Drug designing and docking efficacy assessment of halogen substituted aspirin

[1]Debyani Samantray, and [1]R.K. Sahu
[1]Department of Bioinformatics, B.J.B (A) College, Bhubaneswar, Orissa, India
sahurajani.sahu@gmail.com, debyani.samantray@gmail.com

Abstract: COX-2 inhibitors are a class of drugs which selectively inhibit cox-2, an enzyme involved in inflammation pathway. Prostaglandins induce inflammation, pain and fever. Aspirin blocks the enzyme cyclooxygenase (COX-1,2) which is involved in the ring closure and addition of O_2 to arachidonic acid converting to prostaglandins . The present study was undertaken to analyze the docking efficacy of aspirin with the target molecule (1IGX) and to assess the best ligand for inhibiting COX and to analyze the docking programme by ARGUSLAB. Substituting the –Cl ion in place of –OH group in aspirin showed two hydrogen bonds with phenylalanine and glutamine residues as its docking site where as substituting the –Br ion showed two hydrogen bonds with Histidine and glutamine residues as its docking site. The finding suggests halogen substituted Aspirin to be a better ligand preferably with Bromine. However clinical trials and laboratory investigation will help in marketing the modified drug.
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1. Introduction

Cyclooxygenase (COX) is an enzyme (EC 1.14.99.1) that is responsible for formation of important biological mediators called prostanoids (including prostaglandins, prostacyclin and thromboxane). Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain; this is the method of action of non-steroidal anti-inflammatory drugs, such as the well-known aspirin and ibuprofen. The names "prostaglandin synthase" is still sometimes used to refer to the COX enzyme (Green, 2001). In the course of the search for a specific inhibitor of the negative effects of prostaglandins which spread the positive effects, it was discovered that prostaglandins could indeed be separated into two general classes which could loosely be regarded that as “good prostaglandins” and “bad prostaglandins”, according to the structure of a particular enzyme involved in their biosysnthesis, cyclooxygease. Prostaglandins whose synthesis involves cyclooxygease-1 enzyme, or COX-1, are responsible for maintaince and protection of the gastrointestinal tract, while Prostaglandins whose synthesis involves the cyclooxygease-11 enzyme or COX-2, are responsible for inflammation and pain. The existing non-steroidal anti-inflammatory drugs (NSAIDs) differ in their relative specificities for COX-2 and COX-1; while aspirin in equipotent at inhibiting COX-2 and COX-1 enzymes *in vitro* (Aaron *et al*., 2002).

The first crystal structure of human cyclooxygenase-2, in the presence of a selective inhibitor, is similar to that of cyclooxygenase-1. The structure of the NSAID binding site is also well conserved, although there are differences in its overall size and shape which may be exploited for the further development of selective COX-2 inhibitors. A second COX-2 structure with a different bound inhibitor displays a new, open conformation at the bottom of the NSAID binding site, without significant changes in other regions of the COX-2 structure. These two COX-2 structures provide evidence for the flexible nature of cyclooxygenase, revealing details about how substrate and inhibitor may gain access to the cyclooxygenase active site from within the membrane (Goetzl *et al*., 1995; Smith *et al*., 1991). COX-1 and COX-2 are both targets of nonselective nonsteroidal antiinflammatory drugs (nsNSAIDs) including aspirin and ibuprofen, while COX-2 activity is selectively blocked by COX-2 inhibitors called coxibs (e.g., celecoxib) (Grosser *et al*., 2006). Aspirin is an acetyl derivative of salicylic acid that is a white, crystalline, weakly acidic substance, with melting point 137°C (Fig-1).
Aspirin is unique to a third group of inhibitors that cause a time-dependent, covalent inhibition. Binding of aspirin by PGHS-1 or PGHS-2 leads to an irreversible acetylation of a highly conserved Ser-530 (Rouzer and Marnett, 2003; Schneider *et al*., 2007; Smith, 2008; van der Donk *et al*., 2002). Aspirin acetylates one

