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Synthesis and Phosphodiesterase Inhibitory Activity of New Sildenafil Analogues Containing a Carboxylic Acid Group in the 5′-Sulfonamide Moiety of a Phenyl Ring

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Abstract—New sildenafil analogues possessing a carboxylic acid group in the 5′-sulfonamide of the phenyl ring, **9a–l**, were prepared from the readily available starting compounds **6a–b** and cyclic amines **3–5** in a three-step sequence. In the enzyme assays, it has been shown that all the target compounds **9a–l** proved to be more potent in inhibiting phosphodiesterase type 5 (PDE5) than sildenafil by 4–38-fold. The effects on the IC_{50} values were investigated by varying the alkoxy group (R) of the phenyl ring, the sulfonamide type (X), and the length of the methylene chain linking the carboxylic acid, and the results were discussed in detail. From this study, we have clearly demonstrated that introduction of a carboxylic acid group to the 5′-sulfonamide moiety of the phenyl ring greatly enhanced PDE5 inhibitory activity, probably by mimicking the phosphate group of cGMP. The piperidinyl propionic acid derivative **9i**, which showed the highest PDE5 inhibitory activity and comparable to better selectivity over PDE isozymes in comparison with sildenafil, has been selected for more detailed biological investigations. © 2001 Elsevier Science Ltd. All rights reserved.

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] Ten percent of the respondents claimed complete dysfunction, while 25% and 17% were diagnosed as moderate and minimal dysfunction, respectively. Recent development of sildenafil citrate[3] (Viagra®; Chart 1) as an orally effective agent for the treatment of MED has spurred significant interest in the discovery of additional phosphodiesterase type 5 (PDE5) inhibitors.[4] Sildenafil citrate is a potent, reversible and selective PDE5 inhibitor that blocks cGMP hydrolysis effectively ($K_i \sim 3$ nM). 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 (NO) 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 headache (16%), facial flushing (10%), dyspepsia (7%) and visual disturbances (3%) have been reported, and their incidence is dose-dependent.[3] 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.

It has been our research goal to develop new sildenafil analogues that could alleviate the drawbacks of sildenafil, especially those with a modified phenyl ring, and a couple of our recent results on the sildenafil derivatives

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have been submitted for publication.[7] In the earlier work of the sildenafil series, it was suggested that appropriate substituents on the 5′-position of the phenyl ring could, depending on the actual conformation in the enzyme active site, reproduce the role of the cyclic phosphate of cGMP in binding. In the same report, it was also revealed that two 5′-sulfonamide derivatives (Chart 1) with a hydroxyl (**1**: $IC_{50}=1.9$ nM) or a carboxamide (**2**: $IC_{50}=2.1$ nM) group exhibited higher PDE5 inhibitory activity compared with sildenafil ($IC_{50}=3.6$ nM).[3c] From these data, we deduced that hydrogen bonding or polar interactions might be present inside the space of the enzyme occupied by the phosphate of cGMP, which could be important for increasing the potency and selectivity. On the basis of these findings, it was reasoned that carboxylic acid functionality in the 5′-sulfonamide moiety could enhance the ability to mimic the role of the phosphate of cGMP, probably due to its polar nature and capability for hydrogen bonding interaction. Therefore, we decided to examine if introducing a carboxylic acid group to the 5′-sulfonamide of the phenyl ring could enhance the PDE inhibitory activity and selectivity of new analogues, when compared with sildenafil. In this paper, we disclose the synthesis of new sildenafil analogues possessing a carboxylic acid group in the 5′-sulfonamide of the phenyl ring, **9a**–**l**, and evaluation of their in vitro PDE inhibitory activity.

Results and Discussion

All the requisite piperidine and piperazine derivatives **3**–**5** (Fig. 1) are either commercially available or were readily prepared according to the literature procedures.[8] With the starting cyclic amines **3**–**5** in hand, the target compounds **9a**–**l** were synthesized in a straightforward three-step sequence from the known pyrazole-5-carboxamides **6a**–**b**[9] as shown in Scheme 1. Chlorosulfonylation reactions of compounds **6a**–**b** in neat chlorosulfonic acid at 0 °C proceeded smoothly and selectively at the 5′-position of the phenyl ring to give the desired products **7a**–**b** in 82–83% yields. Two chlorosulfonyl derivatives **7a**–**b** were readily coupled with selected cyclic amines **3**–**5** in EtOH at room temperature to produce the sulfonamides **8a**–**l** in the yields of 61–96%. Cyclization and the concomitant hydrolysis of the ester group, when present in the sulfonamide moiety of the starting mono-cyclic compounds, were efficiently affected under basic conditions at 90 °C using a mixture of aqueous 1 N NaOH solution and EtOH (2:1, v/v) to afford the corresponding pyrazolopyrimidinones **9a**–**l** in 66–93% yields.

Phosphodiesterase inhibitory activity was assessed in vitro against two different forms of PDEs, PDE5 and PDE6, which were isolated from rabbit platelet and bovine retina, respectively, and the IC_{50} values for the

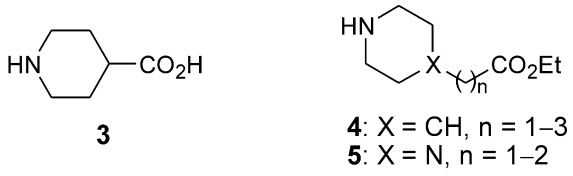


Figure 1. Structures of starting cyclic amines.

