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Design of a potent, soluble glucokinase activator with increased pharmacokinetic half-life

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ABSTRACT

The continued optimization of a series of glucokinase activators is described, including attempts to understand the interplay between molecular structure and the composite parameter of unbound clearance. These studies resulted in the discovery of a new scaffold for glucokinase activators and further exploration of this scaffold led to the identification of **GKA60**. GKA60 maintains an excellent balance of potency and physical properties whilst possessing a significantly different, but complimentary, preclinical pharmacokinetic profile compared with the previously disclosed compound **GKA50**.

Type 2 diabetes is a complex disease which 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] consequently, there is an unmet need for therapeutic agents which deliver superior glycaemic control.

Glucokinase (GK, also known as hexokinase IV or D) is expressed predominantly in the liver, pancreas, brain and gut where its function is to catalyze the conversion of glucose to glucose-6-phosphate. In both the liver, where the action of GK promotes glycogen synthesis, and the pancreas, where the action of GK results in glucose-sensitive insulin release,[3] GK is the rate limiting enzyme in glucose utilisation.[4] Activation of GK is therefore expected to improve glycaemic control by modulating hepatic glucose balance and decreasing the threshold for insulin secretion.[5–7]

Several groups have described small molecule glucokinase activators (GKAs) which act by binding to an allosteric site.[8] In previous letters we have described the identification, and subsequent optimization, of our own series of pyridine acid containing GKAs.[9,10] This optimization required the breaking of an emerging relationship between enzyme potency and binding to plasma proteins in order to achieve the desired profile. The strategy of iterative design, starting from a previously identified outlier to the relationship (**GKA22**), and the use of the composite parameter of unbound clearance (CL_u)[11] to measure progress, was disclosed. These studies resulted in the identification of **GKA50**, which showed excellent in vitro profiles, good pharmacokinetic parameters and excellent in vivo efficacy (Fig. 1).

In addition, we have previously described how knowledge of the plasma compound levels following an oral dose, together with knowledge of the enzyme potency and the plasma protein binding of that compound, can be used to predict in vivo efficacy in an acute animal model.[12] Understanding the importance of the pharmacokinetic (PK) exposure of a compound in relation to in vivo efficacy led us to consider how compounds with different PK profiles might complement each other. In particular we reasoned that a compound with a significantly increased half-life would be worthy of further investigation. Given the impact of plasma protein binding on our in vivo efficacy models we were conscious of the need to avoid driving an increase in half-life simply by increasing plasma protein binding. As such we were keen to explore whether

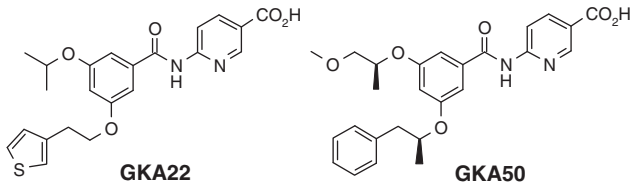

Figure 1. The structures of **GKA22** and **GKA50**.

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targeting a further reduction in unbound clearance would be a more optimal strategy to achieve our goal.

In this Letter we describe our continuing attempts to reduce unbound clearance and to better understand the structural features which impact most on this composite parameter. The extremely low in vitro and in vivo metabolic turnover of our pyridine acid containing GKAs precluded the use of metabolite identification studies to help direct chemistry efforts. Hence an iterative strategy of modifying discrete parts of the molecule to examine the affect on potency,[13] plasma protein binding[14] and rat in vivo clearance[15] was adopted.

Our first step was to investigate modifications to the exposed pendant phenyl ring. A range of substitution patterns incorporating both electron withdrawing and electron donating groups was examined and found to be tolerated, as illustrated in Table 1 (**1–3**). In each case any reduction in clearance observed was accompanied with an increase in plasma protein binding, and vice versa, resulting in no improvement in CL_u. Heterocycles (**4**) and saturated rings (**5**) were also tolerated but again no improvement in CL_u was observed.

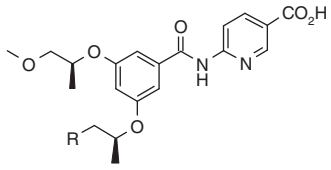
Next we turned our attention to 5-position of the central ring, Table 2. Whilst the isopropyl ether (**6**) is well tolerated this change has a detrimental effect on the unbound clearance. Other modifications designed to reduce metabolism, such as **7** and **8**, were not well tolerated.

