J. Chosun Natural Sci.
Vol. 8, No. 3 (2015) pp. 204 – 208
http://dx.doi.org/10.13160/ricns.2015.8.3.204

Drug Designing for Biologically Important Organic Compound against COX-2 Enzyme: A Computational Approach

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Abstract

Pyrazole, β-lactam, salicidine, pyren and oxazole derivatives exhibit a broad spectrum of biological activities such as antimicrobial, anti-inflammatory and antitumor activities. With growing application on their synthesis and bioactivity, chemists and biologists in recent years have considerable attention on the research of these derivatives. In the view of potential importance of these derivatives, we have crystallized few of the derivatives and its report has been published. The present study focuses on docking studies of these derivatives against COX-2 enzyme. Docking studies using Schrodinger's GLIDE reveals that these derivatives shows better binding energy and score in the defined active site. These results may provide a guiding role to design a lead molecule which may reduce inflamation.

Key words: Pyrazole, Oxazole, β-Lactam, Anti-inflammation, COX-2, Molecular Docking

1. Introduction

Cyclooxygenase (COX-2) is one of the isoforms of Prostaglandin synthase H2 (PGH2) which synthesis prostaglandin necessary for organ and tissue homeostasis. COX-2 is expressed only in certain mammalian tissues in response to inflammatory stimuli. Hence COX-2 is responsible for the elevated prostaglandin levels which cause inflammation[1] and controls cell growth. This enzyme is commonly called as COX after its cyclooxygenase activity[2]. This enzyme helps to elevate the levels of prostaglandin by adding two molecules of O2 to arachidonate fatty acid that generates the cyclopentane ring and the other enzyme peroxidase converts the resulting peroxide intermediate to prostaglandin H2 which is the precursor of other prostaglandins (PGH2).

The three dimensional structure of COX-1 and COX-2 are almost identical, however their amino acid differences make COX-2's active site 20% larger in volume than that of COX-1. Though there are many drugs like aspirin, ibuprofen, celecoxib, rofecxib available in the market which helps to prevents the entry of the substrate, arachidonate from reaching the cyclooxgenase active site.

Due to the adverse side effects of these drugs, many of them were removed from the market and their mechanism of action remained a mystery. Hence structure based drug design program will therefore helps to solve this issue. Hence the Pyrazole[3-5], β-lactam[6], pyrene[7], ester[8,9], oxazole and salicylidine derivatives that has been X-ray crystallographically solved in our lab have been studied for docking analysis against cyclooxygenase-2 of *Mus musculus* [Protein Data Bank (PDB) ID: 3LN1][10] as a drug target. Molecular docking analysis of these derivatives against COX-2 was done using Schrodinger's Glide and these results will helps to give preliminary idea about the derivative which is active against the enzyme.

2. Experimental Section

2.1. Preparation of Protein Structures

Experimentally determined crystal structure of COX-2 (PDB.ID: 3LN1) is retrieved from the Protein Data Bank. The small molecules and all the water molecules present were removed from the co-ordinate file. The resultant PDB co-ordinates were taken for docking studies.

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(Received: July 20, 2015, Revised: September 17, 2015, Accepted: September 25, 2015)

2.2. Preparation of Ligand

The crystal structures 14 compounds from 5 different derivatives (ester, pyrazole, pyrene, β-lactum and salicylidene) determined using X-ray crystallography were taken as inhibitor against the receptor of COX-2 enzyme.

2.3. Determination of Active Site Residues

As structure of COX-2 is crystallographically determined, the active sites information for the above proteins is retrieved from the literature published.

2.4. Docking

Docking was carried out using Schrödinger's Glide. Glide (Grid based LIgand Docking with Energetics)[11] uses a hierarchical series of filters to search for possible locations of the ligand in the active site region of the receptor. The prepared protein is docked using Induced fit protocol. Initially softened potential filter (van der Waals radii scaling) was used to retain 20 geometrically possible poses per ligand and then further evaluated for the coulombic-vdW scores. The structures whose coulombic-Vdw scores less than 100 and H-bond score less than -0.005 were retained.

