Synthesis and Evaluation of Polycyclic Pyrazolo[3,4-*d*]pyrimidines as PDE1 and PDE5 cGMP Phosphodiesterase Inhibitors

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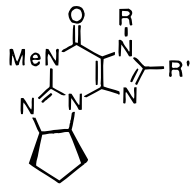
Polycyclic pyrazolo[3,4-*d*]pyrimidines (represented by **3** and **4**) were synthesized as analogues of the recently reported polycyclic guanine phosphodiesterase (PDE) inhibitors. From the structure−activity relationship (SAR) development of a series of compounds, it was discovered that *C*-3 benzyl and *N*-2 methyl disubstitution on the pyrazole ring gave the best combination of potency and selectivity for PDE1 and PDE5 cGMP PDEs as represented by compound **4c**: PDE1, IC_{50} = 60 nM; PDE3, IC_{50} = 55 000 nM; PDE5, IC_{50} = 75 nM. These compounds were also evaluated in vivo and found to be good orally active antihypertensives in laboratory animal models. Finally, comparisons were made of the in vitro and in vivo profiles of the pyrazolo-[3,4-*d*]pyrimidine compound **4c** with those of two representative guanine compounds.

Introduction

The emerging field of cyclic nucleotide level regulation by the isozyme family of phosphodiesterases (PDE) has brought renewed interest in PDE inhibition as a target for therapeutic intervention.[1] Recently the role of cGMP levels and the potential beneficial effects of inhibition of the cGMP PDE for the treatment of cardiovascular diseases have been reviewed.[2] The considerable interest in this area has been the focus of a large number of programs directed at the identification of new chemical classes of inhibitors of cGMP PDE.[3] We have recently disclosed our discovery of a new class of polycyclic guanine PDE inhibitors which are illustrated in Chart 1.[4] These compounds are potent inhibitors of PDE1 and PDE5 cGMP PDEs in vitro and potent oral antihypertensives in vivo. The desirable properties of the polycyclic guanine compounds prompted us to investigate analogues of these guanines to discover other related classes of PDE inhibitors. In particular, we were interested in pyrazolo[3,4-*d*]pyrimidine analogues (such as **3** and **4** in Chart 2) where an isomeric pyrazole ring replaces the imidazole ring in the polycyclic guanine structure.[5] Prior to our work, there was a brief patent description of 5-hexyl-7-methylpyrazolo[3,4-*d*]pyrimidine-4,6-dione as a PDE inhibitor.[6] Since the initiation of our work, there have been several recent reports of pyrazolo[3,4-*d*]pyrimidines and pyrazolo[4,3-*d*]pyrimidines as potent and selective PDE5 inhibitors.[7]

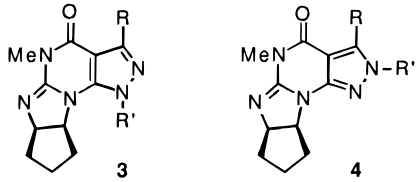
Here we describe the synthesis of polycyclic pyrazolo-[3,4-*d*]pyrimidines and their in vitro and in vivo evaluations as PDE1 and PDE5 cGMP PDE inhibitors. From the structure−activity relationship (SAR) development of this series of compounds, a new class of potent PDE1 and PDE5 cGMP PDE inhibitors with oral antihypertensive activity was discovered.

Chart 1. Polycyclic Guanine PDE Inhibitors



1: R = benzyl; R' = methyl;
2: R = H; R' = benzyl.

Chart 2. Pyrazolo[3,4-*d*]pyrimidine Analogues

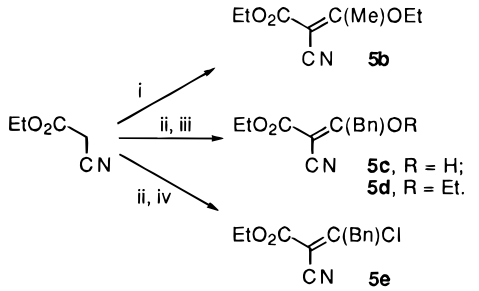


3 **4**

Synthesis

The syntheses of the polycyclic pyrazolo[3,4-*d*]pyrimidine compounds are outlined in Schemes 1−3. Scheme 1 shows the preparation of starting materials **5b**−**e**. Condensation of ethyl cyanoacetate with orthoacetate gave **5b**.[8] Condensation of ethyl cyanoacetate with phenylacetyl chloride gave an enol intermediate (**5c**) which was *O*-alkylated to give **5d** or converted to the chloro intermediate **5e** by treatment with $POCl_3$ and tributylamine.[9]

Scheme 1[a]



[a] Reagents: (i) $CH_3C(OEt)_3$, Ac_2O, reflux, 23%; (ii) BnCOCl, NaH, THF; (iii) Ag_2CO_3, EtI, 41% (from ethyl cyanoacetate); (iv) $POCl_3$, *n*-Bu_3N, 40%.

