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Design, Synthesis, and Pharmacological Evaluation of Monocyclic Pyrimidinones as Novel Inhibitors of PDE5

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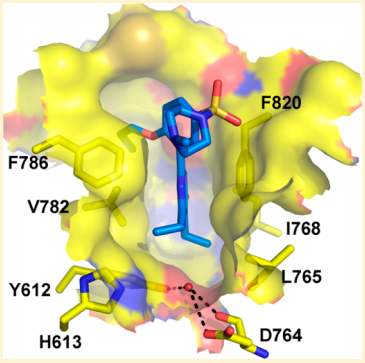
Supporting Information

ABSTRACT: Cyclic nucleotide phosphodiesterase type 5 (PDE5) is a prime drug target for treating the diseases associated with a lower level of the cyclic guanosine monophosphate (cGMP), which is a specific substrate for PDE5 hydrolysis. Here we report a series of novel PDE5 inhibitors with the new scaffold of the monocyclic pyrimidin-4(3*H*)-one ring developed using the structure-based discovery strategy. In total, 37 derivatives of the pyrimidin-4(3*H*)-ones, were designed, synthesized, and evaluated for their inhibitory activities to PDE5, resulting in 25 compounds with IC_{50} ranging from 1 to 100 nM and 11 compounds with IC_{50} ranging from 1 to 10 nM. Compound **5**, 5,6-diethyl-2-[2-*n*-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3*H*)-one, the most potent compound, has an excellent IC_{50} (1.6 nM) in vitro and a good efficacy in a rat model of erection. It thus provides a potential candidate for the further development into a new drug targeting PDE5.



1. INTRODUCTION

Cyclic nucleotide phosphodiesterase type 5 (PDE5), mostly distributed in smooth muscle and found in corpus cavernosum, heart, lung, platelets, prostate, urethra, bladder, liver, brain, and stomach,[1−4] is responsible for specifically cleaving the second messenger, cyclic guanosine monophosphate (cGMP).[5] Inhibitors of PDE5 prevent the hydrolysis of cGMP, leading to their potential to treat the diseases associated with low cGMP levels.[6] PDE5 is thus a prime target for the development of inhibitors which have served as drugs in the treatment of male erectile dysfunction (ED) and cardiovascular diseases. For example, sildenafil, the prototypical member of the PDE5 inhibitors, was approved by FDA in 1998 as the first oral medicine for the treatment of ED[7] and was also launched for the treatment of pulmonary arterial hypertension (PAH) in 2005.[6,8−10] Two other agents, vardenafil and tadalafil, are also used to treat ED and PAH.[4,11] Moreover, tadalafil has received the FDA approval for the treatment of lower urinary tract symptoms (LUTS) in men with benign prostatic hyperplasia (BPH). Another inhibitor, avanafil, as a second-generation PDE5 inhibitor, was also approved for the treatment of ED. Despite deficiencies with these inhibitors,[12−15] such as the lack of sufficient selectivity against other PDE isozymes, most notably PDE6 or PDE11,[16] it has been proved that the inhibition of PDE5 is an effective therapy for the diseases mentioned above and there is continuing interest in discovering novel PDE5 inhibitors. Additionally, the medical application of PDE5 inhibitors for other diseases such as stroke, Raynaud's disease, overactive bladder, and premature ejaculation has also been indicated.[2,17]

The solved crystal structures of the PDE5 catalytic domain in complex with different inhibitors have revealed the characteristic binding interactions of inhibitors with the enzyme. These include mono- or bidentate hydrogen bonds (H-bonds) between inhibitors and the residue Q817, hydrophobic interactions, in particular the $\pi-\pi$ stacking interactions between the aromatic rings of inhibitors and the hydrophobic clamp mainly composed of residues V782 and F820, and occasionally interactions also formed between inhibitors and two metal ions (Zn^{2+} and Mg^{2+} in most cases) coordinated at the active site of the catalytic domain.[9,18−21] For example, in the crystal structure of PDE5/sildenafil (pdb codes 1UDT and 2H42), bidentate H-bonds are formed between the amide moiety of the pyrazolopyrimidinone of sildenafil and the side chain of Q817. A third H-bond is formed between the N2 atom of the pyrazol ring and a water molecule that simultaneously forms H-bonds with the side chain of Y612, the main-chain of D764, and a water molecule coordinating to Zn^{2+} (Figure 1A). The pyrazole ring as well as the ethoxyphenyl group of sildenafil also interact with residues V782, L785, Y612, F820, F786, A783, and L804, in particular, including a face-to-face $\pi-\pi$ stacking interaction with the phenyl ring of F820.[19,21] All these available crystal structures associated with the pharmaco-

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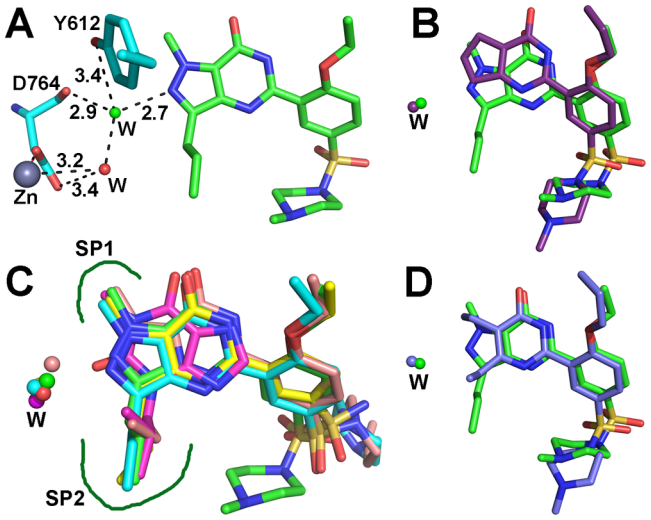
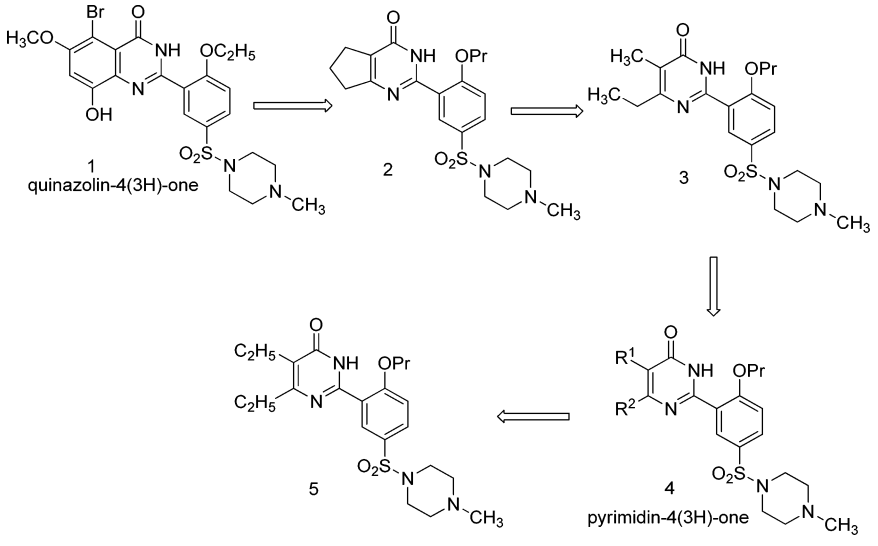


Figure 1. A crucial water molecule in the crystal structures of PDE5 in complex with different inhibitors. The complex structures were superimposed by fitting all the Cα atoms of the enzyme. (A) The H-bond interactions of the water molecule with sildenafil, side chain residues, and the other water molecule in the complex structure of PDE5/sildenafil (pdb code 2H42). (B) Superimposition of compound **2** and sildenafil as well as the crucial water molecule. (C) Superimposition of inhibitors in five complex structures with pdb codes 1UDT (cyan), 2H42 (green), 1RKP (magenta), 3BJC (yellow), and 3B2R (wheat). The water molecules at the same location in these five complex structures as well as in the apo structure (pdb code 2H40, red) are shown as colored balls. SP1 and SP2 represent two subpockets that interact with substituents of the pyrazol, pyrrole, or other heterocyclic rings of the inhibitors. (D) Superimposition of compound **5** with sildenafil as well as the key water molecule.

logical data enable us to carry out structure-based discovery or design of more potent and pharmacologically favorable inhibitors of PDE5.

