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Glucokinase-activating ureas

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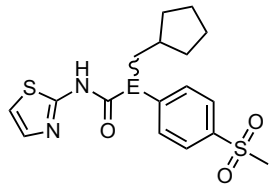
Abstract—The synthesis, SAR and biological evaluation of a series of ureas that activate glucokinase, a target for diabetes therapy as a result of its critical role in the regulation of whole-body glucose homeostasis, are described. Some of the urea-containing glucokinase activators lowered blood glucose levels in vivo following oral dosing to C57BL/6J mice.

Glucokinase (GK) is the characteristic hexokinase isoenzyme found in hepatocytes and in pancreatic β-cells.[1] This enzyme catalyses the first step in glucose (Glc) metabolism, viz., the conversion of Glc to glucose-6-phosphate. As its activity is rate limiting for Glc use, GK functions as a Glc sensor[2] in hepatocytes and β-cells, allowing it to play a critical role in whole-body Glc homeostasis. The recently-described GK activators[3] can alleviate the hyperglycaemia associated with Type 2 Diabetes by increasing hepatic glycogen synthesis and Glc-stimulated pancreatic insulin release. These compounds, such as RO-28-1675 (**1**),[3a] activate GK through an interaction with an allosteric site some 20 Å away from the active site.[4] Here, we describe the synthesis and SAR of urea-containing[5] GK activators in which the chiral α-carbon of **1** is replaced by an achiral nitrogen atom.

Initially, two methods were employed to synthesise the urea-containing GK activators described here (Scheme 1), viz., the three-component condensation[6] of a secondary amine,[7] carbonyldiimidazole and a heteroaromatic amine (Method A) and the reaction of a secondary amine[7] with an isocyanate dimer[8] (Method B). The first of these two methods gave low yields for heteroaromatic amines with poor nucleophilicities, while the second was found not to be generally applicable to the synthesis of a wide range of ureas bearing varied heteroaromatic rings. Subsequently, we discovered that the condensation of the secondary amines[7] with the 4-nitrophenylcarbamate esters[9] of heteroaromatic amines in refluxing MeCN (Method C) permitted the synthesis of a diverse set of ureas in good yields. For poorly nucleophilic secondary amines, this condensation reaction proceeded better in refluxing $MeNO_2$ (Method D) or in $MeNO_2$ under microwave irradiation[10] (Method E).



Cmpd	E	≀
1	CH	≡
2	CH	|
3	N	|

At a Glc concentration of 15 mM, the maximal activation (MA) of GK by urea **3** was 2.8-fold over control levels (Fig. 1a).[11] When the Glc concentration was reduced to 5 mM, the MA value increased, as did the EC_{50} for GK activation (Table 1). A kinetic study with

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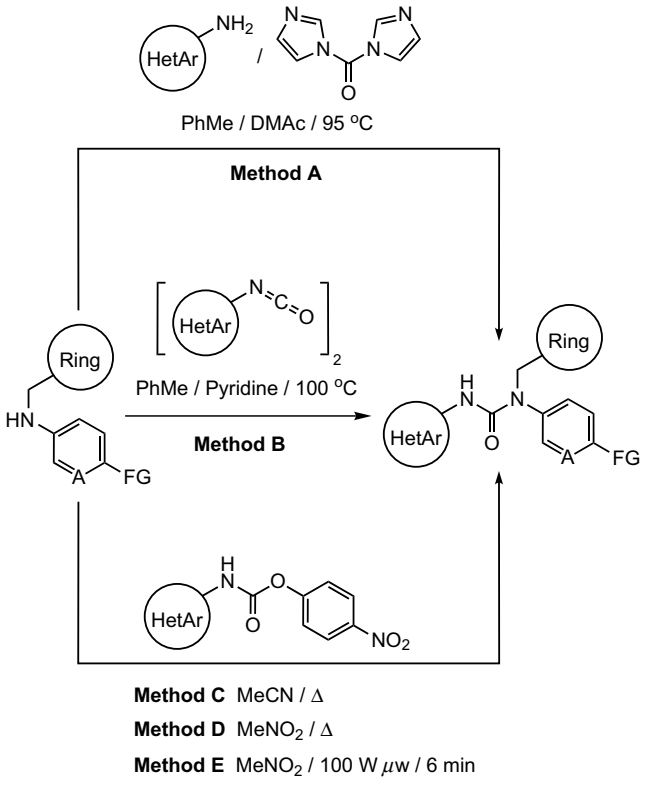


Scheme 1.

