Substituted Pyrazolopyridopyridazines as Orally Bioavailable Potent and Selective PDE5 Inhibitors: Potential Agents for Treatment of Erectile Dysfunction

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Abstract: Novel pyrazolopyridopyridazine derivatives have been prepared as potent and selective PDE5 inhibitors. Compound **6** has been identified as a more potent and selective PDE5 inhibitor than sildenafil (**1**). It is as efficacious as sildenafil in in vitro and in vivo PDE5 inhibition models, and it is orally bioavailable in rats and dogs. The superior isozyme selectivity of **6** is expected to exert less adverse effects in humans when used for erectile dysfunction treatment.

Prior to the introduction of sildenafil [Viagra (**1**), Figure 1] in 1998, erectile dysfunction (ED) was largely an untreated medical disorder.[1,2] Studied originally as a treatment for angina, sildenafil is a potent inhibitor of phosphodiesterase type 5 (PDE5).[3] Its effectiveness in treating ED was discovered serendipitously in phase II angina trials. Despite its success in treating ED, sildenafil has several notable side effects such as headache, nausea, cutaneous flushing, and visual disturbances.[3a,4] These side effects *may* be attributed to the limited selectivity of **1** against other PDE isozymes, most notably PDE1 and PDE6 (Table 1).[3] Thus, the need exists for improved PDE5 inhibitors possessing greater PDE isozyme selectivity, which should potentially lead to drugs with fewer side effects.[2] Recently, compounds **2** (Vardenafil)[5] and **3** (Tadalafil)[6] have been studied in human clinical trials and have demonstrated reduced adverse effects when compared with sildenafil, probably due to their improved isozyme selectivities verses PDE1 and PDE6. Here, we report a new series of potent PDE5 inhibitors represented by the clinical entity **6** with in vivo efficacy comparable to that of **1** but with a much-improved PDE isozyme selectivity profile.

PDE5 is a member of the phosphodiesterase family of enzymes that are responsible for the hydrolysis of cGMP and/or cAMP.[4] PDE5 is the primary cGMP hydrolyzing enzyme present in the corpus cavernosum, the smooth muscle tissue in the penis that is engorged with blood during an erection. Upon sexual stimulation, nitric oxide (NO) is released from nonadrenergic, noncholinergic neurons in the penis. NO activates guanylyl cyclase, which in turn produces cGMP. cGMP initiates a protein phosphorylation cascade, which causes a decrease in the intracellular calcium concentration within corpus cavernosal smooth muscle cells, resulting in vasorelaxation, inflow of arterial blood, and ultimately an erection.[7] Inhibition of PDE5 increases the effective concentration of cGMP in the corpus cavernosum, potentiating the above-described effects, leading to enhanced erectile function.

The therapeutic benefit of sildenafil in ED results from its potent inhibition of PDE5. However, it is only modestly selective toward some other PDE isozymes, most notably PDE1 and PDE6 (Table 1). At relevant therapeutic doses of sildenafil, it is likely that measurable inhibition of PDE1 and PDE6 occurs. As noted earlier, this may be the cause of some of the side effects associated with sildenafil use. Consistent with this hypothesis is the fact that the incidence of side effects associated with sildenafil use is dose-proportional. The visual disturbances associated with sildenafil treatment can be likely linked to inhibition of PDE6, the sole cGMP PDE in the retina. Other side effects may be due, at least in part, to nonspecific inhibition of PDE1, which is abundant throughout most of the vasculature.[8a] Thus, more selective PDE5 inhibitors should be of substantial clinical interest as already exemplified by **2** and **3**.[2,8b] This manuscript details the discovery of a novel series of pyrazolopyridopyridazine derivatives that are potent, selective, efficacious, and orally bioavailable PDE5 inhibitors.

We have recently disclosed **5** as a potent and selective PDE5 inhibitor.[9a] Upon examination of the X-ray structure of **5**, it became apparent that the hydrogen from the benzylic amine (−NH−) formed a hydrogen bond with the amide carbonyl. This observation, coupled with the structure of Eisai's potent PDE5 inhibitor **4**,[10] led us to hypothesize that constraining the conformation of **5** should provide a novel and potent PDE5 scaffold. Thus, the structural combination of **4** and **5** led to the discovery of the pyrazolopyridopyridazine (PPP) scaffold, typified by compounds **6**−**9**, which were determined to be potent and selective PDE5 inhibitors.

