Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Optimising pharmacokinetics of glucokinase activators with matched triplicate design sets – the discovery of AZD3651 and AZD9485

Michael J. Waring,* Stuart N. L. Bennett, Scott Boyd, Leonie Campbell, Robert D. M. Davies, David Hargreaves, Philip MacFaul, Nathaniel G. Martin, Derek J. Ogg, Graeme R. Robb, Gary Wilkinson and J. Matthew Wood.

Supporting Information

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.

pH stability half-lives are quoted at room temperature based on an extrapolation from 37 °C.

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 crystallization, the protein was concentrated to about 10 mg/mL. The protein was crystallized in the presence of 50 mM glucose and compound **14** 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 pH7.0-8.5 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 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 was 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. Subsequent 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: 4IWV)

1. J. W. Pflugrath, *Acta Cryst.* 1999. **D55**, 1718.
2. J. Navaza, *Acta Cryst.* 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 14 complex.

Space Group:	P41	Cell Parameters:	77.94 Å, 77.94 Å, 85.35 Å, 90.0, 90.0, 90.0
Number Observations:	69134	Number Unique Reflections:	28409
Low Resolution:	57.55Å	Outer Shell Low Resolution:	2.18Å
High Resolution:	2.1Å	Outer Shell High Resolution:	2.1Å
Overall Redundancy:	2.43	Outer Shell Redundancy:	2.1
Overall I/Sigma:	10.6	Outer Shell I/Sigma:	2.0
Overall Completeness:	95.2	Outer Shell Completeness:	73.4
Overall R-merge[a]:	0.052	Outer Shell R-merge[a]:	0.275
Resolution range	55.1-2.1	Rwork/Rfree	20.4/25.4
No of refined atoms	3695	Rms deviations Bonds (Å) Angles(degrees) Torsions (degrees)	 0.022 1.91 7.18
Ramachandran Preferred Regions Allowed Outliers	413 (94.5%) 20 (4.6%) 4 (0.9%)	Average B factors (A^2)	38.46

[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.