Bioorganic & Medicinal Chemistry Letters 23 (2013) 4571–4578

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Pyrimidone-based series of glucokinase activators with alternative donor–acceptor motif

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ARTICLE INFO

Article history:
Received 6 May 2013
Revised 7 June 2013
Accepted 11 June 2013
Available online 20 June 2013

Keywords:
Glucokinase activator
Type 2 diabetes mellitus
Pyrimidone

ABSTRACT

Glucokinase activators are a class of experimental agents under investigation as a therapy for Type 2 diabetes mellitus. An X-ray crystal structure of a modestly potent agent revealed the potential to substitute the common heterocyclic amide donor–acceptor motif for a pyridone moiety. We have successfully demonstrated that both pyridone and pyrimidone heterocycles can be used as a potent donor–acceptor substituent. Several sub-micromolar analogs that possess the desired partial activator profile were synthesized and characterized. Unfortunately, the most potent activators suffered from sub-optimal pharmacokinetic properties. Nonetheless, these donor–acceptor motifs may find utility in other glucokinase activator series or beyond.

Type 2 diabetes mellitus is an expanding worldwide health problem with current estimates indicating there are >340 million people with the disease.[1] The disease is characterized by elevated fasting glucose levels, reduced insulin sensitivity, increased hepatic glucose output, and reduced glucose-stimulated insulin secretion.[2] Although several classes of therapies are used as treatment, there is still significant unmet medical need since patients often do not meet treatment goals due to modestly efficacious therapies or undesired side effects such as weight gain.[3]

Glucokinase is an enzyme that catalyzes the rate-limiting step in glucose metabolism, namely the conversion of glucose to glucose-6-phosphate.[4] Its most characterized functions occur in the pancreatic beta cells, where it serves as a 'glucostat' to effectively set the threshold for glucose-stimulated insulin secretion; and in the liver, where it is involved in the regulation of hepatic glucose utilization and output. Since the initial discovery of small molecule allosteric activators of glucokinase, there has been a significant investment to develop these agents as a new class of diabetes therapy.[5,6] In fact, several groups have advanced candidates into clinical development;[7] however, in these clinical studies, hypoglycemia risk and uncertainty regarding durability have emerged as potential challenges.[8]

One strategy that we have pursued is the development of 'partial activators' of the enzyme to increase the dependence of activation on glucose levels and potentially reduce the hypoglycemia risk, thereby increasing the therapeutic index. Glucokinase is a monomeric enzyme with positive cooperativity towards its substrate and its glucose dependency can be justified by several possible conformations that the complex can adapt.[9] One desired profile for an activator with increased glucose-dependency is where the K_m for glucose is not reduced to the extent observed with previous activators, namely α (defined as fold-change in K_m) $\geqslant 0.1$ with minimal change in β (~ 1; defined as fold-change in V_{max}). Previously communicated work towards these efforts resulted in **1** (Fig. 1) as a clinical candidate currently in Phase 2 development.[10]

We subsequently sought to explore whether alternative chemotypes might offer additional enzyme activation profiles with promising therapeutic potential. Of particular interest, an exploratory effort identified **2** as a weak glucokinase activator. This compound was noteworthy in that it did not possess the common heteraromatic amide that was known to provide a key donor–acceptor interaction within the binding site.[6] In comparing the X-ray crystal structures of **1** (PDB code: 4DHY) and **2** (PDB code: 4L3Q) bound in the allosteric pocket of glucokinase (Fig. 2) it became apparent that the pyridone motif of **2** was mimicking the heteroaromatic amide of **1**. Namely, the carbonyl oxygen was forming a H-bond with the backbone NH of Arg63, while the

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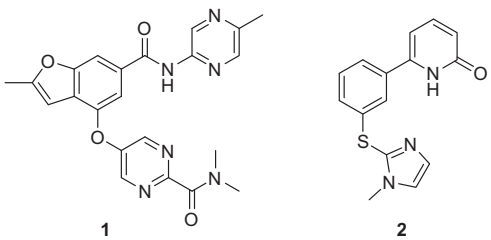
http://dx.doi.org/10.1016/j.bmcl.2013.06.036



1 GK EC_{50} = 0.19 μM, α = 0.10, β = 0.87

2 GK EC_{50} = 2.0 μM, α = 0.20, β = 0.50

Figure 1. Glucokinase activators **1** and **2**.

pyridone NH was forming a H-bond with the backbone carbonyl of the same residue. From this observation we hypothesized that we may be able to replace the heterocyclic amide with a pyridone in other chemical series. We sought to evaluate this idea in the aryloxy series exemplified by **3** (Fig. 3).

