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Design of a potent, soluble glucokinase activator with excellent in vivo efficacy

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Abstract—The optimisation of a series of glucokinase activators is described, including attempts to uncouple the relationship between potency and plasma protein binding, and to better understand the key pharmacokinetic properties of the series. The use of unbound clearance as an optimisation parameter facilitated the identification of **GKA50**, a compound which combines excellent potency and pharmacokinetics with good free drug levels and solubility, and exhibits in vivo efficacy at 1 mg/kg po in an acute rat OGTT model.

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] hence there is a need for new, effective pharmacological agents.

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.[6–8]

Several groups,[9] including those at Hoffmann–La Roche,[10,11] Lilly,[12] Prosidion[13] and Banyu[14], have described small molecule glucokinase activators (**GKA**s) which act by binding to an allosteric site (Fig. 1).[14,15] Further reports of **GKA**s from Novartis[16] and Novo Nordisk[17] have appeared in the recent patent literature.

In a recent letter,[18] we described our own series of **GKA**s and highlighted the interdependence of potency, lipophilicity and physical properties within the series. The thiophene derivative 6-({3-isopropoxy-5-[2-(3-thienyl)ethoxy]benzoyl}amino)nicotinic acid (**GKA22**, Table 1) was identified as having a suitable balance of potency and physical properties, and was shown to demonstrate in vivo efficacy at 30 mg/kg po in an acute rat oral glucose tolerance test (OGTT).

In this paper, we describe our attempts to uncouple the relationship between potency and plasma protein binding, and to further develop our understanding of the DMPK properties of the series. Our key aim for this programme of work was to improve upon **GKA22** by increasing potency and reducing protein binding, whilst lowering clearance to improve oral exposure and in vivo efficacy.[19]

Our first step was to gain greater insight into the DMPK properties of the series. Whilst we have previously reported rat DMPK data for **GKA22**,[18] the accompanying dog data shown in Table 1 proved instructive. As expected of the series, excellent bioavailability and low volume of distribution were maintained, but we were initially surprised to observe that clearance in dog was higher than in rat. This was contrary to that expected on the basis of hepatic blood flow in the respective species[20] and could not be rationalised in terms of in vitro hepatocyte data.

Keywords: Diabetes; Glucokinase; Kinase activator; Unbound clearance; **GKA50**.
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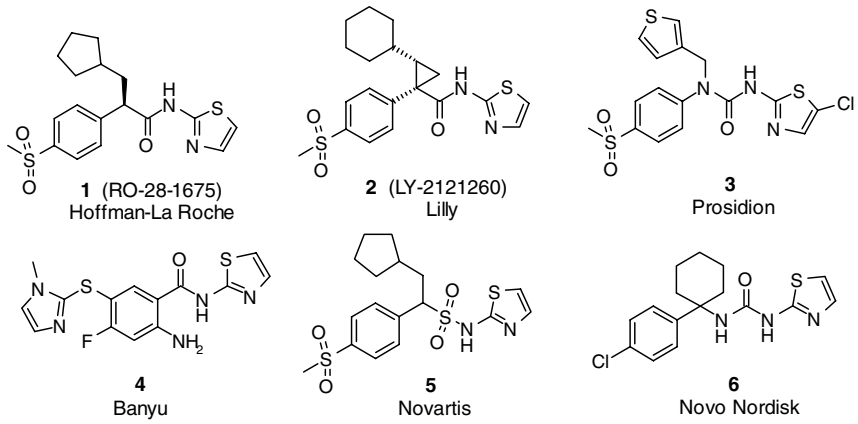
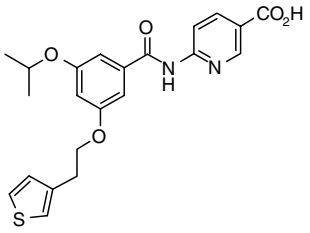


Figure 1. Structures of selected glucokinase activators reported in the literature.

