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Synthesis and phosphodiesterase 5 inhibitory activity of novel pyrido[1,2-*e*]purin-4(3*H*)-one derivatives

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Abstract—Synthesis and primary SAR of a novel series of 2-phenylpyrido[1,2-*e*]purin-4(3*H*)-one derivatives with piperazinyl sulfonamide substituents were described herein. As potential PDE5 inhibitors for erectile dysfunction (ED) treatment, representative compounds exhibit improved selectivity versus PDE1 and PDE6. Meanwhile, compound **3e** demonstrated functional efficacy on rabbit corpus cavernosum strip in vitro.

The phosphodiesterases (PDEs) regulate physiological processes by the hydrolysis of intracellular second messengers, cyclic adenosine monophosphate (cAMP), and cyclic guanosine monophosphate (cGMP, Fig. 1) to their biologically inactive 5′ derivatives.[1] In human corpus carvernosum tissue, type 5 PDE (PDE5) is the major enzyme to degrade cGMP and plays a key role in the control of penile erection.[2] On sexual stimulation, nitric oxide (NO) is released from nonadrenergic, noncholinergic neurons. The NO activates soluble guanylyl cyclase, which cyclizes guanosine triphosphate to generate cGMP. Increased cGMP levels eventually cause a decrease in intracellular calcium concentration, resulting in the relaxation of smooth muscle in the corpus cavernosum. This relaxation leads to an increased arterial blood flow to the penis and ultimately erection. PDE5 inhibition slows cGMP breakdown, increases the half-life of this second messenger, indirectly extending the activity of NO in penile tissue, and facilitates penile erection in patients suffering from erectile dysfunction (ED).[3,4]

Despite the efficacy and commercial success, Viagra® (sildenafil, **1a**, Fig. 1), as a PDE5 inhibitor for ED treatment,

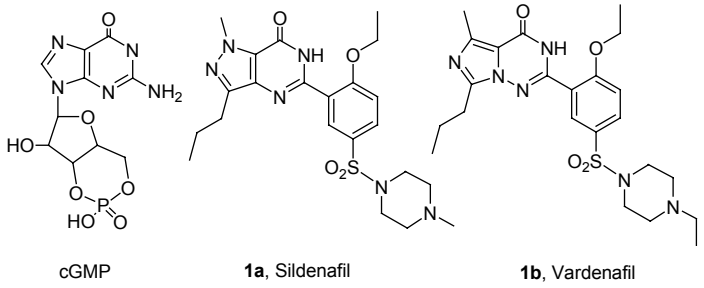

Figure 1. cGMP and PDE5 inhibitors with purine-isosteric heterocyclic systems.

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has shown clinically significant adverse reactions such as headache, flushing, dyspepsia, and visual disturbances, which may be linked to insufficient selectivity versus other PDE isozymes, specifically PDE1 (widely exists in vasculature) and PDE6 (the sole cGMP PDE in the retina).[5,6] Therefore, both the potency toward PDE5 and the selectivity against other PDEs are important for the successful development of new PDE5 inhibitors.[4,7]

In the wide variety of PDE5 inhibitors reported in the past few years, compounds with 6/5-fused ring systems, including **1a** and Levitra® (vardenafil, **1b**), bear more resemblance to the purine base in the substrate cGMP (Fig. 1).[8,9] The 'stretched-out' purine[10] derivatives (**2a** and **2b**) are more potent and selective ones than **1a**, but the derivatives of **2a** did not lead to good result on the functional model (rabbit corpus cavernosum strip) or had a poor oral bioavailability, and no efficacy data of the series of **2b** on the functional model was reported.[11]

Other analogs (**2c**, **2d**, and **2e** shown in Fig. 2) with 5/6/6 or 6/5/6-fused tricyclic systems, being evaluated in preclinical phase, also have potency and highly selectivity toward PDE5.[8] Herein, we report the design and synthesis of a new template of 2-phenyl pyrido[1,2-*e*]purin-4(3*H*)-one, in which, a 'pyridine' ring was fused on the imidazole side of the purine structure. Among the target compounds, the 5′-sulfonamide substituted derivative (**3**, Fig. 2) shown potent and selective inhibitory activity toward PDE5.

