

REVIEW



Glucokinase as an emerging anti-diabetes target and recent progress in the development of its agonists

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ABSTRACT

Type 2 diabetes mellitus is a metabolic disorder with complicated pathogenesis, and mono-target therapy often fails to effectively manage the levels of blood glucose. In recent years, the anti-diabetes target glucokinase (GK) has attracted the attention of researchers. It acts as a glucose sensor, triggering counter regulatory responses following a change in glucose levels to aid restoration of normoglycemia. Activation of GK induces glucose metabolism and reduces glucose levels for the treatment of type 2 diabetes. GK agonists (GKA) are a new class of antidiabetic drugs. Among these agents, dorzagliatin is currently being investigated in phase III clinical trials, while PB-201 and AZD-1656 have reached phase II clinical trials. This article describes the mechanism of action of GK in diabetes and of action of GKA at the protein level, and provides a review of the research, trends, and prospects regarding the use of GKA in this setting.

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1. Introduction

1.1. Structure and function of GK

GK, termed hexokinase 4, is a member of the hexokinase family^{1,2}. It is an inducible enzyme composed of 465 amino acids with a molecular mass of ~52 kDa³. The three-dimensional structure of GK can be divided into three parts: large, small, and connected domains (Figure 1). The connected domain is composed of three segments of linkers and is the main active region of GK. The binding sites of glucose and GKA are located in this region⁴. According to the binding of endogenous substances to the kinase connected domain, GK can be divided into three conformations: closed, open, and super-open forms (Figure 2). The closed and open forms correspond to the open receiving and closed processing states, respectively, and are the two states that determine whether glucose is converted to glucose 6-phosphate⁵. The super-open form is an inactive conformation in which GK does not react with the substrate. Under the condition of low blood glucose levels, GK is abundant in the super-open form.

The GK gene is located on the short arm of human chromosome 7. It is a single-copy gene with a total length of 15.3–15.1 kb. Its complementary DNA has a length of 2,439 bp, including 12 exons and 11 introns⁶. The cDNA of GK in different parts of the human body exhibits different structures. The cDNA of the GK gene expressed in islet cells contains a CCAAT sequence in the upstream regulatory region of the 5' end, while the corresponding cDNA sequence of the GK gene expressed in liver tissues is TATTT. The different structures of these regulatory sequences determine the expression of proteins with certain differences in tissue specificity and activity⁷.

In the human body, GK is mainly concentrated in pancreatic cells and liver cells, as well as the hypothalamus and gastrointestinal tract. It is mainly involved in the first step of glucose metabolism, as the first rate-limiting enzyme. It catalyses the phosphorylation of hexose (e.g. D-glucose, D-fructose, and D-mannitose) to hexose 6-phosphate (e.g. glucose 6-phosphate, fructose 6-phosphate, and mannitose 6-phosphate) (Figure 3)⁸. However, GK has a distinct molecular structure and active function compared with hexokinase. Its molecular weight is usually half or lower than that of other hexokinases and has a higher K_m (Michaelis–Menten constant) value (8 mM). Its reactivity is not affected by the phosphorylated product glucose 6-phosphate, ensuring that the blood glucose within the physiological range is fully phosphorylated.

Located in the beta cells of the pancreas, GK is termed “the glucose receptor”. Its main function is to control the release of insulin according to the concentration of glucose (Figure 4)⁹. Following an increase in blood glucose levels, GK phosphorylates glucose and produces a large amount of ATP through glucose metabolism. It also inactivates the K ATP channels on the surface of the islet cells; consequently, Ca^{2+} influx causes the islet cells to release insulin, thereby reducing the concentration of blood glucose¹⁰.

GK, located in liver cells, mainly plays a role in regulating the glycogen content in the liver. Insulin and glucagon can trigger the transport of GK by glucose transporters, altering the amount of GK in the cytoplasm of hepatocytes and controlling the intracellular glucose content (Figure 5)¹¹. In the liver, GK controls blood sugar by converting glucose to liver sugar; hence, the activity of GK directly determines the glucose conversion rate. GK regulatory protein (GKRP) is a polypeptide (molecular weight: 68 kDa) that

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only exists in mammalian liver. It can competitively bind GK with glucose, prevent GK from catalysing the process of glucose phosphorylation to glucose 6-phosphate, and regulate GK activity in liver cells¹². Under hypoglycaemic conditions, GK forms a complex with GKR (GK-GKR complex) that aggregates in the nucleus. Following an increase in glucose levels, GKR is replaced by glucose, the GK-GKR complex is dissociated, and the levels of GK in the cytoplasm are markedly increased, thereby leading to an increase in GK activity¹³. In mammals, the content of the GK-GKR complex is affected by the levels of glucose, activated by fructose 6-phosphate, and inhibited by fructose 1-phosphate¹⁴⁻¹⁶.

In the hypothalamus, GK is located in glucose-sensing neurons and prevents hypoglycaemic effects caused by overactivation of GK in the liver and pancreas through neuromodulation^{17,18}. Following overexcitation of GK in the hypothalamus, the hypothalamus induces the body to decrease the secretion of adrenaline, norepinephrine, and glucagon, thereby resulting in hypoglycaemia. This can be

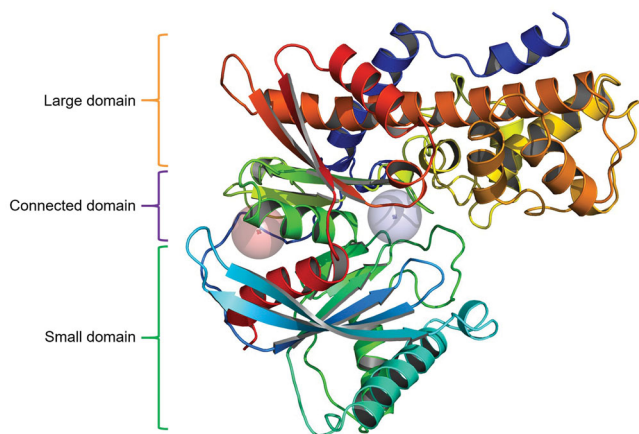


Figure 1. Glucokinase protein structure (PDB ID: 1V4S). This figure was drawn using the PyMOL program (<http://www.pymol.org>).

