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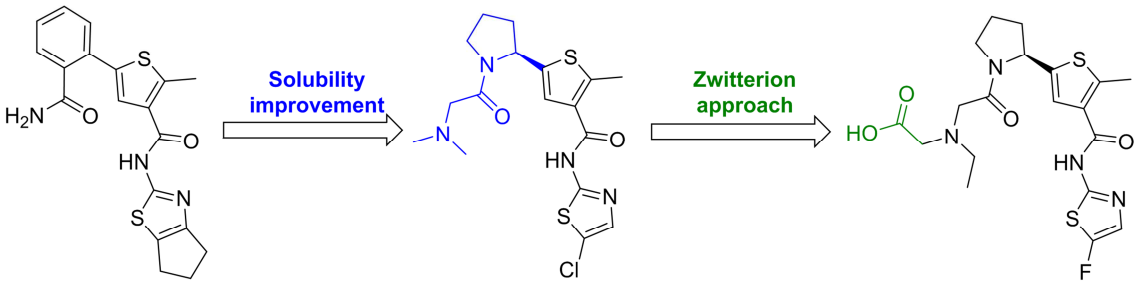

7

38
Orally active GKA

59
Orally active GKA
Low distribution to pancreas

Discovery of a Potent Glucokinase Activator with a Favorable Liver and Pancreas Distribution Pattern for the Treatment of Type 2 Diabetes Mellitus

Hiroki Fujieda,* Masakazu Kogami, Masao Sakairi, Noriyasu Kato, Mitsuhiro Makino, Naoki Takahashi, Toshiyuki Miyazawa, Satoko Harada, Tokuyuki Yamashita

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Abstract

Glucokinase (GK) is an enzyme that plays an important role as a glucose sensor while maintaining whole body glucose homeostasis. Allosteric activators of GK (GKAs) have the potential to treat 2 diabetes mellitus. To identify novel GKAs, a series of compounds based on a thiophenyl-pyrrolidine scaffold were designed and synthesized. In this series, compound **38** was found to inhibit glucose excursion in an oral glucose tolerance test (OGTT) in mice. Optimization of **38** using a zwitterion approach led to the identification of the novel GKA **59**. GKA **59** exhibited potent blood glucose control in the OGTT test as well as a favorable safety profile. Owing to low pancreatic distribution, compound **59** primarily activates GK in the liver. This characteristic could overcome limitations of other GKAs, such as hypoglycemia, increased plasma triglycerides, and loss of efficacy.

Keywords

Type 2 diabetes; Glucokinase; Glucokinase activator; thiophenyl-pyrrolidine scaffold; hERG; Zwitterion

1. Introduction

Diabetes is one of the most common chronic diseases worldwide. According to the International Diabetes Federation, diabetes affected approximately 425 million people in 2017, and this number is expected to rise to 629 million by 2045. The increasing prevalence of diabetes is driven by a variety of factors, including diet, urbanization, and obesity. Type 2 diabetes mellitus (T2DM) is the predominant form of the disease and constitutes the majority of adult cases worldwide [1,2]. T2DM is typically characterized by hyperglycemia, insulin resistance, abnormally elevated hepatic glucose production, and inadequate glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells [3]. Insufficient blood glucose control increases the risk of vascular complications such as coronary artery disease, peripheral arterial disease, stroke, nephropathy, neuropathy, and retinopathy [4]. Although various oral anti-hyperglycemic agents are available, monotherapy or combination regimens are frequently inadequate for maintaining blood glucose levels in the long term. Furthermore, many of these agents exhibit side effects such as hypoglycemia, weight gain, gastrointestinal side effects, and genitourinary infection [5,6]. Therefore, an unmet need exists for more effective therapies offering improved efficacy and safety for the management of diabetes.

GK, also called hexokinase IV or D, is a glycolytic enzyme that converts glucose to glucose-6-phosphate. GK has unique characteristics compared with those of the ubiquitously expressed hexokinase isoforms I–III, and is predominantly expressed in the pancreas and liver. In pancreatic β-cells, GK regulates the threshold for GSIS. In hepatocytes, GK regulates glycogen synthesis and hepatic glucose production, and its activity is controlled by glucokinase regulatory protein (GKRP) [7–9]. Considering that the activation of GK in the pancreas and liver could lead to increased insulin secretion from pancreatic β-cells and glucose uptake in the liver, allosteric activators of GK (GKAs) have emerged as attractive targets for the treatment of T2DM [10].

Following the initial discovery of RO0281675 [11,12], several GKAs, including phenyl acetamides, benzamides, and imidazolylacetamides, have been reported (Figure 1) [13–18].

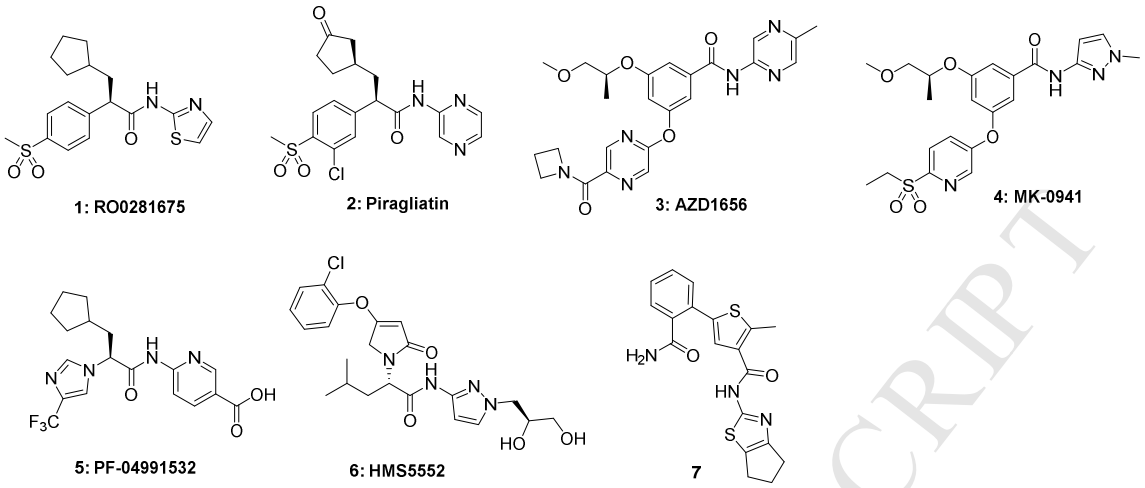


Figure 1. Representative structures of glucokinase activators from the literature and the structure of our lead compound **7**.

To date, several GKAs have advanced to clinical studies and have demonstrated an ability to lower blood glucose levels in both healthy subjects and patients with T2DM. However, in these clinical studies hypoglycemia, increased plasma triglycerides (TG), and loss of efficacy within several months, were determined to be the main obstacles to developing GKAs as therapeutic agents for T2DM [16,19]. It is believed that hypoglycemia is caused by the overstimulation of islet β-cell GK, while increased plasma TG is the result of overstimulation of liver GK. However, the cause of the loss of efficacy in the short term remains to be elucidated [20]. To mitigate these limitations, various types of GKAs have been discovered and have progressed to human clinical trials [18]. Although most were terminated at Phase I or Phase II, some GKAs (including vTv Therapeutics's TTP399 and Hua Medicine's HMS5552) remain currently active and have shown positive results in recent clinical trials [21–23]. These two compounds exhibit characteristics that are unique among GKAs. TTP399 is a liver-selective GKA that does not affect the inhibition of GK by GKRP in the liver. The manufacturer suggests that disruption of the GK-GKRP interaction leads to hypoglycemia and dyslipidemia. HMS5552 has an amino acid-based structure, which is markedly different from that of other GKAs. The development of GKA as a clinical agent for the treatment of T2DM therefore requires the identification of a new type of GKA with characteristics different from those of other GKAs. Herein, we report the discovery of a new type of hepatoselective GKA with a unique structure, robust GK

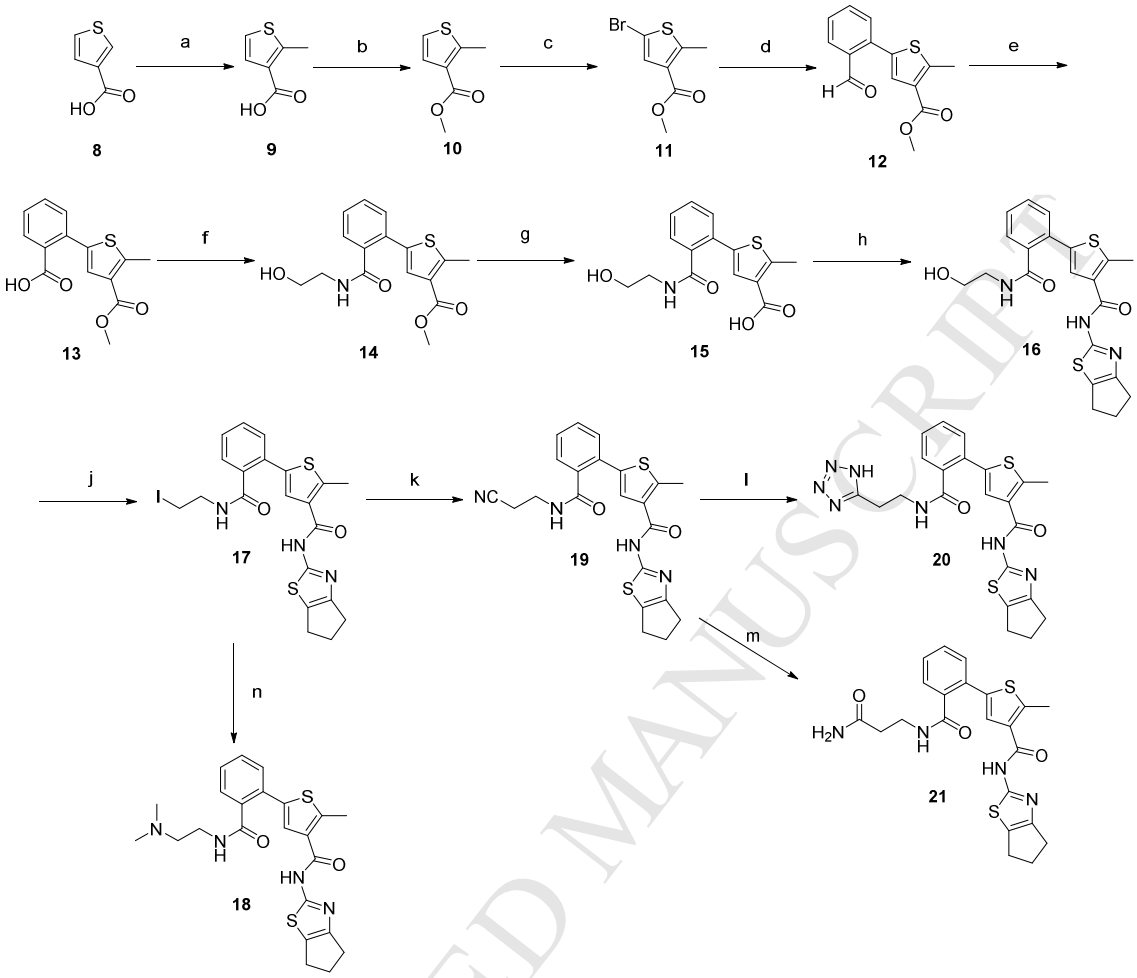
activation potency, and benign safety profile. In addition, a favorable distribution pattern in the pancreas and liver was observed. To the best of our knowledge, an orally active GKA exhibiting such characteristics has not been disclosed to date.

In a previous report, the discovery of novel small molecule **7**, using molecular modeling-aided medicinal chemistry approaches, was described [24]. This compound has a characteristic structure with an amide portion connecting the phenyl thiophene ring and cyclopentathiazole. GKAs with this type of structure are poorly represented in the literature. Therefore, compound **7** was selected as the lead compound for our discovery series.

2. Results and discussion

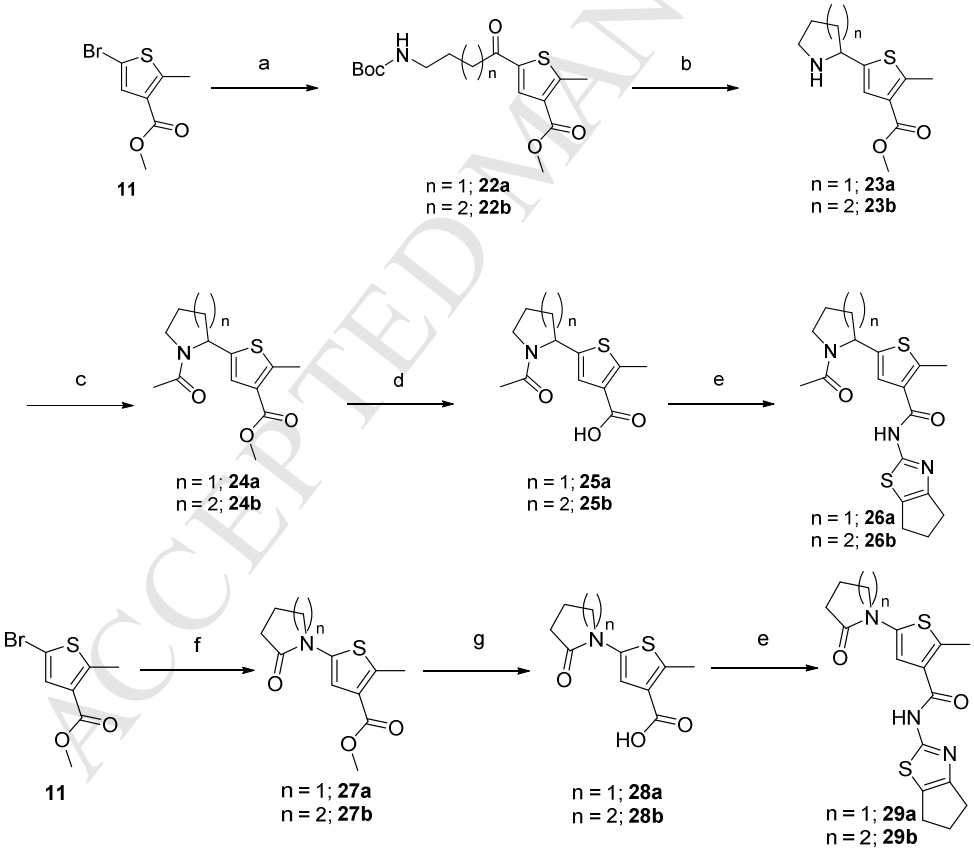
2. 1. Chemistry

The compounds prepared for this study are shown in Tables 1–6. Syntheses were carried out as outlined in Schemes 1–4. The route used for the synthesis of compounds (**16**, **18**–**21**) is described in Scheme 1. Commercially available thiophene-3-carboxylic acid **8** was converted to thiophene methyl ester **10** by methylation of the 2-position of thiophene with methyl iodide and esterification under acidic conditions. Treatment of thiophene **10** with *N*-bromosuccinimide (NBS) and subsequent Suzuki-Miyaura cross-coupling with 2-formylphenylboronic acid gave aldehyde **12**. Aldehyde **12** was converted to carboxylic acid **13** by pinnick oxidation, and subsequent amide coupling of acid **13** with 2-aminoethanol provided ester **14**. Hydrolysis of ester **14** under alkaline conditions gave carboxylic acid **15**. Amidation of **15** with 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine afforded alcohol **16**. Alcohol **16** was converted to iodide **17** via Appel reaction using I_2 and PPh_3, followed by S_N2 reaction of **17** with NaCN to afford nitrile **19**. Nitrile **19** was converted to tetrazole **20** using Bu_3SnN_3 and to amide **21** by hydrolysis of **19** using a platinum-containing catalyst prepared as previously described [25]. The reaction of iodine **17** with dimethylamine gave amine **18**.



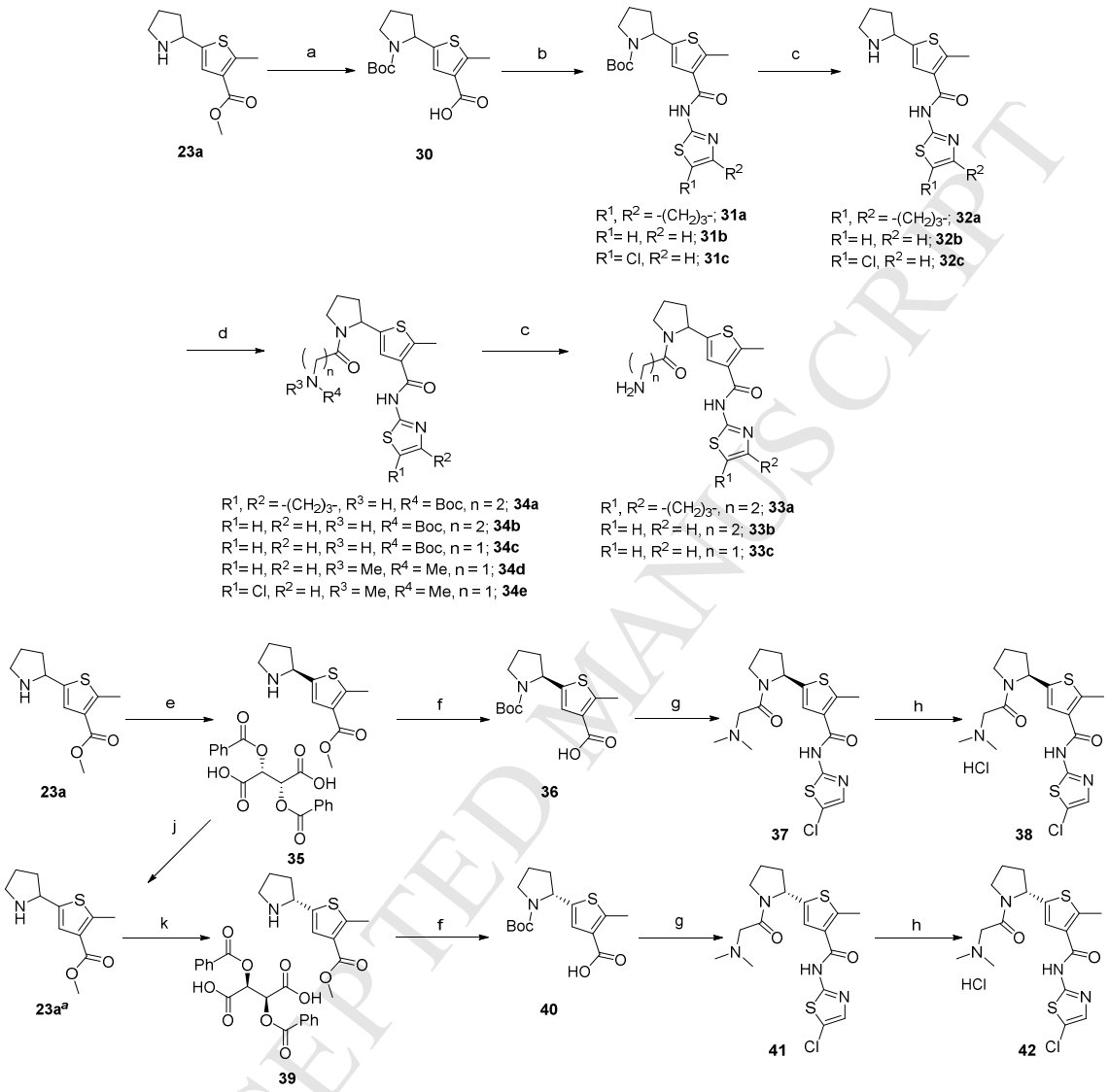
Scheme 1. Synthesis of phenyl thiophenyl derivatives; Reagents and conditions: (a) (i) diisopropylamine, *n*-BuLi, THF, 0 °C, (ii) MeI, −60 °C to rt, 85%; (b) $SOCl_2$, MeOH, reflux, 96%; (c) NBS, DMF, rt, 96%; (d) 2-formylphenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME, reflux, 74%; (e) $NaClO_2$, Na_2HPO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, rt, 68%; (f) 2-aminoethanol hydrochloride, EDCI, HOBt, DIPEA, DMF, rt, 96%; (g) 5 N aqueous NaOH, THF, MeOH, 50 °C, 41%; (h) 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine, EDCI, HOBt, DIPEA, DMF, 50 °C, 39%; (j) I_2, PPh_3, imidazole, THF, 0 °C, 86%; (k) NaCN, DMSO, rt, 21%; (l) Bu_3SnN_3, toluene, 130 °C, 48%; (m) $[PtH(PMe_2OH)(PMe_2O)_2H]$, EtOH, H_2O, reflux, 55%; (n) Me_2NH, THF, rt, 72%.

Scheme 2 shows the preparation of compounds **26a**, **26b**, **29a**, and **29b**. Ketones **22a** and **22b** were prepared from ester **11** in two steps: a Grignard exchange reaction of **11** with *i*-PrMgBr, and reaction of the resulting Grignard reagent with *N*-Boc-protected cyclic amide. Intramolecular reductive amino alkylation of ketones **22a** and **22b** gave pyrrolidine **23a** and piperidine **23b** respectively. After acetylation of amine **23a** and **23b**, amide **24a** and **24b** were hydrolyzed under alkaline conditions followed by coupling with 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine to give **26a** and **26b**. A Cu-catalyzed coupling reaction of **11** with 2-pyrrolidone and 2-piperidone afforded esters **27a** and **27b** [26]. Hydrolysis of esters **27a** and **27b** and subsequent amide coupling with 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine gave **29a** and **29b**, respectively.



Scheme 2. Synthesis of heterocycloalkyl thiophenyl derivatives; Reagents and conditions: (a) (i) *i*-PrMgBr, THF, −40 °C, (ii) 1-Boc-2-pyrrolidone or 1-Boc-2-piperidone, −40 °C to rt, 51–62%; (b) (i) TFA, CH_2Cl_2, 0 °C to rt, (ii) $NaBH_3CN$, conc. HCl, *i*-PrOH, 0 °C, 63–70%; (c) acetyl chloride, pyridine, $CHCl_3$, 0 °C to rt, 85–98%; (d) $LiOH \cdot H_2O$, MeOH, H_2O, 50 °C, 80–98%; (e) 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine, EDCI, HOBt, DIPEA, DMF, rt, 21–43%; (f) 2-pyrrolidone or 2-piperidone, CuI, *N*,*N'*-dimethylethylenediamine, K_2CO_3, toluene, reflux, 17–33%; (g) $LiOH \cdot H_2O$, THF, H_2O, 50 °C, 80–87%.

