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Synthesis and Phosphodiesterase 5 Inhibitory Activity of Novel Phenyl Ring Modified Sildenafil Analogues

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Abstract—New sildenafil analogues containing an ether ring fused into the phenyl moiety, **6a–d** and **7a–d**, were efficiently synthesized from the readily available starting materials, **1a–d** and **2**, in five steps. Ab initio calculations indicated that introduction of a cyclic ether to the phenyl group might enhance the co-planarity of the molecule. The torsional angles were calculated to be 2−3° for the 5-membered cyclic ether derivatives, **6a**, **6c**, **7a**, and **7c**, and 12−16° for the 6-membered ones, **6b**, **6d**, **7b**, and **7d**. On the other hand, sildenafil showed the least co-planarity with the torsional angle of 23° compared with the target compounds, **6a–d** and **7a–d**. In the enzyme assay, however, the in vitro PDE 5 inhibitory activity was found out to be inversely related to the degree of co-planarity. In other words, the least planar sildenafil showed the highest activity, and the most planar 5-membered cyclic ether derivatives were least active by 100–200-fold compared with sildenafil. Our study clearly demonstrated that the open chain 2′-alkoxy group of the phenyl ring, although less effective for inducing the co-planarity, seemed to act as a much better lipophilic requirement than the cyclic alkoxy moiety.

Introduction

Male erectile dysfunction (MED), the persistent inability to achieve or maintain an erection for satisfactory sexual performance, is a common and important medical problem.[1] According to a random community-based sample study, over half of men at 40 to 70 years of age suffered from erectile dysfunction.[2] Recent development of sildenafil citrate[3] (Viagra; Chart 1) as an effective and orally active agent for the treatment of MED has spurred significant interest in the discovery of additional phosphodiesterase type 5 (PDE5) inhibitors.[4] PDE5 is the predominant cGMP-hydrolyzing enzyme present in the corpus cavernosum, the smooth muscle in the penis which helps control vascular tone. Under normal physiological conditions, nitric oxide is released from the cavernosal nerve upon sexual stimulation. This activates soluble guanylyl cyclase in the corpus cavernosum, causing an increase in intracellular cGMP, which is normally hydrolyzed by PDE5. Inhibition of PDE5 elevates levels of the cyclic nucleotide, leading to enhanced relaxation of smooth muscle, increased arterial inflow, venous congestion, and ultimately resulting in improved penile erection in men with erectile dysfunction. Despite the efficacy of sildenafil as a treatment for MED, there are some notable drawbacks associated with its use. Clinically significant adverse effects such as nausea, headache, cutaneous flushing and visual disturbances have been reported, and their incidence is dose-dependent. Certain of these side-effects are thought to be due to nonspecific inhibition of other PDEs, specifically PDE1 and PDE6.[5,6] Therefore, the search for potent and more selective PDE5 inhibitors is of primary interest.

Intramolecular hydrogen bonding has been shown to play an important role for high biological activity by maintaining co-planarity between the phenyl and purine ring in the sildenafil analogues. It has been demonstrated that the 2′-substituent containing a group, which is capable of forming a hydrogen bonding to the pyrimidinone NH, enhances activity of the drugs in the earlier work of antiallergenic 8-azapurines (Chart 1).[7] In the structure–activity relationship study of sildenafil series, it was also suggested that the 2′-alkoxy moiety of

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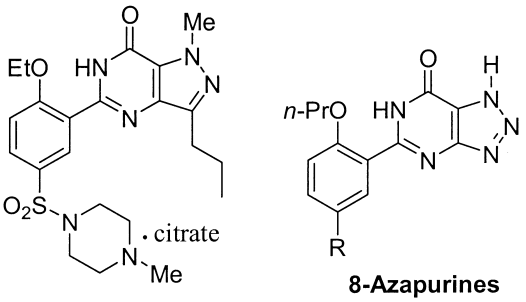
the phenyl ring not only provided a hydrogen bonding between the pyrimidinone NH and oxygen lone pair of the alkoxy group maintaining co-planarity between the phenyl and purine ring, but also served as a requirement for a small lipophilic substituent.[3c] Based on these findings, it was our particular interest to investigate if further modification of the 2′-alkoxy group of the phenyl ring to a fused-ring system could enhance the PDE inhibitory activity and selectivity compared with sildenafil. In this paper, the preparation of new sildenafil analogues possessing an oxygen-containing ring on the phenyl moiety, **6a–d** and **7a–d**, and evaluation of their in vitro PDE inhibitory activity are disclosed.



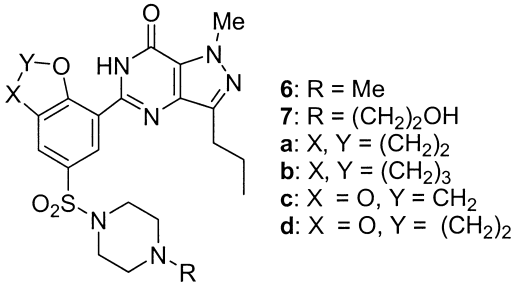
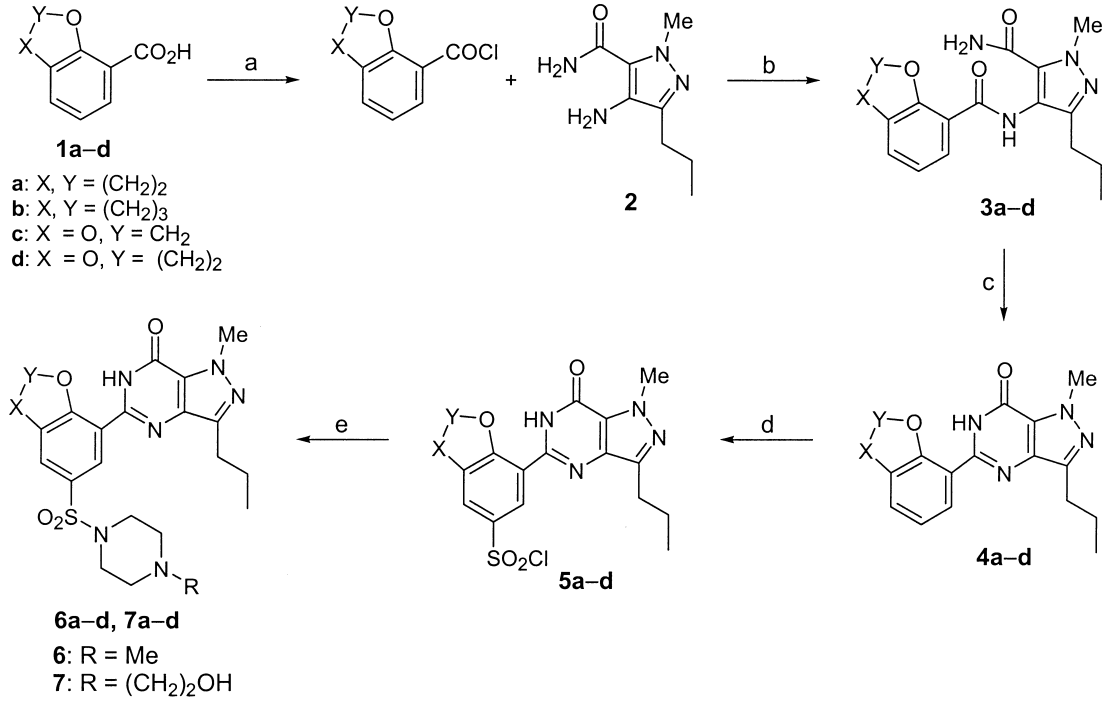