We previously designed and synthesized a series of quinazolin-4(3*H*)-ones by introducing the phenyl ring influenced by the structure of sophoflavescenol instead of the pyrazol ring in sildenafil so as to improve the hydrophobic interactions with the residues of PDE5 and reduce or cancel the interactions of the inhibitor with the metal ions. This finally resulted in inhibitors of PDE5 such as compound **1** (Scheme 1) with similar potency to sildenafil but improved selectivity of PDE5 over PDE6.[22] To reduce the size of the compound but still keep its the hydrophobic interactions with the enzyme, we designed and synthesized compound **2**, bearing a fused cyclopentene ring instead of the sophoflavescenol-derived substituted phenyl ring in compound **1**. However, it turns out that compound **2** displayed a dramatic loss in PDE5 affinity: only 24% inhibition at 1 μM compound concentration. We determined the crystal structure of PDE5 in complex with compound **2** and superimposed it to the structure of PDE5/sildenafil. As we can see from the superimposition of compound **2** and sildenafil in Figure 1B, the scaffold of compound **2** is similar to sildenafil but it is much simplified around the bicyclic rings. Because of its hydrophobic character, the cyclopentene ring in compound **2** binds to the subpocket (SP1) created primarily by residues L765, I768, and F820, resulting in a shift of the whole compound, thus breaking its perfect $\pi-\pi$ stacking interactions with F820 compared to the binding of sildenafil (Figure 1B,C, Figure 2C). However, it is surprising to see that the water molecule which forms H-bonds with both residues (Y612 and D764) and the water molecule coordinating to Zn^{2+} also appear in the complex structure of PDE5/**2**, although there is no H-bond formed between compound **2** and the water molecule (Figure 1B). We also find this water molecule in the apo structure of PDE5 (pdb code 2H40) and five other crystal structures of PDE5 in complex with the inhibitors which have a similar scaffold structure and binding position as the pyrazolopyrimidinone of sildenafil. These structures are PDE5 in complex with sildenafil (pdb codes 1UDT and 2H42), IBMX (pdb code 1RKP), 5-ethoxy-4-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo-[4,3-*d*]pyrimidin-5-yl)thiophene-2-sulfonamide (pdb code 3BJC), and vardenafil (pdb code 3B2R) (Figure 1C). The resolution of these five selected complex structures is all above 2.5 Å in order to see the key water molecules that H-bond with the inhibitors as well as Y612 and D764. It is notable that a water molecule is always present in the structures and that the hydrophobic substituents on the pyrazol, pyrrole, or other heterocyclic ring occupy the same subpockets SP1 and SP2

Scheme 1. Discovery of Monocyclic Pyrimidinones As Novel Inhibitors of PDE5



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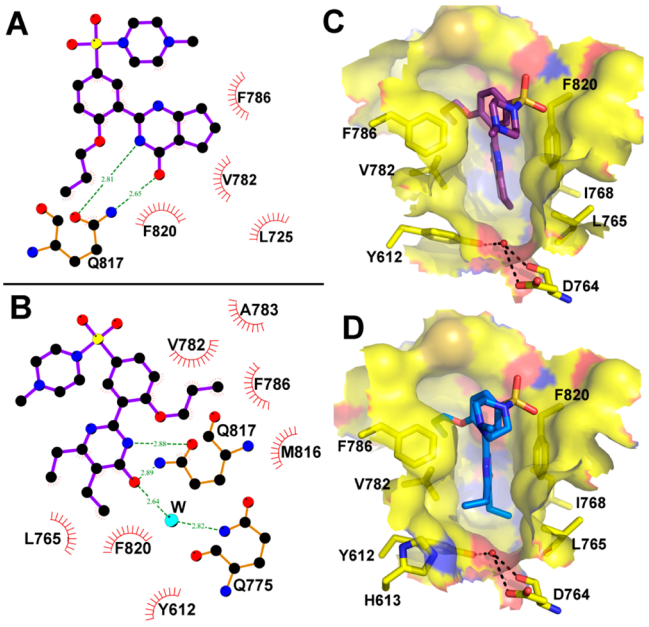

Figure 2. Interactions between two compounds (**2** and **5**) and PDE5 in crystal structures of their complexes. (A,B) LIGPLOT representations of the interactions of compounds **2** (A) and **5** (B) with the enzyme. (C,D) Molecular surface of the binding pockets for compounds **2** (C) and **5** (D) in their complex structures with the PDE5 catalytic domain. The crucial water molecule is shown as a red ball, and the H-bonds formed between it and the residues are labeled with black dash lines.

(Figure 1C). This indicates that the binding of the water molecule to the enzyme is strong and acts essentially as a residue of the enzyme. H-bonding of the polar atom in the inhibitor with this water molecule increases its binding affinity to the enzyme. Meanwhile, the hydrophobic substituents on the heterocyclic ring interact with the two subpockets nearby. Accordingly, the hydrophobic cyclopentene ring of compound **2** has to bind into the subpocket SP1 so as to avoid a close contact with this water molecule.

These structural insights inspired us to design and synthesize compound **3**, opening the cyclopentene ring and retaining only a planar monocyclic structure in the compound so as to enable the two hydrophobic substituent groups on the pyrimidin-4(3*H*)-one ring to fit into the two subpockets SP1 and SP2. Fortunately, compound **3** exhibited a significant increase in the potency to PDE5 compared to that of compound **2**. After that, we designed and synthesized a series of substituted pyrimidones represented by a general structure of **4** to systematically study the monocyclic pyrimidones as inhibitors of PDE5. Among these compounds, compound **5** has an excellent IC_{50} (1.6 nM), good selectivity (29-fold) over PDE6 in vitro, and good efficacy in a rat model of erection. The binding position of compound **5** in its crystal structure with PDE5 is superimposed well on that of sildenafil as shown in Figure 1D, which proved that the ring-opening could fully overcome the disadvantages of the cyclopentene ring in compound **2**, shift the whole compound back to the same binding location as sildenafil, and thus recover or even improve the potency of the compound against PDE5. Accordingly, our study not only obtained more potent inhibitors of PDE5 but also provided a novel scaffold for further selectivity and metabolism optimization of PDE5 inhibitors. This study therefore presents a good example of the structure-based discovery of potent PDE5 inhibitor.

2. CHEMISTRY

We describe herein the synthesis of novel inhibitors of PDE5 bearing a monocyclic pyrimidinone core. To gain a rapid exploration of SAR, two different synthetic routes for bulk preparation of two series of designed derivatives were applied. The synthetic approach to obtain 2-phenylpyrimidin-4(3*H*)-ones **2**, **3**, **5**, and **9**−**31** is outlined in Scheme 2. Compound **7**, 2-propoxy-5-(4-methylpiperazin-1-yl) sulphonylbenzamidine, is the key intermediate, and the synthesis of this compound was reported preciously.[23] The reaction of **7** with diethyl malonate, ethyl cyanoacetate, and the appropriate *β*-ketoesters **8**, which were either commercially available or prepared by the procedure described in the literature,[24] in DMF with the presence of anhydrous potassium carbonate at 100 °C, to obtain substituted pyrimidin-4(3*H*)-ones. In addition, compound **9** was acetylated using acetic anhydride to yield compound **11**, and halogenation of compounds **13** and **15** resulted in compounds **21**−**26**.

The effect of the different substituent at the 2′-position of the phenyl ring of pyrimidin-4(3*H*)-ones was investigated by synthesizing compounds **36**−**39** using an alternative route outlined in Scheme 3. The intermediates, compounds **34**, prepared in the manner described in Scheme 2, were halogenated, then treated with chlorosulfonic acid, and finally followed by *N*-methyl piperazine, resulting in the compounds **36**−**39**.

