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The discovery of UK-369003, a novel PDE5 inhibitor with the potential for oral bioavailability and dose-proportional pharmacokinetics

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ABSTRACT

This paper describes our recent efforts to design and synthesise potent and selective PDE5 inhibitors and the use of in vitro predictors of clearance, absorption and permeability to maximise the potential for dose-proportional pharmacokinetics and good oral bioavailability in man. Optimisation of the preclinical profile resulted in the identification of UK-369003 (**19a**) and its nomination as a clinical candidate. The clinical pharmacokinetic and safety profile has enabled us to progress the compound to test its efficacy in patients with lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH) and a paper describing its efficacy has recently been published.[2,3]

1. Introduction

The launch of Viagra® (Sildenafil citrate, **1**) in 1998 as the first oral treatment of male erectile dysfunction (MED) generated a huge amount of interest in the potential of PDE5 inhibitors to treat MED and other diseases associated with lowering cGMP levels. Kidney, heart and lung are all tissues rich in PDE5 and PDE5 inhibitors have been used extensively in clinical trials to assess their potential to treat diseases associated with vascular tone in these organs.[1] Revatio® was successfully launched for the treatment of pulmonary hypertension in 2005.

2. Chemistry

UK-343664 (**2**) (Fig. 1) was designed as a follow-up to Sildenafil with improved selectivity for PDE5 over PDE6 and was progressed into development. It was shown to have dose-dependent pharmacokinetics in man with supraproportional increases in both AUC and C_{max} with increasing oral dose. The high affinity of UK-343664 for P-glycoprotein was considered to be the main source of the non-proportional pharmacokinetic profile observed in man.[4]

The aim of the current work was to maintain the PDE selectivity profile of UK-343664 whilst maximising the potential for oral bioavailability and dose-proportional pharmacokinetics through minimisation of Pgp affinity and optimisation of physicochemical parameters (such as molecular weight, lipophilicity, solubility and H-bonding capacity).[5] We have been able to explore these changes through modulation of substituents at the N2, C3 and 2′-positions

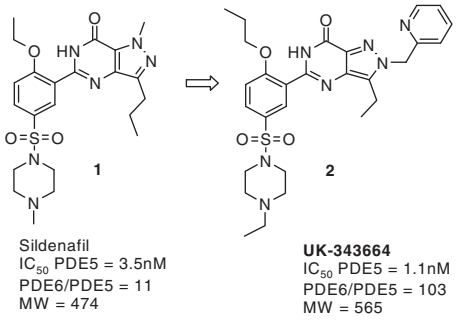

Figure 1.

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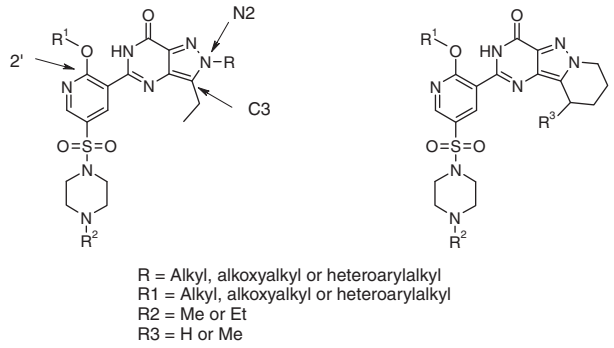


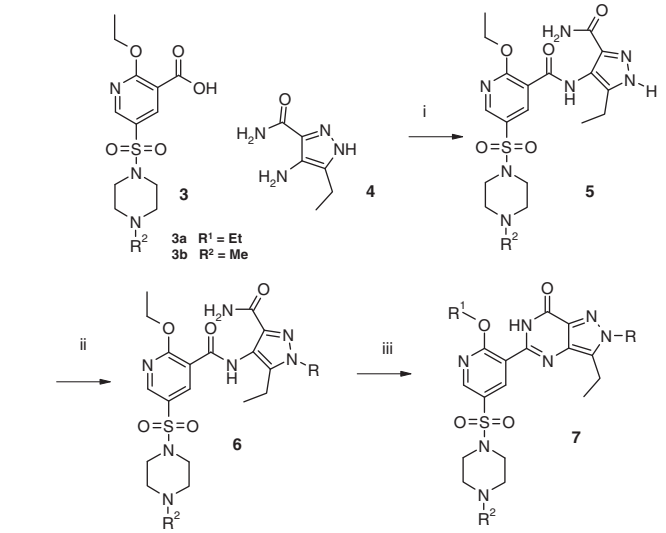
Figure 2.

(see Fig. 2). This has enabled us to discover novel compounds with a significantly improved balance of potency, selectivity, metabolic stability and oral absorption characteristics commensurate with progression to clinical development.

The two classes of compounds which we are described in this paper are shown in Figure 2.

The synthetic route described in Scheme 1 proved to be a versatile and regioselective route to N2 alkylated compounds (**7**).

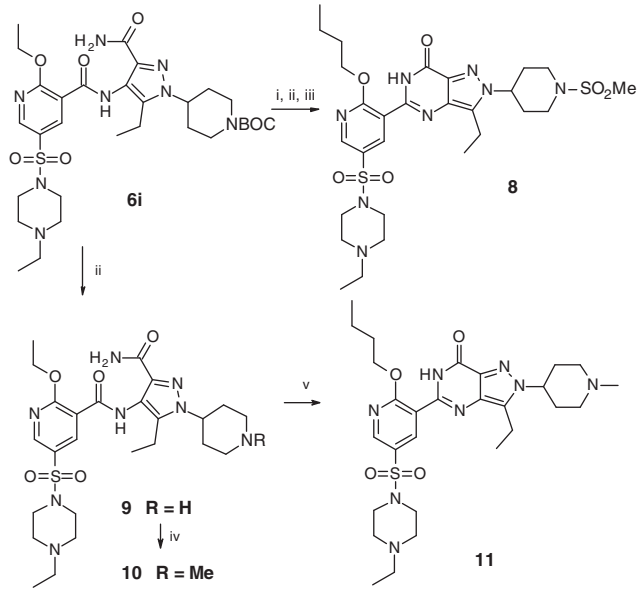
Acid **3** was coupled with aminocarboxamide **4**[6] using standard procedures (either addition of the acid chloride of **3** to amine **4** or via carbodiimide-mediated coupling). A completely regioselective N2 alkylation of **5** could be achieved using an appropriate alkyl-, alkoxyalky- or aminoalkyl-mesylate or chloride to give **6a–6k**. This was followed by a one-pot cyclisation/displacement procedure which facilitated conversion of **6** directly into **7** through use of the appropriate alcoholic solvent (R^1OH) and 4 equiv KHMDS at reflux which resulted in pyrimidinone formation with



R^2 = Et	
7a	R= nPr, R^1= -$(CH_2)_2$OMe
7b	R= iPr, R^1= -$(CH_2)_2$OMe
7c	R= cBu, R^1= -$(CH_2)_2$OMe
7d	R= iBu, R^1= -$(CH_2)_2$OMe
7e	R= cPrCH_2, R^1= -$(CH_2)_2$OMe
7f	R= 4-tetrahydropyran, R^1= -$(CH_2)_2$OMe
7g	R= -$(CH_2)_2NMe_2$, R^1=iBu
7h	R= -$(CH_2)_3NMe_2$, R^1=nBu
7i	R= 4-N-BOCpiperidine, R^1=nBu

R^2 = Me	
7j	R = nPr, R^1 = -$(CH_2)_2$OMe
7k	R = iBu, R^1 = -$(CH_2)_2$OMe

Scheme 1. Reagents and conditions: (i) 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, hydroxybenzotriazole, di-isopropylethylamine, THF, 20 °C, 20 h; (ii) caesium carbonate, alkyl mesylate or alkyl chloride, DMF, 20 °C, 20 h; (iii) KHMDS, R^1OH, 120 °C, 20 h.



Scheme 2. Reagents and conditions: (i) KHMDS, *n*BuOH, 120–130 °C, pressure vessel (ii) TFA, CH_2Cl_2; (iii) methanesulphonyl chloride, NEt_3, CH_2Cl_2; (iv) HOAc, $NaCNBH_3$, CH_2O (v) KHMDS, *n*BuOH, reflux.

concomitant alkoxypyridyl exchange (**7a–7k**). The regiochemistry of the initial alkylation was proved by correlation with compounds made using Scheme 3 (vide infra).

Cyclisation followed by deprotection of **6j** with TFA and sulphonylation furnished **8**. The methylpiperidine analogue (**11**) was formed by deprotection of **6j** with TFA and reductive methylation prior to the cyclisation (Scheme 2).

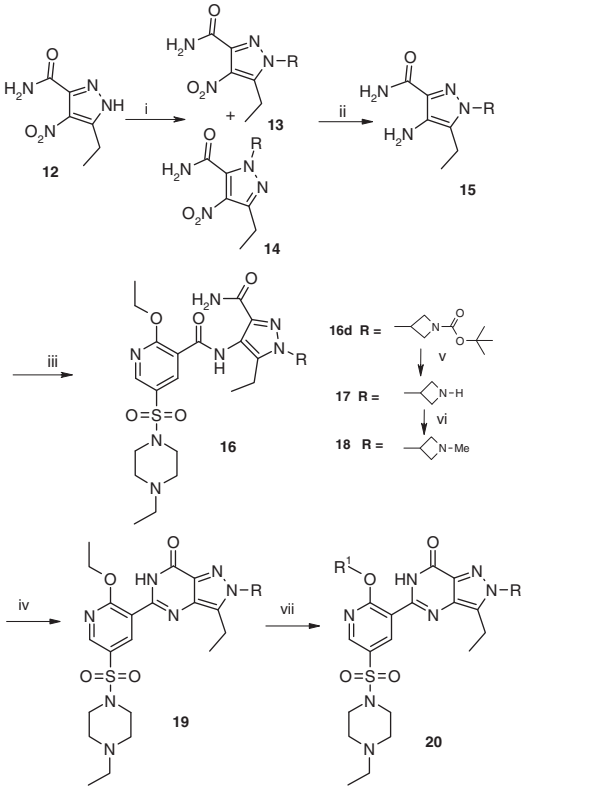
An alternative route to the compounds described in Scheme 1 is shown in Scheme 3. Alkylation of **12** resulted in a separable mixture of regioisomers, **13** and **14**.[7] The desired N2 isomer **15** was made through hydrogenation of **13** and used in the synthesis of **19a–d** and **20a–i**.

