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Identification of novel and potent 2-amino benzamide derivatives as allosteric glucokinase activators

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ABSTRACT

The identification and structure–activity-relationships (SARs) of novel 2-amino benzamide glucokinase activators are described. Compounds in this series were developed to be potent GK activators, and their binding mode to the GK protein was determined by crystal structure analysis. In vivo pharmacokinetic and acute in vivo efficacy studies of compound **18** are also described.

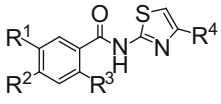
Glucokinase (GK), a member of the hexokinase family,[1] catalyzes the first step in glycolysis involving the phosphorylation of glucose to glucose 6-phosphate. GK plays an important role as a glucose sensor in maintaining plasma glucose homeostasis by enhancing insulin secretion from pancreatic β-cells and glucose metabolism in the liver.[2,3] Therefore activation of GK is expected to be a novel therapeutic strategy for the treatment of type II diabetes. Consistent with this rationale, several groups have reported that the glucokinase activators (GKAs) demonstrated antidiabetic efficacy in rodent models.[4–7] Recently, AstraZeneca[8,9] and OSI Prosidion[10,11] have also defined the SARs of small molecule allosteric activators of GK. In addition, a number of GKAs have been disclosed in the patent literature and reviewed in peer edited journals.[12–15]

As part of our own effort, we have supplied detailed descriptions of in vitro features and mechanisms of action of our GK activator (compound **18**), effects on GK and glucokinase regulatory protein (GKRP) interactions, and the GK activator's effect on pancreatic islets and hepatocytes.[16] In this paper, we describe the discovery and detailed SAR studies of a novel series of potent allosteric GKAs built on a 2-amino benzamide scaffold. We also discuss the SARs based on the binding mode between the GK protein and our own activator as revealed by crystal structure analysis.[17]

A high throughput screen of our compound collection was carried out in order to identify the small molecule GKA and the thiazole amide series (**1a** and **b**) were identified based on their activation of recombinant human GK enzyme.[18] In vitro GK assay was conducted in two different glucose concentration, 2.5 and 10 mM which were simulated to be fasting and postprandial blood glucose conditions, respectively.

At first, with regard to the core structure of the GKAs, both the reduction of the amide linkage to benzylamine and substitution on the amide NH were not tolerated (data not shown). These SARs suggest that the benzamide template would account for the significant GK activation. Preliminary SAR studies on the screening hits (**1a** and **b**) also indicated the importance of the substituent on 5-position of the benzene ring, along with the presence of an NH_2 group in the 2-position, in the activation of the target GK enzyme (Table 1). The removal of a chlorine atom from the 5-position of the

Table 1
Investigation of preliminary SAR on benzamide lead **1**



Compound	R^1	R^2	R^3	R^4	2.5 mM Glc EC_{50} (μM)[a]	10 mM Glc EC_{50} (μM)[a]
1a	Cl	H	NH_2	H	11	2.6
1b	Cl	H	NH_2	Me	6.5	1.4
2	H	H	NH_2	H	>30	>30
3	H	Cl	NH_2	Me	>30	>30
4	Cl	H	H	Me	17	6.3

[a] EC_{50} values are the mean of at least two independent assays (Ref. 18).

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benzene ring (**2**), or the migration of the Cl atom to the 4-position (**3**), resulted in loss of potency. Compound **4**, containing no NH_2 group on the benzene ring, showed reduced GK activation potency. Initially, to explore the SARs in the series, the aniline moiety was kept on the benzamide template and various substitutions were introduced at the 5-position to probe the requirement for potency.

Figure 1 depicts the preparation of compounds using 5-fluoro-2-nitrobenzoic acid as the starting material. Treatment of 5-fluoro-2-nitrobenzoic acid with oxalyl chloride and a catalytic amount of DMF afforded the corresponding benzoyl chloride. The benzoyl chloride then reacted with the appropriate commercially available aromatic amines to give benzamides. Reactions of 2-nitrobenzamides with alkoxide, phenol or thiol nucleophiles (K_2CO_3/DMF or TEA/CH_3CN) provided the 5-substituted-2-nitro benzamides. Subsequently the 2-amino benzamides were obtained by reduction of the nitro compounds catalyzed by iron powder and saturated aqueous NH_4Cl.