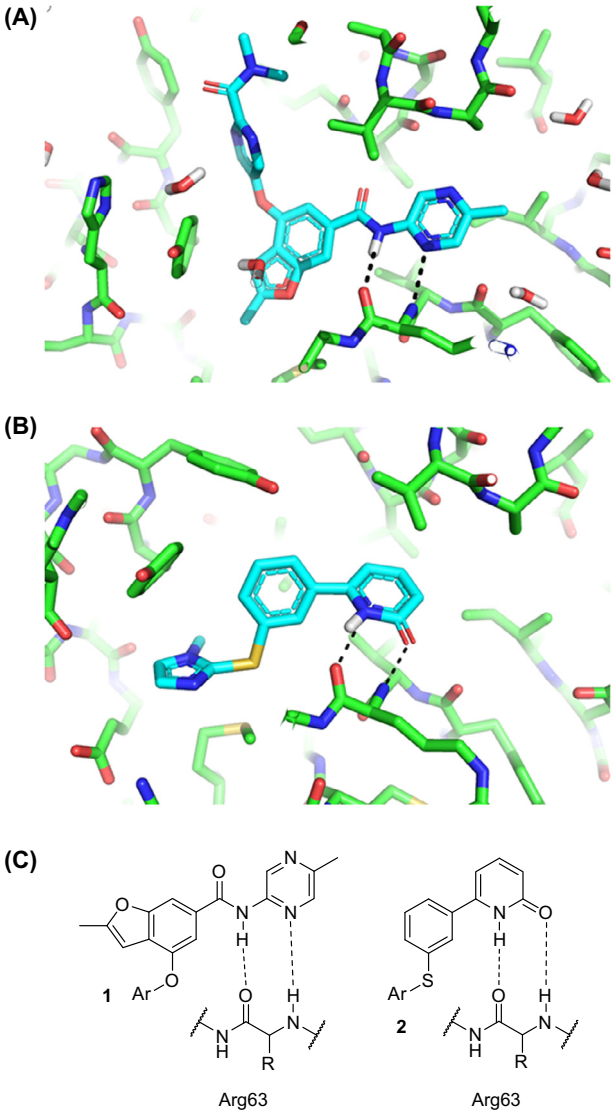


Figure 2. Co-crystal structures of human glucokinase allosteric binding site bound to activators (A) **1** and (B) **2** showing H-bonds to Arg63; and (C) Schematic of donor–acceptor motif for the heterocyclic amide and pyridone motifs.

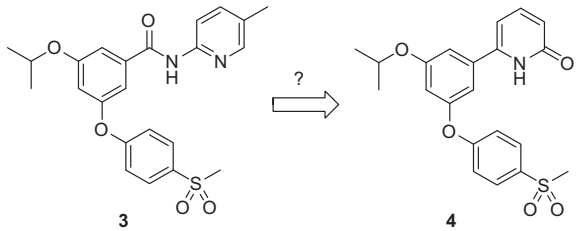
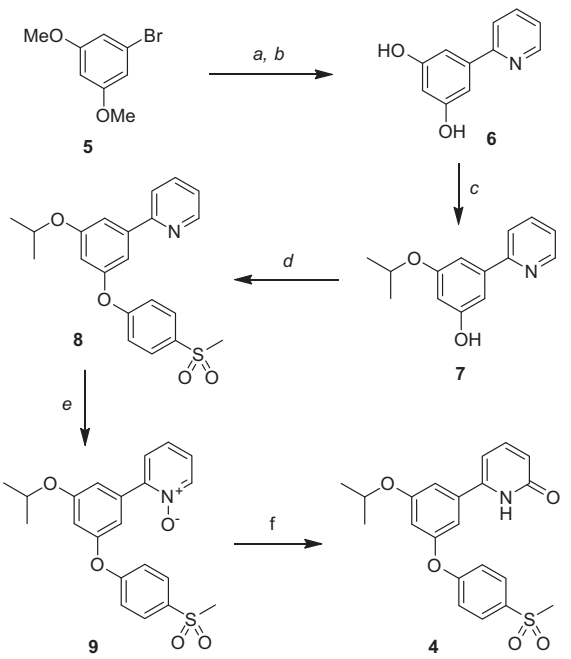


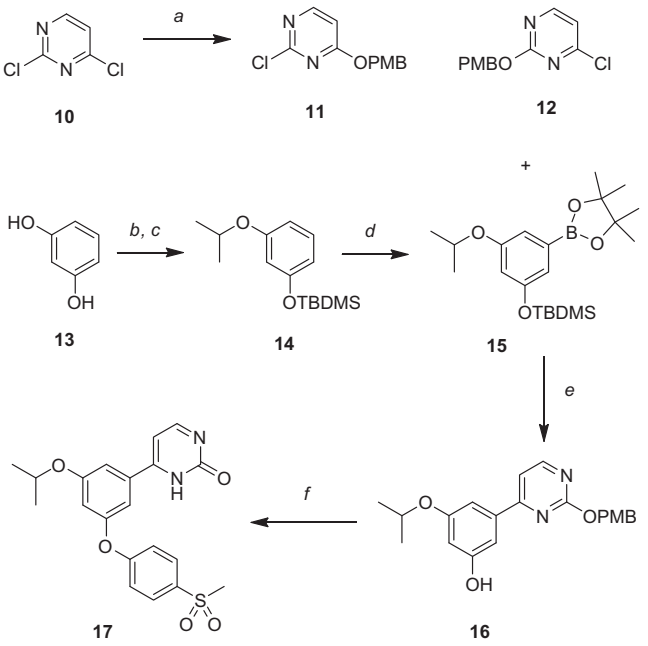
Figure 3. Design hypothesis, incorporating pyridone motif into aryloxy series.



