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Computational analysis of COX-1 & COX-2 and finding out their potent inhibitors

Ankit Singh*, Shilipi Singh, Renukesh Verma and Mayank Agarwal
Department of Biotechnology, MITS, Gwalior, (MP) - India

Abstract

The various NSAID's known to the scientists till date, reduces fever and inflammation when the body gets overzealous in its defenses against infection and damage but it may slows blood flow and blood clotting, reducing the chance of stroke and heart attack in susceptible individuals. Three-dimensional structures of pharmacologically important macromolecules offer a route to the discovery of new drugs. Understanding the macromolecule-ligand interactions and validation of method used for docking and virtual screening of chemical databases is crucial step in structure-based design. We therefore carried out molecular docking for structurally diverse COX-1/COX-2 inhibitors including traditional NSAIDs and Autodock 4.1.2. The complete computational analysis has revealed the best possible ligands combinations for the selective inhibition of COX-2 and COX-1. 3-D Structure of COX-2 has been predicted using the homology modeling tools. Results of docking of bound ligands like Tenoxicam and Valdecoxib have given the best binding scores Autodock.4.1.2. Molecular docking of structurally diverse selective COX-2 and COX-1 inhibitors has been successfully carried out.

Key-Words: Cyclooxygenase (COX-1, COX-2), Classic NSAIDs, Selective COX-2 Inhibitors, Inflammation, Docking, Ligplot, Inhibition

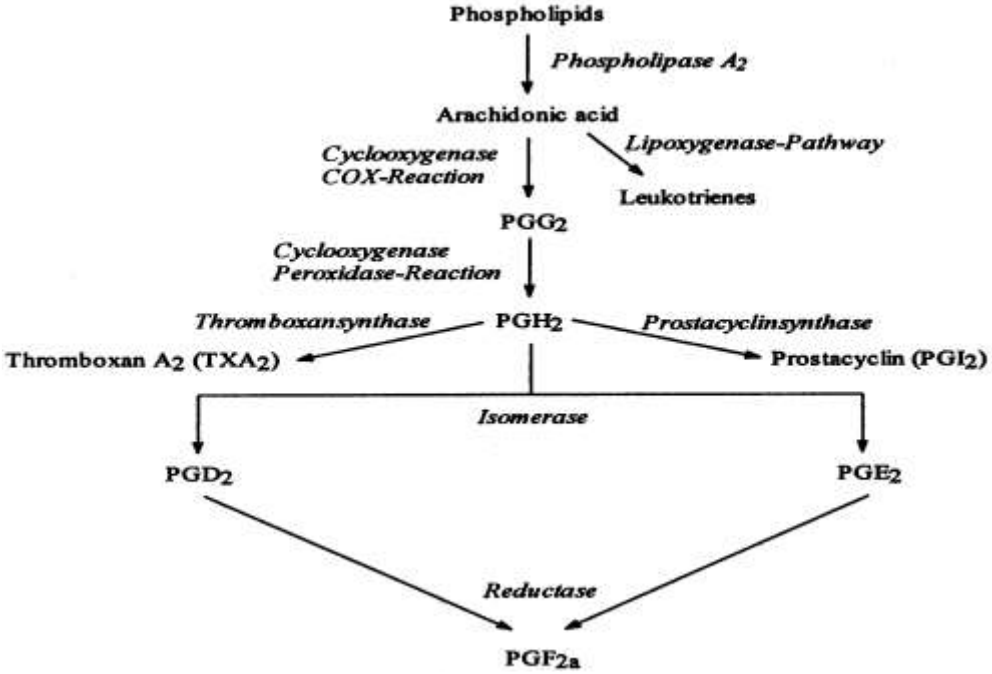
Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are amongst the most widely used therapeutics, primarily for the curing of pain and inflammation, especially arthritis. From a historical point of view, the first NSAID with therapeutic reimbursement was aspirin, which has now been applied for more than 100 years as an NSAID. The generally worldwide production of about 50 000 tons a year reflects the importance of this substance even today [1]. In the 1970s, a scientific breakthrough occurred with the elucidation of the molecular mechanism of aspirin and other NSAIDs. Vane, Samuelson and Bergstrom succeeded in illustrate that these anti-inflammatory matter block the biosynthesis of prostaglandins (PGs) which contribute to a range of physiological and pathophysiological functions. *Figure* 1 recapitulates the biosynthesis of PGs: the preliminary step in the biosynthesis of prostanoids is the emancipation of arachidonic acid (AA) from the phospholipids of the cell film catalyzed by phospholipase A2.

