Selection of Appropriate Inhibitor for Ovarian Type *Cytochrome P450 Aromatase* Gene of *Heteropneustesfossilis* with the Application of Homology Modeling, Molecular Docking and ADMET Analysis

Haleema Sabia and Radha Chaube*
Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India;
chauberadha@rediffmail.com
*Corresponding Author

ABSTRACT:
Background: Cytochrome P450 aromatase (Cyp P450 arom) is an important steroidogenic enzyme responsible for conversion of androgens to estrogens and therefore it plays a critical role in vertebrate reproduction. In contrast to vertebrates, teleost fish have two distinct forms of aromatase. One form predominates in ovaries (ovarian type;*cyp19a1a*), while the other form prevails in brain (brain aromatase;*cyp19a1b*). Aromatase is highly present during the differentiation of ovaries. It is also susceptible to environmental influences, particularly temperature and xenoestrogens, environmental natural and synthetic pollutants.
Methods: In the present study, by applying bioinformatics approach we investigated relationship between catfish ovarian aromatase to its inhibitor related compounds or drugs. The three dimensional (3D) structure of *cyp19a1a*is not predicted experimentally, so its structure is modelled by using comparative modelling approach and further validated by various tools like RAMPAGE, ProSa, Errat. Various bioinformatics approaches such asUniprot, Homology modeling, Molecular docking, ADMET analysis were followed for the selection of appropriate aromatase inhibitor. We mainly emphasised on Docking that was carried out by Autodock 4.2. The ligand molecules docked into the structures of macromolecular targets (aromatase) and as a result of docking we came to know about different Binding Energies of different inhibitor molecules and lastly Docked structures were analysed by Protein-Ligand Interaction Profiler.
Results: Predicted protein model described in this work may be further used for finding interactions with other proteins involved in different types of diseases. Among the drugs Exemestane is highly structurally similar to known active compounds. The selected compounds were further analysed and refined using drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis and with the help of tools we predicted about pharmacokinetic, metabolic, toxicity endpoints.
Conclusion: The study showed that how *in silico* approaches will further increase our ability in the discovery of appropriate drug in the form of Exemestane (which is most stable and perfect aromatase inhibitor among various selected inhibitors). Overall, findings of this study may be helpful in designing the novel therapeutic targets to cure aromatase related cancer disorders.

Keywords : *Catfish, Ovarian Aromatase, Inhibitor, Bioinformatics, Molecular docking,HomologyModeling.*

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I. INTRODUCTION

Aromatase is a member of the Cyp family of synthetic and metabolic enzymes and is encoded by the *cyp*gene (P450arom) that catalyzes the conversion of C19 steroids to estrogens (Callard et al., 2001). The conversion of androgens to estrogens is modulated by the aromatase complex, which consists of a steroid cytochrome P450 aromatase and a ubiquitous flavoprotein, NADPH-dependent cytochrome P450 reductase. CYP19 genes have been identified in mammals (Conley and Hinshelwood, 2001), amphibians (Yu et al., 1993), reptiles (Jeyasuria and Place, 1998), birds (Elbrecht and Smith, 1992; Wartenberg et al., 1992) and teleosts, including freshwater teleosts (Chaube et al. 2015;Tanaka et al., 1992; Trant, 1994; Fukada et al., 1996), marine teleosts (Kitano et al., 2000) and a cartilaginous fishes (Ijiri et al., 2000). In teleosts, there are at least two types of cyp19 isoforms, namely, *cyp19a* and *cyp19b*. *cyp19a*is predominantly expressed in the ovary and plays important roles in sex differentiation and oocyte growth, while *cyp19b* is expressed in neural tissues such as brain and retina and is involved in the developing central nervous system and sex behaviours (Callard et al., 2001).

In teleosts, estrogen is essential for the biosynthesis of yolk protein in the liver and oocyte development in the ovary. In the brain, the synthesis of estrogen by the aromatization of androgen seems to be related to neuroendocrine functions, sexual behaviour and differentiation during the development of the central nervous system.In the last few years the aromatase inhibitors have emerged as a powerful addition to the clinicians' drug against breast cancer. They appear to be more efficacious in the advanced disease setting have become clearly established in the adjuvant setting and are a major focus of ongoing trials in the prevention setting in postmenopausal women.

Bioinformatics is an emerging scientific field which is based on the analysis of biological information using computers and statistical techniques; or we can also say that bioinformatics is the science of developing and utilizing computer algorithms to accelerate and enhance biological research. The number of proteins with known sequences is increasing day by day with the advancement of sequence technologies. A large number of protein structure predictions are very expensive and time consuming. For example structure prediction by using X-Ray crystallography and NMR methods. In today's world of Bioinformatics, we apply different tools for comparative modelling and docking of proteins in a very fast and easy way.

Aromatase activity in*H. fossilis*is the outcomeof seasonal variations and this has been estimated by a microassayanalysis which explain that they are correlated to sex steroids, vitellogenin and ovarian weight during circannual reproductive cycle. In the female catfish, aromatase activity was detectable in the hypothalamus throughout the year whereas in ovary only during active vitellogenesis. In the catfish, hypothalamic aromatase levels increases two times during annual gonadal cycle, once in a fully gravid fish (i.e; in an advanced stage of pregnancy) and then in a reproductively quiescent fish. On the other hand, increase in the ovarian aromatase activity was observed only during vitellogenesis, which showed a direct correlation with plasma levels of sex steroids. Further, hormonal levels of testosterone and estradiol suggested a precursor-product relationship. At the completion of vitellogenesis, ovarian aromatase activity declined sharply resulting in elevation of plasma testosterone levels, which in turn could be utilized as substrate by the hypothalamic aromatase whose activity was the highest in the postvitellogenic catfish. Two isoforms of gene, *cyp19a* and *cyp19b*, coding for aromatase in ovary and brain respectively were expressed in the catfish.