monomer of PGHS-1 to cause a temporally correlated loss of COX activity (Rimon *et al*., 2010). COX-1 and COX-2 are homodimers that exhibit half of sites enzymatic activity (Yuan *et al*., 2006; Yuan *et al*., 2009), and many nsNSAIDs, apparently those involved in time-dependent inhibition, maximally inhibit the enzymes upon binding to only one monomer (Yuan *et a*l., 2006; Kulmacz and Lands, 1985). However, it appears that nsNSAIDs that are reversible, competitive inhibitors of COXs must bind to both monomers to cause enzyme inhibition (Prusakiewicz *et al*., 2009). Similarly, fatty acids are bound to both monomers of a dimer during catalysis (Yuan *et al*., 2009). Fatty acid binding to the first monomer transforms it to an allosteric monomer that, in turn, modulates the substrate specificity of the second, now catalytically active, partner monomer.

Coxibs, at concentrations that do not inhibit COX-1 catalytic activity, have been reported to interfere with the abilities of nsNSAIDs including aspirin to inhibit COX-1 in vitro (Rosenstock *et al*.,1999; Ouellet *et al*., 2001). This suggests that COX-2 inhibitors are able to bind to COX-1 and somehow compete with nsNSAID actions on COX-1 without affecting AA oxygenation. This is a potentially important clinical issue because many elderly patients take a combination of a coxib for pain relief and low-dose aspirin to counterbalance the potential cardiovascular side effects of coxibs (Grosser *et al*.,2002). Extensive clinical trial data on interactions between aspirin and coxibs are only available for one coxib, rofecoxib (Gladding *et al*., 2008), and no significant effect on aspirin inhibition of thrombosis was detected. However, rofecoxib is the COX-2 inhibitor that is least effective in attenuating aspirin inhibition of COX-1 in vitro (Ouellet *et al*., 2001). Currently, the most widely used coxib is celecoxib. In two small trials with healthy human volunteers, the effects of aspirin were found not to be attenuated by celecoxib (Gladding *et al*., 2008); however, in both trials, the volunteers were given a 324 mg daily dose of aspirin, which is four times the dose of 81 mg commonly considered to be "low-dose" aspirin. In a potentially related study, celecoxib did attenuate aspirin inhibition in a dog model of thrombosis (Hennan *et al*., 2001).

In the present study, we have used substituted the halogen compounds (Cl, Br) in place of -OH groups in the best chosen ligand (Aspirin) elaborates the minor change with the best ligand pose of ligand molecule after substitution.

2. Materials and Methods

In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengauer and Rarey, 1996). Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs (Kitchen *et al*., 2004).

In our study the legand Aspirin will docked with receptor molecule by using the softwares and bioinformatics tools, PDB, NCBI, DB, PYMOLE, AUTO DOCK, CHEM SCKTCH and ARUGUS LAB (Lengauer *et al*., 2008). The detail procedure is mentioned in flow chart-1 and 2.

1. Docking of aspirin with the target molecule 1igx was processed in the following manner.
2. Retrieval of receptor from PDB.
3. Retrieval of ligands from (NCBI tool, PUBCHEM compound and Drug Bank database).
4. Interaction of target and ligands in ARGUSLAB.
5. Docking of target and ligand.
6. Visualization of molecule in PYMOL to find out the best ligand.
7. Modifying the ligand to design a drug by using chem scketch. Substituted halogen ions (Cl, Br) in place of oh and h groups.
8. 3-D optimization was performed.
9. Docking of the substituted ligand using ARGUS LAB was performed to get the better result

3. Results and Discussion

Initially the Target, 1IGX is docked with the geometrically optimized ligand Aspirin and the best ligand pose with the hydrogen bonds were tabulated. (Fig-3,4,5 and Table-1)The docking results of 1IGX

and each ligand were found to vary with the best ligand pose and the presence of hydrogen bonds. Aspirin revealed one hydrogen bond with phenylalanine residue at its docking site. We substituted the halogen groups (Cl, Br) in place of -OH groups in the best chosen ligand, (Aspirin) and the changes are revealed with the best ligand pose of halogen substituted ligand molecule. Substituting the -Cl ion in place of –OH group in aspirin showed two hydrogen bonds with phenylalanine and Glutamine residues as its docking site (Fig-6,7,8) whereas, Substituting the -Br ion in place of –OH group in aspirin showed two hydrogen bonds with Histidine and Glutamine residues as its docking site.(Fig-9,10,11).

