Structure –Activity Relationships of PDE5 Inhibitors

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Abstract: cGMP has a short-term effect on smooth muscle tone and a longer-term effect on responses to chronic drug treatment or proliferative signals. cGMP-Phosphodiesterase type 5 (PDE5) hydrolizes cGMP, and the result is smooth muscle contraction. PDE5 is a relatively novel therapeutic target of various diseases, such as erectile dysfunction and pulmonary hypertension. The most intensively examined and marketed PDE5 inhibitor was sildenafil (Viagra) but recently vardenafil (Levitra) and tadalafil (Cialis) were launched with beneficial ADME parameters and PDE5 selectivity. The increasing interest in PDE5 inhibition made it reasonable to collect the available inhibitory data from the scientific literature and set up a structure-activity relationship study. Chemical structures of 438 compounds and their cGMP-PDE5 inhibitory data (IC_{50}) were collected from recently published articles. In this paper physiology, regulation and inhibition of PDE5 (and briefly other PDE-s) are discussed and inhibitors are tabulated by the core structures. Finally, a general QSAR model built from these data is presented. All data used in the QSAR study were summarized in a Supplement (for description please see the online version of the article).

Keywords: PDE5, QSAR, virtual sceening.

INTRODUCTION

Phosphodiesterase 5 (PDE5) is a relatively new therapeutic target in the treatment of erectile disfunction (ED), and there are many other disorders in which PDE5 inhibiton might have therapeutic value. Phosphodiesterases catalyze the degradation of cXMPs to XMPs, thus inhibition of PDEs will result in the prolongation of vasodilatator effects. The first PDE5 inhibitor, sildenafil (Viagra, Pfizer Pharmaceuticals) [1] was launched in 1998 and it revolutionized ED management and market. In 1997 the ED market totalled US $137 million and increased to US $1.8 billion in 2003 [2]. Since then two other drugs were introduced: tadalafil (Cialis, Lilly ICOS LLC) [3] and vardenafil (Levitra, Bayer and GlaxoSmithKline) [4]. The latter two drugs differ from sildenafil in their ADME parameters and PDE5 selectivity.

In this paper we summarize up the recently published results of PDE research, especially of PDE5. Physiology, regulation and inhibition of PDE5 are discussed and the inhibitors collected from the literature are tabulated. Finally, a general QSAR model built from these data is described. The generalization ability of this model is high since many different cores were involved in the model building (the experimental data spans a range of greater than 5 orders of magnitude – the values range from pIC50 = 4.59 – 9.7) and the Q^2 value of the external validation is acceptable (0.69), enabling us to use this model for in silico screening and forecasting PDE5 inhibitory side effects of new drug candidates.

THE PDE SYSTEM

Different factors such as nitric oxide (NO), atrial natriuretic peptide (ANP) and other endogenous vasodilatators stimulate adenylyl- and guanylyl cyclases, which transform ATP and GTP into cAMP and cGMP, respectively in smooth muscle cells (SMCs). NO is produced in vascular endothel cells by nitric oxide synthases, and as NO is a small molecule it can easily cross the cell membranes by diffusion and get into the SMCs. ANP is released into the circulation from the atria in response to hypervolaemia-induced stretch and acts as a hormone [5]. While NO activates the soluble NO-stimulated guanylyl cyclase (NOS-GC) [6], the other factors activate the membrane-bound cyclases [7].

cAMP and cGMP are second messengers and relatively simple molecules. However, their physiological effects are very diverse and cell specific. There are many different cyclases and PDEs which can be expressed in cells which explains how the effects of these simple molecules are cell specific. About 10 different adenylyl cyclase genes and about 20 different PDE genes with varied regulation, physiological properties, etc. have been identified in mammalian species. A human cell can express 1-2 cyclases and 3-4 PDEs, which means that the number of possible combinations is very large [8].

