

SILDENAFIL (VIAGRA™), A POTENT AND SELECTIVE INHIBITOR OF TYPE 5 CGMP PHOSPHODIESTERASE WITH UTILITY FOR THE TREATMENT OF MALE ERECTILE DYSFUNCTION

Nicholas K. Terrett,* Andrew S. Bell, David Brown¹ and Peter Ellis,[‡]

*Departments of Discovery Chemistry and Discovery Biology,[‡]
Pfizer Central Research, Sandwich, Kent, CT13 9NJ, UK*

Abstract: 5-(2'-Alkoxyphenyl)pyrazolo[4,3-d]pyrimidin-7-ones, and in particular our preferred compound, sildenafil (VIAGRA™), discovered through a rational drug design programme, are potent and selective inhibitors of the type 5 cGMP phosphodiesterase from both rabbit platelets and human corpus cavernosum. Sildenafil is currently in the clinic for the oral treatment of male erectile dysfunction.

Copyright © 1996 Elsevier Science Ltd

Introduction: Cyclic guanosine monophosphate (cGMP) is the ubiquitous second messenger for those G-protein coupled receptors activated by endogenous substances such as nitric oxide (NO or EDRF) and atrial natriuretic peptide (ANP). Intracellular levels of cGMP are controlled by activation of cyclic nucleotide cyclases and breakdown by phosphodiesterases (PDE). Specifically, there are a number of PDE isozymes that will hydrolyse cGMP to the inactive GMP and thus cGMP levels may be raised by the use of a selective cGMP PDE inhibitor.² There are at least seven families of PDE³, of which three (types 1, 5 and 6) selectively hydrolyse cGMP relative to cAMP.^{4,5} PDE type 5, the calcium/calmodulin insensitive cGMP phosphodiesterase, occurs in lung, platelets and various forms of smooth muscle.⁶

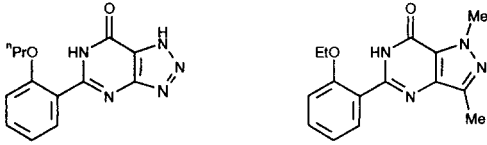
We considered that selective inhibitors of PDE type 5 would be attractive targets for the therapy of a range of cardiovascular disorders. As a consequence of our work on ANP,⁷ we anticipated that a potent inhibitor would have utility in the therapy of hypertension and angina. However, we found that type 5 cGMP PDE is also the predominant cGMP hydrolysing activity in the cytosolic fraction from human corpus cavernosum.⁸ As penile erection is mediated by NO,⁹ and thus cGMP,¹⁰ inhibitors of type 5 PDE improve erection by enhancing relaxation of the corpus cavernosal smooth muscle, and thereby have utility for the treatment of male erectile dysfunction (impotence). We here report the discovery of sildenafil (VIAGRA™) (X), a potent and highly selective inhibitor for the type 5 PDE, that is an orally active treatment for male erectile dysfunction.

Results and Discussion: Prior to our work, very little had been reported on the design, synthesis and screening of selective cGMP PDE inhibitors. Zaprinast (I, M&B 22,948),¹¹ developed as an anti-allergy agent, was one of the first type 5 PDE inhibitors to be reported, albeit only weakly active and a poorly selective

compound. Possibly as a consequence of its PDE inhibitory activity, zaprinast is a vasodilator *in vitro*¹² and lowers mean arterial blood pressure in anaesthetised dogs.¹³ Zaprinast was screened against cGMP PDE type 5 isolated from rabbit platelets, and demonstrated only modest affinity for this cGMP-hydrolysing enzyme and little selectivity over PDE 1.¹⁴

In order to find novel compounds with improved potency and selectivity over zaprinast, we explored a range of novel 2-alkoxyphenyl-substituted heterocyclic systems. Of the many series investigated, we found that derivatives of pyrazolo[4,3-d]pyrimidin-7-one (e.g. **II**, see Figure 1) gave potent cGMP PDE type 5 inhibition. As we sought compounds with type 5 selectivity, all compounds were also screened against the other widespread cGMP degrading PDE, type 1, isolated from rat liver, and cAMP PDE type 3 isolated from rabbit platelets.¹⁵ Type 5 PDE from human corpus cavernosal tissue was obtained and we demonstrated that it was essentially identical to the rabbit platelet enzyme and that standard inhibitors such as zaprinast have similar affinities for both enzymes (corpus cavernosum PDE type 5 IC_{50} = 800nM).