*** Corresponding Author**
E-mail: ankitrules.singh@gmail.com

The following important step is the biotransformation of AA by cyclooxygenase. In a bifunctional action, this first produces the unsteady PGG2, the cyclooxygenase response itself, which is then instantly converted into PGH2 by the same enzyme in a peroxidase reaction. As shown in *figure* 1, the ending products of the AA metabolism are PGs, thromboxanes and prostacyclin [2–5]. PGs are generated by most cells and are also current in tissues, which clarify their lane spectrum of biological responses. PGs reconcile a number of characteristic features of the body's reaction to tissue injury or inflammation. The outstanding effects of the PGs include their cytoprotective properties in the gastrointestinal (GI) tract and arrange of renal tasks in the kidney. PGE2 is the most main PG which mediates the characteristic symptoms of inflammation: rubor, calor, tumor, and dolor. Dilatation of small blood vessels initiates the progress of redness and heat; the increase in vascular permeability causes the characteristic inflammation of tissues. Moreover, PGs sensitize peripheral nerve finish and nociceptors to spread pain signals to the brain and the spinal cord. In adding to the well-accepted proinflammatory role of PGs, there are also details of anti-inflammatory action in certain COX-2-derived PGs in vivo, an experiment lately reported by Gilroy et al. [6]. Like aspirin, all other NSAIDs such as ibuprofen, ketoprofen and naproxen extend their mode of action by blocking

cyclooxygenase. Therefore, group of NSAIDs, for example to luxury inflammatory diseases such as osteoarthritis or rheumatoid arthritis, unavoidably leads to a lack of the prostaglandins requisite for the physiological functions revealed above. Therapeutic effects and side-effects of this class of anti-inflammatory drugs are narrowly related to their biochemical mechanism of action.

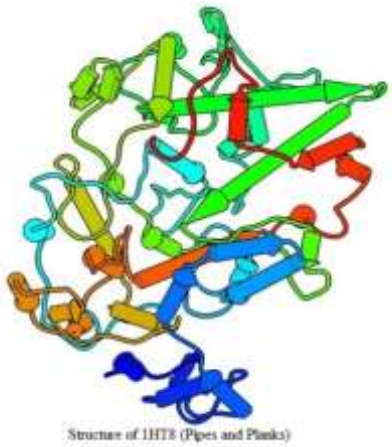


As a outcome, long-term NSAID users endure from a high incidence of GI irritation or, in the worst case, from the progress of life threatening GI ulcers and bleeding. These lesions can lead to improved morbidity in patients [7–9]. Administration of NSAIDs may also lead to renal confusions and have hypertensive effects. Due to a compressed production of PGs, such as PGI2, PGE2 and PDG2, in the ruling of renal blood circulation, the rate of glomeruleric filtration is condensed. Especially in patients with decreased renal function, this leads to maintenance of water, hypertension and, in some cases, to renal failure [10–12]. The reticence of cyclooxygenase in thrombocytes results in decreased production of thromboxane A2. This phenomenon extends bleeding time and leads to inhibition of platelet aggregation. A severe side-effect of NSAIDs is bronchoconstriction with resulting asthmatic events. The condensed amount of bronchodilatating PGE2 on the one hand and a alter in the metabolic lane from the cyclooxygenase pathway to the 5-lipoxygenase pathway on the other hand, seems to be dependable for the bronchoconstriction cause of NSAIDs [13]. The latter pathway metabolizes 'overflow' AA, which cannot be changed by the blocked cyclooxygenase pathway. The resultant leukotrienes act as bronchoconstrictors [14].Because of these problems, a main target of drug research is the progress of NSAIDs with anti-inflammatory and analgesic action but with no side effects.

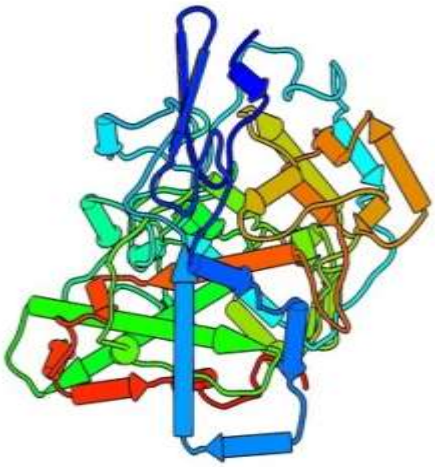
Material and Methods

Steps involved in carrying out this study are as follows:

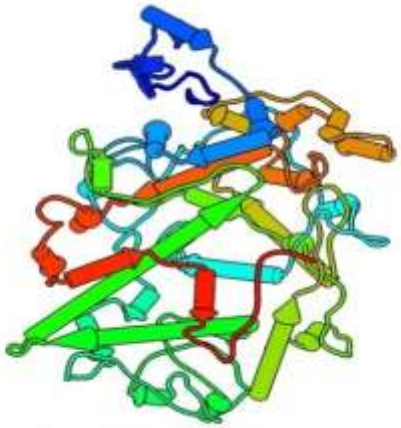
1. Sequence retrieval of COX-1 and COX-2 from GenBank. Protein sequences of COX-1 and COX-2 were retrieved from Genbank that were converted into FASTA format.
2. The sequences were then subjected to BLASTp for identification of local regions and a sequence with maximum similarity. On the basis of the template sequence Homology modeling between the retrieved sequences and the highly similar sequence was done which provides a structure of query sequence (COX-2).
3. After Homology modeling structure refinement was done which is based on energy criteria and other useful parameters for further structure refinement and optimization.
4. The structure are been downloaded from protein data bank (rcsb.org) *i.e.* 1HT8, 3MQE, 3NTG, 1PGF are given below.



