Bioorganic & Medicinal Chemistry Letters 19 (2009) 4450–4454

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel 2-(pyridine-2-yl)-1*H*-benzimidazole derivatives as potent glucokinase activators

Makoto Ishikawa *, Katsumasa Nonoshita, Yoshio Ogino, Yoshikazu Nagae, Daisuke Tsukahara, Hideka Hosaka, Hiroko Maruki, Sumika Ohyama, Riki Yoshimoto, Kaori Sasaki, Yasufumi Nagata, Jun-ichi Eiki, Teruyuki Nishimura

Banyu Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd, Okubo 3, Tsukuba 300-2611, Ibaraki, Japan

ARTICLE INFO

Article history:
Received 24 March 2009
Revised 11 May 2009
Accepted 12 May 2009
Available online 18 May 2009

Keywords:
Glucokinase
Glucokinase activator
Diabetes

ABSTRACT

The synthesis and structure–activity-relationships (SARs) of novel 2-(pyridine-2-yl)-1*H*-benzimidazole glucokinase activators are described. Systematic modification of benzimidazole lead **5a** identified from a high-throughput screening led to the discovery of a potent and metabolically stable glucokinase activator **16p**(***R***) with greater structural diversity from GKAs reported to date. The compound also demonstrated acute oral glucose lowering efficacy in rat OGTT model.

Glucokinase (GK) is a hexokinase isoform (hexokinase IV) which is predominantly expressed in liver and pancreatic beta-cells.[1] It functions as a key initial and rate-limiting step for glucose metabolism, that is, catalyzing the reaction of glucose to glucose-6-phosphate. In the liver, GK activation increases hepatic glucose uptake and utilization, while GK activity is coupled to increase insulin secretion in beta cells.[2,3] Therefore, GK activators (GKAs) can be expected to work as hypoglycemic agents by both increasing glucose uptake in liver and potentiating insulin secretion from pancreatic beta-cells. Consistent with this rationale, several groups have described the structures and SARs of various small-molecule GKAs,[4–9] exemplified by compounds **1**[10] and **2**.[11] We also recently reported the SARs on our own series of 2-aminobenzamide (**3**)[12] and 3,6-disubstituted-2-pyridinecarboxiamide GKAs (**4a**, **4b**),[13] respectively, which function through binding to an allosteric site of GK enzyme.[14]

During our own effort to identify structurally diverse GKAs, a benzimidazole compound **5a** was identified as a lead with μM order of GK activation from a high-throughput screen of our compound collection. As shown in Figure 1, GKAs which have been disclosed in the literature to date commonly possess the heteroaromatic amide structure as an important pharmacophore to bind to the allosteric site of GK and show GK activation. Since the newly identified benzimidazole template is entirely structurally distinct from GKAs previously disclosed, compound **5a** could be an attractive molecule for further modification to improve GK potency and develop structurally diverse GKAs. In this report, the synthesis and SARs on a novel series of GKAs from 2-(pyridine-2-yl)-1*H*-benzimidazole are described.

The general synthetic route of benzimidazole derivatives reported in this Letter is illustrated in Scheme 1. Commercially available 4-bromo-3-fluoroaniline **6** was coupled with 1-Boc-pyrrol-2-boronic acid[15] using Suzuki–Miyaura coupling to yield compound **7**. Reduction of pyrrole **7** with Pt/C under hydrogen atmosphere produced a racemic mixture of pyrrolidine derivative **8**. Manipulation of protecting group of Boc-pyrrolidine **8** and subsequent nitration by fuming nitric acid afforded nitrobenzene compound **9**. The trifluoroacetamide groups were then removed under basic conditions and the resulting pyrrolidine amine was capped by *t*-butylcarbamate group (**10**), followed by displacement of the fluorine atom on 5-position with 4-fluorophenol via nucleophilic aromatic substitution, giving nitroaniline derivative **11**. The nitro group was reduced with Raney-nickel to provide *o*-phenylendiamine **12**. Condensation of the diamine **12** with 2-pyridinecarboxaldehyde and subsequent in situ thermal cyclization afforded the targeted 2-pyridyl-1*H*-benzimidazole structure (**13**).[16] Deprotection of the Boc group on the pyrrolidine portion under acidic conditions gave key intermediate **14a**. Finally, reaction of the amine compound **14a** with appropriate electrophiles gave the corresponding *N*-substituted pyrrolidine derivatives **5a** and **15a–g**.

