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Discovery of novel 3,6-disubstituted 2-pyridinecarboxamide derivatives as GK activators

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ABSTRACT

A novel class of 3,6-disubstituted 2-pyridinecarboxamide derivatives was designed based on X-ray analysis of the 2-aminobenzamide lead class. Subsequent chemical modification led to the discovery of potent GK activators which eliminate potential toxicity concerns associated with an aniline group of the lead structure. Compound **7** demonstrated glucose lowering effect in a rat OGTT model.

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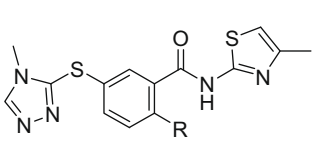
Glucokinase (GK) catalyzes the phosphorylation of glucose to glucose-6-phosphate and is predominantly expressed in liver and pancreatic β-cells. GK acts as a glucose sensor regulating hepatic glucose metabolism and glucose-dependent insulin secretion.[1,2] Based on the dual hepatic and pancreatic effects, GK activators represent novel and promising approach for the treatment of type 2 diabetes.[3–5] In fact, many pharmaceutical companies have actively pursued a program aiming at the development of GK activators.[6–9] Of these, several companies including Roche and OSI/Prosidion have entered their compounds into clinical study.[3,10,11]

We previously reported a 2-aminobenzamide class of allosteric GK activators exemplified by compound **1** (Fig. 1).[12] Compound **1** showed good in vitro GK potency and glucose lowering effect in a rat OGTT model. Moreover, crystal structure of **1** with GK protein revealed a binding mode at an allosteric site distinct from the glucose binding and catalytic sites. This structure elucidation has provided helpful information to further perform SAR study. However, there is a potential toxicity concern associated with the aniline group contained in the structure of **1**.[13,14] Therefore, a new structural class without an aniline group was strongly desired to further develop a promising GK activator. Here we describe the design and synthesis of a novel class of 3,6-disubstituted 2-pyridinecarboxamide GK activators which eliminate the aniline group of the lead compound **1** and thus overcome the potential toxicity concern.



	GK potency (EC_{50})
1 R= NH_2	0.42 uM
2 R= NHMe	1.07 uM

Figure 1. Structures of **1** and **2**.

The crystal structure of GK-compound **1** complex and SAR study around the 2-aminobenzamide class revealed that the aniline NH_2 group has the critical role of showing good GK potency.[12] According to the reported co-crystal structure of GK in complex with **1**, the aniline NH interacted with Tyr215 OH by a hydrogen bond (Fig. 2A). In addition, another aniline NH may form an intra-molecular hydrogen bond with the amide carbonyl group in **1** to fix the conformation. Our continuous efforts provided an additional crystal information on a related compound **2** (Fig. 2B).[15,16] Although **2** still contains an aniline structure and the difference between the two compounds (**1** and **2**) is only the presence or absence of a methyl group on the aniline NH, generation of a new binding pocket surrounding the methyl group was observed in the crystal structure of GK-**2** complex. In contrast to **1**, the methyl group in **2** disrupted the hydrogen bond interaction with Tyr215, as it pushed the tyrosine side chain and consequently generated a new hydrophobic pocket. From these observations, we assumed that an appropriate substituent could replace the aniline group by efficiently occupying the new binding pocket, resulting in the enhancement of GK potency.

Based on this assumption, we designed 3,6-disubstituted 2-pyridinecarboxamide **3** (Fig. 3). The central benzene ring in **1**

