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4-Phenyl-7-azaindoles as potent, selective and bioavailable IKK2 inhibitors demonstrating good in vivo efficacy

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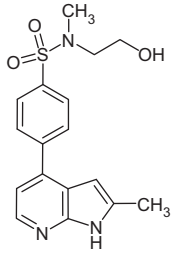
ABSTRACT

The lead optimization of a series of potent azaindole IKK2 inhibitors is described. Optimization of the human whole blood activity and selectivity over IKK1 in parallel led to the discovery of **16**, a potent and selective IKK2 inhibitor showing good efficacy in a rat model of neutrophil activation.

The IκB kinases (IKKs) are essential components of the signaling pathway by which the NF-κB p50/RelA transcription factor is activated in response to pro-inflammatory stimuli such as lipopolysaccharide (LPS) and tumor necrosis factor (TNFα).[1–4] NF-κB signaling results in the expression of numerous genes involved in innate and adaptive immune responses and the pathway is also implicated in chronic inflammatory disorders including rheumatoid arthritis (RA), chronic obstructive pulmonary disorder (COPD) and asthma. The IκB kinase complex contains two catalytic subunits, IKK1 and IKK2, and a regulatory subunit NF-κB essential modulator (NEMO).[5] Although IKK1 and IKK2 are highly homologous serine–threonine protein kinases (61% of amino acids are identical in their aligned kinase domains whereas the ATP binding sites differ by only one amino acid) and contain similar structural domains, studies in knockout mice and derived mouse embryonic fibroblasts suggest that IKK2 is the predominant kinase involved in IkB phosphorylation and hence NF-κB activation.[6,7] IKK1 deficient mice present an unexpected phenotype including skeletal and skin abnormalities.[8] Furthermore, IKK1 appears to be involved in the control of cell cycle through direct phosphorylation of Aurora A in the nucleus and may contribute to inflammatory resolution.[9,10] Therefore, selective inhibition of IKK2 activity offers a promising mechanism for intervention in a range of inflammatory diseases.[11]

Table 1
Profile of lead azaindole **1**



Assay	pIC_{50}
IKK2	7.4
IKK1	5.5
A549 Cell	6.1
LPS/TNF hWB	5.5
HSA binding	91%

pIC_{50} values are reported as mean of ⩾2 experiments.
Assay details are given in Ref. 13. IKK1 and IKK2 activity at 1 μM ATP.
HSA is human serum albumin binding.

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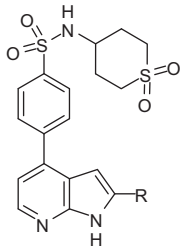
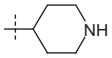
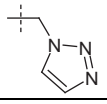
Table 2
In vitro profile of analogues **2–9**



Compd	R	IKK2 pIC_{50}	IKK1 pIC_{50}	LPS/TNFα hWB pIC_{50}
2	CH_3	7.7	7.9	6.4
3		8.3	8.2	6.4
4		8.2	8.2	6.6
5		8.4	8.2	6.6
6		8.1	7.6	6.2
7		7.7	7.0	5.9
8		7.8	7.5	6.2
9		7.8	7.3	5.9

pIC_{50} values are reported as mean of ⩾2 experiments.
Assay details are given in Ref. 13.

We recently reported our early lead optimisation efforts and preliminary SAR around a series of ATP competitive azaindole IKK2 inhibitors.[12] Compound **1** (Table 1) was disclosed as a potent and highly selective inhibitor with good cellular activity in a mechanistic cell assay (inhibition of TNFα induced NF-κB reporter activation in A549 cells).[13] Herein, we report our extended studies on this template and conclude with the discovery of a potent, selective, IKK2 inhibitor with good oral efficacy in a rat in vivo model of lung neutrophil activation.

Although **1** showed good IKK2 enzyme inhibition in the biochemical assay, approximately 100 fold reduction in activity was observed in the whole blood cellular assay (inhibition of LPS stimulated TNFα in human whole blood).[13] The drop-off in activity observed in the whole blood assay reflects a number of factors including plasma protein binding, cellular permeability and competition with endogenous concentrations of ATP. Given that **1** represents an attractive ligand efficient lead (ligand efficiency index = 4.2, log*D* 2.8, MW 345), the aim of the programme was to increase the whole blood activity and introduce adequate pharmacokinetics across preclinical species whilst maintaining the excellent kinase selectivity.[12]

Several sulphonamide analogues were prepared illustrating that excellent IKK2 enzyme inhibition can be achieved with this series of 4-phenyl-7-azaindoles as previously described.[12] However, few analogues had satisfactory whole blood activity (pIC_{50} >5.5). One of the more potent compounds discovered early in the campaign, sulphone **2** (Table 2), was more potent than **1** in the IKK2 biochemical assay and this increase in activity was mirrored with a superior whole blood activity (pIC_{50} 6.4).

