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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2103–2106
Bioorganic & Medicinal Chemistry Letters

Discovery, synthesis and biological evaluation of novel glucokinase activators

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Received 12 November 2004; revised 4 January 2005; accepted 12 January 2005
Available online 22 March 2005

Abstract—The identification, synthesis and SAR of a novel series of glucokinase activators is described. The interplay between lipophilicity, potency and physical properties is discussed, and compound **22** highlighted as having a suitable balance. In vivo pharmacokinetic and acute efficacy studies on this compound are also presented.

Type 2 Diabetes is a complex disease caused by defects in both the action and secretion of insulin, leading to fasting hyperglycaemia and vascular complications. It has become the fourth leading cause of death and affects 150 million people worldwide, with its prevalence expected to double by the year 2025.[1] Current therapies do not achieve adequate glycaemic control,[2] hence there is a need for new, effective pharmacological agents.

The elevated blood glucose levels that characterise Type 2 Diabetes result from a combination of defects in hepatic glucose balance and the failure of pancreatic β-cells to secrete sufficient insulin to overcome insulin resistance. Glucokinase (GK) is the rate-limiting enzyme for glucose utilisation[3] both in the liver, where its function is regulated by a 68 kDa regulatory protein,[4] and in pancreatic β-cells. Phosphorylation of glucose by GK in the liver promotes glycogen synthesis, whilst in the β-cell this results in glucose-sensitive insulin release.[5] Activation of GK is therefore expected to improve glycaemic control by modulating hepatic glucose balance and decreasing the threshold for insulin secretion.

Whilst small molecule kinase inhibitors are well precedented,[6] kinase activation by small molecules is relatively unknown. However, a recent report by Grimsby et al. disclosed the identification and in vivo evaluation of the GK activator RO-28-1675 (**1**).[7,8] The Hoffmann–La-Roche group have also described the co-crystallisation of GK with a bound analogue.[9] Banyu, in collaboration with Merck, have published[10] a similar crystal structure of the bound GK activator **2** (Fig. 1).

Several recent reports of GK activators have also appeared in the patent literature.[11] We have recently described detailed in vitro studies with our own series of GK activators,[12] and herein describe our approach to the identification and SAR of this novel series.[13] The importance of physical properties, and the in vivo profile of a lead candidate, are reported for the first time.

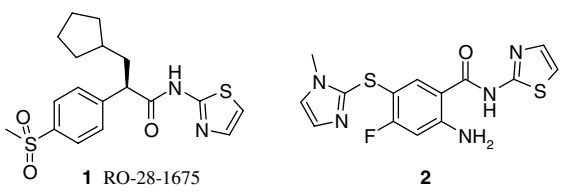

Figure 1. Glucokinase activators reported by Hoffmann–La-Roche (**1**) and Banyu (**2**).

Keywords: Diabetes; Glucokinase; Kinase activator.
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doi:10.1016/j.bmcl.2005.01.087

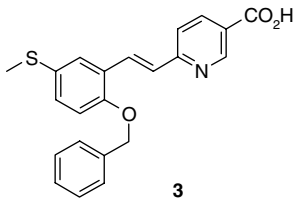

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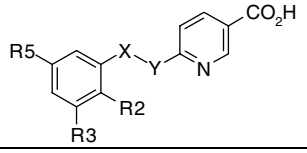
Figure 2. Structure of HTS lead compound.

A high throughput screen (HTS) of the company compound collection was carried out, with hits selected based upon their activation of recombinant human pancreatic enzyme.[14] Of the hits identified, compound **3** was of particular interest, mediating GK activation with an EC_{50} of 3.2 μM[14] (Fig. 2).

We were aware that compounds such as **3** were potential Michael acceptors, and therefore decided to investigate alternatives to the unsaturated linker group. Preliminary studies indicated that whilst saturated alkyl (**4**), ether (**5**), 'reversed' ether (**6**) and 'reversed' amide (**7**, **8**) linkers were not tolerated, amide linkers (**9**) retained activity. As a result, compounds such as **9** represented a more attractive startpoint based on their anticipated chemical stability and synthetic accessibility (Table 1).