Turning our attention to the alkyl linker we discovered that moving the methyl substituent to the β-carbon (**9**), or the introduction of fluorine atoms to that carbon (**10**), resulted in potent compounds (Table 3). However, no reduction in CL_u was observed. Cyclisation of the linker to give an indane ring (**11**) was tolerated but also failed to deliver a reduction in CL_u. Whilst removal of a methylene from the linker did result in a slight reduction of potency (**12**) a significant reduction in CL_u was observed. A further reduction in CL_u was observed when the biphenyl ether moiety was installed (**13**). These results perhaps suggest that metabolism of the carbon chains linking the aromatic rings in this portion of the molecule plays a significant part in the clearance of the compounds. Compound **13** shows a significantly increased pharmacokinetic half-life in rat compared with **GKA50** (10.5 h compared to 4.5 h).

The increased pharmacokinetic half-life observed with **13** compared to **GKA50** encouraged the team to further investigate this novel biphenyl ether scaffold. Studies to understand the effects of substitution on the biphenyl ether on both the potency, and the properties, of the molecule were conducted (Table 4).

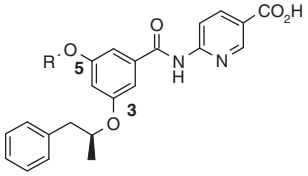
Combining the unsubstituted biphenyl ether with the more optimal alkyl ether side chain (**14**) gave rise to a slight improvement in potency and reduction in plasma protein binding; however, CL_u remained largely unchanged. The introduction of a methoxy substituent generally appeared to improve the potency and reduce plasma protein binding; however, a concomitant increase in clearance rates appears to negate any benefit to CL_u (**15**, **16**). The introduction of a chlorine atom to the ring also resulted in an increase in binding to plasma proteins that was not

Table 1
Relationship of exposed aryl ring to CL_u



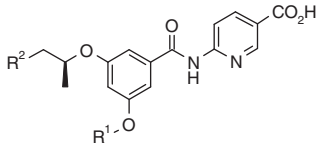
Compd	R	EC_{50} (μM)	% Free (rat)	Rat CL	CL_u
GKA50	Ph	0.03	0.40	1.9	475
1	4-F-Ph	0.04	0.25	1.0	400
2	2-MeO-Ph	0.07	0.42	6.1	1452
3	3-F-5-F-Ph	0.05	0.10	1.1	1100
4	2-Furanyl-5-Cl	0.03	0.25	1.6	640
5	c-Pn	0.09	<0.03	0.4	>1333

Table 2
The relationship between the 5-ether linked substituent and CL_u



Compd	R	EC_{50} (μM)	% Free (rat)	Rat CL	CL_u
6	*i*-Pr	0.02	0.23	3.3	1435
7	$C(Me)_2CH_2OMe$	>10	0.42	—	—
8	4-THP	>10	1.09	—	—

Table 3
The effect of the linker group on CL_u



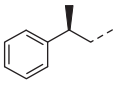
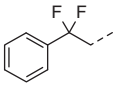
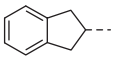
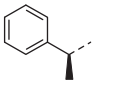
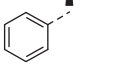
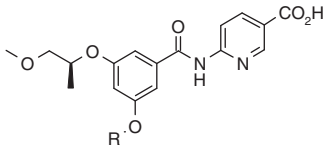
Compd	R^1	R^2	EC_{50} (μM)	% Free (rat)	Rat CL	CL_u
9		OMe	0.07	0.33	2.9	879
10		OMe	0.03	0.37	4.2	1135
11		H	0.10 (n = 1)	0.10	1.6	1600
12		H	0.15 (n = 1)	0.35	0.6	171
13		H	0.21 (n = 1)	0.21	0.2	95

