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Exploration of the 5-bromopyrimidin-4(3*H*)-ones as potent inhibitors of PDE5

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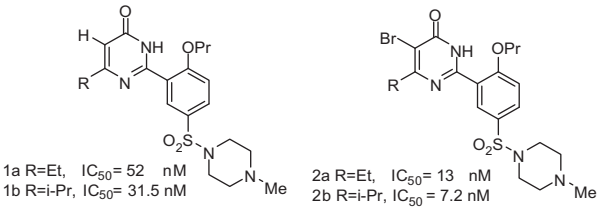
ABSTRACT

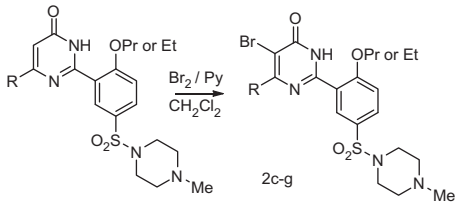
The substituents both at the 6-position of the 5-bromopyrimidinone ring and at the 5′-position of the phenyl ring of 5-bromopyrimidin-4(3*H*)-ones were explored. 5-Bromo-6-isopropyl-2-(2-propoxyphenyl)pyrimidin-4(3*H*)-one was identified as a new scaffold for potent PDE5 inhibitors. The crystal structures of PDE5/**2e** and PDE5/**10a** complexes provided a structural basis for the inhibition of 5-bromopyrimidinones to PDE5. In addition, it was also found that there is a great tolerance for the substitution at the 5′-position of the phenyl ring of 5-bormopyrimidinones and the resulted compound **13a** has the highest inhibition activity to PDE5 (IC_{50}, 1.7 nM).

Since sildenafil launched in 1998, inhibitors of PDE5 have been approved for the treatment of ED, PAH, and lower urinary tract symptoms (LUTS) in men with benign prostatic hyperplasia (BPH).[1–3] In addition, avanafil has received FDA approval to treat ED as a second generation PDE5 inhibitors in April 2012.[4] The clinical success of these inhibitors evidenced that the inhibition of PDE5 is an effective therapy for the diseases mentioned above, resulting in continuing interest in discovering novel PDE5 inhibitors.

In the course of our effort to seek novel inhibitors of PDE5 with improved potency and selectivity, we have disclosed the design, synthesis, and pharmacological evaluation of monocyclic pyrimidinones as novel inhibitors of PDE5.[2] It is interesting that introducing a halogen atom except for fluorine at the 5-position of the pyrimidinone ring led to a remarkable increase in potency compared with that of the 5-hydrogenpyrimidinones (Scheme 1).[5] It has been verified that the increase of potency is attributed to the halogen bond formed between the halogen atom of the compound and the hydroxyl oxygen atom of Y612 in the active site of PDE5.[5] Although the order of halogen-binding contribution to IC_{50} of these compounds to PDE5 is I > Br > Cl > F, the potency of bromo derivative is comparable to that of the iodo ones. Moreover, the bromo derivative is more drug-like than the iodo derivative. Hereupon, we decided to further examine the SAR of this series of 5-bromopyrimidinones as PDE5 inhibitors.



Scheme 1.



Scheme 2.

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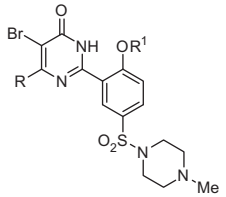
Table 1
SAR of substitution at the 6-position of 5-bromopyrimidinone ring