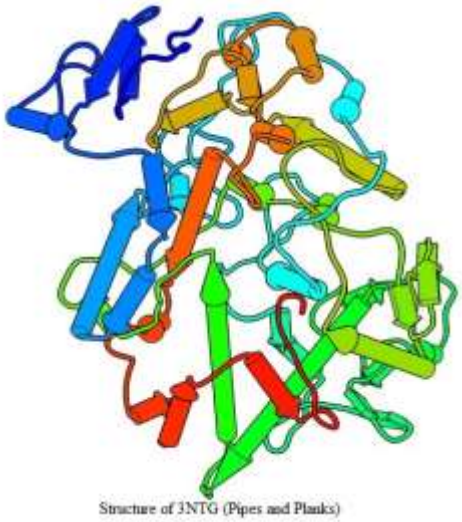
Structure of 1HT8 (Pipes and Planks)



Structure Of 1PGF(Pipes and Planks)



Structure of 3MQE (Pipes and Planks)

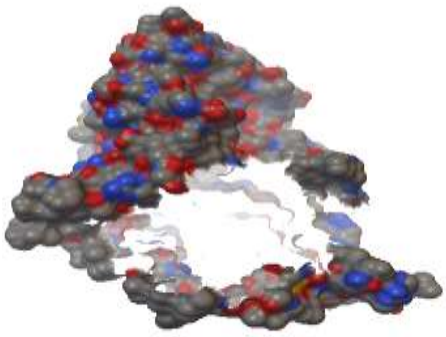


Structure of 3NTG (Pipes and Planks)

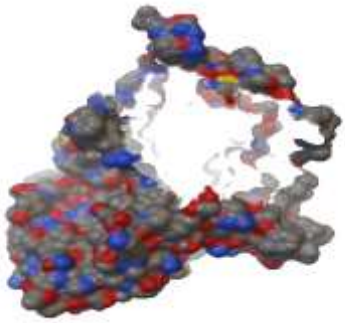
5. Protein cleaning is done with the help of UCSF Chemra (www.cgl.ucsf.edu.chimera/) and PNV.
6. Energy is minimized by SPDBV (www.spdbv.vital-it.ch/).
7. For docking, ligands were retrieved from drug bank and their physicochemical properties were studied. On the basis of these properties targeted ligand molecules were used for docking. Table No. 1
8. A priority among the ligands was generated.
9. Energy parameters, binding affinity, simulations and Autodock 4.2.1, provide the best possible combinations of COX-2, COX-1 and ligand molecules. Showing in table no. 2,3,4,5 respectively.

Binding site Prediction

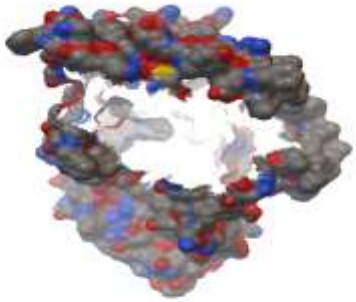
Binding sites were characterized by CASTp [15]Q-Site finder and compared by extensive literature search. By comparing prediction of CASTp algorithm and Q-Site Finder, best active sites were selected. CASTp method was used to identify and measure the binding sites, active sites, surface structural pockets (accessible), interior cavities (inaccessible), shape (alpha complex and triangulation), area and volume (solvent and molecular accessible surface) of each pockets and cavities of proteins. CASTp could be used to measure the number, area, circumference of mouth openings of each pocket in solvent and molecular accessible surface [15].



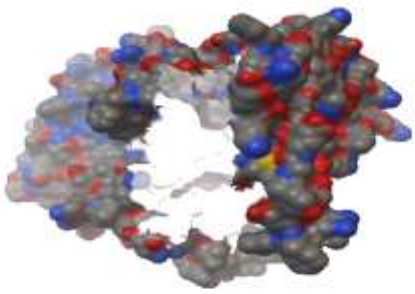
Active site of 1HT8



Active Site of 1PGF



Active Site of 3MQE



Active Site of Valdecoxib

Analyzing the Docking Results

The search for the best ways is to fit ligand molecules into structure, using Autodock 4.2.1 resulted in docking files that contained detailed records of docking. The obtained log files were read in ADT (Auto Dock Tool) to analyze the results of docking. The similarity of docked structures was measured by computing the root mean square deviation (RMSD) between the coordinates of the atoms and creating clustering of the conformations based on the RMSD values. The lowest binding energy conformation in all cluster were considered as the most favourable docking pose. Binding energies that are reported represent the sum of the total intermolecular energy, total internal energy and torsional free energy minus the energy of the unbound system. The top ligands were selected among the 17 based on the energy score after virtual screening Table, 2,3,4,5 of result section.

