INSILICO STUDY ON THE ANTIOXIDANT ACTIVITY OF ISOQUINOLINE FUSED BICYCLES

Dissertation submitted to the University of Kerala in partial fulfillment of the requirement for the award of the Degree of Master of Science in Chemistry

M.Sc. CHEMISTRY

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CONTENT

ABSTRACT

Isoquinoline type alkaloids show biological activities similar to those of morphinane, protoberberine, and benzophenanthridine type alkaloids. Structure-based drug design is the technique to be used in drug design. Structure-based drug design helps in the discovery process of new drugs. Computer-aided drug design (CADD) depends on the extent of structure and other information available regarding the target (enzyme/receptor/protein) and the ligands. Berberine is an important isoquninoline alkaloid generally present inclinically important medical plants which has demonstrated significant in antimicrobial activity against bacteria, fungi, protozoa, viruses, helminthes and chalmydia. Many in vitro and in vivo studies on human and animal models have explained the mechanisms of the chemopreventive effect of COX inhibitor.

Therefore, it is of interest to design and develop new yet effective compounds against COX-2 from medicinal plants such as the natural alkaloid compounds. The anti-COX-1/COX-2, antioxidant and anticancer activities were studied. The molecular docking study was performed using fifty-nine ligands in order to understand the binding interaction of compounds in the active site of cyclooxygenases. Each ligand shows different binding energy due to presence of various functional group in it. The lower the binding energy generally means greater its affinity to bind with the protein. Binding energy calculations revealed that **ligand 3** has obtained the least binding energy from the results obtained after conducting docking with the taken ligands.

CHAPTER 1

INTRODUCTION

1.1 ISOQUINOLINE

Isoquinoline is generally heterocyclic aromatic organic compound. It has general formula C_9H_7N. It is a structural isomer of quinoline. Isoquinoline is an ortho-fused heteroarene, in which *N* atom of benzopyridine is not directly attached to the benzene ring. It has been found generally liquid and solid forms. Solid they generally seem like hygroscopic solid, heavy sweet balsamic and have herbaceous aroma. Liquid or hygroscopic solid it seems colourless with a pungent odour like anise oil mixed with benzaldehyde. Isoqulinoiline plays an important role in pharmacology and biochemistry. It is known as human metabolites that include isoquinoline *N*-oxide. Isoquinoline alkaloids have medical relevance and extensively used in folk medicine. Examples are berberine, palmatine, morphine, codeine, emetine etc.[1-6]

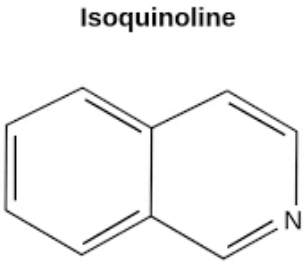


Figure 1. Structure of Isoquinoline

1.2 ISOQUINOLINE FUSED BICYCLE

The isoquinoline fused bicycles are generally synthesized Rhodium(III) catalyzed C-H activation of *O*-Aetyl Ketoximes/*N*-methoxybenzamides. A rhodium(III) catalyzed C-H activation generally approach towards the construction of fused heterocyclic

motifs, such as isoqunoline derivatives, by the reaction of *O*-acteyl ketoximes and *N*-methoxybenzamides, respectively with urea derived from the bicyclic olefins is reported.

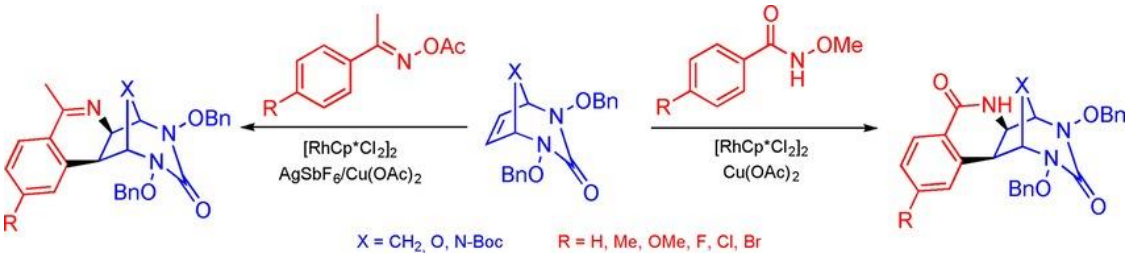


Figure 2. Isoquinoline fused bicycles

An efficient rhodium(III) catalyzed C-H activation approach for the construction of fused heterocyclic motifs with urea-derived bicycle olefins has been demonstrated. A rhodium catalyzed reaction of *O*-acetyl ketoximes with these bicyclic motifs furnished isoquinoline derivatives, whereas isoquinoline–fused bicycles olefins were obtained with the reaction of N-methoxybenzamides. [7,10]

1.3 COMPUTER AIDED DRUG DESIGN (CADD)

Computer-aided drug design (CADD) depends on the extent of structure and other information available regarding the target (enzyme/receptor/protein) and the ligands. The theoretical basis of CADD involves molecular mechanics, quantum mechanics, molecular dynamics, structure-based drug design (SBDD), ligand-based drug design (LBDD), homology modeling, ligplot analysis, molecular docking, de novo drug design, pharmacophore modeling and mapping, virtual screening (VS), quantitative structure-activity relationships (QSARs), In silico ADMET (absorption, distribution, metabolism, excretion and toxicity) prediction etc., CADD center was created to foster collaborative research between biologist, biophysicists, structural biologists and computational scientists. The major goal of the CADD center is to initiate these

collaborations leading to the establishment of research projects to discover novel chemical entities with the potential to be developed into novel therapeutic agents. Drug discovery and developing a new medicine is a long, complex, costly and highly risky process that has few peers in the commercial world. This is why computer-aided drug design (CADD) approaches are being widely used in the pharmaceutical industry to accelerate the process. The cost benefit of using computational tools in the lead optimization phase of drug development is substantial. On an average, it takes 10-15 years and US $500-800 million to introduce a drug into the market, with synthesis and testing of lead analogues being a large contributor to that sum. Therefore, it is beneficial to apply computational tools in hit-to-lead optimization to cover a wider chemical space while reducing the number of compounds that must be synthesized and tested in vitro. Computational methods of drug design are based on a postulate that pharmacologically active compounds act by interaction with their macromolecular targets mainly proteins or nucleic acids. Major factors of such interactions are surfaces of molecules, electrostatic force, hydrophobic interaction and hydrogen bonds formation. These factors are mainly considered during analysis and prediction of interaction of two molecules.[11,16]

1.3.1 STRUCTURAL BASED DRUG DESIGN (SBDD)

Structure-based drug design is the technique to be used in drug design. Structure-based drug design helps in the discovery process of new drugs. The process of structure-based drug design comprises of the following points:

 A preparation of the chosen target should be made in a solution form and its structure should be determined by the help of Crystallography

 Proper analysis of the structure should be made in order to determine the binding sites

 Different compounds from databases should be docked at binding site and then scored regarding its affinity for the site

 Compounds which show the best affinity with the site are selected

 Biochemical assays comprise of application of Leads and Tests which are made to bind at the target sites

a) If the lead is found to be posing as an inhibitor at the site, then it should be analyzed by crystallography regarding its structure

b) It should be further tested for potency and bioavailability in order to launch it

c) In Structure Based Drug Design, the action of the leads can be modified or optimized which would ensure higher success rates[17]

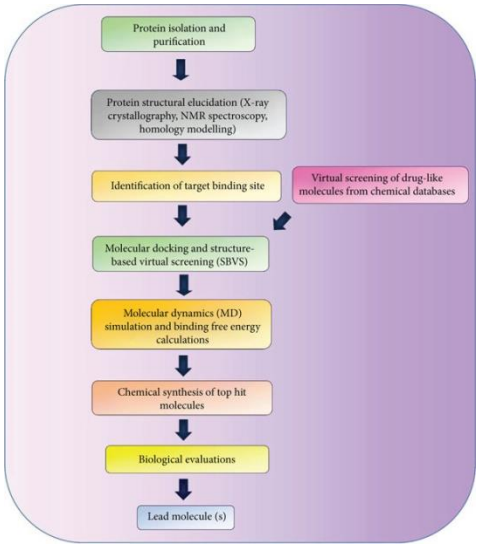


Figure 3. Structure based drug design

1.3.2 LIGAND- BASED DRUG DESIGN (LBDD)

The ligand-based drug design approach involves the analysis of ligands known to interact with a target. These methods use a set of reference structure collected from

compounds known to interact with the target of interest and analysis their 2D or 3D structure. In some cases, usually in which data pertaining to the 3D structure of a target protein are not available, drug design can instead be based on process using the known ligands of a target protein as the starting point. This approach is known as "ligand-based drug design".

a) Ligand Based Drug Designing comprises of the knowledge of molecules which bind to the desired target site[18, 19]

b) These molecules may be used to derive a Pharmacophore model

c) Pharmacophore model is defined as a molecule which is having necessary structural abilities to bind to a desired target site

d) Once the Pharmacophore is identified, it is then determined whether it is fit for the receptor, otherwise Pharmacophore is modified further in order to make it a potential drug