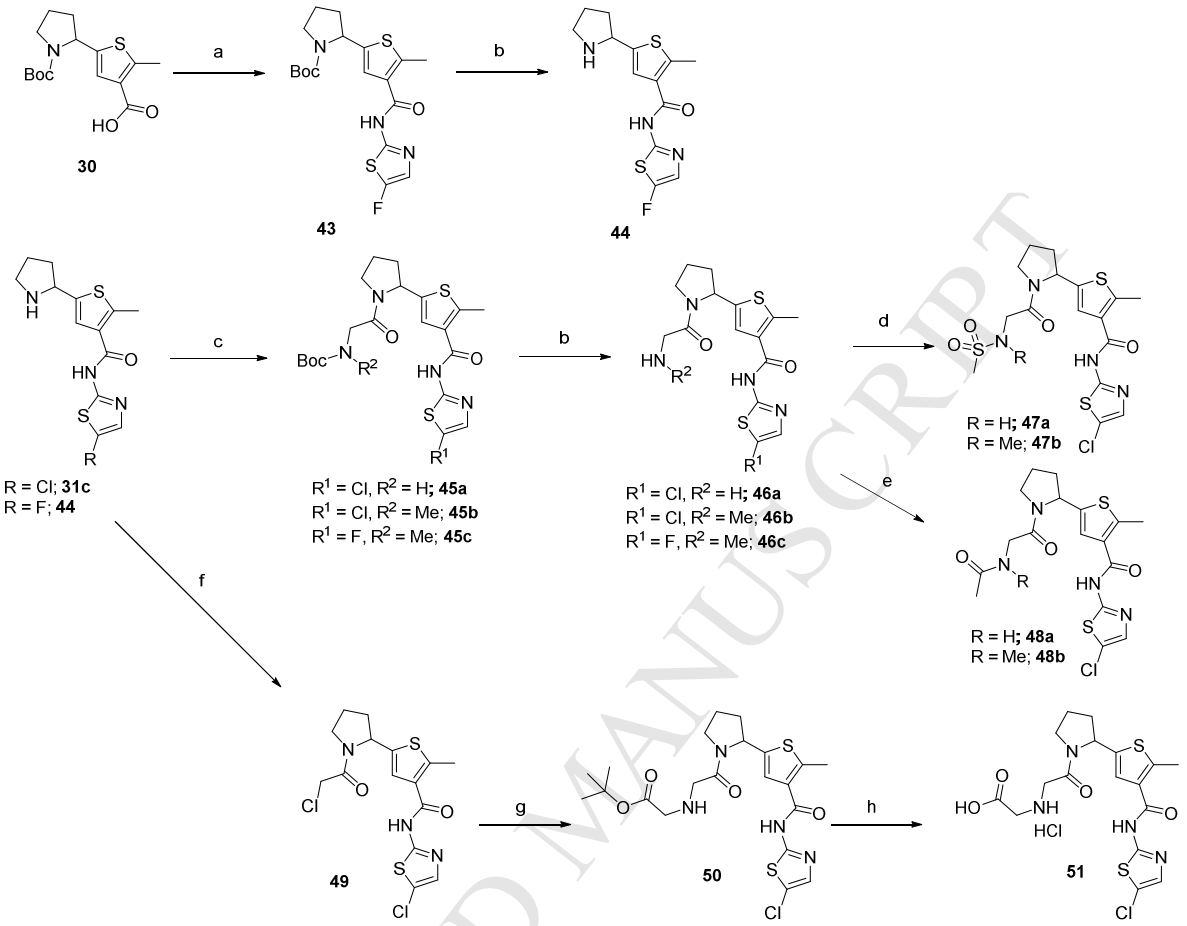
The routes for the synthesis of compounds **33a**–**33c**, **34d**, **34e**, **38**, and **42** are shown in Scheme 3. Compounds **33a**–**33c**, **34d**, and **34e** were synthesized from ester **23a** obtained in Scheme 2. Treatment of **23a** with $(Boc)_2O$ in the presence of triethylamine in CH_2Cl_2 followed by hydrolysis of the ester group by aqueous LiOH gave Boc-carboxylic acid **30**. Next, amidation of **30** with a corresponding thiazole amine afforded amides **31a**–**31c**. Removal of the Boc group of **31a**–**31c** using trifluoroacetic acid followed by coupling with the corresponding carboxylic acid afforded **34a**–**34e**. Deprotection of the Boc group of **34a**–**34c** under acidic conditions gave **33a**–**33c**. To investigate the most efficient chiral-resolving agent for optical resolution of racemic amine **23a**, commercially available acidic resolving agents were used. Optical resolution of racemic ester **23a** was accomplished through diastereomeric salt formation with (−)-dibenzoyl-L-tartaric acid monohydrate (96.6% de), followed by salt decomposition, Boc protection and hydrolysis, to give **36**. Next, (+)-enriched amine **23a** was recovered as a condensate by evaporating the methanolic mother liquor of the first resolution followed by salt decomposition through treatment with saturated aqueous $NaHCO_3$. Optical resolution of liberated (+)-enriched amine **23a** was accomplished through diastereomeric salt formation with (+)-dibenzoyl-D-tartaric acid monohydrate. Compounds **37** and **41** were synthesized in a similar manner to that shown for the synthesis of **34e** by the reaction of chiral carboxylic acids **36** and **40**. Treatment of **37** and **41** with 4 N HCl in 1,4-dioxane afforded hydrochloride salts **38** and **42**, respectively. The crystal structure of the resolved tartaric acid salt **35** was determined by X-ray crystallographic analysis. As a result, the absolute configuration at the chiral center in the pyrrolidine ring of **35** was confirmed to be the *(S)*-configuration (see Supplementary data).

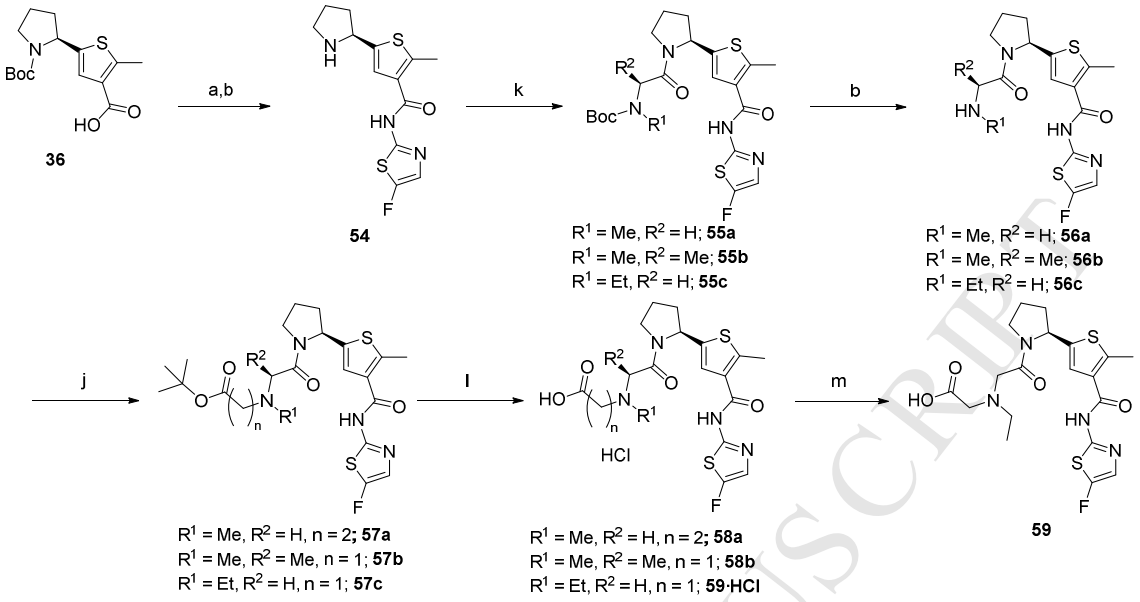


Scheme 3. Synthesis of compound **26a** analogs; Reagents and conditions: (a) (i) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt, (ii) $LiOH{\cdot}H_2O$, MeOH, H_2O, 50 °C, 96% (2 steps); (b) corresponding thiazoleamine, EDCI, HOBt, DIPEA, DMF, 50 °C, 48–88%; (c) TFA, CH_2Cl_2, 0 °C to rt, 27–97%; (d) corresponding carboxylic acid, EDCI, HOBt, DIPEA, DMF, rt, 27–95%; (e) (−)-dibenzoyl-L-tartaric acid monohydrate, MeOH, rt, 48% in theory; (f) (i) saturated aqueous $NaHCO_3$, $CHCl_3$, rt; (ii) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt; (iii) $LiOH{\cdot}H_2O$, MeOH, H_2O, 50 °C, 87–94%; (g) (i) 2-amino-5-chlorothiazole hydrochloride, EDCI, HOBt, DIPEA, DMF, 50 °C, (ii) TFA, CH_2Cl_2, 0 °C to rt, (iii)

N,*N*-dimethylglycine, EDCI, HOBt, DIPEA, DMF, 49–58% (3 steps); (h) 4 N HCl in 1,4-dioxane, CH_2Cl_2, 0 °C to rt, 70–97%; (j) saturated aqueous $NaHCO_3$, $CHCl_3$, rt; (k) (+)-dibenzoyl-D-tartaric acid monohydrate, MeOH, rt, 45% in theory. [a](+)-enriched enantiomer.

The route for the synthesis of compounds **47a**–**48b**, **51**, **53a**–**53e**, and **58a**–**59** is shown in Scheme 4. Compounds **47a**–**48b** and **51** were synthesized from amine **31c**. Condensation of amine **31c** with the corresponding carboxylic acid, followed by deprotection of the Boc group, gave amines **46a** and **46b**. Amines **46a** and **46b** were treated with mesyl chloride to give mesylated compounds **47a** and **47b**. Furthermore, amines **46a** and **46b** were treated with acetyl chloride to give acetylated compounds **48a** and **48b**, respectively. Chloroacetylation of amine **31c**, followed by treatment with glycine *tert*-butyl ester and subsequent removal of the *tert*-butyl ester using hydrochloric acid, afforded carboxylic acid **51**. Fluorothiazole derivative **46c** was synthesized in a similar manner as that for the chlorothiazole counterpart **46b**. Treatment of amines **46b** and **46c** with bromo ester derivatives or *tert*-butyl acrylate followed by deprotection of the ester group gave carboxylic acids **53a**–**53e**. Chiral isomers **58a**, **58b**, and **59** were synthesized from chiral acid **36** obtained in Scheme 3. Carboxylic acid **36** was converted to amines **56a**–**56c** in a four-step sequence: condensation with 2-amino-5-fluorothiazole, deprotection of the Boc group, and condensation with the corresponding *N*-Boc amino acid, followed by deprotection of the Boc group. Compounds **58a**, **58b**, and **59·HCl** were synthesized in a similar manner to that shown for the synthesis of **53a**–**53e** from **46b** and **46c** by the reaction of amines **56a**–**56c**. Finally, we sought to establish a method for isolation of the zwitterion of **59·HCl**. As a result, zwitterion product **59** was crystallized directly from the reaction mixture with 84% yield after pH adjustment to the isoelectric point of pH 5.8 with 1 N aqueous NaOH.





Scheme 4. Synthesis of **47a**–**48b**, **51**, **53a**–**53e**, and **58a**–**59**; Reagents and conditions: (a) (i) $(COCl)_2$, toluene, 0 °C, (ii) 2-amino-5-fluorothiazole hydrochloride, *N*,*N*-diethylaniline, toluene, rt, 76–78% (2 steps); (b) TFA, CH_2Cl_2, 0 °C to rt, 79–99%; (c) glycine derivatives, EDCI, HOBt, DIPEA, DMF, rt, 90–99%; (d) mesyl chloride, pyridine, CH_2Cl_2, 0 °C to rt, 69–81%; (e) acetyl chloride, pyridine, CH_2Cl_2, 0 °C to rt, 74–85%; (f) chloroacetyl chloride, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, 0 °C to rt, 83%; (g) glycine *tert*-butyl ester hydrochloride, DIPEA, DMF, 50 °C, 68%; (h) 4 N HCl in 1,4-dioxane, CH_2Cl_2, 0 °C to rt, or 1 N aqueous NaOH, MeOH, rt, then 4 N HCl in 1,4-dioxane, 0 °C to rt, 68–99%; (j) *tert*-butyl ester derivatives, DIPEA, DMF or *tert*-butyl acrylate, benzyltrimethylammonium hydroxide in MeOH, DMF, rt to 60 °C, 22–77%; (k) corresponding Boc-amino acid, EDCI, HOBt, DIPEA, DMF, rt, 82–99%; (l) 4 N HCl in 1,4-dioxane, CH_2Cl_2 or 4 N HCl in AcOEt, CH_2Cl_2, 0 °C to rt, 71–99%; (m) 1 N aqueous NaOH, MeOH, H_2O, rt, 84%.

2. 2. Identification of a pyrrolidine-thiophene-based glucokinase activator

Since compound **7** had potent glucokinase activity in 5 mM glucose, its glucose-lowering effects were assessed by OGTT in mice. Unfortunately, compound **7** did not exhibit glucose-lowering effects in our OGTT study at 30 mg/kg (p.o.), and had poor bioavailability following oral dosing (C_{max} < 1 μM, data not shown). To convert **7** to the orally bioavailable GKA, we considered two strategies: the first was to significantly improve the GK activation potency of **7**, and the second was to improve the physicochemical properties of **7** affecting bioavailability. In our previous work, the nitrogen atom in the thiazole ring, amide NH, and carboxyl oxygen of terminal carbamoyl group of **7** were expected to form hydrogen bonds with amino acid residues in the GK active site. Given the existing interactions of compound **7** with the GK active site, we chose a strategy to improve the properties of **7** while keeping maintaining these interactions (Figure 2).

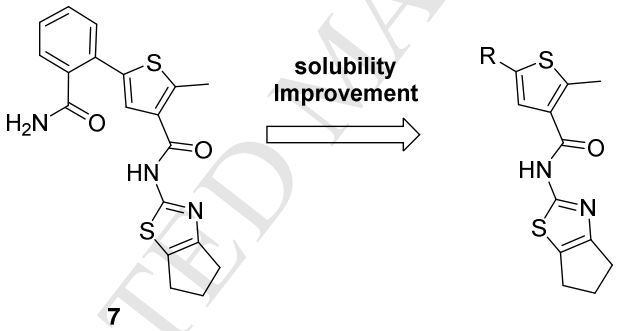


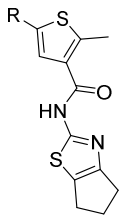
Figure 2. First design of hydrophilic analogs from lead compound **7**.

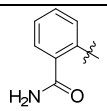
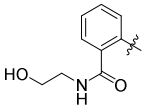
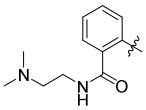
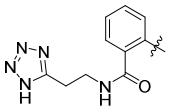
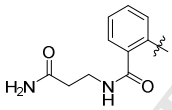
The results of our mouse OGTT study demonstrated that the clearance rate of **7** was not rapid, suggesting that metabolic stability of **7** was not the cause of poor bioavailability. Furthermore, the membrane permeability of **7** was considered adequate (Log *D* value at pH 7.4 was 3.23) [27]. Therefore, we hypothesized that improvements in solubility would lead to increased plasma exposure. From our previous modeling-aided studies, the cavity of the GKA binding site in which the carbamoyl group of **7** binds is composed of hydrophilic amino acid residues, and accommodates the introduction of functional groups. Therefore, we introduced various polar groups with hydrogen bond donors and acceptors to this position. Table 1 details the structure–activity relationship (SAR) of polar groups at the 2-position of the phenyl ring, and the effect of conversion

of the phenyl ring in **7** into a saturated hetero ring. GK activation potency of compounds were determined in a biological assay monitoring the rate of glucose 6-phosphate formation using G6PDH/NADP coupling. Compounds were characterized for relative GK activation using compound **1** (RO0281675) as a reference, and to determine batch variability. Aqueous solubilities were determined experimentally using a nephelometric assay in phosphate buffered saline (pH 7.4). All compounds with a basic amino moiety were used in the free form unless otherwise stated. As shown in Table 1, hydroxyl ethyl derivative **16** and dimethylamino derivative **18** preserved GK activation potency. Unfortunately, **16** and **18** did not show an improvement in solubility relative to that of **7**. Tetrazole derivative **20** and amide derivative **21** showed improvement in solubility, but had decreased GK activation potency compared with that of **7**. Although the introduction of polar groups in this part of the molecule was well tolerated, a promising compound with good solubility and GK activation potency was not identified. Therefore, we sought an alternative scaffold to replace the phenyl core. Lovering and colleagues found previously that carbon bond saturation correlates with solubility [28], we therefore designed molecules with a saturated hetero ring adjacent to the thiophene ring instead of the phenyl group (**26a**, **26b**, **29a**, and **29b**). Although the piperidine and piperidone derivatives **26b** and **29b** exhibited no improvement in solubility relative to that of **7**, the pyrrolidine and pyrrolidone derivatives **26a** and **29a** showed more than a two-fold improvement in solubility compared with that of **7**. These differences were thought to derive from differences in hydrophilicity and molecular weight. Pyrrolidine derivatives have a lower Log *D* value at pH 7.4 and a lower molecular weight than piperidine derivatives. Since **26a** was approximately four-fold more soluble than **7** and maintained GK activation potency to some extent, we focused our attention on the synthesis of pyrrolidine derivatives.

Table 1.

SAR for analogs of compound **7**



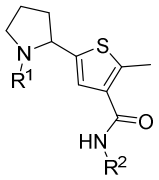
Compound	R	Activation at 5 mM glucose[a]		Solubility[d]
		Fold[b]	Relative[c]	(μM; pH 7.4)
7		6.23 ± 0.03	0.699 ± 0.004	1.95
16		3.89 ± 0.08	0.373 ± 0.011	0.98
18		4.31 ± 0.04	0.428 ± 0.005	1.95
20		0.84 ± 0.01	−0.019 ± 0.001	31.3
21		2.56 ± 0.03	0.187 ± 0.004	3.91
26a		2.53 ± 0.02	0.187 ± 0.002	7.81
26b		1.13 ± 0.00	0.017 ± 0.001	0.98
29a		2.41 ± 0.01	0.168 ± 0.002	3.91
29b		2.96 ± 0.04	0.235 ± 0.004	0.98

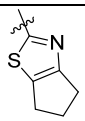
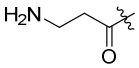
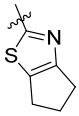
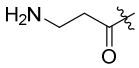
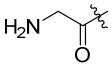
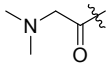
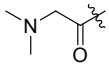
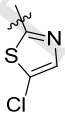
[a]Values are the mean ± SEM for n = 3. [b]Glucokinase activity at 10 μM relative to DMSO control. [c]Glucokinase activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [d]Aqueous solubility was determined experimentally using a nephelometric assay in PBS (pH 7.4).

The optimization of the pyrrolidine derivative **26a** is shown in Table 2. The amino ethyl derivative **33a** was more potent than **26a**, although the solubility of **33a** was low. Considering that the hydrophobicity of cyclopentathiazole moiety was extremely high, the non-substituted thiazole analog **33b** was prepared. The GK-activation potency of **33b** decreased, although the solubility of **33b** improved. Next, the aminomethyl derivative **33c** and the dimethylaminomethyl derivative **34d** were synthesized. These derivatives were comparable to **26a** in solubility, with good GK activation potency. The Cl derivative **34e** was also evaluated. Introduction of a Cl group at the 5-position of the thiazole ring resulted in decreased solubility compared with that of **34d**, but **34e** showed strong GK activation potency.

Table 2.

SAR for analogs of compound **26a**



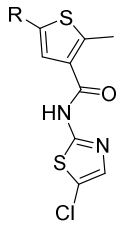
Compound	R^1	R^2	Activation at 5 mM glucose[a]		Solubility[d]
			Fold[b]	Relative[c]	(μM; pH 7.4)
26a			2.53 ± 0.02	0.187 ± 0.002	7.81
33a			3.46 ± 0.02	0.274 ± 0.002	0.98
33b			2.86 ± 0.02	0.175 ± 0.002	15.6
33c			3.67 ± 0.01	0.293 ± 0.001	7.81
34d			4.70 ± 0.04	0.380 ± 0.004	7.81
34e			6.81 ± 0.05	0.598 ± 0.005	3.91

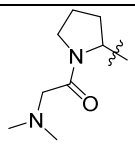
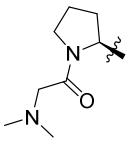
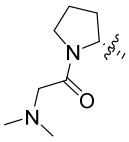
[a]Values are the mean ± SEM for n = 3. [b]Glucokinase activity at 10 μM relative to DMSO control. [c]Glucokinase activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [d]Aqueous solubility was determined experimentally using a nephelometric assay in PBS (pH 7.4).

Thus, with a promising compound showing good GK activation potency and improved solubility identified, chiral isomers of **34e** were synthesized and assessed for GK activation potency to confirm the effect of the pyrrolidine chiral center on GK activity (Table 3). Both isomers **38** and **42** showed GK activation potency, although **38** showed stronger potency than **42**. Therefore, the biologically active form was confirmed to be the *(S)*-configuration. Next, the effect of the compounds on insulin secretion was evaluated in MIN-6 cells, a mouse pancreatic beta-cell line [29]. Compound **38** showed a good insulin secretory effect in this cell line.

Table 3.

The effect of the chiral center of compound **34e**



Compound	R	Activation at 5 mM glucose[a]		Insulin secretion from MIN-6 cells (relative)[a,d]	Solubility[e] (μM; pH 7.4)
		Fold[b]	Relative[c]		
34e		6.81 ± 0.05	0.598 ± 0.005	1.62 ± 0.27	3.91
38[f]		10.4 ± 0.1	0.650 ± 0.010	1.61 ± 0.21	7.81
42[f]		4.99 ± 0.12	0.381 ± 0.011	1.15 ± 0.06	1.95

[a]Values are the mean ± SEM for n = 3. [b]Glucokinase activity at 10 μM relative to DMSO control. [c]Glucokinase activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [d]Insulin secretion activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [e]Aqueous solubility was determined experimentally using a nephelometric assay in PBS (pH 7.4). [f]Hydrochloride salt.

Given the promising in vitro GK activation potency and effect on insulin secretion, **38** was evaluated for the ability to reduce glucose levels in rodents. The effect of **38** on blood glucose was tested using OGTT in non-diabetic (C57BL/6j) mice. Animals were subjected to an overnight fast. Compound **38** was orally administered 15 min prior to the administration of 2 g/kg of glucose. Blood glucose was measured at 0, 20, 40, 60, 90, and 120 min after glucose administration. Compound **38** decreased blood glucose levels dose-dependently in C57BL/6j mice at 10 and 30 mg/kg (Figure 3). As expected,

compound **38**, which demonstrated good solubility, exhibited high bioavailability in this study (C_{max} > 9 μM at 30 mg/kg).

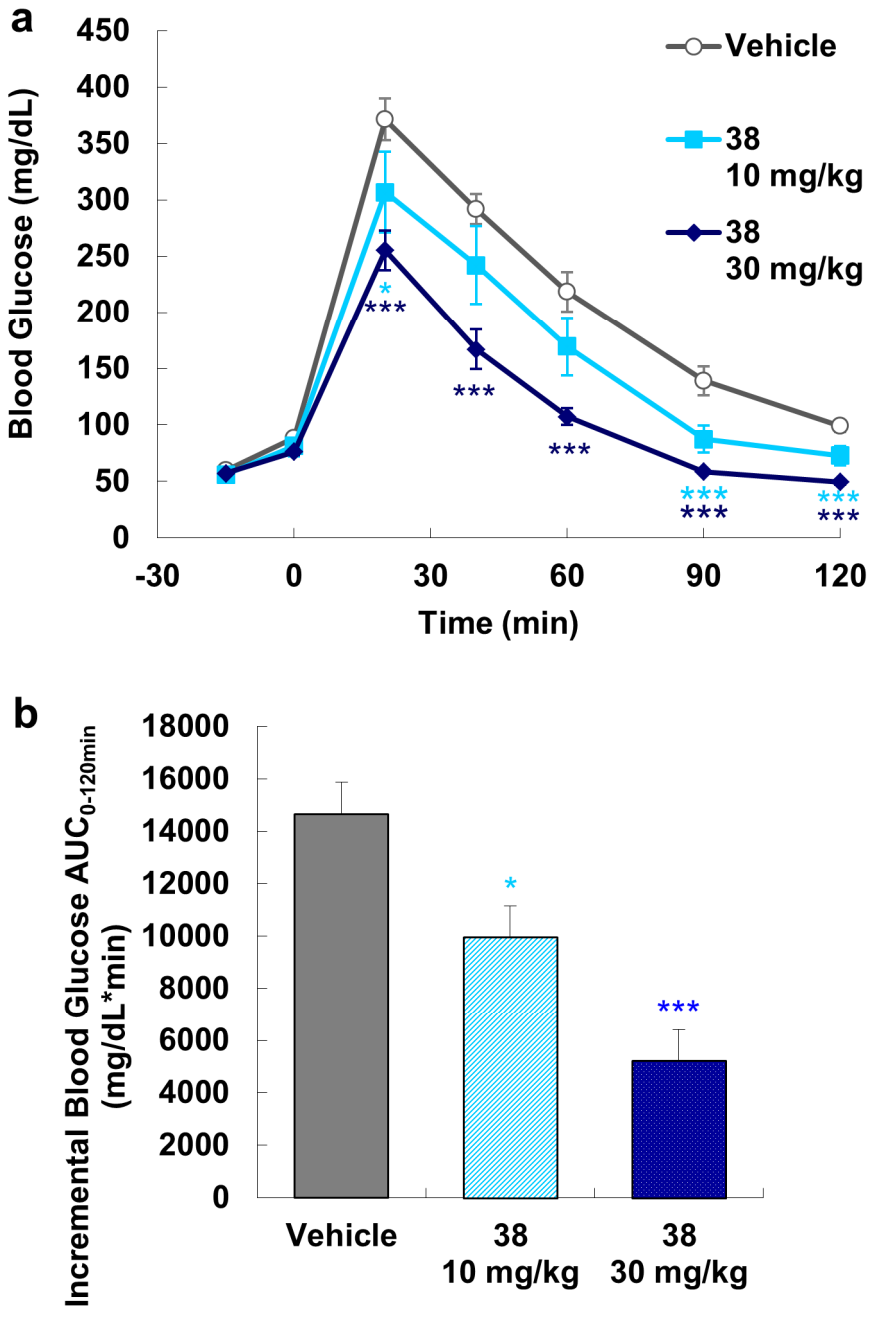


Figure 3. Oral glucose tolerance test (OGTT) of **38** in male C57BL/6j mice. Effect on (a) blood glucose level and (b) incremental blood glucose AUC during OGTT. Data are expressed as (a) mean ± SEM (n = 8) and (b) mean + SEM (n = 8). The data was analyzed using Dunnett's multiple comparison test. *, ***: $p < 0.05$, $p < 0.001$ vs. vehicle control.

For progression of compound **38** as a preclinical development candidate, the cardiovascular safety profile was evaluated. Unfortunately, **38** appeared to bind to the human ether-a-go-go related gene (hERG) channel with high affinity (IC_{50} < 0.3 μM).