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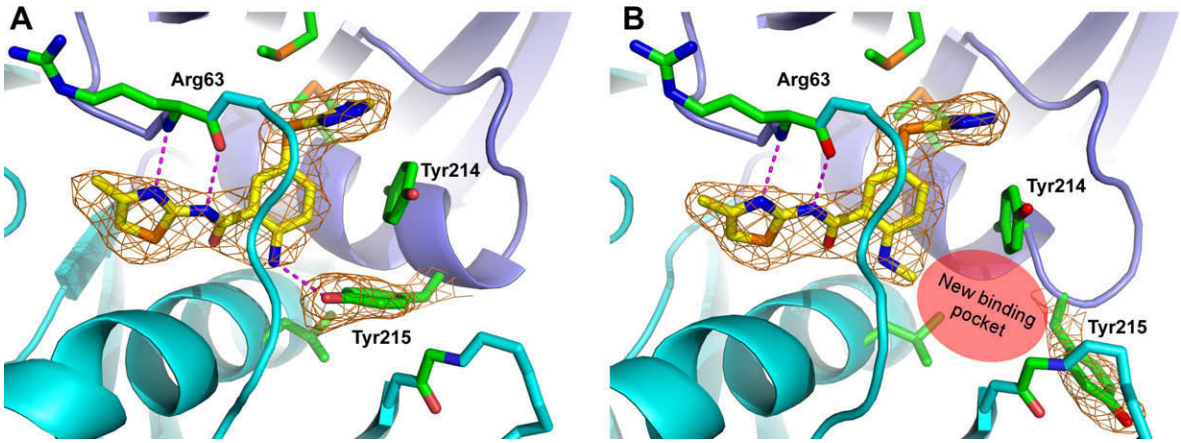


Figure 2. The activator binding mode of GK. Compound **1** (Panel A) and Compound **2** (Panel B) were shown in yellow stick representation. Final 2*Fo-Fc* electron densities (1.2 σ level) for compounds and Tyr215 were shown as orange mesh. This figure was prepared using the PyMOL.[27]

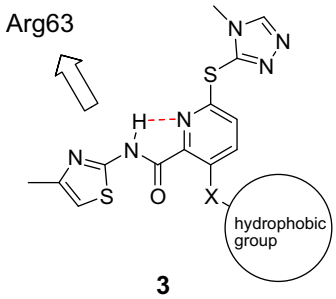


Figure 3.

was replaced by pyridine ring in **3** in order to lock the conformation by intra-molecular hydrogen bonding between the nitrogen atom of the pyridine ring and the amide NH group. The resulting orientation will allow 1) the aminothiazole part to favorably bind to Arg63 and 2) the hydrophobic group at 3-position on the pyridine ring to efficiently occupy the new binding pocket.

As a result of extensive efforts on the modification at the 3-position[17], 4-fluorophenylthio group was eventually discovered as a replaceable structure for the amino group. Compound **4** showed better GK potency[18] (EC_{50} 0.25 μM) than the lead compounds **1** and **2** (EC_{50} 0.42 μM and 1.07 μM). To confirm the predicted binding mode, co-crystal structure of GK with **4** was solved (Fig. 4).[16,19]

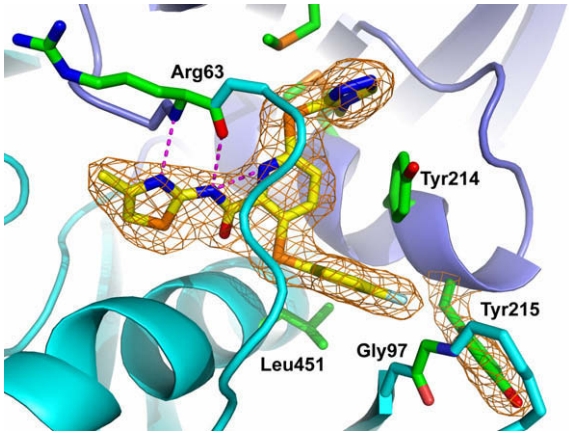


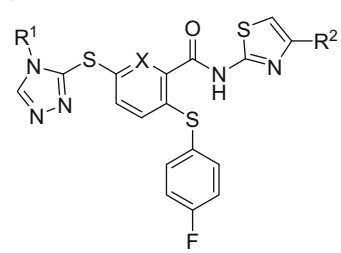
Figure 4. Binding mode of compound **4**. Final 2*Fo-Fc* electron densities (1.2 σ level) for compound **4** and Tyr215 were shown as orange mesh.