The pyrazole compounds of type **3** were prepared as outlined in Scheme 2. Intermediates **7b**−**e** were prepared based on a literature synthesis of pyrazolo[3,4-*d*]pyrimidine **7a** from **5a** via the pyrazole intermediate **6a**.[10] Condensation of **5a,b,d** with methylhydrazine or benzylhydrazine gave pyrazoles **6b**−**e**. Treatment with

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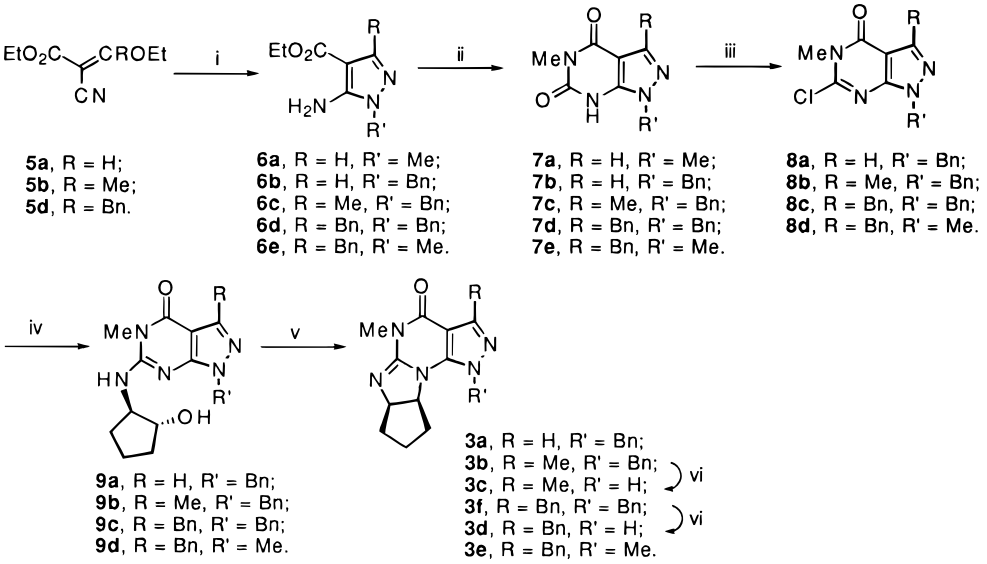
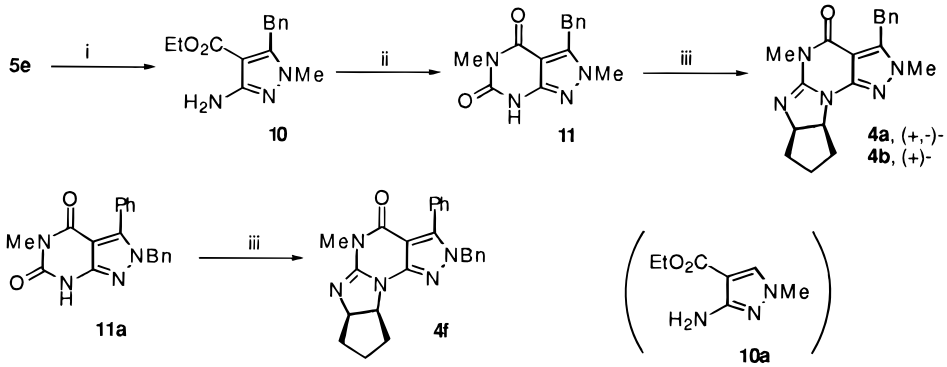
Scheme 2[a]



[a] Reagents: (i) CH_3NHNH_2 or $PhCH_2NHNH_2$, MeOH, reflux, 84%; (ii) MeNCO, NEt_3, PhH, 100 °C; NaOMe, MeOH, reflux, 37%; (iii) $POCl_3$, heat, 67%; (iv) *trans*-2-aminocyclopentanol, DMF, heat, 94%; (v) $SOCl_2$, CH_2Cl_2, 49%; (vi) $Pd(OH)_2/C$, H_2, MeOH, 30%.

Scheme 3[a]



[a] Reagents: (i) benzaldehyde methylhydrazone, PhH; HCl, EtOH, reflux, 73%; (ii) MeNCO, NEt_3, PhH, 100 °C; NaOMe, MeOH, reflux; (iii) same as steps iii–v in Scheme 2.

methyl isocyanate followed by base-catalyzed cyclization formed **7b**–**e**. Intermediates **7b**–**e** were then elaborated to pyrazole compounds **3a**–**e** according to the procedures developed for the polycyclic guanine compounds.[4]

The pyrazole compounds of type **4** were prepared as shown in Scheme 3. It has been reported that condensation of **5a** with benzaldehyde methylhydrazone followed by acid hydrolysis gave **10a**.[10] Our attempt to apply the same reactions using the benzyl-substituted intermediate **5d** did not give the desired pyrazole intermediate **10** in good yield. However, a more reactive chloro intermediate (**5e**) gave **10** in 73% yield under these conditions. Intermediate **10** was converted to the pyrazole compound **4a** according to the aforementioned synthetic sequence (Scheme 2: **6** → **3**). Use of other amino alcohols to replace *trans*-2-aminocyclopentanol in the sequence gave other polycyclic pyrazole compounds (**4c**–**e**) (Table 1). Pyrazole compound **4f** was prepared similarly from the known intermediate **11a**.[11] Use of (−)-*trans*-2-aminocyclopentanol[12] in Schemes 2 and 3 gave the optically active pyrazole compounds **3b**,**c**, and **4b**,**f**.