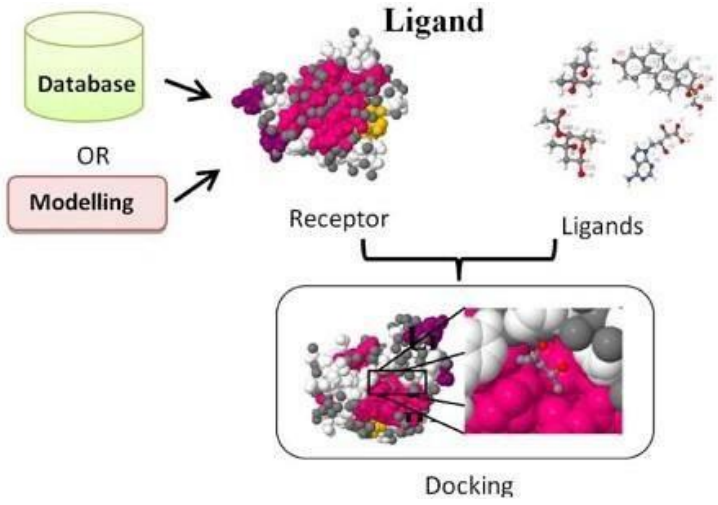


Figure 4. Ligand based drug design

1.4 MOLECULAR DOCKING

Molecular docking has become an increasingly important tool for drug discovery. The differences in and performance of available docking software are also discussed. Flexible receptor molecular docking approaches, especially those including backbone flexibility in receptors, are a challenge for available docking methods. A recently developed Local Move Monte Carlo (LMMC) based approach is introduced as a potential solution to flexible receptor docking problems. Three application examples of molecular docking approaches for drug discovery are provided. The completion of the human genome project has resulted in an increasing number of new therapeutic targets for drug discovery. At the same time, high-throughput protein purification, crystallography and nuclear magnetic resonance spectroscopy techniques have been developed and contributed to many structural details of proteins and protein–ligand complexes.[20]

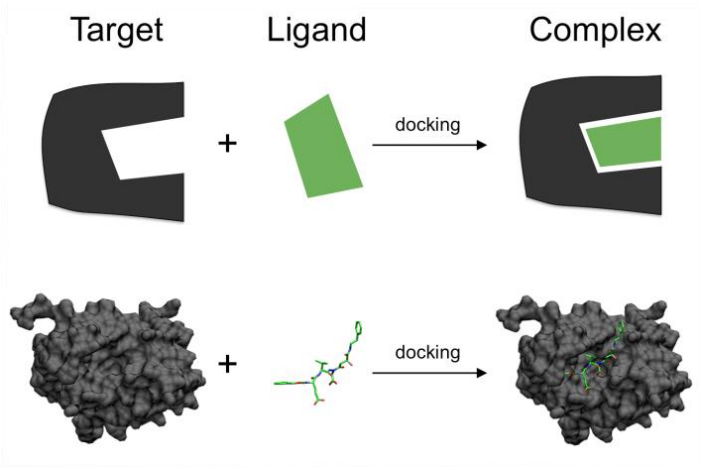


Figure 5. Molecular docking

These advances allow the computational strategies to permeate all aspects of drug discovery today, such as the virtual screening (VS) techniques for hit identification and methods for lead optimization. Compared with traditional experimental high-

throughput screening (HTS), VS is a more direct and rational drug discovery approach and has the advantage of low cost and effective screening. VS can be classified into ligand-based and structure-based methods. When a set of active ligand molecules is known and little or no structural information is available for targets, the ligand-based methods, such as pharmacophore modelling and quantitative structure activity relationship (QSAR) methods can be employed. As to structure-based drug design, molecular docking is the most common method which has been widely used ever since the early 1980s. Programs based on different algorithms were developed to perform molecular docking studies, which have made docking an increasingly important tool in pharmaceutical research. Various excellent reviews on docking have been published in the past, and many comparison studies were conducted to evaluate the relative performance of the programs.[21,22]

The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as pose) and assessment of the binding affinity. These two steps are related to sampling methods and scoring schemes, respectively, which will be discussed in the theory section. Knowing the location of the binding site before docking processes significantly increases the docking efficiency. In many cases, the binding site is indeed known before docking ligands into it. Also, one can obtain information about the sites by comparison of the target protein with a family of proteins sharing a similar function or with proteins co-crystallized with other ligands. In the absence of knowledge about the binding sites, cavity detection programs or

online servers, e.g., GRID, POCKET, Surf Net, PASS and MMC can be utilized to identify putative active sites within proteins. Docking without any assumption about the binding site is called blind docking. The early elucidation for the ligand-receptor binding mechanism is the lock-and-key theory proposed by Fischer, in which the ligand fits into the receptor like lock and key. The earliest reported docking methods were based on this theory and both the ligand and receptor were treated as rigid bodies accordingly. Then the "induced-fit" theory created by Koshland takes the lock-and-key theory a step further, stating that the active site of the protein is continually reshaped by interactions with the ligands as the ligands interact with the protein. This theory suggests that the ligand and receptor should be treated as flexible during docking. Consequently, it could describe the binding events more accurately than the rigid treatment.[23,24]

Considering the limitation of computer resources, docking has been performed with a flexible ligand and a rigid receptor for a long time, and remains the most popular method in use. Recently many efforts have been made to deal with the flexibility of the receptor, however, flexible receptor docking, especially backbone flexibility in receptors, still presents a major challenge for available docking methods. In our study, we propose a Local Move Monte Carlo (LMMC) approach as a potential solution to flexible receptor docking problems.

1.4.1 THEORY OF DOCKING

Essentially, the aim of molecular docking is to give a prediction of the ligand-receptor complex structure using computation methods. Docking can be achieved through two interrelated steps: first by sampling conformations of the ligand in the active site of the protein; then ranking these conformations via a scoring function. Ideally, sampling

algorithms should be able to reproduce the experimental binding mode and the scoring function should also rank it highest among all generated conformations. From these two perspectives, we give a brief overview of basic docking theory. Modelling the interaction of two molecules is a complex problem. Many forces are involved in the intermolecular association, including hydrophobic, van der Waals, or stacking interactions between aromatic amino acids, hydrogen bonding, and electrostatic forces. Modelling the intermolecular interactions in a ligand-protein complex is difficult since there are many degrees of freedom as well as insufficient knowledge of the effect of solvent on the binding association. The process of docking a ligand to a binding site tries to mimic the natural course of interaction of the ligand and its receptor via the lowest energy pathway. There are simple methods for docking rigid ligands with rigid receptors and flexible ligands with rigid receptors, but general methods of docking considering conformation ally flexible ligands and receptors are problematic. The search algorithm should create an optimum number of configurations that include the experimentally determined binding modes. Although a rigorous searching algorithm would go through all possible binding modes between the two molecules, this search would be impractical due to the size of the search space and amount of time it might take to complete. As a consequence, only a small amount of the total conformational space can be sampled, so a balance must be reached between the computational expense and the amount of the search space examined. Some common searching algorithms include molecular dynamics, Monte Carlo methods, genetic algorithms, fragment-based, point complementary and distance geometry methods, Tabu, and systematic searches. On the other hand, scoring function consists of a number of mathematical methods used to predict the strength of the non-covalent interaction called the binding affinity. In all the computational

methodologies, one important problem is the development of an energy scoring function that can rapidly and accurately describe the interaction between the protein and ligand. Several reviews on scoring are available in the ligand. The protein-ligand docking procedure can be typically divided into two parts: rigid body docking and flexible docking.[23,24]

Rigid Docking: This approximation treats both the ligand and the receptor as rigid and explores only six degrees of translational and rotational freedom, hence excluding any kind of flexibility. Most of the docking suites employ rigid body docking procedure as a first step.

Flexible Docking: A more common approach is to model the ligand flexibility while assuming having a rigid protein receptor, considering thereby only the conformational space of the ligand. Ideally, however, protein flexibility should also be taken into account, and some approaches in this regard have been developed. There are three general categories of algorithms to treat ligand flexibility: systematic methods, random or stochastic methods, and simulation methods.[29] Due to the large size of proteins and their multiple degrees of freedom, their flexibility may be the most challenging issue in molecular docking. The methods to address the flexibility of proteins can be grouped into: soft docking, side-chain flexibility, molecular relaxation and protein ensemble docking.

1.4.2 Experimental docking procedures

There are a number of excellent reviews of molecular docking methods and a large number of publications comparing the performance of a variety of molecular docking tools. Following, we will describe the four-step procedure adopted in this study to perform the molecular docking.

Target selection

Ideally, the target structure should be determined experimentally by either X-ray crystallography or nuclear magnetic resonance, which can be downloaded from PDB; however, docking has been performed successfully in comparison to homology models or threading. The model should have good quality. It can be tested using validation software such as Molprobity.[20] After selecting the model, it must be prepared by removing the water molecules from the cavity, stabilizing charges, filling the missing residues, and generating the side chains, all according to the available parameters. The receptor should be at this point biologically active and in the stable state.

Ligand selection and preparation

The type of ligands chosen for docking will depend on the goal. It can be obtained from various databases, e.g., ZINC or/and PubChem, or it can be sketched by means of Chemsketch tool.[21] Often it is necessary to apply filters to reduce the number of molecules to be docked. Examples include the net charge, molecular weight, polar surface area, solubility, commercial availability, similarity thresholds, pharmacophores, synthetic accessibility, and absorption, distribution, metabolism, excretion, and toxicology properties. Many times the researchers design their own molecules such as those generated.

Docking

This is the last step, where the ligand is docked onto the receptor and the interactions are checked. The scoring function generates a score depending on the best selected ligand.

Evaluating docking results

The success of docking algorithms in predicting a ligand binding pose is normally measured in terms of the root-mean-square deviation (RMSD) between the experimentally-observed heavy-atom positions of the ligands and the one(s) predicted by the algorithm. The flexibility of the system is a major challenge in the search for the correct pose. The number of degrees of freedom included in the conformational search is a central aspect that determines the searching efficiency. [22] A good performance is usually considered when the RMSD is less than 2Å.

