d, J = 8.8 Hz), 7.25 (1H, t, J = 2.0 Hz), 7.25 (1H, d, J = 3.6 Hz), 7.40 (1H, t, J = 2.0 Hz), 7.93 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 475 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}N_2O_6S_2$ 475.0998; found 475.0990 $[M+H]^+$; HPLC (a) 99.4%; (b) 99.4%.

Compound ***7i***: HPLC (Chiralpak AS, 0.5 × 25 cm, hexane/EtOH = 1:2, 0.4 mL/min) t_R = 25.2 min, 98% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62–2.08 (6H, m), 3.08 (3H, s), 4.24–4.30 (1H, m), 4.55–4.60 (1H, m), 6.87 (1H, t, J = 2.0 Hz), 7.00 (1H, d, J = 3.6 Hz), 7.14 (2H, d, J = 8.8 Hz), 7.25 (1H, t, J = 2.0 Hz), 7.25 (1H, d, J = 3.6 Hz), 7.40 (1H, t, J = 2.0 Hz), 7.93 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 475 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}N_2O_6S_2$ 475.0998; found 475.0994 $[M+H]^+$; HPLC (a) 99.4%; (b) 99.7%.

6.1.16. 3-{[(*trans*)-2-Hydroxycyclopentyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5j)

Compound **5j** was prepared as a colorless foam from **3** with *cis*-2-{[*tert*-butyl(diphenyl)silyl]oxy}cyclopentanol in a similar manner as described for compound **5g**. Yield: 9%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27–1.35 (6H, m), 3.08 (3H, s), 4.29–4.33 (1H, m), 4.54–

4.56 (1H, m), 6.86 (1H, s), 6.99 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.23 (1H, s), 7.27 (1H, d, J = 3.6 Hz), 7.39 (1H, s), 7.92 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 475 $[M+H]^+$.

6.1.17. 3-{[(1*S*,2*S* or 1*R*,2*R*)-2-Hydroxycyclopentyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (6j) and 3-{[(1*R*,2*R* or 1*S*,2*S*)-2-Hydroxycyclopentyl]oxy}-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (7j)

Optical resolution of **5j** by Chiralpak AD column (2 × 25 cm, EtOH) afforded **6j** (faster, 3.0 mg) as a colorless solid and the antipode **7j** (slower, 3.3 mg) as a colorless solid.

*Compound **6j***: HPLC (Chiralpak AD, 0.5 × 25 cm, EtOH, 0.4 mL/min) t_R = 17.7 min, >99% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27–1.35 (6H, m), 3.08 (3H, s), 4.29–4.33 (1H, m), 4.54–4.56 (1H, m), 6.86 (1H, s), 6.99 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.23 (1H, s), 7.27 (1H, d, J = 3.6 Hz), 7.39 (1H, s), 7.92 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 475 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}N_2O_6S_2$ 475.0998; found 475.0989 $[M+H]^+$; HPLC (a) 99.5%; (b) 99.3%.

*Compound **7j***: HPLC (Chiralpak AD, 0.5 × 25 cm, EtOH, 0.4 mL/min) t_R = 26.2 min, 98% ee; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27–1.35 (6H, m), 3.08 (3H, s), 4.29–4.33 (1H, m), 4.54–4.56 (1H, m), 6.86 (1H, s), 6.99 (1H, d, J = 3.6 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.23 (1H, s), 7.27 (1H, d, J = 3.6 Hz), 7.39 (1H, s), 7.92 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 475 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{23}N_2O_6S_2$ 475.0998; found 475.0993 $[M+H]^+$; HPLC (a) 99.3%; (b) 99.2%.

6.1.18. 3-(3-Hydroxy-1-methylpropoxy)-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5k)

Compound **5k** was prepared as a colorless foam from **3** with 4-{[*t*-butyl(dimethyl)silyl]oxy}butan-2-ol in a similar manner as described for compound **5g**. Yield: 40%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (3H, d, J = 6.1 Hz), 1.87–1.89 (1H, m), 2.00–2.02 (1H, m), 3.10 (3H, s), 3.82–3.85 (2H, m), 4.70–4.71(1H, m), 6.88–6.90 (1H, m), 7.01 (1H, d, J = 3.5 Hz), 7.17 (2H, d, J = 8.9 Hz), 7.22–7.25 (1H, m), 7.35 (1H, d, J = 3.5 Hz), 7.47–7.48 (1H, m), 7.95 (2H, d, J = 8.9 Hz), 11.0 (1H, br s); MS (ESI) m/z = 463 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{23}N_2O_6S_2$ 463.0998; found 463.0999 $[M+H]^+$.