3-(pyridine-2-yl)-(**5b**) and 3-(phenyl-2-yl)-1*H*-benzimidazole derivatives (**5c**) were prepared by the same reaction sequence as those of compound **5a**: coupling and cyclization reaction between

* Corresponding author. Tel.: +81 055 88 2430; fax: +81 055 89 0945.
E-mail address: makoto.ishikawa@mochida.co.jp (M. Ishikawa).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.05.038

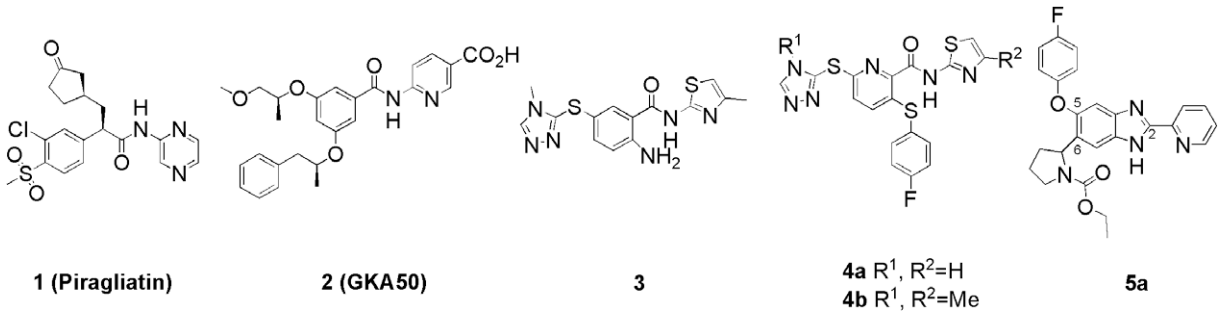
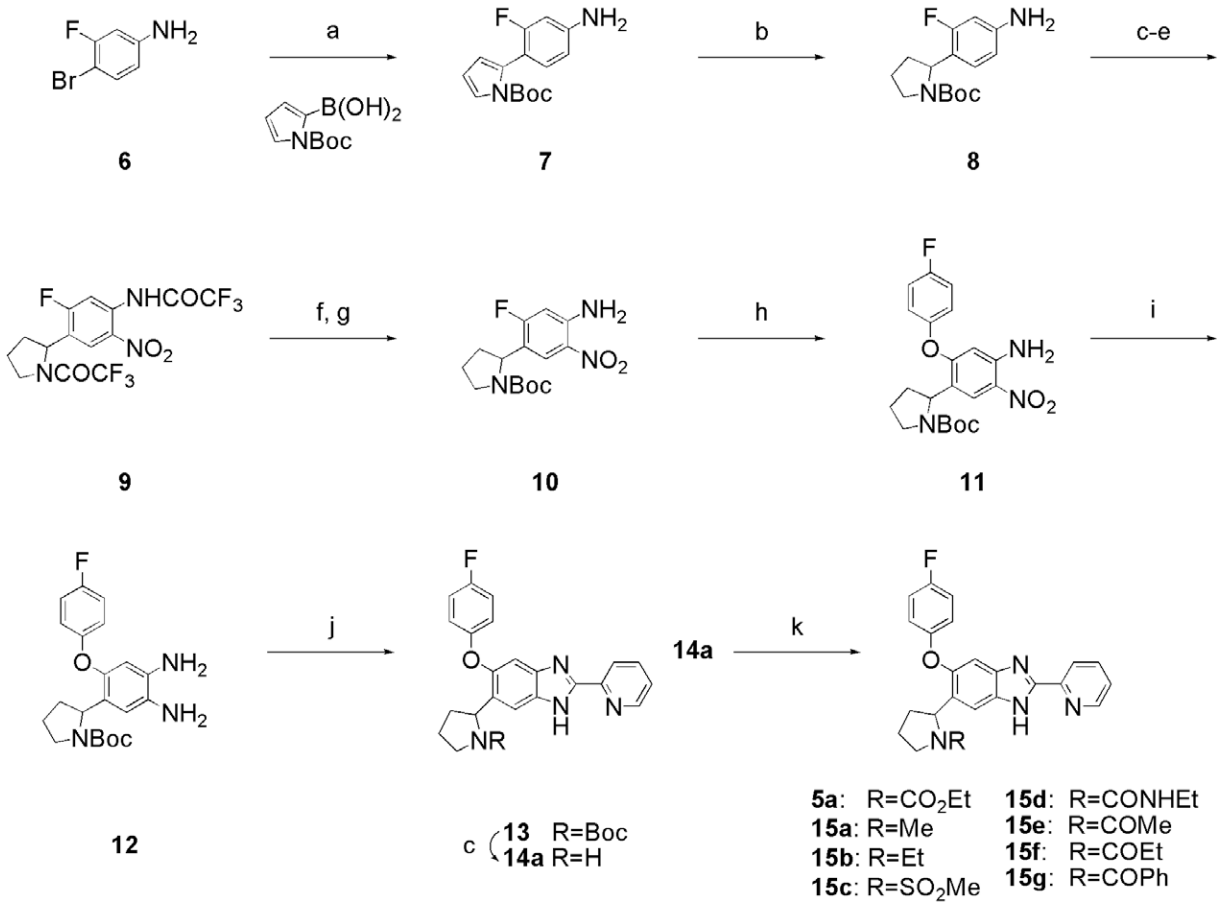


