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8-(3-Chloro-4-methoxybenzyl)-8H-pyrido[2,3-d]pyrimidin-7-one derivatives as potent and selective phosphodiesterase 5 inhibitors



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ABSTRACT

A novel series of highly selective phosphodiesterase 5 (PDE5) inhibitors was found. 8H-Pyrido[2,3-d]pyrimidin-7-one derivatives bearing an (S)-2-(hydroxymethyl)pyrrolidin-1-yl group at the 2-position and a 3-chloro-4-methoxybenzyl group at the 8-position exhibited potent PDE5 inhibitory activities and high PDE5 selectivity over PDE6. Among the synthesized compounds, the 5-methyl analogue (**5b**) showed the most potent relaxant effect on isolated rabbit corpus cavernosum with an EC₃₀ value of 0.85 nM.

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Phosphodiesterase 5 (PDE5) is a cyclic guanosine monophosphate (cGMP)-specific hydrolytic enzyme, and the inhibitors of PDE5 are used for the treatment of erectile dysfunction (ED)^{1,2} and pulmonary arterial hypertension (PAH).³ The first approved orally active PDE5 inhibitor, sildenafil showed high efficacy, but visual side effects such as cyanopsia and visual disturbance were reported in some cases.^{4,5} These adverse effects are indicated to be attributed to inhibition of PDE6.⁶ After the launch of sildenafil, PDE5-selective inhibitors having various frameworks, such as tetrahydro-β-carboline,^{7,8} phthaladine,⁹ quinazoline,¹⁰ quinolone,¹¹ pyrazolopyrimidine,¹² pyridopyrazinone,¹³ and pyrimidin-4(3H)-one¹⁴ have been reported to date.

In previous Letters, we reported findings of 5-(3,4,5-trimethoxybenzoyl)-pyrimidine derivatives **2** as a novel chemical class of highly PDE5-selective inhibitors by a scaffold hopping strategy using isoquinolin-1-one derivative T-1032 (**1**) as a lead compound.¹⁵ Furthermore, transformation to pyrimidine-5-carboxamide derivatives and subsequent optimization of the substituents led to the discovery of avanafil (**3**), which has been approved by the FDA for the treatment of ED (Fig. 1).¹⁶ In the course of this study, 5-benzoylpyrimidine derivative **2a** was found to show a potent PDE5 inhibitory activity (IC₅₀ = 0.21 nM) and a high PDE5 selectivity over

PDE6 (PDE6/PDE5 = 5700), but its relaxant effect on isolated rabbit corpus cavernosum was insufficient (EC₃₀ >100 nM) owing to its high lipophilicity (cLogP = 4.01). Aiming to potentiate the relaxant effect, further synthetic study was carried out using **2a** as a lead compound. First, the trimethoxybenzene moiety was modified to reduce the lipophilicity (compounds **4**). As a next step, the substituents at the 4- and 5-positions of the pyrimidine ring were bound to potentiate PDE5 inhibitory activity (compounds **5**). These efforts led to the finding of a novel chemical series of highly selective PDE5 inhibitors which exhibit potent relaxant effect on isolated rabbit corpus cavernosum. In this Letter, we report the detail of the synthesis and biological activities of novel 8H-pyrido[2,3-d]pyrimidin-7-one derivatives **5**.

In the exploration of the optimal substituent at the 2-position of the pyrimidine derivatives **2**, we found that incorporation of polar substituents to reduce the lipophilicity of the compounds led to an improvement of the relaxant effect.¹⁵ Therefore, we focused on modification of the 3,4,5-trimethoxybenzene moiety at the 5-position, to which high lipophilicity of **2a** (cLogP = 4.01) is attributed, and pyrimidine-5-carbaldehyde **4a** (cLogP = 2.78) and 5-acetylpyrimidine **4b** (cLogP = 3.25) were designed by deletion of the 3,4,5-trimethoxyphenyl group. In addition, **4c** (cLogP = 3.79), **4e** (cLogP = 3.07), and **4f** (cLogP = 3.48) were designed by replacement of the 3,4,5-trimethoxybenzene with N-containing heterocycles.

