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IDENTIFICATION OF A LEAD MOLECULE FOR INFLAMMATION BY MOLECULAR MECHANICS

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ABSTRACT

Inflammation is the first indication of entry of any foreign molecules that enters in to the immune system, during which COX-2 is being produced in high level. If it persists inflammation will be chronic. To nullify the effect of COX-2, Celecoxib (NSAID/ COX-2 Inhibitor) is given. Having known these facts of NSAID, an attempt was made to enhance the efficiency of NSAID by *In-silico* method. 3D structure of NSAID is downloaded from NCBI Pubchem Database, and it is treated as ligand molecule. Crystal structure (3D) of cyclooxygenase - 2 is downloaded from PDB database and treated as target receptor for COX-2 inhibitor. The molecular mechanics were calculated using Poisson-Boltzmann model of Open Eye scientific software. A four-stage protocol was set up for energy minimizations of the protein-inhibitor complex. Minimization at each stage was performed using 100 steps of steepest descent and 2000 steps of conjugate gradient algorithms for minimization. The calculated binding free energies of all 10 analogues of COX-2 inhibitors are compared. The calculated binding affinities of the analogue 10 found to a better inhibitor than other lead analogues. It is predicted to be the most potent inhibitor ($E_{cal\ (MM)}$ = -13.27) to cyclooxygenase-2 enzyme as compared to all other inhibitor considered in this study. Energy components calculated by performing molecular mechanics calculations, both in explicit solvent and complex states are estimated as Energy= 51.96 (Relative binding free energy). The comparison of the calculated binding free energies for structurally similar inhibitors to cox-2, molecule gave us suitable analogues. These results clearly indicate that before synthesis and testing of new analogue, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities to speed up drug discovery process by eliminating less potent compounds from synthesis.

Key Words: COX-2, Inflammation, Celecoxib, Binding free energy, Docking.

INTRODUCTION

Inflammation is a systematic response of the body to protect tissues from infection, injury or disease. Redness, heat, swelling, pain and dysfunction/loss of functions are the characteistics of inflamation. The inflammatory response begins with the production and release of chemical agents by the infected/injured/diseased tissue or cells (*Wolfe et al.*, 1999: *Wallace et al.*, 2007 & 2008). The inflammation at the affected site, releases cytokines like IL - 1 and TNF that inturn activate endothelial cells to upregulate receptors like VCAM-1, ICAM-1, E-selectin, and L-selectin for various immune cells (*Zhu et al.*, 2007). In addition it increases extravasation of neutrophils, monocytes, activated T-helper, T-cytotoxic, memory T cell and B cells to the infected site for immediat recovery (*Qinna et al.*, 2012). Prostanoids (prostaglandins, prostacyclin, thromboxane) are the most important biological mediators produced during inflamation, which are formed by the enzyme Cyclooxygenase (COX). Currently three *COX* isoenzymes are known, they are COX-1, COX-2 and COX-3, they are very similar, they are not identical (*Moor et al.*, 2006: *Michael et al.*, 2006). The COX enzyme that is involved in pain, arthritis, and inflammation in general, is not normally

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present, but is induced on demand by the cells. This inflammatory response usually promotes healing, under some conditions it is uncontrolled and persist, for long duration and become harmful (*Graham et al., 2005*).

Chronic inflammation is characterised by the dominating presence of macrophages in the injured tissue. These cells are powerful defensive agents of the body, but the toxins they release (including reactive oxygen species) are injurious to the organism's own tissues as well as invading agents. That is why chronic inflammation is almost always accompanied by tissue destruction. Finally, an abscess, or a collection of pus, can form in chronic inflammation (*Moor et al.,* 2006: *Michael et al.,* 2006).

There are different causes, symptoms, and treatments for these of inflammation. The normal treatments are rest and inflammatory medicines. Several anti-inflammatory herbs like boswellia, turmeric, licorice root; chamomile and willow are used as cure for inflammation. Drugs such as vitamin C, gamma oryzanol supplements, N-acetyl cysteine, glutamine, arginine, beta-carotene, zinc, and vitamin A are also used to treat inflammation (*Wolfe et al.,* 1999: *Wallace et al.,* 2007 & 2008).