Inhibition of the α-subunit of IKr channels, which is encoded by hERG, can cause delayed ventricular cell repolarization. This phenomenon is observed by electro cardiogram as a prolongation of the QT interval, and represents a major hurdle in drug development [30]. As it was crucial that our GK activation candidate compounds did not exhibit this property, it was necessary to reduce the hERG binding ability of **38**.

2. 3. Reduction of hERG inhibitory activity to discover 59

To reduce the hERG inhibitory activity of **38**, an extensive survey of the medicinal chemistry literature was conducted. Some effective strategies to minimize hERG/IKr activity have been previously reported [31]. Among these, we selected two modification strategies: the attenuation of pKa and the formation of zwitterions (Figure 4).

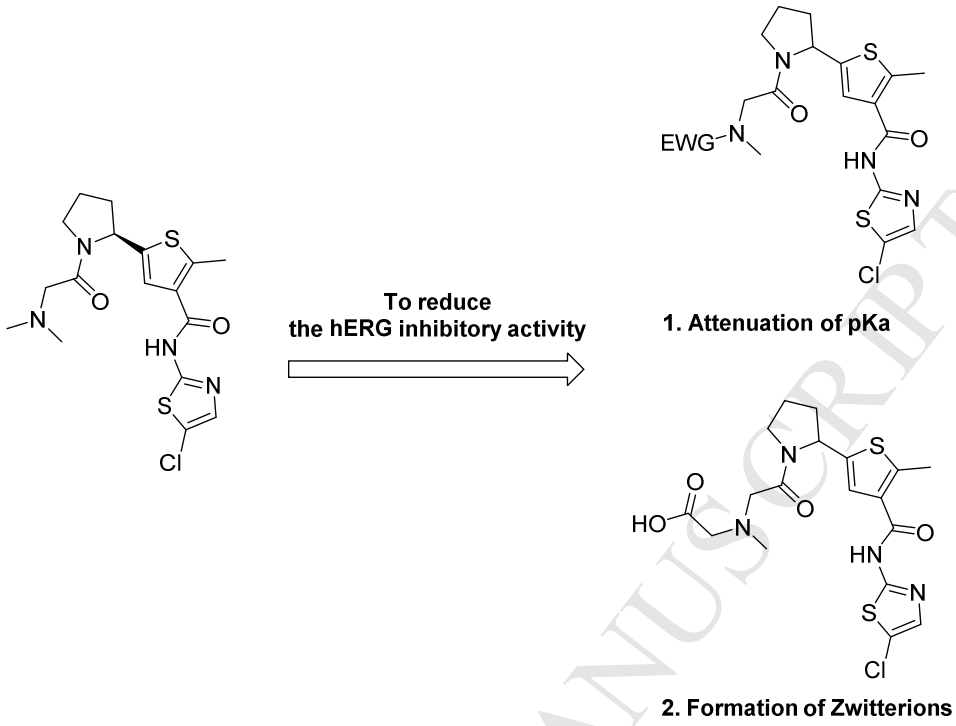


Figure 4. Strategy to reduce the inhibitory activity of hERG

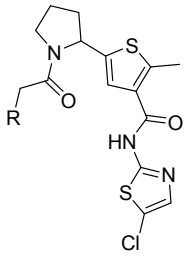
Table 4 shows the result of the modification of **38**. First, we reduced the terminal nitrogen pKa. It is reported that π-cation interactions between aromatic residues within the cavity of the hERG channel and the basic amine moiety in the ligand play a role in binding affinity. Therefore, reducing the protonation of molecules at physiological pH by lowering the pKa of a basic nitrogen could disrupt any putative π-cation interactions between ligand and hERG [32, 33]. To confirm the effect of this strategy, compounds whose nitrogen atom's basicity was weakened by a sulfonyl group (**47a** and **47b**) or acetyl group (**48a** and **48b**) were assessed. Within this series, *N*-methyl derivatives (**47b** and **48b**) showed a significant drop in GK potency, but N-H derivatives (**47a** and **48a**) showed moderate GK activation potency. Furthermore, these N-H derivatives showed a marked decrease in hERG binding affinity.

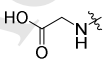
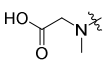
Next, we turned our attention to the introduction of a zwitterionic characteristic to the molecule. The zwitterion approach has been widely employed for the attenuation of hERG activity [34, 35]. Introduction of a carboxylic acid moiety into our potential template led to the synthesis of the zwitterionic compounds **51** and **53a**. These

compounds showed a substantial decrease in hERG binding affinity. Among these, **53a** showed a strong GK activation potency, comparable with **38**.

Table 4.

SAR for analogs of compound **38**



Compound	R	Activation at 5 mM glucose[a]		Insulin secretion from MIN-6 cells (relative)[a,d]	hERG inhibition (%)[a,e]
		Fold[b]	Relative[c]		
38[f,g]		10.4 ± 0.1	0.650 ± 0.010	1.61 ± 0.21	101 ± 1
47a		6.21 ± 0.04	0.460 ± 0.004	0.690 ± 0.226	16 ± 1
47b		3.24 ± 0.03	0.168 ± 0.002	-	-
48a		5.32 ± 0.08	0.395 ± 0.007	1.17 ± 0.16	3 ± 1
48b		2.57 ± 0.04	0.118 ± 0.003	-	-
51[g]		4.75 ± 0.02	0.438 ± 0.003	0.994 ± 0.043	28 ± 1
53a[g]		8.35 ± 0.08	0.718 ± 0.008	1.12 ± 0.22	11 ± 2

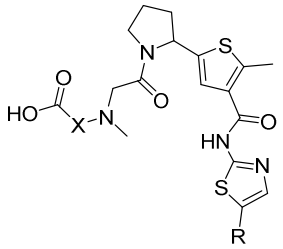
[a]Values are the mean ± SEM for n = 3. [b]Glucokinase activity at 10 μM relative to DMSO control. [c]Glucokinase activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [d]Insulin secretion activity at 10 μM relative to 10 μM of **1** as a

standard activator (DMSO:0; **1**:1). [e]Percentage of inhibition of hERG potassium channel at 30 μM. [f]Chiral isomer. [g]Hydrochloride salt.

Based on these findings, compound **53a** was chosen as the starting point for further optimization (Table 5). We prepared a series of compounds with a 5-F substituted thiazole ring and various tether lengths of the carboxylic acid. 5-F-substituted thiazole analog **53b** was as potent as its 5-Cl-substituted thiazole counterpart **53a** and showed no hERG binding affinity at 30 μM. However, **53b** showed low insulin secretion in MIN-6 cells. The compound whose tether part was extended from ethanoic acid to propanoic acid (**53c**) showed moderate GK activation potency, good MIN6 insulin secretion, and low hERG binding activity. A fine balance was observed between these parameters Compounds with a longer tether than **53c** (**53d** and **53e**) had favorable GK activation potency relative to **53c**, but unfortunately had relatively strong hERG binding affinity. Compounds **53a** and **53c** demonstrated favorable GK activation potency, insulin secretion, and low hERG binding affinity. As a result, the in vivo efficacy of **53a** and **53c** was evaluated by OGTT in normal C57BL/6j mice. Compounds were dosed orally at 30 mg/kg, 30 min prior to glucose loading. Unfortunately, only **53c** showed a significant glucose lowering effect. Incremental blood glucose $AUC_{0\text{-}120\ min}$ values of **53a** and **53c** relative to those of vehicle control were 82% and 47%, respectively. To investigate differences in the hypoglycemic effects of these compounds, plasma concentrations were determined following oral administration. Compound **53c** showed a more than six-fold higher plasma concentration than **53a** [36]. From these results, **53c** was considered the most promising compound in the series. The chiral isomer of this compound was synthesized and evaluated in vitro.

Table 5.

SAR for analogs of compound **53a**



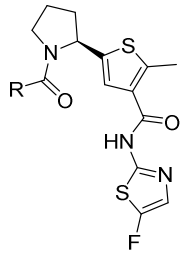
Compound[a]	R	X	Activation at 5 mM glucose[b]		Insulin secretion from MIN-6 cells (relative)[b,e]	hERG inhibition (%)[b,f]
			Fold[c]	Relative[d]		
53a	Cl	$-CH_2-$	8.35 ± 0.08	0.718 ± 0.008	1.12 ± 0.22	11 ± 2
53b	F	$-CH_2-$	6.92 ± 0.02	0.710 ± 0.003	0.379 ± 0.091	− 6 ± 2
53c	F	$-(CH_2)_2-$	4.89 ± 0.04	0.466 ± 0.005	1.19 ± 0.06	17 ± 3
53d	F	$-(CH_2)_3-$	6.86 ± 0.03	0.610 ± 0.003	1.21 ± 0.17	78 ± 2
53e	F	$-(CH_2)_4-$	6.95 ± 0.01	0.619 ± 0.001	0.807 ± 0.119	77 ± 1

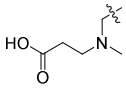
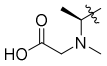
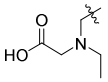
[a]Hydrochloride salt. [b]Values are the mean ± SEM for n = 3. [c]Glucokinase activity at 10 μM relative to DMSO control. [d]Glucokinase activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [e]Insulin secretion activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [f]Percentage of inhibition of hERG potassium channel at 30 μM.

As expected, the chiral isomer of **53c** (**58a**) showed favorable GK activation potency and promoted insulin secretion in MIN6 cells (Table 6). Unexpectedly, this compound showed a stronger hERG binding affinity (49% at 30 μM) relative to racemic compound **53c**. This suggested that the enantiomer of **58a** had a lower hERG binding affinity relative to **53c**, and that the carboxylic acid series proved to be highly sensitive to minor structural changes. Therefore, minor modification of **58a** could decrease hERG binding affinity. Thus, we designed and synthesized two types of ethanoic acid derivatives, **58b** and **59·HCl**. As expected, these derivatives showed good GK activation potency with low hERG binding. **59·HCl** demonstrated higher insulin secretion relative to **58b**, and was selected for further study.

Table 6.

GK activity, insulin secretion, and hERG binding of chiral analogs



Compound[a]	R	Activation at 5 mM glucose[b]		Insulin secretion from MIN-6 cells (relative)[b,e]	hERG inhibition (%)[b,f]
		Fold[c]	Relative[d]		
58a		7.26 ± 0.05	0.686 ± 0.005	0.930 ± 0.017	49 ± 1
58b		8.80 ± 0.15	0.832 ± 0.016	0.436 ± 0.039	4 ± 0
59·HCl		8.60 ± 0.10	0.870 ± 0.011	0.830 ± 0.165	− 1 ± 1

[a]Hydrochloride salt. [b]Values are the mean ± SEM for n = 3. [c]Glucokinase activity at 10 μM relative to DMSO control. [d]Glucokinase activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [e]Insulin secretion activity at 10 μM relative to 10 μM of **1** as a standard activator (DMSO:0; **1**:1). [f]Percentage of hERG potassium channel inhibition at 30 μM.

Based on these encouraging results, **59·HCl** was evaluated for its glucose-lowering effect in rodents via an OGTT study in normal C57BL/6J and diabetic ob/ob mice **59·HCl** induced a significant reduction in blood glucose excursion in a dose-dependent manner in both C57BL/6J and ob/ob mice, and showed a satisfactory PK profile (Figures 5 and 6; Table 7). Moreover, in a 4 week repeated administration study, **59·HCl** decreased blood glucose levels on day 0, 14, and 28 in ob/ob mice (Figure 7). Compound **59** also showed low toxicity in a cell toxicity assay using Chinese hamster ovary cells and rat hepatocyte cells (CC_{50} >100 μM), and showed no significant

inhibition of the hERG channel in patch clamp experiments using human embryo kidney 293 cells (IC_{50} > 300 μM) (Table 8).

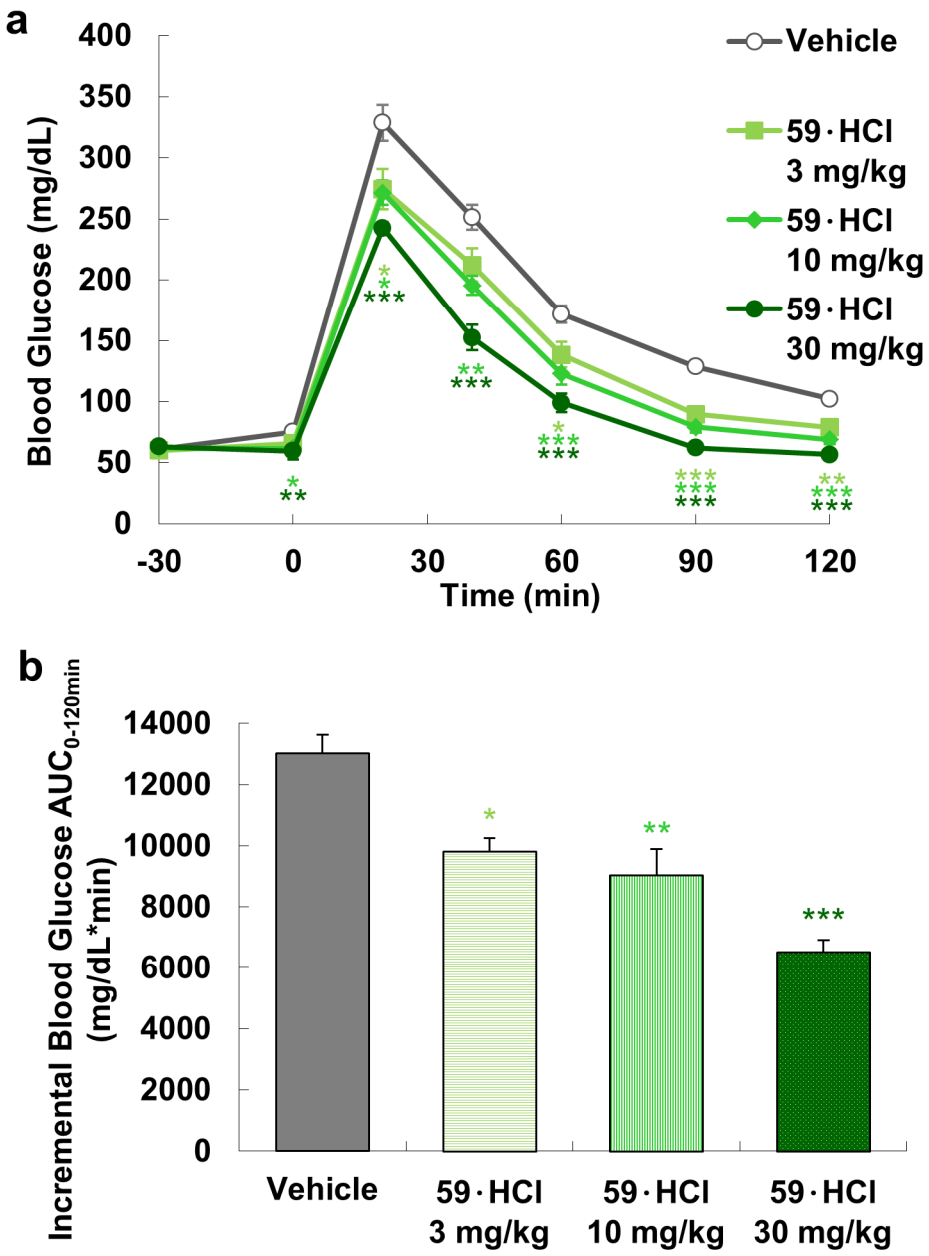


Figure 5. Oral glucose tolerance test (OGTT) for **59·HCl** in male C57BL/6j mice. Effect on (a) blood glucose level and (b) incremental blood glucose AUC during OGTT. Data are expressed as (a) mean ± SEM (n = 8) and (b) mean + SEM (n = 8). The data was analyzed using Dunnett's multiple comparison test. *, **, ***: $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. vehicle control.

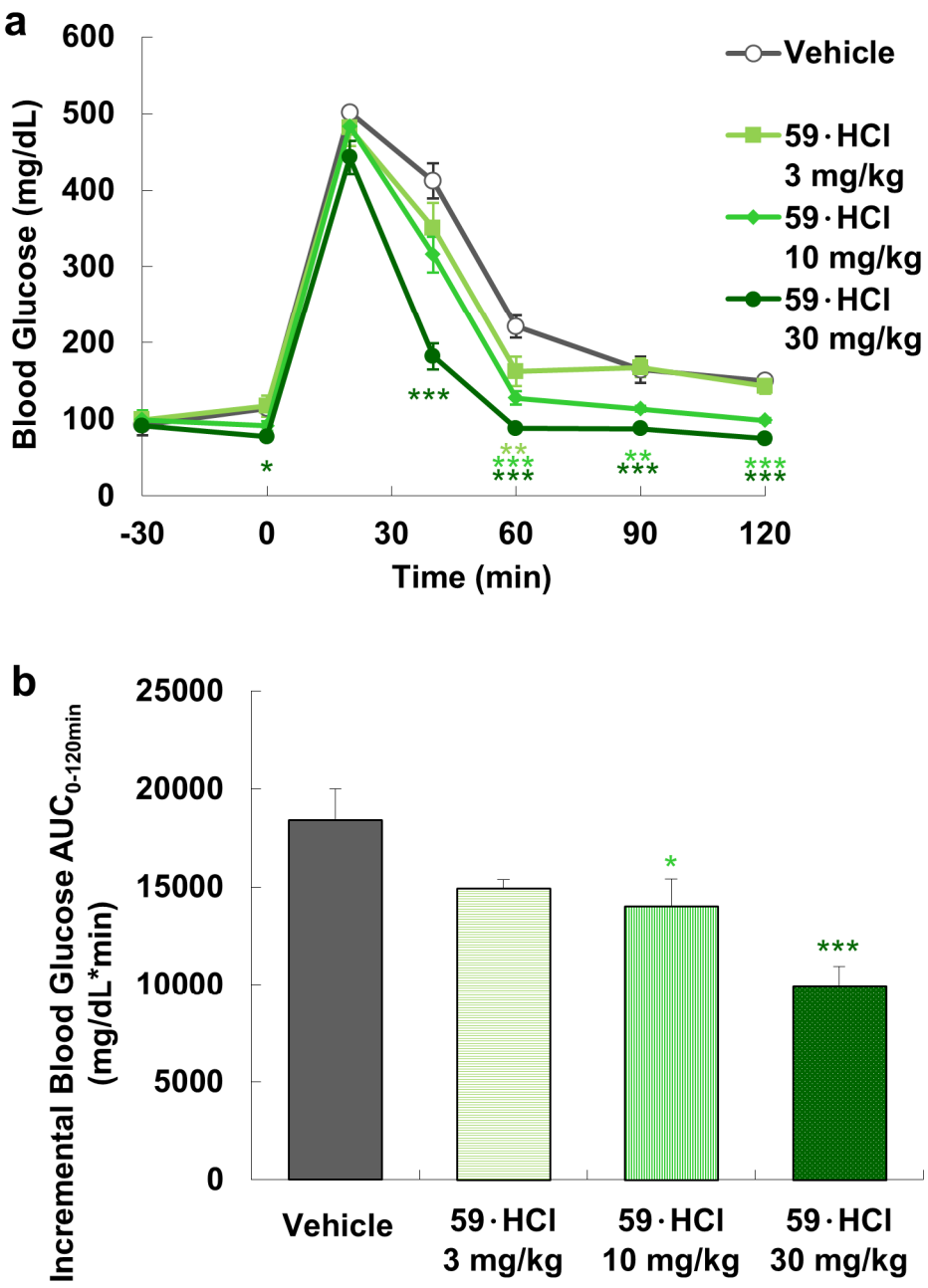


Figure 6. Oral glucose tolerance test (OGTT) for **59·HCl** in male ob/ob mice. Effect on (a) blood glucose level and (b) incremental blood glucose AUC during OGTT. Data are expressed as (a) mean ± SEM (n = 8) and (b) mean + SEM (n = 8). The data was analyzed using Dunnett's multiple comparison test. *, **, ***: $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. vehicle control.

Table 7. Pharmacokinetic parameters of **59** in male ICR mice

Dose (mg/kg)	Route	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-inf} (ng·h/mL)	CL_p (mL/h/kg)	V_{dss} (mL/kg)	$T_{1/2}$(h)		BA (%)
							α	β	
2	IV			8580	233	310	0.47	1.6	
10	PO	3020	0.25	8600				2.0	20.0

Table 8. Toxicological profiles of **59**

	Cell toxicity		hERG patch
	CHO-K1	rat hepatocyte	clamp
59	CC_{50} > 100 μM	CC_{50} > 100 μM	IC_{50} > 300 μM

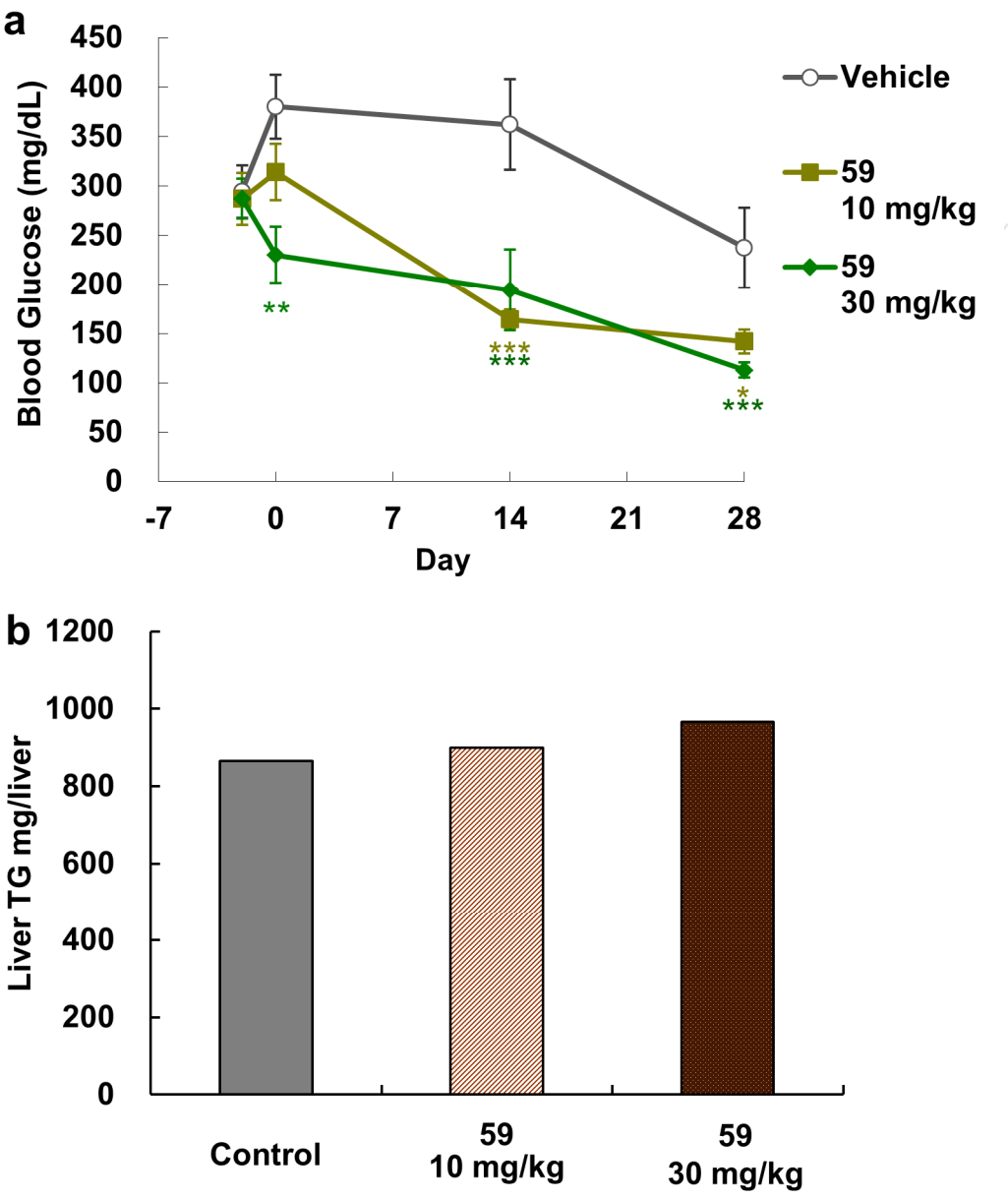


Figure 7. Repeated administration of **59** for 28 days in glucose intolerant male ob/ob mice. Compound **59** was administered orally twice daily for 28 days to male ob/ob mice. Effect on (a) blood glucose level and (b) liver TG level after 28 days treatment. Data are expressed as (a) mean ± SEM (*n* = 10) and (b) mean + SEM (*n* = 10). The data was analyzed using Dunnett's multiple comparison test. *, **, ***: $p < 0.05$, $p < 0.01$, $p < 0.001$ vs. vehicle control.