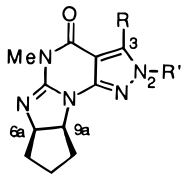
The substitution pattern on the pyrazole ring of representative pyrazole compounds **3e** and **4a** was confirmed by NOE experiments (Chart 3).[13] Irradiation of the pyrazole ring *N*-methyl proton resonance (δ 3.88) of **3e** gave an enhancement of the methine proton signal (δ 4.83) at the C,D-ring junction but not of the benzylic protons (δ 4.15). For compound **4a**, irradiation of the pyrazole ring *N*-methyl proton resonance (δ 3.61) gave an enhancement of the benzylic proton signal (δ 4.28) but not of the methine proton (δ 4.65–4.72) at the C,D-ring junction.

Results and Discussion

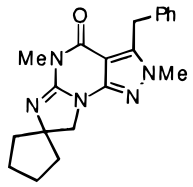
The SAR development of our polycyclic pyrazolo[3,4-*d*]pyrimidine compounds was directed mostly toward substitutions on the pyrazole ring. The choice of substitutions was influenced by the SAR developed for the polycyclic guanine compounds.[4] In the evaluation of selective cGMP PDE inhibitors, we were looking for potent inhibition against the cGMP PDEs (PDE1 and PDE5) and selectivity over the undesired cAMP PDE (PDE3). Table 1 lists the in vitro PDE inhibition results. Similar to the polycyclic guanine compounds, all of the pyrazole compounds are either inactive or weak inhibitors against PDE3, displaying IC_{50} values from 29 μM to greater than 100 μM. Mono *N*-1 benzyl-substituted (**3a**) and mono *C*-3 methyl-substituted (**3c**) compounds gave weak inhibition of PDE1 and PDE5. However, *N*-1 benzyl- and *C*-3 methyl-disubstituted

Table 1. In Vitro PDE Inhibition and in Vivo Antihypertensive Activities of Polycyclic Pyrazolo[3,4-*d*]pyrimidines[a]

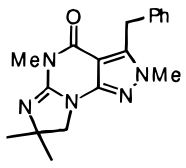
3a-e



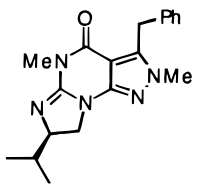
4a,b,f



4c



4d

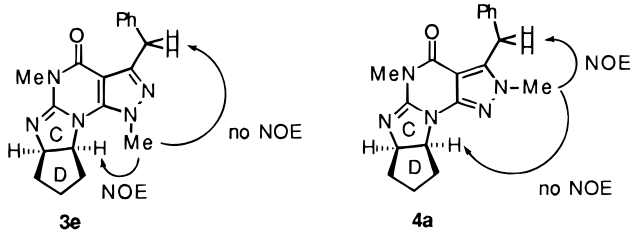


4e

			IC$_{50}$ (nM)			
compd	R	R′	PDE1	PDE3	PDE5	Δ mmHg @ mg/kg
3a	H	benzyl	29000	55000	3100	−14 @ 25
3b	Me	benzyl	2000	50000	1000	−3 @ 10
3c	Me	H	18000	>100000	2900	−7 @ 10
3d	benzyl	H	1100	>100000	190	−16 @ 25
3e	benzyl	Me	850	29000	400	not tested
4a	benzyl	Me	160	85000	140	−18 @ 10
4b	benzyl	Me	55	80000	100	−26 @ 10
4c			60	55000	75	−33 @ 10
4d			18	42000	140	not tested
4e			20	90000	100	not tested
4f	phenyl	benzyl	540	55000	2100	1 @ 10

[a] Compounds **3a**,**d**,**e** and **4a** are racemic mixtures, compounds **3b**,**c** and **4b**,**f** are 6a*R*,9a*S*-enantiomers, and compound **4e** is the 7*R*-enantiomer.