For the novel fused tricyclic inhibitors (**26** and **27** in Scheme 4) the key step was formation of the pyrazole ring from the intermediate **22**.[8] N-nitrosolation of pipecolinic acid (**21a**) (or 3-methylpipecolinic acid (**21b**)) using nitrous acid, followed by reaction of the intermediate with TFAA in ether, gave the betaine **22**. A mixture of isomeric pyrazoles was obtained on heating the betaine in xylene in the presence of ethylpropiolate which were readily separated by chromatography. Nitration and concomitant ester hydrolysis of **23** followed by amide formation and nitro reduction furnished **24**, which was converted to **26** and **27** by the methods described in Schemes 1 and 2. Preparative chiral HPLC was used to separate enantiomeric products.[9]

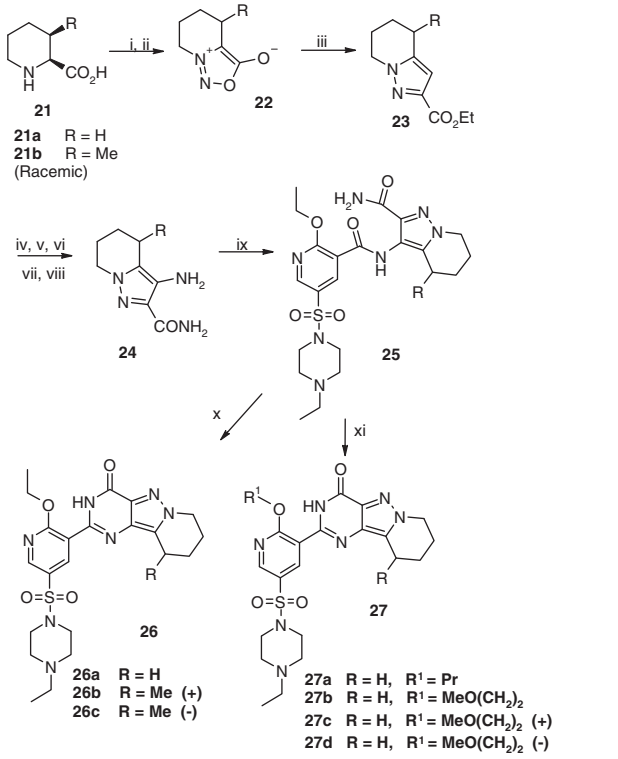
3. Results and discussion

Phosphodiesterase inhibitory activity was assessed on human corpus cavernosum-derived PDE5 with IC_{50} values determined from concentration–response curves using concentrations from 1 nM to 10 μM. Our primary PDE selectivity assay was IC_{50} against $PDE6_{cone}$ derived from dog retina. $PDE6_{cone}$ was chosen for the primary selectivity screen as it has been associated with the low incidence of adverse visual effects associated with high doses of Sildenafil.[10]

We have previously described how improved selectivity for PDE5/6 could be achieved through introduction of a 2′-methoxyethyloxy pyridyl and large N2 pyrazole motifs (**2**). The series of compounds shown in Table 1 combine the 2′-methoxyethoxy functionality with smaller N2 substituents targeting compounds with a



Scheme 3. Reagents and conditions: (i) caesium carbonate, RCl, DMF; (ii) 50 psi H_2, 10% Pd/C (iii) 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, HOBT, di-isopropylethylamine, THF, 20 °C, 20 h; (iv) KHMDS, ethanol, 120 °C, pressure vessel; (v) TFA, CH_2Cl_2; (vi) CH_2O, HOAc, $NaCNBH_3$; (vii) R^1OH, KHMDS, 120 °C.



Scheme 4. Reagents and conditions: (i) $NaNO_2$, HCl, H_2O; (ii) TFAA, Et_2O; (iii) ethyl propynoate, xylene, reflux, 2 h; (iv) NaOH, H_2O, dioxan; (v) HNO_3/H_2SO_4, 40–55 °C; (vi) $(COCl)_2$, CH_2Cl_2, DMF; (vii) NH_3, THF; (viii) 10% Pd/C, EtOH, 60 psi H_2, 20 °C, 14 h; (ix) acid chloride of **3**, NEt_3, CH_2Cl_2; (x) KHMDS, EtOH, 130 °C, 14 h, pressure vessel; (xi) methoxyethanol, KHMDS, reflux, 14 h.

log*D* of 1–3 to maximise the potential for metabolic stability and oral absorption. Replacement of the pyridyl group of **2** with alkyl groups of varying lengths maintained good PDE5 potency and selectivity over PDE6.

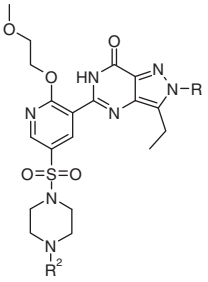
Replacement of the N2 pyridyl substituent of **2** with a methoxyethyl substituent enabled us to explore substituents at the R^1 position with increased lipophilicity whilst maintaining our desired lipophilicity range (Table 2).

Basic functionalities were also tolerated at the N2 position (Table 3) with alkyloxy groups required at the 2′ position to achieve the levels of lipophilicity required for oral absorption. Excellent levels of PDE5 potency could be achieved and PDE5/6 selectivity maintained for the more lipophilic analogues.

A small series of inhibitors was synthesised by cyclising the N2 alkyl and C-3 substituents to form a third ring appended to the pyrazolopyrimidinone system (Table 4). A methyl group was introduced into the fused ring (R^2) to improve PDE5 potency by accessing the lipophilic pocket exploited by Sildenafil.[11] Significant improvements in PDE5 potency were observed (**26b** and **26c, 27c** and **27d**) with **27d** showing good PDE5 potency combined with improved PDE5/6 selectivity.

The compounds shown in Tables 1–4 demonstrate that both the R and R^1 positions on the pyrazolopyrimidinone template are very tolerant to a wide range of substituents, with excellent levels of

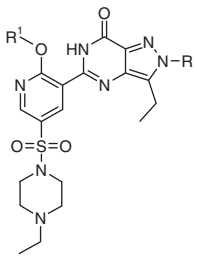
Table 1



Example	R	R^2	MW	IC_{50} PDE5 (nM)	PDE5/6 selectivity
7a	^{n}Pr	Et	534	1.83	170
7b	^{i}Pr	Et	534	1.36	354
7c	^{c}Bu	Et	544	1.76	219
7d	^{i}Bu	Et	548	1.04	331
7e		Et	546	1.01	189
7f		Et	576	1.66	241
7i	^{n}Pr	Me	520	4.35	165
7j	^{i}Bu	Me	534	2.89	237
2		Et	597	1.1	842

PDE5 potency and PDE5/6 selectivity over a narrow lipophilicity range. The selectivity and MW of the compounds described have been plotted in Figure 3 and a general trend for increased selectivity with higher MW can be seen. The desired 'target zone' to max-

Table 2



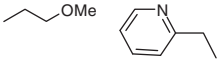
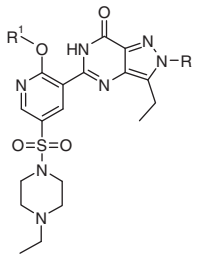
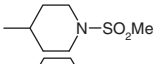
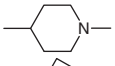
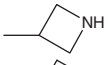
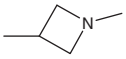
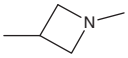
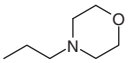
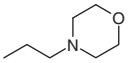
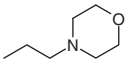
Example	R	R^1	MW	IC_{50} PDE5 (nM)	PDE5/6 selectivity
20a		^{n}Pr	534	0.69	51
20b		^{i}Bu	546	0.69	204
20c			583	0.75	309
20d		MeO	550	1.53	306
19a		Et	534	1.23	117

Table 3



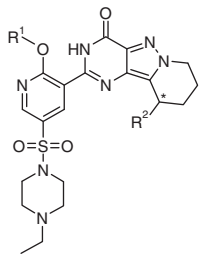
Example	R	R^1	MW	IC_{50} PDE5 (nM)	PDE5/6 selectivity
7g	$-(CH_2)_2NMe_2$	^{n}Bu	561	1.00	171
7h	$-(CH_2)_3NMe_2$	^{i}Bu	575	2.44	404
8		^{n}Bu	651	0.31	100
11		^{n}Bu	587	0.74	184
17		Et	517	15.8	28
18		Et	531	8.2	52
20h		^{n}Bu	559	0.78	209
19b		Et	575	0.86	184
20e		^{c}PrCH$_2$	601	0.26	153
20f		MeO	605	1.27	210

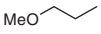
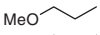
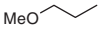
imise the potential for dose-proportional pharmacokinetics (PK) and minimise the incidence of visual side-effects has been shaded.

Further in vitro tests were required to differentiate between inhibitors and select compounds for further development.

The oral bioavailability of most drugs is determined by solubility, permeability across the intestinal wall and hepatic first pass extraction.[12] As solubility is not limiting in this series we have made extensive use of in vitro models of absorption and first pass extraction to select compounds for progression with potential for oral bioavailability.

Table 4



Example	R^2	R^1	MW	IC_{50} PDE5 (nM)	PDE5/6 selectivity
26a	H	Et	488	10.8	70
27a	H	^{n}Pr	502	2.47	55
27b	H		518	8.5	392
26b	Me	Et	502	3.35	125
26c	Me	Et	502	3.70	55
27c	Me		532	2.76	149
27d	Me		532	1.96	490

With the predominant mode of clearance being hepatic P450-mediated metabolic oxidation of the piperazine ring and N-2 position by CYP3A4, we have used human liver microsome incubations as a surrogate for first-pass metabolism.

Caco-2 monolayers have been used extensively as a high throughput screen to assess human intestinal absorption potential and have been very useful in predicting potential for good bioavailability in man from an in vitro system.[13] A high absorptive flux (Papp > 10×10^{-6} cm/s)[14] and an efflux ratio close to unity across the concentration range 10–100 μM increases the potential for concentration independent absorption in man.[4]

Using a panel of in vitro screens we have been able to screen many compounds and progress only those which combine PDE5 potency and PDE5/6 selectivity with good stability in human liver microsomes ($T_{1/2}$ >20 min), good Caco-2 flux (Papps >10×10^{-6} cm/s) and low efflux ratio (A–B/B–A <2) to further PK testing in rat and dog. Tables 5 and 6 summarise some of the SAR trends. A decrease in metabolic stability (associated with increased CYP turnover) and an increase in membrane permeability are both associated with increased lipophilicity as would be expected for compounds metabolised through CYPs.

Within a relatively narrow log$D$ range (1.1–2.9) clear differentiation between similar analogues has been achieved facilitating effective selection of inhibitors to be taken forward and **19a** and **27d** were progressed to rat and dog PK studies.