The three dimensional (3D) structure of the *cyp19a1a*of*Heteropneustesfossilis*is not reported in PDB. In this work, a computational approach is applied to predict the 3D structure of the *cyp19a1a* by homology modeling and to reveal the insights of ***cyp19a*** 3D structure. 3D structure is necessary for the selection of suitable drug and thus without 3D structure, it is very hard to elucidate the inhibitor's binding sites. 3D structure is an effort to create an environment of protein and thenanalyze the drugs. SWISS MODEL is one of the most reliable software for homology modelling. It predicts the authenticity of structure by analysing loops, beta sheets, coils, helices, phi and psi angles by using template structure. This method generates valid and reliable structures by using suitable template having appropriate amino acids identity.Structurally, *cyp19a*1ais a glycoprotein with single peptide chain with subunit having 514 amino acid. Here we emphasise on model (obtained after homology modelling), various attempts were made to build models by evaluating binding ability of different inhibitors to that of aromatase. Moreover, these type of studies also helps in disclosing the molecular mechanism behind various types of diseases, other abnormalities and other strategies which would came into view for the discovery of authentic medicine to regulate the process.

In this paper, we report virtual screening studies to screen selected inhibitors for the protein and to investigate the influence of molecular structure and biological activity involved with Receptor-Inhibitor Complex. Several studies has been carried out in this field and most of them reported that drug receptor compatibility always depends on robustness and domain acceptances of the drug.Nowadays, molecular docking is most commonly used strategy for the drug designing process, to understand the interaction between drug and receptor molecules. The 3D structure of the protein inhibitor complex could be served as a considerable source of understanding the way of protein interact with one another and perform biological function.

II. MATERIALS AND METHODS

Following Tools And Database Used-

UniProt – It is a database for retrieving protein sequence and annotation data. UniProt contains data which is the result of collaborative work of the European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics and the Protein Information Resource (PIR),**BLAST-**The Basic Local Alignment Search Tool (BLAST) is used to identify about functional and evolutionary relationships between sequences as well as it also helps in the identification of members of gene families,**ClustalX2 –** It is a tool used for the multiple sequence alignment program which provides user friendly platform for performing multiple sequence alignments and analysing the results, **MEGA –** This is a tool used for making Phylogenetic tree and as a result of that it helps in the Phylogenetic analysis and establishing evolutionary relationship among organism, **SWISS-MODEL -** It is an online tool for homology-modelling, **QMEAN -** It is a composite scoring function which is able to derive both global (*i.e.* for entire structure) and local (*i.e.* for per residue) error estimates on the basis of one single model, **ProtParam-** It is also an online tool which provide various physical and chemical parameters for a given protein sequence computationaly, **RAMPAGE –** It is also anOnline tool for Ramachandran Plot analysis. It is one of the method for analysis of Protein structure, **Errat-**It is also an online

tool for verifying protein structures determined by crystallography, **ProSa-**Online tool for structure analysis, **ChemSpider**-Itis a free database for chemical structure which provide fast access to various structures, properties and associated information It owned by the Royal Society of Chemistry, **ChemSketch** - ChemSketchFreeware is a drawing package thatallows user to draw chemical structures, **OpenBabel–**It is a chemical toolbox, **Autodock -** It is a suite of automateddocking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure, **Cygwin -** It is a large collection of GNU and Open Source tools which provide functionality similar to a Linux distribution on Windows, **Molinspiration**-Online tool for the analysis of chemical properties and bioactivity of ligand molecule, **UCSF Chimera -** It is a highly extensible tool for interactive visualization and analysis of molecular structures and related data, **Protein-Ligand Interaction Profiler –** Online tool for the analysis of BindingResidues . It is used for easy and fast identification of noncovalent interactions between proteins and small molecule ligands.

Retrieval of target sequence from database-

The complete amino acid (514 aa) sequence of *cyp19a1a* encoding protein was retrieved from Uniprot database with accession number A0A068CLX6 in FASTA format. Further analysis of target sequence has been performed by BLAST and Phylogenetic analysis.

Homology Modeling-

Query sequence and template structure were aligned and preparation of 3D structure of Protein of *cyp19a 1a*is done by using SWISS MODEL. Thus, SWISS MODEL helped in the assessment of 3D structure of proteins.

Model Evaluation –
The recognition of errors in models of protein structures is a major problem in structural bioinformatics. There is no single method that consistently and accurately predicts about the errors in 3D structure. The model that was generated by SWISS MODEL and selected on the basis of SWISS MODEL evaluation score.After modeling, the proteins structure was further evaluated by various other tools like ProtParam, RAMPAGE, Errat and ProSa.

Preparation of Ligands-
In-silico generation of ligands is an important step for the Protein- Ligand Docking and for that we have to filter the drug from one of the best available database and thus we had done filtering of chemical structures screening with database namely ChemSpider.

Ligand preparation was carried out on the ChemSketch tool in the form of .mol format and that was further converted into .pdb format by OpenBabel.

Docking Experiment –
Protein-ligand docking is a process for promising and consistent scoring scheme to evaluate the protein-ligand complex in order to select the best binding conformations in which two molecules fit together in 3D space and it is a key tool in structural biology as well as in computer-aided drug design. The goal of ligand and protein docking is mainly to predict the major binding mode(s) of a ligand with a protein of known three-dimensional structure**.**The molecular docking of various ligands against modelled 3D structure of cyp19a1a proteins performed using Autodock v4.2. Prepared Ligands are rigidly docked to receptors of cyp19a using Glide extra precision function. The conformational search of the ligand is performed by applying the Lamarckian genetic algorithm. Receptor docking is done by Glide [Grid-Based Ligand Docking with Energetics] in AutoDock suite**.** Glide is an integrated platform and asystematic approach for searching conformations, orientations and positions of ligand in the receptor site using a series of hierarchical filters which improves the binding affinities by lowering the penalties.

Virtual screening-Chimera 1.8 used for visualization of docked structure.

ADMET analysis

Molinspiration is used to predict pharmacokinetic properties and drug likeness of the selected ligand. Molinspiration is also used to evaluate the bioavailability of the lead molecules by assessing their physicochemical properties to observe the range of the Lipinski rule for induced molecules**.** These compounds are also evaluated for their chemical behaviour through analysis of pharmacokinetic parameters required for absorption, distribution, metabolism, excretion and toxicity (ADMET). Finally minimized poses are rescored by Glide scoring function and visualized and data recorded through Protein-Ligand Interaction Profiler tool.

