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Discovery of novel pyrazolopyrimidinone analogs as potent inhibitors of phosphodiesterase type-5 ☆

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ABSTRACT

Cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type-5 (PDE5), a clinically proven target to treat erectile dysfunction and diseases associated with lower cGMP levels in humans, is present in corpus cavernosum, heart, lung, platelets, prostate, urethra, bladder, liver, brain, and stomach. Sildenafil, vardenafil, tadalafil and avanafil are FDA approved drugs in market as PDE5 inhibitors for treating erectile dysfunction. In the present study a lead molecule 4-ethoxy-*N*-(6-hydroxyhexyl)-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzenesulfonamide, that is, compound-**4a**, an analog of pyrazolopyrimidinone scaffold has been identified as selective PDE5 inhibitor. A series of compounds was synthesized by replacing *N*-methylpiperazine moiety (ring-C) of sildenafil structure with different *N*-substitutions towards sulfonamide end. Compound-**4a** showed lower IC_{50} value (1.5 nM) against PDE5 than parent sildenafil (5.6 nM) in in vitro enzyme assay. The isoform selectivity of the compound-**4a** against other PDE isoforms was similar to that of the Sildenafil. In corroboration with the in vitro data, this molecule showed better efficacy in in vivo studies using the conscious rabbit model. Also compound-**4a** exhibited good physicochemical properties like solubility, caco-2 permeability, *c*Log*P* along with optimal PK profile having no significant CYP enzyme inhibitory liabilities. Discovery of these novel bioactive compounds may open a new alternative for developing novel preclinical candidates based on this drugable scaffold.

1. Introduction

Phosphodiesterase type 5 (PDE5) enzyme inhibitors such as sildenafil, vardenafil, avanafil, tadalafil (Fig. 1) have achieved remarkable success as the first line of therapy for the management of erectile dysfunction[1] (Fig. 1). PDE5 is an enzyme which is responsible for cGMP degradation in the corpus cavernosum. PDE5 inhibitor enhances the cGMP levels and the vasodilatory effect of nitric oxide (NO) and restores the ability to achieve an erection in a patient with ED.[2] The main therapeutic applications for PDE5 inhibitors are the treatment of erectile dysfunction and idiopathic pulmonary hypertension[3,4] apart from systemic hypertension and prostate hyperplasia.[5] There are at least 11 known PDE families, with each family typically having several different isoforms and splice variants, the prime focus remains to identify more potent and selective PDE5 inhibitors. The selective PDE5 inhibition is important because there is evidence that non-specific inhibition by PDE5 inhibitors can cause an anterior optic neuropathy[6] apart from other adverse effects including hair loss, headache/dizziness, flushing, dyspepsia, nasal congestion or rhinitis. To date there are number of PDE inhibitors that are commercially available, some of the recently developed compounds are of great potential and selective in inhibiting particular PDEs,[7] for example, PDE-1 (vinpocetine),[8] PDE-2 (EHNA (erythro-9-(2-hydroxy-3-nonyl)adenine), PDE-3 (amrinone, anagrelide, cilostazol), PDE-4 (roflumilast, apremilast and rolipram)[9,10] PDE-5 (sildenafil, tadalafil, vardenafil and avanafil).[11–15]

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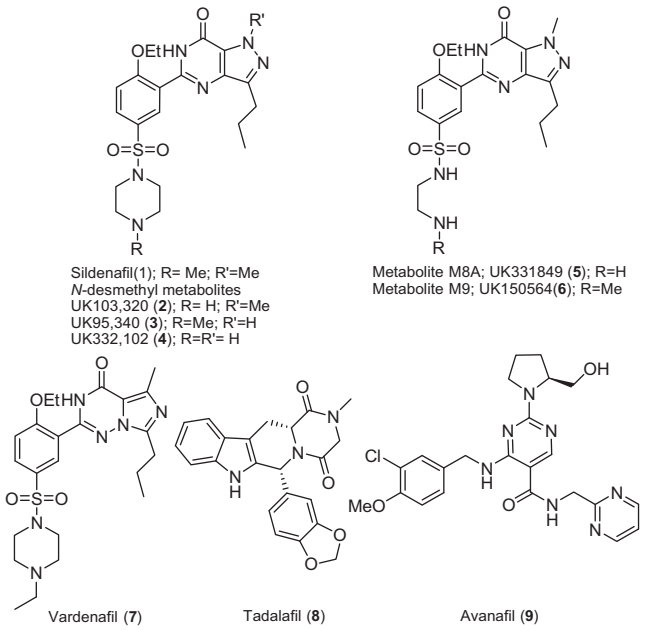

Figure 1. Structures of Sildenafil metabolites[16,17] and some commercially available FDA approved PDE5 inhibitor drugs.

