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Review Article

Phosphodiesterase type 5 inhibitors: discovery and therapeutic utility

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Introduction

Phosphodiesterase type 5 (PDE5) is one member of a superfamily of cyclic nucleotide hydrolyzing enzymes that specifically cleaves cyclic guanosine monophosphate (cGMP), a key intracellular second messenger. Interest in PDE5, originally a target for the treatment of angina and hypertension, has been refocused and stimulated as a result of the success of sildenafil (Viagra™) (**1**, Fig. 1) for the treatment of erectile dysfunction (ED). Sales of Viagra™ are over 1 billion dollars annually and the drug now accounts for over 90% of sales in the ED market.

Erectile dysfunction is defined as the inability to achieve and maintain an erection sufficient to permit satisfactory sexual intercourse (1). ED occurs as a result of a variety of factors such as preexisting disease, including hypertension, diabetes and/or heart disease, smoking, age, psychological influences, hormone levels and spinal cord injury. It may also be secondary to pharmacological treatments for some of the diseases mentioned above, particularly hypertension, as well as following surgery for the treatment of prostate cancer. It has been estimated that up to 30 million men in the United States have some degree of erectile dysfunction (2). Age is one of the most significant risk factors in the disease. A Massachusetts aging study suggested that up to 39% of men at age 40 and 70% at age 70 are affected by ED (3). The prevalence of cardiovascular disease and diabetes in this age group of men combine to accentuate the development and incidence of ED, resulting in the recent estimate that half of all male diabetics suffer from ED (4). A more extensive review of the risk factors and pathophysiology of ED has been published recently (5), as has a summary of the pharmacology and efficacy of sildenafil (6) and readers should consult these excellent sources for detailed information on these topics.

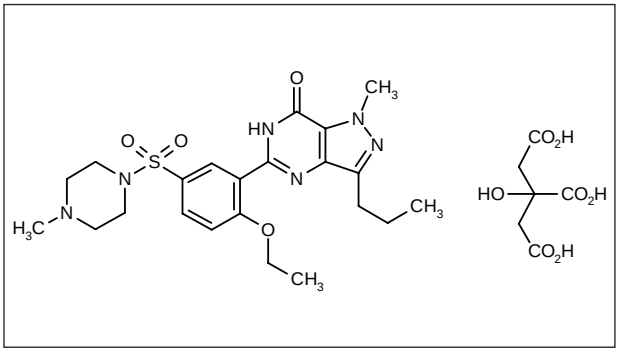


Fig. 1. Sildenafil citrate.

This review will highlight the mechanism of action of PDE5 inhibitors in ED, describe the biology and biochemistry of PDE5, the recent medicinal chemistry (including structure-activity relationships) of new PDE5 inhibitors and other pharmacologic actions of PDE5 inhibitors.

Mechanism of action of PDE5 inhibitors in erectile dysfunction

PDE5 is the primary cGMP hydrolyzing activity found in human corpus cavernosum tissue in the penis. cGMP is one of the primary second messengers that regulates smooth muscle tone in the corpus. Because erection is a hemodynamic event, vascular smooth muscle status is an obvious point for control of the process (Fig. 2). Erection occurs following sexual stimulation, either directly to the penis or psychosomatically. This causes the release of the neurotransmitter nitric oxide (NO) from nonadrenergic, noncholinergic neurons innervating the corpus cavernosum. NO diffuses into the tissue and causes activation of soluble guanylyl cyclase, which converts GTP to

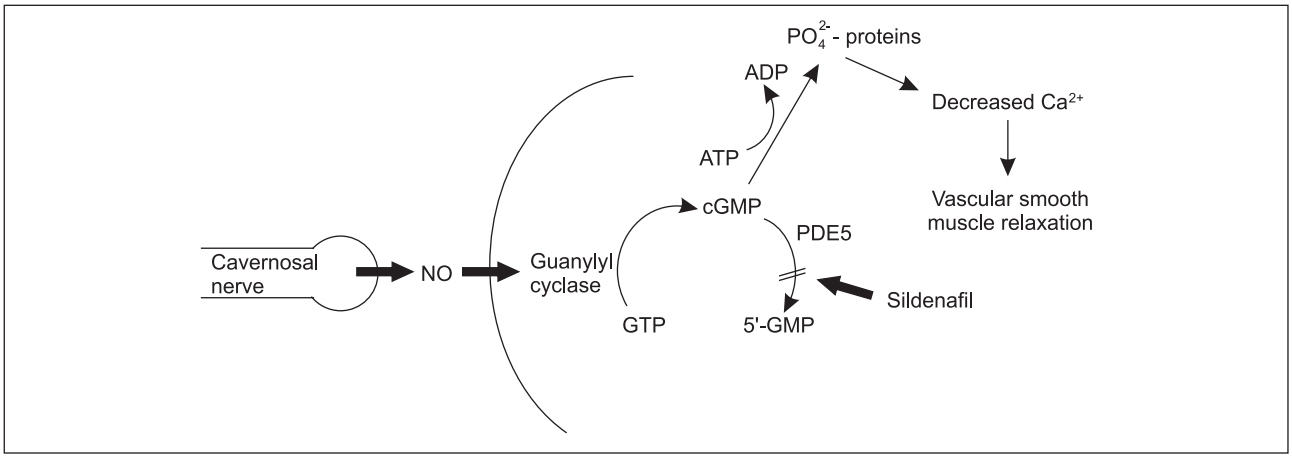


Fig. 2. PDE5 mechanism of action.

cGMP. The cyclic nucleotide activates protein kinase G (PKG), which in turn initiates a phosphorylation cascade. A variety of proteins are activated including phospholamban, the inositol phosphate receptor, Ca^{2+} and K^+ channels, Ca^{2+}-ATPase and others (7). The net result of this process is a decrease in the intracellular concentration of Ca^{2+}. Consequently, vascular tone in the arteries leading to the penis decreases causing increased blood flow and an enlargement of the corpus cavernosum tissue. The increased penile tumescence contracts veins, reduces the outflow of blood and increases intracavernosal pressure, resulting in an erection (5, 7).