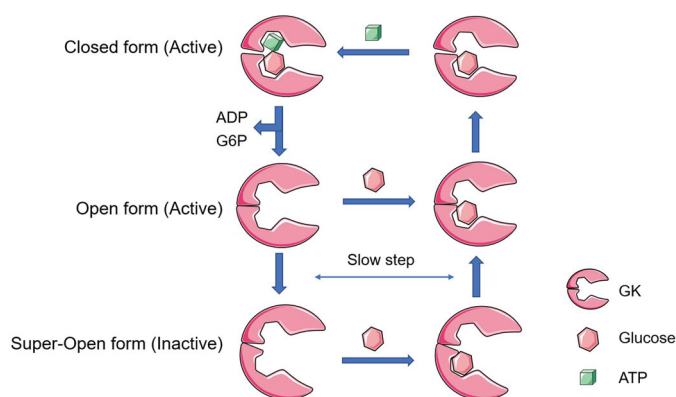


Figure 2. Three conformational transformations of glucokinase. The figures were drawn using Servier Medical Art (<http://www.servier.com>).

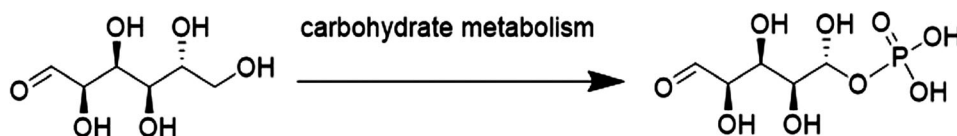


Figure 3. Glucokinase glucose metabolism reaction formula.

applied to the design of GKA to reduce the risk of hypoglycaemia due to overactivation of GK in the hypothalamus by reducing the blood-brain barrier permeability for GKA.

In the intestine, GK is located in endocrine K and L cells and some pituitary cells, and its function may be related to nutrient perception¹⁹⁻²¹.

1.2. Relationship between GK and diabetes mellitus

In a normal physiological state, blood glucose receptors respond to changes in blood glucose levels and promote the synergistic

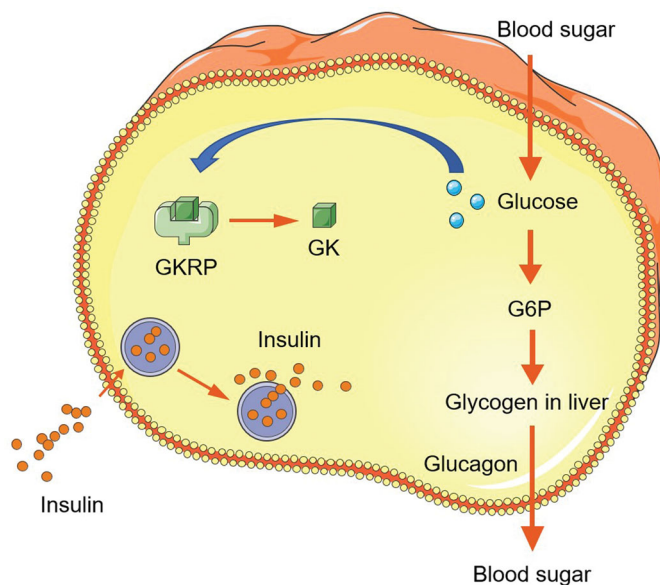


Figure 5. The mechanism of GK action in hepatocytes. The figures were drawn using Servier Medical Art (<http://www.servier.com>).

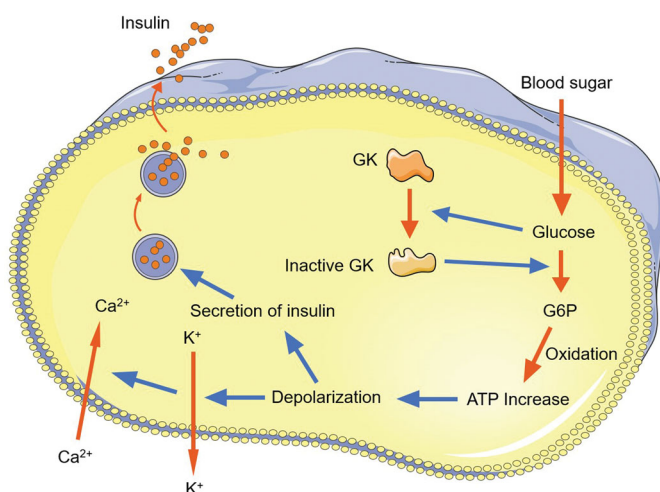


Figure 4. Mechanism of action of GK in pancreatic cells. The figures were drawn using Servier Medical Art (<http://www.servier.com>).

action of human organs and hormones to adjust the blood glucose levels within the normal range. Each step in this regulatory process has the potential to combat hyperglycaemia or hypoglycaemia through blood glucose balance.