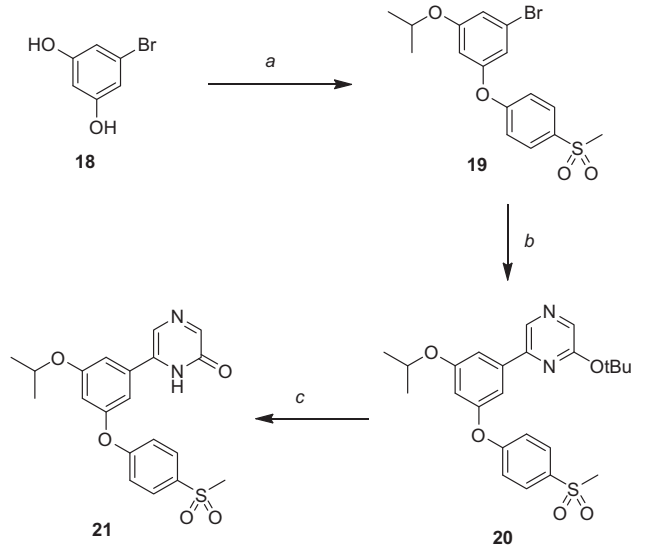
Scheme 1. Synthesis of **4**. Reagents and conditions: (a) (i) 2-bromopyridine, *n*-BuLi (1.6 M in hexanes), THF, −78 °C, 30 min; (ii) $ZnCl_2$, 23 °C, 2 h; (iii) 1-bromo-3,5-dimethoxybenzene, $Pd(PPh_3)_4$, reflux, 20 h, 68%; (b) BBr_3, CH_2Cl_2, −40 to 23 °C, 1 h, 46%; (c) *i*PrI, K_2CO_3, DMF, 80 °C, 4 h, 10%; (d) 4-chlorophenyl methylsulfone, KO*t*Bu, DMSO, 100 °C, 6 h, 30%; (e) *m*CPBA, CH_2Cl_2, 23 °C, 3 h, 69%; (f) (i) Ac_2O, reflux, 20 h; (ii) NaOMe, MeOH, 23 °C, 20 min, 25%.

The synthesis of prototype glucokinase activators are detailed in Schemes 1–6. Scheme 1 shows the synthesis of pyridone **4**. The zincate of 2-bromopyridine was first formed and then coupled to 1-bromo-3,5-dimethoxybenzene (**5**). Demethylation of the methoxy groups yielded bis-phenol **6**, which was mono-alkylated with isopropyliodide to give **7**. Base-mediated nucleophilic displacement of 4-chlorophenyl methylsulfone provided **8**, and finally the pyridine was converted to the pyridone through the two-step procedure of pyridine *N*-oxide formation to give **9**, followed by acylation and rearrangement to result in **4**.

Scheme 2 shows the synthesis of pyrimidin-2(1*H*)-one **17**. *p*-Methoxybenzyl alcohol non-specifically displaced the chlorides of 2,4-dichloropyrimidine **10** to provide a major amount of the more reactive 4-displaced product, **11**, and a minor amount of the 2-displaced product, **12**, as well as some of the bis-ether. Separately, resorcinol, **13**, was mono-alkylated with isopropyl iodide and the remaining phenol protected with a *tert*-butyldimethylsilyl group to provide **14**. This was then regioselectively borylated[11] to yield **15**. Boronate **15** was subsequently coupled to **12**, under which conditions partial removal of the silyl group occurred. TBAF was added to the reaction mixture to fully deprotect the phenol to produce biaryl **16**. Finally, nucleophilic displacement with 4-fluorophenyl methylsulfone, followed by trifluoroacetic acid-mediated deprotection yielded pyrimidone **17**.



Scheme 2. Synthesis of **17**. Reagents and conditions: (a) (i) PMB-OH, NaO*t*Bu, THF, reflux, 1 h; (ii) **10**, DMF, −70 to 23 °C, 2 h, 62% **11** and 10% **12**; (b) *i*PrI, K_2CO_3, DMF, 23 °C, 14 h, 42%; (c) TBDMS-Cl, imidazole, CH_2Cl_2, 23 °C, 1 h, 90%; (d) bis(pinacolato)diboron, (1,5-cyclooctadiene)(methoxy)iridium(I) dimer, 4,4′-di-*tert*-butyl-2,2′-dipyridyl, mtbe, 100 °C, microwave, 10 h; (e) (i) **12**, Pd(dppf)Cl_2, Na_2CO_3, 3:1 DME:H_2O, 70 °C, 13 h; (ii) TBAF, THF, 23 °C, 1 h, 70%; (f) (i) 4-fluorophenyl-methylsulfone, K_2CO_3, DMF, 120 °C, 13 h; (ii) TFA, 23 °C, 30 min, 34%.