To characterize the plasma glucose lowering effect of compound **59**, a comparative study was performed with compound **2** (piragliatin) using normal mice (Figure 8). Compound **2** is a clinical drug candidate, which has progressed to Phase II clinical trial. Although development was terminated at Phase II due to an unfavorable risk-benefit ratio, beneficial information on this compound is available. Compound **2** exhibits activity in both pancreatic β-cells and hepatocytes and demonstrates a glucose-lowering effect in humans. The administration of 100 mg of **2** was associated with a higher incidence of patients requiring glucose infusion to prevent hypoglycemia [19]. In our study, **59** and **2** decreased plasma glucose levels dose-dependently, and **59** showed almost the same efficacy as **2** at a dose of 10 mg/kg. Compound **2** was associated with hypoglycemia at a dose of 30 mg/kg, but **59** did not cause hypoglycemia at doses less than 100 mg/kg. These data suggest that **59** offers a superior therapeutic window compared with that of **2**. To investigate the differences between **59** and **2**, the effects on increasing plasma insulin and tissue distribution of these compounds were evaluated (Figure 9, Table 9). The insulin secretion of **2** at 30 mg/kg was higher than that of **59** at 100 mg/kg. Compound **59** showed a characteristic pattern of distribution (14–23-fold liver to pancreas ratio of tissue distribution) compared with that of **2** (2.9–3.1-fold liver to pancreas ratio of tissue distribution). These results indicate that one of the causes of differences in hypoglycemic risk is the difference in insulin secretion between these compounds, which is thought to derive from their different tissue distribution patterns.

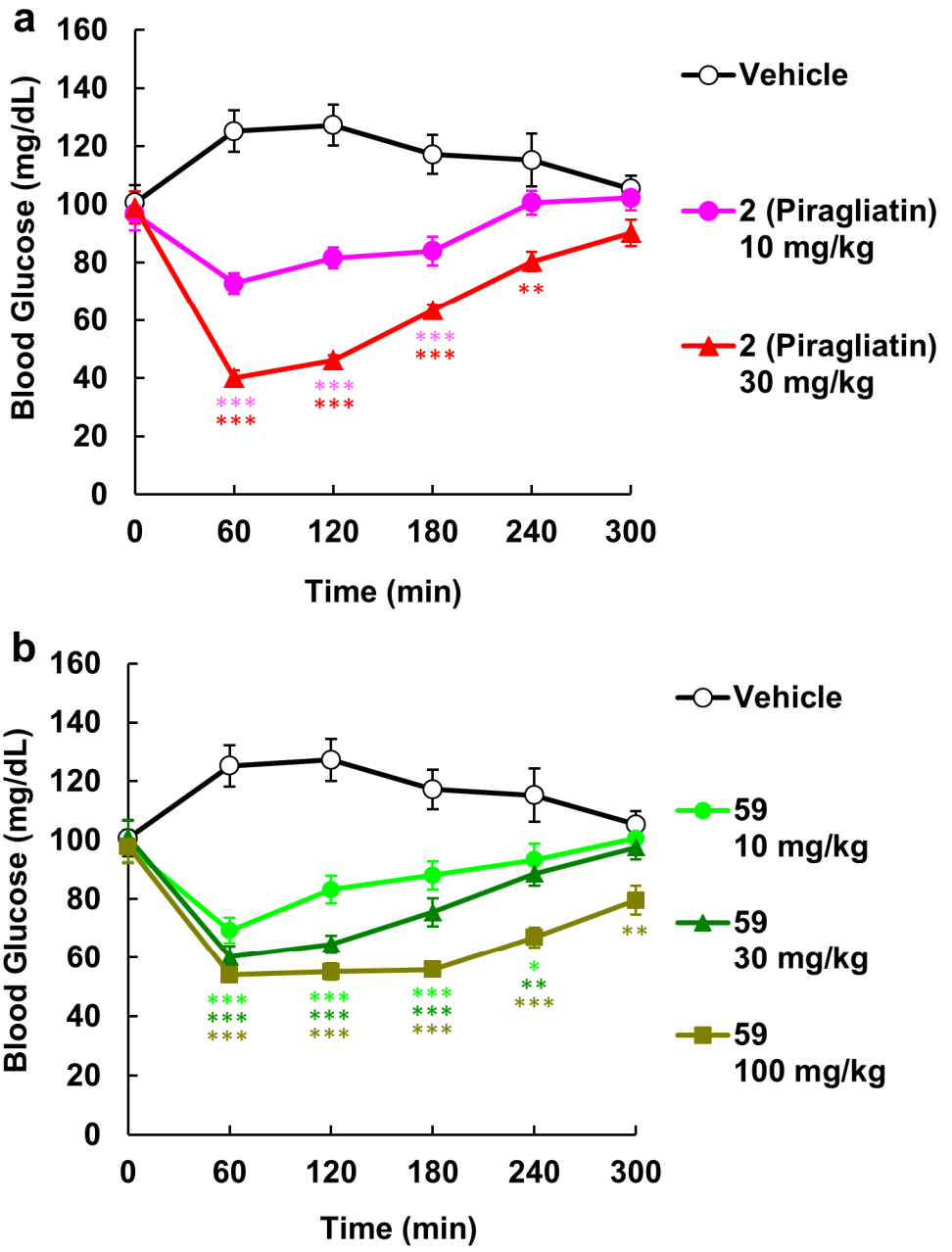


Figure 8. Evaluation of hypoglycemic risk of **2** and **59**. Compounds were administered to 2 h-fasted male C57BL/6j mice. Data are expressed as mean ± SEM (*n* = 8). The data was analyzed using Dunnett's multiple comparison test. *, **, ***: P < 0.05, P < 0.01, P < 0.001 vs. vehicle control. Hypoglycemia was defined as plasma glucose level of < 60 mg/dL.

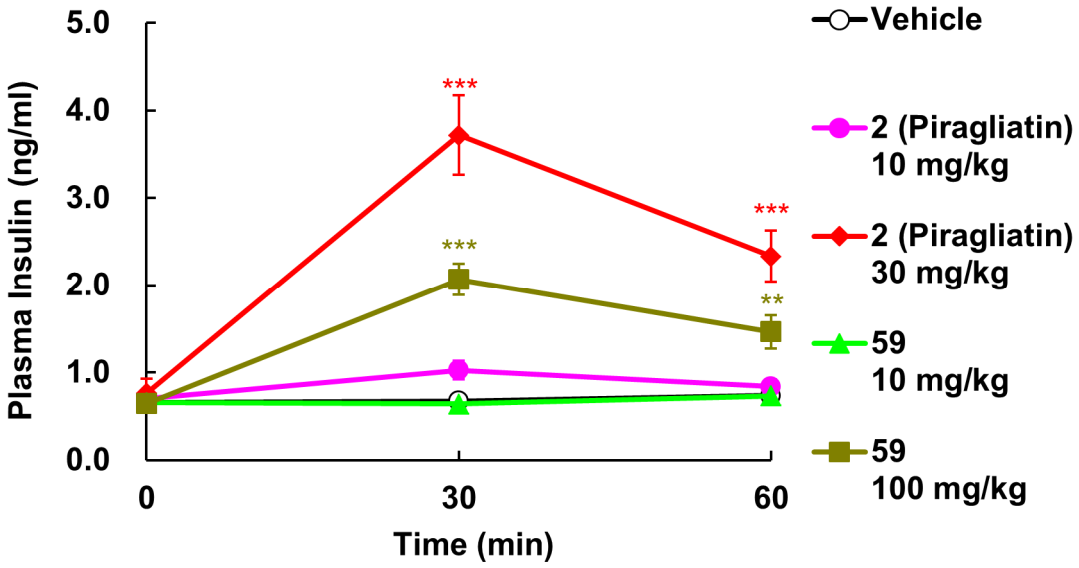


Figure 9. Effects of **2** and **59** on plasma insulin level in male C57BL/6j mice. Compounds were administered to 2 h-fasted male C57BL/6j mice. Data are expressed as mean ± SEM (n = 8). The data was analyzed using Dunnett's multiple comparison test. **, ***: $P < 0.01$, $P < 0.001$.

Table 9. Tissue distribution after a single oral administration of **2** and **59** in male C57BL/6j mice

	Dose	$AUC_{0–1.5h}$ (ng·h/mL or ng·h/g) (K_p)[a]			L/P
	(mg/kg)	Plasma	Liver	Pancreas	ratio[b]
59	10	6750 ± 2200	8910 ± 865 (1.3)	433 ± 75 (0.064)	20.6
	30	17700 ± 4770	32300 ± 7670 (1.6)	1430 ± 599 (0.081)	22.5
	100	86900 ± 4290	148000 ± 24300 (1.7)	10500 ± 577 (0.12)	14.0
2 (Piragliatin)	5	351 ± 70	1470 ± 159 (4.2)	505 ± 94 (1.4)	2.9
	10	574 ± 83	3230 ± 270 (5.6)	1020 ± 37 (1.8)	3.2
	30	2330 ± 78	15700 ± 1210 (6.8)	5030 ± 229 (2.2)	3.1

[a]AUC was expressed as mean ± SD (n = 3). K_p value was calculated using the AUC. ($K_p = AUC_{tissue}/AUC_{plasma}$). [b]L/P ratio; $AUC_{liver}/AUC_{pancreas}$ ratio.

The hepato-selective GKA **59** not only a lowered the risk of hypoglycemia, but also had promising characteristics for development as a therapy for T2DM. Compound **59** has a low distribution volume (V_{dss} is 310 mL/kg), and K_p values of approximately 1.5 and 0.1 in liver and pancreas, respectively. To the best of our knowledge, these values are markedly lower than those of other reported GKAs. Existing hepato-specific GKAs use organic anion transporter (OATP) proteins (OATP1B1, OATP1B3, and OATP2B1) that expressed predominantly in hepatocytes [18, 37]. The use of OATPs is a superior approach to ensure hepato-selective distribution for small molecules, but OATP substrates are frequently involved in drug-drug interactions (DDIs) [38], which could limit the use of OATP specific GKAs in the treatment of chronic disease. Based on the low K_p value in the liver, **59** is not considered a substrate of OATPs. Hepato-selectivity of **59** derives from an extremely low pancreatic distribution. Therefore, **59** has a lower risk of DDI than liver-specific GKAs. Furthermore, in a clinical study of GKAs, increased plasma TG was reported [16]. Although the mechanism of this increase is not fully understood, overstimulation of hepatic GK could be the cause of this adverse effect, considering the experimental findings in transgenic mice with high GK activity [39]. Compound **59** is distributed not only to hepatocytes but also to pancreatic β-cells while its plasma concentration is high, and exerts its hypoglycemic effects through the activation of GK in both the pancreas and liver. Therefore, the risk of overstimulation of hepatic GK by **59** at therapeutic doses is lower than that of other hepato-specific GKAs. Compound **59** did not affect hepatic TG levels in our study in ob/ob mice (Figure 7).

Loss of GKA efficacy within several months has also been reported in clinical trials [16, 40]. This is a significant limitation in the development of GKAs for T2DM. Although the cause of this effect has not been clarified, it has been reported that genetic activation of GK causes apoptosis in pancreatic β-cells in animal studies [41]. Therefore, over-activation of pancreatic GKA for long periods could be a cause of the transient effects of GKAs observed in clinical trials. Compound **59** mainly activates GK in the liver, and activates GK in the pancreas only at high plasma concentration. Therefore, the risk of overstimulating pancreatic β-cells is lower than for other systems (i.e., liver- and pancreas-acting). In our study in ob/ob mice, tachyphylaxis was not observed (Figure 7). Thus, **59** has the potential to overcome the limitations of other GKAs. Therefore, we selected **59** as a candidate for further clinical studies.

The optimum profile of **59** as a GKA derives from its high polarity (log *D* = 0.023 at pH 7.4) [27]. The introduction of a zwitterion characteristic to the molecule, along with

good physical properties, represents an effective approach to the discovery of novel GKAs.

3. Conclusions

To identify an orally active, structurally unique GKA, conversion of the phenyl group of water-insoluble compound **7** led to the discovery of the pyrrolidinyl-thienyl derivative **38**. This compound had favorable solubility and in vivo efficacy in a mouse model for OGTT. Unfortunately, **38** had a high binding affinity to hERG potassium channels. The optimization of **38** using a zwitterion approach led to the discovery of novel GKA **59**. This compound produced a significant reduction in blood glucose levels in normal and diabetic mice, and had a benign safety profile in toxicological studies. Due to favorable in vivo properties and low pancreatic distribution, **59** exhibited a low risk of hypoglycemia as well as properties unique among other reported GKAs. These results prompted us to select **59** as our clinical candidate.

4. Experimental Section

4.1. Chemistry

4.1.1. General

All reagents and solvents were commercially available and used without further purification. Flash column chromatography was performed using silica gel 60 (particle size 0.040–0.050 mm, Kanto Kagaku) or Ultra Pack Columns (silica gel, particle size 0.040 mm, Yamazen Corporation) unless otherwise stated. ^{1}H NMR and ^{13}C NMR were recorded using a JEOL-ECP400 or Varian-400MR spectrometer in solvent as indicated. Data are reported as follows: chemical shift in ppm (δ) relative to tetramethylsilane, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = doublet of doublets, td = triplet of doublets, br = broad, m = multiplet), coupling constant (Hz), integration. Infrared spectra (IR) were recorded on a JASCO FT/IR-4200 spectrometer with a single reflection diamond ATR unit. LC/MS spectra were determined on a Waters ZMD2000 equipped with a Waters 2690 injector and a PDA detector operating at 210–400 nm and interfaced with a Micromass ZMD mass spectrometer or Waters SQD equipped with an Acquity ultra performance liquid chromatography (UPLC) system.

High-resolution mass spectra (HRMS) were obtained using a Thermo LTQ Orbitrap. Melting points were determined using a Yanaco MP-J micro melting point apparatus and are given as uncorrected values. The specific rotation was measured on a Rudolph AUTOPOL IV polarimeter. The purity of the test compounds was determined by UPLC analysis using an Acquity BEH C18 column (1.7 μm, 2.1 × 50 mm) with UV detection at 290 and 300 nm (5 min; 0.4 mL/min flow rate) and elution with binary solvent systems A and B using a gradient elution (A, 0.1% [v/v] HCOOH aqueous solution; B, 0.1% [v/v] HCOOH in MeCN). All tested compounds were confirmed to be ≥ 95% purity via this method.

4.1.2. 2-Methylthiophene-3-carboxylic acid (9).

Compound **9** was synthesized as reported previously [24]. To a solution of diisopropylamine (233 g, 2.30 mol) in THF (2.3 L), a solution of *n*-BuLi (1.6 M in *n*-hexane, 1.50 L, 2.40 mol) was added dropwise at 0 °C. After stirring at the same temperature for 40 min, the reaction mixture was cooled to −60 °C, compound **8** (223 g, 1.74 mol) in THF (500 mL) added dropwise, and stirred at the same temperature for 1 h. After the addition of MeI (254 g, 1.79 mol), the reaction mixture was allowed to warm to room temperature and stirred for 1 h. Next, the mixture was concentrated under reduced pressure, acidified with 6 N aqueous HCl (to pH 1), and extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was crystallized with H_2O/AcOH to give 209 g (85%) of **9** as a pale-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 5.4 Hz, 1H), 7.01 (d, *J* = 5.4 Hz, 1H), 2.78 (s, 3H); MS (ESI) *m/z*: 143 $(M+H)^+$, 141 $(M-H)^-$.

4.1.3. Methyl 2-methylthiophene-3-carboxylate (10).

Thionyl chloride (200 mL, 2.76 mol) was slowly added dropwise to a solution of **9** (100 g, 703 mmol) in MeOH (500 mL). After stirring at reflux for 3 h, the mixture was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2, washed with water, saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure to give 105 g (96%) of

10 as a brown oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 5.4 Hz, 1H), 6.98 (d, *J* = 5.4 Hz, 1H), 3.85 (s, 3H), 2.74 (s, 3H); MS (ESI) *m/z*: 157 $(M+H)^+$.

4.1.4. Methyl 5-bromo-2-methylthiophene-3-carboxylate (11).

To a solution of **10** (156 g, 999 mmol) in DMF (750 mL), NBS (178 g, 1.00 mol) was added at room temperature and stirred overnight. The reaction mixture was diluted with water and extracted with *n*-hexane. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure to give 226 g (96%) of **11** as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 3.83 (s, 3H), 2.67 (s, 3H).

4.1.5. Methyl 5-(2-formylphenyl)-2-methylthiophene-3-carboxylate (12).

A mixture of **11** (100 mg, 0.425 mmol), 2-formylphenylboronic acid (83.0 mg, 0.533 mmol), $Pd(PPh_3)_4$ (24.6 mg, 0.0213 mmol), and K_2CO_3 (176 mg, 1.27 mmol) in DME (650 μL) was stirred at 85 °C for 1.5 h. After cooling to room temperature, the reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was diluted with AcOEt, washed with water and brine, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by preparative TLC on silica gel (AcOEt/*n*-hexane = 1/5) to give 82.0 mg (74%) of **12** as a pale-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.23 (s, 1H), 8.01 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.65–7.59 (m, 1H), 7.53–7.47 (m, 2H), 7.35 (s, 1H), 3.87 (s, 3H), 2.80 (s, 3H); MS (ESI) *m/z*: 261 $(M+H)^+$.

4.1.6. 2-(4-(Methoxycarbonyl)-5-methylthiophen-2-yl)benzoic acid (13).

To a solution of **12** (50.0 mg, 0.192 mmol) and 2-methyl-2-butene (244 μL, 2.30 mmol) in *tert*-BuOH (500 μL), $NaClO_2$ (138 mg, 1.53 mmol) and Na_2HPO_4 (327 mg, 2.30 mmol) in water (500 μL) were added at room temperature. After stirring at the same temperature for 2.5 days, 1 N aqueous HCl was added and the mixture was extracted with AcOEt. The organic layer was washed with H_2O and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue

was triturated with Et_2O/*n*-hexane to give 35.8 mg (68%) of **13** as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95–7.89 (m, 1H), 7.57–7.51 (m, 1H), 7.48–7.40 (m, 2H), 7.35 (s, 1H), 3.83 (s, 3H), 2.75 (s, 3H); MS (ESI) *m/z*: 277 $(M+H)^+$, 275 $(M-H)^-$.

4.1.7. (Methyl 5-(2-((2-hydroxyethyl)carbamoyl)phenyl)-2-methylthiophene-3-carboxylate (14).

To a solution of **13** (216 mg, 0.782 mmol) and 2-aminoethanol hydrochloride (152 mg, 1.56 mmol) in DMF, EDCI (299 mg, 1.56 mmol), HOBt (211 mg, 1.56 mmol), and DIPEA (403 μL, 2.35 mmol) were added. After stirring at room temperature for 2 h, the reaction mixture was diluted with AcOEt and washed with 1 N aqueous HCl, water, and brine, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography ($CHCl_3$/MeOH = 40/1 to 30/1) to give 240 mg (96%) of **14** as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57–7.52 (m, 1H), 7.49 (s, 1H), 7.45–7.34 (m, 3H), 6.19–6.05 (m, 1H), 3.86 (s, 3H), 3.77–3.66 (m, 2H), 3.53–3.43 (m, 2H), 2.74 (s, 3H), 2.54 (br s, 1H); MS (ESI) *m/z*: 320 $(M+H)^+$, 318 $(M-H)^-$.

4.1.8. 5-(2-((2-Hydroxyethyl)carbamoyl)phenyl)-2-methylthiophene-3-carboxylic acid (15).

To a solution of **14** (226 mg, 0.708 mmol) in MeOH (2.00 mL) and THF (2.00 mL), 5 N aqueous NaOH (708 μL, 3.54 mmol) was added. After stirring at 50 °C overnight, the reaction mixture was diluted with water and washed with Et_2O. The resulting aqueous solution was acidified with 1 N aqueous HCl and extracted with $CHCl_3$ and the organic layer was dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure to give 87.6 mg (41%) of **15** as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (br s, 1H), 8.34 (t, *J* = 5.7 Hz, 1H), 7.50–7.43 (m, 2H), 7.42–7.34 (m, 3H), 4.66–4.57 (m, 1H), 3.45–3.38 (m, 2H), 3.22 (q, *J* = 6.4 Hz, 2H), 2.68 (s, 3H); MS (ESI) *m/z*: 306 $(M+H)^+$, 304 $(M-H)^-$.

4.1.9. *N*-(5,6-Dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-5-(2-((2-hydroxyethyl)carbamoyl)phenyl)-2-methylthiophene-3-carboxamide (16).

A mixture of **15** (270 mg, 0.884 mmol), 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine (248 mg, 1.77 mmol), EDCI (339 mg, 1.77 mmol), HOBt (271 mg, 1.77 mmol), and DIPEA (303 μL, 1.77 mmol) in DMF (2.00 mL) was stirred at room temperature for 1 h and then stirred at 50 °C for 13 h. After cooling to room temperature, the reaction mixture was diluted with AcOEt, washed with 1 N aqueous HCl, water, saturated aqueous $NaHCO_3$, and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography (toluene/AcOEt = 1/4 to 1/5) to give 148 mg (39%) of **16** as a pale-yellow foam: t_R = 2.73 min (UPLC purity: 98.4%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.61–7.32 (m, 5H), 3.65 (t, *J* = 5.7 Hz, 2H), 3.43 (t, *J* = 5.7 Hz, 2H), 2.90 (t, *J* = 7.1 Hz, 2H), 2.81–2.71 (m, 5H), 2.59–2.42 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ173.0, 163.6, 163.3, 156.7, 149.3, 139.1, 137.7, 132.5, 131.8, 131.2, 131.1, 129.6, 129.3, 129.0, 126.9, 61.4, 43.5, 28.5, 28.3, 27.4, 15.1; IR (ATR) 1634, 1525 cm^{-1}; MS (ESI) *m/z*: 428 $(M+H)^+$, 426 $(M-H)^-$; HRMS calcd for $C_{21}H_{21}N_3O_3S_2$ $(M+H)^+$ 428.1097, found 428.1093.

4.1.10. *N*-(5,6-Dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-5-(2-((2-iodoethyl)carbamoyl)phenyl)-2-methylthiophene-3-carboxamide (17).

To a solution of **16** (148 mg, 0.346 mmol) in THF (5.50 mL), PPh_3 (182 mg, 0.692 mmol), imidazole (47.1 mg, 0.692 mmol), and I_2 (176 mg, 0.692 mmol) were added at 0 °C and stirred at the same temperature for 40 min. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with saturated aqueous $Na_2S_2O_3$, water, and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography (AcOEt/*n*-hexane = 3/2) to give 160 mg (86%) of **17** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (br s, 1H), 7.58 (dd, *J* = 7.3, 1.6 Hz, 1H), 7.50–7.36 (m, 3H), 7.31 (s, 1H), 6.01 (t, *J* = 5.6 Hz, 1H), 3.70 (q, *J* = 6.1 Hz, 2H), 3.25 (t, *J* = 6.1 Hz, 2H), 2.87 (t, *J* = 7.1 Hz, 2H), 2.81 (s, 3H), 2.66 (t, *J* = 7.2 Hz, 2H), 2.41 (quint, *J* = 7.2 Hz, 2H); MS (ESI) *m/z*: 538 $(M+H)^+$, 536 $(M-H)^-$.

4.1.11. *N*-(5,6-Dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-5-(2-((2-(dimethylamino)ethyl)carbamoyl)phenyl)-2-methylthiophene-3-carboxamide (18).

To a solution of **17** (22.6 mg, 0.0421 mmol) in THF (500 μL), 2.0 M solution of Me_2NH in THF (210 μL, 0.421 mmol) was added at room temperature. After stirring at the same temperature for 2.5 days, the reaction mixture was concentrated under reduced pressure and the residue was purified by preparative TLC on silica gel ($CHCl_3$/MeOH = 8/1) to give 13.7 mg (72%) of **18** as a white foam: t_R = 2.08 min (UPLC purity: 96.7%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.49 (m, 1H), 7.44–7.34 (m, 2H), 7.33–7.25 (m, 2H), 6.43 (t, *J* = 4.6 Hz, 1H), 3.39 (q, *J* = 5.5 Hz, 2H), 2.85 (t, *J* = 7.1 Hz, 2H), 2.80 (s, 3H), 2.59 (t, *J* = 7.1 Hz, 2H), 2.43–2.27 (m, 4H), 2.09 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.3, 161.4, 161.0, 155.5, 149.0, 138.1, 136.4, 131.0, 130.2, 129.9 (2C), 128.4 (2C), 128.3, 125.4, 57.4, 44.8 (2C), 37.2, 27.4, 27.3, 26.7, 15.3; IR (ATR); 1658, 1642, 1548, 1523 cm^{-1}; MS (ESI) *m/z*: 455 $(M+H)^+$, 453 $(M-H)^-$; HRMS (ESI) calcd for $C_{23}H_{26}N_4O_2S_2$ $(M+H)^+$ 455.1570, found 455.1567.

4.1.12. 5-(2-((2-Cyanoethyl)carbamoyl)phenyl)-*N*-(5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methylthiophene-3-carboxamide (19).