3. RESULTS AND DISCUSSION

3.1. Structure Determination of Compounds 2 and 5 in Complex with the PDE5 Catalytic Domain. The crystal structures of compounds **2** and **5** in complex with the catalytic domain of PDE5 have been determined by soaking the compounds into the apo PDE5 crystals (space group, $P3_121$), which diffracted to 2.4, and 2.28 Å, respectively (Supporting Information Table S1). The (F_o − F_c) difference electron-density maps contoured at 3.0 σ for compounds **2** and **5** in two crystal structures were shown in Supporting Information Figure S1. Similar to the binding of sildenafil with PDE5, both compounds fitted into the substrate binding pocket located in the center of the C-terminal helical bundle domain of PDE5. However, as mentioned above, the orientation of the two compounds within the binding pocket is different (Figure 1B,D) and their interactions are different too. The program LIGPLOT was used to plot the interactions between the compounds and PDE5 (Figures 2A,B).[25] Compound **2** only formed the key bidentate H-bonds with residue Q817, while besides these H-bonds compound **5** also interacts with residue Q775 through a water molecule. Compound **5** formed hydrophobic interactions with multiple residues, Y612, L765, V782, A783, F786, M816, and F820 (Figure 2B). The pyrimidinone ring of **5** is located just between the hydrophobic clamp composed of V782 and F820 and has perfect $\pi-\pi$ stacking interactions with the phenyl ring of F820 (Supporting Information Figure S2). Two ethyl groups on the pyrimidinone ring simultaneously occupy two subpockets SP1 (created primarily by residues L765, I768, and F820) and SP2 (created primarily by residues Y612, H613, V782, and F786) (Figure 2D). However, in the complex of PDE5/**2**, as discussed above, the hydrophobic cyclopentene ring of the compound has to

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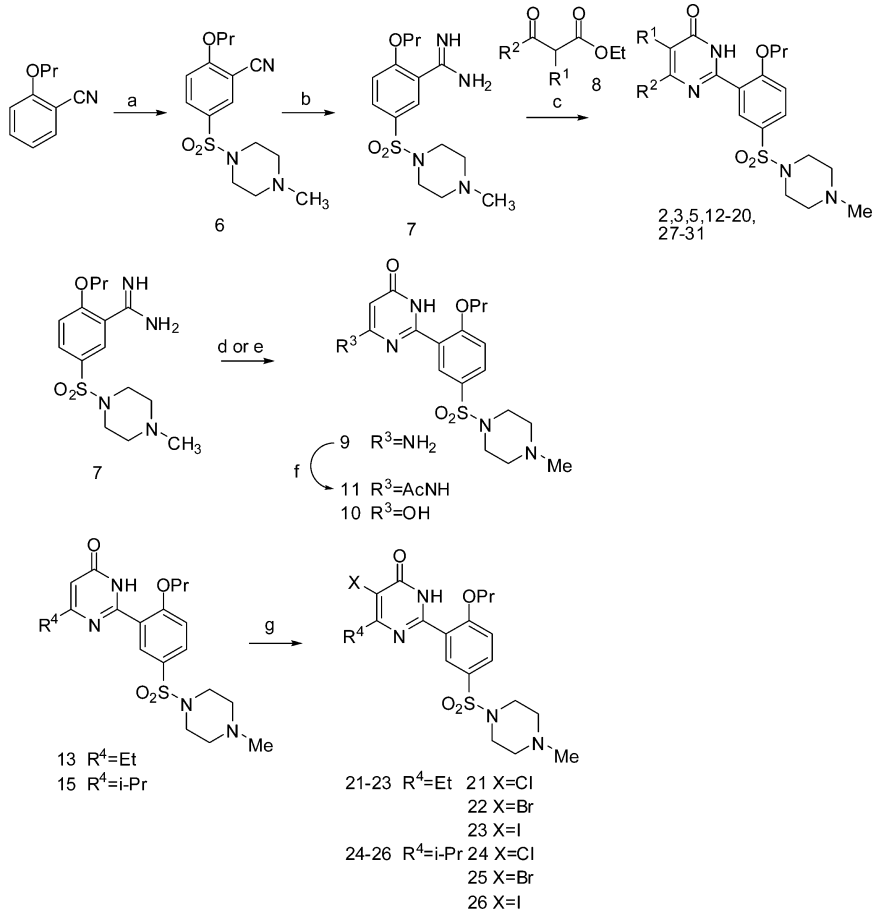
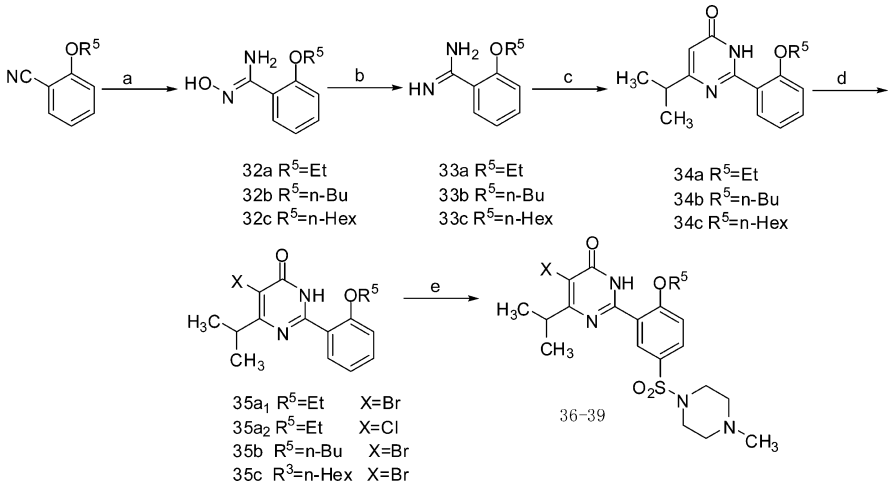
Scheme 2. Synthesis of Compounds with Monocyclic Pyrimidinone Core[a]



[a]Reagents and conditions: (a) (i) HSO_3Cl, 0 °C, (ii) *N*-methyl piperazine, Et_3N, DCM, 0 °C; (b) LiHMDS, THF, room temp; (c) K_2CO_3, DMF, 100 °C; (d) ethyl cyanoacetate, K_2CO_3, DMF, 100 °C; (e) diethyl malonate, K_2CO_3, DMF, 100 °C; (f) Ac_2O, 80 °C; (g) Cl_2, Et_3N, DCM, 0 °C, or Br_2, Et_3N, DCM, 0 °C, or I_2, $AgNO_3$, MeOH, room temperature.

Scheme 3. Synthesis of Compounds 36−39[a]



[a]Reagents and conditions: (a) hydroxylamine hydrochloride, K_2CO_3, methanol, and water; (b) H_2 (3 MPa)/Pd−C; (c) ethyl isobutyrylacetate, K_2CO_3, DMF, 100 °C; (d) Cl_2 (g), Et_3N, DCM, 0 °C, or Br_2, Et_3N, DCM, 0 °C; (e) (i) HSO_3Cl, 0 °C, (ii) *N*-methyl piperazine, Et_3N, DCM, 0 °C.

bind to the subpocket SP1 in order to avoid close contact with the crucial water molecule, which forms H-bonds with two residues and another water molecule coordinating to Zn^{2+} at the active site of the enzyme (Figure 1B). The interaction at SP1 results in a shift of the whole compound and thereby breaks the perfect $\pi-\pi$ stacking interactions between the pyrimidinone ring and F820 seen in both complexes of PDE5/**5** and PDE5/sildenafil. Consequently, hydrophobic interactions

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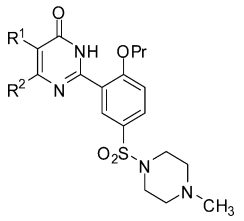
were only formed between compound **2** and four residues L725, V782, F786, and F820 (Figure 2A). Compound **2** formed less hydrophobic and H-bond interactions with PDE5, which leads to its much lower potency (only 24% inhibition of PDE5 at 1 μM concentration) than compound **5** (IC_{50}, 1.6 nM). Therefore, the structural analysis of these two compounds together with other inhibitors including sildenafil binding with PDE5 provide new insights into the substrate binding pocket of the catalytic domain. Consideration of the critical water molecule and the two distinct subpockets provides a rationale for designing more potent PDE5 inhibitors with new scaffold such as the monocyclic pyrimidinone.

