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The design and optimization of a series of 2-(pyridin-2-yl)-1*H*-benzimidazole compounds as allosteric glucokinase activators

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ABSTRACT

The optimization of a series of benzimidazole glucokinase activators is described. We identified a novel and potent achiral benzimidazole derivative as an allosteric GK activator. This activator was designed and synthesized via removal of the chiral center of the lead compound, 6-(*N*-acylpyrrolidin-2-yl)benzimidazole. The activator exhibited good PK profiles in rats and dogs, and significant hypoglycemic efficacy at 1 mg/kg po dosing in a rat OGTT model. The binding site and binding mode of the benzimidazole class of GKA with GK protein was confirmed by X-ray crystallographic analysis.

1. Introduction

Glucokinase (GK), a member of the hexokinase family,[1] is expressed in the liver and in pancreatic β-cells, and catalyzes the first step in glycolysis. This step involves the phosphorylation of glucose to glucose 6-phosphate. GK plays an important role as a glucose sensor in maintaining plasma glucose homeostasis by enhancing both glucose uptake in the liver and insulin secretion from pancreatic β-cells.[2] Therefore, a GK activator (GKA) can be expected to function as a hypoglycemic drug in the treatment of type II (insulin-independent) diabetes mellitus.

It was recently discovered that GKAs bind to an allosteric site on GK, 20 Å away from the glucose binding site.[3–5] These GKAs cause potent hypoglycemic reactions in rodents, both by increasing pancreatic insulin secretion and by augmenting hepatic glucose metabolism.[6]

To date, several amide classes of GKAs, including RO0281675 (Roche),[2,5] GKA50 (AstraZeneca),[7] LY2121260 (Lilly)[8] and PSN-GK1 (Prosidion/OSI)[9] have been disclosed, and some of these compounds have entered human clinical trials (Scheme 1).[10]

Our group has also reported SAR analyses of several structural classes of GKAs.[11] In our previous article, we described the discovery of 6-[(2*R*)-1-acetylpyrrolidin-2-yl]-5-aryloxy-2-pyridine-yl-1*H*-benzimidazole GKAs, represented by compound **1**. In this earlier study, detailed SARs for the pyridine ring at the 2-position and the phenoxy portion at the 5-position of the benzimidazole pharmacophore were examined. However, modification of the *N*-acylpyrrolidine portion of lead compound **1** was not performed due to synthetic complexities caused by the chiral center.[12] From the point of view of drug discovery, removal of the chiral center should expand the scope of modification strategies and further development of potent benzimidazole GKAs. In this paper, we describe detailed SAR analysis of novel benzimidazole GKAs generated by replacing the chiral *N*-acetylpyrrolidin-2-yl group at the 6-position of lead **1** with appropriate achiral substituents. This approach led to the identification of the potent benzimidazole derivative, **3g**. In addition, the binding mode of benzimidazole GKA with GK protein, determined by X-ray crystallographic analysis, is reported.

2. Chemistry

Preparation of compounds **2a–c** and **3a–k** are described in Scheme 2. First, an *o*-nitroanilide **9** was obtained from commercially available 2-fluoro-4-nitrobenzoic acid (**4**) in 60% yield in five steps via esterification, reduction of the nitro group with Raney Ni, amidation with 2-picolinic acid in the presence of WSCI, nitration with fuming HNO_3 and substitution by 4-(ethylsulfonyl)phenol (**27**). Then, reduction of the nitro group in anilide **9** with $SnCl_2$ directly afforded a cyclized benzimidazole product, **10**. Next, benzimidazole

Abbreviations: rt, room temperature; CDI, 1,1′-carbonylbis-1*H*-imidazole; DEAD, diethyl azodicarboxylate; HOBt, 1-hydroxybenzotriazole; LDA, lithium diisopropyl amide; $PdCl_2$(dppf)-CH_2Cl_2, 1,1′-bis(diphenylphosphino)ferrocenedichloro palladium(II) dichloromethane complex; SEM-Cl, 2-(trimethylsilyl)ethoxymethyl chloride; TFA, trifluoroacetic acid; WSCI, 1-ethyl3-(3-dimethylaminopropyl) carbodiimide hydrochloride.

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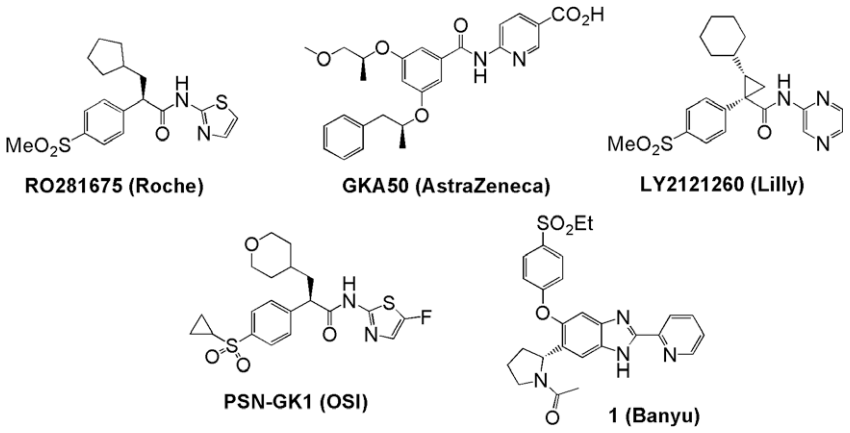
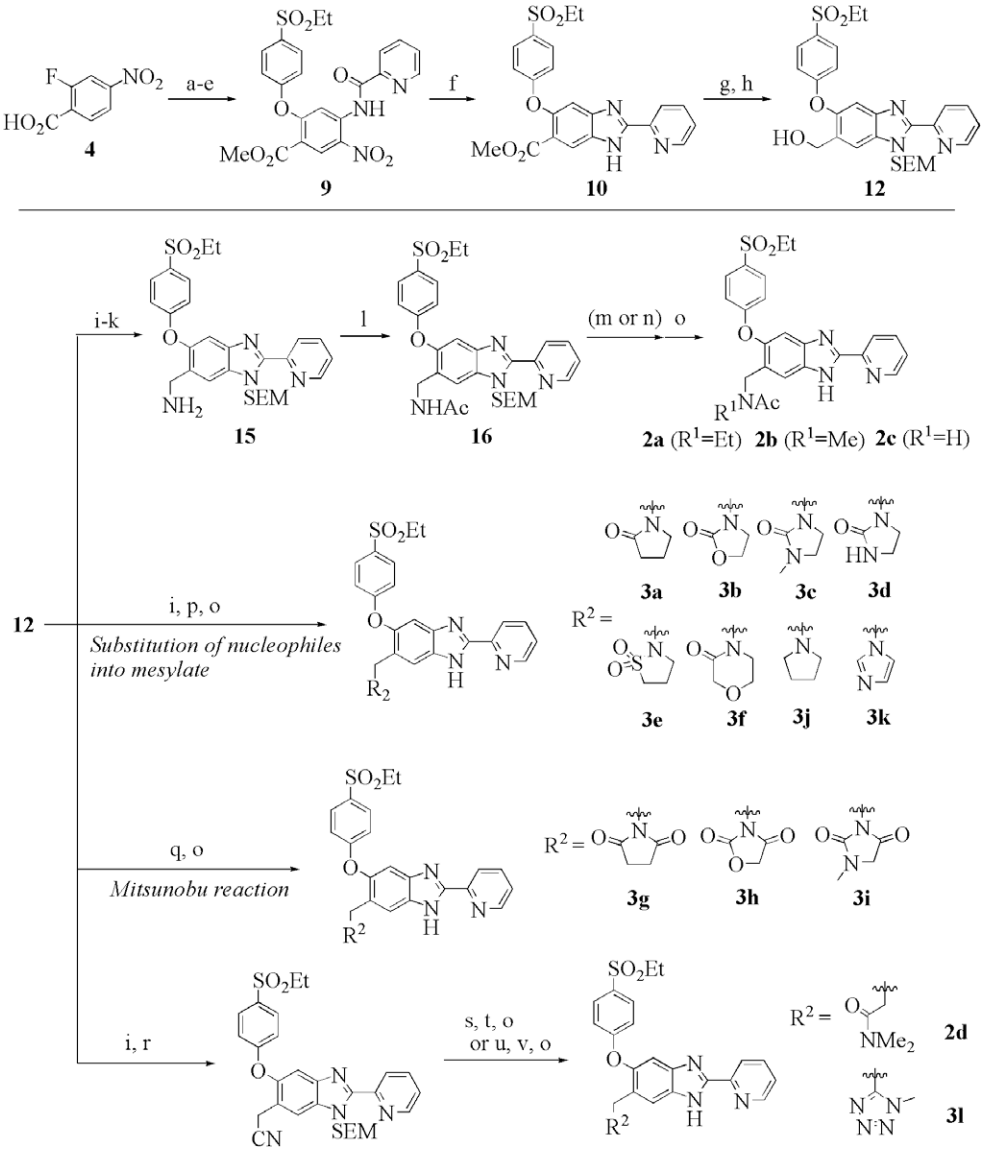