During the course of our optimization studies concerning the 5-position substituent on the benzene ring (Table 2), the presence of hydrophobic groups such as phenoxy (**6**) afforded enhanced GK potency, and introduction of the substitution on the ortho (**7**) position was more effective for GK activation than substitutions on both the meta (**8**) and para (**9**) positions. Introduction of a polar group such as methoxy (**10**) and methylsulfonyl (**11**) onto the ortho position was also tolerated. In order to introduce a wide variety of heteroaromatics with feasible physicochemical properties and metabolic stability, we next examined the thioether derivatives. These studies demonstrated that ethyl-thioether (**12**) was more potent than the corresponding ether compound (**5**); on the other hand, GK activity of phenylthioether (**13**) and 2-pyridinylthioether derivatives (**14**) slightly reduced the potency compared with the phenoxy compound (**6**). Although thio-imidazole (**15**) and 1,2,4-triazole (**17**), both possessing an NH group in their ring structure, significantly reduced the potency, the corresponding *N*-methylated 5-membered ring systems (**16** and **18**) were effective at improving the GK activation.

With these data in hand we elected to focus further on the *N*-Me-triazolylthio compound **18** to achieve a balance between intrinsic potency and physical properties such as aqueous solubility and metabolic stability. Table 3 depicts the SAR associated with the aromatic ring on the amide portion. Among them, 2-thiazolyl (**18**) and [1,2,4]-thiadiazol-5-yl (**19** and **20**) amides are very effective for potent GK activation and the 2-pyridine (**21**) ring system was tolerated. In contrast, 3- and 4-pyridyl (**22** and **23**) and phenyl (**24**) derivatives showed no activation of GK enzyme. Examination of the aromatic ring systems on the amide suggested that the nitrogen atom at the 2-position on the aromatic ring is necessary for GK activation.

Next we examined substitutions on the thiazole ring in the compound (Table 4). A methyl substitution on both 4- and 5-position on the thiazole moiety showed no significant effects on their intrinsic potencies. Results regarding substituents on the 4-position of the thiazole ring in the compound, electron withdrawing groups such as CO_2Et (**27**), CF_3 (**29**) and hydrophilic group like CH_2OH (**28**) showed a significant reduction of potency in comparison with compounds **25** and **18**.

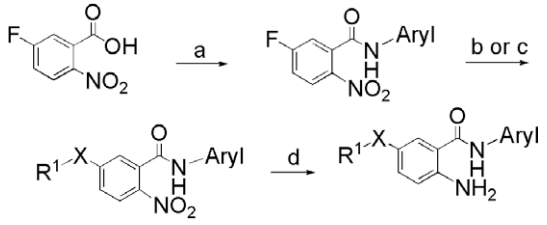
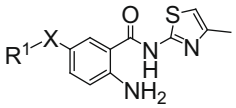


Figure 1. Reagents and conditions: (a) $(COCl)_2$, DMF (cat.), CH_2Cl_2 followed by Aryl-NH_2, TEA, CH_3Cl; (b) EtONa or EtSNa, THF; (c) Phenol or Thiol, K_2CO_3/DMF or TEA/CH_3CN; (d) Fe powder, sat-NH_4Cl, i-PrOH.