INHIBITORS FOR DOCKING

Online Tool - ChemSpider

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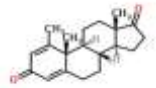
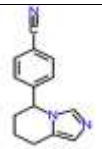
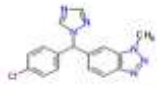
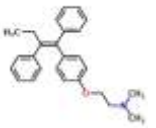
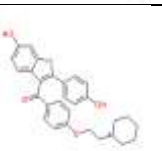
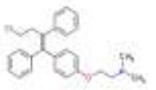
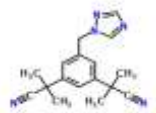
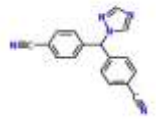
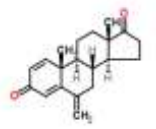
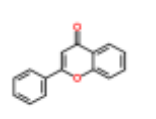
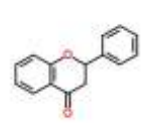
Table 1-List of Inhibitors used in Docking

S.N.	Chem Spider ID	Chemical	Molecular Formula	Molecular Weight (Da)	Chemical Structure
1.	**51438**	Atamestane	$C_{20}H_{26}O_2$	298.419	
2.	**53850**	Fadrazole	$C_{14}H_{13}N_3$	223.273	
3.	**54791**	Varozole	$C_{16}H_{13}ClN_6$	324.7676	
4.	**2015313**	Taremifene	$C_{26}H_{29}NO$	371.515	
5.	**4859**	Raloxifane	$C_{28}H_{27}NO_4S$	473.583	
6.	**2275722**	Tamoxifen	$C_{26}H_{28}ClNO$	405.960	
7.	**2102**	Anastrazole	$C_{17}H_{19}N_5$	293.366	
8.	**3765**	Letrozole	$C_{17}H_{11}N_5$	285.303	
9.	**54278**	Exemestane	$C_{20}H_{24}O_2$	296.403	
10.	**10230**	Flavone	$C_{15}H_{10}O_2$	222.239	
11.	**9833**	Flavanone	$C_{15}H_{12}O_2$	224.255	

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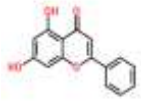
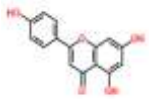
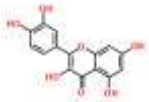
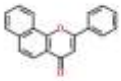
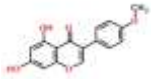
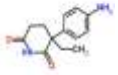
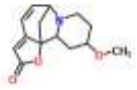
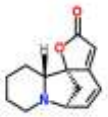
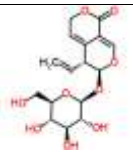
12.	**4444926**	Chrysin	$C_{15}H_{10}O_4$	254.238	
13.	**4444100**	Apigenin	$C_{15}H_{10}O_5$	270.237	
14.	**4444051**	Quercetin	$C_{15}H_{10}O_7$	302.236	
15.	**11297**	7,8-Benzoflavone Or α-Naphthoflavone	$C_{19}H_{12}O_2$	272.297	
16.	**4444068**	Biochanin A	$C_{16}H_{12}O_5$	284.263	
17.	**2060**	Aminoglutethimide	$C_{13}H_{16}N_2O_2$	232.278	
18.	**497156**	Phyllanthine	$C_{14}H_{17}NO_3$	247.2897	
19.	**391181**	Securinine	$C_{13}H_{15}NO_2$	217.264	
20.	**80043**	Gentiopicrin	$C_{16}H_{20}O_9$	356.325	