Table 1. Identification of lead series and investigation of linker SAR



Compd	R2	R3	R5	X–Y	EC_{50} (μM)[14]
3	$–OCH_2Ph$	H	$–SCH_3$	–CH=CH–	3.20
4	H	$–OCH_2Ph$	$–OCH_2Ph$	$–CH_2CH_2–$	>10
5	H	$–OCH_3$	$–OCH_3$	$–OCH_2–$	>10
6	H	$–OCH_2Ph$	$–OCH_2Ph$	$–CH_2O–$	>10
7	H	$–OCH_2Ph$	$–OCH_2Ph$	–NHCO–	>10
8	$–OCH_2Ph$	H	$–SCH_3$	–NHCO–	>10
9	$–OCH_2Ph$	H	$–SCH_3$	–CONH–	4.22

Further SAR studies demonstrated that although the 2-monobenzyl analogue **10** was less active, a single substituent at R3 (**11**) gave a significant increase in potency. Given the disubstituted nature of the original lead, we investigated the effect of adding additional substitution within this series. Whilst 2,3-dibenzyloxy substitution (**12**) led to a drop in potency, 3,5-dialkoxy substitution (**13**) gave a modest improvement. 3,5-Dibenzyloxy (**14**) and the corresponding *ortho*-functionalised analogues (**15**) also showed increased potency, with the latter demonstrating an EC_{50} < 100 nM for the first time (Table 2).

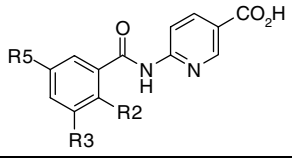
Despite promising potency, compounds such as **14** and **15** suffered from poor aqueous solubility and very high plasma protein binding (>99.9% bound). We therefore sought to decrease lipophilicity in order to achieve improved physical properties.

Our initial attempts focused on replacing the benzyl groups with more polar substituents. Whilst such changes allowed us to demonstrate the expected improvements in both solubility and unbound drug, the data for the tetrahydropyran analogue **16** illustrates that such compounds were significantly less potent (Table 3).

We therefore decided to undertake an iterative approach, seeking to build on the balance of moderate potency and better physical properties exhibited by compounds such as the isopropyl derivative **13**. These studies demonstrated that cyclopentylmethyloxy (**17**) and cyclopentylethyloxy (**18**) substituents were potent, but failed to show an improvement in physical properties. Polar heterocyclic terminal groups such as 3- or 4-pyridyl (**19**, **20**) showed a dramatic reduction in potency despite exhibiting good solubility and unbound levels. In contrast, phenyl (**21**) and, in particular, less polar heterocylic terminal groups such as thiophene (**22**) demonstrated excellent potency whilst retaining acceptable physical properties. Attempts to achieve further improvements by chain length modification (**23**, **24**) or aromatic ring substitution (**25**) proved unfruitful due to the deleterious effects on potency and physical properties, respectively (Table 3).

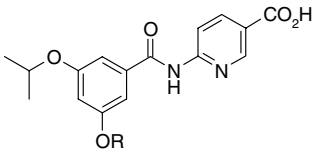
Benzamides **13** and **16**–**25** were synthesised in five steps from methyl 3,5-dihydroxybenzoate according to the

Table 2. Identification of phenyl ring substitution pattern



Compd	R2	R3	R5	EC_{50} (μM)[14]
10	$–OCH_2Ph$	H	H	>10
11	H	$–OCH_2–o$-Cl–Ph	H	0.91
12	$–OCH_2–o$-Cl–Ph	$–OCH_2–o$-Cl–Ph	H	>10
13	H	$–OCH(CH_3)_2$	$–OCH_2CH(CH_3)_2$	0.57
14	H	$–OCH_2Ph$	$–OCH_2Ph$	0.40
15	H	$–OCH_2–o$-F–Ph	$–OCH_2–o$-F–Ph	0.09

