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Synthesis and Phosphodiesterase 5 Inhibitory Activity of New 5-Phenyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one Derivatives Containing an *N*-Acylamido Group on a Phenyl Ring

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Abstract—New sildenafil analogues with an *N*-acylamido group at the 5′-position of the phenyl ring, **6a**–**e**, were prepared from the readily available starting compound **2** in four straightforward steps. Enzyme assays demonstrated that all the target compounds **6a**–**e** showed higher PDE5 inhibitory activities than sildenafil. It was observed that the PDE5 inhibitory activity was enhanced as the chain length of R group increased, but introduction of the branched alkyl groups such as isopropyl (**6d**) and cyclohexyl (**6e**) resulted in the drop of potency compared with **6c**. In particular the *N*-butyrylamido derivative **6c** exhibited the highest PDE5 inhibitory activity, and was about 6-fold more potent than sildenafil. However, all the compounds exhibited somewhat weak selectivity (1–3-fold) over PDE6, indicating that the compounds **6a**–**e** have intrinsically lower selectivity than sildenafil.

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] It has been reported that over half of men at 40–70 years of age suffered from erectile dysfunction according to a random community-based sample study.[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 inhibition is a particularly attractive target because 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 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.

We have been interested in new sildenafil analogues that could alleviate the drawbacks of sildenafil, especially those with a modified phenyl ring, and have just submitted for publication one of our recent results on the sildenafil derivatives containing an ether ring fused into the phenyl group.[7] Earlier, Dumaître and Dodic have published a rather interesting report on new PDE5 inhibitors, 6-phenylpyrazolo[3,4-*d*]pyrimidinones, which feature the isomeric structures of sildenafil.[8] From the

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structure–activity relationship study of 6-phenylpyrazolo[3,4-*d*]pyrimidinone series, it was claimed that a *n*-propoxy group at the 2′-position of the phenyl ring is necessary for inhibitory activity and the 5′-position of the phenyl ring can accommodate a great range of unrelated groups without loss of potency. Among the compounds reported above, the acetamido derivative **1** (Chart 1) caught our attention since it showed very high in vitro PDE5 inhibitory activity as well as the highest in vivo antihypertensive activity, in spite of its somewhat low metabolic stability. Based on these findings, it was our peculiar interest to investigate if the PDE inhibitory activity and selectivity, when compared with sildenafil, could be manipulated by introducing the *N*-acylamido substituent at the 5′-position of the phenyl ring. In this report, preparation of new sildenafil analogues containing an *N*-acylamido moiety on the phenyl ring, **6a**–**e**, and evaluation of their in vitro PDE inhibitory activity are disclosed.

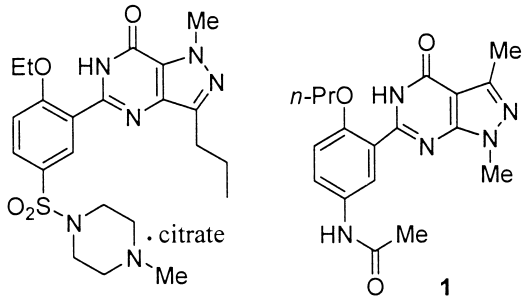
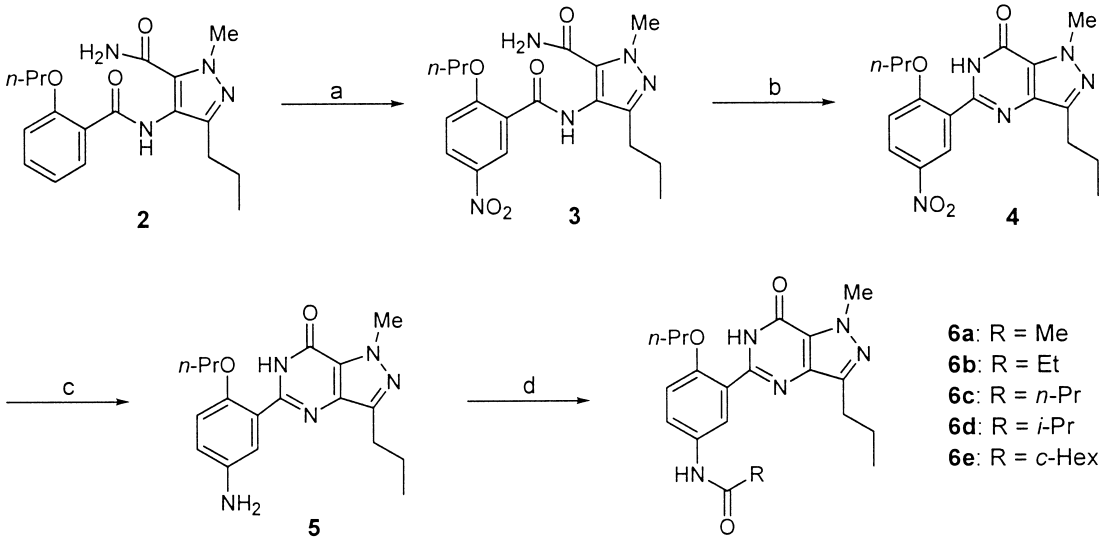


