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Investigation of Phosphodiesterase 5A (PDE5A) Inhibitors by Pharmacophore Modeling, Virtual Screening and Molecular Docking Approach

Manish Sudesh Bhatia[1], Amol Shantinath Sherikar[2*]

[1]Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Near Chitranagri, Kolhapur-416 013 (MS) India.
[2]Department of Pharmaceutical Chemistry, Tatyasaheb Kore College of Pharmacy, Warananagar, Tal-Panhala, Dist-Kolhapur-416 113 (MS) India.

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ABSTRACT

A taxon of accelerator phosphodiesterases (PDEs) corresponds from phosphodiesterase-1 to phosphodiesterase-12 being presently acquainted that inactivates cAMP and cGMP. Owing to this there's no sGC mediate activation of cGMP/cAMP that regulates vasorelaxation. This project was undertaken to grasp perceptions into molecular mechanisms and structural wants that area unit crucial for potential inhibition of PDE5A. During this analysis PDE5A supermolecule was elite and pharmacophore model was generated, virtual screening was completed to urge hit compounds against reference shared feature pharmacophore, the hit compounds were docked with PDE5A proteins. Hydrogen bond acceptor, Hydrogen bond donor and aromatic rings/hydrophobicity are the major phamacophoric features displayed by developed pharmacophore model. The hit compounds were obtained by virtual screening; compounds were further sorted for Lipinski rule of five before docking. Compounds that fulfill all properties of Lipinski rule of five were docked with proteins. They fit appropriately in the pocket of proteins which demonstrated the soundness and stability of ligand compounds. It is suggested that these compounds can be used in the treatment of diseases and disorders of vasculatures.

INTRODUCTION

Phosphodiesterase from vascular smooth muscle catalyzes hydrolyzation of cGMP to inactive product. There are 12 isozymes of phosphodiesterases have been acknowledged in mammalian tissues. Out of 12 isozymes of phosphodiesterase the regulation of cGMP concentration in vascular smooth muscle is associated with phosphodiesterase-5 (PDE5) (Lincoln *et al*., 1989; Beavo *et al*., 1995). Sildenafil and tadalafil are inhibitor of PDE5 that has been used to treat erectile dysfunction in humans (Boolell *et al*., 1996; Boolell *et al*., 1996; Goldstein *et al*., 1998). NO synthases produces nitric oxide (NO) constitutively in the lung from vascular endothelium and the airway epithelia (Bohle *et al*., 2000; German *et al*., 2002). Depending on alveolar ventilation local NO production regulates pulmonary perfusion to guarantee optimized ventilation distribution (Ide *et al*., 1999; Grimminger *et al*., 1995; Sherikar *et al*., 2015). The regulation of nitric oxide synthase activity is based on transcriptional and posttranslational redox-based modulation level (Michelakis *et al*., 2003). NO, prostaglandins and natriuretic peptides activates common signalling pathway of endogenous vasodilators like cAMP and cGMP. Phosphodiesterases with different tissue sharing and substrate specificity inactivate cAMP and cGMP (Ahn *et al*., 1991; Schermuly *et al*., 2001). The PDE inhibitors control the level of cAMP and cGMP differently depending on their selectivity profile. These secondary messengers are stable which boost the activity. Therefore, they might offers as remedial mechanism to boost and lengthen prostanoid and NO related vascular effects.

The applicability and effectiveness of this approach has been verified in numerous experiments (Weimann *et al*., 2000; Tanabe *et al*., 1990). PDE5A was the foremost cGMP-selective PDE to be discovered and is also activated by cGMP. At the present time pharmacophore design approach is a pioneer tool in discovery of drug molecule.