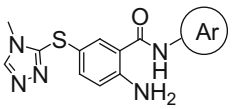
Table 2
Effects of substitution on 5-position of the benzene ring



Compound	R^1	X	2.5 mM Glc EC_{50} (μM)[a]	10 mM Glc EC_{50} (μM)[a]
1b	Cl	–	6.5	1.4
5	Et	O	6.8	1.7
6	C_6H_5	O	0.70	0.13
7	2-F-C_6H_4	O	0.26	0.10
8	3-F-C_6H_4	O	0.60	0.14
9	4-F-C_6H_4	O	1.7	0.40
10	2-MeO-C_6H_4	O	0.41	0.12
11	2-$MeSO_2$-C_6H_4	O	0.51	0.13
12	Et	S	0.78	0.18
13	C_6H_5	S	0.92	0.20
14	2-Pyridine	S	1.2	0.22
15	1H-Imidazol-2-yl	S	1.6	0.38
16	1-Me-1H-Imidazol-2-yl	S	0.23	0.10
17	4H-[1,2,4]-Triazol-3-yl	S	2.4	0.54
18	4-Me-4H-[1,2,4]-Triazol-3-yl	S	0.42	0.14

[a] EC_{50} values are the mean of at least two independent assays (Ref. 18).

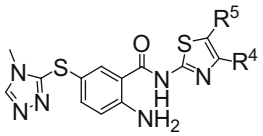
Table 3
SAR around aromatic ring on the amide moiety



Compound	Ar	2.5 mM Glc EC_{50} (μM)[a]	10 mM Glc EC_{50} (μM)[a]
18		0.42	0.14
19		0.49	0.12
20		0.64	0.18
21		1.2	0.23
22		>10	>10
23		>10	>10
24		>10	>10

[a] EC_{50} values are the mean of at least two independent assays (Ref. 18).

Table 4
Effects of substitution on the thiazole ring



Compound	R^5	R^4	2.5 mM Glc EC_{50} (μM)[a]	10 mM Glc EC_{50} (μM)[a]
25	H	H	0.35	0.13
26	Me	H	0.33	0.11
18	H	Me	0.42	0.14
27	H	CO_2Et	1.1	0.42
28	H	CH_2OH	1.6	0.42
29	H	CF_3	2.7	0.93

[a] EC_{50} values are the mean of at least two independent assays (Ref. 18).

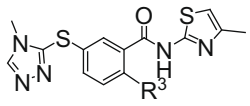
We finally turned our attention to the SARs of the aniline moiety on the core benzene ring (Table 5). However, replacement of NH_2 (**18**) by hydrogen (**30**) or NO_2 group (**31**) resulted in a loss of potency. The introduction of a methyl group (**32**) on the aniline portion reduced GK potency.

In parallel with our SAR studies, the co-crystal structure of GK protein with allosteric activators was solved and the binding mode of our own GKA series was revealed (Fig. 2).[19,20] Compound **18** binds to an allosteric binding site on the hinge region between the small and large domains approximately 20 Å from and opposite the glucose binding site. The crystal data suggested that there are important H-bond and hydrophobic interactions involved in binding to the GK protein and inducing GK activation. The compound makes three hydrogen bonds with the enzyme: both the NH and nitrogen atom in the amino-thiazole moiety pair with the backbone of Arg63, and the NH on aniline pairs with Tyr215. In addition, the aniline moiety on the benzene ring may works as an important functional group which making an intramolecular H-bond interaction with the carbonyl group on its amide linkage, thus fixing the conformation in a beneficial way. The N-Me-triazole moiety on the benzene ring occupies the hydrophobic space on the allosteric binding site.

The X-ray data and revealed binding mode correlate well with our SARs which were developed during the modification of this structure class. In order to bind to the GK enzyme and show potent GK activation, an amino-heteroaromatic amide core possessing the nitrogen atom at the 2-position of the heteroaromatic ring is critical. Furthermore, because the aniline moiety works as both an intra- and intermolecular hydrogen bonding acceptor, introduction of a substitution on the aniline portion and removal of aniline moiety resulted in a loss of potency.