Table 3. Balancing potency and physical properties



Compd	R	Solubility[a] (μM)	% Free[b] (rat)	EC_{50} (μM)[14]
13	$–CH_2CH(CH_3)_2$	198	0.53	0.57
16	$–CH_2CH_2$–4-THP	2380	7.38	1.33
17	$–CH_2$–*c*Pent	44	0.15	0.65
18	$–CH_2CH_2$–*c*Pent	3	0.11	0.17
19	$–CH_2CH_2$–3-pyridyl	3080	5.11	1.26
20	$–CH_2CH_2$–4-pyridyl	ND[c]	6.35	1.78
21	$–CH_2CH_2Ph$	29	0.26	0.13
22	$–CH_2CH_2$–3-thiophene	48	0.26	0.09
23	$–CH_2Ph$	15	0.20	0.29
24	$–CH_2CH_2CH_2Ph$	15	0.13	0.42
25	$–CH_2$–*o*-F–Ph	9	0.14	0.10

[a] Solubility measured in 0.1 M phosphate buffer at pH 7.4.
[b] Protein binding measured by equilibrium dialysis.
[c] Not determined.

route highlighted in Scheme 1. A key feature of the synthesis is the use of sequential alkylation and/or Mitsunobu reactions to install the ether side chains. Benzoate cleavage, followed by amide coupling (via the acid chloride due to the relatively low nucleophilicity of methyl 6-aminonicotinate[17]) and hydrolysis of the methyl ester yielded the final compounds. The chemistry described in Scheme 1 proved to be a quick and robust route to a diverse set of analogues, and as such constituted an attractive strategy for lead optimisation.

Obtaining a suitable balance between potency and physical properties is a common problem in medicinal chemistry,[15] especially when optimising a lipophilic lead. This is emphasised by Figure 3a, which demonstrates the relationship observed for this series between the enzyme pEC_{50} and *c*log *P*. Since we also observed a good correlation between *c*log *P* and plasma protein binding (as

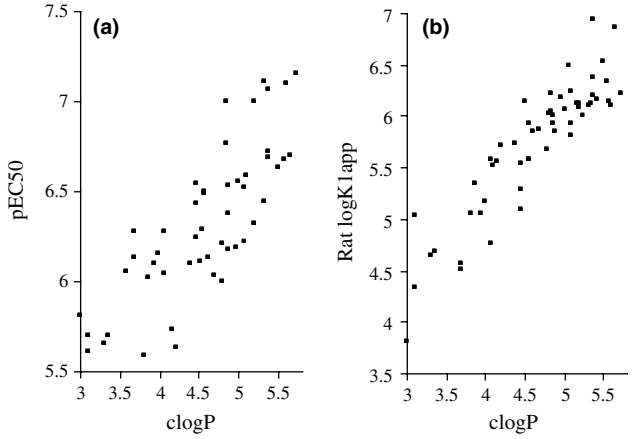


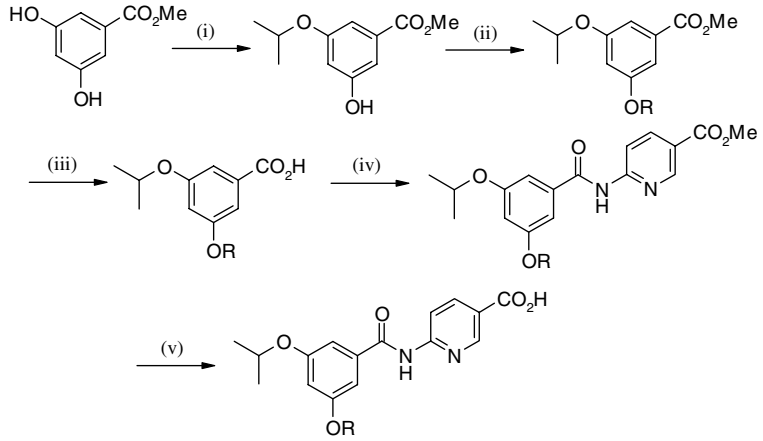
Figure 3. Correlation of lipophilicity with (a) potency, and (b) plasma protein binding.

represented by the first apparent binding constant K_{1app}, Fig. 3b), the challenge faced to simultaneously optimise both potency and free drug levels is evident.

Compound **22** was considered to have a suitable balance of potency and physical properties, and was selected for further evaluation. In vivo pharmacokinetic studies were carried out in female Han-Wistar rats (Charles River Laboratories), which demonstrated that the compound had low clearance and good bioavailability (Table 4).