6.1.19. 3-[4-(Methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-yl-5-[2,2,2-trifluoro-1-(hydroxymethyl)ethoxy]benzamide (5l)

Compound **5l** was prepared as a colorless foam from **3** with 3-{[*tert*-butyl(dimethyl)silyl]oxy}-1,1,1-trifluoropropan-2-ol in a similar manner as described for compound **5g**. Yield: 16%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (3H, s), 4.21–4.27 (2H, m), 4.40–4.41 (1H, m), 6.84 (1H, s), 7.01 (1H, d, J = 3.6 Hz), 7.12 (2H, d, J = 8.7 Hz), 7.28 (1H, d, J = 2.4 Hz), 7.36 (1H, d, J = 1.0 Hz), 7.92 (2H, d, J = 8.7 Hz); MS (ESI) m/z = 503 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{18}N_2O_6F_3S_2$ 503.0558; found 503.0546 $[M+H]^+$.

6.1.20. 3-[1-(Hydroxymethyl)-2-methylpropoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5m)

Compound **5m** was prepared as a colorless foam from **3** with 1-{[*tert*-butyl(dimethyl)silyl]oxy}-3-methylbutan-2-ol in a similar manner as described for compound **5g**. Yield: 33%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95–0.98 (6H, m), 2.04–2.06 (1H, m), 3.07 (3H, s), 3.32–3.85 (2H, m), 4.21–4.23 (1H, m), 6.83–6.85 (1H, m), 6.96 (1H, d, J = 3.7 Hz), 7.11 (2H, d, J = 8.9 Hz), 7.17–7.18 (1H, m), 7.23 (1H, d, J = 3.7 Hz), 7.38–7.40 (1H, m), 7.91 (2H, d, J = 8.8 Hz), 12.0 (1H, br s); MS (ESI) m/z = 477 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{25}N_2O_6S_2$ 477.1154; found 477.1156 $[M+H]^+$.

6.1.21. 3-(2-Amino-1-methylethoxy)-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5o)

To a solution of the above-obtained **5g** (100 mg, 0.22 mmol) in THF (2.2 mL) were added Et_3N (0.19 mL, 1.32 mmol) and methanesulfonyl chloride (0.052 mL, 0.66 mmol) at 0 °C, and the mixture was stirred for 30 min. The reaction was quenched with saturated $NaHCO_3$ solution and the resulting mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated to give the crude mesylate.

To a solution of the above-obtained mesylate in DMF (8.2 mL) was added sodium azide (73 mg, 0.11 mmol), and the mixture was stirred at 60 °C for 4 h. The reaction was cooled, quenched with water and the resulting mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (10% MeOH/$CHCl_3$) to give the azide compound (83.0 mg, 79%) as a colorless oil.

To a solution of the above-obtained azide compound in THF (1 mL) were added Ph_3P (71 mg, 0.27 mmol) and water (0.032 mL, 1.75 mmol), and the mixture was stirred at 60 °C for 2 h. The reaction was cooled and evaporated. The residue was purified by preparative TLC (5% MeOH/$CHCl_3$) to give **5o** (66.8 mg, 85%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (3H, d, J = 6.0 Hz), 2.92 (2H, d, J = 6.0 Hz), 3.09 (3H, s), 4.41 (1H, sextet, J = 6.0 Hz), 6.85–6.87 (1H, m), 6.98 (1H, d, J = 3.5 Hz), 7.14 (2H, d, J = 8.9 Hz), 7.21 (1H, d, J = 3.5 Hz), 7.24–7.26 (1H, m), 7.41–7.43 (1H, m), 8.87 (2H, d, J = 8.9 Hz); MS (ESI) m/z = 448 $[M+H]^+$; HRMS (ESI) calcd for $C_{20}H_{22}N_3O_5S_2$ 448.1001; found 448.1002 $[M+H]^+$.

6.1.22. 3-[2-(Dimethylamino)-1-methylethoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5p)

To a solution of the above-obtained racemate **5o** (10.0 mg, 0.022 mmol) in methanol (0.20 mL) were added formaldehyde solution (0.5 mL) and $NaBH_4$–$ZnCl_2$ (2:1) methanol solution (0.5 mL), and the mixture was stirred at room temperature overnight. The reaction was quenched with saturated $NaHCO_3$ solution and the resulting mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (3% MeOH:$CHCl_3$) to give **5p** (4.6 mg, 44%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, d, J = 6.2 Hz), 2.30 (6H, s), 2.42 (1H, dd, J = 4.4, 13.0 Hz), 2.68 (1H, dd, J = 6.2, 13.0 Hz), 3.09 (3H, s), 4.56 (1H, dt, J = 4.5, 6.2 Hz), 6.88–6.89 (1H, m), 7.00 (1H, d, J = 3.6 Hz), 7.15 (2H, d, J = 8.9 Hz), 7.21–7.23 (1H, m), 7.28 (1H, d, J = 3.6 Hz), 7.40–7.42 (1H, m), 7.93 (2H, d, J = 8.9 Hz), 11.4 (1H, br s); MS (ESI) m/z = 476 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{26}N_3O_5S_2$ 476.1314; found 476.1324 $[M+H]^+$.