Chart 1.

Chemistry

Since it has been reported by Dumaître and Dodic that a *n*-propoxy group at the 2′-position of the phenyl ring is essential for PDE5 inhibitory activity,[8] we decided to focus only on the *n*-propoxy derivatives. Target compounds **6a**–**e** were synthesized in four steps from 1-methyl-4-(2-*n*-propoxybenzamido)-3-*n*-propylpyrazole-5-carboxamide (**2**) which was readily prepared by using known procedures in the literature.[9] As shown in Scheme 1, the synthesis began with nitration of compound **2**, and the reaction proceeded smoothly to produce the desired mono-nitro product **3** in 84% yield when trifluoroacetic acid and concentrated nitric acid were used. On the other hand, it was observed that dinitration of the phenyl ring took place predominantly under the most common reaction conditions using concentrated sulfuric acid and nitric acid even at 0 °C. Cyclization of the nitro compound **3** was carried out under typical basic conditions using NaOH (2.0 equiv) in a mixture of H_2O–EtOH (2:1, v/v) at 60 °C to afford the corresponding pyrazolopyrimidinone **4** in a rather low yield of 41%. Under this basic condition, hydrolysis of the secondary amide bond in compound **3** seemed to be competing with cyclization because a strongly electron-withdrawing nitro group on the phenyl ring would activate the amide moiety toward nucleophilic additions. Thus, this facile hydrolysis of compound **3** might account for the low yield of cyclization reaction. Subsequent reduction of a nitro group of compound **4** occurred readily at room temperature under hydrogenation conditions (10% Pd/C, 1 atm of H_2) to afford the amino derivative **5** in an excellent yield of 97%. Final acylations of the amino compound **5** were performed with an appropriate acylating agent such as an anhydride (acetic anhydride, propionic anhydride,



Scheme 1. (a) Concd HNO_3, CF_3CO_2H, 0 °C to rt, 3 h; (b) 1 N NaOH in H_2O, EtOH, 60 °C, 18 h; (c) H_2 (1 atm), 10% Pd/C, THF–EtOH (1:1), rt, 3 h; (e) $(RCO)_2O$ (for **6a**–**d**), or RCOCl (for **6e**), Et_3N, CH_2Cl_2, rt, 1 h.

butyric anhydride and isobutyric anhydride) or an acyl chloride (cyclohexanecarbonyl chloride). All the acylation reactions using excess amounts of an appropriate acylating agent (1.9 equiv) and Et_3N (2.5 equiv) in anhydrous CH_2Cl_2 at room temperature were almost quantitative, producing the target compounds **6a**–**e** in 98–99% yields.