Figure 1. Structures of glucokinase activators (**1**–**4a**, **b**) reported in the literature and lead compound **5a**.



Scheme 1. Reagents and conditions: (a) $Pd(PPh)_2Cl_2$, Na_2CO_3, 80 °C, DME, H_2O, 63%; (b) Pt/C, H_2, MeOH, 85%; (c) TFA; (d) $(CF_3CO)_2O$, pyridine; (e) $fHNO_3$, 87% for three steps; (f) K_2CO_3, MeOH–H_2O; (g) Boc_2O, Et_3N, $CHCl_3$, 67% for two steps; (h) Cs_2CO_3, 4-fluorophenol, NMP, 80 °C, 85%; (i) Raney-Ni, NH_2NH_2, MeOH, 90%; (j) 2-pyridinecarboxaldehyde, EtOH, AcOH, 80 °C, 65%; (k) electrophile, $CHCl_3$, TEA, room temperature.

the *o*-phenylendiamine intermediate **12** and 3-pyridinecarboxaldehyde for **5b** or benzaldehyde for **5c**, respectively. An *N*-methylbenzimidazole analogue **5d** was prepared by methylation of **5a** with methyl iodide in the presence of NaH. The 5-phenoxy benzimidazole derivatives **16a**–**q** shown in Table 3 were synthesized in a similar manner to those of 4-F-phenyl compound **15e** by reaction of nitroaniline intermediate **10** with appropriate phenols instead of 4-fluorophenol.

All synthesized compounds were tested for their GK activations measured by glucose-6-phosphate dehydrogenase coupled continuous spectrophotometric assay.[17,18] Both EC_{50} values and maximal effective responses (E_{max})[19] are described for comparison of derivatives in each assay. In addition, metabolic stability in microsomes was measured to screen orally-available compounds before in vivo efficacy study.[20,21]

Prior to chemical modification of the benzimidazole lead, the predicted binding mode of lead compound **5a** to the allosteric site of GK enzyme was examined by molecular modelling analysis. Previously we reported the results of co-crystal structure analysis and the binding mode of GKAs **3** and **4b** containing a 2-amino thiazole amide scaffold as a common structural feature. According to the co-crystal structure of GK complex with compound **4b** (Fig. 2), the amino-thiazole moiety makes two critical hydrogen-bonding interactions with the enzyme to achieve potent GK activation: both the NH and nitrogen atom of the compound with the C=O and NH of the backbone Arg63. In contrast, a docking model of compound **5a** into the allosteric binding site of amide GKAs led to the proposed enzyme binding mode shown in Figure 3. This proposed model suggests that the novel 2-(pyridine-2-yl)-1*H*-benzimidazole core also can make key hydrogen-bonding interactions with back-

