Bioorganic & Medicinal Chemistry Letters 23 (2013) 537–542

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a novel phenylethyl benzamide glucokinase activator for the treatment of type 2 diabetes mellitus

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ARTICLE INFO

Article history:
Received 21 September 2012
Revised 16 October 2012
Accepted 7 November 2012
Available online 16 November 2012

Keywords:
Glucokinase
Glucokinase activator
GKAs
YH-GKA
T2DM
Type 2 diabetes mellitus
OGTT

ABSTRACT

Novel benzamide derivatives were synthesized and tested at in vitro assay by measuring fold increase of glucokinase activity at 5.0 mM glucose concentration. Among the prepared compounds, YH-GKA was found to be an active glucokinase activator with EC_{50} of 70 nM. YH-GKA showed similar glucose AUC reduction of 29.6% (50 mg/kg) in an OGTT study with C57BL/J6 mice compared to 29.9% for metformin (300 mg/kg). Acute treatment of the compound in C57BL/J6 and ob/ob mice elicited basal glucose lowering activity. In subchronic study with ob/ob mice, YH-GKA showed significant decrease in blood glucose levels and no adverse effects on serum lipids or body weight. In addition, YH-GKA exhibited high bioavailability and moderate elimination in preclinical species.

Type 2 diabetes mellitus (T2DM) is a rapidly growing public epidemic which affects over 300 million people worldwide. Rates of diabetes have increased noticeably over the last 50 years with the similar trend of increasing rates of obesity, which is thought to be one of the primary causes of type 2 diabetes.[1] The first line oral therapy for type 2 diabetes mellitus (T2DM) is metformin and second line oral therapies[2] are sulfonylureas (SU), dipeptidyl peptidase-4 (DPP-4) inhibitors and thiazolidinediones (TZD) in combination with metformin. However, currently available antidiabetic agents have limited long-term efficacy and trade-offs in efficacy and safety/tolerability. Therefore, the clinical need for improved T2DM therapies remains high and diabetes patients are eager to have novel therapeutic options to safely achieve tight glycemic control.

Glucokinase (GK) is a hexokinase isozyme (hexokinase IV, hexokinase D) with 465 amino acids (molecular weight = 50 kD). Glucokinase facilitates phosphorylation of glucose to glucose-6-phosphate (G6P), which is the first step of both glycogen synthesis and glycolysis. Glucokinase exists in cells in the liver, pancreas, gut, and brain of humans and most other vertebrates. Compared to other hexokinases, glucokinase has a lower affinity for glucose and its activity is localized to a few cell types. Due to this reduced affinity for glucose, the activity of glucokinase varies substantially with the concentration of glucose. Furthermore, unlike other hexokinases, glucokinase is not inhibited by its product, glucose-6-phosphate[3] and distinctively, glucokinase shows moderate cooperativity with glucose with a Hill coefficient (n_H) of about 1.7.[4] Because of this moderate cooperativity, classical Michaelis-Menten kinetics do not apply to the kinetic interaction of glucokinase with glucose.[5] So, instead of using a K_m for glucose, a half-saturation level $S_{0.5}$, which is the concentration at which the enzyme is 50% saturated and active, is used for glucokinase. Glucokinase acts as a glucose sensor regulating hepatic glucose metabolism to provide approximately 95% of the hexokinase activity in hepatocytes.[4] In addition, glucokinase activity serves as a key control for glucose-dependent insulin secretion in islet beta cells.[6] Glucokinase activator (GKA) is expectedly associated with a dual mechanism for lowering blood glucose concentration by the enhancement of insulin secretion from pancreatic beta cell and glucose uptake in the liver. Therefore, Glucokinase has been an attractive target for antidiabetic therapy. Several glucokokinase activators (GKAs) have advanced to clinical studies and have shown to lower both fasting and postprandial glucose in healthy subjects and T2DM patients. Hypoglycemia has been revealed as one of main adverse effects of GKAs. To overcome this hypoglycemia issue, several clinical strategies have been employed including dose titration and more frequent dosing times. Initially, Yuhan adopted this dose titration clinical strategy to reduce the possibility of hypoglycemia caused

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http://dx.doi.org/10.1016/j.bmcl.2012.11.018

by GKA. Recently, two strategies have been employed to reduce the potential for inducing hypoglycemia. One strategy is the design of partial activators that improve the dependence of enzymatic activity on various physiological glucose levels. The other is to make liver selective glucose activators[7–9] that restrict the main enzyme activity at liver since hypoglycemia risk is postulated to result from the increase of pancreatic insulin secretion at low glucose levels.

