Expert Opinion on Therapeutic Patents

Review

Cardiovascular & Renal

Inhibitors of PDE1 and PDE5 cGMP phosphodiesterases: patents and therapeutic potential

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Cyclic 3′5′-guanosine monophosphate (cGMP) is a key second messenger involved in the processes of intracellular signalling. Steady state levels of cGMP are modulated through a balance between the rates of formation and degradation of the nucleotide. A potential therapeutic approach to manipulation of cGMP is the inhibition of the phosphodiesterases PDE1 and PDE5. PDE5 inhibitors have been targeted by many companies and have resulted in a large number of patents. The disclosed inhibitors cover an eclectic range of polycyclic nitrogen heterocycles. Activities reported show IC_{50} values in the low nanomolar to subnanomolar range. A wide range of tissue, cellular and *in vivo* effects are also reported for these PDE5 inhibitors. By contrast, only a very few patents have appeared which claim PDE1 inhibitory activity. The potential use of PDE1 and PDE5 inhibitors in the treatment of coronary artery disease, hypertension, congestive heart failure, erectile dysfunction and pulmonary hypertension is discussed.

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1. Introduction

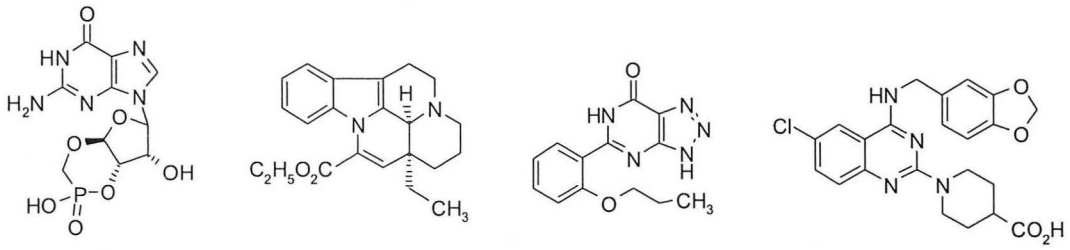
Cyclic 3′5′-guanosine monophosphate (cGMP, **1**, **Figure 1**) is a key second messenger involved in processes of intracellular signalling [1]. cGMP can influence cell function through:

- activation of a distinct family of protein kinases
- modulation of ion conductance *via* cyclic nucleotide gated ion channels
- the regulation of phosphodiesterases which influence steady state levels of cGMP and cAMP

Steady state levels of cGMP are modulated through a balance between the rates of formation and degradation of the nucleotide. Formation of cGMP is governed by soluble and membrane receptor guanylate cyclases. The former is stimulated by nitric oxide (NO) from both endogenous (e.g., *via* activation of nitric oxide synthetases [NOS]) or exogenous (e.g., *via* nitrovasodilators) sources [2]. The receptor guanylate cyclases are activated *via* natriuretic peptides, guanylin and bacterial enterotoxins.

cGMP regulates the function of many cell types. In smooth muscle, cGMP provokes relaxation and inhibition of cell growth [3,4]. In endothelium, nitric oxide and cGMP inhibit the adherence of circulating blood cells [5]. In platelets, cGMP inhibits aggregation [6]. In sensory cells, cGMP stimulates sodium and potassium flux through specific channels leading to changes in transmission of information related to light, smell and taste [7]. In epithelial cells, cGMP stimulates sodium efflux, resulting in diuresis, natriuresis in the kidney, and fluid and electrolyte loss in the gastrointestinal tract [8]. In the heart, cGMP modulates cellular excitability through regulation of potassium and calcium conductances [9,10]. In the CNS, cGMP has been implicated in the mediation of glutamatergic neurotransmission and synaptic plasticity [11].

Figure 1: cGMP and prototype inhibitors of PDE1 and PDE5.



1 cGMP | **2** Vinpocetine | **3** Zaprinast | **4** E4021

Table 1: Major classes of cyclic nucleotide phosphodiesterases.