Chart 3. Confirmation of Structures by NOE Experiments



compound **3b** showed greater PDE1 inhibition. Changing the *C*-3 substituent from methyl to benzyl produced greater inhibition of PDE1 and PDE5 as exemplified by compound **3d**. An additional methyl substitution at the *N*-2 position (**4a,b**) gave the best dual inhibition against PDE1 and PDE5 among the different pyrazole substitutions examined (e.g., **4a** vs **3e**). A comparison of the racemic compound **4a** and the 6a*R*,9a*S*-isomer **4b** reveals the absolute stereochemical preference for the 6a*R*,9a*S*-enantiomer. This absolute stereochemical preference is consistent with our earlier findings in the polycyclic guanine series that the 6a*R*,9a*S*-enantiomer is inherently more potent against PDE1 and PDE5.[4] The *C*-3 phenyl- and *N*-2 benzyl-disubstituted compound **4f** is a mediocre PDE1 and PDE5 inhibitor. Compounds **4c**−**e** represent different substitution patterns of the dihydroimidazole ring of these polycyclic pyrazole compounds. Among these, no substantial effects on in vitro potency and selectivity were observed. The in vivo efficacy of the polycyclic pyrazolo[3,4-*d*]pyrimidine compounds was evaluated in spontaneously hypertensive rats (SHR) by oral administration. As shown in Table 1, compounds **4a**−**c** exhibited good oral antihypertensive activity, roughly consistent with their in vitro potency for PDE1 and PDE5. The less potent compounds **3a**−**d** and **4f**, however, showed weak antihypertensive activity.

Compound **4c** was further evaluated against two other PDE isozymes (PDE2 and PDE4) to get a more comprehensive PDE inhibition profile. The result is listed in Table 2 along with those of the polycyclic guanine compounds **1** and **2** (structures are shown in Chart 1). By comparison, **4c** shows a similar profile to the guanine compound **1** in terms of both in vitro potency for PDE1 and PDE5 and in vivo antihypertensive activity. When comparing **4c** to guanine compound **2**, even though the in vitro profile is similar (except that **4c** appears to be more potent for PDE4 and is thus somewhat less selective), the in vivo antihypertensive activity of **4c** is much weaker than that of **2**. It is also worth noting that the pyrazolo[3,4-*d*]pyrimidine compound **3d**, which is isomeric with guanine compound **2**, also displays much weaker antihypertensive activity than **2**.

Table 2. PDE1, PDE2, PDE3, PDE4, and PDE5 Inhibition of Compound **4c** and Its Comparison with Polycyclic Guanine PDE Inhibitors[a]

	IC$_{50}$ (nM)					Δ mmHg
compd	PDE1	PDE2	PDE3	PDE4	PDE5	@ 10 mg/kg
1[b]	205	not tested	not tested	not tested	225	−28
2[b]	100	1500	80000	8100	80	−47
4c	60	11000	55000	230	75	−33

[a] The polycyclic guanine compounds **1** and **2** are 6a*R*,9a*S*-enantiomers.[4] [b] From ref 4.

In summary, new polycyclic pyrazolo[3,4-*d*]pyrimidine compounds were synthesized as analogues of the polycyclic guanine PDE inhibitors. By varying substitutions on the pyrazole ring, these compounds can achieve similar potency and selectivity for the cGMP PDEs (PDE1 and PDE5). In vivo evaluations using SHR indicated that they are bioavailable. The polycyclic pyrazolo[3,4-*d*]pyrimidine compounds described here represent potential therapeutic agents through cGMP potentiations.

Experimental Section

Melting points were taken on a Thomas-Hoover or Mel-Temp II melting point apparatus and are uncorrected. Chromatography was performed over Universal Scientific or Selecto Scientific flash silica gel (32−63 μm). Analytical HPLC analyses were done on Dynamax columns (silica gel for normal phase and C8 for reverse phase) eluting with appropriate solvents as indicated. ^{1}H NMR spectra were determined with a Varian VXR 200, Gemini 300, or Gemini 400 MHz instrument using either Me_4Si or residual solvent signal as internal

standards. Rotations were determined on a Rudolph Autopol III or Perkin-Elmer 243B polarimeter. Mass spectra were obtained on a VG-ZAB-SE, Extrel-401, HP-MS Engine, JEOL HX-110, or Sciex API 100 mass spectrometer. Elemental analyses were determined by the Physical-Analytical Department of Schering-Plough Research Institute using either CEC 240-HA, CEC CE-440, or Fisons EA 1108 CHNS elemental analyzers and are within 0.4% of the theoretical value unless otherwise noted. Fractional solvent impurities reported in the molecular composition were identified by the characteristic peaks in the ^{1}H NMR and were quantitated by integration of the ^{1}H resonances. Ethyl (ethoxymethylene)cyanoacetate (**5a**) was purchased from Aldrich Chemical Co. and used as such.

Ethyl 2-Cyano-3-ethoxy-2-butenoate (5b). A solution of ethyl cyanoacetate (51 mL, 0.48 mol) and triethyl orthoacetate (88 mL, 0.48 mol) in Ac_2O (150 mL) was refluxed for 3 h. The solution was concentrated in vacuo. The residue was kept in a refrigerator for 3 days to precipitate the product **5b** (20 g, 23%) as white crystals: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (t, 3H, J = 7 Hz), 1.43 (t, 3H, J = 7 Hz), 2.61 (s, 3H), 4.23 (q, 2H, J = 7 Hz), 4.27 (q, 2H, J = 7 Hz).

