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Exploring Structural Parameters for Designing Cox-1 and Cox-2 Inhibitors: Pharmacophoric Modeling, Virtual Screening and Docking Study

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ABSTRACT

Cyclooxygenase-1 (COX-1) and Cyclooxygenase (COX-2) are the two major isoforms of cyclooxygenase responsible mainly for production of prostaglandins. The produced prostaglandins exert function in gastric mucosal defense, renal homeostasis, inflammation and pain. In above research work structure based pharmacophoric methodology is employed for designing inhibitors of COX-1 and COX-2. The structure based pharmacophore models were generated. Virtual screening was done to support hit compounds against reference shared feature pharmacophore. Hydrogen bond acceptor, Hydrogen bond donor and aromatic rings/hydrophobicity are the major pharmacophoric features displayed by developed pharmacophore model. The designed hit molecules then screened by employing Lipinski rule of five. The hit molecules which pass from virtual screening filters were docked into the active site of COX-1 and COX-2. They fit appropriately in the pocket of proteins which demonstrated the soundness and stability of ligand compounds. These hit molecules fulfil most of the structural requirements necessary for inhibiting cyclooxygenase. It is suggested that these hit molecules can be used for the treatment of pain and inflammation.

Keywords: Cyclooxygenase, Prostaglandins, Pharmacophore, Virtual screening, hydrophobicity

INTRODUCTION

Cyclooxygenase (COX) enzymes play an important catalytic role in biogenesis of prostaglandins because of which they are the main pharmacological targets of Nonsteroidal Anti-inflammatory Drugs (NSAIDs) [1]. The enzyme exists in two isomeric forms COX-1 and COX-2 [2]. COX-1 isoenzyme produced PGs which exerts effects like gastric mucosal defense and renal homeostasis, whereas COX-2 synthesizes harmful PGs which are responsible for inflammation and pain [3]. As a consequence, selective COX-2 inhibitors named as "coxibs" were investigated and gained an impressive success [4-8]. Animal and clinical finding data challenged the success of "coxibs" claiming that the selective blocking of COX-2 in tissues interferers with the normal physiologic roles of this isoenzyme, including gastric mucosal defense, renal homeostasis and endothelial PGI_2 production [9-13]. Serious cardiovascular effects have been observed from clinical and pharmacosurveillance study forcing to withdraw all "coxibs" from market [14-18]. This has been considered as a serious disaster which opens the gateway for safer NSAIDs. Novel strategies emerged to improve the therapeutic efficacy and tolerability of these drugs such as targeting COX and 5-Lipoxygenase (5-LOX) [19-21]. For limiting the gastric side effects of NSAIDs, the hybrid NSAID molecules were prepared by combination of these drugs with antisecretory or protective agents such as nitric oxide (NO) and Hydrogen sulphide (HF) [22-28].

The pharmacophore approaches became leading tools in drug discovery. Many ligand and structure based strategies are developed for advanced pharmacophore modelling with success and extensive application in virtual screening, de novo design and lead improvement [29-30]. Pharmacophores are used as queries for getting better likely leads from structural databases for designing molecules with precise needed attributes and for evaluating resemblance and diversity of molecules manipulation pharmacophore fingerprints [31-33]. 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 [34,35].

In this research paper, we account the screening of various compounds from an in-house developed bioactive database, which are having structural similarities with COX-inhibiting NO donors (CINODS). In addition to pharmacophoric screening, other physico-chemical parameters were used to screen the result set such as Lipinski rule of five. Further the screened compounds were docked into the active site of the COX-1 and COX-2 enzyme to study the binding pattern of the molecules. The binding affinities of molecules with COX-1/COX-2 were reported.

MATERIALS AND METHODS

Selection and preparation of proteins

Co-crystallised structures of COX-1 and COX-2 enzyme bound with nimusulide and diclofenac respectively (PDB ID: 3N8X and 1PXX respectively) were retrieved from the Protein Data Bank. The crystallised structures were imported into the compute and Biopredicta module of V-lifeMDS 4.6 software. Wizard was used to optimize and minimize the protein structure which involves removing undesirable water molecules and cofactors. Finally a low energy and structural correct target proteins were achieved. These minimized proteins were used for further analysis.

Preparation of ligand database

A molecular library containing more than 200 molecules was built virtually by considering molecular weight, number of rotatable bonds, calculated logP, number of H-bond donors, number of H-bond acceptors aromatic characteristics.