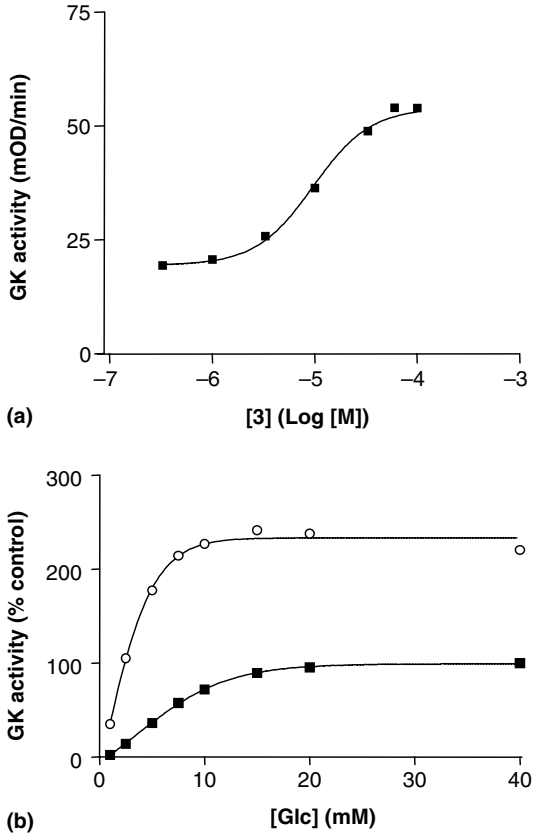


Figure 1. (a) Concentration–response curve highlighting the activation of GK by the urea **3** at a Glc concentration of 15 mM. (b) At 30 μM, urea **3** (open circles) activates GK compared to control (solid squares) by increasing the enzyme's V_{max} and by decreasing the $S_{0.5}$ for Glc.

30 μM **3** revealed (Fig. 1b) that this compound activates GK by increasing this enzyme's V_{max} (221% control) and its affinity for Glc, expressed as a reduction in the Glc $S_{0.5}$ (30% control). Nonetheless, despite maximally activating the enzyme to the same extent as **2**,[3a] a racemic mixture of **1** and its antipode, **3** activated GK less potently. The dramatic difference in potencies between **2** and **3** translated into in vivo activities. In overnight-fasted C57BL/6J mice,[12] **2** lowered basal blood Glc levels by up to 36% compared to vehicle control when administered orally at 50 mg/kg, while **3** showed no blood Glc-lowering effects at a higher dose of 100 mg/kg. Thus, we strove to improve the potencies of the GK-activating ureas, while keeping their physicochemical properties in check to allow reasonable in vivo exposure.

Table 2 portrays the SAR associated with the heteroaromatic amine portion of the ureas. Pyridines, for example, **9**, and thiadiazoles, for example, **4** and **13**, showed poor activity, while thiazoles substituted with a halogen atom at the 5-position, for example, **5**, **8**, **11** and **12**, were the strongest GK activators in terms of both MA and EC_{50}. 5-Chloro- and 5-bromo-substituted thiazoles were equiactive. Despite having slightly better in vitro activity than **2**, chlorothiazole **5** did not cause statistically-significant blood Glc lowering when dosed orally to C57BL/6J mice at 100 mg/kg.

