Synthesis and Cyclic GMP Phosphodiesterase Inhibitory Activity of a Series of 6-Phenylpyrazolo[3,4-*d*]pyrimidones

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A series of 6-phenylpyrazolo[3,4-*d*]pyrimidones is described which are specific inhibitors of cGMP specific (type V) phosphodiesterase. Enzymatic and cellular activity as well as in vivo oral antihypertensive activity are evaluated. A *n*-propoxy group at the 2-position of the phenyl ring is necessary for activity. A series of products substituted at the 5-position in addition to the 2-*n*-propoxy was prepared and evaluated. This position can accommodate many unrelated groups. Amino derivatives were very potent but lacked metabolic stability. Substitution by carbon-linked small heterocycles provided both high levels of activity and stability. Cellular activity very often correlated with in vivo activity. Among the compounds, 1,3-dimethyl-6-(2-propoxy-5-methanesulfonamidophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**38**) and 1-ethyl-3-methyl-6-(2-propoxy-5-(4-methylthiazol-2-yl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**59**) displayed outstanding in vivo activities at 5 mg/kg/os and good metabolic stabilities.

Introduction

The phosphodiesterases (PDEs) which catalyze the hydrolysis of cyclic nucleotides cAMP and cGMP are a group of well-known enzymes that have been classified into several isoenzymes families.[1] Research has been very active and successful in the area of PDE III and IV (cAMP PDEs), whereas at the moment inhibitors of type I (Ca^{2+}-calmodulin PDE) and type II (cGMP-stimulated PDE) with good activity and selectivity have not been reported.[2] Type V PDE (cGMP, calmodulin insensitive) has been subject of much attention, and several, structurally different inhibitors, such as zaprinast, SKF96231, MY5445, and more recently quinazoline derivatives,[3] have been described (Chart 1). PDE V inhibition is a particularly attractive target[4,5] because cGMP mediates the vasorelaxant action of endothelium-derived relaxing factor (NO) as well as the natriuretic and diuretic effect of atrial natriuretic factor (ANF) through activation of PKG (cGMP dependent protein kinase). As such, a potent and selective inhibitor could display vasodilatating, relaxant, and diuretic effects[6,7] and be useful in treatment of hypertension and congestive heart failure.

The known inhibitors being neither very potent nor very specific, we set out to find new compounds with emphasis on selectivity and cellular activity. Since zaprinast and SKF 96231 bear some similarity with the cGMP basic structure, these compounds were chosen as a starting point for structural modifications.

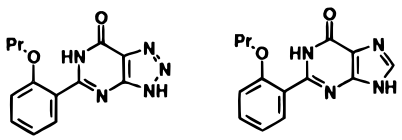
In the patent literature, some examples can be found of compounds where the triazole ring of zaprinast is replaced by imidazole[8] (**2**) and pyrimidine[9] (**3**) with PDE V inhibitory activity in the same range (Table 1). In this paper, we report our results concerning the effect on PDE V inhibitory activity of further modification of the triazole ring and the influence of substituents on the phenyl ring.

Chemistry

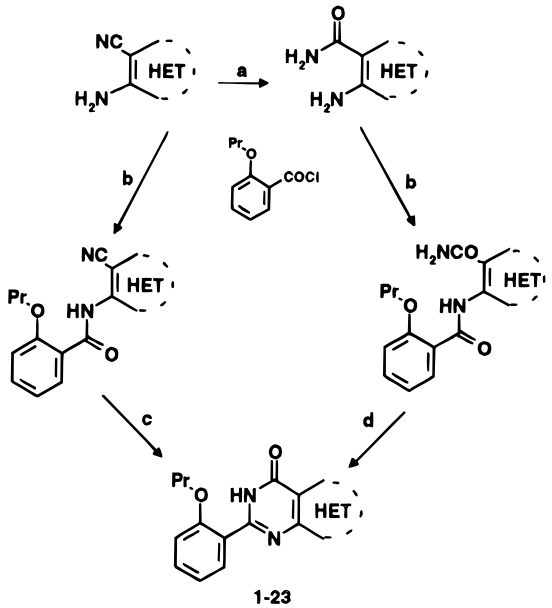
The pyrimidinone basic structure was synthesized according to Scheme 1. The appropriate heterocyclic *o*-aminonitrile was reacted with a substituted benzoyl chloride to give an amide which was cyclized by heating with hydrogen peroxide and dilute NaOH.[10] Alternatively, the starting nitrile was first hydrated with sulfuric acid to the corresponding carboxamide which was condensed with the acid chloride and cyclized with NaOH.

The pyridinopyrimidones **4** and **5** were prepared by condensation of 2-propoxybenzamidine with methyl

Chart 1



Scheme 1[a]



[a] Reagents: (a) H_2SO_4; (b) pyridine; (c) 1 N NaOH, H_2O_2; (d) 1 N NaOH.

[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1996.

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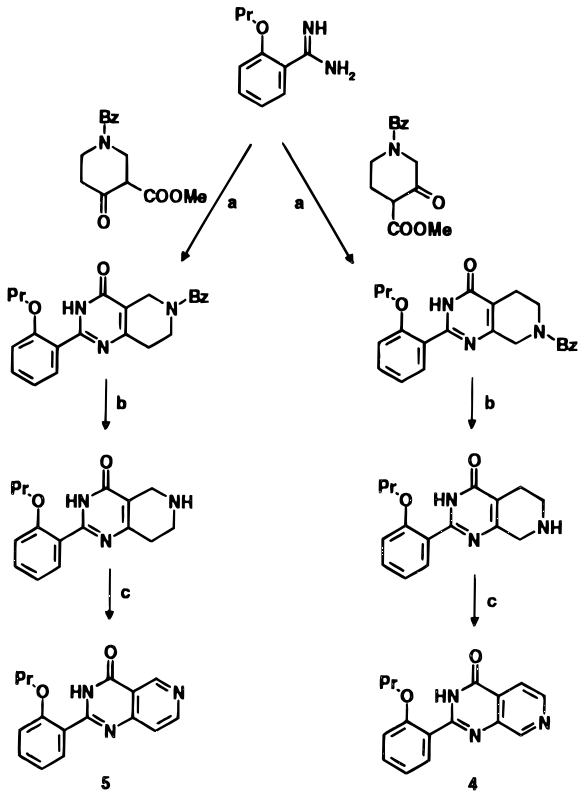
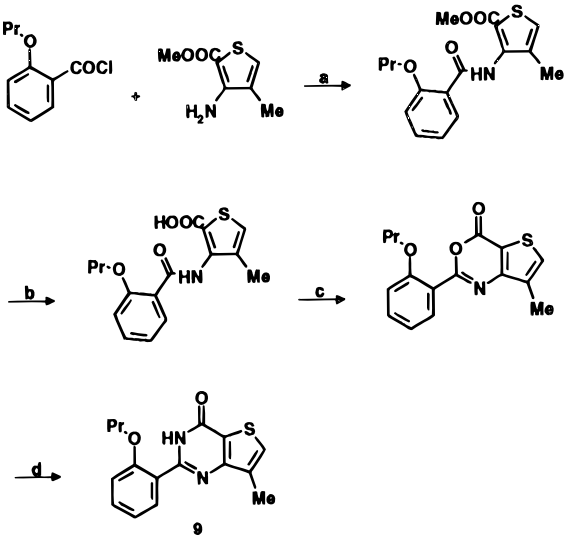
Scheme 2[a]



[a] Reagents: (a) EtONa/EtOH; (b) H_2, Pd/C; (c) Pd/C, xylene.

Scheme 3[a]



[a] Reagents: (a) pyridine; (b) NaOH; (c) $SOCl_2$; (d) NH_3, EtOH.

1-benzyl-3-oxo-4-piperidinecarboxylate and methyl 1-benzyl-4-oxo-3-piperidinecarboxylate, respectively (Scheme 2). The benzyl group was cleaved by hydrogenolysis and the piperidinopyrimidones aromatized by heating in presence of Pd/C in xylene. The thienopyrimidone **9** was prepared in a different way using methyl 3-amino-4-methylthiophene-2-carboxylate as the starting material, according to Scheme 3.

Compounds substituted at the 5-position of the phenyl ring were prepared by standard methods from the 5-nitro derivatives **32** and **33** (Scheme 4) by hydrogenation to the amines **34** and **35** in the presence of Pd/C. Reaction of the amines with sulfonyl or acyl chlorides gave **38**−**43**, with methyl chloroformate **45** and isocyanates **48**−**51**. The unsubstituted ureas and thioureas **46** and **47** were prepared by reaction with KOCN and KSCN, respectively. Reaction of **34** with chloracetyl chloride followed by condensation with *N*-methylpiperazine gave **44**. Methylation of the amines **34** and **35** with HCO_2H/CH_2O gave the *N*,*N*-dimethyl derivatives **36** and **37**. Reaction of **34** with dimethyl (cyanoimino)-dithiocarbonate and subsequent reaction with hydrazine hydrate led to the aminotriazole **52** (Scheme 5). The 5-nitro derivatives **32** and **33** can be prepared either by using 2-alkoxy-5-nitrobenzoyl chloride as in Scheme 1 or by direct nitration of **11** and **18** with nitric acid in trifluoroacetic acid.