Docking software description

There are many algorithms available to assess and rationalize ligand-protein or protein-protein interactions, and their number is constantly increasing. Speed and accuracy are key features for obtaining successful results in docking approaches. Several algorithms share common methodologies with novel extensions focused on obtaining a fast method with accuracy as high as possible. The most common docking programs include AutoDock[23] DOCK, FlexX, GOLD, ICM, ADAM, DARWIN, DIVALI, and DockVision.

AIM AND SCOPE OF STUDY

Berberine is an important isoquninoline alkaloid generally present inclinically important medical plants. This alkaloid has demonstrated significant in antimicrobial activity against bacteria, fungi, protozoa, viruses, helminthes and chalmydia. Recently berberine have been help to reduce blood glucose level in diabetes. Cyclooxygenase inhibitors as anti-inflammatory agents can be used in chemoprevention. Many in vitro and in vivo studies on human and animal models have explained the mechanisms of the chemopreventive effect of COX inhibitor. Cyclooxygenase-2 (COX-2) is liked with breast cancer. Therefore, it is of interest to design and develop new yet effective compounds against COX-2 from medicinal plants such as the natural alkaloid compounds.[24] The anti-COX-1/COX-2, antioxidant and anticancer activities were studied. The molecular docking study was performed in order to understand the binding interaction of compounds in the active site of cyclooxygenases. [25] 15 ligands are selected to check antioxidant activity of isoquinoline fused bicycles by docking was carried out computationally to find out better isoquinoline fused bicycles which decrease the action of favorable protein and prevent its negative effects. The binding energy become higher there will be higher affinity to bind the protein. Because generally consider negative value.

CHAPTER 2

LITERATURE REVIEW

Cyclooxygenase (COX) enzymes catalyze the double dioxygenation of arachidonic acid to prostaglandin endoperoxides, the immediate precursors to prostaglandins and thromboxane. These lipid mediators act through multiple G-protein-coupled receptors to trigger a broad range of physiological and pathophysiological responses. Their biosynthesis is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) which block the binding of arachidonic acid to the COX enzymes. NSAIDs – examples: aspirin, ibuprofen, naproxen, celecoxib are among the most widely prescribed drugs in the world and alleviate a great deal of human suffering. Our laboratory is interested in how COX enzymes carry out their biochemical function. We use X-ray crystallography, site-directed mutagenesis, kinetics, and computational modeling to investigate the molecular determinants that COX enzymes use to bind both substrates and inhibitors. The use this information to design novel inhibitors with enhanced binding or novel functions. Among our recent accomplishments are the design of a novel class of COX-2-selective inhibitors elimination of COX inhibitory activity of several classes of NSAIDs, which enables them to bind to other molecular targets in vivo, and synthesis and validation of COX-2 targeted imaging agents and therapeutics. The latter class of compounds offer great promise for the diagnosis and treatment of cancer because COX-2 is expressed at high levels in many solid tumors but not in surrounding normal tissues. This provides a strategy for directing imaging or therapeutic agents selectivity to tumors.

There are two forms of cyclooxygenases, cyclooxygenase 1 (COX1), which is constitutively expressed, and cyclooxygenase 2 (COX2), which is the product of an immediate early gene capable of being upregulated by diverse stimuli. There is a 60–65% sequence identity between COX1 and COX2 from the same species and 85–90% identity between orthologs from different species. COX functions as a homodimer in which each subunit of the dimer consists of the epidermal growth factor-like domain, the membrane binding domain, and the catalytic domain. The catalytic domain contains the COX and peroxidase active sites on either side of the heme prosthetic group. COX enzyme is present at the luminal surface of the endoplasmic reticulum (ER) and at the inner and outer membranes of the nuclear envelope (NE).[24]

Numerous studies implicate the cyclooxygenase 2 (COX2) enzyme and COX2-derived prostanoids in various human diseases, and thus, much effort has been made to uncover the regulatory mechanisms of this enzyme. COX2 has been shown to be regulated at both the transcriptional and posttranscriptional levels, leading to the development of nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX2 inhibitors (COXIBs), which inhibit the COX2 enzyme through direct targeting. Recently, evidence of posttranslational regulation of COX2 enzymatic activity by s-nitrosylation, glycosylation, and phosphorylation has also been presented. Additionally, posttranslational regulators that actively downregulate COX2 expression by facilitating increased proteasome degradation of this enzyme have also been reported. Moreover, recent data identified proteins, located in close proximity to COX2 enzyme, that serve as posttranslational modulators of COX2 function, upregulating its enzymatic activity. While the precise mechanisms of the protein-protein interaction between COX2 and these regulatory proteins still need to be addressed, it is likely these interactions could regulate COX2 activity either as a result

of conformational changes of the enzyme or by impacting sub cellular localization of COX2 and thus affecting its interactions with regulatory proteins, which further modulate its activity. It is possible that posttranslational regulation of COX2 enzyme by such proteins could contribute to manifestation of different diseases. The uncovering of posttranslational regulation of COX2 enzyme will promote the development of more efficient therapeutic strategies of indirectly targeting the COX2 enzyme, as well as provide the basis for the generation of novel diagnostic tools as biomarkers of disease.[25-28]

Through activation of the corresponding GPCRs and downstream signaling pathways, prostanoids, products of cyclooxygenase activity, have been shown to modulate tumor progression and inflammation and contribute to development of such renal disease as glomerulonephritis through several mechanisms. For example, signaling of these lipid mediators has been shown to directly regulate tumor epithelial cell proliferation, apoptosis, migration, and invasion through activation of downstream mitogenic pathways. Signaling of prostaglandins has also been shown to induce epithelial cells to secrete growth factors, proinflammatory mediators, and angiogenic factors, creating a microenvironment that supports tumor growth and spread. Prostanoids have also been shown to promote cancer progression through inducing an inflammatory microenvironment. Additionally, through direct binding of their receptors on stromal cells, prostanoids have been shown to promote a tumor supportive microenvironment by allowing tumor cells to evade attack by the immune system. Moreover, signaling of prostanoids through their receptors on stromal cells promotes a tumor-supportive microenvironment by inducing angiogenesis.[29,30] Consistent with the mitogenic signaling of these lipid mediators, increased expression of COX2, prostanoid receptors, and elevated COX2-mediated production of prostanoids have been reported

in various human cancers. Beyond elevated levels of COX2 in tumors, in vitro and in vivo studies have further established that COX2 signaling contributes to the development and progression of carcinogenesis.

Due to inflammatory signaling of prostanoids, overexpression of COX2 and increased production of an array of prostaglandins have been also detected in arthritis and in inflammatory bowel disease. Moreover, prostaglandin signaling has been reported to contribute to renal diseases such as proliferative glomerulonephritis by regulating cellular adhesion and proliferation of glomerular mesangial cells.[29-32] Prostaglandin signaling has been shown to increase free cytosolic calcium levels in glomerular mesangial cells promoting glomerular mesangial cell contraction, which can affect glomerular function in diseases such as glomerulonephritis.[33] Additionally, prostaglandin signaling has shown to contribute to glomerulonephritis by progressive accumulation of extracellular matrix (ECM) components, inflammatory changes, and podocytes injury, which further affect the glomerular filtration barrier.[34-36] Consistent with these reports, COX2 has been also shown to be overexpressed in proliferative glomerulonephritis.[37-38] Multiple in vivo animal models have further demonstrated that COX2 signaling contributes to the progression and development of glomerulopathies.[39-43]

CHAPTER 3

MATERIALS AND METHODS

Ligands were selected and its binding capability towards protein 1PXX were observed using software's and there binding energy can be calculated. The following tools used work as follows;

3.1 Marvin Sketch

Marvin Sketch features an extensive set of functionalities to enable the fast and accurate drawing of chemical compounds, reactions, Markus structures and query molecules. Furthermore, Marvin Sketch has built-in structure and valence checkers to provide guidance, and integrated property calculators to pull live results - upon your request. Marvin View is an advanced chemical viewer for single and multiple 2D/3D chemical structures, queries, reactions and their associated data. This lightweight renderer can display even thousands of molecules in a matrix or spreadsheet view along with the on the fly calculated fields, like molecule name, generated IUPAC name and SMILES strings. It supports all common chemistry file formats as well as some document formats. These file types include .doc and .pdf where chemical text names and context are extracted into a Marvin View table.[44]

3.2 Protein data bank

The Protein Data Bank is the single worldwide archive of structural data of biological macromolecules. The Protein Data Bank (PDB) was established at Brookhaven National Laboratories (BNL) (1) in 1971 as an archive for biological macromolecular crystal structures. In the beginning the archive held seven structures, and with each year a handful more were deposited. In the 1980s the number of deposited structures

began to increase dramatically. The mode of access to PDB data has changed over the years as a result of improved technology, notably the availability of the WWW replacing distribution solely via magnetic media. Further, the need to analyze diverse data sets required the development of modern data management systems. Initial use of the PDB had been limited to a small group of experts involved in structural research. Today depositors to the PDB have varying expertise in the techniques of X-ray crystal structure determination, NMR, cryoelectron microscopy and theoretical modeling. Users are a very diverse group of researchers in biology, chemistry and computer scientists, educators, and students at all levels. The tremendous influx of data soon to be fueled by the structural genomics initiative, and the increased recognition of the value of the data toward understanding biological function, demand new ways to collect, organize and distribute the data.[45]

