

***In Silico* Molecular Docking Studies of Lichen Metabolites against Cyclooxygenase-2 Enzyme**

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Abstract

Cyclooxygenase-2 (COX-2) is an inducible enzyme that causes inflammation. COX-2 inhibitors are clinically effective anti-inflammatory agents with less gastrointestinal and renal toxicities. However, they lack anti-thrombotic activity and hence lead to increased incidences of adverse cardiovascular thrombotic events, including myocardial infarction. Therefore, there is still need to develop COX-2 inhibitors with better therapeutic effects and tolerability. The aim of the present study is to explore the anti-inflammatory activity of five lichen metabolites by conducting virtual screenings. In this regard, molecular docking simulations were carried out for the lichen metabolites namely atranorin, diffractic acid, lecanoric acid, salazinic acid and usnic acid with human COX-2 enzyme and the docked results were compared with the standard reference ligands (Celecoxib and Rofecoxib). Among all the docked ligands, the lecanoric acid demonstrated best binding affinity -9.83 kcal/mol followed by atranorin (-8.7 kcal/mol) and diffractic acid (-8.6 kcal/mol) which are comparable to the reference ligands celecoxib (-12.3 kcal/mol) and rofecoxib (-11.2 kcal/mol). The salazinic acid and usnic acid has shown binding affinity of -7.9 kcal/mol and -4.7 kcal/mol, respectively. Moreover, all the ligands except atranorin and diffractic acid satisfied Lipinski's rule of 5. From the docking results it was revealed that the lichen metabolites might have inhibitory activity against COX-2 enzyme, and are expected to be useful in conducting *in vivo* anti-inflammatory screenings on animal model which may lead to the development of more effective and potent new chemical entities with anti-inflammatory properties.

Key words: Lichen metabolites, Cyclooxygenase-2, Molecular docking, Anti-inflammatory.

Introduction

Cyclooxygenase (COX) is an endogenous enzyme involved in production of prostaglandins from arachidonic acid. There are two common isoforms of this enzyme such as COX-1 and COX-2. COX-1 is a constitutive enzyme whereas COX-2 is inducible and is expressed only after an inflammatory stimulus (Vane *et al.*, 1998). COX-2 inhibitors are clinically effective anti-inflammatory agents with less gastrointestinal and renal toxicity. However, they lack anti-thrombotic activity and hence lead to increased incidences of adverse cardiovascular thrombotic events such as myocardial infarction. Therefore, there is still need to develop better therapeutic effect and tolerability of COX-2 inhibitors.

Lichens are symbiotic association of algae and fungi that occur in a wide variety of habitats and natural environmental conditions. A large number of lichen metabolites were reported to exhibit profound analgesic (Okuyama *et al.*, 1995), antipyretic (Okuyama *et al.*, 1995), anti-inflammatory (Vijayakumar *et al.*, 2000), antimicrobial (Rashid *et al.*, 2001) and anti-proliferative or cytotoxic activities (Eugenia *et al.*, 1994; Oksanen 2006; Stocker-Worgotter 2008; Kristmundsdottir *et al.*, 2005).

Rational drug design uses a variety of computational methods to identify novel compounds. One of those methods is molecular docking where interactions between protein receptors and ligands are predicted and analyzed (Akhila *et al.*, 2012). In the present study efforts are made

to identify novel natural lichen metabolites possessing anti-inflammatory activity using bioinformatics tools. In this regard, molecular docking simulations were carried out for the five lichen metabolites (atranorin, diffractic acid, lecanoric acid, salazinic acid and usnic acid) with human COX-2 enzyme.

Methodology

Preparation of target protein X-ray structure: The crystal structure of human COX-2 in complex with celecoxib (PDB code: 3LN1) (Wang *et al.*, 2010) was selected as the protein target model in this virtual screening study. Water molecules, ligands and chain B, C and D were removed from the protein molecule using PyMOL (Schrödinger, version 1.7.4.4). Addition of hydrogen atoms to the protein was conducted by using

PyRx. Energy minimization was performed by applying MM/UFF level of theory in Gaussian 09 software (Frisch *et al.*, 2010).