6.1.23. 3-[4-(Methylsulfonyl)phenoxy]-5-(pyrrolidin-3-yloxy)-*N*-1,3-thiazol-2-ylbenzamide (5n)

Compound **5n** was prepared as a colorless foam from **3** with *tert*-butyl 3-hydroxypyrrolidine-1-carboxylate in a similar manner as described for compound **5g**. Yield: 38%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.04–2.17 (2H, m), 3.08 (3H, s), 3.04–3.28 (4H, m), 4.91 (1H, s), 6.78–6.79 (1H, m), 6.98 (1H, d, J = 3.3 Hz), 7.12 (2H, d, J = 8.8 Hz), 7.21–7.25 (1H, m), 7.29 (1H, d, J = 3.3 Hz), 7.32–7.33 (1H, m), 7.90 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 460 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{22}N_3O_5S_2$ 460.1001; found 460.1000 $[M+H]^+$.

6.1.24. 3-[(1-Methylpyrrolidin-3-yl)oxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5q)

To a solution of **5n** (6.0 mg, 0.013 mmol) in MeOH (0.5 mL) was added 37% HCHO solution (5 drops), and the mixture was stirred at room temperature for 1 h. To the mixture was added $NaBH_4$ (4.0 mg, 0.13 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with water and evaporated. The residue was purified by preparative TLC (10% MeOH/$CHCl_3$) to give **5q** (3.2 mg, 52%) as a colorless foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01–2.02 (1H, m), 2.34–2.46 (2H, m), 2.41 (3H, s), 2.80–2.88 (3H, m), 3.07 (3H, s), 4.96 (1H, s), 6.79–6.80 (1H, m), 6.99 (1H, d, J = 3.6 Hz), 7.12 (2H, d, J = 8.8 Hz), 7.18–7.19

(1H, m), 7.27–7.29 (2H, m), 7.91 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 474 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{24}N_3O_5S_2$ 474.1157; found 474.1156 $[M+H]^+$.

6.1.25. 2-(3-[4-(Methylsulfonyl)phenoxy]-5-{[(4-methyl-1,3-thiazol-2-yl)amino]carbonyl}phenoxy)propanoic acid (5s)

To a solution of **3** (290 mg, 1.0 mmol) in DMF (10 mL) were added NaH (44.0 mg, 1.1 mmol) and *tert*-butyl 2-bromopropanoate (42.0 mg, 2.0 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was quenched with saturated NH_4Cl solution and the resulting mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (10% EtOAc/hexane) to give **12** (374 mg, 89%) as a colorless solid.

Compound **5s** was prepared as a colorless solid from **12** with 4-methylthiazol-2-amine in a similar manner as described for compound **5g**. Yield: 34%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (3H, d, J = 6.8 Hz), 2.28 (3H, s), 3.27 (3H, s), 5.03 (1H, septet, J = 6.8 Hz), 6.81–6.83 (1H, m), 6.92–6.95 (1H, m), 7.25 (2H, d, J = 8.8 Hz), 7.41–7.43 (1H, m), 7.48–7.51 (1H, m), 7.95 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 477 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{21}N_2O_7S_2$ 477.0790; found 477.0794 $[M+H]^+$.

6.1.26. 3-[1-Methyl-2-(methylamino)-2-oxoethoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-(4-methyl-1,3-thiazol-2-yl)benzamide (5t)

