Bioorganic & Medicinal Chemistry Letters 19 (2009) 3247–3252

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Pyridones as glucokinase activators: Identification of a unique metabolic liability of the 4-sulfonyl-2-pyridone heterocycle

Jeffrey A. Pfefferkorn [a,*], Jihong Lou [b], Martha L. Minich [a], Kevin J. Filipski [a], Mingying He [b], Ru Zhou [b], Syed Ahmed [a], John Benbow [a], Angel-Guzman Perez [a], Meihua Tu [a], John Litchfield [a], Raman Sharma [a], Karen Metzler [a], Francis Bourbonais [a], Cong Huang [a], David A. Beebe [a], Peter J. Oates [a]

[a] Pfizer Global Research & Development, Groton Laboratories, Eastern Point Road, Groton, CT 06344, USA
[b] Pfizer Global Research & Development, La Jolla Laboratories, 10770 Science Center Drive, San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 18 March 2009
Revised 16 April 2009
Accepted 21 April 2009
Available online 3 May 2009

Keywords:
Glucokinase
Diabetes
Enzyme activation
Pyridone
Reactive metabolite

ABSTRACT

A promising area of novel anti-diabetic therapy involves identification of small molecule activators of the glucokinase enzyme to reduce blood glucose and normalize glucose stimulated insulin secretion. Herein, we report the identification and optimization of a series of 4-sulfonyl-2-pyridone activators. The activators were evaluated for in vitro biochemical activation and pharmacokinetic properties. As part of these efforts, a unique metabolic liability of the 4-sulfonyl-2-pyridone ring system was identified wherein this heterocycle readily undergoes conjugation with glutathione under non-enzymatic conditions.

Type 2 diabetes mellitus (T2DM) is a rapidly expanding public health problem affecting over 150 million people worldwide.[1] The disease is characterized by elevated fasting plasma glucose (FPG), insulin resistance, abnormally elevated hepatic glucose production and reduced glucose stimulated insulin secretion (GSIS).[2] While several classes of diabetic therapies are available for clinical use, there still remains a significant need for new therapies with improved efficacy and safety to help patients achieve their treatment goals.[3]

A promising area of current research involves the use of small molecule allosteric activators of the glucokinase (GK) enzyme to lower blood glucose and normalize insulin secretion.[4] Glucokinase is responsible for the conversion of glucose to glucose-6-phosphate (G-6-P), and it functions as a key regulator of glucose homeostasis.[5] In the liver, glucokinase regulates hepatic glucose utilization and output whereas in the pancreas it functions as a glucostat establishing the threshold for β-cell glucose-stimulated insulin secretion.[5] Glucokinase is also found in glucose sensing neurons of the ventromedial hypothalamus where it regulates the counter regulatory response (CRR) to hypoglycemia.[6] Finally, glucokinase is reportedly expressed in the endocrine K and L cells where it may help regulate incretin release.[7] Therapeutically, it is anticipated that activation of glucokinase in the liver and pancreas would be an effective strategy for lowering blood glucose by up regulating hepatic glucose utilization, down regulating hepatic glucose output and normalizing glucose stimulated insulin secretion.[4]

Glucokinase (hexokinase IV) is unique among the members of the hexokinase family given its low substrate binding affinity ($S_{0.5}$~8 mM), positive substrate cooperativity and lack of product inhibition.[8] As a monomeric enzyme, glucokinase achieves this cooperativity through equilibration between multiple conformations.[8] In pioneering work, Grimsby and co-workers demonstrated that small molecule activators were capable of binding to glucokinse at an allosteric site 20 Å remote from the active site and influencing the enzyme's kinetic profile by modulating both $S_{0.5}$ and V_{max}.[9] These efforts resulted in the identification of a phenylacetamide series of activators represented by **1** and **2**. A related series of activators represented by **3** was later reported by Fyfe et al.[10] Subsequently, a variety of other structurally diverse glucokinase activators have been identified, including aryl amides **4** and **5**.[11,12] These and other small molecule activators of glucokinase have been recently reviewed.[4] Encouragingly, these small molecule glucokinase activators have been shown to effectively lower blood glucose in a variety of diabetic animal models. Furthermore, **2** was advanced to Phase 2 clinical studies and found to effectively lower fasting and postprandial glucose in T2DM patients (Fig. 1).[13]

* Corresponding author. Tel.: +1 860 686 3421; fax: + 1 860 715 4608.
E-mail address: jeffrey.a.pfefferkorn@pfizer.com (J.A. Pfefferkorn).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.107

