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2,3-Disubstituted acrylamides as potent glucokinase activators

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ABSTRACT

The phenylacetamide **1** represents the archtypical glucokinase activator (GKA) in which only the *R*-isomer is active. In order to probe whether the chiral center could be replaced, we prepared a series of olefins **2** and show in the present work that these compounds represent a new class of GKAs. Surprisingly, the SAR of the new series paralleled that of the saturated derivatives with the exception that there was greater tolerance for larger alkyl and cycloalkyl groups at R^2 region in comparison to the phenylacetamides. In normal Wistar rats, the 2,3-disubstituted acrylamide analog **10** was well absorbed and demonstrated robust glucose lowering effects.

Type 2 diabetes affects over 150 million people worldwide.[1] It is associated with decreased function of pancreatic β-cells, decreased insulin action and excess hepatic glucose production.[2] Glucokinase (GK), a member of the hexokinase family, acts as a β-cell glucose sensor and couples glucose metabolism to insulin release. GK also exerts control over hepatic glucose metabolism by influencing glucose uptake and production.[3,4] As such, we hypothesized that GK activators could represent a novel and promising approach for the treatment of type 2 diabetes.[5,6] Roche scientists discovered the GK activator **1** and showed that it lowers blood glucose levels in rodents and humans.[7,8]

In order to understand the importance of the chiral center in **1** (Fig. 1), we report here an investigation of the effects of replacing it with a double bond resulting in the discovery of 2,3-disubstituted acrylamide GKA series **2**. These analogues were proved to be potent GK activators. We also describe a robust 4-step synthesis of the 2,3-disubstituted acrylamide analogues that allowed for the rapid exploration of the SAR and report the pharmacokinetic properties and potent glucose lowering activity of **10**, a prototypical member of this series.

The preparation of this series is outlined in Scheme 1. Thus, the *trans*-olefin **4** was prepared by taking advantage of a highly regio- and stereoselective 1,4-addition of an alkyl organocopper species[9] to methyl propiolate to obtain, in turn a vinylcopper intermediate, that upon iodinolysis produced the pure trisubstituted olefin **4** where the R^2 and the iodide are *cis* to each other. The required

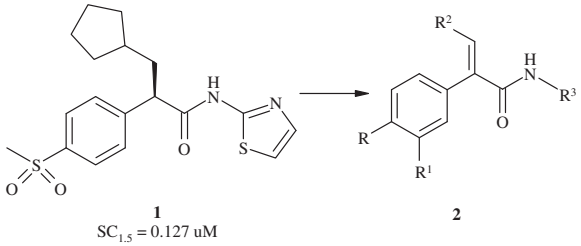

Figure 1.

organocopper reagent could be generated in situ either from an organozinc[10] or organomagnesium[9] intermediate. The intermediate **4** was treated with activated zinc dust and the resulting vinylzinc intermediate was coupled with a substituted bromo or iodobenzene in the presence of $Pd(dba)_2$ and triphenylphosphine to give the desired styrene **5** with retention of the *E*-configuration.[11] Saponification of the methyl ester was achieved with 1.0*N* aqueous sodium hydroxide in ethanol. Finally, the R^3 moiety was introduced via an in situ formed acyl bromide intermediate using *N*-bromosuccinimide and triphenylphosphine at 0 °C for 1.5 h followed by treatment with R^3–NH_2 at 0 °C to room temperature to obtain the target compounds **2**.

