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Novel, Highly Potent Systemic Glucokinase Activators for the Treatment of Type II Diabetes Mellitus

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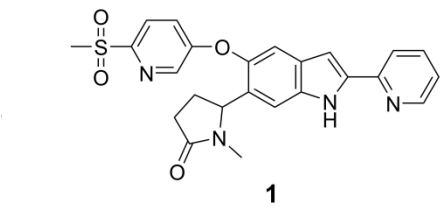
Abstract

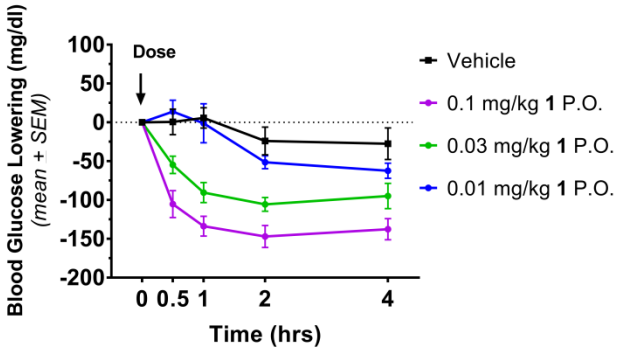
Glucokinase (GK, hexokinase IV) is a unique hexokinase that plays a central role in mammalian glucose homeostasis. Glucose phosphorylation by GK in the pancreatic β-cell is the rate-limiting step that controls glucose-stimulated insulin secretion. Similarly, GK-mediated glucose phosphorylation in hepatocytes plays a major role in increasing hepatic glucose uptake and metabolism and possibly lowering hepatic glucose output. Small molecule GK activators (GKAs) have been identified that increase enzyme activity by binding to an allosteric site. GKAs offer a novel approach for the treatment of Type 2 Diabetes Mellitus (T2DM) and as such have garnered much attention. We now report the design, synthesis, and biological evaluation of a novel series of 2,5,6-trisubstituted indole derivatives that act as highly potent GKAs. Among them, Compound **1** was found to possess high *in vitro* potency, excellent physicochemical properties, and good pharmacokinetic profile in rodents. Oral administration of Compound **1** at doses as low as 0.03 mg/kg led to robust blood glucose lowering efficacy in 3 week high fat diet-fed mice.



1

hGK EC50 (@2.5 mM glucose) = 3.7 nM; Max activity = 784%

hGK EC50 (@10 mM glucose) = 2.4 nM; Max activity = 193%



Keywords

Glucokinase, Glucokinase activator (GKA), hexokinase IV, glucose phosphorylation, glucose homeostasis, Type 2 Diabetes Mellitus, diabetes, antidiabetic, allosteric activator, 2,5,6-trisubstituted indole

Glucose homeostasis is essential to mammalian life and as such it is carefully maintained through a complex network of factors that control both glucose uptake and utilization.[1] Imbalance in these factors can lead to serious disease, including Type 2 Diabetes Mellitus (T2DM). T2DM is characterized by hyperglycemia (elevated blood glucose) due to a combination of insulin resistance and the failure of pancreatic beta-cells to produce sufficient insulin.[2] The long-term complications of uncontrolled T2DM include cardiovascular morbidity and mortality, peripheral neuropathy, retinopathy, and chronic kidney disease.[2] T2DM has become one of the most devastating diseases of mankind, affecting hundreds millions of people worldwide. The estimated cost of diagnosed diabetes in the U.S. alone was $245 billion in 2012, representing a substantial economic burden to society.[3] Treatment of T2DM aims to restore and maintain blood glucose level in the normal range by the use of antidiabetic drugs that have varying mechanisms of action (MOA) which impact different pathways in glucose metabolism.[2, 4] Currently available antihyperglycemic agents include insulin, insulin secretagogues (e.g. sulfonylureas), metformin, insulin sensitizers (PPAR-γ agonists), GLP-1 analogs and the related DPP-4 inhibitors, and SGLT2 inhibitors.[4] Despite these options, many patients are still unable to safely and effectively maintain normal blood glucose levels. Therefore, the search for novel antidiabetic drugs continues.

