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Design and Synthesis of Xanthine Analogues as Potent and Selective PDE5 Inhibitors

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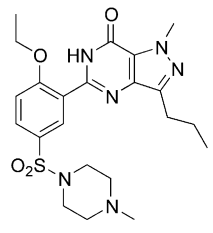
Abstract—We have discovered potent and selective xanthine PDE5 inhibitors. Compound **25** (PDE5 $IC_{50} = 0.6$ nM, PDE6/PDE5 = 101) demonstrated similar functional efficacy and PK profile to Sildenafil (PDE5 $IC_{50} = 3.5$ nM, PDE6/PDE5 = 7).

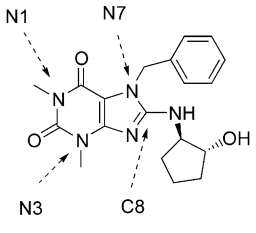
The development of phosphodiesterase type 5 (PDE5) inhibitors for the treatment of male erectile dysfunction (ED) has attracted great attention since the commercial introduction of Sildenafil (**1**, Viagra, Fig. 1).[1] PDE5, a cGMP hydrolytic enzyme, breaks down cGMP in the corpora cavernosa smooth muscle cells. Inhibition of PDE5 has been demonstrated to increase the levels of cGMP induced by nitric oxide (NO) from neuronal and endothelial sources during sexual stimulation. The increased cGMP levels, in turn, enhance the cavernosal smooth muscle relaxation, arterial inflow and venous congestion to achieve penile erection.[2] Despite its commercial success, Sildenafil has showed clinically significant side effects, some of which could be related to its low selectivity versus other PDEs such as PDE6. To discover potent and more selective PDE5 inhibitors for the treatment of male ED, we embarked on the design and synthesis of xanthine analogues based on compound **2** which was identified as a lead structure from high throughput screening. Herein, we would like to report the successful outcome of our efforts directed at discovering potent and selective xanthine PDE5 inhibitors.

As shown in Figure 1, xanthine analogue **2** showed weak PDE5 inhibitory activity. To improve the PDE5 inhibitory activity and selectivity for this series of compounds, the structure–activity relationship (SAR) studies of compound **2** were focused on chemical modification at the C8, N7, N1 and N3 positions.

For the C8 modification, a variety of amino alcohols that have differences in their chirality, steric hindrance and aromatic character were tried. To further identify the SAR role of the hydroxy group of the amino alcohols, two cycloamines were also included. These analogues were prepared as shown in Scheme 1.[3] Commercially available compound **3** was reacted with benzyl bromide to form compound **4**. The bromide atom of compound **4** was displaced with different amines to give the final targets shown in Table 1.



1 (Sildenafil, Viagra™)



2 (PDE5 inhibition: 17% @ 100 nM)

Figure 1. Structures of PDE5 inhibitors.

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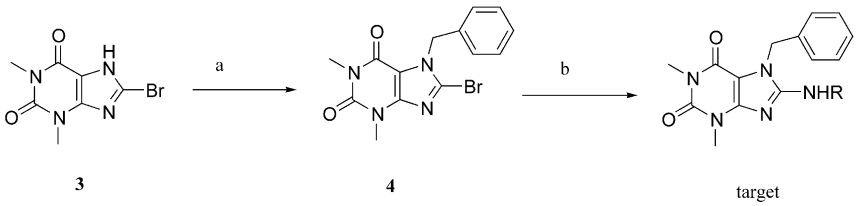
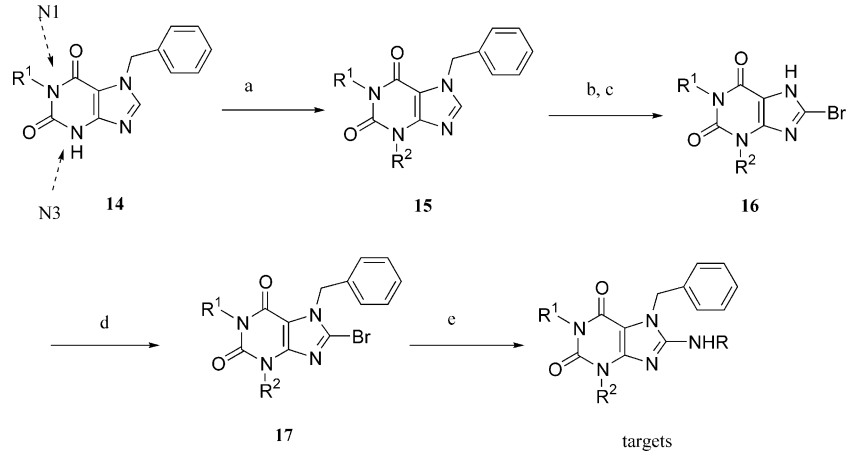
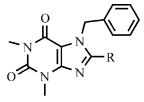