DOCKING

3.3 Biovia Discovery studio

BIOVIA Discovery Studio brings together over 30 years of peer-reviewed research and world class insilico techniques such as molecular mechanics, free energy calculations, bio therapeutics develop ability and more into a common environment. It provides researchers with a complete toolset to explore the nuances of protein chemistry and catalyze discovery of small and large molecule therapeutics from Target ID to Lead Optimization.[46,47]

3.4 Auto dock

Auto Dock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Current distributions of Auto Dock consist of two generations of software:

Auto Dock 4 and Auto Dock Vina. Auto Dock 4 actually consists of two main programs: auto dock performs the docking of the ligand to a set of grids describing the target protein; auto grid precalculates these grids. In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to guide organic synthetic chemists design better binders. Auto Dock Vina does not require choosing atom types and pre-calculating grid maps for them. Instead, it calculates the grids internally, for the atom types that are needed, and it does this virtually instantly.[48,49]

3.5 Cygwin

Cygwin is free software that provides a Unix-like environment and software tool set to users of any modern x86 32-bit and 64-bit versions of MS-Windows.[50]

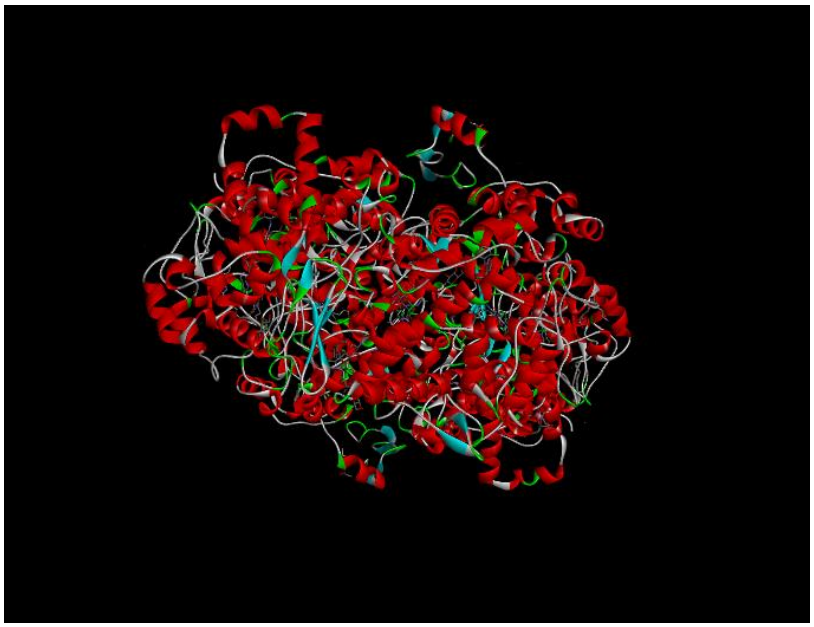
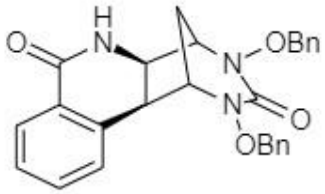
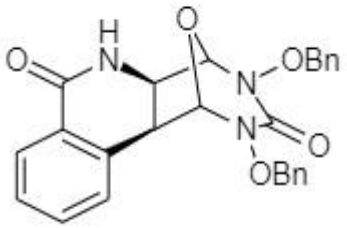
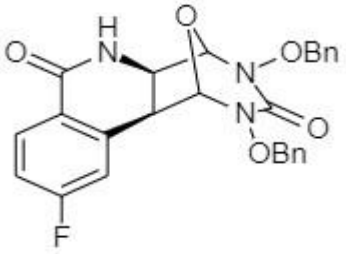
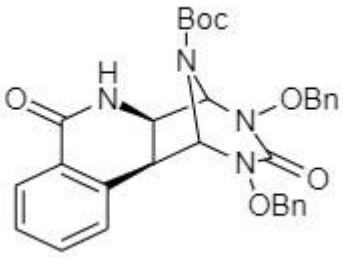
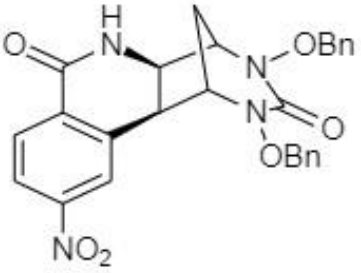
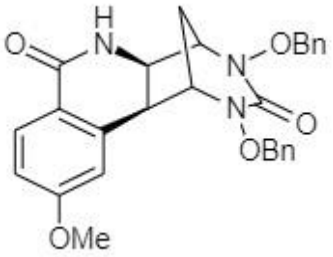
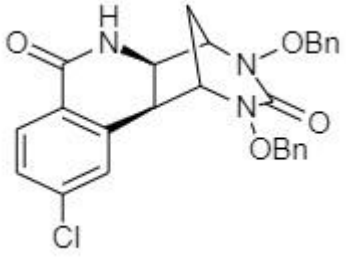
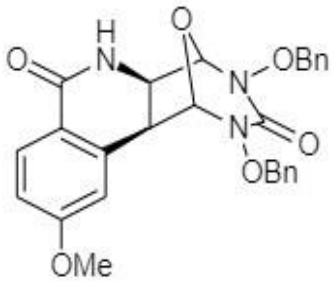
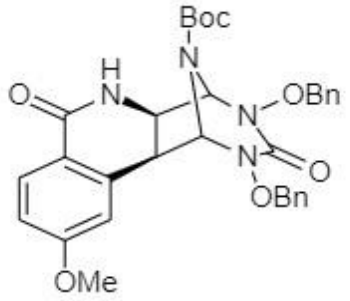
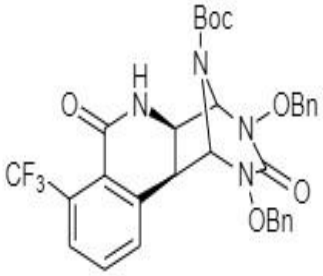
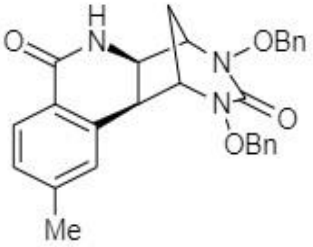
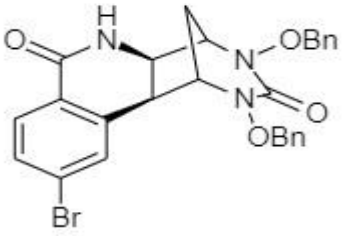
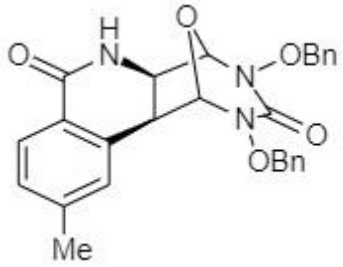
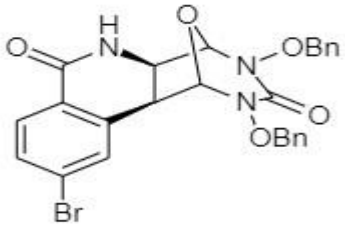
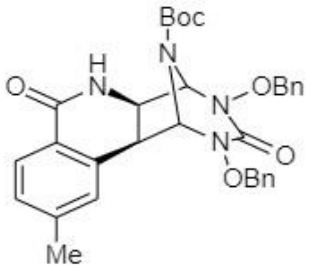
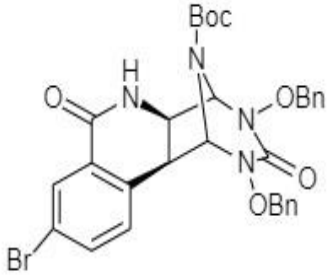
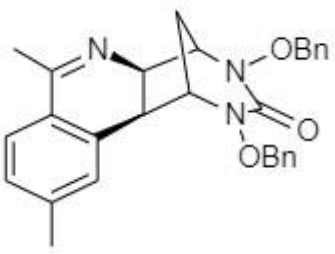
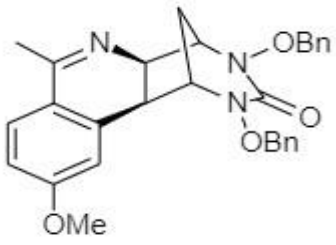
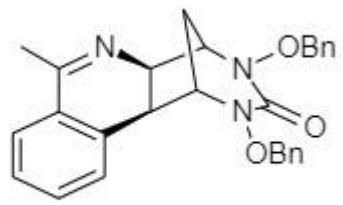
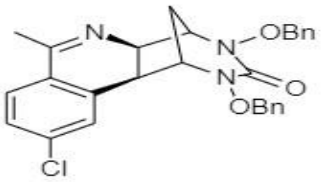
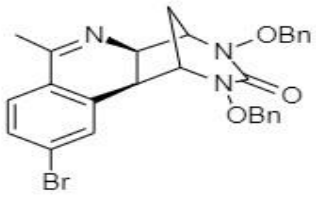
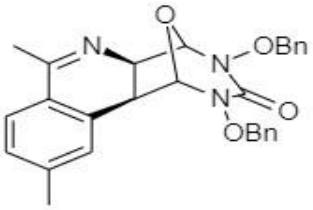
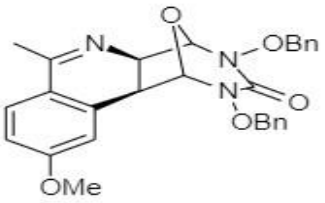
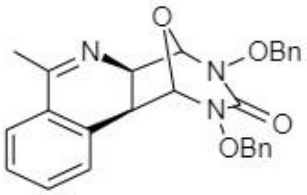
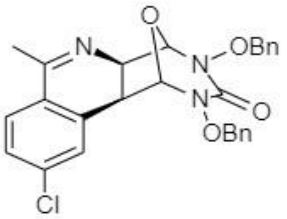
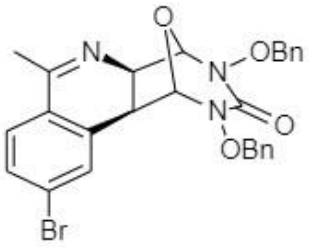
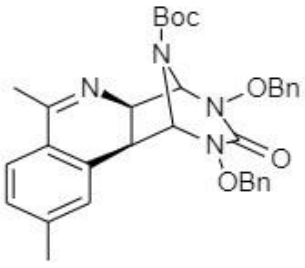