Glucokinase (GK, hexokinase IV) plays a unique role in glucose homeostasis.[5, 6] GK is a monomeric enzyme that catalyzes the ATP-dependent phosphorylation of D-glucose to D-glucose-6-phosphate, which is the initial, as well as rate-limiting step, in cellular glucose uptake and metabolism. GK exhibits positive cooperativity kinetics with respect to glucose, with a $S_{0.5}$ (substrate required for 50% of maximal activity) for D-glucose of 8 mM, which approximates the physiological blood glucose concentration, and a Hill coefficient (n_H) for glucose dose response cooperativity of 1.75.[7-9] GK has limited expression in mammals. In pancreatic beta-cells, GK functions as a "glucose sensor" leading to glucose-dependent insulin release.[10,11] Small molecule activators of GK (GKA) have been identified that increase insulin secretion from pancreatic beta-cells at any given concentration of glucose by lowering the $S_{0.5}$ and/or increasing the maximal reaction velocity (V_{max}) of GK.[6, 12, 13] In hepatocytes, GKAs act to increase D-glucose uptake (and likely decrease hepatic glucose production) by trapping it as glucose-6-phosphate, which is then incorporated into glycogen and/or metabolized by the glycolytic pathway.[14] The activity of hepatic GK is also regulated by glucokinase regulatory protein (GKRP), a GK inhibitory protein present in hepatocytes but not found in pancreatic β-cells.[15] In the past two decades, several direct-acting GKAs, as well as GK-GKRP disruptors, have been identified that lower blood glucose levels in diabetic rodent models.[15-17]

As part of our efforts to identify structurally diverse GKAs,[18-21] we discovered 2,5,6-trisubstituted indole compounds (such a Compound **1**) as a novel series of highly potent GKAs. The general synthetic route to

Compound **1** and its analogues is illustrated in Scheme 1. Zinc iodide mediated homo-Reformatsky reaction[22] of 2-chloro-5-nitrobenzaldehyde and (1-ethoxycyclopropoxy)trimethylsilane, followed by acid catalyzed desilylation-cyclization, afforded lactone **1c** in 70% yield. The lactone ring was opened by DBU in methanol to give γ-hydroxy ester **1d**. Mitsunobu reaction[23] of **1d** with diphenyl phosphorazidate (DPPA) afforded azide **1e** in good yield (83%). The azide group was reduced to an amino group by tributylphosphine in THF/water at room temperature and the product was heated with triethylamine in methanol at 50°C to give lactam **1f** in 71% yield. Methylation of **1f,** followed by substitution of the chloro group with 6-(methylsulfonyl)pyridin-3-ol, gave compound **1h**. The nitro group was reduced by iron/ammonium chloride to give aniline **1i**. Iodination at the ortho-position of the aniline and protection as the ethyl carbamate afforded intermediate **1k**. Sonogashira coupling[24] with 2-ethynylpyridine and base mediated cyclization (Larock indole synthesis[25]) gave target Compound **1** in high yield. By varying the reagent used in the displacement of the chloro group in **1g,** and the alkyne used in the Larock indole synthesis step, we were able to prepare a series of analogues for biological testing.