Scheme 3. Synthesis of **21**. Reagents and conditions: (a) (i) (4-fluorophenyl)-methylsulfone, NaH (60%), DMF, 23–70 °C, 16 h; (ii) *i*PrI, K_2CO_3, 65 °C, 24 h, 14%; (b) 2-(*tert*-butoxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazine, Pd(dppf)Cl_2, K_3PO_4, DMF, H_2O, 80 °C, 16 h, 60%; (c) TFA, CH_2Cl_2, reflux, 1 h, 56%.

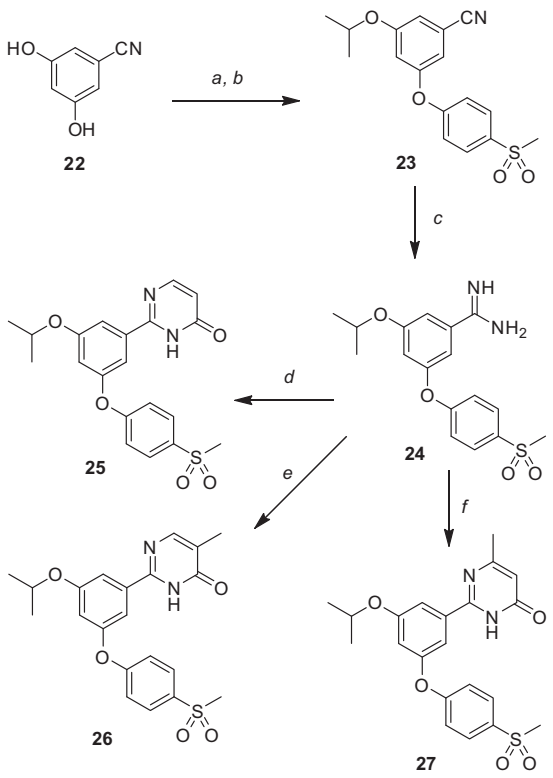
The synthesis of pyrazinone **21** is depicted in Scheme 3. Here, 5-bromobenzene-1,3-diol, **18**, was first mono-arylated with the fluorophenyl sulfone under base-mediated displacement conditions followed by similar alkylation to install the isopropyl moiety to provide **19**. Palladium coupling with 2-(*tert*-butoxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazine provided **20** and finally the *tert*-butyl group was removed with trifluoroacetic acid to give pyrazinone **21**.

Scheme 4 provides a method for the synthesis of several included pyrimidin-4(3*H*)-ones. Similar to above, 3,5-dihydroxybenzonitrile was sequentially mono-alkylated with isopropyliodide and 4-fluorophenyl methylsulfone, respectively, to provide nitrile **23**. The benzonitrile was then converted to the benzamidine under ammonium chloride, trimethylaluminum conditions to yield common intermediate **24**. This amidine was useful in synthesizing several analogs based on the condensation partner used. Ethyl propiolate provided the unsubstituted pyrimidone **25**, ethyl 2-formylpropionate formed the 5-methylated analog **26**, while ethyl acetoacetate yielded the 6-methyl version, **27**.

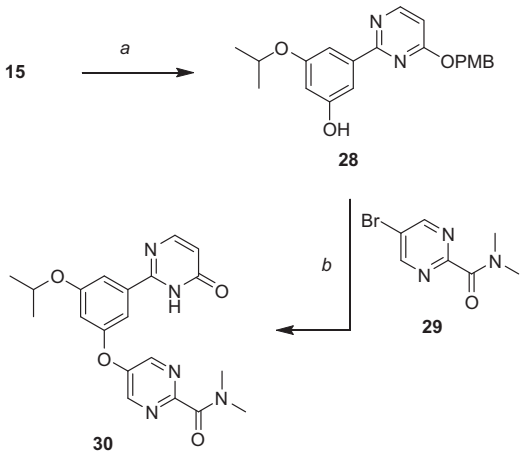
The method shown in Scheme 5 for the synthesis of **30** was used in the preparation of multiple analogs using the appropriate substituents to access a variety of compounds. Boronic ester **15** was first coupled to chloro pyrimidine **11** to provide **28**. This biaryl species was then copper-coupled[12] to bromide **29**, whose synthesis has been detailed previously,[10] followed by *p*-methoxybenzyl deprotection with trifluoroacetic acid to access final compound **30**.

Scheme 6 shows an alternative method that was used in the synthesis of multiple compounds including **36**. 5-Bromobenzene-1,3-diol, **18** was first bis-silyl protected under standard conditions to give **31** and then the boronate was formed and coupled to chloride **11** to provide biaryl **32** after deprotection. Separately, chiral diol **33** was mono-protected to form **34** and then **32** was alkylated with **34** under Mitsunobu conditions to give **35**. Finally, phenol **35** displaced 4-fluorophenyl methylsulfone and the *p*-methoxybenzyl group removed with acid to arrive at analog **36**.