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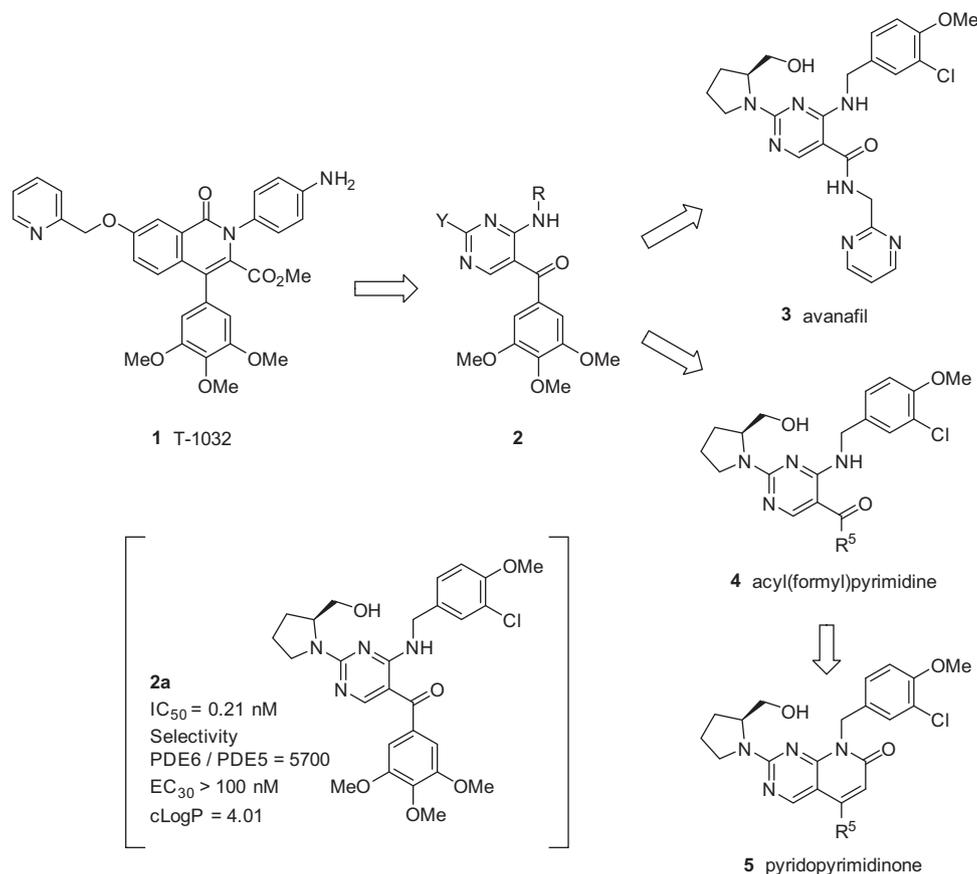


Figure 1. Structures of pyrimidine derivatives (2, 3, 4) and pyridopyrimidinone derivatives 5.

The synthesis of the pyrimidine derivatives (**4a–c**, **4e**, **4f**) was illustrated in Scheme 1. 4-Aminopyrimidine derivative **7** was obtained by condensation of commercially available **6** with 3-chloro-4-methoxybenzylamine. Reduction of the ester group in **7** with $LiAlH_4$ and subsequent oxidation of the resulting alcohol with MnO_2 gave pyrimidine-5-carbaldehyde **8**. The 2-position of the pyrimidine ring was substituted with (*S*)-prolinol after oxidation of the methylsulfanyl group with *m*CPBA to give **4a**. 5-Acylpyrimidine derivatives **4b–d** were obtained by addition of the corresponding R^5 -metal reagents to the formyl group of **4a** and the following oxidation with MnO_2 . The 1-methylimidazol-2-yl analogue **4e** was synthesized via **9** which was prepared from **8** in the same manner as that described above. **4f** was obtained by 1,4-addition of morpholine to 5-acryloylpyrimidine **4d**.