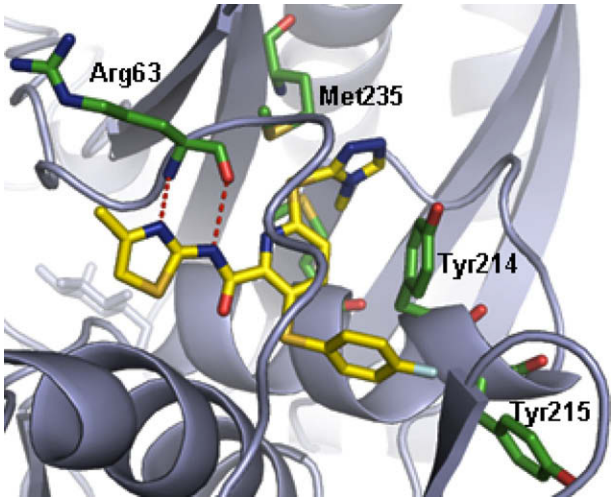


Figure 2. X-ray co-crystal structure of **4b** bound to glucokinase.

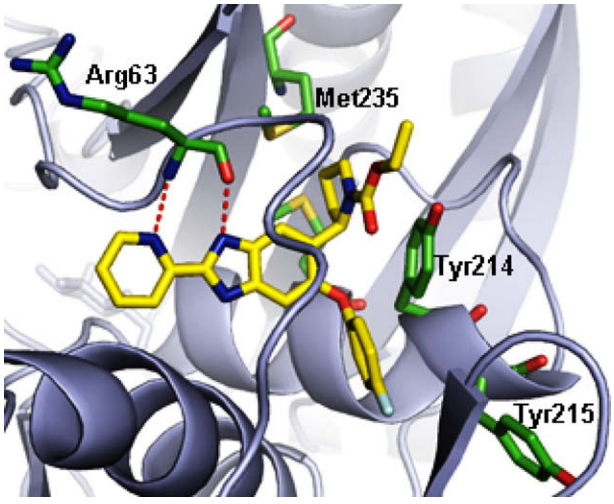


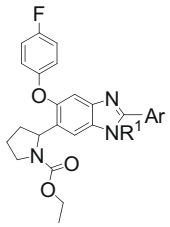
Figure 3. Predicted binding mode for **5a** bound to glucokinase.

bone Arg63 as well as heteroaromatic amide GKAs, and two substituents on the benzimidazole seem to occupy each hydrophobic interaction sites.

To clarify our hypothesis on the binding mode of a benzimidazole GKA (**5a**), we investigated the effect of the position of the nitrogen atom in the aromatic ring (Ar) at the right hand side of the molecule and a substituent (R^1) on the NH in the benzimidazole core to their GK potency (Table 1). The replacement of 2-pyridyl (**5a**) moiety with 3-pyridyl (**5b**) or phenyl (**5c**) resulted in a complete loss of GK potency. In addition, 1-methybenzimidazole derivative **5d** also completely lost its potency. Because these decreases in the GK potency were presumably due to the loss of hydrogen bonding interaction of the nitrogen atom at 2-pyridine moiety or NH in the benzimidazole core with Arg 63 on GK enzyme, the 2-(pyridine-2-yl)-1*H*-benzimidazole template in this lead structure (**5a**) would be an important pharmacophore for GK activation.

To explore the detailed SARs of the substitutions at the 5- or 6-position on the benzimidazole portion, 2-(pyridine-2-yl)-1*H*-benzimidazole core structure was kept based on the predicted binding mode and primary SARs on the lead compound **5a**. Initially, the impact of the substitution (R^2) on the pyrrolidine moiety at the 6-position of the benzimidazole on GK potency and metabolic stability

Table 1
SAR of Ar and R^1 substituent



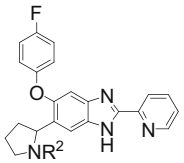
Cpd.	Ar	R^1	EC_{50}[a] (μM)	E_{max}[b] (%)
5a	2-Pyridyl	H	5.5	93
5b	3-Pyridyl	H	NA[c]	NA[c]
5c	Phenyl	H	NA[c]	NA[c]
5d	3-Pyridyl	Me	NA[c]	NA[c]

[a] Values are the mean of two or more independent assays.
[b] See Ref. 19 for detailed description.
[c] No activation at 30 μM.

was examined (Table 2). The NH free pyrrolidine compound **14a** resulted in complete loss of potency. The analogues with *N*-alkyl substituents such as methyl (**15a**) or ethyl (**15b**) also showed no GK activation although they showed improved metabolic stability in human microsomes compare with carbamate lead **5a**. The potency of *N*-methylsulfonylamide (**15c**) and *N*-ethylurea (**15d**) derivatives decreased substantially compared to ethoxycarbonyl compound **5a**. During the course of the modification, acetyl group (**15e**) was identified as an acceptable surrogate for the carbamate group and resulted in improvement of both GK potency and metabolic stability in human microsomes (25% for **15e** vs 8% for **5a**). The increase in bulk of the substituent on the pyrrolidine part such as propionyl (**15f**) and benzoyl group (**15g**) resulted in reduction of potency.