The retained structures enter one round of prime side chain prediction for each of the protein-ligand complex with the given distance of 5 Å. Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non-bonded ligand–receptor interaction energy. Each of the protein-ligand complexes was specified with the lowest energy (30 Kcal/mol) and then the ligand was docked with the receptor molecule. Final scoring is then carried out on the energy-minimized poses. Schrödinger's proprietary GLIDE Score multi ligand scoring function is used to score the poses.

3. Results and Discussion

The three dimensional structure of the enzyme COX-2 of *Mus musculus* is retrieved from the Protein Data Bank is shown in Fig. 1.

The 14 compounds from five different derivatives were subjecetd to docking analysis with the defined active site using Schrodinger's Gide. The schematic representation of all the 14 compounds were shown in

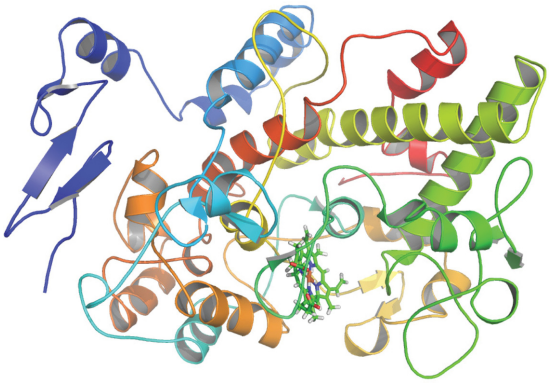

Fig. 1. Three dimensional structure of the enzyme COX-2 of *Mus musculus.*

Fig. 2. Initially all the 14 compounds were screened for docking analysis with the receptor of COX-2 enzyme and from the total of 14, the best three compounds were selected based on their Glide E-model, Glide Energy and hyrogen bod interactions with the active site residues of the drug traget. Based on the above criteria, the best three compounds are discussed hereunder.

The compound 9, compound 12 and compound 5 forms the best interaction with the active site residues of COX-2. The hydrogen bond interaction of the best compounds with the receptor molecule is given in fig. 7.3 and the energy values were tabulated in Table 1. The compound 9 being very lengthy and linear forms the best ineraction with Glide E-model and Glide energy of -72.45 and -60.12 respectively. The ε-2 Nitrogen atom of His 75 forms the strong hydrogen bond interaction with the O3 atom of the compound 9 with the D-H…A distance and angle of 2.8 Å and 178.5° respectively. Similarly the carboxyl oxygen of Leu 338 placed at the right side of the compound 9 forms the hydrogen bond interaction the O2 atom the compound. The N1 atom of compound 9 also forms strong hydrogen bond forming a D-H…A distance and angle of 2.8 Å and 180° with the γ-oxygen of Ser 516 respectively.

The compound 12 forms the second best interaction with the Glide E-model and Glide energy of -60.75 and -56.45 respectively. Though this compound does not interact with the exact active site residues of the enzyme COX-2, this compound binds to the residues that is located nearby to the residues that play a crucial role in bringing about the inhibition of COX-2. This compound forms the hydrogen bonding with the residues that falls