Based on the dual action of hepatic and pancreatic effects, GKAs represent novel and promising approach for the treatment of type 2 diabetes. Many small molecule allosteric activators of this enzyme have been investigated by numerous pharmaceutical companies in the past decade.[10–12] Selected representative small molecule GKAs are shown in Figure 1. Since Grimsby reported small molecule allosteric GKAs in 2003,[13] a phenylacetamide series of activators including clinical candidate 2,[14] have been identified. Also, a variety of other GKAs have been reported, such as benzamides (**1**,**4**–**7**)[15–19] and imidazolylacetamide (**8**).[7] In 2009, Banyu scientists reported the co-crystal structure of glucokinase-compound **1** complex that revealed binding mode at an allosteric site of glucokinase. Having this structural information available to us, the rational compound modification for further improvements to the compound-target binding motifs has been performed in short time. Herein we report the discovery of YH-GKA, a benzamide gulcokinase activator, as a potential preclinical candidate for the treatment of T2DM.

The benzamide scaffold was chosen as a starting point for the synthesis of selective GKAs which would bind to the allosteric binding site of the protein and achieve anti-hyperglycemic effects. Various benzamide derivatives were prepared based on the binding mode analysis from the X-ray structure of the allosteric binding site of glucokinase as shown in Figure 2. The A-part of the molecule is required to have both hydrogen bond donor (NH) and hydrogen bond acceptor (=N) to bind to Arg63 favorably. The B moiety is required to be of small size with potential hydrophobic interactions with Tyr214, Tyr215 and Leu451. The C-pocket of the enzyme is fairly large and long and the end part of C-moiety has the potential for a hydrogen bonding interaction with Arg250 in order to increase binding affinity.

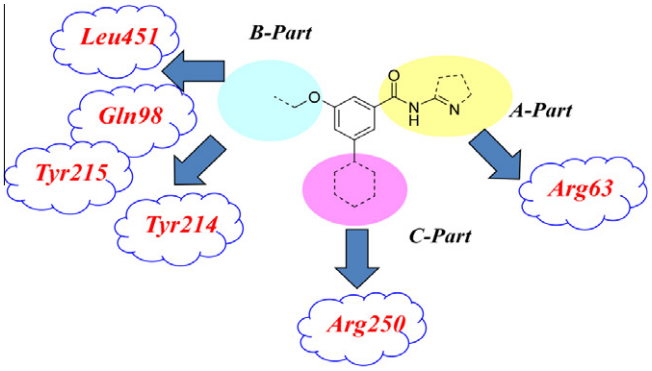


Figure 2. Synthetic strategy for benzamide GKAs.

Hundreds of benzamide derivatives having various A-, B-, and C-part substituents have been synthesized at Yuhan.[20] (*R*)-(−)-1-Methoxy-2-propanoxy group was identified as an optimum moiety through initial diversification of B-part while A- and C-parts were limited. Then A-part had been investigated thoroughly by introducing a variety of aryl or hetero-aromatic groups. Finally, C-part was optimized by prioritizing compounds based on in vitro potency, physicochemical property and in vivo activity. The synthesis of selected compounds, pyrazol benzamide series and carboxypyridine benzamide series, is described in Scheme 1. Mitsunobu reaction of dimethyl 5-hydroxy isophthalate with (*R*)-(−)-1-methoxy-2-propanol gave benzoic acid **9**. Reduction of **9** followed by acetylation provided ester **11**. Amide coupling of **11** with 1-methyl-1*H*-pyrazol-3-ylamine and the hydrolysis of resulting ester **12** followed by the treatment with PBr_3 yielded benzyl bromide **13**. The benzyl bromide **13** was treated with triethylphosphite to give phosphonate **14**. Horner-Wadsworth-Emmons reaction between **14** and a variety of aldehydes provided various E-alkene benzamides **15**. Pyrazol benzoamides **15** were reduced by Pd/H_2 to give final products **16**. Likewise, amide coupling of **11** with 6-aminonicotinic acid methyl ester gave nicotinic ester **17**. E-alkene

