A Novel Series of Potent and Selective PDE5 Inhibitors with Potential for High and Dose-Independent Oral Bioavailability†

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Sildenafil (5-[2-ethoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one), a potent and selective phosphodiesterase type 5 (PDE5) inhibitor, provided the first oral treatment for male erectile dysfunction. The objective of the work reported in this paper was to combine high levels of PDE5 potency and selectivity with high and dose-independent oral bioavailability, to minimize the impact on the C_{max} of any interactions with coadministered drugs in the clinic. This goal was achieved through identification of a lower clearance series with a high absorption profile, by replacing the 5′-piperazine sulfonamide in the sildenafil template with a 5′-methyl ketone. This novel series provided compounds with low metabolism in human hepatocytes, excellent caco-2 flux, and the potential for good aqueous solubility. The in vivo oral and iv pharmacokinetic profiles of example compounds confirmed the high oral bioavailability predicted from these in vitro screens. 5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**2**) was selected for progression into the clinic.

Introduction

The launch of sildenafil (Figure 1) as the first oral treatment for male erectile dysfunction revolutionized the treatment of this disease.[1] Sildenafil is a potent and selective inhibitor of phosphodiesterase type 5 (PDE5), the predominant isozyme responsible for the metabolism of cGMP in the corpus cavernosum of the penis. Following sexual stimulation, nitric oxide is released from the nonadrenergic, noncholinergic nerves within the penis, which activates guanylate cyclase to form cGMP via cyclization of GTP. This increase in intracellular cGMP leads to smooth muscle relaxation, which enables blood to flow into the penis and hence erection to occur. Hydrolysis of cGMP to GMP by PDE5 reverses this process, restoring muscle tone with the consequential loss of erection.[2] Administration of sildenafil inhibits PDE5 activity and therefore slows cGMP breakdown. This enhances the action of nitric oxide and cGMP, and facilitates penile erection in individuals suffering from male erectile dysfunction (MED).

Following the development of sildenafil, we sought PDE5 inhibitors with greater selectivity over phosphodiesterase type 6 (PDE6), since this enzyme is believed to be responsible for the low incidence of adverse visual events, such as abnormalities in color vision, associated with high doses of sildenafil. SAR describing how this selectivity was achieved through the discovery of the pyridyl methyl analogue **1** (Figure 1) has already been published.[3] The objective for the current program was to identify a potent and selective PDE5 inhibitor with high and dose-independent oral bioavailability, to minimize the impact on C_{max} of any interactions with coadministered drugs. In general, good solubility, high absorption across the intestinal wall, and low first-pass clearance are required for high oral bioavailability.[4] We focused on reducing the first-pass clearance of soluble, rule-of-five compliant[5] PDE5 inhibitors to achieve this objective.

The piperazine sulfonamide group is the primary site of metabolism in the sildenafil series.[6] Consequently, we concentrated on finding a more metabolically stable replacement for this functionality. This led to a novel series of potent and selective PDE5 inhibitors, with a methyl ketone at the 5′-position of the 5-(2-alkoxy-3-pyridinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one template. These compounds are relatively small (MW < 500) with moderate lipophilicity, and this results in good oral absorption. In addition, they have low clearance (the predominant metabolite in human hepatocytes being the secondary alcohol) and a basic group attached at the N2 position, which provides good aqueous solubility via salt formation. The combination of these factors leads to high oral bioavailability in vivo. In particular, compound **2** was shown to have the potential for high and dose-independent oral absorption in man and was therefore progressed to the clinic to determine its potential as an oral agent for the treatment of MED. This paper explains the key results that led to its selection.

Chemistry

Initially, the 5′-methyl ketone functionality was introduced into the pyrazolopyrimidinone template via mercury-mediated hydrolysis of an acetylene (Scheme 1). The key intermediate, 5-iodo-2-propoxynicotinic acid **4**, synthesized via iodination of 2-propoxynicotinic acid **3**,[7] was converted to the acid chloride and then coupled with 4-amino-5-ethyl-1-(2-pyridinylmethyl)-1*H*-pyrazole-3-carboxamide.[8] Subsequent cyclization with potassium bis(trimethylsilyl)amide in *n*-propanol yielded the pyrazolopyrimidinone template **6**. The acetylene functionality was then introduced via a Sonagashira reaction with (trimethyl-

† Coordinates have been deposited for the structure of the complex of compound **2** with the catalytic domain of PDE5. PDB ID: 2CHM.

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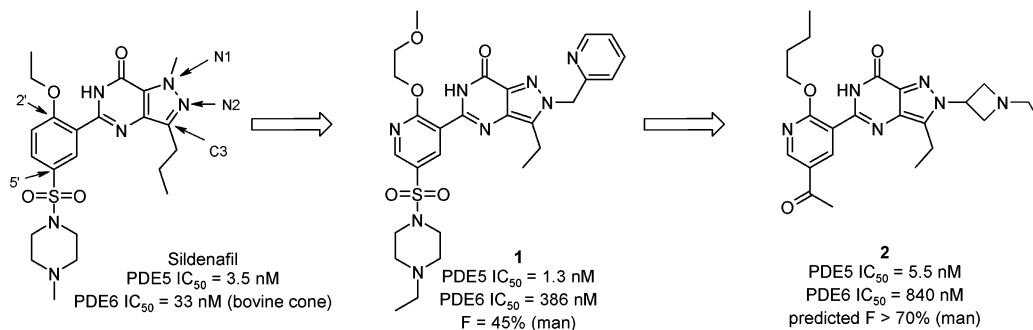


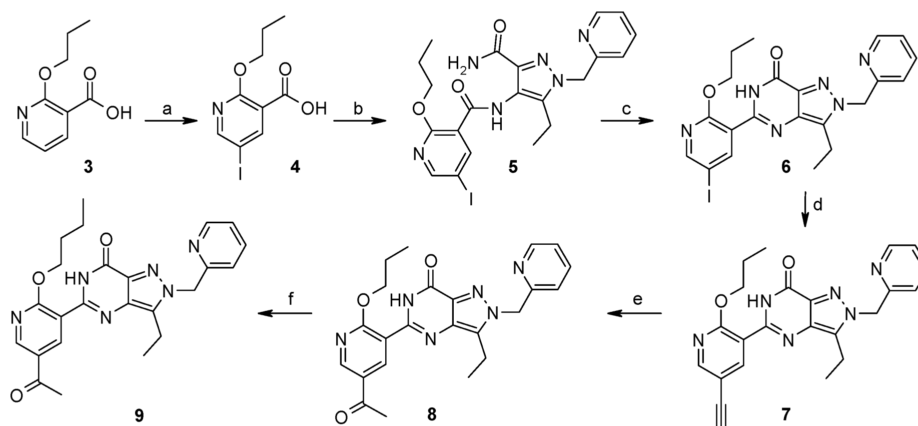
Figure 1. Discovery of 5′-ketone series.

silyl)acetylene,[9] followed by deprotection with potassium fluoride. The acetylene intermediate **7** was hydrolyzed under acidic conditions in the presence of mercury sulfate to yield the methyl ketone **8**. Finally, the 2′-butoxy analogue **9** was synthesized by heating compound **8** in butanol in the presence of potassium bis(trimethylsilyl)amide. The reaction was low yielding due to competing Meerwein reduction[10] of the methyl ketone.

The N2-morpholinoethyl analogue **16** was synthesized by a similar route (Scheme 2). In this case, 4-amino-5-ethyl-1*H*-pyrazole-3-carboxamide[8] was coupled with 2-butoxy-5-iodonicotinic acid **11** and then the N2-substituent was introduced regioselectively via alkylation with *N*-(2-chloroethyl)morpholine.

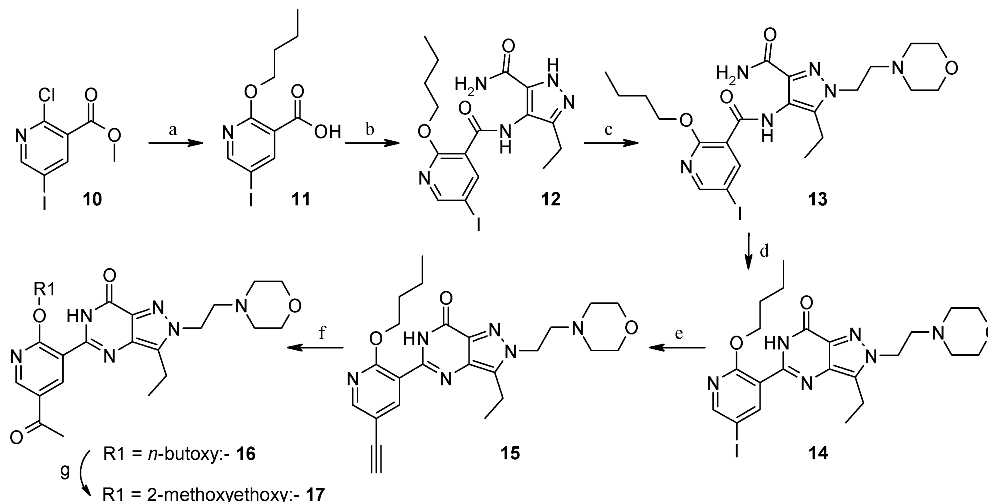
Some of the N2-azetidinyl and N2-piperidinyl analogues were also synthesized by a similar route (Scheme 3). Acidic hydrolysis of the acetylene functionalities in compounds **23**/**24** led to partial BOC deprotection, which was then completed by stirring the crude product in trifluoroacetic acid. Alkyl substituents were then introduced onto the azetidine or piperidine nitrogens via reductive amination. This worked well with formaldehyde and

Scheme 1. Synthesis of 5′-Ketones Using Sonagashira Methodology



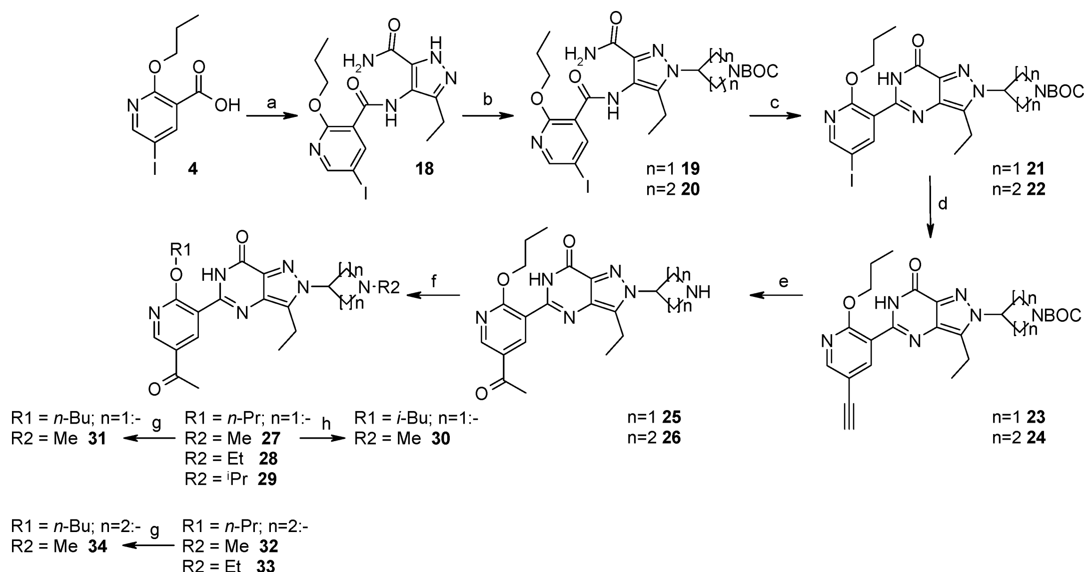
Reagents and conditions*:* (a) NIS, TFA, TFAA, reflux; (b) $(COCl)_2$, CH_2Cl_2, DMF, 0 °C to rt, then 4-amino-5-ethyl-1-(2-pyridinylmethyl)-1*H*-pyrazole-3-carboxamide, pyridine, DCM, rt; (c) KHMDS, *n*-propanol, reflux; (d) (i) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, triethylamine, MeCN, 60 °C; (ii) KF, DMF, H_2O, rt; (e) H_2SO_4, $HgSO_4$, Me_2CO, reflux; (f) KHMDS, *n*-BuOH, 90 °C.