Scheme 1. Structures of GK activators.



Scheme 2. Reagents and conditions: (a) catH_2SO_4, MeOH, reflux, 94%; (b) H_2 (balloon), Raney Ni (cat), MeOH, THF, rt, 99%; (c) 2-picolinic acid, WSCI, pyridine, rt, 92%; (d) fuming HNO_3, 0 °C to rt, 85%; (e) phenol **27**, K_2CO_3, DMF, 80 °C, 82%; (f) $SnCl_2$–$2H_2O$, HCl, MeOH, DMF, 80 °C; (g) SEMCl, NaH, DMF, rt, 73% in two steps; (h) $LiAlH_4$, THF, 0 °C, 80%; (i) MsCl, NEt_3, THF, 0 °C; (j) NaN_3, DMF; (k) $CuSO_4/5H_2O$, $NaBH_4$, MeOH, rt, 51% in two steps; (l) acetyl chloride, NEt_3, $CHCl_3$, rt, 97%; (m) MeI, NaH, DMF, rt, 47%; (n) EtI, NaH, DMF, rt, 28%; (o) TFA, H_2O, rt; (p) Nucleophiles, NaH, DMF, rt. (q) imides, 40 wt % DEAD in toluene, PPh_3, THF, rt; (r) NaCN, DMF, rt, 66%; (s) NaOH, EtOH, reflux, 58%; (t) Me_2NH, WSCI, HOBt, $CHCl_3$, rt, 56%; (u) $TMSN_3$, Bu_2SnO, Toluene; (v) MeI, NaH, DMF, 0 °C.

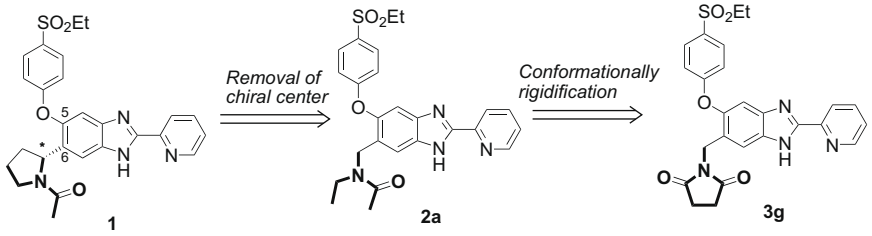


Figure 1. Modification strategy from chiral (*N*-acetylpyrrolidin-2-yl)benzimidazole lead **1** to achiral succinimide derivative **3g**.

10 was protected with a 2-(trimethylsilyl)ethoxymethyl (SEM) group before the carboxyester group was reduced with $LiAlH_4$ to give hydroxymethyl analog **12** as a key intermediate in 58% yield.

The mesylate of **12** was substituted by NaN_3 and reduced with $CuSO_4$–$5H_2O$ to give aminomethyl derivative **15** in 51% yield. Acetylation of **15** followed by alkylation (if necessary) and deprotection of the SEM group afforded acetylamino derivatives **2a**–**c**. On the other hand, the mesylate of **12** was substituted by appropriate nucleophiles followed by deprotection to afford the corresponding targeted compounds **3a**–**f**, **3j** and **3k**. Preparation of compounds **2d** and **3l** were also achieved via cyanation of the mesylate of **12** followed by hydrolysis of the CN group or reaction with TMSN3. Each reaction is described in Section 5.

Mitsunobu reaction of the hydroxymethyl intermediate **12** with succinimide followed by deprotection provided succinimide derivative **3g**. Other imide derivatives (**3h** and **3i**) were also prepared by Mitsunobu reaction with appropriate imides.

3. Results and discussion

In vitro GK assays were conducted at two glucose concentrations, 2.5 mM and 10 mM, which simulate low and high postprandial blood glucose condition, respectively. To remove the chiral center of (*N*-acetylpyrrolidin-2-yl)benzimidazole derivative **1**, we first replaced the chiral *N*-acetylpyrrolidin-2-yl group with the achiral (*N*-acetyl-*N*-ethylamino)methyl group, as shown in Figure 1. *N*-Acetyl-*N*-ethylamino derivative **2a** showed GK activating potency comparable to compound **1**. This result suggested the utility of screening acylamino groups at the 6-position. Noteworthy data are summarized in Table 1. As for substituents on the nitrogen atom, a methyl group (**2b**) resulted in higher potency than an ethyl group (**2a**), whereas removal of the substituent (**2c**) reduced potency. Replacement of the acylamino moiety with the aminocarbonyl group maintained potency (**2d**).

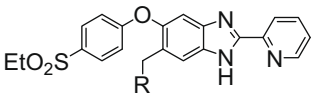
Initial modification of lead **1**, the transformation of the chiral *N*-acetylpyrrolidin-2-yl group to the achiral acetylamino group at the 6-position, led to the identification of two promising compounds, **2a** and **2b**. We then examined rigid cyclic structures, as shown in Figure 1, in order to increase potency. The GK potencies of compounds **3a–l** are summarized in Table 2. The pyrrolidin-2-one (**3a**) and 1,3-oxazolidine-2-one (**3b**) derivatives showed GK potency comparable to the acetyl(methyl)amino derivative (**2b**). Although the 3-methyl-1,3-imidazolidin-2-one (**3c**), 1,1-dioxidoisothiazolidin (**3e**) and morpholin-2-one (**3f**) derivatives also showed acceptable EC_{50} values, their E_{max} values were lower than those of compounds **3a** and **3b**. 1,3-Imidazolidin-2-one derivative (**3d**), which has a proton donor on the nitrogen atom, showed no GK potency.

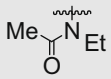
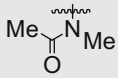
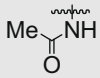
Further SAR development showed that the incorporation of another carbonyl group into the cyclic amide moiety of compounds **3a–c** enhanced potency. Imide derivatives **3g-i** exhibited two to three times higher GK potency than the corresponding amide derivatives **3a–c**. In contrast, removal of the carbonyl group from the pyrrolidine-2-one ring resulted in complete loss of potency. These results suggest that at least one carbonyl group on the substituent is important for potency.

Modification of the substitution at the 6-position of the benzimidazole led to the identification of potent benzimidazole GKAs with an achiral substituent: pyrrolidin-2-one derivative **3a**, 1,3-oxazolidin-2-one derivative **3b** and the corresponding imide analogs, **3g** and **3h**. To select candidates for further evaluation, the in vivo acute glucose lowering efficacies of these compounds were examined in high-fat diet (HFD) mice (Fig. 2). Both the pyrolidin-2-one (**3a**) and succinimide (**3g**) derivatives showed more potent glucose lowering effect than the aliphatic acetylamino derivative **2b** at 10 mg/kg po. On the other hand, the glucose lowering effects of compounds **3b** and **3h,** derivatized with substituents containing the oxazolidine component, were significantly weaker than those of other derivatives (**2b**, **3a** and **3g**).