Inhibition of PDE5 causes cGMP levels to be enhanced, leading to the desired pharmacologic effect. This mechanism of action requires a sexual stimulus, unlike the effects of prostaglandin E_1 that cause a spontaneous erection. Furthermore, when NO levels drop, the effect of a PDE5 inhibitor is significantly reduced because of the resulting decrease in guanylyl cyclase activity. This feature reduces the incidence of priaprism in patients. A precise understanding of the basis for the apparent functional selectivity of sildenafil on penile blood flow compared to the drug's effect on general circulation is not yet available. One possible explanation is based on the requirement for sexual stimulation, which initiates NO release in the corpus cavernosum. This leads to a significant increase in cGMP levels specifically in the penis, which is enhanced by a PDE5 inhibitor. However, the potentially severe interaction between sildenafil and organic nitrates on systemic blood pressure is well known. To better answer the question, a PDE5 inhibitor that is more selective than sildenafil *versus* PDE1 (the primary PDE in the vasculature) will be invaluable.

Phosphodiesterase biology and biochemistry

The mammalian phosphodiesterase superfamily of enzymes is comprised of at least 10 different subfamilies of gene products. Within each of these, there are a variety of splice variants. The known members of this family are listed in Table I.

Table I: Mammalian PDE enzymes.

PDE gene products	Substrate specificity	Consequence of inhibition
1A-C	cGMP, cAMP	Hypotension
2A	cAMP > cGMP	Unknown
3A-B	cAMP > cGMP	+ Inotrope arrhythmia
4A-D	cAMP	Emesis, antiinflammatory
5A	cGMP	Vascular relaxation
6A	cGMP	Visual disturbance, retinitis pigmentosa
7A	cAMP	Inhibits T-cell activation
8A	cAMP	Unknown
9A	cGMP	Unknown
10A	cAMP > cGMP	Unknown

PDEs that specifically hydrolyze cGMP include PDE5, PDE6 and PDE9, while others (PDE1-PDE3 and PDE10) have mixed specificity for other cyclic nucleotides. These enzymes are widely distributed in a variety of tissues; however, some are localized in essentially one site, such as PDE6, which is found in the retina (8). PDE5 is found throughout vascular smooth muscle tissue and to a lesser extent in the kidney, lung and platelets. PDE enzymes exert a critical regulatory function to maintain cyclic nucleotide concentrations within a comparatively narrow range. For example, a 2- to 4-fold increase in cGMP concentration above basal levels will elicit a maximal biological response (7).

PDE5 was first isolated and purified in 1980 (9) and cloned in 1993 (10). Two splice variants of PDE5 have been identified (11, 12) and three forms have recently been found in corpus tissue (13). PDE5 is active as a homodimer, with a molecular weight of approximately 200,000. There are two allosteric cGMP binding sites per subunit, along with a highly conserved Zn^{2+} binding motif. It has been proposed that when cGMP binds to the

catalytic site, this permits improved binding at the allosteric site. When cGMP binds at this locus, a conformational change is induced which leads to phosphorylation of serine 92 (14). The amino acid sequence in these sites (HX_3HX_nD) is critical, as is the conservation of two aspartic acid residues (D714 and D754) downstream from this sequence. It has been hypothesized that these aspartates act as ligands for a required metal, or that they may function in substrate hydrolysis as catalytic bases. Site-directed mutagenesis of 23 conserved amino acids in the catalytic domain was carried out to better define the substrate/inhibitor binding pocket. Analysis of this data indicated that substitution of alanine for Y602 resulted in a 25-fold increase in the IC_{50} value for sildenafil. Four other mutants, H607A, H643A, D754A and E775A, led to 10- to 13-fold increases in the IC_{50} value for sildenafil (15). This work showed that sildenafil, zaprinast (**2**, Fig. 3) and UK-122764 (**3**, Fig. 3) bound to PDE5 competitively with cGMP, indicating that these inhibitors and substrate bind at the same, or closely overlapping site on the protein. Additionally, the data indicated that these inhibitors bound only to the catalytic site, not the allosteric site on PDE5. Turko and coworkers proposed an SAR model for this class of compounds, which indicates that for improved affinity, additional hydrophobic interactions via the aromatic ring attached to the pyrazolopyrimidine core and a lower net charge are desirable. Bovine lung PDE5 was employed in these studies and it was demonstrated that similar K_i values were obtained for sildenafil and zaprinast using human corpus cavernosum PDE5 (16).
In another study, an attempt was made to understand the structural features that influence cyclic nucleotide specificity in PDE5 (17). A hydropathy analysis of the catalytic domain of several PDEs suggested that the particular pattern of hydrophilic and hydrophobic residues near glutamate 775 is critical. In cGMP-specific PDEs, there is a

