

Report on Investigational Drugs

Identification of YH-GKA, a Novel Benzamide Glucokinase Activator as Therapeutic Candidate for Type 2 Diabetes Mellitus

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Glucokinase activator is expectedly associated with a dual mechanism for lowering blood glucose concentration by the enhancement of glucose uptake in the liver and insulin secretion from pancreatic beta cell. Therefore, glucokinase has been an attractive target for anti-diabetic therapy. Novel benzamide derivatives were synthesized and tested using *in vitro* assays 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 and glucose area under the curve reduction of 29.6% at 50 mg/kg in an oral glucose tolerance test. In a 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. Overall, YH-GKA is a promising candidate for the therapy of type 2 diabetes mellitus.

Hyperglycemia is mostly caused by defects of insulin secretion or insulin action and triggers a metabolic disease, diabetes mellitus. The chronic hyperglycemia of diabetes is associated with macrovascular complications (Fowler, 2008) including coronary artery disease, peripheral arterial disease and stroke. Diabetes also causes microvascular complications such as diabetic retinopathy, diabetic nephropathy and diabetic neuropathy.

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 (Kahn et al., 2006). The first line oral therapy for T2DM is metformin and the second line oral therapies are sulfonylureas, dipeptidyl peptidase-4 inhibitors and thiazolidinediones in combination with metformin (Lebovitz, 2005). However, currently available anti-diabetic 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 glyceemic control.

Glucokinase 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, 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 of 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 and distinctively, glucokinase shows moderate cooperativity with glucose with a Hill coefficient (n_H) of about 1.7 (Matschinsky, 1996). Because of this moderate cooperativity, classical Michaelis-Menten kinetics do not apply to the kinetic interaction of glucokinase with glucose (Heredia et al., 2006). 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

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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 (Matschinsky, 1996). In addition, glucokinase activity serves as a key control for glucose-dependent insulin secretion in islet beta cells (Matschinsky et al., 1998). Glucokinase activator (GKA) is expectedly associated with a dual mechanism for lowering blood glucose concentration by the enhancement of glucose uptake in the liver and insulin secretion from pancreatic beta cell (Fig. 1). Therefore, glucokinase has been an attractive target for anti-diabetic therapy.

Based on the dual action of hepatic and pancreatic effects, glucokinase activators (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 (Fyfe and

Procter, 2009; Matschinsky, 2009; Iino et al., 2010; Sidduri et al., 2010). Selected representative small molecule GKAs are shown in Fig. 2. Since Grimsby reported small molecule allosteric GKAs in 2003 (Grimsby et al., 2003), a phenylacetamide series of activators including clinical candidate **2** (Sarabu et al., 2011), have been identified. Also, a variety of other GKAs have been reported, such as benzamides (**1,4,-7**) (Mitsuya et al., 2009; Meininger et al., 2011; Pike et al., 2011; Waring et al., 2011; Winzell et al., 2011) and imidazolylacetamide (**8**) (Pfefferkorn et al., 2012). In 2009, Banyu scientists reported the co-crystal structure of glucokinase-compound **1** complex that revealed binding mode at an allosteric site of glucokinase (Mitsuya et al., 2009). Having this structural information available to us, the rational compound modification for further improvements to the compound-target binding motifs has been performed in a short time. Herein we report the discovery of YH-GKA, a benzamide GKA, 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 Fig. 3. 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.

Hundreds of benzamide derivatives having various A-, B-, and C-part substituents have been synthesized at Yuhan (Yi et al., 2011; WO 2011/081280 A2). The prepared benzamides were tested using *in vitro* assays by measuring fold increase of glucokinase activity at 5.0 mM glucose concentration and YH-GKA was found to be the most active GKA in *in vitro* and *in vivo* assays.