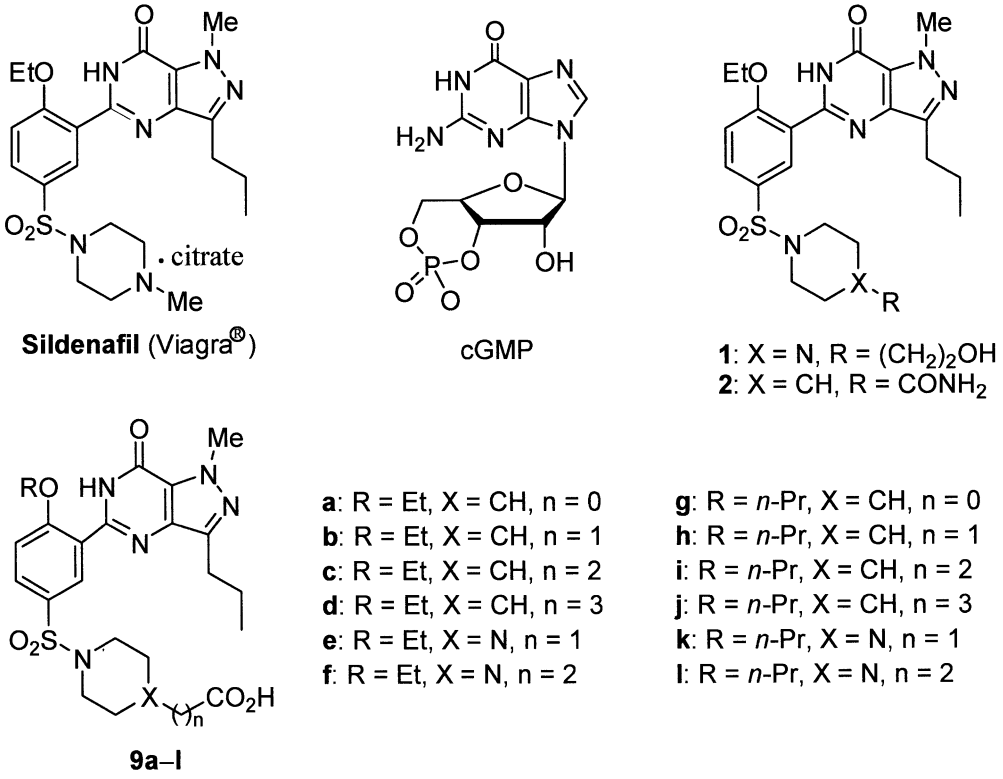
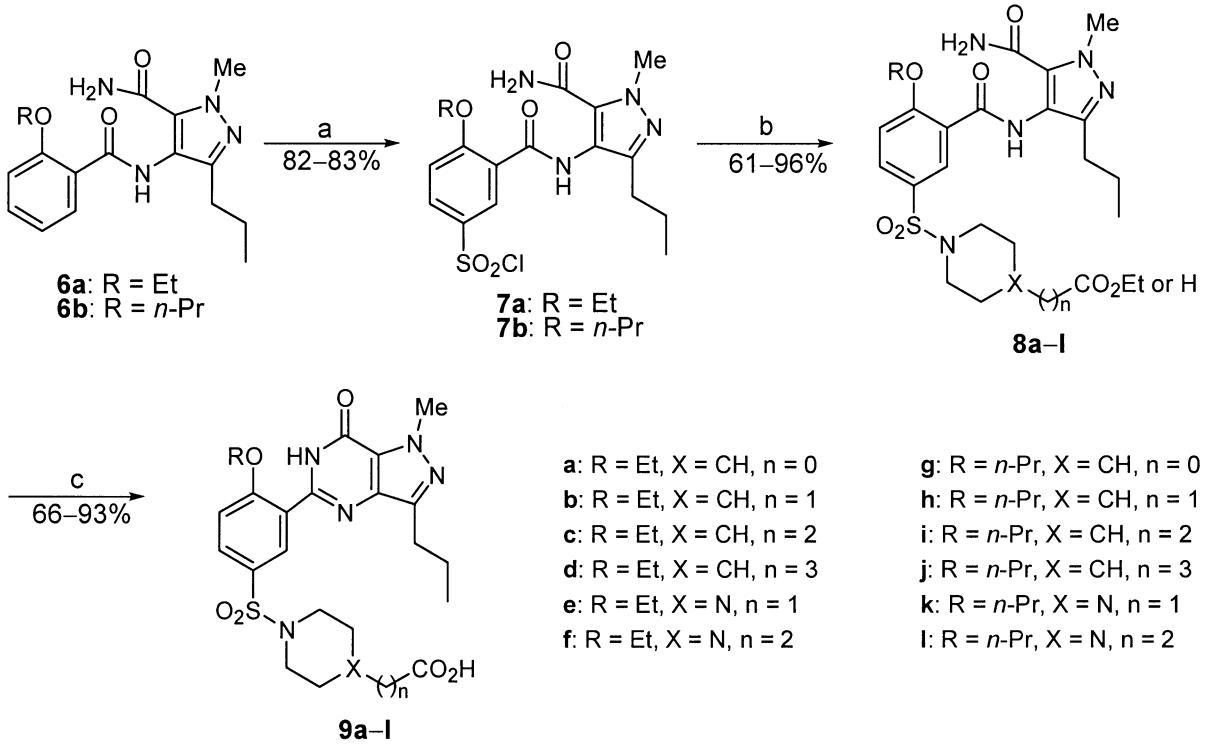


Chart 1.

compounds **9a–l** were determined from concentration–response curves. The effects on the IC_{50} values as a result of varying the alkoxy group (R) of the phenyl ring, the sulfonamide type (X), and the length of the methylene chain linking the carboxylic acid (*n*), are shown in Table 1. Gratifyingly, all the target compounds **9a–l** proved to be more potent (4–38-fold) in inhibiting PDE5 enzyme than sildenafil (IC_{50}: 1.90±0.17 nM), and their IC_{50} values ranged from 0.05 to 0.51 nM. In the view of the structure–activity relationship study, there were three important aspects worth commenting. First, there was about 2–3-fold increase in the activity when the ethoxy group of the phenyl ring was changed into *n*-propyl group (**9a–f** vs **9g–l**). Second, the compounds with the piperidinylsulfonamide group showed higher activity than the corresponding piperazinyl derivatives (e.g., **9h–i** vs **9k–l**). Thirdly, it was clearly observed in the piperidinylsulfonamide series, **9a–d** and **9g–j**, that the PDE5 inhibitory activity was enhanced gradually as the methylene chain length increased from $n=0$ to $n=2$, but the activity decreased with longer chain ($n=3$). In contrast, the effects of the methylene chain length seemed to be negligible in the piperazinylsulfonamide derivatives, **9e–f** and **9k–l**. Among the target compounds, the propionic acid derivative **9i** exhibited the highest PDE5



Scheme 1. (a) $ClSO_3H$, 0 °C to rt, 2 h; (b) **3** (for **8a** and **8g**), **4** or **5** (for the rest), Et_3N, EtOH, rt, 2 h; (c) 1 N NaOH in H_2O–EtOH (2:1), 90 °C, 17 h.

Table 1. In vitro PDE[a] inhibitory activities of compounds **9a–l**

Compd	R	X	*n*	IC_{50} (nM)				IC_{50} ratio
				PDE1	PDE3	PDE5	PDE6	PDE6/PDE5
9a	Et	CH	0	ND	ND	0.51±0.10	ND[b]	ND
9b	Et	CH	1	ND	ND	0.33±0.04	ND	ND
9c	Et	CH	2	ND	ND	0.15±0.04	0.63±0.03	4
9d	Et	CH	3	ND	ND	0.22±0.03	0.72±0.13	3
9e	Et	N	1	ND	ND	0.41±0.05	ND	ND
9f	Et	N	2	ND	ND	0.41±0.03	ND	ND
9g	*n*-Pr	CH	0	93±34	1800±330	0.15±0.03	1.65±0.22	11
9h	*n*-Pr	CH	1	ND	ND	0.11±0.03	0.64±0.19	6
9i	*n*-Pr	CH	2	85±36	1000±310	0.05±0.02	0.49±0.06	10
9j	*n*-Pr	CH	3	ND	ND	0.50±0.12	ND	ND
9k	*n*-Pr	N	1	ND	ND	0.23±0.05	ND	ND
9l	*n*-Pr	N	2	ND	ND	0.21±0.04	0.96±0.07	5
Sildenafil	—	—	—	739±163	14600±2020	1.90±0.17	20.5±2.33	11

[a]PDEs were obtained from bovine heart (PDE1), rabbit platelet (PDE3 and PDE5), and bovine retina (PDE6), respectively, and assayed using [^{3}H]-cGMP or [^{3}H]-cAMP SPA kit. IC_{50} values of sildenafil and test compounds were determined from the logarithmic concentration–inhibition curve. The value is the mean±SEM from three independent experiments.
[b]Not determined.

