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# Pharmacophore elucidation and molecular docking studies on phosphodiesterase-5 inhibitors

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**Abstract:**
cGMP-binding cGMP-specific PDE, PDE5 plays a key role in the hydrolysis of cyclic guanidine monophosphate. Because cGMP mediates vascular functions, a PDE5 inhibitor that elevates cGMP level is an attractive means for vasodilatation and treatment of erectile dysfunction. In this paper we report the elucidation of the common pharmacophore hypothesis of different classes of PDE5 inhibitors. Using LigandScout program, pharmacophore modelling studies were performed on prior reported potent PDE5 inhibitors with a variety of scaffolds in order to identify one common set of critical chemical features of these PDE5 inhibitors **1-52**. The best pharmacophore model, model-1, characterized by four chemical features: one aromatic ring, one hydrophobe, one hydrogen acceptors and one hydrogen donor. Using Dock6 program, docking studies were performed in order to investigate the mode of binding of these compounds. The molecular docking study allowed confirming the preferential binding mode of different classes of PDE5 inhibitors inside the active site. The obtained binding mode was as same as that of vardenafil, X-ray ligand with different orientation with varied PDE5 inhibitors' scaffold.

**Keywords:** pharmacophore, molecular Docking; Phosphodiesterase-5

**Background:**
Phosphodiesterases (PDE) are enzymes that control concentrations of intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanidine monophosphate (cGMP) respectively. PDE-catalyzed cleavage of the phosphodiester bond at the 3'-position cyclic adenosine 3,5-phosphate, cAMP, results in its hydrolysis into their respective 5'-nucleotide monophosphates **[1].** cAMP and cGMP mediate the activation of protein kinase A and protein kinase G that phosphorylate various substrates responsible for regulation of many physiological processes particularly the smooth muscle contraction **[2].** The relative intracellular concentration of cAMP and cGMP is regulated through the synthesis process mediated by adenyl and guanyl cyclases and the hydrolysis process catalyzed by PDEs. PDEs inhibitors block the hydrolysis of cAMP and cGMP resulting in higher levels of these cyclic nucleotides and therefore attractive therapeutic utilities are outcome **[3].** PDEs include 11 identified families, which have been distinguished by their substrate specificities, mechanisms of regulation, and their sensitivities to different pharmacological agents **[1, 3].** cGMP specific phosphodiesterase-5 (PDE5) isoform is expressed in smooth muscle tissue, specifically in the corpus cavernosum **[4].** Being caused by low concentration of cGMP, successful treatment of male erectile dysfunction (MED) was achieved by PDE5 inhibitors that block the hydrolysis of cGMP and elevate its cellular level **[5].** Many PDE5 inhibitors were clinically approved to be marketed as drugs for treatment of human MED. Several PDE5 inhibitors are in wide clinical use for MED treatment, including sildenafil (Viagra®), vardenafil (Levitra®), tadalafil (Cialis®) and udenafil (zydena®). Synthetic and crystal structure study has been reported based on the sildenafil derivatives. Regrettably, the clinical use of PDE5 inhibitors experienced several disadvantages including the

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cross-reactivity with PDE6 and PDE11 resulting in unwanted side effects such as skin rash, blurred vision, headache, back, and muscle pain **[6].**

There are several aryl, biaryl, hetroaryl or heterobiaryl classes of PDE5 inhibitors with different scaffold structures. The aryl class includes substituted nitroanilines and the biaryl class includes substituted naphthalenes. At the same time, the heterobiaryl and heterotriaryl are further sub classified based on its fused system into pyrazolopyrimidinones, triazolopyrimidinones, imidazotriazines, purines, pyrrolopyrimidinones, triazolotrizinones, isoxazolopyrimidinones, β-carbolines, pyrroloquinolones, isoquinolines, quinazolines, imidazoquinazolinones, pyrazolopyridines, pyrazolopyridopyrimidinones **[7-15].** These widely different chemical structures are suggested to have different orientation in the binding site of PDE5 enzyme. In view of these findings and in continuation of our previously published work in the field of design of PDE5 inhibitors **[16]** and in the modelling area of research **[17, 18],** we have been prompted to using the molecular modelling studies in order to investigate the preferential mode of binding of PDE5 inhibitors having different chemical scaffold and to elucidate their common pharmacophore hypothesis as the key pharmacophore of PDE5 inhibitors. These studies aim to be guidance to find new drug candidates having good potency and high selectivity towards the PDE5 inhibitors.