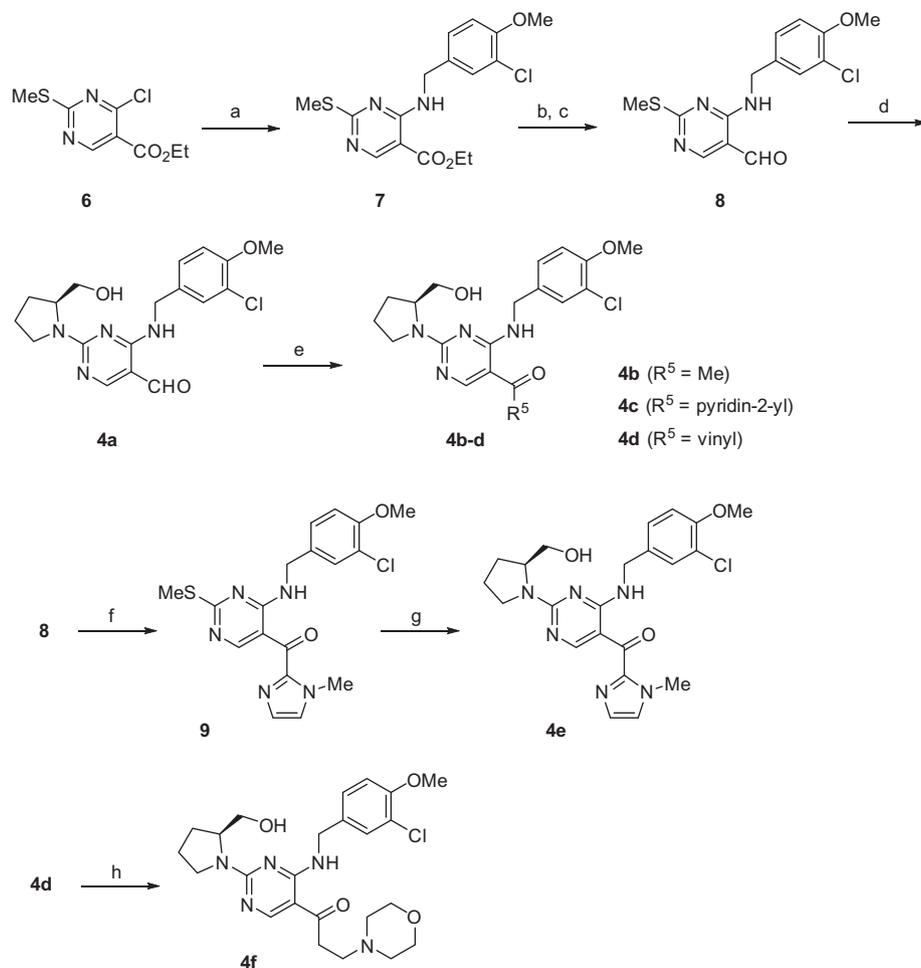
PDE5 inhibitory activities of the synthesized compounds were evaluated using the enzyme isolated from canine lung.¹⁷ As shown in Table 1, the pyrimidine-5-carbaldehyde derivative **4a** showed a moderate PDE5 inhibitory activity ($IC_{50} = 33 \text{ nM}$), and the modification of the formyl group into an acetyl group led to the improvement of the potency (**4b**, $IC_{50} = 5.2 \text{ nM}$). Although the N-containing heterocycles were tolerated on the acyl groups, all of the synthesized 5-position variants were more than ten times less potent than **2a**.

Aiming to improve their PDE5 inhibitory activity, we planned the construction of a bicyclic framework by cyclization between the substituents at the 4- and 5-positions of the pyrimidine ring. Ring closure modifications reduce the flexibility of the molecules and sometimes achieve dramatic changes in potency, selectivity permeability, and so on.^{18,19} Based on this idea, 8*H*-pyrido[2,3-

d]pyrimidin-7-one derivatives were designed and synthesized as described in Scheme 2.

Pyrimidine-5-carbaldehyde **8** was converted into α,β -unsaturated ester **10** by Horner–Wadsworth–Emmons reaction, and **10** was cyclized in the presence of NaH to give pyridopyrimidinone derivative **11a**. Alternatively, **11e** was prepared from 5-acetylpyrimidine **9** by treatment with trimethylphosphonoacetate and excess NaH in toluene at 100°C .²⁰ The 2-methylsulfanyl groups of these compounds were substituted with (*S*)-prolinol in the same manner as that described above to give **5a** and **5e**. 1,4-Addition of a cuprate, which was prepared from MeLi and CuCN, to **10** and concomitant cyclization gave **12**. After substitution of the 2-position, resulting **13** was treated with DDQ to give **5b**. Compound **5c** was obtained from **4c** in 17% yield, accompanied by recovery of 48% of starting material. In the synthesis of **5f**, methyl trimethylsilylacetate was used instead of the Horner–Wadsworth–Emmons reagent,²¹ since **5f** was not obtained by the same procedure as used for preparation of **5c**.

PDE5 inhibitory activities of these compounds were evaluated, and the light-activated bovine retina PDE6 was used for evaluation of PDE6 inhibitory activity. As summarized in Table 2, pyridopyrimidinone derivatives (**5a–c**, **5e**, **5f**) showed high potency, which were superior to the corresponding cyclization precursors, pyrimidine derivatives (**4a–c**, **4e**, **4f**), respectively. These results may be attributed to the restriction of the molecule into a bioactive conformation. Among the synthesized compounds, the 5-methyl analogue (**5b**) showed the most potent PDE5 inhibitory activity ($IC_{50} = 0.86 \text{ nM}$) and the highest selectivity over PDE6 (PDE6/PDE5 = 2300).