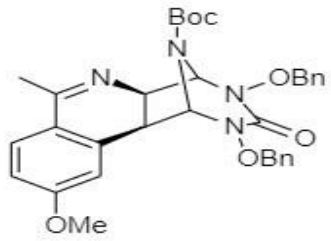
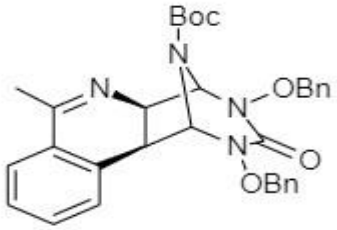
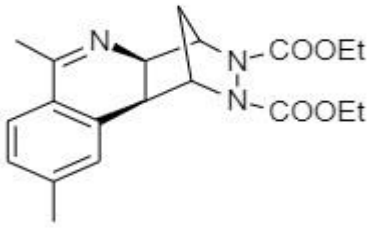
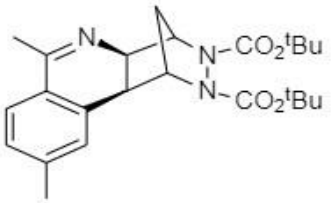
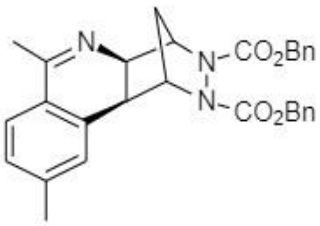
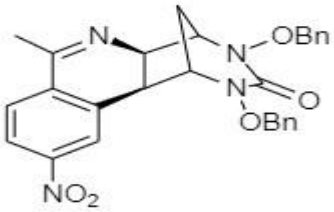
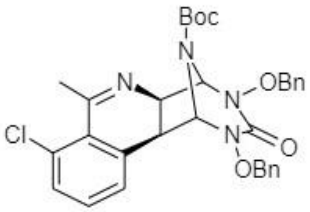
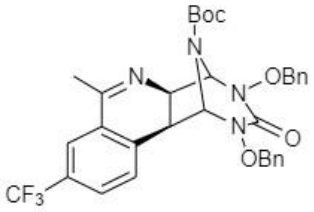
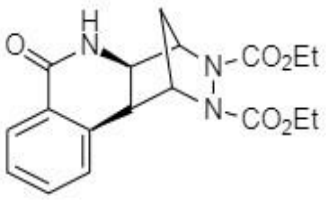
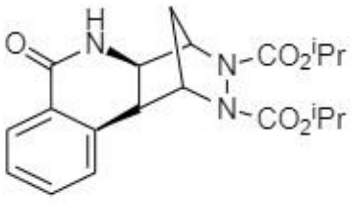
Figure 6. Structure of 1PXX Protein

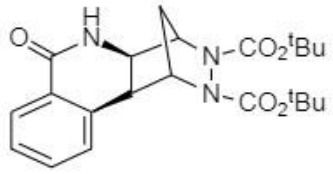
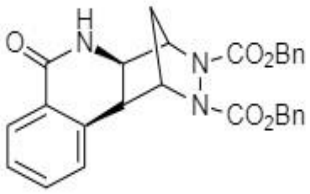
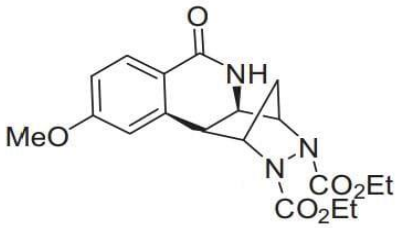
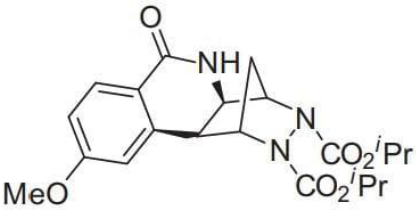
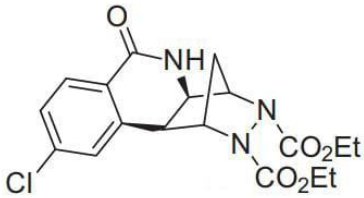
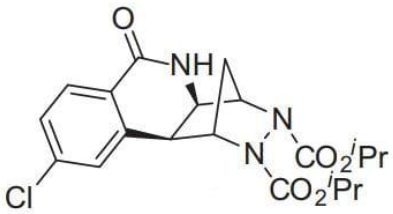
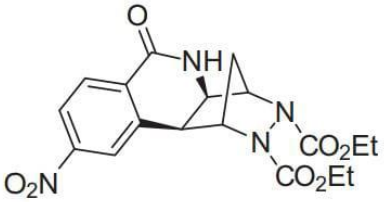
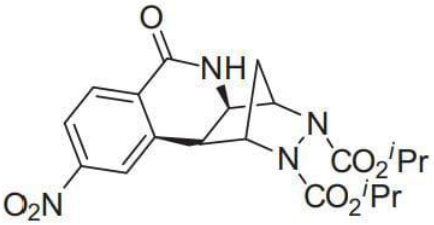
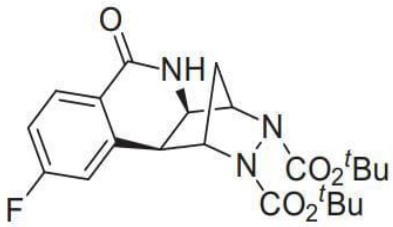
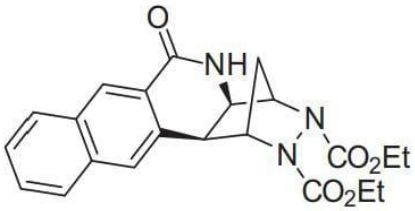
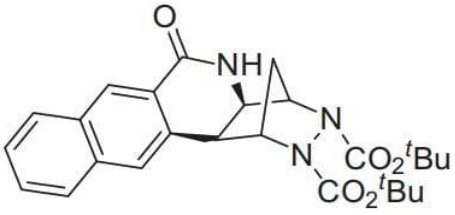
LIGAND STRUCTURES

 **Ligand 1**	 **Ligand 2**
 **Ligand 3**	 **Ligand 4**
 **Ligand 5**	 **Ligand 6**
 **Ligand 7**	 **Ligand 8**
 **Ligand 9**	 **Ligand 10**

 **Ligand 11**	 **Ligand 12**
 **Ligand 13**	 **Ligand 14**
 **Ligand 15**	 **Ligand 16**
 **Ligand 17**	 **Ligand 18**
 **Ligand 19**	 **Ligand 20**

 **Ligand 21**	 **Ligand 22**
 **Ligand 23**	 **Ligand 24**
 **Ligand 25**	 **Ligand 26**
 **Ligand 27**	 **Ligand 28**
 **Ligand 29**	 **Ligand 30**

 **Ligand 31**	 **Ligand 32**
 **Ligand 33**	 **Ligand 34**
 **Ligand 35**	 **Ligand 36**
 **Ligand 37**	 **Ligand 38**
 **Ligand 39**	 **Ligand 40**

 **Ligand 41**	 **Ligand 42**
 **Ligand 43**	 **Ligand 44**
 **Ligand 45**	 **Ligand 46**
 **Ligand 47**	 **Ligand 48**
 **Ligand 49**	 **Ligand 50**
 **Ligand 51**	 **Ligand 52**