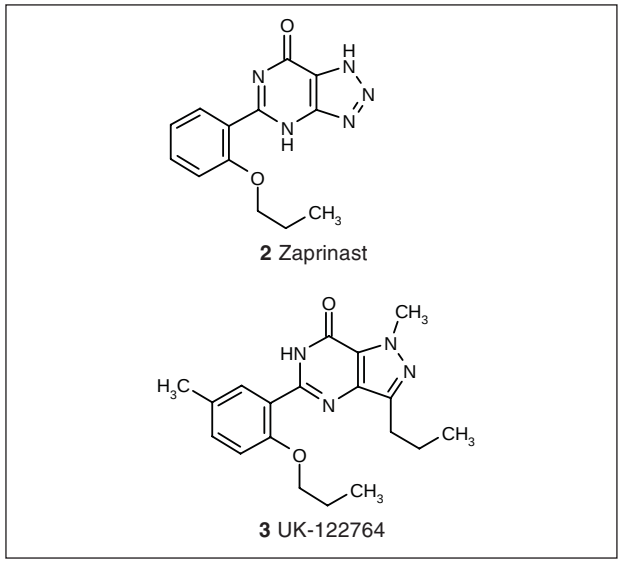


Fig. 3. PDE5 inhibitors.

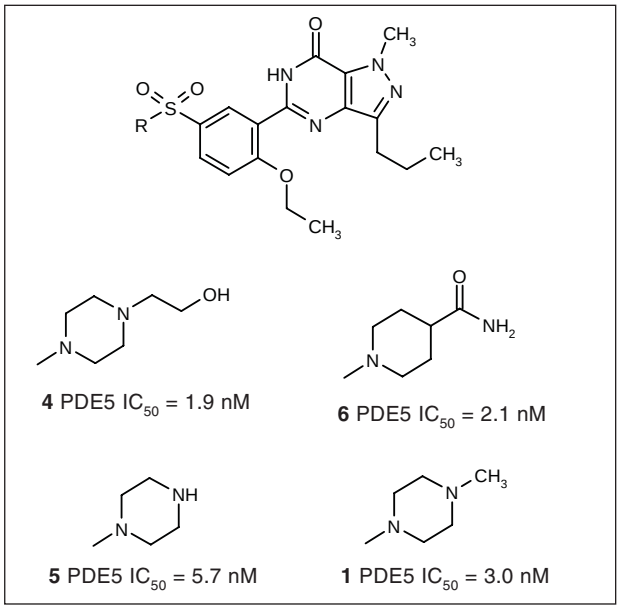


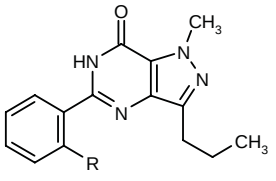
Fig. 4. Pyrazolopyrimidine sulfonamide SAR.

higher proportion of hydrophobic amino acids. This leads not only to enhanced cGMP binding but also discriminates against cAMP binding.

Medicinal chemistry

Until very recently, much of the peer reviewed literature on PDE5 inhibitors has focused on functional activities other than erectile dysfunction. Sildenafil was originally studied clinically for the treatment of angina and hypertension. However, given the success of sildenafil, there is substantial interest in examining PDE5 inhibitors for ED and there are at least four other compounds in clinical testing for this purpose. Wherever possible, the selectivity profile of compounds will be summarized. This feature is an important one for the development of PDE5 inhibitors that may offer improvements over sildenafil. If compounds can be identified that demonstrate significantly improved PDE selectivity, particularly against PDE1 and PDE6, this will reduce the incidence of visual disturbances (PDE6) and possibly also the flushing, headache and organic nitrate interaction (PDE1). With improved selectivity and potency, it may also be possible to expand the dosage window in an attempt to improve on the efficacy of sildenafil, which is currently recommended to be limited to 100 mg per dose.

A limited amount of structure-activity data has been published on sildenafil. Terret and coworkers used zaprinast as the starting point for their work (18). The triazene ring of zaprinast was replaced with a more lipophilic alkylated pyrazole. Addition of a sulfonamide at C5' on the pendant aromatic ring led to a further substantial improvement in PDE5 potency (Fig. 4). Four sulfonamides were reported in this paper and in the information



	R	PDE5 IC_{50} (nM)
7	H	4500
8	OH	1000
9	OEt	27
10	NO_2	4400
11	$NHSO_2CH_3$	780
12		960

Fig. 5. C2' SAR.

disclosed, there was a 3-fold range of PDE5 potency. The most potent analog **4** has a 2-hydroxyethylpiperazine sulfonamide, while the least potent compound was the piperazine *N*-desmethyl derivative **5**. This data was presented without standard errors, and as a result it is possible that sildenafil is in fact equipotent compared to **4** and the isonipecotic amide **6**.

Structure-activity data was also disclosed for the 2'-ethoxy ether substituent (Fig. 5). In this case, there was a significant range of PDE5 activity, with the ethoxy ether clearly preferred. From this information, the authors suggest that this moiety binds in a small lipophilic pocket and that it may also hydrogen bond with the pyrimidinone NH to help maintain a coplanar orientation of the ring systems.

A series of patent applications from Pfizer give a glimpse at other structure-activity relationships in the pyrazolopyrimidinone core. Only PDE5 data was given and thus it was not possible to evaluate the PDE selectivity of these analogs. The sulfonamide linkage can be replaced with both a ketone, *e.g.*, **13** (19) and a secondary alcohol, *e.g.*, **14** (20) resulting in derivatives with similar IC_{50} values (Fig. 6). In the pyrazole ring, the methyl group can be replaced by an ethyl morpholine moiety, *e.g.*, **15** (21). Pyrazole regioisomers **16** (22) and **17** (23) illustrate that pyridyl heterocycles are also tolerated as replacements for the pendant benzene ring and for the alkyl moiety on the pyrazole. Sildenafil analogs **15-17** have similar IC_{50} values, suggesting that the binding pocket for the pyrazole ring is not well defined and can tolerate a variety of substituents.