Scheme 1. C8 modification: (a) K_2CO_3, BnBr, DMF (90%); (b) NH_2R, *i*PrNEt, NMP, sealed tube, 160 °C, overnight, (30–75%).



Scheme 2. N1 and N3 Modification: (a) K_2CO_3, R^2I or R^2 Br, DMF (70–90%); (b) $Pd(OH)_2$, $HCONH_4$, MeOH (60–80%); (c) Br_2, NaOAc, HOAc (70–85%); (d) K_2CO_3, BnBr, DMF (80–90%); (e) NHR, *i*PrNEt, NMP, sealed tube, 160 °C, overnight (75–95%).

Table 1. PDE5 inhibitory results of selected analogues of C8 modification



Compd	R	(% Inhibition @ 100 nM)
2		17
5		10
6		2
7		11
8		0
9		4
10		5
11		0
12		62
13		54

The PDE5 inhibitory activity of the C8 modified analogues were determined according to the reported procedures.[4]

Change of the chiral amino alcohol (**2**) to the achiral amino alcohol (**5**) led to the decrease of the PDE5 inhibitory activity. Introduction of other chiral amino alcohols (**6–10**) also significantly reduced the PDE5 inhibitory activity. However, removal of the hydroxy group increased the PDE5 inhibitory activity from 17% (**2**) to >50% (**12** and **13**). In addition, these C8 replacements (**12** and **13**) resulted in achiral compounds that are synthetically easier to access.

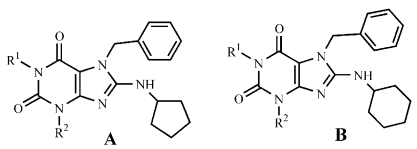
Next, with the cyclohexyl and cyclopentyl amines as the optimal C8 groups, we carried out N1 and N3 modifications. These compounds were prepared as shown in Scheme 2.[3]

Alkylation of the N3 position of commercially available **14** afforded **15** that was hydrogenated and brominated to form **16**. N7 benzylation of **16** produced **17**. The bromide atom of **17** was displaced by the cyclohexyl or cyclopentyl amines to form the target compounds.

The results of PDE5 inhibitory activity of a selected number of N1 and N3 modified analogues are shown in Table 2. The ethyl group at the N1 and N3 positions (**18** and **21**) showed the highest inhibitory activity.

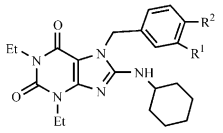
Finally, we modified the N7 position, maintaining ethyl groups at the N1 and N3 positions, while substituting cyclohexyl amine and cyclopentyl amine at the C8 position. The target compounds were prepared as described

Table 2. PDE5 inhibitory results of selected analogues of N1 and N3 modification



Compd	Structure	R^1, R^2	(% Inhibition @ 100 nM)
12	A	R^1R^2 = Me	62
18	A	R^1R^2 = Et	91
19	A	R^1 = Me R^2 = I-Bu	49
20	A	R^1 = Et R^2 = I-Bu	56
13	B	$R^1 = R^2$ = Me	54
21	B	$R^1 = R^2$ = Et	93
22	B	R^1 = Me R^2 = I-Bu	52
23	B	R^1 = Et R^2 = I-Bu	63

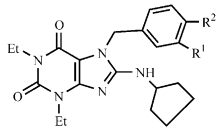
Table 3. PDE5 inhibition and selectivity profile (C8 cyclohexyl analogues)