The chemistry leading to **6**[11] is shown in Scheme 1. Pyrazolopyridine **10** was prepared using methodology that we had previously published.[9b] Reaction of **10** with hydrazine expanded the fused maleimide ring, forming the pyridazine dione **11**. Exposure of **11** to phosphorus oxychloride afforded the dichloropyridazine derivative **12**, which served as the key PPP scaffold allowing independent examination of both the α- and *β*-positions on the pyridazine ring (Scheme 1).

Using the chemistry sequence described in Scheme 1, we have examined substitutions at both positions. As we previously observed,[9a] the 3-chloro-4-methoxyphenylmethylamine[9c] group constituted the best substituent at the α-position (Scheme 1). When the α-sub-

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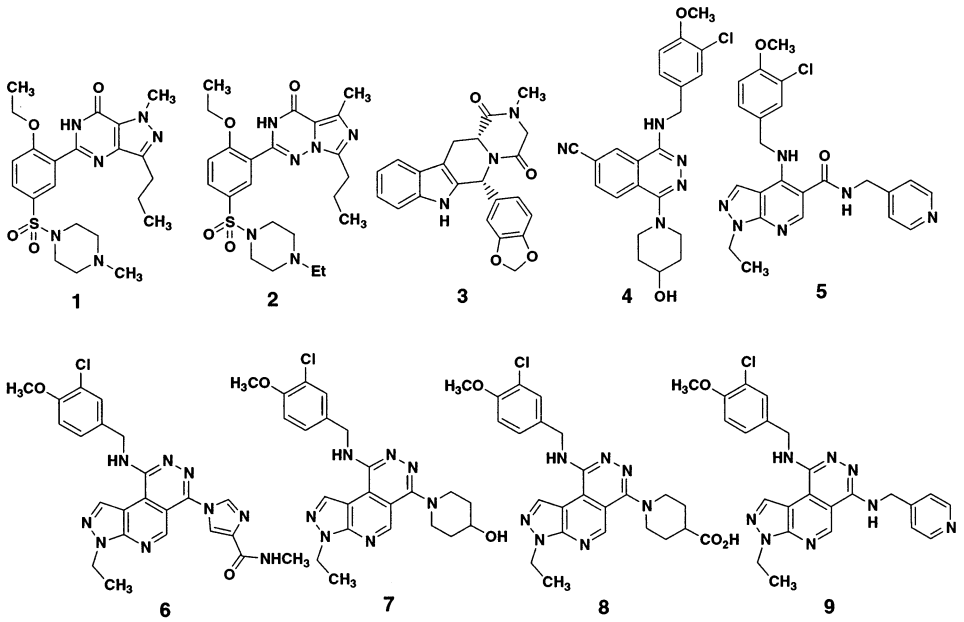


Figure 1. PDE 5 inhibitors.

Table 1. PDE5 Inhibition and Isozyme Selectivities[8]

compd	IC_{50} PDE5 (nM)	IC_{50} ratio of 1/5	IC_{50} ratio of 2/5	IC_{50} ratio of 3/5	IC_{50} ratio of 4/5	IC_{50} ratio of 6/5
1[a]	1.6 ± 0.5	160	>10^4	4100	2900	7
2[5]	0.7	400	>10^4	>10^4	>10^4	49
3[b]	1.3	>10^4	>10^4	>10^4	>10^4	450
4[c]	0.6	>10^4	1600	>10^4	1900	*d*
5[a]	0.8 ± 0.5	6400	9200	3800	600	47
6[a]	0.3 ± 0.1	>10^4	>10^4	>10^4	>10^4	150
7	0.7 ± 0.3	5700	4000	7500	300	80
8[a]	0.03 ± 0.01	>10^4	>10^4	>10^4	7500	30
9[a]	0.10 ± 0.04	>10^4	>10^4	>10^4	4000	210

[a] Determined in house. Enzyme sources: PDE1, bovine heart; PDE2, rat kidney; PDE3, human platelet; PDE4, rat kidney; PDE5, human platelet; PDE6, bovine retina. All IC_{50} determinations are averages based on three determinations. [b] Taken from ref 5. [c] Taken from ref 7. [d] Not available.

stituent as the 3-chloro-4-methoxyphenylmethylamine group was held constant, varied substitution at the β-position provided numerous low nanomolar inhibitors, typified by compounds **6**−**9**. The broad SAR at this position (Table 1, Figure 1) provided us with a unique opportunity to modulate the pharmacokinetic properties of this series of compounds. Eventually, **6** was chosen to be evaluated further.[12]

The in vitro potency and selectivity of **6** were much improved compared to sildenafil (**1**), particularly the significantly improved selectivity for PDE5 versus PDE6 and PDE1 (Table 1).[13]