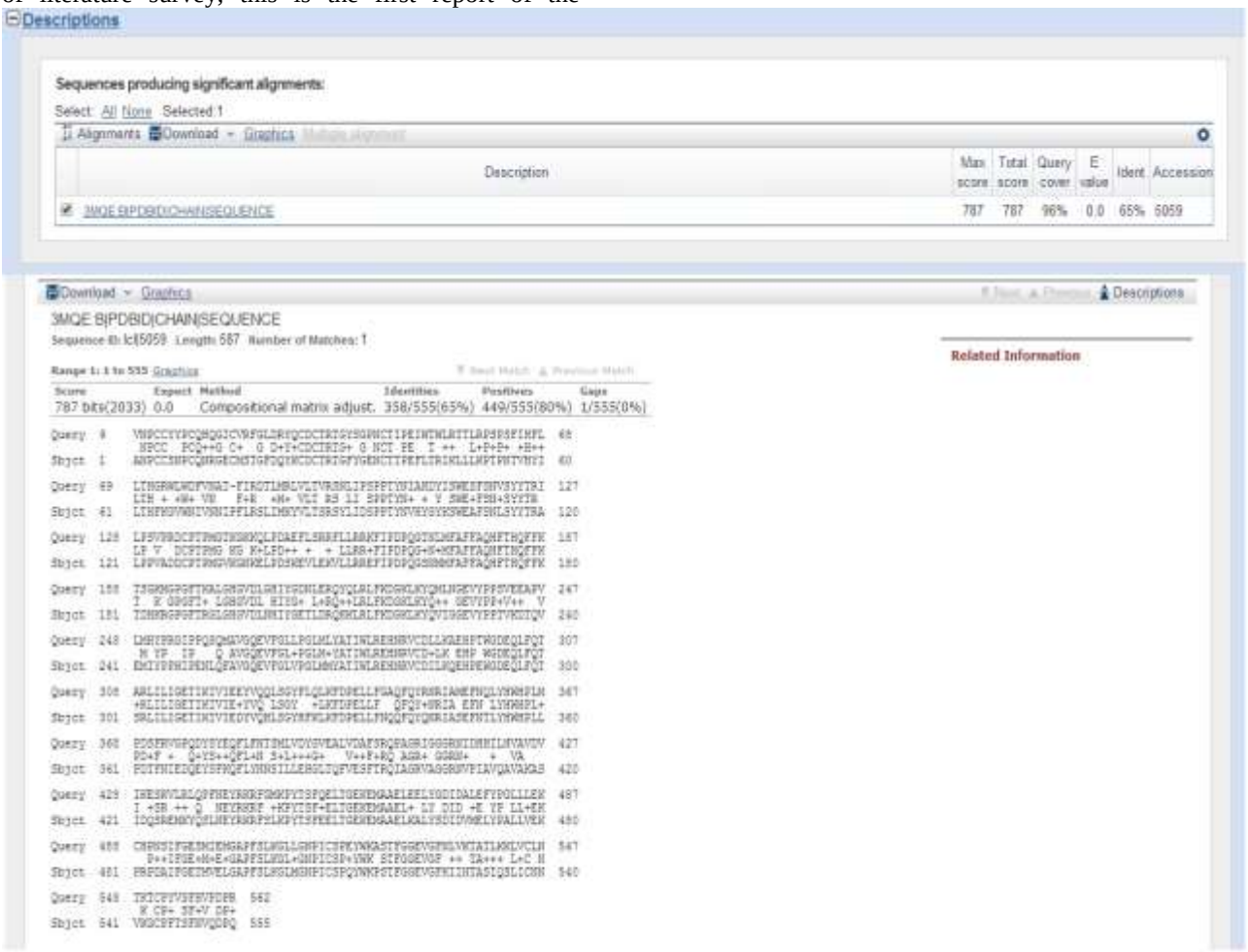
Table 1: List of the Ligands Retrieved from the Drug bank

Ligand	Chemical formula	Molecular wgt.(avg)
Naproxen.	$C_{14}H_{14}O_3$	230.2592
Etoricoxib.	$C_{18}H_{15}CIN_2O_2S$	258.842
Flurbiprofen.	$C_{15}H_{13}FO_2$	244.2609
Ibuprofen	$C_{13}H_{18}O_2$	206.2808
Indomethacin.	$C_{19}H_{16}CINO_4$	357.788
Ketoprofen.	$C_{16}H_{14}O_3$	254.806
Piroxicam.	$C_{15}H1_{13}N_3O_4S$	331.346
Diclofinac.	$C_{12}H_{11}CL_2NO_2$	296.149
Ketorolac.	$C_{15}H_{13}NO_3$	255.2686
Tolmetin	$C_{15}H_{15}NO_3$	257.2845
Tenoxicam.	$C_{13}H_{11}N_3O_4S_2$	337.374
Valdecoxib.	$C_{16}H_{14}N_2O_3S$	314.359
Meloxicam.	$C_{14}H_{13}N_3O_4S_2$	351.401
Phenylbutazone.	$C_{19}H_{20}N_2O_2$	308.3743
Rofecoxib.	$C_{17}H_{14}O_4S$	314.356
Sulindac	$C_{20}H_{17}FO_3S$	356.411
Celecoxib.	$C_{17}H_{14}F_3O_2S$	381.3752