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

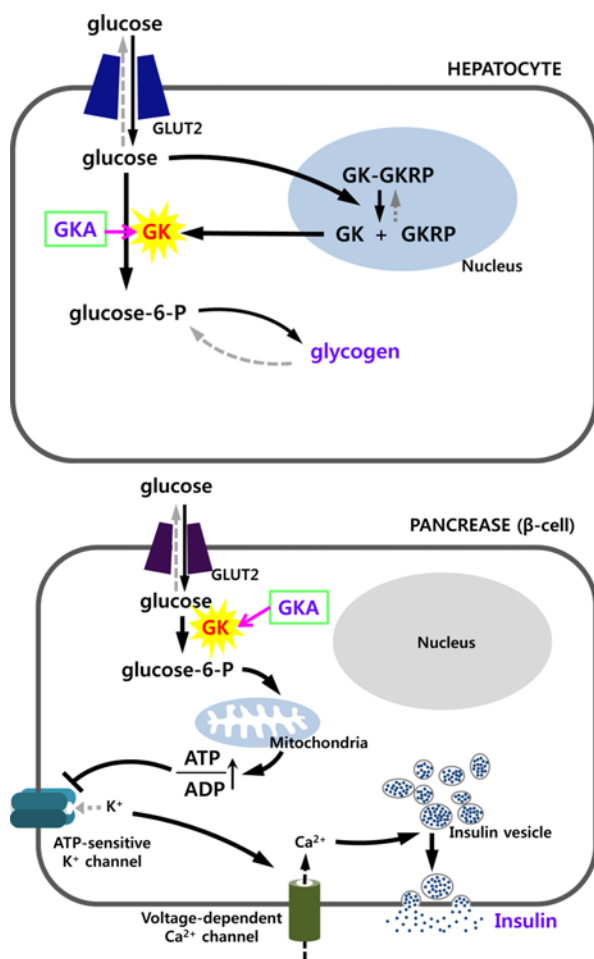


Fig. 1. Mechanism of dual action of GKA in hepatocyte and in β -cell (GK = glucokinase, GKA = glucokinase activator, GKRP = glucokinase regulatory protein, GLUT2 = type 2 glucose transporters).

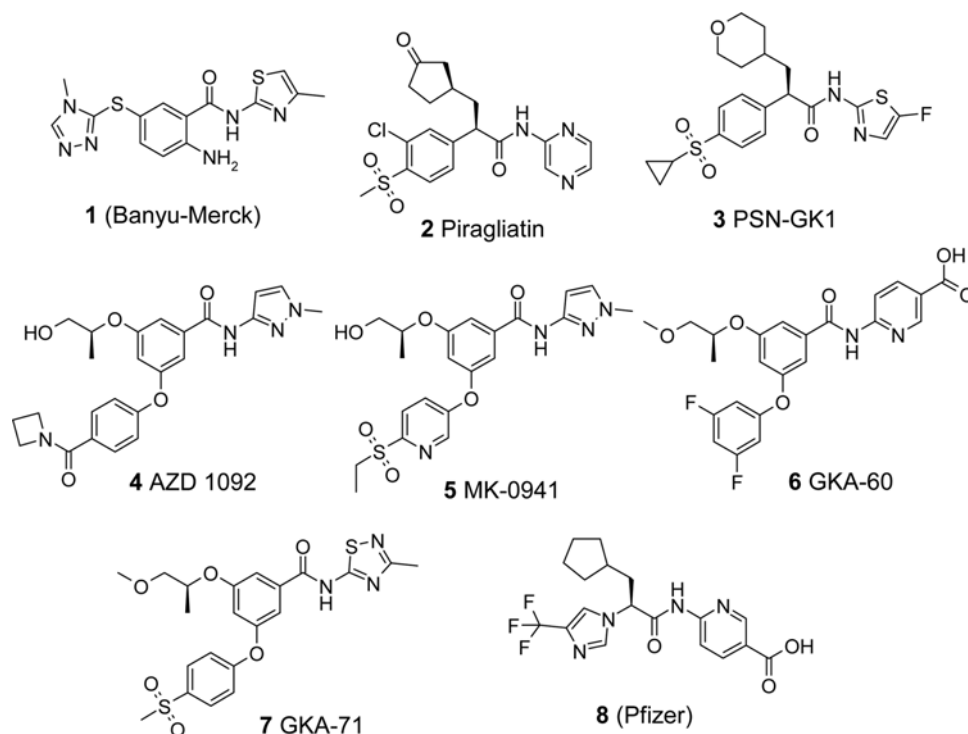


Fig. 2. Representative structures of GKAs.