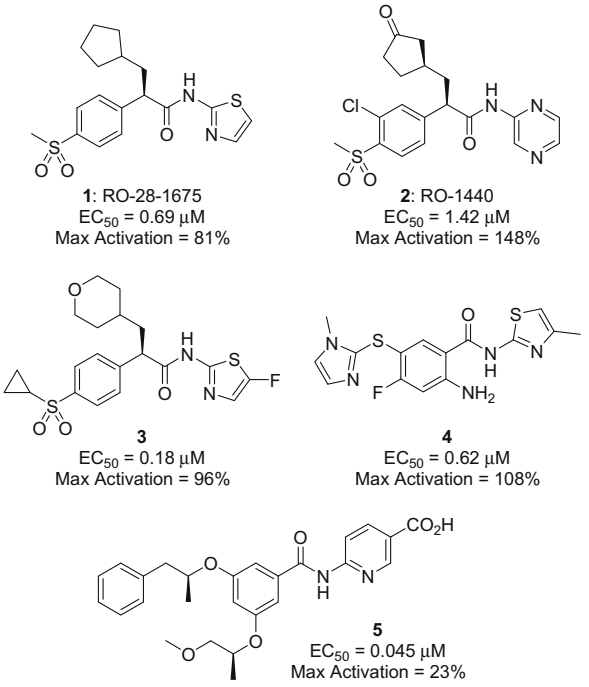


Figure 1. Structures and biochemical properties of representative glucokinase activators. EC_{50} and percent maximum activation (above control) were measured at 6.5 mM glucose and are reported as the mean of $n > 2$ determinations.

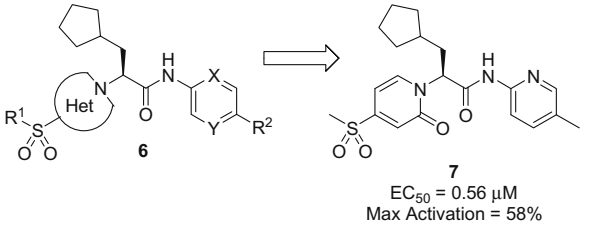
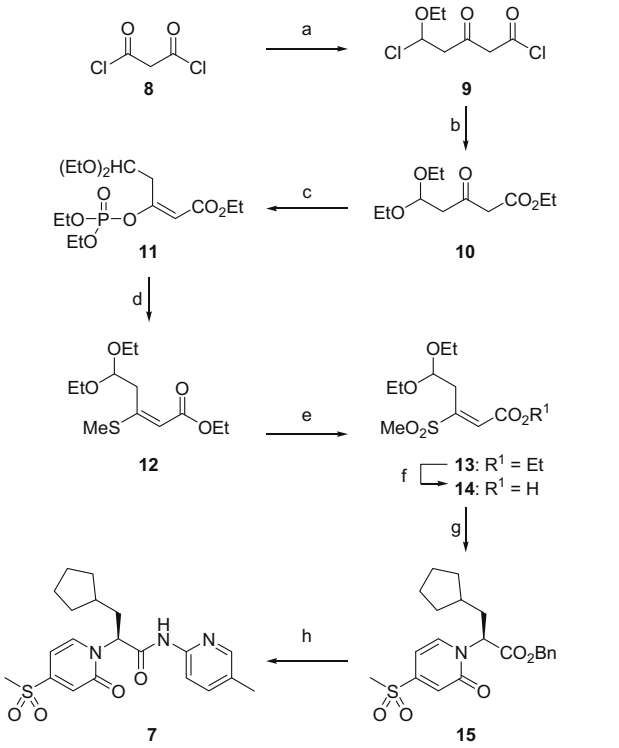


Figure 2. Strategy of evaluating N-linked heterocyclic glucokinase activators affording pyridone **7**.

Given the promise of glucokinase activation for treating T2DM, we have been investigating structurally novel small molecule activators. Of particular interest is the replacement of the aryl ring of the phenylacetamide series with N-linked heterocycles in an effort to improve potency and reduce lipophilicity. Previous literature reports suggested that heterocycles such as hydantoins,[14] isoindolin-1-ones[15] and indazoles[16] are tolerated at this position. Through evaluation of a variety of other heterocycles, we identified the 4-sufonyl-2-pyridone as an effective replacement as illustrated by **7** (Fig. 2).[17] Herein, we describe the evaluation of a series of glucokinase activators based upon this template.