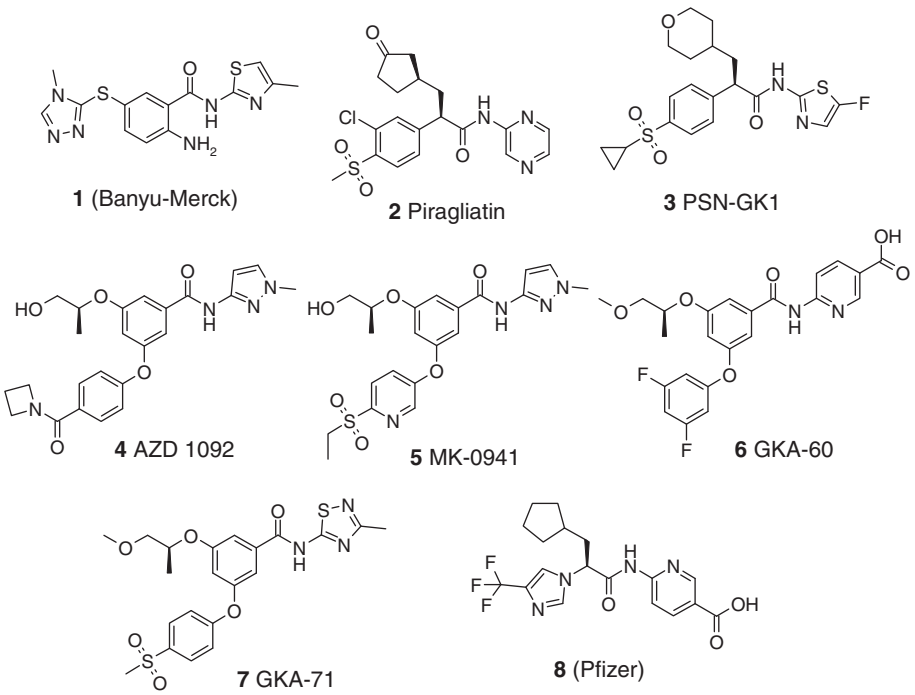
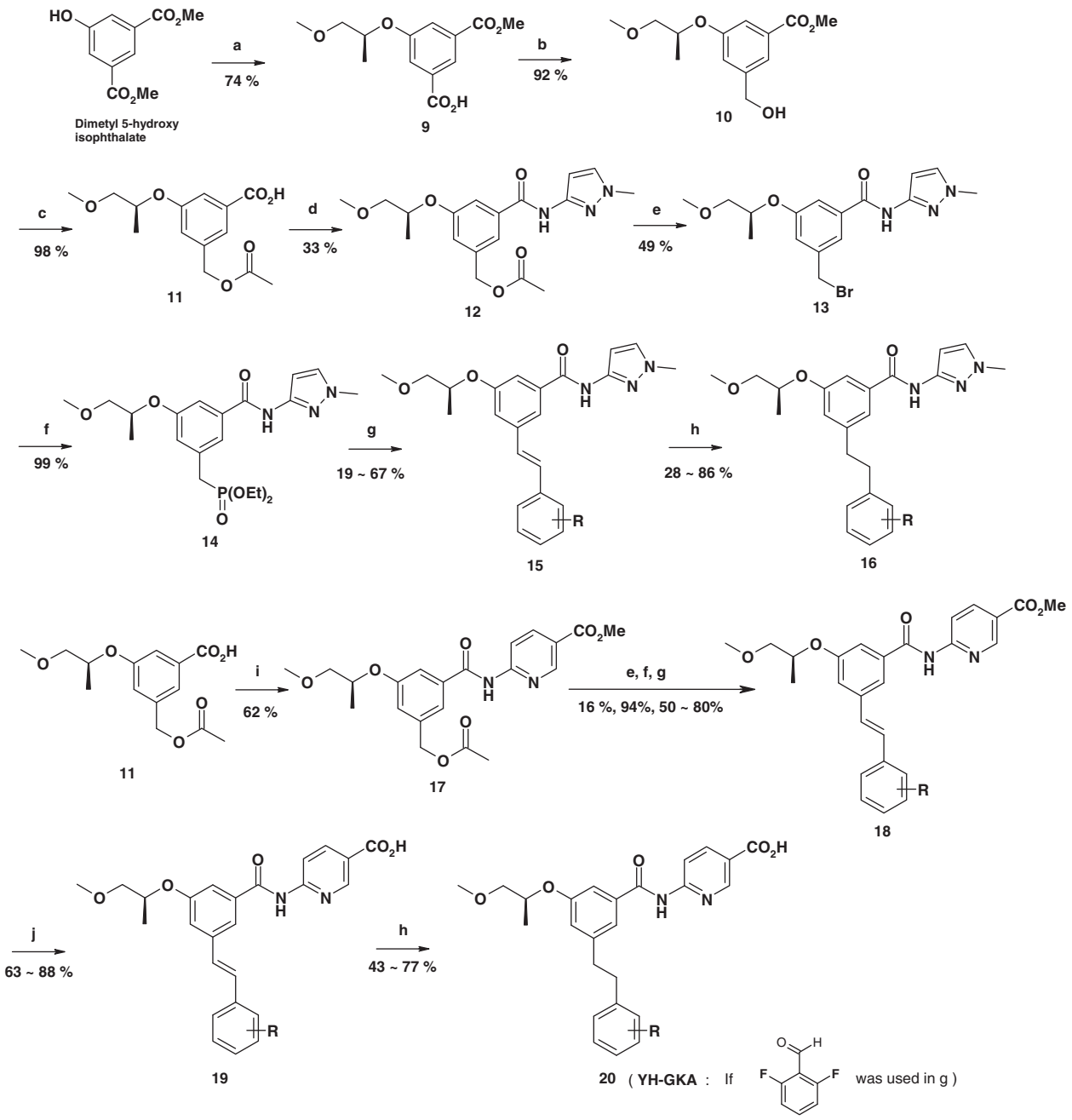