* *Corresponding Author*
Email: amol.sherikar @ gmail.com

Many pharmacophore designing and modelling approaches were successfully and extensively utilized in virtual screening, de novo designing and lead improvement. Many ligand and structure based strategies are also developed (Yang *et al*., 2010; Tripuraneni *et al*., 2016). Pharmacophores are used as queries for getting better likely lead molecules from structural databases. The obtained lead is used for designing molecules with precise structural attributes and for evaluating resemblance and diversity of molecules (Radwan *et al*., 2015; Çifci G *et al*., 2012). Similarly, Virtual screening is a computational process used in the areas of drug discovery and development to explore libraries of small molecules which can be properly bound to their target proteins or enzymes while docking is a phenomenon of predicting the orientations of molecules in the bounded stable complex (Blount *et al*., 2004; Polymeropoulos *et al*., 2006; Chandrasekaran *et al*., 2011; Chien-yu Chen *et al*., 2009; Kayık *et al*., 2017). In this regards, there is necessitate to investigate more molecules specifically targeting PDE/PDE5/PDE5A for its inhibitory potential to obtain a precise structure and phenomenon for vasorelaxation of smooth muscle. Inhibition of PDE5A is the main target for binding of drug molecule to produce smooth muscle relaxation potency.

Herein, we tend to report the application of pharmacophore modeling, virtual screening and molecular docking for PDE5A inhibitors. We undertake above study to recognize intuitions into molecular mechanisms and structural necessities essential for PDE5A inhibition. The novel and potent PDE5A inhibitors can design by using the above information.

MATERIALS AND METHODS

Methodology used in this work is shown in Figure 1.

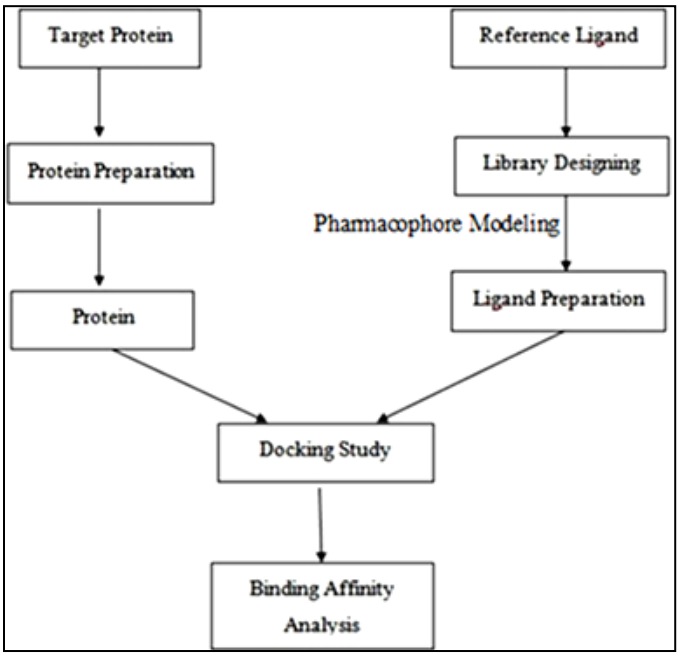


Fig. 1 Docking and binding affinity analysis of the target protein and modified inhibitors.

Selection and Preparation of proteins

RCSB PDB is principally a database that contains X-ray crystallographic and nuclear magnetic resonant 3D structures of proteins and nucleic acids (Berman *et al*., 2008). In protein data bank (RCSB PDB) for structural bioinformatics, the experimental method was selected as x-ray crystallography with an x-ray resolution of 1.0-2.0 A^0. 1XOZ is a 3D structure of PDE5A protein in complex with 6-benzo[1,3]dioxol-5-yl-2-methyl-2,3,6,7,12,12a-hexahydro-pyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione ligand with a molecular formula $C_{22}H_{19}N_3O_4$. The pharmacophore modeling studies were performed on LigandScout 4.1 Essential (Demo Version, Inte:Ligand GmbH, Vienna, Austria) (Wolber *et al*., 2005).

Pharmacophore generation

Pharmacophore hypotheses were generated for reference ligand (Tadalafil) and test ligand using LigandScout software package. Hydrogen bond doner, hydrogen bond acceptor, positive ionizable and aromatic were the common pharmacophoric feature found in every hypothesis. Shared feature pharmacophore was designed which showed the importance of occurrence of common features for inhibition of PDE5A.

Virtual screening of hit compounds against shared feature pharmacophore

All the molecules were aligned with the shared feature pharmacophore model. Virtual screening was done against shared feature pharmacophore to obtain hit compounds.