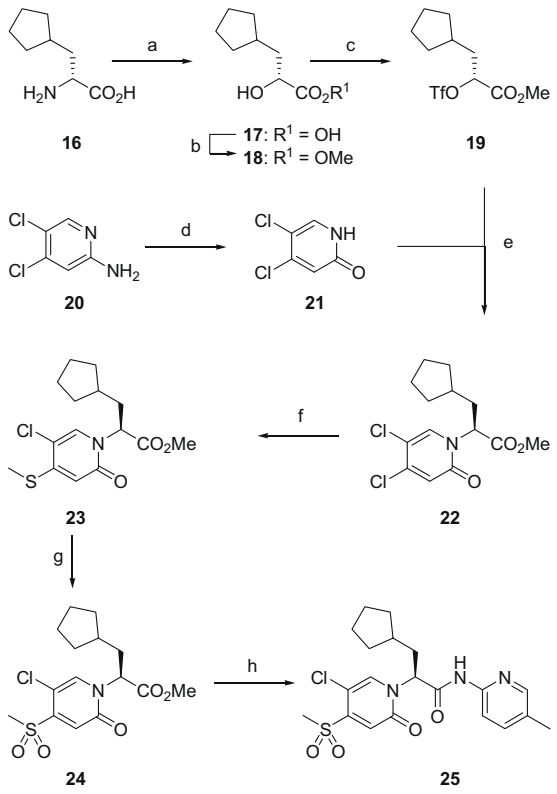
As highlighted in Schemes 1 and 2, two general synthetic strategies (A and B) were employed to construct the activators utilized in the current studies. The first approach (Scheme 1) utilized a cyclization reaction to construct pyridone heterocycles on amino acid derived substrates. Complete synthetic details of this 4-sulfonyl-pyrid-2-one forming reaction have recently been reported.[18] Briefly, this approach required initial construction of a linear pyridone precursor which commenced with reaction of malonyl chloride (**8**) with ethyl vinyl ethers to form intermediate **9** which was subsequently treated with ethanol followed by triethylamine to generate ketoester **10**.[19] The enolate of ketoester **10** was then generated by treatment with sodium hydride and reacted with diethyl chlorophosphate to afford enol phosphate **11** as an inconsequential mixture of geometric isomers.[20] Sodium thiomethoxide



Scheme 1. Synthetic method A. Reagents and conditions: (a) Ethyl vinyl ether, Et_2O, 0 °C, 1 h; (b) (i) EtOH, 0→5 °C; (ii) Et_3N, 10–25 °C, 0.5 h, 63% over two steps; (c) (i) NaH, THF, 0 °C, 10 min; (ii) $(EtO)_2POCl$, THF, 12 h, 89%; (d) NaSMe, NH_4Cl, THF, −60→25 °C, 12 h, 76%; (e) *m*CPBA, CH_2Cl_2, 0–25 °C, 1 h, 91%; (f) LiOH, MeOH, THF, H_2O, 25 °C, 0.5 h, 100%; (g) (i) L-cyclopentylalanine benzyl ester, PyBrOP®, DIPEA, CH_2Cl_2, 0–25 °C, 12 h; (ii) THF, HCl (aq), 80 °C, 43%; (h) 2-amino-5-picoline, $AlMe_3$, 1,2-DCE, 0→25 °C, 12 h, 76%.

was then added to enol phosphate **11** to afford sulfide **12** which was treated with *m*CPBA to afford smooth conversion to the corresponding sulfone **13**. The ester of **13** was saponified to carboxylic acid **14** which was subsequently coupled to L-cyclopentylalanine benzyl ester to produce the corresponding amide. This amide was then treated with aqueous 1 N HCl in THF and heated to reflux resulting in cyclization to generate the desired 4-sulfonyl-2-pyridone **15**. Finally, $AlMe_3$-mediated transamidation of **15** with 2-amino-5-picoline provided glucokinase activator **7**.[21] Substitution of structurally varied thiols, amino acid esters and heterocyclic amines into this synthetic route enabled preparation of a diverse set of activators.

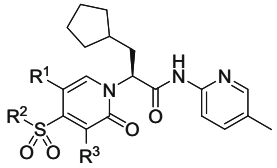
For analogs not accessible via the above approach (particularly those bearing poly-substituted pyridone rings), a complementary pyridone N-alkylation strategy was employed as shown in Scheme 2. This route commenced with conversion of amino acid D-cyclopentylalanine (**16**) to the corresponding hydroxy ester **17** via a diazatization reaction that proceeded with net retention of stereochemistry.[22] Carboxylic acid **17** was then converted to the corresponding methyl ester **18** via treatment with thionyl chloride and methanol. In preparation for a pyridone alkylation reaction, triflate **19** was prepared from **18** via treatment with 2,6-lutidine and trifluoromethanesulfonic anhydride. Separately, the pyridone **21** coupling partner was prepared from 4,5-dichloro-2-amino-pyridine (**20**) via diazatization and subsequent hydrolysis. Alkylation of the newly formed pyridone **21** was accomplished via deprotonation with sodium hydride and reaction with triflate **19** to afford the desired N-alkylated product **22** in modest yield with inversion of stereochemistry along with the O-alkylated side product. Installation of the 4-sulfone moiety was accomplished by reaction of