Next we turned our attention to substitution on the phenoxy moiety at the 5-position of the benzimidazole (**15e**) in order to improve GK potency and identify a candidate for in vivo evaluation (Table 3). First, we confirmed the effect of the regio-chemical structure of a fluoro atom, a methoxy group and a cyano group on GK potency. As a result, introduction of electron donating (**16e** and **f**) or withdrawing (**16h** and **i**) groups onto the 3- or 4-po-

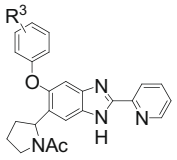
Table 2
SAR of *N*-substituted pyrrolidine derivatives



Cpd.	R^2	EC_{50}[a] (μM)	E_{max}[b] (%)	HM stability[c] % remaining
5a	CO_2Et	5.47	93	8
14a	H	NA[d]	NA[d]	69
15a	Me	NA[d]	NA[d]	77
15b	Et	NA[d]	NA[d]	66
15c	SO_2Me	19.1	132	26
15d	CONHEt	>30	64	37
15e	COMe	1.1	93	26
15f	COEt	6.1	76	6
15g	COPh	>30	44	44

[a] Values are the mean of two or more independent assays.
[b] See Ref. 19 for detailed description.
[c] See Ref. 20 for detailed description.
[d] No activation at 30 μM.

Table 3
Effects of substitution on the benzene ring



Cpd.	R^3	EC_{50}[a] (μM)	E_{max}[b] (%)	HM stability[c] % remaining
16a	H	1.1	110	18
16b	2-F	3.0	87	19
16c	3-F	0.93	138	17
15e	4-F	1.1	93	26
16d	2-OMe	9.6	103	24
16e	3-OMe	2.4	111	28
16f	4-OMe	1.7	109	47
16g	2-CN	>30	28	17
16h	3-CN	1.9	83	46
16i	4-CN	1.9	110	48
16j	3-Ph	7.4	88	62
16k	4-Ph	1.5	89	53
16l	4-$CONH_2$	1.6	100	82
16m	4-CO_2Et	2.7	100	46
16n	4-NO_2	1.9	100	60
16o	4-SO_2Me	1.1	113	76
16p	4-SO_2Et	0.63	98	61
16p(*R*)		0.36	113	41
16p(*S*)		2.3	67	—
16q	4-SO_2iPr	1.5	100	30

[a] Values are the mean of two or more independent assays.
[b] See Ref. 19 for detailed description.
[c] See Ref. 20 for detailed description.

sition of the benzene ring at the phenoxy portion were tolerable. In contrast, installation of these substituents at 2-position of the phenyl ring reduced GK potency (**16b**, **d** and **g**). Incorporation of a bulky substituent like phenyl into the 3-position (**16j**) resulted in substantial loss of GK activity compared to the 4-substituted derivative (**16k**). In addition, *para*-substituted analogues tended to show significantly improved metabolic stability in human microsomes. From these results, our modification effort focused on the 4-position of the phenoxy moiety and we examined hydrophilic substitutions (**16l**–**q**) to improve metabolic stability in human microsomes. Among these analogues, 4-ethylsulfonylphenoxy derivative **16p** exhibited a satisfactory balance between GK potency (EC_{50} = 0.63 μM) and metabolic stability in human microsomes (61% remaining) to address the lead potential of this structure class in animal model.

The next logical step was to synthesize and compare two individual enantiomers of analogue **16p**. Key compound **14b** (Scheme 2) was prepared from intermediate **10** in a similar manner as 4-fluoro-phenoxy compound **14a** using 4-ethylsulfonylphenol in place of 4-fluorophenol. Racemic pyrrolidine compound **14b** was separated by chiral HPLC to identify each enantiomer.[22] Subsequently, both isomers were converted to corresponding acetyl derivatives **16p**(***R***) and **16p**(***S***), respectively. The absolute configuration of **16p**(***R***) and **16p**(***S***) were determined based on authentic samples prepared by reported asymmetric synthesis.[23,24] Between these two enantiomers, one isomer **16p**(***R***) was identified as an active isomer with 0.36 μM EC_{50} value (Table 3).