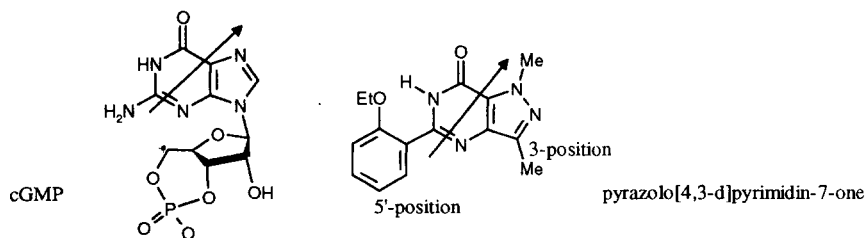
Figure 1 Enzyme inhibitory data. IC_{50} values are in nanomolar unless otherwise stated, and are the mean values of at least 2 determinations.



IC_{50} (nM)	I (zaprinast)	II
PDE 1	9400	3300
PDE 3	>100 μ M	>100 μ M
PDE 5	2000	330

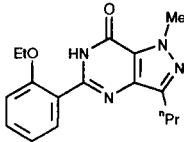
We explored the scope for increasing potency and selectivity with substituents around the pyrazolopyrimidinone structure. Modelling studies suggested that the nucleus may mimic the guanosine base of cGMP, as both are of similar size, shape and have a similar dipole moment (see Figure 2).¹⁶ We considered that

Figure 2 Modelling studies on cGMP and a pyrazolo[4,3-d]pyrimidin-7-one

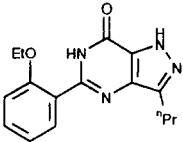


extending the 3-substituent might fill a space in the enzyme active site occupied by ribose, and substituents on the 5'-position of the phenyl ring could, depending on the conformation of cGMP in the enzyme active site, reproduce the role of the phosphate in binding. Replacement of the 3-methyl group in **II** by n-propyl gave a much more potent compound (**III** see Figure 3) with increased selectivity over type 1 PDE. Removal of the 1-methyl group (**IV**) from the pyrazole reduced type 5 activity.

Figure 3 Enzyme inhibitory data. IC_{50} values are in nanomolar unless otherwise stated, and are the mean values of at least 2 determinations.



III

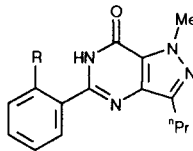



IV

IC ₅₀ (nM)	III	IV
PDE 1	790	860
PDE 3	>100μM	>100μM
PDE 5	27	82

Exploration around the 2'-substituent in this series suggested that ethoxy was preferred over many other groups (see Figure 4). Replacement of ethoxy with hydrogen (**V**) reduced type 5 PDE affinity some 200-fold, and hydroxy, nitro or sulphonamide derivatives (**VI**, **VIII** and **IX** respectively) are all weaker in activity. The SAR suggested that key features in the 2'-alkoxy series were a hydrogen bond between the pyrimidinone NH and oxygen lone pair of the alkoxy group maintaining coplanarity between the phenyl and heterocyclic systems (confirmed by an X-ray crystal structure¹⁷) and a requirement for a small lipophilic substituent.

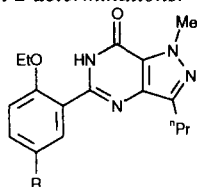
Figure 4 The effect of the 2'-substituent on PDE 5 inhibitory activity. IC_{50} values are in nanomolar unless otherwise stated, and are the mean values of at least 2 determinations. ND = not determined.



Compound	Structure R =	IC ₅₀ (nM)		
		PDE 1	PDE 3	PDE 5 (platelet)
V	H	ND	63,000	4,500
VI	HO	ND	>100μM	1,000
III	EtO	790	>100μM	27
VII		ND	47,000	960
VIII	NO ₂	ND	>100μM	4,400
IX	NHSO ₂ Me	ND	83,000	780

As mentioned above, a 5'-substituent on the 2-ethoxyphenyl ring has the potential to fill a space occupied by the phosphate of cGMP in the PDE active site. Access to 5'-substituted analogues is synthetically straightforward as electrophilic attack occurs selectively at this position and can be effected at a late stage in the synthesis, permitting rapid production of many analogues. Additionally, in order to improve the low solubility of compound **III** ($\log D = 4.0$), we wanted to make analogues with lower lipophilicity. The introduction of polar or charged substituents in 5'-sulphonamides (see Figure 5) gave derivatives with lower values of $\log D$. These were demonstrated to possess greater solubility, as compared with **III** and furthermore we found clear increases in enzyme affinity (see compounds **X** (sildenafil), **XI**, **XII**, and **XIII**). Intriguingly, 5'-substitution of zaprinast with sulphones or sulphonamides enhanced the antiallergic activity of this series,¹⁸ although it was not clear that this activity was mediated through inhibition of cGMP PDE.