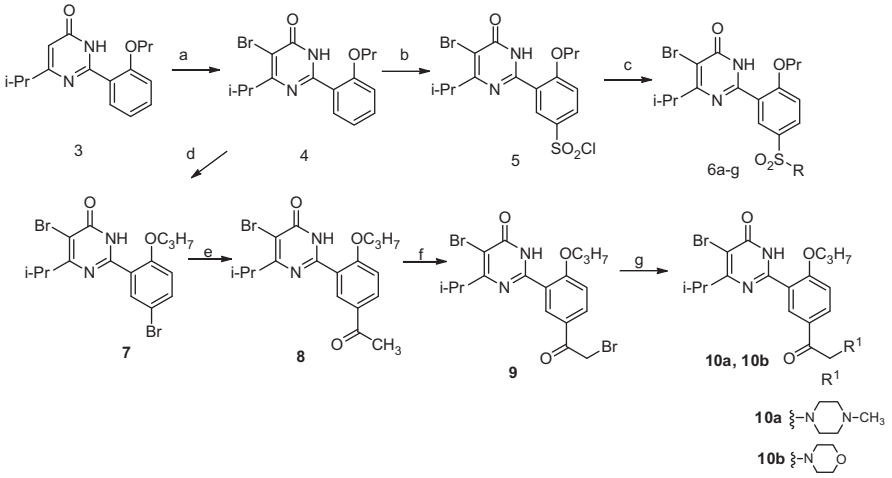


Compound	R^1	R	PDE5 IC_{50} (nM)
Sildenafil	—	—	3.9
2a[a]	*n*-Pr	Et	13
2b[a]	*n*-Pr	*i*-Pr	7.2
2c	*n*-Pr	Me	48
2d	*n*-Pr	*n*-Pr	10.2
2e	Et	*n*-Oct	77
2f	Et	Ph	74
2g	*n*-Pr	CF_3	272

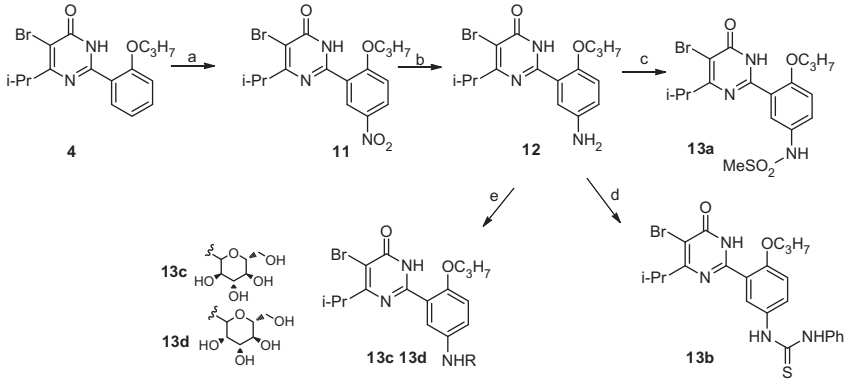
Data are the mean of two independent assays, each performed in duplicate.
[a] Compounds have been reported in our previous study.[2]

Firstly, we devoted our efforts to investigate the substituent at the 6-position of the 5-bromopyrimidinone ring, due to its potential to determine the potency of monocyclic pyrimidin-4(3*H*)-ones to PDE5 activity inhibition. The various types of substituents, such as alkyl, aryl, and the strongly electron-withdrawing trifluoromethyl, were introduced at this position. Synthesis of these compounds is outlined in Scheme 2. 5-Hydrogenpyrimidinones, which were synthesized following the procedure we previously reported,[2] were treated with bromine in the presence of pyridine in dichloromethane to obtain the 5-bromopyrimidinones.

The inhibition activities of these 5-bromopyrimidinones to PDE5 were presented in Table 1. The 5-bromo substituted compounds are more potent to PDE5 than the 5-hydrogen substituted ones. As shown in Scheme 1 and Table 1, the effect of substituents at the 6-position of the 5-bromopyrimidinones to the inhibition potency is similar to that of the 5-hydrogenpyrimidinones, and the compound **2b** bearing an isopropyl group at the 6-position showed the highest potency (IC_{50} = 7.2 nM). Additionally, we have evaluated the effect of the *O*-alkyl side-chain at the 2′-position of the phenyl ring on its hydrophobic interaction with the enzyme.[2] It was found that a 3-carbon chain at this position gives the best efficacy. On the basis of these results, we identified that compound **2b** is the most potent compound and measured the inhibition of **2b** to PDE5 as well as PDE6. The IC_{50} of compound **2b** to PDE6 is 7.4 nM, which is similar to the inhibition of the compound to PDE5. The selectivity of compound **2b** to PDE5 over PDE6 is thus much lower than that of 5,6-diethyl-2-[2-*n*-propoxy-5-(4-