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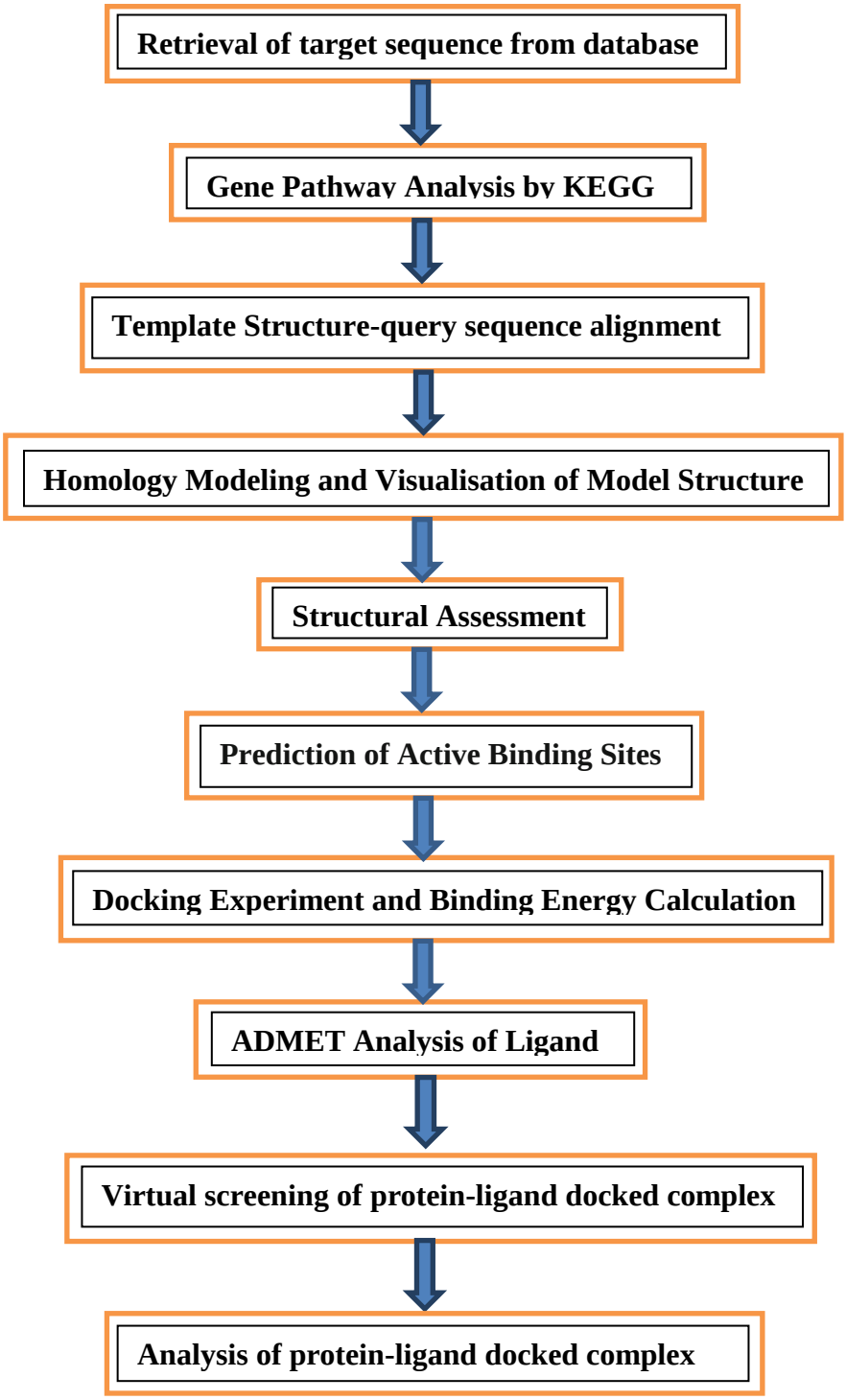
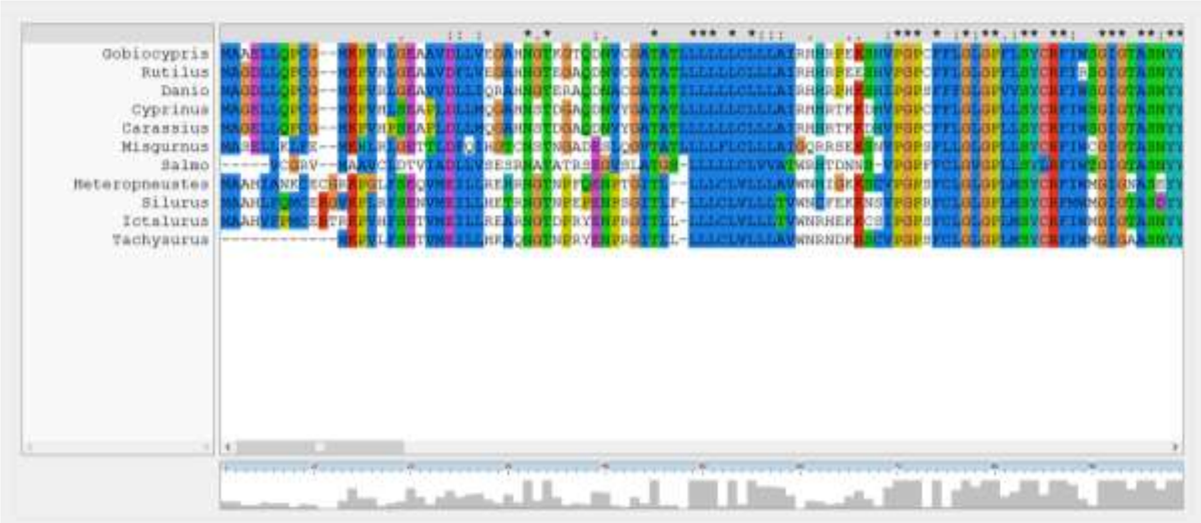
WORK FLOW CHART



RESULTS

Protein sequence similarity network

The sequence set which were downloaded from Uniprot were used for the selection or generation of similar sequence and that were carried out by BLAST and as a result of BLAST we came to know about the E-values which were computed, and the sequence similarity networks were then generated . And from that result of BLAST we get the idea about top ten identical sequence of ***Heteropneustesfossilis*** and they are; *Ictaluruspunctatus*, *Tachysurusfulvidraco*, *Silurusmeridionalis*, *Daniorerio*,*Gobiocyprisrarus*, *Cyprinuscarpio*, *Carassiusauratus*, *Rutilusrutilus*, *Cyprinuscarpio*, *Misgurnusanguillicaudatus* and the selected sequence were analysed in ClustalX and complete alignment were performed .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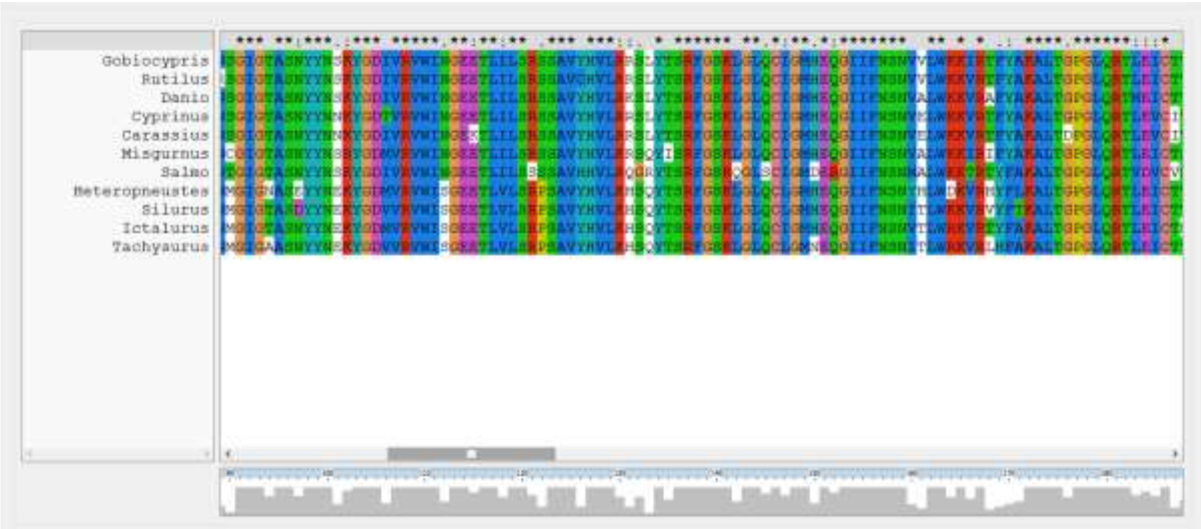
Ictalurus
Tachysurus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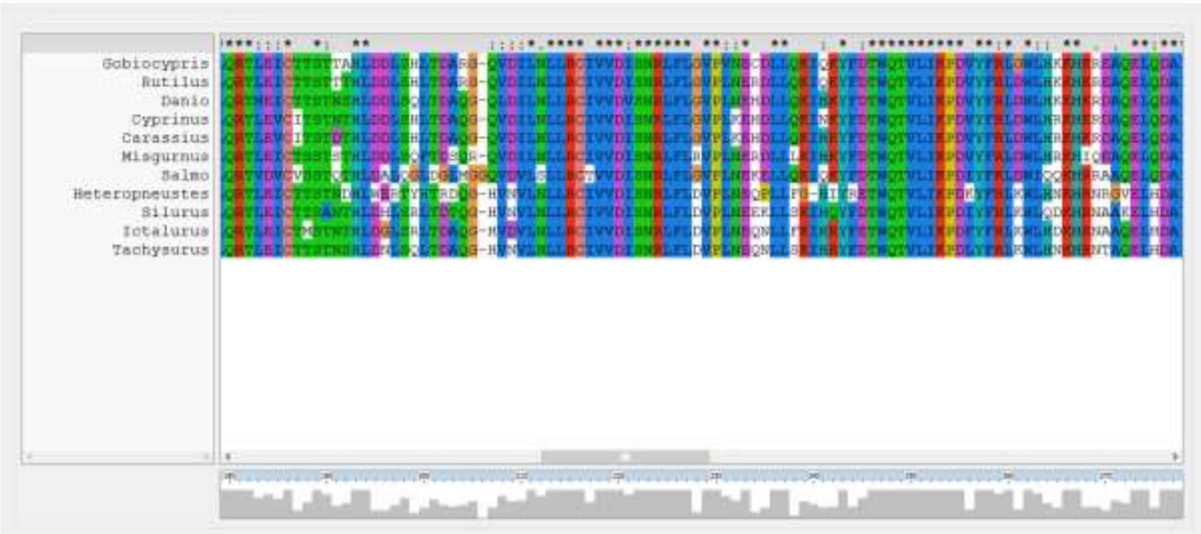
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Misgurnus
Salmo
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Silurus
Ictalurus
Tachysurus