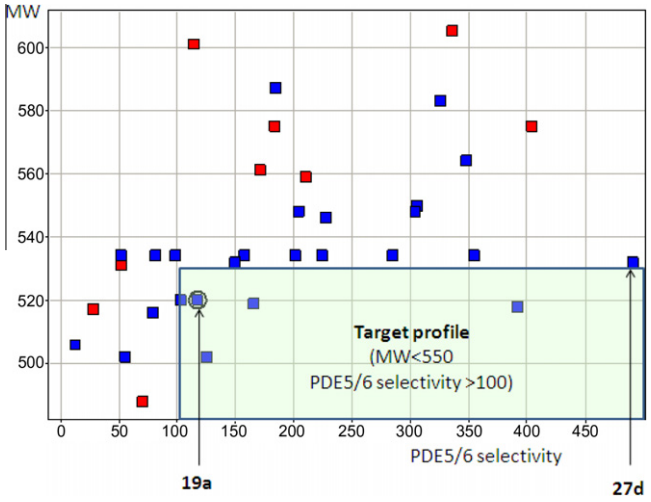
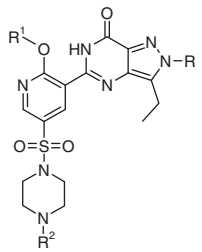
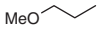
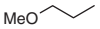
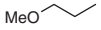
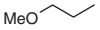
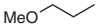
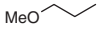
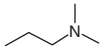
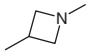
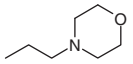
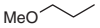
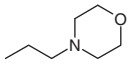


Figure 3. Effect of MW on PDE5/6 selectivity. Red spots represent the dibasic examples in Table 3. Blue spots are the monobasic examples in Tables 1–4.

Table 5



Example	R	R^1	R^2	Log*D* (pH7.4)	HLM[a] (min)	Caco-2[b] *P*app × 10^6	A-B/B-A efflux ratio[c]
20d			Et	1.4	70	9	4.2
19a		Et	Et	2.1	28	16	1.5
7j	iBu		Me	2.3	28	19	2.2
7d	iBu		Et	2.8	11	24	2.5
20b		^{i}Bu	Et	2.9	7	40	1.1
7g		^{n}Bu	Et	3	—	2.8	7
20h		^{n}Bu	Et	2.8	74	6	4
20f			Et	1.1	48	2.8	8.0
19b		Et	Et	1.6	18	2.5	11.2

[a] Half-life of parent compound upon incubation with human liver microsomes.
[b] Apical to basolateral flux across Caco-2 cell monolayer at 25 μM.
[c] Ratio of flux in the apical:basalateral direction to flux in the basolateral:apical direction.

Table 6
In vitro parameters for **27d**

Example	Log*D* (pH 7.4)	HLM (min)	Caco-2 *P*app × 10^6	A-B/B-A efflux ratio
27d	1.6	11.2	24	1.5

Table 7
Pharmacokinetic parameters for **19a** and **27d**

Species	Rat **19a**	Dog **19a**	Dog **27d**
Dose (mg/kg)	1.0 i.v., 2.0 p.o.	0.5 i.v., 1.0 p.o.	0.5 i.v., 1.0 p.o.
i.v. Clearance (ml/min/kg)	26.6	16.0	20.0
V_d (L/kg)	2.2	2.8	2.6
Elimination T1/2 (h)	0.95	2.1	1.5
Oral bioavailability (%)	61	55	18

The PK data for both **19a** and **27d** show clearance values of approximately half hepatic blood flow. The oral bioavailability of **19a** is close to 50%, indicating that the compound is essentially completely absorbed from the g.i. tract. This would be expected from the aqueous solubility (100 mg/ml @ pH 6.7, >14 mg/ml in 0.2 M lactate buffer @pH 4.3) and the Caco-2 cell flux data (Table 7).

The improved bioavailability and reduced synthetic complexity of **19a** compared to **27d** clearly differentiated the two lead compounds **19a** has been progressed to clinical development as a selective, low dose, orally active agent for the treatment of diseases associated with PDE5-modulated levels of cGMP. The PK parameters of **19a** in man are summarised in Table 8 with both rat and dog predicting well for both clearance and volume of distribution. The compound was also well tolerated in healthy volunteers. The preclinical and Phase 1 profile of **19a** encouraged us to progress to further clinical efficacy studies.

Table 8
Pharmacokinetic parameters of **19a** in man

Dose (mg/kg)	0.43 (*iv*), 1.43 (oral)
Clearance (ml/min/kg)	12.8 ± 2.0
V_d (L/kg) (*iv*)	2.8 ± 0.05
Elimination $T_{1/2}$ (h) (*iv*)	2.6 ± 0.43
C_{max} (ng/ml)(oral)	204.8 ± 117.5
T_{max} (h) (oral)	1.0 ± 0.54
AUC (ng h/ml)(oral)	95.2
Oral bioavailability (%)	34 ± 10.6

4. Conclusions

In summary, we report the discovery of several PDE5 inhibitors which show excellent in vitro potency for corpus cavernosum derived PDE5 and selectivity for dog retinal $PDE6_{cone}$. High throughput in vitro assays have been used to optimise bioavailability through determination of oral absorption potential and metabolic stability. Based on in vitro data collected, **19a** and **27d** were selected for progression to dog pharmacokinetics and, based on the results from these studies, **19a** was progressed further to clinical studies.

A paper describing the efficacy of UK-369003 (**19a**) in patients with lower urinary tract symptoms associated with clinical benign prostatic hyperplasia has already been published[3] and a full paper describing the dose dependence of the PK and a full analysis of the metabolites of UK-369003 in man is in preparation.

5. Experimental

^{1}H NMR spectra in deuterochloroform ($CDCl_3$) or dimethyl sulphoxide (DMSO-d_6) were recorded on a Varian Unity Inova-300, a Varian Unity Inova-400, or a Varian Mercury-400 spectrometer. Room temperature means 20–25 °C. Unless otherwise stated,

all reactions were carried out using commercially available anhydrous solvents. Flash column chromatography refers to column chromatography on silica gel (Kieselgel 60, 230–400 mesh). Thin-layer chromatography was performed on either precoated Merck silica gel (60 F254) plates or Macherey–Nagel Polygram Si G/UV (0.2 mm silica gel) plates. Mass spectra were recorded using a Fisons Instruments Trio mass spectrometer in the thermospray ionization mode (TSP) or using a Finnigan navigator with electrospray ionization (ES) in positive and/or negative ionization mode. LRMS means low-resolution mass spectrum and the calculated and observed ions quoted refer to the isotopic composition of lowest mass. Combustion analyses were conducted by Exeter Analytical UK Ltd, Uxbridge, Middlesex.

5.1. Pyridine 2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)-3-carboxylic acid (3a)

(i) 2-Aminopyridine (80 g, 0.85 mol) was added portionwise over 30 min to oleum (320 g) and the resulting solution heated at 140 °C for 4 h. On cooling, the reaction was poured onto ice (200 g) and the mixture stirred in an ice/salt bath for a further 2 h. The resulting suspension was filtered, the solid washed with ice water (200 mL) and cold IMS (200 mL) and dried under suction to afford pyridine-2-amino-5-sulphonic acid as a solid (111.3 g). LRMS (Thermospray): m/z 175 $(M+1)^+$.

(ii) Bromine (99 g, 0.62 mol) was added dropwise over 1 h, to a hot solution of the sulphonic acid (108 g, 0.62 mol) in water (600 mL) so as to maintain a steady reflux. Once the addition was complete the reaction was cooled and the resulting mixture filtered. The solid was washed with water and dried under suction to afford pyridine-2-amino-3-bromo-5-sulphonic acid (53.4 g). ^{1}H NMR (DMSO-d_6) δ: 8.08 (s, 1H), 8.14 (s, 1H).

(iii) A solution of sodium nitrite (7.6 g, 110.0 mmol) in water (30 mL) was added dropwise to an ice-cooled solution of the bromo sulphonic acid (25.3 g, 100.0 mmol) in 20% aqueous hydrochloric acid (115 mL), so as to maintain the temperature below 6 °C. The reaction was stirred for 30 min at 0 °C and for a further 1 h at room temperature. The reaction mixture was evaporated under reduced pressure and the residue dried under vacuum at 70 °C for 72 h. A mixture of this solid, phosphorus pentachloride (30.0 g, 144.0 mmol) and phosphorus oxychloride (1 mL, 10.8 mmol) was heated at 125 °C for 3 h, and then cooled. The reaction mixture was poured onto ice (100 g) and the resulting solid filtered, and washed with water. The product was dissolved in dichloromethane, dried ($MgSO_4$), and evaporated under reduced pressure to afford pyridine-3-bromo-2-chloro-5-sulphonyl chloride as a yellow solid (26.58 g). ^{1}H NMR ($CDCl_3$) δ: 8.46 (s, 1H), 8.92 (s, 1H).

(iv) A solution of 1-ethylpiperazine (11.3 mL, 89.0 mmol) and triethylamine (12.5 mL, 89.0 mmol) in dichloromethane (150 mL) was added dropwise to an ice-cooled solution of the sulphonyl chloride (23.0 g, 79.0 mmol) in dichloromethane (150 mL) and the reaction stirred at 0 °C for 1 h. The reaction mixture was concentrated under reduced pressure and the residual brown oil was purified by column chromatography (dichloromethane and methanol) to afford 3-bromo-2-chloro-5-(4-ethylpiperazine -1-ylsulphonyl)pyridine as an orange solid (14.5 g). ^{1}H NMR ($CDCl_3$) δ: 1.05 (t, 3H), 2.42 (q, 2H), 2.55 (m, 4H), 3.12 (m, 4H), 8.24 (s, 1H), 8.67 (s, 1H).

(v) A mixture of the sulphonyl chloride (6.60 g, 17.9 mmol) and sodium ethoxide (6.09 g, 89.6 mmol) in ethanol (100 mL) was heated under reflux for 18 h, then cooled. The reaction mixture was concentrated under reduced pressure, the residue partitioned between water (100 mL) and ethyl acetate (100 mL), and the layers separated. The aqueous phase was extracted with ethyl acetate (2×100 mL) and the combined organic solutions were dried ($MgSO_4$) and evaporated under reduced pressure to afford 3-bromo-2-ethoxy-5-(4-ethylpiperazine-1-ylsulphonyl) pyridine as a brown solid (6.41 g). ^{1}H NMR ($CDCl_3$) δ: 1.06 (t, 3H), 1.48 (t, 3H), 2.42 (q, 2H), 2.56 (m, 4H), 3.09 (m, 4H), 4.54 (q, 2H), 8.10 (s, 1H), 8.46 (s, 1H). LRMS (Thermospray): m/z 378, 380 $(M+1)^+$.

(vi) A mixture of the bromide (6.40 g, 16.92 mmol), triethylamine (12 mL, 86.1 mmol), and catalytic palladium(0) tris(triphenylphosphine) in ethanol (60 mL) was heated at 100 °C and 200 psi, under a carbon monoxide atmosphere, for 18 h, then cooled. The reaction mixture was evaporated under reduced pressure and the residue purified by column chromatography (dichloromethane and methanol) to afford pyridine 2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)-3-carboxylic acid ethyl ester as an orange oil (6.2 g). ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.39 (t, 3H), 1.45 (t, 3H), 2.40 (q, 2H), 2.54 (m, 4H), 3.08 (m, 4H), 4.38 (q, 2H), 4.55 (q, 2H), 8.37 (s, 1H), 8.62 (s, 1H). LRMS (Thermospray): m/z 372 $(M+1)^+$.

