Synthesis and Pharmacological Evaluations of Sildenafil Analogues for Treatment of Erectile Dysfunction

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Received November 7, 2007

The 5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one, sildenafil, is a cGMP-specific phosphodiesterase-5 (PDE5) inhibitor used for penile erectile dysfunction. In the search for more potent and selective PDE5 inhibitors, new sildenafil analogues (**6a**−**v**), characterized by the presence on the sulfonyl group in the 5′ position of novel *N*-4-substituted piperazines or ethylenediamine moiety, were prepared by traditional and microwave-assisted synthesis and tested in rabbit isolated aorta and corpus cavernosum. Similarly to sildenafil, several analogues showed IC_{50} values in the nanomolar range. In the in vitro studies, all the tested compounds caused concentration-dependent relaxations in both rabbit isolated aorta and corpus cavernosum. All sildenafil analogues potentiated the nitric oxide-dependent vasodilation in endothelium-intact rabbit aorta. Compound **6f** exhibited great pEC_{50} value in corpus cavernosum, and compounds **6r** and **6u** in isolated aorta were found as potent as sildenafil for inhibiting PDE5. Because several analogues were significantly more lipophilic than sildenafil, these compounds may offer a new lead for development of new sildenafil analogues.

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cyclic nucleotides, cAMP and cGMP, to their respective 5′-nucleoside monophosphate by cleavage of the phosphodiester bond at the 3′-position.[1] To date, 11 families of PDEs have been classified according to the sequence homology and biochemical properties.[2,3] Phosphodiesterase 5 (PDE5)[a], a cGMP-binding cGMP-specific PDE, is widely distributed in vascular and visceral smooth muscle cells, platelets, kidney, lung, and corpus cavernosum and plays a key role in the regulation of the cellular level of cGMP. The main therapeutic indications for PDE5 inhibitors are the treatment of erectile dysfunction and idiopathic pulmonary hypertension, although several other potentials have also been identified such as systemic hypertension and prosthate hyperplasia.[4] Common side-effects include headache, facial flushing, nasal congestion, dyspepsia, and transient visual impairment.[5] Although some of these secondary effects such as visual impairment were attributed to unspecific inhibition of PDE6, recent data demonstrate that human retina expresses both PDE5 and PDE6 isoforms.[6]

The compound 5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (sildenafil), is a selective and potent inhibitor of PDE5, which elevates intracellular cGMP levels, enhances NO-dependent corpus cavernosum relaxation in vitro,[7–11] and augments the penile erection in vivo.[11,12] Elevation of cGMP levels causes a decrease in intracellular calcium concentration, leading to enhanced relaxation of smooth muscle, increased arterial inflow, venous congestion, thus ameliorating the erectile dysfunction. PDE5 is a therapeutic target of considerable research interest as can be seen by numerous publications regarding the design, synthesis, and optimization of new PDE5 inhibitors such as 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f][1,2,4]triazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethyl-piperazine (vardenafil), (6*R-trans*)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino[1,2:1,6]pyrido[3,4-b]indole-1,4-dione (tadalafil) and other compounds under clinical trials (see Chart 1).[13–16]

To develop novel PDE5 inhibitors with improved therapeutic efficacy based on the structure of sildenafil, we have explored a series of sildenafil analogues in which the methyl-piperazine moiety was replaced with novel *N*-4-substituted-piperazines. A large number of aryl-, alkyl-, and cycloalkyl- substituents were selected with a large variety of hydrophobic, electronic, and steric properties. Moreover, to investigate the importance of the piperazine moiety, we have designed a novel series of derivatives in which an ethylenediamine structure has been introduced in place of the piperazine ring. These modifications led to a large set of sildenafil analogues (**6a**−**v**), which were prepared by traditional and microwave-assisted synthesis. Because sildenafil is being used not only for treatment of male erectile dysfunction but also for pulmonary hypertension, we have evaluated the effect of the newly synthesized compounds as PDE5 inhibitors in both corpus cavernosum and aorta. The aim

* To whom correspondence should be addressed. Phone: +55-19-35219555. Fax: +55-19-32892968. E-mail: denucci@gdenucci.com. Address: Gilberto De Nucci, Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas-UNICAMP, PO Box 6111, Campinas, SP 13084-971, Brazil.

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[a] Abbreviations: PDE5, phosphodiesterase type-5; DMSO, dimethyl sulfoxide; WP, washed platelets; EDTA, diethylene (2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid; NO, nitric oxide; sGC, soluble guanylate cyclase; PE, phenylephrine; ACh, acetylcholine; PRP, platelets-rich plasma; ACD buffer, acid-citrate dextrose buffer; TEA, triethylamine; DMAP, dimethylaminopyridine; ESI/MS, electrospray ionization mass spectrometry; ^{1}H NMR, nuclear magnetic resonance spectra for proton.

10.1021/jm701400r CCC: $40.75
Published on Web 04/05/2008

Chart 1. Commercially Available PDE5 Inhibitors

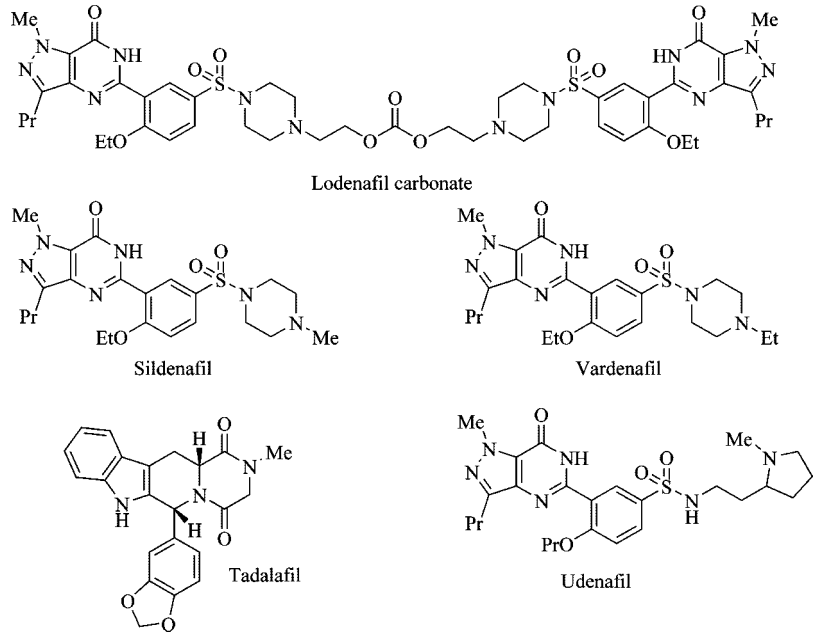


Table 1. Conventional Heating vs Microwave Irradiation for Intermediates **3**−**5** and Final Compounds **6a**−**v**[a]

	conventional heating[b]			microwave irradiation			
compd	yield (%)	time (h)	temp (°C)	yield (%)	time (min)	power (W)	temp (°C)
3	40	2	25	75	5	200	25
4	72	2.5	90	60	25	300	100
5	97	2	25				
6a−**o**	38–70	2	25	65–90[c]	5	200	30
6p	65	2	70	96	15	200	90
6q	70	24	25				
6r−**v**	30–60	2	25	60–92[c]	5	200	30

[a] The experimental conditions used on microwave irradiation were similar to those used by conventional heating, with the same amount of starting reagent and solvent. More details on the experimental conditions of the reactions are reported in the Experimental Section. [b] Oil-bath. [c] Ranging between the reported percentage.

of the present study is also to investigate the effects of compounds **6a**−**v** on PDE5 in human platelets.