Scheme 2. Synthesis of N2-Morpholinoethyl Analogues



Reagents and conditions*:* (a) KHMDS, *n*-BuOH, THF 0 °C to rt; (b) $(COCl)_2$, CH_2Cl_2, DMF, 0 °C to rt, then 4-amino-5-ethyl-1*H*-pyrazole-3-carboxamide, pyridine, DCM, rt; (c) *N*-(2-chloroethyl)morpholine hydrochloride, Cs_2CO_3, DMF, 80 °C; (d) KHMDS, *n*-BuOH, reflux; (e) (i) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, triethylamine, MeCN, 60 °C; (ii) KF, DMF, H_2O, RT; (f) H_2SO_4, $HgSO_4$, Me_2CO, reflux; (g) KHMDS, 2-methoxyethanol, 120 °C.

Scheme 3. Synthesis of N2-Azetidinyl and N2-Piperidinyl Analogues



Reagents and conditions*:* (a) $(COCl)_2$, CH_2Cl_2, DMF, 0 °C to rt, then 4-amino-5-ethyl-1*H*-pyrazole-3-carboxamide, pyridine, DCM, rt; (b) *tert*-butyl-3-iodo-1-azetidinecarboxylate (**19**) or *tert*-butyl 4-[(methylsulfonyl)oxy]-1-piperidinecarboxylate (**20**), Cs_2CO_3, DMF, 100 °C; (c) KHMDS, *n*-PrOH, reflux; (d) (i) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, triethylamine, MeCN, 60 °C; (ii) KF, DMF, H_2O, rt; (e) H_2SO_4, $HgSO_4$, Me_2CO, reflux, then trifluoracetic acid, CH_2Cl_2, rt; (f) HCHO (**27**/**32**) or Me_2CO (**29**), $Na(OAc)_3BH$, CH_2Cl_2, rt or MeCHO, $NaCNBH_3$, NaOAc, MeOH, rt (**28**/**33**); (g) Cs_2CO_3, *n*-BuOH, reflux; (h) Cs_2CO_3, i-BuOH , reflux.

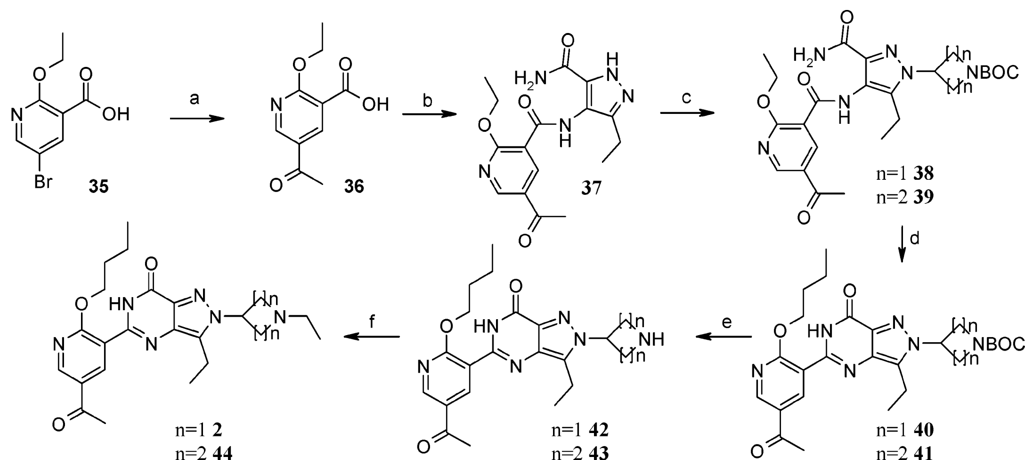
acetone but led to low-yielding reactions with acetaldehyde. As with the synthesis of compound **9**, the 2′-alkoxy substituent could then be varied by performing an exchange reaction in the presence of the required alcohol and cesium carbonate. Cesium carbonate was preferable to potassium bis(trimethylsilyl)amide for the exchange reactions, since it minimized Meerwein reduction of the ketone.

The routes outlined above allowed rapid exploration of SAR, since the versatile 5′-iodo group enabled the introduction of a wide range of functionality at this position (although only 5′-ketones are discussed in this paper). Once the 5′-methyl ketone substituent had been identified as yielding potent, selective PDE5 inhibitors with high bioavailability, we investigated alternative routes directly to this series. Therefore, an alternative route was designed that involved earlier introduction of the 5′-methyl ketone functionality.

A Heck reaction between 5-bromo-2-ethoxynicotinic acid **35** and butyl vinyl ether yielded the key methyl ketone intermediate **36**[11] (Scheme 4). This was coupled with 4-amino-3-ethyl-1*H*-pyrazole-5-carboxamide,[8] and the resulting amide **37** was alkylated to introduce the desired N2 substituents **38**/**39**. Subsequent cyclization with cesium carbonate in *n*-butanol at reflux was accompanied by concomitant 2′-ether exchange to yield the pyrazolopyrimidinones **40**/**41**. BOC deprotection with trifluoroacetic acid followed by either reductive amination or alkylation gave the target compounds **2**/**44**. An identical route, starting from 4-amino-3-propyl-1*H*-pyrazole-5-carboxamide,[12] was followed to synthesize the C3-propyl N2-azetidine analogues **45/46** discussed in Table 3 (see Experimental Section for synthetic details).

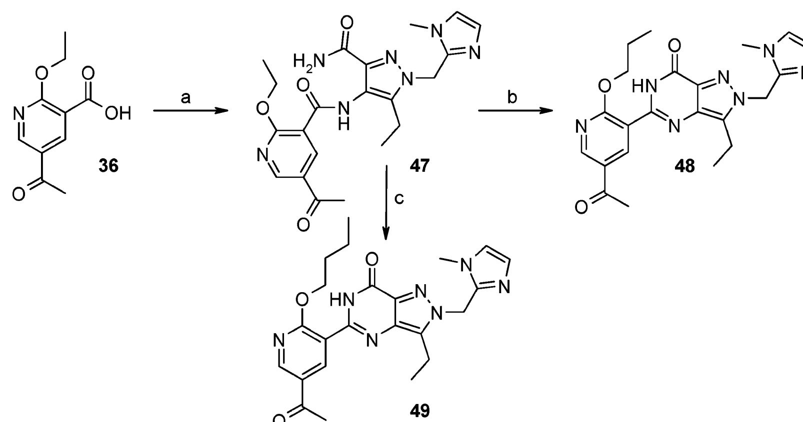
The N2-methylimidazole analogues **48**/**49** were synthesized by a similar procedure, in which the 5-acetyl-2-ethoxynicotinic acid **36** was coupled with 4-amino-5-ethyl-1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-1*H*-pyrazole-3-carboxamide.[7] The cyclization reaction of intermediate **47** with simultaneous 2′-

Scheme 4. Synthesis of 5′-Ketones Using Heck Methodology



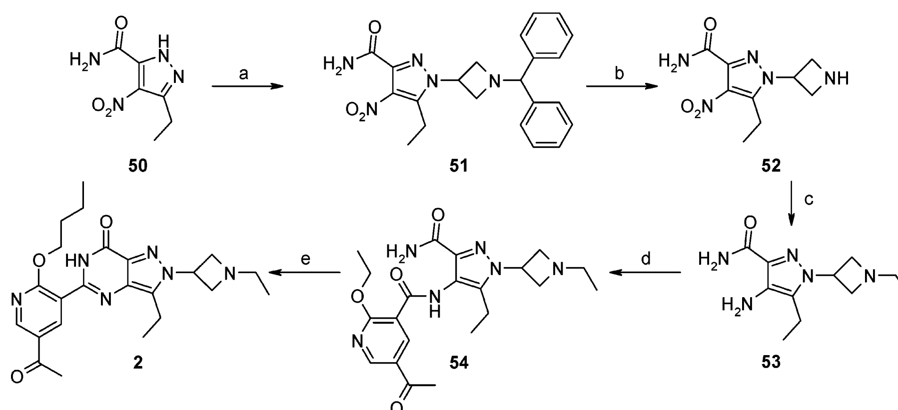
Reagents and conditions*:* (a) butyl vinyl ether, $Pd(OAc)_2$, tri-*o*-tolylphosphine, Et_3N, Na_2CO_3, MeCN, reflux, (65%); (b) 4-amino-3-ethyl-1*H*-pyrazole-5-carboxamide, HATU, CH_2Cl_2, rt; (c) *tert*-butyl-3-iodo-1-azetidinecarboxylate (**38**) or 1-(*tert*-butoxycarbonyl)-4-piperidinyl methanesulfonate (**39**), Cs_2CO_3, DMF, 100 °C; (d) Cs_2CO_3, *n*-BuOH, molecular sieves, reflux; (e) trifluoroacetic acid, CH_2Cl_2, rt; (f) EtI, K_2CO_3, MeCN, 45 to 50 °C.

Scheme 5. Synthesis of N2-Methylimidazole Analogues



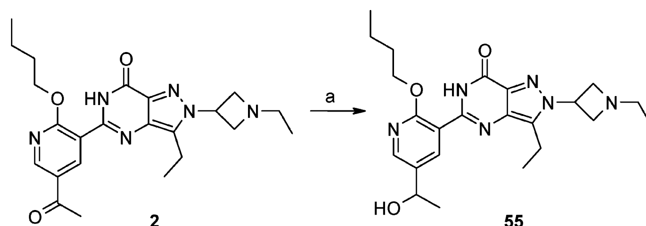
Reagents and conditions: (a) 4-amino-5-ethyl-1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-1*H*-pyrazole-3-carboxamide, HATU, CH_2Cl_2, rt; (b) Cs_2CO_3, *n*-PrOH, molecular sieves, reflux; (c) Cs_2CO_3, *n*-BuOH, molecular sieves, reflux.