PDE	Substrate	Gene products	Regulatory mechanisms	Tissue distribution
1	cGMP cAMP	3	CaM dependent	Vascular tissue, brain, heart, kidney, lung, pancreas, circulating blood cells
2	cGMP cAMP	1	Stimulated by cGMP	Adrenal cortex, platelet, vascular tissue, heart, lung
3	cAMP	2	Inhibited by cGMP	Heart, vascular tissue, liver, platelet, adipocyte
4	cAMP	4	cAMP specific	Heart, kidney, brain, gastrointestinal tract, liver, lung, circulating blood cells
5	cGMP	2	cGMP specific	Platelet, vascular tissue, lung
6	cGMP	3	cGMP specific	Retinal rods, cones, kidney
7	cGMP	1	Unknown	Skeletal muscle, T-cells

cGMP modulation has been exploited for therapeutic purposes for over 100 years. Nitrovasodilators are important drugs in the treatment of cardiovascular, gastrointestinal and urogenital disorders [12]. Although effective, these agents suffer from several limitations, including vasodilator-related side-effects such as hypotension, headache and nausea as well as the potential to develop tolerance with long-term or high dose treatment. Natriuretic peptides have been evaluated for clinical utility in hypertension and congestive heart failure [13]. However, these agents have limited utility due to their peptidic nature, need for injectable administration and, in the case of congestive heart failure, the development of tolerance to their renal effects. Alternative mechanisms for modulating the activity of natriuretic peptides are being explored and may offer novel approaches for treatment of hypertension, congestive heart failure and gastrointestinal disorders.

An alternative approach to the stimulation of guanylate cyclase is the inhibition of phosphodiesterases involved in the degradation of cGMP. At least seven families (**Table 1**) of phosphodiesterases have been identified [14]. These share common structural features, including a highly conserved amino acid sequence at the hydrolytic site. Selective inhibitors have been developed for several of the families of PDE. PDE3 selective inhibitors have been targets for cardiac insufficiency, whereas PDE4 inhibitors are being pursued as a novel approach to asthmatic, pulmonary and inflammatory disorders [15]. The major isoforms involved in the degradation of cGMP are PDE1, 2, 5 and 6.

PDE1 is a calmodulin dependent phosphodiesterase [14,16]. Several isoforms of PDE1 have been identified and are distributed in heart, lung, kidney, circulating blood cells and smooth muscle cells. A distinct PDE1 found in the brain, which hydrolyses both cAMP and cGMP, may play an important role in modulation of neurotransmission. In vascular smooth muscle, PDE1 plays a major role in the hydrolysis of cGMP, and PDE1 inhibitors exert potent vasodilator actions. cGMP binds to and is selectively hydrolysed by PDE5 and 6. PDE5 is distributed in lung, kidney, spleen, platelets, endothelial cells and smooth muscle cells, and plays a key role in hydrolysis of cGMP in these tissues. Potent inhibitors of PDE5 with selectivity over PDE1, 2, 3 and 4 have been discovered and evaluated in both clinical and preclinical settings.

PDE6 is a related, but distinct gene product from PDE5 [16]; it is distributed predominantly in sensory tissues. In retinal tissue, light activates rhodopsin, which in turn signals transducin to activate PDE6, leading to reduction of cGMP levels and closure of the cyclic nucleotide gated ion channel. Mutations which inhibit the function of retinal PDE6 lead to retinal degeneration and blindness in animals and man. PDE5 inhibitors, e.g., E4021 (**4**), also inhibit PDE6 (HS Ahn, unpublished observations). The potential safety implications of this action remain to be defined.

2. Inhibitors of cGMP phosphodiesterases (PDE1 and PDE5)

2.1 PDE5 inhibitors

The alkaloid vinpocetine (**2**) is a weak but selective inhibitor of PDE1 but does not inhibit PDE5 or the cAMP hydrolysing enzyme, PDE3. Zaprinast (also known as M&B 22948) (**3**) is an inhibitor of PDE5 which is selective relative to PDE3. More recently, E4021 (**4**) was reported as a very potent (IC_{50} = 4 nM) inhibitor of PDE5 with high selectivity relative to PDE1, 2, 3 and 4. Very little patent activity or published research has explored the activity of **2**. In contrast to vinpocetine, **3** and **4** have formed the basis for a wide ranging exploration of structure-activity relationships (SAR) related to these core structures.