Preparation of ligands: The initial structure of the lichen metabolites namely Atranorin (CID 68066), Diffractic acid (CID 94870), Lecanoric acid (CID 99613), Salazinic Acid (CID 5320418) and Usnic acid (CID 5646) (Figure 1) were obtained from pubchem (<https://pubchem.ncbi.nlm.nih.gov/search/>). Molecular geometry optimization was then performed with the density functional theory at the B3LYP/Midix level using Gaussian 09 program (Frisch *et al.*, 2010) to obtain the optimum geometry of the structures (Figure 2). The optimized structures of protein and ligands were then saved in PDB format for further analysis.

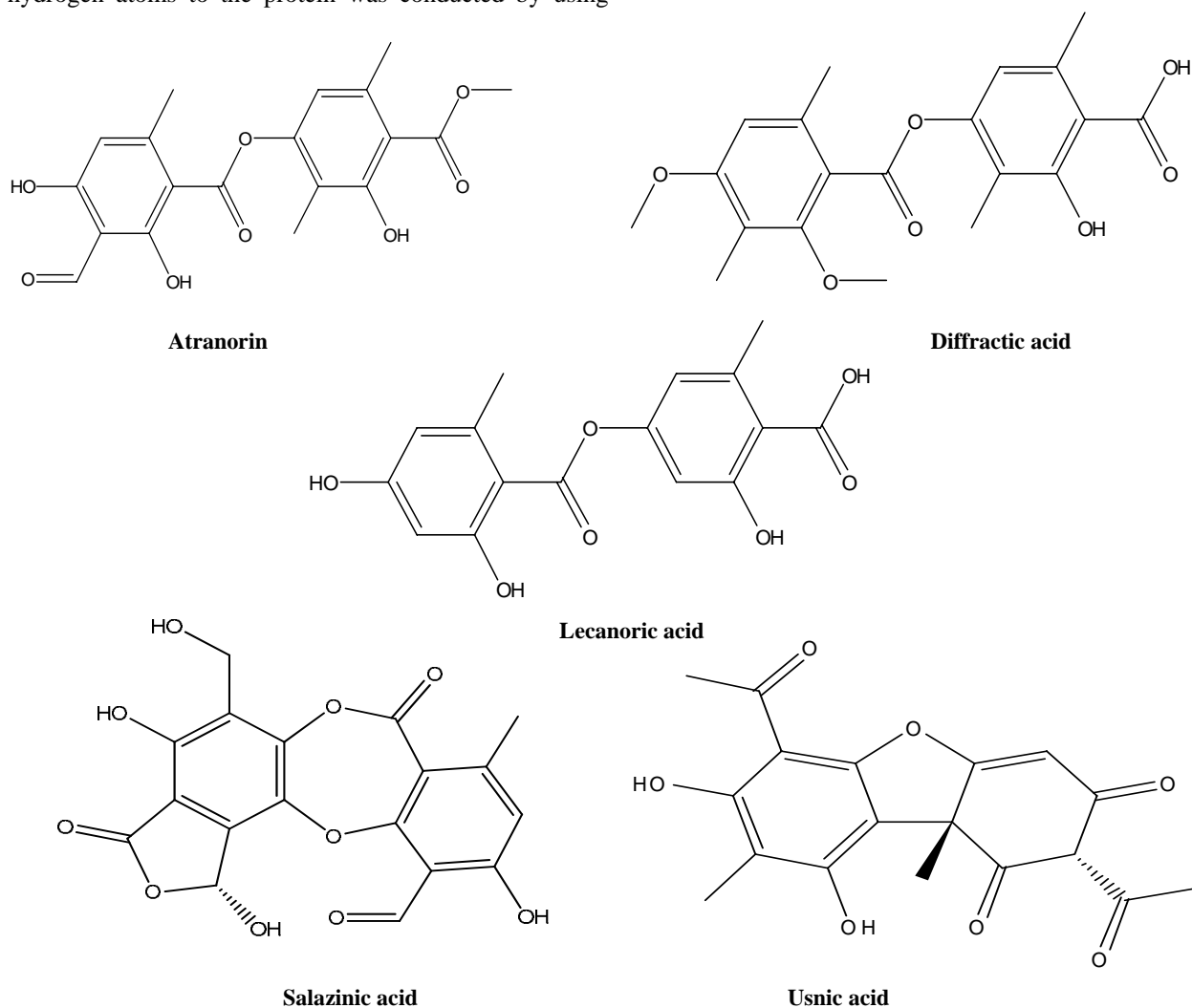


Figure 1. Planar structure of lichen metabolites.