Scheme 6. Optimized Synthetic Route to N2-Azetidinyl Metabolite



Reagents and conditions: (a) 1-benzhydryl-3-azetidinyl methanesulfonate, Na_2CO_3, NaI, THF/H_2O, reflux; (b) 1-chloroethyl chloroformate, CH_2Cl_2, then MeOH, reflux; (c) (i) MeCHO, $Na(OAc)_3BH$, triethylamine, DCM, MeOH, 0 °C to rt; (ii) H_2 (60 psi), 10% Pd/C, EtOH, rt; (d) **36**, 1,1′-carbonyldiimidazole, EtOAc, reflux; (e) Cs_2CO_3, *n*-BuOH, reflux.

Scheme 7. Synthesis of Alcohol Metabolite



Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C to rt.

exchange was performed in *n*-propanol to give **48** and in *n*-butanol to give **49** (Scheme 5).

To supply bulk quantities of **2** to support our development program, we were keen to investigate a more convergent route, which avoided the use of the potential carcinogen ethyl iodide (Scheme 6). Therefore, the nitro pyrazole intermediate **50**[8] was alkylated with 1-(diphenylmethyl)-3-azetidinylmethanesulfonate to give a 2:3 ratio of N1:N2 regioisomers.[13] The N2 regioisomer **51** was separated by trituration and deprotected with 1-chloroethyl chloroformate to yield compound **52**. The ethyl group was then cleanly introduced by reductive amination with acetaldehyde, and the nitro group was reduced to yield amine **53**. Addition of amine **53** to the acyl imidazole derivative of 5-acetyl-2-ethoxynicotinic acid **36** yielded amide **54**, which then underwent cyclization and simultaneous 2′-exchange to yield the target compound **2**.

The alcohol metabolite of **2** was synthesized by reduction of the ketone with sodium borohydride, to yield compound **55** (Scheme 7).

Results and Discussion[14]

Background. Since the development of sildenafil, extensive effort has been focused on identifying PDE5 inhibitors with greater selectivity over PDE6, since this isozyme is responsible for the low incidence of visual disturbances reported with high doses of sildenafil. Previous publications have outlined how greater selectivity was introduced into the sildenafil template by replacing the phenylsulfonamide with pyridylsulfonamide, varying the 2′-substituent, and introducing N-1 or N-2 pyrazole substituents. This led to the development of compound **1** (Figure 1), which is a potent PDE5 inhibitor displaying approximately 300-fold selectivity over canine cone PDE6.[3] In clinical trials, compound **1** showed dose-dependent increases in C_{max} and AUC over the dose range 1−800 mg and increases in these parameters when coadministered with ketoconazole (a potent inhibitor of P-glycoprotein and CYP3A4).[15] Further studies showed that compound **1** is a substrate for human P-glycoprotein and CYP3A4, which explains the variations in pharmacokinetic data that arose when these species were either saturated or inhibited. As a consequence of these clinical data, we desired a PDE5 inhibitor with high and dose-independent oral bioavailability, since this would minimize the impact of any interactions with coadministered drugs in the clinic.

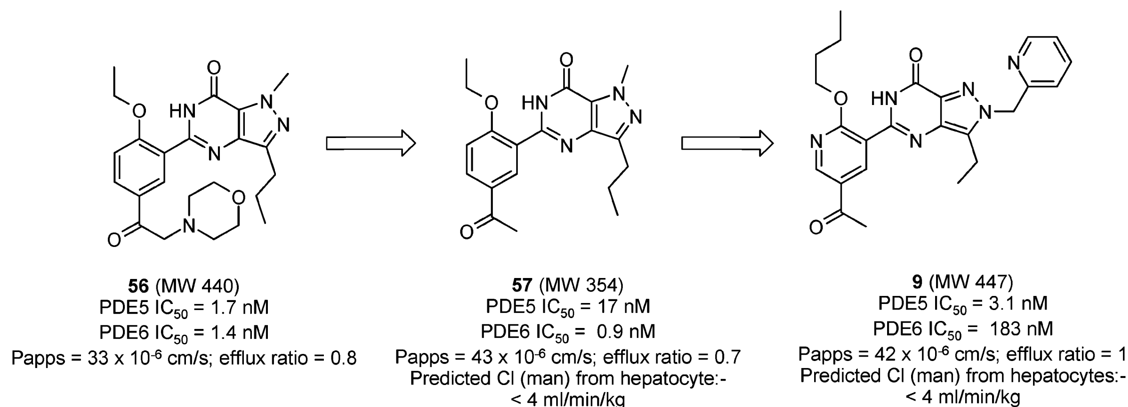


Figure 2. Discovery of 5′-methyl ketone series.

The high oral bioavailability of a compound is generally a result of good solubility, high absorption across the intestinal wall, and low first-pass hepatic extraction.[4] Measured solubilities were used as a filter for our compound selection. Caco-2 monolayers were used to predict human intestinal absorption potential.[16] A high absorptive flux ($P_{app} > 10 \times 10^{-6}$ cm/s)[17] and an efflux ratio close to unity across the concentration range 10−100 μM should ensure that concentration dependence is avoided in man.[15] The metabolism of compounds by human hepatocytes was used as an in vitro prediction of first-pass clearance.[18] These in vitro assays were used to select compounds to profile in vivo. In addition, we only synthesized rule-of-five-compliant compounds to maximize our chances of achieving good oral absorption.[5] With these measures in place, we then focused on reducing first-pass clearance. Since the predominant route of clearance for most sildenafil analogues is cytochrome P-450-mediated metabolic oxidation of the piperazine ring by CYP3A4,[6] we decided to remove this functionality from the template and replace it with a less metabolically vulnerable group.

Discovery of Selective 5′-Ketone Series. An early analogue of sildenafil, compound **56**, contained a ketone at the 5′-position (Figure 2). This compound was a potent PDE5 inhibitor, with an excellent pharmacokinetic profile in man (oral bioavailability > 80%, clearance = 5 mL/min/kg), but since it displayed no in vitro selectivity advantage over sildenafil, it was not progressed further.[19] On the basis of the pharmacokinetic profile of this compound in man, we felt that low clearance and high bioavailablity was achievable in other 5′-ketone series. To introduce PDE6 selectivity into this template, we planned to use the SAR developed in the sildenafil series (outlined above), including the introduction of N-2 substituents. However, before undertaking this work, we were keen to find the minimal pharmacophore required for PDE5 potency in the 5′-ketone series, to ensure that the molecular weight of compounds did not exceed 500.[5] Therefore, we profiled compound **57**, the 5′-methyl ketone analogue of compound **56**, and found that it retained moderate PDE5 potency.[19] This compound provided a low molecular weight, potent template onto which N-2 substituents and larger 2′-groups could be introduced with the aim of enhancing potency and selectivity, while maintaining good Caco-2 flux.

First, we replaced the phenyl ring for a pyridyl and introduced a N-2 pyridylmethyl group. Both of these changes had previously led to higher in vitro selectivity in the sildenafil series (**1**, Figure 1).[3] In addition a 2′-butoxy substituent was introduced, since SAR predicted that this would lead to an increase in potency and selectivity.[3] The resulting compound **9** displayed excellent levels of PDE5 potency and PDE6 selectivity, with Caco-2 cell flux indicative of complete oral absorption (Figure 2). In vitro human hepatocyte stability data predicted this compound would have low first-pass clearance. As expected, compound **9** displayed low total clearance in the dog iv PK study, predictive of 84% bioavailability (Table 1).

Compound **9** showed that low first-pass clearance was achievable within this methyl ketone series. However, the subsequent oral PK study in dog showed the compound to have variable oral bioavailability, due to its poor aqueous solubility (3 μg/mL at pH 7.2). Hence, we decided to introduce more basic substituents at the N-2 position to enable the synthesis of more soluble salt forms (Table 2).

The SAR at the 2′-position (R1) follows that seen in the sildenafil series, with the butoxy substituent giving rise to more potent and selective compounds than the lower alkoxy groups. A small subset of analogues in the C3 propyl series were also synthesized, but since these offered little potency or selectivity advantages over the C3 ethyl series and were more lipophilic, no further analogues were made (Table 3).

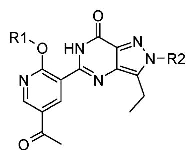
The most potent and selective analogues from Table 2 were profiled through the in vitro pharmacokinetic assays described earlier (Table 4). The morpholine-containing analogue **16** displayed low solubility, and its weakly basic center did not lend itself to salt formation; hence, this compound did not meet our criteria. The imidazole containing analogue **49** showed an unacceptably high rate of metabolism in human hepatocytes, and a low P_{app} value (which may have been due to its low solubility); therefore, this compound was not progressed for future studies. The more basic amine derivatives **29**, **2**, and **44** all displayed low rates of metabolism in human hepatocytes, P_{app} values predictive of complete oral absorption, and adequate

Table 1. Pharmacokinetic Parameters for Compound 9 Administered Intravenously to Dog (mean values for $n = 2$)

Dose (mg/ kg)	$T_{1/2}$ (h)	Vd (L/Kg)	Cl (mL/min/kg)	Tmax (h)	PPB[a] (%)
0.43	5	3	8	2	92

[a] PPB = plasma protein binding.

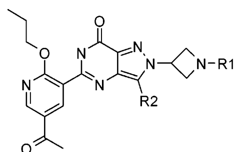
Table 2. SAR for 5′-Methyl Ketones with Basic N2 Substituents



Ex.	R1	R2	PDE5 IC_{50} (nM)	PDE5/6 Selectivity	Ex.	R1	R2	PDE5 IC_{50} (nM)	PDE5/6 Selectivity
48	*n*Pr		2.7	58x	**16**	*n*Bu		2.6	74x
25	*n*Pr		34	55x	**49**	*n*Bu		0.9	142x
27	*n*Pr		9.5	69x	**42**	*n*Bu		21	29x
28	*n*Pr		7.1[a]	61x	**31**	*n*Bu		6.4	43x
29	*n*Pr		5.2	60x	**2**	*n*Bu		5.5	153x
26	*n*Pr		7.0	53x	**43**	*n*Bu		18	57x
32	*n*Pr		12	37x	**34**	*n*Bu		9.1	77x
33	*n*Pr		13	18x	**44**	*n*Bu		3.6	350x
30	*i*Bu		8.2	84x	**17**	$MeOCH_2CH_2$		53	58x

[a] Platelet PDE5 enzyme used.