Table 2 also shows that GK-activating ureas can have a diverse range of substituted aryl and heteroaryl rings attached to the tertiary urea nitrogen. As shown in Table 3, the 4-methanesulfonyl group of **5** can be replaced by a

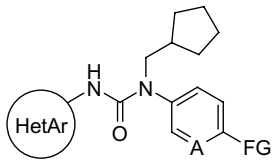
Table 1. In vitro data for the activation of GK by **2** and **3**

Compound	5 mM Glc		15 mM Glc	
	GK EC_{50} (μM)	GK MA	GK EC_{50} (μM)	GK MA
2	2.2	5.0	1.0	1.6
3	16.0	4.7	9.7	2.8

range of electron-withdrawing substitutents, such as sulfonamido (**15**), trifluoromethyl (**16**) or cyano (**17**), to give equipotent compounds with slightly reduced MA values. On the other hand, compounds bearing bulky electron-donating substituents, for example, **18** and **19**, did not activate GK. Compounds with halogen-substituted rings were potent, in some instances furnishing submicromolar GK activators, for example, **20** and **21**. Ureas bearing hydrogen bond-donating substituents, for example, **23** and **24**, were very potent GK activators that strongly activated the enzyme.

As can be seen from Table 4, replacing the cyclopentyl ring of **5** with heterocyclic rings had a detrimental effect on both potency and MA in the in vitro GK assay. Compounds bearing a thiophene ring, such as **26** and **27**, were ca. 4.5-fold less potent than **5** and this reduction in potency became even more dramatic when the cyclopentyl ring was replaced by five-atom, oxygen-

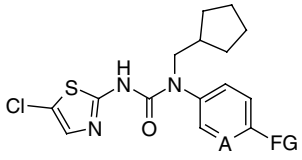
Table 2. SAR around heteroaromatic amine moiety



Compd	Synthesis method/yield (%)	FG	A	HetAr	GK EC_{50} (μM)[a]	GK MA[a]
3	A/25	SO_2Me	CH	2-Thiazolyl	16.0	4.7
4	B/52	SO_2Me	CH	2-(5-Trifluoromethyl-[1,3,4]thiadiazolyl)	34.2	2.4
5	C/94	SO_2Me	CH	2-(5-Chlorothiazolyl)	1.5	7.0
6	C/83	SO_2Me	CH	2-(6-Methanesulfonylbenzothiazolyl)	32.9	2.0
7	C/30	F	CF	2-Thiazolyl	16.3	3.8
8	C/84	F	CF	2-(5-Chlorothiazolyl)	2.6	4.8
9	C/93	F	CF	2-Pyridyl	NA[b]	NA[b]
10	C/13	OMe	N	2-Thiazolyl	19.2	2.7
11	C/95	OMe	N	2-(5-Chlorothiazolyl)	2.7	5.0
12	C/24	OMe	N	2-(5-Bromothiazolyl)	2.0	5.0
13	C/13	OMe	N	2-[1,3,4]Thiadiazolyl	NA[b]	NA[b]
14	C/9	OMe	N	2-(4-Oxo-4,5-dihydrothiazolyl)	29.1	3.1

[a] GK EC_{50} and GK MA values obtained at a Glc concentration of 5 mM.
[b] No activation at 30 μM.

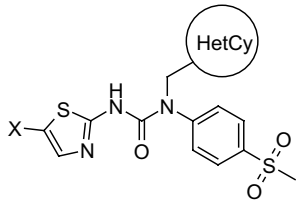
Table 3. SAR around aryl/heteroaryl ring attached to tertiary urea nitrogen



Compd	Synthesis method/yield (%)	FG	A	GK EC_{50} (μM)[a]	GK MA[a]
5	C/94	SO_2Me	CH	1.5	7.0
15	C/90	SO_2NH_2	CH	1.8	5.6
16	C/92	CF_3	CH	1.3	4.9
17	C/61	CN	CH	2.4	5.2
18	B/37	Morpholino	CH	NA[b]	NA[b]
19	C/100	OPh	CH	NA[b]	NA[b]
20	C/73	Me	CCl	0.4	2.2
21	C/92	Br	CF	0.7	4.5
22	A/18	H	C(OMe)	1.4	3.3
23	C/42	H	C(NHAc)	0.3	5.3
24	D/63	H	$C(CONH_2)$	1.0	8.0
25	E/34	CN	N	7.7	6.4

[a] GK EC_{50} and GK MA values obtained at a Glc concentration of 5 mM.
[b] No activation at 30 μM.