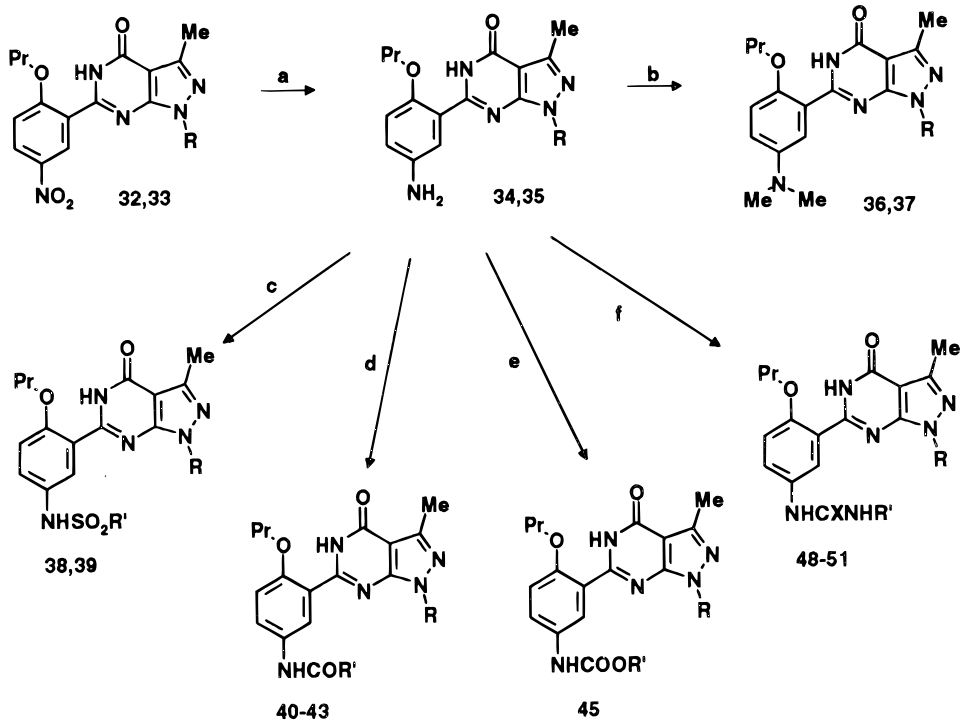
Friedel−Craft reaction of **11** and **18** with bromoacetyl chloride and condensation of the resulting 5-bromoacetyl derivative with a thioamide gave the thiazole analogues **60**−**64** (Scheme 6). Reaction of **11** and **18** with acetyl chloride gave the 5-acetyl derivatives which were transformed in the carboxylic ester by reaction with Br_2/NaOH and esterification with MeOH in the presence of sulfuric acid. Reaction with hydrazine hydrate gave the hydrazide that was cyclized with the appropriate thioamide to give the triazole analogues **53** and **54** (Scheme 6).

2-Thiazoles linked at the 2-position were prepared according to Scheme 7 where **11** and **18** were brominated at the 5-position with bromine in acetic acid and the bromine exchanged with KCN in the presence of 18-crown-6 and tetrakis(triphenylphosphine)palladium(0). Reaction of the nitriles with diethyl dithiophosphate gave the thioamides which were cyclized with a halogenoketone to give **58** and **59** or bromoacetaldehyde to give **57**. Compounds **55**−**56** were prepared by coupling the 5-bromo derivative with the appropriate trimethylstannate in the presence of bis(triphenylphosphine)-palladium(II) chloride (Scheme 8).

Biological Results and Discussion

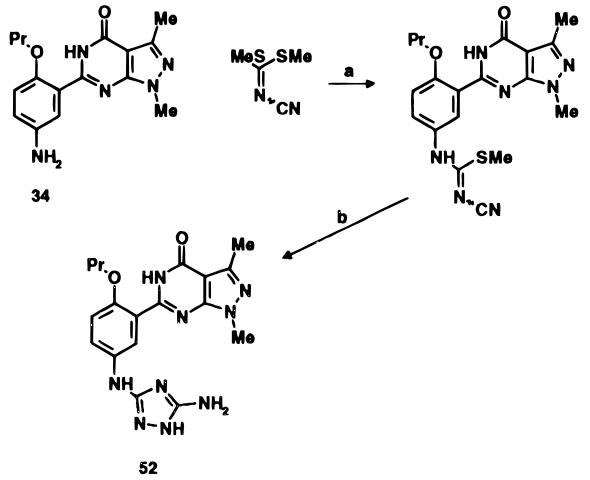
Phosphodiesterase inhibitory activity was assessed on human type V PDE, and the IC_{50} values for the compounds were determined from concentration−response curves using concentrations ranging from 1 nM to 10 μM. The cGMP levels were determined using rat aortic smooth muscle cells (RSMC) stimulated by ANF, and the EC_{50} values are expressed as the dose giving one-half the stimulation shown at saturating concentration. The effects on the IC_{50} and EC_{50} values as a result of varying the heterocycle fused to the pyrimidinone cycle are shown in Table 1. It appeared that in most cases replacement of the triazole ring of zaprinast did not have significant adverse effects and, more interestingly, the pyrazole **11** and the isothiazole **13** proved to be very potent, at both the enzyme and cellular levels. The increase of cGMP in rat smooth muscle cells is particularly vital as this represents the ability of the products to penetrate the cells, and in this way, such a test can be used to forecast in vivo activity. The pyrazolopyrimidone structure **11** was chosen for more detailed structural modification because of its high level of activity and the fact that the 1- and 3-positions of the pyrazole provide an opportunity for further substitution. Since the completion of this work, a patent[11,12] has been published claiming a series of pyrazolo[3,4-*d*]-pyrimidones as PDE V inhibitors confirming the interest of this series. One of the preferred compounds of this patent, **31**, is included in Table 4 for comparison.

Scheme 4[a]



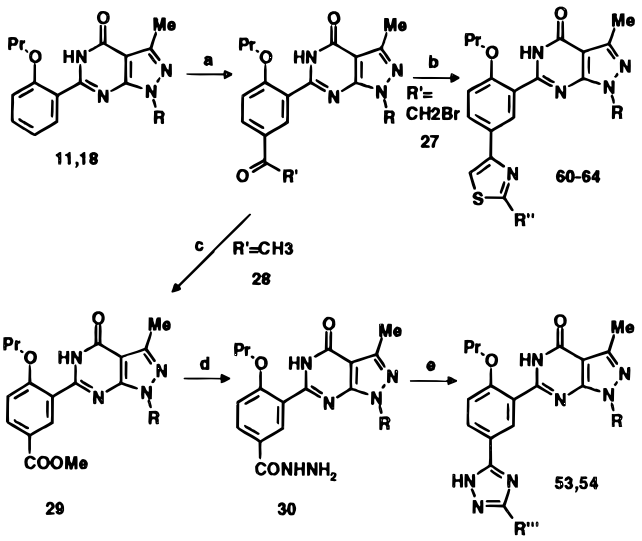
[a] Reagents: (a) Pd/C, H_2, EtOH; (b) CH_2O, HCOOH; (c) R′SO_2Cl, NEt_3, THF; (d) R′COCl, NEt_3, THF; (e) R′OCOCl, NEt_3, THF; (f) R′NC(X), THF.

Scheme 5[a]



[a] Reagents: (a) EtOH; (b) H_2NNH_2, EtOH.

Scheme 6[a]



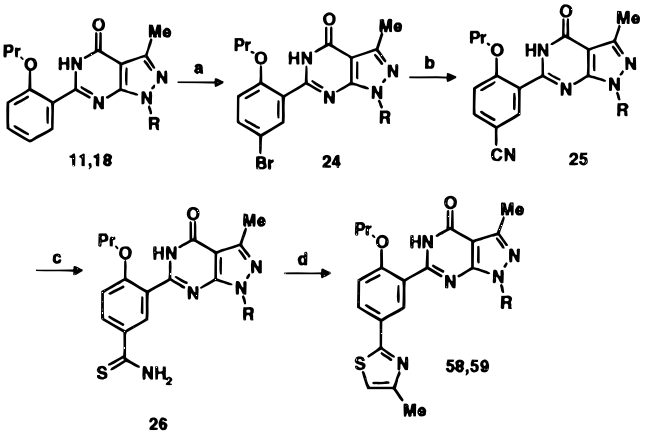
[a] Reagents: (a) R′COCl, $AlCl_3$, CH_2Cl_2; (b) R″$CSNH_2$, EtOH; (c) i, 1 N NaOH, Br_2, ii, MeOH, H^+; (d) H_2NNH_2, EtOH; (e) R‴$CSNH_2$, PhMe.

Table 2 shows the results of modification of the *o*-alkoxy side chain, and it is clear that a 3-carbon chain gives good results for both in vitro and cellular activity. The planarity obtained through hydrogen bonding between the oxygen atom of the side chain and the NH of the pyrimidone ring is also probably important, since the thiopropyl derivative **16** is inactive. Although the 5-nitrogen of the pyrazolopyrimidone is likely to be involved in this hydrogen bond, the possibility of the involvement of the 7-nitrogen can not be totally excluded. Substitution at the 1-position of the pyrazole ring did not affect the activity (Table 3), although a slight decrease in potency was observed with bulky substituents. A methyl or ethyl group seems to be optimum for good activity.

We examined the incorporation of substituents at the 5-position of the 6-phenyl ring. In Table 4 are shown the activities of amino derivatives: In most cases, the activity was conserved or improved. It seems that there is a great tolerance for substitution at this position. It appears that there is no significant difference between small groups like NH_2 and more bulky ones such as the urea (**51**). The thiophenecarboxamide **43** and the 4-fluorobenzenecarboxamide **42** showed outstanding activity on the RSMC test, but unfortunately these products were plagued with poor metabolic stability, and we sought less labile groups.