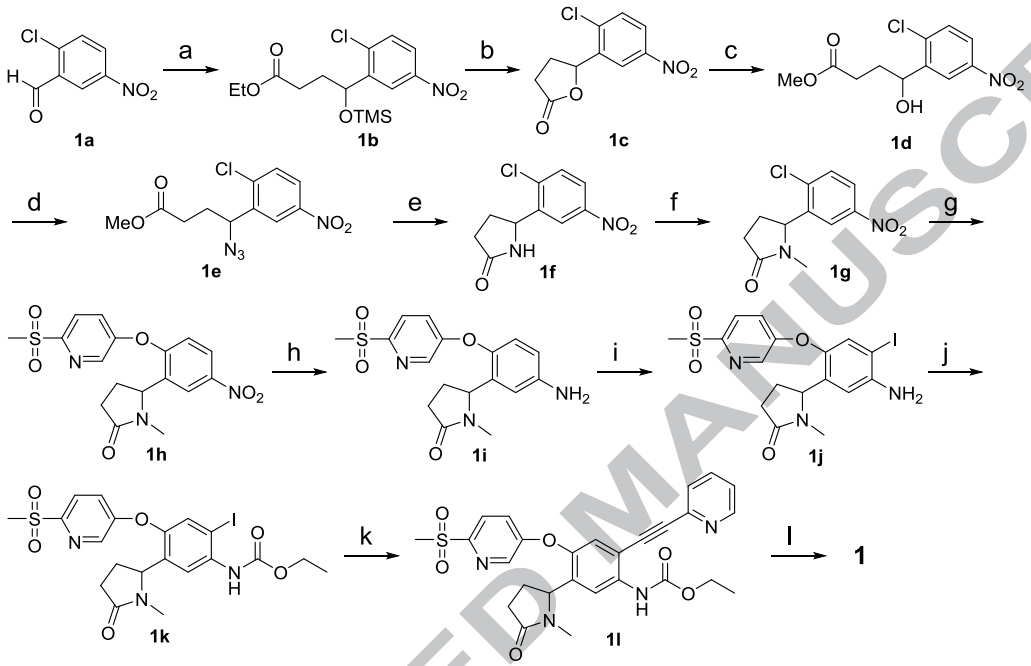
Once synthesized, compounds were tested for their ability to activate recombinant human GK in the presence of glucose at both 2.5 mM and 10 mM. The GK activation EC_{50} and maximum percent velocity increase at saturating GKA values were used to define the *in vitro* potency of the GKAs.[26] Compounds of interest were also evaluated in the GKACA (Glucokinase Activator Cell Activity) assay using both rat INS-1 Clone-13 insulinoma cells and freshly isolated, primary rat hepatocytes in order to determine their ability to activate GK in relevant cell types. In those assays, the ability of a GKA to increase the production of intracellular D-2-deoxyglucose-6-phosphate from exogenous D-2-deoxyglucose was measured by monitoring product formation using HILIC LC-MS.[27] Representative results from these assays are summarized in Table 1.

The 2,5,6-trisubstituted indole derivatives in Table I are potent, low nM GKAs that significantly increase GK activity at both hypoglycemic and hyperglycemic glucose levels. Cellular potencies in the low nM range were commonly observed in this GKA series. Similar to previously reported GKA's series[19], the dual hydrogen-donor/acceptor binding motif between the GKA indole-NH and the N-atom in the adjacent R^1 heterocycle with Arg 63 of GK is presumed to be crucial for good potency. A wide variety of 5- or 6-membered R^1 heterocycles with a N-atom at the 2'-position, including substituted pyridine, thiazole and pyridazine, were well tolerated (compare Compounds **1**, **2** and **3**). Using pyridine as an example, the 4'-position of the ring can be substituted with various groups of differing polarity and steric bulk, such as dimethyl-hydroxymethyl (Compound **4**), -CF_3 (Compound **5**) and methyl ester (Compound **6**).

The chirality at the pyrrolidinone ring has variable impact on enzymatic potency across the series; often one enantiomer is more potent than the other (Compound **3**, **4** and **5** compared to their corresponding enantiomer). Significant differences between enantiomers were also observed in their cellular activities. For example, the enantiomers of Compound **1** have similar EC_{50} values in the enzyme assay (EC_{50} = 3.7/2.4 nM *vs*. 4.2/0.8 nM), but one enantiomer is significantly more potent in the cell assays (EC_{50} = 0.9/7.5 nM *vs*. 33/93 nM). The reason(s) for this are unclear.