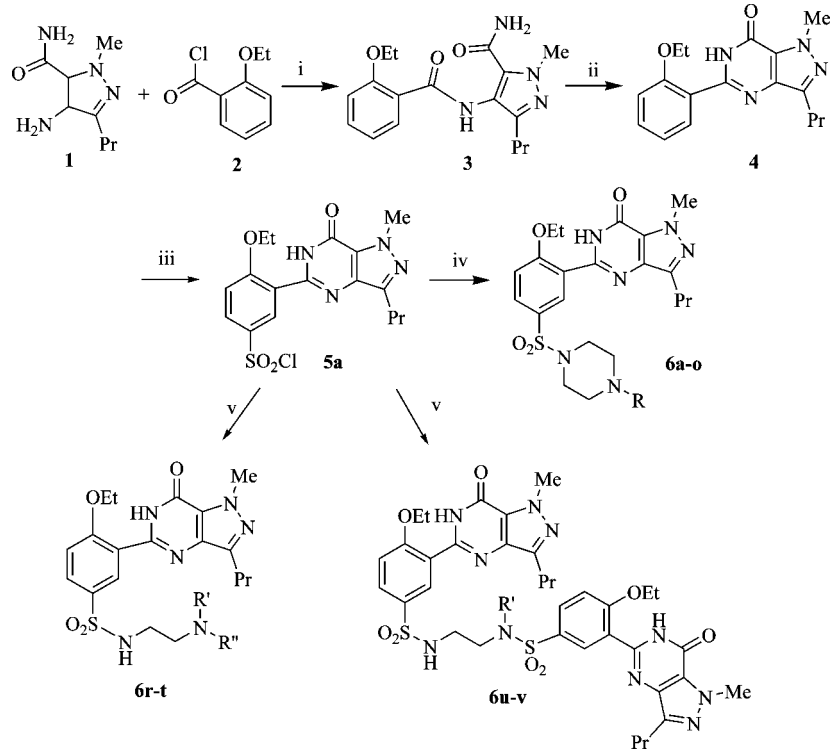
Chemistry

The preparation of some intermediates and final compounds (**6a**−**v**) was performed with traditional and microwave-assisted synthesis using a microwave oven especially designed for organic chemistry. The experimental conditions used in the microwave-assisted synthesis were similar to those used in the conventional heating with the same concentration of starting materials and volume of solvent.

All reactions were performed in sealed vessels using an appropriate microwave program, composed by ramping and holding steps. The optimum profile power/time and temperature for each considered compound is reported in Table 1.

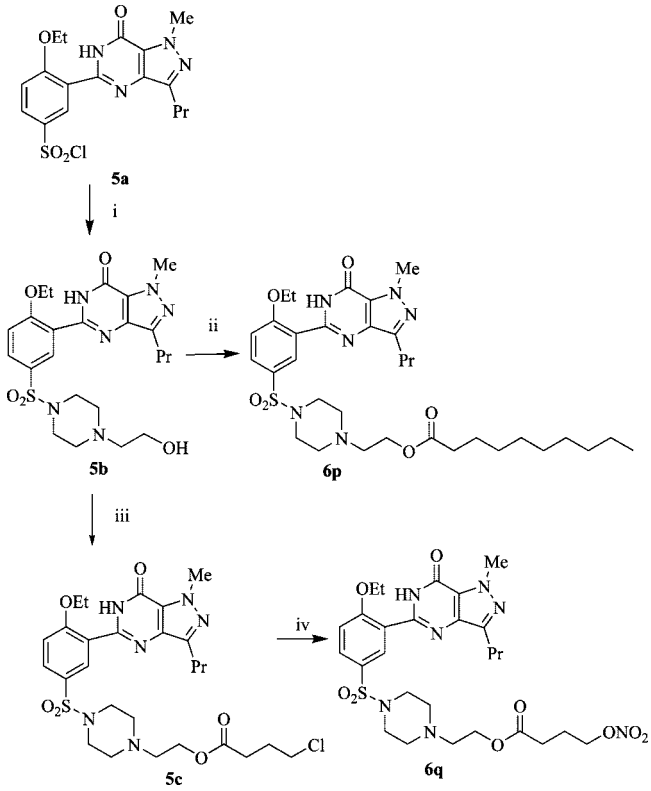
The final compounds **6a**−**v** were prepared following the synthetic routes reported in Schemes 1, 2. The intermediate compounds **3**, **4**, **5a**, and **5b** were synthesized following procedures reported in literature with some modifications.[17] Scheme 1 shows the acylation of the commercially available 4-amino-4,5-dihydro-1-methyl-3-propyl-1*H*-pyrazole-5-carboxamide (**1**) with 2-ethoxy-benzoyl chloride (**2**) in presence of triethylamine (TEA)/4-(dimethylamino) pyridine (DMAP), yielding the desired 4-(2-ethoxybenzamido)-1-methyl-3-propyl-1*H*-pyrazole-5-carboxamide (**3**). Cyclization of intermediate **3**, in the presence of 35% H_2O_2 and NaOH pellets, furnished 5-(2-ethoxyphenyl)-1-methyl-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-7(6*H*)-one (**4**). Reaction of **4** with chlorosulphonic acid yielded the desired key intermediate 4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)benzene-1-sulfonyl chloride (**5a**). Alkylation of intermediate **5a** with the appropriate *N*-4-substituted-piperazine gave the desired final compounds 5-{2-ethoxy-5-[(4-*R*)piperazinylsulphonyl]phenyl}-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-ones (**6a**−**o**). Alternatively, treatment of intermediate **5a** with the appropriate *N*-*N*′-substituted ethylenediamine yielded final compounds 5-(2-ethoxy-5-*R*′,*R*′-ethylenediamine-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo-[4,3d]-pyrimidin-7-ones (**6r**−**t**). It is worth noting that, from the reaction of intermediate **5a** with *N*-*β*-aminoethyl glycine, also the dimer **6u** was isolated. The synthesis of compounds **6r** and **6s** did not provide dimers because of the lack of nucleophilicity of nitrogens linked to the aromatic rings of *N*-phenyl- and *N*-naphthyl-ethylenediamine, respectively. On the other hand, when **5a** was treated with 2-(2-amino-ethylamino)-ethanol only, dimer **6v** was isolated without the formation of the monosubstituted derivative and this could be addressed to the high nucleophilicity of the starting reagent. Scheme 2 represents the synthesis of the final compounds **6p** and **6q**. The key intermediate 5-{2-ethoxy-5-[(4-hydroxyethyl)piperazinylsulphonyl]phenyl}-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (**5b**) was prepared according to the procedure reported in Scheme 1 starting from **5a** and *N*-hydroxyethyl-piperazine. Acylation of **5b** with decanoyl chloride, in anhydrous $CHCl_3$,

Scheme 1[a]



[a] Reagents and conditions: (i) TEA, DMAP/anhydrous CH_2Cl_2, μv; (ii) NaOH pellets, H_2O_2 (35%), H_2O/ETOH, μv; (iii) $ClSO_3H$, 0 °C, N_2; (iv) appropriate 4-*R*-substituted piperazine, TEA/absolute ETOH, μv; (v) *N*,*N*′-substituted-ethylenediamine TEA/ absolute ETOH, μv.