Validation of Hit compounds

After virtual screening the hit compounds were obtained and then validated by screening of Lipinski rule of five. It states that drug-like compound must have hydrogen bond donor (HBD) less than 5, hydrogen bond acceptor (HBA) less than 10, molecular weight not more than 500 Da and logP ranges between 0-5 (Pollastri *et al.*,2010).

Docking of Hit compounds with PDE5A proteins

The hit compounds were docked in PDB ID: 1XOZ by replacing reference inhibitor ligand. A systematic search was performed to obtain the ligand with lowest energy of interaction. The low energy conformations thus obtained, were optimized till they reached gradient energy of 0.001 kcal/mol. Docking studies were carried out on Vlife molecular docking suite 3.5 by using Biopredicta.

RESULT AND DISCUSSION

1XOZ protein ligand binding domain consists of total 364 amino acid residue and composed of A chain. Pharmacophore investigation is measured as an essential segment of drug design. Hydrogen bond acceptor (HBA), hydrogen bond donor (HBD) and aromatic rings (AR) are the major feature of pharmacophore model generated by LigandScout for the selected protein data set of

vascular smooth muscle relaxation. In each pharmacophore model of selected proteins the red arrows represent hydrogen bond acceptor, green arrow represents hydrogen bond donor and yellow spheres represent an aromatic ring. Numerous excluded volumes were also produced in the models to demonstrate the space balancing. The representative pharmacophores of PDE5A protein ligands are shown in Figure 2. Pharmacophore models of selected protein data sets were aligned together on the basis of ligand structure to generate a shared feature pharmacophore and interacting amino acid residues shown in Figure 3.