Scheme 2. Synthetic method B. Reagents and conditions: (a) $NaNO_2$, H_2SO_4, H_2O, 0→25 °C, 19 h, 92%; (b) $SOCl_2$, MeOH, 65 °C, 0.5 h, 72%; (c) trifluoromethanesulfonic anhydride, 2,6-lutidine, CH_2Cl_2, 0 °C, 0.5 h, 96%; (d) $NaNO_2$, HCl, H_2O, 25 °C, 0.5 h, 83%; (e) NaH, THF, 25 °C, 2 h, 67%; (f) NaSMe, THF, 25 °C, 16 h, 57%; (g) Oxone®, THF, H_2O, 48 h, 71%.; (h) 2-amino-5-picoline, $AlMe_2Cl$, 1,2-DCE, 0→25 °C, 3 h, 73%.

dichloride **22** with sodium thiomethoxide affording selective displacement to generate thioether **23**. Oxidation of this thioether with Oxone® then afforded sulfone **24** in good yield. Finally, $AlMe_2Cl$-mediated transamidation[20] of ester **24** with 2-amino-5-picoline provided glucokinase activator **25**. Substitution of alternative amino acids, pyridones and heterocyclic amines into this synthetic route enabled preparation of a diverse set of activators.

Table 1
Biochemical properties of glucokinase activators **7**, **25**–**33**



	R^1	R^2	R^3	Synthetic method	EC_{50} (μM)	Maximum activation (%)
7	H	Me	H	A	0.56	58
26	H	Et	H	A	0.50	73
27	H	iPr	H	A	0.23	65
28	H	cPr	H	A	0.11	76
29	H	cBu	H	A	0.06	67
25	Cl	Me	H	B	0.48	122
30	Me	Me	H	A	0.44	103
31	Cl	*i*Pr	H	B	0.26	69
32	Me	*i*Pr	H	A	0.16	48
33	H	*i*Pr	Cl	B	0.07	57

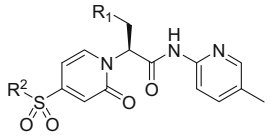
EC_{50} and percent maximum activation (above control) were measured at 6.5 mM glucose and are reported as the mean of $n > 2$ determinations.

All analogs were initially evaluated in a biochemical activation assay with human glucokinase (hexokinase IV) measuring potency and percentage maximum activation (above control) at 6.5 mM glucose.[23] Initially, structure–activity relationships of the pyridone ring were evaluated as highlighted in Table 1. Increasing lipophilicity via steric bulk at the sulfone position afforded increasing potency as illustrated with **29** (R^2 = cyclobutyl) being 10-fold more potent that **7** (R^2 = Me). The changes at the R^2 had a negligible impact on the maximum activation of these analogs. Consistent with previous reports from the phenylacetamide series, addition of small substituents adjacent to the sulfone (R^1 or R^3) afforded slight improvements in potency with the most notable being **33** (R^3 = Cl) which was three fold more potent than its unsubstituted comparator **27** (R^3 = H). Interestingly, in the case of methyl sulfones **25** and **30**, addition of either Cl or Me at the R^1 position afforded a significant increase in maximum activation with only a marginal effect on potency.

Structure–activity studies next examined the effect of modification of the cycloalkyl ring of these pyridone analogs as illustrated in Table 2. Utilizing a relatively consistent set of sulfone and amide substituents, the cyclopentyl (**27**, EC_{50} = 0.23 μM) and cyclohexyl ring (**36**, EC_{50} = 0.24 μM) were shown to offer optimal potency. The smaller cyclobutyl ring of **35** as well as the acyclic isopropyl of **34** were tolerated but tended to be several fold less potent than the cyclopentyl comparator **27**. Introduction of fluorine (e.g., **37**) or an ether linkage (e.g., **38**) into the cyclohexyl ring of **37** resulted in a loss of overall activation. Finally, the evaluation of an aryl ring (e.g., **39**) revealed it to be 14-fold less active than the corresponding saturated cyclohexyl analog **36**.