Table 1. Pharmacokinetic parameters of **GKA22** in rat and dog



	Rat	Dog
Clearance (mL/min/kg)	3.8	8.7
Hepatic blood flow (mL/min/kg)	72	31
Clearance (% hbf)	5.3	28
Cl_{int} (μL/min/10^6 cells)	<1	<1
% Free[a]	0.26	0.64
Unbound clearance[b]	1461	1359

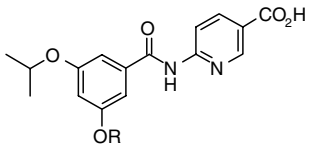
f_u is the fraction unbound, that is, % free/100.
[a] Protein binding determined by equilibrium dialysis.
[b] Unbound clearance is defined as Cl/f_u.[21]

However, we were also aware that the % free drug was higher in dog than in rat and knew that this could influence the observed in vivo clearance.[21] We therefore decided to consider 'unbound clearance' as a composite parameter[21] and observed that this was similar in both species. Since we wanted to increase % free *and* lower clearance,[19] it was clear that minimising unbound clearance was a key objective.

Our strategy was built on the observation that incorporation of an 'α-branch' seemed to lower unbound clearance. This was exemplified by compounds **7** and **8** (Table 2) which differ only in the presence or absence of a methyl group at the benzylic or 'α' position. Whilst the effect on protein binding was minor, the in vivo clearance was significantly reduced. Taken together, the incorporation of the 'α-branch' lowered unbound clearance by an order of magnitude. Although the in vitro hepatocyte data are inconclusive, it is tempting to speculate that incorporation of the 'α-branch' serves to reduce unbound clearance by lowering the potential for benzylic oxidation.

Having identified the beneficial effect of incorporating an α-branch, we next turned our attention to uncoupling the relationship between potency and plasma protein

Table 2. Effect of α-branch incorporation on unbound clearance in rat



	7 (R = CH_2Ph)	**8** (R = (*S*)-$CH(CH_3)Ph$)
% Free (rat)	0.20	0.35
Clearance (mL/min/kg)	3.7	0.6
Cl_{int} (μL/min/10^6 cells)	<1	<1
Unbound clearance	1850	171

binding. We have previously described the interdependence between the enzyme pEC_{50} and plasma protein binding,[18] and needed to uncouple this relationship to deliver improvements in both parameters. Our strategy was to build upon the acceptable balance of properties exhibited by **GKA22**, using iterative optimisation and the knowledge established during our DMPK studies in order to achieve further improvements in both potency and % free (Fig. 2).

We therefore synthesised a range of analogues of **GKA22**, maintaining the isopropoxy group and varying the alkyloxy side chain. We quickly found that incorporation of ether-containing side chains gave compounds

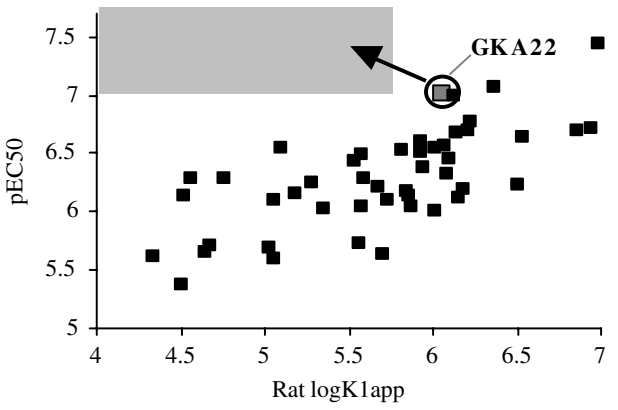


Figure 2. Strategy to uncouple the relationship between potency and plasma protein binding.

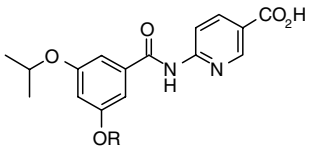
with improvements in % free (compounds **10** and **11**), but also that the (*S*)-enantiomers were consistently more potent than the corresponding (*R*)-isomers (Table 3). In particular, the (1*S*)-1-methyl-2-phenylethoxy side chain (compound **12**) demonstrated a significant improvement in potency. Given that the differences in protein binding between enantiomers were minor, it was clear that the (*S*)-enantiomers demonstrated a much better balance of potency and protein binding, as exemplified in Figure 3.