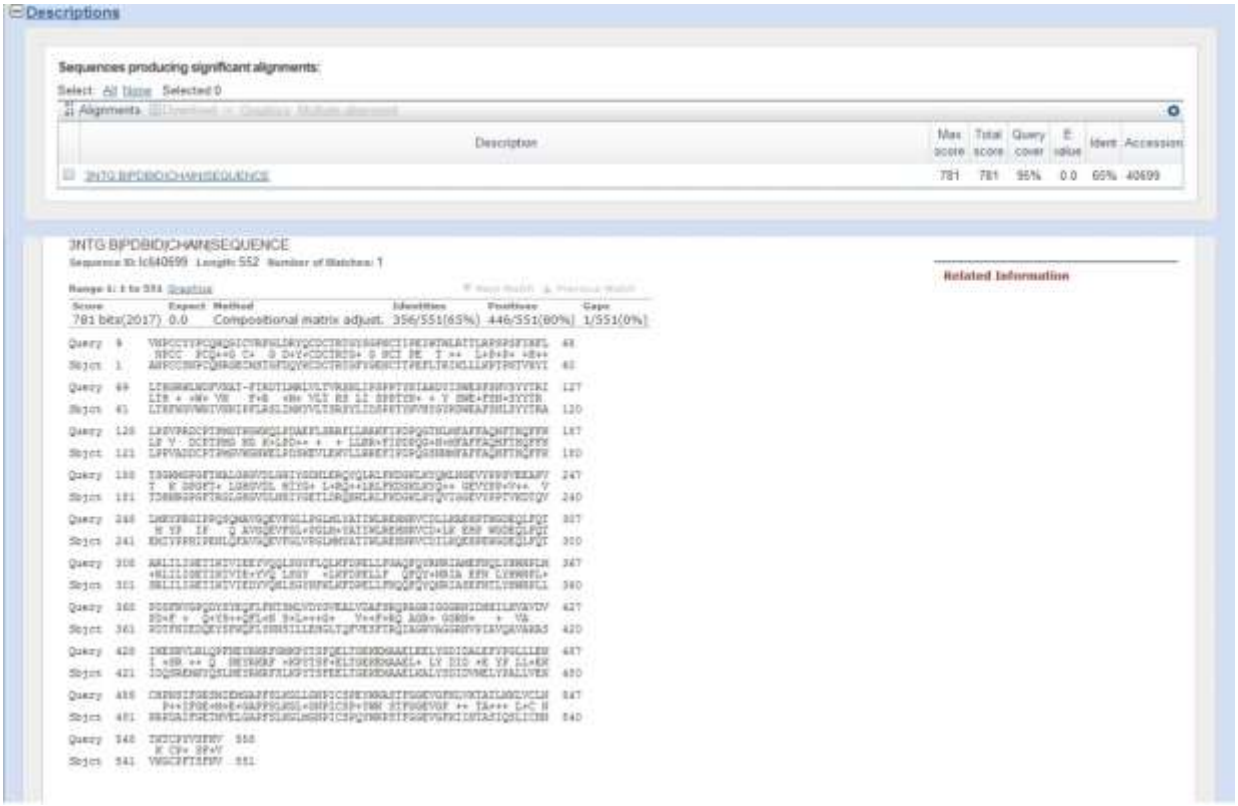
Results and Discussion

We have successfully carried out docking for 17 structurally diverse COX-2 inhibitors. The obtained ADME score was correlated with the biological activities. Some false positives and false negatives were observed but considering the limitations of the available docking program, the results are encouraging. The detailed analysis of the resulted COX-1&COX-2 - ligand complexes may improve our knowledge in understanding the binding interactions in detail. Thus this study will be useful for the design of novel COX-2 inhibitors based on docking and the resulted bioactive conformations of ligands and the results obtained from