Pharmacophore generation

The pharmacophore hypotheses were generated for reference nimusulide (COX-1) and Diclofenac (COX-2) and test ligand using Ligand Scout software package. Each hypothesis was found to contain common features like hydrogen bond donor, hydrogen bond acceptor, positive ionisable and aromatic. The shared feature pharmacophore was designed which indicated the significance of presence of common features for COX-1 and COX-2 inhibition. The pharmacophore modelling studies were performed on Ligand Scout 4.1 Essential (Demo Version, Inte: Ligand GmbH, Vienna, Austria) [36].

Virtual screening and validation against shared feature pharmacophore

All the molecules were aligned with the shared feature pharmacophore model of COX-1 and COX-2. Virtual screening was done against shared feature pharmacophore to obtain hit compounds. The screened result set was further filtered by ligand filtration tool using physicochemical properties such as logP value, H-bond acceptors, H-bond donors and molecular weight and rotation bonds as reported for the reference ligand. The hit compounds obtained were then checked for Lipinski rule of five. Lipinski rule of five states that drug-like compound must have Hydrogen Bond Donor (HBD) less than 5, Hydrogen Bond Acceptor (HBA) less than 10, molecular weight no more than 500 Da and logP ranges between 0-5 [37].

Docking of HIT compounds with COX-1 and COX-2 proteins

The hit compounds were docked in PDB ID: 3N8X and 1PXX for COX-1 and COX-2 respectively by replacing reference inhibitor ligand. A systematic search was performed to obtain the ligand with lowest binding energy. The COX-1/COX-2 binding energy is used for deciding selectivity of hit compounds for COX-1 and COX-2. Docking studies were carried out on Vlife molecular docking suite 3.5 by using Biopredicta [38].

RESULTS AND DISCUSSION

Pharmacophore investigation is measured as an essential segment of drug design. The pharmacophore generated by Ligand Scout for reference ligands for PDB ID: 3N8X and 1PXX for COX-1 and COX-2 respectively. This pharmacophoric model shows three main features HBA, HBD and Aromatic Rings (AR). 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 model contains hydrogen bond acceptors, hydrogen bond donors and aromatic rings. The pharmacophores hypothesis for reference ligands of PDB ID: 3N8X and 1PXX for COX-1 and COX-2 respectively are shown in Figure 1a and b respectively.