Although **2** had excellent whole blood activity, the compound lacked selectivity over IKK1. The azaindole 2-position was generally tolerant of a range of substituents and hence this area was utilised in an attempt to increase IKK2 potency and introduce selectivity over IKK1. 2-Ethyl, isopropyl, cyclopropyl and *t*-butyl analogues **3–6** were more potent than **2** at IKK2, and maintained good activity in the whole blood assay, but offered no advantage in terms of selectivity over IKK1 (Table 2). The increase in IKK2 potency observed with the 2-alkyl substituent is likely due to a hydrophobic interaction within the outer lipophilic pocket of the ATP binding site.

Interestingly, the basic dimethylamino compound **7** was tolerated at IKK2 but showed modest selectivity over IKK1. The 4-piperidine analogue **8** was also potent at both IKK2 and IKK1 with micromolar activity in the whole blood assay. Several analogues containing large 2-substituents were prepared to probe the outer regions of the IKK2/1 catalytic sites in an attempt to exploit any subtle conformational differences and gain selectivity. Although potency at IKK2 was maintained along with good whole blood activity, analogues failed to show an acceptable selectivity window over IKK1. For example, triazole **9** has excellent IKK2 activity but insufficient selectivity over IKK1.

In the absence of an IKK2 protein X-ray structure, we utilised a homology model to dock potential binding modes.[14] As previously

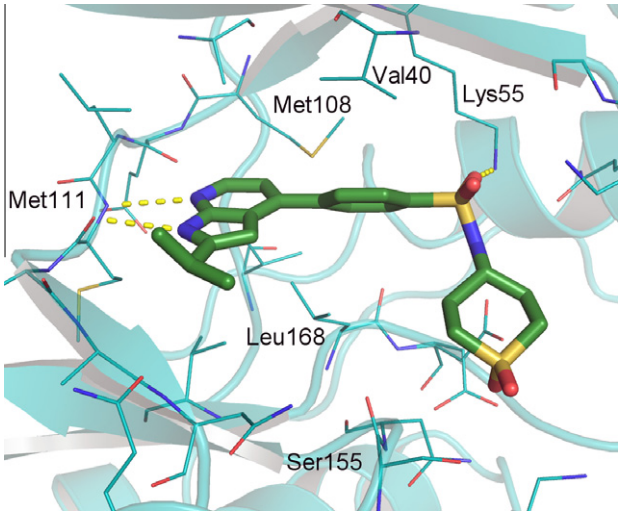
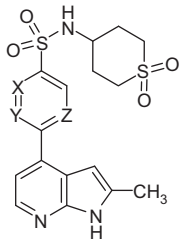


Figure 1. X-ray structure of **4** bound to JNK1, viewed from the same position as the model of IKK2 shown in Figure 1 of Ref. 12.

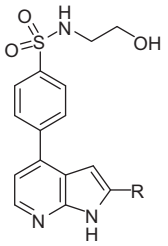
Table 3
In vitro profile of analogues **2**, **10–12**



Compd	X	Y	Z	IKK2 pIC_{50}	IKK1 pIC_{50}	LPS/TNF hWB pIC_{50}
2	CH	CH	CH	7.7	7.9	6.4
10	N	CH	CH	7.9	8.1	6.4
11	CH	N	CH	7.1	7.1	6.0
12	CH	N	N	6.1	6.8	5.5

pIC_{50} values are reported as mean of ⩾2 experiments.
Assay details are given in Ref. 13.

Table 4
In vitro profile of analogues **13–17**



Compd	R	IKK2 pIC_{50}	IKK1 pIC_{50}	LPS/TNF hWB pIC50
13	CH_3	7.7	6.5	5.6
14	CF_3	7.8	6.7	5.6
15		8.1	6.4	5.8
16		8.1	6.7	5.9
17		7.7	6.1	6.0

pIC_{50} values are reported as mean of ⩾2 experiments.
Assay details are given in Ref. 13.