Ethyl 2-Cyano-3-hydroxy-4-phenyl-2-butenoate (5c). NaH (4.0 g, 0.10 mol, 60% dispersion in mineral oil) was added to a solution of ethyl cyanoacetate (10.64 mL, 0.10 mol) in dry THF (100 mL) at room temperature and stirred for 10 min. A solution of phenylacetyl chloride (13.6 g, 0.088 mol, generated from phenylacetic acid and $SOCl_2$ by standard procedures) in dry THF (10 mL) was added dropwise. The mixture was stirred for 1 h at room temperature. The mixture was concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and water. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give crude **5c** (22 g). Distillation at 140 °C/0.5 mmHg gave pure **5c** as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, 3H, J = 7 Hz), 3.81 (s, 2H), 4.25 (q, 2H, J = 7 Hz), 7.10−7.35 (m, 5H), 13.60 (br s, 1H); MS (FAB) m/z 232 (MH^+, 100).

Ethyl 2-Cyano-3-ethoxy-4-phenyl-2-butenoate (5d). The crude **5c** (11 g) was stirred with EtI (19 mL, 0.238 mol) and Ag_2CO_3 (26.27 g, 0.0952 mol) in CH_2Cl_2 (500 mL) at room temperature for 16 h. The mixture was filtered. The filtrate was concentrated in vacuo. Flash chromatography of the residue on a silica gel column with EtOAc−hexane (10−90 then 15−85) as eluent gave **5d** (5.36 g, 41% from ethyl cyanoacetate) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, 3H, J = 7 Hz), 1.33 (t, 3H, J = 7 Hz), 4.22 (q, 2H, J = 7 Hz), 4.26 (q, 2H, J = 7 Hz), 4.50 (s, 2H), 7.19 (d, 2H, J = 7 Hz), 7.25−7.38 (m, 3H); MS (EI) m/z 259 (M^+, 100).

Ethyl 3-Chloro-2-cyano-4-phenyl-2-butenoate (5e). Tri-*n*-butylamine (5.2 mL, 22 mmol) was added dropwise to a solution of pure **5c** (4.62 g, 20 mmol) in $POCl_3$ (10 mL) at 0 °C. The resulting red solution was heated at 88 °C for 1 h. The solution was extracted with ether (200 mL). The extracts were washed with 10% HCl, H_2O, and saturated $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography of the residue on a silica gel column with EtOAc−hexane (5−95) as eluent gave **5e** (2.00 g, 40%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, 3H, J = 7 Hz), 4.29 (q, 2H, J = 7 Hz), 4.41 (s, 2H), 7.18−7.33 (m, 5H).

Ethyl 5-Amino-1-(phenylmethyl)-1*H*-pyrazole-4-carboxylate (6b). A mixture of ethyl (ethoxymethylene)cyanoacetate (**5a**; 1.69 g, 10.0 mmol), benzylhydrazine dihydrochloride (2.14 g, 11.0 mmol), and Et_3N (7.0 mL, 50 mmol) in MeOH (25 mL) was refluxed for 15 h. The mixture was concentrated in vacuo. The residue was partitioned between H_2O and CH_2Cl_2. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on a silica gel column with EtOAc−hexane (2−8 then 3−7) as eluent gave **6b** (2.05 g, 84%) as white solids. Recrystallization from benzene gave white crystal flakes: mp 108−110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, 3H, J = 7 Hz), 4.28 (q, 2H, J = 7 Hz), 4.89 (br s, 2H), 5.18 (s, 2H), 7.18−7.40 (m, 5H), 7.71 (s, 1H); MS (CI) m/z 246 (MH^+, 100). Anal. ($C_{13}H_{15}N_3O_5$) C, H, N.

Ethyl 3-Amino-1-methyl-5-(phenylmethyl)-1*H*-pyrazole-4-carboxylate (10). Methylhydrazine (7.36 g, 0.16 mol) was heated with benzaldehyde (18.8 g, 0.18 mol) in benzene (250 mL) at reflux until no more water separated in the Dean−Stark trap. The intermediate **5e** (20 g, 0.080 mol) was added, and the resulting solution was stirred at room temperature for 2 h. The solution was concentrated in vacuo. The residue was heated with concentrated HCl (14.5 mL) in refluxing EtOH (150 mL) for 45 min. The solution was concentrated in vacuo. The residue was partitioned between EtOAc and $NaHCO_3$ (saturated). The organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography on a silica gel column with EtOAc−hexane (4−6) as eluent gave **10** (9.0 g, 73%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (t, 3H, J = 7 Hz), 3.45 (s, 3H), 4.19 (q, 2H, J = 7 Hz), 4.22 (s, 2H), 4.48 (br s, 2H), 5.18 (s, 2H), 7.01−7.25 (m, 5H).