The synthesized cXMPs activate complex pathways generating different biological effects. cXPMs bind to protein kinases (cAMP binds to the cAMP-dependent protein kinase (PKA) and cGMP binds to the cGMP-dependent protein kinase (PKG)) activating them. They also interact with ion-channels and PDEs. As a result, the intracellular calcium ion concentration decreases and the activity of myosin phosphatase increases, therefore the sensitivity to calcium ions decreases. The short-term output is SMC relaxation (vasodilatation) and there are also longer-term responses. The first is that constant cyclase stimulation increases the expession of PDEs leading to be less responsive to cXMP. This is the major cause of the tolerance to NO-releasing drugs. In human cells only cXPMs induce PDE1C and its inhibition leads to suppression of SMC proliferation [8].

The role of PDEs in this signal transduction process is hydrolyzing the accumulated cXMPs to XMPs, thus PDEs decrease the signal. To date we know 11 PDE gene families [8]. Brief information on PDE families are summarized in Table **1** [8, 9].

A highly conserved catalytic region is located in the C-terminal part of PDEs. According PDEs share an average of about 30% sequence homology in the catalytic domain [9]. We confirmed this information by collecting primary sequences of four PDEs from the Brookhaven Protein Data Bank (PDB) [10] and calculating their sequence homology. The result is shown in Table **2**. PDE5 differs most from the other examined PDEs.

The N-terminal part where the regulation domain(s) are located in PDEs is pretty variable. From the above comparison of the primary sequence of the catalytic domain of PDEs it can be recognized that the enzymes catalyze the same reactions and that they differ in the mode of their regulation.

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Table 1. Human PDE Families

Family	Gene(s)	N-terminal regulation domain(s)	Substrate	Inhibitor(s)	Target disease
PDE1	A, B, C	CBD* (2)	cAMP, cGMP	Vinpocetine	Urge incontinence, low compliance bladder, acute ischemic stroke
PDE2	A	GAF# (2)	cAMP, cGMP		
PDE3	A, B	PMAD%	cAMP, cGMP	Olprinone Cilostazol	Gastric intramuscular acidosis, systemic infalmmation after cardiopulmonary bypass Angiographic restenosis, intermittent claudication
PDE4	A, B, C, D	UCR domain (2)	cAMP	Cilomilast Roflumilast	Asthma, chronic obstructive pulmonary disease, allergic rhinitis
PDE5	A	GAF (2)	cGMP	Sildenafil Vardenafil Tadalafil Exisulind CP461	Pulmonary hypertension, female sexual dysfunction Erectile dysfunction Various cancers
PDE6	A, B, C	GAF (2)	cGMP		
PDE7	A, B	-	cAMP		
PDE8	A, B	PAS domain	cAMP		
PDE9	A	-	cGMB		
PDE10	A	GAF (2)	cAMP, cGMP		
PDE11	A	GAF (2)	cAMP, cGMP		

*Ca/Calmodulin binding domain.
#GAF domain, binds cGMP and/or other proteins.
%Putative membrane-association domain.

Table 2. Sequence Homology (%) of the Catalytic Domain of Four PDEs

	PDE1B	PDE3B	PDE4B	PDE5A
PDE1B	100			
PDE3B	33.5	100		
PDE4B	36.6	31.1	100	
PDE5A	20.5	16.5	18.5	100

PDE5

As Table **1** indicates, the substrate molecule of PDE5 is cGMP. Furthermore, PDE5 is the major cGMP hydrolyzing PDE enzyme [8]. Three isoforms of PDE5 are known, A1, A2 and A3. The isoforms vary in their N-terminal regulatory domain only, and the A1 isoform is predominant. The A3 form is SMC specific, while the other two do not show cell specificity [11].

PDE5 can be isolated from a large variety of tissues, for example it can be found in corpus cavernosum [12], platelets [13], lung [14], brain [15], kidneys [16], vaginal tissues [17], spleen, endothelial cells [18], Purkinje neurons [19], cerebellum, retina, thymus, heart, liver, esophagus, stomach, pancreas, small intestine, colon, prostate and urethra [9].