Scheme 2[a]



[a] Reagents and conditions: (i) 4-hydroxyethyl piperazine, TEA/ absolute ETOH, μv; (ii) decanoyl chloride, anhydrous $CHCl_3$, μv; (iii) chlorobutyryl chloride, anhydrous $CHCl_3$/$NaHCO_3$, 0 °C; (iv) $AgNO_3$, CH_3CN, at reflux under light exclusion.

gave the final compound **6p**. Intermediate **5b** was also reacted with chlorobutyryl chloride in anhydrous $CHCl_3$/$NaHCO_3$, yielding **5c**, which was converted in the desired compound **6q** by treatment with $AgNO_3$ in CH_3CN.

Analytical purification of each product was obtained by chromatography on silica gel column and further crystallization from diethyl ether. All new compounds gave satisfactory elemental analyses (C, H, N) and were characterized by ^{1}H NMR and ESI mass spectrometry. ^{1}H NMR and MS data for all final compounds were consistent with the proposed structures.

Pharmacology

The newly synthesized compounds were tested in vitro in order to evaluate the effects of PDE5 inhibition in human platelets. Phosphodiesterase (PDE) activity was determined as the concentration of cGMP or cAMP measured as a percentage of the inactive PDE (where the control with inactive PDE indicates maximal concentration of cGMP or cAMP present in the sample under the assay conditions). The measurement was performed by LC-MS/MS analysis on a triple quadrupole mass spectrometer (MS) API 4000. IC_{50} is defined as the concentration of inhibitor where 50% of the cGMP or cAMP is present in the sample when compared to the inactive PDE.

Functional studies in rabbit corpus cavernosum and in both endothelium-intact and -denuded rabbit isolated aorta were determined to evaluate smooth muscle relaxation induced by sildenafil analogues (**6a**−**v**). In phenylephrine-contracted preparations, cumulative concentration–response curves for **6a**−**v** were obtained in aortic rings (0.0001–10 μM) and corpus cavernosum (0.001–10 μM). Experimental values were calculated relative to the maximal changes from the contraction produced by phenylephrine in each tissue, which was taken as 100%. Data represent the mean ± SEM of 3–5 experiments.

Results and Discussion

The application of microwave energy to organic compounds for conducting synthetic reactions at highly accelerated rates

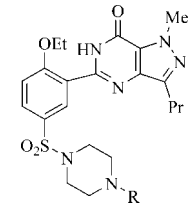
has become a well-known technique.[18] In later years, our interests were directed to the use of microwave irradiation in the field of heterocyclic synthesis;[19] starting from the acquired experience in this area, we performed the preparation of all the final compounds (**6a**−**v**) by microwave flash-heating. The obtained yields were higher (60–96%) than those obtained by conventional heating (Table 1) and the overall reaction times were dramatically reduced from 2−2.5 h to 5−25 min. Similar results were obtained for intermediates **3–5** (Table 1). The obtained yields and time reaction reduction confirm the efficiency of microwave flash-heating chemistry and place this technology as a powerful alternative to conventional heating.

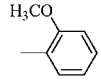
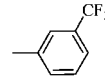
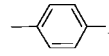
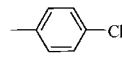
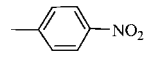
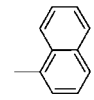
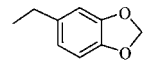
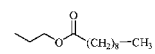
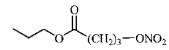
First, the sildenafil analogues were tested in vitro to evaluate the inhibition of PDE5 activity in human platelets. In this assay, the inhibitory potencies (IC_{50} values) of **6c**, **6e**, **6f**, **6 h**, **6i**, **6l**, and **6o** were similar to sildenafil (IC_{50} = 0.053 μM), ranging between 0.05 and 0.15 μM. Compounds **6m**, **6n**, and **6q** showed higher IC_{50} values (0.37, 0.32, and 0.53 μM, respectively), while compounds **6a**, **6b**, **6d**, **6g**, and **6p** presented less than 50% of enzymatic inhibition (Tables 2 and 3). Derivatives embodying ethylenediamine moiety (**6r**, **6s**, **6t**, and **6v**) showed a good activity, with IC_{50} values ranging between 0.20 and 0.51 μM. Interestingly, the analogue **6u** showed an IC_{50} value of 0.038 μM, which represents the lowest obtained value.

Second, we examined the potency of **6a**−**q** analogues in inducing relaxation on rabbit corpus cavernosum. Our data showed that **6a**, **6c**, **6e**, **6f**, **6n**, **6o**, and **6p** were as potent as sildenafil in relaxing the corpus cavernosum, with values of pEC_{50} between 6.36 and 7.08 μM. Particularly, compound **6f** showed the best pEC_{50} value for relaxing corpus cavernosum (7.08 ± 0.09 μM). In contrast, compounds **6b**, **6d**, **6g**, **6 h**, **6i**, **6l**, and **6q** were found to be significantly less potent than sildenafil in this assay. The maximal responses for these compounds (E_{max} values between 100 and 119%) were similar to sildenafil (E_{max} 105 ± 3%). Table 2 shows the pEC_{50} and E_{max} values for these compounds. The compounds containing an ethylenediamine moiety (**6r**−**v**) exhibited activity lower than the corresponding *N*-4-substituted-piperazine analogues in corpus cavernosum. In fact, it should be noted that all compounds exhibited values of pEC_{50} and E_{max} lower than 6.26 ± 0.15 μM and 69 ± 4%, respectively. Overall, our results demonstrated that a set of different *N*-4-substituted piperazine with a large variety of hydrophobic, electronic, and steric properties are able to bind PDE5 enzyme, and several compounds yielded an excellent combination of both enzymatic inhibition and corpus cavernosum relaxation.

In the last set of experiments, functional studies in rabbit isolated aortic rings were performed to evaluate smooth muscle relaxation. Cumulative addition of **6a**−**v** analogues (0.0001–10 μM) evoked sustained relaxations in endothelium-intact and -denuded aortic rings precontracted with phenylephrine (PE, 1 μM) in a concentration-dependent manner. The removal of the endothelium caused a significant rightward shift of the concentration–response curve to **6a**, **6c**, **6d**, **6e**, **6f**, **6g**, **6 h**, **6i**, **6l**, **6n**, **6o**, **6p**, and **6q** (Table 4). Sildenafil showed similar values in both endothelium-intact and -denuded preparations (pEC_{50}: 7.25 ± 0.07 μM, 6.57 ± 0.11 μM, respectively). These results indicate that the tested compounds potentiate the basal NO release from endothelium. In contrast, analogues **6b** and **6m** were less potent than sildenafil in endothelium-intact aortic rings (pEC_{50}: 6.77 ± 0.19 μM, 6.81 ± 0.19 μM, respectively). Table 4 summarizes the pEC_{50} values and maximal responses for sildenafil analogues in both endothelium-denuded and endothelium-intact aorta.