The synthetic route of 2-(2-ethoxyphenyl)pyrido[1,2-*e*]-purin-4(3*H*)-ones (**10a**–**i**) was shown in Scheme 1. Commercially available aminopyridines (**4a**–**i**) were converted into imidazo[1,2-*a*]pyridines (**5a**–**i**) by refluxing in EtOH with ethyl bromopyruvate. The regiospecific nitrations of **5a**–**i** were performed below 0 °C to afford the mono-nitro products (**6a**–**i**).[12] The nitroesters **6a**–**i** were stirred in aqueous ammonia to give the nitroamides **7a**–**i** in high yields. Reductions of **7a**–**i** to the corresponding aminoamides (**8a**–**i**) were achieved by catalytic hydrogenation with 10% Pd–C.[13] Acylations of **8a**–**i** with 2-ethoxylbenzoyl chloride were carried out in the presence of a catalytic amount of DMAP to produce the amides (**9a**–**i**) in middle yields. Using KOtBu as base, dehydrating cyclization of **9a**–**i** in refluxing tBuOH afforded **10a**–**i** in good yields.

According to the SAR study of pyrazolopyrimidinones made by Terrett et al., the attachment of 5′-polar substituents on the 2′-ethoxyphenyl ring will improve the PDE5 inhibitory activity distinctly.[14] We then synthesized a series of 5′-piperazinyl sulfonamide derivatives of **10**. Putatively, these groups may fill a space occupied by the phosphate of cGMP in the PDE5 active site.[9,14] The synthetic method of target compounds **3a**–**g**, **i**, **o** and **p** was shown in Scheme 3. Chlorosulfonylations of **10a**–**g** or **10i** in chlorosulfonic acid at 0 °C gave the 5′-position substituted chlorosulfonyl derivatives, which were coupled with N-substituted piperazines in $CHCl_3$ at room temperature to produce desired sulfonamides in high yields.

To get more SAR information of this pyridopurinones, several additional derivatives were synthesized through modification of pyridinyl moiety in **10**. Compounds **10f** and **10g** were converted to **10j** and **10k**, respectively, by palladium-catalyzed cyanation (Scheme 2). Bromination of **10e** via radical reaction gave **10l**, and the latter was coupled with pyrrolidine or *N*-methylpiperazine to afford **10m** and **10n**, respectively (Scheme 2). We supposed that the polar cyclic amino group of **10m** and **10n** have positive effect on PDE5 inhibitory activity