Table 3. Comparison of SAR for C3 Ethyl and C3 Propyl Methyl Ketones



Ex.	R1	R2	logD	PDE5 IC_{50} (nM)	PDE5/6 Selectivity
25	H	Et	0.3	34.4	55x
45	H	*n*Pr	0.6	18.2	26x
27	Me	Et	1.2	9.5	69x
46	Me	*n*Pr	1.5	14.7	30x

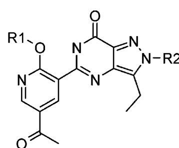
solubility (with the potential to prepare more soluble salt forms). Therefore, since these compounds also displayed low rates of metabolism in dog liver microsomes, they were progressed for dog iv and oral pharmacokinetic studies.

Compound **44** displayed a higher total clearance in dog than the other compounds, which was surprising considering its lower lipophilicity and stability in human hepatocytes (Table 5). Therefore compound **44** was less likely to achieve an oral bioavailability of greater than 75% in man. Both compounds **29** and **2** displayed low total clearances and oral bioavailabilities greater than 75%, with half-life values commensurate with oral, pro re nata dosing.

On the basis of the dog pharmacokinetic studies and the resulting predicted human pharmacokinetic parameters, it was felt that both compounds **29** and **2** were suitable for progression into the clinic. On the basis of clinical data from previous PDE5 inhibitor development candidates, the level of selectivity seen with these compounds will prevent visual disturbances from occurring at all clinically relevant doses. Due to the greater selectivity of compound **2** over canine retinal cone PDE6, this compound was progressed at highest priority. In human hepatocytes, the predominant metabolite from compound **2** was the secondary alcohol **55**,[21] formed by ketone reductase. This secondary alcohol is oxidized back to the 5′-ketone in hepatocytes, so the parent ketone and its alcohol metabolite exist in equilibrium.[22] Some CYP3A4 metabolism was also observed, giving rise to the des-alkyl compound **42** as a minor metabolite (Table 2). Ketone reductase is present in the cytosol in the liver; therefore, the human hepatocyte assay provided us with a good prediction of rate of clearance via this route as well as via cytochrome P-450-mediated metabolism. The alcohol metabolite **55** and the des-alkyl metabolite **42** both showed significant decreases in PDE5 and PDE6 potency, which will prevent any problems associated with active circulating metabolites (Figure 3 and Table 2).

Absorption Profile of Compound 2. To determine whether compound **2** would display the dose-independent oral absorption desired in man, its Caco-2 flux was measured across the

Table 4. In Vitro Pharmacokinetic Data on Key 5′-Methyl Ketones



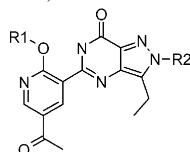
Ex.	R1	R2	MW	LogD (pH7.4)	DLM (min.)	Pred. Cl man (mL/min/kg)[a]	P_{app} (e..r.)[b] (x10^{-6}cm/s)	Solubility[1] (mg/mL)
16	*n*Bu		469	2.4	>120	<4	37 (1.0)	< 0.01 (pH 7.3)
49	*n*Bu		450	2.6	>120	18	5 (1.6)	0.006 (pH 6.95) diHCl salt
29	*n*Pr		439	2.5	>120	5	28 (0.5)	0.17 (pH 7.3) Free base
2	*n*Bu		439	2.8	95	5	30 (0.9)	0.39 (pH 7.4) Free base
44	*n*Bu		467	1.8	>120	5	53 (1.0)	1.10 (pH 7.6) Free base

[a] Predicted from hepatocytes. [b] e.r. = efflux ratio.

concentration range 10−100 μM (Figure 4). For the Pgp substrate **1**, the absorptive flux increased, and hence the efflux ratio decreased, both as the compound concentration increased (due to saturation of the P-glycoprotein efflux mechanism) and in the presence of the P-glycoprotein inhibitor ketoconazole. Hence, when compound **1** was coadministered with ketoconazole in phase II clinical trials, this variability in absorption combined with the expected inhibition of CYP3A4 led to significant increases in C_{max} and AUC.[15] For compound **2**, the absorptive flux in the Caco-2 cell model remained consistently high across the concentration range, and also in the presence of ketoconazole. This predicts that compound **2** will display high and dose independent oral absorption in man. These data are consistent with Lipinski's predictions for good oral absorption, since compound **2** meets all of the rule-of-five criteria. Compound **1**, on the other hand, exceeds the MW rule, and the corresponding increase in molecular size may be the cause of variability in its absorption profile.

Rationale for PDE6 Selectivity of Compound 2. The SAR shows that potent and selective PDE5 inhibitors can be achieved with either basic alkyl or heteroaryl N-2 pyrazole substituents, suggesting that these groups may be pointing out toward bulk solvent rather than making any specific interactions with the PDE5 enzyme as detailed in the X-ray crystal structures of PDE5

Table 5. Dog Pharmacokinetic Data on Key 5′-Methyl Ketones (n = 2)



Ex.	R1	R2	Dose (mg/kg)	$T_{1/2}$ (h)	F (%)	Vd (L/Kg)	Cl (mL/min/kg)	Tmax (h)	PPB (%)
29	*n*Pr		0.5	7.2	91	7.80	13.0	1.9	66
2	*n*Bu		0.5	9.0	76	13.4	17.5	1.4	62
44	*n*Bu		0.5	6.7	_	22	38	_	60

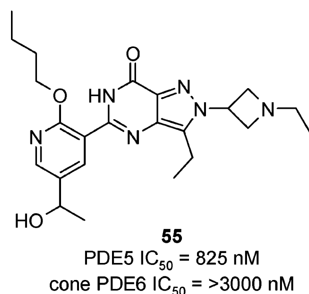


Figure 3. Biological activity of alcohol metabolite.

catalytic domain in complex with inhibitors of this class.[23] The simple addition of a methyl at the N2 position of the series causes a complete "flip" in binding mode compared to sildenafil and helps explain the change in PDE6 selectivity for this N2 substituted series (Figure 5).

The new binding mode adopted by compound **2** clearly makes different interactions with the catalytic site of PDE5 (Figure 6). The major potency-gaining hydrophobic interactions with the Phe820 and Phe376 are maintained, but the hydrogen-bonding pattern with the conserved glutamine Gln817 is changed, and access to a deep hydrophobic pocket (the 2′ substituent pocket) occurs from a different angle. In addition, the 5′-ketone makes an additional hydrogen bond to Gln775, which stabilizes interactions with the absolutely conserved Gln817. As a result of the flipped binding mode adopted by compound **2**, there is an associated shift in the side chain of the Leu804 residue flanking the deep 2′-alkoxy pocket, which enables formation of significant interactions between the inhibitor and Leu804 in PDE5. This interaction does not occur in PDE6, since the Leu804 present in PDE5 is replaced by methionine in PDE6, explaining the increased PDE6 selectivity displayed by compounds adopting the "flipped" binding mode.

Conclusions

In this paper we have reported the discovery of a novel series of PDE5 inhibitors, with excellent in vitro potency for corpus cavernosum PDE5 and selectivity over canine retinal cone PDE6. Replacement of the piperazine sulfonamide functionality in sildenafil with a metabolically more stable methyl ketone led to a series of PDE5 inhibitors with low clearance and dose-independent oral absorption. The predicted high and dose-independent bioavailability for compound **2** should ensure that the impact on C_{max} of any interactions with coadministered drugs in the clinic are minimized. Further data on this compound will be reported in due course.

Figure 5. Crystallographic overlay of structures of sildenafil (green C atoms) and compound **2** (purple C atoms).

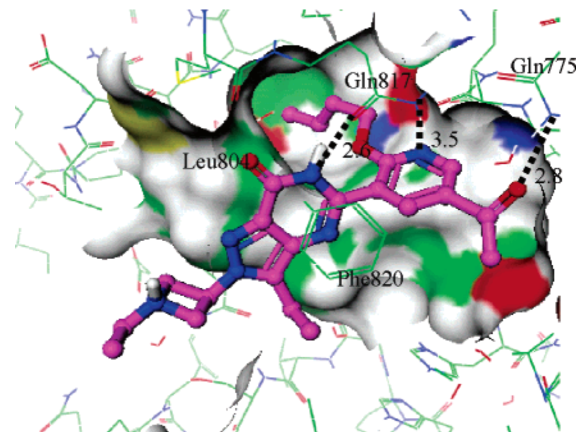


Figure 6. Crystal structure of compound **2** in the active site of the PDE5 catalytic domain.

Experimental Section

Chemistry. 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 pre-coated Merck silica gel (60 F254) plates or Macherey-Nagel Polygram Si G/UV (0.2 mm silica gel) plates. Melting points are uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using a Varian Unity Inova-300, a Varian Unity Inova-400, or a Varian Mercury-400 spectrometer and were in all cases consistent with the proposed structures. 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. High-resolution mass spectra were obtained with a Bruker Apex II FTICR-MS with a 4.7 T magnet using Xmass software. Combustion analyses were conducted by Exeter Analytical

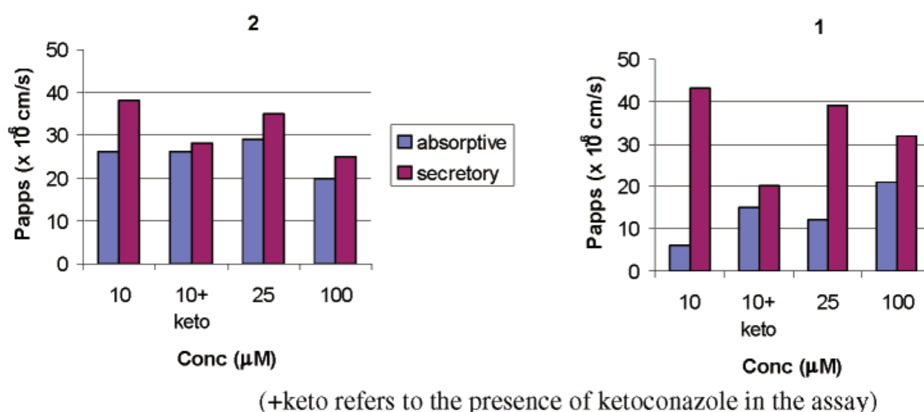


Figure 4. Caco-2 flux of compounds **1** and **2** at increasing concentrations.

U.K. Ltd., Uxbridge, Middlesex. NOE refers to nuclear Overhauser effect, which was recorded using a Varian Inova 500 MHz spectrometer. Other abbreviations are used in conjunction with standard chemical practice.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(2-pyridinylmethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9). To a solution of compound **8** (210 mg, 0.49 mmol) in *n*-butanol (20 mL) was added KHMDS (291 mg, 1.49 mmol). The reaction mixture was heated at 90 °C for 48 h and then concentrated in vacuo. The reaction mixture was then dissolved in ethyl acetate (50 mL), washed with saturated sodium bicarbonate solution (20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated in vacuo. Trituration from ether, followed by flash column chromatography (eluting with 99:1 EtOAc:MeOH), yielded the target compound as a white solid (88 mg, 0.20 mmol, 41%): LRMS (*m/z*) (TSP^+) 447.9 (MH^+). ($C_{24}H_{26}O_3N_6$•$0.25H_2O$•0.1EtOAc) C, H, N.