Dumaitre and Dodic carried out an extensive evaluation of heterocycles appended to the pyrimidinone ring (24). Thiophene, pyridine, pyrimidine, imidazole, pyrazole, isoxazole and isothiazole analogs were prepared and the alkyl pyrazole **18** was shown to be clearly preferable over the others (Fig. 7). This template was then employed to evaluate substituents in the pendant aromatic ring and N-1 of the pyrazole.

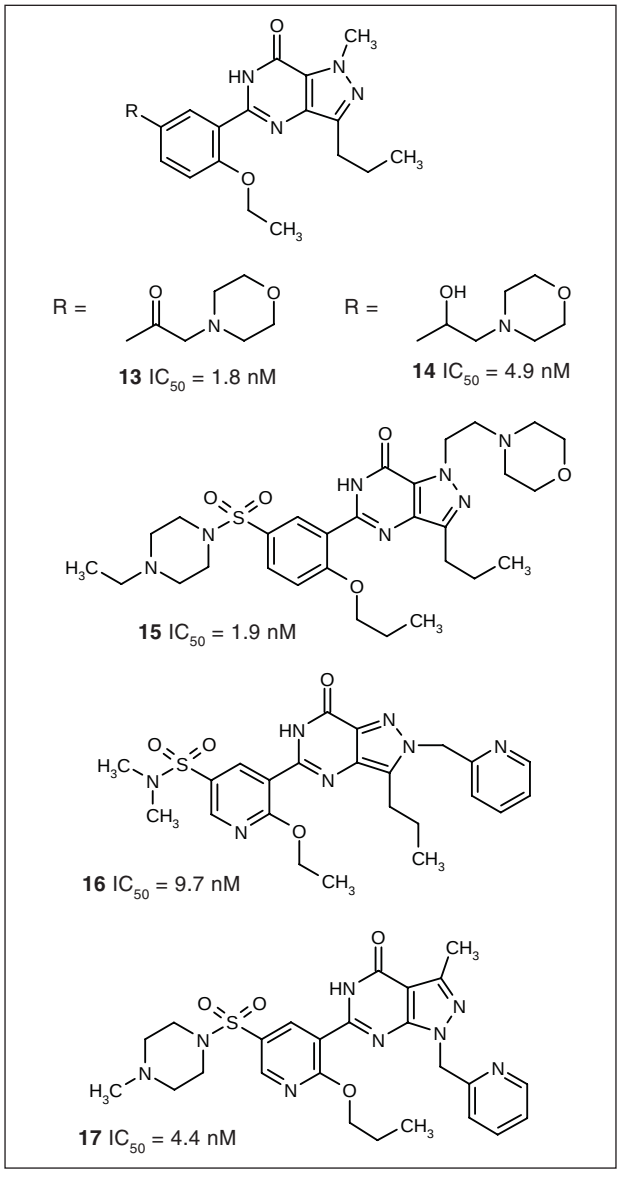


Fig. 6. Additional sildenafil SAR.

At the ether moiety (Fig. 7), it was shown that a propyl ether was preferred (IC_{50} = 8 nM) over an ethoxy ether (IC_{50} = 30 nM) and isopropyl ether or propoxythio ether (IC_{50} = 40 and 2000 nM, respectively). In the pyrazole ring, benzyl and *t*-butyl substituents (Y) are clearly less potent (IC_{50} = 20, 70 nM) than simple alkyl moieties (IC_{50} = 1-8 nM). A nitrogen-based linker at X was used to evaluate amide, alkyl sulfonamide, carbamate, urea, thiourea and heterocyclic moieties. This wide range of analogs produced a comparatively narrow range of PDE5 IC_{50} values, as most of the compounds varied less than 10-fold in activity (IC_{50} = 1-10 nM). From this group, a methanesulfonamide derivative **19** was selected for more detailed evaluation as a potential antihypertensive agent. This choice was based on potency in a whole cell assay

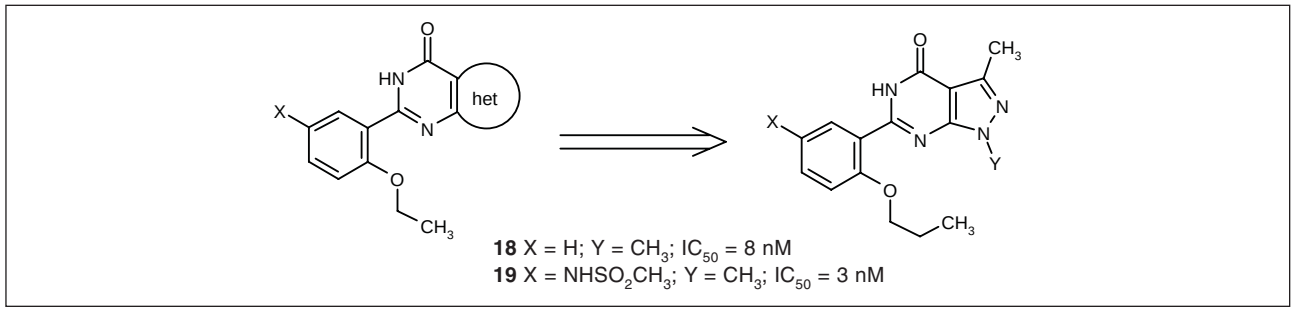


Fig. 7. Pyrazole N-1 and aromatic substituent SAR.