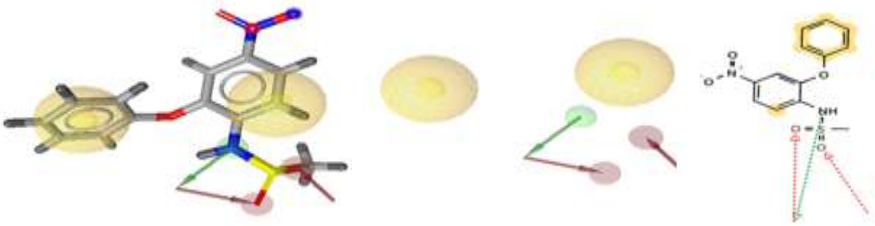
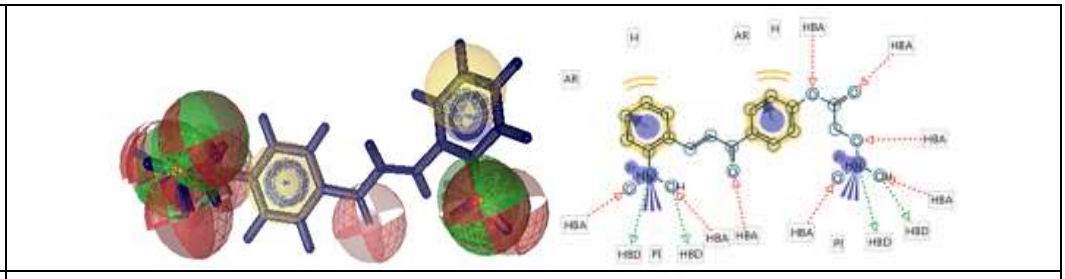
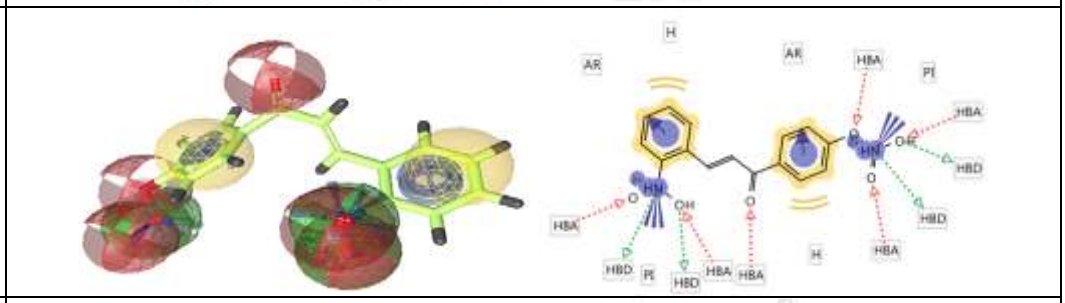
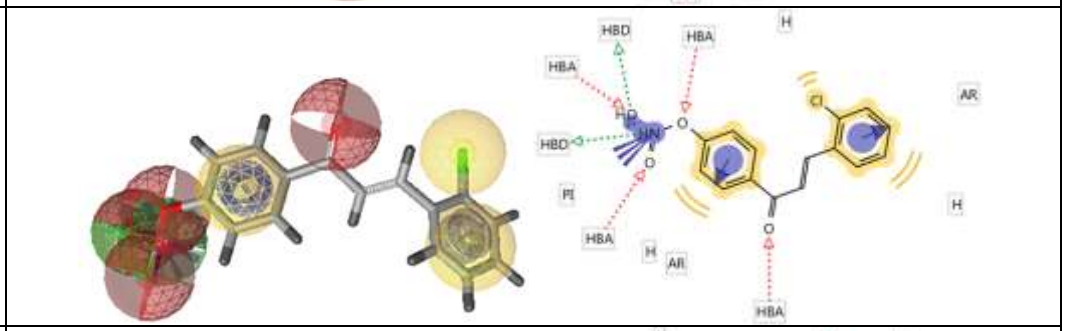
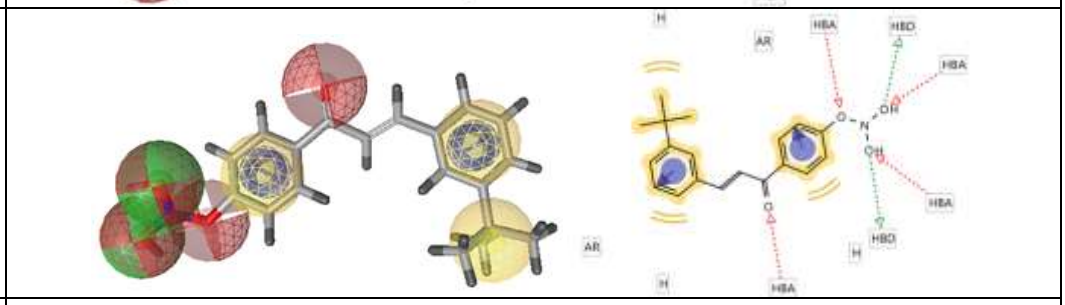
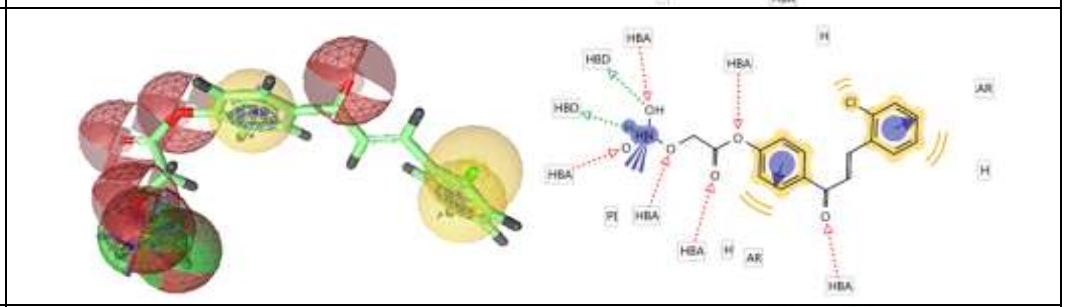
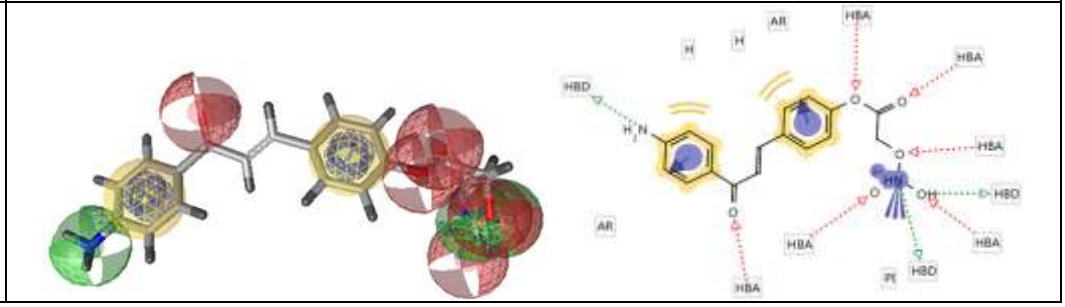