Table 2. IC_{50} (μM) Values for Compounds **6a**−**q** as PDE Inhibitors in Human Platelets and Relaxant Effects (pEC_{50} and E_{max}) of the PDE5 Inhibitor in Rabbit Corpus Cavernosum Preparations Contracted by Phenylephrine

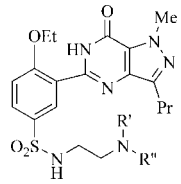


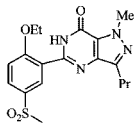
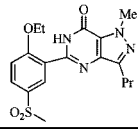
Compd.	R	IC_{50} (μM)	pEC_{50}*	E_{max}*	ClogP
6a		**	6.83 ± 0.15	113 ± 3	5.77
6b		**	6.16 ± 0.15	119 ± 11	5.70
6c		0.15	6.62 ± 0.04	119 ± 8	5.79
6d		**	6.06 ± 0.23	96 ± 7	6.95
6e		0.06	6.52 ± 0.08	119 ± 2	5.63
6f		0.07	7.08 ± 0.09	105 ± 3	6.08
6g		**	5.55 ± 0.15	100 ± 3	6.09
6h		0.10	5.68 ± 0.15	75 ± 5	6.65
6i		0.14	6.14 ± 0.05	97 ± 1	6.65
6l		0.05	6.25 ± 0.10	112 ± 3	5.87
6m		0.37	**	**	6.94
6n		0.32	6.36 ± 0.06	100 ± 8	5.14
6o		0.07	6.57 ± 0.10	105 ± 2	5.79
6p		**	6.86 ± 0.06	108 ± 3	8.41
6q		0.53	6.14 ± 0.08	123 ± 2	4.87
Sildenafil	-CH_3	0.053	6.77 ± 0.08	105 ± 3	3.85

* Experimental values were calculated relative to the maximal changes from the contraction produced by phenylephrine and represented as −log of the molar concentration to produce 50% of the maximal relaxation elicited by sildenafil and analogues in contracted tissues. Data represent the mean ± SEM of *n* experiments. ** Were not obtained values at 50% of enzymatic inhibition. ClogP = octanol-water Partition Coefficient.

The derivatives embodying ethylenediamine moiety (analogues **6r**−**v**), presented pEC_{50} values similar to sildenafil in both endothelium-intact and -denuded preparations (Table 4).

Table 3. IC_{50} (μM) Values for Compounds **6r-v** as PDE Inhibitors in Human Platelets and Relaxant Effects (pEC_{50} and E_{max}) of the PDE5 Inhibitor in Rabbit Corpus Cavernosum Preparations Contracted by Phenylephrine



Compd.	R'	R''	IC_{50} (μM)	pEC_{50}*	E_{max}*	ClogP
6r	H		0.20	6.26 ± 0.15	69 ± 4	4.78
6s	H		0.51	6.11 ± 0.17	51 ± 5	5.95
6t		H	0.50	5.73 ± 0.22	61 ± 5	0.59
6u			0.038	5.72 ± 0.20	60 ± 5	4.07
6v			0.31	5.79 ± 0.16	68 ± 6	3.76

* Experimental values were calculated relative to the maximal changes from the contraction produced by phenylephrine, and represented as −log of the molar concentration to produce 50% of the maximal relaxation elicited by sildenafil and analogues in contracted tissues. Data represent the mean ± SEM of *n* experiments. ClogP = octanol-water Partition Coefficient.

In contrast to the other compounds, the relaxations evoked by the compounds **6r** and **6u** were not affected by the endothelium denudation, whereas those evoked by **6s**, **6t**, and **6v** were greatly attenuated by endothelium removal, producing a marked rightward shift, thus making **6r** and **6u** potential compounds to treat erectile dysfunction. The maximal responses to **6r**, **6s**, **6t**, **6u**, and **6v** were not affected by endothelium denudation.

Thus, our findings suggest that piperazine moiety is not crucial in order to obtain PDE inhibitory activity. The ethylenediamine derivative **6u**, for example, showed an IC_{50} value on PDE5 in human platelets lower than sildenafil (0.038 and 0.053 μM, respectively). The pharmacological results obtained with compounds **6r**−**v** are very interesting because, associated with the absence of the piperazine structure, the newly synthesized derivatives support one or two acidic functions that make the molecules anphoter (**6r**, **6s**, and **6t**) or just acid (**6u** and **6v**). This improved inhibitory activity could be explained assuming that the acidic function mimick the phosphate group of cGMP. These findings are in accordance with recently reported 3D-QSAR studies on sildenafil analogues that indicate that compounds with substituents charged or larger than a methyl group, present on the sildenafil piperazine moiety, are essential for high inhibitory activity.[20] Opening the piperazine ring represents a crucial alteration that gives more flexibility to the molecules allowing different conformations of the supported functional groups.

Therefore, the obtained results show that our molecules, which have been designed by introducing different substituents on piperazine or *N*,*N*′-ethylendiamine moiety, have a good inhibitory activity on PDE-5. The biological profile is due to a combination of the inserted structural modifications and the unmodified sildenafil pharmacophoric portion that binds into the active site pocket mainly by various hydrophobic interactions and two hydrogen bonds.[21]

In conclusion, we have synthesized several compounds that exhibited a good PDE5 inhibitory activity that was as potent as sildenafil. In particular, compound **6f** in rabbit isolated corpus cavernosum, and compounds **6r** and **6u** in aorta, showed the best pharmacological profile and may offer a new lead for development of novel sildenafil analogues. Because these compounds are more lipophilic than sildenafil, they may have better bioavailability and prolonged action in vivo. Further studies on the pharmacokinetic profile of these compounds are required to reveal their therapeutic potential.

Experimental Section

General Chemistry. All starting materials were commercially available and used without further purification. Synthesis was performed using a microwave oven ETHOS 1600, Milestone. All reactions were performed in standard Pyrex glassware with a reflux condenser fitted through the roof of the microwave cavity and were performed by a microwave program that was composed by appropriate temperature ramping and holding steps. Optimum profile power/time and temperature employed for the synthesis is reported in Table 1. Conventional heating (oil bath) and microwave irradiation of the reactions were compared (Table 1). The temperature of the stirred reaction mixture was monitored directly by a microwave-transparent fluoroptic probe inserted into the solution. Organic solutions were dried over anhydrous sodium sulfate. Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Thin-layer chromatography was performed on precoated silica gel Kieselgel 60F254 (E. Merck, AG, Darmstadt, Germany) plates. Column chromatography was performed on 200–400 mesh silica gel. Molecular weights of final compounds were assessed by electrospray ionization mass spectrometry (ESI/MS) on a ThermoFinnigan LCQ Ion-Trap. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a Bruker AM-500 spectrometer. The chemical shift values are expressed in ppm (part per million) relative to tetramethylsilane as internal standard: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Elemental compositions are within ±0.4% of the calculate values. The ClogP values reported in table 2 have been determined using the software "Octanol−Water Partition Coefficients, ClogP for Windows, version 2.0" (BioByte Corp., Claremont, CA).

4-(2-Ethoxybenzamido)-1-methyl-3-propyl-1*H*-pyrazole-5-carboxamide (3). A mixture of 4-amino-4,5-dihydro-1-methyl-3-propyl-1H-pyrazole-5-carboxamide (**1**) (3.0 g, 16.4 mmol), 2-ethoxybenzoyl chloride (**2**) (6.1 g, 33.0 mmol), DMAP (0.02 g, 0.164 mmol), and TEA (3.34 g, 33.0 mmol) in anhydrous dichloromethane (50 mL) was introduced into the reaction vessel and the desired parameters (microwave, power, temperature, and time) were set as reported in Table 1. The solvent was evaporated and the residue dissolved in a mixture (250 mL) of dichloromethane/methanol 19/1 (v/v); the obtained solution was washed with 1 N hydrochloric acid (100 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The obtained residue was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane/methanol 19.4/0.6 (v/v). The combined fractions were evaporated providing the crude product **3** as a pale-pink solid (75%); mp: 153–155 °C.