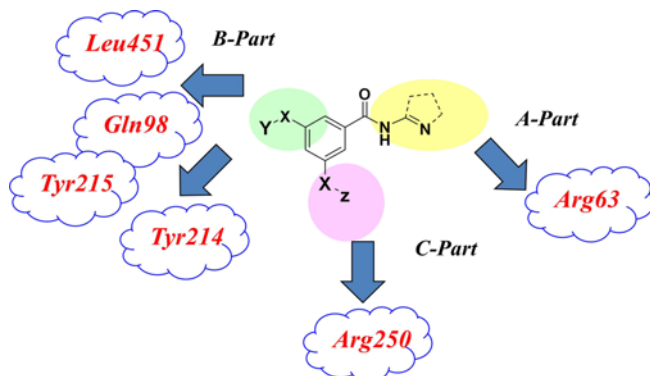


Fig. 3. Synthetic strategy for benzamide GKAs.

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 binding mode analysis of YH-GKA shown in Fig. 4 indicated that A part of YH-GKA could be involved in strong hydrogen bonding interactions with Arg63 in an allosteric site of glucokinase.

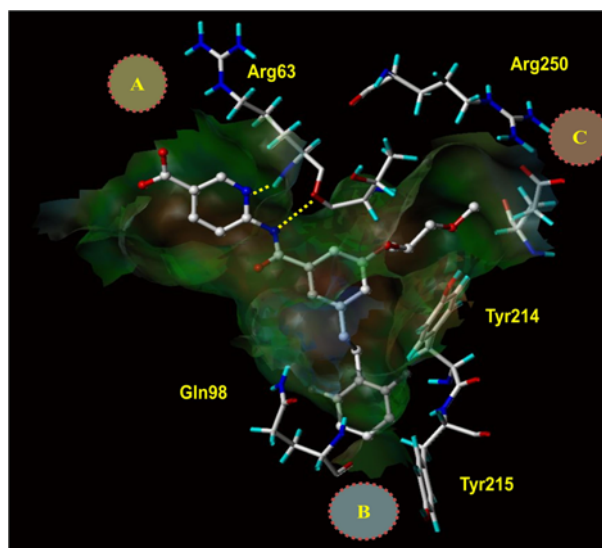


Fig. 4. The binding mode analysis of YH-GKA.

The compound increased insulin secretion from MIN-6 cells in a glucose-dependent manner. It also improved oral glucose tolerance in C57BL/J6 mice in a dose-dependent manner as shown in Fig. 5. Oral glucose tolerance test (OGTT) of YH-GKA at 50 mg/kg in C57BL/6J mice showed similar glucose area under the curve reduction of 29.6%, closely comparable to that of 29.9% for metformin at 300 mg/kg. Acute treatment of YH-GKA in C57BL/J6 and *ob/ob* mice induced basal glu-

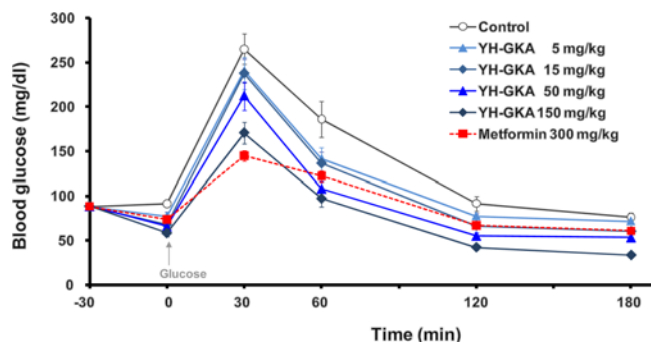


Fig. 5. Plasma blood glucose lowering effect and of YH-GKA in C57BL/J6 mice OGTT.

cose lowering activity. In a 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. These studies strongly support our position that YH-GKA is a promising candidate for the therapy of T2DM.

Several 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. However, more fundamental strategies are required to resolve the hypoglycemia issue that may be involved with the target mechanism of action. 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 GKAs (Bebornitz et al., 2009; Massa et al., 2011; Pfefferkorn et al., 2012) that restrict the main enzyme activity in the liver since hypoglycemia risk is postulated to result from the increase of pancreatic insulin secretion at low glucose levels. YH-GKA is a promising preclinical lead candidate for T2DM. 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. The study results from this new strategy will be reported soon.

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