Scheme 1. Reagents and conditions: (a) 3-chloro-4-methoxybenzylamine, Et₃N, THF, rt (quant); (b) LiAlH₄, THF, rt (83%); (c) MnO₂, CH₂Cl₂, rt (91%); (d) (i) mCPBA, CHCl₃, 0 °C, (ii) (*S*)-prolinol, Et₃N, CHCl₃, 0 °C (83%); (e) (i) MeLi, Et₂O, THF, –78 °C or *n*-BuLi, 2-bromopyridine, Et₂O, THF, –78 °C or vinylmagnesium bromide, THF, 0 °C (ii) MnO₂, CHCl₃, rt (**4b** 60%, **4c** 57%, **4d** 43%, 2 steps); (f) (i) 1-methylimidazole, *n*-BuLi, THF, –78 °C, (ii) MnO₂, CHCl₃, rt (61%, 2 steps); (g) (i) mCPBA, CHCl₃, 0 °C, (ii) (*S*)-prolinol, Et₃N, CHCl₃, rt (86%, 2 steps); (h) morpholine, EtOH, rt (98%).

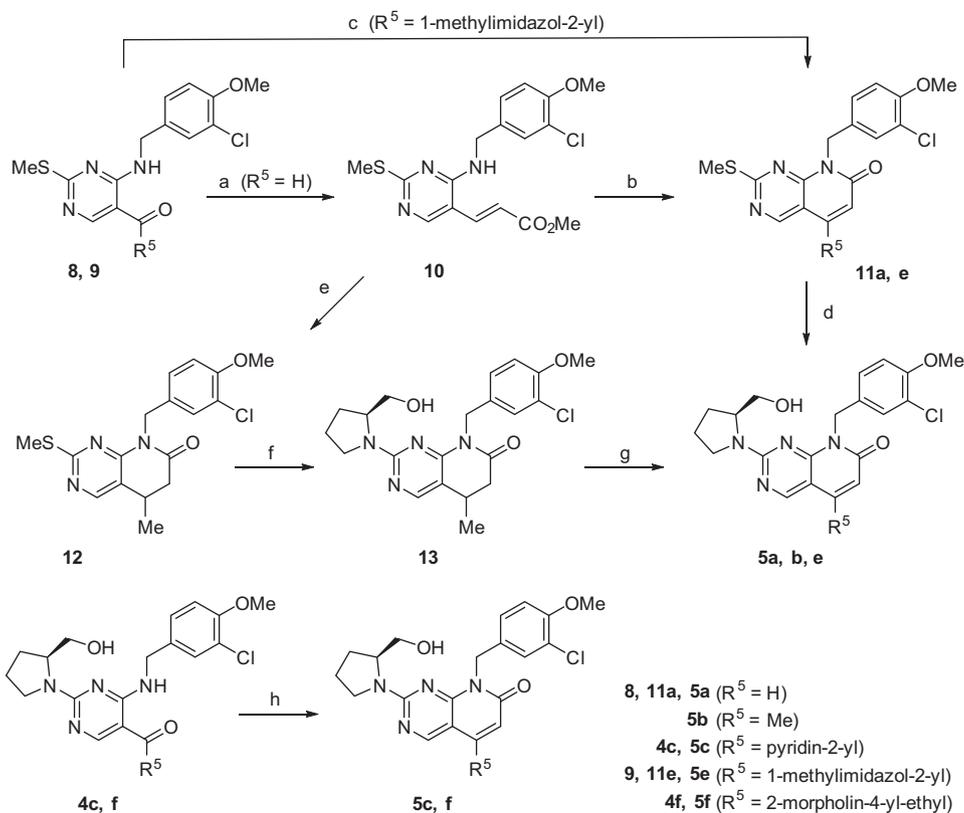
Table 1
PDE5 inhibitory activity of pyrimidine derivatives

4a-c, e, f		
–R ⁵	Compound	IC ₅₀ ^a (nM)
	4a	33
	4b	5.2
	4c	12
	4e	12
	4f	2.9

^a IC₅₀ values of these compounds were calculated by an equation of the first degree using two data; just above and below 50% inhibition. Some of the IC₅₀ values were calculated using Prism[®], version 3.0.