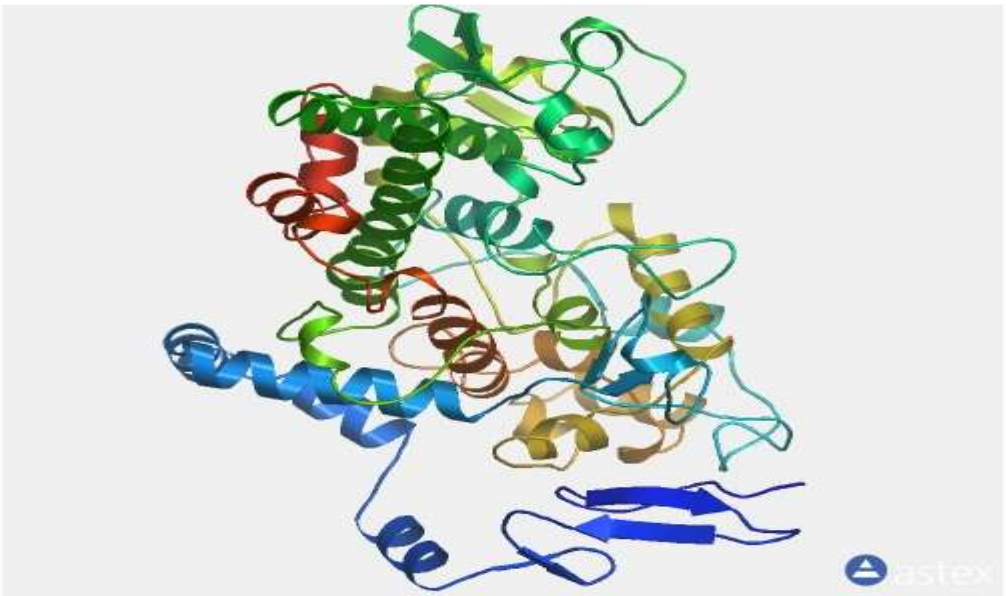
the Autodock of molecular docking and on the basis of binding energy scores we can suggest that tenoxicam and valdecoxib are the best fit ligand combinations which binds selectively with COX-2. This study will provide a platform for the further research and developments of drugs which can selectively suppress COX-2 and would not have any further side effects which were caused earlier due to the inhibition of COX-1 .These drugs will surely help a lot in ailing diseases and genetical disorders like colon cancer and various kinds of arthritis. Agents that inhibit COX-2 while spearing COX-1 represent a new attractive therapeutic development and could represent a major advance in the treatment of arthritis and various diseases. The docking model for the substituted tenoxicam and valdecoxib derivatives with the COX-2 receptor has been developed in this project. To the best of literature survey, this is the first report of the molecular modeling studies of these molecules with the COX-2 receptor. The docking simulation suggested that the modifications in the series that results in better binding potential. The Vander-walls, hydrophobic and charge interactions are responsible for forming the stable compound of the ligands with ligands with receptor. From the **Table.2,3,4,5 (Results)** ligands tenoxicam and valdecoxib do possess minimum dock score i.e. minimum binding energy in kilo joules per mole i.e. these molecule have more affinity for active site of COX-2 enzymes. Clearly, molecules with ester of bulky acids having less affinity for the receptor. Whereas molecules which possesses alcoholic with less bulky function 38-44 are said to have more affinity for COX-2 and can be used as analgesic and anti-inflammatory agents after synthesis.



Results of BLASTp of COX-1(1HT8) AND COX-2(3MQE)



Results of BLASTp of COX-1(1HT8)AND COX-2(3NTG)



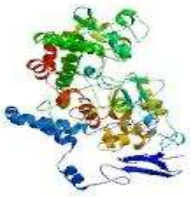
Structure of COX-2 from Swiss model server

SWISS-MODEL Workspace

Workunit: P000002
Title:cox

Model Details: Batch.1

model pic



Target:	
modelled residue range:	18 to 568
based on template	3nt1B (1.73 A)
Sequence Identity [%]:	88.203
Evalue:	0