(vii) A mixture of the ethyl ester (4.96 g, 13.35 mmol) and 2 N NaOH solution (25 mL, 50.0 mmol) in ethanol (25 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure to half it is volume, washed with ether and acidified to pH 5 using 4 N HCl. The aqueous solution was extracted with dichloromethane (3×30 mL), the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound, pyridine 2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)-3-carboxylic acid (**3a**) as a tan coloured solid (4.02 g). Melting point 206–207 °C. ^{1}H NMR (Dmso-d_6) δ: 1.18 (t, 3H), 1.37 (t, 3H), 3.08 (q, 2H), 3.17-3.35 (m, 8H), 4.52 (q, 2H), 8.30 (s, 1H), 8.70 (s, 1H). LRMS (Thermospray): m/z 344 $(M+1)^+$.

5.2. 4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-1*H*-3-ethylpyrazole-5-carboxamide (5a)

A solution of 3-ethyl-1*H*-pyrazole-5-carboxamide (9.2 g, 59.8 mmol) in DMF (60 mL) was added to a solution of **3a** (21.7 g, 62.9 mmol), 1-hydroxybenzotriazole hydrate (10.1 g, 66.0 mmol) and triethylamine (13.15 mL, 94.3 mmol) in dichloromethane (240 mL). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (13.26 g, 69.2 mmol) was added and the reaction stirred at room temperature for 6 h. The dichloromethane was removed under reduced pressure, the remaining solution poured into ethyl acetate (400 mL), and this mixture washed with aqueous $NaHCO_3$ (400 mL). The resulting crystalline precipitate was filtered, washed with ethyl acetate and dried under vacuum, to afford the title compound, as a white powder (22 g, 77%). ^{1}H NMR ($CDCl_3$ + 1 drop DMSO-d_6) δ: 0.96 (t, 3H), 1.18 (t, 3H), 1.50 (t, 3H), 2.25-2.56 (m, 6H), 2.84 (q, 2H), 3.00 (m, 4H), 4.70 (q, 2H), 5.60 (br s, 1H), 6.78 (br s, 1H), 8.56 (d, 1H), 8.76 (d, 1H), 10.59 (s, 1H), 12.10–12.30 (br s, 1H). LRMS (Thermospray) 480 $(M+1)^+$.

5.3. 4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethyl-2-n-propylpyrazole-5-carboxamide (6a)

A mixture of **5a** (1.0 g, 2.09 mmol), *n*-propyl bromide (0.23 mL, 2.5 mmol) and caesium carbonate (374 mg, 1.15 mmol) in DMF (5 mL), was heated at 60 °C for 18 h. The solvent was removed under reduced pressure, the residue suspended in water and extracted with dichloromethane. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. This product was triturated with ether and the resulting white solid filtered and dried to give the title compound (205 mg, 19%). ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, 3H), 1.00 (t, 3H), 1.20 (t, 3H), 1.60 (t, 3H), 1.90 (m, 1H), 2.40 (q, 2H), 2.55 (m, 4H), 3.90 (q, 2H), 3.08 (m, 4H), 4.00 (t, 2H), 4.80 (q, 2H), 5.25 (br s, 1H), 6.65 (br s, 1H), 8.60 (s, 1H), 8.80 (s, 1H), 10.55 (s, 1H).

5.4. 3-Ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-*n*-propyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7a)

A mixture of **6a** (200 mg, 0.38 mmol) and potassium bis(trimethylsilyl)amide (381 mg, 1.91 mmol) in 2-methoxyethanol (3 mL) was heated under reflux for 18 h. The solvent was removed under reduced pressure, the residue partitioned between water and dichloromethane and the mixture neutralised using 2 N HCl. The phases were separated, and the organic layer dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography (dichloromethane and methanol), and the product triturated with ether to give the title compound as a white solid (48 mg, 23%).

^{1}H NMR ($CDCl_3$) δ: 0.95 (t, 3H), 1.05 (t, 3H), 1.4 (t, 3H), 2.00 (q, 2H), 2.42 (q, 2H), 2.55 (m, 4H), 3.02 (q, 2H), 3.10 (m, 4H), 3.55 (s, 3H), 3.86 (t, 2H), 4.25 (t, 2H), 4.75 (t, 2H), 8.62 (s, 1H), 8.98 (s, 1H), 10.75 (br s, 1H). Anal. calcd for $C_{24}H_{35}N_7O_5S.H_2O$: C, 52.25; H, 6.69; N, 17.54. Found: C, 52.25; H, 6.76; N, 17.77.

5.5. 5-[2-*iso*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-2-[2-(dimethylamino)ethyl]-3-ethyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7h)

A mixture of **6g** (127 mg, 0.23 mmol) potassium bis(trimethylsilyl)amide (230 mg, 1.15 mmol) and ethyl acetate (20 mg, 0.23 mmol) in *iso*-butanol (30 mL) was heated at 130 °C in a sealed vessel for 6 h. The solvent was removed under reduced pressure from the cooled mixture, and the residue partitioned between ethyl acetate and $NaHCO_3$ solution. The layers were separated, the aqueous phase extracted with ethyl acetate (3×), and the combined organic extracts dried (Na_2SO_4) and evaporated under reduced pressure. The residual gum was purified by column chromatography (dichloromethane and methanol) to give the title compound, (30 mg, 23%). ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.12 (d, 6H), 1.42 (t, 3H), 2.31 (m, 7H), 2.42 (q, 2H), 2.57 (m, 4H), 2.90 (t, 2H), 3.06 (q, 2H), 3.16 (m, 4H), 4.38–4.47 (m, 4H), 8.61 (d, 1H), 9.01 (d, 1H), 10.60 (s, 1H). Anal. calcd for $C_{26}H_{40}N_8O_4S.0.6H_2O$: C, 54.85; H, 7.25; N, 19.16. Found C, 54.64; H, 7.27; 19.61.

5.6. 3-Ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-*iso*-propyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7b)

The title compound was obtained as a white solid in 21% yield, from **6b**, and 2-methoxyethanol, according to the method of **7a**. Anal. ($C_{24}H_{35}N_7O_5S.0.4H_2O$): C, 53.30; H, 6.67; N, 18.13. Found: C, 53.53; H, 6.64; N, 17.89. ^{1}H NMR ($CDCl_3$) δ: 1.00 (t, 3H), 1.40 (t, 3H), 1.60 (d, 6H), 2.40 (q, 2H), 2.55 (m, 4H), 3.02 (q, 2H), 3.15 (m, 4H), 3.55 (s, 3H), 3.80 (m, 2H), 4.70 (m, 1H), 4.78 (m, 2H), 8.60 (s, 1H), 9.00 (s, 1H), 10.75 (s, 1H). LRMS (Thermospray): *m*/*z* 534.8 $(M+1)^+$.

5.7. 2-Cyclobutyl-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7c)

A mixture of **6c** (450 mg, 0.85 mmol), potassium bis(trimethylsilyl)amide (505 mg, 2.55 mmol) and ethyl acetate (100 μL, 1.28 mmol) in 2-methoxyethanol (8 ml) was heated under reflux for 18 h. The solvent was removed under reduced pressure, and the residue purified by column chromatography (dichloromethane/methanol) to afford the title compound, (133 mg, 29%). Anal. ($C_{24}H_{33}N_7O_4S$): C, 55.90; H, 6.45; N, 19.01. Found: C, 55.98; H, 6.45; N, 18.88. ^{1}H NMR ($CDCl_3$) δ: 1.00 (t, 3H), 1.38 (t, 3H), 1.90 (m, 2H), 2.40 (q, 2H), 2.45 (m, 2H), 2.54 (m, 4H), 2.95 (m, 2H), 3.15 (m, 4H), 3.55 (s, 3H), 3.80 (m, 2H), 4.74 (m, 2H), 4.95 (m, 1H), 8.60 (s, 1H), 8.98 (s, 1H), 10.75 (s, 1H).

5.8. 2-*iso*-Butyl-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7d)

The title compound was obtained as a pale yellow solid in 21% yield, from **6d**, and 2-methoxyethanol, according to the method of **7a**. Anal. ($C_{25}H_{37}N_7O_5S.0.2H_2O$): C, 54.22; H, 6.73; N, 17.61. Found: C, 54.47; H, 6.84; N, 17.79. ^{1}H NMR ($CDCl_3$) δ: 0.95 (d, 6H), 1.05 (t, 3H), 1.40 (d, 3H), 2.40 (m, 3H), 2.55 (m, 4H), 3.00 (q, 2H), 3.10 (m, 4H), 3.55 (s, 3H), 3.85 (t, 2H), 5.05 (d, 2H), 4.80 (t, 2H), 8.60 (s, 1H), 8.95 (s, 1H), 10.80 (s, 1H). LRMS (Thermospray): *m*/*z* 549 $(M+1)^+$.

5.9. 2-Cyclopropylmethyl-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7e)

The title compound was obtained as a white solid in 15% yield, from **6e**, and 2-methoxyethanol, according to the method of **7a**. Anal. ($C_{25}H_{35}N_7O_5S.0.5H_2O$): C, 54.13; H, 6.54; N, 17.68. Found: C, 53.82; H, 6.34; N, 17.61. ^{1}H NMR ($CDCl_3$) δ: 0.43 (m, 2H), 0.60 (m, 2H), 0.80 (m, 1H), 1.00 (t, 3H), 1.40 (t, 3H), 2.40 (q, 2H), 2.54 (m, 4H), 3.00 (q, 2H), 3.07 (m, 4H), 3.50 (s, 3H), 3.80 (m, 2H), 4.20 (d, 2H), 4.78 (m, 2H), 8.60 (s, 1H), 8.97 (s, 1H), 10.75 (br s, 1H).

5.10. 3-Ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(tetrahydropyran-4-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7f)

The title compound was obtained as a pale yellow solid in 21% yield, from **6f**, and 2-methoxyethanol, according to the method of **7a**. Anal. ($C_{26}H_{37}N_7O_6S.1.8H_2O$) C, H, N. ^{1}H NMR ($CDCl_3$) δ: 1.00 (t, 3H), 1.40 (t, 3H), 1.83 (m, 2H), 2.40 (q, 2H), 2.55 (m, 6H), 3.06 (q, 2H), 3.10 (m, 4H), 3.55 (s, 3H), 3.60 (t, 2H), 3.80 (t, 2H), 4.20 (m, 2H), 4.48 (m, 1H), 4.80 (t, 2H), 8.60 (s, 1H), 9.00 (s, 1H), 10.80 (br s, 1H). LRMS (Electrospray): *m*/*z* 576.6 $(M+1)^+$.