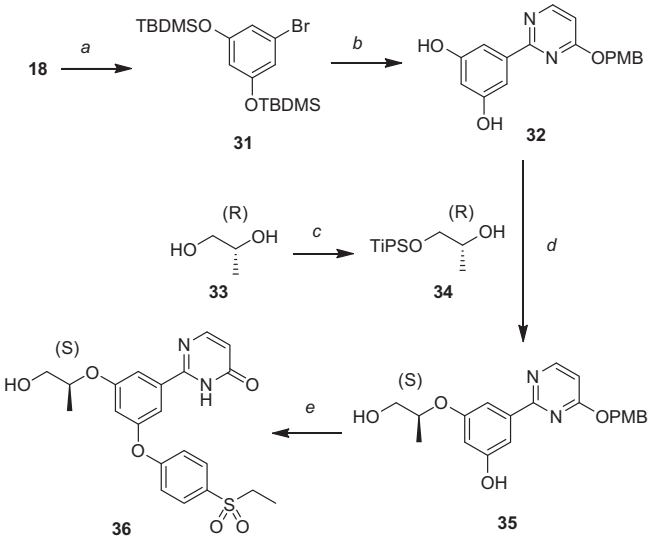
Biochemical evaluation of all analogs utilized a matrix assay that has been previously described.[10] Briefly, the human recombinant glucokinase-containing assay was run at 22 different activator



Scheme 4. Synthesis of **25**, **26**, and **27**. Reagents and conditions: (a) *i*PrI, K_2CO_3, DMF, 65 °C, 16 h, 37%; (b) 4-fluorophenyl-methylsulfone, NaH (60%), DMF, 0–85 °C, 16 h, 78%; (c) NH_4Cl, Me_3Al (2 M in toluene), toluene, 0–110 °C, 36 h; (d) ethyl propiolate, NaOEt (21% in EtOH), EtOH, 0–80 °C, 16 h, sealed tube, 25%; (e) ethyl 2-formylpropionate, NaOEt (21% in EtOH), EtOH, 85 °C, 16 h, 59%; (f) ethyl acetoacetate, NaOEt (21% in EtOH), EtOH, 85 °C, 16 h.



Scheme 5. Synthesis of representative analog **30**. Reagents and conditions: (a) (i) **11**, $Pd(dppf)Cl_2$, Na_2CO_3, 3:1 DME:H_2O, 70 °C, 19 h; (ii) TBAF (1 M in THF), THF, 23 °C, 90 min, 37%; (b) (i) **29**, CuCl, 2,2,6,6-tetramethylheptane-3,5-dione, Cs_2CO_3, DMF, 120 °C, 15 h, sealed tube; (ii) TFA, CH_2Cl_2, 23 °C, 10 min, 65%.



Scheme 6. Synthesis of representative analog **36**. Reagents and conditions: (a) TBDMS-Cl, imidazole, DMF, 23 °C, 3d, 89%; (b) (i) *t*BuLi (1.7 M in pentane), $B(OMe)_3$ THF, −78 to 23 °C, 16 h; (ii) **11**, $Pd(dppf)Cl_2$, Na_2CO_3, DME, H_2O, 65 °C, 16 h; (iii) TBAF, THF, 23 °C, 1 h, 59%; (c) TiPS-Cl, DIEA, imidazole, CH_2Cl_2, DMF 23 °C, 2d, 84%; (d) PPh_3, DIAD, THF, 23 °C, 24 h, 33%; (e) (i) 1-(ethylsulfonyl)-4-fluorobenzene, K_2CO_3, DMF, 80 °C, 3 h; (ii) TFA, 23 °C, 1 h.

concentrations (0–100 μM) and 16 different glucose concentrations (0–100 mM). A nonessential activator model was utilized to determine an activator's maximum fold effect on reducing the glucokinase K_m for glucose, defined as α, and the maximum fold effect on altering the V_{max}, defined as β.[13] The α value is the ratio of the glucokinase K_m at maximum activator concentration divided by the enzyme's K_m in the absence of activator. Values of α range from 0 to 1 with lower values representing more substantial reductions in the enzyme's glucose K_m. The β value is defined as the ratio of the enzyme's velocity at maximum activator concentration divided by the enzyme's velocity in the absence of activator. Values >1 indicate increases in the V_{max} with activator present, $\beta = 1$ indicates no effect on V_{max}, and values <1 indicate suppression of the V_{max} with activator present. The EC_{50} was formally defined as the concentration of activator affording a half-maximal reduction in K_m.

Glucokinase at 20 mg/mL was crystallized in the presence of 40 mM glucose and 1 mM **1** or **2**. Crystallization drops in a 1:1 ratio were set up over wells containing 0.1 M TrisHCl pH 7.0, 80–200 mM glucose, and 19–26% PEG-4000. Structures were solved using molecular replacement method. Human liver microsomal clearance (HLM),[14] rat liver microsomal clearance (RLM), kinetic aqueous solubility, passive permeability flux performed in a low transporter-expressive cell line, MDCKII-LE,[15] and log*D* performed by the shake flask method, were also used to further characterize final compounds. The kinetic solubility assay was performed as follows: 50 mM sodium phosphate buffer, at pH 6.5, was freshly prepared from NaH_2PO_4 and Na_2HPO_4 and filtered. Buffer (294 μL) was combined with 6 μL of 30 mM DMSO stock solution in a Millipore polycarbonate filter solubility plate (part# MSSLBPC10) for a final DMSO concentration of 2%. The plates were heat sealed with a polypropylene seal. After 24 h of 200 rpm shaking at room temperature (23–25 °C), the plates were vacuum filtered into deep well receiver plates. Filtrates were injected into a chemiluminescent nitrogen detector (Antek 8060) for quantification. Apparent concentration was divided by the number of nitrogens in the sample (from the molecular formula) to determine the concentration.