We describe here the glucose lowering efficacy of compound **16p**(***R***) in male Wistar rat. In rat Oral Glucose Tolerance Test (OGTT),[25] oral administration of compound **16p**(***R***) demonstrated glucose lowering efficacy in a dose-dependent manner from 3 mpk to 30 mpk (Fig. 4a), and showed significant reduction of plasma glucose at 3 mg/kg (Fig. 4b).

In summary, we have identified novel 2-(pyridine-2-yl)-1*H*-benzimidazole GKAs which are entirely structurally distinct from GKAs reported so far with heteroaromatic amine templates. Systematic modifications on lead compound **5a** led to an improvement in GK potency and metabolic stability. Further we identified an *N*-acetyl-pyrrolidinylbenzimidazole derivative

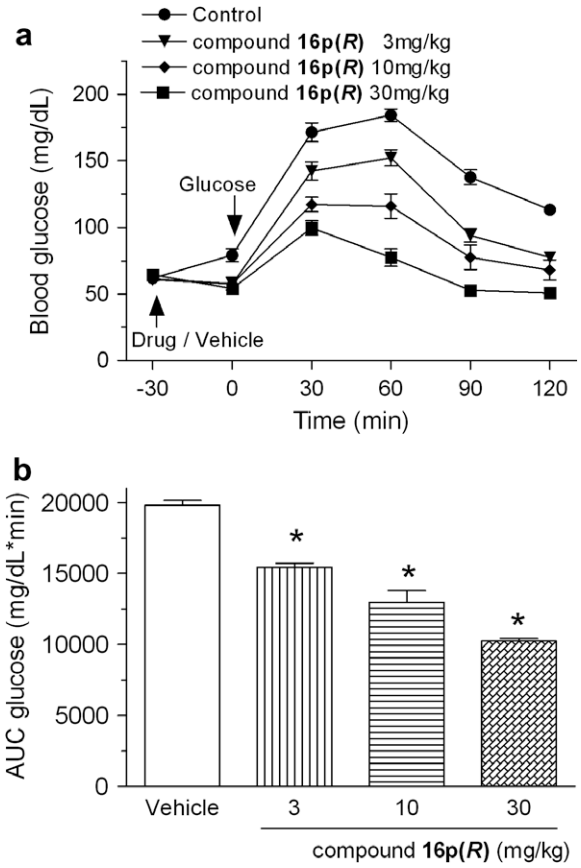
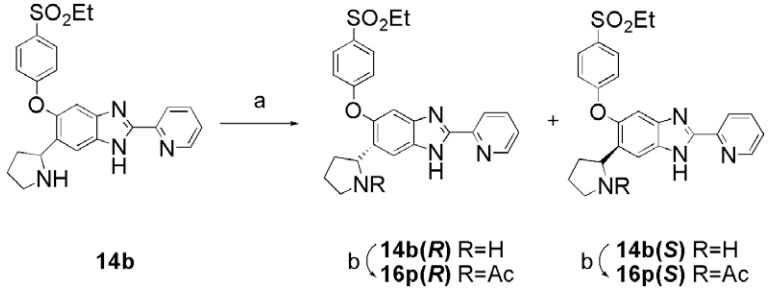


Figure 4. In vivo efficacy data of compound **16p**(***R***) in male Wister rat OGTT.[25] (a) Plasma glucose lowering and (b) AUC reduction. *Values of $p < 0.05$ were considered statistically significant for vehicle group.



Scheme 2. Reagents and conditions: (a) chiral separation by HPLC eluted with *n*-hexane/^{i}PrOH (9/1)/0.1% Et_2NH; (b) Ac_2O, pyridine.

16p(***R***) which showed significant in vivo glucose lowering efficacy in rat OGTT model. The predicted binding mode by molecular modelling and our modification results on lead compound **5a** suggested that the 2-pyridyl-benzimidazole pharmacophore would be an alternative allosteric GKA instead of the heteroaromatic amide template. Co-crystal structure analysis of benzimidazole derivatives with GK protein are underway to confirm the binding mode of this novel structure class of GKAs, and further modification results on this series to improve GK potency will be reported in due course.