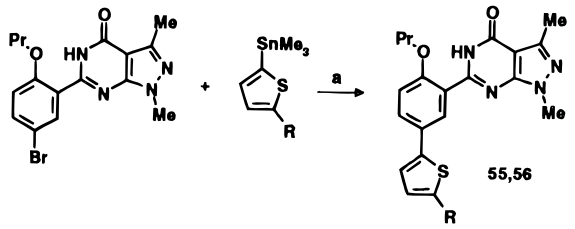
The results of substitution by small heterocycles, most of them carbon linked, are depicted in Table 5. The aminotriazole **52** retained good enzymatic activity but was inactive in the cellular test. On the contrary, the

Scheme 7[a]



[a] Reagents: (a) Br_2, AcOH; (b) KCN, 18-C-6, $Pd(PPh_3)_4$, THF; (c) $(EtO)_2P(S)SH$, H_2O; (d) $ClCH_2COMe$, EtOH.

Scheme 8[a]

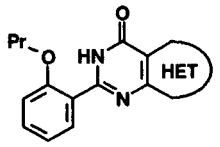


[a] Reagents: (a) $Pd(PPh_3)_2Cl_2$, THF.

phenyltriazoles **53** and **54** were extremely active. To further explore the effect of substitution by heterocycles, thiazoles were linked via their 2- or 4-position. The compounds **57**−**64** were prepared, and these products proved to be potent with no significant differences between them. Thienyl derivatives **55** and **56** were also in the same range of activity, enzymatic and cellular.

For a selection of the best compounds, antihypertensive activity was determined by oral administration in the conscious spontaneously hypertensive rat (SHR), and the results are expressed as the area under the mean arterial blood pressure decrease versus time, curve from 0 to 5 h after administration (AUC in mmHg h) (Table 6). This is a way of evaluating the antihypertensive activity taking into account the fall of blood pressure and the duration of action. The unsubstituted product **11** and the nitro (**32** and **33**) and amino (**35**) derivatives, although being very potent on the RSMC test, displayed weak oral antihypertensive activity. In the case of the amino derivative **34**, pharmacokinetic studies have shown that low concentration of the parent compound was observed in plasma and tissues, but high concentration of a metabolite was present, and this could partly account for the observed in vivo activity. This metabolite has been isolated and identified as the corresponding *N*-acetyl derivative **40**. The latter compound has been synthesized and displayed a potent hypotensive effect with an AUC of 203 mmHg h. However its metabolic stability was low, and its activity was probably due to another unknown metabolite. In general potency was regained by substitution of the amino group except for the urea analogues **46** and **51** which proved to be inactive orally. However poor metabolic stability was observed within these analogues. The sulfonamido **38** was the only amino derivative to provide substantially improved metabolic stability and bioavailability together with a potent hypotensive effect.

Table 1. Variation of the Heterocycle



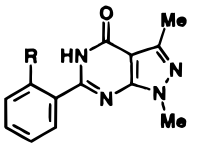
no	HET	formula	mp (°C)	PDE V IC_{50}[a] (nM)	RSMC EC_{50}[b] (μM)
1		$C_{13}H_{13}N_5O_2$	243	200 ± 100	9
2		$C_{14}H_{14}N_4O_2$	262	80 ± 45	2
3		$C_{15}H_{14}N_4O_2$	140	300 ± 45	7.5
4		$C_{16}H_{15}N_3O_2$	184	70 ± 30	2
5		$C_{16}H_{15}N_3O_2$	136	50 ± 30	10
6		$C_{15}H_{14}N_2O_2S$	123	100 ± 45	2.5
7		$C_{16}H_{16}N_2O_2S$	172	60 ± 35	0.7
8		$C_{15}H_{14}N_2O_2S$	145	50 ± 27	1
9		$C_{16}H_{16}N_2O_2S$	96	80 ± 40	1.5
10		C14H14N4O2	180	150 ± 70	>10
11		$C_{16}H_{18}N_4O_2$	139	8 ± 4	0.2
12		C15H15N3O3	176	100 ± 45	>10
13		C15H15N3O2S	160	30 ± 10	1.5

[a] IC_{50} ± SD (mean of three determinations). [b] Single determination.

The substituted triazole **53** was not very potent in vivo in spite of its good activity on the RSMC test. It is noteworthy that not all the products active on the RSMC test are active in vivo, but no product inactive in the cellular test was active in vivo which is good proof of the predictivity of the test. The thiazoles **59**, **61**, and **64** displayed good in vivo activity, especially **59** with an AUC of 198 mmHg h.

The inhibitory activity toward the different PDE isoforms has been determined for some selected compounds and is shown in Table 7. The reference inhibitor

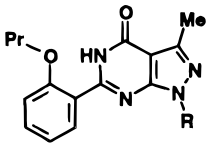
Table 2. Replacement of the Propoxy Chain



no.	R	formula	mp (°C)	PDE V IC_{50}[a] (nM)	RSMC EC_{50}[b] (μM)
14	OEt	$C_{15}H_{16}N_4O_2$	207	30 ± 15	2
11	OPr	$C_{16}H_{18}N_4O_2$	139	8 ± 3.5	0.2
15	O-iPr	$C_{16}H_{18}N_4O_2$	128	40 ± 15	1
16	SPr	$C_{16}H_{18}N_4OS$	176	2000	
17	SO-Pr	$C_{16}H_{18}N_4O_2S$	245	59%[c]	

[a,b] See footnotes in Table 1. [c] Percent inhibition at 10 μM.

Table 3. Substitution of the Pyrazole



no.	R	formula	mp (°C)	PDE V IC_{50}[a] (nM)	RSMC EC_{50}[b] (μM)
11	Me	$C_{16}H_{18}N_4O_2$	139	8 ± 4	0.2
18	Et	$C_{17}H_{20}N_4O_2$	139	2 ± 1	<0.1
19	Pr	$C_{18}H_{22}N_4O_2$	99	1 ± 0.4	0.15
20	Bu	$C_{19}H_{24}N_4O_2$	111	6 ± 2.5	0.9
21	tBu	$C_{19}H_{24}N_4O_2$	194	20 ± 15	0.2
22	CH_2CF_3	$C_{17}H_{17}F_3N_4O_2$	182	7 ± 3	1.5
23	benzyl	$C_{22}H_{22}N_4O_2$	147	70 ± 20	0.35

[a,b] See footnotes in Table 1.

zaprinast exhibited weak selectivity over other PDE isoenzymes. The sulfonamido derivative **38** and the thiazole **59** showed excellent selectivity (over 1000-fold) over PDE I−IV, which makes them the most potent and selective PDE V inhibitors reported up to now.

In conclusion, we have found that 6-(2-propoxyphenyl)pyrazolo[3,4-*d*]pyrimidones display selective activity toward cGMP PDE, the key features being the presence of a substituent at the 5-position. This position can accommodate a great range of unrelated groups without loss of potency, but in vivo activity and metabolic stability require the presence of carbon-linked small heterocycles. The sulfonamido derivative **38** has been selected for more detailed investigations.[15]

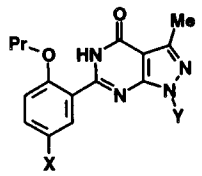
Experimental Section

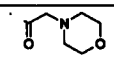
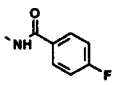
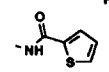
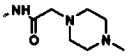
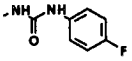
The melting points were determined on a hot-stage Kofler apparatus and are not corrected. Silica gel plates (Merck F254) and silica gel 60 (230−400 mesh) were used for analytical and column chromatography, respectively. Microanalyses were within ±0.4% of the theoretical values, unless stated otherwise. The IR spectra were recorded with a Perkin Elmer FTIR 1600 spectrometer in KBr and are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained with a Bruker AC250 spectrometer at 250 MHz, and shifts are expressed in ppm with TMS as internal standard and are consistent with the proposed structures.

5-Amino-4-cyano-1-ethyl-3-methylpyrazole. A suspension of ethylhydrazine oxalate (25.0 g, 0.166 mol) and sodium methoxide (17 g, 0.34 mol) in methanol (1 L) was stirred and heated under reflux for 15 min. The mixture was filtered, (1-ethoxyethylidene)malononitrile (18.9 g, 0.139 mol) was added to the methanolic solution, and the reflux was continued for 2 h. The solution was then concentrated under reduced pressure and the residue extracted with diethyl ether. The ether extract was washed with water, dried (Na_2SO_4), and concentrated to give the title compound as white crystals (19.0 g, 91%): mp 120−2 °C; IR (KBr) 2209 cm^{-1} (CN); ^{1}H-NMR ($CDCl_3$) δ 4.3 (s, 2H, NH_2), 3.9 (q, 2H, CH_2), 2.3 (s, 3H, CH_3), 1.4 (t, 3H, CH_3).