The tetrazole substituted bromobenzene and iodobenzene starting materials were prepared in two steps following a literature procedure as shown in Scheme 2.[12]

The compounds were assayed as previously described.[7,8] Briefly, the assay format couples the glucokinase mediated formation of

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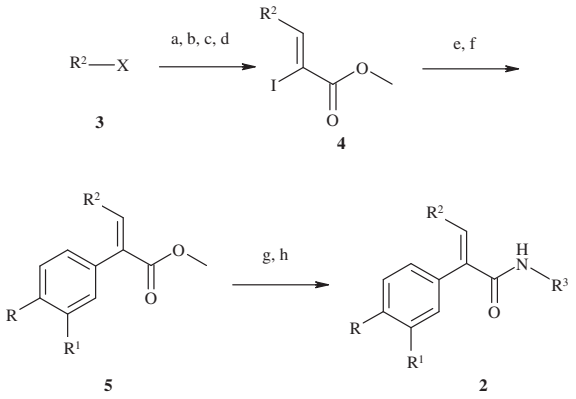
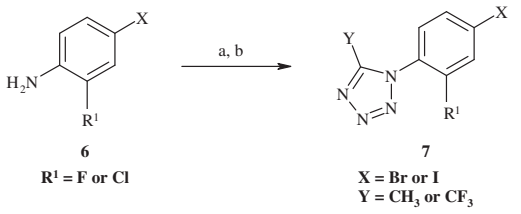


Scheme 1. Reagents and conditions: (a) X = I, Zn dust, 1,2-dibromoethane, TMSCl, THF, rt, 2–5 h, or X = Br, Mg turnings, THF, rt, 4–5 h; (b) CuCN·2LiCl, THF, −70 °C to −10 °C, 10 min; (c) methyl propiolate, THF, −70 °C to −30 °C, 3 h; (d) I_2, THF, −70 °C to rt, 1 h; (e) Zn dust, 1,2-dibromoethane, TMSCl, THF, rt, 3–4 h; (f) substituted bromobenzene or iodobenzene, $Pd(dba)_2$, PPh_3, THF, 40–45 °C, 15 h; (g) 1.0 N aq NaOH, EtOH, 40 °C, 4–5 h; (h) NBS, PPh_3, CH_2Cl_2, 0 °C, 1.5 h, and then 2-amino-R^3, 0 °C to rt, 15 h.



Scheme 2. Reagents and conditions: (a) Acetic anhydride or trifluoroacetic anhydride, THF, 0 °C to rt, 15 h; (b) NaN_3, acetonitrile, CH_2Cl_2, trifluoromethanesulfonic anhydride, 0 °C to rt, 15 h.

glucose-6-phosphate to NADH which is measured spectrophotometrically. Concentration response curves were constructed and the concentration of drug molecule causing a 50% increase ($SC_{1.5}$) in GK activity, relative to baseline, was calculated.

As anticipated from our experience with the saturated derivatives,[8] a significant improvement in potency was observed through the replacement of the lower alkyl moieties at R^2 with cycloalkyl groups (Table 1). Interestingly, the cyclopentyl analog was twofold more potent than that of the corresponding saturated compound (**1** vs **10**). In contrast to the SAR of the saturated derivatives, the cyclohexyl **11** and cycloheptyl **12** were more potent than the cyclopentyl derivative **10**. As a further surprise, the homologated cyclopentylmethyl analog **14** retained equivalent activity to the cyclopentyl analog **10**. A similar modification in the saturated series led to a complete loss of activity.[8] In our experience, the ready accommodation of the R^2 region of larger and homologated cycloalkyl groups is unique to this olefinic class of GK activators.

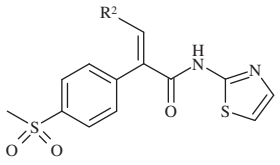
In order to investigate the stereochemical preference of these olefins and the impact of further subsitution, we prepared the *Z*-isomer and tetra-substituted derivatives. The *Z*-regioisomer **15** is ~20-fold less potent than the corresponding *E*-regioisomer **10** and the tetra-substituted olefin **16** was inactive (Fig. 2), possibly due to conformational effects leading to inhibition of essential hydrogen bonding between N–H and the protein.[13]