To study the targeting of GK in the liver, researchers first used standard Cre-LoxP-based gene targeting strategies to knock out the liver-specific expression sequence of GK in mice²². The results showed that the mice were normal at birth, and their fasting blood glucose levels increased with age. Six weeks later, the mice developed hyperglycaemia, and impaired glucose tolerance was detected. In another group of mice, overexpression of liver GK increased the intracellular levels of glucose 6-phosphate and glycogen, as well as the activity of L-pyruvate kinase. These findings suggested that overexpression of GK could directly improve glycolysis and glycogen synthesis *in vivo*²³. It was also found that a high-fat diet in the GK-overexpressing mice did not cause diabetes symptoms in the short term²⁴. However, the mice showed impaired glucose tolerance after 6 months and mild hyperglycaemia, hyperinsulinemia, and hypertriglyceridaemia at 12 months. Moreover, the GK-overexpressing mice gained more weight than those in the control group, eventually leading to glucose intolerance and reduced insulin sensitivity. The results of these experiments indicated that the levels of GK in the liver are closely related to the blood glucose levels, and the absence of GK in the liver leads to the development of hyperglycaemia. Furthermore, high levels of GK expression in the liver can induce hypoglycaemia in the short term; nevertheless, high levels of GK expression in the long term are also associated with the risk of glucose intolerance.

In a study of pancreatic GK, the researchers knocked out the specific expression sequence of GK in islet cells in mice that died shortly after developing severe diabetes in infancy. Studies have shown that GK in islet cells plays an indispensable role in glucose homeostasis²⁵.

2. GKA

2.1. Molecular mechanism of GKA

The GK junction domain is located in the connected domain of the kinase structure, which is the binding site of GKA and glucose. All GKA binding sites identified thus far are all located in the same binding region. In this article, we focussed on human GK and GKA binding conformations for interaction analysis. We analysed the binding conformation of the protein 1V4S and its original ligand of the protein (2-AMINO-4-FLUORO-5-[(1-METHYL-1H-IMIDAZOL-2-YL)SULFANYL]-N-(1,3-THIAZOL-2-YL)BENZAMIDE). At the binding site, GKA forms hydrogen bonds with R65 and binds with Y214 and V455 through hydrophobic interactions (Figure 6).

The combination of GKA and GK can prevent the transformation from the GK conformation to the super-open form, and maintain GK in the two high-affinity conformations (i.e. closed and open forms)²⁶.

Considering the secondary structure of GK, the GKA binding site is located in the green region, while the glucose binding site is located in the red region (Figure 1). Hence, there is a certain distance between the two binding sites. Therefore, the binding of GK to GKA changes the conformation of GK but does not affect the glucose binding site.

By controlling the conformation of GK, GKA maintains GK active, thereby accelerating the conversion of hexose to hexose 6-phosphate.

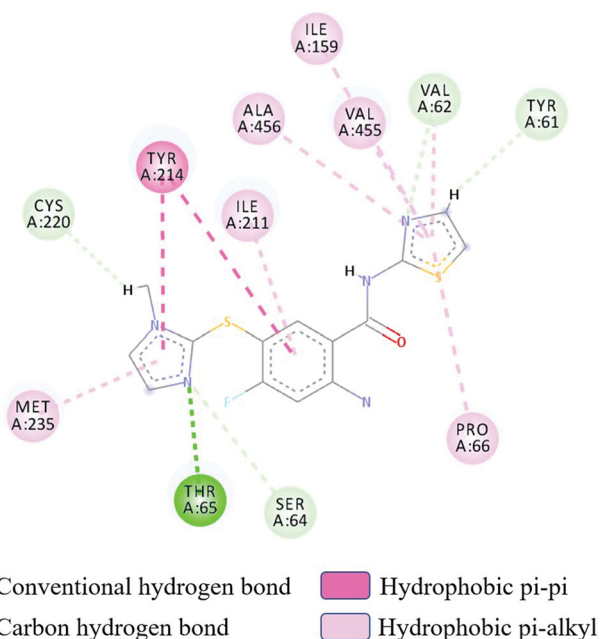


Figure 6. GKA binding sites (PDB ID: 1V4S). This figure was drawn using the Discovery studio program.

For pancreatic beta cells, increased GK activity eventually alters the membrane potential, thereby promoting insulin release.

In the liver, GK junction sites on the GK-GKRP complex can also bind to GKA to promote complex dissociation and GK release²⁷.

2.2. Reported GKA

GKA are a novel class of drugs for the treatment of diabetes, improving the sensitivity of the body to glucose through the joint action of the pancreas and liver. At present, a number of small-molecule GKA have been reported²⁸, and some of those have entered the clinical research stage; the specific progress is shown in Table 1. According to the structural characteristics of these GKA, they can be divided into three types: double-conjugate amides, single-conjugate amides, and others.

2.3. Double-conjugate amide GKA

In this section, double-conjugate amide GKA which are currently under development are introduced in detail (Figure 7).