5-(2-Ethoxyphenyl)-1-methyl-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-7(6*H*)-one (4). 4-(2-Ethoxybenzamido)-1-methyl-3-propyl-1*H*-pyrazole-5-carboxamide (**3**) (2.23 g, 6.76 mmol) was added portion wise to a solution of sodium hydroxide (0.54 g, 13.5 mmol) and 30% hydrogen peroxide solution (2.24 mL) in water (20 mL). Ethanol (70 mL) was added, the reaction mixture was introduced into the reaction vessel, and the desired parameters (microwave, power, temperature, and time) were set as reported in Table 1. The mixture was cooled and evaporated in vacuo. The resulting solid

Table 4. Potency (pEC_{50}) and Maximal Response (E_{max}) Values Derived from Concentration-Response Curves to the Sildenafil Analogues (0.0001−10 μM each) in Endothelium-Intact (E+) and -Denuded Aortic Rings (E−) Contracted with Phenylephrine (1 μM). Data Represent the Mean ± SEM of 5 Experiments

compd	pEC_{50} values	E_{max} values	compd	pEC_{50} values	E_{max} values
6a	E+ 7.18 ± 0.17	59 ± 3	**6m**	E+ 6.81 ± 0.19[b]	66 ± 2
	E− 6.74 ± 0.22[a]	41 ± 3[a]		E− 6.42 ± 0.26[a]	42 ± 2[a]
6b	E+ 6.77 ± 0.19[b]	69 ± 9	**6n**	E+ 7.18 ± 0.14	72 ± 4
	E− 6.48 ± 0.20[a]	49 ± 4[a]		E− 6.69 ± 0.11[a]	61 ± 4
6c	E+ 7.16 ± 0.17	63 ± 8	**6o**	E+ 7.32 ± 0.09	91 ± 5
	E− 6.54 ± 0.31[a]	40 ± 4[a]		E− 6.64 ± 0.10[a]	60 ± 4[a]
6d	E+ 7.22 ± 0.08	86 ± 1	**6p**	E+ 7.46 ± 0.06	93 ± 2
	E− 6.78 ± 0.11[a]	70 ± 3[a]		E− 6.68 ± 0.10[a]	72 ± 6[a]
6e	E+ 7.28 ± 0.10	90 ± 4	**6q**	E+ 7.04 ± 0.09	87 ± 3
	E− 6.68 ± 0.09[a]	75 ± 5[a]		E− 6.54 ± 0.07[a]	88 ± 3
6f	E+ 7.28 ± 0.10	89 ± 3	**6r**	E+ 7.21 ± 0.10	82 ± 4
	E− 6.71 ± 0.10[a]	67 ± 4[a]		E− 7.01 ± 0.11	82 ± 3
6g	E+ 7.40 ± 0.06	91 ± 4	**6s**	E+ 7.28 ± 0.11	70 ± 5
	E− 6.76 ± 0.10[a]	73 ± 4[a]		E− 6.75 ± 0.11[a]	66 ± 7
6h	E+ 7.02 ± 0.14	66 ± 6	**6t**	E+ 7.21 ± 0.10	73 ± 3
	E− 6.49 ± 0.14[a]	47 ± 3[a]		E− 6.73 ± 0.11[a]	69 ± 5
6i	E+ 7.08 ± 0.13	74 ± 6	**6u**	E+ 6.93 ± 0.12	75 ± 5
	E− 6.61 ± 0.12[a]	52 ± 6[a]		E− 6.81 ± 0.09	68 ± 7
6l	E+ 7.18 ± 0.14	77 ± 3	**6v**	E+ 7.07 ± 0.15	69 ± 8
	E− 6.48 ± 0.22[a]	46 ± 4[a]		E− 6.47 ± 0.19[a]	51 ± 5
sildenafil	E+ 7.25 ± 0.07	76 ± 6			
	E− 6.57 ± 0.11[a]	56 ± 1			

[a] $p < 0.05$ compared with the respective control value. [b] $p < 0.05$ compared to sildenafil E+ value.

was treated with 2 N hydrochloric acid (3.8 mL), with external cooling, and the mixture was extracted with dichloromethane. The combined organic extracts were then washed with saturated aqueous sodium carbonate solution and brine and then dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resulting crude residue was purified on silica gel column eluting with a mixture of dichloromethane/ethanol 19.4/0.6 (v/v), and the final compound **4** was obtained as a colorless solid (45% yield); mp: 143–146 °C.

4-Ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)benzene-1-sulfonyl chloride (5a). Carboxamide compound (**4**) (10.0 g, 32.1 mM) was added portion wise to stirred and cooled chlorosulphonic acid (20 mL) in an ice bath under nitrogen atmosphere; the reaction mixture was then warmed to room temperature gradually for 2 h after the addition. The reaction solution was cautiously added to ice–water (150 mL), and the aqueous mixture extracted with a mixture of dichloromethane/methanol 9/1 (v/v). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under vacuum to give the required sulfonyl chloride **5** as a white solid (97% yield); mp: 179–181 °C.

General Procedure for Preparation of Derivatives 6a−o by Condensation of 4-Substituted Piperazines with Intermediate 5a. A mixture of chlorosulphonyl derivative (**5a**) (1 mmol), an appropriate 4-substituted piperazine (1 mmol), and triethylamine (0.20 g, 2 mmol) in anhydrous ethanol (20 mL) was added; the reaction was introduced into the reaction vessel, and the desired parameters (microwave, power, temperature, and time) were set as reported in Table 1. The reaction mixture was dried under reduced pressure, and the resulting crude residue was purified on silica gel column using dichloromethane/methanol 9.7/0.3 (v/v) as eluent, unless otherwise indicated. The crude products **6a−o** were crystallized by diethyl ether. The intermediate compound **5b** was obtained by reaction of **5a** with 4-hydroxyethyl piperazine under the same reaction conditions.[17]

5-{2-Ethoxy-5-[(4-phenyl)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6a). It was synthesized from **5a** and phenylpiperazine. Yield 80%; mp: 203–205 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, J = 7.5 Hz); 1.32 (t, 3 H, OCH_2CH_3, J = 6.9 Hz); 1.69–1.81 (m, 2 H, $CH_2CH_2CH_3$); 2.49 (t, 2 H, $CH_2CH_2CH_3$, J = 7.5 Hz); 3.20–3.60 (m, 8 H, 4 NCH_2); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, J = 6.9 Hz); 6.60 (m, 3H, H-2′, H-4′, and H-6′); 6.92 (d, 1H, H-3′, J = 9.0 Hz); 7.08 (t, 2H, H-3′, and H-5′, J = 7.2); 7.86–7.92 (m, 2H, H-4′, and H-6′); 12.30 (br s, 1H, NH); ESI/MS: 537.44 $[M + H]^+$. Anal. ($C_{27}H_{32}N_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-*o*-tolyl)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6b). It was synthesized from **5a** and *o*-tolylpiperazine. Yield 90%; mp: 233–236 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, J = 7.5 Hz); 1.32 (t, 3H, OCH_2CH_3, J = 6.9 Hz); 1.69–1.81 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, J = 7.5 Hz); 2.76 (s, 3H, CH_3); 3.20–3.60 (m, 8H, 4 NCH_2); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, J = 6.9 Hz); 6.92 (d, 1H, H-3′, J = 9.0 Hz); 7.12 (t, 1H, J = 6.9); 7.39 (d, 2H, J = 7.2); 7.86–7.92 (m, 3H); 12.30 (br s, 1H, NH); ESI/MS: 551.44 $[M + H]^+$. Anal. ($C_{28}H_{34}N_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(1-(2-methoxyphenyl))piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6c). It was synthesized from **5a** and 1-(2-methoxyphenyl) piperazine. Yield 90%; mp: 205–207 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, J = 7.5 Hz); 1.32 (t, 3H, OCH_2CH_3, J = 6.9 Hz); 1.69–1.81 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, J = 7.5 Hz); 3.19 (s, 3H, OCH_3); 3.54–3.66 (m, 8H, 4NCH_2); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, J = 6.9 Hz); 6.60 (m, 2H, H-2′, H-4′, and H-6′); 6.92 (d, 1H, H-3′, J = 9.0 Hz); 7.08 (t, 2H, H-3′, and H-5′, J = 7.2); 7.86–7.92 (m, 2H, H-4′, and H-6′); 12.30 (br s, 1H, NH); ESI/MS: 567.44 $[M + H]^+$. Anal. ($C_{28}H_{34}N_6O_5S$) C, H, N.