Further improvement in potency was achieved by optimizing the heteroaromatic ring (R^3) and the R- and R^1-substituents on the benzene ring while maintaining the cyclopentyl ring at R^2 (Table 2). The SAR at the R^3 region in the current series follows very similar trends to the saturated series. Several smaller substituents such as bromide, chloride, methyl, and cyano were acceptable at the 5-position of the thiazol-2-yl ring. Of these, the chloro **17** and bromo **18** derivatives gave a >5-fold improvement in potency. Other heterocycles such as pyridin-2-yl were also tolerated (**21** vs **22**), but with a small reduction in potency. Replacement of thiazol-2-yl ring with an open chain *N*-methylacetamide resulted a ninefold less potent compound (**25** vs **26**) which is in parallel with results obtained in the saturated series.[8]

Also consistent with our experience with the saturated analogues,[8] the benzene ring accommodates a variety of electron withdrawing groups at the 3- and 4-positions. Some of the 3,4-disubstituted analogues were somewhat more potent than the 4-monosubstituted compound **10**. The methanesulfonyl moiety at the 4-position can be substituted by a 5-methyl tetrazol-1-yl group (**22** vs **27**) without loss of potency. On the other hand, substitution with chloride reduced activity fivefold (**22** vs **24**).

Combination of the optimal substituents at each position R, R^1, R^2, and R^3 led to the very potent analogues described in Table 3. Several GK activators incorporating cyclopentylmethyl, cyclohexyl, and cycloheptyl moieties had $SC_{1.5}$ values in the single digit (**31** and **35**) to low double digit nanomolar range (**29**, **32**, **36** and **37**). The positive impact of addition of strongly electron withdrawing trifluoromethyl- (**28**–**31**) and nitro- (**32**) groups at the R^1 position of the aromatic ring are consistent with parallel observations in saturated series.[14] We also note that the replacement of a 5-methyl by a 5-trifluoromethyl group on the tetrazol-1-yl ring caused a fourfold reduction in potency (**33** vs **34**).

Table 1
SAR summary of R^2 modifications



Compd	R^2	$SC_{1.5}$ (μM)
8	Ethyl	1.2
9	Isopropyl	0.45
10	Cyclopentyl	0.06
11	Cyclohexyl	0.029
12	Cycloheptyl	0.021
13	Cyclooctyl	0.073
14	Cyclopentylmethyl	0.053

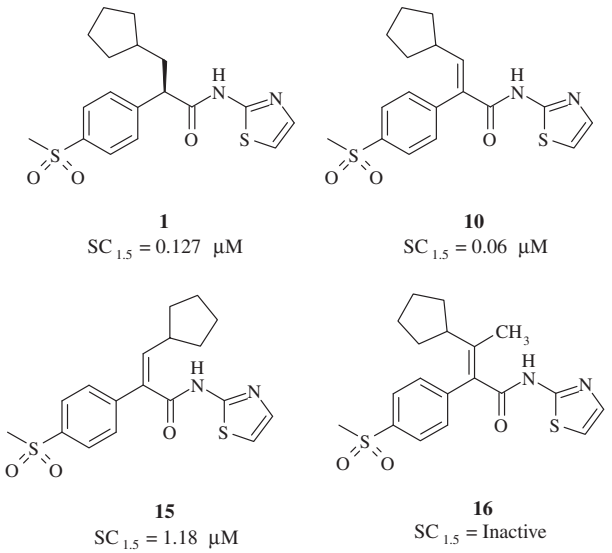
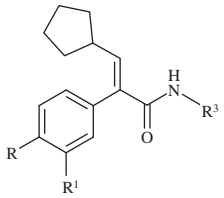


Figure 2. Comparison of *E*-, *Z*- and tetra-substituted olefins.