Results and Discussion

The in vitro inhibitory activity was assessed 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 compounds **6a**–**e** were determined from concentration-response curves using concentrations ranging from 0.03 to 100 nM. It was demonstrated by Terrett et al. that PDE5 from human corpus cavernosal tissue was essentially identical to the rabbit platelet enzyme and sildenafil showed similar affinities for both enzymes ($IC_{50} = 3.0$ nM for corpus cavernosum and 3.6 nM for rabbit).[3c] As summarized in Table 1, all the target compounds **6a**–**e** showed higher PDE5 inhibitory activities than sildenafil (IC_{50}: 1.76 nM), and their IC_{50} values ranged from 0.27 to 0.81 nM. It was observed that the PDE5 inhibitory activity was enhanced as the chain length of R group increased. In other words, there was about a 3-fold increase in the activity when the methyl group (**6a**) was displaced with *n*-propyl chain (**6c**). Especially, the *N*-butyrylamido derivative **6c** exhibited the highest PDE5 inhibitory activity, and was about 6-fold more potent than sildenafil. In contrast, introduction of the branched alkyl groups such as isopropyl (**6d**) and cyclohexyl (**6e**) resulted in a slight decrease in the potency for PDE5 compared with **6c**. The inhibitory activities of these compounds toward PDE6 have been also determined and compared with sildenafil. Unfortunately, all the compounds exhibited somewhat weak selectivity (1–3-fold) over PDE6, indicating that compounds **6a**–**e** have intrinsically lower selectivity than sildenafil. Based on these data, it was concluded that the new sildenafil analogues with an *N*-acylamido moiety at the 5′-position of the phenyl ring, **6a**–**e**, although they showed higher PDE5 inhibitory activity compared with sildenafil, would not be pursued any further due to their disappointingly low selectivity over PDE6.

Table 1. In vitro PDE[a] inhibitory activities of compounds **6a**–**e**

Compound	IC_{50} (nM)		IC_{50} ratio
	PDE5	PDE6	PDE6/5
6a	0.81 ± 0.05	2.09 ± 0.35	3
6b	0.38 ± 0.06	0.69 ± 0.21	2
6c	0.27 ± 0.03	0.43 ± 0.13	2
6d	0.35 ± 0.07	0.49 ± 0.07	1
6e	0.42 ± 0.03	0.79 ± 0.13	2
Sildenafil	1.76 ± 0.12	24.6 ± 6.29	14

[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 ± SEM from three experiments.

Experimental

Melting points were determined on a Thomas-Hoover or Mettler melting point apparatus and are uncorrected. Infra-red 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 $CDCl_3$. 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.

1-Methyl-4-(5-nitro-2-*n*-propoxybenzamido)-3-*n*-propylpyrazole-5-carboxamide (3). To a stirred solution of 1-methyl-4-(2-*n*-propoxybenzamido)-3-*n*-propylpyrazole-5-carboxamide **2** (100 mg, 0.31 mmol) in trifluoroacetic acid (1.0 mL) at 0 °C was added slowly concentrated HNO_3 (0.36 mL), and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured carefully into ice (10 g) and was extracted with $CHCl_3$ (3×20 mL). Combined organic layer was dried ($MgSO_4$) and concentrated in vacuo to afford a yellow oil. Resulting yellow residue dissolved in $CHCl_3$ (1 mL) was added to a solution of hexanes (40 mL) to obtain the titled compound (96 mg, 84%) as a white solid. Analytically pure compound was obtained by crystallization from EtOAc/hexanes: mp 176–176.5 °C; IR (neat) 3469, 3281 (NH), 1679, 1658 (C=O), 1345 (NO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$/TMS) δ 0.94 (t, $J = 7.5$ Hz, 3 H, $CH_2CH_2CH_3$), 1.11 (t, $J = 7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.57–1.71 (m, 2 H, $CH_2CH_2CH_3$), 1.93–2.05 (m, 2 H, $OCH_2CH_2CH_3$), 2.53 (dd, $J = 7.8$ Hz, 7.5 Hz, 2 H, $CH_2CH_2CH_3$), 4.06 (s, 3 H, NCH_3), 4.33 (t, $J = 6.6$ Hz, 2 H, $OCH_2CH_2CH_3$), 5.67 (br s, 1 H, $CONH_2$), 7.19 (d, $J = 9.0$ Hz, 1 H, H-3′), 7.60 (br s, 1 H, $CONH_2$), 8.41 (dd, $J = 9.0$ Hz, 3.0 Hz, 1 H, H-4′), 9.15 (d, $J = 3.0$ Hz, 1 H, H-6′), 9.20 (s, 1 H, NH); MS (FAB) *m*/*z* 390 (MH^+). Anal. calcd for $C_{18}H_{23}N_5O_5$: C, 55.52; H, 5.95; N, 17.98. Found: C, 55.69; H, 6.02; N, 17.83.