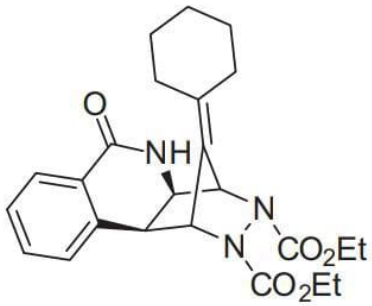
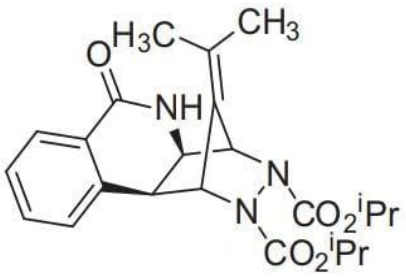
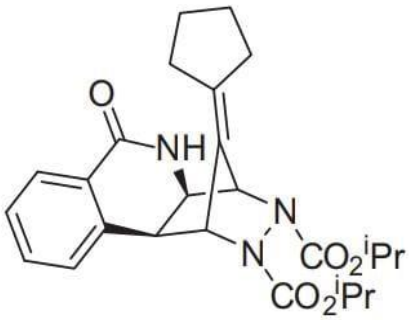
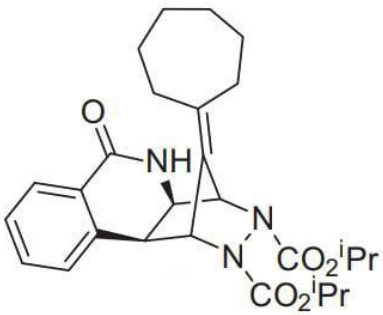
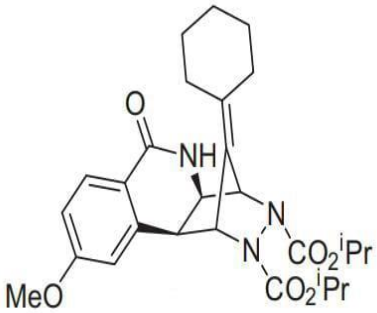
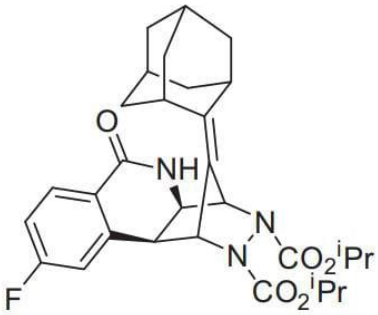
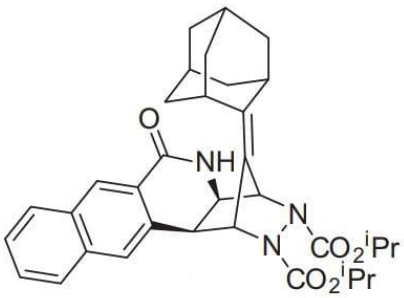
 **Ligand 53**	 **Ligand 54**
 **Ligand 55**	 **Ligand 56**
 **Ligand 57**	 **Ligand 58**
 **Ligand 59**	

Table 1. Structure of ligands

Methodology

Cycloxygenase receptor protein 1PXX was retrieved from protein data bank. The protein was downloaded in PDB format. Then using Maestro 12.3 the protein was splitted into ligand, water etc and the ligand and protein are saved as .pdb format. This protein was cleaned in Discovery studio and all hetero atoms and unwanted things are deleted in this and saved as disport pdb. the above saved disport is converted into pdbqt. Similarly our ligands[34] (given structure) which was drawn using Marvin sketch are cleaned in 2D, 3D and geometric optimization was done with Avogadro. Both the protein and ligands were saved in pdbqt format using Autodock. The active site residue taken is VAL 349 which is selected by reading various research papers.[35] The grid box was generated with coordinates (16.937, 168.068, 142.637) with dimensions 60 x 60 60 Å. Then is saved as gpf format. Similarly docking is done with 50 Genetic algorithm runs and saved in dpf format. Using cigwin the commands were given and docking was completed for 50 runs. 2D diagram was observed for further investigations.

CHAPTER 4

RESULT AND DISCUSSION

Molecular docking was carried out using a software Autodock.the cyclooxygenase receptor protein 1PXX was selected, and natural ligand was isolated. The 59 selected ligands were used for the docking proces. In docking the active site of proteins was blocked by using the set of ligands. Blocking will inhibit further cell division and growth by preventing the mechanism. Autodock provide more information about the binding energy in which the ligand perfectly fit to the protein. The binding energy obtained with the Autodock software is given in table 2.

LIGAND NAME	BINDING ENERGY	LIGAND NAME	BINDING ENERGY
Ligand 1	-12.24	Ligand 31	-6.19
Ligand 2	-12.13	Ligand 32	-5.99
Ligand 3	**-13.52**	Ligand 33	-5.84
Ligand 4	-11.34	Ligand 34	-6.72
Ligand 5	-12.68	Ligand 35	-7.04
Ligand 6	-12.07	Ligand 36	-10.16
Ligand 7	-12.31	Ligand 37	-6.70
Ligand 8	-11.65	Ligand 38	-6.44
Ligand 9	-12.07	Ligand 39	-7.44
Ligand 10	-11.45	Ligand 40	-7.18
Ligand 11	-12.08	Ligand 41	-6.03
Ligand 12	-9.57	Ligand 42	-8.16
Ligand 13	-10.43	Ligand 43	-7.66
Ligand 14	-11.74	Ligand 44	-5.79
Ligand 15	-12.16	Ligand 45	-6.03
Ligand 16	-13.13	Ligand 46	-7.72
Ligand 17	-12.44	Ligand 47	-8.10
Ligand 18	-12.99	Ligand 48	+1.37
Ligand 19	-12.01	Ligand 49	-9.71
Ligand 20	-11.37	Ligand 50	-5.31
Ligand 21	-12.02	Ligand 51	-4.44
Ligand 22	-11.84	Ligand 52	-2.39
Ligand 23	-11.88	Ligand 53	-0.93
Ligand 24	-12.26	Ligand 54	-8.27
Ligand 25	-11.83	Ligand 55	-4.20
Ligand 26	-12.06	Ligand 56	-9.51
Ligand 27	-12.14	Ligand 57	-6.50
Ligand 28	-11.30	Ligand 58	-8.69
Ligand 29	-6.02	Ligand 59	-5.15
Ligand 30	-10.29		

Table 2. Binding energy obtained with the Autodock software

From the table we can say that different ligands obtained different binding energy in Autodock. Using Autodock calculations **ligand 3** (-13.52 kcal/mol) has minimum amount of binding energy. **Ligand 48** has maximum binding energy (+1.37 kcal/mol) and hence have minimum stability among the ligands.

Ligand 3(**Figure 7**) shows various interactions like Van der waals interaction, conventional hydrogen bond, carbon hydrogen bond, π-sigma, π-sulphur, π-π stacked, amide π- stacked, alkyl, π-alkyl interactions. Van der waals interaction are shown by Leu2534, Leu2359, Met2113, Tyr2355, Arg2513, His2090, Gln2192 and Ile2517. Conventional hydrogen bond is exhibited by Ser2530. Carbon hydrogen bond shown by Ser2553 and Trp2387. π-sigma interactions exhibited by Leu2531, Val2349, Val2325 and Gly2526. π-sulphur interactions exhibited by Met2522. Phe2518 show π-π stacked and amide-π stacked interactions. Alkyl and π-alkyl interactions exhibited by Ile2345, Leu2352, Leu2384, Ala2527, Tyr2385 and Phe2381. The amount of binding energy obtained is -13.52kcal/mol.

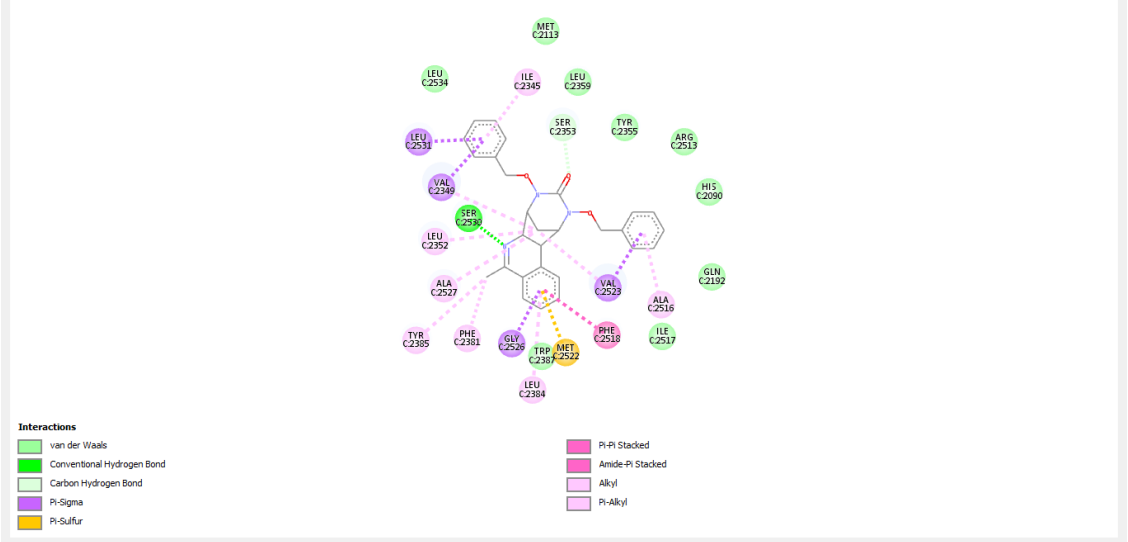


Figure 7. Ligand 3

Ligand 16(**Figure 8**) shows various interactions like Van der waals interaction, conventional hydrogen bond, carbon hydrogen bond, Unfavarable donor donor, π-

sigma, π-sulphur, π-π stacked, amide π-stacked, alkyl, π-alkyl interactions. Van der waals interactions shown by Val2344, Leu2534, Leu2359, Met2113, Tyr2355, His2090, Arg2513 and Gln2192. Ser2530 shows conventional hydrogen bond interactions. Carbon hydrogen bond interactions exhibited by Ser2353, Ile2517, Trp2387, Tyr2385 and Phe2381. π-sigma interactions shown by Leu2531, Val2349, Val2523 and Gly2526. Met2522 exhibited by π-sulfur interactions. Phe2518 show π-π stacked and amide π stacked interactions. Alkyl and π-alkyl interactions exhibited by Ile2345, Leu2352, Leu2384, Ala2516. The amount of binding energy obtained is -13.13kcal/mol.