Table 2
SAR summary of R, R^1, and R^3 modifications



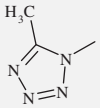
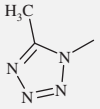
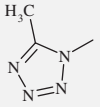
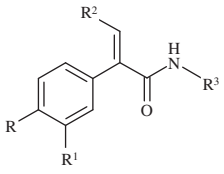
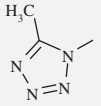
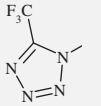
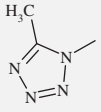
Compd	R	R^1	R^3	$SC_{1.5}$ (μM)
17	SO_2Me	H	5-Chloro-thiazol-2-yl	0.012
18	SO_2Me	H	5-Bromo-thiazol-2-yl	0.008
19	SO_2Me	H	5-Methyl-thiazol-2-yl	0.049
20	SO_2Me	H	5-Cyano-thiazol-2-yl	0.097
21	SO_2Me	Cl	Pyridin-2-yl	0.096
22	SO_2Me	Cl	Thiazol-2-yl	0.045
23	SO_2Me	Br	Thiazol-2-yl	0.033
24	Cl	Cl	Thiazol-2-yl	0.215
25		F	Thiazol-2-yl	0.102
26		F		0.904
27		Cl	Thiazol-2-yl	0.04

Table 3
SAR summary of R, R^1, R^2, and R^3 modifications



Compd	R	R^1	R^2	R^3	$SC_{1.5}$ (μM)
28	SO_2Me	CF_3	$cC_5H_9CH_2$	Thiazol-2-yl	0.037
29	SO_2Me	CF_3	cC_6H_{11}	Thiazol-2-yl	0.010
30	SO_2Me	CF_3	cC_6H_{11}	5-Bromo-pyridin-2-yl	0.036
31	SO_2Me	CF_3	cC_6H_{11}	5-Bromo-thiazol-2-yl	0.004
32	SO_2Me	NO_2	cC_6H_{11}	Thiazol-2-yl	0.013
33		Cl	cC_6H_{11}	Thiazol-2-yl	0.025
34		Cl	cC_6H_{11}	Thiazol-2-yl	0.092
35	SO_2Me	H	cC_7H_{13}	5-Bromo-thiazol-2-yl	0.004
36	SO_2Me	CF_3	cC_7H_{13}	5-Bromo-thiazol-2-yl	0.011
37		Cl	cC_7H_{13}	5-Bromo-thiazol-2-yl	0.012

Based on these results, we believe that the working model proposed previously for chiral compounds such as **1** would be applicable: donor–acceptor hydrogen bonding from the 2-amino heteroaromatic ring, a hydrophobic cyclic aliphatic R^2 and an aromatic ring which tolerates a range of electron withdrawing groups in the 3- and 4-positions. [8]

One concern with this class of compounds was the potential formation of a GSH adduct by a 1,4-Michael addition of GSH to the α,β-unsaturated acrylamide. To understand whether this possibility constituted a liability for this series, compound **10** was labeled with ^{14}C at the carbonyl carbon and the resulting analog was incubated with primary cryopreserved hepatocytes from rat, dog, monkey, and human for 30 min. During this study, six major metabolites were identified (Fig. 3). Four of them are the products of mono-oxidation at the 2- and 3-positions of the cyclopentane ring (M1 and M2) and their corresponding ketones (M3 and M4). The other two metabolites were identified as the thiourea derivatives (M5 and M6) resulting from oxidative cleavage of the thiazole ring of the M1 and M2 metabolites. Basically, the total metabolism varies widely from species to species as shown in Table 4, although the major metabolites form in all species tested were M5 and M6. Interestingly, we found no trace of a GSH adduct formed during this study.

Based on the in vitro and metabolic stability data, compound **10** was selected for pharmacokinetic and in vivo efficacy studies in rats to evaluate the potential of this class of compounds.

As shown in Table 5 and **10** was completely absorbed and displayed a high clearance and moderately high volume of distribution resulting in an elimination half-life of 1.7 h in rats (Table 5). The oral bioavailability (F%) in mice (10 mg/kg), dogs (10 mg/kg), and monkey (100 mg/kg) was 58%, 88%, and 37%, respectively.