**Methodology:**

*General*

The pharmacophore and docking studies were performed on PC windows Vista Home Premium Intel(R) Core(TM)2 Duo, 1.83 GHz using LigandScout program v3.1 (G. Wolber and Inte:Ligand GmbH 1999-2013) **[19]** and Dock6.4 program **[20].** The chemical structures of different classes of PDE5 inhibitors used in this study are shown in **Table 1 (Available with authors**). The biological data are cited from the literature **[7-15].**

*Common Feature-Based Pharmacophore Models*

The study was carried out using the software LigandScout (version 3.0). Using the default settings, LigandScout program **[19]** was used to derive the 3D chemical feature-based pharmacophores from the structural data of the compounds **1-52 [7-15]** are included in the modelling method. Prior to the generation of pharmacophore hypotheses, the training set compounds [**1-38]** in **Table 1 (Available with authors**) were converted to 3D structure and were used to generate diverse conformations using the best conformation model generation method using the default parameters including maximum number of 500 conformers, and a conformer energy threshold value of 20 kcal/mol.

*Hypothesis validation using test set*

Compounds **39-52 Table 1 (Available with authors**) were selected as a test set in order to clarify whether the generated pharmacophore hypothesis is capable to make real prediction of the activities of compounds other than the training set. The conformation generation for the test set compounds was carried out in a similar way, like the training set compounds using BEST conformation analysis algorithm, implemented within the LigandScout program with setting values, as same as those used with the training set. The compounds associated with their conformations were subsequently carried out for pharmacophore mapping **[18].**

*Docking procedure*

All compounds were generated in the protonation state under physiological condition. The xray structure of 3`,5`-cyclic phosphodiesterase enzyme (PDE5) complexed with its co-crystallised ligand vardenafil inhibitor is published in the Protein Data Bank (1XP0) **[6].** The coordinates were taken from the Protein Data Bank and co-crystallised ligand was docked in its original protein structure using default settings. A 10-A° sphere around the centre of the binding pocket was defined as binding pocket for the docking runs. All torsion angles in each compound were allowed to rotate freely. In order to get a population of possible conformations and orientations for compounds **7, 23, 28,** and **36** at the binding site, docking procedure was performed with default settings.

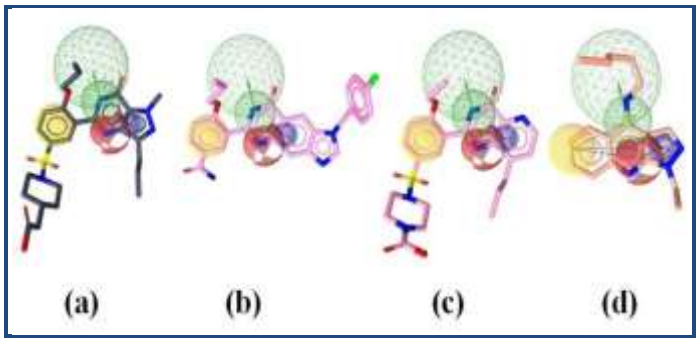


**Figure 1**: Best aligned pose of compounds 7 (IC50 = 0.0001µM) (a), 23 (I C50 = 0.00048 µM), 28 (IC50 = 0.0012 µM) and 36 (IC50 = 0.0022 µM), superposed with the query (model-1).

**Results & Discussion:**

*Common Feature-Based Pharmacophore Models*

Table 1 shows the structures of training **1–38** and test set **39-52** compounds that are reported as PDE5 inhibitors **[7-15].** Assuming that the most active compounds bind in a similar fashion at the enzyme's active site, we employed the LigandScout approach to evaluate the common features required for binding and the hypothetical geometries adopted by these ligands in their most active forms **[19].** Ten pharmacophore models were obtained having score in the range of 0.87–0.70. The top-ranked chemical feature-based pharmacophore model identified in this study is shown in **Figure 1.**

The successful pharmacophore run resulted in generation of ten pharmacophore hypotheses, based on its highest rank score and mapping into all training set molecules, model-1 was considered statistically as the best hypothesis and was selected for further investigation and analysis is shown in **Figure 1.** This pharmacophore model contains four chemical features: an aromatic ring (A), a hydrophobe (BH), a hydrogen acceptors (HA) and a hydrogen donor (HD). As a quick and primary validation of model-1, the compounds of the training set were mapped onto the model, and the orientation of the mapped compounds relative to the proposed pharmacophore was scored ("fit value") at a range of 34.3-47.78 **Table 1 (Available with authors).** Initial investigation of the obtained results revealed a moderate correlation between the fit value and the biological activity of each of the compounds understudy. This


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initial correlation encouraged us to generate a linear model based on "fit value" to predict the biological activity of the compounds under investigation. The generated model (Equation 1) showed good statistics.

pIC50 = 0.1343 Fit value - 2.7117 (1)

n = 38, SE = 0.0919, and $R^2$ = 0.966.