The search to identify novel potent and isoform specific PDE inhibitors is being pursued in many laboratories worldwide. In the current study, we report discovery of potent and novel PDE5 inhibitors. The efforts in the direction towards optimizing the lead molecule are described.

2. Results and discussion

Sildenafil possesses a pyrazolopyrimidinone structure and is metabolized by cytochrome P450 (CYP3A4 (major route) and CYP2C9 (minor route)) via *N*-demethylation to its main metabolite, UK-103,320 (**2**),[16,17] and some other metabolites like UK95,340 (**3**), UK332,102 (**4**), M8A or UK331,849 (**5**), M9 or UK150564(**6**), as shown in Figure 1. Structurally these metabolites can be classified into two groups depending upon the fact if the piperazine ring is still intact (**2**, **3** and **4**) or opened (**5** and **6**). The major circulating *N*-desmethyl metabolite **2** of sildenafil is reported to be active and keeps its selectivity and in vitro activity for PDE5 approximately up to 50%.[18] We were interested to design the sildenafil analogs with open chain structures resembling the metabolite **5** and **6**.

2.1. Molecular docking studies

In order to design the open chain analogs similar to the metabolites **5** and **6** we carried out the binding site analysis of PDE5 (PDB code 2H42) with respect to the standard molecule. As can be seen from Figure 2 there is scope of modification at *N*-methyl piperazine ring position with the attachment of a hydrophobic moiety and then donor and/or acceptor group towards end. Based on this concept we generated analogs of sildenafil in silico by the replacement of *N*-methylpiperazine moiety with open chain substitutions and different modifications at the N-terminal of sulfonamide functional group. The analogs (compound-**4a** to **4m**) which showed promising docking results were synthetically prepared using conventional procedures and screened by the in vitro enzyme based assay. In this study, compound-**4a** turned out to be the most potent molecule, when screened in vitro having IC_{50} value much lower than the sildenafil itself (discussed later in detail). From the interaction figure generated for this compound (Fig. 3), it was observed that the amide moiety of pyrazolopyrimidinone group is involved in a bidentate interaction with the conserved Gln817 residue which is involved in H-bond at 1.6 Å and 1.97 Å, respectively. The $-SO_2NH$ group from sulfonamide end is engrossed into the hydrophobic clamp within the PDE5 binding pocket. There is also a π–π interaction of this moiety with Tyr612. The six-carbon aliphatic chain occupies the hydrophobic space identified through the binding site analysis as described above and the attachment of hydroxyl group at the end is proposed to provide extra stability to the complex with the H-bond at 2.1 Å with Asn662 and 2.04 Å with Arg667.

2.2. Chemistry

The synthesis of novel inhibitors of PDE5 with modifications at N-terminal of sulfonamide functionality of sildenafil structure was

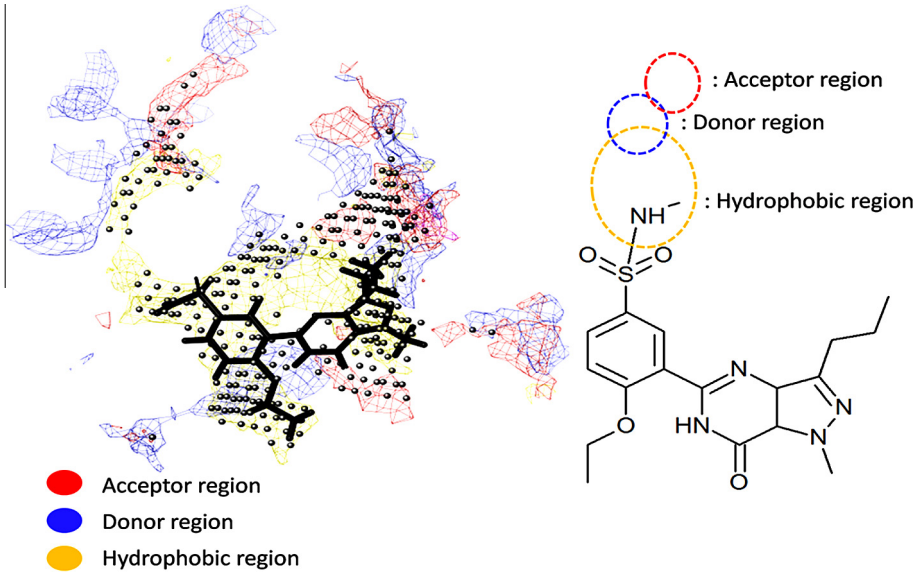

Figure 2. Binding site analysis of PDE5 with respect to the standard molecule showing the sites for modifications, provides a basis for design of new analogs towards $-SO_2NH$ end as shown.