inhibitory activity, and was about 38-fold more potent than sildenafil. The inhibitory activities toward PDE6 have been also determined for some of selected compounds with relatively high PDE5 inhibitory activity, and their PDE6/PDE5 selectivity was compared with that of sildenafil. Whereas two compounds, **9g** and **9i**, showed comparable selectivity (10–11) to sildenafil (11), the other four derivatives exhibited somewhat lower selectivity (3–6-fold) over PDE6. Furthermore, selectivity over other isozymes (PDE1 and PDE3) was obtained for two analogues, **9g** and **9i**, which showed high PDE5 inhibitory activity and comparable selectivity over PDE6. As shown in Table 1, there were small increases in their selectivity over PDE1 (1.6–4.3-fold) and PDE3 (1.5–2.6-fold) compared with those of sildenafil, and compound **9i** exhibited better selectivity than **9g** and sildenafil. In conclusion, we have clearly demonstrated that the presence of a carboxylic acid group in the 5′-sulfonamide moiety of the phenyl ring indeed enhanced PDE5 inhibitory activity to the great extent, presumably by mimicking the phosphate group of cGMP. The piperidinyl propionic acid derivative **9i**, which showed the highest PDE5 inhibitory activity and comparable to better selectivity over PDE isozymes in comparison with sildenafil, has been selected for more detailed biological investigations.

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 (FAB-MS) 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 chlorosulfonyl derivatives 7a–b: chlorosulfonylation reactions

To a stirred and cooled chlorosulfonic acid (30 mL) in an ice bath under nitrogen atmosphere was added portionwise carboxamide compound **6a–b** (45.40 mmol), and the reaction mixture was warmed to room temperature gradually for 2 h after the addition. The resulting mixture was added, carefully and dropwise, to the cooled mixture of $CHCl_3$ (500 mL) and ice water (100 g) in an ice bath, and the organic layer was separated. The aqueous layer was extracted further with $CHCl_3$ (2×100 mL), and the combined extracts were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure to give the desired sulfonyl chloride as an off-white solid. The crude product was solidified by dissolving in CH_2Cl_2 (20 mL) and pouring CH_2Cl_2 solution to a large quantity of hexanes (800 mL) to afford the titled compound **7a–b** as a white solid, which was crystallized from a suitable solvent.

4-(5-Chlorosulfonyl-2-ethoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (7a). Yield 82%; mp 158–159 °C ($CHCl_3$/hexanes); IR (neat) 3350, 3190 (NH), 1665, 1641 (C=O), 1176 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J=7.4 Hz, 3H, $CH_2CH_2CH_3$), 1.60–1.72 (m, 2H, $CH_2CH_2CH_3$), 1.62 (t, J=6.9 Hz, 3H, OCH_2CH_3), 2.54 (dd, J=7.8 Hz, 7.2 Hz, 2H, $CH_2CH_2CH_3$), 4.06 (s, 3H, NCH_3), 4.46 (q, J=6.9 Hz, 2H, OCH_2CH_3), 5.71 (br s, 1H, $CONH_2$), 7.26 (d, J=9.0 Hz, 1H, H-3′), 7.61 (br s, 1H, $CONH_2$), 8.19 (dd, J=9.0 Hz, 2.7 Hz, 1H, H-4′), 8.95 (d, J=2.7 Hz, 1H, H-6′), 9.19 (br s, 1H, NH); MS (FAB) m/z 429 (MH^+).

4-(5-Chlorosulfonyl-2-*n*-propoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (7b). Yield 83%; mp 140–141 °C (CH_2Cl_2/hexanes); IR (neat) 3469, 3286 (NH), 1683, 1651 (C=O), 1177 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.11 (t, J=7.5 Hz, 3H, $OCH_2CH_2CH_3$), 1.59–1.72 (m, 2H, $CH_2CH_2CH_3$), 1.94–2.06 (m, 2H, $OCH_2CH_2CH_3$), 2.52 (t, J=7.5 Hz, 2H, $CH_2CH_2CH_3$), 4.06 (s, 3H, NCH_3), 4.34 (t, J=6.6 Hz, 2H, $OCH_2CH_2CH_3$), 5.68 (br s, 1H, $CONH_2$), 7.27 (d, J=9.0 Hz, 1H, H-3′), 7.56 (br s, 1H, $CONH_2$), 8.19 (dd, J=9.0 Hz, 2.7 Hz, 1H, H-4′), 8.96 (d, J=2.7 Hz, 1H, H-6′), 9.19 (br s, 1H, NH); MS (FAB) m/z 443 (MH^+).

General procedures for the preparation of the sulfonamides, 8a–l: coupling reactions

A mixture of chlorosulfonyl derivative **7a–b** (1.51 mmol), an appropriate cyclic amine **3–5** (1.81 mmol), and triethylamine (0.63 mL, 4.53 mmol) in anhydrous EtOH (25 mL) was stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was evaporated to dryness under reduced pressure, and the resulting residue was purified by MPLC on silica gel using MeOH in $CHCl_3$ to afford the titled compound **8a–l**, which was crystallized from a suitable solvent.

4-(2-Ethoxy-5-((4-hydroxycarbonyl)piperidinylsulfonyl)-benzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8a). Yield 97%; mp 247 °C dec (MeOH/$CHCl_3$/hexanes); IR (neat) 3345, 3160 (NH, CO_2H), 1706, 1656, 1640 (C=O), 1156 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.90 (t, J=7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.42 (t, J=6.9 Hz, 3H, OCH_2CH_3), 1.51–1.65 (m, 4H, $2CH_{ax}$ and $CH_2CH_2CH_3$), 1.84–1.94 (m, 2H, 2 CH_{eq}), 2.17–2.27 (m, 1H, $CHCO_2$), 2.36–2.52 (m, 4H, $2NCH_{ax}$ and $CH_2CH_2CH_3$), 3.40–3.52 (m, 2H, $2NCH_{eq}$), 3.92 (s, 3H, NCH_3), 4.30 (q, J=6.9 Hz, 2H, OCH_2CH_3), 7.34 (br s, 1H, $CONH_2$), 7.41 (d, J=8.7 Hz, 1H, H-3′), 7.81 (br s, 1H, $CONH_2$), 7.84 (dd, J=8.7 Hz, 2.4 Hz, 1H, H-4′), 7.88 (d, J=2.4 Hz, 1H, H-6′), 9.61 (br s, 1H, NH); MS (FAB) m/z 522 (MH^+). Anal. calcd for $C_{23}H_{31}N_5O_7S$: C, 52.96; H, 5.99; N, 13.43. Found: C, 53.12; H, 6.08; N, 13.27.