5-Methyl-1-(phenylmethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione (7b). The pyrazole intermediate **6b** (2.28 g, 9.3 mmol) was heated with methyl isocyanate (2.2 mL, 37 mmol) and Et_3N (0.26 mL, 1.9 mmol) in benzene (20 mL) at 100 °C for 10 h. The solution was concentrated in vacuo. The residue was heated with NaOMe (1.5 g, 28 mmol) in refluxing MeOH (50 mL) for 1 h. The solution was concentrated in vacuo. The residue was dissolved in water, washed with CH_2Cl_2, and adjusted to pH 6 with 10% HCl. The precipitates were filtered, washed with water and ether, and dried in vacuo to give **7b** (0.87 g, 37%) as white solids: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.38 (s, 3H), 5.43 (s, 2H), 7.35 (s, 5H), 7.98 (s, 1H), 12.07 (br s, 1H); MS (CI) m/z 257 (MH^+, 100). Anal. ($C_{13}H_{12}N_4O_2$) C, H, N.

2,5-Dimethyl-3-(phenylmethyl)-2*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione (11): similarly prepared from **10**; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.12 (s, 3H), 3.65 (s, 3H), 4.32 (s, 2H), 7.15−7.32 (m, 5H), 11.57 (s, 1H).

6-Chloro-1,5-dihydro-5-methyl-1-(phenylmethyl)-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8a). The intermediate **7b** (7.00 g, 27.3 mmol) was heated with $POCl_3$ (100 mL) at reflux for 48 h. The solution was cooled and diluted with hexane (1000 mL). The liquid layer was decanted. The residue was dissolved in CH_2Cl_2, washed (carefully!) with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated in vacuo. Recrystallization from benzene gave **8a** (5.05 g, 67%) as white solids: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (s, 3H), 5.45 (s, 2H), 7.26−7.34 (m, 5H), 8.06 (s, 1H); MS (CI) m/z 275 (MH^+). Anal. ($C_{13}H_{11}ClN_4O$) C, H, N.

(±)-1,5-Dihydro-6-[(2-hydroxycyclopentyl)amino]-5-methyl-1-(phenylmethyl)-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (9a). The chloro intermediate **8a** (4.60 g, 16.7 mmol) was heated with (±)-*trans*-2-aminocyclopentanol (3.38 g, 33.4 mmol) in DMF (100 mL) at 100 °C under N_2 for 15 h. The solution was concentrated in vacuo. The residue was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography of the residue on a silica gel column with $CHCl_3$−MeOH (95−5) as eluent gave **9a** (5.32 g, 94%) as white solids: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.51−2.36 (m, 6H), 3.43 (s, 3H), 3.98−4.11 (m, 1H), 4.88 (br s, 1H), 5.31 (d, 1H, J = 18 Hz), 5.36 (d, 1H, J = 18 Hz), 7.23−7.36 (m, 5H), 7.96 (s, 1H); MS (CI) m/z 340 (MH^+). Anal. ($C_{18}H_{21}N_5O_2$) C, H, N.

***rac*-(6a*R**,9a*S**)-5,6a,7,8,9,9a-Hexahydro-5-methyl-1-(phenylmethyl)cyclopent[4,5]imidazo[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(1*H*)-one (3a).** Thionyl chloride (3.2 mL, 44 mmol) was added dropwise to a solution of intermediate **9a** (5.00 g, 14.7 mmol) in CH_2Cl_2 (200 mL) and stirred at room temperature for 11 h. The solution was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography of the residue on a silica gel column with $CHCl_3$−MeOH (99−1) as eluent gave **3a** (2.31 g, 49%) as white solids: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55−2.07 (m, 6H), 3.34 (s, 3H), 4.55 (t, 1H, J = 7 Hz), 4.66 (t, 1H, J = 7 Hz), 5.31 (d, 1H, J = 17 Hz), 5.58 (d, 1H, J = 18 Hz), 7.06 (d, 2H, J = 6 Hz), 7.32−7.40 (m, 3H), 7.91 (s, 1H); MS (FAB) m/z 322 (MH^+). Anal. ($C_{18}H_{19}N_5O$) C, H, N.

Similarly prepared were the following compounds.

(6a*R*,9a*S*)-3,5-Dimethyl-5,6a,7,8,9,9a-hexahydro-1-(phenylmethyl)cyclopent[4,5]imidazo[1,2-*a*]pyrazolo-[4,3-*e*]pyrimidin-4(1*H*)-one (3b): $[\alpha]^{22}_D$ +200.7° (*c* 0.27,

EtOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48−2.05 (m, 6H), 2.47 (s, 3H), 3.32 (s, 3H), 4.51 (t, 1H, J = 6 Hz), 4.64 (t, 1H, J = 6 Hz), 5.24 (d, 1H, J = 17 Hz), 5.48 (d, 1H, J = 17 Hz), 7.04 (d, 2H, J = 8 Hz), 7.28−7.40 (m, 3H); MS (FAB) m/z 336 (MH^+, 100%); Anal. ($C_{19}H_{21}N_5O$) C, H, N.