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Figure 1-Multiple sequence alignment of cyp19a gene with various organism sequences using ClustalX method. Dashes represent gaps introduced to maximize alignment. The colouring intensity represents the degree of conservation

As a result of Complete Alignment, Phylogenetic tree were obtained in the form of .dnd file and thus on the basis of BLAST E-value we came to know about the idea of evolutionary relationship with different organisms.

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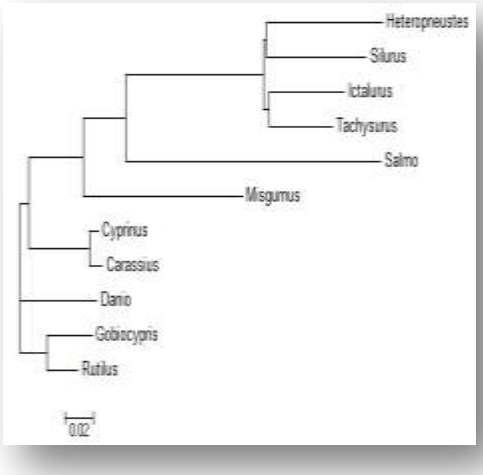

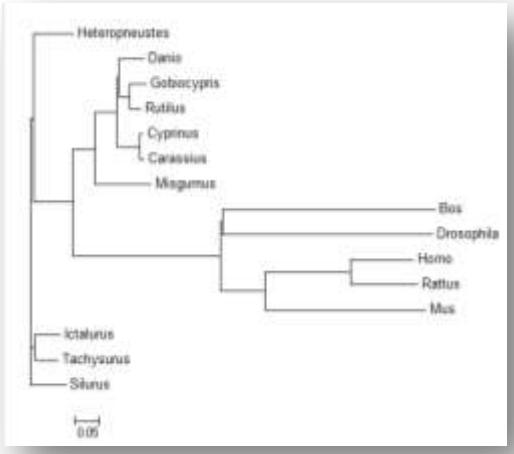

Figure 2 **Figure 3**

Figure 2-Phylogenetic Analysis of related species on the basis of E-value.

Figure 3-Phylogenetic Analysis of random sequences.

In Figure 2 Phylogenetic relationship has been described in the form of Phylogenetic tree and it shows about the closest relationship of ***Heteropneustesfossilis*** with*Silurusmeridionalis.*After that random sequence of cytochrome family were selected and on the basis of that we came to know about the relationship of *Heteropneustesfossilis* Cytochrome P450 with other organisms like *Homo sapiens*Aromatase ,*Rattusnorvegicus* Cytochrome P450 , *Drosophila melanogaster* Cytochrome P450 etc.

In the Figure 3Phylogenetic Analysis of random sequences has been explained and along with other organism it shows result on the basis of sequence alignment.

Prediction of 3D Structure by SWISS MODEL -

SWISS MODEL generated 3D models by using the sequence of **cyp19a** of *Heteropneustesfossilis*which was downloaded from Uniprot.