Figure 5 The lipophilicity of 5-(2'-alkoxyphenyl)pyrazolo[4,3-d]pyrimidin-7-ones could be varied by the use of polar or charged 5'-sulphonamide substituents. IC_{50} values are in nanomolar unless otherwise stated, and are the mean values of at least 2 determinations.



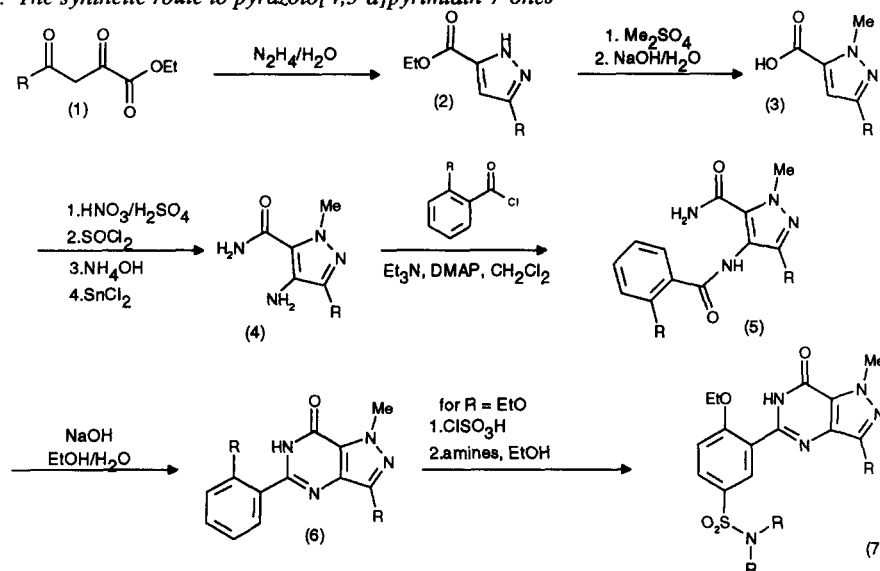
Compound	Structure R =	IC_{50} (nM)			Log D
		PDE 1	PDE 3	PDE 5 (platelet)	
III	H	790	>100 μ M	27	4.0
X Sildenafil (VIAGRA TM)		260	65,000	3.6 (platelet) 3.0 (corpus cavernosum)	2.7
XI		460	62,000	1.9	2.0
XII		110	34,000	2.1	2.3
XIII		390	>100 μ M	5.7	1.5

Overall, our results demonstrated that a range of different 5'-substituents are tolerated by PDE type 5, and amongst these, sildenafil (**X**) gave an excellent combination of enzyme inhibitory potency, selectivity, solubility and *in vivo* characteristics.

The synthesis of pyrazolo[4,3-d]pyrimidin-7-ones commenced with the preparation of the pyrazole ring from the diketoeester (1) and hydrazine (see Figure 6). Following the regioselective N-methylation of the pyrazole, hydrolysis gave the carboxylic acid (3). Nitration followed by carboxamide formation and nitro group

reduction gave the key substituted pyrazole intermediate (4). Acylating the amine with a 2-substituted benzoyl chloride and cyclisation under basic conditions produced the pyrazolopyrimidinone (6). For the preferred 2'-ethoxy series, chlorosulphonylation proceeds selectively on the 5'-position of the phenyl ring, allowing ready coupling with a range of amines to afford the sulphonamide products (7).

Figure 6. The synthetic route to pyrazolo[4,3-d]pyrimidin-7-ones



In summary, 5-(2'-alkoxyphenyl)pyrazolo[4,3-d]pyrimidin-7-ones, and sildenafil in particular, are potent and selective inhibitors of the type 5 cGMP phosphodiesterase from both rabbit platelets and human corpus cavernosum. We have demonstrated that structural modification has achieved a 500-fold increase in cGMP PDE affinity over our early leads. The efficacy of sildenafil (VIAGRA™) for the oral therapy of male erectile dysfunction is currently being assessed through clinical trials, and results from these studies will be published in the near future.

Acknowledgements: The authors are grateful to S.F. Campbell and D.A. Roberts for initial suggestions and modelling work with zaprinast and cGMP, to M.F. Burslem for enzyme isolation and screening, and also to J. Bordner (Pfizer Central Research, Groton, CT, USA) for X-ray crystallographic studies.

References and Notes

1. Current address, Medicinal Sciences, Glaxo Wellcome Research and Development, Medicines Research Centre, Gunnel Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
2. Goy, M.F. cGMP: the Wayward Child of the Cyclic Nucleotide Family. *Trends Neurosci.* **1991**, *14*, 293-299
3. Beavo, J.A.; Conti, M.; Heasley, R.J. Multiple Cyclic Nucleotide Phosphodiesterases. *Mol. Pharmacol.* **1994**, *46*, 399-400

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.