2.3.1. MK-0941

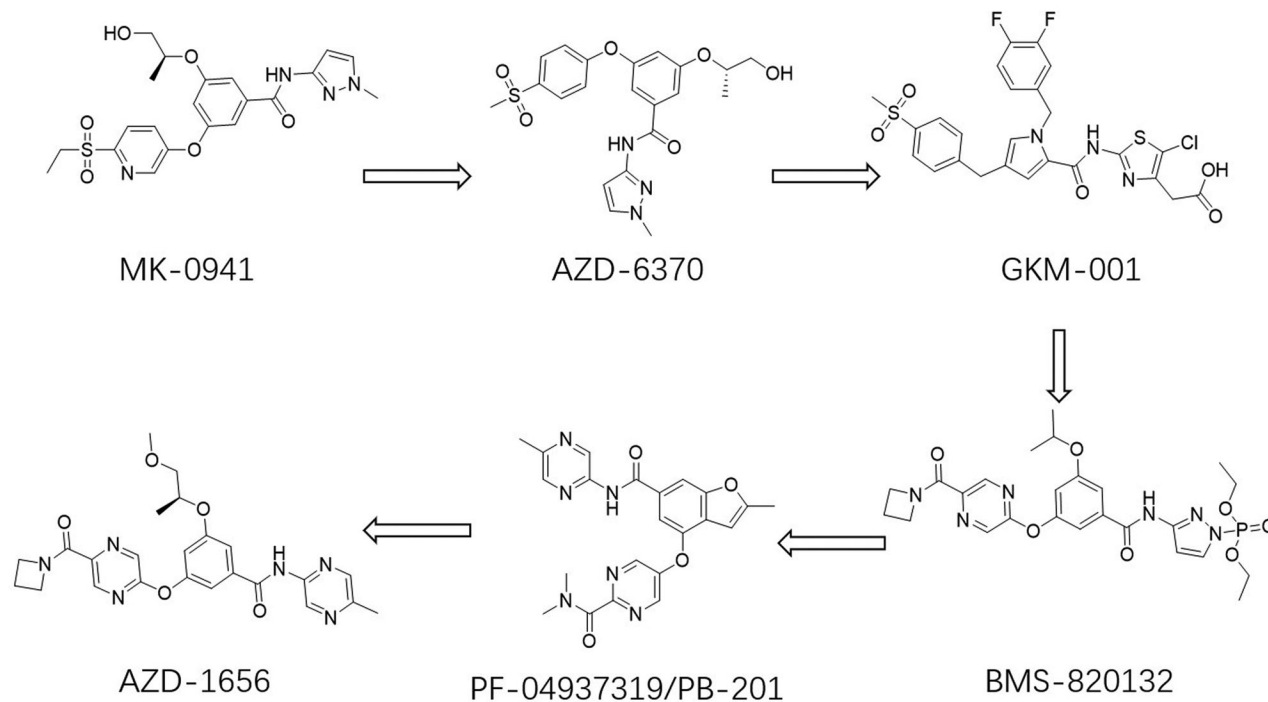
MK-0941 (Figure 7) is a GKA developed by Merck Sharp & Dohme (Kenilworth, NJ, USA). A phase II trial was conducted to study the dose range of MK-0941 in patients with type 2 diabetes mellitus receiving insulin (NCT00767000)²⁹. In the low-dose group (MK-0941 10 mg thrice daily), 80.7% of participants experienced at least one adverse event. These findings led to termination of the study. MK-0941 was not investigated further due to its unfavourable safety profile.

2.3.2. AZD-6370

AZD-6370 (Figure 7), a GK activator, is being developed by AstraZeneca (London, UK). A dose-ranging, randomised, single-blind, placebo-controlled, crossover assignment phase I study

Table 1. Clinical status of glucokinase agonists (GKA).

Clinical stage	Amount	Molecule
Phase III	1	Dorzagliatin
Phase II	13	TMG-123; AZD-1656; TTP547; PSN-010; GKM-002; GKM-001; PF-04991532; ARRY-403; MK-0941; AZD-6370; TTP-399; PB-201; piragliatin; LY2608204
Phase I	11	TTP-355; AZD-6714; MK-0599; TAK-329; BMS-820132; AZD-5658; DS-7309; RO-0281675; ZYGK1; RO-4597014
Application	1	Recombinant human glucokinase adenovirus

**Figure 7.** Development of double-conjugate amide glucokinase agonists (GKA).

(NCT00690287) was initiated in patients with type 2 diabetes (estimated $n = 24$) in Sweden³⁰. The study was designed to evaluate fasting and postprandial P-glucose levels, safety, and tolerability after the twice- or four times-daily oral administration of AZD-6370. Clinically indicated AZD-6370 resulted in dose-dependent reductions in plasma glucose levels by $\leq 30\%$ versus placebo ($p < 0.001$ at 60 mg and 180 mg doses). In January 2011, research on AZD-6370 was discontinued by AstraZeneca due to undisclosed reasons. Investigators suggested that the development program for AZD-6370 may have been terminated due to the lack of an advantage over the competing compound AZD-1650 and a similar safety profile.

2.3.3. GKM-001

GKM-001 (Figure 7) is a liver-selective GKA developed by Advinus Therapeutics (Karnataka, India)³¹. Its unique liver-selective mechanism enables this agent to avoid the increased insulin secretion caused by the activation of thymic GK, thereby preventing the occurrence of hypoglycaemia. In 2011, Advinus Therapeutics launched a phase II trial that enrolled 60 patients with type 2 diabetes (CTRI/2011/04/001661). The entire clinical trial was conducted over a 14-day period to examine the efficacy and safety of GKM-001 at incremental doses (25, 50, 200, 600, 1,000 mg). The clinical trial results showed that GKM-001 was effective in decreasing glucose levels at all examined doses. The area under the curve for insulin of the subjects at 24 h was reduced in a dose-dependent manner (from 9% to 20%) compared with that measured

before treatment. The placebo group exhibited a reduction of only 2%. Fasting glucose levels were reduced by 23–4,623 mg/dL, and there was no change in the area under the curve for C-peptide. C-peptide is a peptide fragment containing 31 amino acids (molecular weight: 3 kDa) that is cleaved during the transformation of proinsulin into insulin under the action of proteolytic enzymes. The clinical significance of serum C-peptide is similar to that of insulin. Thus, the C-peptide data showed that the hypoglycaemic mechanism of GKM-001 was independent of insulin secretion. The trial confirmed the previous hypothesis that liver-selective GKA are effective in reducing glucose levels and do not cause hypoglycaemia^{32,33}. Impetis Biosciences (Mumbai, India) acquired the drug discovery business of originator Advinus Therapeutics and development of GKM-001 in 2017 and continues to develop a range of GKAs³⁴.