Chart 1.

Chemistry

Requisite starting materials, 7-carboxy-2,3-dihydrobenzofuran (**1a**),[8] 8-chromancarboxylic acid (**1b**),[9] 2,3-methylenedioxybenzoic acid (**1c**),[10] 2,3-ethylenedioxybenzoic acid (**1d**)[11] and 4-amino-1-methyl-3-*n*-propylpyrazole-5-carboxamide (**2**),[3c,12] were readily available by using known procedures in the literature. As shown in Scheme 1, the synthesis commenced with the conversion of each carboxylic acid **1a–d** to the corresponding acyl chloride in refluxing thionyl chloride ($SOCl_2$), and the resulting acyl chloride was used for the next step after complete removal of excess thionyl chloride under reduced pressure. Selective acylations of the amine moiety in **2** with cyclic benzoyl chlorides were carried out in CH_2Cl_2 in the presence of a catalytic amount of DMAP (0.1 equiv) at 0 °C to produce the amides **3a–d** in high yields of 82–99%. Cyclization reactions of the amides **3a–d** were efficiently affected under basic conditions at reflux temperature using NaOH (2.0 equiv) in a mixture of H_2O–EtOH (3:1, v/v) to afford the corresponding pyrazolopyrimidinones **4a–d** in 70–77% yields. It was observed that under basic cyclization conditions, the primary amide group of **3a–d** was also hydrolyzed, and the corresponding crude carboxylic acid as a major by-product was isolated in



Scheme 1. (a) $SOCl_2$, reflux, 3 h; (b) DMAP, Et_3N, CH_2Cl, 0 °C, 1 h; (c) NaOH, H_2O/EtOH (3:1), 110 °C, 7–12 h; (d) $ClSO_3H$, 0 °C, 2 h; (e) 1-methylpiperazine (for **6a–d**), or 1-(2-hydroxyethyl)piperazine (for **7a–d**), EtOH, room temperature, overnight.

about 20–29%. Chlorosulfonylations of the pyrazolopyrimidinones **4a–d** in chlorosulfonic acid at 0 °C proceeded smoothly and selectively at the 5′-position of the phenyl ring to give the desired compounds **5a–d** in 50–95% yields. In these reactions, the yields were found to be highly variable, seemingly depending on the structure of the substrate and the reaction scale. These chlorosulfonyl derivatives **5a–d** were readily coupled with two cyclic amines to produce the sulfonamides as the target compounds. 1-Methylpiperazine and 1-(2-hydroxyethyl)piperazine were selected as starting materials since they have been reported to provide most suitable PDE5 inhibitory activities and physical properties in the earlier related work of Terrett and his co-workers.[3c] The coupling reactions with chlorosulfonyl derivatives **5a–d** were performed using excess amounts of an appropriate cyclic amine (3–4 equiv) in EtOH at room temperature to afford the target products, **6a–d** and **7a–d**, in 70–97% yields.

Results and Discussion

Prior to actual synthesis, ab initio calculations using Gaussian program[13] were carried out for sildenafil and the target compounds, **6a–d** and **7a–d**, to examine the co-planarity between the phenyl and the heterocyclic ring at the HF/3-21G and HF/6-31G(d) levels. In the processes, the geometries of the total nine compounds were first optimized using 3-21G basis set, and then the resulting structures were taken for geometry optimization calculation at HF/6-31G(d) level. The fully optimized structures and their key parameters are shown in Figure 1 and Table 1, respectively, and the torsional angles depicted in Figure 1 can be used to evaluate the co-planarity. The torsional angles from HF/3-21G calculation featured the perfect planar structures for all nine compounds since the angle values were between −1.1 and 1.3°. On the other hand, when the higher level of theory, HF/6-31G(d), was implemented into calculation,

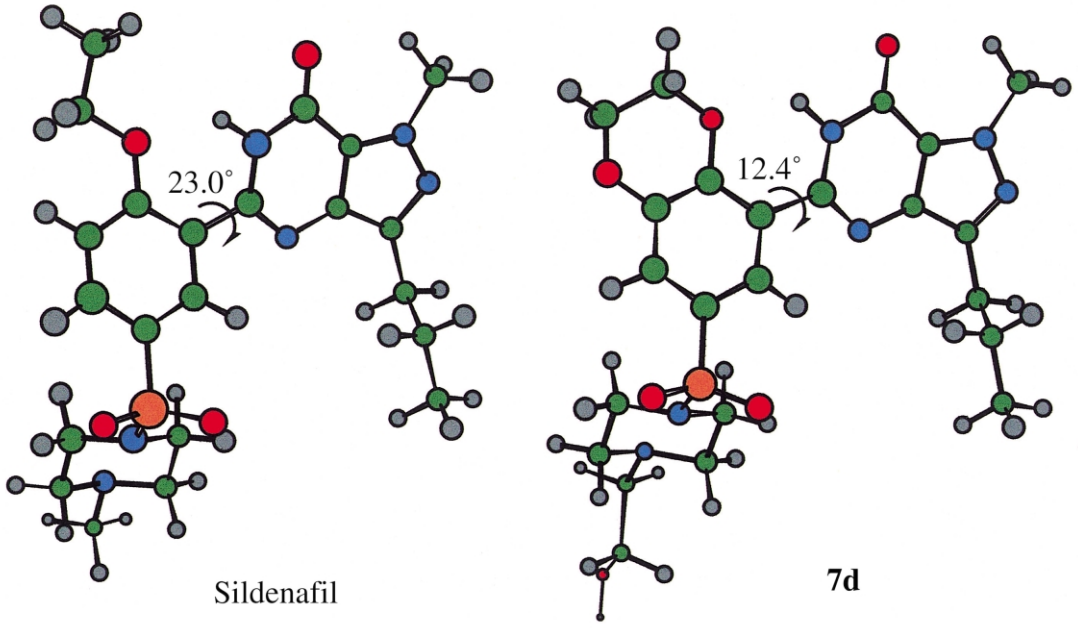


Figure 1. Optimized structures of sildenafil and **7d** using HF/6-31G(d) method as visualized by CS Chem3D Pro.