5-[5-Acetyl-2-(2-methoxyethoxy)-3-pyridinyl]-3-ethyl-2-[2-(4-morpholinyl)ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (17) was prepared in 46% yield from the butoxy analogue **16** using 2-methoxyethanol as solvent and heating to 120 °C, following a procedure similar to that described for compound **9**: LRMS (*m/z*) (TSP^+) 471.1 (MH^+). Anal. ($C_{23}H_{30}O_5N_6$•$0.25H_2O$) C, H, N. Irradiation of the pyrazolopyrimidinone C3-CH_2CH_3 and pyrazole $NCH_2CH_2NR_2$ showed mutual NOE enhancements, confirming the regiochemistry.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-[2-(4-morpholinyl)-ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (16). Sulfuric acid (1 N, 0.25 mL) was added to a stirred solution of **15** (78.0 mg, 0.17 mmol) in acetone (5 mL) at room temperature. Mercury sulfate (8 mg, 0.03 mmol) was added and the mixture heated at reflux for 4 h. Further aliquots of mercury sulfate (8 mg, 0.03 mmol), 1 N sulfuric acid (0.25 mL), and acetone (5 mL) were then added, and the reaction mixture was heated at reflux for a further 4 h before being left at room temperature overnight. The reaction mixture was concentrated in vacuo, and the residue partitioned between ethyl acetate (10 mL) and saturated sodium bicarbonate solution (10 mL). The organic layer was separated and the aqueous layer was extracted further with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by flash column chromatography (eluting with 100:0 to 99:1 EtOAc:MeOH) yielded the target compound as a white solid (48 mg, 0.10 mmol, 59%): LRMS (*m/z*) (ES^+) 469 (MH^+). Anal. ($C_{24}H_{32}O_4N_6$•$0.25H_2O$•$0.2Et_2O$) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-[(1-methyl-1*H*-imidazol-2-yl)methyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (48) was prepared in 31% yield from the amide **47** using *n*-propanol as solvent, following a procedure similar to that described for compound **2** (route B): mp 243.1−245.0 °C; LRMS (*m/z*) (TSP^+) 436.1 (MH^+). Anal. ($C_{22}H_{25}O_3N_7$•$0.7H_2O$) C, H, N.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-[(1-methyl-1*H*-imidazol-2-yl)methyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (49) was prepared in 25% yield from the amide **47** using *n*-butanol as solvent, following a similar procedure to that described for compound **2** (route B) : mp 251.7−253.0 °C; LRMS (*m/z*) (TSP^+) 450.2 (MH^+). Anal. ($C_{23}H_{27}O_3N_7$•$0.3H_2O$) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-2-(3-azetidinyl)-3-ethyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (25). Sulfuric acid (1 N, 6 mL) was added to a stirred solution of acetylene **23** (1.44 g, 3.01 mmol) in acetone (50 mL) at room temperature. Mercury sulfate (268 mg, 0.90 mmol) was added and the mixture heated at reflux for 6 h. The reaction mixture was poured into saturated aqueous sodium bicarbonate solution (20 mL). The aqueous was then extracted with DCM (6 × 20 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), and concentrated in vacuo to yield a brown oil. The oil was dissolved in 40% TFA in DCM and stirred at room temperature for 1 h. The reaction mixture was then concentrated in vacuo, and the crude product was purified by flash column chromatography (eluting with 95:5:1−80:20:1 CH_2Cl_2:MeOH:0.88 N NH_3) to yield the trifluoroacetate salt of the title compound as a white, hygroscopic foam (1.65 g), which was taken onto the next stage crude: LRMS (*m/z*) (TSP^+) 397.5 (MH^+). Anal. ($C_{20}H_{24}O_3N_6$•$2.0H_2O$•TFA) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-methyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (27). Formaldehyde (400 μL of a 37−41% aq solution, 5.04 mmol) was added to a stirring solution of amine **25** (500 mg, 1.26 mmol) in DCM (30 mL). Sodium triacetoxyborohydride (670 mg, 3.15 mmol) was added after 15min. After a further 30 min the reaction mixture was diluted with DCM (15 mL) and washed with saturated aqueous sodium bicarbonate solution (15 mL). The layers were separated, and the aqueous layer was extracted further with DCM (15 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by flash column chromatography (eluting with 95:5:1 CH_2Cl_2:MeOH:0.88 N NH_3) to yield the title compound as a white, hygroscopic solid (288 mg, 0.70 mmol, 56%): mp 175.9−177.0 °C; LRMS (*m/z*) (TSP^+) 411.6 (MH^+). Anal. ($C_{21}H_{26}O_3N_6$•$0.7H_2O$) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (28). Sodium cyanoborohydride (92 mg, 1.47 mmol) was added to a stirring solution of amine **25** (500 mg, 0.98 mmol), acetaldehyde (64 μL, 1.18 mmol), and sodium acetate (161 mg, 1.96 mmol) in MeOH (10 mL) under nitrogen at room temperature. After 1 h the mixture was poured into saturated aqueous sodium bicarbonate solution (20 mL), and extracted with DCM (3 × 15 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash column chromatography (eluting with 95:5:0.5−80:20:1 EtOAc:MeOH:0.88 N NH_3) to yield the title compound as a white solid (140 mg, 0.33 mmol, 34%): LRMS (*m/z*) (TSP^+) 425.3 (MH^+).

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (29) was prepared in 31% yield from the secondary amine **25** and acetone, following a similar procedure to that described for compound **27**: mp 162.8−163.6 °C; LRMS (*m/z*) (TSP^+) 439.6 (MH^+). Anal. ($C_{23}H_{30}O_3N_6$) C, H, N.

5-(5-Acetyl-2-isobutoxy-3-pyridinyl)-3-ethyl-2-(1-methyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (30). The 2′-propoxy analogue **27** (70 mg, 0.17 mmol) and cesium carbonate (167 mg, 0.51 mmol) were dissolved in isobutyl alcohol (2 mL) and heated in a reactivial at 90 °C with 4 Å molecular sieves for 24 h. A further aliquot of cesium carbonate (56 mg, 0.17 mmol) and isobutyl alcohol (0.5 mL) were added, and heating was continued for a further 24 h. The reaction mixture was then poured into DCM (10 mL) and a saturated aqueous sodium bicarbonate solution (5 mL). The organic layer was separated and the aqueous layer extracted further with DCM (3 × 10 mL). The combined organics were dried ($MgSO_4$) and concentrated in vacuo. Purification by flash column chromatography (eluting with 97.5:2.5:0.5 CH_2Cl_2:MeOH:0.88 N NH_3) yielded an inseparable mixture of title compound and starting material. The mixture was dissolved in isobutyl alcohol (2 mL), cesium carbonate (167 mg, 0.51 mmol) was added, and the reaction mixture was heated at 90 °C for 48 h. The reaction was then worked up as described previously and purified by flash column chromatography (eluting with 95:5:0.5−85:15:0.5 EtOAc:MeOH:0.88 N NH_3 and then repeated eluting with 99:1:0.1−95:5:0.5 CH_2Cl_2:MeOH:0.88 N NH_3) to yield the title compound as a white solid (32 mg, 0.08 mmol, 44%): LRMS (*m/z*) (TSP^+) 425.5 (MH^+); HRMS ($C_{22}H_{28}N_6O_3$) calcd for $(M + 1)^+$ 425.2296, found 425.2287.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-2-(3-azetidinyl)-3-ethyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (42). TFA (25 mL, 31% vol) was added to a solution of the carbamate **40** (13.4 g, 262 mmol) in DCM (80 mL) at 0 °C, and the mixture was then stirred at room temperature for 1 h. The reaction mixture was poured into toluene (100 mL) and concentrated in vacuo to yield an oil. The oil was azeotroped again with toluene (50 mL) and the residue taken up in isopropyl acetate. The resulting precipitate was removed by filtration and dried in vacuo to yield the trifluoroacetate salt of the

title compound as a white solid (11.2 g, 17.5 mmol, 67%): LRMS (*m/z*) (ES^+) 411.0 (MH^+), (ES^-) 409.0 (MH^-). Anal. ($C_{21}H_{26}O_3N_6$•2.0H_2O•0.05DIPE) C, H, N.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-methyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (31) was prepared in 33% yield from 2′ propoxy analogue **27** and *n*-butanol, following a similar procedure to that described for compound **30**: LRMS (*m/z*) (ES^+) 425.0 (MH^+), (ES^-) 423.0 (MH^-); HRMS ($C_{22}H_{28}N_6O_3$) calcd for $(M + 1)^+$ 425.2296, found 425.2294.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (2). Route A. Potassium carbonate (4.80 g, 34.7 mmol) and ethyl iodide (1.4 mL, 17.5 mmol) were added to a cloudy solution of the secondary amine **42** (11.1 g, 17.4 mmol) in MeCN (600 mL), and then the reaction mixture was heated to 45−50 °C for 2.5 h. The solvent was then removed in vacuo and the residue dissolved in 95:5:0.5 CH_2Cl_2:MeOH:0.88 N NH_3 (50 mL). The resulting solution was filtered and then purified by flash column chromatography (eluting with 95:5:0.5−92:8:1 CH_2Cl_2:MeOH:0.88 N NH_3). The product was crystallized from diisopropyl ether to yield the title compound as white crystals (4.90 g, 11.2 mmol, 64%): mp 143.0−144.0 °C.

Route B. Cesium carbonate (38.6 g, 119 mmol) was added to a solution of the amide **54** (25.4 g, 59.3 mmol) in *n*-butanol (400 mL) in the presence of powdered 4-Å molecular sieves (10 g). The reaction mixture was then heated to reflux and 20 mL of solvent removed via distillation into a splash trap. Refluxing was then continued for 4 h, after which the reaction mixture was cooled and filtered. The filtrate was concentrated in vacuo and then purified by flash column chromatography (eluting with 95:5:0.5 CH_2Cl_2:MeOH:0.88 N NH_3) to yield a green oil. The crude product was then purified by crystallization from ethyl acetate, to yield the title compound as a white solid (9.00 g, 20.5 mmol, 35%): mp 143.0−144.0 °C; LRMS (*m/z*) (TSP^+) 439.2 (MH^+). Anal. ($C_{23}H_{30}O_3N_6$) C, H, N. Inversion of the pyrazolopyrimidinone C3-CH_2CH_3 and azetidine-C3-H showed mutual NOE enhancements, confirming the regiochemistry.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(4-piperidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (26) was prepared in 65% yield from the acetylene **24**, following a similar procedure to that described for compound **25**: LRMS (*m/z*) (TSP^+) 425.5 (MH^+). Anal. ($C_{22}H_{28}O_3N_6$•1.45DCM) C; H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-methyl-4-piperidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (32) was prepared in 66% yield from the secondary amine **26**, following a similar procedure to that described for compound **27**: mp 219.0−220.0 °C; LRMS (*m/z*) (TSP^+) 439.5 (MH^+). Anal. ($C_{23}H_{30}O_3N_6$•0.2H_2O•0.1DCM) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-4-piperidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (33) was prepared in 28% yield from the secondary amine **26**, following a similar procedure to that described for compound **28**: LRMS-(*m/z*) (TSP^+) 453.8 (MH^+). Anal. ($C_{24}H_{32}O_3N_6$•0.2H_2O•0.1DCM•0.1MeOH) C, H, N.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(4-piperidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (43) was prepared in 90% crude yield (contained ∼10% impurity) from the carbamate **41**, following a similar procedure to that described for compound **42**: LRMS (*m/z*) (TSP^+) 439 (MH^+). HRMS ($C_{23}H_{30}N_6O_3$) calcd for $(M + 1)^+$ 439.2452, found 439.2446.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-methyl-4-piperidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (34) was prepared in 18% yield from the 2′-propoxy analogue **32** using *n*-butanol as solvent, following a similar procedure to that described for compound **30**: mp 198.0−199.0 °C; LRMS (*m/z*) (TSP^+) 453-(MH^+). Anal. ($C_{24}H_{32}O_3N_6$•0.25H_2O•0.1DIPE) C, H, N.