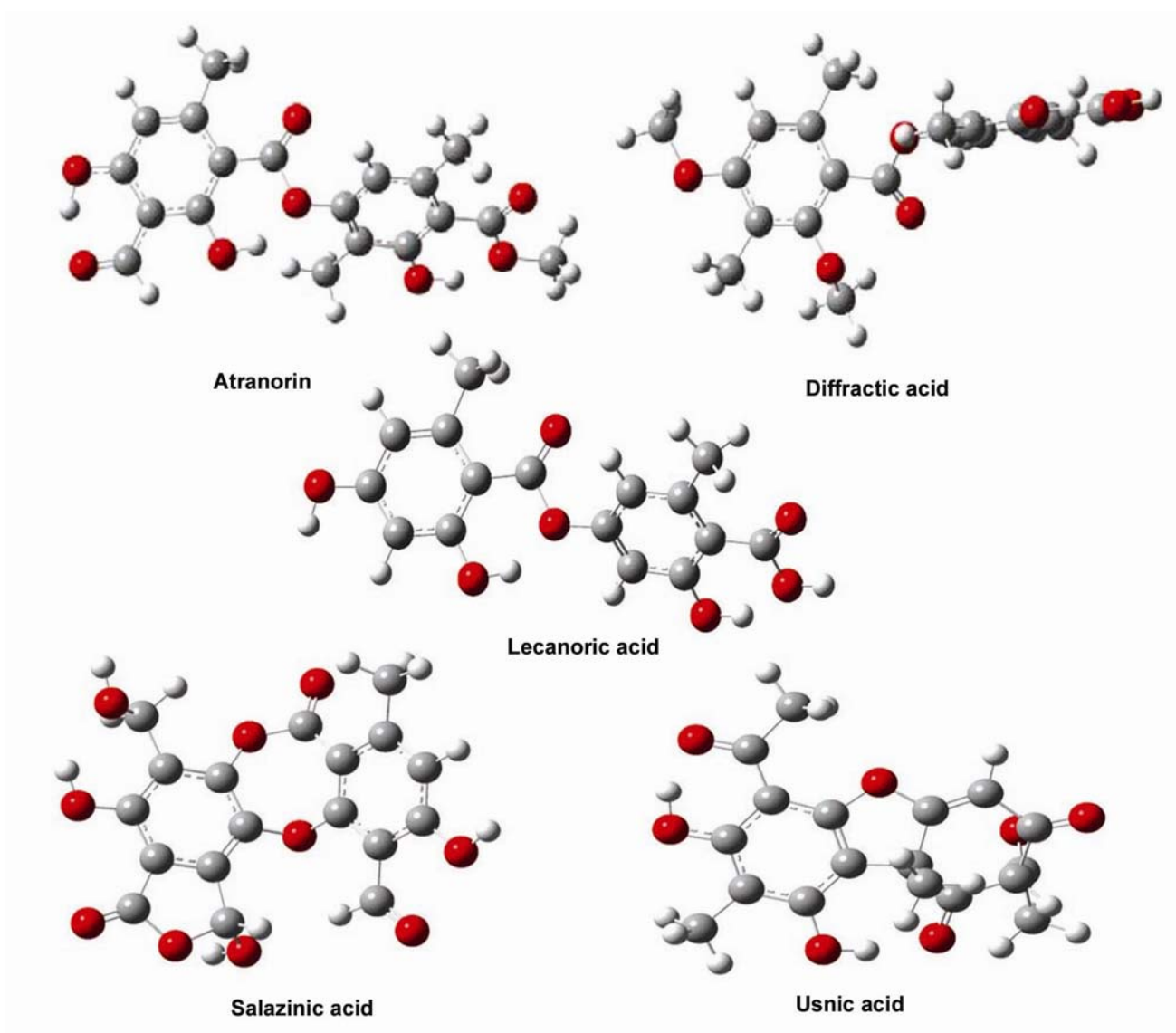


Figure 2. Three dimensional optimized (B3LYP/Midix) structure of lichen metabolites.

Protein-ligand docking: The docking of the target protein with the ligand was performed using the AutoDock vina (Trott and Olson, 2010) in PyRx platform. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Using PyRx software, the macromolecule (COX-2) and ligands are prepared and then docking was performed using a grid whose centers are 34.8544, -29.0810, -9.1090 and dimensions are 25.00, 25.00, 25.00

Angstrom. Throughout the docking study the macromolecule was kept as rigid and ligand molecules were flexible. The best conformation was chosen with the lowest docked energy or binding affinity pose, after the docking search was completed. The interactions of complex protein-ligand conformations, including hydrogen bonds and the bond lengths were analyzed by using PyMOL (Figure 3).