5-Amino-4-cyano-1,3-dimethylpyrazole. Methylhydrazine (8.5 g, 0.185 mol) was added dropwise to a stirred solution of (1-ethoxyethylidene)malononitrile (25.0 g, 0.184 mol) in ethanol (200 mL) at room temperature. The mixture was then

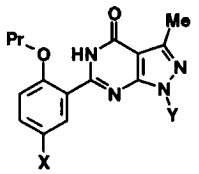
Table 4. Substitution of the Phenyl by Amino Derivatives

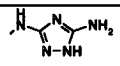
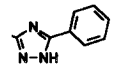
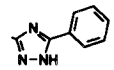
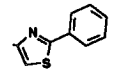
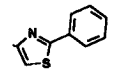
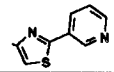


no	X	Y	formula	mp (°C)	PDE V IC_{50}[a] (nM)	RSMC EC_{50}[b] (μM)
31		Me	$C_{22}H_{27}N_5O_4$	195	4 ± 2	0.3
32	NO_2	Me	$C_{16}H_{17}N_5O_4$	228	4 ± 3	0.1
33	NO_2	Et	$C_{17}H_{19}N_5O_4$	203	0.1 ± 0.05	0.05
34	NH_2	Me	$C_{16}H_{19}N_5O_2$	141	10 ± 6	0.3
35	NH_2	Et	$C_{17}H_{21}N_5O_2$	167	7 ± 4	0.02
36	$N(Me)_2$	Me	$C_{18}H_{23}N_5O_2$	180	5 ± 3	0.3
37	$N(Me)_2$	Et	$C_{19}H_{25}N_5O_2$	153	4 ± 2	1.5
38	$NHSO_2Me$	Me	$C_{17}H_{21}N_5O_4S$	236	3 ± 1	0.35
39	$NHSO_2Me$	Et	$C_{18}H_{23}N_5O_4S$	245	4 ± 2	0.2
40	NHCOMe	Me	$C_{18}H_{21}N_5O_3$	155	0.8 ± 0.5	0.04
41	NHCOMe	Et	$C_{19}H_{23}N_5O_3$	239	2 ± 1.5	0.03
42		Me	$C_{23}H_{22}FN_5O_3$	259	2 ± 1.3	< 0.01
43		Me	$C_{21}H_{21}N_5O_3S$	263	1.5 ± 1	< 0.01
44		Me	$C_{23}H_{31}N_7O_3$	163	3.5 ± 2	0.4
45	NHCOOMe	Me	$C_{18}H_{21}N_5O_4$	253	2 ± 1	0.1
46	$NHCONH_2$	Me	$C_{17}H_{20}N_6O_3$	300	2 ± 1	0.9
47	$NHCSNH_2$	Me	$C_{17}H_{20}N_6O_2S$	290	1 ± 0.6	1
48	NHCONHEt	Me	$C_{19}H_{24}N_6O_3$	275	3 ± 2	0.05
49	NHCSNHEt	Me	$C_{19}H_{24}N_6O_2S$	261	3 ± 2	0.06
50	NHCSNH-COOEt	Me	$C_{20}H_{24}N_6O_4S$	241	2 ± 1	0.04
51		Me	$C_{23}H_{23}FN_6O_3$	280	0.2 ± 0.15	0.04

[a,b] See footnotes in Table 1.

Table 5. Substitution of the Phenyl by Heterocycles



no	X	Y	formula	mp (°C)	PDE V IC_{50}[a] (nM)	RSMC EC_{50}[b] (μM)
52		Me	$C_{18}H_{21}N_9O_2$	300	5 ± 3	> 10
53		Me	$C_{24}H_{23}N_7O_2$	250	20 ± 8	< 0.1
54		Et	$C_{25}H_{25}N_7O_2$[c]	224	1.7± 0.5	< 0.01
55		Me	$C_{20}H_{20}N_4O_2S$	184	5 ± 2	0.3
56		Me	$C_{21}H_{22}N_4O_2S$	178	4 ± 2	< 0.1
57		Me	$C_{19}H_{19}N_5O_2S$	209	3 ± 1	< 0.1
58		Me	$C_{20}H_{21}N_5O_2S$	230	2.5 ± 2	0.45
59		Et	$C_{21}H_{23}N_5O_2S$	218	2 ± 1	< 0.1
60		Me	$C_{20}H_{21}N_5O_2S$	204	2.5 ± 2	0.2
61		Et	$C_{21}H_{23}N_5O_2S$	183	3 ± 1	< 0.1
62		Me	$C_{25}H_{23}N_5O_2S$	200	2.5 ± 2	0.2
63		Et	$C_{26}H_{25}N_5O_2S$	217	3 ± 2	0.2
64		Et	$C_{25}H_{24}N_6O_2S$	219	2 ± 2	< 0.1

[a,b] See footnotes in Table 1. [c] N: calcd, 22.21; found, 18.78.

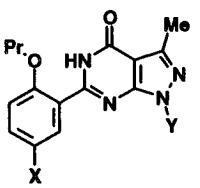
heated under reflux for 2 h and cooled in ice. The resulting crystals were filtered, washed with ethanol and ether, and dried to give the title compound as white crystals (21.0 g, 84%): mp 193 °C; ^{1}H-NMR ($CDCl_3$) δ 4.2 (s, 2H, NH_2), 3.55 (s, 3H, NCH_3), 2.2 (s, 3H, CH_3).

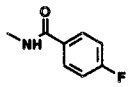
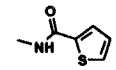
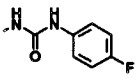
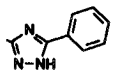
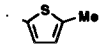
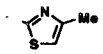
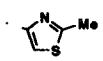
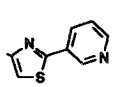
2-Amino-3-carbamoylthiophene. A mixture of thioacetaldehyde (6.0 g, 0.077 mol), cyanoacetamide (8.4 g, 0.1 mol) and triethylamine (10 mL) in ethanol (50 mL) was heated at 70 °C for 2 h, after which time the dissolution was complete. One-half of the ethanol was evaporated and the solution cooled in ice. The crystals formed were filtered, washed with a little cold ethanol, and dried. After crystallization from water (100 mL), the title product was obtained as pale gray crystals (6.8 g, 62%): mp 160 °C.

3-Amino-4-cyano-5-methylisoxazole. Hydroxylamine hydrochloride (14.0 g, 0.2 mol) was added to a solution of 10% NaOH (80 mL), and (1-ethoxyethylidene)malononitrile (27.2 g, 0.2 mol) was added with vigorous stirring while keeping the temperature below 50 °C. The mixture was then stirred at room temperature for 2 h; the resultant precipitate was filtered, washed with water, and dried to give the title compound as white crystals (20.7 g, 84%): mp 222−4 °C.

3-Amino-4-cyano-5-methylisothiazole. To a stirred solution of sodium (3 g, 0.125 mol) in ethanol (125 mL) were added

Table 6. Antihypertensive Activity in SHR Rats



no	X	Y	RSMC EC_{50} (μM)	Vivo[a] AUC mmHg.h
1	zaprinast		9	28 ± 15[d]
11	H	Me	0.2	92 ± 23[d]
32	NO_2	Me	0.1	74 ± 48[d]
33	NO_2	Et	< 0.1	69 ± 58[c]
34	NH_2	Me	0.3	128 ± 52[d]
35	NH_2	Et	0.02	77 ± 28[b]
38	$NHSO_2Me$	Me	0.35	129 ± 11[b]
40	NHCOMe	Me	0.04	203 ± 59[b]
42		Me	< 0.01	144 ± 44[b]
43		Me	< 0.01	123 ± 28[b]
45	$NHCO_2Me$	Me	0.1	120 ± 25[b]
46	$NHCONH_2$	Me	0.9	0[c]
50	NHCSNH-COOEt	Me	< 0.1	109 ± 30[c]
51		Me	0.04	0[c]
53		Me	< 0.1	81 ± 18[b]
56		Me	< 0.1	100 ± 28[b]
59		Et	< 0.1	198 ± 31[b]
61		Et	< 0.1	155 ± 46[b]
64		Et	< 0.1	145 ± 25[b]

[a] Hypotensive effect in SHR rats; AUC is the area of the change in MABP versus time curve, relative to solvent, recorded from 0 to 5 h. Mean ± SD of six animals. [b] 5 mg/kg/po. [c] 10 mg/kg/po. [d] 30 mg/kg/po.

dropwise simultaneously (two dropping funnels) malononitrile (8.25 g, 0.125 mol) and ethyl dithioacetate (13 mL, 0.125 mol) each in ethanol (50 mL) at room temperature. The mixture was then heated at reflux for 3 h and concentrated to dryness. The residue was dissolved in water (150 mL) and added to a solution of chloramine prepared by mixing NaOCl at 5% Cl (250 mL) with a solution of NH_4OH (20%, 30 mL) in water

Table 7. Selectivity Data[a]

	I	II	III	IV	V
zaprinast	3 ± 0.6	70 ± 0.5	100	70 ± 7	0.2 ± 0.1
38	1 ± 0.5	3 ± 0.2	10 ± 1.5	22 ± 3	0.003 ± 0.001
59	1 ± 0.5	21%[b]	48%[b]	28%[b]	0.002 ± 0.001

[a] IC_{50}, μM. [b] Percent inhibition at 10 μM.

(250 mL) while keeping the temperature below 5 °C. The yellow precipitate that formed was filtered, washed with water, and dried. Recrystallization from ethanol afforded the title compound as pale yellow crystals (11.4 g, 65%): mp 179−80 °C.

