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Pharmacophore based virtual screening of natural product database to identify potential lead Cyclooxygenase-2 inhibitors (COX-2)

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ABSTRACT

Inflammation is a key factor linked to almost all chronic and degenerative diseases, including arthritis, heart disease, asthma, neurodegeneration, cancer, kidney and bowel diseases. Since ancient time's natural products such as turmeric, ginger root extract and some of the essential oils have been used to relieve pain. Till today around 135 Indian medicinal plants screened for their activity on the basis of ethanopharmacology on random basis. The active constituents from these plants are also reported to be selective inhibitors of enzymes such as COX-1, COX-2 which is responsible for inflammation. Such natural product scaffolds find applications in the treatment of inflammation and can also be used for the development of new generation anti-inflammatory drugs with low toxicity and higher therapeutic value. In this paper, we report screening of various natural compounds from an in-house developed bioactive database containing around 10^3 bioactives. As a first step, known crystallized COX-2 inhibitor SC-558 was used to generate pharmacophore, which was used for initially screening of the database. The result set was further filtered using physico-chemical properties similar to that of the known inhibitor SC-558. Our screening approach identified around 347 structurally similar molecules, which were further docked using the GLIDE software module. The docking studies resulted into 15 compounds with comparable docking score with respect to known inhibitor SC-558.Binding interactions were further studied to get insights into binding pattern of these molecules with respect to binding site of COX-2 enzyme.

Keywords: cyclooxygenase; anti-inflammatory; docking; natural product; pharmacophore; COX-2 inhibitor; virtual screening.

INTRODUCTION

Inflammation is mainly caused due to the over production of prostaglandins during the biogenesis of arachidonic acid to prostaglandins which are synthesized by cyclooxygenase enzymes. The enzyme exists in two isomeric forms COX-1 and COX-2[1]. COX-1 is constitutively expressed in virtually all tissues under basal conditions. It produces prostanoids that mediate homeostatic functions especially in the gastric mucosa, small and large bowel mucosa, the kidney, in platelets and in the vascular endothelium. On the other hand COX-2 is induced by growth factors (FGF, PDGF, and EGF), hormones (LH) and other physiological stimuli. These findings lead to the initial hypothesis that COX-2 produces the "bad" prostaglandins during inflammatory processes [2].

Today, a number of selective COX-2 inhibitors are known which primarily are sulphonamide non-steroidal anti-inflammatory drugs (NSAID).These non-steroidal anti-inflammatory drugs (NSAIDs) used in inflammatory disorders may be either with analgesic and insignificant anti-inflammatory effects or with analgesic and mild to moderate anti-inflammatory activity. Most of these drugs cause adverse effects such as gastric or intestinal ulceration that can sometimes cause secondary anaemia [3].

On the other hand now-a-days herbal drugs are routinely used for curing diseases rather than chemically derived drugs having side effects. For centuries, natural remedies such as white willow bark (*Salix alba*) and myrtle (*Myrtuscommunis*), turmeric provided some pain relief. Other plants such as Ananas comosu, Curcuma longa, Tripterygium wilfordii, Boswellia serrata etc are known for their anti-inflammatory properties[4]. Diverse groups of natural products from these plants possess anti-inflammatory properties. But screening of such a large number of natural compounds either for their anti-inflammatory activity or for inhibiting the COX-2 enzyme responsible for inflammation using *in vivo* experiments is not an effective approach, particularly since this strategy makes the whole process eco-unfriendly, time consuming and centered on the sacrifice of a huge number of test animals. Recent approaches of using virtual screening and molecular docking in the development, research and drug design has proved to be significant in terms of developing alternative methodologies wherein the animal sacrifices is minimized quite drastically.

In this paper, we report the screening of various natural compounds from an in-house developed bioactive database, which are structurally similar to the known COX-2 inhibitor SC-558. In addition to pharmacophoric screening, other physico-chemical parameters were used to screen the result set. Further the screened compounds were docked into the active site of the COX-2 enzyme to study the binding pattern of the molecules, the results of which are presented herein.

MATERIALS AND METHODS

The co-crystallized structure of the COX-2 enzyme bound with the ligand SC-558 (PDB code 1CX2) was selected as the receptor-ligand model. The structural pharmacophoric features and physico-chemical properties of the ligand SC-558 were used to screen a library containing around 10,000 bioactive compounds.