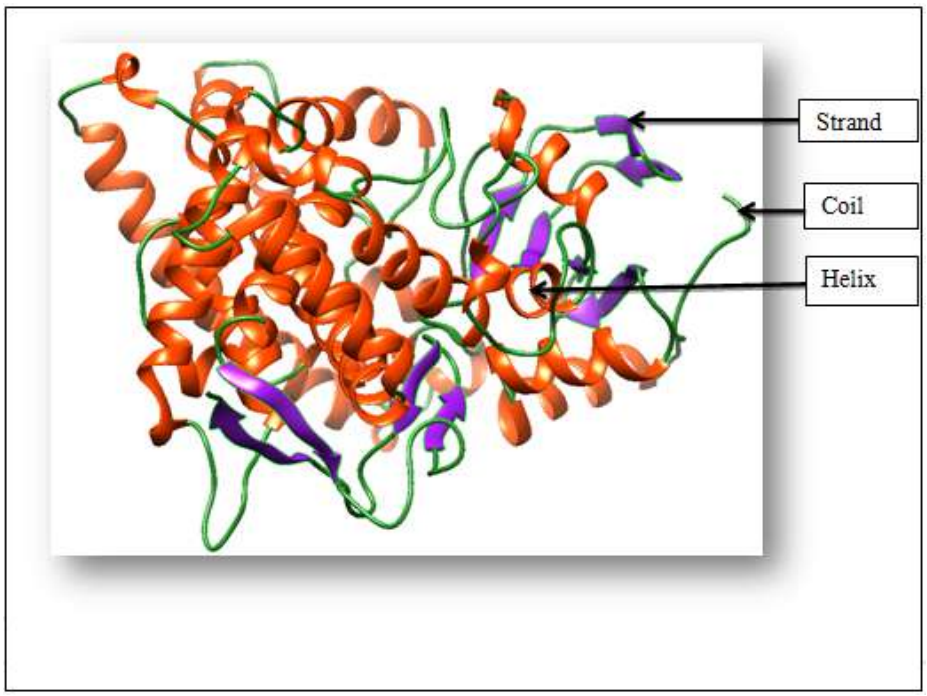

Figure 4- Visualisation of Model obtained after Homology Modeling in Chimera.

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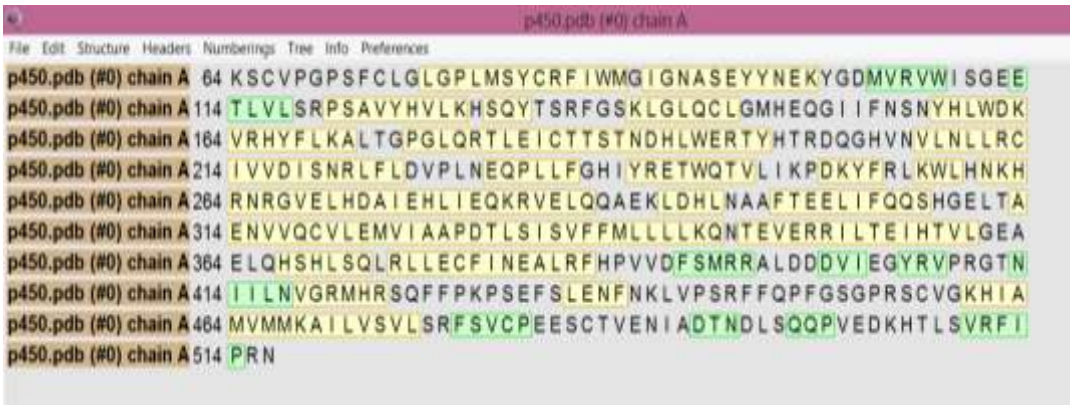

Figure 5-Sequence Analysis in Chimera

Structural Assessment-For further evaluation of the predicted 3D structure, model was submitted into various tools like QMEAN,RAMPAGE, Errat, ProSa.

Model validation by QMEAN-

This will provide structural information about the model structure and this is described in following graphs.

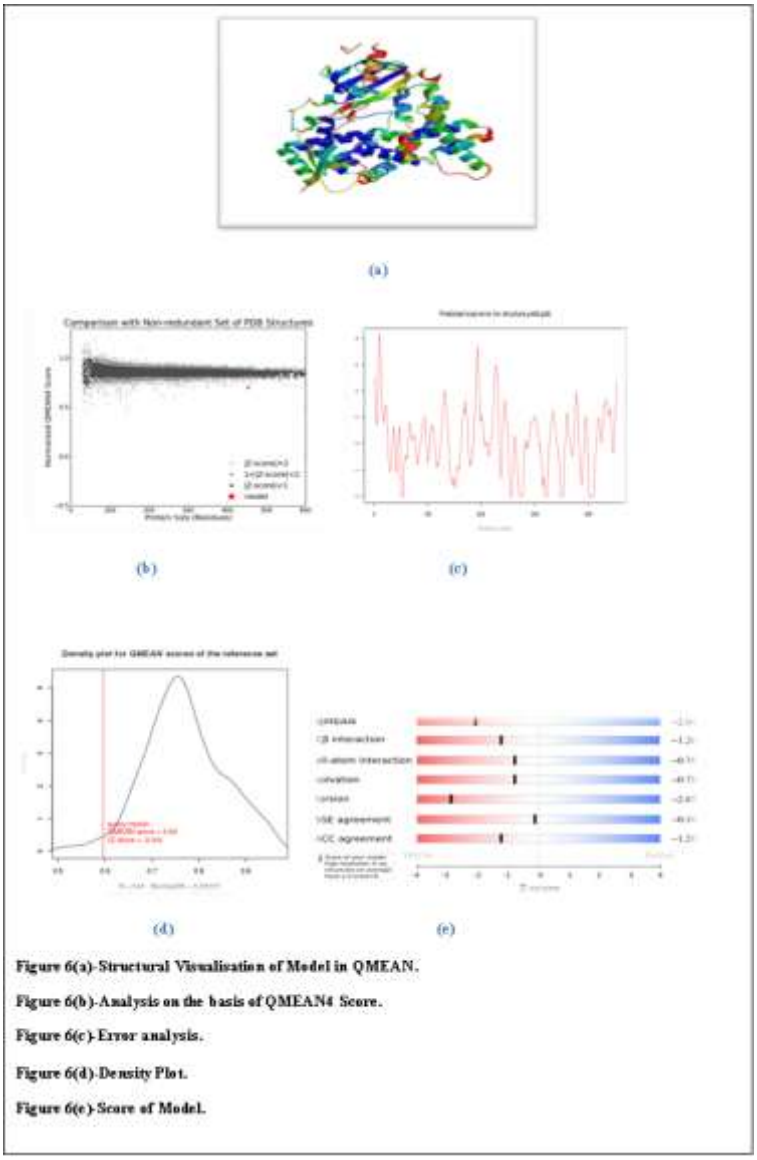

Figure 6(a)-Structural Visualisation of Model in QMEAN.

Figure 6(b)-Analysis on the basis of QMEAN4 Score.

Figure 6(c)-Error analysis.