The final area of structural modification was the amino heterocycle of the template's amide moiety. Previous precedent has established that a 2-amino nitrogen containing heterocycle is required to act as a donor-acceptor engaging the glucokinase protein in a pair of hydrogen bonding interactions.[4] Consistent with these reports, preliminary efforts (analogs not shown) to remove this motif in this series resulted in complete loss of biological activity. Hence, a variety of 2-amino heterocycles were examined as high-

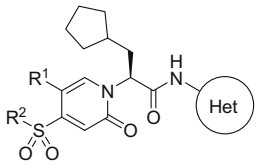
Table 2
Biochemical properties of glucokinase activators **27**, **34**–**39**

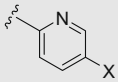
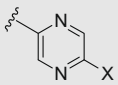
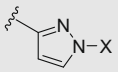


	R^1	R^2	EC_{50} (μM)	Maximum activation (%)
34		*i*Pr	0.51	37
35		*i*Pr	0.67	72
27		*i*Pr	0.23	65
36	 X = CH_2	*i*Pr	0.24	46
37	X = CF_2	*i*Pr	0.16	15
38	X = O	Et	1.5	26
39		*i*Pr	3.3	30

EC_{50} and percent maximum activation (above control) were measured at 6.5 mM glucose and are reported as the mean of $n > 2$ determinations. All compounds were prepared by synthetic Method A.

Table 3
Biochemical properties of glucokinase activators **27**, **40**–**51**



	R^1	R^2	Heterocycle	Heterocycle substituent	Synthetic method	EC_{50} (μM)	Maximum activation (%)
40	H	*i*Pr		X = H	A	0.60	43
27	H	*i*Pr		X = Me	A	0.23	65
41	H	*i*Pr		X = CF_3	A	0.33	35
42	Cl	Me		X = Cl	B	0.14	82
43	H	*c*Pr		X = H	A	1.3	94
44	H	*c*Bu		X = H	A	0.59	66
45	Cl	*i*Pr		X = Me	B	0.70	104
46	H	Me		X = Me	A	3.5	101
47	H	*i*Pr		X = H	A	0.72	50
48	H	*i*Pr		X = Me	A	0.91	57
49	H	*c*Pr		X = Me	A	1.5	84
50	H	*c*Bu		X = Me	A	0.26	67
51	H	*i*Pr			A	1.3	42

EC_{50} and percent maximum activation (above control) were measured at 6.5 mM glucose and are reported as the mean of $n > 2$ determinations.

lighted in Table 3. Generally, 2-amino pyridine amides (**27** and **40**–**42**) afforded the greatest potency while the 2-amino pyrazoles (**47**–**50**) exhibited similar levels of activation but were several fold less potent. Relative to their pyridine comparators, compounds containing 2-aminopyrazine amides (**43**–**46**) were generally two fold less potent but offered increased levels of maximum activation.

Having evaluated the structure activity relationships of this series and identified a set of analogs with promising biochemical activity, we next sought to evaluate the ADME properties of selected analogs. Generally, compounds in this series exhibited high permeability (data not shown) and reasonable aqueous solubility (>50 μM). The microsomal metabolic stability of a representative set of analogs is shown in Table 4 examining both human and rat liver microsomes. As shown, the analogs exhibited good stability in human microsomes and moderate stability in rat microsomes.

Table 4
Metabolic stability of selected glucokinase activators in human and rat liver microsomes

	Structure	HLM		RLM	
		Cl_h (mL/min/kg)	$T_{1/2}$ (min)	CL_h (mL/min/kg)	$T_{1/2}$ (min)
7	Table 1	5	>120	58	9
37	Table 2	8	73	36	42
38	Table 2	<5	>120	<18	>120
46	Table 3	<5	>120	31	56
48	Table 3	10	93	58	8

Human liver (HLM) and rat liver (RLM) microsomal stability was determined via incubation of 1.0 μM of compound with 0.8 mg/mL of protein.

Table 5
Pharmacokinetic parameters for compounds **46** and **48**

	F (%)	$T_{1/2}$ (h)	V_{dss} (mL/kg)	Cl (mL/min/kg)	PPB F_u
46	2	0.2	4.6	337	0.35
48	8	0.2	2.0	164	0.59

Pharmacokinetic parameters expressed as geometric mean ($n = 2$) of Sprague–Dawley rats dosed 0.5 mg/kg iv and 5 mg/kg po. Rat plasma protein binding (PPB) is reported as fraction unbound.

We next selected two representative compounds (**46** and **48**) for evaluation in in vivo PK experiments as shown in Table 5. Somewhat unexpectedly, both **46** and **48** exhibited very high clearance (Cl > 100 mg/min/kg), short half-lives ($T_{1/2}$ < 0.5 h) and poor bioavailabilities (F% < 10%).