Scoring functions are fast approximate mathematical method used to predict the strength of the non-covalent interactions between two molecules after they have been docked. Most commonly one of the molecules is a drug and second is target molecule such as protein receptor. It can also predict the strength of inter molecular interaction. In the present study the scoring function of the halogen substituted aspirin is higher than normal aspirin (Table-1). Among the two halogen groups Br demonstrated higher scoring function in comparison to Cl. This gives us a clue regarding the stronger affinity and strength of inter molecular Vanderwaals and electrostatic interaction inside the molecular complex and the energies of two binding partners (Jain, 2006; lensik *et al* 2007; Bohm, 1998)**.**

Conclusion

It is concluded that the structural modification done to the aspirin has undergone few changes with the help of docking software. The structural and functional relationship with the target is well established and reveals more hydrogen bonds and scoring function which function with better affinity with target molecule. Hence the substitution of halogen substituted aspirin appears to be a better ligand among which substitution of bromine proves to be better than chlorine .However pharmacokinetics activity and toxicological investigation in experimental approach will authenticate the positive effect of the modified aspirin.

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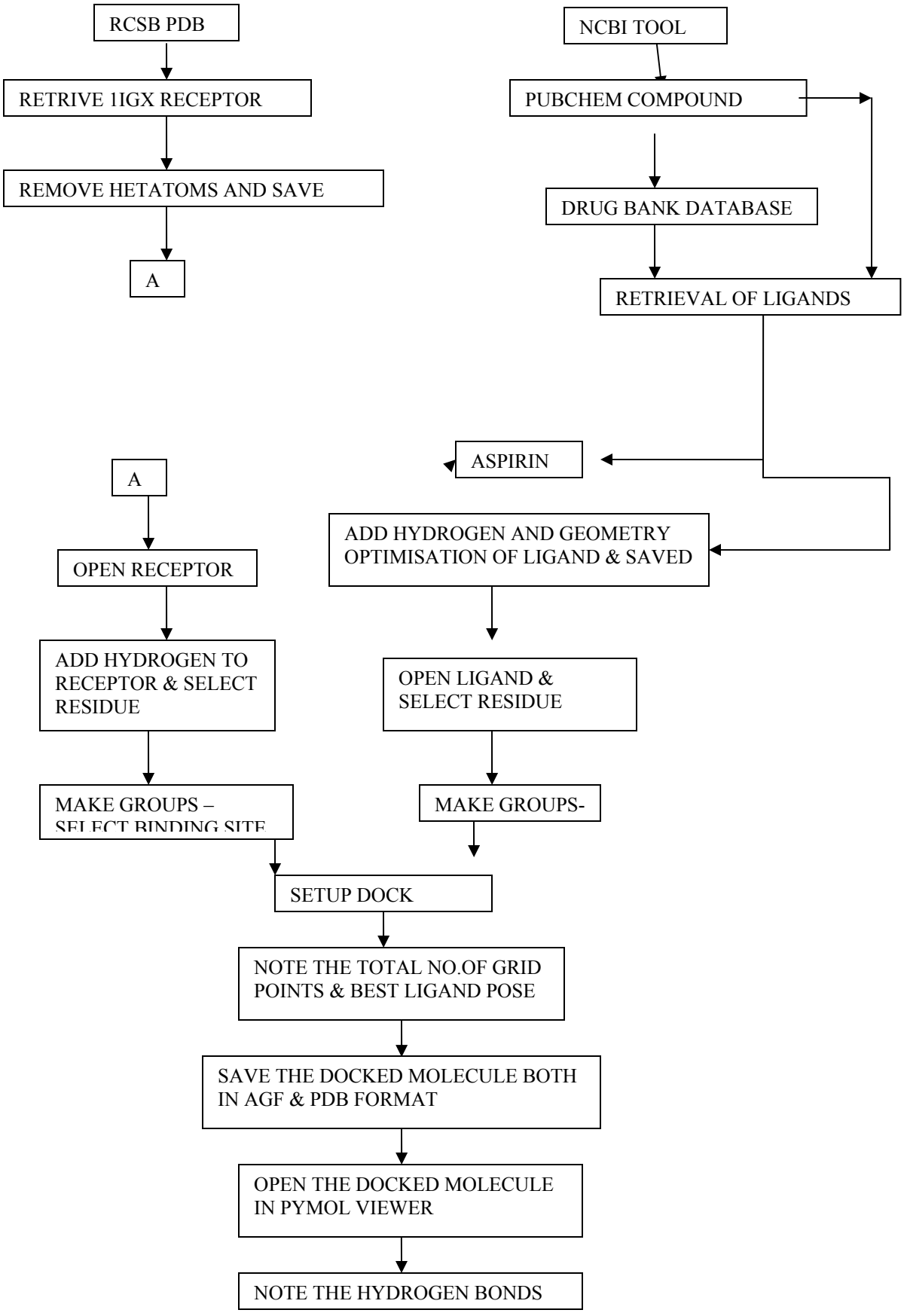
Table-1