2.3.4. BMS-820132

BMS-820132 (Figure 7) is a GKA developed by Bristol-Myers Squibb (New York City, [New York] USA)³⁵. The researchers examined the effects of daily administration of BMS-820132 for 1 month in rats with different blood sugar levels. The results showed that the effective concentration 50 (EC_{50}) in rats with hyperglycaemia (12 mM) was 39 nM. In rats with normal blood glucose levels (5 mM), the EC_{50} was 73 nM³⁵. In 2011, Bristol-Myers Squibb conducted two phase II clinical studies (NCT01105429, NCT01290575) to study the single/multiple-dose administration, tolerability, and

safety of BMS-820132. Thus far, results of this trial have not been published.

2.3.5. PF-04937319/PB-201

PF-04937319 (Figure 7) is a partial GKA developed by Pfizer (New York City, [New York] USA) that acts simultaneously on the liver and pancreas³⁶. However, As PF-04937319 reduces the V_{max} of GK while activating GK, the ability of excitation is reduced, resulting in a limited hypoglycaemic effect on type 2 diabetes.

In 2011, Pfizer conducted a randomised, double-blind, phase II clinical trial (NCT01475461) to evaluate the safety and efficacy of PF-04937319 on glycemic control in adult patients with type 2 diabetes mellitus inadequately controlled on metformin. At the same time, glimepiride and sitagliptin were also studied in parallel. The results showed that the reduction rate of haemoglobin A1c (HbA1c) in the PF-04937319 (100 mg), sitagliptin, and glimepiride groups was -0.47 , -0.43 , and -0.83% , respectively. In terms of side effects, 5.1, 2.5, 1.8, and 34.4% of subjects developed hypoglycaemia in the PF-04937319 (100 mg), placebo, sitagliptin, and glimepiride groups, respectively. Therefore, the addition of PF-04937319 (100 mg) to treatment with metformin was shown to be effective and well tolerated. However, the incidence of adverse events caused by PF-04937319 was 3.5-fold higher than that of metformin^{36–39}.

In 2016, pegbio (Suzhou, China) purchased the PF-04937319 compound and renamed it PB-201. In 2019, PB-201 was investigated in a phase I clinical study involving Chinese patients with type 2 diabetes. The safety, tolerability, pharmacokinetics, and pharmacodynamics of PB-201 at three doses (50, 100, and 150 mg) were investigated in a randomised, double-blind,

placebo-controlled, phase IV, and case-crossover study which is currently ongoing.

2.3.6. AZD-1656

AZD-1656 (Figure 7) is a GKA developed by AstraZeneca (London, UK). In 2010, a randomised, double-blind, placebo-controlled, phase II clinical study (NCT01152385) was conducted in Japan. In that trial, 224 patients with type 2 diabetes received treatment with high-(daily titration 200 mg), medium-(daily titration 140 mg), or low-dose (daily titration 80 mg) AZD-1656 or placebo to observe changes in HbA1c levels within 4 months and evaluate drug safety and tolerability. The results showed that, after 2 months of treatment, the levels of HbA1c in the patients were decreased by 0.3–0.8% compared with those recorded at baseline; of note, the levels of HbA1c in the placebo group were decreased by only 0.1%. At 4 months, the reduction in HbA1c in the 40–200 mg group was similar to that observed in the placebo group. This study showed that AZD-1656 significantly reduced the levels of glycosylated haemoglobin after short-term treatment; however, the efficacy diminished over time^{39,40}. In 2011, AstraZeneca terminated the AZD-1656 program due to unsatisfactory results.

2.4. Single-conjugate amide GKA

Piragliatin, developed by F. Hoffmann-La Roche AG (Basel, Switzerland), was the first single-conjugate amide GKA to enter the clinical stage. Also, HMS5552 developed by Huazhong Pharmaceutical co., Ltd (Guangdong, China) is the most advanced and has entered Phase III trials. In this section, some single-conjugate amide GKA are described in detail (Figure 8).