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-4-piperidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (44) was prepared in 65% yield from the secondary amine **43**, following a similar procedure to that described for compound 2 (route A). A sample was crystallized from ethyl acetate for analysis: mp 182.0−183.0 °C; LRMS (*m/z*) (TSP^+) 467.3 (MH^+). Anal. ($C_{25}H_{34}O_3N_6$) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-2-(3-azetidinyl)-3-propyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (45) was prepared in 67% yield from the carbamate **58**, using a similar procedure to that described for compound **42**. An analytical sample was prepared by crystallization from diethyl ether: mp 185 °C (dec); LRMS (*m/z*) (ES^+) 411 $(MH)^+$; (ES^-) 409 $(M - H)^-$. Anal. ($C_{21}H_{26}N_6O_3$•0.3H_2O) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-2-(1-methyl-3-azetidinyl)-3-propyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (46) was prepared in 20% yield from the secondary amine **45**, using a similar procedure to that described for compound **27**: mp 118−122 °C; LRMS (*m/z*) (ES^+) 447 $(MNa)^+$, 425 $(MH)^+$; (ES^-) 423 $(M - H)^-$. Anal. ($C_{22}H_{28}N_6O_3$•0.3H_2O•0.5EtOAc) C, H, N.

5-[2-Butoxy-5-(1-hydroxyethyl)-3-pyridinyl]-3-ethyl-2-(1-ethyl-3-azetidinyl) -2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (55). Sodium borohydride (17 mg, 0.46 mmol) was added to an ice-cooled suspension of ketone **2** (400 mg, 0.91 mmol) in MeOH (10 mL) under a nitrogen atmosphere, and the solution was allowed to warm to room temperature, with stirring, over 1 h. Additional sodium borohydride (17 mg, 0.46 mmol) was then added, and the reaction stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure, the residue was partitioned between ethyl acetate (30 mL) and water (20 mL), and the layers were separated. The aqueous phase was extracted with ethyl acetate (2 × 20 mL), and the combined organic solutions were dried ($MgSO_4$) and concentrated in vacuo. The residual yellow foam was purified by flash column chromatography (eluting with DCM: MeOH:0.88 N NH_3 95:5:0.5). The resulting foam was crystallized from Et_2O to afford the title compound as a white solid (285 mg, 0.65 mmol, 71%): mp 117−119 °C; LRMS (*m/z*) (TSP^+) 441.2 (MH^+). Anal. ($C_{23}H_{32}N_6O_3$) C, H, N.

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(2-pyridinylmethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (8) was prepared in 12% yield from the acetylene **7**, using a similar procedure to that described for compound **16**: LRMS (*m/z*) (TSP^+) 433.4 (MH^+). Anal. ($C_{23}H_{24}O_3N_6$•0.5H_2O•0.5DCM) C, H, N.

3-Ethyl-5-(5-ethynyl-2-propoxy-3-pyridinyl)-2-(2-pyridinylmethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7). Potassium fluoride (239 mg, 4.12 mmol) was added to a stirring solution of trimethylsilylacetylene **59** (1.00 g, 2.06 mmol) in DMF (20 mL) and water (2 mL) at 0 °C. The reaction mixture was then stirred at room temperature for 4 h. The reaction mixture was then concentrated in vacuo, and the residue was taken up in water (50 mL) and ethyl acetate (50 mL) and filtered through Arbocel. The organic layer was separated, and the aqueous layer was extracted further with ethyl acetate (3 × 50 mL) and DCM (50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to give the title compound as a beige solid (700 mg, 1.69 mmol, 82%): mp 189 °C; LRMS (*m/z*) (TSP^+) 415.6 (MH^+). Anal. ($C_{23}H_{22}O_2N_6$•0.7H_2O) C, H, N.

3-Ethyl-5-(2-propoxy-5-[(trimethylsilyl)ethynyl]-3-pyridinyl)-2-(2-pyridinylmethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (59). $Pd(PPh_3)_2Cl_2$ (60.0 mg, 0.08 mmol), trimethylsilylacetylene (615 μL, 4.44 mmol), and cuprous iodide (15 mg, 0.08 mmol) were added to a stirred slurry of iodo analogue **6** (1.50 g, 2.90 mmol) in triethylamine (23 mL) and MeCN (1 mL) at room temperature under nitrogen. The mixture was stirred at room temperature for 3 h, after which a further aliquot of trimethylsilylacetylene (300 μL, 2.17 mmol) was added. After stirring for a further 1 h, another aliquot of trimethylsilylacetylene (300 μL, 2.17 mmol) was added. After 1 h the reaction was concentrated in vacuo and then partitioned between ethyl acetate (50 mL) and water (50 mL). The organic layers were separated, and the aqueous layer was extracted further with ethyl actetate (2 × 50 mL). The combined organic layers were washed with brine (75 mL), dried ($MgSO_4$), and concentrated in vacuo to give a yellow solid. Trituration from ether yielded the title compound as an off-white solid (873 mg, 1.80 mmol, 62%): LRMS (*m/z*) (TSP^+) 487.5 (MH^+).

3-Ethyl-5-(5-iodo-2-propoxy-3-pyridinyl)-2-(2-pyridinylmethyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6). KHMDS (3.65 g, 18.3 mmol) was added to a stirring solution of the amide **5** (6.50 g, 12.2 mmol) in 3-methyl-3-pentanol (50 mL) at room temperature under nitrogen. The reaction mixture was then heated at 120 °C for 42 h, after which a further aliquot of KHMDS (3.65 g, 18.3 mmol) was added. Heating at 120 °C was continued for a further 24 h, followed by another addition of KHMDS (3.65 g, 18.3 mmol). After a further 3 h of heating at 120 °C, the reaction mixture was concentrated in vacuo. The residue was partitioned between ethyl acetate (200 mL) and saturated aqueous sodium bicarbonate solution (200 mL). The organic layer was separated and the aqueous layer extracted further with ethyl acetate (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude product was triturated in ether to yield some of the target compound. The residue was purified by flash column chromatography (eluting with 99.8:0.2 to 99:1 CH_2Cl_2:MeOH) to yield the title compound (which was combined with that obtained earlier) as a solid (1.40 g, 2.71 mmol, 22%): mp 228.9−233.8 °C; LRMS (*m*/*z*) (ES^+) 517 (MH^+). Anal. ($C_{21}H_{21}O_2N_6I$) C, H, N.

***N*-[3-(Aminocarbonyl)-5-ethyl-1-(2-pyridinylmethyl)-1*H*-pyrazol-4-yl]-5-iodo-2-propoxynicotinamide (5).** Oxalyl chloride (3.93 mL, 45 mmol) was added dropwise to a solution of acid **4** (4.61 g, 15 mmol) in DCM (100 mL) and DMF (100 μL) under nitrogen at 0 °C. The reaction mixture was allowed to warm to room temperature and then stirred for 16 h. The reaction mixture was then concentrated in vacuo and azeotroped three times with toluene, to give a yellow/brown solid. This solid was then dissolved in DCM (20 mL) and added to a solution of 4-amino-5-ethyl-1-(2-pyridinylmethyl)-1*H*-pyrazole-3-carboxamide[8] (3.68 g, 15 mmol) in pyridine (70 mL) stirring under nitrogen. After 4 h the reaction mixture was concentrated in vacuo, and the product was partitioned between ethyl acetate (200 mL) and saturated aqueous sodium bicarbonate solution (200 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo to yield the target compound as a peach-colored compound (7.70 g, 14.4 mmol, 96%): LRMS (*m*/*z*) (ES^+) 535 (MH^+), (ES^-) 533 (MH^-). Anal. ($C_{21}H_{23}O_3N_6I$) C, H, N.

2-Propoxy-5-iodonicotinic Acid (4). 2-Propoxynicotinic acid 3 (9.30 g, 51.3 mmol) was dissolved slowly in TFA (75 mL) and trifluoroacetic anhydride (19 mL). NIS (18.6 g, 82.7 mmol) was then added portionwise, and the red/brown solution was heated at reflux for 6 h followed by 16 h at room temperature. The reaction mixture was then concentrated in vacuo and water (150 mL) added. The aqueous mixture was extracted with DCM (3 × 150 mL). The combined organic layers were extracted with aqueous sodium hydroxide solution (1 N, 200 mL), and then the aqueous layer was acidified with concentrated hydrochloric acid. The acidic aqueous layer was then extracted with DCM (4 × 150 mL), and the combined organic layers were washed with brine (150 mL), dried ($MgSO_4$), and concentrated in vacuo. Trituration from pentane yielded the target compound as an off-white solid (11.3 g, 36.8 mmol, 72%): LRMS (*m*/*z*) (ES^-) 306 (MH^-).

5-(2-Butoxy-5-ethynyl-3-pyridinyl)-3-ethyl-2-[2-(4-morpholinyl)ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (15) was prepared in 78% yield from the trimethylsilyl analogue **60**, following a similar procedure to that described for compound **7**: LRMS (*m*/*z*) (ES^+) 451 (MH^+).

5-{2-Butoxy-5-[(trimethylsilyl)ethynyl]-3-pyridinyl}-3-ethyl-2-[2-(4-morpholinyl)ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (60) was prepared in 68% yield from the iodo analogue **14**, following a similar procedure to that described for compound **59**: LRMS (*m*/*z*) (ES^+) 523 (MH^+).

5-(2-Butoxy-5-iodo-3-pyridinyl)-3-ethyl-2-[2-(4-morpholinyl)ethyl]-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (14) was prepared in 67% yield from the amide **13** using *n*-butanol as solvent, following a similar procedure to that described for compound **6**: LRMS (*m*/*z*) (ES^+) 553 (MH^+).