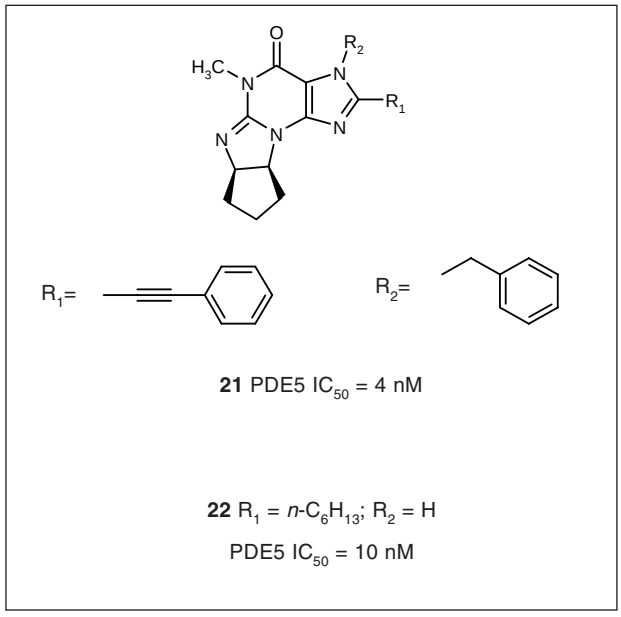


Fig. 8. C2 SAR of Schering-Plough PDE5 inhibitors.

using rat aortic smooth muscle, *in vitro* metabolic stability and blood pressure reduction in spontaneously hypertensive rats. It was stated that **19** showed greater than 1000-fold selectivity *versus* PDE1-PDE4.

Schering-Plough has reported on a series of tetracyclic dual PDE1/PDE5 inhibitors that have potential as antihypertensive agents. These two PDE isoforms are widely distributed in the vasculature and inhibition of both can result in blood pressure lowering in animal models. One report in the series from Schering-Plough described structure-activity relationships at C2 of the imidazole ring (Fig. 8) (25).

In this series, lipophilic moieties at R_1 were clearly preferred over tertiary amines, imidazole or alkoxy ethers for PDE5 activity. The preferred compound **21** had a PDE5 IC_{50} of 4 nM. An alkyl chain was also acceptable, with *n*-hexyl optimal (PDE5 IC_{50} = 10 nM). The presence or absence of a benzyl group at R_2 had variable effects on PDE5 activity. With the same group at R_1, in some cases the benzyl analog was significantly more potent, while in others it was markedly less active. At R_2, a smaller range of substituents was studied with a benzyl group at R_1. The most active analog in this set was substantially less active than **21** and **22**.

A later report (26) looked in greater detail at analogs related to **22** (Fig. 9). This work showed clearly that PDE5 affinity improved as the length of the alkyl chain was extended (6 carbons optimal) and that minimal distal branching (*e.g.*, **23**) was acceptable, but that branching closer to the tetracyclic core (*e.g.*, **24**) was unfavorable. The carbocyclic ring could be replaced equally well by a spiro system (**25**), gem-dimethyl (**26**) or isopropyl substituents (**27**).

Regioisomeric tricyclic pyrazolopyrimidines were also prepared and evaluated by the group at Schering Plough

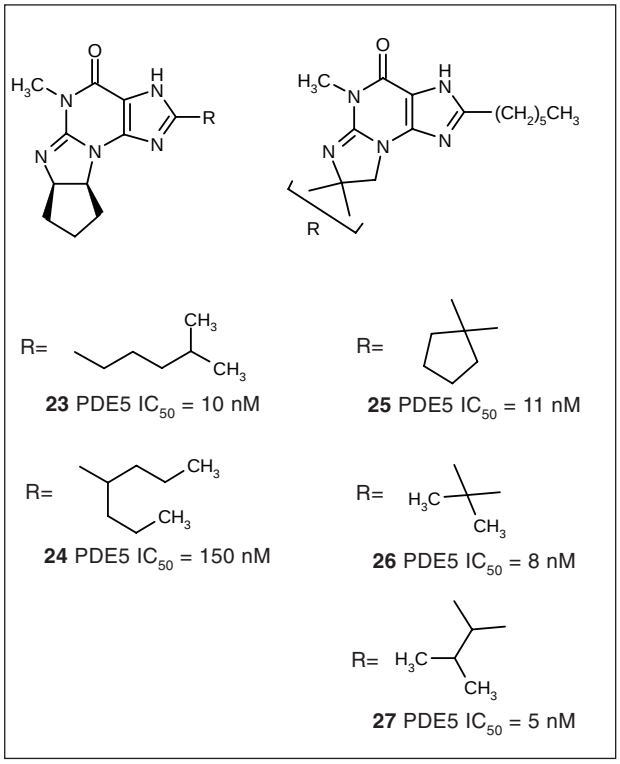


Fig. 9. C2 alkyl and carbocyclic ring SAR.