3.2. SAR of Substitution at the 6-Position of the 4(3*H*)-Pyrimidinone Ring. The initial investigation on the structure−activity relationship (SAR) of the pyrimidin-4(3*H*)-one derivatives was carried out by substituting the 6-position of the pyrimidinone ring and fixing the 5-[(4-methyl-1-piperazinyl)sulfonyl]-2-propoxyphenyl ring. The inhibition activities of these substituted compounds to PDE5 were presented in Table 1. Compounds **9** and **10** with an amino and a hydroxyl group, respectively, at the 6-position of the 4(3*H*)-pyrimidinone ring showed little inhibition of PDE5, while compound **11** bearing an acetamido group had a great increase in potency, with an IC_{50} of 450 nM, suggesting that a hydrophilic substitution at this position is unfavorable to the interaction of inhibitors with the residues in the substrate binding pocket. After the hydrophilic group was replaced by other hydrophobic groups, the resulted compounds **12**−**16** displayed more potent inhibition activity to PDE5 than those of **2**, **9**, and **10**, which is consistent with our structural understanding mentioned above that the substitute on the 6-position of the pyrimidinone ring tends to form hydrophobic interactions with the enzyme and it indeed binds into the SP2 subpocket, which has been seen in the PDE5/**5** complex structure. Compounds **13** and **15** showed higher PDE5 inhibitory activity than **12**, **14**, and **16**, indicating that the length of alkyl maybe influence the interaction with the subpocket and the 2-carbon substituent such as an ethyl group or *i*-propyl group may be suitable for the pocket. Furthermore, replacing the alkyl group with a phenyl group resulted in a decrease in potency (compound **17**), which also implies that the subpocket is more favorable in holding an alkyl substituent than an aromatic ring. In the case of compound **18**, a significant loss of potency in comparison with that of **12** might be ascribed to the partial loss of the stacking interaction of the phenyl ring of F820 with the pyrimidinone moiety because of the lack of electron in the pyrimidinone ring caused by the substitution of the strongly electron-withdrawing trifluoromethyl group in this compound.

Table 1. SAR of Substitution at the 6-Position of the 4(3*H*)-Pyrimidinone Ring

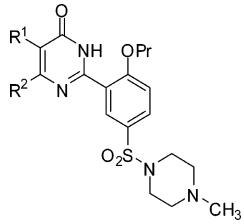


Compound	R^1	R^2	IC_{50}(nM)
Sildenafil	-	-	3.9
2			>1000
9	H	NH_2	>2000
10	H	OH	>2000
11	H	AcNH	450
12	H	Me	71
13[a]	H	Et	52
14	H	n-Pr	65
15	H	i-Pr	31.5
16	H	i-Bu	88
17	H	Ph	104
18	H	CF_3	312

[a]Compound has been reported in our previous study (ref 23).

3.3. SAR of Substitution at the 5-Position of the 4(3*H*)-Pyrimidinone Ring. On the basis of the above results, we decided to retain the 2-carbon chain, the ethyl or *i*-propyl group, at the 6-position while surveying various 5-substituted pyrimidin-4(3*H*)-ones, and the results are summarized in Table 2. The introduction of an acetylamino group at the 5-position of pyrimidin-4(3*H*)-one drastically decreased the potency in comparison with that of **13**. It was quickly discovered that introducing a halogen atom except for fluorin at the 5-position led to a remarkable increase in potency comparable to or even higher than that of **13** and **15**. Among them, the iodo derivatives, compounds **23** and **26**, have the highest IC_{50} of 7.2 nM and 5.8 nM, respectively. Most significantly, we find that

Table 2. SAR of Substitution at the 5-Position of the 4(3*H*)-Pyrimidinone Ring



compd	R^1	R^2	IC_{50} (nM)
sildenafil			3.9
19	AcNH	Et	>2000
20[a]	F	Et	91
21[a]	Cl	Et	36
22[a]	Br	Et	13
23[a]	I	Et	7.2
24	Cl	*i*-Pr	6.6
25	Br	*i*-Pr	7.2
26	I	*i*-Pr	5.8
3	Me	Et	12.5
5	Et	Et	1.6
27	*n*-Pr	Et	34.3
28	Me	*i*-Pr	4.8
29	Et	*i*-Pr	3.0
30	Et	Me	13.7
31	Et	*n*-Pr	4.8

[a]Compounds have been reported in our previous study (reference 23).

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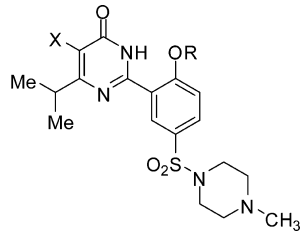
compound **5** with an ethyl group at both the 6- and 5-position of the pyrimidin-4(3*H*)-one ring exhibited better PDE5 inhibitory activity than sildenafil and showed the highest IC_{50} (1.6 nM) among all the compounds reported in the present study. Compounds **3** and **27** with a shorter and longer alkyl group than 2-carbon one, respectively, both had less inhibitory activity to PDE5 than that of **5**, indicating that the subpocket of the enzyme to accommodate the substituted group at the 5-position of the pyrimidin-4(3*H*)-one ring is also favorable in interaction with an 2-carbon alkyl group, which is verified by the crystal structure of PDE5/**5** complex. Although the introduction of an *i*-propyl group at the 6-position of the pyrimidinone ring in compound **15** resulted in better inhibitor activity than that of **13** with an ethyl group at the same position, compound **29** with an *i*-propyl group at the 6-position of the pyrimidinone ring showed a potency 2-fold less than **5**. Subsequently, the ethyl group was selected for the 5-position substituent and the methyl and *n*-propyl groups were individually chosen for the substituent at the 6-position. The compounds **29**, **30**, and **31** had ∼2-fold, 8-fold, and 3-fold, respectively, loss in potency in comparison with that of **5**. Therefore, the SAR results presented above indicate that the substituent at both the 5- and 6-position of the pyrimidin-4(3*H*)-one moiety are pivotal for determining the inhibition potency of the compounds to PDE5 and, consequently, the 5, 6-diethyl-pyrimidin-4(3*H*)-one showed the strongest inhibition potency among the series of pyrimidin-4(3*H*)-one analogues.

Using compounds **13** (H), **20** (F), **21** (Cl), **22** (Br), and **23** (I), we have previously reported that the halogen bond formed between the halogen atom of the compound and the hydroxyl oxygen atom of Y612 in the active site of PDE5 plays an important role in binding of the inhibitors with PDE5.[23] The order of halogen-binding contribution to IC_{50} of these compounds to PDE5 is **23** (I) > **22** (Br) > **21** (Cl) > **20** (F). Here, the chemical structure of compound **5** is similar to those of the four compounds except the substitution at the 5-position of the 4(3*H*)-pyrimidinone ring is an ethyl group instead of the halogen atoms. The IC_{50} of compound **5** is even higher than that of compound **23**. It is suggested that the hydrophobic interactions of the ethyl group of **5** with the subpocket SP2, mainly composed of the residues F786, V782, Y612, and H613, compensate the missed halogen bonding interactions in PDE5/**5** complex, contributing greatly to the binding affinity of compound **5** with PDE5. It is thereby concluded that the optimization of the substitutions at the 5-position of the 4(3*H*)-pyrimidinone is important for the potency of the pyrimidin-4(3*H*)-ones.

3.4. SAR of Substitution at the 2′-Position of the Phenyl Ring. In parallel to establishing SAR at the 6- and 5-position substitutions of the pyrimidinone moiety, we devoted a part of our effort to evaluating the effect of the *o*-alkyl side-chain at the 2′-position of the phenyl ring on its hydrophobic interaction with the enzyme. As shown in Table 3, it is clear that a 3-carbon chain in compounds **24** and **25** resulted in high inhibition potency to PDE5. Therefore, the *n*-propoxyl group is selected for the substitution at the 2′-position of the phenyl ring of the inhibitors, which contributes the proper hydrophobic interaction with PDE5.

3.5. Selectivity and in Vivo Study of Compound 5. The inhibitions of compound **5** versus 11 PDEs were measured by using sildenafil as a control (Table 4). The inhibition of the compound to PDE2, PDE3, PDE4, PDE7A1, PDE8A1, PDE9A2, and PDE10A2 is very weak (IC_{50} > 10000 nM).