Table 1. Estimated co-planarity of sildenafil and target compounds, **6a–d** and **7a–d**, by ab initio calculations[a]

	Method	
	HF/3-21G	HF/6-31G(d)
Compd	Torsion (°)	Torsion (°)[a]
Sildenafil	14.3	23.0
6a	0.4	3.2
6b	−0.9	16.5
6c	0.5	2.4
6d	−1.3	12.5
7a	0.4	3.2
7b	−0.9	16.0
7c	0.5	2.3
7d	−1.1	12.4

[a]Torsional angles are shown in Figure 1.

Table 2. In vitro PDE[a] inhibitory activities of **6a–d** and **7a–d**

Compd	IC_{50} (nM)		IC_{50} ratio
	PDE5	PDE6	PDE6/PDE5
6a	238	ND[b]	ND
6b	12.8	53.9	4
6c	423	ND	ND
6d	7.90	10.6	1
7a	204	ND	ND
7b	12.7	41.1	3
7c	330	ND	ND
7d	6.25	7.34	1
Sildenafil	1.90	20.5	11

[a]PDE5 and PDE6 were prepared from rabbit platelet and bovine retina, respectively, and assayed using [^{3}H]-cGMP SPA kit. IC_{50} values were determined from the logarithmic concentration-inhibition curve. The value is the mean from three experiments.

the torsional difference of the object compounds became clear. The computed results showed that introduction of a cyclic ether to the phenyl group indeed enhanced the co-planarity between the phenyl and the heterocyclic ring. Particularly, the co-planarity was greater in the compounds, **6a**, **6c**, **7a**, and **7c**, which contain the 5-membered cyclic ether, than in the 6-membered ring compounds, **6b**, **6d**, **7b**, and **7d**. The torsional angles were 2–3° for the 5-membered cyclic ether derivatives, **6a**, **6c**, **7a**, and **7c**, and 12–16° for the 6-membered ones, **6b**, **6d**, **7b**, and **7d**. On the other hand, sildenafil showed the least co-planarity with the torsional angle of 23° compared with the target compounds, **6a**–**d** and **7a**–**d**. As expected, the structural variation of the 5′-substituent of the phenyl ring in the compounds **6** and **7** did not affect the planarity of the molecule. We found these results from ab initio calculations to be really encouraging since it has been known that greater co-planarity of the molecule could improve the biological activity.

Target compounds, **6a**–**d** and **7a**–**d**, were evaluated for in vitro inhibitory activities against two different forms of PDEs, PDE5 and PDE6, and their IC_{50} values were determined from concentration–response curves (Table 2). Disappointingly, all the compounds with the modified alkoxy group of the phenyl ring showed lower PDE5 inhibitory activities than sildenafil. Replacement of the alkoxy side chain with 5-membered ether ring (**6a** and **7a**) resulted in about 100-fold drop of PDE5 inhibitory activities, and the activities decreased even further (about 200-fold) with the addition of one more oxygen into the ether ring (**6c** and **7c**). In contrast, compounds with a 6-membered ring (**6b** and **7b**) showed about 6-fold decrease in PDE5 inhibitory activities compared with sildenafil, and introduction of additional oxygen (**6d** and **7d**) slightly improved their potency. Substituents on the piperazine did not seem to produce any significant change in their PDE5 inhibitory activities, although slight increases in potency were observed with the 2-hydroxyethyl moiety. In addition, the inhibitory activity toward PDE6 has been determined for the selected compounds, but they exhibited no (**6d** and **7d**) or rather low (**6b** and **7b**) selectivity over PDE6, indicating that these series of compounds have intrinsically lower selectivity than sildenafil.

It should be noted that while the modification of the alkoxy chain to a ring system increased the degree of co-planarity according to the quantum mechanical calculations, it significantly reduced the enzyme inhibitory activity. In other words, compounds with the 5-membered ether ring (**6a**, **6c**, **7a** and **7c**) were expected to have the highest co-planarity but they exhibited the least activity, which is contrary to our initial anticipation that the higher co-planarity would produce higher inhibitory activity through more favorable hydrogen bonding interaction. Although highly speculative, this discrepancy might be explained in terms of the spatial rearrangement of the lipophilic 2′-substituent on the phenyl ring. By scrutinizing the structures of sildenafil and the representative compound **7d** in Figure 1, the ethyl group of sildenafil seems to be freely rotating and spans away from the phenyl ring, which could help reach into the right lipophilic pocket of the enzyme. On the other hand, the ethylenedioxy unit of compound **7d** is tied onto the phenyl ring and therefore might not provide proper spatial orientation for the appropriate lipophilic interactions with the enzyme, which could lead to the diminished activity.

In conclusion, our extensive studies on new sildenafil analogues containing an ether ring fused into the phenyl moiety, **6a**–**d** and **7a**–**d**, clearly demonstrated that the open chain 2′-alkoxy group of the phenyl ring, although less effective for inducing the co-planarity, seemed to fulfill an important role as a lipophilic requirement much more efficiently than the cyclic ones.