Figure 1a: Structure-based hypotheses of reference ligand nimusulide for PDB ID: 3N8X for COX-1. Red arrows represent hydrogen bond acceptor, green arrow represents hydrogen bond donor and yellow spheres represent an aromatic ring

Figure 1b: Structure-based hypotheses of reference ligand diclofenac for PDB ID: 1PXX for COX-2. Red arrows represent hydrogen bond acceptor, green arrow represents hydrogen bond donor and yellow spheres represent an aromatic ring

The pharmacophores of all the compounds were then matched and a unique pharmacophores were identified after a detailed analysis. Similar features were identified after analysing the pharmacophores of all compounds. The pharmacophores of test set compounds are shown in Figure 2.

Ca2	
AI2	
AI4	
AI8	
Ca4	
Cb2	

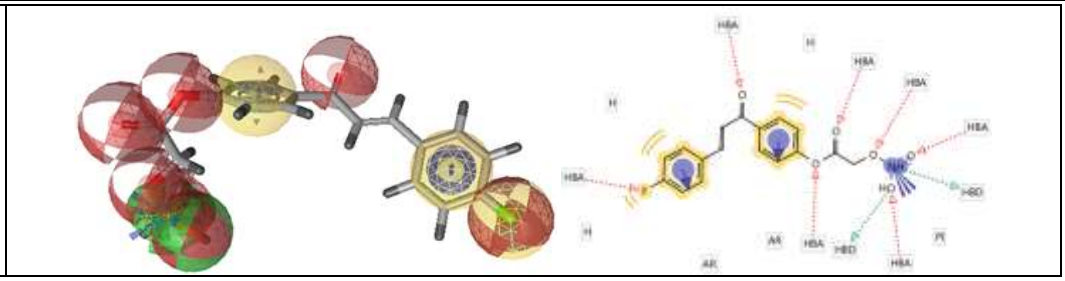
Ca5	

Figure 2: Structure-based hypotheses of selected test molecules. Red arrows represent hydrogen bond acceptor, green arrow represents hydrogen bond donor and yellow spheres represent an aromatic ring

Pharmacophore models of selected protein data sets were aligned together with hit compounds on the basis of ligand structure to generate a shared feature pharmacophore, combined feature pharmacophore hypothesis is generated which will be the proposed pharmacophoric hypothesis for inhibition of COX-1 and COX-2 protein databases shown in Figure 3a and b respectively.

Figure 3a: Common feature alignment shared pharmacophoric features and combined pharmacophoric features for PDB ID: 3N8X for COX-1. Red spheres represent hydrogen bond acceptor, green spheres represents hydrogen bond donor and yellow spheres represent an aromatic ring