These results led to the choice of succinimide derivative **3g** for pharmacokinetic studies and oral glucose tolerance tests (OGTTs). The PK profiles determined in male SD rats and beagle dogs are shown in Table 3. Compound **3g** exhibited moderate and acceptable bioavailability in both species. In Wister rat OGTTs, oral administration of compound **3g** demonstrated hypoglycemic efficacy at 1 and 3 mg/kg, resulting in 19% and 27% reduction in the area under the glucose curve, respectively (Fig. 3).

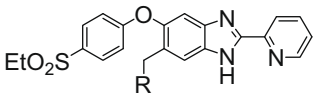
Table 1
EC_{50} of the benzimidazole derivatives with an aliphatic amide moiety at the 6-position[6]

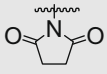
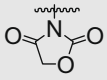
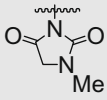


Compounds	R	2.5 mM Glc EC_{50} (E_{max}) μM (fold)[a]	10 mM Glc EC_{50} (E_{max}) μM (fold)[a]
2a		0.50 (1.04)	0.11 (0.95)
2b		0.30 (1.02)	0.07 (1.13)
2c		1.41 (0.98)	0.22 (0.94)
2d		0.62 (1.00)	0.12 (0.98)

[a] Values are the means of those obtained from two or more independent assays. Compound **A** (see Section 5) was used as the internal control across all assay plates for data validation. The EC_{50} values of A were 0.42 ± 0.09 μM and 0.14 ± 0.04 μM at 2.5 mM and 10 mM glucose conditions, respectively. E_{max} values of each compound were calculated as a ratio of the maximal response evoked by the compound compared to compound **A** at each concentration.

Table 2
EC_{50} of the benzimidazole derivatives with a pyrrolidin-2-one, azolidin-2-ones, morpholin-2-one, imides, pyrrolidine or aromatic moiety at the 6-position[6]



Compounds	R	2.5 mM Glc EC_{50} (E_{max}) μM (fold)[a]	10 mM Glc EC_{50} (E_{max}) μM (fold)[a]
3a		0.35 (1.07)	0.06 (1.00)
3b		0.25 (1.00)	0.06 (1.03)
3c		0.32 (0.96)	0.08 (0.69)
3d		>5	>5
3e		0.46 (0.80)	0.09 (0.63)
3f		0.28 (0.88)	0.10 (0.85)
3g		0.16 (1.00)	0.04 (1.09)
3h		0.09 (1.08)	0.02 (1.02)
3i		0.18 (0.90)	0.06 (0.74)
3j		>5	>5
3k		0.43 (1.03)	0.12 (0.99)
3l		0.41 (1.10)	0.07 (1.07)

[a] Values are the means of those obtained from two or more independent assays. Compound **A** (see Section 5) was used as the internal control across all assay plates for data validation. The EC_{50} values of A were 0.42 ± 0.09 μM and 0.14 ± 0.04 μM at 2.5 mM and 10 mM glucose conditions, respectively. E_{max} values of each compound were calculated as a ratio of the maximal response evoked by the compound compared to compound **A** at each concentration.

The co-crystal structure of GK protein with GKA **3g** was determined, revealing the binding mode of benzimidazole GK activators to GK protein, as well as the structural requirements for efficacy (Fig. 4). 2-(Pyridine-2-yl)-1*H*-benzimidazole **3g** binds to the same allosteric site of GK protein as heteroaromatic amide GKAs bind, as described in our earlier report.[11a] The NH of the imidazole and the nitrogen atom in the pyridine ring make key hydrogen bonds with the backbone C=O and N–H of Arg63, respectively. In addition, the plenary succinimide moiety and the aromatic ring of Tyr214 are parallel to each other, which probably increase potency

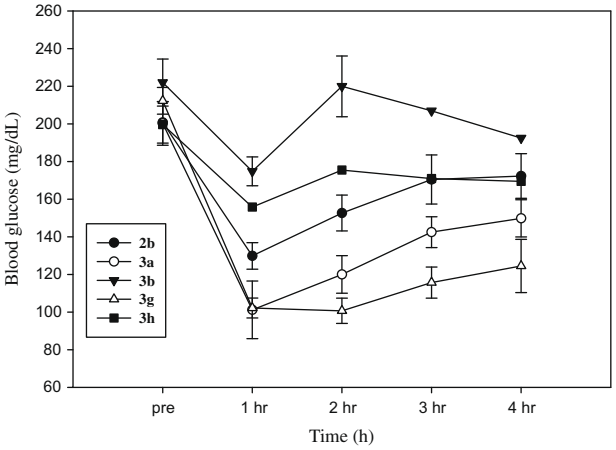


Figure 2. Acute glucose lowering effect in HFD mice of compounds **2b**, **3a**, **3b**, **3g** and **3h**.

Table 3
PK data for **3g**

Species	AUC (μM h)	CLp (mL/min/kg)	C_{max} (μM)	$T_{1/2}$ (h)	*F* (%)
Rat[a]	0.5	32	0.4	0.6	16
Dog[b]	4.0	3.6	0.4	6.4	41

[a] Doses: 1 mg/kg *iv*, 3 mg/kg *po*.
[b] Doses: 0.3 mg/kg *iv*, 1 mg/kg *po*.

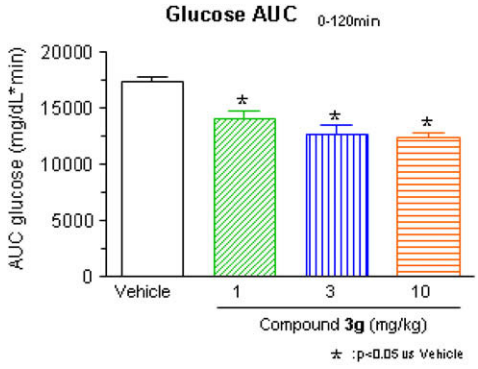


Figure 3. Wister Rat OGTT using compound **3g**.

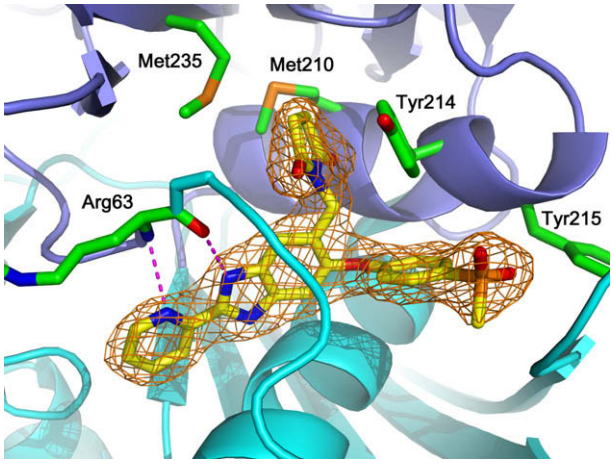


Figure 4. GK activator **3g** bound to Glucokinase. Final 2*Fo–Fc* electron density (1.2 σ level) for compound **3g** is shown as orange mesh. This figure was prepared using the CCP4 package[13] and PyMOL.[14]

due to van der Waals interactions. In fact, the imide derivatives **3g–i,** which have an additional carbonyl group in the ring structure, showed higher potency than the corresponding amide derivatives, 3a–**c**. The carbonyl group in the ring system at the 6-position of our GKAs is important functional group to support the planarity of the ring structure and interact with Tyr214 on GK enzyme. The introduction of plenary heteroaromatic rings such as imidazole (**3k**) and tetrazole (**3l**) in place of the imide moiety was also compatible with potency (Table 2).