***N*-{3-(Aminocarbonyl)-5-ethyl-1-[2-(4-morpholinyl)ethyl]-1*H*-pyrazol-4-yl}-2-butoxy-5-iodonicotinamide (13).** Cesium carbonate (880 mg, 2.70 mmol) was added to a stirring solution of amide **12** (750 mg, 1.60 mmol) and *N*-(2-chloroethyl)morpholine hydrochloride (370 mg, 1.99 mmol) in DMF (5 mL) under a nitrogen atmosphere. The mixture was stirred at room temperature for 16 h and then heated at 60 °C for 24 h. Further aliquots of *N*-(2-chloroethyl)morpholine hydrochloride (185 mg, 0.99 mmol) and cesium carbonate (486 mg, 1.49 mmol) were then added and heating continued for a further 16 h. The reaction mixture was cooled and poured into water (50 mL), and the aqueous layer was extracted with ether (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by flash column chromatography (eluting with 99:1 to 98:2 CH_2Cl_2:MeOH) yielded the title compound (310 mg, 0.54 mmol, 34%): LRMS (*m*/*z*) (ES^+) 571 (MH^+).

***N*-[3-(Aminocarbonyl)-5-ethyl-1*H*-pyrazol-4-yl]-2-butoxy-5-iodonicotinamide (12)** was prepared in 98% yield from acid **11** and 4-amino-3-ethyl-1*H*-pyrazole-5-carboxamide,[8] following a similar procedure to that described for compound **5**: LRMS (*m*/*z*) (TSP^+) 457.9 (MH^+).

2-*n*-Butoxy-5-iodonicotinic Acid (11). *n*-Butanol (2.96 g, 40.0 mmol) was added dropwise to a stirring suspension of KHMDS (7.96 g, 40.0 mmol) in THF (30 mL), with cooling. The reaction mixture was then stirred at room temperature for 15 min. A solution of methyl-2-chloro-5-iodonicotinate **10**[24] (1.98 g, 6.60 mmol) in THF (10 mL) was then added dropwise over 10 min. After 2 h a solution of sodium hydroxide (532 mg, 13.2 mmol) in water (3 mL) was added dropwise over 5 min. The reaction was then neutralized to pH 8−9 with 2 M HCl, and the bulk of solvent was removed in vacuo. The pH of the remaining solution was adjusted to pH 8−9 again and then partitioned with ethyl acetate (100 mL). The resulting precipitate was separated by filtration and shown to be the title compound (640 mg, 1.99 mmol, 30%). The organic and aqueous layers were separated, and the aqueous layer was re-extracted with ethyl acetate (100 mL). The combined organic layers were washed with 2 M HCl (50 mL), dried ($MgSO_4$), and concentrated in vacuo to give a brown solid. This solid was combined with those separated earlier to yield the title compound (730 mg, 2.27 mmol, 34%): LRMS (*m*/*z*) (TSP) 321.9 (MH^+).

5-Acetyl-*N*-{3-(aminocarbonyl)-5-ethyl-1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-1*H*-pyrazol-4-yl}-2-ethoxynicotinamide (47) was prepared in 70% yield from acid **36** and 4-amino-5-ethyl-1-[(1-methyl-1*H*-imidazol-2-yl)methyl]-1*H*-pyrazole-3-carboxamide,[7] following a similar procedure to that described for compound **37**: LRMS (*m*/*z*) (TSP^+) 440.7 (MH^+).

5-Acetyl-2-ethoxynicotinic Acid (36). Triethylamine (354 mL, 2.54 M) was added to a slurry of 5-bromo-2-ethoxynicotinic acid[25] (250 g, 1.02 M) in MeCN (1 L). To this reaction mixture was added palladium(II) acetate (4.56 g, 20.3 mmol), butyl vinyl ether (305 g, 3.05 M), and tri-*o*-tolylphosphine (12.4 g, 40.6 mmol), each addition being washed in with MeCN. The remaining solvent was then added and the reaction mixture refluxed under nitrogen for 22 h. The reaction mixture was left at room temperature for 16 h, and then the precipitate was removed by filtration and discarded. The filtrate was concentrated in vacuo to give a brown gum, which was then stirred for 1 h in water (1 L) and concentrated HCl (1 L). The reaction mixture was diluted with water (6.25 L) and extracted with DCM (6 × 500 mL). The combined organic layers were extracted with 5% aqueous sodium bicarbonate solution (1.2 L, 2 × 400 mL). The basic aqueous extracts were washed with DCM (250 mL) and then acidified to pH 3. After stirring for 30 min the precipitated product was removed by filtration, washed with water (250 mL), and dried at 50 °C in vacuo to yield the target compound as a white solid (134 g, 64.1 mmol, 63%): LRMS (*m*/*z*) (ES^-) 208 (MH^-).

***tert*-Butyl 3-[3-ethyl-5-(5-ethynyl-2-propoxy-3-pyridinyl)-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-azetidinecarboxylate (23)** was prepared in 85% yield from the trimethylsilyl

analogue **61**, following a similar procedure to that described for compound **7**: LRMS (*m/z*) (TSP$^+$) 496.6 (MNH$_4^+$), 379.7 (MH$^+$ − BOC).

***tert*-Butyl 3-(3-ethyl-7-oxo-5-{2-propoxy-5-[(trimethylsilyl)-ethynyl]-3-pyridinyl}-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)-1-azetidinecarboxylate (61)** was prepared in 83% yield from the iodo analogue **21**, following a similar procedure to that described for compound **59**: LRMS (*m/z*) (TSP$^+$) 568.6 (MNH$_4^+$), 452.0 (MH$^+$). Anal. ($C_{28}H_{38}O_4N_6Si$) C, H, N.

***tert*-Butyl 3-[3-ethyl-5-(5-iodo-2-propoxy-3-pyridinyl)-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-azetidinecarboxylate (21)** was prepared in 63% yield from the amide **19** using *n*-propanol as solvent, following a similar procedure to that described for compound **6**: LRMS (*m/z*) (TSP$^+$) 598.1 (MNH$_4^+$). Anal. ($C_{23}H_{29}O_4N_6I$) C, H, N. Irradiation of the pyrazolopyrimidinone C3-CH_2CH_3 and azetidine-C3-H showed mutual NOE enhancements, confirming regiochemistry.

***tert*-Butyl 3-(3-(aminocarbonyl)-5-ethyl-4-{[(5-iodo-2-propoxy-3-pyridinyl)carbonyl]amino}-1*H*-pyrazol-1-yl)-1-azetidinecarboxylate (19)** was prepared in 65% yield from unsubstituted pyrazole **18** and *tert*-butyl-3-iodo-1-azetidinecarboxylate,[26] following a similar procedure to that described for compound **13**: LRMS (*m/z*) (TSP$^+$) 373.2 (MH$^+$ − BOC and I). Anal. ($C_{23}H_{31}O_5N_6I\cdot 0.2DCM$) C, H, N.

***N*-[3-(Aminocarbonyl)-5-ethyl-1*H*-pyrazol-4-yl]-5-iodo-2-propoxynicotinamide (18)** was prepared in 74% yield from acid **4** and 4-amino-3-ethyl-1*H*-pyrazole-5-carboxamide,[8] following a similar procedure to that described for compound **5**: LRMS (*m/z*) (TSP$^+$) 444.3 (MH$^+$). Anal. ($C_{15}H_{18}O_3N_5I$) C, H, N.

***tert*-Butyl 3-[5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-azetidinecarboxylate (40)** was prepared in 46% crude yield from amide **38** using *n*-butanol as solvent, following a similar procedure to that described for compound **2** (route B). Crystallization from diisopropyl ether yielded the title compound, containing a 10% impurity, as white crystals: LRMS (*m/z*) (ES$^+$) 433 (MNa$^+$), (ES$^-$) 509 (MH$^-$).

***tert*-Butyl 3-[4-{[(5-acetyl-2-ethoxy-3-pyridinyl)carbonyl]-amino}-3-(aminocarbonyl)-5-ethyl-1*H*-pyrazol-1-yl]-1-azetidinecarboxylate (38)** was prepared in 63% yield from unsubstituted pyrazole **37** and *tert*-butyl-3-iodo-1-azetidinecarboxylate,[26] following a similar procedure to that described for compound **13**: mp 220−223 °C; LRMS (*m/z*) (ES$^+$) 523.0 (MNa$^+$), (ES$^-$) 499.0 (MH$^-$). Anal. ($C_{24}H_{32}O_6N_6$) C, H, N.

5-Acetyl-*N*-[3-(aminocarbonyl)-5-ethyl-1*H*-pyrazol-4-yl]-2-ethoxynicotinamide (37). A solution of acid **36** (5.70 g, 27.3 mmol) and HATU (10.9 g, 28.6 mmol) in DCM (100 mL) was added to a solution of 4-amino-3-ethyl-1*H*-pyrazole-5-carboxamide[8] (4.20 g, 27.3 mmol) and *N*,*N*-diisopropylethylamine (23.7 mL, 136.2 mmol) in DCM (115 mL) under nitrogen. After 1 h the mixture was diluted with brine (100 mL) and washed with saturated aqueous sodium bicarbonate solution (100 mL) and 2 N HCl (100 mL). Each aqueous layer was extracted with DCM (100 mL), and the combined organics were washed with brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo. An analytical sample of the title compound was obtained by trituration with ethyl acetate, followed by crystallization from ethanol, while the remainder was purified by flash column chromatography (eluting with 95:5 CH_2Cl_2:MeOH) to yield the title compound as a white solid (total weight = 7.8 g, 22.5 mmol, 83%): mp 217−219 °C; LRMS (*m/z*) (TSP$^+$) 346.2 (MH$^+$). Anal. ($C_{16}H_{19}O_4N_5$) C, H, N.

5-Acetyl-*N*-[3-(aminocarbonyl)-5-ethyl-1-(1-ethyl-3-azetidinyl)-1*H*-pyrazol-4-yl]-2-ethoxynicotinamide (54). 1,1-Carbonyldiimidazole (13.9 g, 85.8 mmol) was added to a suspension of acid **36** (17.1 g, 81.8 mmol) in ethyl acetate (140 mL) under nitrogen, and the reaction mixture was stirred at 45 °C for 45 min and refluxed for 90 min. The reaction mixture was then cooled to room temperature and a slurry of amine **53** (19.4 g, 81.8 mmol) in ethyl acetate (70 mL) was added. The reaction mixture was then refluxed for 16 h, after which a precipitate had formed. The suspension was cooled to room temperature and the solid removed by filtration. The solid was washed with water:ethanol 90:10 and then dried in vacuo to yield the title compound as a white solid (24.0 g, 56.0 mmol, 69%): mp 230−233 °C; LRMS (*m/z*) (TSP$^+$) 429.2 (MH$^+$). Anal. ($C_{21}H_{28}O_4N_6$) C, H, N.