***rac*-(6a*R**,9a*S**)-5,6a,7,8,9,9a-Hexahydro-1,5-dimethyl-3-(phenylmethyl)cyclopent[4,5]imidazo[1,2-*a*]pyrazolo-[4,3-*e*]pyrimidin-4(1*H*)-one (3e):** ^{1}H NMR (300 MHz, CD_3OD) δ 1.55−2.10 (m, 6H), 3.32 (s, 3H), 3.88 (s, 3H), 4.15 (AB q, 2H), 4.77 (t, 1H, J = 7 Hz), 4.83 (t, 1H, J = 7 Hz), 7.15−7.47 (m, 5H); MS (CI) m/z 336 (MH^+, 100). Anal. ($C_{19}H_{21}N_5O$) C, H; N: calcd, 20.88; found, 19.80.

***rac*-(6a*R**,9a*S**)-1,3-Bis(phenylmethyl)-5,6a,7,8,9,9a-hexahydro-5-methylcyclopent[4,5]imidazo[1,2-*a*]pyrazolo-[4,3-*e*]pyrimidin-4(1*H*)-one (3f):** ^{1}H NMR (300 MHz, CD_3OD) δ 1.50−2.10 (m, 6H), 3.34 (s, 3H), 4.17 (d, 1H, J = 14 Hz), 4.28 (d, 1H, J = 14 Hz), 4.52 (t, 1H, J = 7 Hz), 4.64 (t, 1H, J = 7 Hz), 5.27 (d, 1H, J = 17 Hz), 5.55 (d, 1H, J = 17 Hz), 7.00−7.50 (m, 10H); MS (EI) m/z 411 (M^+, 15). Anal. ($C_{18}H_{19}N_5O$·0.24$CHCl_3$) C, H; N: calcd, 15.91; found, 15.42.

***rac*-(6a*R**,9a*S**)-5,6a,7,8,9,9a-Hexahydro-2,5-dimethyl-3-(phenylmethyl)cyclopent[4,5]imidazo[1,2-*a*]pyrazolo-[4,3-*e*]pyrimidin-4(1*H*)-one (4a):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44−2.24 (m, 6H), 3.38 (s, 3H), 3.61 (s, 3H), 4.28 (s, 2H), 4.65−4.72 (m, 2H), 7.12−7.27 (m, 5H); MS (FAB) m/z 336 (MH^+, 100). Anal. ($C_{19}H_{21}N_5O$) C, H, N.

(6a*R*,9a*S*)-5,6a,7,8,9,9a-Hexahydro-2,5-dimethyl-3-(phenylmethyl)cyclopent[4,5]imidazo[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(1*H*)-one (4b): $[\alpha]^{22}_D$ +187.9° (c 0.74, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44−2.24 (m, 6H), 3.38 (s, 3H), 3.61 (s, 3H), 4.28 (s, 2H), 4.65−4.72 (m, 2H), 7.12−7.27 (m, 5H); MS (EI) m/z 335 (M^+, 39), 306 (100). Anal. ($C_{19}H_{21}N_5O$·0.10H_2O) C, H, N.

2′,5′-Dimethyl-3′-(phenylmethyl)spiro[cyclopentane-1,7′(8′*H*)-[2*H*]imidazo[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin]-4′-(5′*H*)-one (4c): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52−1.95 (m, 8H), 3.34 (s, 3H), 3.56 (s, 3H), 3.70 (s, 2H), 4.28 (s, 2H), 7.10−7.25 (m, 5H); MS (ESI) m/z 350 (MH^+, 100). Anal. ($C_{20}H_{23}N_5O$) C, H, N.

7,8-Dihydro-3-(phenylmethyl)-2,5,7,7-tetramethyl-[2*H*]-imidazo[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(5*H*)-one (4d): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 6H), 3.40 (s, 3H), 3.65 (s, 3H), 3.69 (s, 2H), 4.36 (s, 2H), 7.21−7.31 (m, 5H); MS (ESI) m/z 324 (MH^+, 100). Anal. ($C_{18}H_{21}N_5O$) C, N; H: calcd, 6.67; found, 6.25.

(7*R*)-7,8-Dihydro-2,5-dimethyl-7-(1-methylethyl)-3-(phenylmethyl)-[2*H*]imidazo[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(5*H*)-one (4e): $[\alpha]^{25}_D$ +89° (c 0.28, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (d, 3H, J = 6.7 Hz), 1.02 (d, 3H, J = 6.7 Hz), 1.89 (m, 1H), 3.43 (s, 3H), 3.65 (s, 3H), 3.69 (m, 1H), 3.96 (t, 1H, J = 9.5 Hz), 4.07 (m, 1H), 4.36 (s, 2H), 7.21−7.32 (m, 5H); MS (ESI) m/z 338 (MH^+, 100). Anal. ($C_{19}H_{23}N_5O$) C, H, N.