4. Conclusion

In conclusion, we have identified several potent GK activators with a cyclic amide or imide moiety on the benzimidazole structure. These compounds, exemplified by **3g**, were designed and discovered by removing the chiral center of (*N*-acetylpyrrolidin-2-yl)benzimidazole lead **1,** followed by conformational rigidification of the resulting acetylamino group in compound **2a**. Succinimide derivative **3g** demonstrated significant glucose lowering effect in rat OGTT at 1 mg/kg po, and showed acceptable pharmacokinetic profiles in beagle dogs. Further in-depth evaluation of compound **3g** is ongoing. Furthermore, X-ray crystallographic analysis of the GKA **3g**/GK protein complex revealed the binding mode of this novel benzimidazole GK activator to the allosteric binding site of GK.

5. Experimental

5.1. Chemistry

All solvents, chemicals and reagents were commercially available unless otherwise noted. ^{1}H NMR spectra were recorded on a Varian MERCURY-400 (400 MHz) or a JEOL JMN-AL400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm units relative to tetramethylsilane, methanol-d_4 or dimethylsulfoxide-d_6 as the internal standard. Coupling constants are expressed in units of hertz (Hz). Splitting patterns are designated as: s, singlet; d, doublet; t. triplet; q, quartet; m, multiplet; br, broad. Mass spectra were recorded on a Waters Micromass ZQ2000 spectrometer under electronspray ionization (ESI) or atmospheric pressure chemical ionization (APCI) conditions. High resolution mass spectra (HRMS) were recorded on a Micromass Q-Tof-2 spectrometer under ESI conditions. Column chromatography was performed with pre-packed silica gel columns (KP-Sil silica) purchased from Biotage Japan, Ltd. Preparative thin-layer chromatography (TLC) was performed on a TLC Silica gel 60 F (Merck5744) purchased from Merck KGaA. Preparative HPLC purification was performed on a YMC-CombiPrep Pro C18 (50 mm × 30 mm id, S-5 μm, 12 nm), eluting with a gradient of 0.1% aqueous TFA/CH_3CN at a flow rate of 40 mL/min under appropriate conditions.

5.1.1. A mixture of (5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl) methanol and (6-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl) methanol (12): Preparation of the key intermediate

5.1.1.1. Methyl 2-fluoro-4-nitrobenzoate (5). To a stirred solution of 2-fluoro-4-nitrobenzoic acid (**4**, 140 g, 756 mmol) in MeOH (1.30 L) was added H_2SO_4 (5.00 mL), and the mixture was refluxed for 48 h. After concentration in vacuo, the residue was triturated with water. The resulting precipitate was collected, dried in vacuo to give **5** (141 g, 708 mmol, 94%) as a yellow solid.

5.1.1.2. Methyl 4-amino-2-fluorobenzoate (6). A solution of **5** (141 g, 708 mmol) in MeOH (1.00 L) and THF (400 mL) was hydrogenated at rt overnight in the presence of Raney Ni (20.0 g). After filtration through Celite, the mixture was concentrated in vacuo to give **6** (119 g, 704 mmol, 99%).

5.1.1.3. Methyl 2-fluoro-4-[(pyridin-2-ylcarbonyl)amino]benzoate (7). To a stirred solution of **6** (18.9 g, 112 mmol) and 2-picolinic acid (16.5 g, 134 mmol) in pyridine (500 mL) was added WSCI (32.0 g, 167 mmol), and the mixture was stirred at rt for 2 h. After concentration in vacuo, the residue was dissolved into EtOAc (600 mL), and washed with 0.25 M aq HCl, 0.25 M aq NaOH and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was triturated with EtOAc/*n*-hexane and the resulting participate was collected, dried in vacuo to give **7** (28.3 g, 103 mmol, 92%) as a white solid.

5.1.1.4. Methyl 2-fluoro-5-nitro-4-[(pyridin-2-ylcarbonyl)amino] benzoate (8). Fuming HNO_3 (110 mL) was slowly added to **7** (27.7 g, 101 mmol) at 0 °C, and the mixture was stirred at rt for 1.5 h. Then the mixture was poured into a cooled solution of Na_2CO_3 (138 g) in water (2.00 L), and the resulting precipitate was collected, dried in vacuo to give **8** (27.5 g, 86.1 mmol, 85%) as a yellow solid.

5.1.1.5. Methyl 2-[4-(ethylsulfonyl)phenoxy]-5-nitro-4-[(pyridin-2-ylcarbonyl)amino]benzoate (9). To a stirred solution of **8** (6.00 g, 18.8 mmol) and 4-(ethylsulfonyl)phenol (**27**, 3.48 g, 18.7 mmol) in DMF (110 mL) was added K_2CO_3 (3.50 g, 25.3 mmol), and the mixture was stirred at 80 °C for 30 min. The mixture was cooled and poured into water (300 mL). The resulting precipitate was collected, dried in vacuo to give **9** (7.46 g, 15.4 mmol, 82%) as a yellow solid.

5.1.1.6. Methyl 5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazole-6-carboxylate (10). To a stirred suspension of **9** (7.46 g, 15.4 mmol) in DMF (37.0 mL) and MeOH (37.0 mL) were added $SnCl_2$–$2H_2O$ (17.3 g, 76.7 mmol) and HCl (15.0 mL), and the mixture was stirred at 80 °C for 40 min. After cooling, the mixture was neutralized with aq Na_2CO_3, diluted with EtOAc and stirred at rt for 30 min. The resulting insoluble solid was filtered off. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo to give a crude product containing **10** (6.90 g) as a yellow solid. This was used in the next step without further purification.

5.1.1.7. A mixture of methyl 5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazole-6-carboxylate and methyl 6-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazole-5-carboxylate (11). To a solution of **10** (6.90 g) in DMF (70.0 mL) were added SEM-Cl (4.00 mL, 22.6 mmol) and NaH (70% dispersion in paraffin liquid, 920 mg, 26.8 mmol), and the mixture was stirred at rt for 30 min. After cooling, the mixture was partitioned between saturated aq NH_4Cl and EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with EtOAc/*n*-hexane to give **11** (6.43 g, 11.3 mmol, 73% in two steps) as a yellow solid.

5.1.1.8. A mixture of (5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl)methanol and (6-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl)methanol (12a and 12b). To a stirred cooled solution of $LiAlH_4$ (990 mg, 26.1 mmol) in THF (60.0 mL) was slowly added a solution of **11** (5.90 g, 10.4 mmol) in THF (50.0 mL), and the mixture was stirred at rt for 15 min. The mixture was cooled to 0 °C, and sodium sulfate decahydrate was slowly added until the foaming quieted down. After diluting with EtOAc, the mixture was stirred at rt for

1 h. The resulting insoluble solid was filtered off. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with EtOAc/*n*-hexane to give **12** (4.50 g, 8.34 mmol, 80%) as a yellow amorphous mass. In some cases, regiomixture **12** was separated by column chromatography on silica gel to give **12a** and **12b** as a pale yellow solid respectively.

5.1.2. *N*-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)-*N*-methylacetamide (2b)

5.1.2.1. (5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl)methyl methanesulfonate (13a). To a stirred solution of **12a** (100 mg, 0.186 mmol) and Et_3N (52.0 μL, 0.370 mmol) in THF (1.00 mL) was added MsCl (29.0 μL, 0.370 mmol) at 0 °C, and then the mixture was stirred at the same temperature for 30 min. The mixture was partitioned between water and EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude product containing **13a**. This was used in the next step without further purification.

5.1.2.2. 6-(Azidomethyl)-5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazole (14). To a stirred cooled solution of **13a** in DMF (1.00 mL) was added NaN_3 (60.0 mg, 0.930 mmol), and then the mixture was stirred at rt. The mixture was partitioned between water and EtOAc, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude product containing **14** (136 mg). This was used in the next step without further purification.