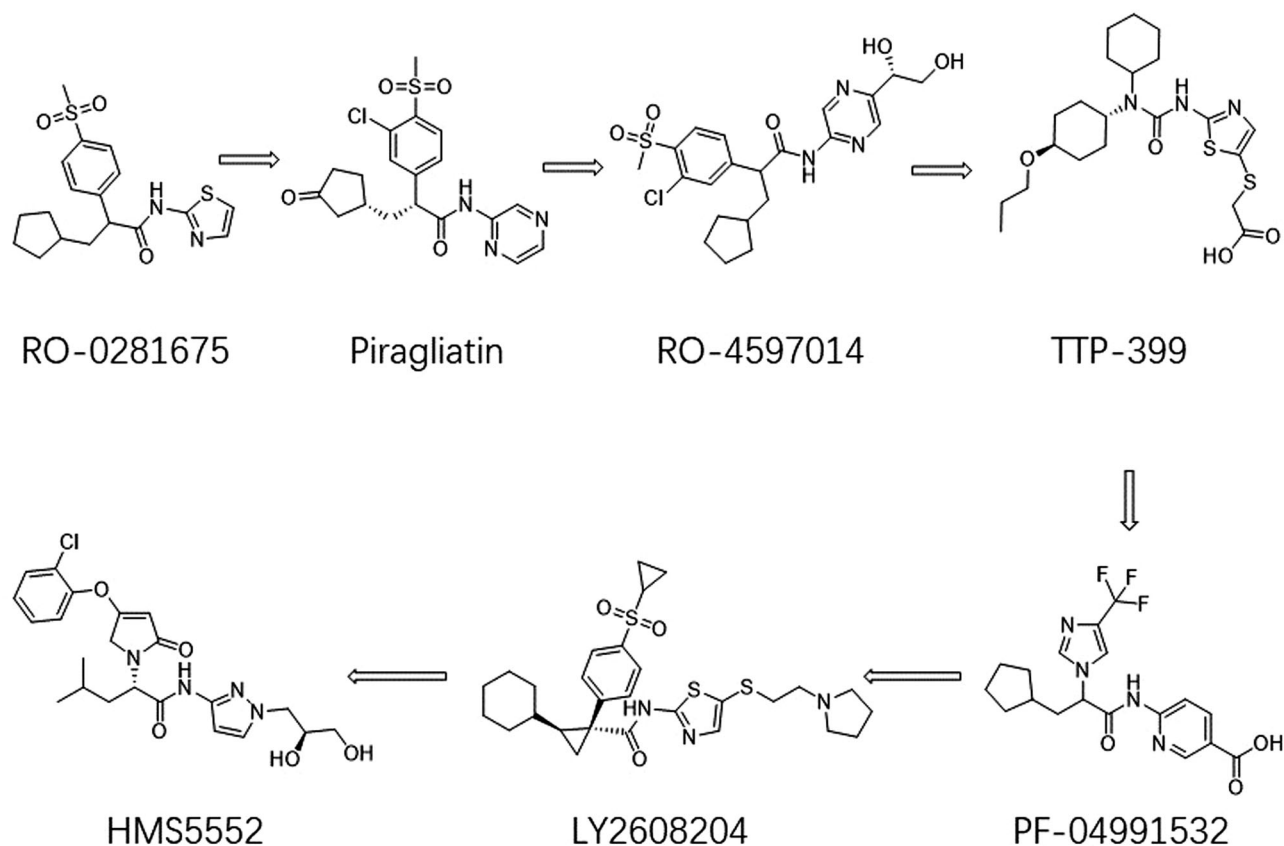


Figure 8. Development of single-conjugate amide GKA.

2.4.1. RO-281675

In 2003, F. Hoffmann-La Roche AG first reported that GK could be excited by small molecules (RO-281675) (Figure 8). The evidence demonstrated that GK could be a potential target for diabetes, and these small molecules could be developed into oral drugs⁴¹. At a concentration of 3 μ M, RO-281675 induced a 1.5-fold increase in the maximum metabolic rate (V_{\max}) of GK. In addition, the substrate concentration $[S]_{0.5}$ (glucose) at half of the V_{\max} was decreased from 86 mM to 20 mmol/L⁴¹.

RO-281675 is a mixed agonist. An insulin release experiment was conducted on freshly isolated fused rat islets. These cells were treated with 0.3, 1, 3, and 10 μ M RO-281675. To observe the lowest glucose concentration at which insulin is released, the glucose concentration was gradually increased from 0 to 20 mM, at a rate of 1 mM every 20 min. Finally, the glucose sensitivity threshold of islet cells was decreased from 7 to 3 mM, when islets were treated with 0 to 10 μ M RO-281675⁴². However, due to the potential cardiovascular hazards, the compound did not enter the clinical stage. Although this compound did not progress to clinical development, it paved the way for pharmaceutical companies to develop efficient and safe GKA.

2.4.2. Piragliatin

F. Hoffmann-La Roche AG avoided the cardiovascular hazards associated with the first-generation GKA through a series of structural modifications of the compound RO-281675 and, subsequently, produced the second-generation GKA piragliatin⁴³.

Piragliatin, also termed RO4389620 (Figure 8), is a mixed GKA which can simultaneously stimulate the GK in both the pancreas and liver⁴⁴. Piragliatin (110 μ M) increases the maximum metabolic rate of GK from 10.6 to 17.9 μ M^{43,45}. Piragliatin is the first GKA to enter the clinical study phase.

However, the drug has a cyclopentanone structure that is metabolised to produce cyclopentyl alcohol, which is toxic to liver cells. In 4-week toxicity studies (40, 80, and 120 mg/kg, orally, once daily), rats fed with any dose of piragliatin metabolite developed hepatic lipodosis. A phase II clinical study (NCT00266240) also found that long-term use of this product can cause greater liver load. Research on piragliatin was terminated because of its potential liver-poisoning effects in patients who regularly use the drug for the treatment of chronic conditions, such as type 2 diabetes^{42,44,45}.

2.4.3. RO-4597014

RO-4597014 (Figure 8) is the second GKA, produced by F. Hoffmann-La Roche AG (Basel, Switzerland), to enter clinical development. The company prioritised RO-4597014 as back-up compound of piragliatin. In April 2009, research on this drug was terminated because its cyclopentane structure may result in the accumulation of metabolites.