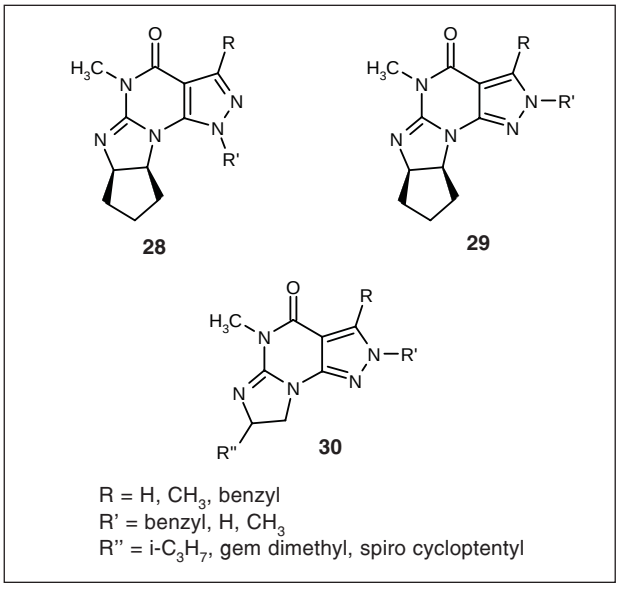


Fig. 10. Schering-Plough pyrazolopyrimidines.

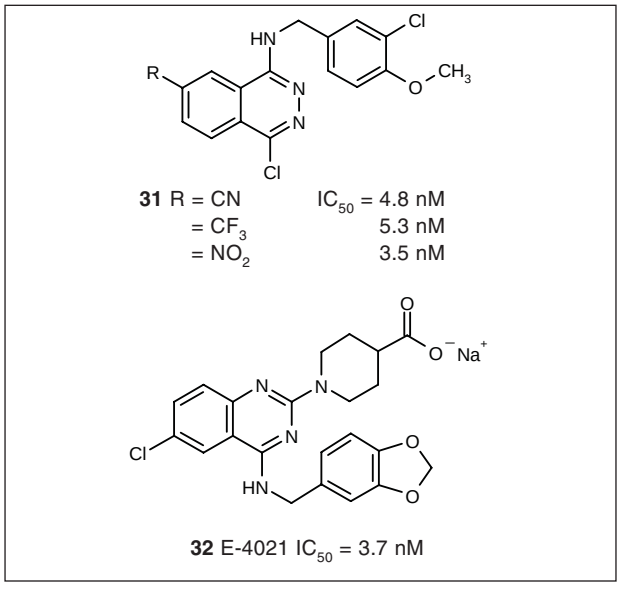


Fig. 11. Benzylamino phthalazines.

(27). The compounds reported in this paper evaluated benzyl and methyl analogs of the template structure (Fig. 10). None of the compounds were as potent as **23** or **25-27**; however, the activity of analogs with optimized substituents was not reported. The most active compound in the series (**30**, R = benzyl, R' = methyl, R''= spirocyclopentyl) had a PDE5 IC_{50} of 75 nM and an IC_{50} of 60 nM *versus* PDE1.

Watanabe and coworkers have reported a series of 4-benzylamino-6-substituted phthalazines as potent PDE5 inhibitors (28). These compounds were derived from their earlier work on a series of quinazolines, which resulted in E-4021 (**32**, Fig. 11) (28). An electron withdrawing group at the 6-position of the phthalazine was essential for activity, and CN, NO_2 or CF_3 were equally good *in vitro*. Substitution on the benzylamino group proved to be a particularly critical determinant of PDE5 activity. In this series, the benzene ring must be a 3-chloro-4-methoxy analog. This pattern was at least 10-fold better than 3,4-methylenedioxy.

The Eisai group used these chloro phthalazines for further derivatization (30). They built on their earlier work and used the 3-chloro-4-methoxybenzylamino and 6-cyano-substituted phthalazine core as a substrate for displacement of the 1-chloro group with a variety of amines (Fig. 12). Among the most potent analogs were the 4-hydroxy piperidine E-4010 (**33**), thiomorpholine **34**, 4-keto piperidine **35** and isonipecotamide **36**, all of which had IC_{50} values ≤ 0.5 nM *versus* PDE5. The PDE selectivity of E-4010 was measured and it was found that **33** was a micromolar inhibitor of PDE1-4, giving a selectivity ratio of at least 1800 in favor of PDE5. E-4010 is reported to be in phase I clinical trials for ED. Phenyl piperazines, benzylamines and other alkyl amines gave less potent derivatives.

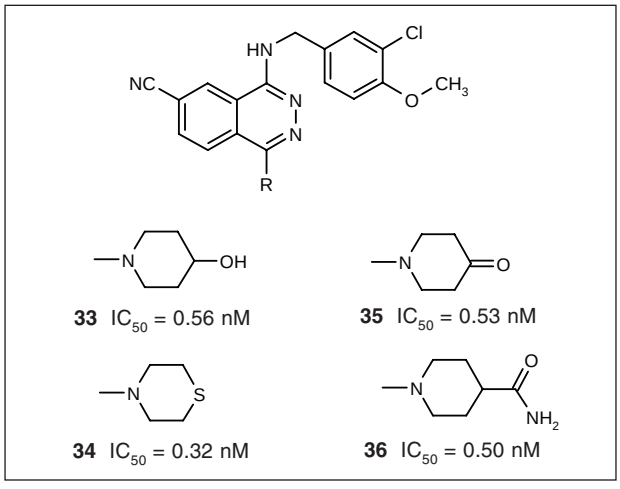
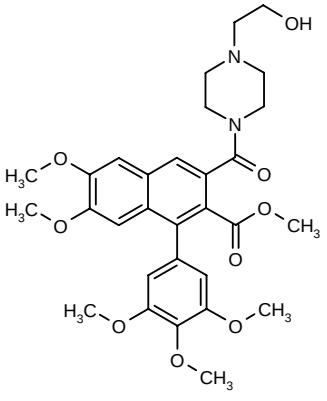
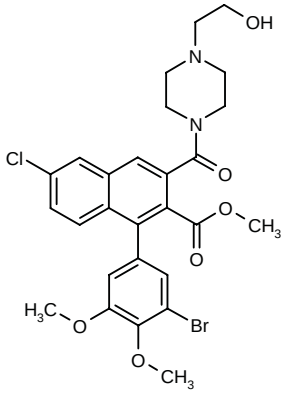


Fig. 12. 1-Amino substituted phthalazines.