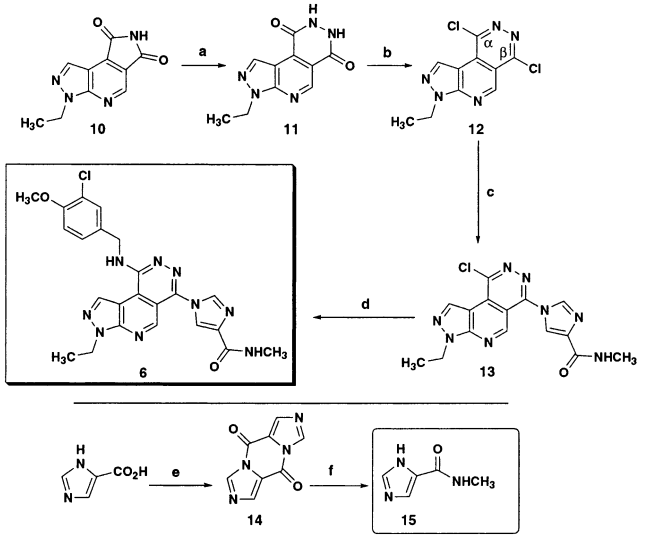
The ability of a compound to potentiate relaxation of corpus cavernosal tissue strips in vitro has been used as a functional measure of the PDE5 inhibition.[9a] This model requires that the drug penetrates corpus cavernosal cells, since PDE5 is an intracellular enzyme. When **6** was evaluated in rabbit corpus cavernosum tissue strips, it demonstrated efficacy equivalent to that of sildenafil (**1**, Table 2). Thus, in addition to being a more potent and selective PDE5 inhibitor in vitro (compared with sildenafil), compound **6** was at least equivalent to sildenafil in its in vitro functional PDE5 activity in rabbit corpus cavernosal tissue.

Since sildenafil was identified in human clinical trials and not as a result of a preclinical studies, we needed to develop our own preclinical model of erectile function. To accomplish this, we used drug-induced increases in the intracavernosal blood pressure in the penis of an anesthetized rabbit as our in vivo measure of erectile function.[14] Accordingly, compound **6** was then examined in vivo in anesthetized male rabbits, using sildenafil as a control. Compound **6** demonstrated equal efficacy with sildenafil in its ability to elevate mean blood pressure in the corpus cavernosum (Table 2). This animal model suggested that **6** and sildenafil were equivalent in vivo in rabbits.

Further profiling of **6** was pursued given the excellent in vitro and in vivo results obtained. Thus, examination of the pharmacokinetic profile of **6** in rats (2.0 μmol/kg) and dogs (5.1 μmol/kg) demonstrated equal or better exposure upon oral dosing of the drug in rats and dogs when compared with **1** (Table 2). The relatively short terminal half-life of **6** in both species was consistent with the on-demand dosing that is likely best suited for a medication for this quality-of-life disorder (Table 2). These results gave confidence to the expectation that **6** would be orally active in humans.

Appropriate toxicity studies of **6** necessarily followed the pharmacokinetic studies. Accordingly, the safety profile of **6** in rats was evaluated at doses of 10, 50, and 250 mg/kg, and there were no drug-related adverse effects at 10 and 50 mg/kg. The only adverse effect observed at 250 mg/kg was limited to lower body weight in male rats (6% lower than control). There were no drug-related gross-pathologic lesions observed at any

Scheme 1[a]



[a] Reagents: (a) hydrazine hydrate (2.0 equiv), EtOH/water, room temperature, 2 h (100%); (b) $POCl_3$ (3.0 equiv, neat), reflux 3 h (85%); (c) **15** (1.05 equiv), $(i\text{-}Pr)_2NEt$ (3.0 equiv), NMP, at 80 °C for 3 h (40%); (d) 3-Cl−4-MeO−phenylmethylamine·HCl (1.1 equiv), $(i\text{-}Pr)_2NEt$ (3.0 equiv), NMP, at 110 °C for 3 h (85%); (e) $SOCl_2$ (3.0 equiv), DMF (cat.), toluene, reflux 2 h (100%); (f) CH_3NH_2 (5.0 equiv), THF at room temperature (90%).