As Table **1** shows, the N-terminal part of PDE5 contains two, so called GAF domains, GAF A and GAF B. Originally, GAF domain was found to be present in cGMP-regulated phosphodiesterases, adenyl cyclases and bacterial transcription factor called FhlA. The initial letters of these proteins gave the name to the GAF domain [20]. The GAF domain can bind cGMP, which directly activates the enzyme.

PKG can phosphorylate PDE5 on serine 92 if the GAF domain binds cGMP. This phosphorylation does not directly enhance the activity of PDE5, but it increases the apparent affinity of PDE5 for cGMP binding [21]. It is suspected that the role of the phosphorylation is stabilizing PDE5 in its cGMP-bound, active state. The existence of two states (inactive and active) of PDE5 explains why the inhibitory effect of inhibitors depends on the cGMP concentration - the two states have different affinity for the same inhibitor [8]. The mechanism of action and regulation of PDE5 is depicted in Fig. (**1**).

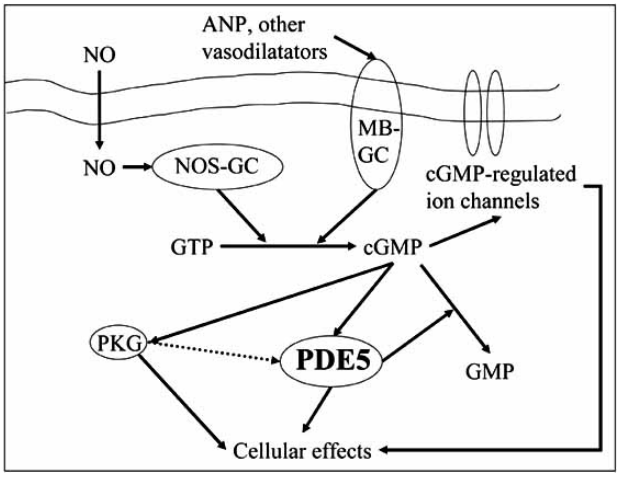


Fig. (1). The effects and regulation of PDE5.

NO: nitric oxide, NOS: NO-stimulated Guanylyl Cyclase, MB-GC: Membrane-bound Gunaylyl Cyclase, ANP: Atrial Natriuretic Peptide. The dotted arrow represents the phosphorylation of PDE5 on Ser-92 by PKG.

INHIBITION OF PDE5

The main effects of PDE5 inhibition are cardiovascular. Sildenafil was originally tested as an antianginal agent [22], but it

quickly turned out that it only slightly decreases the systemic blood pressure [23]. It has also been demonstrated that administration of a PDE5 inhibitor in a therapeutic dose does not affect the electrocardiogram [24] and does not have any inotropic effect [25].

Pulmonary artery pressure, on the other hand, is significantly decreased by sildenafil suggesting that PDE5 inhibitors might be used for the treatment of pulmonary hypertension [14, 26].

The effective PDE5 inhibitor is a possible therapeutic agent for the treatment of congestive heart failure [18]. In healthy heart, sildenafil moderately caused relaxing effects on the coronary blood vessels. In contrast, this effect is significantly increased in the presence of myocardial ischaemia [27].

Admittedly the most important cardiovascular effect of PDE5 inhibitors takes place in the corpus cavernosum. ED is the prevailing yet undertreated state in which the patients have difficulties in producing and/or maintaining erection.

The risk factors of ED (hypertension, diabetes, smoking, lipid abnormality, obesity and lack of physical activity) are common in those with coronary artery disease, 75 % of men with chronic coronary artery disease also suffer from ED [28].

Before developing Viagra, the treatment of ED was much less effective and it consisted of injection therapies, prosthetic implants, vacuum devices and less effective oral agents [2]. The introduction the PDE5 inhibitors will enhance the treatment of ED, which entails the improvement of mood and reduction of depression [28].