5.1.2.3. 1-(5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl)methanamine (15). To a stirred solution of **14** (136 mg) in MeOH (2.00 mL) were added $CuSO_4/10H_2O$ (2.00 mg, 8.00 μmol) and $NaBH_4$ (35.0 mg, 0.930 mmol), and the mixture was stirred at rt for 30 min. The mixture was partitioned between aq NH_4Cl, aq NH_3 and EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with MeOH/$CHCl_3$ to give **15** (81.5 mg, 0.151 mmol, 51% in two steps) as yellow oil.

5.1.2.4. *N*-[(5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl)methyl] acetamide (16). To a stirred solution of **15** (400 mg, 0.744 mmol) in $CHCl_3$ (4.00 mL) were added Et_3N (0.208 mL, 1.49 mmol) and AcCl (0.104 mL, 1.49 mmol), and the mixture was stirred at rt for 30 min. The mixture was partitioned between water and EtOAc, and diluted with aq $NaHCO_3$. The organic layer was separated, washed with brine, dried over $MgSO_4$ and, and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give **16** (387 mg, 0.718 mmol, 97%) as a yellowish brown amorphous mass.

5.1.2.5. *N*-[(5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl)methyl]-*N*-methylacetamide (17). To a stirred cooled solution of **16** (38.0 mg, 0.0650 mmol) in DMF (0.300 mL) were added NaH (55% dispersion in paraffin liquid, 5.20 mg, 0.130 mmol) and MeI (18.4 mg, 0.130 mmol), and the mixture was stirred at rt for 2 h. The mixture was partitioned between aq NH_4Cl and EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by preparative HPLC followed by usual work-up to give **17** (18.1 mg, 0.030 mmol, 47%) as colorless oil.

5.1.2.6. *N*-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)-*N*-methylacetamide (2b). TFA (0.500 mL) was added to the compound **17** (18.1 mg, 30.0 μmol), and the mixture was stirred at rt for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved into $CHCl_3$, and then Et_3N was added. The solvent was evaporated under reduced pressure. The residue was purified by preparative TLC eluted with MeOH/$CHCl_3$ to give **2b** (13.2 mg, 28.0 μmol, 93%) as a colorless amorphous mass. ^1H NMR ($CDCl_3$) δ: 10.8 (1H, br), 8.70–8.65 (1H, m), 8.42–8.37 (1H, m), 7.90–7.82 (3H, m), 7.73–7.17 (total 3H, m), 7.10–7.06 (2H, m), 4.67–4.56 (total 2H, m), 3.16–3.09 (2H, m), 2.96 and 2.99 (total 3H, s), 2.05 and 2.08 (total 3H, s), 1.33–1.24 (3H, m). HRMS *m/z*: Calcd for $C_{24}H_{25}N_4O_4S$ ($[M+H]^+$) 465.1597. Found: 465.1593.

5.1.3. *N*-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)acetamide (2c)

Compound **2c** was prepared in a similar manner to the step of Section 5.1.2.6 using **16** instead of **17**. ^1H NMR ($CDCl_3$) δ: 10.8 (1H × 1/2, br), 10.7 (1H × 1/2, br), 8.70–8.65 (1H, m), 8.39–8.36 (1H, m), 7.90–7.83 (3H, m), 7.90–7.83 (1H × 1/2, m), 7.65 (1H × 1/2, s), 7.46 (1H × 1/2, s), 7.41–7.38 (1H, m), 7.15 (1H × 1/2, s), 5.97 (1H × 1/2, br), 5.83 (1H × 1/2, br), 4.50–4.48 (2H, m), 3.11 (2H, q, *J* = 7.4 Hz), 1.95 (3H, s), 1.30 (3H, t, *J* = 7.4 Hz). HRMS *m/z*: Calcd for $C_{23}H_{23}N_4O_4S$ ($[M+H]^+$) 451.1440. Found: 451.1437.

5.1.4. *N*-Ethyl-*N*-({5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)acetamide (2a)

To a stirred cooled solution of **16** (90.0 mg, 0.155 mmol) in DMF (1.00 mL) were added NaH (55% dispersion in paraffin liquid, 12.0 mg, 0.310 mmol) and EtI (37.0 mg, 0.310 mmol), and the mixture was stirred at rt for 2 h. The mixture was partitioned between aq NH_4Cl and EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by preparative HPLC to give *N*-ethyl-*N*-[(5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]-methyl}-1*H*-benzimidazol-6-yl)methyl]acetamide (**18**). TFA (0.500 mL) was added to **18**, and the mixture was stirred at rt for 2 h. After concentration, the residue was dissolved into $CHCl_3$ and neutralized with aq $NaHCO_3$. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by preparative TLC eluted with MeOH/$CHCl_3$ to give **2a** (12.5 mg, 26.0 μmol, 17% in two steps) as a white amorphous mass.

^1H NMR ($CDCl_3$) δ: 10.8 (1H, br), 8.70–8.64 (1H, m), 8.41–8.39 (1H, m), 7.90–7.00 (1H × 3/5, br m), 7.88–7.82 (3H, m), 7.64 (1H × 3/5, s), 7.42–7.38 (1H, m), 7.42–7.38 (1H × 2/5, m), 7.11–7.00 (2H, m), 7.11–7.00 (1H × 2/5, m), 4.66 (2H × 3/5, s), 4.56 (2H × 2/5, s), 3.48 (2H × 2/5, q, *J* = 7.2 Hz), 3.30 (2H × 3/5, q, *J* = 7.2 Hz), 3.16–3.09 (2H, m), 2.11 (3H × 3/5, s), 2.07 (3H × 2/5, s), 1.32–1.27 (3H, m), 1.15–1.07 (3H, m). HRMS *m/z*: Calcd for $C_{25}H_{27}N_4O_4S$ ($[M+H]^+$) 479.1753. Found: 479.1751.

5.1.5. 1-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)pyrrolidin-2-one (3a): General Procedure A

5.1.5.1. (6-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl)methyl methanesulfonate (13b). To a stirred solution of **12b** (100 mg, 0.186 mmol) and Et_3N (50.0 μL, 0.360 mmol) in THF (1 mL) was added MsCl (28.0 μL, 0.360 mmol) at 0 °C, and the mixture was stirred at the same temperature for 30 min. The mixture was partitioned between water and EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude product containing **13b** (110 mg) as pale orange oil. This was used in the next step without further purification.

5.1.5.2. 1-[(6-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl)methyl]pyrrolidin-2-one (19). To a stirred solution of **13b** (110 mg) and 2-pyrrolidone (68.0 μL, 0.900 mmol) in DMF (2.00 mL) was added NaH (55% dispersion in paraffin liquid, 21.6 mg, 0.495 mmol) at 0 °C, and the mixture was stirred at rt for 30 min. The mixture was partitioned between aq NH_4Cl and EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude product containing **19** as yellow oil. This was used in the next step without further purification.

5.1.5.3. 1-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)pyrrolidin-2-one (3a). TFA (1.00 mL) was added to the compound **19**, and the mixture was stirred at rt for 1 h. The solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC (YMC-CombiPrep Pro C18, eluting CH_3CN/water + 0.1%TFA) followed by usual work-up to give **3a** (26.5 mg, 0.0556 mmol, 31%) as a white solid. ^1H NMR ($CDCl_3$) δ: 10.86 (1H, br s), 8.64 (1H, d, J = 4.3 Hz), 8.39 (1H, d, J = 7.8 Hz), 7.91–7.78 (2H, m), 7.84 (2H, d, J = 8.4 Hz), 7.61–7.45 (1H, m), 7.42–7.37 (1H, m), 7.06 (2H, d, J = 8.4 Hz), 4.54 (2H, s), 3.30 (2H, t, J = 7.1 Hz), 3.11 (2H, q, J = 7.4 Hz), 2.34 (2H, t, J = 8.1 Hz), 1.93 (2H, tt, J = 8.1, 7.1 Hz), 1.29 (3H, t, J = 7.4 Hz). HRMS m/*z*: Calcd for $C_{25}H_{25}N_4O_4S$ ($[M+H]^+$) 477.1597. Found: 477.1591.