2.4.4. TTP399

TTP399 (Figure 8) is an oral GKA designed by vTv Therapeutics LLC (High Point, USA). In animal studies, TTP399 did not activate GK in pancreatic cells, affect insulin secretion, or cause insulin-related hypoglycemia^{39,46,47}. The researchers examined the effects of TTP399 on patients with different blood glucose levels. At hyperglycaemic levels (15 mM), the EC_{50} was 304 nM. At normal blood glucose levels (5 mM), the EC_{50} was 762 nM⁴⁸. The drug is designed to activate GK only in the liver for optimal control of glucose levels⁴⁸. The company conducted a multicenter,

randomised, double-blind, phase II clinical trial (NCT02405260) to evaluate the safety, tolerability, pharmacokinetic characteristics, and pharmacodynamic effect of TTP399 as adjuvant treatment for diabetes. In this 6-month study, TTP399 (800 mg/day) was directly associated with a decrease in HbA1c levels, with an average 0.9% reduction from baseline compared with placebo ($p < 0.01$). TTP399 (800 mg/day) also increased the levels of HDL cholesterol (3.2 mg/dL) compared with placebo. It also reduced the levels of fasting plasma glucagon by 20 pg/mL ($p < 0.05$), and induced weight loss of 3.4 kg in patients with body weight of ≥ 100 kg ($p < 0.05$)^{48,49}.

2.4.5. PF-04991532

PF-04991532 (Figure 8) is a liver-selective GKA developed by Pfizer (New York City, NY, USA). A randomised, double-blind, placebo-controlled, dose-varying phase II clinical study (NCT01336738) showed that the levels of HbA1c in patients were decreased by 0.49%, compared with those recorded at baseline (PF-04991532: 750 mg once daily). Research on PF-04991532 was discontinued due to poor clinical activity. Notably, treatment with sitagliptin (100 mg once daily) decreased the levels of HbA1c in patients by 0.79% compared with baseline.

2.4.6. LY2608204

LY2608204 (Figure 8) is a GKA developed by Eli Lilly (Indianapolis, USA). A phase II clinical trial (NCT01247363) recruited 20 eligible patients who received treatment orally with LY2608204 capsules once daily. The study examined the safety and tolerability of LY2608204 in patients with type 2 diabetes receiving increasing dosages of medicine. The starting dose was 160 mg, which was gradually increased to 240, 320, and 400 mg every 7 days of treatment (total duration of treatment: 28 days). The results showed that the drug exerted a good hypoglycaemic effect without serious adverse events; the incidence rate of other adverse events was 47.37%. Common adverse events included gastrointestinal diseases, site reactions, headache, and hypertriglyceridaemia caused by high dosages. The maximum hypoglycaemic effect on the glucose area under the curve was 42% compared with the control group at high doses (30 mg/kg). Interpolation analysis showed that, when the average concentration of LY2608204 in plasma was 99 ng/mL (179 nM) (equivalent to 6.9 mg/kg LY2608204), the glucose area under the curve concentration decreased by 20%.

In 2019, a parallel, randomised, double-blind, phase II clinical trial (CRT20192351) was conducted in China to assess the safety, efficacy, and tolerability of LY2608204 in patients with type 2 diabetes (Active with enrolment of 200 volunteers).

2.4.7. HMS5552

F. Hoffmann-La Roche AG developed the fourth-generation GKA HMS5552, also termed Dorzagliatin (Figure 8), to address the problems linked to the use of second-generation GKA (liver cell damage or inflammation in human metabolism)⁵⁰. This is a mixed agonist, which can simultaneously activate GK in the pancreas and liver, promote pancreatic insulin secretion and liver glucose transformation in patients with type 2 diabetes, and exert multiple hypoglycaemic effects by activating intestinal GK to regulate the secretion of GLP-1. The most striking feature of this compound is that it increased GK activity at low levels of blood sugar and does not enhance insulin secretion, thus avoiding the development of hypoglycaemic symptoms.

In 2017, the results of a phase II clinical trial (NCT02561338) of HMS5552 were published. The data showed a distinct curative effect, as well as an excellent tolerability and safety profile with low risk of hypoglycaemia and absence of the most common side effects associated with the pharmacological treatment of diabetes. In this multicenter, randomised, double-blind, placebo-controlled, multi-dose study, 258 patients with diabetes were divided into the placebo group and four groups of patients who received different doses of GKA. After 12 weeks, the mean change in HbA1c least-squares was -0.35% in the placebo group, -0.39% in the 75 mg once-daily group, -0.65% in the 100 mg once-daily group, -0.79% in the 50 mg twice-daily group, and -1.12% in the 75 mg twice-daily group. Patients in the 50 mg or 75 mg twice-daily groups exhibited more significant changes in HbA1c than those in the placebo group⁵¹. The incidence of adverse events was similar between the treatment groups and placebo group, and there were no reports of drug-related serious adverse events or severe hypoglycaemia. Treatment with HMS5552 showed significant advantages over similar GKA, no significant changes in liver enzymes, blood lipids, or other laboratory parameters compared with placebo, and no abnormalities in physical examination, vital signs, or electrocardiogram findings^{52,53}.

In 2018, a multicenter, randomised, double-blind, placebo-controlled, 24-week treatment, phase III clinical study (NCT03141073) was conducted to evaluate the efficacy and safety of GKA HMS5552 in combination with metformin in patients with type 2 diabetes; the study also add-on to metformin with additional 28-week open-label treatment. By July 2019, 718 patients were enrolled in the trial. The primary clinical outcome was achieved within the first 24 weeks of the trial. At 24 weeks, patients receiving the combination therapy had a 1.07% decrease in HbA1c levels compared with baseline (8.35%). Of note, those receiving the placebo had a 0.50% decrease in HbA1c levels compared with baseline (8.37%). The reduction in HbA1c noted in the combination group was statistically significant compared with that observed in the placebo group ($p < .001$)^{54,55}.