Compounds **10** and **12** were selected for further evaluation and could be considered as complementary to each other. Compound **10** was a moderately potent GK activator but exhibited excellent solubility and % free, whilst compound **12** was a potent GK activator but was less soluble and more bound to plasma proteins. Pleasingly, both compounds showed excellent DMPK properties and demonstrated significant glucose lowering in a rat OGTT model (Table 4).[23]

We therefore sought to combine the complementary profiles of compounds **10** and **12** into a single compound by combining the fragments that had delivered the advantageous properties. In other words, we attempted to hybridise the (1*S*)-1-methyl-2-phenylethoxy side chain which had delivered excellent in vitro potency, with the (1*S*)-2-methoxy-1-methylethoxy ether side chain that had delivered excellent solubility and % free. We decided to use the chiral ether side chain of compound **10** as a replacement for the isopropyl group of compound **12** and were pleased to discover that the resulting compound, **GKA50**, demonstrated an excellent balance of potency, solubility and % free. In addition, **GKA50** demonstrated excellent in vivo pharmacokinetics in both rat and dog (Table 5).

GKA50 was a clear outlier on the plot of potency against plasma protein binding (Fig. 4) and showed excellent,

Table 3. Identification of chiral side chains and stereochemical influence on potency



Compound	R	% Free (rat)	EC_{50}[22] (μM)
8	(*S*)-$CH(CH_3)Ph$	0.35	0.11
9	(*R*)-$CH(CH_3)Ph$	0.48	0.95
10	(*S*)-$CH(CH_3)CH_2OCH_3$	5.34	0.61
11	(*R*)-$CH(CH_3)CH_2OCH_3$	6.22	5.51
12	(*S*)-$CH(CH_3)CH_2Ph$	0.23	0.02
13	(*R*)-$CH(CH_3)CH_2Ph$	0.23	0.09

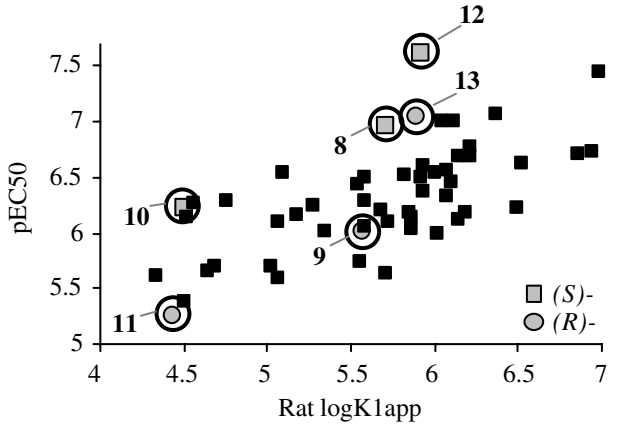
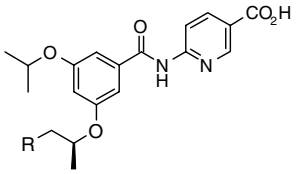


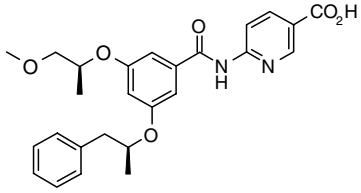
Figure 3. Identification of compound **12** with better balance of potency and protein binding.