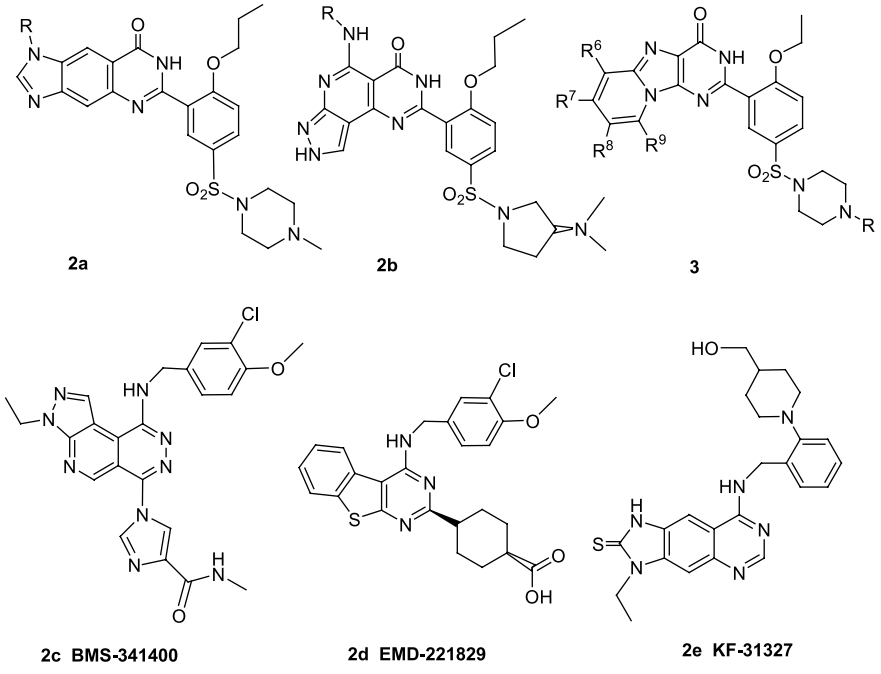
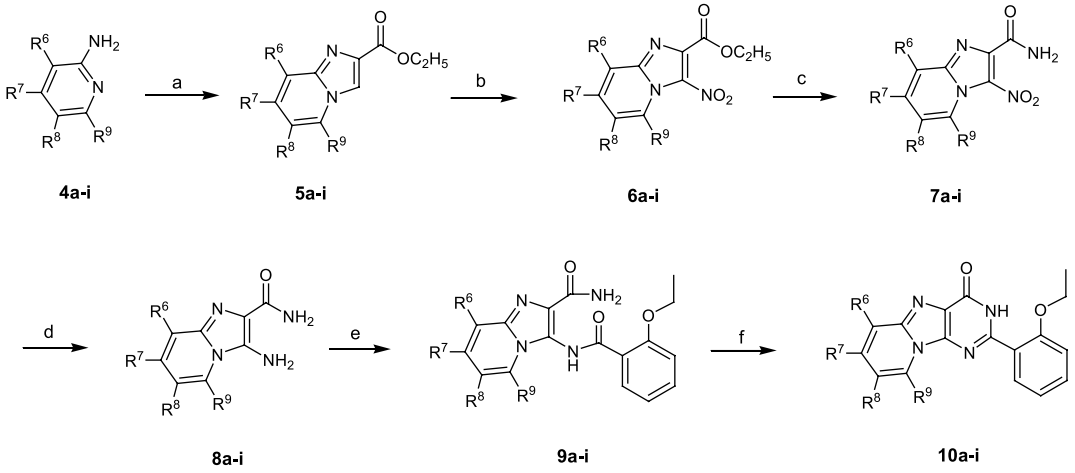
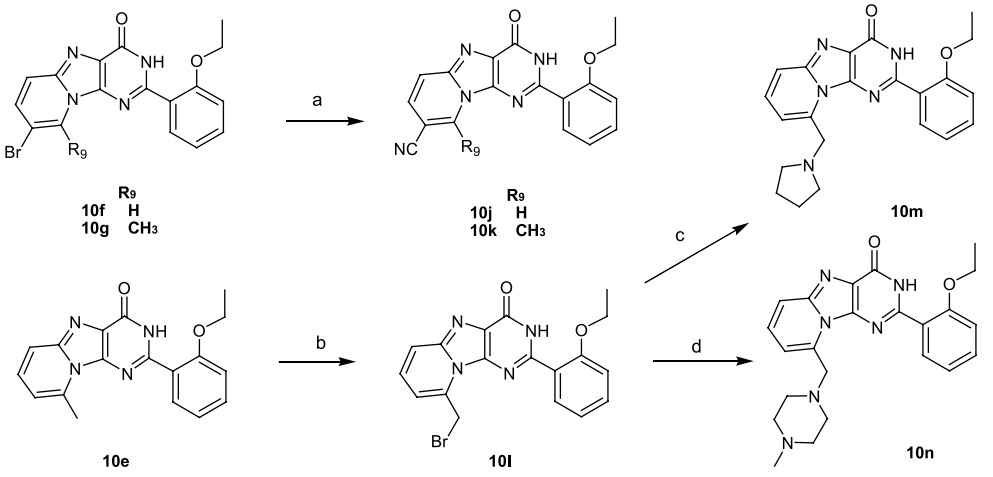


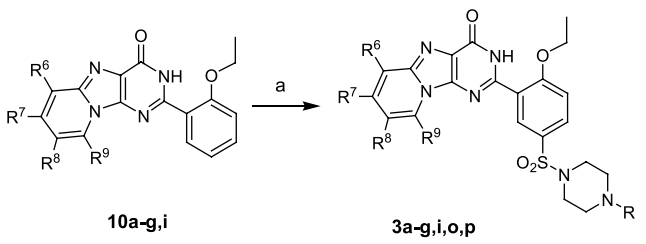
Figure 2. PDE5 inhibitors under development with 'stretched-out' purine and other tricyclic skeletons.