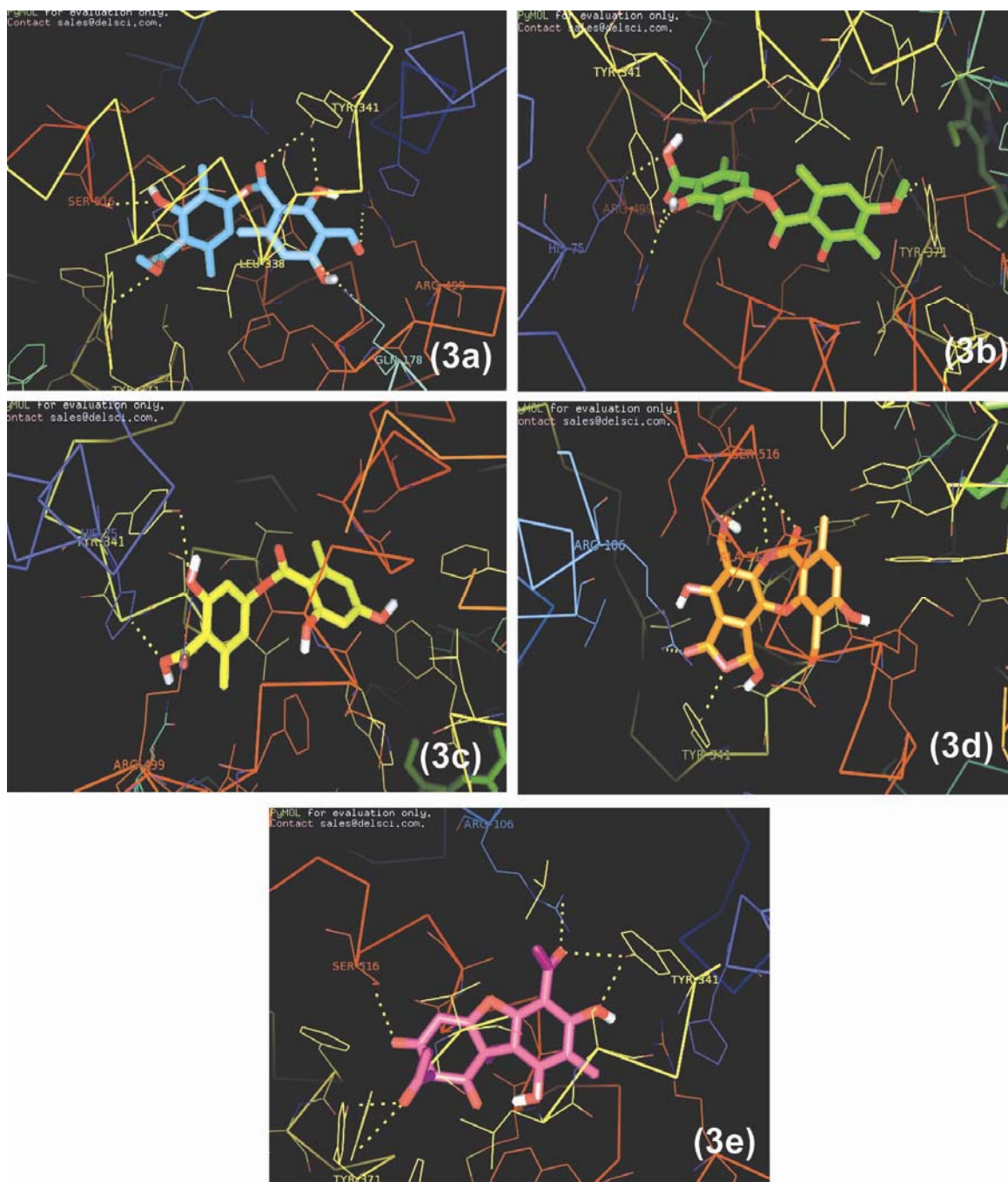


Figure 3. Binding mode of Atranorin (3a), Diffractic acid (3b), Lecanoric acid (3c), Salazinic acid (3d) and Usnic acid (3e) with cyclooxygenase-2 enzyme, yellow dots (-----) indicates H bonding.

Before screening the ligands, the docking protocol was validated by redocking celecoxib ligand into its binding pocket within the COX-2 crystal structure to obtain the docked pose and RMSD. The result showed that the optimized celecoxib almost exactly superimposed with

the experimental crystal structure of celecoxib (Figure 4). Thus, the protocol is good in reproducing the X-ray crystal structure and can be applied for further docking experiments.