As we predicted, the 4-fluorophenylthio group occupied the new binding pocket by making hydrophobic interactions with Tyr214, Leu451 and Gly97. Moreover, intra-molecular hydrogen bonding interaction of the pyridine nitrogen with the amide NH was observed, suggesting that the pyridine nitrogen locks the conformation and makes the aminothiazole part preferable for binding to Arg63. This importance of the pyridine nitrogen was verified by the fact that phenyl analog **5** exhibited reduced potency. Thus, compound **4** was found to be a potent GK activator without the aniline group and selected for further optimization.

The effects of methyl groups on the thiazole and triazole rings were examined to improve the potency of **4** (Table 1). Removal of the methyl group on the thiazole (**6**) slightly increased the potency. Additional enhancement of the potency was obtained by removal of the methyl group on the triazole ring (**7**). Compound **7** displayed good GK potency with EC_{50} of 0.076 μM.

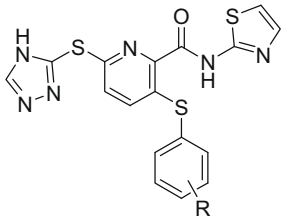
Next, SAR at the 4-fluorophenylthio part in **7** was investigated by incorporating commercially available thiophenols. As shown in Table 2, most of the compounds tested were tolerable in terms of GK potency regardless of substituents on the phenyl ring. Although SAR data was limited, there was no clear correlation between the GK potency and the nature of electron density of the phenyl ring. Bulkiness of the substituents slightly affected the potency. Compounds bearing small sized substituents such as fluorine (**8**, **9**) and hydrogen (**10**) showed better potencies, however their metabolic stability predicted from in vitro screening method using rat hepatocytes was less than that of **7**.[20]

Table 1
In vitro GK potency for **4**–**7**



Compound	X	R^1	R^2	GK potency[18] EC_{50} (μM)
4	N	Me	Me	0.25
5	CH	Me	Me	0.97
6	N	Me	H	0.12
7	N	H	H	0.076

Table 2
In vitro GK potency and metabolic stability for **7**–**14**



Compound	R	GK potency[18] EC_{50} (μM)	Metabolic stability[20] F_H[a] (%)
7	4-F	0.076	100
8	2-F	0.057	71
9	3-F	0.040	89
10	H	0.038	77
11	4-MeO	0.12	35
12	4-CF_3	0.16	NT[b]
13	3-MeO	0.16	NT[b]
14	3-Me	0.10	69

[a] In vitro hepatic availability in rat hepatocyte.
[b] Not tested.

Based on the balance of in vitro GK potency and metabolic stability, compound **7** was selected and tested in pharmacokinetic and in vivo efficacy studies to see the potential of this class for further drug development. With respect to the pharmacokinetic profile, **7** exhibited moderate oral bioavailability (23%) with $T_{1/2}$ of 4.0 h in rats (Fig. 5). The reason for the moderate bioavailability of **7** may be due to its low absorption. In a rat OGTT model,[21] **7** demonstrated significant glucose lowering effect after oral dosing at 30 mpk, indicating that **7** is an orally active GK activator (Figs. 6 and 7).

Synthesis of the representative compound **4** is shown in Scheme 1. As a key synthetic intermediate, 3,6-dichloro-2-pyridinecarboxamide **16** was selected in terms of its feasibility to quickly explore SAR. Coupling of acid **15**[22] with 4-methyl-2-aminothiazole gave **16**. Substitution of **16** by 4-fluorothiophenol under basic conditions (K_2CO_3/DMF) and chromatographic separation of the regio-isomer afforded compound **17** in 77% yield. Subsequent treatment of **17** with 3-mercapto-4-methyl-1,2,4-triazole produced **4** in 75% yield. Other analogs (**6–14**) were similarly prepared by using the corresponding thiophenols.

	$AUC_{0-\infty}$ (μM·hr)	$T_{1/2}$ (hr)	CL_p (mL/min/kg)	V_{dss} (L/kg)	F (%)
iv (1 mg/kg)	4.9	4.0	8.8	0.5	
po (3 mg/kg)	3.3				23

Figure 5. Pharmacokinetic profile of **7** in rats.