Scheme 1. Reagents and conditions: (a) ethyl bromopyruvate, EtOH, reflux, 6 h; (b) HNO_3, H_2SO_4, −5–0 °C, 2 h; (c) NH_4OH, H_2O, THF, rt overnight; (d) H_2, 10% Pt–C, 50 °C; (e) 2-ethoxybenzoyl chloride, pyridine, DMAP, CH_2Cl_2; (f) KO*t*-Bu, *t*-BuOH, reflux.



Scheme 2. Reagents and conditions: (a) NaCN, CuI, $Pd(PPh_3)_4$, DMF, reflux, 24 h; (b) NBS, CCl_4, *hv*, 2 h; (c) pyrrolidine, K_2CO_3, acetone, rt, 20 h; (d) *N*-methylpiperazine, K_2CO_3, acetone, rt, 20 h.



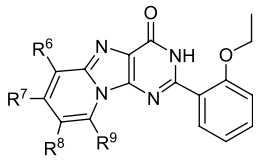
Scheme 3. Reagents and conditions: (a) (i) chlorosulfonic acid, 0–5 °C, 3 h; (ii) N-substituted piperazine, Et_3N, $CHCl_3$, rt, overnight.

like the 5′-piperazinyl sulfonamide substituents mentioned above.

Using [^{3}H]-cGMP SPA kit, the pyridopurinones (**10a**–**k**, **m**, **n**) and its sulfonamide derivatives (**3a**–**g**, **i**, **o**, **p**) were evaluated for the inhibitory activities against PDE5 isolated from human platelet.[15] Table 1 summarizes the structure and PDE5 inhibitory potency of **10a**–**k**, **m**, **n**. Introduction of methyl group in the pyridinyl ring (**10b**–**e**) led to more potent activity than **10a** (IC_{50} >10 μM), and the order of potencies was 9-methyl (**10e**, IC_{50} = 0.116 μM) >8-methyl (**10d**, IC_{50} = 0.381 μM) >7-methyl (**10c**, IC_{50} = 0.849 μM) >6-methyl (**10b**, IC_{50} = 1.05 μM). Introduction of electron-withdrawing substitutents (Br, CN) at 8-position and 6-position resulted in the diminishing of activity. IC_{50} of **10f** (8-Br analog of **10d**) was only 2.16 μM. Both 8-Br and 6,8-di Br derivatives of **10e** decrease potency (**10g**, IC_{50} = 0.710 μM, **10h**, IC_{50} = 2.10 μM). The replacement of Br in **10f** and **10g** with cyano group caused further loss of activities (**10j**, IC_{50} = 3.34 μM, **10k**, IC_{50} = 4.87 μM). Unexpectedly, introduction of tertiary amine on 9-methyl group resulted in a marked loss of PDE5 inhibitory activities (**10m**, IC_{50} = 1.33 μM and **10n**, IC_{50} = 1.57 μM).

As shown in Table 2, the inhibitory potencies toward PDE5 of all the piperazinyl sulfonamide derivatives (**3a**–**g**, **i**, **o**, **p**) were correspondingly higher than the parent ones (**10a**–**g**, **i**). The position of methyl group on pyridinyl ring affects the activity. The potency of

Table 1. Structure and PDE5 inhibitory potency of 2-(2-ethoxyphenyl) pyrido[1,2-*e*]purin-4(3*H*)-ones (**10a–k**, **10m** and **10n**)[a]