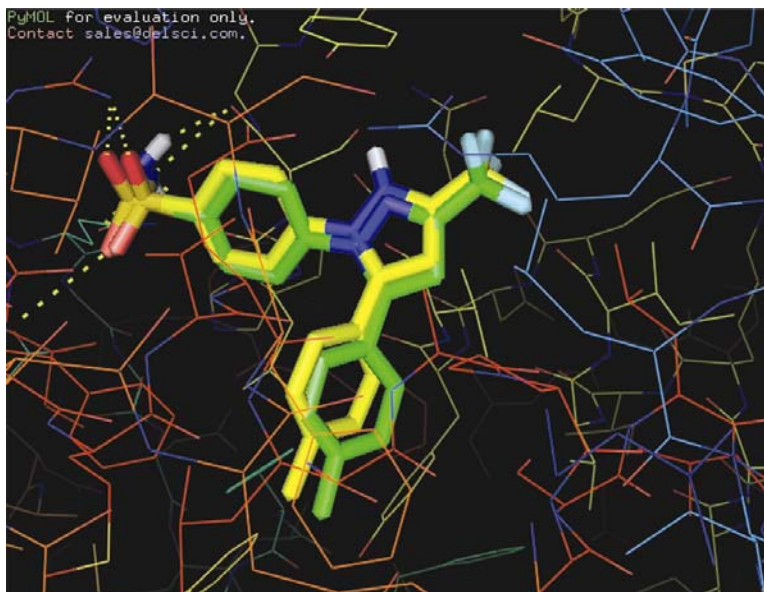


Figure 3. The superimposition of the best docking structure of Celecoxib with the X-ray structure.

(■) Docked Celecoxib, (■) Experimental Celecoxib, yellow dots (-----) indicates H bonding.

Lipinski's rule of 5 screening: Lipinski's rule of 5 is widely implemented to analyze the "druglikeness" of the proposed ligand. It states that poor absorption or permeation is more likely when a ligand molecule violates these rules (Lipinski *et al.*, 2001). The properties of the ligands are calculated for the screening using Chemaxon's MarvinSketch software 15.6.29.