(6a*R*,9a*S*)-5,6a,7,8,9,9a-Hexahydro-5-methyl-3-phenyl-2-(phenylmethyl)cyclopent[4,5]imidazo[1,2-*a*]pyrazolo-[4,3-*e*]pyrimidin-4(1*H*)-one (4f): $[\alpha]^{22}_D$ +147° (c 0.46, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45−2.30 (m, 6H), 3.35 (s, 3H), 4.72 (t, 1H, J = 7 Hz), 4.79 (t, 1H, J = 7 Hz), 5.15 (s, 2H), 6.95−7.45 (m, 10H); MS (EI) m/z 397 (M^+, 51), 368 (100). Anal. ($C_{24}H_{23}N_5O$) C, H, N.

***rac*-(6a*R**,9a*S**)-5,6a,7,8,9,9a-Hexahydro-5-methyl-3-(phenylmethyl)cyclopent[4,5]imidazo[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(1*H*)-one (3d).** A mixture of **3f** (425 mg, 1.03 mmol) and 20% $Pd(OH)_2$/C (0.41 g) was hydrogenated at 60 psi for 24 h. The solids were filtered and washed with CH_2Cl_2−MeOH (5−1). The filtrate and washings were combined and concentrated in vacuo. Flash chromatography of the residue on a silica gel column with CH_2Cl_2−MeOH (95−5 then 90−10) as eluent gave **3d** (100 mg, 30%) as white solids: ^{1}H NMR (300 MHz, CD_3OD) δ 1.50−2.30 (m, 6H), 3.31 (s, 3H), 4.25 (s, 2H), 4.70 (t, 1H, J = 7 Hz), 4.83 (t, 1H, J = 7 Hz), 7.17−7.38 (m, 5H); HRMS EI ($C_{18}H_{19}N_5O$) calcd 321.1590 (M^+), found 321.1588. Anal. ($C_{18}H_{19}N_5O$·0.05CH_2Cl_2) C, H, N.

(6a*R*,9a*S*)-3,5-Dimethyl-5,6a,7,8,9,9a-hexahydrocyclopent[4,5]imidazo[1,2-*a*]pyrazolo[4,3-*e*]pyrimidin-4(1*H*)-one (3c): similarly prepared from **3b**; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20−2.05 (m, 6H), 2.32 (s, 3H), 3.09 (s, 3H), 4.48 (t, 1H, J = 6 Hz), 4.55 (t, 1H, J = 7 Hz), 12.64 (br s, 1H); MS (CI) m/z 246 (MH^+, 100). Anal. ($C_{12}H_{15}N_5O$·0.08CH_2Cl_2) C, H, N.

PDE Inhibition Assays. PDE assays were performed in a reaction medium containing 50 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 0.1 mg/mL BSA, and 1 μM cGMP or cAMP, respectively. Assays were carried out for 30 min at 30 °C using the methods described by Thompson.[14] Reaction mixture for assay of PDE1 activity also contained 1 mM $CaCl_2$ and 0.1 μM calmodulin. The reaction mixture for assay of PDE2 contained 5 μM cGMP. [^{3}H]cGMP was used as the substrate in the assays for PDE1 and PDE5, while [^{3}H]cAMP was used as the substrate for PDE2, PDE3, and PDE4. The concentration of compounds that produce 50% inhibition of enzyme activity (IC_{50}) was determined from the curve of percentage inhibition of enzyme activity vs log molar concentration of the compounds. All assays were carried out in duplicate, and reported values represent the mean of the two determinations. Measurements were reproducible on the average to ±25%, except for the very weakest inhibitors where solubility limits were occasionally exceeded. PDE1, PDE2, PDE3, PDE4, and PDE5 utilized in the assays were purified from bovine aorta,[15] recombinant bovine adrenal cortex,[16] bovine heart,[17] canine kidney,[18] and bovine lung,[19] respectively. These preparations were free of substantial contaminating phosphodiesterase activities.

Antihypertensive Activity. PDE inhibitors were evaluated in the spontaneously hypertensive rat using the methodology previously described by Smith et al.[20] and more recently by Vemulapalli et al.[21] Reported values reflect peak changes in mean arterial pressure in comparison to a control group to which vehicle was administered. The compounds were adminstered orally as aqueous solutions or suspensions in 0.4% methylcellulose as the vehicle. In general differences of ≥10 mmHg are considered statsitically signficant. The drugs verapamil and nifedipine were routinely run as positive controls in this assay. Two hours after a 30 mg/kg oral dose of verapamil or nifedipine, reproducible falls in blood pressure of 70 ± 4 and 58 ± 5 mmHg (mean ± SEM), respectively, were produced.

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Supporting Information Available: HPLC chromatograms (normal and reverse phases) of compounds **3c**−**e** and **4b** (8 pages). Ordering information is given on any current masthead page.

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