5-{2-Ethoxy-5-[(4-(3-(trifluoromethyl)phenyl)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6d). It was synthesized from **5a** and 3-(trifluoromethyl)phenylpiperazine. Yield 85%; mp: 184–186 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, J = 7.5 Hz); 1.32 (t, 3H, OCH_2CH_3, J = 6.9 Hz); 1.69–1.81 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, J = 7.5 Hz); 3.20–3.60 (m, 8H, 4NCH_2); 4.14 (s, 3H, NCH_3); 4,20 (q, 2H, OCH_2CH_3, J = 6.9 Hz); 6.92 (d, 2H, J = 9.0 Hz); 7.09–7.19 (m, 2H); 7.86–7.92 (m, 4H, H-4′, and H-6′); 12.30 (br s, 1H, NH); ESI/MS: 606.44 $[M + H]^+$. Anal. ($C_{28}H_{31}F_3N_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(2-fluorophenyl)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6e). It was synthesized from **5a** and 2-(fluorophenyl) piperazine. Yield 90%; mp: 208–210 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, J = 7.5 Hz); 1.32 (t, 3 H, OCH_2CH_3, J = 6.9 Hz); 1.69–1.81 (m, 2 H, $CH_2CH_2CH_3$); 2.49 (t, 2 H, $CH_2CH_2CH_3$, J = 7.5 Hz); 3.20–3.60 (m, 8 H, 4NCH_2); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, J = 6.9 Hz); 6.60 (m, 2H, H-4′, and H-6′); 6.92 (d, 1H, H-3′, J = 9.0 Hz); 7.08 (t, 2H, H-3′,

and H-5′, $J = 7.2$); 7.86–7.92 (m, 2H, H-4′, and H-6′); 12.21 (br s, 1H, NH); ESI/MS: 555.44 $[M + H]^+$. Anal. ($C_{27}H_{31}FN_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(4-fluorophenyl)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6f). It was synthesized from **5a** and 4-(fluorophenyl) piperazine. Yield 85%; mp: 195–197 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.28 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.62–1.73 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 3.20–3.60 (m, 8H, $4NCH_2$); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.60 (m, 2H, H-4′, and H-6′); 6.92 (d, 1H, H-3′, $J = 9.0$ Hz); 7.08 (t, 2H, H-3′, and H-5′, $J = 7.2$); 7.86–7.92 (m, 2H, H-4′, and H-6′); 12.20 (br s, 1H, NH); ESI/MS: 555.44 $[M + H]^+$. Anal. ($C_{27}H_{31}FN_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(2-chlorophenyl))piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6g). It was synthesized from **5a** and (2-chlorophenyl) piperazine. Yield 65%; mp: 213–215 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.92 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.32 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.74 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 2.74–3.05 (m, 8H, $4NCH_2$); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.60 (m, 2H, H-4′, and H-6′); 6.92 (d, 1H, H-3′, $J = 9.0$ Hz); 7.08 (t, 2H, H-3′, and H-5′, $J = 7.2$); 7.86–7.88 (m, 2H, H-4′, and H-6′); 12.21 (br s, 1H, NH); ESI/MS: 572.44 $[M + H]^+$. Anal. ($C_{27}H_{31}ClN_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(3-chlorophenyl))piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6h). It was synthesized from **5a** and (3-chlorophenyl) piperazine. Yield 77%; mp: 200–202 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.92 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.30 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.74 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 2.74–3.05 (m, 8H, $4NCH_2$); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.78 (m, 2H, H-4′, and H-6′); 6.91 (d, 1H, H-3′, $J = 9.0$ Hz); 7.16 (t, 2H, H-3′, and H-5′, $J = 7.2$); 7.86–7.88 (m, 2H, H-4′, and H-6′); 12.18 (br s, 1H, NH); ESI/MS: 572.44 $[M + H]^+$. Anal. ($C_{27}H_{31}ClN_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(4-chlorophenyl))piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6i). It was synthesized from **5a** and (4-chlorophenyl) piperazine. Yield 66%; mp: 212–214 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.92 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.32 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.74 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 2,74–3,02 (m, 8H, $4NCH_2$); 4,10 (s, 3H, NCH_3); 4,21 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.89 (m, 2H, H-4′, and H-6′); 6.92 (d, 1H, H-3′, $J = 9.0$ Hz); 7.37 (t, 2H, H-3′, and H-5′, $J = 7.2$); 7.83–7.86 (m, 2H, H-4′, and H-6′); 12.21 (br s, 1H, NH); ESI/MS: 572.44 $[M + H]^+$. Anal. ($C_{27}H_{31}ClN_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(4-nitrophenyl))piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6l). It was synthesized from **5a** and (4-nitrophenyl) piperazine and eluted with dichloromethane/methanol 9/1 (v/v). Yield 90%; mp: 221–223 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.91 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.27 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.68–1.73 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 2.71–2.74 (m, 8H, $4NCH_2$); 3.32 (s, 3H, NCH_3); 4.14 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.98 (m, 2H, H-4′, and H-6′); 7.31 (d, 1H, H-3′, $J = 9.0$ Hz); 7.81 (t, 2H, H-3′, and H-5′, $J = 7.2$); 8.01–8.02 (m, 2H, H-4′, and H-6′); 12.21 (br s, 1H, NH); ESI/MS: 572.44 $[M + H]^+$. Anal. ($C_{27}H_{31}N_7O_6S$) C, H, N.

5-{2-Ethoxy-5-[4-(1-naphthyl)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6m). It was synthesized from **5a** and 1-naphthylpiperazine. Yield 89%; mp: 260–262 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.32 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.69–1.81 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 3.20–3.60 (m, 8H, $4NCH_2$); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.92 (d, 1H, H-3′, $J = 9.0$ Hz); 6.62–7.47 (m, 9H); 12.30 (br s, 1H, NH); ESI/MS: 587.44 $[M + H]^+$. Anal. ($C_{31}H_{34}N_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-(1-piperonyl)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6n). It was synthesized from **5a** and 1-piperonylpiperazine. Yield 85%; mp: 193–195 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.94 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.32 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.69–1.81 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 3.28–3.34 (m, 10H, $4NCH_2$, CH_2); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 5.94 (s, 2H); 6.66–6.79 (m, 3H); 7.36 (d, 1H, H-3′, $J = 9.0$ Hz); 7.79–7.81 (m, 2H, H-4′, and H-6′); 12.30 (br s, 1H, NH); ESI/MS: 595.44 $[M + H]^+$. Anal. ($C_{28}H_{32}N_6O_6S$) C, H, N.