Table 4. Complementary profiles of compounds **10** and **12**



	10 (R = CH_3O)	**12** (R = Ph)
Enzyme EC_{50} (μM)	0.61	0.02
Solubility (μM)	>3140	8
% Free (rat)	5.34	0.23
Rat clearance (mL/min/kg)	2.3	3.3
Unbound clearance (rat)	43	1435
Rat bioavailability (%)	100	85
OGTT dose	1 mg/kg	3 mg/kg

Table 5. Potency, physical properties and pharmacokinetic characteristics of **GKA50**



Enzyme EC_{50} (μM)	0.03
Solubility (μM)	1350
% Free (rat, dog and human)	0.40, 1.46, 0.44
Rat clearance (mL/min/kg)	1.9
Rat bioavailability (%)	99
Dog clearance (mL/min/kg)	4.4
Dog bioavailability (%)	100
Cl_{int}: rat, dog and human (μL/min/10^6 cells)	<1, <1, <1
Unbound clearance (rat and dog)	475, 301

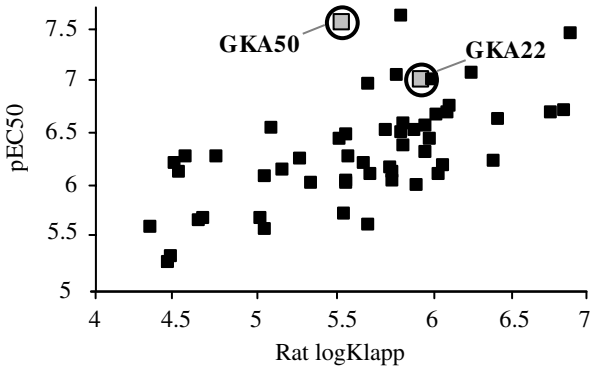


Figure 4. Balance of potency and protein binding exhibited by **GKA50**.

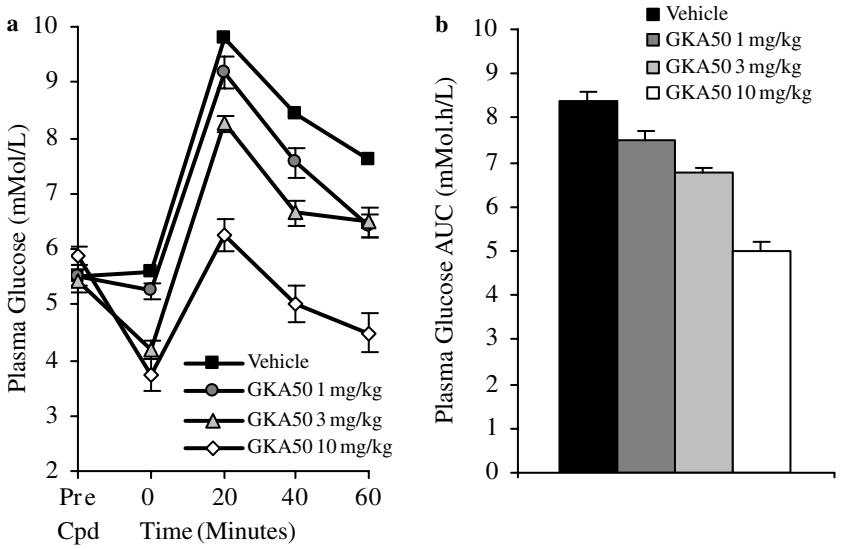


Figure 5. (a) Glucose lowering and (b) AUC reduction exhibited by **GKA50** in a Zucker rat OGTT at 10, 3 and 1 mg/kg po.

dose-dependent efficacy in the high fat fed female Zucker rat OGTT model,[23] demonstrating significant glucose lowering (Fig. 5a) and reduction in area under the glucose curve (Fig. 5b) at oral doses as low as 1 mg/kg. In terms of OGTT activity, **GKA50** is the most potent **GKA** described in the literature and, as such, was selected for further pre-clinical evaluation.

In summary, we have described the ongoing development of our series of glucokinase activators, our understanding of the key DMPK parameters and our attempts to optimise against unbound clearance. We have also described our attempts to uncouple the relationship between potency and plasma protein binding, and highlighted the identification of **GKA50** which successfully combines excellent potency with good free drug levels and solubility. **GKA50** has been shown to exhibit good pharmacokinetics in both rat and dog, and demonstrated excellent in vivo efficacy in an acute rat model.

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