Alignment

```
TARGET     18    ANPCCSHP CQNRGVCMSV GFDQYKCDCT RTGFYGENCS TPEFLTRIKL
3nt1B      33    anpccsnp cqnrgvcmst gfdqykcdct rtgfygenct tpefltrikl

TARGET           hh       aaaaa    aaaaa    aaa      aaahhhhhhh
3nt1B            hh       aaaaa    aaaaa    aaa      aaahhhhhhh

TARGET     66    FLKPTPNTVH YILTHFKGFW NVVNNIPFLR NAIMSYVLTS RSHLIDSPPT
3nt1B      81    llkptpntvh yilthfkgvw nivnnipflr alimkyvlts rsylidsppt

TARGET           hh   hhhhh hhh      hhhh hhh     hhhh hhhhhhhhhh hhh
3nt1B            hh   hhhhh hhh      hhhh hhh     hhhh hhhhhhhhhh hhh

TARGET     116   YNADYGYKSW EAFSNLSYYT RALPPVPDDC PTPLGVKGKK QLPDSNEIVG
3nt1B      130   ynvhygykaw eafsnlsyvt ralppvaddc ptpmgvkgnk elpdskeyle

TARGET                 h hhh    aaa                                hhhhhh
3nt1B                  h hhh    aaa                                hhhhhh

TARGET     166   KLLLRRKFIP DPQGSNMMFA FFAQHFTHQF FKTDHKRGPA FTNGLGHGVD
3nt1B      180   kvllrrefip dpqgsnmmfa ffaqhfthqf fktdhkrgpg ftnglghgvd

TARGET           h      ss s    ssssbhh bhhhhhhh       aaa    a aaa
3nt1B            h      ss s    ssssbhh bhhhhhhh       aaa    a aaa
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TARGET    216  LNHIYGETLA RQRKLRLFKD GKMKYQIIDG EMYPPTVKDT QAEMIYPPQV
3nt1B     230  lnhiygetld rqhklrlfkd gklkyqviqg evypptvkdt qvemiypphi

TARGET         hhhh   hh hhhhh        sss   sss ssshhh
3nt1B          hhhh   hh hhhhh        sss   sss ssshhh

TARGET    266  PEHLRFAVGQ EVFGLVPGLM MYATIWLREH NRVCDVLKQE HPEWGDEQLF
3nt1B     280  penlqfavgq evfglvpglm myatiwlreh nrvcdilkqe hpewgdeqlf

TARGET         sss    hhh   hhh hhhhhhhhh hhhhhhhhh          hhhhh
3nt1B          sss          hhh hhhhhhhhh hhhhhhhhh          hhhhh

TARGET    316  QTSRLILIGE TIKIVIEDYV QHLSGYHFKL KFDPELLFNK QFQYQNRIAA
3nt1B     330  qtsrlilige tikiviedyv qhlsgyhfkl kfdpellfnq qfqyqnriaa

TARGET         hhhhhhhhhh hhhhhhhhh hhh            hhh           h