Scheme 3. Reagents and conditions: (a) Br_2, Et_3N, DCM, 0 °C, 95%; (b) HSO_3Cl, 0 °C; (c) the corresponding amine moiety, Et_3N, DCM, 0 °C, 59–86%; (d) Br_2, AcOH, 60 °C, 76%; (e) (i) vinyl *n*-butyl ether, $Pd(OAc)_2$, bis(diphenylphosphino)butane, Et_3N; (ii) hydrochloric acid (10%); (f) Br_2, AcOH, 30 °C, 18%; (g) *N*-methylpiperazine or morpholine, Et_3N, 39–50%.



Scheme 4. Reagents and conditions: (a) H_2SO_4, HNO_3, 90%; (b) Fe, HCl, 82%; (c) CH_3SO_2Cl, Et_3N, 36%; (d) phenyl isothiocynate, Et_3N, 89%; (e) CH_3COOH, *n*-butanol, glucose or mannose, 21–35%.

methyl-1-piperazinylsulfonyl)phenyl]pyrimid-4(3*H*)-one.[2]
According to our previous study, modifying the sulfamide group may improve the selectivity over PDE6 of PDE5 inhibitors using sildenafil as a starting template.[6]

We fixed the 5-bromo-6-isopropyl-2-(2-propoxyphenyl)pyrimidin-4(3*H*)-one and focused on exploration of the effect of substituents at the 5′-position of the phenyl ring to obtain both potent and selective PDE5 inhibitors. The solved crystal structures of PDE5 catalytic domain in complex with the inhibitors also indicate that there might be a great tolerance for the substitution at this position.[7–10] We therefore synthesized three types of inhibitors bearing various series of substitutions and evaluated their bioactivities.

Compounds **6a**–**g** were synthesized as shown in Scheme 3. The key intermediate **4**, which was prepared following the procedure we previously reported,[2] was treated with chlorosulfonic acid, and then followed by the corresponding amine moiety, resulting in the compounds **6a**–**g**. The intermediate **7**, prepared through bromation of **4** in acetic acid at 60 °C,[11] was coupled with vinyl *n*-butyl ether by $Pd(OAc)_2$-catalyzed coupling reaction in the presence of bis(diphenylphosphino)butane and triethylamine, and then the resulted products were treated with hydrochloric acid (10%) to obtain **8**.[12] The solution of **8** and bromine in acetic acid was stirred for 3 h at 30 °C, giving the intermediate **9**.[13] Compounds **10a** and **10b** were obtained by the reaction of **9** with *N*-methylpiperazine or morpholine.[14]

The intermediate **4** was nitrated and hydrogenated to give **12**.[15] Compound **13a** was obtained by the sulfonylation of **12** employing methanesulfonyl chloride.[11]

The alcoholic solution of **12** was treated with triethylamine and phenylisothiocyanate to give **13b**.[16] Compounds **13c** and **13d** were formed through the glucosylation and mannosylation of the amine group in **12**, respectively (Scheme 4).[17]

The inhibition activities of those substituted compounds to PDE5 were summarized in Table 2. Although the value of IC_{50} varied in a wide range from 1.7 to 29.3 nM, there is at least one potent inhibitor (IC_{50} <10 nM) for each type of substituents at the 5′-position of the phenyl ring. This result is consistent with the previous suggestion that there is tolerance for such substitution. Two compounds, **6e** and **13a**, were chosen to test the selectivity of PDE5 over PDE6, and compound **13a** showed an excellent selectivity (PDE6/5 = 941).