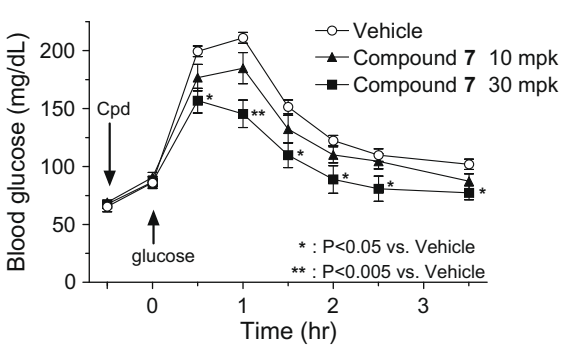


Figure 6. Plasma glucose lowering effect of **7**.

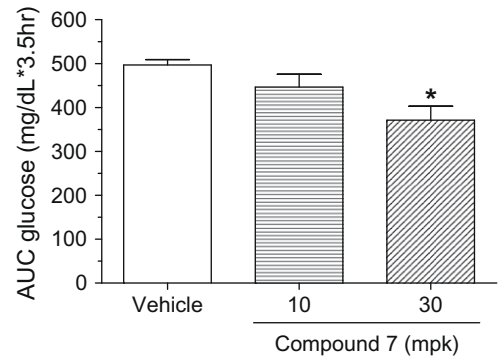
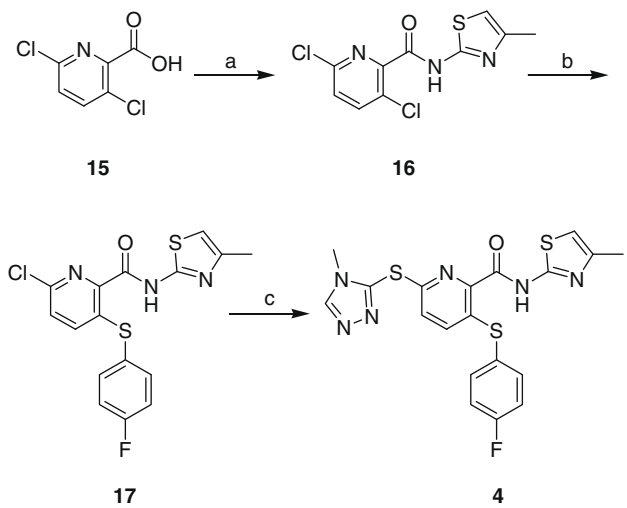
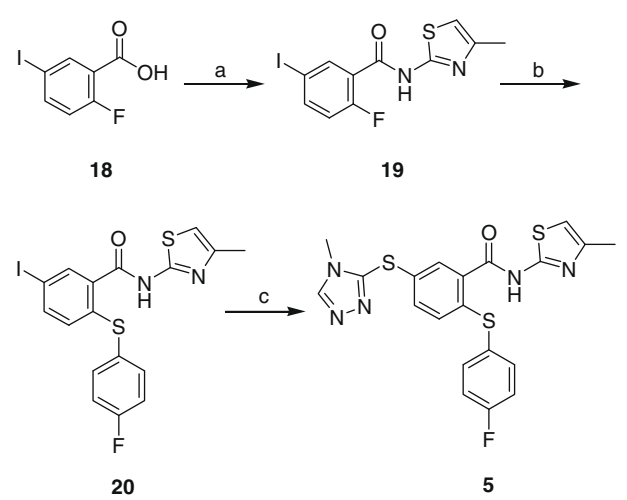


Figure 7. AUC levels in a rat OGTT model.



Scheme 1. Reagents and conditions: (a) EDCI, HOBt, 2-amino-4-methylthiazole, $CHCl_3$, rt, 24 h, 64%; (b) 4-fluorothiophenol, K_2CO_3, DMF, rt, 3 h, 77%; (c) 3-mercapto-4-methyl-1,2,4-triazole, *t*-BuOK, DMA, 120 °C, 20 h, 75%.