These SAR studies allowed the identification of several potent activators of human glucokinase. In addition, an activator which provided an acceptable pharmacokinetic and metabolic profile was identified and evaluated in animals. We describe here the pharmacokinetic profile and glucose-lowering efficacy of compound **18** in rats. During an in vivo pharmacokinetic study of male Wistar rats, compound **18** demonstrated excellent bioavailability (Table 6). In a Wistar rat Oral Glucose Tolerance Test

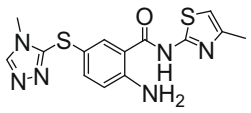
Table 5
SAR of the aniline moiety of compound **18**



Compound	R^3	2.5 mM Glc EC_{50} (μM)[a]	10 mM Glc EC_{50} (μM)[a]
18	NH_2	0.42	0.14
30	H	7.3	1.5
31	NO_2	>10	>10
32	NHMe	1.1	0.33

[a] EC_{50} values are the mean of at least two independent assays (Ref. 18).

Table 6
Pharmacokinetic parameter of compound **18** in male Wistar rats



Distribution volume (L/kg)	Clearance (mL/min/kg)	$t_{1/2}$ (iv) (h)	C_{max} (μM)	t_{max} (h)	F_{po} (%)
0.4	14.3	0.9	8.5	0.5	~100

Doses: 1 mg/kg (iv) and 3 mg/kg (po).

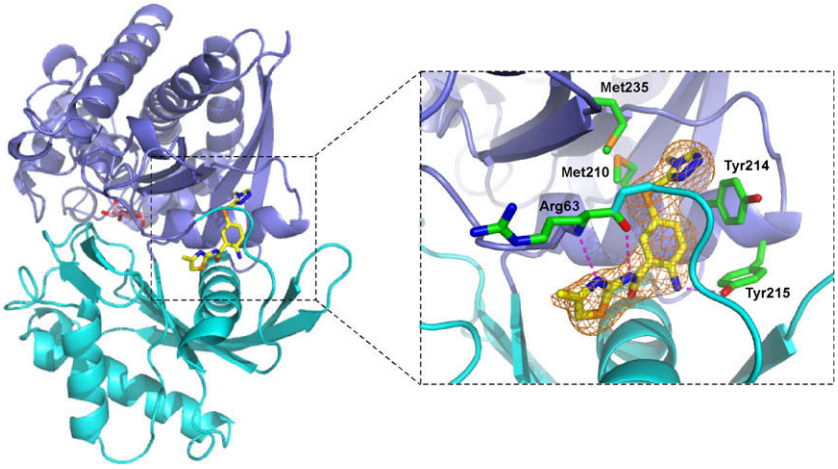


Figure 2. Overall structure of GK protein with compound **18** and binding mode of compound **18**; Final $2F_o - F_c$ electron density (1.2 σ level) for compound **18** is shown as orange mesh. This figure was prepared using the CCP4 package[21] and PyMOL[22]

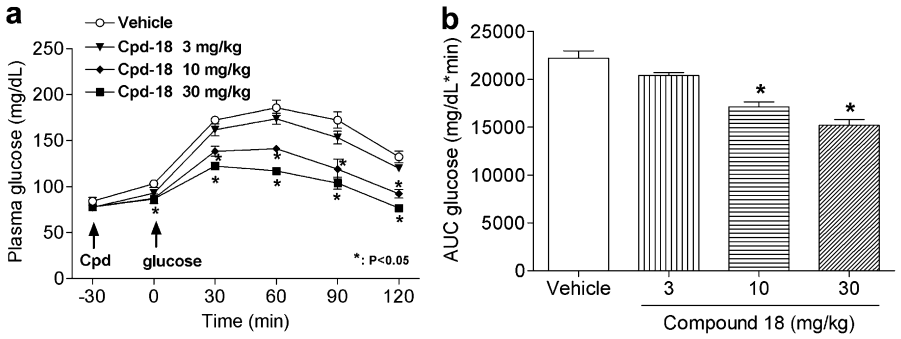


Figure 3. In vivo efficacy data of compound **18** in male Wistar rat OGTT[23]. (a) Plasma glucose lowering and (b) AUC reduction. '∗' Values of $p < 0.05$ were considered statistically significant for vehicle group.