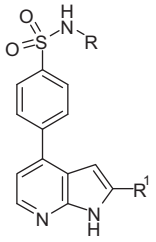
reported, the model suggests that the 7-azaindole binds at the hinge region of the ATP site with a pair of hydrogen-bonds to the backbone of Cys99, with the 2-position pointing towards the outer edge of the site. The sulfonamide substituent was predicted to lie close to the conserved lysine (Lys44).[12]

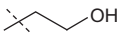
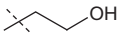
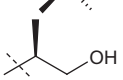
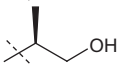
To gain confidence in this model, crystallisation was attempted with several surrogate kinases. A structure was solved with **4** bound to JNK1 (JNK1 pIC_{50} of 6.0).[15] In this structure (Fig. 1), the azaindole does indeed form the hydrogen-bonding pair of interactions with the hinge (Met111, the JNK1 equivalent of Cys99 in IKK2). The sulfonamide adopts the same orientation in the IKK2 model as in the JNK1 crystal structure, interacting with the conserved lysine (Lys55 in JNK1). The cyclic sulfone makes no specific interactions with JNK1, although one of the SO_2 oxygens does lie within 3.0 Å of the sidechain oxygen of Ser155. In IKK2, the Ser residue is replaced by the glutamic acid Glu149, therefore no direct hydrogen-bonding interaction is likely. The lack of any obvious favourable IKK2 interactions is consistent with the relatively flat SAR observed with the sulphonamide moiety.

Introduction of heteroatoms into the central aryl ring did not improve IKK2 potency or selectivity (Table 3). The pyridyl compound **10** maintained good activities at both IKK2 and IKK1. The southern pyridyl isomer **11** lost activity at both IKK2 and IKK1 and was essentially equipotent at both isoforms. The pyrimidyl compound **12** was a weak IKK2 inhibitor (pIC_{50} 6.1) and showed a marginal preference for IKK1. These observations probably result from a combination of factors. The first is that polar nitrogen atoms are disfavoured at the southern position because of the proximity to the hydrophobic sidechains of Val29, Met96, and Ile165 in IKK2 (equivalent to Val40, Met108 and Leu168 in JNK1, Fig. 1). The second is that nitrogen atoms adjacent to the bi-aryl bond would favour a more planar torsion angle than 30°, which was observed in the JNK1 X-ray complex.

Although the initial hydroxyethyl sulphonamides **1** and **13** had moderate whole blood activity, they were the most selective over the IKK1 isoform. The reasons for this are unclear, since all of the residues in the ATP site of IKK2 and IKK1 are identical, with the exception of one position located next to the hinge (Gln100 in IKK2, Ser99 in IKK1). However, this points away from the binding site. Any selectivity must be due to conformational effects transmitted to the binding site from the outer region. The SAR observed at the azaindole 2-position of the 2-hydroxyethyl sulphonamides (Table 4) was similar to that observed with the sulphones in Table

Table 5
In vitro profile and rat PK profile of compounds **15**, **16** and **18–21**



Compd	R	R^1	IKK1 pIC_{50}	IKK2 pIC_{50}	LPS/TNF hWB pIC_{50}	*c*Log*P*	Rat PK summary		
							IV Cl mL/min/kg	IV $t_{1/2}$ (h)	*F*%
15			8.1	6.4	5.8	2.9	39	0.45	18
16			8.1	6.7	5.9	2.4	13	0.6	49
18			8.2	6.5	6.0	3.1	14	0.7	100
19		CH_3	8.0	6.7	6.0	2.7	70	0.6	32
20			8.1	6.6	6.3	2.7	15	0.9	29
21		CF_3	7.7	6.5	5.9	2.8	7	3.2	83

pIC_{50} values are reported as mean of ⩾2 experiments. Assay details are given in Ref. 13.
IV administration (n = 2) 1 mg/kg. Oral administration (n = 1) 1 mg/Kg. Cl is the in vivo clearance from IV administration.

