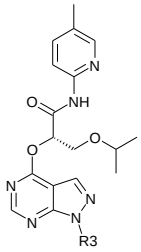
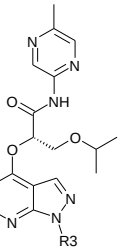
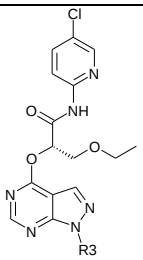
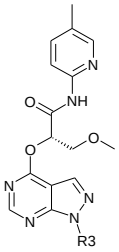
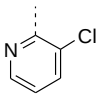
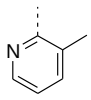
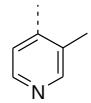
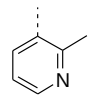
Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Matched triplicate design sets in the optimisation of glucokinase activators –maximising medicinal chemistry information content

Michael J. Waring,* Stuart N. L. Bennett, Scott Boyd, Leonie Campbell, Robert D. M. Davies, Stefan Gerhardt, David Hargreaves, Nathaniel G. Martin, Graeme R. Robb and Gary Wilkinson.

Supporting Information

Compound details and data summarised in Table 3

R3		Mean change[a]	 **10**		 **14**		 **4**
	GK pEC_{50}	Δ +0.7	6.9		7.1		6.5
	LogD	Δ +1.0	3.9		3.6		3.4
	LLE	Δ –0.7	3.0		3.5		3.1
	hERG pIC_{50}						
					15		**5**
	GK pEC_{50}	Δ 0	6.8	6.5	6.8	6.5	5.8
	LogD	Δ 0	2.9	2.1	2.6	3.0	2.3
	LLE	Δ 0	3.9	4.4	4.2	3.5	3.5
	GK pEC_{50}	Δ –0.2	6.5	6.5		6.3	
	LogD	Δ –0.3	2.6	2.3		2.7	
	LLE	Δ 0.0	3.9	4.2		3.6	
	GK pEC_{50}	Δ –0.8	6.0		5.9	5.8	
	LogD	Δ 0.0	2.9		2.5	3.1	
	LLE	Δ –0.8	3.1		3.4	2.7	
	GK pEC_{50}	Δ –1.1	5.2		5.7	6.0	
	LogD	Δ +0.3	3.2		2.8	3.3	
	LLE	Δ –1.3	2.0		2.9	2.7	
	GK pEC_{50}	Δ –0.7	6.0		6.2	6.0	
	LogD	Δ 0.0	2.9		2.5	3.0	
	LLE	Δ –0.6	3.1		3.7	3.0	

[a]Mean change refers to the average change in value across the matched triplicate set relative to 6-chloropyridin-2-yl

Synthesis and glucokinase assay

The syntheses of all compounds described in this paper and details of the glucokinase assay are detailed in: PCT Int. Appl. (2010), WO 2010/015849

ADMET assays

Protocols for generation of relevant ADMET data are described in:

D. Buttar, N. Colclough, S. Gerhardt *et al*. *Bioorg. Med. Chem.* 2010, **18**, 7486.

G. Camenisch, J. Alsenz, H. van de Waterbeemd and G. Folkers, *Eur. J. Pharm. Sci*., 1998, **6**, 313.

M. H. Bridgland-Taylor, A. C. Hargreaves, A. Easter *et al*. *J. Pharmacological and Toxicological Methods*, 2006, **54**, 189.

Data Analysis

All data analyses were carried in SAS JMP (www.jmp.com).

X-ray Crystallography

Material & Methods

Recombinant human glucokinase comprising residues 11 to 465 (triple mutant E27A E28A/E51A E52A), fused at the *N*-terminus with a six-residue HIS-tag, was expressed over night in *E. coli* at 20°C. The protein was purified by Ni-NTA affinity, and after cleavage of the His-tag with TEV protease, further by ion exchange and size exclusion chromatography. For crystallisation, the protein was concentrated to about 10 mg/mL. The protein was crystallized in the presence of 50 mM glucose and compound **11** which was added to a final concentration of 2 mM from a 100 mM stock solution in DMSO. Crystals were obtained from 10-18% Peg8000, 200 mM sodium acetate, MMT 100 mM at pH 7.0-8.5 by sitting drop vapour diffusion, appearing after 3-4 days and growing to the final size within one week. Crystals were flash-frozen in liquid nitrogen with 25% (v/v) glycerol as cryoprotectant.

Data from crystals were collected in-house on a Rigaku MicroMax-007 rotating anode X-ray generator with and a Saturn944 CCD detector using 1º oscillations. The resulting diffraction data were integrated and scaled using Dtrek[1]. Molecular replacement was successfully used to solve the structure in spacegroup P41 using AMORE[2] and 1V4S as the original search model. Model building and refinement were conducted using COOT[3] and Refmac[4] respectively. Table 1 gives a summary of the data collection and refinement statistics.

The refined coordinates have been deposited with PDB (code: 4IXC)

1. J. W. Pflugrath, *Acta Cryst.* 1999. **D55**, 1718.
2. J. Navaza, *Acta Cryst.a* 1994, **A50**, 157.
3. P. Emsley, K. Cowtan, *Acta Cryst. Section D-Biological Crystallography* 2004, 60: Iss. 1 Part 12, 2126.
4. G. N. Murshudov, A. A. Vagin, E. J. Dodson, , *Acta Cryst.* 1997, **D53**, 240.

Table 1. Crystallographic Data Collection and Refinement Statistics for the compound 11 complex.

Space Group:	P41	Cell Parameters:	77.63 Å, 77.63 Å, 85.82 Å, 90.0, 90.0, 90.0
Number Observations:	184124	Number Unique Reflections:	31632
Low Resolution:	57.75Å	Outer Shell Low Resolution:	2.07Å
High Resolution:	2.0Å	Outer Shell High Resolution:	2.00Å
Overall Redundancy:	5.82	Outer Shell Redundancy:	1.84
Overall I/Sigma:	9.9	Outer Shell I/Sigma:	1.7
Overall Completeness:	91.9	Outer Shell Completeness:	51.3
Overall R-merge[a]:	0.078	Outer Shell R-merge[a]:	0.309
Resolution range	54.8-2.0	Rwork/Rfree %	21.3/26.6
No of refined atoms	3799	Rms deviations Bonds (Å) Angles(degrees) Torsions (degrees)	 0.012 1.65 6.49
Ramachandran Preferred Regions (%) Allowed Outliers	419 (95.9) 14 (3.2) 4 (0.9)	Average B factors (A^2)	28.6

[a] R-merge = $\sum|(I_{hkl}) - \langle I\rangle|/(\sum(I_{hkl})$ where I_{hkl} is the integrated intensity of a given reflection.
[b] Rwork = $\sum_h|F_o(h) - F_c(h)| / \sum_h|F_o(h)|$ where $F_o(h)$ and $F_c(h)$ are observed and calculated structure factors.