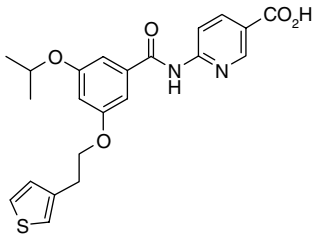
The acute in vivo efficacy of compound **22** was also evaluated in an Oral Glucose Tolerance Test (OGTT) in female Zucker rats fed with a high fat diet to render them insulin resistant.[16] Administration of a 30 mg/kg oral dose of compound **22** delivered a statistically significant reduction in plasma glucose levels following the oral glucose challenge (Fig. 4a), resulting in a 14% reduction in the area under the glucose curve[16] (Fig. 4b).

In summary, we have described the discovery, synthesis and detailed SAR studies of a novel series of glucokinase activators, and highlighted the interdependence of



Scheme 1. General synthetic route to benzamides **13** and **16**–**25**. Reagents and conditions: (i) $(CH_3)_2$ CHBr, K_2CO_3, DMF (35%); (ii) Ph_3P, DEAD, ROH, THF *or* RBr, K_2CO_3, DMF (60–80%); (iii) 1 M NaOH, THF/H_2O (90%); (iv) $(COCl)_2$, CH_2Cl_2, DMF (cat.) followed by methyl 6-aminonicotinate, THF, pyridine (70%); (v) 1 M NaOH, THF/H_2O (90%).

Table 4. Pharmacokinetic parameters of compound **22**[a]



Volume of distribution (L/kg)	Clearance (mL/min/kg)	Half life (h)	Bioavailability (%)
0.3	3.8	0.9	100

[a] Doses 1 mg/kg (iv) and 10 mg/kg (po).

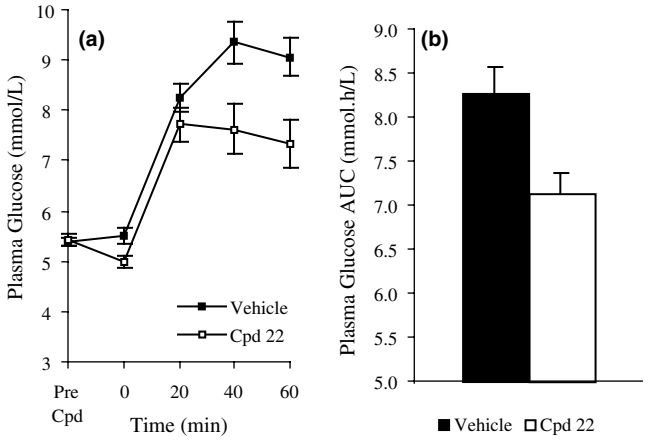


Figure 4. In vivo OGTT efficacy data for compound **22** (30 mg/kg po).

potency, lipophilicity and physical properties within the series. Thiophene **22** was identified as having a suitable balance of potency and physical properties, and has been shown to demonstrate low clearance, good bioavailability and promising in vivo efficacy in an acute rat model. Further studies, including our attempts to uncouple the relationship between potency and plasma protein binding, will be communicated in due course.

Acknowledgements

The authors would like to acknowledge the contributions of colleagues in bioscience, physical chemistry and pharmacokinetics in generating the data reported in this communication. Drs. K. G. Pike and M. J. Waring are also thanked for helpful discussions and assistance with preparation of the manuscript.