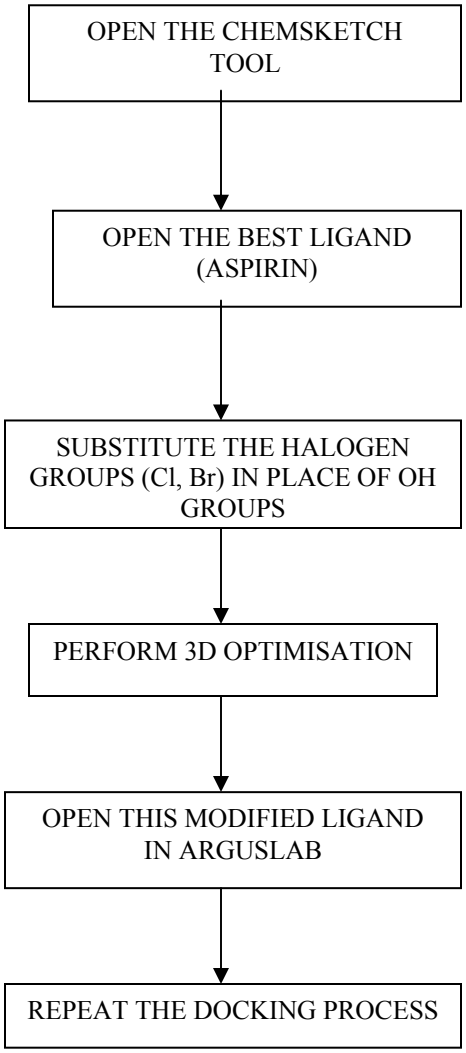
The docking efficacy of halogen substituted cox inhibitor

Sl.No	Target	Ligand	Total No. of grid points		Best ligand pose Kcal/mol	No. of Hydrogen bonds
1	1IGX	Aspirin	Not Substituted	3442951	-9.93768kcal/mol	1
2	1IGX	Aspirin	Substituted with Bromine	3442951	-10.4621 kcal/mol	2
3	1IGX	Aspirin	Substituted with Chlorine	3442951	-10.3571 kcal/mol	2

Flow chart-1: Description of the retrieval and optimization for docking



Flow chart-2. To analyze the docking efficacy of halogen substituted cox- inhibitors