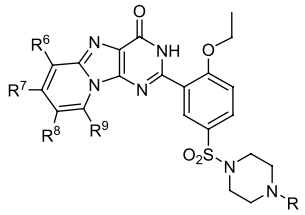


Compd	R^6	R^7	R^8	R^9	IC_{50} (μM)[b]
10a	H	H	H	H	>10
10b	CH_3	H	H	H	1.05
10c	H	CH_3	H	H	0.849
10d	H	H	CH_3	H	0.381
10e	H	H	H	CH_3	0.116
10f	H	H	Br	H	2.16
10g	H	H	Br	CH_3	0.710
10h	Br	H	Br	CH_3	2.10
10i	H	H	H	C_2H_5	0.634
10j	H	H	CN	H	3.34
10k	H	H	CN	CH_3	4.87
10m	H	H	H	CH_2-N-Pr	1.33
10n	H	H	H	CH_2-N-Pi	1.57

[a] Assays carried out as described in Ref. 15b. PDE5 was isolated from human platelets.

[b] The IC_{50} values were determined from the logarithmic concentration–inhibition curve (at least five points). The value is given as the mean of at least two duplicate experiments.

Table 2. Structure and PDE inhibitory potency of 5′-sulfonamide substituted derivatives of 2-(2-ethoxyphenyl)pyrido[1,2-*e*]purin-4(3*H*)-ones (**3a–g**, **i**, **o**, **p**)[a]



Compd	Starting material	R	IC_{50} (μM)[b]		
			PDE5	PDE1	PDE6
3a	**10a**	CH_3	0.878	—	5.13
3b	**10b**	CH_3	0.080	—	0.472
3c	**10c**	CH_3	0.112	—	1.05
3d	**10d**	CH_3	0.048	>100	0.693
3e	**10e**	CH_3	0.013	>100	0.184
3f	**10f**	CH_3	0.146	—	0.860
3g	**10g**	CH_3	0.033	>100	0.289
3i	**10i**	CH_3	0.038	>100	0.455
3o	**10e**	C_2H_5	0.009	>100	0.204
3p	**10e**	$CH(CH_3)_2$	0.015	>100	0.275
1a			0.003	0.561	0.025

[a] Assays carried out as described in Ref. 15b. enzyme sources: PDE5: human platelets; PDE1: bovine heart; PDE6: bovine retina.

[b] The IC_{50} values were determined from the logarithmic concentration–inhibition curve (at least five points). The value is given as the mean of at least two duplicate experiments.

9-methyl derivative (**3e**, IC_{50} = 13 nM) is 5-fold, 8-fold, and 11-fold higher than that of 8-methyl, 6-methyl, and 7-methyl derivatives (**3d**, IC_{50} = 48 nM; **3b**, IC_{50} = 80 nM; **3c**, IC_{50} = 112 nM), respectively. Meanwhile, it seems that one carbon elongation of 9-position alkyl chain did not improve the activity (the 9-ethyl derivative **3i** were less potent than **3e**). Almost equivalent potency was shown by the activity data of **3e**, **3o**, and **3p**, indicating that the *N*-alkyl substituents on the piperazine moiety play a limited role in the PDE5 inhibitory activity. It was consistent with the results reported by J. D. Corbin recently.[16]

Besides PDE5 inhibitory activity, the piperazinyl sulfonamide derivatives (**3a–g**, **i**, **o**, **p**) were also investigated for other inhibitory activity against two isoforms of PDEs isolated from bovine heart (PDE1) and bovine retina (PDE6).[17] All the compounds displayed an improved selectivity over PDE1, while most of them over PDE6 (Table 2). Compared with **1a**, compound **3o** was 2.7-fold higher in its selectivity over PDE6 (**3o**, IC_{50} ratio of PDE6/PDE5 = 22.6; **1a**, IC_{50} ratio of PDE6/PDE5 = 8.3).

