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Original Article

Molecular docking and binding studies of bioactive compounds from elite coastal flora on their interaction with cyclooxygenase-2 and 5-lipooxygenase protein: a search for novel anti inflammatory drug

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ABSTRACT

Cyclooxygenase-2(COX-2) and 5-lipooxygenase (5-LOX) enzymes involved in the arachidonic acid pathway has become an important target for the pharmacological intervention of chronic inflammation. Naturally occurring bioactive compounds are gaining importance as potential drug candidates for a number of pathological conditions including chronic inflammatory diseases. Computer based predictions have become more reliable, the synergic effects of the drug molecules in the crude can be identified faster using *in silico* tools. Four bioactive compounds derived from coastal flora were taken for the present study and docked with target proteins. The results inferred that the all four molecules are promising hits as inflammatory inhibitors of natural origin. Notably betulinic acid was identified as potential dual inhibitor of COX-2/5-LOX. Therefore, further studies can be carried out on these natural compounds to identify lead molecule against chronic inflammation.

Key words: Elite Coastal flora, COX-2/5-LOX, chronic inflammation

INTRODUCTION

Chronic inflammatory disease is characterized by persistent inflammation. Clinical studies indicate that inflammation is a significant risk factor to develop various human diseases such as inflammatory bowel disease (IBD), chronic asthma, rheumatoid arthritis, multiple sclerosis, and psoriasis [1]. Arachidonic acid (AA) Pathway is a major component of inflammatory pathway. AA is oxygenated by the enzymes cyclooxygenase (COX) and lipooxygenase (LOX) and further transformed into a variety of products which mediate or modulate inflammatory reactions [2]. COX exists in two distinct isozymes (COX-1 and COX-2), one of which, COX-2, is primarily responsible for inflammation but apparently not for gastrointestinal integrity or platelet aggregation [3]. Leucotrienes which are synthesized from the activity of 5-lipooxygenase (5-LOX) have a major role in inflammatory process. Modulation of AA metabolism by inhibiting these enzymes (COX-2/5-LOX) has been considered as an effective mechanism for chemoprevention [4]. Recently, dual COX-2/5-LOX enzyme inhibitors are considered significant in managing the molecular mechanisms during chronic inflammation and possesses minimal adverse effects compared to NSAIDs [5]. The market withdrawal of selective COX-2 inhibitors namely rofecoxib in 2004 and valdecoxib in 2005 due to their cardiovascular toxicity [6] have led to identify compounds with dual COX/LOX inhibitory effect.

Most of the modern synthetic medicines are associated with severe adverse effects. In comparison to modern drugs, herbal medicines are frequently used to treat chronic diseases [7]. Coastal flora is a rich source of phytochemicals such as steroids, triterpenes, saponins, flavonoids, alkaloids and tannins. Many coastal plants such as mangroves, sand dune plants have ethnopharmacological relevance and have also been exploited by the local people in the search for remedies for various ailments [8]. Some coastal flora contain a wide range of compounds namely

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agallochaol O, betulinic acid, mimosol D, and eugenol (Fig 1-4) which has been reported for their anti-inflammatory activity [9]. Therefore, the impact of these four selected ligands derived from coastal flora on COX-2/5-LOX enzyme inhibition is worthy investigating. Traditionally, pharmacologists strive to optimize and accelerate the new drugs by developing new *in vivo* and *in vitro* investigation strategies which is a long and costly process [10]. Alternatively, virtual screening of chemical libraries by docking analysis will allow a faster and cheaper identification of promising drug candidates to be finally tested on the bench [11]. Docking of small molecules in the receptor binding site and estimation of binding affinity of the complex is a vital part of structure based drug design [12]. In the present study, the binding modes of selected four anti-inflammatory compounds from coastal flora were evaluated by *in silico* molecular docking studies on COX-2 and 5-LOX as chronic inflammatory therapeutic targets.