The 1-aryl naphthalene lignan nucleus, previously reported to be useful for the synthesis of PDE4 inhibitors (31), was reexamined for utility as a PDE5 inhibitor template (32). A series of 2-carboxy-3 carbomethoxy analogs were examined initially, leading to the selection of a 2-hydroxyethyl piperazine amide **37** as the most potent compound, with good (> 400-fold) selectivity *versus* PDE1 and PDE3 (Fig. 13). Substituents on the 1-aryl ring were then examined, along with substituents on the naphthalene ring. A 3-bromo-4,5-dimethoxy benzene ring was found to be similar in PDE5 potency to the 3,4,5-trimethoxy analog. Replacement of the dimethoxy substituents on the naphthalene ring with a 6-chloro group



37 IC_{50} = 9.4 nM



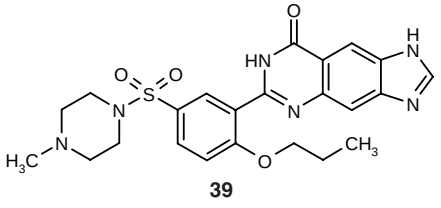
38 IC_{50} = 6.2 nM

Fig. 13. 1-Aryl lignan PDE5 inhibitors.

gave a compound (**38**) with significantly improved PDE1 and PDE4 selectivity (IC_{50}s > 100 mM) and equal PDE5 potency. Compound **38** had a superior selectivity profile compared to E-4021 (32) with only slightly less PDE5 activity.

Bristol-Myers Squibb has reported details on the discovery, selectivity and functional activity of imidazoquinazolinones based – like sildenafil – on zaprinast (33, 34). The starting point for this research was *N*-methyl piperazine sulfonamide **39** (Fig. 14), a potent but nonselective PDE5 inhibitor. It was discovered that a benzyl moiety at N-3 of the imidazoquinazoline significantly improved PDE selectivity while maintaining PDE5 potency. Optimization of substituents on the benzyl group and at C5' on the pendant aromatic ring led to the identification of a series of compounds which were both more potent and selective *in vitro* compared to sildenafil, especially *versus* PDE6.

Rabbit corpus cavernosum tissue strips were employed in an *in vitro* functional assay for efficacy. This test measures potentiation of the normal relaxation of smooth muscle following electrically stimulated contraction. At 30 nM, sulfonamides **39** and **41** and 4-fluorobenzyl carboxamide **43** had efficacy similar to sildenafil (Table II). Sulfonamides **40** and **42**, as well as other *N*-benzyl sulfonamides, were either weakly active or inactive in this assay. It was hypothesized that the comparatively high molecular weight (> 570) of these compounds was a primary contributing factor to the poor efficacy in the corpus strip assay. Carboxamide **43** was chosen for pharmacokinetic evaluation in fasted dogs. The compound had insufficient oral bioavailability (10%) for further study in an *in vivo* model of ED.



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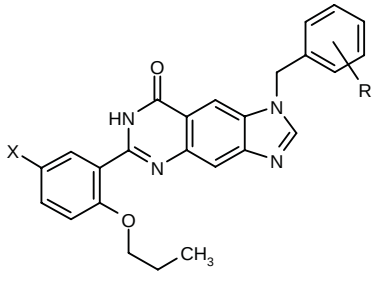
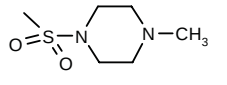
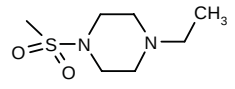
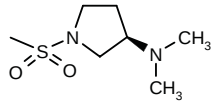




40 R = 3-Cl



41 R = 2-OCH_3



42 R = 4-F

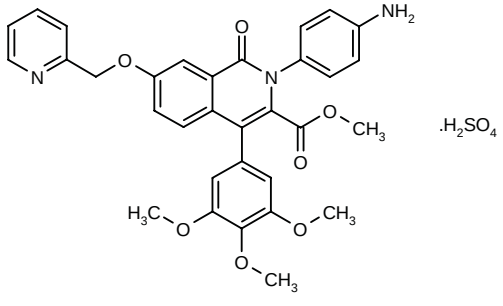
43 X = $CONH_2$ R = 4-F

Fig. 14. N-3-Benzyl imidazoquinazolines.

Table II: PDE5 inhibition, selectivity and functional activity.

Cpd	PDE IC_{50} nM	PDE6 selectivity ratio	% Control relaxation integral (30 nM)[1]
39	5.6	2	150
40	0.71	90	130
41	0.62	80	140
42	0.20	70	120
43	0.48	60	150
1	1.6	7	150

[1]Untreated control = 100%.