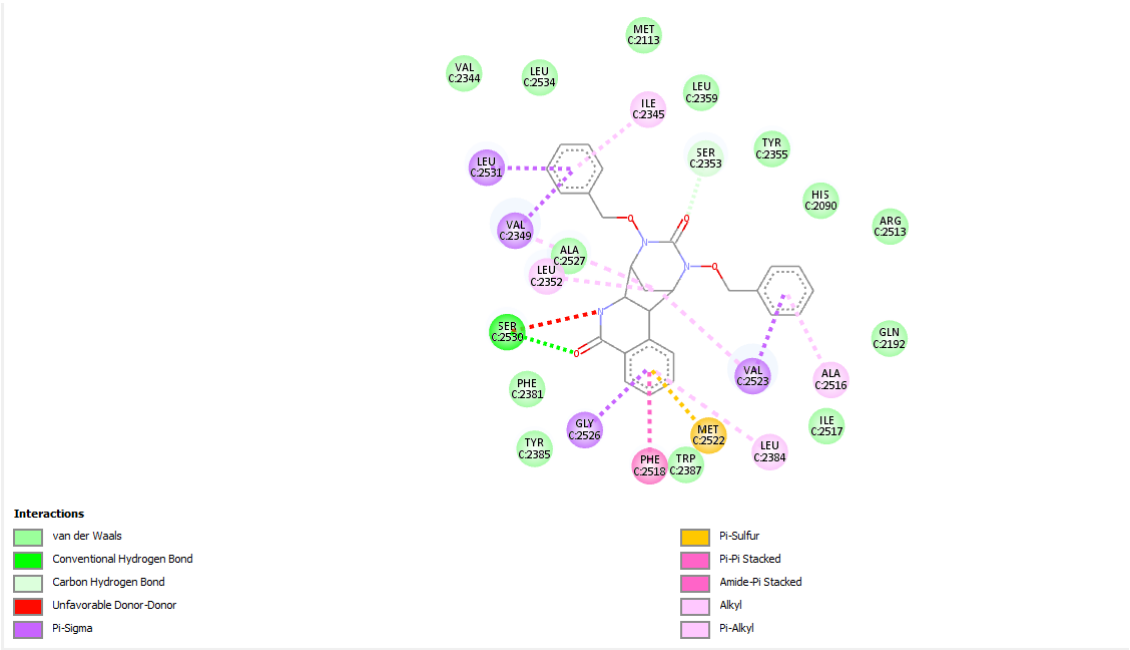


Figure 8. Ligand 16

Ligand 18 (**Figure 9**) shows various interactions like Van der waals interaction, conventional hydrogen bond, carbon hydrogen bond, π-sigma, π- sulphur, π-π stacked, amide π- stacked, alkyl, π-alkyl interactions. Van der waals interaction are shown by Leu2534, Leu2359, Leu2353, Met2113, Tyr2355, Arg2513, His2090, Gln2192 and Ile2517. Conventional hydrogen bond is exhibited by Ser2530 and Tyr2385. Carbon

hydrogen bond shown by Phe2381, Ser2353 and Trp2387. π-sigma interactions exhibited by Leu2531, Val2349, Val2325 and Leu2531. π-sulphur interactions exhibited by Met2522. Phe2518 and Gly2526 show π-π stacked and amide- π stacked interactions. π-alkyl interactions exhibited by Ile2345, Leu2384, Ala2516. The amount of binding energy obtained is -12.99kcal/mol.

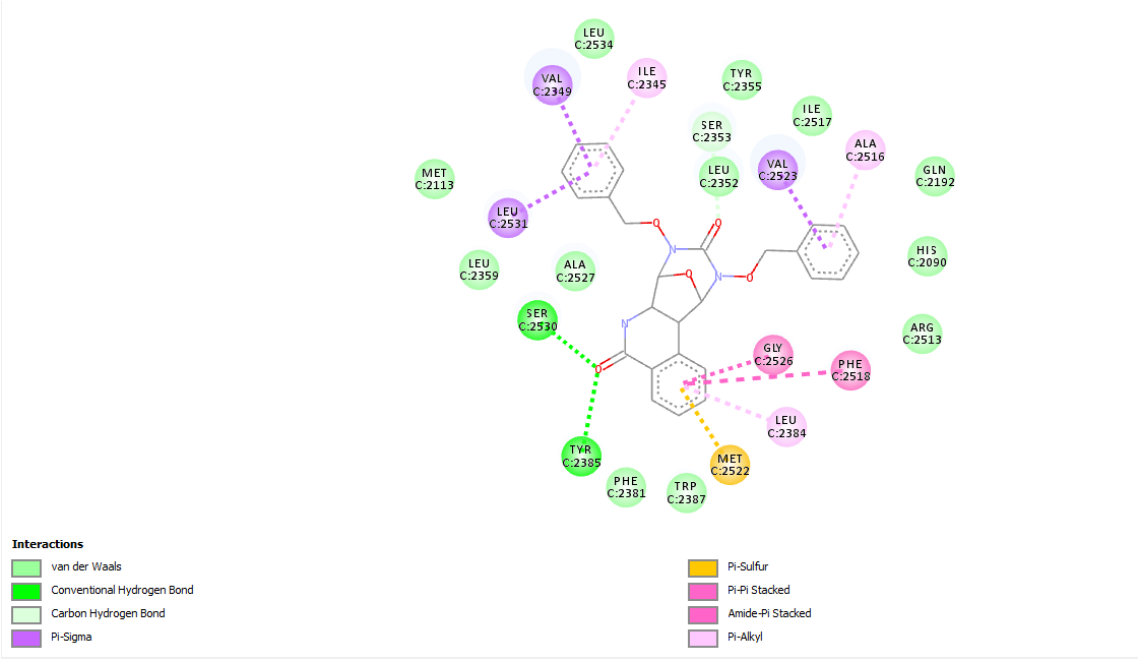


Figure 9. Ligand 18

Ligand 5 (**Figure 10**) shows various interactions like Van der waals interaction, carbon hydrogen bond, π-sigma, alkyl, π-alkyl interactions. Van der waals interaction are shown by Ser2353, Ser2350, Tyr2355, Tyr2385, Arg2120, Arg2513, Gln2192, Ile2517, His2090, Gly2526, Phe2205, Leu2359, Leu2534 and Ser2530. Carbon hydrogen bond shown by Val2523. π-sigma interactions exhibited by Leu2531, Val2349, Phe2518 and Leu2532. Alkyl and π-alkyl interactions exhibited by Ile2345, Trp2387, Tyr2348, Val2344, Met2522, Ala2527, Ala2516. The amount of binding energy obtained is -12.68kcal/mol.

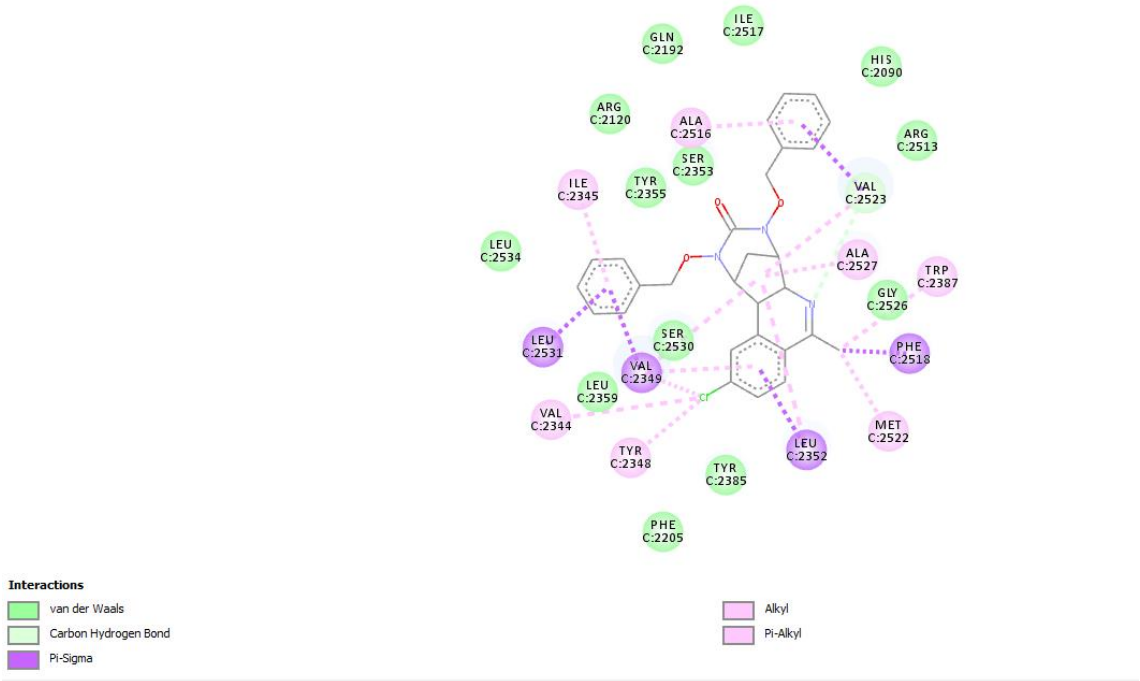


Figure 10. Ligand 5

Ligand 48 (**Figure 11**) shows various interactions like van der waals interaction, halogen(fluorine), π-sigma, alkyl, π-alkyl interactions. Van der waals interaction are shown by Tyr2385, Tyr2348, Phe2381, Gly2526, Gln2192, Ser2353, Ser2530 and Arg2120. Halogen (Fluorine) shown by Ala2527. π-sigma interactions exhibited by Val2349. Alkyl and π-alkyl interactions exhibited by Tyr2355, Trp2387, Val2523, Leu2384, Leu2352, Leu2531 Met2522, Ile2517, Phe2518, Ala2516 . The amount of binding energy obtained is +1.37kcal/mol.