Patent activity relating to cGMP phosphodiesterases from 1992 to 1995 has been extensively reviewed in two recent articles [16,18]. This review will focus on those new inhibitors of cGMP hydrolysing PDEs which have emerged, primarily in the patent literature, between 1995 and early 1997. Most of the activity has surrounded inhibitors of PDE5 (**Table 2**) with a much smaller representation for PDE1. Structures shown in **Table 2** represent, for the most part, the most potent PDE5 inhibitors exemplified within the patent description. The structures which have been described represent an eclectic collection of polycyclic nitrogen heterocylic compounds. Some of these follow well know lead structures such as zaprinast (**3**) and E4021 (**4**), but many strike into new structural territory.

Pyrazolo analogues (**5** - **9**) of zaprinast are potent and selective inhibitors of PDE5. Sanofi-Winthrop has patented 6-benzyl (**5**) and 6-phenyl (**6**) derivatives with IC_{50} values as low as 1.8 nM. A significant amount of SAR is disclosed in these series with *in vitro* PDE5 activity reported for about 80 analogues between the two patents [100,101]. These inhibitors are reported to decrease blood pressure and reverse nitroglycerine tolerance in the SHR. However, since the drugs were administered only by the intravenous route, the potential for these PDE5 inhibitors to be orally effective is unknown. Zaprinast (**3**) has been shown to be effective only at very high oral doses, e.g., 200 mg/kg/day [19].

Urea (**7**) or sulfonamide (**8**,**9**) substitution on the pendant 6-(2-propyloxy)-phenyl is a strategy disclosed by three companies [102-105]. In each case, the best inhibitors have IC_{50} values < 5 nM for PDE5. These patents' claimed biological activities suggest that this type of modification results in good oral activity. Indeed, Glaxo has published extensively on the SAR of compounds related to **8**, and many compounds are reported to reduce blood pressure over a five hour period when administered to SHR at 5 mg/kg p.o. [20]. Pfizer has advanced one member of this class, sildenafil (**9**), to clinical trials for the treatment of male impotence [21]. In an aggressive strategy to protect this potentially large commercial franchise, Pfizer has filed broad use patents on pyrazolopyrimidinones, bicyclic heterocycles, and, in fact, essentially all PDE5 inhibitors claimed in a wide variety of patents filed by other pharmaceutical companies [106].

The potent and selective PDE5 inhibitor E4021 (**4**) has been the prototype for new structures (**10** - **14**) which have appeared in five recent patents. Ono has explored novel quinazolines (**10**), bicyclic pyrimidines (**11**), and pyrimidines (**12**) as inhibitors of PDE5 [107-111].

An interesting additional claim within these patents is the inhibition of TXA_2 synthetase activity. The synthesis and SAR of structures related to **10** have also been detailed in a recent publication [22]. The best compounds in this series show IC_{50} values in the low nanomolar range and more than 1000-fold selectivity for PDE5 relative to the other four PDE types. The reported TXA_2 synthetase inhibitor activity is, however, somewhat more modest, with inhibition only being significant in the μM range. Because of this separation in *in vitro* activity, it is not clear whether both enzymes would be inhibited upon administration of a drug at doses which would show effects from PDE5 inhibition. Eisai continues to follow-up E4021 (**4**) with additional patent filings on heterocyclic variants (**13**) and ring opened analogues (**14**) of the 1,3,7-trisubstituted quinazoline lead [112,113]. Of particular note are the structures related to **13** (IC_{50} = 12 pM) of which several are claimed to have highly potent PDE5 activity. Compound **12**, as well as **13**, clearly suggests that the bicyclic nitrogen heterocycles so commonly associated with PDE5 inhibitors are not an absolute requirement for potent inhibitory activity.

The other PDE5 inhibitors which have been patented in this period represent an eclectic group with no common structural theme. Glaxo has disclosed novel

Table 2: PDE5 inhibitors: structures and biological properties.