4-(2-Ethoxy-5-(4-(ethoxycarbonylmethyl)piperidinylsulfonyl)benzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8b). Yield 93%; mp 177–178 °C (EtOAc/hexanes); IR (neat) 3357, 3180, 3075 (NH), 1733, 1670, 1640 (C=O), 1168 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.15 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.16–1.29 (m, 2H, $2CH_{ax}$), 1.42 (t, $J=6.9$ Hz, 3H, OCH_2CH_3), 1.53–1.65 (m, 3H, CH and $CH_2CH_2CH_3$), 1.71 (br d, $J=12.6$ Hz, 2H, 2 CH_{eq}), 2.18–2.26 (m, 2H, $2NCH_{ax}$), 2.21 (d, $J=6.6$ Hz, 2H, CH_2CO_2), 2.46 (dd, $J=7.8$, 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, $J=11.4$ Hz, 2H, $2NCH_{eq}$), 3.92 (s, 3H, NCH_3), 4.02 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.30 (q, $J=6.9$ Hz, 2H, OCH_2CH_3), 7.33 (br s, 1H, $CONH_2$), 7.41 (d, $J=8.7$ Hz, 1H, H-3′), 7.83 (dd, $J=8.7$, 2.4 Hz, 1H, H-4′), 7.87 (br s, 1H, $CONH_2$), 7.88 (d, $J=2.4$ Hz, 1H, H-6′), 9.60 (br s, 1H, NH); MS (FAB) m/z 564 (MH^+). Anal. calcd for $C_{26}H_{37}N_5O_7S$: C, 55.40; H, 6.62; N, 12.42. Found: C, 55.22; H, 6.69; N, 12.58.

4-(2-Ethoxy-5-(4-(2-ethoxycarbonylethyl)piperidinylsulfonyl)benzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8c). Yield 90%; mp 174–175 °C (EtOAc/hexanes); IR (neat) 3358, 3202, 3180 (NH), 1730, 1668, 1640 (C=O), 1170 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.09–1.21 (m, 3H, CH and $2CH_{ax}$), 1.15 (t, $J=6.9$ Hz, 3H, $CO_2CH_2CH_3$), 1.39–1.47 (m, 2H, $CHCH_2CH_2$), 1.42 (t, $J=6.9$ Hz, 3H, OCH_2CH_3), 1.52–1.65 (m, 2H, $CH_2CH_2CH_3$), 1.70 (br d, $J=9.3$ Hz, 2H, 2 CH_{eq}), 2.16 (br t, $J=10.5$ Hz, 2H, $2NCH_{ax}$), 2.25 (t, $J=7.5$ Hz, 2H, CH_2CO_2), 2.46 (dd, $J=8.1$, 7.2 Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, $J=11.1$ Hz, 2H, $2NCH_{eq}$), 3.92 (s, 3H, NCH_3), 4.01 (q, $J=6.9$ Hz, 2H, $CO_2CH_2CH_3$), 4.30 (q, $J=6.9$ Hz, 2H, OCH_2CH_3), 7.33 (br s, 1H, $CONH_2$), 7.41 (d, $J=8.7$ Hz, 1H, H-3′), 7.84 (dd, $J=8.7$, 2.4 Hz, 1H, H-4′), 7.87 (br s, 1H, $CONH_2$), 7.88 (d, $J=2.4$ Hz, 1H, H-6′), 9.60 (br s, 1H, NH); MS (FAB) m/z 578 (MH^+). Anal. calcd for $C_{27}H_{39}N_5O_7S$: C, 56.14; H, 6.80; N, 12.12. Found: C, 56.29; H, 6.91; N, 11.90.

4-(2-Ethoxy-5-(4-(3-ethoxycarbonylpropyl)piperidinylsulfonyl)benzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8d). Yield 76%; mp 168–168.5 °C (MeOH/Et_2O); IR (neat) 3365, 3179, 3074 (NH), 1733, 1670, 1639 (C=O), 1167 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 1.15 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.06–1.18 (m, 5H, $CHCH_2CH_2$ and $2CH_{ax}$), 1.40–1.51 (m, 2H, $CHCH_2CH_2$), 1.42 (t, $J=6.9$ Hz, 3H, OCH_2CH_3), 1.52–1.62 (m, 2H, $CH_2CH_2CH_3$), 1.70 (br d, $J=9.9$ Hz, 2H, 2 CH_{eq}), 2.18 (br t, $J=10.8$ Hz, 2H, $2NCH_{ax}$), 2.23 (t, $J=7.2$ Hz, 2H, CH_2CO_2), 2.46 (dd, $J=8.1$, 7.2 Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, $J=11.4$ Hz, 2H, $2NCH_{eq}$), 3.92 (s, 3H, NCH_3), 4.02 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.30 (q, $J=6.9$ Hz, 2H, OCH_2CH_3), 7.33 (br s, 1H, $CONH_2$), 7.41 (d, $J=8.7$ Hz, 1H, H-3′), 7.82 (br s, 1H, $CONH_2$), 7.84 (dd, $J=8.7$, 2.4 Hz, 1H, H-4′), 7.88 (d, $J=2.4$ Hz, 1H, H-6′), 9.60 (br s, 1H, NH); MS (FAB) m/z 592 (MH^+). Anal. calcd for $C_{28}H_{41}N_5O_7S$: C, 56.83; H, 6.98; N, 11.84. Found: C, 56.99; H, 7.07; N, 11.71.

4-(2-Ethoxy-5-(4-(ethoxycarbonylmethyl)piperazinylsulfonyl)benzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8e). Yield 68%; mp 178–178.5 °C ($CHCl_3$/EtOAc/hexanes); IR (neat) 3359, 3182 (NH), 1747, 1674, 1640 (C=O), 1169 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.90 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.16 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.42 (t, $J=6.9$ Hz, 3H, OCH_2CH_3), 1.53–1.65 (m, 2H, $CH_2CH_2CH_3$), 2.46 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.60 (br s, 4H, 2 NCH_2), 2.88 (br s, 4H, 2 NCH_2), 3.24 (s, 2H, NCH_2CO_2), 3.92 (s, 3H, NCH_3), 4.05 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.31 (q, $J=6.9$ Hz, 2H, OCH_2CH_3), 7.31 (br s, 1H, $CONH_2$), 7.43 (d, $J=8.7$ Hz, 1H, H-3′), 7.78–7.90 (m, 3H, H-4′, H-6′ and $CONH_2$), 9.64 (br s, 1H, NH); MS (FAB) m/z 565 (MH^+). Anal. calcd for $C_{25}H_{36}N_6O_7S$: C, 53.18; H, 6.43; N, 14.88. Found: C, 53.01; H, 6.32; N, 15.03.

4-(2-Ethoxy-5-(4-(2-ethoxycarbonylethyl)piperazinylsulfonyl)benzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8f). Yield 95%; mp 160.5–161 °C (EtOAc/hexanes); IR (neat) 3365, 3191 (NH), 1726, 1673, 1643 (C=O), 1169 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 1.22 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.60 (t, $J=7.2$ Hz, 3H, OCH_2CH_3), 1.60–1.72 (m, 2H, $CH_2CH_2CH_3$), 2.42 (t, $J=7.2$ Hz, 2H, $CH_2CH_2CH_3$), 2.52–2.57 (m, 6H, 3 NCH_2), 2.69 (t, $J=7.2$ Hz, 2H, CH_2CO_2), 3.05 (br s, 4H, 2 NCH_2), 4.07 (s, 3H, NCH_3), 4.10 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.40 (q, $J=7.2$ Hz, 2H, OCH_2CH_3), 5.62 (br s, 1H, $CONH_2$), 7.17 (d, $J=9.0$ Hz, 1H, H-3′), 7.69 (br s, 1H, $CONH_2$), 7.91 (dd, $J=9.0$, 2.4 Hz, 1H, H-4′), 8.63 (d, $J=7.2$ Hz, 1H, H-6′), 9.28 (br s, 1H, NH); MS (FAB) m/z 579 (MH^+). Anal. calcd for $C_{26}H_{38}N_6O_7S$: C, 53.96; H, 6.62; N, 14.52. Found: C, 54.03; H, 6.52; N, 14.39.