Table 6
In vitro profile and physicochemical properties of **16**

Parameter	Result
IKK2/IKK1 (pIC_{50})	8.1/6.7
A549 cell (pIC_{50})	6.7
Whole blood LPS-TNFα (pIC_{50})	5.9 (human), 5.2 (rat)
MW/clog *P*	357/2.4
CYP450 (IC_{50})	7 μM (2C9), >10 μM (3A4, 2D6, 1A2)
Intrinsic clearance (hepatocytes)	<1.0 (human), <1.0 (rat) (mL/min/g)
hERG (pIC_{50})	<4.3
Plasma protein binding (%)	97.6 (rat), 93.4 (human)
Aqueous solubility	64 μM

Assay details are given in Ref. 13.

2. The trifluoromethyl analogue **14** was essentially equipotent to **13** in the biochemical and whole blood assay. Isopropyl and cyclopropyl derivatives **15** and **16** showed an increase in IKK2 potency with a modest improvement in whole blood activity. The t-butyl analogue **17** was equipotent to **13** but again marginally more active in the whole blood assay.

Compounds **15** and **16** had favourable physicochemical properties, good selectivity and whole blood potency and hence were profiled in a rat pharmacokinetic study alongside several close analogues listed in Table 5. Interestingly, the 2-substituent generally had a significant effect on the rat in vivo clearance and hence on oral bioavailability. The 2-methyl and isopropyl analogues generally had higher clearance than the 2-cyclopropyl and 2-CF_3 analogues; the isopropyl analogue **15** had moderate clearance (~50% liver blood flow) and poor bioavailability of 18%. The corresponding cyclopropyl compound **16**, which is of similar lipophilicity to **15**, had lower rat in vivo clearance and good oral bioavailability of 49%. Both **15** and **16** had a low volume of distribution (0.8 and 0.6 L/Kg, respectively) leading to a short half life of less than one hour. The high in vivo clearance consistently observed with the 2-methyl and 2-isopropyl analogues may reflect a greater propensity for oxidation on the pseudo-benzylic CH and hence phase I metabolism.

Methyl substitution was tolerated on the hydroxyethyl chain but had marginal affect on the biochemical and cellular activity of the series. Good rat pharmacokinetic profiles were obtained

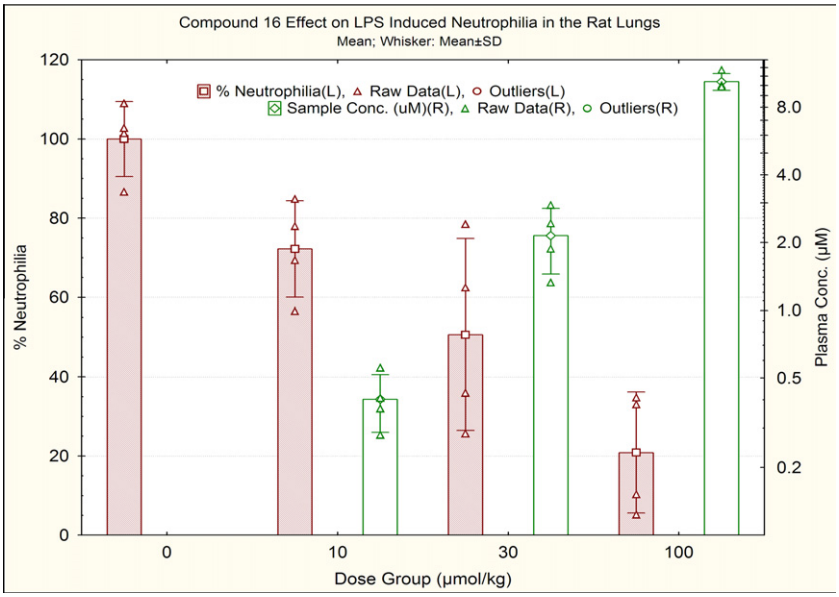
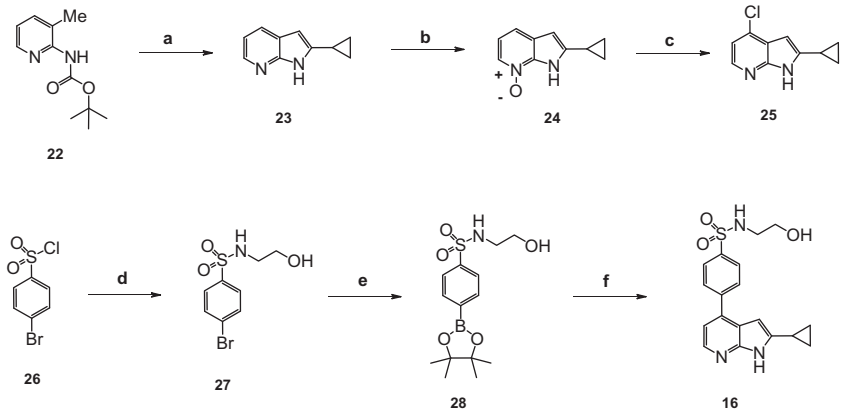


Figure 2. Dose dependent inhibition of neutrophil infiltration in rat lungs with compound **16**.