The crystal structures of the catalytic domain of PDE5 in complex with compounds **2e** and **10a** were determined by soaking the compounds into the apo crystals of the protein (Fig. 1). The protocol for solving the 3D structures of PDE5/**2e** and PDE5/**10a** complexes was the same as that described in our previous publications.[2,5] The complete statistics, as well as the quality of the two solved structures, are shown in Supplementary data (Supplementary Table S1). The binding position of the two compounds inside the catalytic domain of PDE5 is very similar to that of sildenafil as well as our previously reported PDE5 inhibitors. Briefly, the protein–ligand interactions include the conserved bidentate hydrogen bonds (H-bonds) between the pyrimidinone rings and the side chain of Q817, a H-bond between the carbonyl oxygen of the pyrimidinone ring and the side chain of Q775 via a water molecule, a face-to-face π–π stacking interactions between the pyrimidinone ring and the phenyl ring of F820, and hydrophobic interactions between the compounds and other residues nearby. Moreover, a halogen bond between the bromo atom substituted at the 5-position of the pyrimidinone ring and the hydroxyl oxygen of the residue Y612 which simultaneously H-bonds to D764 through a water molecule is formed (Fig. 1C). For the compound **2e**, its long alkyl chain substituted at the 6-position of the pyrimidinone ring fits into the cavity toward the hydrophobic residue L725 as shown in Figure 1A. Hydrophobic interactions are formed between the alkyl chain and residues such as L725. In the complex

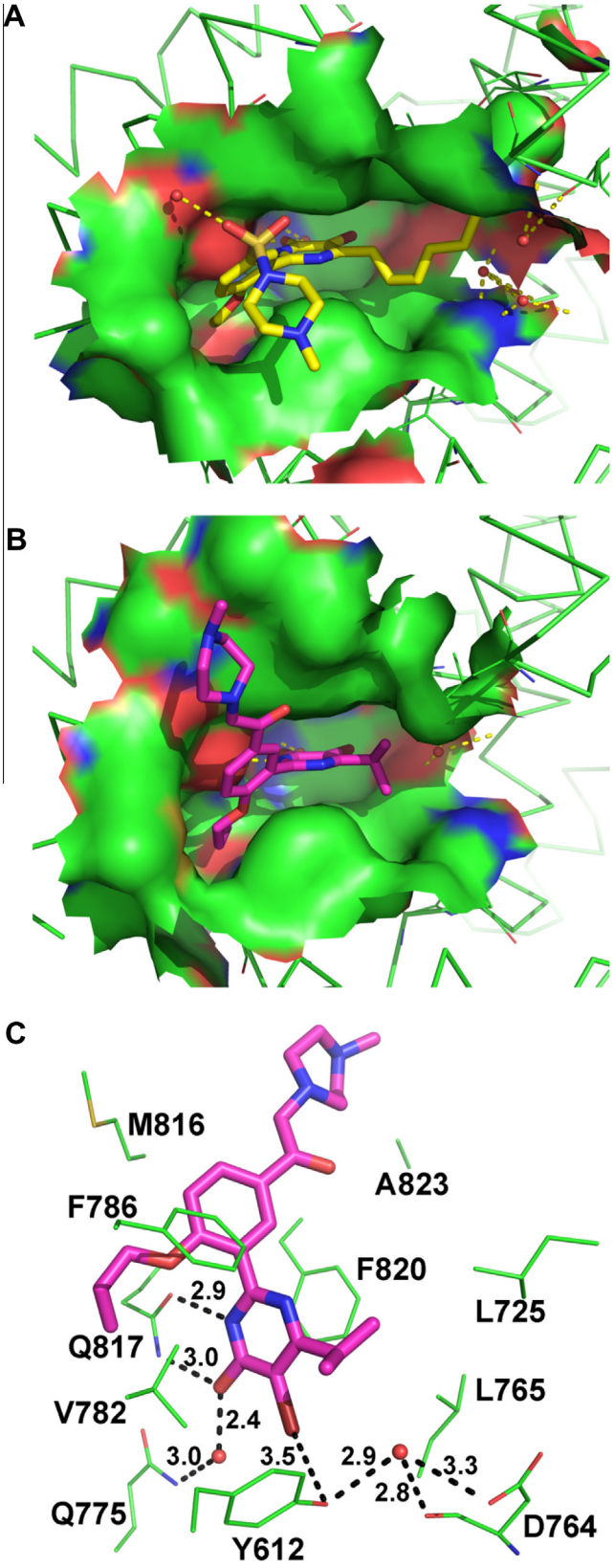
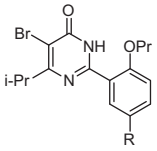
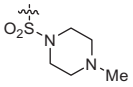
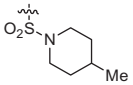
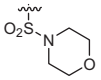
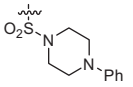
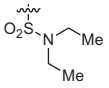
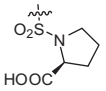
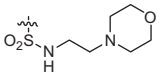
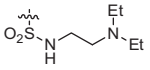
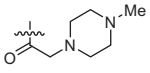
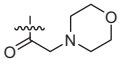
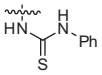
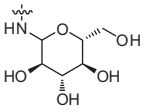
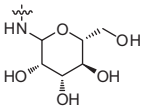