Compd	R^1	R^2	PDE5 IC_{50} (nM)	PDE6/PDE5	PDE 1–4/PDE5
21	H	H	12	35	na[a]
24	H	OMe	2.6	51	na
25	H	OH	0.6	101	>3000
26	OMe	H	100	na	na
27	OH	H	1.2	36	na
28	Me	OMe	19	58	na
29	Me	OH	4.5	133	na
30	–OCH_2O—		3.7	14	na
31	Cl	OMe	0.58	345	>9500
32	Cl	OH	2.0	250	
33	F	OMe	0.30	350	>28,000
34	F	OH	0.33	364	>12,000
35	Br	OMe	1.7	219	>10,000

[a]na: Not available.

Table 4. PDE5 binding and selectivity profile (C8 cyclopentyl analogues)



Compd	R^1	R^2	PDE5 IC_{50} (nM)	PDE6/PDE5	PDE 1–4/PDE5
18	H	H	10	21	na[a]
36	H	OMe	2.1	33	na
37	H	OH	1.5	46	na
38	Cl	OMe	1.1	118	na
39	Cl	OH	1.8	261	na
40	F	OMe	0.69	89	na
41	F	OH	0.82	182	>4,400

in Scheme 1 and Scheme 2. The complete PDE5 inhibition and selectivity profile for the selected analogues with C8 cyclohexyl amine is shown in Table 3.

Since rapid clearance of PDE5 inhibitors is a desirable property as illustrated by Sildenafil, a metabolically labile methoxy group was introduced to the para or the meta position of the N7 benzyl ring. Compounds **24**, **25**, and **29** as well as the halogen substituted analogues **31**–**35** showed dramatic improvement of PDE5 inhibition and selectivity. It is especially worth noting that the halogen atoms increased PDE5 selectivity versus other PDE isozymes significantly. These analogues demonstrated superior PDE5 inhibition and selectivity over Sildenafil (PDE5 IC_{50} = 3.5 nM, PDE6/PDE5 = 7). This SAR trend was also observed for the analogues with C8 cyclopentyl amine (Table 4).

Compound **25**,[5] the first compound to achieve 100-fold PDE6/5 ratio, was tested in the functional efficacy assay and the pharmacokinetic assay. In the electrical frequency stimulated (EFS)-induced relaxation of rabbit corpus cavernosum test,[6] compound **25** demonstrated efficacy similar to that of Sildenafil (Fig. 2).

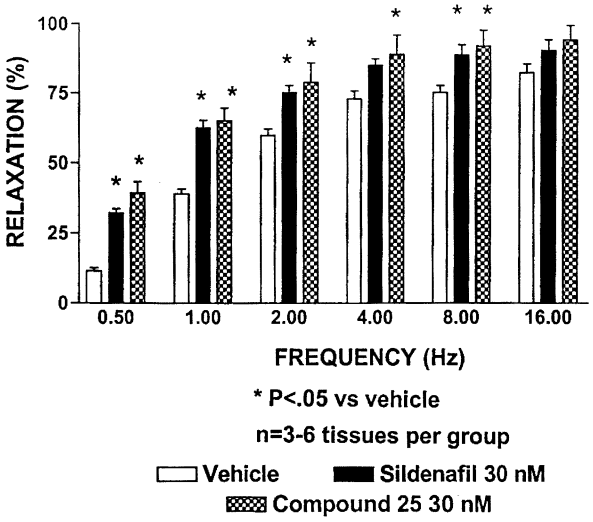
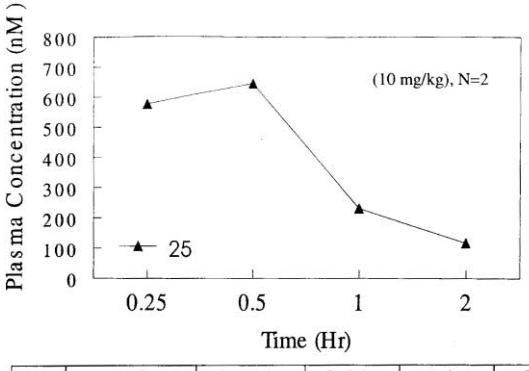


Figure 2. EFS-induced relaxation of rabbit corpus Cavernosum.