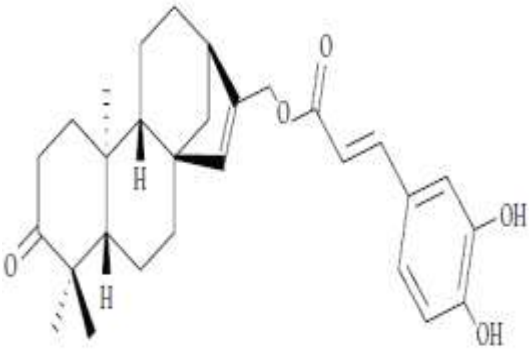


Figure: 1 Agallochaol O, an anti-inflammatory diterpene isolated from the stems and twigs of milky mangrove *Excoecaria agallocha* [13]

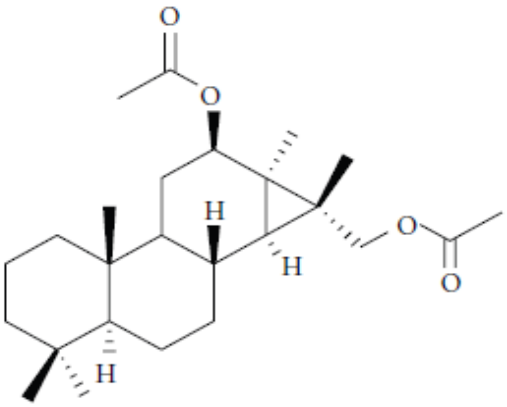


Figure:3 Mimosol D, an anti inflammatory diterpenoid isolated from the roots of *Caesalpinia mimosoides* [15]

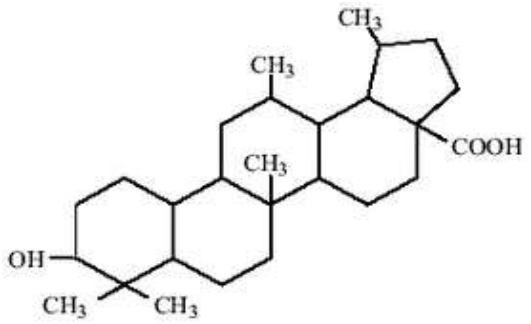


Figure:2 Betulinic acid, an anti-inflammatory compound isolated from *Avicennia marina*[14]