Table 3. SAR of Substitution at the 2′-Position of the Phenyl Ring



compd	X	R	IC_{50} (nM)
sildenafil			3.9
25	Br	*n*-Pr	7.2
36	Br	Et	8.5
37	Br	*n*-Bu	41
38	Br	*n*-Hex	58
24	Cl	*n*-Pr	6.6
39	Cl	Et	12.6

Table 4. Inhibition Selectivity of Compound 5 and Sildenafil over 11 PDEs

	IC_{50} (nM)	
PDEs	**5**	sildenafil
PDE5A	1.6	3.9
PDE1	741	230
PDE2	>10000	>10000
PDE3	>10000	7310
PDE4	>10000	5830
PDE6C	45.6	39.2
PDE7A1	>10000	4690
PDE8A1	>10000	>10000
PDE9A2	>10000	>10000
PDE10A2	>10000	>10000
PDE11A4	3360	4210

The IC_{50} of **5** to PDE11A4, PDE1, and PDE6C are 2127-, 469-, and 29-fold higher than the IC_{50} of this compound to PDE5A, respectively. The selectivity profile of sildenafil to the 11 PDEs is similar to that of compound **5**.

With excellent enzyme inhibition of PDE5 and also good selectivity over other PDEs in vitro, compound **5** was selected for further testing in vivo. The pharmacokinetic data of compound **5** is summarized in Table 5, and the profile generated based on these data is shown in Figure 3. After oral administration of a 10 mg/kg dose of **5** to male rats, a C_{max} of 468 ng/mL was obtained at 20 min, and the oral bioavailability of the compound was 10.0%, lower than that of sildenafil (23%).[26]

In vivo efficacy of compound **5** was evaluated in the rat model of erection (Figure 4). Intracavernous pressure (ICP) and arterial blood pressure (BP) were simultaneously monitored during electric stimulation before and after the oral administration of compound **5**. At a dose of 10 mg/kg, compound **5** significantly increased the ICP/BP value at 30 min, compared to vehicle. It showed a good efficacy as sildenafil did.

4. CONCLUSIONS

The structural analysis based on crystal structures of the PDE5 catalytic domain in complex with our compounds as well as other well-known PDE5 inhibitors revealed new characteristics

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Table 5. Pharmacokinetic Data of Compound 5 in Rats[a]

dose (mg/kg)	route	CL (L/h/kg)	V (L/kg)	MRT_{0-10h} (h)	AUC_{0-10h} (μg/L·h)	C_{max} (ug/L)	$t_{1/2}$ (h)	T_{max} (h)	F (%)
5	iv	1.17 ± 0.35	2.07 ± 0.13	0.81 ± 0.44	4474 ± 1147		1.29 ± 0.35		
10	oral			3.09 ± 0.48	895 ± 376	468 ± 300	3.94 ± 0.65	0.25 (0.25−0.5)	10.0

[a]Data are expressed as mean ± SD (n = 3), or median (range).

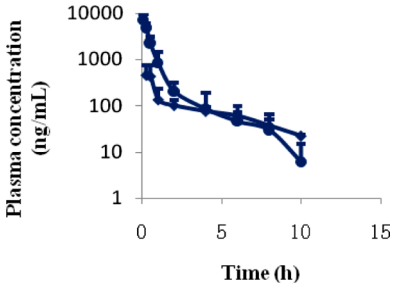

Figure 3. Mean plasma concentrations of compound **5** in male rat, after single po 10 mg/kg (◆) and iv 5 mg/kg (●). Data used to generate the profile are the mean ± SD from three male rats (individual samples at all time points).

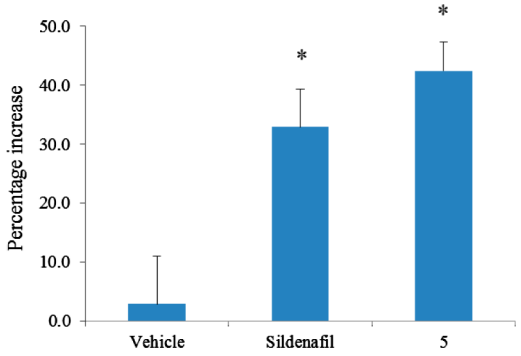

Figure 4. Effect of compound **5** on ICP/BP 30 min after po administration (10 mg/kg) in the rat erection model. Data are expressed as mean ± SD (n = 3). Percentage increase, $((ICP/BP)_{after} - (ICP/BP)_{before}) / (ICP/BP)_{before} 100\%$; *, compared with vehicle $P < 0.05$, student's t test.

of the inhibitor binding pocket. This includes the two separated subpockets and a crucial water molecule which enable us to design the monocyclic pyrimidin-4(3*H*)-one as a new scaffold to synthesize the novel, potent, and selective PDE5 inhibitors. Systematic exploration of SAR of the substitutions on the pyrimidine-4(3*H*)-one moiety resulted in the identification of 25 compounds with IC_{50} ranging from 1 to100 nM and 11 compounds with IC_{50} ranging from 1 to10 nM. In particular, the compound **5** has the best IC_{50}, with a value of 1.6 nM, among all these compounds. The crystal structures of PDE5/**5**, PDE5/**2**, and PDE5/sildenafil complexes confirmed our rationale to design simpler monocyclic pyrimidin-4(3*H*)-ones working as potent inhibitors of PDE5. The structures also revealed that simultaneously occupying two subpockets by two ethyl groups of monocyclic pyrimidin-4(3*H*)-ones compensated the H-bond formed with the crucial water molecule which has been seen in the PDE5/sildenafil complex but missed in pyrimidin-4(3*H*)-ones in complex with PDE5. Moreover, in vivo efficacy studies have revealed that compound **5** exhibited a significant effect on intracavernosal pressure in rats. Further investigation to improve the druggability of this compound through structural modification to seek a potential candidate for clinical study is ongoing.

5. EXPERIMENTAL SECTION

5.1. Chemistry. ^{1}H NMR spectra was determined using a Mercury 300 MHz, FT NMR spectrometer. HRMS were performed on a Finnigan MAT95 mass spectrometer. Reaction solvents were purchased and used without further purification. HPLC conditions were as follows: column, YMC-Pack CN 5 μM, 4.6 mm × 250 mm; solvent system, (A) MeCN, (B) 0.02 M KH_2PO_4 (pH 6.0); step gradient, time 0, 30% A; time 20 min, 70% A; stop time, 25 min; flow rate 1.0 mL/min; UV detection, 220 nm; injection volume, 5 μL; temperature, 30 °C. The purity of all target compounds was >95% as confirmed by HPLC.

*5.1.1. General Procedure for the Synthesis of **2**, **3**, **5**, **9**, **10**, **12**−**20**, and **27**−**31**. 2-[2-n-Propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]cyclopentapyrimid-4(3H)-one (**2**).* A mixture of 5-(4-methyl-piperazin-1-ylsulfonyl)-2-propoxybenzimidamide (340 mg, 1 mmol), ethyl 2-oxo-cyclopentane-1-carboxylate (312 mg, 2 mmol), and potassium carbonate (276 mg, 2 mmol) in *N*,*N*-dimethylformamide (5 mL) was stirred at 100 °C for 4 h. After cooled to room temperature, the reaction mixture was poured into ice water and extracted with dichloromethane (3 × 10 mL). The organic layer was washed with saturated brine, dried over anhydrous sodium sulfate, and concentrated. The residue was purified by flash column chromatography. After crystallization from ethyl acetate, compound **2** was obtained as a white solid (84 mg, yield 19%).

^{1}H NMR ($CDCl_3$) δ: 10.96 (1H, br), 8.84 (1H, d), 7.82 (1H, dd), 7.15 (1H, d), 4.25 (2H, t), 3.09 (4H, br), 2.93 (2H, t), 2.85 (2H, t), 2.51 (4H, br), 2.28 (3H, s), 2.16−1.98 (4H, m), 1.15 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{21}H_{29}N_4O_4S$ 433.1910, found 433.1916.

*5-Methyl-6-ethyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**3**).* Compound **3** was prepared in 63% yield from ethyl 2-methylpropionylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 11.02 (1H, br), 8.78 (1H, d), 7.80 (1H, dd), 7.14 (1H, d), 4.22 (2H, t), 3.10 (4H, br), 2.65 (2H, q), 2.54 (4H, br), 2.30 (3H, s), 2.09 (3H, s), 1.99 (2H, m), 1.23 (3H, t), 1.12 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{21}H_{31}N_4O_4S$ 435.2066, found 435.2066.

*5,6-Diethyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**5**).* Compound **5** was prepared in 65% yield from ethyl 2-ethylpropionylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR (DMSO-d_6) δ: 12.06 (1H, br), 7.88 (1H, d), 7.82 (1H, dd), 7.38 (1H, d), 4.12 (2H, t), 2.88 (4H, br), 2.57 (2H, q), 2.46 (2H, q), 2.36 (4H, br), 2.14 (3H, s), 1.75 (2H, m), 1.17 (3H, t), 1.04 (3H, t), 0.96 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{22}H_{33}N_4O_4S$ 449.2223, found 449.2227.