Where n: number of compounds; $R^2$: square of the coefficient of multiple correlation; SE: standard error.

**Figure 1a-d** showed the alignment of the hypothesis model with compounds **7, 23, 28,** and **36** as representative examples different classes of PDE5 inhibitors. A closer look at the mapped structures revealed the importance of certain structural features for activity. The obtained model suggests that the scaffold essential for the activity should contains at least one central aromatic or heteroaromatic ring linked with one hydrophobic group while, the hydrogen donor and acceptor groups are included as substituent groups on an aromatic ring or as heteroatoms of a heterocylic ring. **Figure 1**

***Hypothesis validation using test***

The obtained pharmacophore model-1 was validated using fourteen structurally diverse compounds as test set. It is a measure how well it could make real prediction of the activity of external compounds. **Table 1 (Available with authors**) shows the experimental and predicted activity. The regression of the predicted activity against the experimental one of the test set showed a correlation coefficient value $R^2$ of 0.89 (Equation 2).

pIC50 = 0.209 Fit value - 6.2127….(2)

n = 14, SE = 0.30856, and $R^2$ = 0.89. Where n: number of compounds; $R^2$: square of the coefficient of multiple correlation; SE: standard error.

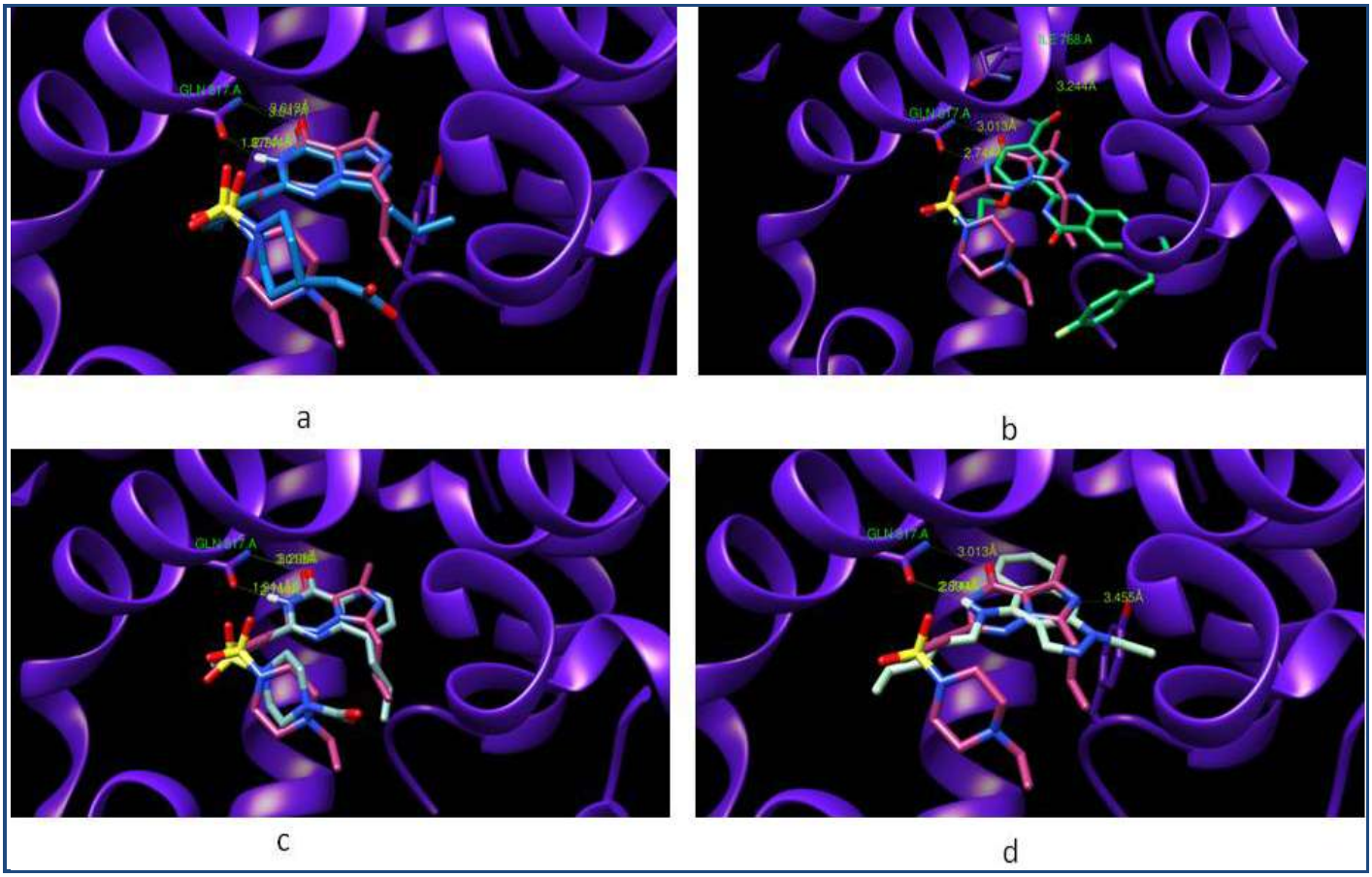


**Figure 2a-d:** Phosphodiesterase 5 (1XP0) binding site: the docked compound 7 (colored blue): **a)**, the docked compound 23 (colored green); **b),** the docked compound 28 (colored cyan); **c),** the docked compound 36 (colored blue) (d), vardenafil, xray ligand, (colored rose) Hydrogen bond is displayed in green.

***Docking procedure***

Docking study was undertaken using Dock6.4 **[20]** to make comparable study of possible interactions of different classes of PDE5 inhibitors and the active site of PDE5. The RMSD value difference of 0.485274 Å of the pose of the non-restricted redocking of the X-ray structure of the PDE5 inhibitor (vardenafil) from itself also confirmed the approach. The docking poses of compounds **7, 23, 28,** and **36** as representatives of different classes of PDE5 inhibitors, compared with that of the vardenafil, X-rary structure, are shown in **Figures 2a**-d respectively. Compound **7 (Figure 2a)** showed same orientation of vardenafil inside the binding site. Its pyrazolopyrimidinone scaffold adopts the same position as the triazolopyrimidinone moiety of the vardenafil. Also, they showed similar hydrogen bonding to the amino group of the side chain of GLN817. Their propyl and piprazinyl substituents are oriented similarly inside the hydrophobic pockets of the active site. Occupying same binding site, compound **23** showed different orientation than