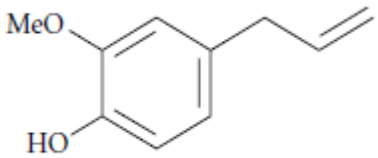


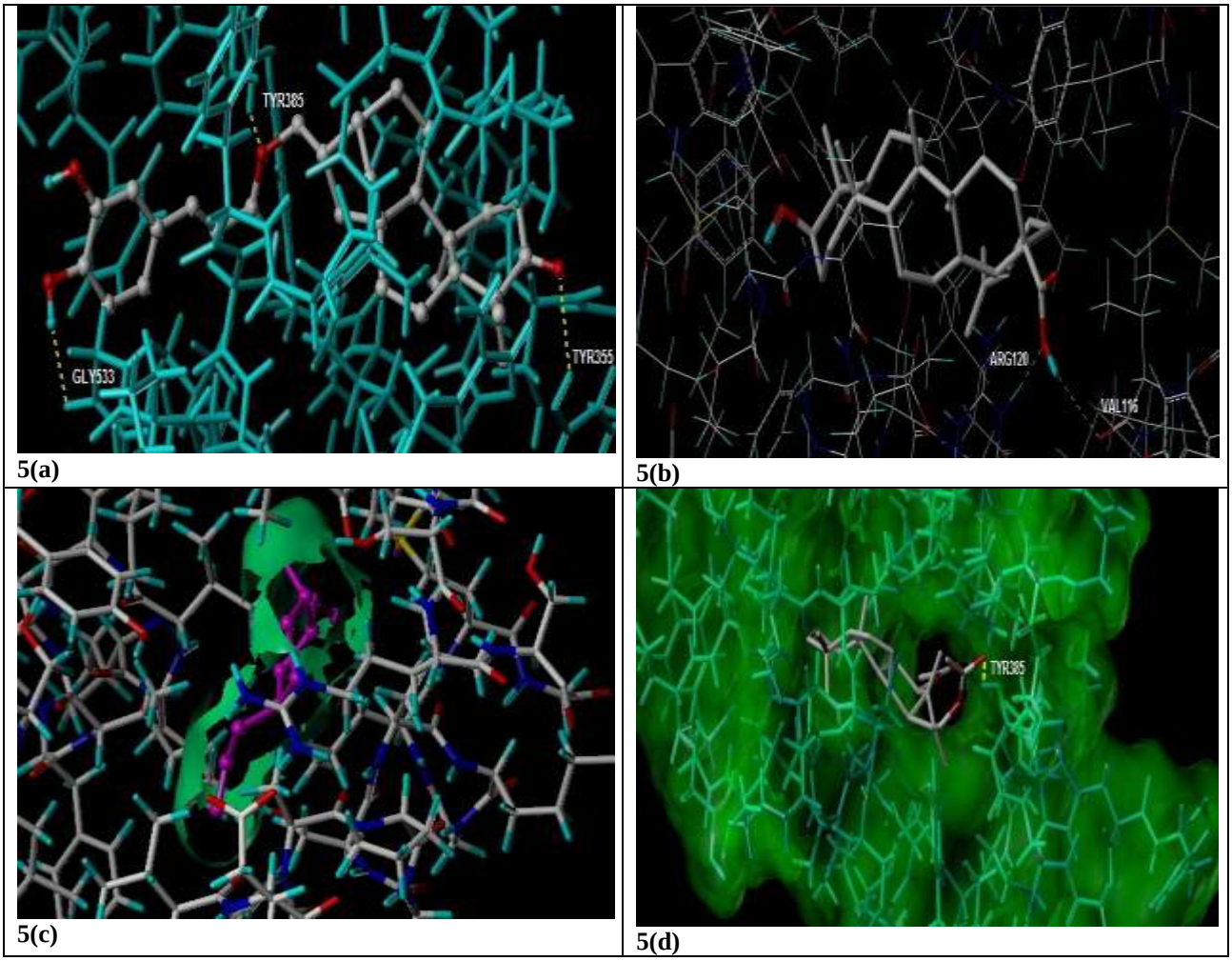
Figure: 4 Eugenol, an analgesic and anti-inflammatory compound isolated from *Ipomoea-pes-caprae* [16]

MATERIALS AND METHODS

The crystal structures from PDB of cyclooxygenase-2(3OLT), Lipoxygenase (3V99) was used for the study [17]. The protein was prepared by removing all water molecules and adding all hydrogen atoms. The structure of ligands agallochaol O, betulinic acid, mimosol D, eugenol was retrieved from PubChem database [18]. The ligands were docked into the active site using the molecular docking software SYBYL ver 7.3 (Tripos, L.P.) Surflex-Dock (BioPharmics LLC.) with the default parameters. The proprietary software is licensed to Manipal Institute of Technology, Manipal University, India. Surflex-Dock is a program for calculating the docking modes of small molecules into protein-binding sites. In this study we have used ChemScore, a scoring function that is derived from regression against ligand-receptor binding free energies. In the docking process the active site was defined. For each ligand, 20 conformations were generated (40x20=800 conformations) and then docked into active sites of target proteins.