The apparent disconnect between the moderate predicted clearance (based on in vitro microsomal data) for compound **46** and its very high in vivo clearance prompted us to more closely examine the metabolism of this compound. Biotransformation studies of **46** were conducted in both microsomes and hepatocytes to examine Phase 1 and Phase 2 metabolism, respectively.[24] First, incubation of **46** in both human and rat microsomes revealed minor hydroxylation of the cyclopentyl ring or the 5-methyl group of the pyrazine consistent with the low microsomal clearance observed for this compound (i.e., Table 4). Subsequently, as highlighted in Figure 3, incubation of **46** with rat hepatocytes resulted in complete consumption of parent and the formation of a single major metabolite which was confirmed to be glutathione conjugate **52** via extracted ion chromatography (Fig. 4).

To better understand this propensity for bioconjugate formation, in a separate experiment, **46** was incubated directly with aqueous glutathione (5 mM) at 37 °C for 1.0 h and the same conjugate **52** was observed in the absence of any metabolic activation. Subsequently, analogs **26**, **27**, **28**, **30** and **42** were evaluated under these same conditions. These additional analogs contained increasing steric bulk either directly on the sulfone or adjacent to it thus enabling an evaluation as to whether or not steric hindrance might mitigate this conjugation liability. Unfortunately, all of these analogs also underwent facile reactions with glutathione suggesting that steric considerations were not necessarily important and that this was a general liability inherent to the 4-sulfonyl-2-pyridone motif of these activators. This liability prevented further evaluation of this series of glucokinase activators in models of diabetic efficacy. Interestingly, a review of the literature did not afford similar reports of the instability of the 4-sulfonyl-2-pyridone ring system[25] despite the use of this heterocycle in other chemotypes

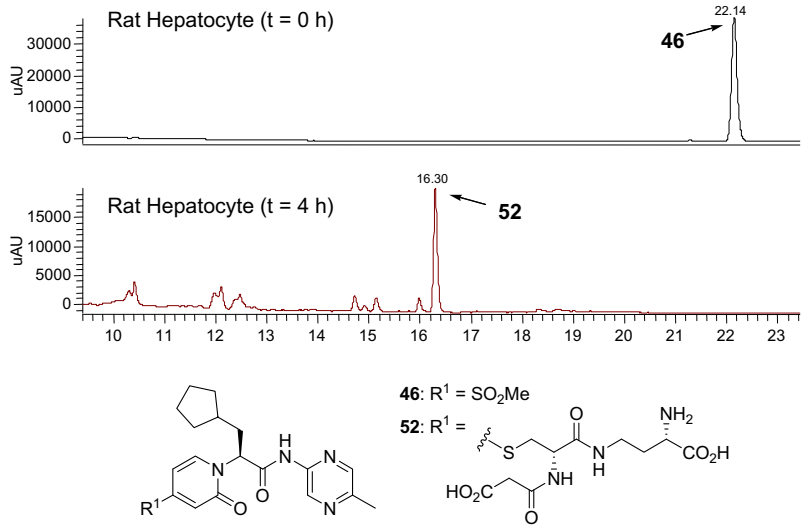


Figure 3. Results of rat hepatocyte incubation with compound **46**.

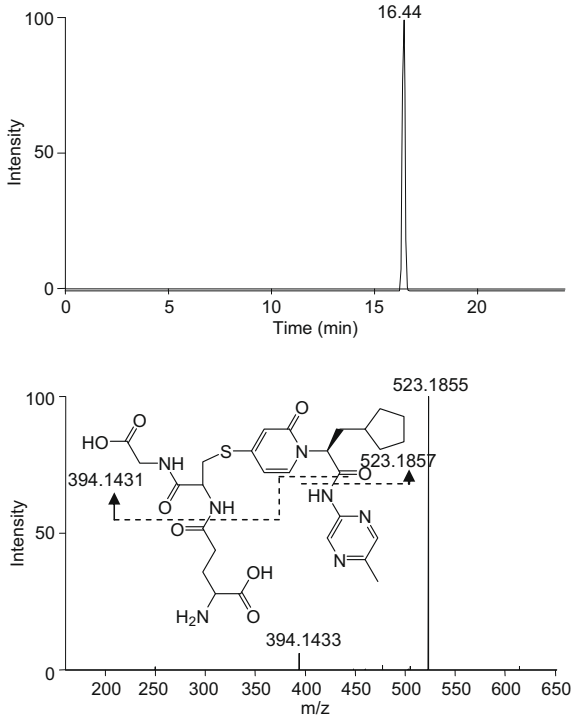


Figure 4. Confirmation of the structure of glutathione conjugate **52** by extracted ion chromatography. Extracted ion chromatogram of GSH conjugate **52** (top panel) and exact mass product ion spectra obtained by CID of the MH^+ ion (m/z 632) with the origins of the diagnostic ions are as indicated.