5.11. 5-[2-*n*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-2-[3-(dimethylamino)-*n*-propyl]-3-ethyl-2,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (7g)

The title compound was obtained in 44% yield, from **6h** and n-butanol, by the method of **7g**. Anal. ($C_{27}H_{42}N_8O_4S.H_2O$): C, 55.76; H, 7.53; N, 18.88. Found: C, 55.55; H, 7.42; N, 19.20. ^{1}H NMR ($CDCl_3$) δ: 1.01 (t, 6H), 1.40 (t, 3H), 1.56 (m, 2H), 1.95 (m, 2H), 2.17 (m, 2H), 2.21 (s, 6H), 2.24 (t, 2H), 2.40 (q, 2H), 2.57 (m, 4H), 3.06 (q, 2H), 3.17 (m, 4H), 4.37 (t, 2H), 4.65 (t, 2H), 8.61 (d, 1H), 9.02 (d, 1H), 10.59 (s, 1H).

5.12. 3-Ethyl-5-[2-(2-methoxyethoxy)-5-(4-methylpiperazin-1-ylsulphonyl)pyridin-3-yl]-2-*n*-propyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7i)

The title compound was synthesised from **6j** according to the method of **7a**.

Anal. ($C_{23}H_{33}N_7O_5S.0.5H_2O$): C, 53.13; H, 6.69; N, 18.07. Found: C, 53.15; H, 6.56; N, 18.03. ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, 3H), 1.40 (t, 3H), 2.00 (q, 2H), 2.25 (s, 3H), 2.50 (m, 4H), 3.00 (q, 2H), 3.18 (m, 4H), 3.82 (m, 2H), 4.22 (t, 2H), 4.80 (m, 2H), 8.60 (s, 1H), 9.00 (s, 1H), 10.75 (br s, 1H). LRMS (Thermospray): *m*/*z* 520.2 $(M+1)^+$.

5.13. 2-*iso*-Butyl-3-ethyl-5-[2-(2-methoxyethoxy)-5-(4-methylpiperazin-1-ylsulphonyl)pyridin-3-yl]-2,6-dihydro-7H-pyrazolo[4,3-*d*]pyrimidin-7-one (7j)

The title compound was obtained as a white solid in 5% yield, from **6k**, and 2-methoxyethanol, according to the method of **7a**. Anal. ($C_{24}H_{35}N_7O_5S.2.5H_2O$): C, 54.02; H, 6.61; N, 18.37. Found: C, 49.70; H, 6.99; N, 16.88. ^{1}H NMR ($CDCl_3$) δ: 0.97 (d, 6H), 1.40 (t, 3H), 2.28 (m, 4H), 2.52 (m, 4H), 3.02 (q, 2H), 3.16 (m, 4H), 3.57 (s, 3H), 3.86 (t, 2H), 4.10 (d, 2H), 4.78 (t, 2H), 8.61 (d, 1H), 8.98 (d, 1H), 10.79 (s, 1H). LRMS (Thermospray): *m*/*z* 534 $(M+1)^+$.

5.14. 5-[2-*n*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[1-(methylsulphonyl)piperidin-4-yl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (8)

(i) 5-[2-*n*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-2-(1-*tert*-butoxycarbonylpiperidin-4-yl)-3-ethyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**7i**) was obtained in 69% yield, from **6i** and n-butanol, by the method of **7g**. ^{1}H NMR ($CDCl_3$): 1.01 (t, 6H), 1.34-1.60 (m, 14H), 1.93 (m, 4H), 2.41 (m, 4H), 2.57 (m, 4H), 2.90 (m, 2H), 3.00–3.20 (m, 6H), 4.38 (m, 3H), 4.66 (t, 2H), 8.61 (d, 1H), 9.00 (s, 1H), 10.58 (s, 1H).

(ii) Trifluoroacetic acid (1.5 mL) was added to a solution **7i** (150 mg, 0.22 mmol) in dichloromethane (1.5 mL), and the solution stirred for 2½ h at room temperature. The mixture was evaporated under reduced pressure, the residue triturated well with ether, and the resulting precipitate, filtered and dried to give a white powder (156 mg).

(iii) Methanesulphonyl chloride (23 μL, 0.30 mmol) was added to a solution of this intermediate in dichloromethane (4 mL) and triethylamine (110 μL, 0.79 mmol), and the reaction stirred at room temperature for 1½ h. The mixture was treated with saturated aqueous $NaHCO_3$ solution (10 mL), and extracted with ethyl acetate (2 × 10 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography (dichloromethane and methanol) to afford the title compound as a white solid (50 mg, 34%). ^{1}H NMR ($CDCl_3$) δ: 1.01 (t, 6H), 1.40 (t, 3H), 1.55 (m, 2H), 1.95 (m, 2H), 2.08 (m, 2H), 2.42 (q, 2H), 2.57 (m, 6H), 2.90 (s, 3H), 3.01-3.18 (m, 8H), 4.01 (m, 2H), 4.42 (m, 1H), 4.66 (t, 2H), 8.62 (d, 1H), 9.01 (d, 1H), 10.60 (s, 1H). Anal. calcd for $C_{28}H_{42}N_8O_6S_2$: C, 51.67; H, 6.50; N, 17.22. Found C, 51.39; H, 6.62; N, 17.05.

5.15. 4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethyl-2-(piperidin-4-yl)pyrazole-5-carboxamide ditrifluoroacetate (9)

Trifluoroacetic acid (3 mL) was added to a solution of the piperidine **6i** (309 mg, 0.47 mmol) in dichloromethane (4 mL), and the solution stirred for 2½ h. The reaction was evaporated under reduced pressure and the residue triturated well with ether. The resulting solid was sonicated in ether for 1 minute, the resulting precipitate filtered and dried to afford the title compound as a white solid (278 mg, 75%). ^{1}H NMR (DMSO-d_6) δ: 1.15 (m, 6H), 1.46 (t, 3H), 2.04 (m, 2H), 2.20 (m, 2H), 2.40–2.84 (m, 6H), 3.00-3.22 (m, 6H), 3.25–3.60 (m, 4H), 3.76 (m, 1H), 4.62 (m, 4H), 7.27 (s, 1H), 7.40 (s, 1H), 8.41 (m, 2H), 8.70 (m, 2H), 10.24 (s, 1H).

5.16. 4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethyl-2-(1-methylpiperidin-4-yl)pyrazole-5-carboxamide (10)

Formaldehyde (217 μL, 37% aqueous, 2.90 mmol) was added to a solution of **9**, in dichloromethane (8 mL), and the solution stirred vigorously for 30 min. Acetic acid (88 μL, 1.69 mmol) was added, the solution stirred for a further 30 min, then sodium triacetoxyborohydride (169 mg, 0.80 mmol) was added and the reaction stirred at room temperature for 16 h. The reaction mixture was poured into aqueous $NaHCO_3$ solution, and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography (dichloromethane and methanol and 0.88 ammonia) to afford the title compound (70 mg, 35%). ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.22 (t, 3H), 1.58 (t, 3H), 1.92 (m, 2H), 2.14 (m, 2H), 2.25–2.45 (m, 7H), 2.54 (m, 4H), 2.91 (q, 2H), 2.99–3.16 (m, 6H), 4.08 (m, 1H), 4.78 (q, 2H), 5.11 (br s, 1H), 6.65 (br s, 1H), 8.63 (d, 1H), 8.83 (d, 1H), 10.53 (s, 1H). LRMS (Thermospray): *m*/*z* 344 $(M+1)^+$.

5.17. 5-[2-*n*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (11)

The title compound was obtained in 50% yield, from **10** by the method of **7g** using *n*-butanol as the solvent. ^{1}H NMR ($CDCl_3$) δ: 1.02 (m, 6H), 1.40 (t, 3H), 1.57 (m, 2H), 1.94 (m, 4H), 2.16 (m, 2H), 2.37 (s, 3H), 2.41 (q, 2H), 2.56 (m, 6H), 3.03 (m, 4H), 3.15 (m, 4H), 4.22 (m, 1H), 4.66 (t, 2H), 8.62 (d, 1H), 9.01 (d, 1H), 10.55 (s, 1H). Anal. calcd for $C_{28}H_{42}N_8O_4S.0.75H_2O$: C, 56.01; H, 7.27; N, 18.51. Found C, 56.03; H, 7.30; N, 18.67.

5.18. 3-Ethyl-1-(2-methoxyethyl)-4-nitropyrazole-5-carboxamide (14a) and 3-ethyl-2-(2-methoxyethyl)-4-nitropyrazole-5-carboxamide (13a)

A mixture of **12** (1.7 g, 8.8 mmol), 2-bromoethyl methyl ether (0.85 mL, 8.85 mmol) and caesium carbonate (2.9 g, 9.0 mmol) in DMF (20 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between ethyl acetate (125 mL) and brine (100 mL). The phases were separated, and the organic layer was dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/methanol) to afford the title compound **14a** (831 mg, 39%).

^{1}H NMR (DMSO-d_6) δ: 1.19 (t, 3H), 2.82 (q, 2H), 3.20 (s, 3H), 3.68 (t, 2H), 4.22 (t, 2H), 8.18 (s, 1H), 8.38 (s, 1H). LRMS (Thermospray): *m*/*z* 260 $(M+18)^+$ and the title compound of **13a**, (793 mg, 37%).

^{1}H NMR ($CDCl_3$) δ: 1.18 (t, 3H), 2.98 (q, 2H), 3.22 (s, 3H), 3.70 (t, 2H), 4.28 (t, 2H), 7.65 (s, 1H), 7.94 (s, 1H). LRMS (Thermospray): *m*/*z* 243 $(M+1)^+$.

5.19. 4-Amino-3-ethyl-2-(2-methoxyethoxy)pyrazole-5-carboxamide (15a)

A mixture of the title compound from **14a** (500 mg, 2.07 mmol) and 10% palladium on charcoal (50 mg) in ethanol (20 mL) was hydrogenated at 50 psi and room temperature for 18 h. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to afford the title compound as a white solid. ^{1}H NMR (DMSO-d_6) δ: 1.03 (t, 3H), 2.57 (q, 2H), 3.20 (s, 3H), 3.63 (t, 2H), 4.09 (t, 2H), 4.39 (s, 2H), 6.90 (s, 1H), 7.01 (s, 1H). LRMS (Thermospray): *m*/*z* 213 $(M+1)^+$.