5-Amino-4-carbamoyl-1,3-dimethylpyrazole. 5-Amino-4-cyano-1,3-dimethylpyrazole (10.0 g, 0.073 mol) was slowly added with stirring to concentrated sulfuric acid (50 mL) while maintaining the temperature below 10 °C. The mixture was then heated at 60 °C for 2 h and poured into crushed ice. The solution was made slightly alkaline (pH = 9) with 20% NH_4OH, and the resulting crystals were filtered to give the title compound as white crystals (9.0 g, 80%): mp 202 °C; ^{1}H-NMR (DMSO) δ 6.45 (brs, 2H, $CONH_2$), 6.15 (s, 2H, NH_2), 3.45 (s, 3H, NCH_3), 2.2 (s, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxyphenyl)-1,5-dihydropyrazolo-[3,4-*d*]pyrimidin-4-one (11). 2-Propoxybenzoyl chloride (22.5 g, 0.113 mol) was added dropwise to a solution of 5-amino-4-cyano-1,3-dimethylpyrazole (15.0 g, 0.11 mol) in pyridine (150 mL) while maintaining the temperature below 10 °C. The mixture was then stirred at room temperature for 2 h and then at 45 °C for 0.5 h. After pouring into iced water, the resulting precipitate was filtered, washed with water, and dried.

This material was heated at 90 °C with stirring in 1 N sodium hydroxide (220 mL), H_2O (450 mL), and 30% hydrogen peroxide (60 mL) for 3 h. Further H_2O_2 (25 mL) was added and the mixture heated for a further 5 h. The solution was filtered to remove a small amount of insoluble material and then acidified with diluted HCl. The precipitate was collected, washed with water, and dried. Recrystallization from ethanol yielded **11** as white crystals (12.1 g, 37%): mp 139−40 °C; IR (KBr) 1692.8 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 11.05 (s, 1H, NH), 8.5 (dd, 1H, Ar), 7.5 (td, 1H, Ar), 7.15 (t, 1H, Ar), 7.05 (d, 1H, Ar), 4.2 (t, 2H, OCH_2), 3.95 (s, 3H, NCH_3), 2.6 (s, 3H, CH_3), 2 (m, 2H, CH_2), 1.2 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-nitrophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (32). Compound **11** (33.0 g, 0.11 mol) was added slowly to a stirred mixture of trifluoroacetic acid (300 mL) and nitric acid (69%, d = 1.42, 70 mL) while keeping the temperature below 5 °C, and the mixture was stirred overnight at room temperature. The reaction mixture was poured into ice and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated to give a solid. Recrystallization of this material from ethanol gave **32** as pale yellow crystals (32.0 g, 85%): mp 228−9 °C; IR (KBr) 3317 (NH), 1698 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 10.75 (s, 1H, NH), 9.35 (sd, 1H, Ar), 8.35 (dd, 1H, Ar), 7.2 (d, 1H, Ar), 4.35 (t, 2H, OCH_2), 4 (s, 3H, NCH_3), 2.65 (s, 3H, CH_3), 2.1 (m, 2H, CH_2), 1.2 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-nitrophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (33). Method A: 2-Propoxy-5-nitrobenzoyl chloride (2.43 g, 10 mmol) was added to a solution of 5-amino-4-cyano-1-ethyl-3-methylpyrazole (1.5 g, 10 mmol) in pyridine (25 mL). The mixture was heated at 60 °C for 3 h and then poured into water. The resulting precipitate was filtered, washed with water and then ethanol, and dried. This crude product was heated with stirring at 100 °C in a mixture of 1 N sodium hydroxide (50 mL), water (30 mL), and 30% hydrogen peroxide (3 mL) for 18 h. The solution was filtered and acidified with dilute HCl. The resulting precipitate was collected by filtration, washed with water, and dried. Purification by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 95:5) and recrystallization from ethanol gave **33** as pale yellow crystals (330 mg, 9.2%): mp 202−4 °C; ^{1}H-NMR (DMSO) δ 2.1 (s, 1H, NH), 8.5 (sd, 1H, Ar), 8.4 (dd, 1H, Ar), 7.4 (d, 1H, Ar), 4.3 (q, 2H, NCH_2), 4.2 (t, 2H, OCH_2), 2.45 (s, 3H, CH_3), 1.8 (m, 2H, CH_2), 1.4 (t, 3H, CH_3), 1 (t, 3H, CH_3).

Method B: 2-Propoxy-5-nitrobenzoyl chloride (2.43 g, 10 mmol) was added to a solution of 5-amino-4-carbamoyl-1,3-dimethylpyrazole (1.54 g, 10 mmol) in pyridine (20 mL). The mixture was stirred overnight at room temperature and poured into water. The resulting precipitate was filtered, washed with water and ethanol, and dried. This material was dissolved in 1 N sodium hydroxide (50 mL) and ethanol (10 mL) and heated at reflux for 4 h with stirring. The solution was then poured into water and acidified with dilute HCl, and the resulting precipitate was filtered, washed with water, and dried. Purification by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 95:5) followed by recrystallization from ethanol afforded **33** as pale yellow crystals (210 mg, 6.1%): mp 202−4 °C.

1,3-Dimethyl-6-(2-propoxy-5-aminophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (34). A solution of **32** (7.1 g, 0.02 mol) in THF (250 mL) and ethanol (250 mL) was hydrogenated in the presence of 10% Pd/C (0.7 g) at room temperature. After absorption of the theoretical volume of hydrogen, the mixture was filtered through a Celite pad, and the filtrate was concentrated in vacuo to give a yellow solid. Recrystallization of this material from acetonitrile gave **34** as pale yellow crystals (6 g, 92%): mp 140−2 °C; IR (KBr) 3413 (NH), 3344−3286 (NH_2), 1691 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 11.25 (s, 1H, NH), 7.9 (s, 1H, Ar), 6.8−6.95 (m, 2H, Ar), 4.1 (t, 2H, OCH_2), 3.95 (s, 3H, NCH_3), 3.7 (brs, 2H, NH_2), 2.6 (s, 3H, CH_3), 2 (q, 2H, CH_2), 1.15 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-methanesulfonamidophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (38). Methanesulfonyl chloride (0.3 mL, 3.9 mmol) was added to a solution of **34** (625 mg, 2 mmol) in THF (50 mL) and triethylamine (0.6 mL), and the reaction mixture was stirred at room temperature for 1 h. The mixture was then concentrated and the residue treated with water and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was recrystallized from ethanol to give **38** as white crystals (300 mg, 38%): mp 235−7 °C; IR (KBr) 3302 (NH), 1679 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 10.95 (s, 1H, NH), 8.3 (sd, 1H, Ar), 7.5 (dd, 1H, Ar), 7.05 (d, 1H, Ar), 6.95 (s, 1H, NH), 4.15 (t, 2H, OCH_2), 3.95 (s, 3H, NCH_3), 3 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 1.95 (m, 2H, CH_2), 1.1 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-acetamidophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (40). Acetyl chloride (0.3 mL, 4.2 mmol) was added to a solution of **34** (625 mg, 2 mmol) and triethylamine (0.6 mL) in THF (30 mL), and the mixture was stirred at room temperature for 0.5 h. The resultant precipitate was filtered off and the solution evaporated to dryness. The residue was taken up in water and the mixture extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and concentrated. Recrystallization from ethanol afforded **40** as white crystals (400 mg, 56%): mp 254−6 °C; IR (KBr) 3304 (NH), 1707, 1664 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 11.1 (s, 1H, NH), 8.4 (sd, 1H, Ar), 7.95 (dd, 1H, Ar), 7.35 (s, 1H, NH), 7.05 (d, 1H, Ar), 4.15 (t, 3H, OCH_2), 3.95 (s, 3H, NCH_3), 2.6 (s, 3H, CH_3), 2.2 (s, 3H, CH_3), 1.95 (m, 2H, CH_2), 1.15 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-((methoxycarbonyl)amino)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (45). Methyl chloroformate (0.2 mL, 2.6 mmol) was added to a solution of **34** (500 mg, 1.59 mmol) in THF (50 mL) and triethylamine (0.5 mL), and the reaction mixture was stirred at room temperature for 1 h. The mixture was concentrated and the residue treated with water and extracted with CH_2Cl_2. The organic extract was washed with water, dried (SO_4Na_2), and evaporated to dryness. The residue was recrystallized from THF to give **45** as white crystals (280 mg, 47%): mp 253 °C; IR (KBr) 3323 (NH), 1735, 1674 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 11.1 (s, 1H, NH), 8.4 (sd, 1H, Ar), 7.75 (dd, 1H, Ar), 7.35 (s, 1H, NH), 7.05 (d, 1H, Ar), 4.20 (t, 3H, OCH_2), 4.0 (s, 3H, NCH_3), 3.80 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 2 (m, 3H, CH_3), 1.6 (s, 3H, CH_3), 1.15 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-ureidophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (46). Potassium cyanate (1 g, 12.3 mmol) was added to a solution of **34** (500 mg, 1.5 mmol) in water (5 mL) and acetic acid (5 mL). After stirring at room temperature for 1 h, the crystals that formed