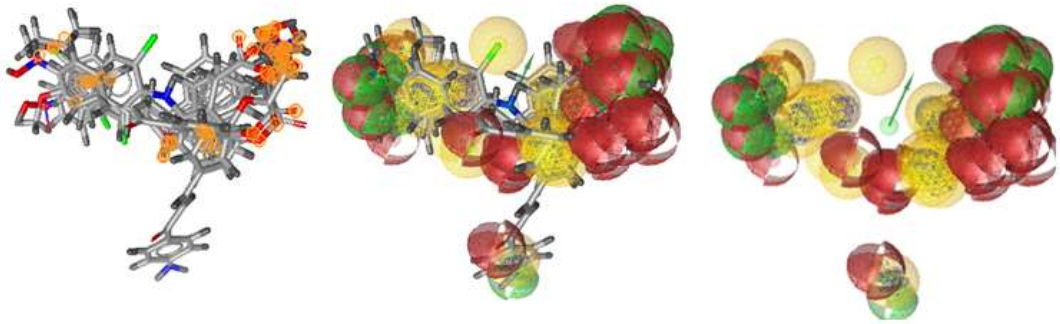


Figure 3b: Common feature alignment shared pharmacophoric features and combined pharmacophoric features for PDB ID: 1PXX for COX-2. Red spheres represent hydrogen bond acceptor, green spheres represents hydrogen bond donor and yellow spheres represent an aromatic ring

The hit compounds were then screened for Lipinski rule of five which states that molecular weight < 500 Da, HBD < 5, HBA < 10 and logP between 0-5. The representative of hit compounds which fulfilled Lipinski rule of five are shown in Table 1.

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

Compound	Molecular formula	Molecular weight	LogP	HBD	HBA
Reference Ligand (Nimusulide)	$C_{13}H_{12}N_2O_5S_1$	308	3.839	1	2
Reference Ligand (Diclofenac)	$C_{14}H_{10}N_1O_2Cl_2$	295	1.959	0	2
Ca2	$C_{19}H_{17}NO_8$	388	3.510	04	08
AI2	$C_{17}H_{15}NO_6$	330	3.426	04	06
AI4	$C_{17}H_{16}N_2O_4$	313	3.824	02	07
AI8	$C_{19}H_{19}NO_4$	326	3.839	2	4
Ca4	C_{22} H_{19} N_3 O_4	390	3.432	2	6
Cb2	$C_{19}H_{17}NO_8$	388	3.839	3	6
Ca5	$C_{17}H_{15}FNO_6$	346	3.839	2	7

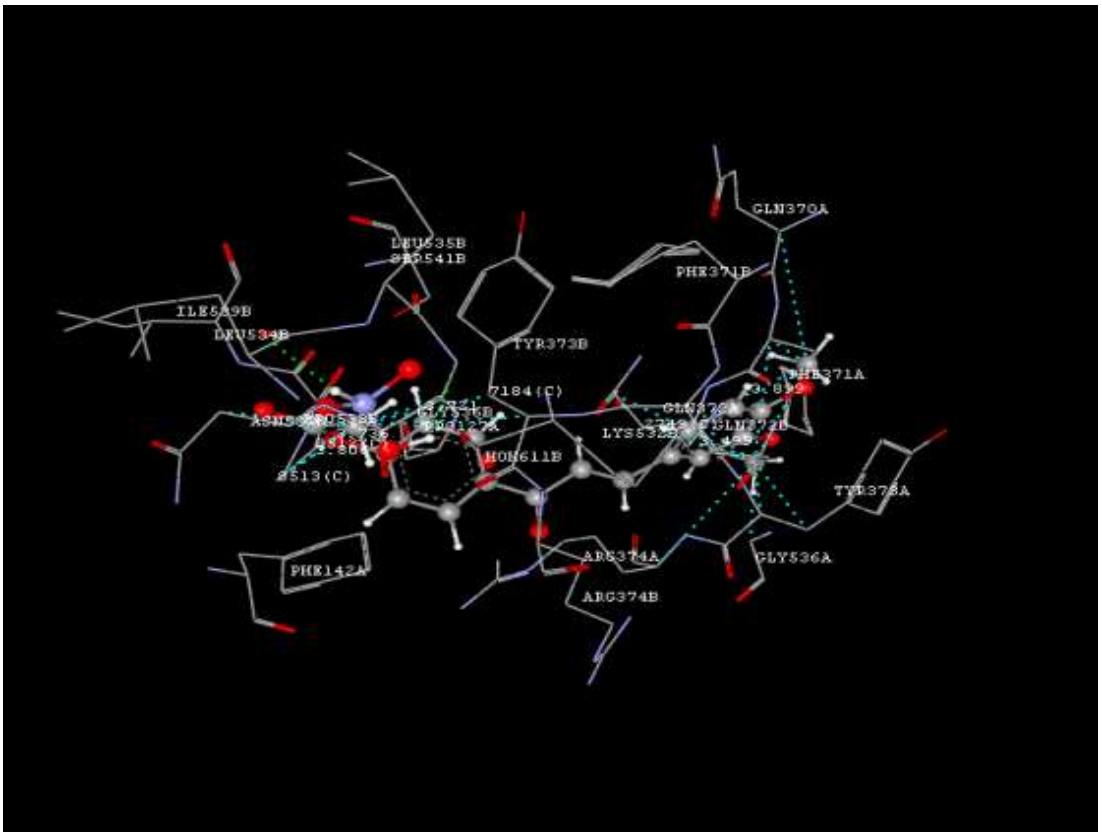
Molecular docking is a crucial tool in the structural molecular biology and the computer-aided drug design. The aim of ligand-protein docking is to predict the principal binding modes of a ligand with a known 3D structure of protein. All satisfied compounds were docked with PDE-5A