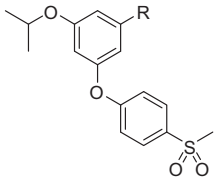
Our design hypothesis of replacing the heterocyclic amide with a pyridone was confirmed with **4** (Table 1) having sub-micromolar potency, while reducing molecular weight and increasing polarity as compared to amide **3**, leading to a modestly higher lipophilic efficiency (LipE).[16] Several other properties also were improved, namely passive permeability, kinetic solubility, and human microsomal clearance. Gratifyingly, this compound also had a higher α (i.e., less substantial reduction in the enzyme's K_m for glucose), indicating the potential for increased glucose dependency relative benchmark candidate **3**, as discussed above.

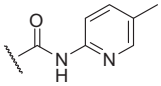
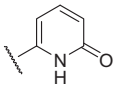
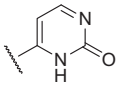
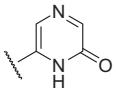
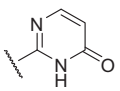
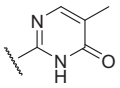
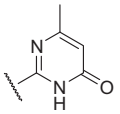
A nitrogen-walk around the pyridine of **4** was first performed. The pyrimidin-2(1*H*)-one, **17**, and pyrazinone, **21**, were much less active, while the pyrimidin-4(3*H*)-one, **25**, was equipotent with still desirable activation profile. Since the log*D* was reduced from the pyridone lead, this compound had improved LipE, as well as improved permeability and solubility, and so was deemed to be a superior motif and was used in subsequent optimization. Based on the crystal structure and the overlap with the heterocyclic amide motif it was envisioned that building off the 5-position would be sterically tolerated while the 6-position appeared less promising. This hypothesis played out with the 5-methyl analog, **26**, having improved potency and ligand efficiency (LE = 0.33 for **26** vs 0.31 for **25**),[17] and maintained LipE; while the 6-methyl homolog, **27**, lost significant activity. Therefore additional substitutions were examined in the 5-position as shown in Table 2.

An SAR trend with small alkyl substituents, **26**, **37**, and **38**, shows that ethyl, **37**, is optimal for potency. Replacing the ethyl with the isosteric methoxy group, **39**, shows a loss in potency. Noteworthy, is that electron-donating substituents, as in **26** and **37**–**39**, are significantly more potent than electron-withdrawing substituents **40** and **41**, even for the similarly sized and lipophilic chloro compared to methyl or ethyl. This suggested the importance of the strength of the H-bonding between the pyrimidone carbonyl and NH with the binding site residue. Specifically, the anticipated pK_a of the pyrimidone NH with an electron-withdrawing group present is low enough where at physiological pH a significant percentage of the deprotonated compound will be present; eliminating the H-bond interaction and creating an electronic repulsion.[18]

Exploration of SAR around the *O*-aryl ring is shown in Table 3. Several heterocycles and substituents were incorporated as was done in related series.[10,19] Sulfone (**25**), sulfonamide (**42**), and amide substituents (**30**, **43**, and **44**) all showed good to moderate activity, while large improvements in LipE and kinetic solubility were demonstrated with pyridyl (**43**), pyrimidyl (**30**), and

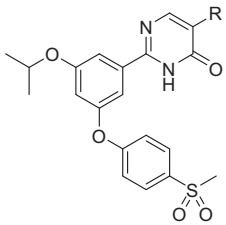
Table 1
Properties of glucokinase activators **3**, **4**, **17**, **21**, **25–27**[a]



	R	EC_{50}[b] (μM)	α[b]	β[b]	MW	log*D*	LipE[d]	Permeability (10^{-6} cm/s)	Solubility (μM)	HLM Cl_{int} (mL/min/kg)	RLM Cl_{int} (mL/min/kg)
3		0.11[c]	0.07[c]	1.09[c]	440	4.05	3.22	4.4	1.4	17	97
4		0.74 ± 0.33	0.14 ± 0.01	0.75 ± 0.03	399	2.82	3.62	18	11	<8.0	349
17		>100[c]	—	—	400	ND	—	ND	ND	ND	ND
21		23.0 ± 4.3	<0.37 ± 0.05	0.83 ± 0.08	400	2.27	2.66	94	118	11	61
25		0.83 ± 0.33	0.10 ± 0.01	1.12 ± 0.02	400	2.14	4.25	24	25	<9.8	105
26		0.20 ± 0.05	0.14 ± 0.03	0.83 ± 0.07	414	2.68	4.33	17	1.9	<8.0	189
27		10.3[c]	0.49[c]	0.40[c]	414	2.43	2.86	4.8	15	<8.0	96

[a] ND = Not Determined.
[b] Biochemical data reported as the geometric mean ± SD of ⩾2 determinations except where indicated.
[c] Data from $n = 1$ determination.
[d] LipE = −log (EC_{50}) − log*D*.