5.20. 4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethyl-2-(2-methoxyethyl)pyrazole-5-carboxamide (16a)

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.26 g, 27.4 mmol) was added to a solution of **3a** (7.25 g, 21.1 mmol), and **15a** (4.45 g, 20.9 mmol), 1-hydroxybenzotriazole

hydrate (3.71 g, 27.4 mmol), and *N*-diisopropylethylamine (10.96 mL, 63.3 mmol) in dichloromethane (70 mL), and the reaction stirred for 18 h. The reaction mixture was diluted with dichloromethane (100 mL), washed with water (100 mL), saturated aqueous $NaHCO_3$ solution (100 mL), and brine (100 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by column chromatography (dichloromethane and methanol) to give the title compound as a foam, (10.1 g, 94%). ^{1}H NMR ($CDCl_3$) δ: 1.03 (t, 3H), 1.20 (t, 3H), 1.58 (t, 3H), 2.40 (q, 2H), 2.54 (m, 4H), 2.95 (q, 2H), 3.10 (m, 4H), 3.37 (s, 3H), 3.80 (t, 2H), 4.26 (t, 2H), 4.78 (q, 2H), 5.27 (s, 1H), 6.66 (s, 1H), 8.65 (s, 1H), 8.85 (s, 1H), 10.51 (s, 1H). LRMS (Thermospray): *m*/*z* 538 $(M+1)^+$.

5.21. 4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethyl-2-(1-methylazetidin-3-yl)pyrazole-5-carboxamide (17)

Trifluoroacetic acid (2.5 mL) was added to a solution of **16d** (700 mg, 1.1 mmol) in dichloromethane (3.5 mL) and the solution stirred at room temperature for 2½ h. The reaction mixture was evaporated under reduced pressure and the residue triturated well with ether and dried under vacuum. The solid was suspended in saturated aqueous $NaHCO_3$ solution, extracted with ethyl acetate, and the combined organic extracts evaporated under reduced pressure.

Formaldehyde (280 μL, 37% aqueous, 4.4 mmol) was added to a solution of the intermediate amine in dichloromethane (8 mL), and the solution stirred vigorously for 30 min. Acetic acid (53 μL, 1.1 mmol) was added, the solution stirred for a further 30 min, then sodium triacetoxyborohydride (238 mg, 1.12 mmol) was added and the reaction stirred at room temperature for 16 h. The reaction mixture was poured into aqueous $NaHCO_3$ solution (30 mL), and extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography (dichloromethane/methanol/0.88 ammonia) to afford the title compound (470 mg, 78%). ^{1}H NMR ($CDCl_3$) δ: 1.01 (t, 3H), 1.18 (t, 3H), 1.58 (t, 3H), 2.40 (q, 2H), 2.48 (s, 3H), 2.54 (m, 4H), 2.85 (q, 2H), 3.10 (m, 4H), 3.59 (t, 2H), 3.82 (t, 2H), 4.79 (q, 2H), 4.96 (m, 1H), 5.32 (br s, 1H), 6.79 (br s, 1H), 8.64 (d, 1H), 8.82 (d, 1H), 10.52 (s, 1H).

5.22. 5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(2-methoxyethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (19a)

The title compound was obtained in 27% yield, after recrystallisation from ethyl acetate, from **16a** and ethanol, by the method of **7g**.

mp 161–162 °C. ^{1}H NMR ($CDCl_3$) δ: 1.04 (t, 3H), 1.40 (t, 3H), 1.58 (t, 3H), 2.41 (q, 2H), 2.57 (m, 4H), 3.08 (q, 2H), 3.14 (m, 4H), 3.30 (s, 3H), 3.92 (t, 2H), 4.46 (t, 2H), 4.75 (q, 2H), 8.62 (d, 1H), 9.04 (d, 1H), 10.61 (s, 1H). Anal. calcd for $C_{24}H_{35}N_7O_6S.0.2EtOAc$: C, 53.12; H, 6.47; N, 18.15. Found C, 53.21; H, 6.49; N,18.25. LRMS (Thermospray): *m*/*z* 520 $(M+1)^+$.

5.23. 5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-[2-(morpholin-4-yl)ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (19b)

The title compound was obtained as a solid, from **16b** and ethanol, by the method of **7g**. Anal ($C_{26}H_{38}N_8O_5S$): C, 54.16; H, 6.66; N, 19.34. Found C, 54.33; H, 6.66; N,19.50. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.42 (t, 3H), 1.57 (t, 3H), 2.40 (q, 2H), 2.54 (m, 8H), 2.98 (t, 2H), 3.04 (q, 2H), 3.15 (m, 4H), 3.66 (m, 4H), 4.40 (t, 2H), 4.76 (q, 2H), 8.62 (d, 1H), 9.04 (d, 1H), 10.61 (s, 1H). LRMS (Thermospray): *m*/*z* 574 $(M+1)^+$.

5.24. 5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-2-ethyl-3-ethyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (19c)

The title compound was obtained as a solid, from **16c** and ethanol, by the method of **7g**, in the absence of ethyl acetate, and after trituration from ether. Anal. ($C_{23}H_{33}N_7O_5S.0.2Et_2O$): C, 53.29; H, 6.26; N, 18.19. Found C, 53.49; H, 6.60; N, 18.35. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.40 (t, 3H), 1.58 (m, 6H), 2.41 (q, 2H), 2.55 (m, 4H), 3.00–3.18 (m, 6H), 4.38 (q, 2H), 4.75 (q, 2H), 8.63 (s, 1H), 9.04 (s, 1H), 10.63 (s, 1H). LRMS: *m*/*z* 490 $(M+1)^+$.

5.25. 5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylazetidin-3-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (19d)

A mixture of **18** (470 mg, 0.86 mmol) and potassium bis(trimethylsilyl)amide (600 mg, 3.0 mmol) in ethanol (45 mL) was heated at 130 °C for 16 h. The cooled mixture was concentrated under reduced pressure, the solution diluted with aqueous $NaHCO_3$ solution to give pH 8, and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography (dichloromethane:methanol:0.88 ammonia) to give the title compound, (170 mg, 37%). ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.38 (t, 3H), 1.58 (m, 3H), 2.40 (q, 2H), 2.50 (s, 3H), 2.57 (m, 4H), 3.01 (q, 2H), 3.16 (m, 4H), 3.79 (t, 2H), 3.90 (t, 2H), 4.78 (q, 2H), 5.12 (m, 1H), 8.62 (d, 1H), 9.01 (d, 1H), 10.62 (s, 1H). Anal. calcd for $C_{24}H_{34}N_8O_4S.1.33\ H_2O$: C, 52.31; H, 6.53; N, 19.80. Found C, 51.97; H, 6.66; N,20.20.

5.26. 3-Ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-n-propoxypyridin-3-yl]-2-(2-methoxyethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20a)

Potassium bis(trimethylsilyl)amide (153 mg, 0.77 mmol) was added to a solution of **19a** (100 mg, 0.19 mmol) in *n*-propanol (5 mL), and the reaction heated under reflux for 18 h. The solvent was removed under reduced pressure, the residue partitioned between water and dichloromethane and the mixture neutralised using solid CO_2. The layers were separated, the aqueous phase extracted with dichloromethane, and the combined organic extracts dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by column chromatography (dichloromethane and methanol) to give the title compound (68 mg, 66%). ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.40 (t, 3H), 1.52 (t, 3H), 1.98 (m, 2H), 2.40 (q, 2H), 2.57 (m, 4H), 3.14 (m, 6H), 3.32 (s, 3H), 3.94 (t, 2H), 4.46 (t, 2H), 4.62 (t, 2H), 8.61 (s, 1H), 9.02 (s, 1H), 10.62 (s, 1H). Anal. calcd for $C_{24}H_{35}N_7O_5S.H_2O$: C, 53.81; H, 6.61; N, 18.27. Found C, 54.02; H, 6.61; N,18.27. LRMS (Thermospray): *m*/*z* 534 $(M+1)^+$.

5.27. 5-[2-*iso*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl) pyridin-3-yl]-3-ethyl-2-(2-methoxyethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20b)

The title compound was obtained as a solid, from **19a** and 2-methyl-*n*-propanol, by the method of **20a**. Anal. ($C_{25}H_{37}N_7O_5S$): C, 54.84; H, 6.84; N, 17.65. Found C, 54.83; H, 6.81; N,17.90. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.12 (d, 6H), 1.40 (t, 3H), 2.30 (m, 1H), 2.42 (q, 2H), 2.57 (m, 4H), 3.08 (q, 2H), 3.13 (m, 4H), 3.30 (s, 3H), 3.90 (t, 2H), 4.46 (m, 4H), 8.62 (s, 1H), 9.02 (s, 1H), 10.60 (s, 1H). LRMS (Thermospray): *m*/*z* 548 $(M+1)^+$.

5.28. 3-Ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-[(pyridin-2-yl)methyl]pyridin-3-yl]-2-(2-methoxyethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20c)

The title compound was obtained as a solid, from **20a** and pyridine-2-methanol, by the method of **20a**. Anal. ($C_{27}H_{34}N_8O_5S.0.5H_2O$): C, 55.20; H, 5.88; N, 18.97. Found C, 55.31; H, 5.91; N, 19.11. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.42 (t, 3H), 2.42 (q, 2H), 2.57 (m, 4H), 3.12 (m, 6H), 3.30 (s, 3H), 3.94 (t, 2H), 4.46 (t, 2H), 5.90 (s, 2H), 7.35 (m, 2H), 7.78 (m, 1H), 8.59 (s, 1H), 8.84 (m, 2H), 12.70 (s, 1H). LRMS (Thermospray): *m*/*z* 583 $(M+1)^+$.

5.29. 3-Ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(2-methoxyethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20d)

The title compound was obtained as a solid, from **19a** and 2-methoxyethanol, by the method of **20a**. Anal. ($C_{25}H_{37}N_7O_6S$): C, 52.45; H, 6.42; N, 17.84. Found C, 52.26; H, 6.46; N,17.56. ^{1}H NMR ($CDCl_3$) δ: 1.02 (m, 6H), 1.84 (m, 2H), 2.42 (q, 2H), 2.56 (m, 4H), 3.01 (t, 2H), 3.15 (m, 4H), 3.29 (s, 3H), 3.57 (s, 3H), 3.88 (m, 4H), 4.44 (t, 2H), 4.78 (t, 2H), 8.61 (s, 1H), 8.98 (s, 1H), 10.76 (s, 1H). LRMS (Thermospray): *m*/*z* 564 $(M+1)^+$.