5-{2-Ethoxy-5-[(4-(1-(cyclohexyl))piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6o). It was synthesized from **5a** and 1-(cyclohexyl) piperazine. Yield 90%; mp: 209–211 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.92 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.12 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.29–1.32 (m, 10H); 1.65–1.74 (m, 2H, $CH_2CH_2CH_3$); 2.49 (t, 2H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 2.70–2.83 (m, 1H); 3.20–3.60 (m, 8H, $4NCH_2$); 4.14 (s, 3H, NCH_3); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 7.36 (d, 1H, H-3′, $J = 9.0$ Hz); 7.79–7.80 (m, 2H, H-4′, and H-6′); 12.30 (br s, 1H, NH); ESI/MS: 543.44 $[M + H]^+$. Anal. ($C_{27}H_{38}N_6O_4S$) C, H, N.

5-{2-Ethoxy-5-[(4-decanoic acid ethyl ester)piperazinylsulphonyl]phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6p). A mixture of 5-{2-ethoxy-5-[(4-hydroxyethyl)piperazinylsulphonyl]phenyl}-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (**5b**) (0.5 g, 1 mmol) and decanoyl chloride (0.19 g, 1 mmol) in anhydrous chloroform (20 mL) was added; the reaction mixture was introduced into the reaction vessel, and the desired parameters (microwave, power, temperature, and time) were set as reported in Table 1. The solvent was evaporated in vacuo, the residue was dissolved in H_2O (50 mL) and brine, and then the solution was washed with ethyl acetate (70 mL), dried with Na_2SO_4, and evaporated under vacuum. The resulting crude residue was purified on silica gel column using dichloromethane/methanol 15/1 (v/v) as eluent. The product was **6p** crystallized as white solid from diethyl ether. Yield (90%); mp: 149–151 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.81–0.93 (m, 6H); 1.16–1.43 (m, 15H); 1.72–1.73 (m, 4H); 2.74–2.86 (m, 6H); 3.20–3.60 (m, 8H, $4NCH_2$); 4.03–4.20 (m, 5H); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.92 (d, 1H, H-3′, $J = 9.0$ Hz); 7.86–7.92 (m, 2H, H-4′, and H-6′); 12.30 (br s, 1H, NH); ESI/MS: 659.85 $[M + H]^+$. Anal. ($C_{33}H_{50}N_6O_6S$) C, H, N.

5-{2-Ethoxy-5-[(4-chlorobutyric acid ethyl ester)piperazinylsulphonyl] phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (5c). A mixture of 5-{2-ethoxy-5-[(4-hydroxyethyl)piperazinylsulphonyl]phenyl}-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (**5b**) (0.5 g, 1 mmol), chlorobutyryl chloride (0.1 g mmol) in anhydrous chloroform (20 mL), and a solution of saturated $NaHCO_3$ (10 mL) was stirred at 0 °C for 2 h. The solvent was evaporated under vacuum, the residue dissolved in H_2O (50 mL) and brine, and then the organic phase was separated, dried over anhydrous Na_2SO_4, and evaporated under vacuum. (90%); mp: 169–170 °C.

5-{2-Ethoxy-5-[(4-nitro-butyric acid ethyl ester)piperazinylsulphonyl] phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d] pyrimidin-7-one (6q). A mixture of **5c** (0.6 g, 1 mmol) with $AgNO_3$ (0.34 g, 2 mmol) in acetonitrile (30 mL) was stirred at reflux under light exclusion. The mixture was filtered on Celite, the solvent was evaporated in vacuo, the residue was dissolved in H_2O (50 mL) and brine, and then the organic phase was separated, dried with Na_2SO_4, and evaporated in vacuo. The resulting crude residue was purified on silica gel column using diethyl ether/ethanol, 8:2 (v/v) as eluent. Yield 90%; mp: 153–155 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.92 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.32 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.72–1.73 (m, 2H, $CH_2CH_2CH_3$); 1.85–1.87 (m, 2H); 2.35–2.37 (m, 4H); 2.49–2.52 (m, 8H, $4NCH_2$); 2.74–2.77 (m, 2H); 3.30–3.31 (m, 2H); 4.05–4.19 (m, 5H); 4.20 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz); 6.92 (d, 1H, H-3′, $J = 9.0$ Hz);

7.86–7.92 (m, 2H, H-4′, and H-6′); 12.30 (br s, 1H, NH); ESI/MS: 636.44 $[M + H]^+$. Anal. ($C_{27}H_{37}N_7O_9S$) C, H, N.

General Procedure for Synthesis of Ethylenediamine Derivatives 6r−v. A mixture of chlorosulphonyl derivative (**5a**) (0.410 g, 1 mmol), an appropriate *N*,*N*′-substituted ethylendiamine (1 mmol), and TEA (0.202 g, 2 mmol) in anhydrous ethanol (25 mL) was added; the mixture was introduced into the reaction vessel, and the desired parameters (microwave, power, temperature, and time) were set as reported in Table 1. The reaction mixture was dried under reduced pressure, and the resulting crude residue was purified on a silica gel column using dichloromethane/ethanol, 19.4/0.6 (v/v), as eluent, unless otherwise indicated. The final compounds **6r−v**, obtained as solids, were crystallized by diethyl ether.

5-(2-Ethoxy-5-(*N*-phenyl-ethylenediamine-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6r). It was synthesized from **5a** and *N*-phenylethylenediamine. Yield 92%; mp: 165–167 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.96 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.31 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.73 (m, 2H, $CH_2CH_2CH_3$); 2.08 (d, 1H); 2.74–2.77 (m, 2H); 2.87–2.88 (m, 2H); 3.08–3.41 (m, 6H); 4.15–4.17 (m, 3H); 5.52 (t, 2H); 6.47–6.48 (d, 1H); 6.99–7.02 (t, 1H); 7.29–7.31 (d, 1H); 7.74–7.96 (m, 2H); 12.13 (br s, 1H, NH). ESI/MS: 510.64 $[M + H]^+$. Anal. ($C_{25}H_{30}N_6O_4S$) C, H, N.

5-(2-Ethoxy-5-(*N*-naphthyl-ethylenediamine-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6s). It was synthesized from **5a** (0.410 g, 1 mmol) and *N*-naphthylethylenediamine (0.259 g, 1 mmol). Yield 55%; mp: 192–194 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.96 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.31 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.73 (m, 2H, $CH_2CH_2CH_3$); 2.08 (d, 1H); 2.74–2.77 (m, 2H); 2.87–2.88 (m, 2H); 3.08–3.41 (m, 6H); 4.15–4.17 (s, 3H); 5.52 (t, 2H); 6.42 (d, 1H); 7.07–7.39 (m, 3H); 7.70 (d, 1H); 7.87 (d, 1H); 8.00 (d, 1H); 12.13 (br s, 1H, NH); ESI/MS: 560.44 $[M + H]^+$. Anal. ($C_{29}H_{32}N_6O_4S$) C, H, N.

5-(2-Ethoxy-5-(*N*-acetic acid ethylenediamine-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one (6t), 5-(2-Ethoxy-5-(*N*-acetic acid,*N*′-5-(2-ethoxy-5-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one-ethylenediamine-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo [4,3-d]pyrimidin-7-one (6u). Compounds **6t** and **6u** were synthesized in one step from **5a** (0.410 g, 1 mmol) and *N*-β-aminoethyl glycine (0.118 g, 1 mmol).