2.5. Other GKA

2.5.1. ARRY-403

ARRY-403, also termed AMG-151 (Figure 9), is a mixed agonist that simultaneously activates GK in the pancreas and liver⁵⁶. Amgen conducted a phase II clinical study (NCT01464437) to evaluate the dose-effect relationship of AMG 151 compared with placebo, on fasting plasma glucose in subjects with type 2 diabetes treated with metformin⁵⁷. Fasting plasma glucose levels were assessed in 236 patients with type 2 diabetes treated with

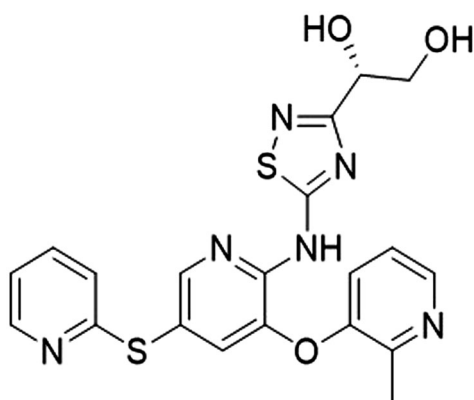


Figure 9. Structure of ARRY-403.

metformin (33–35 patients per group) to evaluate the dose-response relationship between ARRY-403 and placebo. A significant linear dose-response relationship between efficacy and dose was observed in subjects who received 50, 100, and 200 mg ARRY-403 orally twice daily ($p = 0.004$). After 7 days of treatment, fasting plasma glucose levels were reduced by 1.38 mM. The reduction remained constant until the last day of administration. However, the incidence of adverse events associated with ARRY-403 was 1.5-fold higher than that linked to metformin, mainly due to elevated serum triglyceride levels which increased by 17–25% from baseline; however, there was no evidence of dose dependence^{57–60}. Amgen eventually discontinued the research on this compound.

2.5.2. TMG-123

TMG-123 was developed by Teijin Limited (Chiyoda, Japan); the definite chemical structure of this compound has not been reported⁶¹. At glucose levels of 5 nM, the EC_{50} value for the human liver and pancreas was 0.35 and 0.32 μ M respectively, indicating that TMG-123 could effectively activate GK in both organs. Following the administration of 30 nM of TMG-123, the $[S]_{0.5}$ was 0.5 nM and there was no change in V_{max} ⁶¹. TMG-123 may exert a favourable hypoglycaemic effect on type 2 diabetes.

At present, TMG-123 has entered the phase II clinical research (JapicCTI173698). In this study, involving 90 patients with type 2 diabetes (age: 20–74 years), the researchers investigated the efficacy, safety, and dose-response of TMG-123 for 12 weeks versus placebo. The patients received TMG-123 orally daily for 24 weeks in a fasting state at a gradient range from 1 to 160 mg. TMG-123 did not cause serious adverse events or hypoglycaemia. Electrocardiography, vital signs, and laboratory testing did not show abnormality. When the blood glucose concentration was less than 200 ng/mL, TMG-123 exerted a significant dose-dependent hypoglycaemic effect. At 30 min after receiving TMG-123 (80 mg), the serum insulin levels were significantly increased. The results showed that the oral administration of the liver GKA TMG-123 was well tolerated, and the agent could be rapidly absorbed on an empty stomach without being affected by eating. The use of TMG-123 was safe and effective in healthy individuals.

2.5.3. ZYGK1

ZYGK1 was developed by Zydus Cadila (Ahmedabad, India); its specific chemical structure has not been reported. Studies using a variety of preclinical models have shown that ZYGK1 is safe and effective in controlling fasting and non-fasting blood glucose levels. ZYGK1 reduces blood sugar levels by enhancing the ability of the pancreas to sense the concentration of glucose, thereby increasing insulin production and improving the net uptake of blood sugar by the liver. In 2011, the company conducted a randomised, double-blind, placebo-controlled, phase I clinical trial (NCT01472809). The trial recruited 96 healthy volunteers and patients with type 2 diabetes from India to evaluate the safety, pharmacokinetics, and pharmacodynamics of ZYGK1. However, the clinical trial was suspended because the results in the 4 mg single-dose group did not meet the researchers' expectations.

3. Conclusion

Typically, the treatment of diabetes requires long-term administration of medications, resulting in great burden to patients and society. Long-term use of metformin – a first-line antidiabetic drug –

is associated with the development of drug resistance. In addition, the traditional oral hypoglycaemic drugs (e.g. sulfonylureas, acarbose, and pyrazolidines) have many disadvantages (e.g. poor efficacy) and are linked to severe side effects. Therefore, the development of new antidiabetic drugs is urgently warranted.

In this article, we reviewed the GKA that have been investigated in clinical studies thus far. The currently available clinical data suggest that GKA can effectively control the levels of HbA1c in patients with type 2 diabetes and can be used to treat this disease. In addition, GKA were well tolerated by patients and associated with a low risk of developing minor adverse effects ($p > 0.05$).

There are two major challenges in the development of GKA, namely overcoming the rapid development of drug resistance and reducing the occurrence of treatment-related adverse effects (e.g. hypoglycaemia and abnormal serum triglyceride levels).

Both types of parent ring structures of GKA described herein have good research potential. Double-conjugated amide PB-201 and single-conjugated amides TTP 399 and HMS 5552 are characterised by high activity and low toxicity. However, the usefulness of MK-0941, AZD-6370, and AZD-1656 is limited by the rapid development of resistance, as a result of the introduction of propane-1, 2-diol. Treatment with LY 2608204 and AMG-151 has been linked to elevation in the levels of serum triglycerides.

Owing to their favourable safety profile, GKA can be used for the routine treatment of diabetes and as a temporary hypoglycaemic agent for diabetic patients with other diseases. As a new target, this type of therapeutic agents is suitable for combination with other hypoglycaemic drugs to achieve a better effect in the treatment of diabetes.

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