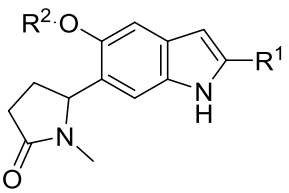
SAR of the aryl ether moiety reveal that good enzymatic potency can be retained with a variety of substituted phenyl or pyridyl groups. However, methyl sulfonylpyridine appears to be a preferred group

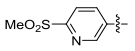
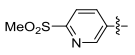
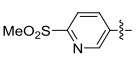
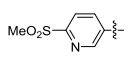
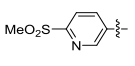
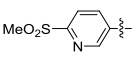
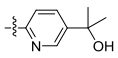
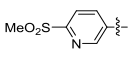
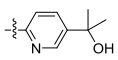
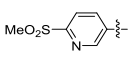
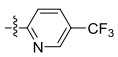
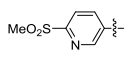
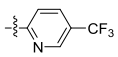
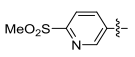
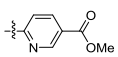
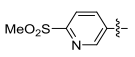
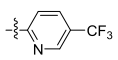
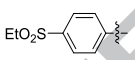
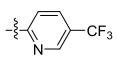
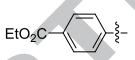
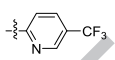
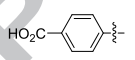
at that position. Compared to Compound **5**, phenyl analogue Compound **7** is equipotent in the enzyme assays, but 10-fold less potent in hepatocyte assay. Similarly, Compound **8,** with a replacement of the methyl sulfonylpyridine of **7** with an ethyl carboxylic ester, is also less potent in the cell assays. Its acid analogue, Compound **9**, which has good solubility at pH 7 [SOLY (pH2, 7) = 5, 113 µM], also has weak activity in cells, despite being reasonably potent against the enzyme. Presumably, this difference between the enzymatic and cellular activities is due to the much lower membrane permeability of the carboxylic acid salt of Compound **9** at physiological pH.



Scheme 1. *a.* ZnI_2, (1- ethoxycyclopropoxy)trimethylsilane, DCM, rt, 24 h; *b.* TFA, DCM/H_2O (100:1), rt, 2 d, 70% two steps; *c*. DBU, MeOH, rt, 2 h, 88%; *d*. PPh_3, DEAD, DPPA, THF, 0 ^oC to rt, 83%; *e*. PBu_3, H_2O, THF, rt, 30 min; then, TEA, methanol, 50 ^oC, 24 h, 71%; *f*. NaH, MeI, THF/DMF (1/1), rt, 30 min, 99%; *g*. K_2CO_3, 6-(methylsulfonyl)pyridin-3-ol, DMA, 120 ^oC, 16 h, 66%; *h*. Fe, NH_4Cl, isopropanol/water (1/1), 60 ^oC, 40 min, 99%; *i*. KI, KIO_3, HCl (5 M), MeOH/dioxane/water (4/3/3), 50 ^oC, 15 min, 99%; *j*. Ethyl chloroformate, pyridine, rt, 16 h, 99%; Chiral separation by HPLC, 36%; *k*. 2-Ethynylpyridine, bis(triphenylphosphine) palladium(II) dichloride, CuI, TEA, THF, 55 ^oC, 30 min, 99%; *l*. TBAF, THF, 55 ^oC, 1 h, 97%.

Table 1. *Structure activity relationships of the* 2,5,6-trisubstituted indole series GKAs



Compound	R^1	R^2	EC_{50} (nM) @ 2.5mM glucose	Maximum activity (vs. DMSO)	EC_{50} (nM) @ 10mM glucose	Maximum activity (vs. DMSO)	GKACA assay Rat INS-1 Cells EC_{50} (nM)	GKACA assay Rat Hepatocytes EC_{50} (nM)
1			3.7	784%	2.4	193%	0.9	7.5
1-en			4.2	628%	0.8	158%	33	93
2			4.5	620%	3.8	168%	21	16
2-en			5.5	482%	1.0	132%	37	77
3			4.5	713%	4.2	188%	7.2	23
3-en			48	577%	18	155%	247	1510
4			4.8	739%	2.1	173%	3.9	5.2
4-en			31	581%	9.8	132%	38	34
5			2.4	764%	<0.85	174%	2.5	2.7
5-en			86	583%	31	141%	375	199
6-rac			8.2	739%	3.4	184%	7.8	20
7			2.0	602%	1.4	158%	1.7	174
8			4.8	452%	9.2	123%	22	232
9			24	683%	9.6	179%	2111	728

Data are from a single titration.