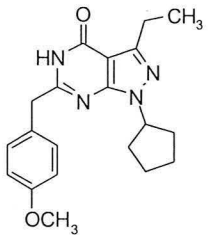
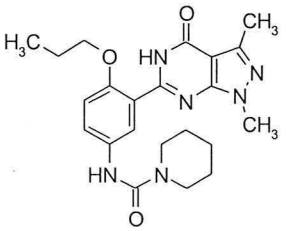
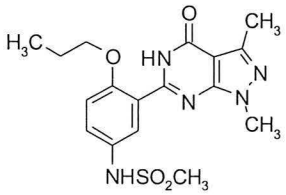
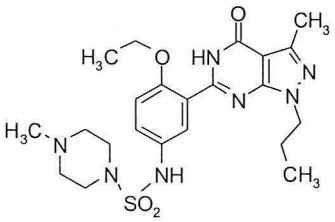
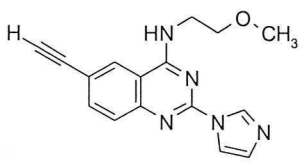
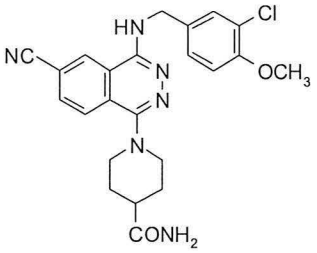
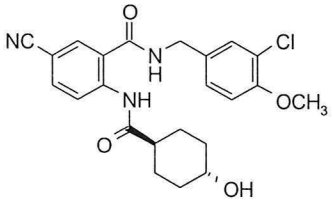
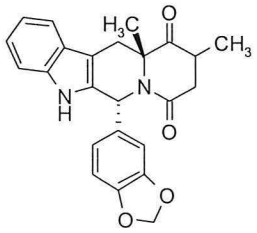
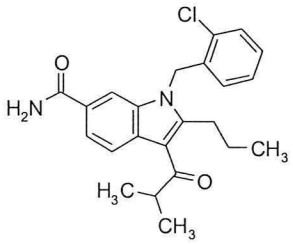
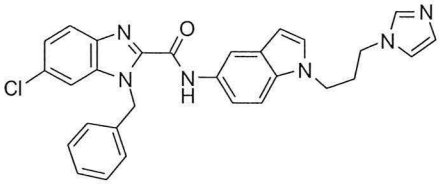
Compound number	Structure	PDE5 IC_{50} (nM)	Additional biological data
5		23	SHR: 30% reduction in MAP after iv. dosing at 10 mg/kg SHR: 69% reversal of nitroglycerin tolerance after iv. dosing @ 1.0 mg/kg
6		1.6	SHR: 1% reduction in MAP after iv. dosing at 10 mg/kg SHR: 49% reversal of nitroglycerin tolerance after iv. dosing @ 1.0 mg/kg
7		1.5	
8		3	EC_{50} = 0.35 µM for relaxation of rat aortic smooth muscle SHR: AUC = 129 mmHg.h for 0 - 5 h after p.o. dosing @ 5.0 mg/kg
9		3.6	PDE1 : IC_{50} = 260 nM PDE3 : IC_{50} = 65000 nM
10		46	TXA_2 synthetase: IC_{50} = 2.4 µM
11		24	TXA_2 synthetase: 63% inhibition @ 10 µM

Table 2: PDE5 inhibitors: structures and biological properties *(continued)*.

Compound number	Structure	PDE5 IC_{50} (nM)	Additional biological data
12		14	TXA_2 synthetase: 90% inhibition @ 10 μM
13		≤ 0.11	
14		0.7	
15		2	EC_{50} = 0.2 μM for elevation of cGMP in rat aortic smooth muscle SHR: AUC = 135 mmHG.h for 0 - 5 h after p.o. dosing @ 5 mg/kg
16		< 100	IC_{50} < 100 nM for relaxation of precontracted rat VSM IC_{50} < 100 nM for inhibition of proliferation of rat vSMC Inhibition of vasopressin induced vasospasm > 50% @ 10 mg/kg p.o.
17		10	IC_{50} = 0.5 μM for inhibition of proliferation of rat A10 cells IC_{50} = 0.04 μM for inhibition of proliferation of human fibroblasts IC_{50} = 3.0 μM for inhibition of proliferation of mouse T-cells IC_{50} = 0.79 μM for inhibition of proliferation of rat mesangial cells

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