Scheme 1. Reagents and conditions: (a) *N*-Methoxy-*N*-methylcyclopropanecarboxamide, *n*BuLi, THF, −5 °C, 1 h then 5 M HCl, 50 °C, 18 h, 86%. (b) *m*-Chloroperoxybenzoic acid, DME, 0–20 °C, 7 h, 58%. (c) Methanesulphonyl chloride, DMF, 60 °C, 2 h, 50%. (d) Ethanolamine, triethylamine, DCM, 0 °C, 3 h then 20 °C, 16 h, 81%. (e) 4,4,4′,4′,5,5,5′,5′-octamethyl-2-2′-bi-1,3,2-dioxaborolane, palladium acetate, potassium acetate, DMF, 55 °C, 4 h, 77%. (f) **25**, potassium phosphate, 2′-(dimethylamino)-2-biphenyl-palladium(II) chloride dinorbonylphosphine, dioxane, water, 100 °C, 1 h, 60%.

with several analogues. For example, the lipophilic (*clog P* 3.1) gem-dimethyl analogue **18** maintained low rat in vivo clearance (14 mL/min/kg), typical of the majority of 2-cyclopropyl analogues, and a high bioavailability of approximately 100%. The corresponding 2-methyl analogue **19** had high in vivo clearance (70 mL/min/kg), and a moderate bioavailability of 32%. The homochiral analogues **20** and **21** also had attractive in vitro profiles. However, **20** had poor oral bioavailability of 29% in the rat. **21** had excellent bioavailability of 83% in the rat when dosed at 1 mg/kg but suffered from poor aqueous solubility compared to other analogues. Such low solubility is likely to preclude achieving sufficiently high systemic concentrations required for in vivo toxicity evaluation.

Compound **16** had the preferred overall profile including low molecular weight (357), optimal log *P* (2.4), good solubility and a free fraction of approximately 7% in human blood (Table 6). Furthermore, **16** had an acceptable P450 profile (2C9 IC_{50} = 7 μM, other isoforms IC_{50} >10 μM) with no evidence of time dependent inhibition at CYP3A4 and 2D6. Importantly, **16** had low intrinsic clearance when incubated with human hepatocytes.

Compound **16** also had excellent kinase selectivity. When screened against over 60 in-house kinases (including ROCK, JNK1, Aurora A and B), only IKK1 inhibition was within 30 fold of IKK2 inhibition (fold selectivity based on comparative IC_{50} values).

The in vivo efficacy of **16** was evaluated in a rat model of neutrophil activation, a standard animal model of inflammation. **16** was dosed orally at 10, 30 and 100 μmol/kg to male rats 30 min prior to the rats being exposed to aeorosolised LPS.[16] **16** was found to inhibit neutrophil lung infiltration in a dose dependent manner giving approximately 50% reduction with a corresponding plasma concentration of approximately 2 μM (Fig. 2). At 30 μmol/kg po (equivalent to approximately 11 mg/kg), there was a significant reduction in neutrophil infiltration as compared to those animals receiving the vehicle.[17] This data is consistent with the potency value obtained using the rat LPS/TNFα cellular assay (pIC_{50} = 5.2)

The compounds described in this paper were prepared by previously reported chemistry.[13] Analogues were generally prepared via a divergent route involving a final Suzuki reaction coupling the azaindole core to the phenylsulphonamide moiety. The synthetic route to **16** is shown in Scheme 1. An initial lithiation of a Boc-protected 2-amino-3-methyl pyridine and condensation with *N*-methoxy-*N*-methylcyclopropanecarboxamide furnished the cyclopropyl-azaindole **23**. Oxidation of the azaindole followed by addition of methane sulphonyl chloride yielded the 4-chloroazaindole **25**. Routine chemistry furnished the borane ester **28** and subsequent Suzuki reaction gave **16** in 60% yield.