To a solution of **17** (160 mg, 0.298 mmol) in DMSO (2.70 mL), NaCN (29.2 mg, 0.596 mmol) was added at room temperature and stirred at the same temperature for 4 h. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with water and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography ($CHCl_3$/MeOH = 80/1 to 70/1) to give 27.0 mg (21%) of **19** as a pale-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60–7.55 (m, 1H), 7.49–7.33 (m, 4H), 6.26–6.15 (m, 1H), 3.56 (q, *J* = 6.3 Hz, 2H), 2.85 (t, *J* = 7.0 Hz, 2H), 2.80 (s, 3H), 2.76 (s, 1H), 2.68–2.59 (m, 4H), 2.40 (quint, *J* = 7.2 Hz, 2H); MS (ESI) *m/z*: 437 $(M+H)^+$, 435 $(M-H)^-$.

4.1.13. 5-(2-((2-(1*H*-Tetrazol-5-yl)ethyl)carbamoyl)phenyl)-*N*-(5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methylthiophene-3-carboxamide (20).

To a solution of **19** (60.2 mg, 0.138 mmol) in toluene (900 μL), Bu_3SnN_3 (190 μL, 0.690 mmol) was added at room temperature. After stirring at 130 °C for 5 h, the

reaction mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The resulting residue was purified by flash column chromatography (10% w/w anhydrous K_2CO_3-silica; $CHCl_3$/MeOH = 1/0 to 9/1) to give 32.0 mg (48%) of **20** as a pale-yellow solid: mp 254–256 °C; t_R = 2.71 min (UPLC purity: 99.7%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.16 (br s, 1H), 8.61 (t, *J* = 5.6 Hz, 1H), 7.78 (s, 1H), 7.61–7.56 (m, 1H), 7.51 (td, *J* = 7.5, 1.5 Hz, 1H), 7.42 (td, *J* = 7.4, 1.2 Hz, 1H), 7.35 (dd, *J* = 7.6, 1.3 Hz, 1H), 3.57 (q, *J* = 6.6 Hz, 2H), 3.11 (t, *J* = 7.1 Hz, 2H), 2.85 (t, *J* = 7.0 Hz, 2H), 2.74–2.65 (m, 5H), 2.45–2.35 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.8, 161.2, 161.0, 155.1, 154.0, 147.5, 136.6, 136.2, 130.7, 129.8, 129.5, 129.2, 127.8, 127.6, 126.9, 126.5, 37.2, 27.0, 26.7, 26.1, 23.0, 14.8; IR (ATR) 1636, 1524 cm^{-1}; MS (ESI) *m/z*: 480 $(M+H)^+$, 478 $(M-H)^-$; HRMS (ESI) calcd for $C_{22}H_{21}N_7O_2S_2$ $(M+H)^+$ 480.1271, found 480.1266.

4.1.14. 5-(2-((3-Amino-3-oxopropyl)carbamoyl)phenyl)-*N*-(5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methylthiophene-3-carboxamide (21).

To a solution of **19** (10.9 mg, 0.0250 mmol) in EtOH (600 μL) and water (300 μL) $[PtH(PMe_2OH)(PMe_2O)_2H]$ (2.00 mg, 0.00464 mmol) was added at room temperature and refluxed for 19 h. The reaction mixture was concentrated under reduced pressure and the residue was washed with $CHCl_3$ to give 6.20 mg (55%) of **21** as a white solid: mp 246–247 °C; t_R = 2.63 min (UPLC purity: 96.3%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 8.42 (t, *J* = 5.6 Hz, 1H), 7.78 (s, 1H), 7.60–7.55 (m, 1H), 7.50 (td, *J* = 7.5, 1.6 Hz, 1H), 7.44–7.33 (m, 2H), 6.99 (s, 1H), 3.43–3.28 (m, 2H), 2.85 (t, *J* = 7.0 Hz, 2H), 2.76–2.65 (m, 5H), 2.46–2.36 (m, 2H), 2.33 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.7, 168.5, 161.2, 155.2, 155.0, 147.5, 136.7, 136.6, 130.6, 129.8, 129.3, 129.2, 127.8, 127.6, 126.9, 126.3, 35.7, 34.3, 27.0, 26.7, 26.1, 14.9; IR (ATR); 1668, 1638, 1556 cm^{-1}; MS (ESI) *m/z*: 455 $(M+H)^+$, 453 $(M-H)^-$; HRMS (ESI) calcd for $C_{22}H_{22}N_4O_3S_2$ $(M+H)^+$ 455.1206, found 455.1204.

4.1.15. Methyl 5-(4-((*tert*-butoxycarbonyl)amino)butanoyl)-2-methylthiophene-3-carboxylate (22a).

To a solution of **11** (40.1 g, 170 mmol) in THF (150 mL), a solution of *i*-PrMgBr (0.78 M in THF, 229 mL, 179 mmol) was added dropwise at −40 °C and stirred at the same temperature for 1.5 h. Next, a solution of 1-*tert*-butoxylcarbonyl-2-pyrrolidinone (31.6 g, 170 mmol) in THF (180 mL) was added dropwise at −40 °C. After stirring at the same temperature for 1.5 h and then stirring at room temperature for 1 h, the reaction was quenched with saturated aqueous NH_4Cl. The reaction mixture was extracted with AcOEt, and organic layer was washed with water and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was recrystallized with AcOEt/*n*-hexane to give 29.5 g (51%) of **22a** as a pale-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 4.64 (br s, 1H), 3.87 (s, 3H), 3.21 (q, *J* = 6.3 Hz, 2H), 2.92 (t, *J* = 7.2 Hz, 2H), 2.78 (s, 3H), 1.92 (quint, *J* = 7.0 Hz, 2H), 1.43 (s, 9H); MS (ESI) *m/z*: 340 $(M-H)^-$.

4.1.16. Methyl 5-(5-((*tert*-butoxycarbonyl)amino)pentanoyl)-2-methylthiophene-3-carboxylate (22b).

Compound **22b** was prepared from **11** using the same procedure as described for **22a** to yield a yellow oil (yield 62%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 4.57 (br s, 1H), 3.88 (s, 3H), 3.21–3.08 (m, 2H), 2.89 (t, *J* = 7.3 Hz, 2H), 2.78 (s, 3H), 1.76 (quint, *J* = 7.5 Hz, 2H), 1.70–1.38 (m, 11H); MS (ESI) *m/z*: 356 $(M+H)^+$.

4.1.17. Methyl 2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxylate (23a).

To a solution of **22a** (29.5 g, 86.4 mmol) in CH_2Cl_2 (120 mL), trifluoroacetic acid (17.1 mL, 216 mmol) was added dropwise at 0 °C and stirred at room temperature for 3 h. The mixture was then concentrated under reduced pressure, diluted with $CHCl_3$, and washed with saturated aqueous $NaHCO_3$. The organic layer was separated and dried over Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure and the residue was dissolved in *i*-PrOH (450 mL). Conc. HCl (45.0 mL) and $NaBH_3CN$ (10.9 g 173 mmol) were added slowly to the mixture at 0 °C and stirred at the same temperature for 5 h. Next, the reaction mixture was basified with 50% aqueous NaOH to pH 9, evaporated to remove *i*-PrOH and extracted with $CHCl_3$. The organic layer was washed with brine and dried over Na_2SO_4. After filtration, the solvent was concentrated

under reduced pressure and the residue was purified by flash column chromatography ($CHCl_3$/MeOH = 9/1 to 4/1) to give 13.6 g (70%) of **23a** as a pale-yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 4.32 (t, *J* = 7.1 Hz, 1H), 3.82 (s, 3H), 3.19–3.09 (m, 1H), 3.06–2.96 (m, 1H), 2.68 (s, 3H), 2.25–2.12 (m, 1H), 2.01–1.72 (m, 3H); MS (ESI) *m/z*: 226 $(M+H)^+$.

4.1.18. Methyl 2-methyl-5-(piperidin-2-yl)thiophene-3-carboxylate (23b).

Compound **23b** was prepared from **22b** using the same procedure as described for **23a** to yield a colorless oil (yield 63%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (s, 1H), 3.85–3.76 (m, 4H), 3.19–3.12 (m, 1H), 2.82–2.72 (m, 1H), 2.68 (s, 3H), 1.97–1.81 (m, 2H), 1.57–1.40 (m, 4H); MS (ESI) *m/z*: 240 $(M+H)^+$.

4.1.19. Methyl 5-(1-acetylpyrrolidin-2-yl)-2-methylthiophene-3-carboxylate (24a).

To a mixture of **23a** (50.0 mg, 0.222 mmol) and pyridine (53.6 μL, 0.666 mmol) in $CHCl_3$ (1.00 mL), acetyl chloride (47.3 μL, 0.666 mmol) was added at 0 °C and stirred at room temperature for 30 min. The reaction mixture was diluted with $CHCl_3$, washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography (AcOEt/*n*-hexane = 3/1 to 9/1) to give 58.0 mg (98%) of **24a** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (s, 1H), 5.02 (d, *J* = 7.7 Hz, 1H), 3.83 (s, 3H), 3.74–3.45 (m, 2H), 2.69 (s, 3H), 2.37–1.91 (m, 7H); MS (ESI) *m/z*: 268 $(M+H)^+$.

4.1.20. Methyl 5-(1-acetylpiperidin-2-yl)-2-methylthiophene-3-carboxylate (24b).

Compound **24b** was prepared from **23b** using the same procedure as described for **24a** to yield a colorless oil (yield 85%): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (s, 1H), 6.03 (br s, 1H), 5.16 (br s, 1H), 4.65–4.50 (m, 1H), 3.83 (s, 3H), 3.71–3.58 (m, 1H), 3.24–3.09 (m, 1H), 2.76–2.62 (m, 3H), 2.25–2.12 (m, 4H), 1.99–1.36 (m, 3H); MS (ESI) *m/z*: 282 $(M+H)^+$.

4.1.21. 5-(1-Acetylpyrrolidin-2-yl)-2-methylthiophene-3-carboxylic acid (25a).

A mixture of **24a** (34.6 mg, 0.129 mmol), $LiOH \cdot H_2O$ (16.3 mg, 0.388 mmol), and water (200 μL) in MeOH (1.00 mL) was stirred at 50 °C for 19 h. The mixture was then neutralized with 1 N aqueous HCl at 0 °C and extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure to give 32.0 mg (98%) of **25a** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 5.36 (d, *J* = 6.7 Hz, 1H), 3.76–3.46 (m, 2H), 2.67 (s, 3H), 2.42–1.88 (m, 7H); MS (ESI) *m/z*: 254 $(M+H)^+$, 252 $(M-H)^-$.

4.1.22. 5-(1-Acetylpiperidin-2-yl)-2-methylthiophene-3-carboxylic acid (25b).

Compound **25b** was prepared from **24b** using the same procedure as described for **25a** to yield a colorless oil (yield 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (s, 1H), 6.04 (br s, 1H), 5.18 (br s, 1H), 4.59 (d, *J* = 12.7 Hz, 1H), 3.66 (d, *J* = 14.1 Hz, 1H), 3.17 (t, *J* = 12.7 Hz, 1H), 2.78–2.62 (m, 3H), 2.28–2.14 (m, 3H), 2.00–1.38 (m, 4H); MS (ESI) *m/z*: 268 $(M+H)^+$, 266 $(M-H)^-$.

Compounds **26a** and **26b** were prepared from 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine and the appropriate carboxylic acid using the same procedure as described for **16**.

4.1.23. 5-(1-Acetylpyrrolidin-2-yl)-*N*-(5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methylthiophene-3-carboxamide (26a).

Yield 26%; White solid: mp 173–175 °C; t_R = 2.91 min (UPLC purity: 95.5%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.23 (s, 1H), 5.34–5.26 (m, 1H), 3.81–3.46 (m, 2H), 2.90 (t, *J* = 7.1 Hz, 2H), 2.81–2.63 (m, 5H), 2.53–1.91 (m, 9H); ^{13}C NMR (101 MHz, CD_3OD) δ 172.3, 163.5, 163.3, 156.8, 147.4, 144.3, 130.6, 129.5, 124.3, 57.5, 47.4, 34.6, 28.5, 28.3, 27.4, 25.0, 22.5, 15.1; IR (ATR); 1620, 1557, 1532 cm^{-1}; MS (ESI) *m/z*: 376 $(M+H)^+$, 374 $(M-H)^-$; HRMS (ESI) calcd for $C_{18}H_{21}N_3O_2S_2$ $(M+H)^+$ 376.1148, found 376.1151.

4.1.24. 5-(1-Acetylpiperidin-2-yl)-*N*-(5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methylthiophene-3-carboxamide (26b).

Yield 21%: mp 182–183 °C; t_R = 3.25 min (UPLC purity: 97.2%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.63 (br s, 1H), 6.97 (s, 1H), 6.05 (br s, 1H), 5.19 (br s, 1H), 4.58 (d, *J* = 11.8 Hz, 1H), 3.67 (d, *J* = 11.7 Hz, 1H), 3.14 (t, *J* = 12.8 Hz, 1H), 2.90 (t, *J* = 7.1 Hz, 2H), 2.79–2.57 (m, 5H), 2.46 (q, *J* = 7.3 Hz, 2H), 2.18 (s, 3H), 2.00–1.38 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.5, 161.3, 160.9, 155.4, 147.4, 141.3, 129.3, 128.5, 123.3, 47.7, 42.8, 28.4, 27.3(2C), 26.7, 25.9, 21.7, 19.7, 15.3; IR (ATR) 1621, 1531 cm^{-1}; MS (ESI) *m/z*: 390 $(M+H)^+$, 388 $(M-H)^-$; HRMS (ESI) calcd for $C_{19}H_{23}N_3O_2S_2$ $(M+H)^+$ 390.1304, found 390.1296.

4.1.25. Methyl 2-methyl-5-(2-oxopyrrolidin-1-yl)thiophene-3-carboxylate (27a).

A mixture of **11** (500 mg, 2.13 mmol), 2-pyrrolidone (210 mg, 2.47 mmol), CuI (20.0 mg, 0.105 mmol), *N*,*N'*-dimethylethylenediamine (18.5 mg, 0.210 mmol), and K_2CO_3 (580 mg, 4.20 mmol) in toluene (2.50 mL) was refluxed for 12 h. After cooling to room temperature, the reaction mixture was diluted with AcOEt and filtered through celite. The filtrate was washed with saturated aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was triturated with 50% (v/v) solution of AcOEt/*n*-hexane to give 166 mg (33%) of **27a** as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.73 (s, 1H), 3.89–3.82 (m, 5H), 2.66 (s, 3H), 2.62 (t, *J* = 8.1 Hz, 2H), 2.25 (quint, *J* = 7.7 Hz, 2H); MS (ESI) *m/z*: 240 $(M+H)^+$.

4.1.26. Methyl 2-methyl-5-(2-oxopiperidin-1-yl)thiophene-3-carboxylate (27b).

Compound **27b** was prepared from **11** using the procedure described for **27a** to yield a white solid (yield 17%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (s, 1H), 3.84 (s, 3H), 3.79 (t, *J* = 6.2 Hz, 2H), 2.67–2.58 (m, 5H), 2.08–1.97 (m, 2H) 1.94–1.84 (m, 2H); MS (ESI) *m/z*: 254 $(M+H)^+$.

Compounds **28a** and **28b** were prepared from the appropriate ester using the same procedure as described for **25a**.

4.1.27. 2-Methyl-5-(2-oxopyrrolidin-1-yl)thiophene-3-carboxylic acid (28a).

Yield 87%; white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 6.82 (s, 1H), 3.91 (t, *J* = 7.2 Hz, 2H), 2.63 (s, 3H), 2.60 (t, *J* = 8.1 Hz, 2H), 2.24 (quint, *J* = 7.7 Hz, 2H); MS (ESI) *m/z*: 226 $(M+H)^+$, 224 $(M-H)^-$.

4.1.28. 2-Methyl-5-(2-oxopiperidin-1-yl)thiophene-3-carboxylic acid (28b).

Yield 80%; colorless oil; ^{1}H NMR (400 MHz, CD_3OD) δ 6.98 (s, 1H), 3.82 (t, *J* = 6.1 Hz, 2H), 2.61 (s, 3H), 2.57 (t, *J* = 6.6 Hz, 2H), 2.05–1.96 (m, 2H), 1.91–1.82 (m, 2H); MS (ESI) *m/z*: 240 $(M+H)^+$, 238 $(M-H)^-$.

Compounds **29a** and **29b** were prepared from 5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-amine and the appropriate carboxylic acid using the same procedure as described for **16**.

4.1.29. *N*-(5,6-Dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methyl-5-(2-oxopyrrolidin-1-yl)thiophene-3-carboxamide (29a).

Yield 43%; white solid; mp 235–237 °C; t_R = 2.89 min (UPLC purity: 97.9%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (br s, 1H), 6.58 (s, 1H), 3.78 (t, *J* = 7.2 Hz, 2H), 2.95–2.85 (m, 2H), 2.74 (s, 3H), 2.71–2.59 (m, 4H), 2.52–2.39 (m, 2H), 2.26 (quint, *J* = 7.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3, 161.6, 161.2, 155.4, 140.0, 136.9, 128.4, 126.0, 108.7, 48.1, 31.0, 27.4, 27.3, 26.8, 17.9, 14.6; IR (ATR) 1679, 1654, 1559, 1526 cm^{-1}; MS (ESI) *m/z*: 348 $(M+H)^+$, 346 $(M-H)^-$; HRMS (ESI) calcd for $C_{16}H_{17}N_3O_2S_2$ $(M+H)^+$ 348.0835, found 348.0840.

4.1.30. *N*-(5,6-Dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methyl-5-(2-oxopiperidin-1-yl)thiophene-3-carboxamide (29b).

Yield 33%; white solid; mp 192–193 °C; t_R = 3.04 min (UPLC purity: 97.4%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (br s, 1H), 6.69 (s, 1H), 3.66 (t, *J* = 6.1 Hz, 2H), 2.93–2.85 (m, 2H), 2.71 (s, 3H), 2.67–2.59 (m, 4H), 2.48–2.38 (m, 2H), 2.05–1.96 (m, 2H), 1.94–1.85 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 162.7, 161.9, 155.1, 141.5, 138.9, 128.1, 125.3, 109.5, 48.4, 32.7, 27.5, 27.1, 26.7, 22.9, 20.5, 14.2; IR (ATR) 1647,

1557, 1523 cm^{-1}; MS (ESI) *m/z*: 362 $(M+H)^+$, 360 $(M-H)^-$; HRMS (ESI) calcd for $C_{17}H_{19}N_3O_2S_2$ $(M+H)^+$ 362.0991, found 362.0993.

4.1.31. 5-(1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxylic acid (30).

To a solution of **23a** (1.13 g, 5.02 mmol) in CH_2Cl_2 (25.0 mL), Et_3N (839 μL, 6.02 mmol) and $(Boc)_2O$ (1.38 mL, 6.02 mmol) were added at room temperature. After stirring at the same temperature for 1 h, the mixture was evaporated under reduced pressure and azeotroped with toluene. The residue was dissolved in MeOH (15.0 mL) and water (2.50 mL) and $LiOH \cdot H_2O$ (634 mg, 15.1 mmol) were added. After stirring at 50 °C for 9 h, the reaction mixture was cooled to 0 °C and neutralized with 1 N aqueous HCl. The precipitate was collected by filtration, rinsed with H_2O, and dried to give 1.49 g (96%) of **30** as a white solid: ^1H NMR (400 MHz, CD_3OD) δ 7.11 (s, 1H), 5.06–4.94 (m, 1H), 3.54–3.36 (m, 2H), 2.65 (s, 3H), 2.36–2.16 (m, 1H), 2.08–1.87 (m, 3H), 1.53–1.22 (m, 9H); MS (ESI) *m/z*: 310 $(M-H)^-$.

Compounds **31a**–**31c** were prepared from **30** and an appropriate amine using the same procedure as described for **16**.

4.1.32. *tert*-Butyl 2-(4-((5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidine-1-carboxylate (31a).

Yield 69%; white solid; ^1H NMR (400 MHz, $CDCl_3$) δ 9.89 (br s, 1H), 6.97 (br s, 1H), 5.15–4.86 (m, 1H), 3.62–3.30 (m, 2H), 2.93–2.84 (m, 2H), 2.74 (s, 3H), 2.65–2.57 (m, 2H), 2.48–2.38 (m, 2H), 2.23 (br s, 1H), 2.03–1.86 (m, 3H), 1.56–1.22 (m, 9H); MS (ESI) *m/z*: 434 $(M+H)^+$, 432 $(M-H)^-$.

4.1.33. *tert*-Butyl 2-(5-methyl-4-(thiazol-2-ylcarbamoyl)thiophen-2-yl)pyrrolidine-1-carboxylate (31b).

Yield 88%; white foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.49 (br s, 1H), 7.43 (d, *J* = 3.6 Hz, 1H), 7.05–6.95 (m, 2H), 5.19–4.93 (m, 1H), 3.63–3.33 (m, 2H), 2.76 (s, 3H), 2.34–2.16 (m, 1H), 2.08–1.87 (m, 3H), 1.62–1.21 (m, 9H); MS (ESI) *m/z*: 394 $(M+H)^+$, 392 $(M-H)^-$.

4.1.34. *tert*-Butyl 2-(4-((5-chlorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidine-1-carboxylate (31c).

Yield 48%; white foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (br s, 1H), 7.18 (br s, 1H), 7.07–6.91 (m, 1H), 5.13–4.91 (m, 1H), 3.60–3.34 (m, 2H), 2.74 (s, 3H), 2.34–2.18 (m, 1H), 2.07–1.89 (m, 3H), 1.53–1.24 (m, 9H); MS (ESI) *m/z*: 430, 428 $(M+H)^+$, 428, 426 $(M-H)^-$.

4.1.35. *N*-(5,6-Dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxamide (32a).

To a solution of **31a** (100 mg, 0.231 mmol) in CH_2Cl_2 (1.00 mL), trifluoroacetic acid (266 μL, 3.47 mmol) was added at 0 °C. After stirring at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was diluted with $CHCl_3$, washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure to give 75.0 mg (97%) of **32a** as a pale-yellow foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (s, 1H), 4.33 (t, *J* = 7.1 Hz, 1H), 3.18–2.98 (m, 2H), 2.89 (t, *J* = 7.1 Hz, 2H), 2.74 (s, 3H), 2.67 (t, *J* = 7.3 Hz, 2H), 2.50–2.39 (m, 2H), 2.22–2.12 (m, 1H), 2.00–1.68 (m, 3H); MS (ESI) *m/z*: 334 $(M+H)^+$, 332 $(M-H)^-$.

Compounds **32b** and **32c** were prepared from **31b** and **31c**, respectively, using the same procedure as described for **32a**.

4.1.36. 2-Methyl-5-(pyrrolidin-2-yl)-*N*-(thiazol-2-yl)thiophene-3-carboxamide (32b).

Yield 97%; pale-yellow foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 3.6 Hz, 1H), 7.11 (s, 1H), 6.96 (d, *J* = 3.6 Hz, 1H), 4.34 (t, *J* = 7.0 Hz, 1H), 3.20–3.09 (m, 1H), 3.07–2.97 (m, 1H), 2.76 (s, 3H), 2.24–2.12 (m, 1H), 2.00–1.70 (m, 3H); MS (ESI) *m/z*: 294 $(M+H)^+$, 292 $(M-H)^-$.

4.1.37. *N*-(5-chlorothiazol-2-yl)-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxamide (32c).

Yield 70%; pale-yellow foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 7.03 (s, 1H), 4.34 (t, *J* = 7.1 Hz, 1H), 3.18–2.95 (m, 2H), 2.75 (s, 3H), 2.28–2.12 (m, 1H), 1.99–1.70 (m, 3H); MS (ESI) *m/z*: 330, 328 $(M+H)^+$, 328, 326 $(M-H)^-$.

4.1.38. *tert*-Butyl (3-(2-(4-((5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-3-oxopropyl)carbamate (34a).

A mixture of **32a** (50.0 mg, 0.150 mmol), *N*-(*tert*-butoxycarbonyl)-β-alanine (56.8 mg, 0.300 mmol), EDCI (57.5 mg, 0.300 mmol), HOBt (45.9 mg, 0.300 mmol), and DIPEA (103 μL, 0.600 mmol) in DMF (1.00 mL) was stirred at room temperature for 18 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by preparative TLC ($CHCl_3$/MeOH = 9/1) to give 20.2 mg (27%) of **34a** as a white foam: ^{1}H NMR (400 MHz, CD_3OD) δ 7.21 (s, 1H), 5.33 (d, *J* = 7.0 Hz, 1H), 3.79–3.46 (m, 2H), 3.37–3.20 (m, 2H), 2.90 (t, *J* = 7.1 Hz, 2H), 2.75 (t, *J* = 7.2 Hz, 2H), 2.71–1.96 (m, 11H), 1.36 (m, 9H); MS (ESI) *m/z*: 505 $(M+H)^+$, 503 $(M-H)^-$.

4.1.39. *tert*-Butyl (3-(2-(5-methyl-4-(thiazol-2-ylcarbamoyl)thiophen-2-yl)pyrrolidin-1-yl)-3-oxopropyl)carbamate (34b).