protein; in every docked complex the common interacting amino acid residues were same as that of pharmacophore model. The compounds which clears virtual screening filter and fulfilled Lipinski rule of five, their best docking results with COX-1 and COX-2 with their binding energy selectivity are shown in Table 2.

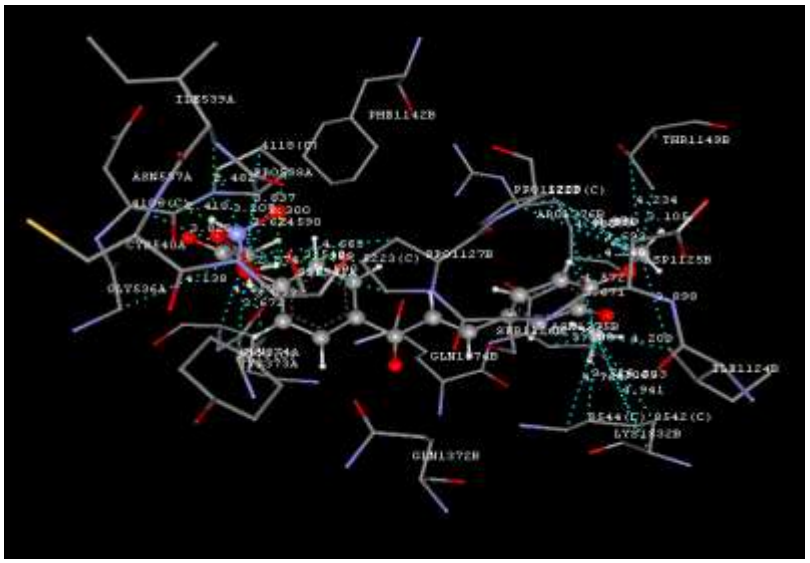
Table 2: Comparison of bonding types of compounds which demonstrated ideal docking results along with binding energy selectivity

Compound Code	Binding Energy and interactions with COX-1	Binding Energy and interactions with COX-2	Binding Energy Selectivity COX-1/COX-2
Reference Ligand Nimusulide	-58.406 VDW, hydrogen bonding and hydrophobic interaction	-22.019 VDW, hydrogen bonding and hydrophobic interaction	2.652
Reference Ligand Diclofenac	-17.210 VDW, hydrogen bonding and hydrophobic interaction	-70.832 VDW, hydrogen bonding and hydrophobic interaction	0.242
Ca2	-21.250 VDW, hydrogen bonding and hydrophobic interaction	-84.142 VDW, hydrogen bonding and hydrophobic interaction	0.252
AI2	-82.612 VDW, hydrogen bonding and hydrophobic interaction	-9.254 VDW and hydrophobic interaction	8.927
AI4	-81.034 VDW, hydrogen bonding and hydrophobic interaction	-12.256 VDW and hydrophobic interaction	6.611
AI8	-72.153 VDW, hydrogen bonding and hydrophobic interaction	-12.256 VDW and hydrophobic interaction	5.887
Ca4	-72.238 VDW, hydrogen bonding and hydrophobic interaction	-15.526 VDW and hydrophobic interaction	4.652
Cb2	-66.593 VDW, hydrogen bonding and hydrophobic interaction	-17.952 VDW and hydrophobic interaction	3.709
Ca5	-61.623 VDW, hydrogen bonding and hydrophobic interaction	-28.246 VDW and hydrophobic interaction	2.181