Figure 1. Representative structures of GKAs.

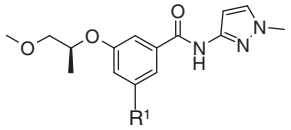


Scheme 1. Reagents and conditions: (a) (i) (*R*)-(−)-1-methoxy-2-propanol, PPh_3, DIAD, THF, (ii) KOH, MeOH, reflux; (b) 1 M BH_3/THF; (c) (i) 3 N NaOH, THF, (ii) acetyl chloride, CH_2Cl_2, pyridine, water; (d) 1-methyl-1*H*-pyrazol-3-ylamine, HOBT, EDAC, TEA, CH_2Cl_2, (e) (i) NaOMe, MeOH/THF, (ii) PBr_3, THF; (f) triethylphosphite, reflux; (g) aldehyde, *t*-BuOK, THF; (h) Pd/C, H_2-gas, MeOH; (i) (i) $SOCl_2$, reflux, (ii) 6-aminonicotinic acid methyl ester, pyridine; (j) 3 N NaOH, THF.

benzamides **18** were prepared by using the same reactions that were used for the synthesis of **15**. Nicotinic esters **18** were hydrolyzed to form carboxylic acids **19** and **19** were reduced by Pd/H_2 to give final products **20**. The prepared benzamides were tested at in vitro assay by measuring fold increase of glucokinase activity at 5.0 mM glucose concentration.

Introduction of nitro, fluorine or methoxy groups in the terminal phenyl ring of pyrazol benzamide derivatives increased potency with EC_{50} of submicromolar activity (Table 1). Replacement of the terminal phenyl ring with methyl pyridine group (**16k**) sustained a good potency. 4′-Substituent (**16c**) tended to give somewhat lower potency than 2′- or 3′-nitro substituent (**16a**, **16b**). Tri-fluorine substituted compound **16i** exhibited a considerable decrease in potency compared to mono- or di-fluorinated analogs (**16d–h**). Reduction of E-alkenes (**16d–j**) to the corresponding alkanes (**16l–q**) resulted in retained potency. In order to improve physicochemical property, pyrazol moiety in benzamide derivatives (**16**) was replaced with nicotinic acid group (**20**) (Table 2). All mono-nitro substituted analogs (**20a–c**) exhibited a good activity with EC_{50} <300 nM. Like pyrazol benzamide derivatives, tri-fluorine substituted compound **20i** exhibited a 6- to 14-fold decrease in potency relative to mono- or di-fluorinated analogs (**20d–h**). The E-alkenes that showed an EC_{50} <100 nM (**20d**, **20f**, **20h**, **20j**) were further reduced to the corresponding alkanes. Among the reduced compounds, only **20k** (YH-GKA) exhibited a similar potency to the corresponding E-alkene **20h**. YH-GKA (6-[3-[2-(2,6-difluoro-phenyl)-ethyl]-5-(2-methoxy-1-methyl-ethoxy)-benzoyl-amino]-nicotinic acid) was found to be the most active GKA with favorable physicochemical property.