were filtered, washed with water, and dried. Recrystallization from ethanol provided **46** as white crystals (300 mg, 54%): mp 300 °C; IR (KBr) 3307−3427 (NH, NH_2), 1701, 1668 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.65 (s, 1H, NH), 8.55 (s, 1H, NH), 7.8 (sd, 1H, Ar), 7.55 (dd, 1H, Ar), 7.05 (d, 1H, Ar), 5.8 (s, 2H, NH_2), 4 (t, 2H, OCH_2), 3.85 (s, 3H, NCH_3), 2.45 (s, 3H, CH_3), 1.7 (m, 2H, CH_2), 0.95 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-thioureidophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (47). A mixture of potassium thiocyanate (2 g, 20.5 mmol) and **34** (500 mg, 1.5 mmol) in water (5 mL) and acetic acid (5 mL) was heated at reflux with stirring for 3 h. The mixture was then diluted with water, and the crystals that formed were filtered, washed with water and ethanol, and dried. Recrystallization from dimethylformamide afforded **47** as white crystals (240 mg, 41%): mp 290 °C; ^{1}H-NMR (DMSO) δ 11.7 (s, 1H, NH), 9.65 (s, 1H, NH), 7.65 (d, 1H, Ar), 7.5 (dd, 1H, Ar), 7.1 (d, 1H, Ar), 4H (t, 2H, OCH_2), 3.8 (s, 3H, NCH_3), 2.4 (s, 3H, CH_3), 1.7 (m, 2H, CH_2), 0.95 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-((4-fluorophenyl)ureido)-phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (51). 4-Fluorophenyl isocyanate (0.15 mL, 1.3 mmol) was added to a solution of **34** (330 mg, 1 mmol) in THF (40 mL). Crystallization occurred after a few minutes, and the mixture was stirred for 30 min at room temperature. The crystals were collected by filtration, washed with ether, and dried. Recrystallization from THF gave **51** as white crystals (200 mg, 44%): mp 280 °C; IR (KBr) 3301 (NH), 1702, 1668 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.65 (s, 1H, NH), 8.65 (d, 2H, Ar), 7.85 (sd, 1H, Ar), 7.55 (dd, 1H, Ar), 7.4 (m, 2H, NH), 7.1 (m, 3H, Ar), 4 (t, 2H, OCH_2), 3.8 (s, 3H, NCH_3), 2.4 (s, 3H, CH_3), 1.7 (m, 2H, CH_2), 0.95 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-(dimethylamino)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (36). Formaldehyde solution (3 mL, 35%) was added to a solution of **34** (0.5 g, 1.52 mmol) in formic acid (3 mL), and the reaction mixture was heated overnight at 80 °C. The resultant solution was then poured into water and neutralized with dilute NaOH and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and concentrated to give a yellow oil that was purified by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 95:5). Recrystallization from ethanol gave **36** as white crystals (125 mg, 24%): mp 180 °C; IR (KBr) 3287 (NH), 1689 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 11.2 (s, 1H, NH), 7.4 (sd, 1H, Ar), 6.95 (m, 2H, Ar), 4.1 (t, 2H, OCH_2), 3.95 (s, 3H, NCH_3), 3 (s, 6H, $N(CH_3)_2$), 2.6 (s, 3H, CH_3), 1.95 (m, 2H, CH_2), 1.15 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(5-cyano-2-propoxyphenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (25). A mixture of **24** (350 mg, 0.9 mmol), sodium cyanide (45 mg, 0.9 mmol), 18-crown-6 (0.24 g, 0.9 mmol), and tetrakis(triphenylphosphine)-palladium(0) (0.52 g, 0.45 mmol) in THF (20 mL) and EtOH (20 mL) was heated under reflux for 4 h. The reaction mixture was evaporated to dryness, and the residue was diluted with water and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4) and concentrated to give a solid that was purified by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 98:2). After crystallization from 2-propanol, **25** was obtained as white crystals (0.14 g, 46%): mp 190 °C; IR (KBr) 3318 (NH), 2232 (CN), 1701 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.95 (s, 1H, NH), 8.05 (sd, 1H, Ar), 7.95 (dd, 1H, Ar), 7.3 (d, 1H, Ar), 4.2 (q, 2H, NCH_2), 4.1 (t, 2H, OCH_2), 2.4 (s, 3H, CH_3), 1.7 (m, 2H, CH_2), 1.35 (t, 3H, CH_3), 0.9 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-thiocarbamoylphenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (26). A mixture of **25** (850 mg, 2.5 mmol), 85% diethyl dithiophosphate (1 mL, 6 mmol), and water (3 drops) was stirred at room temperature for 24 h. By this time the mixture was homogeneous. It was taken up with water, and the precipitate was filtered, washed with water, and dried. After crystallization from methanol, **26** was obtained as yellow crystals (580 mg, 62.5%): mp 256 °C; IR (KBr) 3330, 3184 (NH, NH_2), 1662 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.8 (s, 1H, NH), 9.75, 9.45 (2s, 2H, NH_2), 8.2 (sd, 1H, Ar), 8.1 (dd, 1H, Ar), 7.15 (d, 1H, Ar), 4.25 (q, 2H, NCH_2), 4.1 (t, 2H, OCH_2), 2.4 (s, 3H, CH_3), 1.7 (m, 2H, CH_2), 1.35 (t, 3H, CH_3), 0.9 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-(4-methylthiazol-2-yl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (59). A mixture of **26** (450 mg, 1.2 mmol) and chloroacetone (0.2 mL, 2.4 mmol) in ethanol (30 mL) was heated under reflux for 16 h. After concentration, the residue was treated with water and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 95:5). After recrystallization from 2-propanol, **59** was obtained as white crystals (160 mg, 32%): mp 218 °C; IR (KBr) 3103−3314 (NH), 1706 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.85 (s, 1H, NH), 8.1 (sd, 1H, Ar), 7.95 (dd, 1H, Ar), 7.25 (s, 1H, Ar), 7.2 (d, 1H, Ar), 4.2 (q, 2H, NCH_2), 4 (t, 2H, OCH_2), 2.4 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 1.65 (m, 2H, CH_2), 1.3 (t, 3H, CH_3), 0.85 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-(thiazol-2-yl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (57). A mixture of 1,3-dimethyl-6-(2-propoxy-5-thiocarbamoylphenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (350 mg, 0.98 mmol), potassium carbonate (406 mg, 2.94 mmol), and bromoacetaldehyde (240 mg, 1.96 mmol) in DMF (20 mL) was stirred at room temperature overnight. After concentration under reduced pressure, the residue was treated with water and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4) and evaporated to dryness.

A mixture of the above product and trifluoroacetic anhydride (1 mL) in CH_2Cl_2 (20 mL) was stirred at room temperature for 1 h. After dilution with CH_2Cl_2, the organic phase was washed with water, dried (Na_2SO_4), and concentrated to leave an oil which was purified by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 95:5). After crystallization from H_2O/DMF, **57** was obtained as cream crystals (0.12 g, 32%): mp 209 °C; IR (KBr) 3520, 3319 (NH), 1697.5 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.8 (s, 1H, NH), 8.15 (sd, 1H, Ar), 8 (dd, 1H, Ar), 7.8 (d, 1H, Ar), 7.65 (d, 1H, Ar), 7.2 (d, 1H, Ar), 4 (t 2H, OCH_2), 3.75 (s, 3H, NCH_3), 2.35 (s, 3H, CH_3), 1.65 (m, 2H, CH_2), 0.9 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-(2-phenylthiazol-4-yl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (62). A mixture of **27** (400 mg, 0.95 mmol) and thiobenzamide (130 mg, 0.95 mmol) in ethanol (50 mL) was heated under reflux for 1 h. The solution was concentrated, treated with water, and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and concentrated to give a solid which was purified by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 95:5). Crystallization from methanol gave **62** as white crystals (0.12 g, 28%): mp 200 °C; IR (KBr) 3318, 3083 (NH), 1700, 1683 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.9 (s, 1H, NH), 8.35 (sd, 1H, Ar), 8.2 (dd, 1H, Ar), 8.15 (s, 1H, ar), 8 (m, 2H, Ar), 7.5 (m, 3H, Ar), 7.25 (d, 1H, Ar), 4.1 (t, 2H, OCH_2), 3.85 (s, 3H, NCH_3), 2.4 (s, 3H, CH_3), 1.75 (m, 2H, CH_2), 0.95 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-(5-methylthien-2-yl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (56). A solution of butyllithium in hexane (2.5 M, 21 mL, 52.5 mmol) was added dropwise to a solution of 2-methylthiophene (5 g, 51 mmol) and TMEDA (5.82 g, 51 mmol) in anhydrous THF (100 mL) under argon at room temperature, and the mixture was heated at 80 °C for 2 h. After cooling to −70 °C, trimethyltin chloride in THF (1 M, 60 mL, 60 mmol) was added dropwise, and the reaction mixture was stirred at −70 °C for 4 h. After warming to room temperature, diethyl ether was added and the organic layer washed with water. After drying (Na_2SO_4) and concentration, 2-(trimethylstannyl)-5-methylthiophene was obtained as an oil (13.9 g) and used without further purification.

A mixture of the above product (1.7 g), 1,3-dimethyl-6-(2-propoxy-5-bromophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (1 g, 2.65 mmol), and bis(triphenylphosphine)palladium-(II) chloride (140 mg, 7 mol%) in THF (100 mL) was heated at reflux with stirring for 16 h. After evaporation of the THF, the mixture was chromatographed on silica gel 60 (CH_2Cl_2−MeOH, 99:1). After crystallization from diisopropyl ether containing 10% 2-propanol, **56** was obtained as white crystals (140 mg, 13.5%): mp 178 °C; IR (KBr) 3319 (NH), 1698 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 10.9 (s, 1H, NH), 8.6 (sd, 1H, Ar),

7.6 (dd, 1H, Ar), 7.1 (d, 1H, Ar), 7 (d, 1H, Ar), 6.7 (d, 1H, Ar), 4.15 (t, 2H, OCH_2), 3.95 (s, 3H, NCH_3), 2.55 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 1.95 (m, 2H, CH_2), 1.15 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-bromophenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (24). A solution of bromine (0.9 mL) in acetic acid (10 mL) was added dropwise to a solution of **18** (3.3 g, 10.6 mmol) in acetic acid (100 mL), and the solution was heated at 100 °C with stirring for 10 h. After concentration under reduced pressure, the mixture was poured into water and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and concentrated to give a thick oil which crystallized from diisopropyl ether to give **24** as cream-colored crystals (3.35 g, 81%): mp 143−4 °C; IR (KBr) 3292 (NH), 1695 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.7 (s, 1H, NH), 7.75 (sd, 1H, Ar), 7.6 (dd, 1H, Ar), 7.05 (d, 1H, Ar), 4.15 (q, 2H, NCH_2), 3.9 (t, 2H, OCH_2), 2.35 (s, 3H, CH_3), 1.6 (m, 2H, CH_2), 1.25 (t, 3H, CH_3), 0.85 (t, 3H, CH_3).