Moreover, representative compound was evaluated in vitro for efficacy on rabbit corpus cavernosum strip.[17b,18] To show activity in this function model, molecules must efficiently diffuse into the smooth muscle cell of corpus cavernosum. Because molecular weight (MW) influences cell penetration ability of molecules,[11a] **3e** (MW = 482, IC_{50} ratio of PDE6/PDE5 = 14.2, PDE1/PDE5 >7690), with similar MW to **1a** (MW = 474) and higher isozyme selectivity than **1a** (IC_{50} ratio of PDE6/PDE5 = 8.3, PDE1/PDE5 = 187), was chosen as the first compound in this test. As shown in Figure 3, compound **3e** significantly enhanced the electrical frequency stimulation (EFS)-induced relaxation of rabbit corpus cavernosum. The slight difference in efficacy of **3e** and **1a** reflects in part the potency difference of the two compounds.

In summary, novel pyrido[1,2-*e*]purin-4(3*H*)-one derivatives were discovered as a new class of potent and selective PDE5 inhibitors. The improved selectivities of representative compounds may translate into decreasing of side-effect in vivo. Furthermore, compound **3e** demonstrated functional efficacy on rabbit corpus cavernosum strip in vitro. Encouraged by these primary results, we are continuing to optimize the potency of this

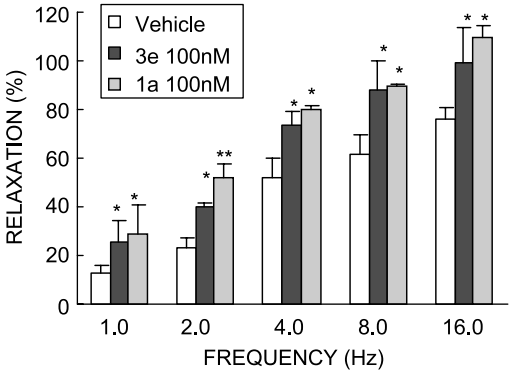


Figure 3. Electrical frequency stimulated (EFS)-induced relaxation of rabbit corpus cavernosum. (*p <0.05 vs vehicle, **p <0.01 vs vehicle).

series of molecules, and further SAR study and pharmalogical results will be reported later.