*6-Amino-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**9**).* Compound **9** was prepared in 38% yield from ethyl cyanoacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR (DMSO-d_6) δ: 11.25 (1H, br), 7.90 (1H, d), 7.81 (1H, dd), 7.38 (1H, d), 6.56 (2H, br), 5.00 (1H, s), 4.12 (2H, t), 2.87 (4H, br), 2.36 (4H, br), 2.14 (3H, s), 1.75 (2H, m), 0.97 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{18}H_{26}N_5O_4S$ 408.1706, found 408.1709.

*6-Hydroxy-2-[2-n-propoxy-5-(4-methyl-1-piperazinytsulfonyl)-phenyl]pyrimid-4(3H)-one (**10**).* Compound **10** was prepared in 41% yield from diethyl malonate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR (DMSO-d_6) δ: 8.09 (1H, d), 7.76 (1H, dd), 7.36 (1H, d), 4.52 (1H, s), 4.15 (2H, t), 2.87 (4H, br), 2.36 (4H,

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br), 2.13 (3H, s), 1.79 (2H, m), 1.01 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{18}H_{25}N_4O_5S$ 409.1546, found 409.1549.

*6-Methyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**12**).* Compound **12** was prepared in 62% yield from ethyl acetoacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 11.03 (1H, br), 8.83 (1H, d), 7.85(1H, dd), 7.16 (1H, d), 6.23 (1H, s), 4.25 (2H, t), 3.07 (4H, br), 2.48 (4H, br), 2.35 (3H, s), 2.26 (3H, s), 2.03 (2H, m), 1.14 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{19}H_{27}N_4O_4S$ 407.1753, found 407.1744.

*6-Ethyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**13**).* Compound **13** was prepared in 60% yield from ethyl propionylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR (DMSO-d_6) δ: 7.89 (1H, d), 7.84 (1H, dd), 7.39 (1H, d), 4.15 (2H, t), 2.89 (4H, br), 2.45 (2H, q), 2.36 (4H, br), 2.16 (3H, s), 1.75 (2H, m), 1.13 (3H, t), 0.96 (3H, t). HRMS (ESI) calcd [M + Na]$^+$ for $C_{20}H_{29}N_4O_4NaS$ 443.1729, found 443.1736.

*6-n-Propyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**14**).* Compound **14** was prepared in 58% yield from ethyl butyrylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 11.01 (1H, br), 8.85 (1H, d), 7.85 (1H, dd), 7.15 (1H, d), 6.22 (1H, s), 4.25 (2H, t), 3.07 (4H, br), 2.56 (2H, t), 2.48 (4H, br), 2.26 (3H, s), 2.03 (2H, m), 1.74 (2H, m), 1.14 (3H, t), 0.98 (3H, t). HRMS (ESI) calcd [M + Na]$^+$ for $C_{21}H_{30}N_4O_4SNa$ 457.1885, found 457.1898.

*6-Isopropyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**15**).* Compound **15** was prepared in 55% yield from ethyl isobutyrylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 10.99 (1H, br), 8.87 (1H, d), 7.86 (1H, dd), 7.15 (1H, d), 6.24 (1H, s), 4.25 (2H, t), 3.08 (4H, br), 2.83 (1H, m), 2.49 (4H, br), 2.26 (3H, s), 2.03 (2H, m), 1.26 (6H, d), 1.15 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{21}H_{31}N_4O_4S$ 435.2066, found 435.2069.

*6-Isobutyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**16**).* Compound **16** was prepared in 65% yield from ethyl isovalerylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 11.02 (1H, br), 8.86 (1H, d), 7.86 (1H, dd), 7.15 (1H, d), 6.19 (1H, s), 4.25 (2H, t), 3.07 (4H, br), 2.48 (4H, br), 2.44 (2H, d), 2.26 (3H, s), 2.13 (1H, m), 2.01 (2H, m), 1.15 (3H, t), 0.96 (6H, d). HRMS (ESI) calcd [M + H]$^+$ for $C_{22}H_{33}N_4O_4S$ 449.2223, found 449.2210.

*6-Phenyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimid-4(3H)-one (**17**).* Compound **17** was prepared in 67% yield from ethyl benzoylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR (DMSO-d_6) δ: 12.44 (1H, br), 8.10−8.05 (2H, m), 8.01 (1H, d), 7.87 (1H, dd), 7.53−7.47 (3H, m), 7.43 (1H, d), 6.91 (1H, s), 4.15 (2H, t), 2.92 (4H, br), 2.37 (4H, br), 2.14 (3H, s), 1.76 (2H, m), 0.95 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{24}H_{28}N_4O_4S$ 469.1910, found 469.1916.

*6-Trifluoromethyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**18**).* Compound **18** was prepared in 37% yield from ethyl 4,4,4-trifluoroacetoacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 8.84 (1H, d), 7.92 (1H, dd), 7.19 (1H, d), 6.75 (1H, s), 4.29 (2H, t), 3.08 (4H, br), 2.49 (4H, br), 2.27 (3H, s), 2.04 (2H, m), 1.17 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{19}H_{24}F_3N_4O_4S$ 461.1470, found 461.1470.

*5-Acetamido-6-ethyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**19**).* Compound **19** was prepared in 42% yield from ethyl 2-acetamidopropionylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR (DMSO-d_6) δ: 12.48 (1H, br), 9.22 (1H, br), 7.88 (1H, d), 7.84 (1H, dd), 7.39 (1H, d), 4.13 (2H, t), 2.91 (4H, br), 2.43 (2H, q), 2.40 (4H, br), 2.16 (3H, s), 2.02 (3H, s), 1.76 (2H, m), 1.13 (3H, t), 0.96 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{22}H_{32}N_5O_5S$ 478.2124, found 478.2126.

*5-Fluoro-6-ethyl-2-[2-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**20**).* Compound **20** was prepared in 72% yield from methyl 2-fluoro-3-oxopentanoate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 8.80 (1H, d), 7.86 (1H, dd), 7.16 (1H, d), 4.26 (2H, t), 3.09 (4H, br), 2.74 (2H, q), 2.51 (4H, br), 2.28 (3H, s), 2.03 (2H, m), 1.28 (3H, t), 1.15 (3H, t). HRMS (ESI) calcd [M + Na]$^+$ for $C_{20}H_{27}N_4O_4NaSF$ 461.1635, found 461.1630.

*5-Propyl-6-ethyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**27**).* Compound **27** was prepared in 39% yield from ethyl 2-propylpropionylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 10.97 (1H, br), 8.84 (1H, d), 7.82 (1H, dd), 7.13 (1H, d), 4.23 (2H, t), 3.07 (4H, br), 2.65 (2H, q), 2.52 (2H, t), 2.49 (4H, br), 2.26 (3H, s), 2.02 (2H, m), 1.55 (2H, m), 1.26 (3H, t), 1.15 (3H, t), 0.99 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{23}H_{35}N_4O_4S$ 463.2379, found 463.2379.

*5-Methyl-6-isopropyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**28**).* Compound **28** was prepared in 54% yield from ethyl 2-methylisobutyrylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 10.93 (1H, br), 8.86 (1H, d), 7.83 (1H, dd), 7.13 (1H, d), 4.24 (2H, t), 3.16 (1H, m), 3.08 (4H, br), 2.48 (4H, br), 2.26 (3H, s), 2.12 (3H, s), 2.02 (2H, m), 1.22 (6H, d), 1.15 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{22}H_{33}N_4O_4S$ 449.2223, found 449.2227.

*5-Ethyl-6-isopropyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**29**).* Compound **29** was prepared in 41% yield from ethyl 2-ethylisobutyrylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 10.95 (1H, br), 8.85 (1H, d), 7.82 (1H, dd), 7.13 (1H, d), 4.23 (2H, t), 3.15 (1H, m), 3.07 (4H, br), 2.58 (2H, q), 2.47 (4H, br), 2.25 (3H, s), 2.02 (2H, m), 1.23 (6H, d), 1.14 (3H, t), 1.12 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{23}H_{35}N_4O_4S$ 463.2379, found 463.2379.