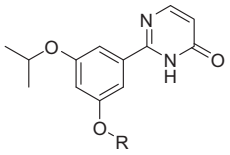
Table 2
Properties of glucokinase activators **37–41**[a]

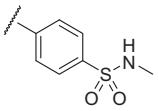
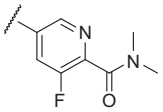
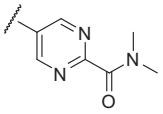
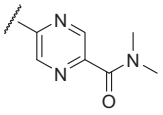


	R	EC_{50}[b] (μM)	α[b]	β[b]	MW	log*D*	LipE[d]	Permeability (10^{-6} cm/s)	Solubility (μM)	HLM Cl_{int} (mL/min/kg)	RLM Cl_{int} (mL/min/kg)
37	Et	0.07 ± 0.01	0.16 ± 0.01	0.66 ± 0.06	428	3.11	4.33	4.0	5.7	<8.0	171
38	*i*Pr	0.59[c]	0.20[c]	0.43[c]	442	ND	—	ND	ND	ND	ND
39	OMe	0.94 ± 0.19	0.26 ± 0.03	0.73 ± 0.01	430	2.21	4.12	3.9	7.1	<8.0	<25.4
40	Cl	7.5[c]	0.14[c]	1.00[c]	418	2.04	3.39	8.3	24	<8.0	<25.4
41	CN	9.2[c]	0.37[c]	0.78[c]	425	ND	—	ND	ND	ND	ND

[a] ND = Not Determined.
[b] Biochemical data reported as the geometric mean ± SD of ⩾2 determinations except where indicated.
[c] Data from $n = 1$ determination.
[d] LipE = −log (EC_{50}) − log*D*.

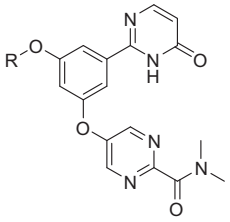
Table 3
Properties of glucokinase activators **30**, **42**–**44**

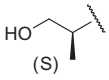
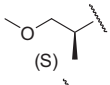
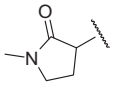


	R	EC_{50}[a] (μM)	α[a]	β[a]	MW	log*D*	LipE[b]	Permeability (10^{-6} cm/s)	Solubility (μM)	HLM Cl_{int} (mL/min/kg)	RLM Cl_{int} (mL/min/kg)
42		1.4 ± 0.5	0.09 ± 0.03	1.12 ± 0.07	415	2.63	3.52	15	117	<8.0	112
43		0.23 ± 0.08	0.12 ± 0.02	0.79 ± 0.02	412	1.54	5.40	13	558	8.3	102
30		0.85 ± 0.04	0.12 ± 0.01	0.86 ± 0.01	395	0.97	5.32	7.1	332	<8.0	<25.4
44		0.50 ± 0.11	0.12 ± 0.01	0.76 ± 0.04	395	1.34	5.26	16	373	<8.0	47

[a] Biochemical data reported as the geometric mean ± SD of ⩾2 determinations.
[b] LipE = −log (EC_{50}) − log*D*.

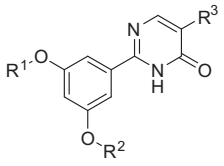
Table 4
Properties of glucokinase activators **45**–**50**[a]

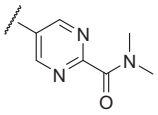
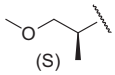
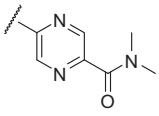
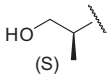
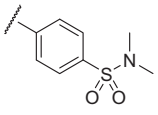
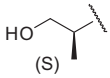
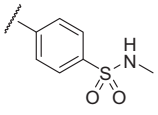
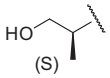
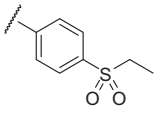


	R	EC_{50}[b] (μM)	α[b]	β[b]	MW	log*D*	LipE[d]	Permeability (10^{-6} cm/s)	Solubility (μM)	HLM Cl_{int} (mL/min/kg)	RLM Cl_{int} (mL/min/kg)
45		0.23 ± 0.03	0.13 ± 0.01	0.84 ± 0.01	423	1.91	5.04	17	469	22.4	91
46		0.44 ± 0.16	0.13 ± 0.02	0.95 ± 0.07	421	1.74	4.92	14	398	<8.0	106
47		1.1 ± 0.2	0.13 ± 0.01	0.78 ± 0.02	411	−0.02	6.27	0.81	339	<8.0	26
48		1.0 ± 0.1	0.14 ± 0.01	0.76 ± 0.01	425	0.43	5.85	ND	342	<8.0	38
49		7.5 ± 0.1	0.18 ± 0.01	0.88 ± 0.01	423	ND	—	ND	ND	ND	ND
50		3.8[c]	0.19[c]	1.00[c]	423	ND	—	ND	ND	ND	ND

[a] ND = Not Determined.
[b] Biochemical data reported as the geometric mean ± SD of ⩾2 determinations except where indicated.
[c] Data from *n* = 1 determination.
[d] LipE = −log (EC_{50}) − log*D*.