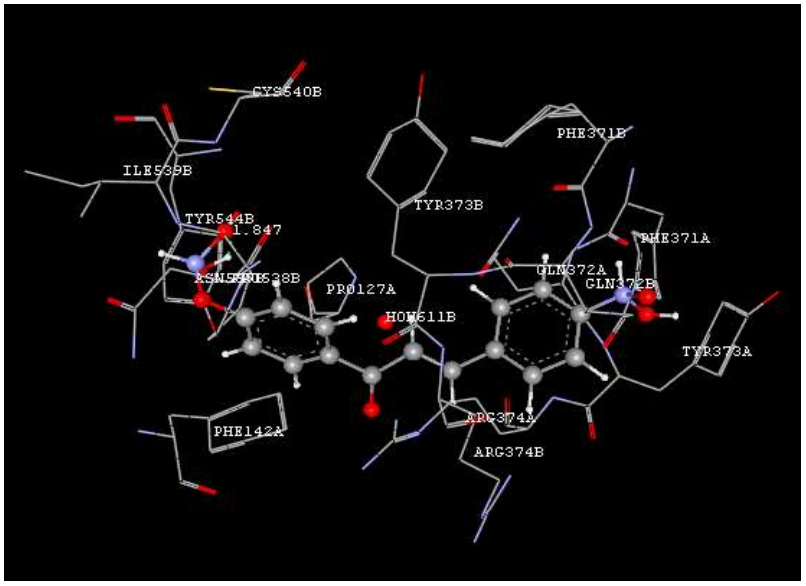
The best docking poses with interaction of these compounds with COX-1 and COX-2 are shown in Figure 4. All the molecules fit properly into the active site of proteins. The docking is carried out by replacing original ligands by HIT molecules.



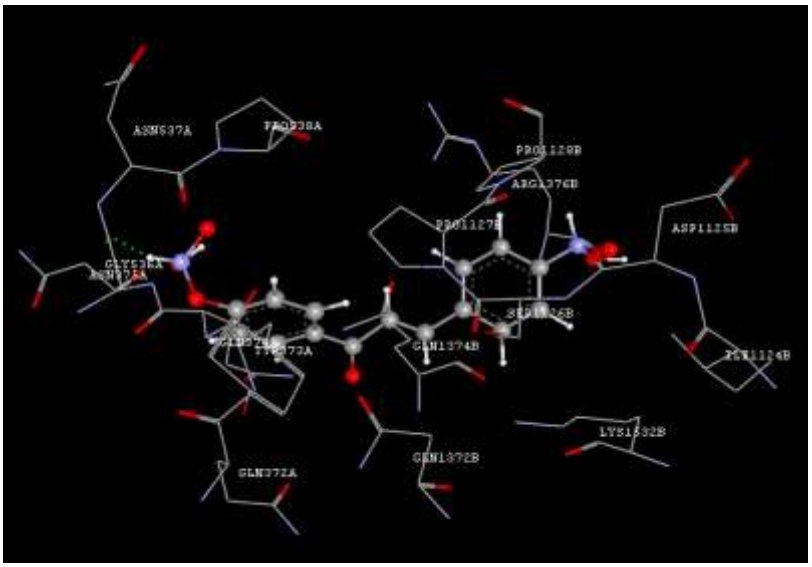
Ca2 binding interactions with COX-1



Ca2 binding interactions with COX-2



AI2 binding interactions with COX-1



AI2 binding interactions with COX-2

Figure 4: Docking interactions of HIT molecules into the active site of COX-1 and COX-2

CONCLUSION

Present strategy of via virtual screening and molecular docking in the development, research and drug design has proved to be significant in terms of developing alternative methodologies where in the animal scarification. Using these approaches we have successfully screened a library of around 200 bioactive molecules which leads to around 6 bioactive as COX-1 inhibitors and 01 bioactive as COX-2 inhibitors. The virtual screening and applying Lipinski rule of five increases probability of selecting more potent hit molecules. The approach of deciding COX-1/COX-2 selectivity based on binding energy increase the chances of reducing potential side effects of Non-selective NSAIDs. The ligand Ca2 was found to have COX-1/COX-2 selectivity of 0.252, which means that it is having affinity more towards COX-2 but still it binds to COX-1 also. This signifies the concept of dual inhibitor which may reduce the potential side effect of selective COX-2 inhibitors. We would like to screen the above ligands for Anti-inflammatory, Analgesic and Ulcerogenic activity.

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