Figure 1. Crystal structures of PDE5/**2e** and PDE5/**10a** complexes. (A, B) Molecular surface representation of the binding pocket of compounds **2e** (A) and **10a** (B) in the catalytic domain of PDE5. (C) The H-bonding and hydrophobic interactions between the compound **10a** and the residues of PDE5.

of PDE5/**10a**, there are no strong interaction observed between the substituent at 5′-position of the phenyl ring of **10a** and the

Table 2
SAR of substitution at the 5′-position of the phenyl ring



Compound	R	IC_{50} (nM)	
		PDE5	PDE6
Sildenafil	–	3.9	39.2
2b		7.2	7.4
6a		25.5	–
6b		6.2	–
6c		29.3	–
6d		11.1	–
6e		2.8	2.2
6f		4.5	–
6g		9.6	–
10a		18.2	–
10b		8.8	–
13a		1.7	1600
13b		4.0	–
13c		21.3	–
13d		17.4	–

Data are the mean of two independent assays, each performed in duplicate.

protein except for the hydrophobic interactions between the substituent and A823. Accordingly, the crystal structure of PDE5/**2e** and PDE5/**10a** complexes revealed the detailed binding mode of the two inhibitors with the catalytic domain of PDE5, though only the structural data are not enough to fully address the difference in inhibition activities (77 and 18.2 nM) of the two compounds.

In summary, we have designed as well as synthesized a series of 5-bormopyrimidinones, and identified the 5-bromo-6-isopropyl-2-(2-propoxy-phenyl)pyrimidin-4(3*H*)-one as a new scaffold of potent PDE5 inhibitors. The different substitutions at the 5′-position of the phenyl ring were also explored, which verified that there is a great tolerance for the substitution at this position and the substituents play an important role in the selectivity of PDE5 over PDE6. The resulted 5-bromo-6-isopropyl-2-(2-propoxyphenyl)pyrimidin-4(3*H*)-one analog **13a** showed the highest activity with an IC_{50} value of 1.7 nM and an excellent selectivity over PDE6 (PDE6/5 = 941). The crystal structures of PDE5/**2e** and PDE5/**10a** complexes give insight into the binding mode of 5-bormopyrimidinones with the catalytic domain of PDE5. With this available structure information, further modifications on the compound **13a** are ongoing.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.062.

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