Compound **1** was analyzed for its solubility, liver microsomal stability, plasma protein binding and cell permeability. The results are summarized in Table 2. The compound has excellent solubility at pH 2 and 7 (194 and 142 μM, respectively). It has reasonable stability upon incubation with human and rat liver microsome preparations (75% and 79% remaining after 30 min, respectively). A significant fraction of Compound **1** remains unbound in 100% human and C57BL/6 mouse plasma (8.7% and 7.2%, respectively). Compound **1** is also highly cell permeable, with a P_{app} = 40 x10^{-6} cm/s. These characteristics suggested that Compound **1** might have an attractive *in vivo* pharmacokinetic profile. Indeed, Compound **1** was found to have low clearance (18 mL/min/kg) in the rat, resulting in an acceptable half-life of 2.8 h. The oral bioavailability (%F) of Compound **1** was 30% (Table 3).

Table 2. Selected properties of Compound **1**

Solubility (μM)		Liver Microsomal Stability % remaining @ 1 μM after 30 min		Plasma Protein Binding % Unbound		LLC-PK1 MDCK P_{app} (10^{-6}cm/s)
pH 2	pH 7	Human	Rat	Human	Mouse	
194	142	75	79	8.7	7.2	40

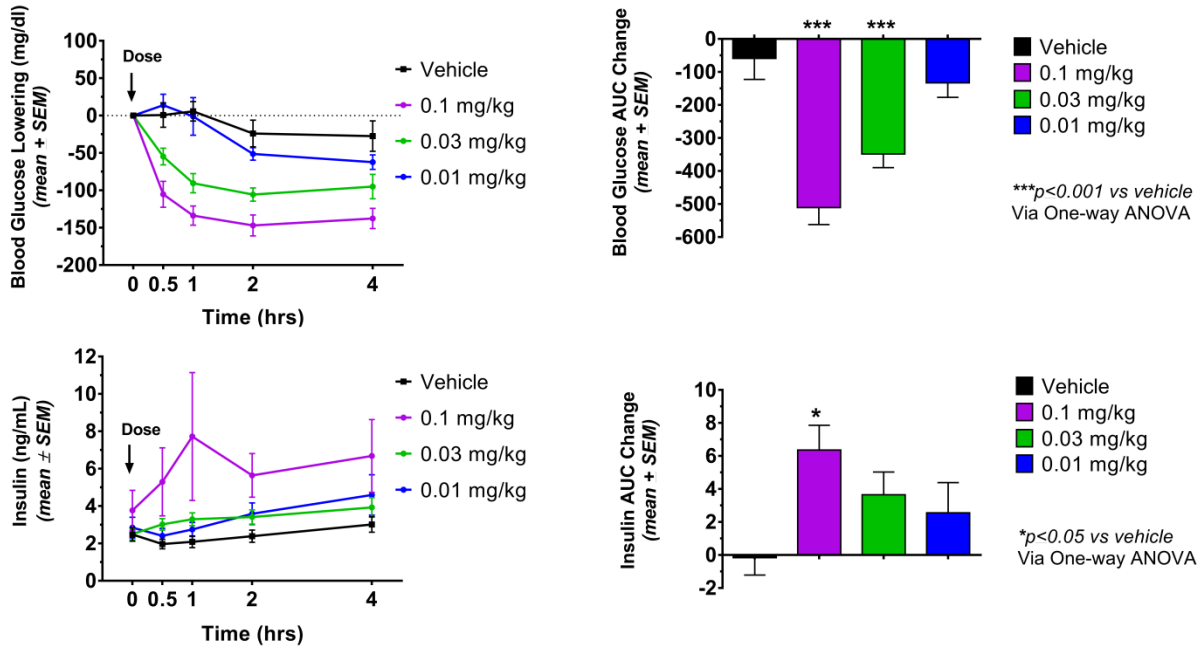
Table 3. Pharmacokinetics of Compound **1** in the rat