Table 2. Characteristics of **6** Compared to Sildenafil (**1**)[a]

compd	EC_{50} in vitro strip assay (nM)	ED_{50} rabbit in vivo model (nmol/kg)	*F* (%) in male rats (R) or dogs (D)	half-life (h) in male rats (R) or dogs (D)
1 (sildenafil)	19	110	15 (R)	0.3 (R)
			54 (D)	5.2 (D)
6	13	100	29 (R)	1.2 (R)
			42 (D)	2.0 (D)

[a] Oral bioavailability (% *F*) and half-life of sildenafil taken from the Viagra NDA.

dose in both male and female rats. Because of its excellent in vitro and in vivo potency, selectivity, and safety profile, compound **6** was progressed into human clinical trials and was found to be well tolerated at the highest dose tested (50 mg) in healthy human volunteers.[15]

In summary, we have identified a series of novel and potent PDE5 inhibitors based on a pyrazolopyridopyridazine template (i.e., **6**).[12] These compounds were characterized by potent PDE5 inhibition in vitro and potent efficacy in vivo. Compound **6** distinguished itself by its selectivity profile and in vivo efficacy. Its PDE1 and PDE6 isozyme selectivities were superior to that of **1** (sildenafil). Additional studies demonstrated that **6** had a desirable pharmacokinetic profile in two animal species with no safety concerns. Accordingly, studies in healthy volunteers were conducted, and no safety concerns were revealed. Thus, because of its improved PDE isozyme selectivity profile compared with that of sildenafil, compound **6** demonstrated fewer PDE-related side effects, such as visual disturbances, in healthy volunteers. A detailed SAR examination of this series and the effects of **6** in human subjects will be reported in due course.

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Supporting Information Available: Experimental details, X-ray structure of **6**, and PDE assay protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The characterization of compound **6** was as follows. Compound **6**: ^1H NMR (400 MHz, CD_3Cl) δ 1.56 (t, 3H), 3.03 (d, 3H), 3.87 (s, 3H), 4.75 (q, 2H), 4.97 (d, 2H), 5.79 (bs, 1H, NH), 6.90 (d, 2H), 7.25 (m, 1H), 7.37 (m, 1H), 7.48 (s, 1H), 8.02 (d, 2H), 8.47 (s, 1H), 9.01 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 15.0, 25.8, 43.3, 45.6, 56.2, 99.9, 104.1, 112.3, 113.8, 118.1, 122.5, 122.8, 127.6, 129.9, 130.6, 131.1, 137.3, 138.4, 143.0, 146.2, 149.3, 154.2, 162.7. Anal. Calcd for $C_{23}H_{22}ClN_9O_2$: C, 56.16; H, 4.51; N, 25.63; Cl, 7.21. Found: C, 55.72; H, 4.32; N, 25.94; Cl, 7.23. Mp 181−183 °C and 206−207 °C.

(12) A more comprehensive examination of the SAR within this series will be presented separately.

(13) Enzyme inhibition assays were carried out as described in the following. Rotella, D.; Sun, Z.; Zhu, Y.; Krupinski, J.; Pongrac, R.; Seliger, L.; Normandin, D.; Macor, J. E. N-3-Substituted imidazoquinazolinones: potent and selective PDE5 inhibitors as potential agents for treatment of erectile dysfunction. *J. Med. Chem.* **2000**, *43* (7), 1257−1263.

(14) Rabbits were anesthetized with a combination of ketamine (10 mg/kg, im) followed by urethane (0.5 g/kg, iv). Arterial and venous accesses were obtained via the femoral artery and vein for the measurement of blood pressure and the administration of compounds, respectively. Intracavernosal pressure (ICP) was monitored continuously during the course of the experiment. After a baseline ICP was measured in an animal, SNAP was injected intracavernosally (ic). The total amount of SNAP injected ranged from 1 to 5 μg/kg in a final volume of 100 μL of Dulbecco's phosphate-buffered saline (DPBS). The catheter was flushed with 200 mL of DPBS containing heparin (5 units/mL) to ensure that all of the SNAP was injected into the corpus. ICP responses to SNAP after the initial transient injection artifact were characterized by the peak pressure that developed (in mmHg), the duration of the response (in seconds (s)), and most importantly the area under the curve of the response (AUC in mmHg s). Control responses to SNAP in each animal were measured in triplicate before the iv administration of **6** or sildenafil. After determination of control SNAP-dependent ICP responses, increasing doses of either **6** or sildenafil were given followed by SNAP to determine the effect of the PDE5 inhibitors on the NO-dependent changes in ICP. Increasing doses of the compound (10, 30, 100, 300, and 1000 nmol/kg) were given 30 min apart, and SNAP was injected 10 min after the administration of each dose. Standard dose-dependent curves were generated, and the ED_{50} values were determined using these curves.

(15) The results of the human clinical trials of **6** will be reported elsewhere in due course.

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