Table 1
In vitro glucokinase activity of pyrazole benzamide derivatives **16**



16a - q

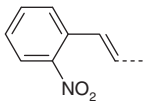
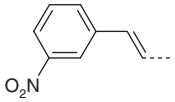
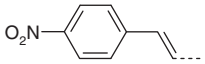
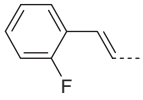
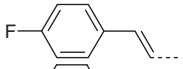
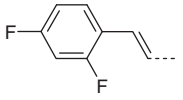
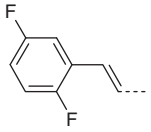
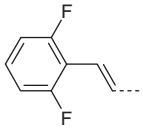
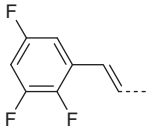
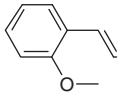
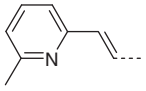
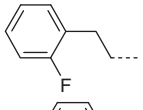
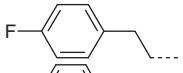
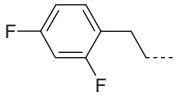
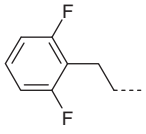
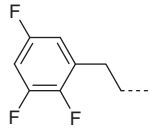
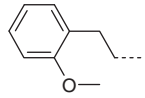
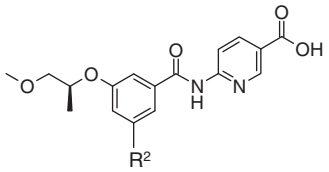
Compound	R^1	hGK EC_{50}[a] (nM)
16a		283
16b		239
16c		852
16d		80
16e		119
16f		109
16g		200
16h		161
16i		848
16j		115
16k		132
16l		217
16m		216
16n		184
16o		131

Table 1 (*continued*)

Compound	R^1	hGK EC_{50}[a] (nM)
16p		855
16q		135

[a] Mean of at least duplicate runs.

Table 2
In vitro glucokinase activity of carboxypyridine benzamide derivatives **20**



20a - k

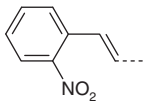
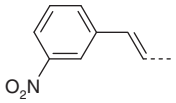
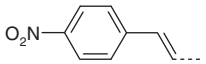
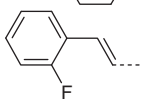
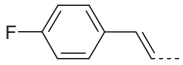
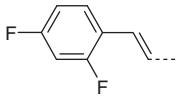
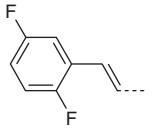
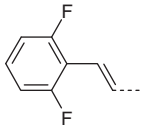
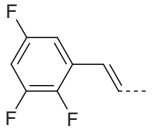
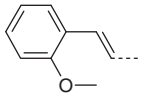
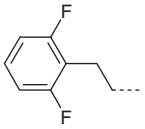
Compound	R^2	hGK EC_{50} (nM)[a]
20a		296
20b		165
20c		284
20d		56
20e		120
20f		95
20g		134
20h		90
20i		778
20j		83

Table 2 (*continued*)

Compound	R^2	hGK EC_{50} (nM)[a]
20k (YH-GKA)		70

[a] Mean of at least duplicate runs.