(OGTT), oral administration of compound **18** demonstrated glucose-lowering efficacy in a dose-dependent manner, and showed significant reduction of plasma glucose levels at 10 and 30 mg/kg (Fig. 3).[23]

In conclusion, we have identified a series of 2-amino benzamides as small molecule allosteric activators of glucokinase. The binding mode of these molecules was revealed and detailed SAR on GK activation was developed and analyzed. The triazole compound **18** was developed and has demonstrated promising in vivo glucose-lowering effects in an acute rat model. Further modifications and SAR studies based on the crystal structure analyses are subjects for future investigation and will be reported in due course.

References and notes

1. Printz, R. L.; Magnuson, M. A.; Granner, D. K. *Annu. Rev. Nutr.* **1993**, *13*, 463.
2. Matschinsky, F. M. *Diabetes* **1996**, *45*, 223.
3. Matschinsky, F. M.; Glaser, B.; Magnuson, M. A. *Diabetes* **1998**, *47*, 307.
4. Grimsby, J.; Sarabu, R.; Corbett, W. L.; Haynes, N. E.; Bizzarro, F. T.; Coffey, J. W.; Guertin, K. R.; Hilliard, D. W.; Kester, R. F.; Mahaney, P. E.; Marcus, L.; Qi, L. D.; Spence, C. L.; Tengi, J.; Magnuson, M. A.; Chu, C. A.; Dvorozniak, M. T.; Matschinsky, F. M.; Grippo, J. F. *Science* **2003**, *301*, 370.
5. Efanov, A. M.; Barrett, D. G.; Brenner, M. B.; Briggs, S. L.; Delaunois, A.; Durbin, J. D.; Giese, U.; Guo, H. H.; Radloff, M.; Gil, G. S.; Sewing, S.; Wang, Y.; Weichert, A.; Zaliani, A.; Gromada, J. *Endocrinology* **2005**, *146*, 3696.
6. Coope, G. J.; Atkinson, A. M.; Allott, C.; McKerrecher, D.; Johnstone, C.; Pike, K. G.; Holme, P. C.; Vertigan, H.; Gill, D.; Coghlan, M. P.; Leighton, B. *Br. J. Pharmacol.* **2006**, *149*, 328.
7. Fyfe, M. C. T.; White, J. R.; Taylor, A.; Chatfield, R.; Wargent, E.; Printz, R. L.; Sulpice, T.; McCormack, J. G.; Procter, M. J.; Reynet, C.; Widdowson, P. S.; Wong-Kai-In, P. *Diabetologia* **2007**, *50*, 1277.
8. McKerrecher, D.; Allen, J. V.; Bowker, S. S.; Boyd, S.; Caulkett, P. W. R.; Currie, G. S.; Davies, C. D.; Fenwick, M. L.; Gaskin, H.; Grange, E.; Hargreaves, R. B.; Hayter, B. R.; James, R.; Johnson, K. M.; Johnstone, C.; Jones, C. D.; Lackie, S.; Rayner, J. W.; Walker, R. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2103.
9. McKerrecher, D.; Allen, J. V.; Caulkett, P. W. R.; Donald, C. S.; Fenwick, M. L.; Grange, E.; Johnson, K. M.; Johnstone, C.; Jones, C. D.; Pike, K. G.; Rayner, J. W.; Walker, R. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2705.
10. Castelhano, A. L.; Dong, H. Q.; Fyfe, M. C. T.; Gardner, L. S.; Kamikozawa, Y.; Kurabayashi, S.; Nawano, M.; Ohashi, R.; Procter, M. J.; Qiu, L.; Rasamison, C. M.; Schofield, K. L.; Shah, V. K.; Ueta, K.; Williams, G. M.; Witter, D.; Yasuda, K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1501.
11. Bertram, L. S.; Black, D.; Briner, P. H.; Chatfield, R.; Cooke, A.; Fyfe, M. C. T.; Murray, P. J.; Naud, F.; Nawano, M.; Procter, M. J.; Rakipovski, G.; Rasamison, C. M.; Reynet, C.; Schofield, K. L.; Shah, V. K.; Spindler, F.; Taylor, A.; Turton, R.; Williams, G. M.; Wong-Kai-In, P.; Yasuda, K. *J. Med. Chem.* **2008**, *51*, 4340.
12. Sarabu, R.; Grimsby, J. *Curr. Opin. Drug Discov. Dev.* **2005**, *8*, 631.