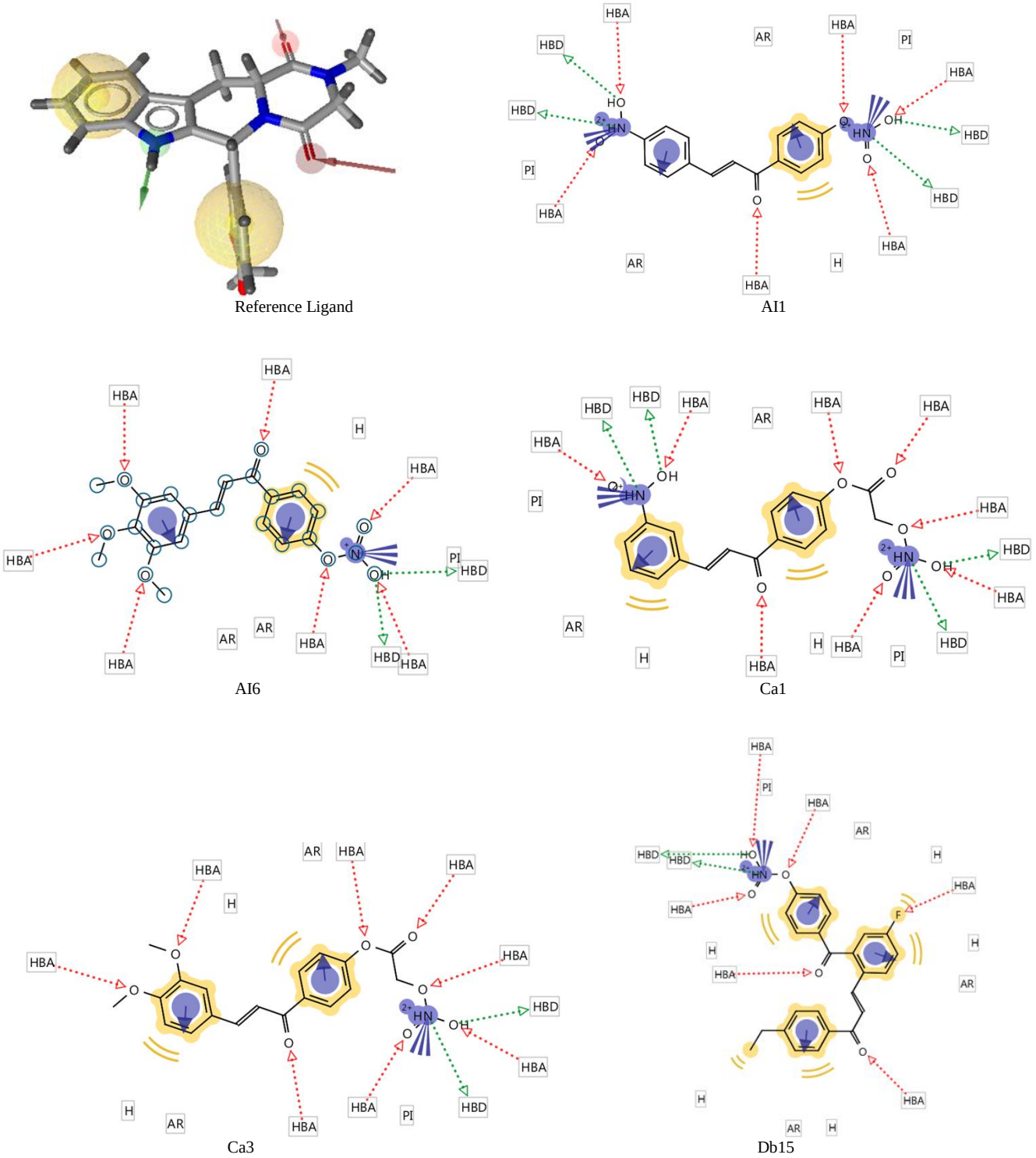
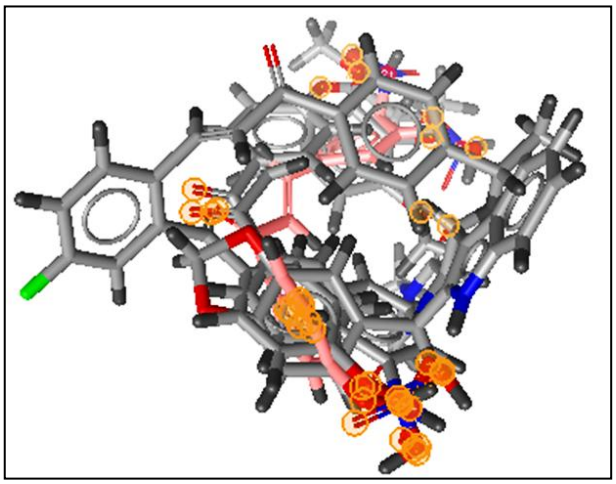
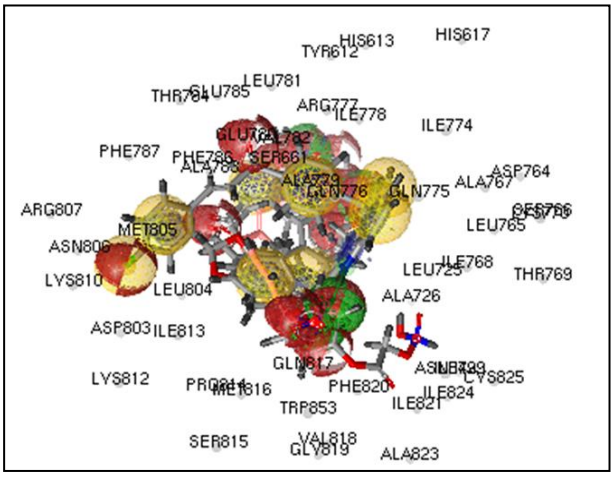


Fig. 2: Pharmacophore model of 1XOZ protein with reference ligand, AI1, AI6, Ca1, Ca3 and Db15.