Compound **34b** was prepared from **32b** using the same procedure as described for **34a** to yield a white foam (yield 62%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.50–7.46 (m, 1H),

7.27–7.23 (m, 1H), 7.15–7.10 (m, 1H), 5.33 (d, *J* = 6.9 Hz, 1H), 3.81–3.18 (m, 4H), 2.78–1.93 (m, 9H), 1.41 (s, 9H); MS (ESI) *m/z*: 465 $(M+H)^+$, 463 $(M-H)^-$.

4.1.40. *tert*-Butyl (2-(2-(5-methyl-4-(thiazol-2-ylcarbamoyl)thiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (34c).

Compound **34c** was prepared from **32b** using the same procedure as described for **34a** to yield a white foam (yield 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (br s, 1H), 7.38 (d, *J* = 2.8 Hz, 1H), 7.23 (s, 1H), 7.02–6.93 (m, 1H), 5.85 (br s, 1H), 5.38 (d, *J* = 6.4 Hz, 1H), 4.10–3.87 (m, 2H), 3.80–3.41 (m, 2H), 2.70 (s, 3H), 2.41–1.93 (m, 4H), 1.44 (s, 9H); MS (ESI) *m/z*: 451 $(M+H)^+$, 449 $(M-H)^-$.

4.1.41. 5-(1-(Dimethylglycyl)pyrrolidin-2-yl)-2-methyl-*N*-(thiazol-2-yl)thiophene-3-carboxamide (34d).

Compound **34d** was prepared from **32b** using the same procedure as described for **34a** to yield a white foam (yield 80%): t_R = 1.51 min (UPLC purity: 96.5%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.51–7.45 (m, 1H), 7.31–7.25 (m, 1H), 7.16–7.10 (m, 1H), 5.35 (d, *J* = 6.5 Hz, 1H), 3.82–3.50 (m, 2H), 3.37–3.19 (m, 2H), 2.68 (s, 3H), 2.44–1.92 (m, 10H); ^{13}C NMR (101 MHz, CD_3OD) δ 170.6, 163.8, 160.4, 147.6, 144.4, 138.5, 130.5, 124.5, 114.6, 62.3, 57.8, 47.6, 45.9 (2C), 34.3, 25.2, 15.1; IR (ATR) 1646, 1536 cm^{-1}; MS (ESI) *m/z*: 379 $(M+H)^+$, 377 $(M-H)^-$; HRMS (ESI) calcd for $C_{17}H_{22}N_4O_2S_2$ $(M+H)^+$ 379.1257, found 379.1260.

4.1.42. *N*-(5-Chlorothiazol-2-yl)-5-(1-(dimethylglycyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxamide (34e).

Compound **34e** was prepared from **32c** using the same procedure as described for **34a** to yield a colorless oil (yield 82%): t_R = 2.05 min (UPLC purity: 98.8%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.37–7.34 (m, 1H), 7.31–7.26 (m, 1H), 5.34 (d, *J* = 6.2 Hz, 1H), 3.82–3.53 (m, 2H), 3.35–3.17 (m, 2H), 2.67 (s, 3H), 2.43–1.93 (m, 10H); ^{13}C NMR (101 MHz, CD_3OD) δ 170.6, 163.8, 158.2, 148.3, 144.4, 136.5, 129.8, 124.6, 124.4,

62.4, 57.8, 47.6, 45.9 (2C), 34.3, 25.2, 15.2; IR (ATR); 1653, 1619, 1534 cm^{-1}; MS (ESI) *m/z*: 415, 413 $(M+H)^+$, 413, 411 $(M-H)^-$; HRMS (ESI) calcd for $C_{17}H_{21}ClN_4O_2S_2$ $(M+H)^+$ 413.0867, found 413.0868.

Compounds **33a**–**33c** were prepared from **34a**–**34c**, respectively, using the same procedure as described for **32a**.

4.1.43. 5-(1-(3-Aminopropanoyl)pyrrolidin-2-yl)-*N*-(5,6-dihydro-4*H*-cyclopenta[*d*]thiazol-2-yl)-2-methylthiophene-3-carboxamide (33a).

Yield 65%; pale-yellow foam; t_R = 2.12 min (UPLC purity: 100%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.29–7.19 (m, 1H), 5.34 (d, *J* = 7.5 Hz, 1H), 3.79–3.50 (m, 2H), 3.02–2.84 (m, 4H), 2.79–2.55 (m, 7H), 2.53–1.93 (m, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 170.9, 163.5, 161.8, 148.6, 144.1, 143.9, 129.7, 125.0, 124.6, 57.7, 48.0, 37.1, 36.8, 32.2, 28.3 (2C), 27.5, 25.0, 15.2; IR (ATR) 1623, 1533 cm^{-1}; MS (ESI) *m/z*: 405 $(M+H)^+$, 403 $(M-H)^-$; HRMS (ESI) calcd for $C_{19}H_{24}N_4O_2S_2$ $(M+H)^+$ 405.1413, found 405.1408.

4.1.44. 5-(1-(3-Aminopropanoyl)pyrrolidin-2-yl)-2-methyl-*N*-(thiazol-2-yl)thiophene-3-carboxamide (33b).

Yield 27%; pale-yellow foam; t_R = 1.64 min (UPLC purity: 96.9%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (d, *J* = 3.6 Hz, 1H), 7.27 (s, 1H), 7.15–7.11 (m, 1H), 5.35 (d, *J* = 6.8 Hz, 1H), 3.79–3.51 (m, 2H), 3.05–2.86 (m, 2H), 2.78–1.94 (m, 9H); ^{13}C NMR (101 MHz, DMSO-*d*6) δ 170.0, 161.6, 158.7, 145.6, 142.4, 137.4, 128.7, 123.1, 113.3, 56.3, 45.5, 37.3, 37.0, 35.3, 23.6, 14.8; IR (ATR); 1617, 1536 cm^{-1}; MS (ESI) *m/z*: 365 $(M+H)^+$, 363 $(M-H)^-$; HRMS (ESI) calcd for $C_{16}H_{20}N_4O_2S_2$ $(M+H)^+$ 365.1100, found 365.1103.

4.1.45. 5-(1-Glycylpyrrolidin-2-yl)-2-methyl-*N*-(thiazol-2-yl)thiophene-3-carboxamide (33c).

Yield 54%; white foam; t_R = 1.57 min (UPLC purity: 99.0%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.50–7.46 (m, 1H), 7.30–7.27 (m, 1H), 7.15–7.10 (m, 1H), 5.36 (d, *J* = 6.6 Hz, 1H), 3.80–3.45 (m, 4H), 2.68 (s, 3H), 2.47–1.93 (m, 4H); ^{13}C NMR (101 MHz,

DMSO-d_6) δ 161.4, 161.3, 158.3, 145.9, 142.0, 137.4, 128.5, 123.3, 113.5, 55.3, 44.9, 42.3, 32.1, 23.5, 14.8; IR (ATR); 1644, 1533 cm^{-1}; MS (ESI) *m/z*: 351 $(M+H)^+$, 349 $(M-H)^-$; HRMS (ESI) calcd for $C_{15}H_{18}N_4O_2S_2$ $(M+H)^+$ 351.0944, found 351.0948.

4.1.46. Methyl *(S)*-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxylate (2R,3R)-2,3-bis(benzoyloxy)succinate (35).

(−)-Dibenzoyl-L-tartaric acid monohydrate (6.68 g, 17.8 mmol) was dissolved in MeOH (167 mL) at room temperature. A solution of **23a** (4.00 g, 17.8 mmol) in MeOH (100 mL) was added to the above solution. After 10 min, the mixture was inoculated with the optically active salt (> 98% de) of **35**. The resulting mixture was sealed with a stopper and held at room temperature for 9 h. The precipitated solid was collected by suction filtration and rinsed with a small amount of MeOH to give 2.62 g (48% in theory) of **35** as a white solid with 96.6% de (HPLC)*: mp 188–190 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01–7.94 (m, 4H), 7.69–7.61 (m, 2H), 7.52 (t, *J* = 7.7 Hz, 4H), 7.41 (s, 1H), 5.70 (s, 2H), 4.72–4.62 (m, 1H), 3.77 (s, 3H), 3.26–3.08 (m, 2H), 2.61 (s, 3H), 2.31–2.17 (m, 1H), 2.03–1.78 (m, 3H); MS (ESI) *m/z*: 226 $(M+H)^+$.

4.1.47. Methyl *(R)*-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxylate (2S,3S)-2,3-bis(benzoyloxy)succinate (39).

The methanolic mother liquors of the first resolution were evaporated to dryness to give 7.71 g (43.6% de) of (+)-enriched diastereometic solid, whereby (+)-enriched **23a** was liberated by the extraction of $CHCl_3$-saturated aqueous $NaHCO_3$. The resultant (+)-enriched **23a** was optically resolved with (+)-dibenzoyl-D-tartaric acid monohydrate using the same procedure as described for **35** to give **39** (2.47 g, 45% in theory) to yield a white solid with 96.6% de (HPLC)*: mp, NMR, MS data are the same as for **35**.

*The analytical conditions were as follows: column, Daicel OD-H; eluent, hexane/isopropanol/diethylamine = 95/5/0.1; rate, 0.5 mL/min; and detection, 254 nm. The retention times for (+)- and (−)-**23a** were 14.9 and 16.6 min, respectively.

4.1.48. *(S)*-5-(1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxylic acid (36).

Diastereomeric salt **35** (1.96 g, 3.25 mmol) was liberated by the extraction of $CHCl_3$-saturated aqueous $NaHCO_3$. The resulting organic layer was washed with saturated aqueous $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure to give liberated amine methyl *(S)*-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxylate as a yellow oil. To a solution of methyl *(S)*-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxylate in CH_2Cl_2 (15.0 mL), $(Boc)_2O$ (860 μL, 3.90 mmol) and Et_3N (540 μL, 3.90 mmol) were added at room temperature. After stirring at the same temperature for 4 h, the reaction mixture was concentrated under reduced pressure and azeotroped with toluene. MeOH (12.0 mL), water (2.00 mL), and $LiOH \cdot H_2O$ (480 mg, 11.4 mmol) were added to the resulting mixture, which was stirred at 50 °C for 18 h. After cooling, water was added and the mixture was neutralized with 1 N aqueous HCl at 0 °C. The precipitate was collected to give 950 mg (94%) of **36** as a white solid: ^{1}H NMR, MS data are the same as for **30**.

4.1.49. *(R)*-5-(1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxylic acid (40).

Compound **40** was prepared from **39** using the same procedure as described for **36** to yield a white solid (Yield 87%). ^{1}H NMR, MS data are the same as for **30**.

Compounds **37** and **41** were prepared from **36** and **40**, respectively, using the same procedure as described for **31c**, **32c**, and **34e**.

4.1.50. *(S)*-*N*-(5-Chlorothiazol-2-yl)-5-(1-(dimethylglycyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxamide (37).

Yield 49%; white foam. ^{1}H NMR, MS data are the same as for **34e**.

4.1.51. *(R)*-*N*-(5-Chlorothiazol-2-yl)-5-(1-(dimethylglycyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxamide (41).

Yield 58%; white foam. ^{1}H NMR, MS data are the same as for **34e**.

4.1.52. *(S)*-*N*-(5-Chlorothiazol-2-yl)-5-(1-(dimethylglycyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxamide hydrochloride (38).

To a solution of **37** (583 mg, 1.41 mmol) in CH_2Cl_2 (8.00 mL), 4 N HCl in 1,4-dioxane (1.76 mL, 7.05 mmol) was added at 0 °C. After stirring at room temperature for 1.5 h, Et_2O was added and the resulting precipitate was collected to give 617 mg (97%) of **38** as a white solid: mp 157–159 °C; t_R = 2.05 min (UPLC purity: 97.6%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.38–7.36 (m, 1H), 7.34–7.32 (m, 1H), 5.40 (d, *J* = 5.8 Hz, 1H), 4.29–4.17 (m, 2H), 3.85–3.45 (m, 2H), 3.05–2.61 (m, 9H), 2.50–1.97 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 164.5, 163.6, 158.1, 148.7, 143.4, 136.5, 129.9, 125.2, 124.9, 59.5, 58.3, 47.2, 45.0, 44.9, 34.3, 25.1, 15.2; IR(ATR) 1644, 1533 cm^{-1}; MS (ESI) *m/z*: 415, 413 $(M+H)^+$, 413, 411 $(M-H)^-$; HRMS (ESI) calcd for $C_{17}H_{21}ClN_4O_2S_2$ $(M+H)^+$ 413.0867 found 413.0865; $[\alpha]_{23}^{D}$ −77.7 (c 0.556, MeOH).

4.1.53. *(R)*-*N*-(5-Chlorothiazol-2-yl)-5-(1-(dimethylglycyl)pyrrolidin-2-yl)-2-methylthiophene-3-carboxamide hydrochloride (42).

Compound **42** was prepared from **41** using the same procedure as described for **38** to yield a white solid (yield 70%): t_R = 2.07 min (UPLC purity: 95.7%); ^{1}H NMR, ^{13}C NMR, IR, MS, HRMS data are the same as for **38**; $[\alpha]_{20}^{D}$ +80.9 (c 1.00, MeOH).

4.1.54. *tert*-Butyl 2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidine-1-carboxylate (43).

To a suspension of **30** (1.56 g, 5.02 mmol) in toluene (16.0 mL), oxalyl chloride (4.30 mL, 50.2 mmol) was added at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was evaporated under reduced pressure. The residue was azeotroped with toluene and dissolved with toluene (16.0 mL), 2-amino-5-fluorothiazole hydrochloride (1.16 g, 7.53 mmol) and *N*,*N*-diethylaniline (3.20 mL, 20.9 mmol), which were added at room temperature. After stirring at the same temperature overnight, the

reaction mixture was diluted with AcOEt, washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography (AcOEt/*n*-hexane = 2/3) to give 1.62 g (78%) of **43** as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (br s, 1H), 7.12 (d, *J* = 2.6 Hz, 1H), 5.10–4.99 (m, 1H), 3.59–3.41 (m, 2H), 2.69 (s, 3H), 2.39–2.22 (m, 1H), 2.13–1.89 (m, 3H), 1.61–1.24 (m, 9H); MS (ESI) *m/z*: 412 $(M+H)^+$, 410 $(M-H)^-$.

4.1.55. *N*-(5-Fluorothiazol-2-yl)-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxamide (44).

Compound **44** was prepared from **43** using the same procedure as described for **32a** to yield a white foam (yield 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.87 (d, *J* = 2.6 Hz, 1H), 4.33 (t, *J* = 7.0 Hz, 1H), 3.17–3.07 (1H, m), 3.06–2.94 (m, 1H), 2.73 (s, 3H), 2.24–2.09 (m, 1H), 2.00–1.64 (m, 3H); MS (ESI) *m/z*: 312 $(M+H)^+$, 310 $(M-H)^-$.

Compounds **45a**, **45b**, and **45c** were prepared from **31c**, **31c**, and **44**, respectively, using the procedure described for **34a**.

4.1.56. *tert*-Butyl (2-(2-(4-((5-chlorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (45a).

Yield 99%; white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 (s, 1H), 7.34 (s, 1H), 5.36–5.27 (m, 1H), 3.99–3.84 (m, 2H), 3.78–3.50 (m, 2H), 2.66 (s, 3H), 2.48–1.89 (m, 4H), 1.48–1.37 (m, 9H); MS (ESI) *m/z*: 487, 485 $(M+H)^+$, 485, 483 $(M-H)^-$.

4.1.57. *tert*-Butyl (2-(2-(4-((5-chlorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)carbamate (45b).

Yield 90%; pale-yellow foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 7.12 (s, 1H), 5.38 (d, *J* = 7.3 Hz, 1H), 4.22–3.45 (m, 4H), 2.96 (s, 3H), 2.76 (s, 3H), 2.40–1.81 (m, 4H), 1.59–1.34 (m, 9H); MS (ESI) *m/z*: 501, 499 $(M+H)^+$, 499, 497 $(M-H)^-$.

4.1.58. *tert*-Butyl (2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)carbamate (45c).

Yield 98%; white foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (br s, 1H), 7.11 (s, 1H), 7.01–6.92 (m, 1H), 5.38 (d, *J* = 6.8 Hz, 1H), 4.16–4.05 (m, 1H), 3.97–3.86 (m, 1H), 3.82–3.44 (m, 2H), 2.96 (s, 3H), 2.75 (s, 3H), 2.41–1.73 (m, 4H), 1.53–1.34 (m, 9H); MS (ESI) *m/z*: 483 $(M+H)^+$, 481 $(M-H)^-$.

Compounds **46a**–**46c** were prepared from **45a**–**45c**, respectively, using the same procedure as described for **32a**.

4.1.59. *N*-(5-Chlorothiazol-2-yl)-5-(1-glycylpyrrolidin-2-yl)-2-methylthiophene-3-carboxamide (46a).

Yield 93%; white foam; ^{1}H NMR (400 MHz, CD_3OD) δ 7.35–7.27 (m, 2H), 5.34 (d, *J* = 7.0 Hz, 1H), 3.78–3.39 (m, 4H), 2.66 (s, 3H), 2.43–1.91 (m, 4H); MS (ESI) *m/z*: 387, 385 $(M+H)^+$, 385, 383 $(M-H)^-$.

4.1.60. *N*-(5-Chlorothiazol-2-yl)-2-methyl-5-(1-(methylglycyl)pyrrolidin-2-yl)thiophene-3-carboxamide (46b).

Yield 89%; white foam; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 1H), 7.17 (s, 1H), 5.39 (d, *J* = 7.3 Hz, 1H), 3.80–3.34 (m, 4H), 2.71 (s, 3H), 2.49–1.81 (m, 7H); MS (ESI) *m/z*: 401, 399 $(M+H)^+$, 399, 397 $(M-H)^-$.

4.1.61. *N*-(5-Fluorothiazol-2-yl)-2-methyl-5-(1-(methylglycyl)pyrrolidin-2-yl)thiophene-3-carboxamide (46c).

Yield 99%; white foam; ^{1}H NMR (400 MHz, CD_3OD) δ 7.29–7.24 (m, 1H), 7.12 (d, *J* = 2.5 Hz, 1H), 5.35 (d, *J* = 6.6 Hz, 1H), 3.79–3.41 (m, 4H), 2.67 (s, 3H), 2.47–1.93 (m, 7H); MS (ESI) *m/z*: 383 $(M+H)^+$, 381 $(M-H)^-$.

4.1.62. *N*-(5-Chlorothiazol-2-yl)-2-methyl-5-(1-((methylsulfonyl)glycyl)pyrrolidin-2-yl)thiophene-3-carboxamide (47a).

To a solution of **46a** (96.2 mg, 0.250 mmol) in CH_2Cl_2 (1.00 mL), methanesulfonyl chloride (39.0 μL, 0.500 mmol) and pyridine (41.0 μL, 0.500 mmol) were added at 0 °C. After the reaction mixture was stirred at room temperature for 6 h, it was diluted with $CHCl_3$, washed with water, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by preparative TLC on silica gel ($CHCl_3$/MeOH = 10/1) gave 93.5 mg (81%) of **47a** as a white solid: mp 208–210 °C; t_R = 2.95 min (UPLC purity: 97.8%);^{1}H NMR (400 MHz, CD_3OD) δ 7.37–7.24 (m, 2H), 5.36–5.27 (m, 1H), 4.10–3.95 (m, 2H), 3.79–3.51 (m, 2H), 2.96 (s, 3H), 2.67 (s, 3H), 2.44–1.92 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.4, 163.4, 158.0, 148.8, 143.8, 136.4, 129.4, 124.5, 121.1, 58.1, 47.8, 47.1, 46.1, 34.3, 25.0, 15.3; IR (ATR); 1649, 1537 cm^{-1}; MS (ESI) *m/z*: 465, 463 $(M+H)^+$, 463, 461 $(M-H)^-$; HRMS (ESI) calcd for $C_{16}H_{19}ClN_4O_4S_3$ $(M+H)^+$ 463.0329, found 463.0331.

4.1.63. *N*-(5-Chlorothiazol-2-yl)-2-methyl-5-(1-(*N*-methyl-*N*-(methylsulfonyl)glycyl)pyrrolidin-2-yl)thiophene-3-carboxamide (47b).

Compound **47b** was prepared from **46b** using the same procedure as described for **47a** to yield a white solid (yield 69%): mp 225–226 °C; t_R = 3.14 min (UPLC purity: 98.3%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.4 (br s, 1H), 7.22 (s, 1H), 7.11 (s, 1H), 5.39–5.31 (m, 1H), 4.19–3.97 (m, 2H), 3.80–3.43 (m, 2H), 3.05–2.98 (m, 6H), 2.74 (s, 3H), 2.44–1.86 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 161.3, 156.9, 148.1, 142.9, 134.7, 128.2, 123.5, 122.6, 56.6, 52.2, 46.2, 38.5, 35.7, 33.0, 24.5, 15.3; IR (ATR); 1643, 1534 cm^{-1}; MS (ESI) *m/z*: 479, 477 $(M+H)^+$, 477, 475 $(M-H)^-$; HRMS (ESI) calcd for $C_{17}H_{21}ClN_4O_4S_3$ $(M+H)^+$ 477.0486, found 477.0484.

4.1.64. 5-(1-(Acetylglycyl)pyrrolidin-2-yl)-*N*-(5-chlorothiazol-2-yl)-2-methylthiophene-3-carboxamide (48a).

To a solution of **46a** (96.2 mg, 0.250 mmol) in CH_2Cl_2 (1.00 mL), acetyl chloride (36.0 μL, 0.500 mmol) and pyridine (41.0 μL, 0.500 mmol) were added at 0 °C. After stirring

at room temperature for 6 h, the reaction mixture was diluted with $CHCl_3$, washed with water, and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by preparative TLC on silica gel ($CHCl_3$/MeOH = 10/1) to give 90.6 mg (85%) of **48a** as a white solid: mp 122–124 °C; t_R = 2.75 min (UPLC purity: 99.8%);^{1}H NMR (400 MHz, $CDCl_3$) δ 10.67 (br s, 1H), 7.45–7.34 (m, 1H), 7.19 (s, 1H), 5.35–5.29 (m, 1H), 4.34–4.23 (m, 1H), 4.05–3.87 (m, 1H), 3.77–3.41 (m, 2H), 2.65 (s, 3H), 2.39–1.79 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 167.6, 161.2, 161.0, 156.4, 148.6, 141.8, 134.9, 127.8, 123.2, 56.7, 46.0, 42.4, 33.2, 29.7, 22.9, 15.4; IR (ATR) 1651, 1614, 1530 cm^{-1}; MS (ESI) *m/z*: 429, 427 $(M+H)^+$, 427, 425 $(M-H)^-$; HRMS (ESI) calcd for $C_{17}H_{19}ClN_4O_3S_2$ $(M+H)^+$ 427.0660 found 427.0661.

4.1.65. 5-(1-(*N*-Acetyl-*N*-methylglycyl)pyrrolidin-2-yl)-*N*-(5-chlorothiazol-2-yl)-2-methylthiophene-3-carboxamide (48b).

Compound **48b** was prepared from **46b** using the same procedure as described for **48a** to yield a white solid (yield 74%): mp 141–142 °C; t_R = 2.87 min (UPLC purity: 98.7%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.17 (br s, 1H), 7.50 (d, *J* = 1.0 Hz, 1H), 7.29–7.22 (m, 1H), 5.35 (d, *J* = 6.9 Hz, 1H), 4.62 (d, *J* = 15.4 Hz, 1H), 3.97–3.87 (m, 1H), 3.59–3.41 (m, 2H), 3.22 (s, 3H), 2.75 (s, 3H), 2.37–1.74 (m, 7H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 167.7, 162.0, 161.6, 156.8, 149.2, 140.3, 135.2, 125.6, 124.3, 56.8, 51.3, 46.0, 39.0, 33.2, 23.7, 21.2, 15.5; IR (ATR); 1647, 1622, 1533 cm^{-1}; MS (ESI) *m/z*: 443, 441 $(M+H)^+$, 441, 439 $(M-H)^-$; HRMS (ESI) calcd for $C_{18}H_{21}ClN_4O_3S_2$ $(M+H)^+$ 441.0816, found 441.0818.

4.1.66. 5-(1-(2-Chloroacetyl)pyrrolidin-2-yl)-*N*-(5-chlorothiazol-2-yl)-2-methylthiophene-3-carboxamide (49).

To a solution of **31c** (250 mg, 0.763 mmol) in CH_2Cl_2 (8.00 mL), 2,6-di-*tert*-butylpyridine (359 μL, 1.60 mmol) and chloroacetyl chloride (122 μL, 1.53 mmol) were added at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was diluted with $CHCl_3$ and washed with water and saturated aqueous $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography

(AcOEt/*n*-hexane = 1/1) to give 257 mg (83%) of **49** as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (br s, 1H), 7.24 (s, 1H), 7.13 (s, 1H), 5.42–5.37 (m, 1H), 4.14–4.09 (m, 1H), 4.01–3.83 (m, 1H), 3.82–3.73 (m, 1H), 3.70–3.60 (m, 1H), 2.71 (s, 3H), 2.49–1.94 (m, 4H); MS (ESI) *m/z*: 406, 404 $(M+H)^+$, 404, 402 $(M-H)^-$.