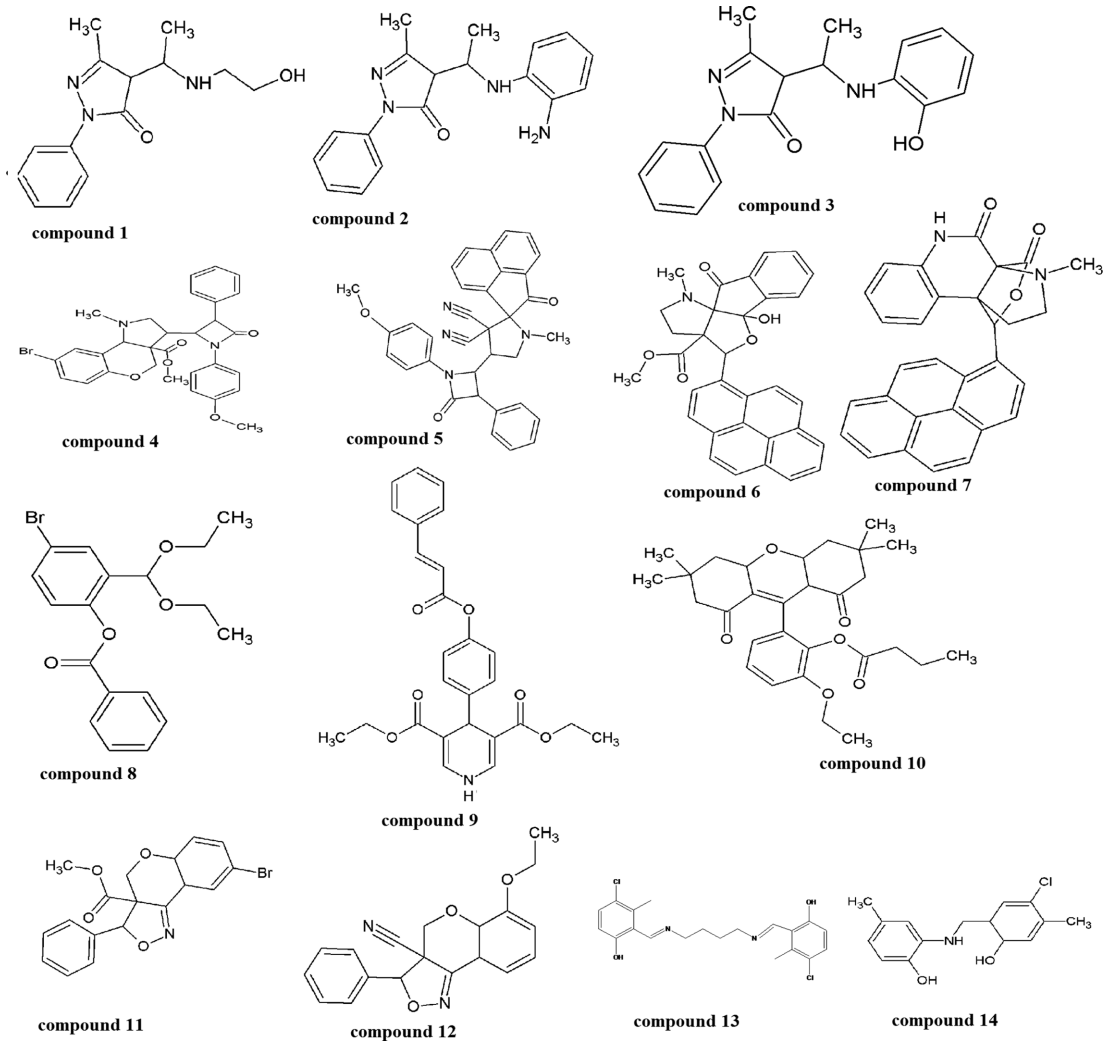

Fig. 2. Schematic representation of the compounds.

Table 1. Hydrogen bond interaction and docking score of best compounds with the COX-2

Sl. No	Ligand Name	Glide E-model	Glide Energy	D-H…A distance (Å)	Interactions
1	Compound 9	-72.45	-60.12	2.8	N(E)2 His 75…O3
				3.1	O Leu 338…O2
				2.8	N1-H…O(G) Ser 516
2	Compound 12	-60.75	-50.45	3.4	N(D)1 His 75…N2
				2.8	OH Tyr 341…N2
				3.5	Arg 106 N-H2…O3
				3.0	O Val 335…N1
3	Compound 5	-55.86	-45.34	2.9	O(D)1 Asp 501…O1
				3.0	O Asp 501…O1
				2.7	O(D)2 Asp 501…N4
				2.6	O Pro 500…N4
				2.8	O Thr 79…N1