Table 5
Properties of glucokinase activators **36**, **51**–**54**[a]



	R^1	R^2	R^3	EC_{50}[b] (μM)	α[b]	β[b]	MW	log*D*	LipE[c]	Permeability (10^{-6} cm/s)	Solubility (μM)	HLM Cl_{int} (mL/min/kg)	RLM Cl_{int} (mL/min/kg)
51			Me	0.17 ± 0.01	0.15 ± 0.01	0.59 ± 0.03	409	1.48	5.59	11	218	<8.0	<25.4
52			H	0.20 ± 0.07	0.15 ± 0.01	0.68 ± 0.01	425	0.81	6.19	2.2	728	<8.0	<25.4
53			Me	0.09 ± 0.01	0.09 ± 0.01	0.80 ± 0.01	459	2.26	5.11	14	197	12	ND
54			Me	0.11 ± 0.05	0.09 ± 0.01	0.88 ± 0.02	445	2.01	5.26	1.6	104	<8.0	<25.4
36			H	0.15 ± 0.07	0.09 ± 0.01	1.05 ± 0.05	430	1.42	5.71	2.8	390	<8.0	<25.4

[a] ND = Not Determined.
[b] Biochemical data reported as the geometric mean ± SD of ⩾2 determinations.
[c] LipE = −log (EC_{50}) − log*D*.

Table 6
Pharmacokinetic profiles of glucokinase activators **25**, **26**, **36**, **47**[a]

	Cl[b] (mL/min/kg)	V_{dss}[b] (L/kg)	$t_{1/2}$[b] (h)	C_{max}[c] (ng/mL)	AUC[c] (ng h/mL)	*F* (%)
25	61	6.41	1.5	150	703	29
26	13	0.746	5.3	31	141	2
45	44	5.79	6.0	412	580	32
36	19	0.746	1.8	74	253	6

[a] Wistar-Han rats with $n = 3$ animals/group.
[b] iv administration dosed at 1 mg/kg.
[c] p.o. administration dosed at 5 mg/kg.

pyrazinyl (**44**) heterocycles. The pyrimidyl dimethyl-carboxamide was chosen as an efficient moiety for exploration of alternative *O*-alkyl substituents (Table 4). Although some improvement in potency was observed with large alkyl substituents (**45** and **46**), overall lipophilic efficiency went down. LipE was improved, however, with propanol **47** and propylether **48,** albeit at the low lipophilicity range, where oral absorption could potentially be negatively impacted.[20] Other polar moieties such as ether **49** and lactam **50** lost activity.

Table 5 shows several compounds with good potency that combine some of the results from the above work. These possess calculated log*D* values that would suggest favorable physicochemical properties and contain moieties that had previously demonstrated good activity. Despite having excellent potency and good permeability, pyrimidine **51** and pyrazine **52** have low β values, indicating potential inhibition of enzymatic activity under high glucose conditions; an unfavorable profile. Propanols **36**, **53**, and **54** increased β closer to desired levels around 1. The dimethyl sulfonamide, **53**, showed some HLM turnover, while the methyl sulfonamide, **54**, suffered from low permeability. Ethyl sulfone **36** provided an improved yet still modest permeability value.

Several compounds with ideal activation profiles were chosen for in vivo rat pharmacokinetic (PK) characterization (Table 6). As a summary, compounds **25** and **45**, which were less potent glucokinase activators, had acceptable PK characteristics with higher plasma exposures and near 30% oral bioavailability, while the more potent compounds **26** and **36** showed very poor oral bioavailabilities of 2% and 6%, respectively. The poor bioavailability for **36** is likely a result of its poor permeability due to an additional H-bond donor, while that for **26** may be due to poor solubility and/or high rat oxidative clearance. The latter two compounds were also run in an oral glucose tolerance test (OGTT) in vivo efficacy study in rats

and neither demonstrated glucose lowering. Subsequent PK analyses of these samples showed free drug levels below their in vitro EC_{50}'s, further confirming the insufficient PK profiles for these more potent analogs. This series showed both an HLM/RLM in vitro disconnect and rat in vitro/in vivo clearance disconnect, the origin of which remains unknown. This contributed to the difficulty of finding a compound with sufficient PK properties to show an in vivo pharmacodynamic effect.

In summary, a substitution of the common heterocyclic amide donor–acceptor motif present in many glucokinase activators for a pyridone or pyrimidone moiety provided a structurally novel series of partial activators with favorable activation profiles. This change resulted in a better lipophilic efficiency compared to the heterocyclic amides. Although an acceptable balance of potency and PK was not achieved through optimization of this series to provide oral efficacy in a rat model, it is envisioned that this structural change may benefit other glucokinase activator series, or more broadly in other programs where a similar donor–acceptor motif is required.

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