4-(5-(4-(Hydroxycarbonyl)piperidinylsulfonyl)-2-*n*-propoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8g). Yield 99%; mp 171–172 °C (MeOH/$CHCl_3$); IR (neat) 3346, 3183, 3075 (NH, CO_2H), 1673, 1641 (C=O), 1167 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 0.99 (t, $J=7.5$ Hz, 3H, $OCH_2CH_2CH_3$), 1.51–1.65 (m, 4H, $2CH_{ax}$ and $CH_2CH_2CH_3$), 1.76–1.85 (m, 4H, 2 CH_{eq} and $OCH_2CH_2CH_3$), 2.00–2.02 (m, 1H, $CHCO_2$), 2.41–2.52 (m, 4H, $2NCH_{ax}$ and $CH_2CH_2CH_3$), 3.38–3.43 (m, 2H, $2NCH_{eq}$), 3.91 (s, 3H, NCH_3), 4.19 (t, $J=6.6$ Hz, 2H, $OCH_2CH_2CH_3$), 7.40 (d, $J=8.7$ Hz, 1H, H-3′), 7.44 (br s, 1H, $CONH_2$), 7.83 (dd, $J=8.7$, 2.4 Hz, 1H, H-4′), 7.81–7.87 (m, 2H, $CONH_2$ and H-6′), 9.67 (br s, 1H, NH); MS (FAB) m/z 536 (MH^+). Anal. calcd for $C_{24}H_{33}N_5O_7S$: C, 53.82; H, 6.21; N, 13.08. Found: C, 54.01; H, 6.16; N, 12.96.

4-(5-(4-(Ethoxycarbonylmethyl)piperidinylsulfonyl)-2-*n*-propoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8h). Yield 95%; mp 171–172 °C (EtOAc/hexanes); IR (neat) 3348, 3182, 3074 (NH), 1741, 1670, 1641 (C=O), 1168 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 0.99 (t, $J=7.5$ Hz, 3H, $OCH_2CH_2CH_3$), 1.15 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.17–1.29 (m, 2H, $2CH_{ax}$), 1.52–1.65 (m,

3H, CH and $CH_2CH_2CH_3$), 1.71 (br d, $J=12.3$ Hz, 2H, 2 CH_{eq}), 1.78–1.88 (m, 2H, $OCH_2CH_2CH_3$), 2.17–2.27 (m, 2H, $2NCH_{ax}$), 2.22 (d, $J=6.6$ Hz, 2H, CH_2CO_2), 2.46 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, $J=11.4$ Hz, 2H, $2NCH_{eq}$), 3.92 (s, 3H, NCH_3), 4.02 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.20 (t, $J=6.6$ Hz, 2H, $OCH_2CH_2CH_3$), 7.33 (br s, 1H, $CONH_2$), 7.41 (d, $J=8.7$ Hz, 1H, H-3′), 7.83 (dd, $J=8.7$, 2.4 Hz, 1H, H-4′), 7.86 (br s, 1H, $CONH_2$), 7.87 (d, $J=2.4$ Hz, 1H, H-6′), 9.57 (br s, 1H, NH); MS (FAB) m/z 578 (MH^+). Anal. calcd for $C_{27}H_{39}N_5O_7S$: C, 56.14; H, 6.80; N, 12.12. Found: C, 56.27; H, 6.78; N, 11.99.

4-(5-(4-(2-Ethoxycarbonylethyl)piperidinylsulfonyl)-2-*n*-propoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8i). Yield 91%; mp 170–171 °C (EtOAc/hexanes); IR (neat) 3349, 3208, 3077 (NH), 1731, 1669, 1640 (C=O), 1166 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 0.97 (t, $J=7.5$ Hz, 3H, $OCH_2CH_2CH_3$), 1.15 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.10–1.20 (m, 3H, CH and $2CH_{ax}$), 1.39–1.47 (m, 2H, $CHCH_2CH_2$), 1.52–1.62 (m, 2H, $CH_2CH_2CH_3$), 1.65–1.72 (m, 2H, 2 CH_{eq}), 1.75–1.86 (m, 2H, $OCH_2CH_2CH_3$), 2.17 (br t, $J=10.5$ Hz, 2H, $2NCH_{ax}$), 2.26 (t, $J=7.5$ Hz, 2H, CH_2CO_2), 2.45 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, $J=11.1$ Hz, 2H, $2NCH_{eq}$), 3.92 (s, 3H, NCH_3), 4.02 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.19 (t, $J=6.3$ Hz, 2H, $OCH_2CH_2CH_3$), 7.33 (br s, 1H, $CONH_2$), 7.41 (d, $J=8.7$ Hz, 1H, H-3′), 7.81–7.86 (m, 3H, $CONH_2$, H-4′ and H-6′), 9.57 (br s, 1H, NH); MS (FAB) m/z 592 (MH^+). Anal. calcd for $C_{28}H_{41}N_5O_7S$: C, 56.83; H, 6.98; N, 11.84. Found: C, 56.66; H, 6.91; N, 11.97.

4-(5-(4-(3-Ethoxycarbonylpropyl)piperidinylsulfonyl)-2-*n*-propoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8j). Yield 75%; mp 153–154.5 °C (MeOH/Et_2O); IR (neat) 3350, 3184, 3074 (NH), 1735, 1668, 1640 (C=O), 1167 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 0.99 (t, $J=7.2$ Hz, 3H, $OCH_2CH_2CH_3$), 1.12–1.21 (m, 5H, $CHCH_2CH_2$ and $2CH_{ax}$), 1.15 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.43–1.52 (m, 2H, $CHCH_2CH_2$), 1.52–1.65 (m, 2H, $CH_2CH_2CH_3$), 1.70 (br d, $J=10.2$ Hz, 2H, 2 CH_{eq}), 1.76–1.88 (m, 2H, $OCH_2CH_2CH_3$), 2.19 (br t, $J=11.1$ Hz, 2H, $2NCH_{ax}$), 2.23 (t, $J=7.2$ Hz, 2H, CH_2CO_2), 2.45 (dd, $J=7.8$, 7.2 Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, $J=11.4$ Hz, 2H, $2NCH_{eq}$), 3.92 (s, 3H, NCH_3), 4.02 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.20 (t, $J=6.6$ Hz, 2H, $OCH_2CH_2CH_3$), 7.33 (br s, 1H, $CONH_2$), 7.42 (d, $J=8.7$ Hz, 1H, H-3′), 7.80 (br s, 1H, $CONH_2$), 7.83 (dd, $J=8.7$, 2.7 Hz, 1H, H-4′), 7.87 (d, $J=2.7$ Hz, 1H, H-6′), 9.57 (br s, 1H, NH); MS (FAB) m/z 606 (MH^+). Anal. calcd for $C_{29}H_{43}N_5O_7S$: C, 57.50; H, 7.16; N, 11.56. Found: C, 57.63; H, 7.10; N, 11.45.