1,3-Dimethyl-6-(2-propoxy-5-(bromoacetyl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (27). Aluminum chloride (2.25 g, 16.9 mmol) was added to an ice-cooled solution of **11** (1 g, 3.36 mmol) and bromoacetyl chloride (1.4 mL, 16.9 mmol) in CH_2Cl_2 (80 mL). The reaction mixture was stirred at room temperature for 48 h, poured into water, and stirred for 15 min to complete the hydrolysis. Extraction with CH_2Cl_2, washing with H_2O, drying (Na_2SO_4), and concentration gave **27** as a solid that was purified by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 95:5). Recrystallization from methanol gave **27** (0.98 g, 72%): mp 189 °C; IR (KBr) 3319 (NH), 1694 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.9 (s, 1H, NH), 8.2 (sd, 1H, Ar), 8.1 (dd, 1H, Ar), 7.2 (d, 1H, Ar), 4.8 (s, 2H, CH_2Br), 4.05 (t, 2H, OCH_2), 3.75 (s, 3H, NCH_3), 2.35 (s, 3H, CH_3), 1.65 (m, 2H, CH_2), 0.85 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-acetylphenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (28). Aluminum chloride (3.6 g, 27 mmol) was slowly added to an ice-cooled solution of **18** (1.7 g, 5.45 mmol) and acetyl bromide (2 mL, 40 mmol) in CH_2Cl_2 (100 mL), and the mixture was stirred at room temperature for 48 h. The reaction mixture was poured into water and stirred for 15 min to complete the hydrolysis. Extraction with CH_2Cl_2, washing with water, drying (Na_2SO_4), and concentration gave an oil which was triturated with isopropyl ether to afford **28** as pale yellow crystals (1.72 g, 89%): ^{1}H-NMR (DMSO) δ 11.95 (s, 1H, NH), 8.25 (sd, 1H, Ar), 8.15 (dd, 1H, Ar), 7.3 (d, 1H, Ar), 4.3 (q, 2H, NCH_2), 4.15 (t, 2H, OCH_2), 2.6 (s, 2H, $COCH_3$), 2.45 (s, 3H, CH_3), 1.75 (m, 2H, CH_2), 1.4 (t, 3H, CH_3), 0.95 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-(methoxycarbonyl)-phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (29). Bromine (0.75 mL) was added slowly to a solution of NaOH (1.92 g of NaOH pellets, 80 mL of H_2O), and to this solution was added dropwise a solution of **28** (1.7 g, 4.8 mmol) in dioxane (80 mL). The mixture was stirred at room temperature for 2 h. The dioxane was evaporated under reduced pressure and the residue acidified with dilute HCl. The resulting precipitate was filtered, washed with water, and dried. This product was esterified by heating at reflux in methanol (80 mL) containing concentrated H_2SO_4 (0.5 mL) for 18 h. The solution was concentrated, taken up with water, and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and concentrated to afford **29** as a cream-colored solid (0.94 g, 68%): mp 200−2 °C; ^{1}H-NMR (DMSO) δ 11.75 (s, 1H, NH), 8.1 (sd, 1H, Ar), 7.95 (dd, 1H, Ar), 7.15 (d, 1H, Ar), 4.15 (q, 2H, NCH_2), 3.95 (t, 2H, OCH_2), 3.75 (s, 3H, $COOCH_3$), 2.3 (s, 3H, CH_3), 1.6 (m, 2H, CH_2), 1.25 (t, 3H, CH_3), 0.85 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-(hydrazinocarbonyl)-phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (30). A mixture of **29** (0.9 g, 2.43 mmol) and hydrazine hydrate (1 mL) in ethanol (100 mL) was heated under reflux for 16 h. The solution was evaporated and the residue treated with water and extracted with CH_2Cl_2. The organic extract was washed with water, dried (Na_2SO_4), and concentrated to afford **30** as cream-colored crystals (0.76 g, 84%). The analytical sample was obtained by recrystallization from methanol as white crystals: mp 264 °C; IR (KBr) 3295 (NH), 3214, 3095 (NH_2), 1704, 1637 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 11.85 (brs, 1H, NH), 9.8 (s, 1H, NH), 8.2 (sd, 1H, Ar), 8 (dd, 1H, Ar), 7.25 (d, 1H, Ar), 4.55 (brs, 2H, NH_2), 4.3 (q, 2H, NCH_2), 4.1 (t, 2H, OCH_2), 2.5 (s, 3H, CH_3), 1.75 (m, 2H, CH_2), 1.4 (t, 3H, CH_3), 0.95 (t, 3H, CH_3).

1-Ethyl-3-methyl-6-(2-propoxy-5-(5-phenyl-1,2,4-triazol-3-yl)phenyl)-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (54). A mixture of the hydrazide **30** (0.5 g, 1.35 mmol) and thiobenzamide (138 mg, 135 mmol) in xylene (20 mL) was heated under reflux for 24 h. The mixture was evaporated to dryness, taken up in water, and extracted with CH_2Cl_2 (2 × 100 mL). The organic extract was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2−MeOH (98:2 and then 95:5) to give **54** as an off-white solid (150 mg, 24%). The analytical sample was obtained by recrystallization from 2-propanol: mp 224 °C; IR (KBr) 3332 (NH), 1705 cm^{-1} (CO); ^{1}H-NMR (DMSO) δ 8.25 (sd, 1H, Ar), 8.15 (dd, 1H, Ar), 8.05 (m, 2H, Ar), 7.55 (m, 3H, Ar), 7.35 (d, 1H, Ar), 4.2 (q, 2H, NCH_2), 4.05 (t, 2H, OCH_2), 2.4 (S, 3H, CH_3), 1.7 (m, 2H, CH_2), 1.3 (t, 3H, CH_3), 0.9 (t, 3H, CH_3).

3-Methyl-6-(2-propoxyphenyl)-5*H*-thieno[4,5-*d*]pyrimidin-4-one (9). (a) 2-Propoxybenzoyl chloride (5.9 g, 3 mmol) was added to a solution of methyl 3-amino-4-methylthiophene-2-carboxylate (5 g, 2.9 mmol) in pyridine (60 mL) with stirring and cooling. The mixture was stirred overnight, poured into water, and acidified with dilute HCl. The resulting precipitate was filtered, washed with H_2O, and dried (6.8 g): mp 106−8 °C.

(b) This product was heated under reflux with 1 N NaOH (100 mL) and ethanol (40 mL) for 1.5 h. The ethanol was evaporated and the remaining solution poured into iced water and neutralized with 1 N HCl (100 mL). The resulting precipitate was filtered, washed with water, and dried to give the free acid (5.5 g): mp 183 °C.

(c) This acid was dissolved in CH_2Cl_2 (250 mL), and thionyl chloride (3.7 g) was added dropwise with stirring at room temperature. The mixture was stirred overnight, and then the solution was washed with water, dried (Na_2SO_4), and concentrated to give a white solid (4 g).

(d) The latter material (1 g) was dissolved in ethanol (50 mL) saturated with NH_3 gas and placed in a pressure bomb which was closed and heated overnight at 120 °C. The reaction mixture was evaporated to give an oil which was purified by chromatography on silica gel 60 (CH_2Cl_2−MeOH, 97:3). Recrystallization from acetonitrile gave **9** as white crystals (0.2 g, 23%): mp 95−7 °C; IR (KBr) 1670 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 8.6 (dd, 1H, Ar), 7.5 (td, 1H, Ar), 7.45 (s, 1H, Ar), 7.15 (t, 1H, Ar), 7.05 (d, 1H, Ar), 4.2 (t, 2H, OCH_2), 2.5 (s, 3H, CH_3), 2 (m, 2H, CH_2), 1.15 (t, 3H, CH_3).

2-(2-Propoxyphenyl)-3*H*-pyrido[4,3-*d*]pyrimidin-4-one (5). (a) Methyl 1-benzyl-4-oxo-3-piperidinecarboxylate hydrochloride (6.36 g, 22.4 mmol) was added to sodium ethoxide (prepared from Na (2.12 g) and ethanol (100 mL)) maintaining the temperature below 10 °C. 2-Propoxybenzamidine oxalate (6 g, 22.4 mmol) was then added and the mixture heated at reflux for 16 h. After evaporation to dryness, the residue was taken up in water and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4) and concentrated to give a white solid (6.5 g).

(b) The latter material (1.7 g) was hydrogenated in acetic acid (50 mL) in the presence of 10% Pd/C (0.2 g) at 90 °C for 2 h. The catalyst was then filtered off and the solution concentrated to give an oil that crystallized upon treatment with diisopropyl ether.