To a solution of **5s** (10.0 mg, 0.025 mmol) in DMF (0.1 mL) and CH_2Cl_2 (0.5 mL) were added HOBT (10.0 mg, 0.075 mmol), methylamine hydrochloride (15.0 mg, 0.23 mmol), Et_3N (0.031 mL, 0.23 mmol) and EDCI (10.0 mg, 0.075 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with 10% citric acid solution and the resulting mixture was extracted with $CHCl_3$-MeOH. The organic phase was dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (10% MeOH/$CHCl_3$) to give **5t** (7.3 mg, 60%) as a colorless foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (3H, s), 2.26 (3H, s), 2.86 (3H, d, J = 4.7 Hz), 3.10 (3H, s), 4.73 (1H, q, J = 6.6 Hz), 6.47 (1H, br s), 6.56–6.58 (1H, m), 6.81–6.84 (1H, m), 7.12 (2H, d, J = 8.8 Hz), 7.20–7.23 (1H, m), 7.30–7.32 (1H, m), 7.93 (2H, d, J = 8.8 Hz), 11.0 (1H, br s); MS (ESI) m/z = 490 $[M+H]^+$; HRMS (ESI) calcd for $C_{22}H_{24}N_3O_6S_2$ 490.1107; found 490.1099 $[M+H]^+$.

6.1.27. 3-[2-(Dimethylamino)-1-methyl-2-oxoethoxy]-5-[4-(methylsulfonyl)phenoxy]-*N*-(4-methyl-1,3-thiazol-2-yl)benzamide (5u)

Compound **5u** was prepared as a colorless oil from **3** with dimethylamine hydrochloride in a similar manner as described for compound **5t**. Yield: 6%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62 (3H, d, J = 6.7 Hz), 2.35 (3H, s), 2.98 (3H, s), 3.10 (3H, s), 3.14 (3H, s), 5.06 (1H, q, J = 6.7 Hz), 6.56–6.59 (1H, m), 6.81–6.84 (1H, m), 7.15 (2H, d, J = 8.8 Hz), 7.19–7.22 (1H, m), 7.28–7.30 (1H, m), 7.95 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 504 $[M+H]^+$; HRMS (ESI) calcd for $C_{23}H_{26}N_3O_6S_2$ 504.1263; found 504.1262 $[M+H]^+$.

6.1.28. Methyl 3-hydroxy-5-iodobenzoate (9)

To a solution of **8** (400 mg, 2.45 mmol) in 1,4-dioxane (2.0 mL) and H_2O (3.0 mL) were added 4 N HCl in 1,4-dioxane solution (1.80 mL, 7.20 mmol) and $NaNO_2$ (186 mg, 2.70 mmol) in H_2O (2.0 mL) at 0 °C, and the mixture was stirred at 0 °C for 15 min. To the mixture was added KI (489 mg, 2.94 mmol) in H_2O (4.0 mL) at 0 °C, and the mixture was stirred at 10 °C for 10 min. Et_2O (10 mL) was added to the mixture at 10 °C, and the mixture was stirred at room temperature for 2 h. The reaction was quenched with $KHSO_4$ solution and extracted with EtOAc. The organic phase was washed with NH_4Cl solution and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (33% EtOAc/hexane) to give **9** (380 mg, 56%) as a colorless solid. ^{1}H NMR (300 MHz, CD_3OD) δ 3.91 (3H, s), 5.38 (1H, br s), 7.42 (1H, s), 7.47 (1H, s), 7.94 (1H, s); MS (ESI) m/z = 277 $[M-H]^-$.

6.1.29. 3-Iodo-5-[4-(methylsulfonyl)phenoxy]-N-(1,3-thiazol-2-yl)benzamide (10)

To a solution of **9** (869 mg, 3.13 mmol) in CH_2Cl_2 (32 mL) were added 4-$MeSO_2$–$PhB(OH)_2$ (1.25 g, 6.25 mmol), $Cu(OAc)_2$ (568 mg, 3.13 mmol) and Et_3N (2.18 mL, 15.6 mmol), and the mixture was stirred under oxygen atmosphere at room temperature overnight. The mixture was filtered through a Celite pad and the filtrate was evaporated. The residue was partitioned with H_2O and $CHCl_3$. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (33% EtOAc/hexane) to give methyl 3-iodo-5-[4-(methylsulfonyl)phenoxy]benzoate (913 mg, 68%) as a white solid.

Compound **10** was prepared as a colorless foam from the above obtained ester in a similar manner as described for compound **5g**. Yield: 54%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.09 (3H, s), 7.04 (1H, s), 7.15 (2H, d, J = 8.6 Hz), 7.32 (1H, s), 7.62–7.68 (2H, m), 7.97 (2H, d, J = 8.6 Hz), 8.10 (1H, s).