*5-Ethyl-6-methyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**30**).* Compound **30** was prepared in 42% yield from ethyl 2-ethylacetoacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 11.00 (1H, br), 8.77 (1H, d), 7.80 (1H, dd), 7.12 (1H, d), 4.22 (2H, t), 3.05 (4H, br), 2.55 (2H, q), 2.48 (4H, br), 2.37 (3H, s), 2.25 (3H, s), 1.98 (2H, m), 1.13 (3H, t), 1.11 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{21}H_{31}N_4O_4S$ 435.2066, found 435.2068.

*5-Ethyl-6-propyl-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**31**).* Compound **31** was prepared in 38% yield from ethyl 2-ethylbutyrylacetate and 5-(4-methylpiperazin-1-ylsulfonyl)-2-propoxybenzimidamide following a similar procedure to that described for synthesis of **2**. ^{1}H NMR ($CDCl_3$) δ: 10.95 (1H, br), 8.82 (1H, d), 7.82 (1H, dd), 7.13 (1H, d), 4.24 (2H, t), 3.07 (4H, br), 2.63−2.55 (4H, m), 2.49 (4H, br), 2.27 (3H, s), 2.03 (2H, m), 1.74 (2H, m), 1.15 (3H, t), 1.14 (3H, t), 1.00 (3H, t). HRMS (ESI) calcd [M + H]$^+$ for $C_{23}H_{35}N_4O_4S$ 463.2379, found 463.2379.

*5.1.2. 6-Acetamido-2-[2-n-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3H)-one (**11**).* The compound **9** (0.20 g, 0.5 mmol) was suspended in acetic anhydride (5 mL), and the mixture was stirred at 100 °C for 1 h. The cooled reaction mixture was poured into ice water to generate a white solid. The solid was collected by filtration, washed with clear water (3 × 10 mL), and dried at 60 °C to give compound **11** (0.12 g, yield 54%). ^{1}H NMR (DMSO-d_6) δ: 12.16 (1H, br), 10.54 (1H, br), 7.92 (1H, d), 7.84 (1H, dd), 7.40 (1H, d), 6.89 (1H, s), 4.12 (2H, t), 2.88 (4H, br), 2.36 (4H, br),

2.14 (3H, s), 2.08 (3H, s), 1.74 (2H, m), 0.95 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{20}H_{28}N_5O_5S$ 450.1811, found 450.1811.

*5.1.3. General Procedure for the Synthesis of **21**–**26**. 5-Chloro-6-ethyl-2-[2-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]-pyrimidin-4(3H)-one (**21**).* Chlorine gas was bubbled into an ice-cold solution of **13** (100 mg, 0.24 mmol) and pyridine (40 μL, 0.5 mmol) in dichloromethane (10 mL) for 1 min. The resulting mixture was washed with 1N $Na_2S_2O_3$ (aq, 5 mL) and water (5 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. After crystallization from ethyl acetate, compound **21** was obtained as white crystals (93 mg, yield 85%). ^{1}H NMR ($CDCl_3$) δ: 11.25 (1H, br), 8.85 (1H, d), 7.87 (1H, dd), 7.16 (1H, d), 4.27 (2H, t), 3.08 (4H, br), 2.85 (2H, q), 2.49 (4H, br), 2.27 (3H, s), 2.03 (2H, m), 1.29 (3H, t), 1.15 (3H, t). HRMS (ESI) calcd $[M + Na]^+$ for $C_{20}H_{27}N_4O_4NaSCl$ 477.1339, found 477.1339.

*5-Bromo-6-ethyl-2-[2-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**22**).* Bromine (40 mg, 0.27 mmol) was added to an ice-cold solution of **13** (100 mg, 0.24 mmol) and pyridine (22 μL, 0.27 mmol) in dichloromethane (10 mL). The mixture was stirred for 15 min at 0 °C and washed with 1 N $Na_2S_2O_3$ (aq, 5 mL) and water (5 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. After crystallization from ethyl acetate, compound **22** was obtained as a white solid (107 mg, yield 90%). ^{1}H NMR ($CDCl_3$) δ: 11.12 (1H, br), 8.86 (1H, d), 7.88 (1H, dd), 7.16 (1H, d), 4.27 (2H, t), 3.09 (4H, br), 2.88 (2H, q), 2.50 (4H, br), 2.28 (3H, s), 2.03 (2H, m), 1.29 (3H, t), 1.15 (3H, t). HRMS (ESI) calcd $[M + Na]^+$ for $C_{20}H_{27}N_4O_4NaSBr$ 521.0834, found 521.0834.

*5-Iodo-6-ethyl-2-[2-propoxy-5-(4-methyl-1-piperazinylsulfonyl)-phenyl]pyrimidin-4(3H)-one (**23**).* Iodine (254 mg, 1 mmol) was added to a solution of **13** (420 mg, 1 mmol) and silver nitrate (170 mg, 1 mmol) in methanol (10 mL) at 0 °C. The mixture was stirred at room temperature for 30 min. After filtration, the filtrate was poured into water (25 mL) and extracted with dichloromethane (20 mL). The organic layer was washed with 1N $Na_2S_2O_3$ (aq, 5 mL) and water (5 mL), dried, and concentrated. After crystallization from ethyl acetate, **23** was obtained as a white solid (410 mg, 75%). ^{1}H NMR (CDCl3) δ: 11.12 (1H, br), 8.88 (1H, d), 7.88 (1H, dd), 7.16 (1H, d), 4.27 (2H, t), 3.10 (4H, br), 2.92 (2H, q), 2.52 (4H, br), 2.30 (3H, s), 2.03 (2H, m), 1.28 (3H, t), 1.16 (3H, t). HRMS (ESI) calcd $[M + Na]^+$ for $C_{20}H_{27}N_4O_4NaSI$ 569.0695, found 569.0704.

*5-Chloro-6-isopropyl-2-[2-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**24**).* Compound **24** was prepared in 72% yield from **15** following a similar procedure to that described for synthesis of **21**. ^{1}H NMR ($CDCl_3$) δ: 11.20 (1H, br), 8.87 (1H, d), 7.89 (1H, dd), 7.17 (1H, d), 4.28 (2H, t), 3.50 (1H, m), 3.10 (4H, br), 2.51 (4H, br), 2.28 (3H, s), 2.05 (2H, m), 1.27 (6H, d), 1.16 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{21}H_{30}N_4O_4SCl$ 469.1676, found 469.1676.

*5-Bromo-6-isopropyl-2-[2-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**25**).* Compound **25** was prepared in 68% yield from **15** following a similar procedure to that described for synthesis of **22**. ^{1}H NMR ($CDCl_3$) δ: 11.16 (1H, br), 8.86 (1H, d), 7.89 (1H, dd), 7.16 (1H, d), 4.28 (2H, t), 3.50 (1H, m), 3.09 (4H, br), 2.49 (4H, br), 2.27 (3H, s), 2.03 (2H, m), 1.26 (6H, d), 1.15 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{21}H_{30}N_4O_4SBr$ 513.1711, found 513.1715.

*5-Iodo-6-isopropyl-2-[2-propoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**26**).* Compound **26** was prepared in 62% yield from **15** following a similar procedure to that described for synthsis of **23**. ^{1}H NMR ($CDCl_3$) δ: 8.90 (1H, d), 7.89 (1H, dd), 7.16 (1H, d), 4.27 (2H, t), 3.46 (1H, m), 3.09 (4H, br), 2.49 (4H, br), 2.27 (3H, s), 2.04 (2H, m), 1.25 (6H, d), 1.16 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{21}H_{30}N_4O_4SI$ 561.1032, found 561.1035.

*5.1.4. 5-Bromo-6-isopropyl-2-[2-ethoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**36**).* A mixture of 2-ethoxybenzonitrile (20.5 g, 0.14 mol), potassium carbonate (38.5 g, 0.28 mol), and hydroxylamine hydrochloride (19.2 g, 0.28 mol) in a mixed solution of ethanol (100 mL) and water (100 mL) was refluxed for 10 h, and the ethanol was removed by vacuum distillation. After the residue was cooled, white solid **32a** (18.1 g, 72%) was separated out and collected by filtration.