1-Methyl-5-(5-nitro-2-*n*-propoxyphenyl)-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (4). A suspension of 1-methyl-4-(5-nitro-2-*n*-propoxybenzamido)-3-*n*-propylpyrazole-5-carboxamide **3** (76 mg, 0.20 mmol) in 1 N NaOH aqueous solution (0.41 mL, 0.41 mmol) and EtOH (0.5 mL) was heated at 60 °C under nitrogen atmosphere for 18 h. The reaction mixture was cooled and acidified to about pH 2–3 with 1.0 N aqueous HCl solution. Resulting mixture was extracted with $CHCl_3$ (3×5 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 a yellow solid. The crude product was purified by MPLC on silica gel (gradient elution: 1/3 EtOAc in $CHCl_3$ followed by 1/2 EtOAc in $CHCl_3$) to afford the titled compound (30 mg, 41%) as a white solid. Analytically pure compound was obtained by

crystallization from EtOAc/hexanes: mp 199–199.5 °C; IR (neat) 3319 (NH), 1699 (C=O), 1343 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$/TMS) δ 1.05 (t, $J=7.5$ Hz, 3 H, $CH_2CH_2CH_3$), 1.20 (t, $J=7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.82–1.94 (m, 2 H, $CH_2CH_2CH_3$), 2.01–2.13 (m, 2 H, $OCH_2CH_2CH_3$), 2.96 (dd, $J=7.8$ Hz, 7.2 Hz, 2 H, $CH_2CH_2CH_3$), 4.28 (s, 3 H, NCH_3), 4.31 (t, $J=7.5$ Hz, 2 H, $OCH_2CH_2CH_3$), 7.16 (d, $J=9.3$ Hz, 1 H, H-3′), 8.33 (dd, $J=9.3$ Hz, 3.0 Hz, 1 H, H-4′), 9.34 (d, $J=3.0$ Hz, 1 H, H-6′), 10.80 (br s, 1 H, NH); MS (FAB) m/z 372 (MH^+). Anal. calcd for $C_{18}H_{21}N_5O_4$: C, 58.21; H, 5.70; N, 18.86. Found: C, 58.40; H, 5.79; N, 18.69.

5-(5-Amino-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (5). A mixture of 1-methyl-5-(5-nitro-2-*n*-propoxyphenyl)-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one **4** (2.29 g, 6.17 mmol) and 10% Pd/C (0.20 g) in THF (70 mL) and EtOH (70 mL) was purged with hydrogen gas three times, and stirred vigorously under hydrogen atmosphere (a balloon) at room temperature for 3 h. The mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness under reduced pressure. Resulting yellow residue was purified by MPLC on silica gel (gradient elution: 1/4 EtOAc in hexanes, 1/2 EtOAc in hexanes, followed by 1/1 EtOAc in $CHCl_3$) to afford the titled compound (2.08 g, 99%) as a pale yellow solid. Analytically pure compound was obtained by crystallization from EtOAc/hexanes: mp 110–110.5 °C; IR (neat) 3422, 3349, 3279 (NH), 1694 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$/TMS) δ 1.04 (t, $J=7.5$ Hz, 3 H, $CH_2CH_2CH_3$), 1.14 (t, $J=7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.81–1.92 (m, 2 H, $CH_2CH_2CH_3$), 1.90–2.01 (m, 2 H, $OCH_2CH_2CH_3$), 2.93 (dd, $J=7.8$ Hz, 7.5 Hz, 2 H, $CH_2CH_2CH_3$), 4.08 (t, $J=6.6$ Hz, 2 H, $OCH_2CH_2CH_3$), 4.27 (s, 3 H, NCH_3), 6.79 (dd, $J=8.7$ Hz, 3.0 Hz, 1 H, H-4′), 6.89 (d, $J=8.7$ Hz, 1 H, H-3′), 7.83 (d, $J=3.0$ Hz, 1 H, H-6′), 11.30 (br s, 1 H, NH); MS (FAB) m/z 342 (MH^+). Anal. calcd for $C_{18}H_{23}N_5O_2$: C, 63.32; H, 6.79; N, 20.51. Found: C, 63.15; H, 6.88; N, 20.66.