References and notes

1. (a) Francis, S. H.; Turko, I. V.; Corbin, J. D. *Prog. Nucleic Acid Res. Mol. Biol.* **2000**, *65*, 1; (b) Rotella, D. P. *Nat. Rev. Drug Discov.* **2002**, *1*, 674.
2. (a) Lin, C. S.; Lau, A.; Tu, R.; Lue, T. F. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 628; (b) Yanaka, N.; Kotera, J.; Ohtsuka, A.; Akatsuka, H.; Imai, Y.; Michibata, H.; Fujishige, K.; Kawai, E.; Takebayashi, S.; Okumura, K.; Omori, K. *Eur. J. Biochem.* **1998**, *255*, 391.
3. Boolell, M.; Gepi-Attee, S.; Gingell, J. C.; Allen, M. J. *Br. J. Urol.* **1996**, *78*, 257.
4. Lanter, J. C.; Sui, Z.; Macielag, M. J.; Fiordeliso, J. J.; Jiang, W.; Qiu, Y.; Bhattacharjee, S.; Kraft, P.; John, T. M.; Haynes-Johnson, D.; Craig, E.; Clancy, J. *J. Med. Chem.* **2004**, *47*, 656.
5. (a) Martel, A. M.; Graul, A.; Rabasseda, X.; Castaner, R. *Drugs Fut.* **1997**, *22*, 138; (b) Beavo, J. A. *Physiol. Rev.* **1995**, *75*, 725.
6. Fox, D. A.; Campbell, M. L.; Blocker, Y. S. *Neurotoxicology* **1997**, *18*, 645.
7. (a) Jiang, W.; Alford, V. C.; Qiu, Y.; Bhattacharjee, S.; John, T. M.; Haynes-Johnson, D.; Kraft, P. J.; Lundeen, S. G.; Sui, Z. *Bioorg. Med. Chem.* **2004**, *12*, 1505; (b) Ukita, T.; Sugahara, M.; Terakawa, Y.; Kuroda, T.; Wada, K.; Nakata, A.; Kikkawa, H.; Ikezawa, K.; Naito, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2341.
8. Sui, Z. *Expert Opin. Therap. Pat.* **2003**, *13*, 1373.
9. Haning, H.; Niewöhner, U.; Schenke, T.; Es-Sayed, M.; Schmidt, G.; Lampe, T.; Bischoff, E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 865.
10. 'Stretched-out' purine, first used as dimensional probe to investigate the requirements of enzymatic reaction, was defined as a tricyclc system consisting of a purine and a spacer. The spacer, usually a benzene ring or pyridine ring, is formally 'inserted' between the pyrimidine ring and the imidazole ring of purines (see Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128). The similar design was also used in anti-viral agents (Harris, P. A.; Pendergast, W. *J. Heterocycl. Chem.* **1996**, *33*, 319) and non-specific PDE inhibitors (Schneller, S. W.; Ibay, A. C.; Martinson, E. A.; Wells, J. N. *J. Med. Chem.* **1986**, *29*, 972).
11. (a) Rotella, D. P.; Sun, Z.; Zhu, Y.; Krupinski, J.; Pongrac, R.; Seliger, L.; Normandin, D.; Macor, J. E. *J. Med. Chem.* **2000**, *43*, 1257; (b) Rotella, D. P.; Sun, Z.; Zhu, Y.; Krupinski, J.; Pongrac, R.; Seliger, L.; Normandin, D.; Macor, J. E. *J. Med. Chem.* **2000**, *43*, 5037; (c) Bi, Y.; Stoy, P.; Adam, L.; He, B.; Krupinski, J.; Normandin, D.; Pongrac, R.; Seliger, L.; Watson, A.; Macor, J. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2461.
12. Teulade, J. C.; Escale, R.; Rossi, J. C.; Chapat, J. P.; Grassy, G.; Payard, M. *Aust. J. Chem.* **1982**, *35*, 1761.
13. Pinguet, F.; Mavel, S.; Galtier, C.; Gueiffier, A. *Pharmazie* **1999**, *54*, 876.
14. Terrett, N. K.; Bell, A. S.; Brown, D.; Ellis, P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1819.
15. (a) Seiler, S.; Gillespie, E.; Arnold, A. J.; Brassard, C. L.; Meanwell, N. A.; Fleming, J. S. *Thrombosis Res.* **1991**, *62*, 31; (b) Kim, D. K.; Lee, J. Y.; Lee, N.; Ryu, D. H.; Kim, J. S.; Lee, S.; Choi, J. Y.; Ryu, J. H.; Kim, N. H.; Im, G. J.; Choi, W. S.; Kim, T. K. *Bioorg. Med. Chem.* **2001**, *9*, 3013.
16. Corbin, J. D. *Neurochem. Int.* **2004**, *45*, 859.
17. (a) Berger, A. L.; Cerione, R. A.; Erickson, J. W. *J. Biol. Chem.* **1997**, *272*, 2714; (b) Ballard, S. A.; Gingell, C. J.; Tang, K.; Turner, L. A.; Price, M. E.; Naylor, A. M. *J. Urol.* **1998**, *159*, 2164.
18. Strips of corpus cavernosum tissue were immersed in organ chambers containing physiological salt solution, maintained at 37 °C and aerated with carbon dioxide and oxygen (pH 7.4) in the presence of 10 μM indomethacin. In all experiments, the strips were incubated with atropine (1 μM) and guanethidine (5 μM). After the tissues were equilibrated, they were subjected to electrical field stimulation (24 V, 0.7 ms pulse duration) at 1–16 Hz. The tissues were then incubated with vehicle, or test compound (100 nM) for 30 min contracted with phenylephrine. The electrical stimulation was repeated. The relaxation responses were expressed as the percentage of total relaxation (loss in tone) induced by the addition of 0.1 mM papaverine HCl to the chambers at the end of the experiment. All data are expressed as the mean ± standard error of the mean. (Angulo, J.; Cuevas, P.; Fernandez, A.; Gabancho, S.; Saenz de Tejada, I. *Urology* **2001**, *57*, 585).