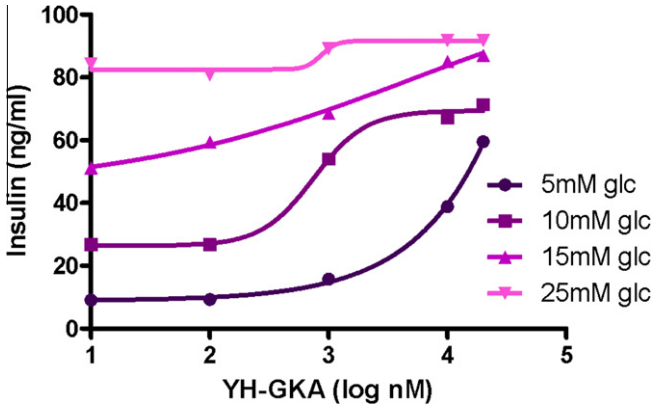


Figure 3. Glucose dependent insulin secretion.

An enzymatic glucokinase assay using purified recombinant human pancreatic glucokinase and liver glucokinase was used to evaluate compounds. Selectivity against hexokinase 1 and 2 was tested using enzymatic hexokinase 1 and 2 assays. MIN-6 cells, mouse pancreatic beta-cell line, were used to evaluate the effect of glucokinase activity on glucose dependent insulin secretion. The change of basal blood glucose levels and oral glucose tolerance (OGT) in non-diabetic (C57BL/J6) and diabetic (DIO, ob/ob) mice after oral administration was evaluated. The levels of blood glucose and OGT were measured in mice after repeated oral dosing. YH-GKA showed human pancreatic glucokinase activity of EC_{50} = 70 nM at 5.0 mM glucose with a half maximum saturation concentration ($S_{0.5}$) of 1.27 mM glucose and maximum reaction rate (V_{max}) of 130%. YH-GKA also activated human hepatic glucokinase with an EC_{50} of 85 nM and did not affect hexokinase 1 and 2. The compound increased insulin secretion from MIN-6 cells in a glucose dependent manner (Fig. 3). It also improved oral glucose tolerance in C57BL/J6 mice in a dose-dependent manner (Fig. 4).

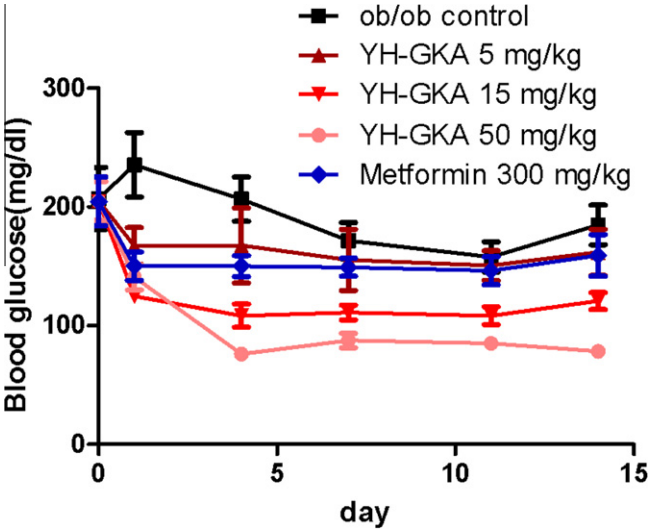


Figure 5. Glucose lowering activity of YH-GKA in ob/ob mice.

Oral glucose tolerance test (OGTT) of YH-GKA at 50 mg/kg in C57BL/J6 mice showed similar glucose area under the curve (AUC) reduction of 29.6%, closely comparable to that of 29.9% for metformin at 300 mg/kg. As shown in Figure 4, YH-GKA showed the indication of hypoglycemia risk at 150 mg/kg. However, the hypoglycemia risk diminished at 50 mg/kg and this dose was chosen for further study in disease animal models. Acute treatment of YH-GKA in C57BL/J6 and ob/ob mice induced basal glucose lowering activity (data not shown). In a subchronic study with ob/ob mice, YH-GKA showed significant decrease in blood glucose levels (Fig. 5) and no adverse effects on serum lipids or body weight. These studies strongly support our position that YH-GKA is a promising candidate for a study in humans as a therapeutic agent for type 2 diabetes mellitus.