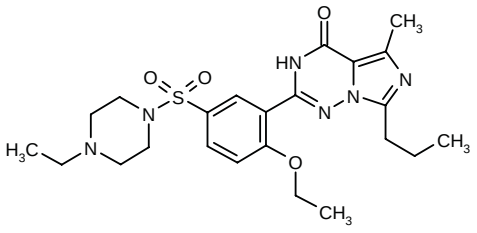
44

PDE IC_{50} (nM)	
5	1
1	3000
2	10000
3	$>10^5$
4	3000
6	30

Fig. 15. T-1032.

Tanabe has recently reported the *in vivo* activity of T-1032 (**44**, Fig. 15) in a dog model of ED (35). This highly substituted isoquinoline derivative is more potent and selective than sildenafil *in vitro*. The compound demonstrated efficacy comparable to sildenafil in an *in vitro* functional assay using canine penile tissue. When T-1032 was given intravenously to dogs, nerve-induced tumescence was potentiated over a dose range of 10-100 mg/kg, without a significant effect on blood pressure. Interestingly, when the compound was administered intracavernosally, it was active but did not cause an increase in intracavernosal pressure like sildenafil. T-1032 is reportedly in clinical development for ED.

Vardenafil (Bay-38-9456) (**45**, Fig. 16) is in phase III trials for the treatment of ED. It was recently reported that the compound is efficacious *in vitro* and *in vivo* in conscious rabbits (36, 37). Vardenafil is more potent and selective than sildenafil *in vitro*, with a subnanomolar IC_{50} value. In rabbits, the drug was administered over a dose range of 0.1-3 mg/kg i.v. and potentiated erection induced by the NO donor sodium nitroprusside.

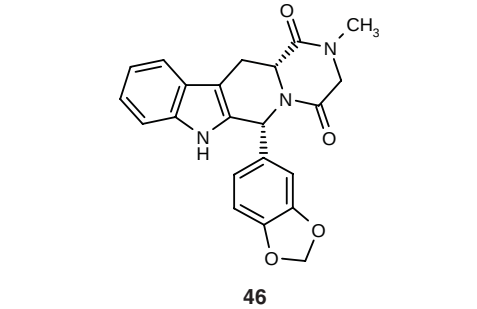


45

PDE IC_{50} (nM)	
5	0.7
1	180
2	2500
3	4000
4	$>10^4$
6	157

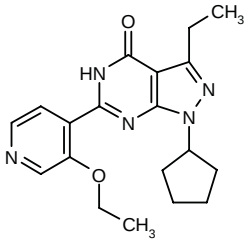
Fig. 16. Vardenafil.

IC-351 (Cialis™) (**46**, Fig. 17) is another PDE5 inhibitor in phase III trials for efficacy as a treatment for ED. This compound was originally discovered by Icos, which subsequently partnered with Eli Lilly for clinical development. While no *in vitro* data on potency and selectivity have been published at this writing, clinical results from a variety of studies have been reported (38-40). On demand doses of 2-25 mg in 212 men over 8 weeks led to “significant” improvement in a number of parameters of the international index of erectile function at doses ≥ 5 mg. Similar results were obtained in 179 men at a different center. A larger study used 294 men and doses up to 100 mg. In this group, a larger percentage of participants reported improved erectile function compared to the lower dose studies. Adverse events reported in these trials include headache, dyspepsia and back pain, and were mild, reversible and dose-related. There was no significant incidence of visual disturbances reported, suggestive of measurably greater PDE6 selectivity for IC-351. It has been projected that Eli Lilly will file an NDA on IC-351 in 2001.



46

Fig. 17. IC-351.



47

Fig. 18. WIN-65529/SR-265579.

Other activities of PDE5 inhibitors

Although much of the current interest in PDE5 inhibitors is associated with their potential use for the treatment of ED, there have been a number of reports in recent years on other activities associated with PDE5 inhibition. Recall that Schering Plough has investigated dual PDE1/PDE5 inhibitors in animal models of hypertension and shown that selected compounds demonstrated oral activity (24-26). Blood pressure lowering activity has also been demonstrated using WIN-65579 (**47**, Fig. 18) in conscious, spontaneously hypertensive rats at an oral dose of 10 mg/kg, without a significant change in heart rate (41). This same compound, described as SR-265579, was reported by another group to dilate bronchioles in guinea pigs induced by histamine (42). Following i.v. dosing, an EC_{50} of 0.63 mg/kg was measured.

The phthalazine E-4010 was reported to improve survival in monocrotaline-induced pulmonary hypertension in rats. The drug was administered in the diet at 0.01% or 0.1% for 23 days. A statistically significant improvement in survival was measured in the higher dose group (43).

Watkins and coworkers have shown that in diabetic mice, sildenafil can reverse delayed gastric emptying (44). This process is dependent on relaxation of the pyloric muscle which is stimulated by NO and cGMP as the intracellular second messenger. Diabetic gastroparesis is one of the long-term side effects of poor blood sugar control and occurs in a significant number of people with diabetes. If this finding can be replicated in man, it would expand the medically justifiable use of a PDE5 inhibitor. This would move the drug beyond a simple “quality of life” treatment.

Summary and outlook

The wide variety of chemotypes and potent, selective PDE5 inhibition illustrated above are indicative of a substantial level of interest in the area. Vardenafil and IC-351 are in advanced clinical development, while T-1032 and E-4010 are at an earlier stage of clinical evaluation. The reports of additional beneficial properties associated with PDE5 inhibitors, if they can be replicated in man, will extend the market for this group of compounds. This clearly is an area of drug development to be closely followed for the next several years.

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