Ligand bioactive database:
A library of 10,000 bioactive compounds was build in-house by collating information from published scientific literature and patent documents. All the bioactives which were shown to possess therapeutic activity either in vitro, in vivo or in human clinical trials were considered to create the database. These bioactives belonged to around 500 Indian medicinal plants which were already screened for their toxicity and were traditionally known for their ethanopharmacological activities. Molecules in the database are annotated by molecular weight, number of rotatable bonds, calculated logP, number of H-bond donors, number of H-bond acceptors etc. This database is searchable either by the physico-chemical properties, structure or the activity.

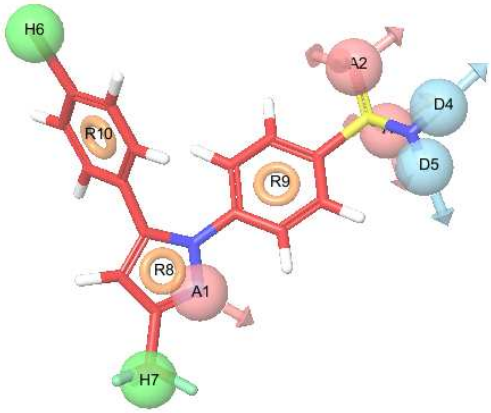
Protein Preparation:
Co-crystallised structure COX-2 enzyme bound with SC_558 (PDB NO: 1CX2) was retrieved from the Protein Data Bank. The crystallised structure was imported into the protein preparation wizard of Schrodinger Software (Ver 9.4). The enzyme exists in the tetrameric form in the crystal. The monomeric chain/pocket which had the active site to which the SC-558 ligand was bound was retained. Wizard was used to optimize and minimize the protein structure which involves removing undesirable water molecules and cofactors, optimization with OPLS_2005 force filed using standard parameters and other defects in the target protein. Finally a low energy and structural correct target protein was achieved. This minimized protein was used for further docking analysis.

Grid generation:
As the energy minimised protein structure already had the active site for SC-558, the grid was generated by selecting SC-558 ligand as the reference ligand. Active site consists of His 90, Arg 120, Tyr 355, Tyr 385, Arg 513, Val 523 and Ser 530 residues. Amino acids within 6.5 A of the inhibitor SC-558 were included in grid generation. The generated grid was further used for docking studies.

Ligand preparation:
The ligand structure of SC-558 as retrieved from the co-crystallised protein structure was prepared using the LigPrep module [4]. It was subjected to OPLS-2005 force field to generate single low energy 3-D structure.

Virtual Screening:
Prepared SC_558 inhibitor was used to generate simplified pharmacophore model using Phase module [5]. The pharmacophoric model highlighted different pharmacophoric features essential for activity. Generated pharmacophoric model of SC_558 consists of 10 features as shown in figure 1. All the pharmacophoric features of SC_558 were selected to screen in-house natural product database using “Find Matches” option in Phase module. The screened result set was further filtered by Ligand filtration tool using physico-chemical properties such as log P value, H-bond acceptors, H-bond donors, molecular weight and rotation bonds as reported for the ligand SC-558.

Figure 1: Pharmacophoric features of SC_558 ligand



This filtration resulted into 367 structurally similar compounds from the bioactive database which were further optimised and used for docking studies.

Docking:

The generated grid was used for docking reference ligand SC_558 and screened structurally similar ligands using Glide Module [6]. Docking protocol was carried using Extra precision and write XP descriptor information. This generated favourable ligand poses which are further screened through filters to examine spatial fit of the ligand in the active site. Ligand poses which pass through initial screening are subjected to evaluation and minimization of grid approximation. Scoring is then carried on energy minimized poses to generate Glide score.

RESULTS AND DISCUSSION

Natural products which were shortlisted by pharmacophore and physico-chemical parameter screening were successfully docked against the binding site of COX-2 target (PDB: 1CX2) with SC_558 as the reference ligand. SC_558 is diaryl heterocyclic inhibitor and has selectivity for COX-1 & COX-2 target. It shows specific interactions in the binding pocket of 1CX2. This includes H-bond formation with His-90. Further the bromophenyl ring of SC_558 is bound in the hydrophobic cavity of formed by Phe 381, Leu 384, Tyr 385, Phe 518, Val 523 and Ser 530. These features of SC_558 are quite important in studying the binding modes of different ligands.