4.1.67. *tert*-Butyl (2-(2-(4-((5-chlorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)glycinate (50).

A mixture of glycine *tert*-butyl ester hydrochloride (93.9 mg, 0.560 mmol) and DIPEA (115 μL, 0.672 mmol) in DMF (1.00 mL) was stirred at 50 °C for 5 min. After the addition of **49** (45.4 mg, 0.112 mmol) in DMF (1.00 mL) at 50 °C, the reaction mixture was stirred at the same temperature for 2 h, diluted with water, and extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by preparative TLC on silica gel ($CHCl_3$/MeOH = 10/1) to give 37.7 mg (68%) of **50** as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.14 (m, 2H), 5.39 (d, *J* = 6.5 Hz, 1H), 3.79–3.19 (m, 6H), 2.68 (s, 3H), 2.41–1.84 (m, 4H), 1.44 (s, 9H); MS (ESI) *m/z*: 501, 499 $(M+H)^+$, 499, 497 $(M-H)^-$.

4.1.68. (2-(2-(4-((5-Chlorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)glycine hydrochloride (51).

To a solution of **50** (112 mg, 0.224 mmol) in CH_2Cl_2 (3.00 mL), 4 N HCl in 1,4-dioxane (1.70 mL, 6.72 mmol) was added at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was diluted with Et_2O and stirred for 30 min. The precipitate was collected and rinsed with Et_2O to give 85.0 mg (79%) of **51** as a white solid: mp 174–176 °C; t_R = 2.17 min (UPLC purity: 95.0%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.41–7.37 (m, 1H), 7.35 (s, 1H), 5.39 (d, *J* = 6.2 Hz, 1H), 4.14 (s, 2H), 4.02–3.44 (m, 4H), 2.68 (s, 3H), 2.61–1.95 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 169.0, 165.4, 162.9, 159.3, 150.7, 143.3, 133.7, 128.6, 125.4, 124.9, 58.4, 48.4, 47.3, 34.5, 24.9, 22.7, 15.6; IR (ATR); 1647, 1547 cm^{-1}; MS (ESI) *m/z* 445, 443 $(M+H)^+$, 443, 441 $(M-H)^-$; HRMS (ESI) calcd for $C_{17}H_{19}ClN_4O_4S_2$ $(M+H)^+$ 443.0609, found 443.0610.

4.1.69. *tert*-Butyl *N*-(2-(2-(4-((5-chlorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-*N*-methylglycinate (52a).

To a solution of **46b** (30.0 mg, 0.0752 mmol) in DMF (1.00 mL), *tert*-butyl chloroacetate (11.9 μL, 0.0827 mmol) and DIPEA (38.7 μL, 0.226 mmol) were added at room temperature and further stirred at 60 °C for 4 h. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine and dried over Na_2SO_4. After filtration, the solvent was concentrated under reduced pressure and the residue was purified by preparative TLC on silica gel (AcOEt/MeOH = 10/1) to give 28.0 mg (73%) of **52a** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.26 (br s, 1H), 7.28–7.11 (m, 2H), 5.37 (d, *J* = 6.6 Hz, 1H), 3.82–3.55 (2H, m), 3.50–3.15 (4H, m), 2.72 (3H, s), 2.55–1.89 (7H, m), 1.46 (9H, s); MS (ESI) *m/z*: 515, 513 $(M+H)^+$, 513, 511 $(M-H)^-$.

4.1.70. *tert*-Butyl *N*-(2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-*N*-methylglycinate (52b).

Compound **52b** was prepared from **45c** using the same procedure as described for **52a** to yield a white solid (yield 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.09 (br s, 1H), 7.12 (1H, s), 6.92 (d, *J* = 2.6 Hz, 1H), 5.36 (d, *J* = 6.2 Hz, 1H), 3.83–3.55 (m, 2H), 3.51–3.13 (m, 4H), 2.71 (s, 3H), 2.52–1.90 (m, 7H), 1.46 (s, 9H); MS (ESI) *m/z*: 497 $(M+H)^+$, 495 $(M-H)^-$.

4.1.71. *tert*-Butyl 3-((2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)amino)propanoate (52c).

To a solution of **46c** (46.0 mg, 0.120 mmol) in 1,4-dioxane (1.00 mL), *tert*-butyl acrylate (87.7 μL, 0.602 mmol) and 1 portion of benzyltrimethylammonium hydroxide (40 wt.% in MeOH) were added at room temperature. After stirring at 60 °C overnight, the mixture was evaporated under reduced pressure and azeotroped with toluene. The residue was purified by preparative TLC on silica gel (AcOEt/MeOH = 9/1) to give 13.8 mg (22%) of **52c** as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.32–7.26 (m, 1H),

7.14–7.10 (m, 1H), 5.32 (d, *J* = 7.3 Hz, 1H), 3.89–3.49 (m, 2H), 3.41–3.17 (m, 2H), 2.86–2.59 (m, 5H), 2.50–1.92 (m, 9H), 1.43 (s, 9H); MS (ESI) *m/z*: 511 $(M+H)^+$, 509 $(M-H)^-$.

4.1.72. *tert*-Butyl 4-((2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)amino)butanoate (52d).

Compound **52d** was prepared from **46c** using the same procedure as described for **52a** to yield a colorless oil (yield 36%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.32–7.23 (m, 1H), 7.14–7.08 (m, 1H), 5.33 (d, *J* = 6.1 Hz, 1H), 3.88–3.46 (2H, m), 3.44–3.19 (2H, m), 2.66 (s, 3H), 2.57–1.93 (m, 11H), 1.82–1.57 (2H, m), 1.50–1.37 (9H, m); MS (ESI) *m/z*: 525 $(M+H)^+$, 523 $(M-H)^-$.

4.1.73. Methyl 5-((2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)amino)pentanoate (52e).

Compound **52e** was prepared from **46c** using the same procedure as described for **52a** to yield a white solid (Yield 77%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 (s, 1H), 7.14–7.10 (m, 1H), 5.36–5.30 (m, 1H), 3.88–3.49 (m, 5H), 3.42–3.17 (m, 2H), 2.66 (s, 3H), 2.55–1.82 (m, 11H), 1.74–1.25 (m, 4H); MS (ESI) m/z: 497 $(M+H)^+$, 495 $(M-H)^-$.

Compounds **53a**–**53d** were prepared from **52a**–**52d**, respectively, using the same procedure as described for **51**.

4.1.74. *N*-(2-(2-(4-((5-Chlorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-*N*-methylglycine hydrochloride (53a).

Yield 76%; white solid; mp 162–164 °C; t_R = 2.23 min (UPLC purity: 97.7%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.42–7.29 (m, 2H), 5.41 (d, *J* = 7.1 Hz, 1H), 4.59–3.99 (m, 4H), 3.86–3.45 (m, 2H), 3.06 (s, 3H), 2.69 (s, 3H), 2.51–1.98 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 168.3, 164.6, 162.9, 159.8, 151.1, 143.1, 132.7, 128.6, 125.5, 124.8, 58.4, 57.0, 48.0, 44.3, 34.4, 24.8, 22.7, 15.5; IR (ATR); 1650, 1567 cm^{-1}; MS (ESI) m/z: 459,

457 $(M+H)^+$, 457, 455 $(M-H)^-$; HRMS (ESI) calcd for $C_{18}H_{21}ClN_4O_4S_2$ $(M+H)^+$ 457.0765, found 457.0766.

4.1.75. *N*-(2-(2-(4-((5-Fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)-*N*-methylglycine hydrochloride (53b).

Yield 97%; white solid; mp 164–165 °C; t_R = 2.00 min (UPLC purity: 98.0%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.34–7.28 (m, 1H), 7.20–7.13 (m, 1H), 5.40 (d, *J* = 6.9 Hz, 1H), 4.56–4.00 (m, 4H), 3.67–3.49 (m, 2H), 3.05 (s, 3H), 2.74–2.65 (m, 3H), 2.50–1.98 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 176.1, 168.2, 164.5, 163.1, 159.4 (d, *J* = 294 Hz), 150.6, 143.1, 129.1, 124.9, 116.6 (d, *J* = 16 Hz), 58.4, 58.2, 48.1, 47.1, 44.2, 34.3, 24.9, 15.3; IR (ATR); 1664, 1648, 1558, 1206 cm^{-1}; MS (ESI) *m/z*: 441 $(M+H)^+$, 439 $(M-H)^-$; HRMS (ESI) calcd for $C_{18}H_{21}FN_4O_4S_2$ $(M+H)^+$ 441.1061, found 441.1060.

4.1.76. 3-((2-(2-(4-((5-Fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)amino)propanoic acid hydrochloride (53c).

Yield 68%; pale-yellow solid; mp 142–144 °C; t_R = 2.02 min (UPLC purity: 97.9%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.36–7.30 (m, 1H), 7.19–7.12 (m, 1H), 5.39 (d, *J* = 7.5 Hz, 1H), 4.52–4.17 (m, 2H), 3.92–3.40 (m, 4H), 3.04–2.63 (m, 8H), 2.51–1.97 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.7, 164.7, 164.6, 163.2, 159.9 (d, *J* = 276 Hz), 149.5, 143.0, 129.3, 124.8, 118.4 (d, *J* = 13 Hz), 58.4, 54.1, 47.2, 42.9, 34.3, 29.6, 25.0, 22.7, 15.3; IR (ATR); 1652, 1558, 1196 cm^{-1}; MS (ESI) *m/z*: 455 $(M+H)^+$, 453 $(M-H)^-$; HRMS (ESI) calcd for $C_{19}H_{23}FN_4O_4S_2$ $(M+H)^+$ 455.1217, found 455.1214.

4.1.77. 4-((2-(2-(4-((5-Fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)amino)butanoic acid hydrochloride (53d).

Yield 99%; white solid; mp 125–126 °C; t_R = 2.03 min (UPLC purity: 95.5%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.38–7.31 (m, 1H), 7.23–7.17 (m, 1H), 5.39 (d, *J* = 6.6 Hz, 1H), 4.49–4.17 (m, 2H), 3.90–3.50 (m, 2H), 3.39–3.03 (m, 2H), 2.99–2.89 (m, 3H), 2.74–2.65 (m, 3H), 2.54–1.78 (m, 8H); ^{13}C NMR (101 MHz, CD_3OD) δ 174.6, 164.6, 162.9, 159.0 (d, *J* = 289 Hz), 157.9, 151.0, 143.4, 128.6, 124.7, 115.4 (d, *J* = 11 Hz),

58.3, 58.2, 58.1, 47.2, 42.9, 34.3, 31.4, 24.8, 20.8, 15.5; IR (ATR); 1648, 1557 cm^{-1}; MS (ESI) *m/z*: 469 $(M+H)^+$, 467 $(M-H)^-$; HRMS (ESI) calcd for $C_{20}H_{25}FN_4O_4S_2$ $(M+H)^+$ 469.1374, found 469.1368.

4.1.78. 5-((2-(2-(4-((5-Fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)amino)pentanoic acid hydrochloride (53e).

To a solution of **52e** (63.0 mg, 0.127 mmol) in MeOH (1.00 mL), 1 N aqueous NaOH (500 μL, 0.500 mmol) was added at room temperature. After stirring at room temperature for 5 h, the reaction mixture was acidified with 4 N HCl in 1,4-dioxane (500 μL, 2.00 mmol) at 0 °C. After stirring at room temperature for 1 h, the mixture was evaporated and the residue was diluted with CH_2Cl_2. The mixture was filtered and the filtrate was concentrated under reduced pressure to give 57.6 mg (87%) of **53e** as a pale-yellow solid: mp 126–128 °C; t_R = 2.02 min (UPLC purity: 100%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.29 (s, 1H), 7.15–7.11 (m, 1H), 5.37 (d, *J* = 6.3 Hz, 1H), 4.38–4.10 (m, 2H), 3.84–3.48 (m, 2H), 3.24–3.06 (m, 2H), 2.98–2.86 (m, 3H), 2.83–2.60 (m, 3H), 2.47–1.96 (m, 6H), 1.84–1.46 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.5, 164.7, 164.6, 163.1, 159.6 (d, *J* = 283 Hz), 150.0, 143.4, 129.2, 124.8, 116.9 (d, *J* = 12 Hz), 58.6, 58.4, 58.3, 47.4, 42.7, 34.4, 33.9, 24.9, 27.8, 20.6, 15.4; IR (ATR); 1648, 1560, 1279 cm^{-1}; MS (ESI) *m/z*: 483 $(M+H)^+$, 481 $(M-H)^-$; HRMS (ESI) calcd for $C_{21}H_{27}FN_4O_4S_2$ $(M+H)^+$ 483.1530, found 483.1525.

4.1.79. *(S)*-*N*-(5-Fluorothiazol-2-yl)-2-methyl-5-(pyrrolidin-2-yl)thiophene-3-carboxamide (54).

Compound **54** was prepared from **36** using the procedure described for **43** and **44** to yield a white foam (58% 2 steps): ^{1}H NMR, MS data are the same as for **44**.

Compounds **55b** and **55c** were prepared from **54** and the appropriate carboxylic acid using the procedure described for **34a**.

4.1.80. *tert*-Butyl ((*(S)*-1-((*(S)*-2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-1-oxopropan-2-yl)(methyl)carbamate (55b).

Yield 82%; white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (s, 1H), 7.14–7.12 (m, 1H), 5.35–5.20 (m, 1H), 4.98–4.70 (m, 1H), 3.84–3.72 (m, 1H), 3.71–3.56 (m, 1H), 2.85 (s, 3H), 2.67 (s, 3H), 2.37–1.91 (m, 4H), 1.47 (s, 9H), 1.39–1.17 (m, 3H); MS (ESI) *m/z*: 497 $(M+H)^+$, 495 $(M-H)^-$.

4.1.81. *tert*-Butyl *(S)*-ethyl(2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)carbamate (55c).

Yield 83%; white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.34 (br s, 1H), 7.18–7.10 (m, 1H), 6.96–6.78 (m, 1H), 5.38 (d, *J* = 7.1 Hz, 1H), 4.17–3.87 (m, 2H), 3.79–3.14 (m, 4H), 2.75 (s, 3H), 2.42–1.77 (m, 4H), 1.52–1.33 (m, 9H), 1.16–0.98 (m, 3H); MS (ESI) *m/z*: 497 $(M+H)^+$, 495 $(M-H)^-$.

Compounds **56b** and **56c** were prepared from **55b** and **55c**, respectively, using the procedure described for **32a**.

4.1.82. *N*-(5-Fluorothiazol-2-yl)-2-methyl-5-((*(S)*-1-(methyl-*L*-alanyl)pyrrolidin-2-yl)thiophene-3-carboxamide (56b).

Yield 88%; white solid; ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (s, 1H), 7.15–7.10 (m, 1H), 5.45–5.37 (m, 1H), 3.85–3.68 (m, 2H), 3.62–3.47 (m, 1H), 2.66 (s, 3H), 2.48–1.95 (m, 7H), 1.33–1.21 (m, 3H); MS (ESI) *m/z*: 397 $(M+H)^+$, 395 $(M-H)^-$.

4.1.83. *(S)*-5-(1-(Ethylglycyl)pyrrolidin-2-yl)-*N*-(5-fluorothiazol-2-yl)-2-methylthiophene-3-carboxamide (56c).

Yield 87%; white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.52 (br s, 1H), 9.05 (br s, 1H), 7.45 (s, 1H), 7.03–6.91 (m, 1H), 5.13 (d, *J* = 7.0 Hz, 1H), 4.16–3.58 (m, 4H), 3.31–3.00

(m, 2H), 2.78–2.57 (m, 3H), 2.35–1.79 (m, 4H), 1.35 (t, *J* = 7.2 Hz, 3H); MS (ESI) *m/z*: 397 $(M+H)^+$, 395 $(M-H)^-$.

4.1.84. *tert*-Butyl *N*-((*S*)-1-((*S*)-2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl) pyrrolidin-1-yl)-1-oxopropan-2-yl)-*N*-methylglycinate (57b).

Compound **57b** was prepared from **56b** using the same procedure as described for **52a** to yield a white solid (32%): ^{1}H NMR (400 MHz, CD_3OD) δ 7.24–7.22 (m, 1H), 7.14–7.11 (m, 1H), 5.36–5.30 (m, 1H), 4.05–3.94 (m, 1H), 3.85–3.61 (m, 3H), 3.57–3.24 (m, 2H), 2.66 (s, 3H), 2.44–1.93 (m, 7H), 1.47 (s, 9H), 1.20 (d, *J* = 6.8 Hz, 3H); MS (ESI) *m/z*: 511 $(M+H)^+$, 509 $(M-H)^-$.

4.1.85. *tert*-Butyl (*S*)-*N*-ethyl-*N*-(2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)glycinate (57c).

Compound **57c** was prepared from **56c** using the same procedure as described for **52a** to yield a white solid (60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.60 (br s, 1H), 7.12 (s, 1H), 6.85 (d, *J* = 2.6 Hz, 1H), 5.37–5.31 (m, 1H), 3.83–3.54 (m, 2H), 3.51–3.19 (m, 4H), 2.81–2.58 (m, 5H), 2.38–1.84 (m, 4H), 1.52–1.34 (m, 9H), 1.08 (t, *J* = 7.2 Hz, 3H); MS (ESI) *m/z*: 511 $(M+H)^+$, 509 $(M-H)^-$.

4.1.86. (*S*)-3-((2-(2-(4-((5-Fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)(methyl)amino)propanoic acid hydrochloride (58a).

Compound **58a** was prepared from **54** using the same procedure as described for **45c**, **46c**, **52c**, and **53c** to yield a white solid (yield 38% 4 steps): mp 140–141 °C; t_R = 1.92 min (UPLC purity: 96.2%); ^{1}H NMR, ^{13}C NMR, IR, MS, HRMS data are same as **53c**; $[\alpha]_{23}^{D}$ −91.4 (c 1.00, MeOH).

4.1.87. *N*-((*S*)-1-((*S*)-2-(4-((5-Fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl) pyrrolidin-1-yl)-1-oxopropan-2-yl)-*N*-methylglycine hydrochloride (58b).

Compound **58b** was prepared from **57b** using the same procedure as described for **51** to yield a pale-yellow solid (yield 71%): mp 155–157 °C, t_R = 2.03 min (UPLC purity: 95.3%); ^{1}H NMR (400 MHz, CD_3OD): δ 7.30 (s, 1H), 7.21–7.15 (m, 1H), 5.47–5.34 (m, 1H), 4.64–4.51 (m, 1H), 4.25–3.93 (m, 2H), 3.79–3.65 (m, 2H), 3.04 (s, 3H), 2.76–2.61 (m, 3H), 2.53–1.96 (m, 4H), 1.67–1.53 (m, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.4, 168.6 (2C), 163.5, 160.0 (d, *J* = 296 Hz), 148.4, 143.4, 130.1, 124.8, 118.7 (d, *J* = 13 Hz), 63.4, 58.6, 58.4, 48.3, 34.3, 25.5, 20.5, 15.4, 15.2; IR (ATR); 1644, 1561, 1221 cm^{-1}; MS (ESI) *m/z*: 455 $(M+H)^+$, 453 $(M-H)^-$; HRMS (ESI) calcd for $C_{19}H_{23}FN_4O_4S_2$ $(M+H)^+$ 455.1222, found 455.1216; $[\alpha]_{23}^{D}$ −74.0 (c 0.973, MeOH).

4.1.88. *(S)*-*N*-Ethyl-*N*-(2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)glycine hydrochloride (59·HCl).

Compound **59·HCl** was prepared using the same procedure as described for **51** to yield a white solid (yield 99%). Here, 4 N HCl in AcOEt was used instead of 4 N HCl in 1,4-dioxane: mp 165–167 °C, t_R = 2.08 min (UPLC purity: 98.8%); ^{1}H NMR (400 MHz, CD_3OD): δ 7.39 (br s, 1H), 7.24 (d, *J* = 2.1 Hz, 1H), 5.40 (d, *J* = 6.5 Hz, 1H), 4.61–4.02 (m, 4H), 3.87–3.32 (m, 4H), 2.73–2.62 (m, 3H), 2.50–2.26 (m, 1H), 2.20–1.94 (m, 3H), 1.47–1.27 (m, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 168.5, 165.1, 163.1, 162.8, 159.4 (d, *J* = 290 Hz), 150.6, 143.0, 128.7, 125.0, 117.5 (d, *J* = 15 Hz), 58.6, 57.0, 55.0, 54.3, 48.1, 34.5, 22.7, 15.4, 9.7; IR (ATR) 1651, 1568, 1195 cm^{-1}; MS (ESI) *m/z*: 455 $(M+H)^+$, 453 $(M-H)^-$; HRMS (ESI) calcd for $C_{19}H_{23}FN_4O_4S_2$ $(M+H)^+$ 455.1217 found 455.1213; $[\alpha]_{22}^{D}$ −110 (c 1.00, MeOH).

4.1.89. *(S)*-*N*-Ethyl-*N*-(2-(2-(4-((5-fluorothiazol-2-yl)carbamoyl)-5-methylthiophen-2-yl)pyrrolidin-1-yl)-2-oxoethyl)glycine (59).

A solution of **59·HCl** (51.2 g, 102 mmol) in MeOH (5.10 L) and Milli-Q water (0.570 L) was adjusted at pH 5.81 (isoelectric point) by the addition of 1 N aqueous NaOH at room temperature. The resulting solution was evaporated to remove MeOH and diluted with Milli-Q water (1.70 L). The mixture was inoculated with **59** and stirred at room temperature for 10 h, and the resulting precipitate was corrected and rinsed with Milli-Q

water to give **59** (39.1 g, 84 %) as a white solid: mp 221–223 °C, t_R = 2.08 min (UPLC purity: 98.9%); ^{1}H NMR (400 MHz, CD_3OD) δ 7.32 (br s, 1H), 7.12 (d, *J* = 2.5 Hz, 1H), 5.40–5.33 (m, 1H), 4.35–4.17 (m, 2H), 3.92–3.50 (m, 4H), 3.40–3.19 (m, 2H), 2.66 (s, 3H), 2.48–1.95 (m, 4H), 1.32 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ 170.2, 165.8, 165.6, 163.4, 160.1 (d, *J* = 291 Hz), 148.6, 143.3, 129.9, 125.0, 118.9 (d, *J* = 13 Hz), 58.4, 57.8, 56.3, 52.8, 47.2, 34.4, 25.1, 15.3, 10.1; IR (ATR) 1639, 1556, 1190 cm^{-1}; MS (ESI) *m/z*: 455 $(M+H)^+$, 453 $(M-H)^-$; HRMS (ESI) calcd for $C_{19}H_{23}FN_4O_4S_2$ $(M+H)^+$ 455.1217, found 455.1213; $[\alpha]_{25}^{D}$ −123 (c 1.00, DMSO).

4.2. Biology

4.2.1. Glucokinase assay

The in vitro GK activation potency of compounds was assessed as described previously [24]. In 96-well UV plates, 178 μL/well of GK assay mixture (25 mM HEPES buffer [pH 7.1], 25 mM KCl, 2 mM $MgCl_2$, 1 mM NADP, 1 mM dithiothreitol, 2 unit/mL G6PDH, 5 mM D-glucose, and human GK) was added. After the addition of 2 μL of test compound, positive control (**1**), or negative control (DMSO), the mixture was incubated at room temperature for 10 min. Reactions were initiated by the addition of 20 μL of 20 mM ATP. An absorbance of 340 nm was measured using a Spectra Max Plus 384 Microplate Reader (Molecular Devices, CA, USA) for 5 min at intervals of 30 s at room temperature and the slope of absorption against time was calculated at the first 3 min.

4.2.2. Measurement of insulin secretion

MIN6 cells were seeded onto 24-well plates and precultured in DMEM containing 10% FBS and 61.6 μM of β-mercaptoethanol for 2 days. The cells were washed with HEPES balanced Krebs-Ringer bicarbonate buffer (KRH) with 2 mM glucose and incubated in KRH at 37 °C under 5% CO_2 in air for 45 min. Next, the KRH buffer containing glucose was replaced with test compounds, positive control (**1**), or negative control (DMSO). After incubation at 37 °C under 5% CO_2 in air for 45 min, released insulin was measured according to the manufacture's protocol (Mouse Insulin ELISA KIT [TMB]; AKRIN-011T, Shibayagi, Gunma, Japan).

4.2.3. Solubility test

Five microliters of compound at various concentrations (serial dilutions 8 μM–2 mM) was transferred to measurement plates (Coster, no. 3631) and 95 μL of PBS was added. The solubility of compounds was measured by nephelometry using a NEPHELOstar microplate reader (BMG LABTECH GmbH, Ortenberg, Germany). Before the experiment, blank values of the wells were measured. The data from the well in which blank value was lower than the mean blank value + 2SD was used.