Table 4. SAR of compounds where the cyclopentyl ring of **5** is replaced by a heterocycle



Compd	Synthesis method/yield (%)	HetCy	X	GK EC_{50} (μM)[a]	GK MA[a]
5	C/94	Cyclopentyl	Cl	1.5	7.0
26	C/87	2-Thienyl	Cl	6.6	3.3
27	E/13	3-Thienyl	Cl	6.8	3.0
28	E/32	3-Thienyl	Br	6.5	3.9
29	E/19	2-Thiazolyl	Br	24.1	2.2
30	D/5	2-Furyl	Cl	11.5	3.8
31	E/28	2-Tetrahydrofuryl	Cl	21.6	5.0
32	E/45	3-Tetrahydrofuryl	Cl	21.9	5.9
33	E/18	4-Tetrahydropyranyl	Cl	4.9	3.0

[a] GK EC_{50} and GK MA values obtained at a Glc concentration of 5 mM.

containing heterocycles, such as in **30**–**32**. Nonetheless, a large amount of the potency lost was regained in **33**, which contains a six-atom, 4-tetrahydropyranyl ring. Urea **26** lowered blood Glc levels by up to 34% compared to vehicle control when administered orally at 100 mg/kg to overnight-fasted C57BL/6J mice. The furan-containing urea **30** demonstrated slightly reduced hypoglycaemic effects in vivo compared to its thiophene-containing counterpart **26**. This compound lowered blood Glc levels by up to 22% compared to vehicle control following oral administration to overnight-fasted C57BL/6J mice at 100 mg/kg.

In summary, we have discovered a series of ureas that activate GK, an enzyme critical for the control of whole-body Glc homeostasis. Some of the GK activators lowered blood Glc levels following oral dosing to mice, giving further credence to the premise that GK activators could be a useful addition to the physician's arsenal in the fight against Type 2 Diabetes.[13]

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11. The in vitro GK assay was performed at 30 °C in a flat bottom 96-well assay plate with a final incubation volume of 100 μL. The assay buffer contained 25 mM Hepes buffer (pH 7.4), 12.5 mM KCl, 5 or 15 mM D-Glc, 5 mM ATP, 6.25 mM NADP, 25 mM $MgCl_2$, 1 mM dithiothreitol, test compound or 5% DMSO, 3.0 unit/mL glucose-6-phosphate dehydrogenase and 0.4 μL/mL human liver GST-GK fusion protein. The test compounds were dissolved in DMSO, before being added to the assay buffer without ATP. This mix was pre-incubated in the temperature controlled chamber of a SPECTRAmax 250 microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA, USA) for 10 min, then the reaction was started by the addition of 10 μL ATP solution. After starting the reaction, the increase in optical density (OD) at 340 nm was monitored over a 10 min incubation period as a measure of GK activity. The 50% efficacy concentration (EC_{50}) values were calculated from sigmoidal dose–response curves by the least squares method, using GraphPad Prism 3.0 (GraphPad Software, Inc., San Diego, CA, USA).
12. In vivo assay for GK activators: The compounds were dissolved in EtOH–Gelucire 44/14–PEG400 q.s. (4:66:30 v/v/v), at a concentration of 13.3 mg/mL, before being given orally to C57BL/6J mice ($n = 5$)—which had been weighed and fasted for 18 h beforehand—at 50 or 100 mg/kg. Immediately prior to dosing, pre-dose (time zero) blood Glc readings were acquired by snipping off a small portion of the animals' tails (<1 mm) to allow 15 μL of blood to be collected for analysis. After compound treatment, further blood Glc readings were taken at 1, 2, 4 and 6 h post-dose from the same tail wound. Results were interpreted by comparing the mean blood Glc values of five vehicle treated mice with the five compound treated mice over the 6 h study duration. Compounds were considered active when they exhibited a statistically significant decrease in blood Glc compared to vehicle for two consecutive assay time points. The percentage blood Glc reduction at each time point was calculated using the following relationship: $\{([\text{Blood Glc}]_{\text{vehicle}} - [\text{Blood Glc}]_{\text{compound}})/[\text{Blood Glc}]_{\text{vehicle}}\} \times 100\%$.
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