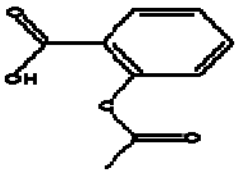


Fig-1 Structure of Aspirin

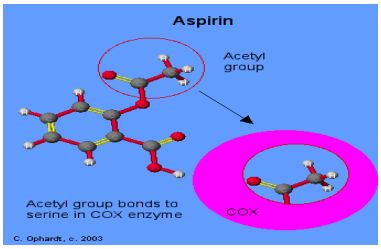


Fig-2 Aspirin blocks the enzyme Cyclooxygenase

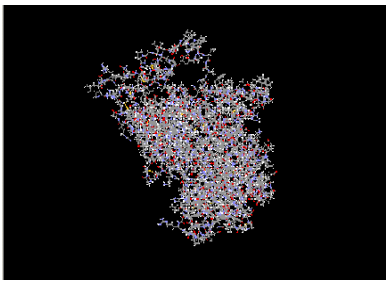


Fig-3 1IGX –target
(Removal of H atoms and addition of hydrogen)

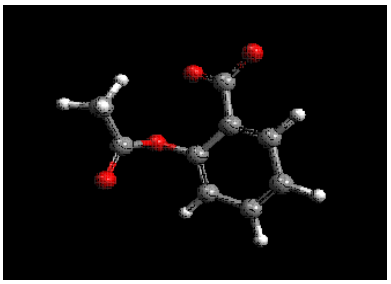


Fig-4 Aspirin – geometry optimization

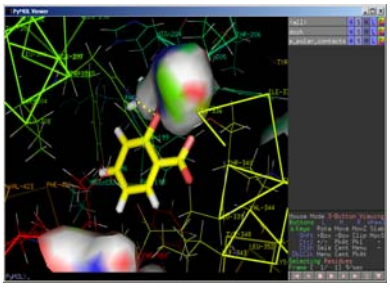


Fig-5 Aspirin docked with 1IGX –Pymol viewer

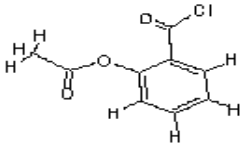


Fig-6 Aspirin substituted with chlorine

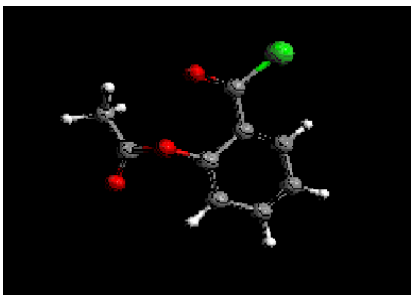


Fig-7 Aspirin substituted with chlorine- geometry optimization docked with 1IGX-Pymo

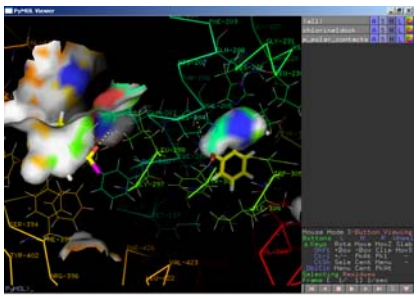


Fig-8 Chlorine substituted Aspirin

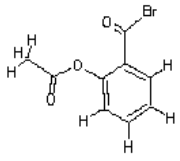


Fig-9 Aspirin substituted with bromine optimizatio

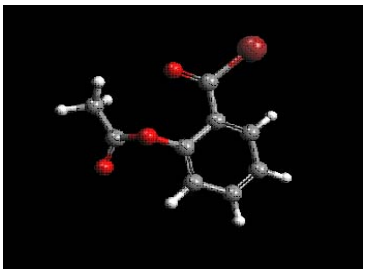


Fig-10 Aspirin substituted with bromine – geometry

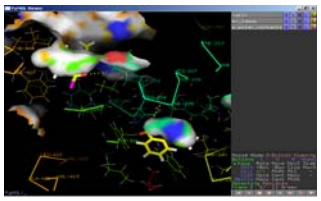


Fig-11 Bromine substituteed aspirin docked with 1IGX- Pymol

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