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

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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.

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 PDE[3], 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]

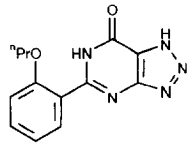
We considered that selective inhibitors of PDE type 5 would be attractive targets for the therapy of a range of cardiovascular disorders. As a consequence of our work on ANP,[7] we anticipated that a potent inhibitor would have utility in the therapy of hypertension and angina. However, we found that 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.

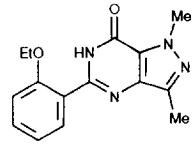
Results and Discussion: Prior to our work, very little had been reported on the design, synthesis and screening of selective cGMP PDE inhibitors. Zaprinast (**I**, M&B 22,948),[11] developed as an anti-allergy agent, was one of the first type 5 PDE inhibitors to be reported, albeit only weakly active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

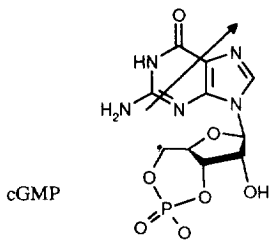




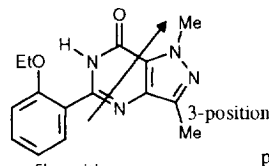
IC_{50} (nM)	**I** (zaprinast)	**II**
PDE 1	9400	3300
PDE 3	>100μM	>100μM
PDE 5	2000	330

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*



cGMP

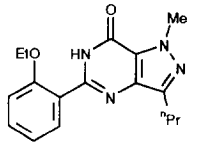


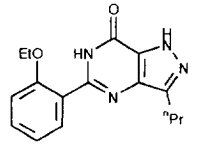
5'-position

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.*

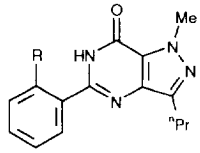




IC_{50} (nM)	**III**	**IV**
PDE 1	790	860
PDE 3	>100μM	>100μM
PDE 5	27	82

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 structure[17]) and a requirement for a small lipophilic substituent.

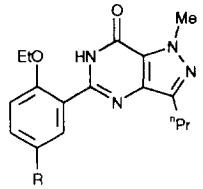
Figure 4 *The effect of the 2'-substituent on PDE 5 inhibitory activity. IC_{50} values are in nanomolar unless otherwise stated, and are the mean values of at least 2 determinations. ND = not determined.*

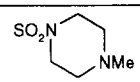
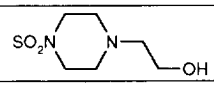
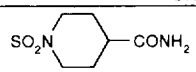
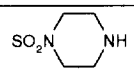


Compound	Structure R =	IC_{50} (nM)		
		PDE 1	PDE 3	PDE 5 (platelet)
V	H	ND	63,000	4,500
VI	HO	ND	>100μM	1,000
III	EtO	790	>100μM	27
VII		ND	47,000	960
VIII	NO_2	ND	>100μM	4,400
IX	$NHSO_2Me$	ND	83,000	780

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.*



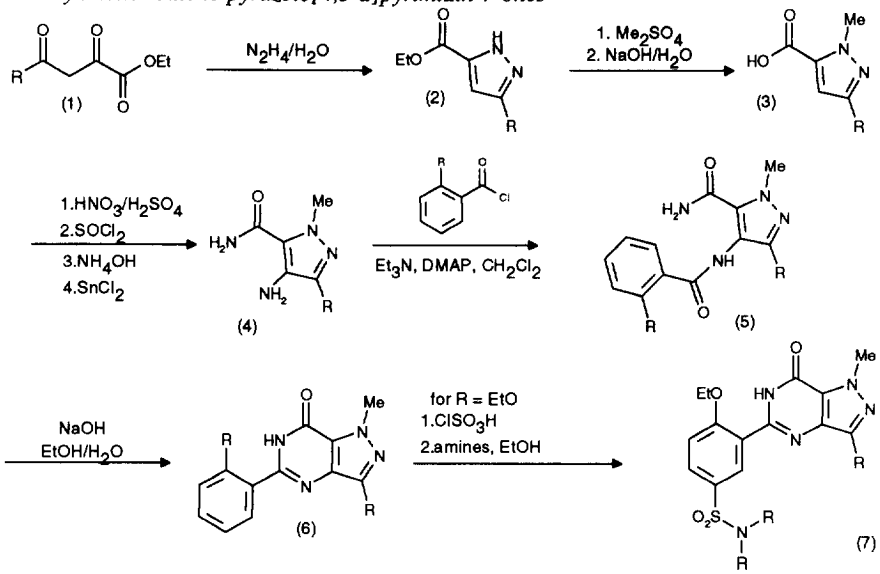
Compound	Structure R =	IC_{50} (nM)			Log D
		PDE 1	PDE 3	PDE 5 (platelet)	
III	H	790	>100μM	27	4.0
X Sildenafil (VIAGRA™)		260	65,000	3.6 (platelet) 3.0 (corpus cavernosum)	2.7
XI		460	62,000	1.9	2.0
XII		110	34,000	2.1	2.3
XIII		390	>100μM	5.7	1.5

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 diketoester (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.

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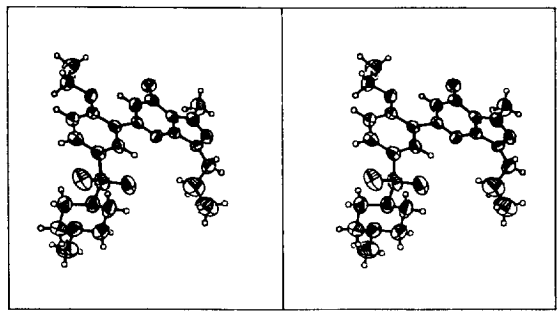
15. Compound affinities for the PDEs were assessed by determination of their IC_{50} values. The PDE enzymes were isolated from rat kidney (type 1), rabbit platelets (types 3 and 5) and human corpus cavernosum (type 5) by the method of Thompson, W.J. et al. *Biochem.*, **1971**, 10, 311, and assays performed by the 'batch' method of Thompson, W.J.; Appleman, M.M.; *Biochem.*, **1971**, 18, 5228. Inhibition studies were performed using a substrate concentration of 500nM. Inhibitors were dissolved in DMSO and concentration-response curves were constructed over the range $3x10^{-10}$ to $1x10^{-4}$ in half log increments. IC_{50} values were calculated using a sigmoidal curve fitting algorithm.
16. Dipole moments were calculated using SPARTAN, Wavefunction, Inc., Irvine, CA, USA.
17. X-ray crystallographic studies of sildenafil (see below) and related compounds demonstrated that in the solid state at least, this hydrogen bond was important.



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