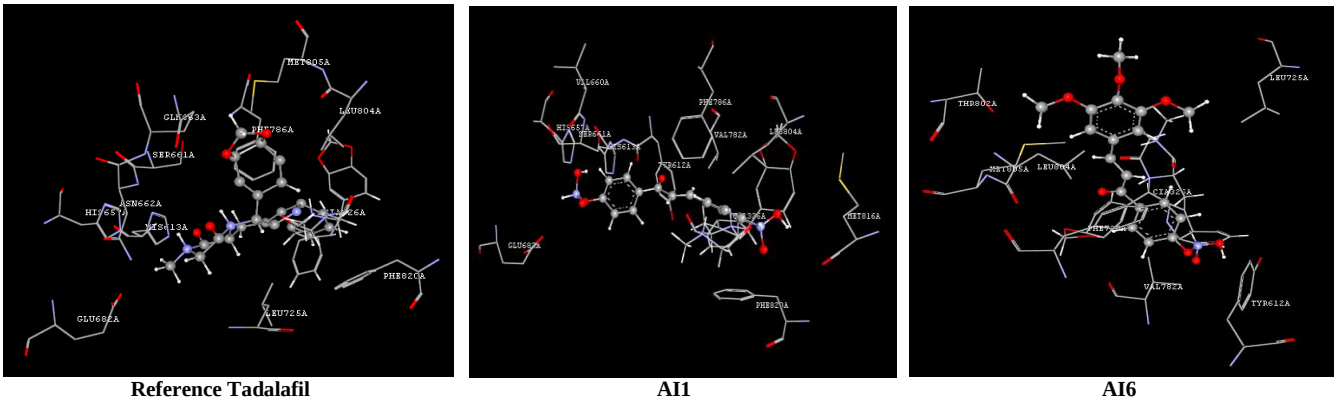


Alignment of reference and ligands with shared feature pharmacophores

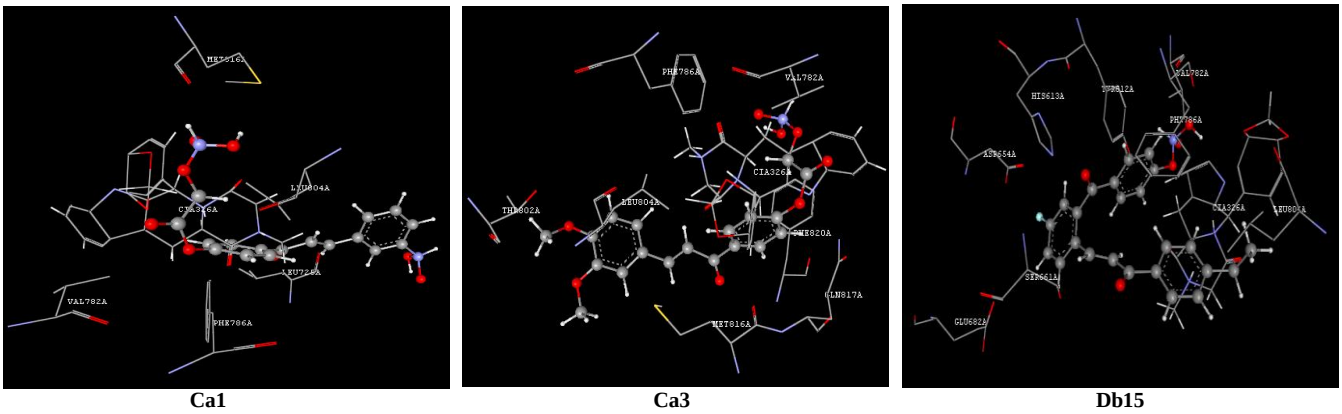


Shared feature pharmacophores and interacting amino acid residues

Fig. 3: Model of shared feature pharmacophore of 1XOZ protein structures ligands.



Reference Tadalafil **AI1** **AI6**



Ca1 **Ca3** **Db15**

Fig. 4 Docking results of Reference (Tadalafil), AI1, AI6, Ca1, Ca3 and Db15 with 1XOZ protein.

In the Virtual screening hit compounds analogous to share feature pharmacophore model were obtained. The hit compounds were then checked for Lipinski rule of five, only five compounds were fulfilling all the rules of Lipinski, i.e., molecular weight<500 Da, HBD<5, HBA<10 and logP between 0-5. The hit compounds which fulfilled Lipinski rule of five are shown in Table 1.

The docking study is implemented to calculate and predict the different interaction between drug and receptor. All satisfied compounds were docked with PDE 5A protein; in every docked complex the common interacting amino acid residues were same as that of pharmacophore model. The five compounds which fulfilled Lipinski rule of five and their best docking results are shown in Table 2. The best arrival poses with receptor interaction of these molecules are shown in figure 4.