RESULTS

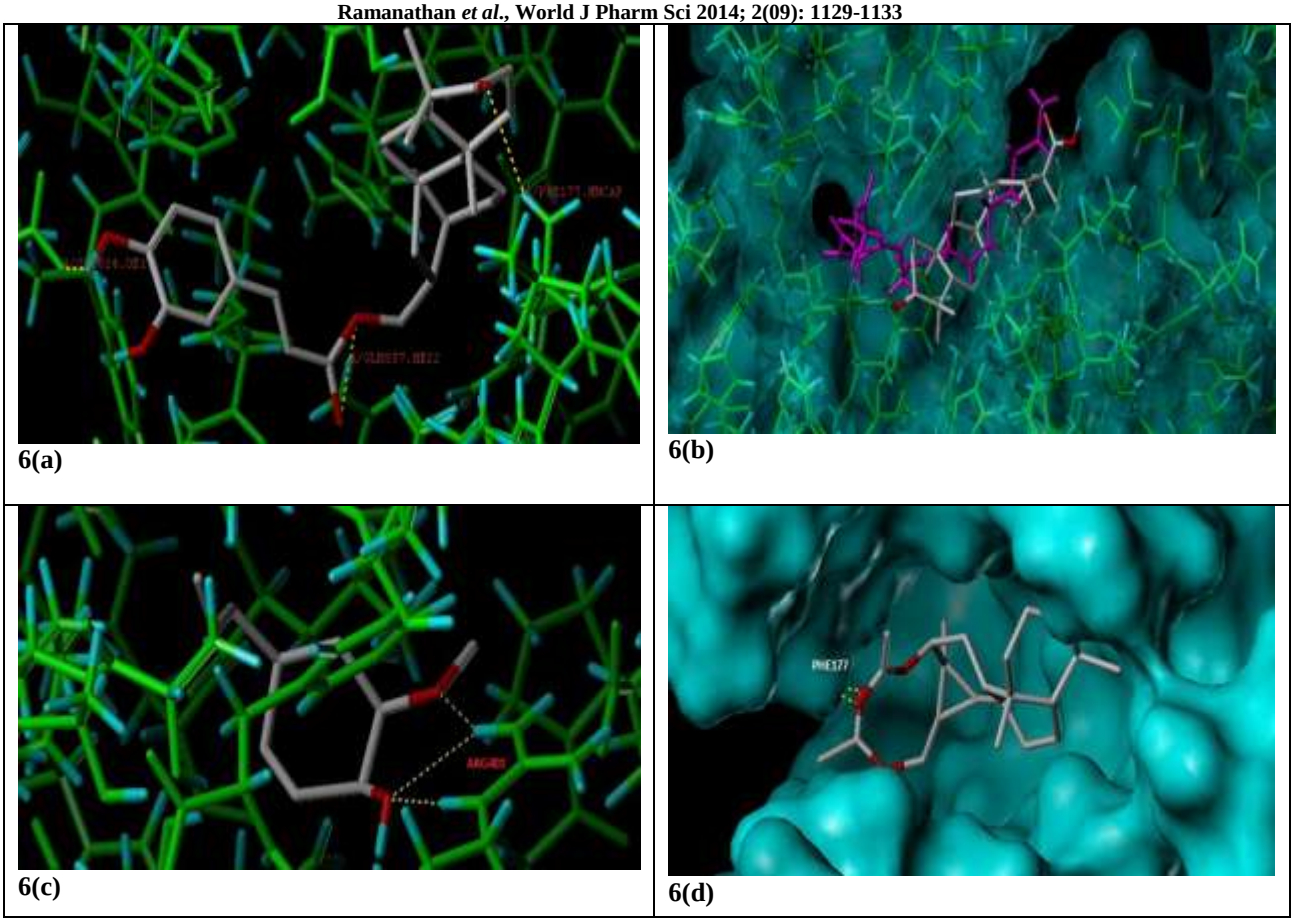
Docking studies revealed that all four ligands interact in the active site of COX-2 and 5-LOX. Among the potent compounds, agallochaol O showed better interaction stabilized by three hydrogen bonds within the active site amino acids GLY533, TYR385, TYR355 of COX-2(Fig 5a) and GLY614, GLN557 and PHE177 aminoacids of LOX-5 targets(Fig 6a) respectively. Similarly the amino acids of COX-2, TYR385, ARG120 and VAL116 showed single and double hydrogen bond interactions with mimosol D and betulinic acid (Fig 5b and 5d). The aminoacids PHE177, ARG401 of 5-LOX showed single hydrogen bond interactions mimosol D and Eugenol (Fig 6c and 6d). Non bonded interactions were observed for eugenol and betulinic acid within the binding cavity of COX-2 and 5-LOX respectively (Fig 5c and 6b). The binding affinity of the ligands with COX-2 and 5-LOX proteins has been analyzed from the ChemScore. Based on the ChemScore, the COX-2 inhibitory activity of the selected compounds was found to be decreased in the order of betulinic acid, Agallochaol O, Mimosol D, Eugenol. With COX-2 enzyme, Betulinic acid was found to have very high ChemScore value when compared with all other compounds. Docking study with ligands on binding site of 5-LOX, shows that all ligands are good 5-LOX inhibitors. Out of 4 docked compounds betulinic acid binds well within 5-LOX binding site. Based on the ChemScore, the 5-LOX inhibitory activity of the selected compounds was found to be decreased in the order of betulinic acid, Eugenol, Agallochaol O, Mimosol D. From this result, it is inferred that the all 4 molecules are promising hits as inflammatory inhibitors of natural origin.