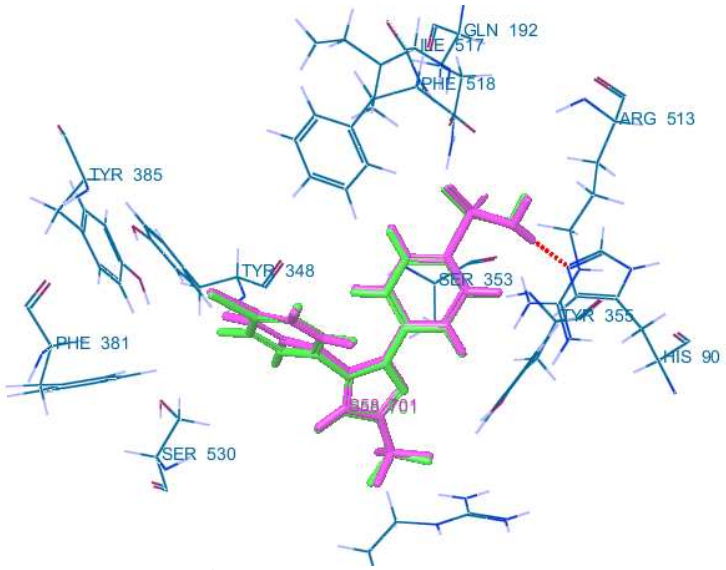


Figure 2. Comparative binding orientation of the Crystallographic SC_558 (green) and docked SC_558 (purple) as predicted by Schrodinger Glide software.

As the docking grid was generated using Glide module, the docking protocol was firstly validated wherein separately prepared ligand SC_558 was re-docked into the binding pocket of the protein through the generated grid. Figure 2 shows comparison between the original binding mode of crystallographic SC_558 against docked binding mode as predicted by Schrodinger Glide software. Figure 2 clearly shows that adapted Schrodinger methodology successfully predicted the binding mode of crystallographic mode with Root mean square deviation of 0.5 °A.

Further both the docked conformer and crystallographic SC_558 were found of have specific hydrogen bonding with His-90 residue in the binding pocket of 1CX2 target.

Similarly the screened 347 compounds were docked against the generated grid using Extra precision and write XP descriptor information. The docking resulted into 8 molecules whose Glide score was comparable to that of the reference ligand SC_558 with Glide score close to -10. Therefore molecules close to or having lower docking score than -10 were considered of high interest. Binding energies of docked ligands ranges from (-41.97 to -39.46 Kcal/mol). Ligand1, Ligand2, and Ligand3 were predicted to have better binding efficiency with respect to SC_558. The predicted binding energies of all the 8 ligands is listed in table no. 1

Table No 1. Docking scores of SC_558 and 8 new compounds with respect to 1CX2 protein

Ligands	MW	GScore	Glide Energy	logPo/w	logS	PSA
SC_558	446.241	-10.12	-56.36	3.545	-6.036	79.412
Lig 1	310.306	-10.1	-41.97	2.944	-4.484	81.868
Lig 2	307.305	-9.53	-39.87	1.925	-3.467	79.833
Lig 3	244.29	-9.11	-32.24	2.178	-3.328	62.772
Lig 4	341.276	-8.62	-39.28	2.463	-3.327	108.703
Lig 5	298.251	-8.42	-33.49	1.262	-2.702	126.048
Lig 6	284.225	-8.34	-36.77	0.942	-2.603	142.614
Lig 7	279.295	-8.33	-35.58	2.549	-3.63	60.264
Lig 8	271.272	-8.34	-39.46	1.633	-3.161	87.436

Similarly the binding interaction of ligands within binding site of COX-2 was compared with that of SC_558. The binding mode and H-bond interactions of ligands (Lig 1, Lig 2, Lig 3) as predicted by the Glide software as represented in Figure 3. According to the docking models, all the molecules are predicted to bind in the binding site of COX-2 protein with comparatively good Glide score. Mol 56 with the predicted conformation shows hydrogen bond formation with Tyr 385 residue in binding pocket. Similarly Mol 281 & Mol 311 formed H-bonds with Tyr 355 respectively.