Table 4
The effect of substitution on the biphenyl ether motif



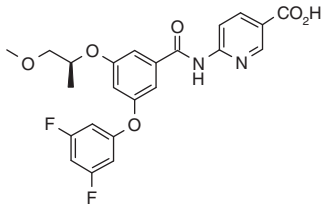
Compd	R	EC_{50} (μM)	% Free (rat)	Rat CL	CL_u
14	Ph	0.16 (n = 1)	0.55	0.4	73
15	3-MeO-Ph	0.04	0.66	2.8	424
16	4-MeO-Ph	0.06	0.69	3.2	464
17	3-Cl-Ph	0.11	0.16	0.8	500
18	4-Cl-Ph	0.10	0.12	0.9	750
19	3-F-Ph	0.18	0.21	0.2	95
20	3-NMe_2-Ph	0.40	1.22	—	—
21	4-SO_2Me-Ph	0.06	7.38	8.2	111

compensated for by a sufficient reduction in clearance rates (**17**, **18**); however, the introduction of fluorine to the 3-position did appear to offer a good balance of properties (**19**). The introduction of more polar functionality resulted in a significant reduction of plasma protein binding but was accompanied by either a reduction in potency (**20**), or an increase in clearance which negated any significant benefit to CL_u (**21**). We have previously reported the dependency of both potency and plasma protein binding on lipophilicity for our pyridine acid GKAs and highlighted the challenge this posed for subsequent optimisation of the series.[9,10] For the subset of biphenyl ether containing compounds, such as **14**–**21** and additional closely related structural analogues, the binding to plasma proteins also appears related to lipophilicity, as evaluated by *C log P*.[16] In contrast the potency of these compounds appears to be largely independent of lipophilicity (Fig. 2).

Biphenyl ether compounds, such as **14**–**21**, could be prepared according to the route outlined in Scheme 1. A key feature of the synthesis is the use of a copper mediated coupling of a phenol with a phenylboronic acid to install the biphenyl ether motif.[17] The synthetic availability of a range of substituted phenylboronic acids allowed the rapid exploration of the SAR outlined above.

To build on the promising profile of **19** the effect of disubstitution was investigated. These investigations resulted in the identification of **GKA60**, a potent, soluble GKA possessing a further reduction in unbound clearance. The profile of **GKA60** compared to that of **GKA50** is summarized in Table 5.

Table 5
The structure of **GKA60** is shown along with a comparison of its properties compared to **GKA50**



Compd	GKA50	GKA60
EC_{50} (μM)	0.03	0.09
Solubility (μM)	1350	841
% Free (rat, dog, human)	0.40, 1.46, 0.44	0.23, 1.59, 0.55
Rat clearance (mL/min/kg)	1.9	0.1
Rat half-life (h)	4.5	15.8
Rat bioavailability (%)	99	97
Dog clearance (mL/min/kg)	4.4	5.2
Dog half-life (h)	1.4	4.9
Dog bioavailability (%)	100	>100
Hepatic Clint: rat, dog, human (μL/min/10^6 cells)	<1, <1, <1	<1, <1, <1
CL_u (rat, dog)	475, 301	43, 327

As can be seen, both **GKA50** and **GKA60** possess excellent potency, solubility and pharmacokinetic profiles in both rat and dog. In particular, the significantly increased pharmacokinetic half-life of **GKA60** in rat results in a different shape of PK profile. Following identical 3 mg/kg oral doses of **GKA50** and **GKA60** to CR-Han Wistar rats maximal concentrations (C_{max}) of 4.23 μg/mL and 16.2 μg/mL, respectively, were observed. When differences in plasma protein binding and potency are taken into account these C_{max} levels would be anticipated to result in similar activation of GK (Fig. 3). However, **GKA60** retains significantly higher plasma levels at later time-points (24 h post dose a concentration of 7.06 μg/mL of **GKA60** is observed compared with a concentration of 0.116 μg/mL for **GKA50**). As such we believe this compound possesses a significantly different, but complementary, pharmacokinetic profile to that of **GKA50**.

In summary, we have described our continued efforts to reduce CL_u within a series of GKAs in the absence of structural in vitro metabolism data. We have outlined the systematic investigation into the relationship between the molecular structure and CL_u which, in turn, highlighted the importance of the alkyl linker