(c) A mixture of this material (1.2 g) and 10% Pd/C (0.7 g) in xylene (50 mL) was heated at reflux for 2 h. The catalyst was then filtered off and the solution evaporated to give **5** as a solid which was recrystallized from ethanol (0.36 g, 22%): mp 135−7 °C; IR (KBr) 1696 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 11.4 (s, 1H, NH), 9.5 (s, 1H, Ar), 8.8 (d, 1H, Ar), 8.6 (dd, 1H, Ar), 7.6 (m, 2H, Ar), 7.2 (t, 1H, Ar), 7.1 (d, 1H, Ar), 4.25 (t, 2H, OCH_2), 2.05 (m, 2H, CH_2), 1.2 (t, 3H, CH_3).

2-(2-Propoxyphenyl)-3*H*-pyrido[3,4-*d*]pyrimidin-4-one (4). (a) Ethyl 1-benzyl-3-oxo-4-piperidinecarboxylate hydrochloride (4.68 g, 15.7 mmol) was added to sodium

ethoxide (prepared from Na (1.48 g) and ethanol (60 mL)) maintaining the temperature below 10 °C. 2-Propoxybenzamidine oxalate (4.22 g, 15.7 mmol) was then added and the mixture heated at reflux for 15 h. After evaporation to dryness, the residue was taken up in water and extracted with CH_2Cl_2. The organic extract was dried (Na_2SO_4) and concentrated to give a white solid (4 g).

(b) The above product (3 g) was hydrogenated in acetic acid (90 mL) in the presence of 10% Pd/C (0.3 g) at 90 °C during 4 h. The catalyst was filtered off and the solution concentrated to give an oil that crystallized by trituration with diisopropyl ether (2.5 g): mp 167−9 °C.

(c) A solution of the latter product (1.8 g) in xylene (100 mL) together with 10% Pd/C (1 g) was heated at reflux for 2 h. The catalyst was filtered off and the solution concentrated to give **4** as a solid which was recrystallized from ethanol (0.8 g, 31%): mp 184−5 °C; IR (KBr) 1695 cm^{-1} (CO); ^{1}H-NMR ($CDCl_3$) δ 11.4 (s, 1H, NH), 9.2 (s, 1H, Ar), 8.65 (d, 1H, Ar), 8.6 (dd, 1H, Ar), 8.05 (d, 1H, Ar), 7.5 (td, 1H, Ar), 7.2 (t, 1H, Ar), 7.1 (d, 1H, Ar), 4.2 (t, 2H, OCH_2), 2.05 (m, 2H, CH_2), 1.2 (t, 3H, CH_3).

PDE assays. The PDE assay[13,15] was based on the use of multiscreen plates (Millipore) and a vacuum manifold (Millipore). In such plates, both the reaction and the subsequent separation between substrates and products can be achieved. The assay (100 μL) contained 50 mM Tris-HCl, 5 mM Mg acetate, 1 mM EGTA, and 250 μg/mL snake venom nucleotidase, pH 7.5; 50 nM [8-^{3}H]-cGMP (15 Ci/mmM; Amersham) was added. Reactions were started by the addition of 25 μL of the enzyme preparation. The assays were incubated for 30 min at 30 °C. Microcolumns were prepared by aliquoting 300 μL of QAE Sephadex previously swollen for 2 h in water (12 mL/g). At the end of the incubation, the total volume of each assay was loaded onto microcolumn plates by filtration. The elution of free radioactivity was effected by 200 μL of water from which 50 μL aliquots were analyzed by scintillation counting.

In this PDE assay, the substrate concentration never exceeded 30% of the K_m of the enzyme tested. Under such conditions, the IC_{50} obtained for any given compound closely corresponds to the K_i for such a compound. In addition, all enzyme studies were performed under conditions of initial velocity (maximal substrate hydrolysis of 10−15%). Stock solutions of PDE inhibitors were prepared in dimethyl sulfoxide. The final solvent concentration in each assay was 2% (v/v).

cGMP Measurements. Rat aortic smooth muscle cells (RSMC) were prepared according to Chamley.[14] Cells were cultured in Dubelcco's modified Eagle medium (GIBCO) containing 10% fetal calf serum, 1% glutamine, and 1% penicilin−streptomycin at 37 °C in a 95% air−5% CO_2-humidified atmosphere.

Cells were seeded in 24-well culture dishes at a density of $(2-5) \times 10^4$ cells/well. Experiments were performed after 3−5 days in culture when cells reached confluence. Media were aspired and replaced by 0.5 mL of PBS containing the PDE inhibitor. After 30 min at 37 °C, soluble or particular guanylate cyclase was stimulated by addition of SNP (0.5 μM) or ANF (0.1 μM), respectively, at 37 °C. At the end of the incubation, the medium was removed and stored at −20 °C for extracellular cyclic nucleotide determinations. Intracellular cyclic nucleotides were extracted by two ethanolic (65%) washes at 4 °C for 5 min. The ethanolic extracts were pooled, evaporated to dryness using a Speed-Vac system, and stored at −20 °C. cGMP and cAMP were measured by scintillation proximity immunoassay (Amersham). In all cases, any given treatment with effectors was performed in duplicate or triplicate wells. Stock solutions of PDE inhibitors were made in dimethyl sulfoxide. In the assays, the final concentration of dimethyl sulfoxide never exceeded 0.1% (v/v).

Determination of Metabolic Stability: In Vitro. Rats (OFA, Charles River France) were sacrificed, and microsomal pellets (100.000 g) from rat liver were prepared. The microsomal suspension was diluted to bring the protein concentration to 10 mg/mL. The incubation medium contained microsomal protein (2 mg/mL), $MgCl_2$ (5 mmol), NADPH (0.5 mmol), UDPGA (0.5 mmol), and NADH (0.5 mmol) in Tris (0.1 M) buffer, pH 7, with 3% BSA. Incubations were conducted at 25 °C with 5 μg/mL of product, and the rate of disappearance was determined by HPLC.

In Vivo. The products were administrated per os at 5 mg/kg in DMF/olive oil, 1:9. Animals were sacrificed at different times. Unchanged compound was monitored by HPLC after liquid/liquid extraction in blood, liver, and kidneys.

Effect on Blood Pressure of Conscious SHR. The experiments were performed in hypertensive rats (SHR, Charles River France) weighing 340−380 g. The day before experiment, the left carotid artery was catheterized under pentobarbital anesthesia. On the day of the experiment, the catheter was connected to a pressure tranducer for blood pressure measurement. After an equilibration time of ca. 30 min, the product in this vehicle (10% 1 N NaOH/90% saline) was administered per os in a volume of 1 mL. Arterial blood pressure was monitored continuously over 7 h.

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References

(1) Beavo, J. A.; Reifsnyder, D. H. Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. *Trends Pharmacol. Sci.* **1990**, *11*, 150−155.
(2) Erhardt, P. W. Second generation phosphodiesterase inhibitors; structure-activity relationships and receptor models. *Cyclic Nucleotide Phosphodiesterases*: *Structure, Regulation and Drug Action*; John Wiley and Sons Ltd.: New York, 1990; pp 317−332.
(3) Yasutaka, T.; Takao, S.; Masatoshi, F.; Isao, S. Cyclic GMP Phosphodiesterase Inhibitors. 1. The discovery of a novel potent inhibitor, 4-((3,4-(methylenedioxybenzyl)amino)-6,7,8-trimethoxyquinazoline. *J. Med. Chem.* **1993**, *36*, 3765−3770.
(4) Tremblay, J.; Gerrer, R.; Hamet, P. Cyclic GMP in cell function. *Adv. Second Messenger Phosphoprotein Res.* **1988**, *22*, 319−383.
(5) Waldman, M.; Murad, F. Cyclic GMP, synthesis and function. *Pharmacol. Rev.* **1987**, *39*, 163−196.
(6) Lincoln, T. M. Cyclic GMP and mechanisms of vasodilatation. *Pharmacol. Ther.* **1989**, *41*, 479−502.
(7) Nicholson, C.; Challis, R.; Shakio, M. Differential modulation of tissue function and therapeutic potential of selective inhibitors of cyclic nucleotide phosphodiesterase isoenzymes. *Trends. Pharmacol. Sci.* **1991**, *12*, 19−27.
(8) Coates, W. J. Eur. Pat. EP32960, 1990; *Chem. Abstr.* **1990**, *113*, 40713.
(9) Coates, W. J. Eur. Pat. EP351058, 1990; *Chem. Abstr.* **1990**, *113*, 40711.
(10) Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Bristol, J. A. Synthesis and Structure-Activity Relationships of pyrazolo[4,3-d]pyrimidin-7-ones as Adenosine Receptor antagonists. *J. Med. Chem.* **1987**, *30*, 91−96.
(11) Bell, A. S.; Terret, N. K. PCTApp WO93/07149, 1993.
(12) Terret, N. K. PCTApp WO94/00453, 1994.
(13) Wells, J. N.; Baird, C. E.; Wu, Y. J.; Hardmann, J. G. Cyclic nucleotide phosphodiesterase activities of pig coronary arteries. *Biochim. Biophys. Acta* **1975**, *384*, 430−442.
(14) Chamley, J. H.; Campbell, G. R.; McConnel, J. D.; Groschel, S. U. Comparison of vascular smooth muscle cells from adult human, monkey and rabbit in primary culture and in subculture. *Cell Tissue Res.* **1977**, *177*, 503−522.
(15) Coste, H.; Grondin, P. Characterisation of a novel potent and specific inhibitor of type V phosphodiesterase. *Biochem. Pharmacol.* **1995**, *50*, 1577−1585.

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