The compounds **3b–3f, 3j** and **3k** were prepared in a similar manner to that described for the preparation of **3a**.

5.1.6. 3-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)-1,3-oxazolidin-2-one (3b)

1,3-Oxazolidin-2-one instead of 2-pyrrolidone was used at the step of Section 5.1.5.2 followed by Section 5.1.5.3. ^1H NMR ($CDCl_3$) δ: 10.68–10.59 (1H, br m), 8.70–8.61 (1H, m), 8.43–8.35 (1H, m), 7.93–7.81 (1H, m), 7.85 (2H, d, J = 8.8 Hz), 7.65 (1H × 1/2, s), 7.51 (1H × 1/2, s), 7.44–7.38 (1H × 1/2, m), 7.44–7.38 (1H, m), 7.12–7.04 (1H × 1/2, m), 7.09 (2H, d, J = 9.8 Hz), 4.52 (2H, s), 4.24 (2H, t, J = 8.3 Hz), 3.54–3.40 (2H, m), 3.12 (2H, q, J = 7.4 Hz), 1.30 (3H, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{24}H_{23}N_4O_5S$ ($[M+H]^+$) 479.1389. Found: 479.1386.

5.1.7. 1-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)-3-methylimidazolidin-2-one (3c)

1-Methylimidazolidin-2-one instead of 2-pyrrolidone was used at the step of Section 5.1.5.2 followed by Section 5.1.5.3. ^1H NMR ($CDCl_3$) δ: 10.84 (1H × 1/2, br s), 10.81 (1H × 1/2, br s), 8.64 (1H × 1/2, d, J = 4.7 Hz), 8.62 (1H × 1/2, d, J = 4.7 Hz), 8.40 (1H × 1/2, d, J = 7.8 Hz), 8.37 (1H × 1/2, d, J = 7.8 Hz), 7.89–7.84 (1H, m), 7.80 (2H, d, J = 9.0 Hz), 7.61 (1H × 1/2, s), 7.47 (1H × 1/2, s), 7.40–7.37 (1H, m), 7.16 (1H × 1/2, s), 7.06 (1H × 1/2, s), 7.03 (2H, d, J = 9.0 Hz), 4.43 (2H × 1/2, s), 4.43 (2H × 1/2, s), 3.20–3.16 (4H, m), 3.10 (2H, q, J = 7.4 Hz), 2.75 (3H × 1/2, s), 2.73 (3H × 1/2, s),1.28 (3H, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{25}H_{26}N_5O_4S$ ($[M+H]^+$) 492.1706. Found: 492.1704.

5.1.8. 1-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)imidazolidin-2-one (3d)

Imidazolidin-2-one instead of 2-pyrrolidone was used at the step of Section 5.1.5.2 followed by Section 5.1.5.3. ^1H NMR ($CDCl_3$, one drop of CD_3OD) δ: 8.65–8.61 (1H, m), 8.37 (1H, d, J = 7.4 Hz), 7.90–7.85 (1H, m), 7.82 (2H, d, J = 8.2 Hz), 7.77 (1H × 1/2, s), 7.59 (1H × 1/2, s), 7.45 (1H × 1/2, s), 7.41–7.37 (1H, m), 7.20 (1H × 1/2, s), 7.05 (2H, d, J = 8.2 Hz), 4.65 (1H × 1/2, s), 4.63 (1H × 1/2, s), 4.44 (2H × 1/2, s), 4.41 (2H × 1/2, s), 3.28–3.25 (4H, m), 3.11 (2H, q, J = 7.2 Hz), 1.28 (3H, t, J = 7.2 Hz). HRMS *m/z*: Calcd for $C_{24}H_{24}N_5O_4S$ ($[M+H]^+$) 478.1549. Found: 478.1559.

5.1.9. 6-[(1,1-Dioxidoisothiazolidin-2-yl)methyl]-5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazole (3e)

Isothiazolidine 1,1-dioxide[15] instead of 2-pyrrolidone was used at the step of Section 5.1.5.2 followed by Section 5.1.5.3. ^1H NMR ($CDCl_3$) δ: 10.7 (1H, br), 8.70–8.65 (1H, m), 8.41–8.39 (1H, m), 7.96 (1H × 1/2, br s), 7.91–7.86 (1H, m), 7.84 (2H, d, J = 8.9 Hz), 7.71 (1H × 1/2, br s), 7.50 (1H × 1/2, br s), 7.43–7.39 (1H, m), 7.19 (1H × 1/2, br s), 7.08 (2H, d, J = 8.9 Hz), 4.28 (2H, br s), 3.20–3.05 (4H, m), 3.12 (2H, q, J = 7.4 Hz), 2.30–2.20 (2H, m), 1.30 (3H, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{24}H_{25}N_4O_5S_2$ ($[M+H]^+$) 513.1266. Found: 513.1265.

5.1.10. 4-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)morpholin-3-one (3f)

Morpholin-3-one[16] instead of 2-pyrrolidone was used at the step of Section 5.1.5.2 followed by Section 5.1.5.3. ^1H NMR ($CDCl_3$) δ: 10.8 (1H × 1/2, br), 10.7 (1H × 1/2, br), 8.70–8.66 (1H, m), 8.42–8.37 (1H, m), 7.84 (2H, d, J = 8.8 Hz), 7.90–7.80 (1H, m), 7.90–7.80 (1H × 1/2, m), 7.68 (1H × 1/2, s), 7.49 (1H × 1/2, s), 7.42–7.39 (1H, m), 7.18 (1H × 1/2, s), 7.09–7.05 (2H, m), 4.73–4.70 (2H, m), 4.18–4.13 (2H, m), 3.85–3.81 (2H, m), 3.36–3.34 (2H, m), 3.11 (2H, q, J = 7.4 Hz), 1.30 (3H, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{25}H_{25}N_4O_5S$ ($[M+H]^+$) 493.1546. Found: 493.1546.

5.1.11. 5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-6-(pyrrolidin-1-ylmethyl)-1*H*-benzimidazole (3j)

Pyrrolidine instead of 2-pyrrolidone was used at the step of Section 5.1.5.2 followed by Section 5.1.5.3. ^1H NMR ($CDCl_3$) δ: 10.73 (1H, br s), 8.65 (1H, d, J = 3.9 Hz), 8.40 (1H, d, J = 7.3 Hz), 8.06–7.11 (1H, m), 7.91–7.78 (2H, m), 7.82 (2H, d, J = 8.8 Hz), 7.42–7.36 (1H, m), 7.05 (2H, d, J = 8.8 Hz), 3.77 (2H, br s), 3.12 (2H, q, J = 7.5 Hz), 2.65 (4H, br s), 1.79 (4H, s), 1.29 (3H, t, J = 7.5 Hz). MS *m/z*: 463 ($[M+H]^+$).

5.1.12. 5-[4-(Ethylsulfonyl)phenoxy]-6-(1*H*-imidazol-1-ylmethyl)-2-(pyridin-2-yl)-1*H*-benzimidazole (3k)

Imidazole instead of 2-pyrrolidone was used at the step of Section 5.1.5.2 followed by Section 5.1.5.3. ^1H NMR ($CDCl_3$) δ: 10.8 (1H × 1/2, br), 10.7 (1H × 1/2, br), 8.70–8.60 (1H, m), 8.44–8.35 (1H, m), 7.94–7.80 (3H, m), 7.80–7.70 (1H, m), 7.60–7.45 (2H, m), 7.45–7.40 (1H, m), 7.20–6.88 (4H, m), 5.20 (2H, s), 3.13 (2H, q, J = 7.4 Hz), 1.34 (3H, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{24}H_{22}N_5O_3S$ ($[M+H]^+$) 460.1443. Found: 460.1447.