Acknowledgement

We would like to acknowledge the contributions of colleagues in biochemistry, pharmacology and pharmacokinetics in generating and interpreting the data reported in this communication. We thank Yukari Tachibana for the binding mode prediction of GKAs.

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. Sarabu, R.; Grimsby, J. *Curr. Opin. Drug Disc. Dev.* **2005**, *8*, 631.
5. 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.
6. 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.
7. Guertin, K. R.; Grimsby, J. *Curr. Med. Chem.* **2006**, *13*, 1839.
8. Sarabu, R.; Berthel, S. J.; Kester, R. F.; Tilley, J. W. *Expert Opin. Ther. Patents* **2008**, *18*, 759.
9. 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.
10. Daniewski, A. R.; Liu, W.; Radinov, R. N. WO Patent 2007/115968.
11. 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.
12. Nishimura, T.; Iino, T.; Mitsuya, M.; Bamba, M.; Watanabe, H.; Tsukahara, D.; Kamata, K.; Sasaki, K.; Ohyama, S.; Hosaka, H.; Futamura, M.; Nagata, Y.; Eiki, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1357.
13. Mitsuya, M.; Kamata, K.; Bamba, M.; Watanabe, H.; Sasaki, Y.; Sasaki, K.; Ohyama, S.; Hosaka, H.; Nagata, Y.; Eiki, J.; Nishimura, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2718.
14. Kamata, K.; Mitsuya, M.; Nishimura, T.; Eiki, J. I.; Nagata, Y. *Structure* **2004**, *12*, 429.
15. Kelly, T. A.; Fuchs, V. U.; Perry, C. W.; Snow, R. J. *Tetrahedron* **1993**, *49*, 1009.
16. Sharghi, H.; Asemani, O.; Khalifeh, R. *Synth. Commun.* **2008**, *38*, 1128.
17. 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 mM glucose. Compound **3** was used an internal control across all assay plates for data validation. The EC_{50} value of compound **3** is 0.42 ± 0.09 μM. Detailed assay methods and conditions are described in Ref. 18.
18. Futamura, M.; Hosaka, H.; Kadotani, A.; Shimazaki, H.; Sasaki, K.; Ohyama, S.; Nishimura, T.; Eiki, J.; Nagata, Y. *J. Biol. Chem.* **2006**, *281*, 37668.
19. The maximal activating response was defined as E_{max} equal to 100% efficacy of compound **3** and the maximal response of test compounds was compared to that of compound **3**.
20. Human microsomal stability was determined by % parent compound (1 μM) remaining after 30 min (37 °C) incubation with human liver microsomes (0.25 mg protein/ml).
21. Shibata, Y.; Takahashi, H.; Ishii, Y. *Drug Metab. Dispos.* **2000**, *28*, 1518.
22. The racemic **14b** was optically resolved using chiral HPLC (CHIRALCEL AD-H, 2 cm × 25 cm, *n*-hexane/^{i}PrOH (9:1) with 0.1% Et_2NH, flow rate = 5 mL/min) to give optically pure enantiomers **14b**(***R***) and **14b**(***S***), respectively. Chiral analytical HPLC indicated that enantiomeric excess (ee) of each enantiomer was >99% (CHIRALCEL AD-H, 0.46 cm × 25 cm, *n*-hexane/^{i}PrOH (9:1) with 0.1% Et_2NH, flow rate = 0.4 mL/min, t_R = 11.5 min for **14b**(***R***), 23.7 min for **14b**(***S***).
23. Campos, K. R.; Klapars, A.; Waldman, J. H.; Dormer, P. G.; Chen, C. Y. *J. Am. Chem. Soc.* **2006**, *128*, 3538.
24. Klapars, A.; Campos, K. R.; Waldman, J. H.; Zewge, D.; Dormer, P. G.; Chen, C. Y. *J. Org. Chem.* **2008**, *73*, 4986.
25. Eight-week old male Wistar rats were fasted overnight before performing the test. The rats were orally administered compound **16p**(***R***) or vehicle alone (0.5% methylcellulose solution) followed 30 min later by an oral glucose challenge (2 g/kg). Blood glucose concentrations were measured by OneTouch Ultra (LifeScan, Inc., Milpitas, CA) just prior to and following the glucose challenge (30, 60, 90 and 120 min). AUC values were calculated from the data (from −30 min to 120 min).