including NHE-3 inhibitors (**53**, Fig. 5), CB2 agonists (**54**) and angiotensin II receptor antagonists (**55**).[26–28] Mechanistically, this transformation can be viewed as a nucleophilic addition–elimination reaction on an α,β-unsaturated amide. While the local electronic environment of a particular pyridone ring will affect the propensity for such a displacement reaction, our observations suggest that this liability should nevertheless be evaluated when utilizing this heterocyclic system in medicinal chemistry applications. Additional studies on the factors influencing glutathione conjugate formation with the 2-pyridone ring system are in progress and will be reported separately.

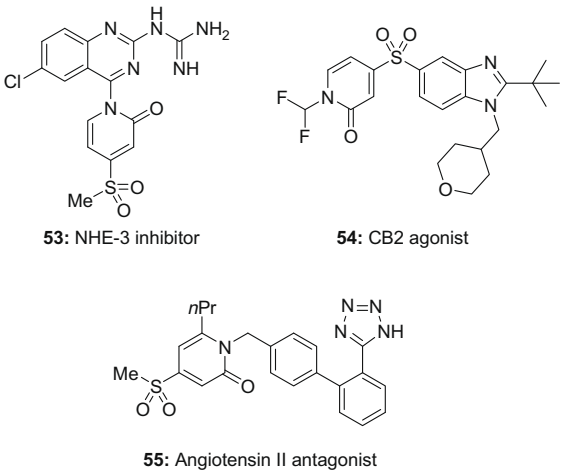


Figure 5. Representative examples of other 4-sulfonyl-2-pyridones.

Acknowledgment

We would like to thank Amit Kalgutkar for helpful discussions regarding the biotransformation data.

Supplementary data

Supplementary data (experimental procedures for the glucokinase biochemical activation assay and metabolite identification experiments) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.107.

References and notes

1. (a) Zimmet, P.; Alberti, K. G.; Shaw, J. *Nature* **2001**, *414*, 782; (b) Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. *Diabetes Care* **2004**, *27*, 1047.
2. Saltiel, A. R.; Kahn, C. R. *Nature* **2001**, *14*, 799.
3. (a) Xu, J. *IDrugs* **2004**, *7*, 249; (b) Nathan, D. M. *N. Eng. J. Med.* **2002**, *347*, 1342; (c) Mona, A.; Smith, R. E. Y. *Nat. Rev. Drug Disc.* **2007**, *6*, 777.
4. For recent reviews, see: (a) Grimsby, J.; Berthel, S. J.; Sarabu, R. *Curr. Topics Med. Chem.* **2008**, *8*, 1524; (b) Coghlan, M.; Leighton, B. *Exp. Opin. Investig. Drugs* **2008**, *17*, 145; (c) Sarabu, R.; Berthel, S. J.; Kester, R. F.; Tilley, J. W. *Exp. Opin. Ther. Patents* **2008**, *18*, 759.
5. (a) Cardenas, M. L. *Glucokinase: Its regulation and Role in Liver Metabolism*; R.G. Landes Co.: Austin, 1995. pp 21–40; (b) Iynedjian, P.B. *Glucokinase and Glycemic*