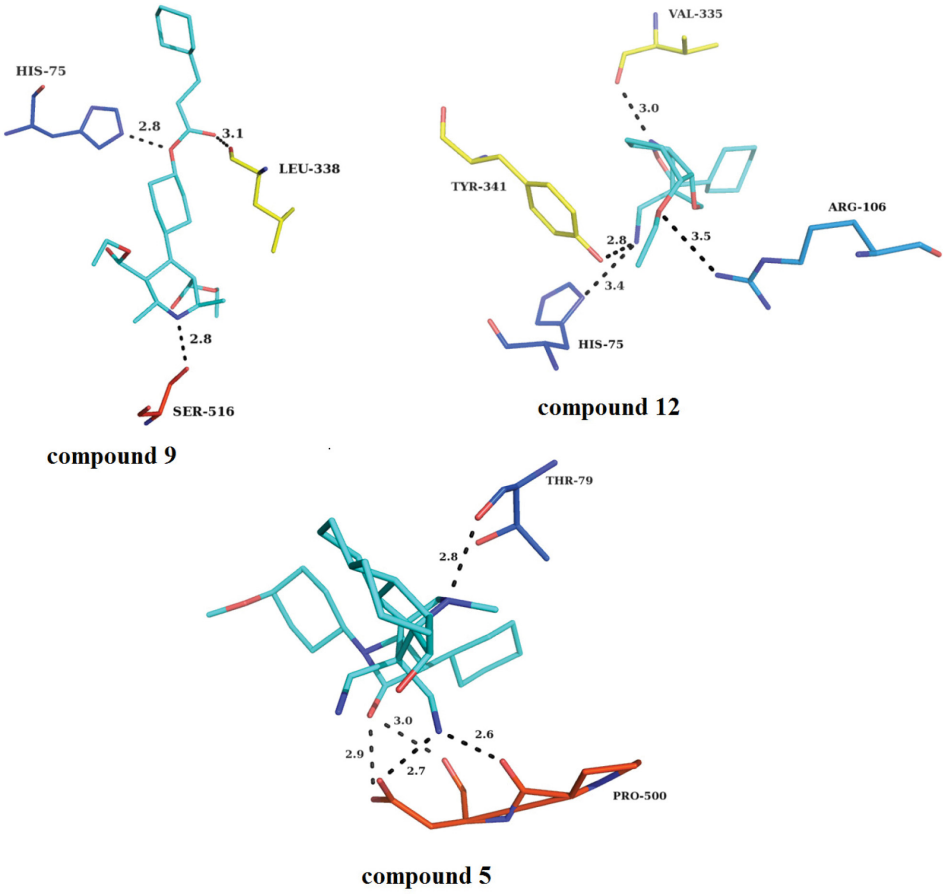

Fig. 3. Hydrogen bond interaction of the best compounds with the active site residues of COX-2.

on the same pocket where the active sites are located. The carboxyl oxygen of Val 335 forms hydrogen bond inetraction with the N1 atom of compound 12 with the D-H…A distance of 3.0 Å. The N2 atom of the compound 12 forms bifurcated hydrogen bond interaction with the δ-1 nitrogen atom of His 75 and hydroxyl group of Tyr 341 that is attached to the zeta carbon of the aromatic ring forming a D-H…A distance of 3.4 Å and 2.8 Å respectively. The amine group (NH1) of Arg 106 which is placed at the lower right corner of this compound forms a hydrogen bond interaction with the O3 atom of compound forming a D-H…A distance of 3.5 Å (Fig. 3).

The compound 5 forms the third best inetraction with the Glide E model and Glide energy of -55.86 and -47.34 respectively. Similarly the compound 5 also cannot able to form hydrogen bonding with the active site residues. The O1 of this compound forms bifurcated hydrogen bond with the carboxyl oxygen and δ1-oxygen of Asp 501 forming a D-H…A distance of 2.9 Å and 3.0 Å respectively. The N3 atom of the compound 5 forms strong bifurcated hydrogen bond interaction with the δ-2 oxygen of Asp 501 and carboxyl oxygen of Pro 500 forming a D-H…A distance of 2.6 Å and 2.8 Å respectively. The carboxl oxygen of Thr 79 placed at the right top corner of the the compound forms hydrogen bond interaction with the N1 atom of the compound forming a D-H….A distance of 2.8 Å. Among the fourteen compounds, the compound (9,12and 5) shows better inhibition on the defined active site residues of COX-2 of *Mus musculus*. Thus in-short this study will be useful for designing novel anti-inflammatory agents.

4. Conclusions

Docking analysis of all the fourteen derivatives with

the protein targets, COX-2 brought important interactions operating at the molecular level. Hence targeting the above proteins is necessary for the design of broad spectrum of anti- inflammation. In order to study the binding mode and enzyme-inhibitor interaction with the active site residues of the target proteins, we have compared our compound with all ready co-crystallized ligand. The above established enzyme-inhibitor complex is taken as a model for explaining our interaction the active sites of the enzyme The docking studies with the all derivatives showed that our derivatives also established a similar way of binding with the target protein which is explained by their comparable binding energy and interaction with the active site residues of the enzymes. Thus in short this study will be useful for the design of novel anti- inflammatory agents based on docking methods.

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