Common anti-inflammatory drugs like aspirin block the function of the COX-1 enzyme along with another enzyme, COX-2. Cyclooxygenase-2 inhibitors are newly developed drugs for inflammation that selectively block the COX-2 enzyme. Blocking this enzyme impedes the production of the chemical messengers (prostaglandins- a fatty-acid derivatives located all over the body) that cause the pain and swelling of arthritis inflammation. They are the new class of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Aspirin, indomethacin (Indocin), ibuprofen (Motrin), naproxen (Naprosyn), piroxicam (Feldene), and nabumetone (Relafen) are some of the NSAID drugs (Wolfe *et al*., 1999: Wallace *et al*., 2007 & 2008).

The COX-2 inhibitors, that do not affect COX-1, but selectively block only COX-2. This selective action provides the benefits of reducing inflammation without irritation. These drugs pose an advantage in comparison to previous anti- inflammatory drugs because their mechanism of action carries nowhere near the risk of stomach ulceration and bleeding. The COX-2 inhibitor is now on the market in the form of celecoxib (Celebrex). It is widely expected that COX-2 inhibitors will be of great value to people with arthritis and variety of pain or painful conditions.

Whilst many NSAID's are considered to have equivalent efficacy, there is now no doubt that NSAID's have different side effects also. The investigation to understand about the different cyclooxygenase enzymes has given us more fundamental understanding of the actions and side effects of these drugs. Having known these facts, an attempt was made in this study to develop new COX-2 inhibitor by Computer Aided Drug Designing (CADD) followed by binding free energy calculations.

MATERIALS & METHODS

Ligand Molecule

Several anti-inflammatory drugs exist in market, among those, Celecoxib - is classified as a nonsteroidal anti-inflammatory drug (NSAID) (*Trells et al.,* 2011: *Pilatti et al.,* 2006). It is used to treat rheumatoid arthritis, osteoarthritis, and familial adenomatous polyposis (FAP). The mechanism of action of celecoxib is believed to be due to inhibition of prostaglandin synthesis. Unlike most NSAIDs, which inhibit both types of cyclooxygenases (COX-1 and COX-2), celecoxib is a selective noncompetitive inhibitor of cyclooxygenase-2 (COX-2) enzyme (Mackenzie *et al.,* 2010). It can be used for relief and management of osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis, acute pain, primary dysmenorrhea and oral adjunct to usual care for patients with familial adenomatous polyposis. The structure of which is downloaded from PubChem Database (Figure-1 A).

Drug Target

Since, Cyclooxygenase - 2 have a role as a major mediator of inflammation and/or a role for prostanoid signaling in activity-dependent plasticity (*Pilatti et al.,* 2006), it is used as drug target for Celecoxib. Hence, the structure of Cyclooxygenase - 2 was downloaded from PDB (Figure -1 B)

SOFTWARES USED

(I) *Open Eye Scientific Software*

OpenEye Scientific Software develops large-scale modeling applications and toolkits. Primarily geared towards drug discovery and design, areas of application include structure generation, docking, shape comparison, electrostatics, chemical informatics and visualization. The software is designed for scientific rigor, as well as speed, scalability and platform independence.

Ligand –solvent interactions (inter-solvent) (-solventpb)

For optimization of small molecules in solution, the electrostatic part of molecule-solvent interactions was calculated using Poisson-Boltzmann model of Open Eye scientific software.

(II) Gold

GOLD is a program for calculating the docking modes of small molecules into protein binding sites. It is a product of collaboration between the University of Sheffield, GlaxoSmithKline plc and CCDC, GOLD is very highly regarded within the molecular modeling community for its accuracy and reliability.

Ligand –protein interactions (Inter-protein) (Docking)

For Docking of small molecules into the protein active site, the VDW, Hydrogen bonds and hydrophobic energies of ligand-protein interactions will be calculated using GA of Gold software.