The lack of sexual activity decreases testosteron concentration in blood [29]. Testosteron levels increase with PDE5 inhibition.

Another sexual problem which can be related to PDE5 inhibition is rapid ejaculation [30].

As PDE5 is also present in the female genitals, it is supposed that it has an important role in the female sexual response as well [17].

In kidney sildenafil stimulates renin secretion, this may explain why PDE5 inhibitors have only a moderate effect on the blood pressure [16].

In the central nerveous system sildenafil activates the serotonin transformer (SERT) which stimulates serotonin uptake [31]. This can be major source of the CNS side effects of sildenafil.

It has been shown that sildenafil induces neurogenesis and promotes functional recovery after stroke in rats [15].

There are other diseases in which PDE5 inhibitors might be useful such as in respiratory system diseases (sildenafil has also antiinflammatory effects) [32] and benign prostatic hyperplasia associated urinary dysfunction [9]. PDE5 inhibitors may be potential therapeutical agents in the management of preeclampsia, which is the major cause of perinatal and maternal morbidity [33]. In rat experiments zaprinast, which is another PDE5 inhibitor, has been shown to promote recovery from ischemic acute renal failure [9].

PDE5 INHIBITORS AS DRUGS

All clinically useful PDE5 inhibitor drugs have nanomolar IC50 against PDE5. The agents differ in their selectivity towards other phosphodiesterases and in pharmacokinetics.

The first PDE5 inhibitor on the market for the treatment of ED was sildenafil [1]. For example, it has been shown that Viagra, and any other PDE5 inhibitor drug, do not block the HERG channel [34], do not induce apoptosis [35] and do not contribute to the development of myocardial infarction or ischemia [23]. Despite of the success of Viagra there are a number of problems which inspired the research for further PDE5 inhibitory drugs.

Research resulted in two more PDE5 inhibitor drugs, vardenafil (Levitra, Bayer and GlaxoSmithKline) [4] and tadalafil (Cialis, Lilly ICOS LLC) [36]. The selectivity of these drugs are better than that of sildenafil, but the side effect profile of them is similar to sildenafil, except the vision-related side effects. Contraindication for new drugs are similar to sildenafil, but vardenafil and tadalafil certainly have beneficial properties.

Vardenafil is a potent PDE5 inhibitor (IC50 = 0.7 nM) and its effect develops quickly [37]. This increases its efficacy and reliability even in the case of the difficult to treat patient groups (diabetics, patients with severe ED, prostatectomized men, etc) [2]. Fatty foods do not influence the relative bioavailability, but delays the absorption of vardenafil. An important warning concerning the administration of vardenafil is that CYP3A4 inhibitors, for example ritonavir, can affect the hepatic metabolism of vardenafil [37]. Vardenafil is more selective than sildenafil, but it blocks PDE6. The two drugs have very similar chemical structure: the main difference is the position of one nitrogen atom in the heterocyclic core which causes the different selectivity of the compounds. The extra methylene group in Vardenafil do not interfere with binding, but can enhance the permeability.

The effect of tadalafil lasts the longest of these three drugs, up to 36 hours (the effect of sildenafil and vardenafil lasts for 4-8 hours). Its effect is not influenced by food, and develops very quickly (in 16-17 minutes) [2]. Tadalafil is a selective PDE5 inhibitor, but it also inhibits PDE11, an enzyme with unknown physiological functions [38].

General side effects of the three drugs are vasodilatation (due to PDE1 inhibition), vision related disturbance (due to PDE6 inhibition), increased heart rate and inhibition of platelet aggregation (due to PDE3 inhibition) [38], nasal congestion [37], headache, flushing, dyspepsia. Although these side effects are tolerable, a chronic use of a PDE5 inhibitor can enhance them [39]. Sildenafil can cause migrain [40] and it may have certain central side effects associated with its SERT stimulation such as dizziness, depression, insomnia, abnormal dreams, anxiety and aggressive behavior [31]. The rationally designed second generation PDE5 inhibitors will lack these side effects [41].