4-Amino-5-ethyl-1-(1-ethyl-3-azetidinyl)-1*H*-pyrazole-3-carboxamide (53). A mixture of nitropyrazole **62** (22.0 g, 82.3 mmol) and 10% palladium on charcoal (2.0 g) in ethanol (500 mL) was hydrogenated at 60 psi and room temperature for 4 h. The reaction mixture was then filtered through Arbocel under nitrogen, and the filtrate was concentrated in vacuo to yield the title compound as a cream solid (19.6 g, 82.6 mmol, 100%): mp 155−157 °C; LRMS (*m/z*) (TSP$^+$) 238.2 (MH$^+$). Anal. ($C_{11}H_{19}O_1N_5$) C, H, N.

5-Ethyl-1-(1-ethyl-3-azetidinyl)-4-nitro-1*H*-pyrazole-3-carboxamide (62). To a stirring solution of the secondary amine **52** (31.1 g, 113 mmol) and triethylamine (14.1 mL, 102 mmol) in DCM (400 mL) and MeOH (400 mL) at 0 °C was added sodium triacetoxyborohydride (60 g, 282 mmol) in one portion. Acetaldehyde (19 mL, 339 mmol) was then added dropwise over 2 min. The reaction mixture was then allowed to warm to room temperature over 30 min. The solvent was then removed in vacuo and the residue partitioned between DCM (500 mL) and water (300 mL). The organic layer was separated, and the aqueous layer was basified with solid sodium bicarbonate and extracted with DCM (500 mL) and DCM:MeOH (95:5, 500 mL; 90:10, 500 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The residue was triturated from hot ethyl acetate and a white solid separated by filtration. The filtrate was concentrated in vacuo and purified by flash column chromatography (eluting with 95:5:0.5 CH_2Cl_2:MeOH:0.88 N NH_3) to give a white solid which was combined with the previous batch to yield the title compound (23.3 g, 86.8 mmol, 77%): mp 177−179 °C; LRMS (*m/z*) (TSP$^+$) 268.3 (MH$^+$). Anal. ($C_{11}H_{17}O_3N_5$) C, H, N.

1-(3-Azetidinyl)-5-ethyl-4-nitro-1*H*-pyrazole-3-carboxamide (52). To a suspension of the benzhydryl compound **51** (35.3 g, 87.1 mmol) in DCM (700 mL) at 0 °C under nitrogen was added 1-chloroethyl chloroformate (10.4 mL, 95.8 mmol) dropwise. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 18 h. The reaction mixture was then concentrated in vacuo, and the oily residue dissolved in MeOH (700 mL) and refluxed for 1 h. The solvent was then removed in vacuo, and the crude product triturated from ethyl acetate (200 mL) and acetone (200 mL) to yield the dihydrochloride salt of the title compound as a beige solid (21.3 g, 77.3 mmol, 89%): mp 164−167 °C; LRMS (*m/z*) (TSP$^+$) 240.3 (MH$^+$). Anal. ($C_9H_{13}O_3N_5\cdot 2HCl\cdot 0.2Me_2CO$) C, H, N.

1-(1-Benzhydryl-3-azetidinyl)-5-ethyl-4-nitro-1*H*-pyrazole-3-carboxamide (51). 5-Ethyl-4-nitro-1*H*-pyrazole-3-carboxamide **50**[8] (25.0 g, 136 mmol), sodium carbonate (57.6 g, 543 mmol), sodium iodide (40.7 g, 272 mmol), and 1-benzhydryl-3-azetidinyl methanesulfonate[27] (86.2 g, 272 mmol) were dissolved in THF (338 mL) and water (38 mL) and refluxed for 5 days. The reaction mixture was then concentrated in vacuo and taken up in ethyl acetate (500 mL) and water (300 mL). The resulting precipitate was filtered and washed with ethyl acetate and water to yield the title compound as a white solid (17.0 g, 41.9 mmol, 31%): mp 257−260 °C; LRMS (*m/z*) (TSP$^+$) 406.2 (MH$^+$). Irradiation of pyrazole-CH_2CH$_3$ and azetidine-C3-*H* showed mutual NOE enhancements, confirming the regiochemistry.

***tert*-Butyl 4-[3-ethyl-5-(5-ethynyl-2-propoxy-3-pyridinyl)-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-piperidinecarboxylate (24)** was prepared in 100% yield from the trimethylsilyl analogue **63**, following a similar procedure to that described for compound **7**: mp 221 °C; LRMS (*m/z*) (TSP$^+$) 507.7 (MH$^+$), 524.3 (MNH$_4^+$). Anal. ($C_{27}H_{34}O_4N_6\cdot 0.5H_2O$) C, H, N.

***tert*-Butyl 4-(3-ethyl-7-oxo-5-{2-propoxy-5-[(trimethylsilyl)-ethynyl]-3-pyridinyl}-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)-1-piperidinecarboxylate (63)** was prepared in 75% yield from the iodo analogue **22**, following a similar procedure to that described for compound **59**: LRMS (*m/z*) (TSP$^+$) 580 (MH$^+$), 479.7 (MH$^+$ − BOC). Anal. ($C_{30}H_{42}O_4N_6Si\cdot 0.2H_2O$) C, H, N.

***tert*-Butyl 4-[3-ethyl-5-(5-iodo-2-propoxy-3-pyridinyl)-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-piperidinecarboxylate (22)** was prepared in 16% yield from the amide **20** using *n*-propanol as solvent, following a similar procedure to that described for compound **6**: LRMS (*m/z*) (TSP^+) 609.7 (MH^+), 509.0 ($MH^+ - BOC$).

***tert*-Butyl 4-(3-(aminocarbonyl)-5-ethyl-4-{[(5-iodo-2-propoxy-3-pyridinyl)carbonyl]amino}-1*H*-pyrazol-1-yl)-1-piperidinecarboxylate (20)** was prepared in 52% yield from the unsubstituted pyrazole **18** and *tert*-butyl 4-[(methylsulfonyl)oxy]-1-piperidinecarboxylate,[28] following a similar procedure to that described for compound **13**: LRMS (*m/z*) (TSP^+) 627.4 (MH^+). Anal. ($C_{25}H_{35}O_5N_6I{\cdot}0.3H_2O$) C, H, N.

***tert*-Butyl 4-[5-(5-acetyl-2-butoxy-3-pyridinyl)-3-ethyl-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-piperidinecarboxylate (41)** was prepared in 40% yield from amide **39** using *n*-butanol as solvent, following a similar procedure to that described for compound **2** (route B). Trituration from diethyl ether gave the title compound as a white powder: mp 194−195 °C; LRMS (*m/z*) (TSP^+) 539 (MH^+), 439 ($MH^+ - BOC$). Anal. ($C_{28}H_{38}O_5N_6$) C, H, N.

***tert*-Butyl 4-[4-{[(5-acetyl-2-ethoxy-3-pyridinyl)carbonyl]amino}-3-(aminocarbonyl)-5-ethyl-1*H*-pyrazol-1-yl]-1-piperidinecarboxylate (39)** was prepared in 57% yield from unsubstituted pyrazole **37** and 1-(*tert*-butoxycarbonyl)-4-piperidinyl methanesulfonate,[28] following a similar procedure to that described for compound **13**: mp 197−202 °C; LRMS (*m/z*) (ES^+) 529 (MH^+). Anal. ($C_{26}H_{36}O_6N_6{\cdot}0.5H_2O$) C, H, N.

***tert*-Butyl 3-[5-(5-acetyl-2-propoxy-3-pyridinyl)-7-oxo-3-propyl-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-azetidinecarboxylate (58)** was prepared in 16% yield from amide **64** using *n*-propanol as solvent, following a similar procedure to that described for compound **2** (route B): mp 195−198 °C; LRMS (*m/z*) (ES^+) 533 $(MNa)^+$; (ES^-) 509 $(M - H)^-$. Anal. ($C_{26}H_{34}N_6O_5$) C, H, N. Irradiation of pyrazole-C$H_2CH_2CH_3$ and azetidine-C3-*H* showed mutual NOE enhancements, confirming the regiochemistry.

***tert*-Butyl 3-[4-{[(5-acetyl-2-ethoxy-3-pyridinyl)carbonyl]amino}-3-(aminocarbonyl)-5-propyl-1*H*-pyrazol-1-yl]-1-azetidinecarboxylate (64)** was prepared as an indistinguishable mixture of regioisomers in 83% yield from the unsubstituted pyrazole **65** and 3-iodo-1-azetidinecarboxylic ^{t}butyl ester,[26] following a similar procedure to that described for compound **13**: mp 200−203 °C; LRMS (*m/z*) (EI^+) 537 $(MNa)^+$, 515 $(MH)^+$. Anal.($C_{25}H_{34}N_6O_6$) C, H, N.

5-Acetyl-*N*-[3-(aminocarbonyl)-5-propyl-1*H*-pyrazol-4-yl]-2-ethoxynicotinamide (65) was prepared in 48% yield from acid **36** and 4-amino-5-propyl-1*H*-pyrazole-3-carboxamide,[8] following a similar procedure to that described for compound **37**: mp 196−199 °C; LRMS (*m/z*) (ES^+) 382 $(MNa)^+$, 360 $(MH)^+$; (ES^-) 358 (MH^-). Anal. ($C_{17}H_{21}N_5O_4{\cdot}0.9H_2O$) C, H, N.

PDE5 Enzyme Inhibitor Assays. Phosphodiesterase type 5 was prepared from human corpus cavernosum tissue as previously described.[29] 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 μm 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.[30] 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 ^{3}H-labeled at a concentration $\sim 1/3K_m$) such that $IC_{50} \cong K_i$. The final assay volume was made up to 102 μL 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 μL 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.

Structural Biology. The structure of the *Escherichia coli* engineered PDE5 was solved by molecular replacement (MR) using a model of PDE5.[23] X-ray diffraction data were collected with a RaxisIV image plate detector on an in-house RU200HB rotating anode (Rigaku), with Blue Osmic mirrors (MSC). All data were processed using the HKL package.[31] Data collection statistics for compound **2** are available in the Supporting Information. The crystals belong to the monoclinic space group *C*2, with cell dimensions $a = 55.84$ Å, $b = 76.66$ Å, $c = 81.27$ Å, $\beta = 102.584°$. There is one molecule per asymmetric unit ($M_w = 37\,562.41$ kDa) and a calculated solvent content of 45.14% ($V_M = 2.26$).[32] Molecular replacement was performed using AMORE (CCP4).[33] The resulting map was of good quality and the structure was refitted using QUANTA.[34] Refinement was carried out in the resolution range 35−1.6 Å using CNX[35] with the "*mlf*" maximum likelihood target function. Partial structure factors from a flat bulk-solvent model and anisotropic *B*-factor correction were supplied throughout the refinement. The *R*-factor for the current model is 0.31 (free *R*-factor, 5% of data, 0.32) for all data in the resolution range 35−1.6 Å. The refinement statistics for compound **2** are available in the Supporting Information.

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Supporting Information Available: Tables of ^{1}H NMR data, elemental analysis data for target compounds, X-ray data collection statistics for compound **2**, and crystallographic refinement statistics of deposited model for compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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