References and notes

1. Tadayyon, M.; Smith, S. A. *Expert Opin. Investig. Drugs* **2003**, *12*, 307.
2. Turner, R. C.; Cull, C. A.; Frighi, V.; Holmann, R. *J. Am. Med. Assoc.* **1999**, *281*, 2005.
3. Matschinsky, F. M.; Magnuson, M. A. In *Frontiers in Diabetes*; Matschinsky, F. M., Magnuson, M. A., Eds.; Karger: Basel, 2004; Vol. 16, pp 1–17.
4. Van Schaftingen, E.; Veigha da Cunha, M. In *Frontiers in Diabetes*; Matschinsky, F. M., Magnuson, M. A., Eds.; Karger: Basel, 2004; Vol. 16, pp 193–207.
5. Matschinsky, F. M. *Diabetes* **2002**, *51*, S394.
6. Cohen, P. *Nature Rev. Drug. Discov.* **2002**, *1*, 309.
7. 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.; Spence, C. L.; Tengi, J.; Magnuson, M. A.; Chu, C. A.; Dvorozniak, M. T.; Matschinsky, F. M.; Grippo, J. F. *Science* **2003**, *301*, 370.
8. Grimsby, J.; Matschinsky, F. M.; Magnuson, M. A. In *Frontiers in Diabetes*; Matschinsky, F. M., Grippo, J. F., Eds.; Karger: Basel, 2004; Vol. 16, pp 360–378.
9. Dunten, P.; Swain, A.; Kammlott, U.; Crowther, R.; Lukacs, C. M.; Levin, W.; Reik, L.; Grimsby, J.; Corbett, W. L.; Magnuson, M. A.; Matschinsky, F. M.; Grippo, J. F. In *Frontiers in Diabetes*; Matschinsky, F. M., Magnuson, M. A., Eds.; Karger: Basel, 2004; Vol. 16, pp 145–154.
10. Kamata, K.; Mitsuya, M.; Nishimura, T.; Eiki, J.-i.; Nagata, Y. *Structure* **2004**, *12*, 429.
11. GK activators reported in the recent patent literature include: (a) Fyfe, M. C. T.; Gardner, L. S.; Nawano, M.; Procter, M. J.; Rasamison, C. M.; Schofield, K. L.; Shah, V. K.; Yasuda, K. PCT Int. Appl. WO2004072031, 2004; *Chem. Abstr*. **2004**, *141*, 225496; (b) Weichert, A. G.; Barrett, D. G.; Heuser, S.; Riedl, R.; Tebbe, M. J.; Zaliani, A. PCT Int. Appl. WO2004063179, 2004; *Chem. Abstr*. **2004**, *141*, 157108; (c) Bebernitz, G. R. PCT Int. Appl. WO2004050645, 2004; *Chem. Abstr*. **2004**, *141*, 54350; (d) Polisetti, D. R.; Kodra, J. T., Lau; J.; Bloch, P.; Valcarce-Lopez, M. C.; Blume, N.; Guzel, M.; Santhosh, K. C.; Mjalli, A. M. M.; Andrews, R. C.; Subramanian, G.; Ankersen, M.; Vedsø, P.; Murray, A.; Jeppesen, L. PCT Int. Appl. WO2004002481, 2004; *Chem. Abstr*. **2004**, *140*, 77140.
12. Brocklehurst, K. J.; Payne, V. A.; Davies, R. A.; Carroll, D.; Vertigan, H. L.; Wightman, H. J.; Aiston, S.; Waddell, I. D.; Leighton, B.; Coghlan, M. P.; Agius, L. *Diabetes* **2004**, *53*, 535.
13. Preliminary aspects of this work have previously been presented in poster form: McKerrecher, D.; Allen, J. V.; Block, M. H.; Bowker, S. S.; Boyd, S.; Caulkett, P. W. R.; Currie, G. S.; Davies, C. D.; Fenwick, M. L.; Gaskin, H.; Hargreaves, R. B.; Hayter, B. R.; James, R.; Johnson, K. M.; Johnstone, C.; Jones, C. D.; Rayner, J. W.; Walker, R. P. *Abstracts of Papers*, 227th National Meeting of the American Chemical Society, Anaheim, CA, Mar 28–Apr 1, 2004; American Chemical Society: Washington, DC, 2004; MEDI 341.
14. EC_{50} values were determined at 10 mmol/L glucose, and are quoted as the geometric mean of at least three independent experiments. Assay to assay variability was within ±2-fold based on the results of a standard compound. All compounds in this series investigated exhibited a negligible effect on V_{max}. Detailed in vitro investigations and assay conditions are described in Ref. 12.
15. (a) Van de Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. *J. Med. Chem.* **2001**, *44*, 1313; (b) Van de Waterbeemd, H.; Smith, D. A.; Jones, B. C. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 273.
16. Further details of the high fat fed female Zucker OGTT model will be described in a future publication.
17. Aranapakam, V.; Albright, J. D.; Grosu, G. T.; Delos Santos, E. G.; Chan, P. S.; Coupet, J.; Ru, X.; Saunders, T.; Mazandarani, H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1737.