Coadministration of PDE5 inhibitors with nitrates or NO generating molecules is contraindicated because it causes significant vasodilatation and reduction of blood pressure. Alpha-adrenergic blockers can be administered with precaution with sildenafil and tadalafil, but are contraindicated with vardenafil [23].

PDE5 INHIBITORS

All PDE5 inhibitors published so far act by binding to the active site of PDE5. Theoretically it might be possible to find a new group of PDE5 inhibitors binding to the GAF domain. This idea could lead to the discovery of more selective PDE5 inhibitors [8].

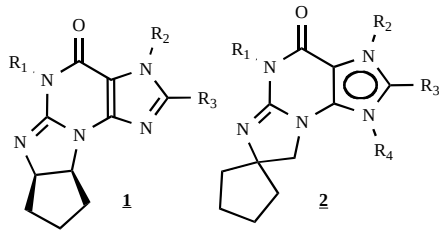
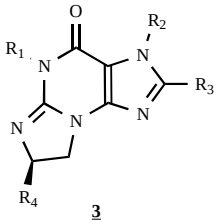
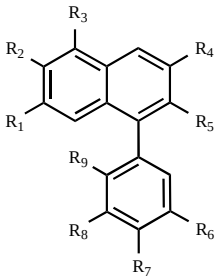
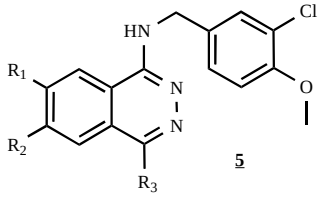
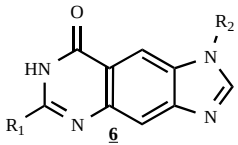
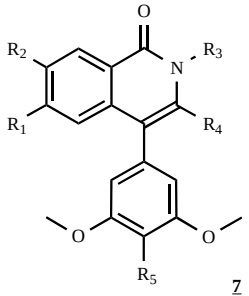
In Table **3** we listed the PDE5 inhibitory compound types found in the literature by June of 2007 (note that not all compounds were used in the QSAR modeling).

The X-ray structure of PDE5A co-crystallized with GMP (1t9s.pdb [88]), sildenafil (1tbf.pdb [88]), tadalafil (1xoz.pdb [89]) and vardenafil (1xp0.pdb [89]) has unraveled the binding mode of the inhibitors. Fig. (**2**) shows the four superimposed structures, we visualized the superimposition with Sybyl 7.0 software [90].

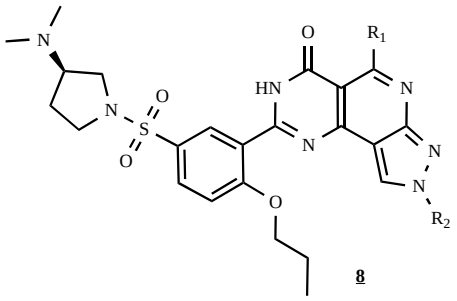
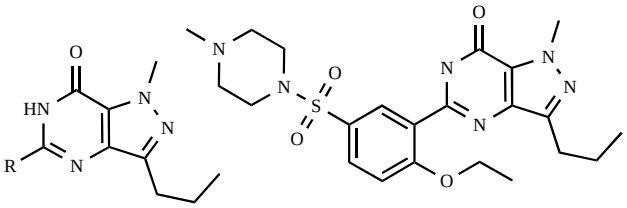
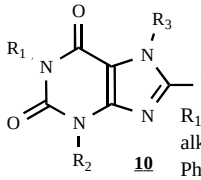
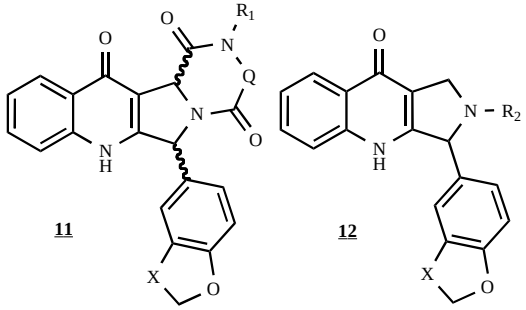
QSAR MODELING

