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

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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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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.

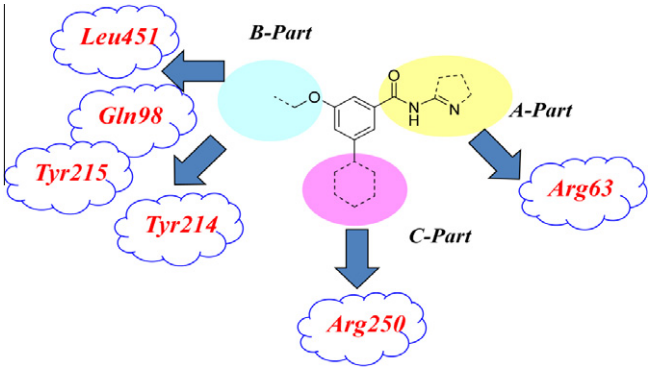

Figure 2. Synthetic strategy for benzamide GKAs.

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

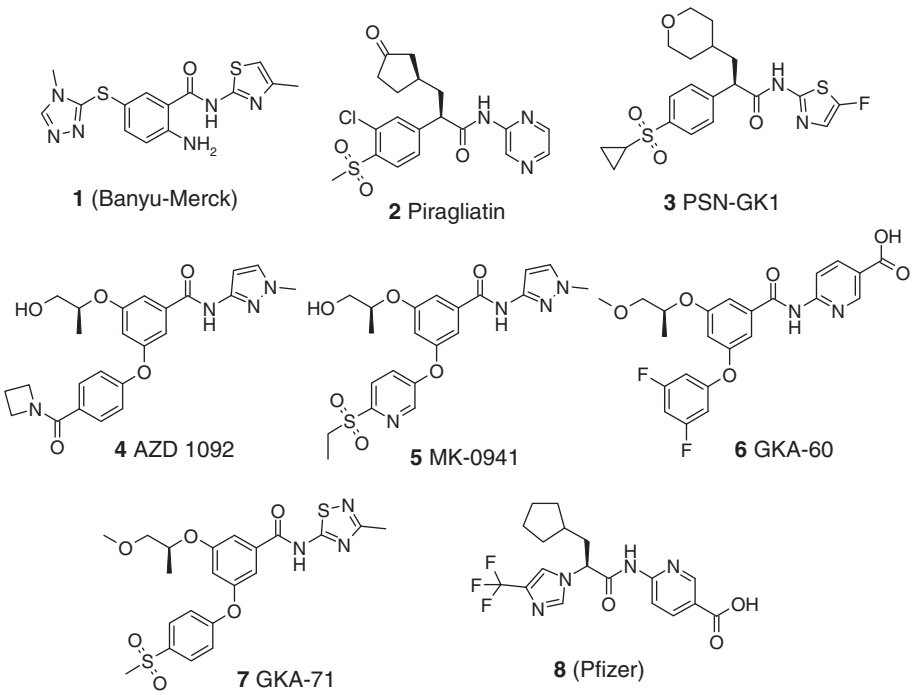

Figure 1. Representative structures of GKAs.