4-(5-(4-(Ethoxycarbonylmethyl)piperazinylsulfonyl)-2-*n*-propoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8k). Yield 61%; mp 130 °C dec (EtOAc/hexanes); IR (neat) 3345, 3186 (NH), 1739, 1671, 1642 (C=O), 1171 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 0.99 (t, $J=7.5$ Hz, 3H, $OCH_2CH_2CH_3$), 1.16 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.53–1.65 (m, 2H, $CH_2CH_2CH_3$), 1.76–1.88 (m, 2H, $OCH_2CH_2CH_3$), 2.46 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 2.59 (br s, 4H, 2 NCH_2), 2.89 (br s, 4H, 2 NCH_2), 3.23 (s, 2H, NCH_2CO_2), 3.92 (s, 3H, NCH_3), 4.05 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.22 (t, $J=6.3$ Hz, 2H, $OCH_2CH_2CH_3$), 7.32 (br s, 1H, $CONH_2$), 7.43 (d, $J=9.6$ Hz, 1H, H-3′), 7.78–7.84 (m, 3H, H-4′, H-6′ and $CONH_2$), 9.62 (br s, 1H, NH); MS (FAB) m/z 579 (MH^+). Anal. calcd for $C_{26}H_{38}N_6O_7S$: C, 53.96; H, 6.62; N, 14.52. Found: C, 54.11; H, 6.60; N, 14.39.

4-(5-(4-(2-Ethoxycarbonylethyl)piperazinylsulfonyl)-2-*n*-propoxybenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (8l). Yield 90%; mp 177–177.5 °C (EtOAc/hexanes); IR (neat) 3422, 3190 (NH), 1723, 1670, 1643 (C=O), 1170 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 1.10 (t, $J=7.2$ Hz, 3H, $OCH_2CH_2CH_3$), 1.22 (t, $J=7.2$ Hz, 3H, $CO_2CH_2CH_3$), 1.60–1.72 (m, 2H, $CH_2CH_2CH_3$), 1.91–2.03 (m, 2H, $OCH_2CH_2CH_3$), 2.42 (t, $J=7.2$ Hz, 2H, $CH_2CH_2CH_3$), 2.52–2.57 (m, 6H, 3 NCH_2), 2.69 (t, $J=7.2$ Hz, 2H, CH_2CO_2), 3.05 (br s, 4H, 2 NCH_2), 4.07 (s, 3H, NCH_3), 4.10 (q, $J=7.2$ Hz, 2H, $CO_2CH_2CH_3$), 4.28 (t, $J=6.6$ Hz, 2H, $OCH_2CH_2CH_3$), 5.60 (br s, 1H, $CONH_2$), 7.18 (d, $J=9.0$ Hz, 1H, H-3′), 7.64 (br s, 1H, $CONH_2$), 7.91 (dd, $J=9.0$, 2.4 Hz, 1H, H-4′), 8.63 (d, $J=7.2$ Hz, 1H, H-6′), 9.27 (br s, 1H, NH); MS (FAB) m/z 593 (MH^+). Anal. calcd for $C_{27}H_{40}N_6O_7S$: C, 54.71; H, 6.80; N, 14.18. Found: C, 54.55; H, 6.88; N, 14.31.

General procedures for the preparation of the pyrazolopyrimidinones 9a–l: cyclization reactions

A suspension of carboxamide **8a–l** (4.79 mmol) in 1 N NaOH aqueous solution (34 mL, 34.00 mmol) and EtOH (17 mL) was heated at 90 °C under nitrogen atmosphere for 17 h. The reaction mixture was cooled, evaporated in vacuo and acidified to about pH 2–3 with 2 N aqueous HCl solution. The resulting solution was extracted with 10% MeOH in $CHCl_3$ (3×20 mL), and the combined extracts were washed once with brine (20 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated to dryness in vacuo to afford an off-white solid. The crude product was purified by MPLC on silica gel using MeOH in $CHCl_3$ to afford the titled compound as a white solid, which was crystallized from a suitable solvent.

5-(2-Ethoxy-5-(4-(hydroxycarbonyl)piperidinylsulfonyl)-phenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9a). Yield 68%; mp 203–204.5 °C (MeOH/$CHCl_3$/hexanes); IR (neat) 3294, 3101 (NH, CO_2H), 1706, 1684 (C=O), 1164 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 1.33 (t, $J=6.9$ Hz, 3H, OCH_2CH_3), 1.53–1.63 (m, 2H, $2CH_{ax}$), 1.64–1.80 (m, 2H, $CH_2CH_2CH_3$), 1.81–1.89 (m, 2H, 2 CH_{eq}), 2.16–2.23 (m, 1H, $CHCO_2$), 2.47 (br t, $J=9.0$ Hz, 2H, $2NCH_{ax}$), 2.78 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 3.43 (br d, $J=11.7$ Hz, 2H, $2NCH_{eq}$), 4.16 (s, 3H, NCH_3), 4.22 (q, $J=6.9$ Hz, 2H, OCH_2CH_3), 7.36 (d, $J=8.7$ Hz, 1H, H-3′), 7.83 (dd, $J=8.7$, 2.4 Hz, 1H, H-4′), 7.87 (d, $J=2.1$ Hz, 1H, H-6′),

12.20 (br s, 1H, NH); MS (FAB) *m/z* 504 (MH^+). Anal. calcd for $C_{23}H_{29}N_5O_6S$: C, 54.86; H, 5.80; N, 13.91. Found: C, 54.98; H, 5.72; N, 13.77.

5-(2-Ethoxy-5-(4-(hydroxycarbonylmethyl)piperidinylsulfony)phenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9b). Yield 84%; mp 219–220 °C ($CHCl_3/Et_2O$); IR (neat) 3306, 3123 (NH, CO_2H), 1729, 1674 (C=O), 1163 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.14–1.27 (m, 2H, $2CH_{ax}$), 1.33 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 1.51–1.65 (m, 1H, CH), 1.68–1.80 (m, 4H, 2 CH_{eq} and $CH_2CH_2CH_3$), 2.12 (d, *J* = 6.9 Hz, 2H, CH_2CO_2), 2.26 (br t, *J* = 12.0 Hz, 2H, $2NCH_{ax}$), 2.78 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, *J* = 12.0 Hz, 2H, $2NCH_{eq}$), 4.16 (s, 3H, NCH_3), 4.21 (q, *J* = 6.9 Hz, 2H, OCH_2CH_3), 7.36 (d, *J* = 8.7 Hz, 1H, H-3′), 7.82 (dd, *J* = 8.7, 2.4 Hz, 1H, H-4′), 7.86 (d, *J* = 2.4 Hz, 1H, H-6′), 12.18 (br s, 1H, NH); MS (FAB) *m/z* 518 (MH^+). Anal. calcd for $C_{24}H_{31}N_5O_6S$: C, 55.69; H, 6.04; N, 13.53. Found: C, 55.83; H, 5.96; N, 13.36.