6.1.30. 3-[2-(Hydroxymethyl)prop-2-en-1-yl]-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (11)

To a solution of **10** (57.6 mg, 0.12 mmol) in DMF (2.0 mL) were added $NaHCO_3$ (19.0 mg, 0.23 mmol), 2-methylprop-2-en-1-ol (0.097 mL, 1.15 mmol), nBu_4NBr (19.0 mg, 0.058 mmol) and $Pd(PPh_3)_2Cl_2$ (8.0 mg, 0.012 mmol), and the mixture was stirred at 110 °C overnight. After cooling, the reaction was quenched with H_2O and EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (75% EtOAc/hexane) to give **11** (5.6 mg, 11%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.08 (3H, s), 3.49 (2H, s), 4.06 (2H, s), 4.91 (1H, s), 5.19 (1H, s), 7.00 (1H, d, J = 3.3 Hz), 7.11 (2H, d, J = 9.0 Hz), 7.13 (1H, d, J = 3.3 Hz), 7.20 (1H, s), 7.55 (1H, s), 7.67 (1H, s), 7.92 (2H, d, J = 9.0 Hz); MS (ESI) m/z = 445 $[M+H]^+$.

6.1.31. 3-(3-Hydroxy-2-methylpropyl)-5-[4-(methylsulfonyl)phenoxy]-*N*-1,3-thiazol-2-ylbenzamide (5r)

To a solution of **11** (4.2 mg, 0.0094 mmol) in MeOH (2.0 mL) was added 10% Pd–C (4.0 mg), and the mixture was stirred under hydrogen atmosphere for 3 h. The mixture was filtered through a Celite pad and the filtrate was evaporated. The residue was purified by preparative TLC (75% EtOAc/hexane) to give **5r** (1.0 mg, 25%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (6H, d, J = 6.7 Hz), 1.97–2.05 (1H, m), 2.50–2.94 (2H, m), 3.08 (3H, s), 3.50–3.56 (2H, m), 7.03 (1H, d, J = 3.5 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.17 (1H, s), 7.42 (1H, d, J = 3.5 Hz), 7.52 (1H, s), 7.63 (1H, s), 7.93 (2H, d, J = 8.8 Hz); MS (ESI) m/z = 447 $[M+H]^+$; HRMS (ESI) calcd for $C_{21}H_{23}N_2O_5S_2$ 447.1048; found 447.1055 $[M+H]^+$.

6.2. Log *D*

Log *D* values were measured using reported methods.[18–20]

6.3. Solubility

Solubility data were measured using HPLC-UV method. To 1 mg of sample was added 1 ml of 200 mM phosphate buffer (pH 7.4), and the mixture was stirred at 25 °C for 1 h and left 25 °C for 24 h without stirring. After centrifugation, UV area of the skimming was measured and calculated using HPLC-UV method based on standard curve prepared previously.

6.4. Biology

6.4.1. In vitro GK assay

The recombinant human liver GK used in this assay was expressed in *E. coli* as a FLAG fusion protein. GK activity was measured by the glucose-6-phosphate dehydrogenase coupled continuous spectrophotometric assay. GK was incubated with DMSO solution in assay buffer containing 25 mM Hepes, pH 7.2, 1 mM dithiothreitol, 0.5 mM thionicotinamide adenine dinucleotide, 2 mM $MgCl_2$, 1 mM ATP, 2 U/mL glucose-6-phosphate dehydrogenase and 2.5 or 10 mM glucose at 30 °C. Reaction velocities were obtained from the rate of increase in absorbance at 405 nm after 5 min of reaction. The OD values were measured at each concentration of the evaluated compound, using the OD value of the DMSO control as 100%. The EC_{50} (μM) values were calculated from the OD value at each concentration, and used as indices of GK activator potency of the compound.

6.4.2. In vivo assay: in normal fed mice

Ten-to-eleven-week old male ICR mice were freely fed before performing the test. The mice were orally administered compounds **2a**, **6g** or vehicle alone (0.5% methylcellulose solution). Blood glucose concentrations were measured just prior to and following oral dosing (0.5, 1, 1.5, 2, 3 and 4 h). AUC values were calculated from the data (from 0 h to 4 h).

6.4.3. In vivo assay: in HFD mice

Forty-one-week old male high-fat diet (HFD) mice were freely fed before performing the test. The mice were orally administered either compound **6g** or vehicle alone (0.5% methylcellulose solution). Blood glucose concentrations were measured just prior to and following oral dosing (1, 2, 4, and 6 h). AUC values were calculated from the data (from 0 h to 6 h).

6.4.4. In vivo assay: in KKAy mice

Nine-week old male KKAy mice were fasted 4 hours before performing the test. The mice were orally administered either compound **6g** or vehicle alone (0.5% methylcellulose solution). Blood glucose concentrations were measured just prior to and following oral dosing (0.5, 1, 2, and 4 h). AUC values were calculated from the data (from 0 h to 4 h).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.04.040.

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