General procedures for the preparation of the pyrazolopyrimidinones 6a–e. To a stirred solution of 5-(5-amino-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one **5** (0.92 mmol) and triethylamine (0.33 mL, 2.34 mmol) in CH_2Cl_2 (4 mL) was added an appropriate anhydride (1.76 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness under reduced pressure, and the resulting yellow residue was purified by MPLC on silica gel (gradient elution: 2% MeOH in $CHCl_3$ followed by 5% MeOH in $CHCl_3$) to afford the titled compound as a white solid, which was crystallized from EtOAc/hexanes.

5-(5-Acetylamino-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6a). Yield 99%; mp 233–233.5 °C; IR (neat) 3310, 3285 (NH), 1703, 1661 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$/TMS) δ 1.03 (t, $J=7.5$ Hz, 3 H, $CH_2CH_2CH_3$), 1.16 (t, $J=7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.80–1.92 (m, 2 H, $CH_2CH_2CH_3$), 1.93–2.05 (m, 2 H, $OCH_2CH_2CH_3$), 2.21 (s, 3 H, CH_3CO), 2.92 (dd, $J=8.1$ Hz, 7.5 Hz, 2 H, $CH_2CH_2CH_3$), 4.16 (t, $J=6.5$ Hz, 2 H, $OCH_2CH_2CH_3$), 4.27 (s, 3 H, NCH_3), 7.02 (d, $J=9.0$ Hz, 1 H, H-3′), 7.35 (br s, 1 H, CONH), 8.01 (dd, $J=9.0$ Hz, 3.0 Hz, 1 H, H-4′), 8.20 (d, $J=3.0$ Hz, 1 H, H-6′), 11.20 (br s, 1 H, 6-NH); MS (FAB) m/z 384 (MH^+). Anal. calcd for $C_{20}H_{25}N_5O_3$: C, 62.65; H, 6.57; N, 18.26. Found: C, 62.77; H, 6.71; N, 18.11.

1-Methyl-5-(5-propionylamino-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6b). Yield 99%; mp 212–213 °C: IR (neat) 3314, 3288 (NH), 1705, 1659 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$/TMS) δ 1.03 (t, $J=7.5$ Hz, 3 H, $CH_2CH_2CH_3$), 1.16 (t, $J=7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.28 (t, $J=7.5$ Hz, 3 H, CH_3CH_2CO), 1.80–1.92 (m, 2 H, $CH_2CH_2CH_3$), 1.93–2.05 (m, 2 H, $OCH_2CH_2CH_3$), 2.43 (q, $J=7.5$ Hz, 2 H, CH_3CH_2CO), 2.92 (dd, $J=7.8$ Hz, 7.5 Hz, 2 H, $CH_2CH_2CH_3$), 4.16 (t, $J=6.6$ Hz, 2 H, $OCH_2CH_2CH_3$), 4.27 (s, 3 H, NCH_3), 7.02 (d, $J=9.0$ Hz, 1 H, H-3′), 7.34 (br s, 1 H, CONH), 8.07 (dd, $J=9.0$ Hz, 2.7 Hz, 1 H, H-4′), 8.18 (d, $J=2.7$ Hz, 1 H, H-6′), 11.20 (br s, 1 H, 6-NH); MS (FAB) m/z 398 (MH^+). Anal. calcd for $C_{21}H_{27}N_5O_3$: C, 63.46; H, 6.85; N, 17.62. Found: C, 63.63; H, 6.70; N, 17.49.

5-(5-Butyrylamino-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6c). Yield: 98%; mp 207–207.5 °C; IR (neat) 3317, 3291 (NH), 1704, 1656 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$/TMS) δ 1.03 (t, $J=7.5$ Hz, 6 H, $CH_2CH_2CH_3$ and $CH_3CH_2CH_2CO$), 1.16 (t, $J=7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.73–1.93 (m, 4 H, $CH_2CH_2CH_3$ and $CH_3CH_2CH_2CO$),1.93–2.05 (m, 2 H, $OCH_2CH_2CH_3$), 2.37 (t, $J=7.5$ Hz, 2 H, $CH_3CH_2CH_2CO$), 2.92 (t, $J=7.5$ Hz, 2 H, $CH_2CH_2CH_3$), 4.16 (t, $J=6.6$ Hz, 2 H, $OCH_2CH_2CH_3$), 4.27 (s, 3 H, NCH_3), 7.02 (d, $J=9.0$ Hz, 1 H, H-3′), 7.29 (br s, 1 H, CONH), 8.07 (dd, $J=9.0$ Hz, 3.0 Hz, 1 H, H-4′), 8.18 (d, $J=3.0$ Hz, 1 H, H-6′), 11.20 (br s, 1 H, 6-NH); MS (FAB) m/z 412 (MH^+). Anal. calcd for $C_{22}H_{29}N_5O_3$: C, 64.21; H, 7.10; N, 17.02. Found: C, 63.99; H, 7.22; N, 17.17.