5-(2-Ethoxy-5-(4-(2-hydroxycarbonylethyl)piperidinylsulfonyl)phenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9c). Yield 93%; mp 208–209 °C ($CHCl_3/Et_2O$); IR (neat) 3294, 3103 (NH, CO_2H), 1706, 1689 (C=O), 1164 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.05–1.25 (m, 3H, CH and $2CH_{ax}$), 1.33 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 1.36–1.47 (m, 2H, $CHCH_2CH_2$), 1.62–1.81 (m, 4H, 2 CH_{eq} and $CH_2CH_2CH_3$), 2.12–2.29 (m, 4H, $2NCH_{ax}$ and CH_2CO_2), 2.78 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, *J* = 11.1 Hz, 2H, $2NCH_{eq}$), 4.16 (s, 3H, NCH_3), 4.20 (q, *J* = 6.9 Hz, 2H, OCH_2CH_3), 7.36 (d, *J* = 8.7 Hz, 1H, H-3′), 7.82 (dd, *J* = 8.7 Hz, 2.4 Hz, 1H, H-4′), 7.85 (d, *J* = 2.4 Hz, 1H, H-6′), 11.97 (br s, 1H, CO_2H), 12.19 (br s, 1H, NH); MS (FAB) *m/z* 532 (MH^+). Anal. calcd for $C_{25}H_{33}N_5O_6S$: C, 56.48; H, 6.26; N, 13.17. Found: C, 56.63; H, 6.24; N, 13.04.

5-(2-Ethoxy-5-(4-(3-hydroxycarbonylpropyl)piperidinylsulfonyl)phenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9d). Yield 91%; mp 215.5–216.5 °C ($MeOH/CHCl_3/Et_2O$); IR (neat) 3293 (NH, CO_2H), 1705 (C=O), 1164 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2CH_3$), 1.06–1.20 (m, 5H, $CHCH_2CH_2$ and $2CH_{ax}$), 1.33 (t, *J* = 7.2 Hz, 3H, OCH_2CH_3), 1.40–1.50 (m, 2H, $CHCH_2CH_2$), 1.68–1.80 (m, 4H, 2 CH_{eq} and $CH_2CH_2CH_3$), 2.14 (t, *J* = 7.2 Hz, 2H, CH_2CO_2), 2.22 (br t, *J* = 11.1 Hz, 2H, $2NCH_{ax}$), 2.78 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.62 (br d, *J* = 11.1 Hz, 2H, $2NCH_{eq}$), 4.16 (s, 3H, NCH_3), 4.21 (q, *J* = 7.2 Hz, 2H, OCH_2CH_3), 7.36 (d, *J* = 8.7 Hz, 1H, H-3′), 7.82 (dd, *J* = 8.7, 2.4 Hz, 1H, H-4′), 7.86 (d, *J* = 2.4 Hz, 1H, H-6′), 12.12 (br s, 1H, NH); MS (FAB) *m/z* 546 (MH^+). Anal. calcd for $C_{26}H_{35}N_5O_6S$: C, 57.23; H, 6.47; N, 12.83. Found: C, 57.11; H, 6.50; N, 12.97.

5-(2-Ethoxy-5-(4-(hydroxycarbonylmethyl)piperazinylsulfonyl)phenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9e). Yield 83%; mp 212 °C dec ($CHCl_3/Et_2O$); IR (neat) 3311 (NH, CO_2H), 1735, 1701 (C=O), 1169 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.42 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 1.69–1.81 (m, 2H, $CH_2CH_2CH_3$), 2.78 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.20–3.60 (m, 10H, $5NCH_2$), 4.17 (s, 3H, NCH_3), 4.23 (q, *J* = 6.9 Hz, 2H, OCH_2CH_3), 7.43 (d, *J* = 9.0 Hz, 1H, H-3′), 7.86–7.92 (m, 2H, H-4′ and H-6′), 12.30 (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, 53.12; H, 5.90; N, 16.38.

5-(2-Ethoxy-5-(4-(2-hydroxycarbonylethyl)piperazinylsulfonyl)phenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9f). Yield: 74%; mp 236 °C dec ($CHCl_3$/MeOH/hexanes); IR (neat) 3318, 3068 (NH, CO_2H), 1730, 1693 (C=O), 1161 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.95 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2CH_3$), 1.34 (t, *J* = 6.9 Hz, 3H, OCH_2CH_3), 1.69–1.81 (m, 2H, $CH_2CH_2CH_3$), 2.75–2.81 (m, 4H, $CH_2CH_2CH_3$ and CH_2CO_2), 3.09–3.90 (m, 10H, $5NCH_2$), 4.17 (s, 3H, NCH_3), 4.22 (q, *J* = 6.9 Hz, 2H, OCH_2CH_3), 7.42 (d, *J* = 8.7 Hz, 1H, H-3′), 7.88–7.93 (m, 2H, H-4′ and H-6′), 12.28 (br s, 1H, NH); MS (FAB) *m/z* 533 (MH^+). Anal. calcd for $C_{24}H_{32}N_6O_6S$: C, 54.12; H, 6.06; N, 15.78. Found: C, 54.31; H, 6.00; N, 15.55.

5-(5-(4-(Hydroxycarbonyl)piperidinylsulfonyl)-2-*n*-propoxypheny)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9g). Yield 91%; mp 222–223 °C (EtOAc/hexanes); IR (neat) 3312 (CO_2H), 1707 (C=O), 1162 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2CH_3$), 0.95 (t, *J* = 7.5 Hz, 3H, $OCH_2CH_2CH_3$), 1.53–1.67 (m, 2H, $2CH_{ax}$), 1.69–1.80 (m, 4H, 2 $CH_2CH_2CH_3$), 1.85–1.95 (m, 2H, 2 CH_{eq}), 2.19–2.29 (m, 1H, $CHCO_2$), 2.24–2.45 (m, 2H, $2NCH_{ax}$), 2.78 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.46 (br d, *J* = 11.7 Hz, 2H, $2NCH_{eq}$), 4.11 (t, *J* = 6.3 Hz, 2H, $OCH_2CH_2CH_3$), 4.16 (s, 3H, NCH_3), 7.37 (d, *J* = 9.0 Hz, 1H, H-3′), 7.83 (dd, *J* = 9.0, 2.1 Hz, 1H, H-4′), 7.87 (d, *J* = 2.1 Hz, 1H, H-6′), 12.18 (br s, 1H, NH); MS (FAB) *m/z* 518 (MH^+). Anal. calcd for $C_{24}H_{31}N_5O_6S$: C, 55.69; H, 6.04; N, 13.53. Found: C, 55.88; H, 5.98; N, 13.33.

5-(5-(4-(Hydroxycarbonylmethyl)piperidinylsulfonyl)-2-*n*-propoxypheny)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9h). Yield 66%; mp 179–180 °C (EtOAc/hexanes); IR (neat) 3286, 3079 (NH, CO_2H), 1729, 1705 (C=O), 1167 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.93 (t, *J* = 7.2 Hz, 3H, $CH_2CH_2CH_3$), 0.94 (t, *J* = 7.2 Hz, 3H, $OCH_2CH_2CH_3$), 1.15–1.27 (m, 2H, $2CH_{ax}$), 1.53–1.65 (m, 1H, CH), 1.66–1.80 (m, 6H, 2 CH_{eq} and 2 $CH_2CH_2CH_3$), 2.11 (d, *J* = 6.9 Hz, 2H, CH_2CO_2), 2.27–2.31 (m, 2H, $2NCH_{ax}$), 2.77 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, *J* = 11.4 Hz, 2H, $2NCH_{eq}$), 4.11 (t, *J* = 6.3 Hz, 2H, $OCH_2CH_2CH_3$), 4.16 (s, 3H, NCH_3), 7.37 (d, *J* = 8.7 Hz, 1H, H-3′), 7.82 (dd, *J* = 8.7, 2.4 Hz, 1H, H-4′), 7.86 (d, *J* = 2.4 Hz, 1H, H-6′), 12.18 (br s, 1H, NH); MS (FAB) *m/z* 532 (MH^+). Anal. calcd for $C_{25}H_{33}N_5O_6S$: C, 56.48; H, 6.26; N, 13.17. Found: C, 56.62; H, 6.00; N, 13.06.