5(a) **5(b)**

5(c) **5(d)**

Figure 5: shows the binding modes of Agallochaol O(5a), Betulinic acid(5b), Eugenol(5c), Mimosol D (5d) in the allosteric binding site pocket of COX-2 enzyme



6(a) **6(b)** **6(c)** **6(d)**

Figure 6 shows the binding modes of Agallochaol O (6a), Betulinic acid (6b), Eugenol (6c), Mimosol D (6d) in the allosteric binding site pocket of 5-LOX enzyme

DISCUSSION

Chronic pain conditions affect at least 116 million US adults and more than one-third of adults worldwide [19] [20]. NSAIDs are used extensively for the treatment of chronic pain due to their efficacy as anti-inflammatory and analgesic agents [21]. NSAIDs are generally chiral molecules (except diclofenac), but mostly a single enantiomer is pharmacologically active [22]. The mechanism of action of NSAIDs is based on inhibition of PGs and LT synthesis. It has been observed that NSAIDs are effective against pain because of their ability to inhibit PG-mediated cerebral vascular vasodilation. However, long-term administration of NSAIDs causes adverse gastrointestinal (GI) symptoms including mucosal lesions, bleeding, peptic ulcer, and inflammation in intestine leading to perforation, strictures in small and large intestines, leading to chronic problems [22].

The 5-LOX/COX-2 blockers have an excellent preclinical gastrointestinal pharmacological safety profile [4]. Several clinically effective NSAIDs have been structurally modified to yield potent dual COX–LOX inhibitors [23]. In the past few decades, several compounds have been developed to block both COX-2 and 5-LOX, but their uses were abandoned owing to liver toxicity [4]. A number of studies reported that plant-derived extracts or plant derivatives show anti-inflammatory activity by controlling the levels of various inflammatory cytokines or inflammatory mediators including IL-1, IL-6, IL-10, TNF-α, NF-κB, NO, iNOS and COX-2. The anti-inflammatory activity of several plant extracts and isolated compounds has already been scientifically demonstrated [24].

Herbal medications are becoming increasingly popular because of their relatively few side effects. Extracts from coastal flora have been used worldwide for medicinal purposes, and having been recorded around 349 metabolites it turns out to be a rich source of steroids, diterpenes and triterpenes, saponins, flavonoids, alkaloids and tannins [25]. Coastal flora derived compounds such as Agallochaol O, Betulinic acid, Eugenol and Mimosol D has already been reported for their anti inflammatory activity [9]. Based on previous reports, we sought to investigate bioactive compounds from coastal flora for their anti depressant effects by bioinformatics approach. From our study, we find that betulinic acid can be used as potent COX/LOX inhibitor. All ligands showed inhibiting activity with COX-2 and 5-LOX

thereby preventing the AA metabolism and can be good drugs for chronic pain diseases.

CONCLUSION

Our computational results indicate that bioactive compounds from coastal flora possess dual inhibitory effect on COX-2/5-LOX enzyme activity. The results obtained from computational studies may trigger the interest of medicinal chemist and cell biologist for identifying novel structural analogs from coastal flora in future as dual COX-2/5-LOX inhibitors.

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