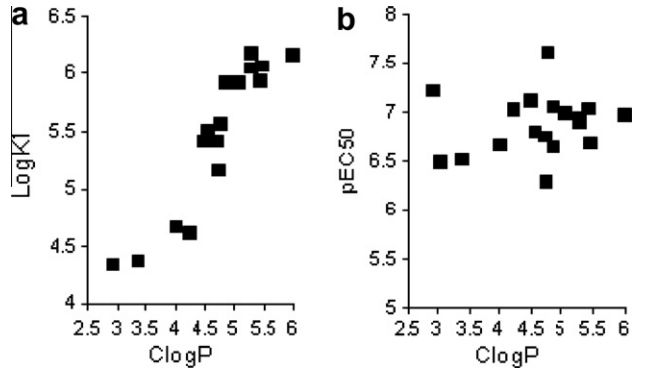
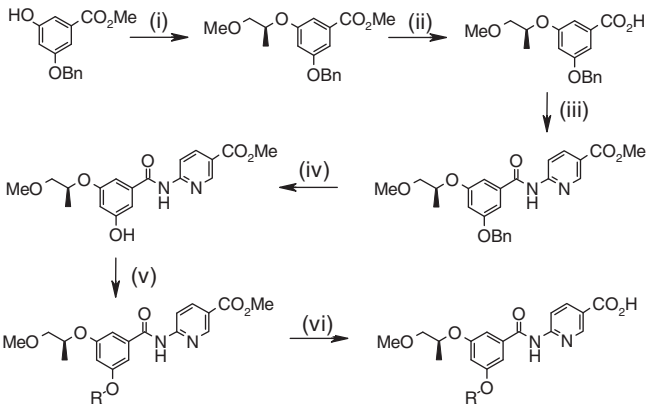


Figure 2. Plots of (a) plasma protein binding ($\log K1_{app}$) and (b) enzyme potency (pEC_{50}) against lipophilicity ($C \log P$) for a range of biphenyl ether compounds.



Scheme 1. General synthetic route to biphenyl ethers **14**–**21**. Reagents and conditions: (i) PS-PPh3, DIAD, (*R*)-1-methoxypropan-2-ol, THF; (ii) 2 M NaOH, THF/MeOH/H_2O (73% over two steps); (iii) $(COCl)_2$, CH_2Cl_2, DMF (cat) followed by methyl 6-aminonicotinate, THF, pyridine (50%); (iv) Pd/C, MeOH/THF, H_2(g) (72%); (v) $RB(OH)_2$, $Cu(OAc)_2$, Et_3N, molecular sieves, CH_2Cl_2 (40–80%); (vi) 0.5 M NaOH, THF/H_2O (95%).

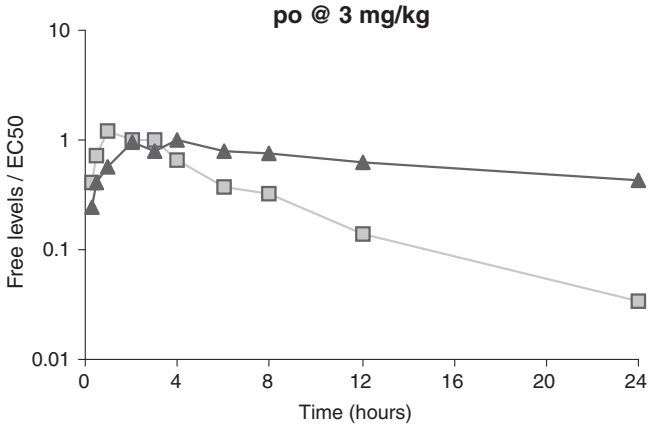


Figure 3. Pharmacokinetic profiles, normalised for plasma protein binding and enzyme potency, for **GKA50** (grey squares) and **GKA60** (black triangles).

moiety and resulted in the discovery of a novel biphenyl ether scaffold. Compounds adhering to this scaffold have been identified that possess a reasonable balance of potency and physicochemical properties whilst also delivering an increased pharmacokinetic half-life in rat. In particular, **GKA60** has been identified as a potent, soluble GKA displaying a significantly different, but complementary, pharmacokinetic profile to **GKA50**, in rat. Pleasingly **GKA60** also shows significant activity in our animal models and was selected for further pre-clinical evaluation.

Acknowledgements

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13. EC_{50} values were determined at 10 mmol/L glucose, and are quoted as the geometric mean of at least three independent experiments (unless otherwise indicated). Assay to assay variability was within two fold based on the results of a standard compound. All compounds in this series exhibited a negligible effect on V_{max}. Detailed in vitro studies and assay conditions are described in: 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.
14. The plasma protein binding of compounds was measured using equilibrium dialysis technique. More detailed description of the assay conditions are described in Ref.[11].
15. Rat clearance values were obtained following an IV dose of 1 mg/kg to CR-Han Wistar rats. The compounds were dosed as a solution and the clearance values are quoted in mL/min/kg.
16. *C* log *P* values were calculated using the commercially available package *C* log *P* v4.3 from BioByte Corp, 201 W. 4th St., #204 Claremont, CA 91711-4707.
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