Disease. From Basics to Novel Therapeutics; Matschinsky, F.M., Magnuson, M.A., Eds.; Karger: Basel, **2004**; Vol. 16, p 155.; (c) Matschinsky, F. M. *Diabetes* **1996**, *45*, 223; (d) Matschinsky, F. M.; Glaser, B.; Magnuson, M. A. *Diabetes* **1998**, *47*, 307.
6. (a) Dunn-Meynell, A. A.; Routh, V. H.; Kang, L.; Gaspers, L.; Levin, B. E. *Diabetes* **2002**, *51*, 2056; (b) Kang, L.; Dunn-Meynell, A. A.; Routh, V. H.; Gaspers, L. D.; Nagata, Y.; Nishimura, T.; Eiki, J.; Zhang, B. B.; Levin, B. E. *Diabetes* **2006**, *55*, 412.
7. Reimann, F.; Gribble, F. M. *Diabetes* **2002**, *51*, 2757.
8. For a review, see: Iynedjian, P. B. *Cell. Mol. Life Sci.* **2009**, *66*, 27.
9. 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.
10. Fyfe, M. C.; 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.
11. (a) Nishimura, T.; Iino, T.; Nagata, Y.; Eiki, J. WO2003080585.; (b) 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.* **2009**, doi: 10.1016/j.bmcl.2009.03.137..
12. 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.
13. Zhi, J.; Zhai, S.; Mulligan, M. -E.; Grimsby, J.; Arbet-Engels, C.; Boldrin, M.; Balena, R. Presentation 42, 2008 EASD 44th Annual Meeting, Rome, Italy..
14. Goodnow, R. J.; Le, K. F. WO200183465..
15. Guertin, K. R. F. WO200248106..
16. Cao, S. X.; Feng, J.; Gwaltney, S. L.; Hosfield, D. J.; Skene, R. J.; Stafford, J. A.; Tang, M. US20072811942..
17. A patent application describing structurally similar pyridone-based glucokinase activators has recently published, see: Cheruvallath, Z.; Feng, J.; Guntupalli, P.; Gwaltney, S. L.; Miura, J.; Sabat, M.; Tang, M.; Wang, H. WO2008079787..
18. Minich, M. L.; Watson, I. D.; Filipski, K. J.; Pfefferkorn, J. A. *Tetrahedron Lett.* **2009**, *50*, 2094.
19. (a) Effenberger, F.; Maier, R.; Schoenwaelder, K.-H.; Ziegler, T. *Chem. Ber.* **1982**, *115*, 2766; (b) Effenberger, F.; Ziegler, T.; Schoenwaelder, K.-H. *Chem. Ber.* **1985**, *118*, 741.
20. Beszant, S.; Giannini, E.; Zanoni, G.; Vidari, G. *Eur. J. Org. Chem.* **2003**, *20*, 3958.
21. Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *48*, 4171.
22. Menche, D.; Hassfeld, J.; Li, J.; Rudolph, S. *J. Am. Chem. Soc.* **2007**, *129*, 6100.
23. Biochemical activation assay was conducted using purified recombinant human glucokinase prepared as previously described, see: Ralph, E. C.; Thompson, J.; Sun, S. *Biochemistry* **2008**, *47*, 5028. The 6.5 mM glucose concentration was selected to offer an optimal window to measure activation relative to basal enzyme activity. Evaluation of selected activators at higher glucose concentrations confirmed that these analogs continued to activate the enzyme up to 100 mM glucose. An assay protocol is provided in the Supplementary data..
24. Experimental procedures for microsomal and hepatocyte incubations and metabolite identification are provided in the Supplementary data..
25. A review of the literature did not reveal similar examples of glutathione conjugate formation with 4-sulfonyl-2-pyridones. However, glutathione displacement of a sulfonamides have been reported on other heterocycles, for examples see: (a) Graham, S. L.; Shepard, K. L.; Anderson, P. S.; Baldwin, J. J.; Best, D. B.; Christy, M. E.; Freedman, M. B.; Gautheron, P.; Habecker, C. N.; Hoffman, J. M.; Lyle, P. A.; Michelson, S. R.; Ponticello, G. S.; Robb, C. M.; Schwam, H.; Smith, A. M.; Smith, R. L.; Sondey, J. M.; Strohmaier, K. M.; Sugrue, M. F.; Varga, S. L. *J. Med. Chem.* **1989**, *32*, 2548; (b) Graham, S. L.; Hoffman, J. M.; Gautheron, P.; Michelson, S. R.; Scholz, T. H.; Schwam, H.; Shepard, K. L.; Smith, A. M.; Smith, R. L.; Sondey, J. M. *J. Med. Chem.* **1990**, *33*, 749; (c) Zhao, Z.; Koeplinger, K. A.; Peterson, T.; Conradi, R. A.; Burton, P. S.; Suarato, A.; Heinrikson, R. L.; Tomasselli, A. G. *Drug Metab. Dispos.* **1999**, *27*, 992; Additionally, a glutathione conjugate of a 2-pyridone ring system was reported during biotransformation studies of the cardiotonic agent amrinone; however, in this case, the conjugate occurred at the 6-position of the 2-pyridone rather than the 4-position observed in the current studies. See: Baker, J. F.; Chalecki, D. P.; Benziger, P. E.; O'Melia, S. D.; Clemans, S. D.; Edelson, J. *Drug Metab. Dispos.* **1982**, *10*, 168.
26. Gericke, R.; Beirer, N.; Wilm, C. WO2003051866..
27. Gijsen, H. J. M.; De Cleyn, M. A. J.; Surkyn, M.; Verbist, B. M. P. WO2008003665..
28. Bantick, J. R.; Beaton, H. G.; Cooper, S. L.; Hill, S.; Hirst, S. C.; McInally, T.; Spencer, J.; Tinker, A. C.; Willis, P. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 121.