(III) Hyperchem

HyperChem is a versatile molecular modeler and editor and a powerful computational package. It offers many type of molecular and quantum mechanics calculations. For optimization of small molecules in solution and protein complex the intra molecular energies of ligand-solvent and ligand protein was calculated using molecular mechanics calculations of Hyperchem software.

(IV) Computer aided drug design approaches

Computational assessment for the binding affinity of enzyme inhibitors prior to synthesis is an important component of computer-aided drug design (CADD) paradigms. In this study, the molecular mechanics (MM) method is used for the estimation of relative binding affinities of inhibitors to an enzyme (*Cruz-Lopez et al., 2007*).

RESULTS

In this work, the binding modes of the putative/proposed (Table - 1) inhibitors were obtained by carefully aligning them with the known crystal structures of inhibitors in the active site of the **S58 (**Fig. 1 A**).** The target for inhibitor COX-2 molecule were downloaded and shown in Fig. 1B. These inhibitors (Fig. 1 A and B) are evaluated by performing minimization calculations both in solvent (water) and in complex using the AMBER force field (Weiner *et al*, 1984). The details of relative binding affinities using energy components obtained from minimizations of each inhibitor are given in Table – 3. It binds with its polar sulfonamide side chain to a hydrophilic side pocket region close to the active COX-2 binding site (Figure-2 A & B). Both COX-1 and COX-2 catalyze the conversion of arachidonic acid to prostaglandin (PG) H2, the precursor of PGs and thromboxane (*Atzori et al.,* 2005).

A four-stage protocol was set up for energy minimizations of the protein-inhibitor complex. Minimization at each stage was performed using 100 steps of steepest descent and 2000 steps of conjugate gradient algorithms for minimization. The binding energy of each ligand with COX-2 is given in the table 2.

The minimized structures for all the 8 inhibitors in the complex and solvated states were used for calculating the following energy variables:

$$E_{bfe}(intra) = E_{com}(intra) - E_{sol}(intra)$$

$$E_{bfe}(inter) = E_{com}(inter) - E_{sol}(inter)$$

Where, E_{bind} (intra) and E_{bind} (inter) are relative intra and intermolecular binding interaction energies of a ligand, respectively, and where E_{com}(intra), E_{com}(inter), E_{sol}(intra), and E_{sol}(inter) are intra and intermolecular interaction energies of a ligand in the complexed and solvated states, respectively (*Cruz-Lopez et al., 2007*).

It is better to study the combined effect of ligands. Hence relative binding free energies of inhibitors 1 to 8 were calculated (*Interne* 2009: *Mackenzie et al.,* 2010). The binding affinities of the inhibitors between one with other are shown in Table – 3. The results revealed that, the inhibitor 8 has more potential binding efficiency than others. Inhibitor 8 (Ligand 1 & 10) is found to be a better inhibitor to cyclooxygenase-2 enzyme as compared to all the other inhibitors considered in this study.

Table 1. List of Inhibitors Developed

S. No.	Inhibitor	Substituents
1.	Inhibitor-1	$-CH_3$
2	Inhibitor2	$-CH_2OH$
3	Inhibitor-3	$-CH_2CH_3$
4	Inhibitor-4	CF_2OH
5	Inhibitor-5	$-CF_3$
6	Inhibitor-6	-H
7	Inhibitor-7	$-NH_2$
8	Inhibitor-8	$-CCl_2OH$
9	Inhibitor-9	-Cl
10	Inhibitor-10	-OH

Table 2. Energy Calculations

Ligand	Energy
Ligand 1	Energy= 51.216
Ligand 2	Energy= 51.218
Ligand 3	Energy= 53.42
Ligand 4	Energy= 52.491
Ligand 5	Energy= 50.378

Ligand 6	Energy= 44.91
Ligand 7	Energy= 34.86
Ligand 8	Energy= 44.78
Ligand 9	Energy= 50.56
Ligand 10	Energy= 51.96