In vitro metabolism studies for CYP inhibition and CYP induction were conducted using human liver microsomes and a PXR reporter gene assay. Metabolic stability studies were also performed using liver microsomes from mouse, dog and human. Pharmacokinetics of YH-GKA in mice and dogs was determined following single intravenous and oral administration. YH-GKA showed acceptable metabolic stability across species with that more than 60% of compound remained after 1 h incubation. In vitro CYP inhibition (CYP1A2, 2C9, 2C19, 2D6, 3A) and CYP induction (CYP3A4) suggested no inhibitory or induction effect up to 25–50 μM concentration of YH-GKA. Pharmacokinetic study of YH-GKA in mice

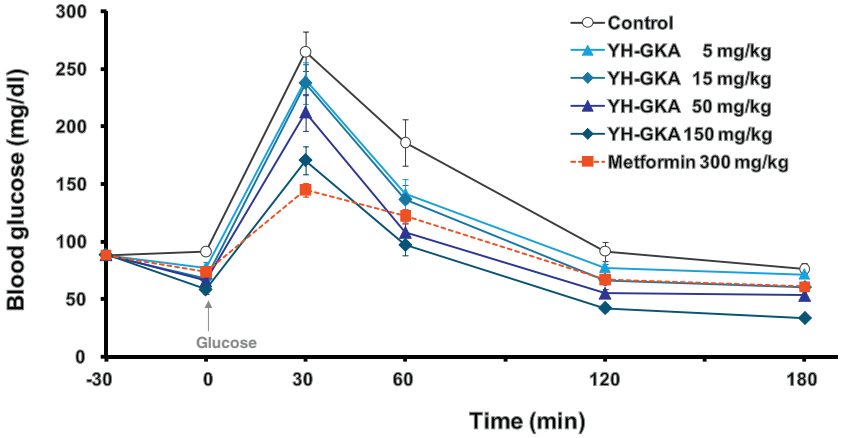


Figure 4. Plasma blood glucose lowering effect of YH-GKA in C57BL/J6 mice OGTT.

and dogs established mean clearance ranging from 0.15 to 0.43 L/h/kg, distribution volume ranging from 0.33 to 0.49 L/kg, elimination half-life ranging from 2.6 to 3.7 h, and bioavailability (F) ranging from 66% to 144%. There was no significant pharmacokinetic difference observed between mice and dogs. Overall, YH-GKA exhibited high bioavailability and moderate elimination in preclinical species. In vitro examination of CYP inhibition and induction suggested that YH-GKA has low risk of drug–drug interactions in humans. These results indicate that YH-GKA has a highly favorable pharmacokinetic profile for an oral anti-diabetic agent. Unsurprisingly, YH-GKA (EC_{50} = 70 nM, $T_{1/2}$ = 2.6 h and F = 85% in dog) possesses comparable potency, physicochemical property and pharmacokinetic profiles with **6** (GKA-60, EC_{50} = 90 nM, $T_{1/2}$ = 4.9 h and F = 100% in dog)[18] presumably due to the structural similarity. Since Waring et al. recently reported that pyridine-5-carboxylic acid containing GKAs (e.g., **6**, GKA-60) might cause testicular toxicology,[21] the testicular toxicity test for YH-GKA will be monitored carefully and the results will be reported in due course.

In summary, YH-GKA was found to be an active GKA with EC_{50} of 70 nM and showed glucose reduction of 29.6% (50 mg/kg) in an OGTT study, equivalent to 300 mg/kg metformin. Acute treatment in C57BL/J6 and ob/ob mice elicited basal glucose lowering activity. Also YH-GKA showed significant decrease in blood glucose levels and no adverse effects on serum lipids or body weight at a subchronic study in ob/ob mice. In addition, YH-GKA exhibited high bioavailability and moderate elimination in mice and dogs. In conclusion, YH-GKA is a promising preclinical lead candidate for type 2 diabetes mellitus. To identify additional preclinical GKA candidates with better efficacy and improved safety profile without hypoglycemia risk, we are performing further lead optimization of the benzamide scaffold by utilizing an innovative and translational screening strategy containing optimized in vitro, ex vivo, in vivo biological assays. The study results from this new strategy will be reported soon.

Acknowledgments

This study was supported by a Grant of Chungcheong Leading Industry Office for Medicine & Bio Projects, Ministry of Knowledge Economy, Republic of Korea (C-2-1, 500108, 7007618). We thank Mr. Kyeong Bae Kim and Dr. Su Youn Nam for their helpful discussion.

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