Experimental

Melting points were determined on a Thomas–Hoover or Mettler melting point apparatus and are uncorrected. Infrared spectra were recorded on a Magna 750 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity 300 spectrometer. The chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane in $CDCl_3$ or DMSO-d_6. Fast-atom bombardment mass spectra (FABMS) were obtained on a VG Quattro mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254 glass plates. Medium-pressure chromatography (MPLC) was performed using Merck silica gel 60 (230–400 mesh) with a VSP-2200 ceramic pump (Eyela). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

General procedures for the preparation of the amides 3a–d: acylation reactions

A solution of a cyclic benzoic acid **1a**–**d** (46.0 mmol) in $SOCl_2$ (45 mL) was refluxed at 90 °C for 3 h under N_2 atmosphere. Excess $SOCl_2$ was distilled out from the reaction mixture, and the residual $SOCl_2$ was removed completely under vacuum for 1 h. The crude acyl chloride was dissolved in anhydrous CH_2Cl_2 (110 mL), and was slowly added to a cooled mixture of **2** (7.28 g, 40.0 mmol), Et_3N (11.2 mL, 80.0 mmol) and a catalytic amount of DMAP (0.49 g, 4.0 mmol) in anhydrous CH_2Cl_2 (110 mL) at 0 °C under N_2 atmosphere over 30 min. After additional stirring for 1 h at 0 °C, the mixture was washed with 1 N HCl aqueous solution (150 mL), and the aqueous layer was further extracted with 3% MeOH in $CHCl_3$ (2×150 mL). The combined organic layer was dried ($MgSO_4$) and evaporated to dryness under reduced pressure to afford the desired amide as a yellow to brown solid. The crude product was purified by MPLC over SiO_2 using a mixture of MeOH/$CHCl_3$ as eluent to give the titled compound, which was crystallized from a suitable solvent.

4-((2′,3′-Dihydrobenzofuran-7′-carbonyl)amino)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (3a). Yield 99%; mp 169.4–170.2 °C (EtOAc); IR (neat) 3399, 3335, 3306 (NH), 1677, 1647 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.2 Hz, 3H, $CH_2CH_2CH_3$), 1.57–1.70 (m, 2H,

$CH_2CH_2CH_3$), 2.54 (dd, $J=7.8$ Hz, 7.2Hz, 2H, $CH_2CH_2CH_3$), 3.36 (t, $J=8.7$ Hz, 2H, OCH_2CH_2), 4.06 (s, 3H, NCH_3), 4.81 (t, $J=8.7$ Hz, 2H, OCH_2CH_2), 5.55 (br s, 1H, $CONH_2$), 7.03 (dd, $J=7.8$ Hz, 7.2 Hz, 1H, H-5′), 7.43 (dd, $J=7.2$ Hz, 1.5 Hz, 1H, H-4′), 7.89 (br s, 1H, $CONH_2$), 7.95 (dd, $J=7.8$ Hz, 1.5 Hz, 1H, H-6′), 8.96 (br s, 1H, NH); MS (FAB) m/z 329 (MH^+). Anal. calcd for $C_{17}H_{20}N_4O_3$: C, 62.18; H, 6.14; N, 17.06. Found: C, 62.35; H, 6.27; N, 16.87.

4-((8′-Chromancarbonyl)amino)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (3b). Yield 82%; mp 167.1–168.1 °C (EtOAc/hexane); IR (neat) 3427, 3231 (NH), 1670, 1649 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.58–1.70 (m, 2H, $CH_2CH_2CH_3$), 2.09–2.17 (m, 2H, $OCH_2CH_2CH_2$), 2.54 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.92 (t, $J=6.3$ Hz, 2H, $OCH_2CH_2CH_2$), 4.06 (s, 3H, NCH_3), 4.41 (dd, $J=5.4$ Hz, 5.1 Hz, 2H, $OCH_2CH_2CH_2$), 5.58 (br s, 1H, $CONH_2$), 7.01 (t, $J=7.8$ Hz, 1H, H-6′), 7.27 (dd, $J=7.8$ Hz, 1.8 Hz, 1H, H-5′), 7.98 (br s, 1H, $CONH_2$), 8.06 (dd, $J=7.8$ Hz, 1.8 Hz, 1H, H-7′), 9.37 (br s, 1H, NH); MS (FAB) m/z 343 (MH^+). Anal. calcd for $C_{18}H_{22}N_4O_3$: C, 63.14; H, 6.48; N, 16.36. Found: C, 63.33; H, 6.71; N, 16.31.

4-((2′,3′-Methylenedioxybenzoyl)amino)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (3c). Yield 91%; mp 158–159 °C (EtOAc); IR (neat) 3303, 3177 (NH), 1671, 1639 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.60–1.70 (m, 2H, $CH_2CH_2CH_3$), 2.53 (t, $J=7.8$ Hz, 2H, $CH_2CH_2CH_3$), 4.06 (s, 3H, NCH_3), 5.59 (br s, 1H, $CONH_2$), 6.18 (s, 2H, OCH_2O), 7.01 (t, $J=7.8$ Hz, 1H, H-5′), 7.08 (dd, $J=7.8$ Hz, 1.5 Hz, 1H, H-4′), 7.61 (dd, $J=7.8$ Hz, 1.5 Hz, 1H, H-6′), 7.67 (br s, 1H, $CONH_2$), 8.27 (br s, 1H, NH); MS (FAB) m/z 331 (MH^+). Anal. calcd for $C_{16}H_{18}N_4O_4$: C, 58.17; H, 5.49; N, 16.96. Found: C, 58.01; H, 5.23; N, 17.18.

4-((2′,3′-Ethylenedioxybenzoyl)amino)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (3d). Yield 94%; mp 135–137 °C (EtOAc/hexane); IR (neat) 3579, 3351, 3277 (NH), 1676, 1632 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.55–1.75 (m, 2H, $CH_2CH_2CH_3$), 2.54 (dd, $J=7.8$ Hz, 7.2 Hz, 2H, $CH_2CH_2CH_3$), 4.06 (s, 3H, NCH_3), 4.37–4.39 (m, 2H, OCH_2), 4.49–4.51 (m, 2H, OCH_2), 5.59 (br s, 1H, $CONH_2$), 7.01 (dd, $J=8.1$ Hz, 7.8 Hz, 1H, H-5′), 7.11 (dd, $J=8.1$ Hz, 1.8 Hz, 1H, H-4′), 7.79 (dd, $J=7.8$ Hz, 1.8 Hz, 1H, H-6′), 7.84 (br s, 1H, $CONH_2$), 9.01 (br s, 1H, NH); MS (FAB) m/z 345 (MH^+). Anal. calcd for $C_{17}H_{20}N_4O_4$: C, 59.29; H, 5.85; N, 16.27. Found: C, 59.55; H, 5.97; N, 16.01.

General procedures for the preparation of the pyrazolopyrimidinones 4a–d: cyclization reactions. A suspension of the amide **3a–d** (28.26 mmol) and NaOH (2.26 g, 56.51 mmol) in a mixture of H_2O (96 mL) and EtOH (32 mL) was refluxed at 110 °C for 7–12 h, cooled to room temperature, and followed by EtOH evaporation in vacuo. The pH of the reaction mixture was adjusted to 1L with 2 N HCl aqueous solution (18 mL), and extraction with CH_2Cl_2 (2×350 mL) was performed. The combined extracts were dried ($MgSO_4$) and evaporated to dryness under reduced pressure to afford the desired pyrazolopyrimidinone as a white solid. The crude product was purified by MPLC on SiO_2 using a mixture of $MeOH/CHCl_3$ as eluent to give the titled compound, which was crystallized from a suitable solvent.