Preparation of phenyl analog **5** is depicted in Scheme 2. Starting from 2-fluoro-5-iodobenzoic acid **18**,[23] coupling with 4-methyl-2-aminothiazole gave amide **19** in 69% yield. The amide **19** was treated with 4-fluorothiophenol in the presence of K_2CO_3 to yield compound **20**. Coupling of the iodide **20** with 3-mercapto-



Scheme 2. Reagents and conditions: (a) EDCI, HOBt, 2-amino-4-methylthiazole, $CHCl_3$, rt, 20 h, 69%; (b) 4-fluorothiophenol, K_2CO_3, DMF, rt, 1 h, 98%; (c) 3-mercapto-4-methyl-1,2,4-triazole, CuI, K_2CO_3, DMF, 125 °C, 14 h, 56%.

4-methyl-1,2,4-triazole by copper-catalyzed reaction produced **5** in 56% yield.

In conclusion, we designed a novel class of 3,6-disubstituted 2-pyridinecarboxamide GK activators based on the crystal information from a GK-allosteric activator complex. Chemical modification led to the discovery of a series of potent GK activators which eliminates the aniline group of the lead compound **1** and serve as a good source for the development of GK activators suitable for clinical study. Further optimization of this series is ongoing to improve drug-like properties such as ADME/PK and in vivo efficacy. Detailed SAR and biological profiles of this novel class will be reported elsewhere.

Acknowledgment

We thank Dr. Norikazu Ohtake and Dr. Junichi Sakaki for helpful discussion.

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15. Crystallographic statistics for the GK-compound **2** complex are as follows: space group $P6_522$, unit cell 80.1, 80.1, 324.0 Å, resolution 2.52 Å, 21,669 reflections from 439,153 observations give 98.3% completeness with R_{merge} of 4.7% and mean I/σ *(I)* of 71.2. The final model containing 3506 protein, 128 water, 1 salt, 12 glucose and 26 compound atoms has an *R*-factor of 22.1% (R_{free} using 5% of the data 28.2%). Mean temperature factors for the protein and the ligand are 30.8 and 28.0 $Å^2$, respectively.
16. Protein and crystals were obtained according to established procedures.[24] Crystals were soaked in 0.5 mM compound **2** or compound **4** overnight in mother liquor containing 5% DMSO. Diffraction data were collected on beamline BL6B of the Photon Factory in KEK, at 100 K. Data processing and data reduction were carried out using programs from the HKL2000 (HKL Research, Inc.) and the CCP4 package.[25] Compounds were modeled into the electron density using AFITT (OpenEye Scientific Software). The protein-compound complex models were refined using CNX (Accelrys) and REFMAC5 from the CCP4 package. The final structures have been deposited in the Protein Data Bank with the deposition code 3A0I and 3GOI together with structure factors and detailed experimental conditions.
17. Substituents such as alkyloxy, alkylthio and aryloxy groups at the 3-position were tested. However, they reduced GK potency.
18. According to the literature,[26] EC_{50} values were measured at 2.5 mM glucose concentration and are the means of at least two or more independent assays. Compound **1** was used as an internal control across all assay plates for data validation. The EC_{50} values of **1** are 0.42 ± 0.09 μM. Maximal GK activation by compounds **4–14** was in the same range as compound **1** (8.0–8.3-fold over control levels).
19. Crystallographic statistics for the GK-compound **4** complex are as follows: space group $P6_522$, unit cell 79.8, 79.8, 322.0 Å, resolution 2.20 Å, 30,342 reflections from 111,693 observations give 94.5% completeness with R_{merge} of 6.4% and mean I/σ *(I)* of 16.5. The final model containing 3506 protein, 137 water, 1 salt, 12 glucose and 30 compound atoms has an *R*-factor of 22.6% (R_{free} using 5% of the data 27.7%). Mean temperature factors for the protein and the ligand are 36.1 and 24.8 $Å^2$, respectively.
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21. Nine-week old male Wistar rats were fasted overnight before performing the test. The rats were orally administrated either compound **7** or vehicle alone (0.5% methylcellulose solution), followed 30 min later by an oral glucose challenge (2 g/kg). Blood glucose concentrations were measured just prior to and following the glucose challenge (30, 60, 90, 120, 150 and 210 min).
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