The solid **32a** (18 g, 0.5 mol) was solved in acetic acid (200 mL) and degassed. A catalytic amount of 10% Pd/C was added, and the solution was placed on the shaker under 3 MPa hydrogen at 65 °C for 8 h. After the solvent was removed by vacuum distillation, the residue was **33a** acetate (13.2 g, 80%), which was not purified.

A mixture of **33a** acetate (9.4g, 42 mmol), ethyl isobutyrylacetate (7.3 g, 46 mmol), and potassium carbonate (11.6 g, 84 mmol) in *N*,*N*-dimethylformamide (80 mL) was stirred at 100 °C for 4 h. The mixture was then cooled to room temperature and taken up in water (400 mL), and the solid was collected by filtration and purified by recrystallization from ethyl acetic to yield **34a** (7.7 g, 71%) as white solid. ^{1}H NMR ($CDCl_3$) δ: 11.22 (1H, br), 8.52 (1H, dd), 7.48 (1H, t), 7.12 (1H, t), 7.03 (1H, d), 6.20 (1H, s), 4.29 (2H, q), 2.82 (1H, m), 1.59 (3H, t), 1.27 (6H, d).

Bromine (1.9g, 12 mmol) was added to an ice-cold solution of **34a** (3.2 g, 12 mmol) and pyridine (1 mL) in dichloromethane (150 mL). The mixture was stirred for 10 min at 0 °C and washed with 1 N $Na_2S_2O_3$ (aq, 45 mL) and water (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. After crystallization from ether–acetonitrile, **35a1** (3.3 g, yield 82%) was obtained. ^{1}H NMR ($CDCl_3$) δ: 8.53 (1H, d), 7.51 (1H, t), 7.13 (1H, t), 7.04 (1H, d), 4.32 (2H, q), 3.51 (1H, m), 1.59 (3H, t), 1.27 (6H, d).

Compound **35a1** (0.34 g, 1 mmol) was added portionwise to an ice-cold chlorosulfonic acid (2 mL). The resulting mixture was stirred for 2 h at 0 °C and then was added dropwise to crushed ice. The mixture was extracted by dichloromethane, and the organic layer was added dropwise to a solution of 1-methylpiperazine (0.11 g, 1.1 mmol) and triethylamine (1 mL) in dichloromethane (30 mL) at 0 °C. After stirred at 0 °C for 10 min, the reaction mixture was washed with water, dried over anhydrous sodium sulfate, and concentrated to give a crude product. After crystallization from ethyl acetate, **36** was yielded (0.38 g, 76%). ^{1}H NMR (DMSO-d_6) δ: 7.91 (1H, d), 7.85 (1H, dd), 7.39 (1H, d), 4.21 (2H, q), 3.36 (1H, m), 2.90 (4H, br), 2.36 (4H, br), 2.14 (3H, s), 1.35 (3H, t), 1.17 (6H, d). HRMS (ESI) calcd $[M + H]^+$ for $C_{20}H_{28}N_4O_4SBr$ 499.1015, found 499.1017.

*5.1.5. 5-Bromo-6-isopropyl-2-[2-butoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**37**).* Compound **37** was prepared from 2-butoxybenzonitrile following a similar procedure to that described for **36**. ^{1}H NMR (DMSO-d_6) δ: 7.89 (1H, d), 7.85 (1H, dd), 7.39 (1H, d), 4.14 (2H, t), 3.37 (1H, m), 2.90 (4H, br), 2.37 (4H, br), 2.14 (3H, s), 1.71 (2H, m), 1.35 (2H, m), 1.23 (3H, m), 1.15 (6H, d), 0.81 (3H, t). HRMS (ESI) calcd $[M + H]^+$ for $C_{22}H_{32}N_4O_4SBr$ 527.1328, found 527.1330.

*5.1.6. 5-Bromo-6-isopropyl-2-[2-hexoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**38**).* Compound **38** was prepared from 2-hexoxybenzonitrile following a similar procedure to that described for **36**. ^{1}H NMR (DMSO-d_6) δ: 7.89 (1H, d), 7.85 (1H, dd), 7.39 (1H, d), 4.14 (2H, t), 3.37 (1H, m), 2.90 (4H, br), 2.37 (4H, br), 2.14 (3H, s), 1.70 (2H, m), 1.35 (2H, m), 1.28–1.19 (4H, m), 1.15 (6H, d), 0.81 (3H, t). HRMS calcd $[M + H]^+$ for $C_{24}H_{36}N_4O_4SBr$ 555.1641, found 555.1639.

*5.1.7. 5-Chloro-6-isopropyl-2-[2-ethoxy-5-(4-methyl-1-piperazinylsulfonyl)phenyl]pyrimidin-4(3H)-one (**39**).* Chlorine gas was bubbled into an ice-cold solution of **34a** (0.52 g, 2 mmol) and pyridine (0.5 mL) in dichloromethane (20 mL) for 3 min. The resulting mixture was washed with 1N $Na_2S_2O_3$ (aq, 5 mL) and 1 M hydrochloric acid (5 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. After crystallization from ether–acetonitrile, **35a2** was obtained (0.57 g, 98%). ^{1}H NMR ($CDCl_3$) δ: 8.52 (1H, d), 7.50 (1H, t), 7.13 (1H, t), 7.04 (1H, d), 4.31 (2H, q), 3.49 (1H, m), 1.59 (3H, t), 1.27 (6H, d).

Compound **39** was prepared in 70% from **35a2** following a similar procedure to that described for **36**. ^{1}H NMR (DMSO-d_6) δ: 7.91 (1H, d), 7.84 (1H, dd), 7.39 (1H, d), 4.21 (2H, q), 3.36 (1H, m), 2.90 (4H, br), 2.37 (4H, br), 2.14 (3H, s), 1.34 (3H, t), 1.17 (6H, d). HRMS (ESI) calcd $[M + H]^+$ for $C_{20}H_{28}N_4O_4SCl$ 455.1520, found 455.1523.

5.2. Protein Purification and Crystallization. Production of the catalytic domain of recombinant human PDE5A1 followed the

protocols of Wang et al.[9] with certain modifications. Briefly, a cDNA fragment encoding PDE5A1 residues 535−860 was cloned into the vector pET15b and the protein was expressed in *Escherichia coli*. The expressed PDE5A1 was passed through a Ni-NTA column (Qiagen), subjected to thrombin cleavage, and further purified by Q-Sepharose and Superdex75 (GE). The recombinant PDE5A1 with a purity of >95% was concentrated to ∼10 mg/mL for further crystallization. Crystallization of apo PDE5A1 was performed at room temperature using the hanging drop vapor-diffusion method by mixing equal volumes of the protein and of the precipitant (19−20% (w/v) PEG 3350/200 mM $MgSO_4$/100 mM Tris-HCl at pH 7.5. To obtain a crystalline complex with **2** and **5**, a 5 mM solution of the inhibitor in DMSO was diluted 10-fold, to 0.5 mM, in the precipitant solution so as to generate a soaking drop. The crystal was transferred into the soaking drop and left for 24 h prior to data collection.

5.3. Structure Determination and Refinement. Data were collected at 100 K on beamline BL17U at the Shanghai Synchrotron Radiation Facility (SSRF) and were processed with the XDS[27] software package. The structures were solved by molecular replacement, using the program PHASER[28] with the search model of pdb code 1T9R.[29] The structure was refined with the simulated-annealing protocol implemented in the program PHENIX.[30] With the aid of the program Coot,[31] inhibitors, water molecules, and others were fitted into to the initial $F_o - F_c$ map. The complete statistics, as well as the quality of the two solved structures, are shown in Supporting Information Table S1.

5.4. Bioassay. The synthesized compounds were evaluated for their inhibitory activities against PDE5 isolated from rabbit platelet using [^{3}H]cGMP SPA kit. The experiments were detailed in the Supporting Information.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Data collection and refinement statistics, ($F_o - F_c$) difference electron-density maps contoured at 3.0 σ for compounds **2** and **5** in their complex structure with PDE5, superimposition of the binding modes of the compounds **2** and **5** within PDE5, PDE assays, in vivo efficacy in the rat model, pharmacokinetics, and HPLC of novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

PDE5, phosphodiesterase type 5; cGMP, cyclic guanosine monophosphate; ED, erectile dysfunction; PAH, pulmonary arterial hypertension; LUTS, lower urinary tract symptoms; BPH, benign prostatic hyperplasia; ICP, Intracavernous pressure; BP, blood pressure

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