Figure 6(d)-Density Plot.

Figure 6(e)-Score of Model.

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Model validation by RAMPAGE-

Ramachandran plots showed Φ and Ψ distributions of non-Glycine, non-Proline residues (Figure 12(a) and Figure 12(b)) and gave residues distribution (Table 3). The phi and psi angles originated were plotted against each other to differentiate the favourable and unfavourable regions. These angles were used to evaluate the quality of regions.

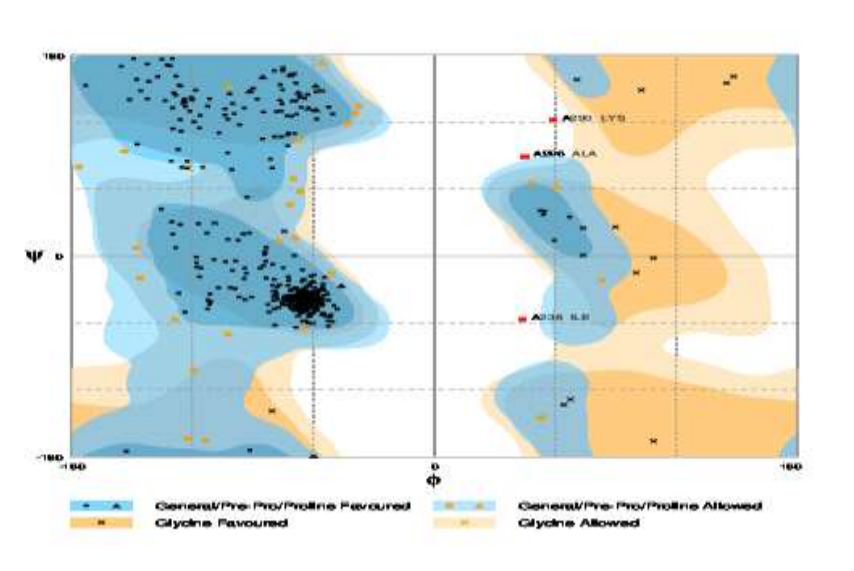

Figure 7(a)-Ramachandran plot

Model validation by Ramachandran Plot-

It provide information about permitted value of *phi* and *psi* and there distributions indicated on 2-D map.As a result of that we came to know about the favoured region(also known as low energy region) correspond to conformations where there are no steric clashes and allowed region correspond to conformations having outer limit van der Waals distances where atoms are allowed to come a little closer.

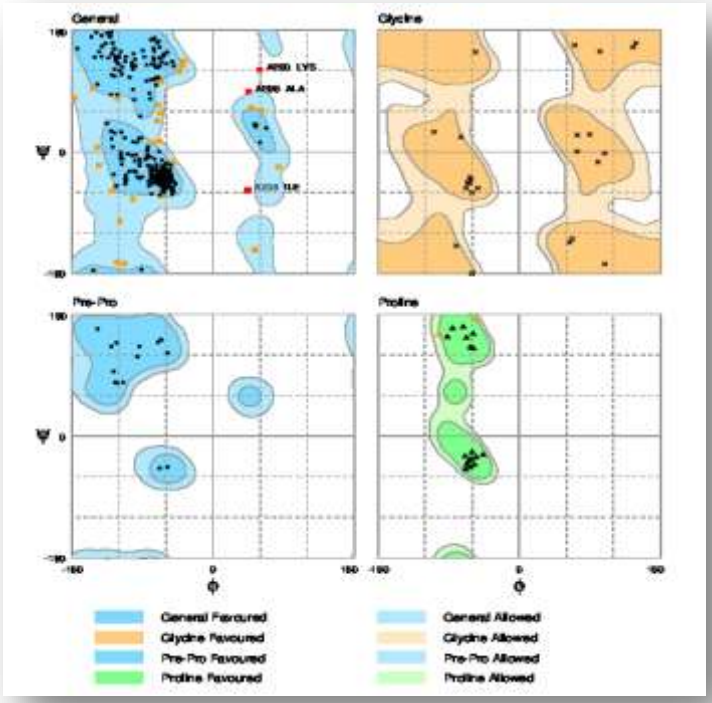

Figure 7-Allowed region in Ramachandran Plot.

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Table 2-Analysis of Ramachandran Plot

Residues Distribution	% Residues Expected	Residues in Model
Number of residues in favoured region	(~98.0% expected)	419 (92.9%)
Number of residues in allowed region	(~2.0% expected)	29 (6.4%)
Number of residues in outlier region		3(0.7%)

Model validation by Errat –

3D predicted model of***cyp19a*** was subjected into the Errat protein structure verification server. Errat provided an Overall quality factor of model as 84.49%, which is near about satisfactory (Figure 8).

On the error axis, two lines were drawn to indicate the confidence with which it is possible to reject the regions that exceed the error value. It expressed as the percentage of the protein for the calculated error value falls below the 95% of rejection limit. Good high resolution structure generally produce values around 95% higher. For lower resolutions (2.5 to 3.0 A), the average overall quality factor is around 91%. Errat produces a plot that gives the value of error function which showed confidence limits by comparing with statistical analysis from highly refined predicted structures. So, 84.49%, overall value is not much reliable but can be satisfactory for further analysis because we are using modelled structure.