Compound 6t. Yield 60%; mp: 198–200 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.96 (t, 3H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.31 (t, 3H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.73 (m, 2H, $CH_2CH_2CH_3$); 2.08 (d, 1H); 2.74–2.77 (m, 2H); 2.87–2.88 (m, 2H); 3.08–3.41 (m, 6H); 3.31–3.39 (s, 2H); 4.15–4.17 (m, 3H); 5.52 (t, 2H); 12.13 (br s, 1H, NH); ESI/MS: 493.6 $[M + H]^+$. Anal. ($C_{21}H_{28}N_6O_6S$) C, H, N.

Compound 6u. Yield 20%; mp: 167–169 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.96 (t, 6H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.31 (t, 6H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.73 (m, 4H, $CH_2CH_2CH_3$); 2.08 (d, 2H); 2.74–2.77 (m, 4H); 2.87–2.88 (m, 4H); 3.08–3.41 (m, 6H); 3.31–3.39 (s, 2H); 4.15–4.17 (m, 3H); 5.52 (t, 4H); 7.07–7.28 (m, 1H); 7.83–7.94 (m, 1H); 12.13 (br s, 2H, NH); ESI/MS: 868.20 $[M + H]^+$. Anal. ($C_{38}H_{46}N_{10}O_{10}S_2$) C, H, N.

5-(2-Ethoxy-5-(*N*-ethanol,*N*′-5-(2-ethoxy-5-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-d]pyrimidin-7-one-ethylenediamine-sulfonyl)phenyl)-1-methyl-3-*N*-propyl-1,6-dihydro-7*H*-pyrazolo [4,3-d]pyrimidin-7-one (6v). It was synthesized from **5a** (0.410 g, 1 mmol) and 2-(2-amino-ethylamino)-ethanol (0,104 g, 1 mmol). Yield 45%; mp: 211–213 °C. ^{1}H NMR (500 MHz, DMSO) δ: 0.96 (t, 6H, $CH_2CH_2CH_3$, $J = 7.5$ Hz); 1.31 (t, 6H, OCH_2CH_3, $J = 6.9$ Hz); 1.70–1.73 (m, 4H, $CH_2CH_2CH_3$); 2.08 (t, 2H); 2.48 (t, 2H); 2.74–2.77 (m, 4H); 2.87–2.88 (m, 4H); 3.08–3.41 (m, 6H); 3.31–3.39 (s, 2H); 4.15–4.17 (m, 3H); 5.52 (t, 4H); 7.07–7.28 (m, 1H); 7.83–7.94 (m, 1H); 12.13 (br s, 2H, NH); ESI/MS: 854.20 $[M + H]^+$. Anal. ($C_{38}H_{48}N_{10}O_9S_2$) C, H, N.

In Vitro Studies: Preparation of PDE Crude Extracts from Human Platelets. Blood samples from healthy male volunteers were collected in 0.1 vol of 3.13% sodium citrate and centrifuged at 200*g* for 15 min at 10 °C. Platelet-rich plasma (PRP) was removed and followed by addition of 10 mM EDTA. Next, platelet-rich plasma (PRP) was centrifuged at 900*g* for 15 min at 10 °C. The pellets were then washed in calcium-free Krebs buffer containing 10 mM EDTA. The pellets were resuspended in Krebs buffer containing 0.32 M sucrose to a final concentration of 1×10^9 cells mL^{-1} and subsequently sonicated for 10 s. The protein concentration was determined by a Protein Assay kit (BioRad) using bovine serum albumin as the standard. The enzyme sample was frozen and stored at −80 °C until experimentation.

Determination of PDE Activity by LC-MS/MS Analysis. The standard enzymatic reaction mixture (total volume of 200 μL) contained 50 mM Tris-HCl (pH 8.0), 100 mM $MgCl_2$, and PDE enzyme sample (final protein concentration 0.5 mg/mL).[22,27] Increasing concentrations (0.005–1 μM) of the agents under study (sildenafil analogues) were prepared in dimethylsulfoxide (DMSO) and preincubated in the enzymatic mixture for 5 min at room temperature. Reaction was initiated by the addition of the substrate cGMP (5 μM) at 35 °C for 30 min. The reaction was stopped by immersing the sample in boiling water for 2 min. Samples were stored at −20 °C. DMSO alone was added in the samples used to measure 100% of enzyme activity. The chromatographic system consisted of an analytical column (Genesis C_{18}, 4 μmol, 100 mm × 2.1 mm, i.d., reversed phase). The mobile phase was a mixture of eluent A (water containing 10 mM formic acid) and eluent B (acetonitrile containing 10 mM formic acid).[27] The flow rate was 350 μL/min and the injection volume was 40 μL of each sample, previously diluted with water. The total run time was 10 min. The triple quadrupole mass spectrometer (MS) API 4000, AB/MDS Sciex Instruments (Toronto, Canada), was equipped with an electrospray source. To optimize all of the MS parameters, analysis ionization was carried out using standard solutions of GMP and cGMP infused directly into the mass spectrometer in positive ion mode, specific monitoring compounds were identified by multiple reaction monitoring (MRM) of ion GMP *m*/*z* 363.9 and its fragment *m*/*z* 152.1 and ion cGMP *m*/*z* 346.0 and its fragment *m*/*z* 152.2. A six-point calibration curve was constructed.

Preparation of Rabbit Isolated Corpus Cavernosum and Aorta. Male New Zealand white rabbits (2–3 kg) were anaesthetized with sodium pentobarbital (Hypnol, 50 mg/kg, i.v.), exsanguinated via the carotid artery, and aorta and penis removed. The protocol was approved by the University Ethics Committee. The aorta was cleaned of all loosely adherent tissue. Corpus cavernosum preparations were obtained, following dissection of the tunica albuginea and surrounding connective tissues. Tissues were immediately placed in chilled Krebs solution of the following composition (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.17; $CaCl_2 \cdot 2H_2O$, 2.5. In some aortic rings, the endothelium was removed mechanically by rubbing the lumen of the aorta with a closed pair of fine-tipped forceps. Endothelium removal was confirmed by the lack of a relaxant response to acetylcholine (ACh, 1 μM) at the start of the experiments. Aortic rings and cavernosal strips were mounted under tension 10 mN in 10 mL organ chambers, as previously described.[23–25] Isometric force was recorded using a PowerLab 400 data acquisition system (software chart, version 4.2, AD Instruments, MA). The tissues were allowed to equilibrate for 1 h before the start of the experiments, and phenylephrine (PE, 1 and 10 μM) was added to increase the basal tone. Thereafter, when reaching the submaximal contraction, sildenafil analogues (**6a−v**) and sildenafil itself were tested in the concentration range of 0.0001–10 μM (aortic rings) or 0.001–10 μM (corpus cavernosum). One concentration–response curve to **6a−v** was obtained for each preparation owing to the lower contractile effect of PE observed in the second curve.

Acknowledgment. Haroldo Flores Toque is thankful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support. The NMR spectral data were

provided by Centro di Ricerca Interdipartimentale di Analisi Strumentale, Universita degli Studi di Napoli "Federico II". The assistance of the staff is gratefully appreciated.

Supporting Information Available: Elemental analysis data for compounds **6a–v**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM701400R