5-(2′,3′-Dihydrobenzofuran-7′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (4a). Yield 77%; mp 168.0–168.9 °C (EtOAc/hexane); IR (neat) 3306 (NH), 1703 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.81–1.94 (m, 2H, $CH_2CH_2CH_3$), 2.93 (dd, $J=7.8$ Hz, 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.32 (t, $J=8.7$ Hz, 2H, OCH_2CH_2), 4.27 (s, 3H, NCH_3), 4.82 (t, $J=8.7$ Hz, 2H, OCH_2CH_2), 7.03 (dd, $J=7.8$ Hz, 7.5 Hz, 1H, H-5′), 7.33 (dd, $J=7.2$ Hz, 1.2 Hz, 1H, H-4′), 8.26 (dd, $J=7.8$ Hz, 1.2 Hz, 1H, H-6′), 10.81 (br s, 1H, NH); MS (FAB) m/z 311 (MH^+). Anal. calcd for $C_{17}H_{18}N_4O_2$: C, 65.79; H, 5.85; N, 18.05. Found: C, 65.92; H, 5.99; N, 18.01.

5-(Chroman-8′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (4b). Yield 73%; mp 159.9–160.6 °C (EtOAc/hexane); IR (neat) 3263 (NH), 1705 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.81–1.93 (m, 2H, $CH_2CH_2CH_3$), 2.05–2.15 (m, 2H, $OCH_2CH_2CH_2$), 2.89 (t, $J=6.3$ Hz, 2H, $OCH_2CH_2CH_2$), 2.93 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 4.27 (s, 3H, NCH_3), 4.30 (dd, $J=5.4$ Hz, 5.1Hz, 2H, $OCH_2CH_2CH_2$), 7.01 (t, $J=7.8$ Hz, 1H, H-6′), 7.18 (dd, $J=7.8$ Hz, 1.8 Hz, 1H, H-5′), 8.28 (dd, $J=7.8$ Hz, 1.8Hz, 1H, H-7′), 10.98 (br s, 1H, NH); MS (FAB) m/z 325 (MH^+). Anal. calcd for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.58; H, 6.31; N, 17.11.

5-(2′,3′-Methylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (4c). Yield 70%; mp 186–187 °C (CH_2Cl_2/hexane); IR (neat) 3177 (NH), 1686 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 1.80–1.93 (m, 2H, $CH_2CH_2CH_3$), 2.93 (dd, $J=7.8$ Hz, 7.5 Hz, 2H, $CH_2CH_2CH_3$), 4.27 (s, 3H, NCH_3), 6.17 (s, 2H, OCH_2O), 6.96 (dd, $J=7.8$ Hz, 1.8 Hz, 1H, H-4′), 7.02 (t, $J=7.8$ Hz, 1H, H-5′), 7.90 (dd, $J=7.8$ Hz, 1.8 Hz, 1H, H-6′), 10.07 (br s, 1H, NH); MS (FAB) m/z 313 (MH^+). Anal. calcd for $C_{16}H_{16}N_4O_3$: C, 61.53; H, 5.16; N, 17.94. Found: C, 61.71; H, 5.39; N, 17.82.

5-(2′,3′-Ethylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (4d). Yield 74%; mp 154.5–157.0 °C (EtOAc/hexane); IR (neat) 3279 (NH), 1700 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.73–1.93 (m, 2H, $CH_2CH_2CH_3$), 2.92 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 4.27 (s, 3H, NCH_3), 4.34–4.37 (m, 2H, OCH_2), 4.49–4.52 (m, 2H, OCH_2), 6.97–7.04 (m, 2H, H-4′ and H-5′), 7.99 (dd, $J=6.6$ Hz, 3.0 Hz, 1H, H-6′), 10.67 (br s, 1H, NH); MS (FAB) m/z 327 (MH^+). Anal. calcd for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found: C, 62.36; H, 5.67; N, 17.41.

General procedures for the preparation of the chlorosulfonyl derivatives 5a–d: chlorosulfonylation reactions. To a well-stirred and cooled chlorosulfonic acid (3 mL) in an ice bath under N_2 atmosphere was added portionwise the pyrazolopyrimidinone **4a–d** (3.16 mmol) over 30 min in order to prevent clumping, and the resulting brownish solution was stirred in an ice bath for an additional 2 h. The reaction mixture was carefully poured into the well-stirred ice–water (200 g), and was extracted with 10% MeOH in CH_2Cl_2 (2×200 mL). The combined organic phase was washed with brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure to afford the desired chlorosulfonyl compound as a yellowish solid. The crude product was triturated with Et_2O to give the titled compound, which was crystallized from a suitable solvent.

5-(5′-Chlorosulfonyl-2′,3′-dihydrobenzofuran-7′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (5a). Yield 61%; mp 228 °C dec (CH_2Cl_2/hexane); IR (neat) 3337 (NH), 1695 (C=O), 1174 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.80–1.93 (m, 2H, $CH_2CH_2CH_3$), 2.96 (dd, J=7.8 Hz, 7.2Hz, 2H, $CH_2CH_2CH_3$), 3.47 (t, J=8.9 Hz, 2H, OCH_2CH_2), 4.28 (s, 3H, NCH_3), 5.04 (t, J=8.9 Hz, 2H, OCH_2CH_2), 7.95 (d, J=2.1 Hz, 1H, H-4′), 9.00 (d, J=2.1 Hz, 1H, H-6′), 10.52 (br s, 1H, NH); MS (FAB) m/z 409 (MH^+). Anal. calcd for $C_{17}H_{17}ClN_4O_4S$: C, 49.94; H, 4.19; N, 13.70. Found: C, 49.63; H, 4.46; N, 13.99.