Compound	CL (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)	MRT (h)	AUCN (μM.h.kg/mg)	C_{max}/dose (μM.kg/mg)	F (%)
1	18	3.05	2.8	2.85	0.59	0.235	30

AUCN = normalized area under the curve; Vdss = volume of distribution; MRT = mean residence time

Based on its promising properties, Compound **1** was evaluated for its acute glucose lowering efficacy *in vivo* using a 3 week high fat diet (HFD)-fed C57BL/6 mouse model.[27] In this study, the compound was administered orally at 0.01, 0.03 and 0.1 mg/kg doses to mice having a baseline blood glucose level of ~200 mg/dl. Compound **1** was extremely efficacious in this model, with highly significant blood glucose lowering observed as early as 30 min post administration at doses as low as 0.03 mg/kg (Figure **1**). The effect persisted throughout the 4 h experimental period, resulting in a large reduction in blood glucose area under the curve (AUC) at the two highest doses. As expected, statistically significant increases in plasma insulin were observed at the 0.1 mg/kg dose. There was a trend towards increased plasma insulin levels at the 0.03 mg/kg dose. The observed glucose lowering efficacy in the absence of statistically significant insulin elevation at this dose may be due solely to the hepatic effects of GKAs to lower glucose. This data suggests that despite the high potency of Compound 1 as a GKA, a clinically useful therapeutic window might exist between its ability to meaningfully lower glucose while avoiding hypoglycemia. The plasma levels of Compound **1** (total drug) at 4 hours post dosing were 3.8 –18 nM and roughly dose-proportional (Table 4).

Figure 1. Acute effect of varying doses of orally administered Compound **1** on blood glucose and plasma insulin levels in HFD-fed mice.



N = 7 mice for each treatment group.

Table 4. Total Compound **1** drug levels in HFD mouse plasma at 4 h post dosing

Dose	Total [Compound **1**] in plasma (μM)
0.01 mg/kg	0.0038
0.03 mg/kg	0.012
0.10 mg/kg	0.018

In conclusion, a series of 2,5,6-trisubstituted indole compounds have been prepared and demonstrated to be novel, highly potent GK activators both *in vitro* and *in vivo*. SAR studies revealed excellent tractability in this series of GKAs. Optimization efforts led to the discovery of Compound **1**, which activates GK *in vitro* both in the enzymatic assays and in pancreatic β-cells and hepatocytes with high potency. Compound **1** exhibited good oral bioavailability and PK. Oral administration of Compound **1** at doses as low as 0.03 mg/kg led to robust blood glucose lowering in 3 week HFD-fed mice. Additional studies of this series of GKAs will be reported in due course.

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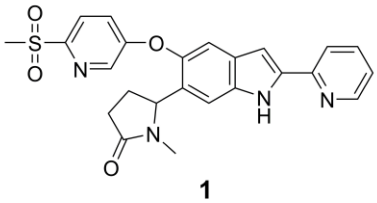
26. Glucokinase activity was measured by determining the ATP-dependent production of D-glucose-6-phosphate from D-glucose at 2.5 and 10 mM using a continuous glucose-6-phosphate dehydrogenase coupled spectrophotometric assay. All GKA were titrated using a final assay concentration of 1% DMSO, which served as the control. EC_{50} values are defined as concentration of GKA which gave half of maximal increase in glucokinase-catalyzed product formation at either 2.5 or 10 mM glucose.
27. Detailed experimental procedures will be published separately.

Graphical abstract



1

hGK EC50 (@2.5 mM glucose) = 3.7 nM; Max activity = 784%

hGK EC50 (@10 mM glucose) = 2.4 nM; Max activity = 193%