13. Guertin, K. R.; Grimsby, J. *Curr. Med. Chem.* **2006**, *13*, 1839.
14. Coghlan, M.; Leighton, B. *Expert Opin. Investig. Drugs* **2008**, *17*, 145.
15. Sarabu, R.; Berthel, S. J.; Kester, R. F.; Tilley, J. W. *Expert Opin. Ther. Patents* **2008**, *18*, 759.
16. Futamura, M.; Hosaka, H.; Kadotani, A.; Shimazaki, H.; Sasaki, K.; Ohyama, S.; Nishimura, T.; Eiki, J.; Nagata, Y. *J. Biol. Chem.* **2006**, *281*, 37668.
17. Kamata, K.; Mitsuya, M.; Nishimura, T.; Eiki, J.; Nagata, Y. *Structure* **2004**, *12*, 429.
18. Glucokinase activities were measured by the glucose-6-phosphate dehydrogenase coupled continuous spectrophotometric assay. EC_{50} values were designated as concentration of compounds which give half of maximal increased enzymatic activities of glucokinase by the compound at 2.5 and 10 mM glucose. Compound **18** was used an internal control across all assay plates for data validation. The EC_{50} values of this compound are 0.42 ± 0.09 and 0.14 ± 0.04 μM at 2.5 and 10 mM glucose, respectively. All compounds in this series investigated exhibited equivalent E_{max} values to the maximal response evoked by compound **18** (8.0- to 8.3-fold and 1.6- to 1.8-fold over control levels at 2.5 and 10 mM glucose). Detailed assay methods and conditions are described in Ref. 16.
19. Protein and crystals were obtained according to established procedures.[17] Crystals were soaked in 0.5 mM compound **18** overnight in mother liquor containing 5% DMSO. Diffraction data were collected on beamline BL32B2 at the SPring-8, at 100 K (a Rigaku *R*-axis V image plate). Data processing and data reduction were carried out using programs from the HKL2000 (HKL Research, Inc.) and the CCP4 package. Compound **18** was modeled into the electron density using Afitt (OpenEye Scientific Software). The protein–compound complex model was refined using CNX (Accelrys), and the final structure has been deposited in the Protein Data Bank with the deposition code 3FR0 together with structure factors and detailed experimental conditions.
20. Crystallographic statistics for the GK-compound 18 complex are as follows: space group $P6_522$, unit cell 84.1, 84.1, 323.2 Å, resolution 2.70 Å, 17,901 reflections from 361,813 observations give 99.8% completeness with R_{sym} of 6.2% and mean $I/\sigma(I)$ of 9.2. The final model containing 3506 protein, 135 water, 1 salt, 12 glucose and 26 compound atoms has an *R*-factor of 22.1% (R_{free} using 5% of the data 27.5%). Mean temperature factors for the protein and the ligand are 44.4 and 33.3 $Å^2$, respectively.
21. Collaborative. *Acta Crystallogr. D* **1994**, *50*, 760.
22. PyMOL molecular graphics system, DeLano Scientific, San Carlos, CA. Available from: <http://www.pymol.org>.
23. Oral glucose tolerance test (OGTT); Nine-week-old male Wistar rats fasted overnight before performing the test. The rats were orally administered compound **18** or vehicle alone (0.5% methylcellulose solution) followed 30 min later by an oral glucose challenge (2 g/kg). Plasma glucose concentrations were measured just prior to and following the glucose challenge (30, 60, 90, and 120 min). AUC values were calculated from the data (from −30 to 120 min).