5-(6′-Chlorosulfonylchroman-8′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (5b). Yield 86%; mp 190.2–190.9 °C dec (EtOAc/hexane); IR (neat) 3327 (NH), 1711 (C=O), 1168 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.83–1.93 (m, 2H, $CH_2CH_2CH_3$), 2.17–2.24 (m, 2H, $OCH_2CH_2CH_2$), 2.95 (t, J=7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.02 (t, J=6.3 Hz, 2H, $OCH_2CH_2CH_2$), 4.28 (s, 3H, NCH_3), 4.58 (dd, J=5.4 Hz, 5.1 Hz, 2H, $OCH_2CH_2CH_2$), 7.85 (d, J=2.4 Hz, 1H, H-5′), 8.94 (d, J=2.4 Hz, 1H, H-7′), 10.64 (br s, 1H, NH); MS (FAB) m/z 369 [(M–H_2O–Cl)$^+$]. Anal. calcd for $C_{18}H_{19}ClN_4O_4S$: C, 51.12; H, 4.53; N, 13.25. Found: C, 51.46; H, 4.81; N, 12.93.

5-(5′-Chlorosulfonyl-2′,3′-methylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (5c). Yield 50%; mp 203 °C dec (CH_2Cl_2/hexane); IR (neat) 3236 (NH), 1680 (C=O), 1173 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.80–1.93 (m, 2H, $CH_2CH_2CH_3$), 2.95 (dd, J=7.8 Hz, 7.5Hz, 2H, $CH_2CH_2CH_3$), 4.28 (s, 3H, NCH_3), 6.39 (s, 2H, OCH_2O), 7.53 (d, J=1.8 Hz, 1H, H-4′), 8.67 (d, J=1.8 Hz, 1H, H-6′), 9.93 (br s, 1H, NH); MS (FAB) m/z 393 [(M–H_2O)H^+]. Anal. calcd for $C_{16}H_{15}ClN_4O_5S$: C, 46.78; H, 3.68; N, 13.64. Found: C, 46.45; H, 3.83; N, 13.86.

5-(5′-Chlorosulfonyl-2′,3′-ethylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (5d). Yield 95%; mp 209 °C dec (CH_2Cl_2/hexane); IR (neat) 3361 (NH), 1704 (C=O), 1178 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.67–1.79 (m, 2H, $CH_2CH_2CH_3$), 2.79 (t, J=7.5 Hz, 2H, $CH_2CH_2CH_3$), 4.16 (s, 3H, NCH_3), 4.28–4.37 (m, 4H, 2 OCH_2), 7.16 (d, J=1.8 Hz, 1H, H-4′), 7.39 (d, J=1.8 Hz, 1H, H-6′), 7.57 (br s, 1H, NH); MS (FAB) m/z 407 [(M–H_2O)H^+]. Anal. calcd for $C_{17}H_{17}ClN_4O_5S$: C, 48.06; H, 4.03; N, 13.19. Found: C, 48.33; H, 4.29; N, 13.01.

General procedures for the preparation of the sulfonamides, 6a–d and 7a–d: coupling reactions. A mixture of the chlorosulfonyl derivative **5a–d** (0.4 mmol) and an appropriate cyclic amine (1.2 mmol) in absolute EtOH (12 mL) was stirred overnight at room temperature, and then the reaction mixture was concentrated in vacuo to afford the crude sulfonamides, **6a–d** and **7a–d**, as a yellow solid. The residue was purified by MPLC on SiO_2 using a mixture of MeOH/$CHCl_3$ as eluent to give the titled compound, which was crystallized from a suitable solvent.

5-(5′-(4-Methylpiperazinyl)sulfonyl-2′,3′-dihydrobenzofuran-7′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo 4,3-*d*]pyrimidin-7-one (6a). Yield 97%; mp 188.8–189.6 °C (MeOH/Et_2O); IR (neat) 3327 (NH), 1706 (C=O), 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.95 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.70–1.82 (m, 2H, $CH_2CH_2CH_3$), 2.18 (br s, 3H, NCH_3), 2.42 (br s, 4H, 2 NCH_2), 2.80 (t, J=7.5 Hz, 2H, $CH_2CH_2CH_3$), 2.93 (br s, 4H, 2 SO_2NCH_2), 3.40 (t, J=8.7 Hz, 2H, OCH_2CH_2), 4.16 (s, 3H, NCH_3), 4.84 (t, J=8.7 Hz, 2H, OCH_2CH_2), 7.74 (d, J=1.8 Hz, 1H, H-4′), 7.95 (d, J=1.8 Hz, 1H, H-6′), 11.78 (br s, 1H, NH); MS (FAB) m/z 473 (MH^+). Anal. calcd for $C_{22}H_{28}N_6O_4S$: C, 55.92; H, 5.97; N, 17.78. Found: C, 56.13; H, 5.86; N, 17.92.

5-(6′-(4-Methylpiperazinyl)sulfonylchroman-8′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]-pyrimidin-7-one (6b). Yield 97%; mp 221–222 °C dec (EtOAc/hexane); IR (neat) 3313 (NH), 1680 (C=O), 1164 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.93 (t, J=7.2 Hz, 3H, $CH_2CH_2CH_3$), 1.65–1.79 (m, 2H, $CH_2CH_2CH_3$), 1.95–2.05 (m, 2H, $OCH_2CH_2CH_2$), 2.15 (s, 3H, NCH_3), 2.37 (br s, 4H, 2 NCH_2), 2.77 (t, J=7.5 Hz, 2H, $CH_2CH_2CH_3$), 2.85–2.95 (m, 6H, 2 SO_2NCH_2 and $OCH_2CH_2CH_2$), 4.15 (s, 3H, NCH_3), 4.31 (dd, J=5.1 Hz, 4.8 Hz, 2H, $OCH_2CH_2CH_2$), 7.59 (d, J=2.1 Hz, 1H, H-5′), 7.65 (d, J=2.1 Hz, 1H, H-7′), 12.20 (br s, 1H, NH); MS (FAB) m/z 487 (MH^+). Anal. calcd for $C_{23}H_{30}N_6O_4S$: C, 56.77; H, 6.21; N, 17.27. Found: C, 56.89; H, 6.39; N, 17.07.

5-(5′-(4-Methylpiperazinyl)sulfonyl-2′,3′-methylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]-pyrimidin-7-one (6c). Yield 90%; mp 187–188 °C (MeOH/hexane); IR (neat) 3335 (NH), 1701 (C=O), 1172 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.69–1.82 (m, 2H, $CH_2CH_2CH_3$), 2.15 (s, 3H, NCH_3), 2.33–2.40 (m, 4H, 2 NCH_2), 2.79 (t, J=7.5 Hz, 2H, $CH_2CH_2CH_3$), 2.90–2.98 (m, 4H, 2 SO_2NCH_2), 4.16 (s, 3H, NCH_3), 6.32 (s, 2H, OCH_2O), 7.36 (d, J=1.8 Hz, 1H, H-4′), 7.64 (d, J=1.8 Hz, 1H, H-6′), 12.15 (br s, 1H, NH); MS (FAB) m/z 475 (MH^+). Anal. calcd for $C_{21}H_{26}N_6O_5S$: C,

53.15; H, 5.52; N, 17.71. Found: C, 53.11; H, 5.60; N, 17.66.