5.30. 5-[2-Cyclopropylmethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-[2-(morpholin-4-yl)ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20e)

The title compound was obtained as a white solid in 38% yield, after recrystallisation from ethyl acetate, from **19b** and cyclopropanemethanol, by the method of **20a**. Anal. ($C_{28}H_{40}N_8O_5S.0.5H_2O$): C, 55.36; H, 6.78; N, 18.24. Found C, 52.26; H, 6.78; N, 17.56. ^{1}H NMR ($CDCl_3$) δ: 0.46 (m, 2H), 0.77 (m, 2H), 1.01 (t, 3H), 1.42 (t, 3H), 2.40 (q, 2H), 2.54 (m, 8H), 2.97 (t, 2H), 3.01–3.20 (m, 4H), 3.68 (m, 4H), 4.40 (t, 2H), 4.50 (d, 2H), 8.60 (d, 1H), 9.05 (d, 1H), 10.76 (s, 1H). LRMS (Thermospray) 601 $(M+1)^+$.

5.31. 3-Ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-[2-(morpholin-4-yl)ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20f)

The title compound was obtained as a white solid in 48% yield, from **19b** and 2-methoxyethanol, by the method of **20a**. Anal. ($C_{27}H_{40}N_8O_6S.0.5H_2O$): C, 52.72; H, 6.76; N, 18.11. Found C, 52.84; H, 6.73; N,18.25. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.42 (t, 3H), 2.41 (q, 2H), 2.53 (m, 8H), 2.96 (t, 2H), 3.05 (q, 2H), 3.16 (m, 4H), 3.57 (s, 3H), 3.66 (m, 4H), 4.40 (t, 2H), 4.78 (t, 2H), 8.62 (d, 1H), 8.99 (d, 1H), 10.78 (s, 1H).

5.32. 2-Ethyl-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20g)

The title compound was obtained as a solid, from **19c** and 2-methoxyethanol, by the method of **20a**, using ethyl acetate/diethylamine for column chromatography. Anal. ($C_{23}H_{33}N_7O_5S$): C, 53.29; H, 6.26; N, 18.19. Found C, 53.49; H, 6.60; N, 18.35. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.42 (t, 3H), 1.59 (t, 3H), 2.44 (q, 2H), 2.57 (m, 4H), 3.05 (q, 2H), 3.16 (m, 4H), 3.58 (s, 3H), 3.86 (t, 2H), 4.28 (q, 2H), 4.79 (t, 2H), 8.62 (s, 1H), 8.99 (s, 1H). LRMS (Thermospray): *m*/*z* 520 $(M+1)^+$.

5.33. 5-[2-*iso*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylazetidin-3-yl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (20h)

The title compound was obtained in 45% yield, from **19d** and *n*-butanol, by the method of **20a**. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 6H), 1.38 (t, 3H), 1.57 (m, 2H), 1.96 (m, 2H), 2.41 (q, 2H), 2.50 (s, 3H), 2.56 (m, 4H), 3.00 (q, 2H), 3.15 (m, 4H), 3.79 (t, 2H), 3.94 (t, 2H), 4.68 (t, 2H), 5.12 (m, 1H), 8.62 (d, 1H), 9.01 (d, 1H), 10.61 (s, 1H). Anal. ($C_{26}H_{38}N_8O_4S.1.25H_2O$): C, 54.13; H, 6.75; N, 18.86. Found C, 53.73; H, 7.02; N, 19.28.

5.34. *cis*-3-Methyl-2-piperidinecarboxylic acid (21b)

A mixture of 2-cyano-3-methylpyridine (20.0 g, 169 mmol) and conc. HCl (50 mL) in water (50 mL) was heated under reflux for 8 h. The reaction was evaporated under reduced pressure, the residue azeotroped with water, then triturated with acetone. The resulting pink solid was recrystallised from acetonitrile/water (50:50 by volume) to give an off-white solid (13.8 g).

A mixture of this intermediate acid, and platinum oxide (5.0 g) in ethanol (70 mL) and water (70 mL) was hydrogenated at 60 °C and 60 psi for 24 h. The cooled mixture was filtered through Arbocel® and the filtrate concentrated under reduced pressure to a volume of 50 mL. This solution was cooled in ice, and the resulting precipitate filtered and dried to afford the title compound, (8.15 g, 34%). LRMS (Thermospray) *m*/*z*: 144.1 $(M+1)^+$.

5.35. 4,5,6,7-Tetrahydro[1,2,3]oxadiazolo[3,4-*a*]pyridin-8-ium-3-olate (22a)

A solution of sodium nitrite (14.0 g, 203 mmol) in water (30 mL) was added dropwise over 1 h, to a solution of pipecolinic acid (25.0 g, 193 mmol) in water (90 mL) and conc HCl (16.5 mL, 198 mmol), cooled in an ice/acetone bath. Once addition was complete, the solution was stirred at 0 °C for 1 h, then allowed to warm to room temperature. The reaction was extracted with dichloromethane (2×200 ml), the combined organic extracts dried (Na_2SO_4), and evaporated under reduced pressure. The residual oil was dissolved in ether (400 mL), this solution cooled in ice, trifluoroacetic anhydride (28 mL, 200 mmol) was added dropwise over 1 h, and the reaction stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and the residual oil was purified by column chromatography (ethyl acetate), to give the title compound as a white solid (13.0 g, 43%). ^{1}H NMR ($CDCl_3$) δ: 1.95 (m, 2H), 2.10 (m, H), 2.62 (t, 2H), 4.43 (t, 2H). LRMS (Thermospray) *m*/*z*: 158.1 $(M+1)^+$.

5.36. Ethyl 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxylate (23a)

A mixture of **22a** (13.0 g, 93 mmol), and ethyl propiolate (28.5 mL, 278 mmol), in xylene (250 mL) was heated under reflux for 2 h. The cooled mixture was evaporated under reduced pressure. The residual oil was purified by column chromatography (diethylether), to afford the title compound as an oil, (5.82 g, 32%). ^{1}H NMR ($CDCl_3$) δ: 1.39 (t, 3H), 1.88 (m, 2H), 2.06 (m, 2H), 2.82 (t, 2H), 4.20 (t, 2H), 4.39 (q, 2H), 6.55 (s, 1H). LRMS (Thermospray) *m*/*z*: 195.2 $(M+1)^+$.

5.37 3-Amino-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxamide (24a)

(i) Sodium hydroxide solution (20 mL, 2 M, 40 mmol) was added to a solution of **23a** (5.8 g, 30 mmol) in dioxan (100 mL),

and the reaction stirred at room temperature for 18 h. The mixture was evaporated under reduced pressure and HCl (2 M, 21 mL) was added. The mixture was evaporated under reduced pressure, the residue azeotroped with toluene, and the product dried under vacuum.

(ii) Fuming nitric acid (5 mL) was added to concentrated sulphuric acid (30 mL) with ice cooling. The resulting mixture was warmed to 40 °C and the intermediate acid (5.0 g) was added portionwise over 1 h so as to keep the internal temperature between 50 and 60 °C. The reaction was stirred at 60 °C for 1 h followed by 4 h at 50 °C. The mixture was allowed to cool to room temperature and stirred overnight. The mixture was poured onto ice and the resulting precipitate filtered off, washed with water, and dried to afford the acid intermediate, 3-nitro-4,5,6,7-tetrahydropyrazolo[1,5-*a*] pyridine-2-carboxylic acid (4.6 g). ^{1}H NMR ($CDCl_3$) δ: 2.00 (m, 2H), 2.15 (m, 2H), 3.20 (t, 2H), 4.28 (t, 2H). LRMS (Thermospray) *m*/*z*: 229.3 $(M+18)^+$.

(iii) Oxalyl chloride (5.7 mL, 65 mol) was added over 1 min to an ice-cooled solution of this acid in dichloromethane (100 mL) and DMF (100 μL) and the reaction was allowed to warm to room temperature over 2.5 h. The mixture was evaporated under reduced pressure, dried under vacuum and the resulting solid was dissolved in tetrahydrofuran (100 mL) and cooled in an ice bath. Ammonia gas was bubbled through for 10 min and the reaction stirred a room temperature for 5 h. The mixture was evaporated under reduced pressure and the resulting solid was treated with water (20 mL), washed with water (5 mL) and dried to give 3-nitro-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxamide, as a beige solid (4.2 g). ^{1}H NMR ($CDCl_3$) δ: 1.96 (m, 2H), 2.10 (m, 2H), 3.17 (t, 2H), 4.21 (m, 2H), 6.04 (br s, 1H), 7.76 (br s, 1H). LRMS (Thermospray) *m*/*z*: 211.1 $(MH)^+$.

(iv) A mixture of the carboxamide, and 10% Pd/C (500 mg) in methanol (200 mL) was hydrogenated at 60 psi and room temperature for 5 h. The mixture was diluted with methanol (200 mL) with warming, in order to dissolve the residual solid then filtered through Arbocel and the filtrate evaporated under reduced pressure to afford the title compound as a brown solid (3.15 g, 58%). ^{1}H NMR (DMSO-d_6) δ: 1.75 (m, 2H), 1.90 (m, 2H), 2.55 (t, 2H), 3.96 (t, 2H), 4.40 (br s, 2H), 6.86 (br s, 1H), 7.04 (br s, 1H). LRMS (Thermospray) *m*/*z*: 181.5 $(M+1)^+$.

5.38. 3-[({2-Ethoxy-5-[(4-ethyl-1-piperazinyl) sulfonyl]-3-pyridinyl}carbonyl)amino]-4-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-2-carboxamide (25a)

Oxalyl chloride (4.4 mL, 50.0 mmol) was added to an ice-cooled solution of **3a** (5.0 g, 13.0 mmol) in dichloromethane (100 mL) and DMF (0.2 mL), and the reaction stirred for 1 h at 0 °C, then for 4 h at room temperature. The solvent was removed under reduced pressure, and the residue azeotroped with toluene, and dried under vacuum. The resulting acid chloride was suspended in dichloromethane (200 mL), cooled in an ice-bath, **24a** (2.37 g, 13.0 mmol) and Et_3N (5.0 mL, 36.0 mmol) added, and the reaction stirred at room temperature for 18 h. The reaction was diluted with dichloromethane, washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated under reduced pressure. The crude was purified by column chromatography (dichloromethane and methanol) to give the title compound as a white powder (6.0 g, 91%). ^{1}H NMR ($CDCl_3$) δ: 1.01 (t, 3H), 1.58 (t, 3H), 1.90 (m, 2H), 2.27 (m, 2H), 2.39 (q, 2H), 2.53 (m, 4H), 3.00 (t, 2H), 3.10 (m, 4H), 4.17 (t, 2H), 4.79 (q, 2H), 5.30 (br s, 1H), 6.63 (br s, 1H), 8.62 (s, 1H), 8.81 (s, 1H), 10.74 (s, 1H). LRMS (Thermospray) *m*/*z*: 506.9 $(M+1)^+$.