5.1.13. 1-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)pyrrolidine-2,5-dione (3g): General Procedure B

5.1.13.1. A mixture of 1-[(5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl) methyl]pyrrolidine-2,5-dione and 1-[(6-[4-(ethylsulfonyl)-phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl)methyl]pyrrolidine-2,5-dione (20). To a stirred solution of **12** (2.40 g, 4.45 mmol), succinimide (1.30 g, 13.1 mmol) and PPh_3 (13.3 mmol) in THF (24.0 mL) was added a 40 wt % solution of DEAD in toluene (5.80 mL, 12.7 mmol) at 0 °C, and the mixture was stirred at rt for 1 h. After concentration in vacuo, the residue was purified by column chromatography on silica gel eluted with EtOAc/*n*-hexane to give **20** (2.30 g, 3.70 mmol, 83%) as a yellow amorphous mass.

5.1.13.2. 1-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)pyrrolidine-2,5-dione (3g). TFA (15.0 mL) was added to the compound **20** (2.30 g, 3.70 mmol), and the mixture was stirred at rt for 2 h. After concentration, the residue was purified by column chromatography on silica

gel eluted with MeOH/$CHCl_3$ followed by crystallization from EtOAc to give **3g** (1.02 g, 2.08 mmol, 68%) as a white crystal. ^{1}H NMR ($CDCl_3$) δ: 10.58 (1H × 1/2, s), 10.52 (1H × 1/2, s), 8.65 (1H × 1/2, d, J = 4.9 Hz), 8.63 (1H × 1/2, d, J = 4.1 Hz), 8.38 (1H × 1/2, d, J = 7.8 Hz), 8.36 (1H × 1/2, d, J = 8.0 Hz), 7.90–7.81 (1H, m), 7.86 (2H × 1/2, d, J = 8.8 Hz), 7.83 (2H × 1/2, d, J = 9.0 Hz), 7.76 (1H × 1/2, s), 7.64 (1H × 1/2, s), 7.45 (1H × 1/2, s), 7.42–7.37 (1H, m), 7.12 (1H × 1/2, s), 7.11 (2H × 1/2, d, J = 8.8 Hz), 7.08 (2H × 1/2, d, J = 9.0 Hz), 4.80 (2H × 1/2, s), 4.79 (2H × 1/2, s), 3.12 (2H × 1/2, q, J = 7.4 Hz), 3.11 (2H × 1/2, q, J = 7.4 Hz), 2.65 (2H, s), 2.54 (2H, s), 1.30 (3H × 1/2, t, J = 7.4 Hz), 1.29 (3H × 1/2, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{25}H_{23}N_4O_5S$ ([M+H]$^+$) 491.1389. Found: 491.1387.

The compounds **3h** and **3i** were prepared in a similar manner to that described for the preparation of **3g**.

5.1.14. 3-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)-1,3-oxazolidine-2,4-dione (3h)

1,3-Oxazolidine-2,4-dione[17] instead of succinimide was used at the step of Section 5.1.13.1 followed by Section 5.1.13.2. 1H NMR ($CDCl_3$) δ: 10.90 (1H × 1/2, br s), 10.85 (1H × 1/2, br s), 8.67–8.62 (1H, m), 8.42–8.37 (1H, m), 7.92–7.83 (1H × 1/2, m), 7.92–7.83 (3H, m), 7.70 (1H × 1/2, s), 7.47 (1H × 1/2, s), 7.44–7.38 (1H, m), 7.11 (1H × 1/2, s), 7.10 (2H, d, J = 8.2 Hz), 4.83 (2H × 1/2, s), 4.81 (2H × 1/2, s), 4.59 (2H × 1/2, s), 4.52 (2H × 1/2, s), 3.12 (2H, q, J = 7.4 Hz), 1.30 (3H, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{24}H_{21}N_4O_6S$ ([M+H]$^+$) 493.1182. Found: 493.1176.

5.1.15. 3-({5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}methyl)-1-methylimidazolidine-2,4-dione (3i)

1-Methylhydantoin instead of succinimide was used at the step of Section 5.1.13.1 followed by Section 5.1.13.2. ^{1}H NMR ($CDCl_3$) δ: 10.67 (1H × 1/2, br s), 10.63 (1H × 1/2, br s), 8.65–8.60 (1H, m), 8.37 (1H, t, J = 8.2 Hz), 7.89–7.79 (1H × 1/2, m), 7.89–7.79 (3H, m), 7.66 (1H × 1/2, s), 7.45 (1H × 1/2, s), 7.40–7.36 (1H, m), 7.10 (1H × 1/2, s), 7.06 (2H, d, J = 9.0 Hz), 4.78 (2H × 1/2, s), 4.77 (2H × 1/2, s), 3.74 (2H × 1/2, s), 3.59 (2H × 1/2, s), 3.11 (2H, q, J = 7.0 Hz), 2.92 (3H × 1/2, s), 2.85 (3H × 1/2, s), 1.29 (3H, t, J = 7.0 Hz). HRMS *m/z*: Calcd for $C_{25}H_{24}N_5O_5S$ ([M+H]$^+$) 506.1498. Found: 506.1502.

5.1.16. 2-{5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}-*N*,*N*-dimethylacetamide (2d)

5.1.16.1. A mixture of (5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl)acetonitrile and (6-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl)-acetonitrile (21). Compound **13** was prepared in a similar manner to the step of Section 5.1.2.1 using **12** instead of **12a**. Then the compound **13** was dissolved into DMF (20.0 mL) at 0 °C and NaCN (254 mg, 5.19 mmol) was added. The resulting mixture was stirred at rt for 3 h. The mixture was partitioned between aq $NaHCO_3$ and EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with EtOAc/*n*-hexane to give **21** (623 mg, 1.14 mmol, 66%).

5.1.16.2. A mixture of (5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol- 6-yl)acetic acid and (6-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl)-acetic acid (22). To a solution of **21** (30.0 mg, 55.0 μmol) in EtOH (1.00 mL) was added 2.5 M aq NaOH (1.00 mL), and the mixture was refluxed overnight. The mixture was cooled to 0 °C and acidified with 10% citric acid. The resulting insoluble solid was filtered off. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative HPLC to give **22** (17.7 mg, 32.0 μmol, 58%).

5.1.16.3. A mixture of 2-(5-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-6-yl)-*N*,*N*-dimethylacetamide and 2-(6-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazol-5-yl)-*N*,*N*-dimethylacetamide (23). To a suspension of **22** (17.7 mg, 32.0 μmol) in $CHCl_3$ (1.00 mL) were added HOBt (8.70 mg, 64.0 μmol) and WSCI (12.3 mg, 0.064 mmol), and the mixture was stirred at rt for 10 min. Then to the mixture was added 2.0 M solution of $(CH_3)_2NH$ in THF (48.0 μL, 96.0 μmol), and the resulting mixture was stirred at rt for 1.5 h. The mixture was cooled to 0 °C, and partitioned between $CHCl_3$ and water. The organic layer was separated, washed with aq $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC eluted with MeOH/$CHCl_3$ to give **23** (10.8 mg, 18.0 μmol, 56%).

5.1.16.4. 2-{5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-1*H*-benzimidazol-6-yl}-*N*,*N*-dimethylacetamide (2d). TFA (1.00 mL) was added to the compound **23** (10.8 mg, 18.0 μmol), and the mixture was stirred at rt for 1 h. After concentration, the residue was dissolved into $CHCl_3$, neutralized with aq $NaHCO_3$ and extracted with $CHCl_3$. The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC eluted with MeOH/$CHCl_3$ to give **2d** (7.00 mg, 15.1 μmol, 84%) as an amorphous mass. ^{1}H NMR ($CDCl_3$) δ: 10.94 (1H × 1/2, br s), 10.88 (1H × 1/2, br s), 8.65–8.61 (1H, m), 8.42–8.36 (1H, m), 7.82 (2H, d, J = 9.0 Hz), 7.89–7.80 (1H, m), 7.77 (1H × 1/2, s), 7.55 (1H × 1/2, s), 7.44 (1H × 1/2, s), 7.40–7.37 (1H, m), 7.12 (1H × 1/2, s), 7.08 (2H, d, J = 9.0 Hz), 3.74 (2H × 1/2, s), 3.73 (2H × 1/2, s), 3.11 (2H, q, J = 7.4 Hz), 2.98 (3H, s), 2.91 (3H × 1/2, s), 2.90 (3H × 1/2, s), 1.29 (3H, t, J = 7.4 Hz). HRMS *m/z*: Calcd for $C_{24}H_{25}N_4O_4S$ ([M+H]$^+$) 465.1597. Found: 465.1600.