5-(5′-(4-Methylpiperazinyl)sulfonyl-2′,3′-ethylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]-pyrimidin-7-one (6d). Yield 98%; mp 218.4–221.4 °C (MeOH/$CHCl_3$/hexane); IR (neat) 3337 (NH), 1679 (C=O), 1164 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.67–1.80 (m, 2H, $CH_2CH_2CH_3$), 2.15 (s, 3H, NCH_3), 2.36 (br s, 4H, 2 NCH_2), 2.78 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.80 (br s, 4H, 2 SO_2NCH_2), 4.16 (s, 3H, NCH_3), 4.36–4.47 (m, 4H, 2 OCH_2), 7.30 (d, $J=2.1$ Hz, 1H, H-4′), 7.42 (d, $J=2.1$ Hz, 1H, H-6′), 12.13 (br s, 1H, NH); MS (FAB) m/z 489 (MH^+). Anal. calcd for $C_{22}H_{28}N_6O_5S$: C, 54.09; H, 5.78; N, 17.20. Found: C, 53.88; H, 5.95; N, 17.37.

5-(5′-(4-(2-Hydroxyethyl)piperazinyl)sulfonyl-2′,3′-dihydrobenzofuran-7′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7a). Yield 91%; mp 213.6–214.8 °C (MeOH/Et_2O); IR (neat) 3391, 3327 (NH and OH), 1707 (C=O), 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.95 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.70–1.82 (m, 2H, $CH_2CH_2CH_3$), 2.37 (br s, 2H, NCH_2CH_2OH), 2.51 (br s, 4H, 2 NCH_2), 2.80 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.90 (br s, 4H, 2 SO_2NCH_2), 3.30–3.42 (m, 4H, NCH_2CH_2OH and OCH_2CH_2), 4.16 (s, 3H, NCH_3), 4.36 (br s, 1H, OH), 4.84 (t, $J=8.7$ Hz, 2H, OCH_2CH_2), 7.73 (d, $J=1.8$ Hz, 1H, H-4′), 7.94 (d, $J=1.8$ Hz, 1H, H-6′), 11.78 (br s, 1H, NH); MS (FAB) m/z 503 (MH^+). Anal. calcd for $C_{23}H_{30}N_6O_5S$: C, 54.96; H, 6.02; N, 16.72. Found: C, 54.91; H, 6.13; N, 16.85.

5-(6′-(4-(2-Hydroxyethyl)piperazinyl)sulfonylchroman-8′-yl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]-pyrimidin-7-one (7b). Yield 85%; mp 211.7–212.3 °C dec (EtOAc/hexane); IR (neat) 3561, 3380 (NH and OH), 1675 (C=O), 1174 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.93 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 1.67–1.80 (m, 2H, $CH_2CH_2CH_3$), 1.95–2.05 (m, 2H, $OCH_2CH_2CH_2$), 2.36 (t, $J=6.0$ Hz, 2H, NCH_2CH_2OH), 2.40–2.55 (m, 4H, 2 NCH_2), 2.77 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.83–2.98 (m, 6H, 2 SO_2NCH_2 and $OCH_2CH_2CH_2$), 3.39–3.45 (m, 2H, NCH_2CH_2OH), 4.15 (s, 3H, NCH_3), 4.31 (dd, $J=5.1$ Hz, 4.8 Hz, 2H, $OCH_2CH_2CH_2$), 4.38 (t, $J=5.4$ Hz, 1H, OH), 7.59 (d, $J=2.1$ Hz, 1H, H-5′), 7.65 (d, $J=2.1$ Hz, 1H, H-7′), 12.19 (br s, 1H, NH); MS (FAB) m/z 517 (MH^+). Anal. calcd for $C_{24}H_{32}N_6O_5S$: C, 55.80; H, 6.24; N, 16.27. Found: C, 55.62; H, 6.45; N, 16.09.

5-(5′-(4-(2-Hydroxyethyl)piperazinyl)sulfonyl-2′,3′-methylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7c). Yield 88%; mp 232–234 °C dec (MeOH/hexane); IR (neat) 3362, 3308 (NH and OH), 1704 (C=O), 1168 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.69–1.82 (m, 2H, $CH_2CH_2CH_3$), 2.37 (t, $J=6.3$ Hz, 2H, NCH_2CH_2OH), 2.45–2.52 (m, 4H, 2 NCH_2), 2.79 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.93 (br s, 4H, 2 SO_2NCH_2), 3.39–3.43 (m, 2H, NCH_2CH_2OH), 4.16 (s, 3H, NCH_3), 4.37 (t, $J=5.4$ Hz, 1H, OH), 6.32 (s, 2H, OCH_2O), 7.36 (d, $J=1.8$ Hz, 1H, H-4′), 7.64 (d, $J=1.8$ Hz, 1H, H-6′), 12.15 (br s, 1H, NH); MS (FAB) m/z 505 (MH^+). Anal. calcd for $C_{22}H_{28}N_6O_6S$: C, 52.37; H, 5.59; N, 16.66. Found: C, 52.47; H, 5.68; N, 16.51.

5-(5′-(4-(2-Hydroxyethyl)piperazinyl)sulfonyl-2′,3′-ethylenedioxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7d). Yield 96%; mp 207.4–209.2 °C (MeOH/$CHCl_3$/hexane); IR (neat) 3439, 3335 (NH and OH), 1689 (C=O), 1169 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.67–1.80 (m, 2H, $CH_2CH_2CH_3$), 2.36 (t, $J=6.0$ Hz, 2H, NCH_2CH_2OH), 2.48 (br s, 4H, 2 NCH_2), 2.78 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.80 (br s, 4H, 2 SO_2NCH_2), 3.41 (t, $J=6.0$ Hz, 2H, NCH_2CH_2OH), 4.16 (s, 3H, NCH_3), 4.35–4.44 (m, 5H, 2 OCH_2 and OH), 7.30 (d, $J=2.1$ Hz, 1H, H-4′), 7.42 (d, $J=2.1$ Hz, 1H, H-6′), 12.25 (br s, 1H, NH); MS (FAB) m/z 519 (MH^+). Anal. calcd for $C_{23}H_{30}N_6O_6S$: C, 53.27; H, 5.83; N, 16.21. Found: C, 53.47; H, 5.70; N, 16.48.