3nt1B          hhhhhhhhhh hhhhhhhhh hhh            hhh           h

TARGET    366  EFNTLYHWHP LLPDTFQIHD QKYNYQQFIY NNSILLEHGI TQFVESFTRQ
3nt1B     380  efntlyhwhp llpdtfqied qeysfkqfly nnsillehgl tqfvesftrq

TARGET         hhhhh          sss   sss hhhh     hhhhh     hhhhhhhhhhs
3nt1B          hhhhh          sss   sss hhhh     hhhhh     hhhhhhhhhhs

TARGET    416  IAGRVAGGRN VPPAVQKVSQ ASIDQSRQMK YQSFNEYRKR FMLKPYESFE
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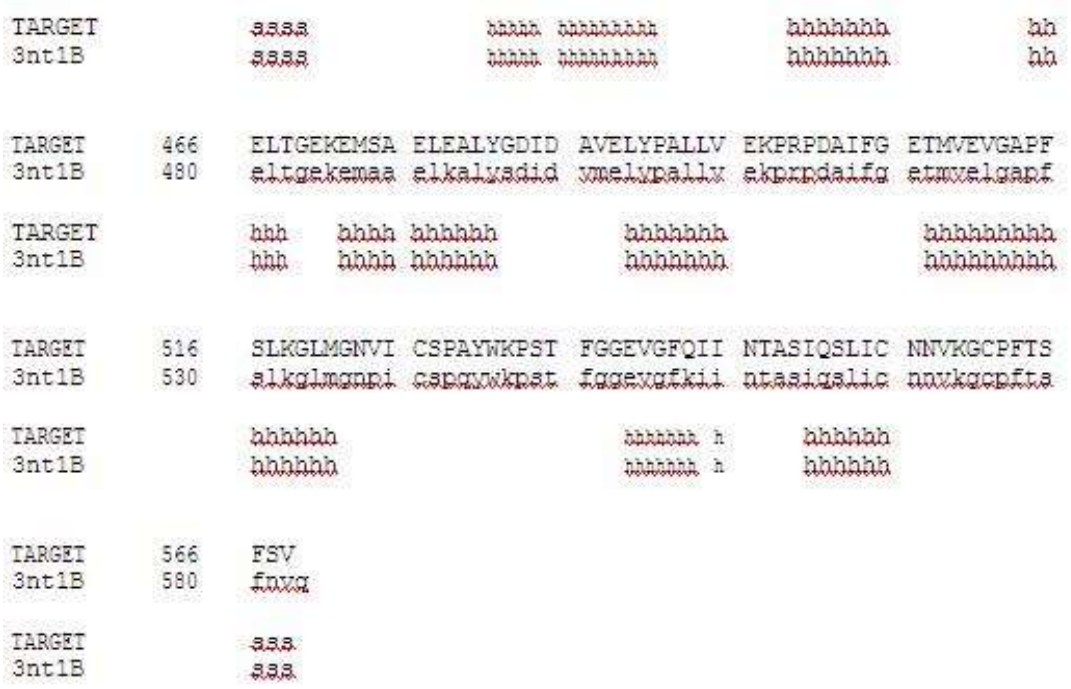


Docking and ADME

Table 2: Binding energy and other parameters of the ligands with 1HT8

Ligand	Binding Energy	RMSD	Inhibition Constant	H Bonds
Celecoxib.	-9.28	0	257.36	4
Tenoxicam	-12.29	0	988.24	4

Table 3: Binding energy and other parameters of the ligands with 3MQE

Ligand	Binding Energy	RMSD	Inhibition Constant	H Bonds
Tenoxicam	-12.37	0	856.71	3
Valdecoxib	-12.75	0	452.7	9

Table 4: Binding energy and other parameters of the ligands with 3NTG

Ligand	Binding Energy	RMSD	Inhibition Constant	H Bonds
Ketoprofen	-9.22	0	173.52	3
Telometin	-9.13	0	204.19	3
Valdecoxib	-13.4	0	150.74	7

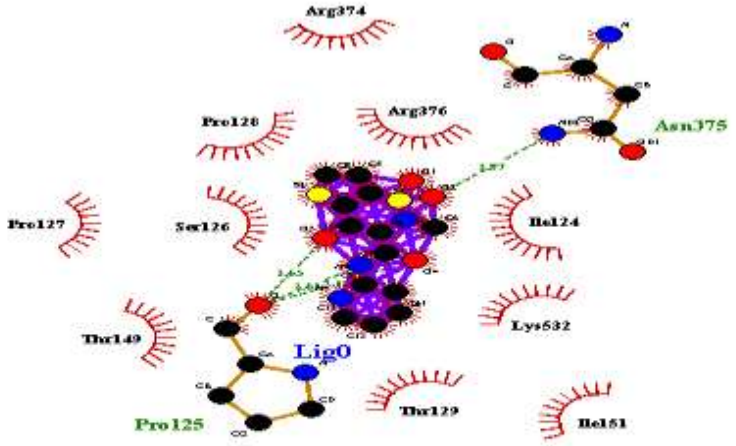
Table 5: Binding energy and other parameters of the ligands with 1PGF

Ligand	Binding Energy	RMSD	Inhibition Constant	H Bonds
Piroxicam	-16.59	0	690.85	4
Tenoxicam	-13.65	0	98.64	3

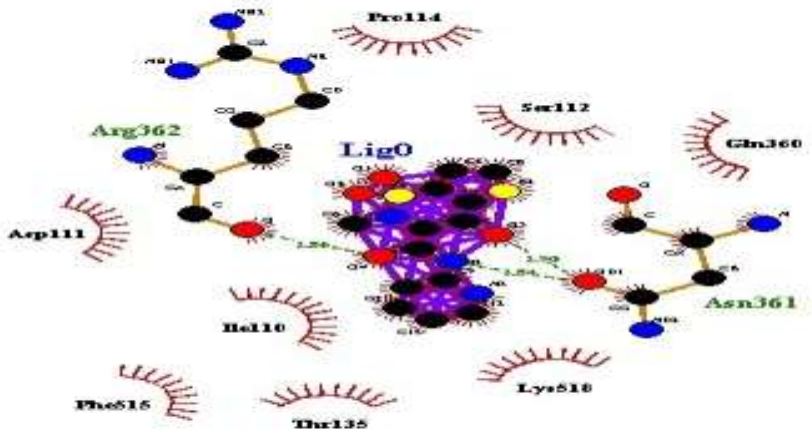
Table 6: Drug Likeliness Perdection(ADME)

Ligand	Intestinal absorbtion	Blood brain barrier	Caco-2 permeable	Ames Test
Tenoxicam	+0.9955	-0.9455	+0.8867	Negative
Piroxicam	+0.9898	-0.9659	+0.8867	Negative
Valdecoxib	+1	+0.9386	+0.5	Negative

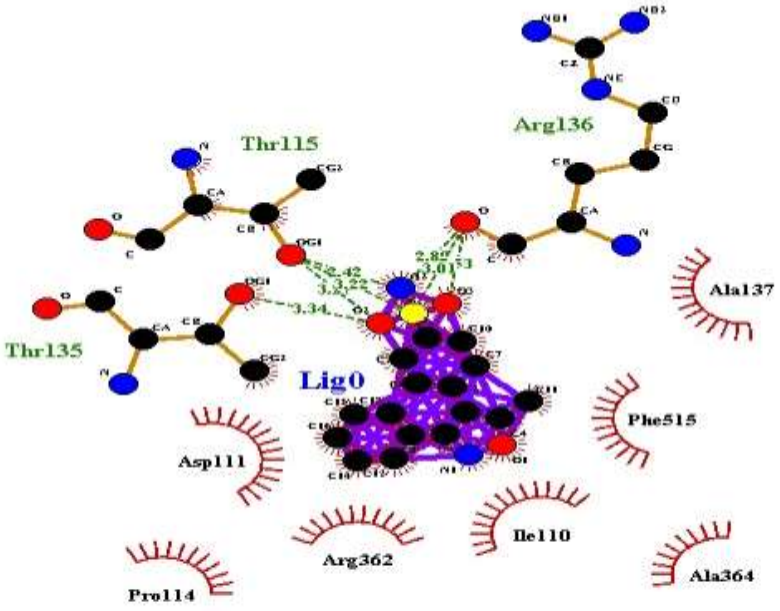
Ligplot



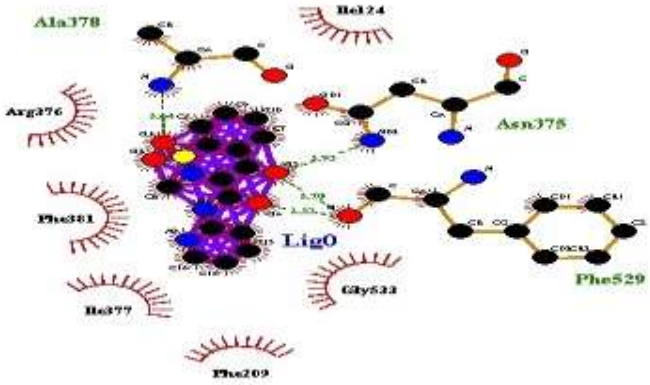
Hydrogen Bond Between 1HT8 and Tenoxicam



Hydrogen Between 3MQE and Tenoxicam



Hydrogen Bonds Between 3NTG and valdecoxib



Hydrogen Bonds Between 1PGF and ligand (Piroxicam)

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