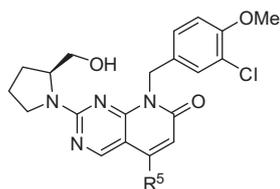
The relaxant effects on isolated rabbit corpus cavernosum were evaluated for selected compounds, and the results were summarized in Table 3.²² **5a** (cLogP = 1.91), **5b** (cLogP = 2.41), and **5f** (cLogP = 2.03), which were less lipophilic compared to **2a**, showed improved relaxant effects despite of the slightly reduced PDE5 inhibitory activity. Among them, **5b** showed the most potent relaxant effect on isolated rabbit corpus cavernosum with an EC₃₀ value of 0.85 nM. These values were superior to those of sildenafil (EC₃₀ = 8.7 nM, PDE6/PDE5 = 500).

In conclusion, we found 8*H*-pyrido[2,3-*d*]pyrimidin-7-one derivatives as a novel series of highly selective PDE5 inhibitors, which were designed by the modification to reduce the lipophilicity and subsequent cyclization to constrain the conformation of the pyrimidine derivatives. A series of pyridopyrimidinone derivatives showed superior potency in comparison to the corresponding pyrimidine derivatives, respectively; therefore, a methyl group and basic functionalities were tolerated at the 5-position of the pyridopyrimidinone ring with potent PDE5 inhibitory activities. By reducing lipophilicity while maintaining potent PDE5 inhibitory activity, pyridopyrimidinone derivatives showed improved relaxant effects compared to that of **2a**. Among the synthesized compounds, **5b** bearing a methyl group at the 5-position of the pyridopyrimidinone ring showed the most potent relaxant effect on isolated rabbit corpus cavernosum (EC₃₀ = 0.85 nM) and the highest PDE5 selectivity over PDE6 (PDE6/PDE5 = 2300). These findings encouraged us to carry out further optimization of **5b**.



Scheme 2. Reagents and conditions: (a) $(\text{MeO})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{Me}$, NaH, THF, rt (59%); (b) NaH, MeOH, rt (50%); (c) $(\text{MeO})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{Me}$, NaH, toluene 100 °C (13%); (d) (i) *m*CPBA, CHCl_3 , 0 °C, (ii) (*S*)-prolinol, Et_3N , CHCl_3 , 0 °C (**5a** 77%, **5e** 96%, 2 steps); (e) MeLi, CuCN, Et_2O , THF, 0 °C, (67%); (f) (i) *m*CPBA, CHCl_3 , 0 °C, (ii) (*S*)-prolinol, Et_3N , CHCl_3 , 60 °C (75%, 2 steps); (g) DDQ, dioxane, 60 °C (30%); (h) $(\text{MeO})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{Me}$, NaH, toluene, 100 °C (**5c** 17%) or $\text{TMSCH}_2\text{CO}_2\text{Me}$, dicyclohexylamine, *n*-BuLi, THF, rt (**5f** 8%).

Table 2
PDE5 inhibitory activity and selectivity against PDE6 of pyridopyrimidinone derivatives



$-\text{R}^5$	Compound	IC_{50}^a (nM)	Selectivity (PDE6/5)
H	5a	3.3	850
Me	5b	0.86	2300
	5c	1.7	1900
	5e	7.2	NT
	5f	0.98	1600

^a IC_{50} values of these compounds were calculated by an equation of the first degree using two data; just above and below 50% inhibition. Some of IC_{50} values were calculated using Prism[®], version 3.0.

Table 3
Relaxant effect on isolated rabbit corpus cavernosum

Compound	Relaxant effect EC_{30}^a (nM)
5a	2.2
5b	0.85
5f	8.6
Sildenafil	8.7

^a EC_{30} values were determined from the logarithmic concentration–inhibition curve. The value is given as the average of at least two experiments, where the variation from the mean value is $\pm 30\%$ or less.

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22. The initial resting isometric tension of each tissue strip was adjusted to 1.5 g by gradual incremental stretching in 10 mL of organ bath chambers containing physiological salt solution (PSS) at 37 \pm 0.5 °C, continuously aerated with 95% O₂ and 5% CO₂. The contractile response to high-KCl (120 mM) PSS was checked twice. Phenylephrine (PE) (5 \times 10⁻⁶ M) was added into each organ bath in order to obtain a tonic contraction. After the PE contractile response was stabilized, test compound (10⁻¹⁰–10⁻⁶ M) or vehicle was added to the preparation at an interval of 30 min. Papaverine hydrochloride was added into each organ bath chamber (final concentration, 10⁻⁴ M) to confirm the maximal relaxation of the tissue strips at the end of experiment. The composition of PSS was as follows (mM): NaCl 118, KCl 4.7, MgSO₄ 1.2, CaCl₂ 1.5, KH₂PO₄ 1.2, NaHCO₃ 25.0, dextrose 11.0, EDTA-2Na 0.023 (pH 7.3 or 7.4).