Determination of PDE5 and PDE6 inhibitory activity. PDE5 was prepared from the rabbit platelet using the method described by Hidaka et al.[14] with minor modifications. Fresh rabbit whole blood was centrifuged at 360×*g* to obtain the platelet-rich plasma (PRP). Platelets were isolated from PRP by centrifugation at 1200×*g*, sonicated (20 s per mL) in 50 mM Tris–HCl buffer (pH 7.4) containing 1 mM $MgCl_2$, and then centrifuged at 40,000×g for 2 h at 4 °C. The supernatant was loaded on the DEAE-cellulose column with a bed volume of 35 mL (Sigma Co., St. Louis, MO, USA) pre-equilibrated with equilibration buffer (50 mM Tris-acetate containing 3.75 mM 2-mercaptoethanol, pH 6.0). After the column was washed with 60 mL of equilibration buffer, PDE5 was eluted using a continuous gradient of 0–600 mM sodium acetate in equilibration buffer with a total volume of 60 mL. The bovine retina PDE6 was prepared using the method described by Ballard et al.[15] with minor modifications. Bovine retinas were minced and homogenized in the homogenization buffer (20 mM HEPES containing 0.25 M sucrose, 1 mM EDTA, 1 mM phenylmethyl sulfonylfluoride [PMSF], pH 7.2) using a Polytron PT 10/35 homogenizer (Kinematica AG, Switzerland) at 5000 rpm with 2 bursts for 10 s. The homogenate was then centrifuged at 40,000×*g* for 60 min at 4 °C. The supernatant was recovered and filtered through 0.2 μm filter. The filtered sample was loaded on the Hitrap Q column with a bed volume of 5 mL (Pharmacia, Uppsala, Sweden) pre-equilibrated with 20 mM HEPES buffer (pH 7.2) containing 1 mM EDTA and 0.5 mM PMSF. The column was then washed with 25 mL of equilibration buffer. PDE6 was eluted using a continuous gradient of 0–600 mM NaCl in equilibration buffer with a total volume of 60 mL. Fractions (1.0 mL each) were collected at a flow rate of 60 mL/h and characterized for cGMP & cAMP hydrolytic PDE activities as described below. Fractions comprising the main peaks of cGMP hydrolytic PDE activity were pooled and stored at

−20 °C in 50% glycerol until the enzyme assay. Enzymatic activity was determined using a PDE scintillation proximity assay (SPA) kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) according to the protocol supplied by the manufacturer. The reaction buffer contained [^{3}H]-cGMP (5 μCi/mL), 1.7 mM EGTA, and 8.3 mM $MgCl_2$ in 50 mM Tris–HCl buffer (pH 7.5). After PDE was added to the reaction buffer, the mixtures were incubated at 30 °C for 30 min. The reaction was then stopped by the addition of 50 μL of SPA beads, and the radioactivity was counted on the liquid scintillation counter (Tri-Carb 1500, Packard Inc., Meriden, CT, USA) after each sample was settled for 20 min. For the inhibitor studies, sildenafil and test compounds were dissolved in DMSO and diluted with distilled water. The final concentration of DMSO was less than 0.2% (v/v). All the inhibition experiments were conducted under the conditions where the level of cGMP hydrolysis did not exceed 15%, and the product formation increased linearly with time and amount of enzyme. IC_{50} was defined as the concentration of compounds to produce a 50% inhibition of enzyme activity and calculated by quantal probit analysis in Pharmacological Calculation System.[16]

References

1. (a) Saenz de Tejada, I. *Contemp. Urol.* **1992**, *7*, 52. (b) Andersson, K.-E.; Wagner, G. *Physiol. Rev.* **1995**, *75*, 191.
2. Feldman, H. A.; Goldstein, I.; Hatzichristou, D. G.; Krane, R. J.; McKinlay, J. B. *J. Urol.* **1994**, *151*, 54.
3. (a) Moreland, R. B.; Goldstein, I.; Kim, N. N.; Traish, A. *Trends Endocrinol. Metab.* **1999**, *10*, 97. (b) Martel, A. M.; Graul, A.; Rabasseda, X.; Castaner, R. *Drugs Future* **1997**, *22*, 138. (c) Terrett, N. K.; Bell, A. S.; Brown, D.; Ellis, P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1819. (d) Boolell, M.; Allen, M. J.; Ballard, S. A.; Gepi-Attee, S.; Muirhead, G. J.; Naylor, A. M.; Osterloh, I. H.; Gingell, C. *Int. J. Urol. Res.* **1996**, *8*, 47.
4. (a) Eardley, I. *Exp. Opin. Invest. Drugs* **1997**, *6*, 1803. (b) Garcia-Reboll, L.; Mulhall, J. P.; Goldstein, I. *Drugs Aging* **1997**, *11*, 140. (c) Truss, M. C.; Stief, C. G. *Drugs Today* **1998**, *34*, 805.
5. Beavo, J. A. *Physiol. Rev.* **1995**, *75*, 725.
6. *Scrip* **1997**, October 9.
7. Wilson, S. R.; Wilson, B. W.; Shoemaker, K. R.; Wooldridge, K. R. H.; Hodgson, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 259.
8. Murphy, R.; Kung, H. F.; Kung, M.; Billings, J. *J. Med. Chem.* **1990**, *33*, 171.
9. (a) Okumura, K.; Inoue, I. *J. Pharm. Soc. Jpn.* **1960**, *81*, 453. (b) Stanetty, P.; Koller, H.; Purstinger, G. *Monatsche. Chem.* **1990**, *121*, 883.
10. Perkin, W. H.; Trikojus, V. M. *J. Chem. Soc.* **1926**, 2925.
11. Fuson, R. C.; Gaertner, R.; Chadwick, D. H. *J. Org. Chem.* **1948**, *13*, 489.
12. US Patent 5,346,901. **1994**.
13. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*; Gaussian, Inc.: Pittsburgh, PA, 1995.
14. Hidaka, H.; Asano, T. *Biochim. Biophys. Acta.* **1976**, *429*, 485.
15. Ballard, S. A.; Gingell, C. J.; Tang, K.; Turner, L. A.; Price, M. E.; Naylor, A. M. *J. Urol.* **1998**, *159*, 2164.
16. Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculations with Computer Program*; Springer-Verlag: New York, 1987; pp 3135.