The graphical 2D database of PDE5 inhibitors containing structures of 438 molecules with their IC50 data was built in ISISBase software [91]. We have not found a QSAR study of PDE5 inhibiton from such a large dataset of diverse structures in the literature. The negative logarithm of IC50, i.e., the pIC50 values were used for modeling. 3D structures of the molecules were calculated by the CONCORD algorithm of the Tripos software package [92]. The

Table 3. The Structures of Published PDE5 Inhibitors

Compound group	IC_{50} (nM)	Structure	Ref.
Polycyclic guanines	0.3 -16000	 **1** **2**  **3** R_1: CH_3, C_2H_5, i-Pr R_2: H, alkyl, (subst.)-aryl-CH_3, -CH_2CH_2OH R_3: H, alkyl, (subst.)-aryl-alkyl,alkyl-oxy, CH_2OCH_3, $SO_2C_2H_5$,(subst.)-amino-alkyl, CF_3, SC_2H_5, CN, $COOCH_3$, $COONH_2$, $COONHCH_3$, NH_2, triazino R_4: H, CH_2-Phe	[42, 43]
Naphthalenes	6.2 - >10000	 **4** R_1, R_2: H, Cl, CH_3, H_3CO, H_3CCH_2O R_3: H, Cl R_4,R_5: $COOCH_3$, CH_2OH, NH_2,-$COOCH_2$-, CO(disubst.)N R_6, R_7: H, H_3CO, Cl, -OCH_2O- R_8: H, H_3CO, Cl, Br, NH_2 R_9: H, CH_3	[18]
Aminophthalazines	0.56 -600	 **5** R_1: H, CN, NO_2, CF_3, Cl, $COOHCONH_2$, $CON(CH_3)_2$ R_2: H, Cl R_3: Cl, subst.-amino	[44]
Imidazoquinazo-linones	0.2 - 40	 **6** R_1: subst.-Ph R_2: H, (subst.)-CH_2-Phe	[45, 46, 47]
4-Aryl-1-isoquinolinones	1 - >10000	 **7** R_1: H, alkyloxy R_2: alkyloxy R_3: H, alkyl, subst.-amino,(subst.)-aryl, CH_2-Phe R_4: COOH, $COOCH_3$, $COOC_2H_5$, $CONH_2$, $CONHCH_3$, $CON(CH_3)_2$ R_5:CH_3, OH, OCH_3, Br	[48]

(Table 3). Contd…..

Compound group	IC_{50} (nM)	Structure	Ref.
Pyrazolo-pyrido-pyrimidines	0.31 - 13	 **8** R_1: H, Cl, subst.-CH_2-Phe-amino R_2: H, CH_2-Phe-4-OH	[49, 52]
Pyrazolo-pyrimidinones	0.05 - 40	 **9** R: subst.-Phe **9b** Sildenafil	[50 , 51, 52]
Xanthines	0.3 - >10000	 **10** R_1: H, alkyl, (subst.)-amino-alkyl, (subst.)-aryl-alkyl, H_3CCH_2O,C_2H_5OH, Phe-(O,S)-$(CH_2)_2$ R_2: alkyl, subst.-CH_2-Phe R_3: H, (subst.)-CH_2-Phe R_4: (subst.)-aryl, 4-Br-CH_2-Phe,c-alkyl-amino, H_3COCH_2	[53, 54, 55, 42]
Pyrrolo-quinolones	0.019 - 148 (K_i)	 **11** **12** X = CH_2, O Q = CH_2, CH_2CH_2 R_1 = CH_3, CH_2-Phe, 2-pyridyl-CH_2 R_2 = subst.-aryl, CO-subst.-aryl, COO-CH_2-Phe	[18, 56, 57, 58, 59]

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