5-(5-(4-(2-Hydroxycarbonylethyl)piperidinylsulfonyl)-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9i). Yield 87%; mp 181–182 °C (EtOAc/hexanes); IR (neat) 3314, 3052 (NH, CO_2H), 1702 (C=O), 1163 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 0.95 (t, $J=7.5$ Hz, 3H, $OCH_2CH_2CH_3$), 1.10–1.25 (m, 3H, CH and $2CH_{ax}$), 1.36–1.45 (m, 2H, $CHCH_2CH_2$), 1.68–1.78 (m, 6H, 2 CH_{eq} and 2 $CH_2CH_2CH_3$), 2.12–2.25 (m, 4H, $2NCH_{ax}$ and CH_2CO_2), 2.77 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 3.61 (br d, $J=11.1$ Hz, 2H, $2NCH_{eq}$), 4.11 (t, $J=6.3$ Hz, 2H, $OCH_2CH_2CH_3$), 4.16 (s, 3H, NCH_3), 7.36 (d, $J=8.7$ Hz, 1H, H-3′), 7.82 (dd, $J=8.7$, 2.4 Hz, 1H, H-4′), 7.86 (d, $J=2.4$ Hz, 1H, H-6′), 12.10 (br s, 1H, NH); MS (FAB) m/z 546 (MH^+). Anal. calcd for $C_{26}H_{35}N_5O_6S$: C, 57.23; H, 6.47; N, 12.83. Found: C, 57.03; H, 6.55; N, 13.01.

5-(5-(4-(3-Hydroxycarbonylpropyl)piperidinylsulfonyl)-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9j). Yield 71%; mp 183.5–184.5 °C (MeOH/$CHCl_3$/Et_2O); IR (neat) 3290 (NH, CO_2H), 1732, 1706 (C=O), 1165 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 0.95 (t, $J=7.2$ Hz, 3H, $OCH_2CH_2CH_3$), 1.07–1.20 (m, 5H, $CHCH_2CH_2$ and $2CH_{ax}$), 1.41–1.52 (m, 2H, $CHCH_2CH_2$), 1.68–1.78 (m, 6H, 2 CH_{eq} and 2 $CH_2CH_2CH_3$), 2.15 (t, $J=7.5$ Hz, 2H, CH_2CO_2), 2.22 (br t, $J=10.5$ Hz, 2H, $2NCH_{ax}$), 2.77 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 3.62 (br d, $J=11.1$ Hz, 2H, $2NCH_{eq}$), 4.11 (t, $J=6.3$ Hz, 2H, $OCH_2CH_2CH_3$), 4.16 (s, 3H, NCH_3), 7.37 (d, $J=9.0$ Hz, 1H, H-3′), 7.82 (dd, $J=9.0$, 2.4 Hz, 1H, H-4′), 7.86 (d, $J=2.4$ Hz, 1H, H-6′), 11.92 (br s, 1H, CO_2H), 12.16 (br s, 1H, NH); MS (FAB) m/z 560 (MH^+). Anal. calcd for $C_{27}H_{37}N_5O_6S$: C, 57.94; H, 6.66; N, 12.51. Found: C, 58.11; H, 6.58; N, 12.34.

5-(5-(4-(Hydroxycarbonylmethyl)piperazinylsulfonyl)-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9k). Yield 87%; mp 189 °C dec ($CHCl_3$/Et_2O); IR (neat) 3317 (NH, CO_2H), 1733, 1701 (C=O), 1169 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 0.95 (t, $J=7.5$ Hz, 3H, $OCH_2CH_2CH_3$), 1.65–1.82 (m, 4H, 2 $CH_2CH_2CH_3$), 2.78 (t, $J=7.5$ Hz, 2H, $CH_2CH_2CH_3$), 3.18–3.75 (m, 10H, $5NCH_2$), 4.13 (t, $J=6.6$ Hz, 2H, $OCH_2CH_2CH_3$), 4.17 (s, 3H, NCH_3), 7.44 (d, $J=8.7$ Hz, 1H, H-3′), 7.88 (dd, $J=8.7$ Hz, 2.4 Hz, 1H, H-4′), 7.91 (d, $J=2.4$ Hz, 1H, H-6′), 12.26 (br s, 1H, NH); MS (FAB) m/z 533 (MH^+). Anal. calcd for $C_{24}H_{32}N_6O_6S$: C, 54.12; H, 6.06; N, 15.78. Found: C, 54.30; H, 5.85; N, 15.61.

5-(5-(4-(2-Hydroxycarbonylethyl)piperazinylsulfonyl)-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (9l). Yield 79%; mp 220 °C dec ($CHCl_3$/MeOH/hexanes); IR (neat) 3315, 3061 (NH, CO_2H), 1728, 1693 (C=O), 1161 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.94 (t, $J=7.5$ Hz, 3H, $CH_2CH_2CH_3$), 0.95 (t, $J=7.2$ Hz, 3H, $OCH_2CH_2CH_3$), 1.66–1.80 (m, 4H, 2 $CH_2CH_2CH_3$), 2.70–2.82 (m, 4H, $CH_2CH_2CH_3$ and CH_2CO_2), 3.10–3.90 (m, 10H, $5NCH_2$), 4.13 (t, $J=6.3$ Hz, 2H, $OCH_2CH_2CH_3$), 4.17 (s, 3H, NCH_3), 7.42 (d, $J=8.7$ Hz, 1H, H-3′), 7.87–7.93 (m, 2H, H-4′ and H-6′), 12.26 (br s, 1H, NH); MS (FAB) m/z 547 (MH^+). Anal. calcd for $C_{25}H_{34}N_6O_6S$: C, 54.93; H, 6.27; N, 15.37. Found: C, 55.02; H, 6.42; N, 15.21.

Determination of PDE1, PDE3, PDE5 and PDE6 inhibitory activity

Bovine heart PDE1 was purchased from Sigma (St. Louis, MO, USA). PDE3 and PDE5 were prepared from the rabbit platelet using the method described by Hidaka et al.[10] 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.) 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, PDE3 and PDE5 were eluted using a continuous gradient of 0 to 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.[11] 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 two 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 to 600 mM NaCl in equilibration buffer with a total volume of 60 mL.

Fractions (1.0 mL each) collected at a flow rate of 60 mL/h were characterized for cGMP (PDE5 and PDE6) or cAMP (PDE3) 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 [^3H]-cGMP (5 μCi/mL) or [^3H]-cAMP (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 or cAMP 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.[12]

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