#	AUC (ng.hr/mL)	0.25-hr	0.5-hr	1-hr	2-hr
25	303	578	646	231	117

Figure 3. Rapid rat pharmacokinetics.

The oral PK profile of compound **25** was also examined in a rapid rat PK assay.[7] The result is shown in Figure 3. Compound **25** was rapidly absorbed and cleared.

In summary, we have discovered potent and selective PDE5 inhibitors. Compounds such as **25** have demonstrated good efficacy in the EFS induced relaxation assay. Compound **25** also showed a desirable oral PK profile with rapid onset and fast clearance. Further development of this series of compounds will be reported in the future.

Acknowledgements

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References and Notes

1. 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 and references cited there .

2. (a) Corbin, J. D.; Francis, S. H. *J. Biol. Chem.* **1999**, *274*, 13729. (b) Gibson, A. *Eur. J. Pharmacol.* **2000**, *411*, 1. (c) Langtry, H. D.; Markham, A. *Drugs* **1999**, *57*, 967.

3. All of the target compounds were purified by preparative TLC and showed satisfactory results in the analysis by NMR, MS and HRMS.

4. Human PDEs 5 and 2 isolated from corpus cavernosum and PDE3 isolated from platelets were assayed as described previously (Wang, P.; Wu, P.; Myers, J. G.; Stamford, A.; Egan, R. W.; Billah, M. M. *Life Sci.* **2001**, *68*, 1997). Recombinant human PDE1A3 was assayed at the final concentration of cAMP of 10 μM. Recombinant human PDE1A3 was assayed at the final concentration of cAMP of 10 μM. Recombinant human PDE4B2 was assayed at the final concentration of cAMP of 1 μM. Human PDE6 isolated from retina was assayed at the final concentration of cGMP of 0.5μM. All the PDE assays were performed in the presence of 2% DMSO and 0.1% BSA. All the assays were done in duplicate, and data shown are means from 2–4 experiments (SEM $<15\%$).

5. Analytical data for compound **25**: ^{1}H NMR (400 Hz, $CDCl_3$): δ 0.87 (m, 1H), 1.02–1.34 (m, 10H), 1.57 (m, 4H), 1.90 (d, $J=10.4$ Hz, 2H), 3.71 (m, 1H), 3.94 (d, $J=7.6$ Hz, 1H), 4.07 (m, 4H), 5.21 (s, 2H), 6.73 (d, $J=8.4$ Hz, 2H), 7.03 (d, $J=8.4$ Hz, 2H). EI–MS: m/z 412 (M + 1). HRMS calcd for $C_{22}H_{30}N_5O_3$: 412.2349, found: 412.2332.

6. The corpus cavernosum from anesthetized male New Zealand rabbits was mounted in organ bath chambers containing physiological salt solution. The optimum length-tension curves were obtained by gradual stretching and contracting the tissues with phenylephrine 10 μM. The tissues were equilibrated in a solution containing atropine (1 μM), guanethideine (5 μM) and indomethacin (5 μM). After the tissues were equilibrated, they were subjected to electrical field stimulation (10 V, 0.5 ms pulse duration) at 0.5–16 Hz. The tissues were then incubated with vehicle, or test compound (30 nM) for 30 min contracted with phenylephrine. The electrical stimulation was repeated. Relaxation was calculated as a percentage of phenylephrine-induced contraction. For details, see: Vemulapalli, S.; Kurowski, S. *Fundamental & Clinical Pharmacol.* **2001**, *15*, 1.

7. Compound 25 was formulated in PG/TW80 due to its low water solubility. For the reference of the rapid rat assay, see: Cox, K. A.; Dunn-Meynell, K.; Korfmacher, W. A.; Broske, L.; Nomeir, A. A.; Lin, C. C.; Cayen, M. N.; Barr, W. H. A. *Drug Discov. Today* **1999**, *4*, 232.