4.2.4. SPA-based dofetilide binding assay (hERG binding assay)

A scintillation proximity assay (SPA) was carried out in assay buffer consisting of 50 mM Tris-Cl, 1.0 mM $MgCl_2$, and 10 mM KCl, pH 7.4. Test compounds were mixed with [^{3}H]-dofetilide at a final concentration 10 nM. Ysi poly-L-lysine SPA beads, and hERG-HEK293 cell membrane and shaken for 1 hour. Beads were allowed to settle for 3 hours before radioactivity was determined by scintillation counting using a MicroBeta TRILUX scintillation counter (PerkinElmer Life and Analytical Sciences, CT, USA). Negative control (DMSO, 0% inhibition) and positive control (E-4031, nonspecific binding, 100% inhibition) were used to normalize the raw data.

4.2.5. Cell viability measurements (CHO-K1 cells)

CHO-K1 cells were seeded onto a 96-well plate at a density of 6000 cells/well in Ham's F12K media containing 2% FBS, 1 mM L-glutamine, 25 units/mL penicillin, and 25 μg/mL streptomycin and incubated overnight at 37 °C under 5% CO_2 in air. Next, cells were exposed to each concentration of **59** (final concentrations 1–100 μM), positive control (benzbromarone), or vehicle control (1% DMSO). Following drug incubation for 24 h, cell viability was assessed using WST-1 reagent according to the manufacturer's instructions.

4.2.6. Cell viability measurements (primary rat hepatocytes)

Rat primary hepatocytes were isolated from male Crj: CD (SD) rat (7 weeks old) using a two-step collagenase perfusion method. After washing in cold MEM media, hepatocytes were suspended in modified-WE media in the presence of 10% FBS, 10^{-10} M dexamethasone, 10^{-9} M insulin, 4 mM L-glutamine, 50 units/mL penicillin, and 50 μg/mL streptomycin. Viable hepatocytes were seeded onto a collagen-coated 96-well plate at a density of $2–3\times10^4$ cells/well and incubated at 37 °C under 5% CO_2 in air. After 4 h, the culture medium was removed from each well and replaced with modified-WE media in the absence of FBS. At 20–24 h after seeding, hepatocytes were exposed to each concentration of **59** (final concentrations 1–100 μM), positive control (benzbromarone), or vehicle control (1% DMSO). Following drug incubation for 24 h, cell viability was assessed using alamarBlue® according to the manufacturer's instructions.

4.2.7. hERG patch clamp assay

Experiments were performed using HEK293 cells stably expressing the hERG potassium channel. hERG currents were measured using a whole-cell patch clamp technique. Cells were placed in the recording chamber and continuously perfused at a rate of 120 mL/h with perfusing solution at 26.0 ± 2.0 °C. The perfusing solution was composed of the following (mM): NaCl 137; KCl 4; $CaCl_2$ 1.8; $MgCl_2$ 1; HEPES 10; D-glucose 10; pH 7.4. The pipette solution was composed of the following (mM): KCl 130; EGTA 5; HEPES 10; $MgCl_2$ 1; MgATP 5; pH 7.2. The membrane potential was held at −80 mV. A depolarization pulse at −40 mV to +20 mV (4 steps) was applied for 1.5 seconds, with a subsequent repolarization pulse at −50 mV for 1.5 seconds. This cycle was repeated every 15 seconds. Each concentrations of **59** (3, 10, 30, 100, and 300 μM), a positive control (E-4031), and a vehicle control (0.1% DMSO) was applied to the cells. The hERG currents were measured with an amplifier EPC 8 (HEKA Elektronik Dr. Schulze GmbH, Lambrecht, Germany), and the electric signals were recorded using analysis software (pClamp 9, Axon Instruments, Inc.).

4.2.8. Animal Study

All Experiments were approved by the Animal Care and Use Committee of the testing facility, SANWA KAGAKU KENKYUSHO. Male C57BL/6J mice (8 weeks old),

B6.Cg-Lepob/J (ob/ob) mice (8 or 9 weeks old) or male Crlj:CD1 (ICR) mice (8 weeks old) were obtained from Charles River Laboratories Japan, Inc.

4.2.9. Oral Glucose Tolerance Test in mice

Male C57BL/6J mice (8 weeks old) or B6.Cg-*Lep*^{ob}/J (ob/ob) mice (8 weeks old) were obtained from Charles River Laboratories Japan, Inc. Vehicle (0.5% methyl cellulase) was administered orally to fasted animals (n = 8/group) 15 or 30 min before glucose loading (2 g/kg). Tail vein blood glucose levels were measured at −15 or −30, 0, 20, 40, 60, 90, and 120 min using a portable glucometer (GLUTEST ProR; Sanwa Kagaku Kenkyusho, Japan).

4.2.10. Repeated administration study of 59 in ob/ob mice

Male ob/ob mice (9 weeks old) were administered vehicle (0.5% MC) or **59** orally twice a day for 4 weeks. Plasma glucose and insulin levels at 2 weeks and 4 weeks were measured using a portable glucometer (GLUTEST ProR; Sanwa Kagaku Kenkyusho, Japan) and Ultra Sensitive Mouse Insulin ELISA kit (Morinaga Institute of Biological Science, Inc, Japan) respectively. At the end of the study, circulating level of TG was analyzed by biochemistry automatic analyzer 7170S (Hitachi High-Technologies, Japan).

4.2.11. Assessment of hypoglycemia risk in mice

Compounds were administered orally to 2 h-fasted male C57BL/6J mice (8 weeks old) at time 0 min (n = 8/group). Tail vein blood glucose levels were measured at 0, 60, 120, 180, 240, and 300 min using a portable glucometer (GLUTEST ProR; Sanwa Kagaku Kenkyusho, Japan).

4.2.12. Assessment of casual plasma insulin in mice

Compounds were administered orally to 2 h-fasted male C57BL/6J mice (8 weeks old)

at time 0 min (n = 8/group). Tail vein blood insulin levels were measured at 0, 30, and 60 min using an Ultra Sensitive Mouse Insulin ELISA kit (Morinaga Institute of Biological Science, Inc, Japan).

4.2.13. Pharmacokinetic study

Male Crlj:CD1 (ICR) mice (8 weeks old) were housed under standard conditions with a 12 h light/dark cycle and allowed free access to water and a commercial diet (CRF-1, Oriental Yeast Co., LTD., Japan). Mice were fasted overnight before dosing, and fed 7 h post-dose. A single intravenous dose of **59** (2 mg/kg) in saline or a single oral dose of **59** (10 mg/kg) in 0.5 w/v% MC was administrated to each mouse. Blood samples were collected using a heparinized capillaries via the orbital venous plexus under anesthesia at serial time points for 24 h post-dose. Plasma was obtained by centrifugation at 10700 × g for 10 min at 4 °C and stored at −70 °C until analysis. Plasma samples were mixed with acetonitrile and methanol containing an internal standard. The mixtures were vortexed and centrifuged, and the supernatant was diluted three times with 0.1% acetic acid and subjected to LC/MS/MS analysis. Peak areas were calculated by Xcalibur software (Thermo Electron Corporation, UK).

4.2.14. Tissue distribution study

Male C57BL/6J mice (8 weeks old) were housed under standard conditions and allowed free access to water and a commercial diet. On the day before the experiment, mice were fasted until the end of the experiment. A single oral dose of **59** (10, 30, 100 mg/kg) or piragliatin (10, 30 mg/kg) in 0.1 w/v% Tween80 and 0.5 w/v% MC was administrated to each mouse. Blood samples were collected using heparinized syringes via the postcaval vein at 0.5, 1, and 1.5 h post-dose. Plasma was obtained by centrifugation. The liver and pancreas were dissected out and gently washed with ice-cold saline. The dissected tissues were homogenized and stored at −70 °C until analysis. Plasma, liver homogenate, and pancreas homogenate samples were mixed with acetonitrile and methanol containing an internal standard, followed by centrifugation. The supernatant was subjected to LC/MS/MS analysis. Peak areas were calculated using

Xcalibur software (Thermo Electron Corporation, UK) and AUC values were calculated by the trapezoidal rule.

4.2.15. Statistical analysis

The data was analyzed using Dunnett's multiple comparison test. All statistical analyzes were performed using the EXSUS statistical software package version 8.1.0 (CAC Croit Corporation, Tokyo, Japan).

Abbreviations

n-BuLi, *n*-butyllithium; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; GK, glucokinase; GKA, glucokinase activator; GSIS, glucose-stimulated insulin secretion; hERG, human ether-a-go-go related gene; NBS, *N*-bromosuccinimide; OATP, organic anion transporting polypeptide; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.

Appendix A. Supplementary data

Supplementary data related to this article can be found at...

References

[1] IDF Diabetes Atlas. http://www.idf.org/diabetesatlas (accessed Feb. 7, 2018).

[2] T. Scully, Diabetes in Numbers, Nature 485 (2012) S2–3.

[3] C.J. Nolan, P. Damm, M. Prentki, Type 2 diabetes across generations: from pathophysiology to prevention and management, Lancet 378 (2011) 169–181.

[4] M.J. Fowler, Microvascular and macrovascular complications of diabetes, Clin. Diabetes 26 (2008) 77–82.

[5] J.R. Banegas, E. López-García, J. Dallongeville, E. Guallar, J.P. Halcox, C. Borghi, E.L. Massó-González, F.J. Jiménez, J. Perk, P.G. Steg, G. De Backer, F. Rodríguez-Artalejo, Achievement of treatment goals for primary prevention of cardiovascular disease in clinical practice across europe: the EURIKA study, Eur. Heart J. 32 (2011) 2143–2152.

[6] S.E. Inzucchi, R.M. Bergenstal, J.B. Buse, M. Diamant, E. Ferrannini, M. Nauck, A.L. Peters, A. Tsapas, R. Wender, D.R. Matthews, Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the american diabetes association and the european association for the atudy of diabetes, Diabetes Care 38 (2015) 140–149.

[7] F.M. Matschinsky, A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm, Diabetes 45 (1996) 223–241.

[8] F.M. Matschinsky, B. Glaser, M.A. Magnuson, Pancreatic β-cell glucokinase: closing the gap between theoretical concepts and experimental realities, Diabetes 47 (1998) 307–315.

[9] L. Agius, Glucokinase and molecular aspects of liver glycogen metabolism, Biochem. J. 414 (2008) 1–18.

[10] F.M. Matschinsky, M.A. Magnuson, D. Zelent, T.L. Jetton, N. Doliba, Y. Han, R. Taub, J. Grimsby, The network of glucokinase-expressing cells in glucose homeostasis and the potential of glucokinase activators for diabetes therapy, Diabetes 55 (2006) 1–12.

[11] J. Grimsby, R. Sarabu, W.L. Corbett, N.-E. Haynes, F.T. Bizzarro, J.W. Coffey, K.R. Guertin, D.W. Hilliard, R.F. Kester, P.E. Mahaney, L. Marcus, L. Qi, C.L. Spence, J. Tengi, M.A. Magnuson, C.A. Chu, M.T. Dvorozniak, F.M. Matschinsky, J.F. Grippo, Allosteric activators of glucokinase: potential role in diabetes therapy, Science 301 (2003) 370–373.

[12] N.E. Haynes, W.L. Corbett, F.T. Bizzaro, K.R. Guertin, D.W. Hillard, G.W. Holland, R.F. Kester, P.E. Mahaney, L. Qi, C.L. Spence, J. Tengi, M.T. Dvorozniak, A. Railkar, F.M. Matschinsky, J.F. Grippo, J. Grimsby, R. Sarabu, Discovery, structure–activity relationship, pharmacokinetics, and efficacy of glucokinase activator (2R)-3-cyclopentyl-2-(4-methanesulfonylphenyl)-N-thiazol-2-yl-propionamide (RO0281675), J. Med. Chem. 53 (2010) 3618–3625.

[13] F.M. Matschinsky, Assessing the potential of glucokinase activators in diabetes therapy, Nat. Rev. Drug Discov. 8 (2009) 399–416.

[14] R. Sarabu, F.T. Bizzarro, W.L. Corbett, M.T. Dvorozniak, W. Geng, J.F. Grippo, N.E. Haynes, S. Hutchings, L. Garofalo, K.R. Guertin, D.W. Hilliard, M. Kabat, R.F. Kester, W. Ka, Z. Liang, P.E. Mahaney, L. Marcus, F.M. Matschinsky, D. Moore, J. Racha, R. Radinov, Y. Ren, L. Qi, M. Pignatello, C.L. Spence, T.

Steele, J. Tengi, J. Grimsby, Discovery of piragliatin–first glucokinase activator studied in type 2 diabetic patients, J. Med. Chem. 55 (2012) 7021–7036.

[15] M.J. Waring, D.S. Clarke, M.D. Fenwick, L. Godfrey, S.D. C. Groombridge, Johnstone, D. McKerrecher, K.G. Pike, J.W. Rayner, G.R. Robb, I. Wilson, Property based optimizsation of glucokinase activators–discovery of the phase IIb clinical candidate AZD1656, Med. Chem. Commun. 3 (2012) 1077–1081.

[16] G.E. Meininger, R. Scott, M. Alba, Y. Shentu, E. Luo, H. Amin, M.J. Davies, K.D. Kaufman, B.J. Goldstein, Effects of MK-0941, a novel glucokinase activator, on glycemic control in insulin–treated patients with type 2 diabetes, Diabetes Care 34 (2011) 2560–2566.

[17] N. Yoshikawa, F. Xu, J.D. Arredondo, T. Itoh, A large-scale synthesis of potent glucokinase activator MK-0941 via selective O-Arylation and O-Alkylation, Org. Process Res. Dev. 15 (2011) 824–830.

[18] J.A. Pfefferkorn, A. Guzman-Perez, J. Litchfield, R. Aiello, J.L. Treadway, J. Pettersen, M.L. Minich, K.J. Filipski, C.S. Jones, M. Tu, G. Aspnes, H. Risley, J. Bian, B.D. Stevens, P. Bourassa, T. D'Aquila, L. Baker, N. Barucci, A.S. Robertson, F. Bourbonais, D.R. Derksen, M. MacDougall, O. Cabrera, J. Chen, A.L. Lapworth, J.A. Landro, W.J. Zavadoski, K. Atkinson, N. Haddish-Berhane, B. Tan, L. Yao, R.E. Kosa, M.V. Varma, B. Feng, D.B. Duignan, A. El-Kattan, S. Murdande, S. Liu, M. Ammirati, J. Knafels, P. DaSilva-Jardine, L. Sweet, S. Liras, T.P. Rolph, Discovery of (S)-6-(3-cyclopentyl-2-(4-(trifluoromethyl)-1H-imidazol-1-yl)propanamido)nicotinic acid as a hepatoselective glucokinase activator clinical candidate for treating type 2 diabetes mellitus, J. Med. Chem. 55 (2012) 1318–1333.

[19] R.C. Bonadonna, T. Heise, C. Arbet-Engels, C. Kapitza, A. Avogaro, J. Grimsby, J. Zhi, J.F. Grippo, R. Balena, Piragliatin (RO4389620), a novel glucokinase activator, lowers plasma glucose both in the postabsorptive state and after a glucose challenge in patients with type 2 diabetes mellitus: a mechanistic study, J. Clin. Endocrinol. Metab. 95 (2010) 5028–5036.

[20] L. Agius, Lessons from glucokinase activators: the problem of declining efficacy, Expert Opin. Ther. Patents 24 (2014) 1155–1159.

[21] (a) vTv Therapeutics Completes Enrollment of Phase 2b Trial Evaluating TTP399 for the Treatment of Type 2 Diabetes [press release]. http://ir.vtvtherapeutics.com/phoenix.zhtml?c=254081&p=irol-newsArticle&ID=2136914 (accessed Feb. 7, 2018). (b) C. Valcarce, I. Grimes, J. Freeman.

TTP399, a Novel, Liver Selective Glucokinase Activator: Results from a 10 Day Pilot Study in Patients with Type 2 Diabetes Mellitus (T2DM) Naïve to Drug. Presented at 76th American Diabetes Association Meeting, New Orleans, LA, 2016. (c) C. Valcarce. The Importance of Tissue Selectivity and Preservation of the Physiological Regulation when Targeting Key Metabolic Regulators as Glucokinase. Presented at the Keystone Symposia on New Therapeutics for Diabetes and Obesity, La Jolla, CA, 2016. (d) C. Valcarce. TTP399, A Liver Selective Glucokinase Activator Increases Efficacy of Currently Marketed Therapies for Type 2 Diabetes. Presented at 75th American Diabetes Association Meeting, Boston, MA, 2015. (e) C. Valcarce. TTP399, a Liver-Selective Glucose Kinase Activator (GKA), Lowers Glucose and Does NOT Increase Lipids in Subjects with Type 2 Diabetes Mellitus (T2DM). Presented at 74th American Diabetes Association Meeting, San Francisco, CA, 2014. (f) M.G. Rees, D. Ng, S. Ruppert, C. Turner, N.L. Beer, A.J. Swift, M.A. Morken, J.E. Below, I. Blech; NISC Comparative Sequencing Program, J.C. Mullikin, M.I. McCarthy, L.G. Biesecker, A.L. Gloyn, F.S. Collins, Correlation of rare coding variants in the gene encoding human glucokinase regulatory protein with phenotypic, cellular, and kinetic outcomes, J. Clin. Invest. 122 (2012) 205–217.

[22] (a) Hua Medicine Announces First-Ever Successful Phase 2 Monotherapy Trial for New Class of Diabetes Drug [press release]. http://www.huamedicine.com/show_news.asp?s_id=1 (accessed Feb. 7, 2018). (b) M.S. Dzyurkevich, D.A. Babkov, N.V. Shtyrlin, O.Y. Mayka, A.G. Iksanova, P.M. Vassiliev, K.V. Balakin, A.A. Spasov, V.V. Tarasov, G. Barreto, Y.G. Shtyrlin, G. Aliev, Pyridoxine dipharmacophore derivatives as potent glucokinase activators for the treatment of type 2 diabetes mellitus., Sci. Rep. 22 (2017) 16072.

[23] H. Xu, L. Sheng, W. Chen, F. Yuan, M. Yang, H. Li, X. Li, J. Choi, G. Zhao, T. Hu, Y. Li, Y. Zhang, L. Chen, Safety, tolerability, pharmacokinetics, and pharmacodynamics of novel glucokinase activator HMS5552: results from a first-in-human single ascending dose study, Drug Des. Devel. Ther. 10 (2016) 1619–1626.

[24] M. Lang, M.H. Seifert, K.K. Wolf, A. Aschenbrenner, R. Baumgartner, T. Wieber, V. Trentinaglia, M. Blisse, N. Tajima, T. Yamashita, D. Vitt, H. Noda,

Discovery and hit-to-lead optimization of novel allosteric glucokinase activators, Bioorg. Med. Chem. Lett. 21 (2011) 5417–5422.

[25] T. Ghaffer, A.W. Parkins, A new homogeneous platinum containing catalyst for the hydrolysis of nitriles, Tetrahedron. Lett. 36 (1995) 8657–8660.

[26] A. Klapars, X. Huang, S.L. Buchwald, A general and efficient copper catalyst for the amidation of aryl halides, J. Am. Chem. Soc. 124 (2002) 7421–7428.

[27] Log *D* value at pH 7.4 is calculated using Pipeline Pilot ver.9.5.0.831.

[28] F. Lovering, J. Bikker, C. Humblet, Escape from flatland: increasing saturation as an approach to improving clinical success, J. Med. Chem. 52 (2009) 6752–6756.

[29] K. Minami, H. Yano, T. Miki, K. Nagashima, C.-Z. Wang, H. Tanaka, J. Miyazaki, S. Seino, Insulin secretion and differential gene expression in glucose-responsive and -unresponsive MIN6 sublines, Am. J. Physiol. Endocrinol. Metab. 272 (2000) E773–E781.

[30] B. Fermini, A.A. Fossa, The impact of drug-induced QT interval prolongation on drug discovery and development, Nat. Rev. Drug Discov. 2 (2003) 439–447.

[31] C. Jamieson, E.M. Moir, Z. Rankovic, G. Wishart, Medicinal chemistry of hERG optimizations: highlights and hang-ups, J. Med. Chem. 49 (2006) 5029–5046.

[32] S.R. Fletcher, F. Burkamp, P. Blurton, S.K.F. Cheng, R. Clarkson, D. O¢Connor, D. Spinks, M. Tudge, M.B. van Niel, S. Patel, K. Chapman, R. Marwood, S. Shepheard, G. Bentley, G.P. Cook, L.J. Bristow, J.L. Castro, P.H. Hutson, A.M. MacLeod, 4-(Phenylsulfonyl)piperidines: novel, selective, and bioavailable 5-HT_{2A} receptor antagonists, J. Med. Chem. 45 (2002) 492–503.

[33] J. Liang, R.M. Brochu, C.J. Cohen, I.E. Dick, J.P. Felix, M.H. Fisher, M.L. Garcia, G.J. Kaczorowski, K.A. Lyons, P.T. Meinke, B.T. Priest, W.A. Schmalhofer, M.M. Smith, J.W. Tarpley, B.S. Williams, W.J. Martin, W.H. Parsons, Discovery of potent and use-dependent sodium channel blockers for treatment of chronic pain, Bioorg. Med. Chem. Lett. 15 (2005) 2943–2947.

[34] J. Xu, L. Wei, R. Mathvink, J. He, Y.J. Park, H. He, B. Leiting, K.A. Lyons, F. Marsilio, R.A. Patel, J.K. Wu, N.A. Thornberry, A.E. Weber, Discovery of potent and selective phenylalanine based dipeptidyl peptidase IV inhibitors, Bioorg. Med. Chem. Lett. 15 (2005) 2533–2536.

[35] S.D. Edmondson, A. Mastracchio, M. Beconi, L.F.Jr. Colwell, B. Habulihaz, H. He, S. Kumar, B. Leiting, K.A. Lyons, A. Mao, F. Marsilio, R.A. Patel, J.K.

Wu, L. Zhu, N.A. Thornberry, A.E. Weber, E.R. Parmee, Potent and selective proline derived dipeptidyl peptidase IV inhibitors, Bioorg. Med. Chem. Lett. 14 (2004) 5151–5155.

[36] Plasma concentration of compounds was confirmed through a single time point 2 h after the compound was dosed.

[37] J. Xu, S. Lin, R.W. Myers, M.E. Trujillo, M.J. Pachanski, S. Malkani, H-s. Chen, Z. Chen, B. Campbell, G.J. Eiermann, N. Elowe, B.T. Farrer, W. Feng, Q. Fu, R. Kats-Kagan, M. Kavana, D.R. McMasters, K. Mitra, X. Tong, L. Xu, F. Zhang, R. Zhang, G.H. Addona, J.P. Berger, B. Zhang, E.R. Parmee, Discovery of orally active hepatoselective glucokinase activators for treatment of type II diabetes mellitus, Bioorg. Med. Chem. Lett. 27 (2017) 2063–2068.

[38] International Transporter Consortium, K.M. Giacomini, S.M. Huang, D.J. Tweedie, L.Z. Benet, K.L. Brouwer, X. Chu, A. Dahlin, R. Evers, V. Fischer, K.M. Hillgren, K.A. Hoffmaster, T. Ishikawa, D. Keppler, R.B. Kim, C.A. Lee, M. Niemi, J.W. Polli, Y. Sugiyama, P.W. Swaan, J.A. Ware, S.H. Wright, S.W. Yee, M.J. Zamek-Gliszczynski, L. Zhang, Membrane transporters in drug development, Nat. Rev. Drug Discov. 9 (2010) 215–236.

[39] T. Ferre, E. Riu, S. Franckhauser, J. Agudo, F. Bosch, Long-term overexpression of glucokinase in the liver of transgenic mice leads to insulin resistance, Diabetologia. 46 (2003) 1662–1668.

[40] J.P. Wilding, M. Leonsson-Zachrisson, C. Wessman, E. Johnsson, Dose-ranging study with the glucokinase activator AZD1656 in patients with type 2 diabetes mellitus on metformin, Diabetes Obes. Metab. 15 (2013) 750–759.

[41] S. Tornovsky-Babeay, D. Dadon, O. Ziv, E. Tzipilevich, T. Kadosh, Schyr-Ben. R. Haroush. A. Hija, M. Stolovich-Rain, J. Furth-Lavi, Z. Granot, S. Porat, L.H. Philipson, K.C. Herold, T.R. Bhatti, C. Stanley, F.M. Ashcroft, P. In't Veld, A. Saada, M.A. Magnuson, B. Glaser, Y. Dor, Type 2 diabetes and congenital hyperinsulinism cause DNA double-strand nreaks and p53 activity in β cells, Cell Metab. 19 (2014) 109–121.

Highlights

- Novel thiophene derivatives as GKAs were designed and synthesized.
- Several GKAs exhibited high GK activation potency.
- GKA **59** showed potent activity in the OGTT in normal and diabetic mouse.
- GKA **59** has a favorable safety profile.