5.39. 2-{2-Ethoxy-5-[(4-ethyl-1-piperazinyl)sulfonyl]-3-pyridinyl}-7,8,9,10-tetrahydropyrido[2′,1′:5,1]pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (26a)

A mixture of **25a** (1.0 g, 1.98 mmol) and potassium bis(trimethylsilyl)amide (1.20 g, 6.0 mmol) and ethyl acetate (100 μL, 1.0 mmol) in ethanol (80 mL) was heated in a sealed vessel at 130 °C for 18 h. The solvent was removed under reduced pressure, the residue partitioned between dichloromethane and water and the mixture neutralised by the addition of solid CO_2. The layers were separated, the aqueous phase extracted with dichloromethane (2 × 100 mL), and the combined organic extracts dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by column chromatography (dichloromethane and methanol) to afford the title compound (490 mg, 51%). ^{1}H NMR ($CDCl_3$) δ: 1.01 (t, 3H), 1.57 (t, 3H), 2.01 (m, 2H), 2.18 (m, 2H), 2.40 (q, 2H), 2.55 (m, 4H), 3.14 (m, 6H), 4.40 (m, 2H), 4.75 (q, 2H), 8.61 (s, 1H), 9.04 (s, 1H), 10.66 (s, 1H). Anal. calcd for $C_{22}H_{29}N_7O_4S.H_2O$: C, 52.65; H, 6.02; N19.46. Found C, 52.25; H, 6.18; N,19.39.

5.40. 2-{2-Ethoxy-5-[(4-ethyl-1-piperazinyl)sulfonyl]-3-pyridinyl}-10-methyl-7,8,9,10-tetrahydropyrido[2′,1′:5,1]-pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (26b)

The title compound was made by the method of **26a** and the enantiomers were separated by chiral preparative HPLC Chiralpak IA, 50/50 EtOH:MeOH. Anal. calcd for ($C_{23}H_{31}N_7O_4S.H_2O$): C, 53.16; H, 6.40; N, 18.87. Found C, 53.48; H, 6.28; N, 18.76. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.59 (m, 7H), 2.02–2.28 (m, 3H), 2.41 (q, 2H), 2.57 (m, 4H), 3.15 (m, 4H), 3.38 (m, 1H), 4.34 (m, 1H), 4.43 (m, 1H), 4.77 (q, 2H), 8.62 (d, 1H), 9.03 (d, 1H), 10.65 (s, 1H).

5.41. 2-{2-Ethoxy-5-[(4-ethyl-1-piperazinyl)sulfonyl]-3-pyridinyl}-10-methyl-7,8,9,10-tetrahydropyrido[2′,1′:5,1]-pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (26c)

The title compound was made by the method of **26a** and the enantiomers were separated by chiral preparative HPLC Chiralpak IA, 50/50 EtOH:MeOH. Anal. calcd for ($C_{23}H_{31}N_7O_4S.H_2O$): C, 53.16; H, 6.40; N, 18.87. Found C, 53.37; H, 6.24; N, 18.87. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.59 (m, 7H), 2.02–2.28 (m, 3H), 2.41 (q, 2H), 2.57 (m, 4H), 3.15 (m, 4H), 3.38 (m, 1H), 4.34 (m, 1H), 4.43 (m, 1H), 4.77 (q, 2H), 8.62 (d, 1H), 9.03 (d, 1H), 10.65 (s, 1H).

5.42. 2-{5-[(4-Ethyl-1-piperazinyl)sulfonyl]-2-*n*-propoxy-3-pyridinyl}-7,8,9,10-tetrahydropyrido[2′,1′:5,1]pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (27a)

A solution of **25** (250 mg, 0.51 mmol) in n-propanol (20 mL) was heated to reflux, and some solvent allowed to evaporate off. The solution was cooled to room temperature, potassium bis(trimethylsilyl)amide (510 mg, 2.6 mmol) was added, and the reaction heated under reflux for 18 h. The cooled mixture was concentrated under reduced pressure and the residue partitioned between dichloromethane and water. This mixture was neutralised using CO_2 pellets, the layers separated, and the aqueous phase extracted with dichloromethane (3×). The combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by column chromatography (dichloromethane/methanol) to afford the title compound as a solid, (130 mg, 51%). Anal. ($C_{23}H_{31}N_7O_4S.0.5H_2O$): C, 54.10; H, 6.31; N, 19.20. Found C, 54.09; H, 6.27; N, 19.14. ^{1}H NMR (CD_3OD) δ: 1.02 (m,

6H), 1.83 (m, 2H), 2.00 (m, 2H), 2.18 (m, 2H), 2.41 (q, 2H), 2.57 (m, 4H), 3.10 (m, 4H), 4.38 (t, 2H), 4.54 (t, 2H), 8.44 (d, 1H), 8.64 (d, 1H). LRMS (Thermospray): m/z 502 $(M+1)^+$.

5.43. 2-{5-[(4-Ethyl-1-piperazinyl)sulfonyl]-2-(2-methoxyethoxy)-3-pyridinyl}-7,8,9,10-tetrahydropyrido[2′,1′:5,1]pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (27b)

The title compound was obtained as a white solid in 59% yield, from **29a**, by the method of **31a**. Anal. ($C_{23}H_{31}N_7O_5S;0.5H_2O$): C, 52.45; H, 6.12; N, 18.62. Found C, 52.32; H, 6.11; N, 18.54. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.00 (t, 3H), 2.00 (m, 2H), 2.18 (m, 2H), 2.40 (q, 2H), 2.56 (m, 4H), 3.12 (6H, m), 3.57 (s, 3H), 3.84 (t, 2H), 4.40 (t, 2H), 4.78 (t, 2H), 8.60 (d, 1H), 8.98 (d, 1H), 10.80 (s, 1H). LRMS (Thermospray): m/z 518.1 $(MH)^+$.

5.44. 2-{5-[(4-Ethyl-1-piperazinyl)sulfonyl]-2-(2-methoxyethoxy)-3-pyridinyl}-10-methyl-7,8,9,10-tetrahydropyrido[2′,1′:5,1]pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (27c)

The title compound was made by the method and the enantiomers were separated by chiral preparative HPLC Chiralpak IA, 50/50 EtOH:MeOH. Anal. calcd for ($C_{24}H_{33}N_7O_5S.0.5H_2O$): C, 53.32; H, 6.34; N, 18.14. Found C, 53.23; H, 6.31; N, 18.03. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.58 (d, 6H), 2.07 (m, 1H), 2.20 (m, 2H), 2.40 (q, 2H), 2.55 (m, 4H), 3.15 (m, 4H), 3.38 (m, 1H), 3.57 (s, 3H), 3.86 (t, 2H), 4.32 (m, 1H), 4.42 (m, 1H), 4.78 (t, 2H), 8.61 (d, 1H), 8.98 (d, 1H), 10.77 (s, 1H). LRMS (Thermospray): m/z: 532 $(M+1)^+$.

5.45. 2-{5-[(4-Ethyl-1-piperazinyl)sulfonyl]-2-(2-methoxyethoxy)-3-pyridinyl}-10-methyl-7,8,9,10-tetrahydropyrido[2′,1′:5,1]pyrazolo[4,3-*d*]pyrimidin-4(3*H*)-one (27d)

The title compound was made by the method and the enantiomers were separated by chiral preparative HPLC on Chiralpak IA, 50/50 EtOH:MeOH. Anal. calcd for ($C_{24}H_{33}N_7O_5S.H_2O$): C, 52.44; H, 6.42; N, 17.84. Found C, 52.51; H, 6.36; N, 17.93. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H), 1.58 (d, 6H), 2.07 (m, 1H), 2.20 (m, 2H), 2.40 (q, 2H), 2.55 (m, 4H), 3.15 (m, 4H), 3.38 (m, 1H), 3.57 (s, 3H), 3.86 (t, 2H), 4.32 (m, 1H), 4.42 (m, 1H), 4.78 (t, 2H), 8.61 (d, 1H), 8.98 (d, 1H), 10.77 (s, 1H). LRMS (Thermospray): m/z: 532 $(M+1)^+$.

6. PDE5 and PDE6 enzyme inhibitor assays

Phosphodiesterase type 5 was prepared from human corpus cavernosum tissue as previously described. Phosphodiesterase type 6 (cone) was prepared from canine retina. Following dissection, retinas were suspended in cold 20 mM Hepes, 1 mM EDTA, 250 mM sucrose, pH 7.8 buffer, to which a Complete Inhibitor Cocktail tablet (Roche Molecular Biologicals) had been added prior to use. Retinas were homogenized on ice using 5_5-s bursts of an Ultra-Turrax hand-held homogenizer. The homogenate was filtered through two layers of surgical gauze and centrifuged at 100 000*g* for 60 min at 4 °C.

The supernatant was filtered through a 0.22 *ί*m filter and applied to a 1 mL Resource Q anion exchange column equilibrated in 20 mM HEPES, 5 mM $MgCl_2$, 0.2 mM EGTA, pH 7.4, using a Pharmacia FPLC system (Amersham Pharmacia Biotech Ltd, Little Chalfont, UK). The bound protein was washed with 5 column volumes and eluted using a 0–300 mM NaCl linear gradient over 65 mL, followed by a steeper gradient of 300–500 mM NaCl over 10 mL, and a final 1 M NaCl wash for 10 mL. The flow rate was 5 mL/min, and the fractions were 2 mL. Cone PDE6 eluted at approximately 150 mM NaCl. The column fractions comprising the highest level of cGMP-hydrolytic activity (determined as below) were pooled, aliquotted, and stored in liquid nitrogen until use. PDE activity was measured using a scintillation proximity assay (SPA)-based method as previously described. The effect of PDE inhibitors was investigated by assaying a fixed amount of enzyme in the presence of varying inhibitor concentrations and low substrate (cGMP in a 3:1 ratio unlabeled to 3H-labeled at a concentration_1/3*K*m) such that $IC_{50} = Ki$. The final assay volume was made up to 102 *ί*L with assay buffer (20 mM Tris–HCl pH 7.4 at 30 °C, 5 mM $MgCl_2$, 1 mg/mL bovine serum albumin). Reactions were initiated with substrate, incubated for 30–60 min at 30 °C to give <30% substrate turnover, and terminated with 50 *ί*L of yttrium silicate SPA beads (Amersham Pharmacia Biotech Ltd, Little Chalfont, UK). Plates were resealed and shaken for 20 min, after which the beads were allowed to settle for 20 min in the dark and then counted on a TopCount plate reader (Packard, Meridien, CT). Radioactivity units were converted to percent activity of an uninhibited control (100%) and plotted against inhibitor concentration, and inhibitor IC_{50} values were obtained using the 'Fit Curve' Microsoft Excel extension.

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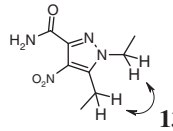

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14. *P*app (cm/s) is apparent permeability coefficient and is calculated using the following equation:
$P\text{app} = (F \times V_D)/SA \times M_D)$
where SA is the surface area for transport, V_D is the donor volume and M_D is the donor amount at $t = 0$. Efflux ratio is the ratio of secretory flux to absorptive flux.