All these five compounds are best suitable to use as drugs as they fulfill all the properties of Lipinski's rule so they will demonstrate fewer side effects as compared to the drugs available in the market. It is suggested that these five compounds can be used in the treatment of vasculature diseases and disorders.

Table 1: Chemical structures, molecular formulae, molecular weights, logP, HBD and HBA of reference and hits fulfilling the Lipinski rule of five.

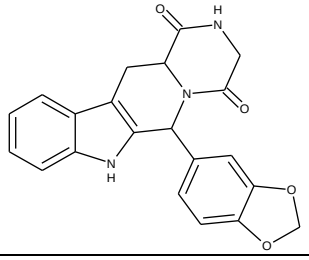
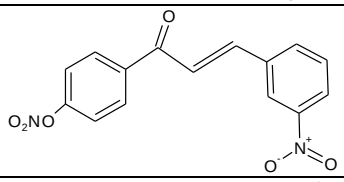
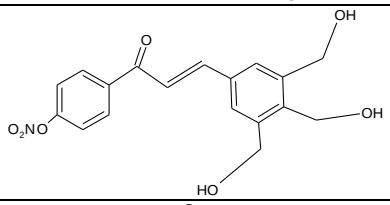
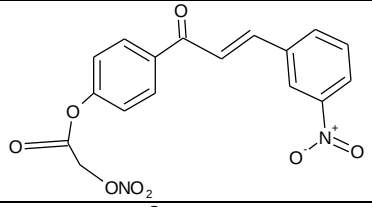
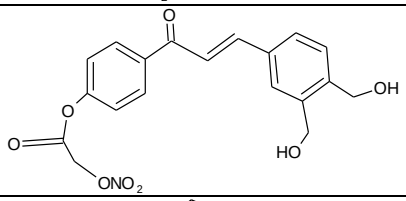
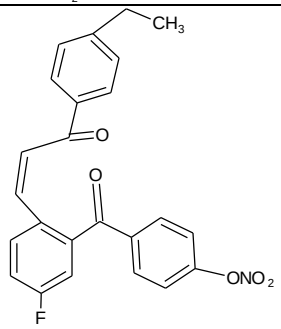
	Compound Structure	Molecular formula	Molecular Weight	LogP	HBD	HBA
Reference Ligand		$C_{21}H_{17}N_3O_4$	375.37738	389.411	01	02
AI1		$C_{15}H_{10}N_2O_6$	314.2497	3.426	04	06
AI6		$C_{18}H_{17}NO_7$	359.33008	3.824	02	07
Ca1		$C_{17}H_{12}N_2O_8$	372.28578	3.452	04	08
Ca3		$C_{19}H_{17}NO_8$	387.34018	3.250	02	08
Db15		$C_{24}H_{18}FNO_5$	419.4018232	3.542	02	06

Table 2: Comparison of bonding types of compounds which demonstrated ideal docking results along with binding energy.

Compound Code	Bonding Interactions	Binding Energy
Reference Ligand	Hydrophobic and VDW Interactions	-49.978155
AI1	Hydrogen bond and VDW Interactions	-43.784469
AI6	VdW and hydrophobic interaction	-28.377318
Ca1	VdW, Hydrogen bonding and hydrophobic interaction	-43.28555
Ca3	VdW, Hydrogen bonding and hydrophobic interaction	-41.577673
Db15	VdW, Hydrogen bonding and hydrophobic interaction	-40.925281

CONCLUSION

The pharmacophore model is a very promising tool for new lead compounds discovery and development. Two common approaches used in pharmacophore modelling are ligand based and structured based. Ligand-based pharmacophore modelling adapts the superposition of potent compounds and isolating shared structural features necessary for the biological activity of molecule whiles structure based pharmacophore modelling adapts the mechanism of examining promising interactions between receptor and ligand (Yang *et al.,* 2010). The ligand based pharmacophore modelling approach is utilized in this research work. Combinations of the pharmacophore modelling, virtual screening and molecular docking positively give possible inhibitors that can have endless influence for various experimental studies in vasculature diseases.

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Conflict of Interests: There are no conflicts of interest.

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