Table 3. Relative Binding Affinities using MM

Name	Ligands	Ecal (MM)
Inhi-1	L1--→L2	-12
Inhi-2	L1-----L3	-8.78
Inhi-3	L1------L4	-6.96
Inhi-4	L1--→L5	-6.87
Inhi-5	L1--→L6	-6.93
Inhi-6	L1--→L7	-9.06
Inhi-7	L1--→L8	-0.08
Inhi-8	L1--→L10	-13.27

Fig 1. Structure of Ligand (Celecoxib) and Target (COX-2) Molecules

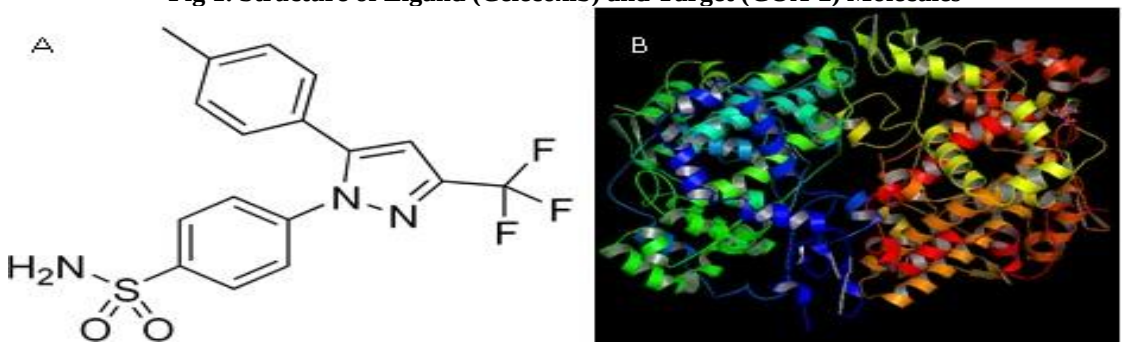
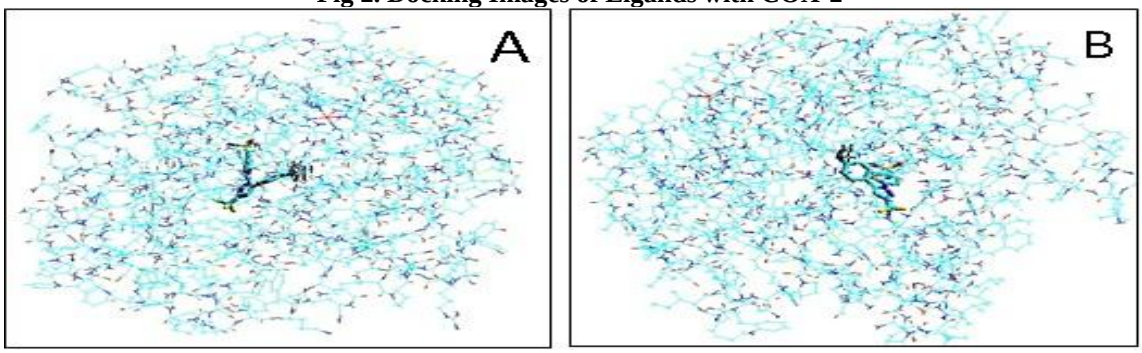


Fig 2. Docking Images of Ligands with COX-2



CONCLUSION

Inflammatory response is always being the frontline protective measures for any kind of infection by the immune system. Though it is supportive, under some conditions it becomes chronic. In order to overcome this issue, different kind of anti-inflammatory drugs are used. SAID and NSAID are the drugs widely used as anti-inflammatory agents (*Cronstein et al.,* 2009). Still they have poor sensitivity and side effects. Hence there is a need of searching better anti-inflammatory drugs. In this study an anti-inflammatory drug was selected, a comparison of calculated binding free energies were done for structurally similar inhibitors to cox - 2, in addition molecular mechanics methods gave suitable analogues among the selected inhibitors (*Cornejo-Garcia et al.,* 2009: *Canto et al.,* 2009). These results clearly indicated that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis. The inhibitor 10 (celecoxib, *Andersohn et al.,* 2006) with the substituent –OH is identified as the most suitable analogues of cyclooxygenase-2 in the present study, this need to be further evaluated in the laboratory.

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