5-(5-Isobutyrylamino-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6d). Yield 98%; mp 223–223.5 °C; IR (neat) 3314 (NH), 1703, 1661 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$/TMS) δ 1.04 (t, $J=7.5$ Hz, 3 H, $CH_2CH_2CH_3$), 1.16 (t, $J=7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.29 (d, $J=6.9$ Hz, 6 H, $CH(CH_3)_2$), 1.81–1.91 (m, 2 H, $CH_2CH_2CH_3$), 1.91–2.05 (m, 2 H, $OCH_2CH_2CH_3$), 2.55 (septet, $J=6.9$ Hz, 1 H, $CH(CH_3)_2$), 2.93 (dd, $J=7.8$ Hz, 7.5 Hz, 2 H, $CH_2CH_2CH_3$), 4.17 (t, $J=6.6$ Hz, 2 H, $OCH_2CH_2CH_3$), 4.27 (s, 3 H, NCH_3), 7.03 (d, $J=9.0$ Hz, 1 H, H-3′), 7.28 (br s, 1 H, CONH), 8.11 (dd, $J=9.0$ Hz, 2.7 Hz, 1 H, H-4′), 8.16 (d, $J=2.7$ Hz, 1 H, H-6′), 11.20 (br s, 1 H, 6-NH); MS (FAB) m/z 412 (MH^+). Anal. calcd for $C_{22}H_{29}N_5O_3$: C, 64.21; H, 7.10; N, 17.02. Found: C, 64.40; H, 7.19; N, 16.88.

5-(5-Cyclohexanecarbonylamino-2-*n*-propoxyphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6e). Yield 99%; mp 213–214 °C; IR (neat) 3314, 3290 (NH), 1703, 1657 (C=O) cm^{-1}; ^1H NMR

($CDCl_3$/TMS) δ 1.03 (t, $J=7.5$ Hz, 3 H, $CH_2CH_2CH_3$), 1.16 (t, $J=7.5$ Hz, 3 H, $OCH_2CH_2CH_3$), 1.24–1.40 (m, 3 H, c-Hex), 1.50–1.59 (m, 2 H, c-Hex), 1.70–1.76 (m, 1 H, c-Hex), 1.81–1.93 (m, 4 H, $CH_2CH_2CH_3$ and c-Hex), 1.93–2.05 (m, 4 H, $OCH_2CH_2CH_3$ and c-Hex), 2.44 (tt, $J=15.0$ Hz, 3.3 Hz, 1 H, CHCO), 2.93 (dd, $J=8.1$ Hz, 7.2 Hz, 2 H, $CH_2CH_2CH_3$), 4.16 (t, $J=6.5$ Hz, 2 H, $OCH_2CH_2CH_3$), 4.27 (s, 3 H, NCH_3), 7.02 (d, $J=9.0$ Hz, 1 H, H-3′), 7.27 (br s, 1 H, CONH), 8.11 (dd, $J=9.0$ Hz, 3.0 Hz, 1 H, H-4′), 8.16 (d, $J=3.0$ Hz, 1 H, H-6′), 11.20 (br s, 1 H, 6-NH); MS (FAB) m/z 452 (MH^+). Anal. calcd for $C_{25}H_{33}N_5O_3$: C, 66.50; H, 7.37; N, 15.51. Found: C, 66.71; H, 7.53; N, 15.34.

Determination of PDE 5 and PDE6 inhibitory activity

PDE5 was 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., 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 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 0–600 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 and 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.[12]

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