Result and Discussion

Molecular docking studies of five lichen metabolites namely usnic acid, atranorin, diffractic acid, lecanoric acid and salazinic acid were carried out with COX-2 enzyme using AutoDock Vina, to identify the binding mode of ligands and the intermolecular hydrogen bond interaction between ligands and the target protein. Among all the docked ligands, the lecanoric acid satisfied the Lipinski's rule of 5 and exhibited best binding affinity (-9.83 kcal/mol) which was comparable to the reference ligands celecoxib (-12.3 kcal/mol) and rofecoxib (-11.2 kcal/mol). Although atranorin and diffractic acid showed good binding affinity (-8.7 kcal/mol and -8.6 kcal/mol, respectively) but they failed to satisfy the Lipinski's rule of 5. On the other hand, the salazinic acid and usnic acid have shown binding affinity of -7.9 kcal/mol and -4.7

kcal/mol, respectively and satisfied the Lipinski's rule of 5 (Table 1).

Cyclooxygenase plays a key role in the conversion of arachidonic acid to prostaglandins (Kurumbail *et al.*, 1996). Prostaglandins regulate pathological processes such as inflammatory and cardiovascular responses (Smith *et al.*, 1993). COX-1, a constitutive enzyme is present in mammalian cells and COX-2, an inducible enzyme is found in inflammatory sites (Howe *et al.*, 200). Thus, suppressing levels of COX-2 will be an effective method for inhibiting inflammation. Currently, NSAID used widely to control inflammation, and it has been estimated that 30-60% of NSAID users have gastrointestinal side effects and abdominal discomfort (Mofleh and Rashed, 2007). The current study focuses on the *in silico* investigation of natural compounds from the lichen metabolites for anti-inflammatory property to avoid any undesirable side effects. From the results of our docking studies and the Lipinski's rule of 5 screenings, it can be concluded that the lecanoric acid and salazinic acid are potent COX-2 inhibitors which hold lots of promise to develop newer COX-2 inhibitors.

Table 1. The ligand parameters to satisfy Lipinski's rule of 5 and binding affinity (Kcal/mol) with Cyclooxygenase-2 (3LN1).

Compound name	Binding affinity (Kcal/mol)	Interacting residues of COX-2	Molecular formula	Molecular weight (<500 Da)	LogP (<5)	H bond donar (5)	H bond acceptor (<10)
Celecoxib	-12.3	GLN160, LEU320, SER321, ARG481, PHE486	C ₁₇ H ₁₄ F ₃ N ₃ O ₂ S	381.08	4.0	1	3
Rofecoxib	-11.2	HIS57, ARG481	C ₁₇ H ₁₄ O ₄ S	314.06	2.6	0	3
Atranzorin	-8.7	GLN160, LEU320, TYR323, TYR353, ARG481, SER498	C ₁₉ H ₁₈ O ₈	374.35	6.6	3	6
Diffractic acid	-8.6	HIS57, TYR323, TYR353, ARG481	C ₂₀ H ₂₂ O ₇	374.14	5.4	2	6
Lecanoric acid	-9.1	HIS57, TYR323, ARG481	C ₁₆ H ₁₄ O ₇	318.07	4.7	4	6
Salazinic Acid	-7.9	ARG88, TYR323, ALA495, SER498	C ₁₈ H ₁₂ O ₁₀	388.04	2.3	4	7
Usnic acid	-4.7	ARG88, TYR316, TYR323, TYR353, SER498	C ₁₈ H ₁₆ O ₈	360.09	2.4	2	8

Conclusion

The lichen metabolites showed better binding features with the COX-2 enzyme. Thus, these compounds can be effectively used as drugs for treating inflammation which is predicted on the basis of docking study. However, further investigations and *in vivo* studies are needed for the development of natural COX-2 inhibitors for the treatment of inflammatory disorders.

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