In normal Wistar rats, **10** caused a robust dose-dependent reduction in blood glucose levels after oral doses of 3–30 mg/kg. At the higher dose, the effects persisted more than 8 h (Fig. 4).

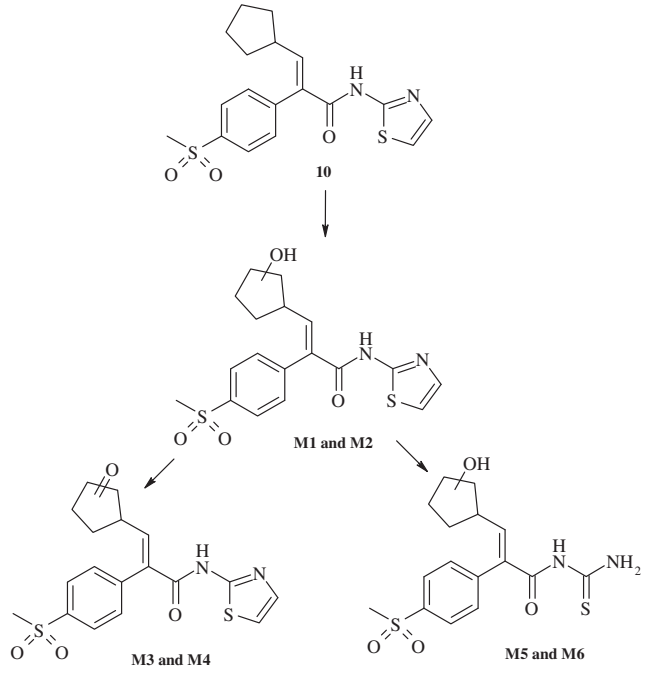


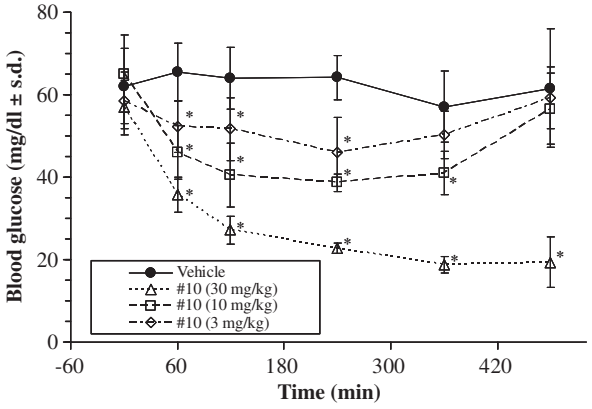
Figure 3. Metabolites of compound **10**.

Table 4
Metabolites profile of ^{14}C radiolabelled compound **10** at 30 μM concentration

Primary cryopreserved hepatocytes	M1 and M2	M3 and M4	M5 and M6	Total % metabolism
Rat	8.0	2.9	5.2	16.1
Dog	0.6	0.0	0.2	0.8
Monkey	37.1	12.3	16.5	65.9
Human	13.7	7.3	15.6	36.6

Table 5
Pharmacokinetic parameters of compound **10** in rats following intravenous and oral administration

	AUC_{0-24} (ng h)	$T_{1/2}$ (h)	CL (mL/h/kg)	V_{dss} (mL/kg)	*F* (%)
iv (5 mg/kg)	3150	1.7	1543	2643	
po (30 mg/kg)	23998				100



Rats (n=5) were fasted for two hours prior to dose.
*, $p < 0.05$ compared to Vehicle using a Student's *t*-test.

Figure 4. Efficacy with compound **10** in Wistar rats.

In conclusion, we prepared a novel class of 2,3-disubstituted acrylamide GK activators as achiral derivatives of the chiral phenylacetamide derivative **1**. This modification resulted in the identification of a number of potent GK activators with good exposure in a variety of species and robust efficacy in lowering glucose levels in vivo.

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