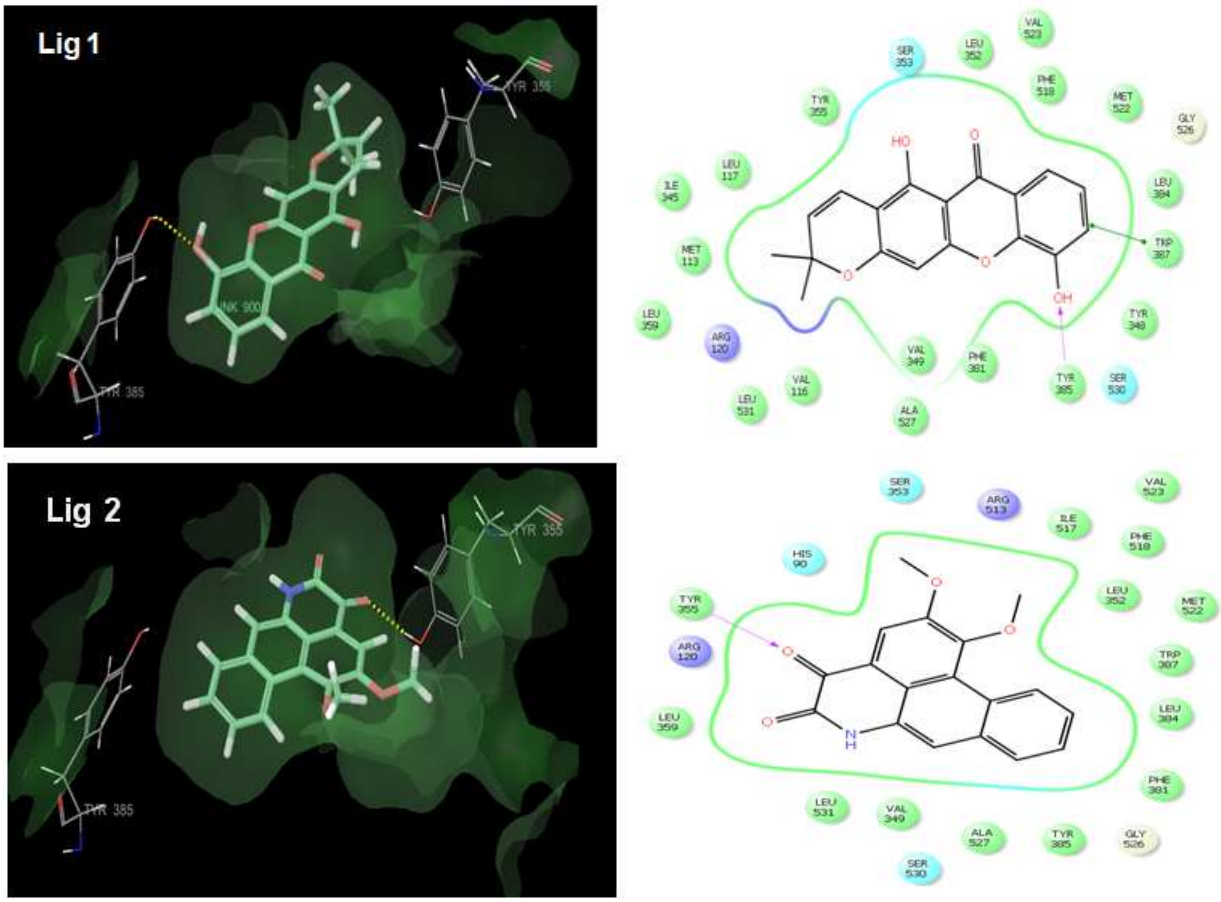


Fig 3. H-bond fomation indicated by yellow dotted for Lig 1 with residue Tyr385 and in case of Lig 2 & Lig 3 with Tyr355 residue respectively

Further ADME prediction was carried out to predict partition coefficient (log P o/w), polar surface area (PSA), and aqueous solubility (log S) properties. These results have been listed in Table No. 1. All 3 top ligands (Lig 1, 2 & 3) have properties are in the acceptable range. This clearly indicates that the ligands show similar binding mode and ADME properties as compared to SC_558 and hence can be evaluated in laboratory for in-vivo anti-inflammatory studies.

CONCLUSION

Recent approaches of using 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 sacrifices is either totally not involved or minimized quite drastically. Using these approaches we have successfully screened a library of around 10^3 bioactives which lead to around 8 bioactives as COX-2 inhibitors. The docked poses of the three bioactives resembles similar orientation as observed with SC-558 ligand. All the three bioactives bind with at least one of the amino acid in the active pocket via H-bonds. Out of the three ligands the Ligand 2 is an alkaloid from the stems of Fissistigma oldhamii [7]. The alkaloids from the stems of this plant were reported in literature for their anti-inflammatory activity. The docking results obtained in the present study shows that ligand 2 which is a phytoconstituent of this plant shows interactions with COX-2 which are comparable to that of the known COX-2 inhibitors. The ligand 4 from the table 1 is a triterpenoid present in the resinous exudates of Commiphora myrrha [8]. Ligands 4 and 3 were evaluated for their anti-tumor activities in the published scientific literature articles. Research studies have reported that overexpression of COX-2 is also responsible for causing various human tumors. Since these two bioactives (ligand 4 and 3) are reported for their anti-tumor activity, the findings from the docking study reveal that these ligands may possess anti-tumor activity by inhibiting the overexpression of COX-2.

As the bioactives obtained in this study show interactions with COX-2 which are comparable to that of the know inhibitor SC-558, these compounds may serve as scaffolds for further development of natural product based selective COX-2 inhibitors.

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