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that of vardenafil **(Figure 2b).** Its imidazoquinazolinone moiety showed similar hydrophobic interaction as the propyl side chain of the vardenafil, while the phenyl ring occupied the same orientation of the imidazotriazinone moiety of the vardenafil and its carboxamide substituent explored similar hydrogen bonding to the amino group of the side chain of GLN817 while its propoxyl substituent showed same orientation as the piperazine moiety of vardenafil. Compound **28 (Figure 2c)** was oriented in the active site similar to vardenafil. Its pyridopyrimidinone scaffold adopted the same position as the imidazotriazinone moiety of the vardenafil, X-ray structure. Also, they showed similar hydrogen bonding to the amino group of the side chain of GLN817. Their propyl and piprazinyl substituents are oriented similarly inside the hydrophobic pockets of the active site. Compound **36 (Figure 2d)** oriented its pyrazoloquinoline scaffold so that the pyridine ring and pyrazole ring adopt the same position of the imidazole ring and propyl substituent of vardenafil respectively. However, compound **36** and vardenafil showed similar hydrogen bonding to the amino group of the side chain of GLN817, an additional hydrogen bond was shown between the quinoline nitrogen atom and TYR612. The pentyl substituent on compound **36** is oriented in the same hydrophobic pocket of the piprazine moiety of vardenafil **(Figure 2a-d).**

**Conclusion:**

In conclusion, a computational approach using pharmacophore modeling and docking analysis was applied to PD5 inhibitors with widely different scaffolds in order to identify the common structural features required for an effective inhibition of PDE5, in an aim to be a future guide to discover drugs for treatment of male erectile dysfunction. A reliable pharmacophore model was generated based on 38 training set compounds, which consists of na aromatic ring (A), a hydrophobe (HB), a hydrogen acceptor (HA) and a hydrogen donor (HD). This model revealed internal ($R^2$ = 0.966) prediction of training set as well as external (pred $R^2$ = 0.89) prediction of fourteen compounds of test set. The PDE5 inhibitors with different structural scaffolds, **7, 23, 28,** and **36** were docked in the active site of PDE5. Compound **7** and **28** oriented in the binding site similarly to the vardenafil. The pyrazolopyrimidinone and pyridopyrimindinone of compounds **7** and **28** respectively occupied the same orientation of imidazotriazinone scaffold of vardenafil. While compounds **23** and **36** having fused tricyclic scaffold showed different orientation of their fused aromatic rings from that of the imidazotriazine moiety of vardenafil. All of the compounds explored hydrogen bonding to the amino group of the side chain of GLN817 as well as the vardenafil. In addition, the scaffold essential for the activity should contains at least one central aromatic or heteroaromatic ring linked with one hydrophobic group while, the hydrogen donor and acceptor groups are included as substituent groups on an aromatic ring or as heteroatoms of a heterocylic ring.These findings could be exploited for future ligand design in order to obtain novel derivatives as inhibitors of PDE5.

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