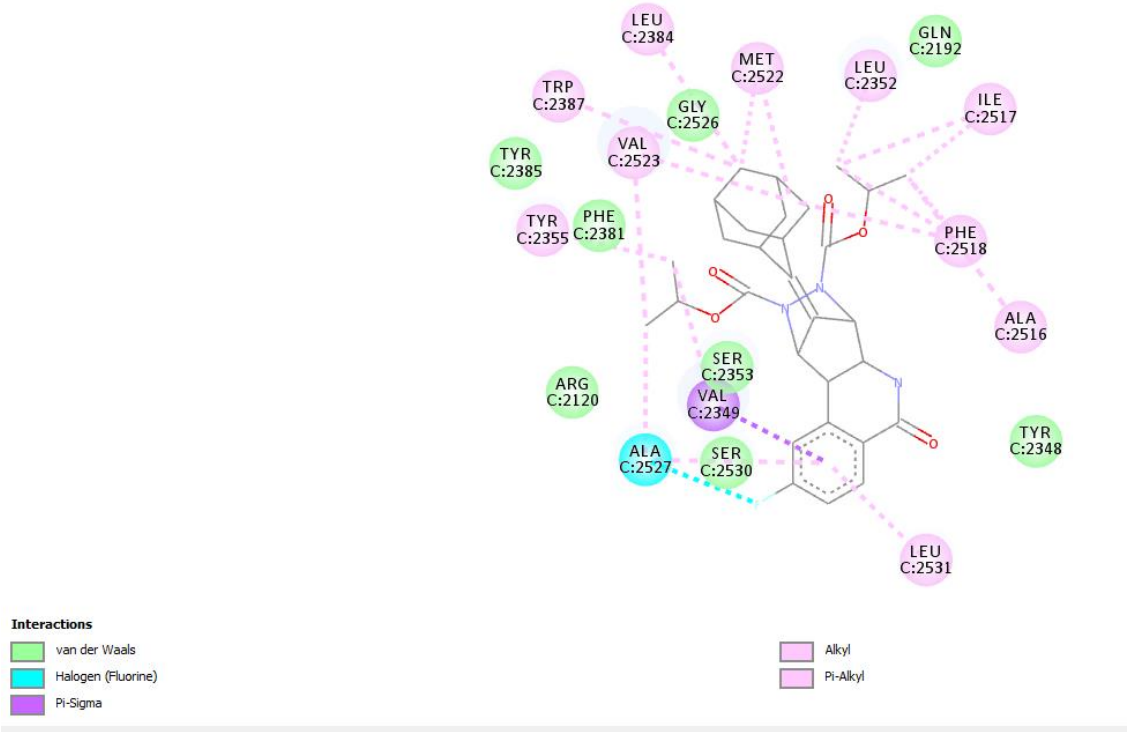


Figure 11. Ligand 48

From this information about 59 ligands which are docked to the protein, **ligand 3** showed more negative binding energy (-13.52kcal/mol). **Ligand 5** has a binding energy of -12.68kcal/mol. On comparing the structures of the ligands, **ligand 1**, **4**, **6**, **7**, **8**, **10**, **11**, **12**, **19**, **22**, **25**, **26**, **27** and **28** same scaffold structures with **ligand 5**. **Ligand 1**, **4**, **6**, **7**, **8**, **10**, **11**, **12**, **19**, **22**, **25**, **26**, **27** and **28** have binding energies -12.24, -11.34, -12.07, -12.31, -11.65, -11.45, -12.08, -9.57, -12.01, -11.84, -12.26, 12.06, -12.14 and -11.30kcal/mol respectively. Among this, **ligand 5** which is free of substituents has the most negative binding energy followed by **ligand 7** which has –OMe as the substituent.

Ligand 2 has a binding energy of -12.13kcal/mol. **Ligand 2** having an oxygen atom at the bridge head. Thus we see that the presence of oxygen atom on the bridge head increases the binding energy a small amount. **Ligand 16**, **17**, **20**, **21**, **23** and **24** have

the same scaffold structure with **ligand 2**, having different functional groups attached such as –S, -OMe, -F, -Me, and –Br respectively. **Ligand 16**, **17**, **20**, **21**, **23** and **24** have binding energies -13.13, -12.44, -11.37, -12.02, -11.88 and -12.26 kcal/mol respectively. Among these, **ligand 16** which has –S as the substituent has the most negative binding energy. The reason for this is may be due to the presence of the highest electronegative atom 'S'. It is followed by **ligand 17** which has –F as the substituent.

Ligand 9 has a binding energy of –12.07kcal/mol. On comparing with **ligand 18** it has same scaffold structure with **ligand 9**. **Ligand 18** having corresponding binding energy -12.99 kcal/mol. The binding energy of **ligand 9** is – 12.07 kcal/mol which is higher than **ligand 13** which has a binding energy of -13.57 kcal/mol. The increase in binding energy of **ligand 9** may be due to the presence of the bulkier –Boc group attached to the nitrogen atom. Among these, **ligand 18** which has –S as the substituent has the most negative binding energy, followed by **ligand 9** due to the lone pair attached to benzene ring of the compound.

Ligand 15 has a binding energy of –12.16 kcal/mol. The different functional groups attached to **ligand 15** such as -NO_2, OMe, respectively. On comparing the structures of the ligands, ligand 13 and 14 have same scaffold structures with **ligand 15**. **Ligand 13** and **14** have binding energies -10.43 and -11.74 16 kcal/mol respectively. The increase in binding energy of **ligand 9** may be due to the presence of the bulkier –Boc group attached to the nitrogen atom, followed by **ligand 14** which has –OMe as the substituent.

Ligand 29 has a binding energy of -6.02 kcal/mol. It has the same scaffold structure as **ligand 35**, **39** and **45**. The binding energies corresponding to the **ligand 35**, **39** and

45 are -7.04, -7.44 and -6.03 respectively, having different functional groups attached such as –S, -OMe, -F, -Me, and –Br respectively. **Ligand 39** shows higher negative binding energy. It is due to the presence of double oxygen as acceptor. Followed by **ligand 35** has – OMe as the substituent.

Ligand 30 has binding energy of –10.29 kcal/mol. The different functional groups attached such as –S, -OMe, -F, -Me, and -NO respectively. It has the same scaffold structure as **ligand 32**, **36**, **37**, **38** and **40** respectively. The binding energies corresponding to the **ligand 32**, **36**, **37**, **38** and **40** are -5.99, -10.6, -6.70, -6.44, and 7.18 kcal/mol. From these, **ligand 36** shows most negative binding energy due to NO as substituent followed by **ligand 40** has OMe as the substituent.

Ligand 31 has binding energy -6.19 kcal/mol. The different functional groups attached such as –Cl, -OMe, -Me, and -NO respectively. It has the same scaffold structure as **ligand 33**, **34**, **41** and **49** respectively. The binding energies corresponding to the **ligand 33**, **34**, **41** and **49** are -5.84, -6.72, -6.03 and –9.71 kcal/mol. From these **ligand 49** shows most negative binding energy due to NO as substituent through bridge followed by **ligand 34** has pi- cation in as the substituent.

Ligand 42 has binding energy -8.16 kcal/mol. It has the same scaffold structure as **ligand 43**, **56**, **57** and **58** respectively. The different functional groups attached such as –NO,-Br, -F -OMe –Me respectively. The binding energies corresponding to the **ligand 43**, **56**, **57** and **58** are -7.66, -9.51, -6.50 and -8.69 kcal/mol. From here **ligand 56** has highest negative binding energy. It is due to the presence of -F in the structure. It is followed by **ligand 58** due to the presence of –Br with in the structure. That is higher electro negativity character have negative binding energy value.

Ligand 44 has binding energy –5.79 kcal/mol. The different functional groups attached such as –NO, -F -OMe –Me respectively. **Ligand 44** has the same scaffold structure as **ligand 46**, **47**, **48** and **59**. The binding energies corresponding to the **ligand 46**, **47**, **48** and **59** are -7.72, -8.10, +1.37 and -5.15 kcal/mol respectively. **Ligang 47** has highest negative binding energy followed by **ligand 46** due to presence of –OMe in as the substituent. **Ligand 48** shows positive binding energy value or highest binding energy value. The binding energies of **ligand 48** with substituents such as –OMe and $–CF_3$ tend to positive values. This may be due to the highly electron-withdrawing nature of these groups and the presence of the bulky –Boc group.

Ligand 50 has binding energy -5.31 kcal/mol. The different functional groups attached such as –NO, -F, -OMe, –Me respectively. **Ligand 50** the same scaffold structure as **ligand 51**, **52, 53**, **54** and **55** respectively. The binding energies corresponding to the **ligand 51**, **52**, **53**, **54** and **55** are -4.44, -2.39, -0.93, -8.27 and -4.20 respectively. **Ligand 54** has highest negative binding energy value followed by **ligand 50**, the high binding energy of this is due to the presence of unfavorable bump which interacts with various amino acids.

CHAPTER 5

CONCLUSION

The molecular docking studied and conducted by Autodock COX-2 protein and various isoquinoline fused bicycles. Each ligand shows different binding energy due to presence of various functional group in it. The lower the binding energy generally means greater its affinity to bind with the protein. If the ligands containing bulkier group (tertiary butyl, Benzyl, isopropyl, ethyl and Boc groups) the binding energy of its shifted to positive value. When electron withdrawing group was attached to this scaffold the binding energy was shifted to negative. Binding energy calculations revealed that **ligand 3** has obtained the least binding energy from the results obtained after conducting docking with the taken ligands.

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