5.1.17. 5-[4-(Ethylsulfonyl)phenoxy]-6-[(1-methyl-1*H*-tetrazol-5-yl)methyl]-2-(pyridin-2-yl)-1*H*-benzimidazole (3l)

5.1.17.1. 5-[4-(Ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-6-(2*H*-tetrazol-5-ylmethyl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazole (24a) and 6-[4-(ethylsulfonyl)phenoxy]-2-(pyridin-2-yl)-5-(2*H*-tetrazol-5-ylmethyl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazole (24b). To a stirred solution of **21** (100 mg, 0.183 mmol) in toluene (1.00 mL) were added $TMSN_3$ (0.0485 mL, 0.365 mmol) and dibutyltin(IV) oxide (9.20 mg, 0.037 mmol), and the mixture was stirred at 115 °C for 24 h. The mixture was cooled to 0 °C, diluted with aq $NaHCO_3$ and extracted with $CHCl_3$ repeatedly. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with MeOH/$CHCl_3$ to give **24a** (14.0 mg, 0.024 mmol, 13%) and **24b** (13.3 mg, 0.022 mmol, 12%).

5.1.17.2. 5-[4-(Ethylsulfonyl)phenoxy]-6-[(1-methyl-2*H*-tetrazol-5-yl)methyl]-2-(pyridin-2-yl)-1-{[2-(trimethylsilyl)ethoxy]methyl}-1*H*-benzimidazole (25). To a stirred solution of **24a** (14.0 mg, 0.0240 mmol) in DMF (0.500 mL) were added NaH (55% dispersion in paraffin liquid, 1.60 mg, 0.0360 mmol) and MeI (4.60 mg, 32.0 μmol), and the mixture was stirred at rt for 1 h. The mixture was partitioned between aq NH_4Cl and EtOAc. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by preparative TLC eluted with MeOH/$CHCl_3$ to give **25** (3.50 mg, 5.80 μmol, 24%) and its regioisomer **26** (4.10 mg, 6.80 μmol, 28%).

5.1.17.3. 5-[4-(Ethylsulfonyl)phenoxy]-6-[(1-methyl-1*H*-tetrazol-5-yl)methyl]-2-(pyridin-2-yl)-1*H*-benzimidazole (3l). Compound **3l** was prepared in a similar manner to the step of Section 5.1.2.6 using **25** instead of **17**. ^{1}H NMR ($CDCl_3$) δ: 10.65 (1H, br s), 8.67–8.63 (1H, m), 8.40–8.35 (1H, m), 7.91–7.85 (1H, m), 7.84 (2H, d, J = 8.6 Hz), 7.70 (1H × 1/2, s), 7.48 (1H × 1/2, s), 7.46 (1H × 1/2, s), 7.43–7.39 (1H, m), 7.17 (1H × 1/2, s), 7.05 (2H × 1/2, d, J = 8.6 Hz), 7.02 (2H × 1/2, d, J = 8.6 Hz), 4.34 (2H, s), 3.91 (3H × 1/2, s), 3.90 (3H × 1/2, s), 3.13 (2H, q, J = 7.4 Hz),1.31 (3H, t, J = 7.4 Hz). HRMS m/z: Calcd for $C_{23}H_{22}N_7O_3S$ ([M+H]$^+$) 476.1505. Found: 476.1507.

5.1.18. 4-(Ethylsulfonyl)phenol (27)

To a stirred cooled solution of 4-mercaptophenol (5.00 g, 39.6 mmol) in acetone (50.0 mL) was added K_2CO_3 (5.74 g, 41.6 mmol) and EtI (4.75 mL, 59.4 mmol), and the mixture was stirred at rt for 3 h. The insoluble solid was filtered off and the filtrate was concentrated in vacuo. The residue was dissolved into Et_2O and extracted with 2 M aq NaOH. The aqueous layer was separated, acidified with 6 M aq HCl, and then extracted with Et_2O. The organic layer was separated, washed with brine, dried over $MgSO_4$, concentrated in vacuo to give a crude product containing 4-(ethylsulfanyl)phenol (7.21 g) as brown oil. This was dissolved into AcOH (50.0 mL), and 30% aq H_2O_2 was carefully dropped. And the reaction mixture was stirred at 100 °C (bath temperature) for 1 h. Then the mixture was cooled to rt, poured into saturated aq $NaHCO_3$ on crushed ice and repeatedly extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluted with EtOAc/*n*-hexane to give **27** (8.29 g, 44.5 mmol, 112%) as pale yellow oil.

5.2. Biology and pharmacology

5.2.1. GK assay

The recombinant human liver GK used in this assay was expressed in *Escherichia 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 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. The maximal activating response (E_{max}) elicited by the compounds was expressed as a ratio compared to the maximal response evoked by compound **A**, 2-amino-*N*-(4-methyl-1,3-thiazol-2-yl)-5-[(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]benzamide, as described in our earlier report[6] at each concentration.

5.2.2. Acute in vivo efficacy study

Forty-two to forty-five week old or twenty-five week old male mice fed a high-fat diet (HFD) were freely fed before performing the test. To the mice were orally administrated each compound **2b**, **3a**, **3b**, **3g**, **3h** or vehicle alone (0.5% methylcellulose solution). Blood glucose concentrations were measured just prior to and following oral dosing (1, 2, 3 and 4 h).

5.2.3. Rat OGTT study

Nine-week-old male Wistar rats were fasted overnight before performing the test. The rats were orally administrated glucose (2 g/kg) and either compound **3g** or vehicle alone (0.5% methylcellulose solution) at the same time. Blood glucose concentrations were measured by OneTouch Ultra (LifeScan, Inc., Milpitas, CA) just prior to and following the glucose challenge (30, 60, 90, and 120 min). The area under the curve (AUC) values were calculated from the data (from 0 min to 120 min). Statistical analysis was performed using Prism Software (version 4.00; GraphPad Software Inc.). One-way analysis of variance was used followed by Dunnett's test for multiple comparisons. Values of $p < 0.05$ were considered statistically significant.

5.3. X-ray crystallography

Protein and protein crystals were obtained according to established procedures.[3] Crystals were soaked in 0.5 mM compound **3g** overnight in mother liquor containing 5% DMSO. Diflayer data were collected on beamline BL32B2 at the SPring-8, at 100 K (a Rigaku R-axis V imaging plate). Data processing and data reduction were carried out using programs from the HKL2000 (HKL Research, Inc.) and the CCP4 package. Compound **3g** was modeled into the electron density using Afitt (OpenEye Scientific Software). The protein-compound complex model was refined using REFMAC5 of the CCP4 package. The final structure has been deposited in the Protein Data Bank under deposition code 3H1 V, together with structure factors and detailed experimental conditions.

Crystallographic statistics for the GK-compound **3g** complex are as follows: space group $P6_522$, unit cell 79.8, 79.8, 326.1 Å, resolution 2.11 Å, 33,083 reflections from 185,069 observations give 94.7% completeness with R_{sym} of 4.8% and mean I/σ (I) of 31.9. The final model containing 3493 protein, 120 water, 1 salt, 12 glucose and 34 compound atoms has an R-factor of 22.1% (R_{free} using 5% of the data 27.5%). Mean temperature factors for the protein and the ligand are 40.2 and 31.5 Å^2, respectively.

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