In summary, we have presented the lead optimisation of a series of potent and selective azaindole IKK2 inhibitors. The medicinal chemistry strategy was focussed on maintaining the excellent physicochemical properties of the lead **1** whilst optimising the whole blood activity and rodent pharmacokinetics; this led to the discovery of **16** which had an excellent physicochemical and in vitro profile. Furthermore, **16** had good rat pharmacokinetics and demonstrated in vivo efficacy in a rat model of neutrophil activation.

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13. The IKK2 enzyme assay, NF-κB cellular mechanistic assay, human whole blood assay and azaindole syntheses are described in WO2008/034860.
14. A homology model of IKK2 was built as previously described: Christopher, J. A.; Avitabile, B. G.; Bamborough, P.; Champigny, A. C.; Cutler, G. J.; Dyos, S. L.; Grace, K. G.; Kerns, J. K.; Kitson, J. D.; Mellor, G. W.; Morey, J. V.; Morse, M. A.; O'Malley, C. F.; Patel, C. B.; Probst, N.; Rumsey, W.; Smith, C. A.; Wilson, M. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3972.
15. *E. coli* expressed GST-tagged truncated human Jnk1alpha (1-364) was purified to homogeneity as detailed in *Bioorg. Med. Chem. Lett.* **2009**, *19*, 360. The protein–ligand complex, concentrated to between ~8–11 mg/ml in 50 mM Tris pH 7.6, 150 mM NaCl, 10 mM DTT, was crystallized against a well solution consisting of 0.05 M ammonium sulfate, 0.05 bis-Tris pH 6.5, 30% v/v Pentaerythritol (15/4 EO/OH). Crystals were improved by use of seeding. Data from a single frozen crystal was collected at the European Synchrotron Radiation Facility, Grenoble and processed using MOSFLM and scaled using SCALA to give a 1.90 Å dataset. The P212121 cell (a = 50.438 Å, b = 72.747 Å, c = 110.155 Å, $a = b = g = 90°$) has a single molecule in the ASU. After rigid body refinement using a previously determined in house structure, model building was performed using Coot and refined using REFMAC. There was clear difference density for the ligand in the ATP binding site and the binding mode could be unambiguously determined. The final model (R/Rfree = 18.8/21.9%) has been deposited in the protein databank under the accession codes 4awi.pdb.
16. Compound **16** was dissolved in 2% DMSO with 0.5% methylcellulose for a final concentration of 10 μmol/kg (3.57 mg/ml). 200 μl 1 N NaOH was added to assist in solubilisation. Dilutions were made with 0.5% methylcellulose to obtain concentrations of 3 and 1 μmol/ml. Male Lewis rats were dosed orally at a volume of 10 ml/kg 30 min prior to LPS aerosol. Final doses were 10, 30 and 100 μmol/kg. Each group, including vehicle, was n = 4. Thirty minutes following the compound dose, the rats were exposed to aerosolized 0.1 mg/ml LPS solution from *E. coli*, serotype 026:B6 (Sigma, St. Louis) at a rate of 4.5 L/min for 20 min. At 4 h-post LPS exposure the rats were euthanized by Fatal Plus i.p. (100 mg/kg). Bronchoalveolar lavage (BAL) was performed through a 14 gauge blunt needle inserted into the exposed trachea in five, 5 ml washes of Dulbecco's phosphate buffered saline (DPBS) to collect a total of 20–23 ml of BAL fluid. Blood was taken via cardiac puncture for DMPK analysis. BAL fluid was centrifuged at 1000–1500 rpm for 10 min. Supernatant was aspirated and discarded. Cell pellet was resuspended in 5 ml PBS. An aliquot of 70–100 μl was centrifuged on a Shandon cytospin at 300 rpm for 5 min. Slides were stained with Diff-Quick (a Wright-Giemsa stain) for differential cell counts. Total cell counts were performed with a haemocytometer. Data were calculated as % inhibition of each dose as compared to vehicle-treated animals and and ED50 was calculated of 28.2 μmol/kg (10.1 mg/kg). Plasma concentrations at 4.5 h post-compound administration were determined.
17. Statistical analysis using Anova followed by Post Hoc Dunnett's test confirmed that the 30 μmol/kg dose led to significant inhibition compared to control.
The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.
All animal studies were ethically reviewed and carried out in accordance with animals (scientific procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.