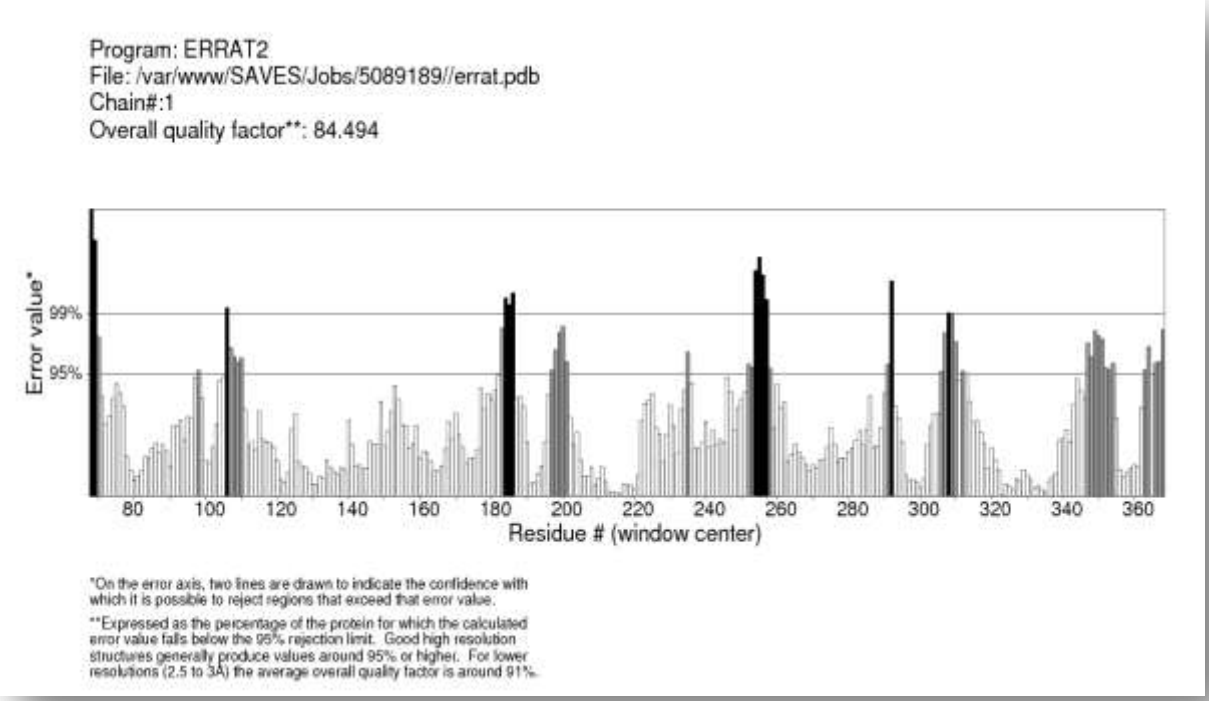

Figure 8- ERRAT result showing an overall quality factor of model (error-axis showing the error values to reject regions that exceed error value).

Model validation by ProSa-

ProSa gave Z-score plot (Figure 9a) of protein structure to determine the overall model quality. The Z-score of the predicted model was -8.17 which represents a good quality model. The local model quality was judged by plotting energies as functions of amino acids in ProSa residue score plot (Figure 9b).

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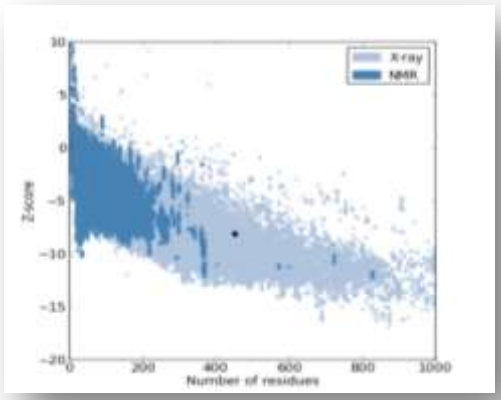
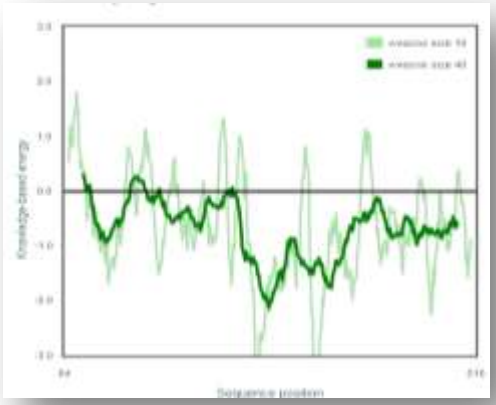
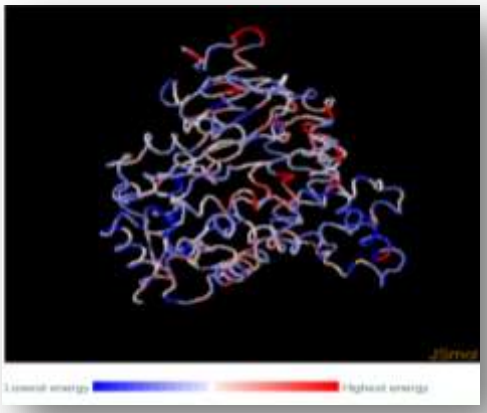
Z Score- -8.17



(a)



(b)



(c)

Figure 9(a)-Overall Model Quality

Figure 9(b)-Local Model Quality

Figure 9(c)-Visualisation of Model Structure

RESULT-

Docking Study-AutoDock 4.0.2 tool was employed to explore how the ligand binds to the respective protein, best structural information, functionally interacting residues and the binding conformation. The list of ligand for **cyp19a** is described in Table 1 and after docking there energies are observed as:-

Table 3- Ascending Order of Molecule's Binding Energy.

RANK	S.No.	Name of Drug	Ascending Order Of Min. Binding Energy(kcal)
1	9	Exemestane	-8.91
2	1	Atamestane	-7.9
3	15	7,8-Benzoflavone	-7.66
4	3	Varozole	-7.45
5	12	Chrysin	-7.39
6	14	Quercetin	-7.03
7	13	Apigenin	-6.98
8	16	Biochanin A	-6.95
9	10	Flavone	-6.86
10	11	Flavanone	-6.74
11	7	Anastrazole	-6.63
12	8	Letrozole	-6.57
13	2	Fadrazole	-6.1
14	19	Securinine	-6.08
15	5	Raloxifane	-6.02
16	20	Gentiopicrin	-5.93
17	17	Aminoglutethimide	-5.39
18	18	Phyllanthine	-5.21
18	6	Tamoxifen	-5.21
20	4	Taremifene	-4.7

Minimum binding energy is an important concept in Molecular Docking and thus it plays crucial role in binding of ligand with receptor and according to the above experiment top 5 ligands are Exemestane,Atamestane,7,8-Benzoflavone,Varozole,Chrysin and there binding energies are -8.91 ,-7.90 ,-7.66,-7.45,-7.39 kcal respectively.

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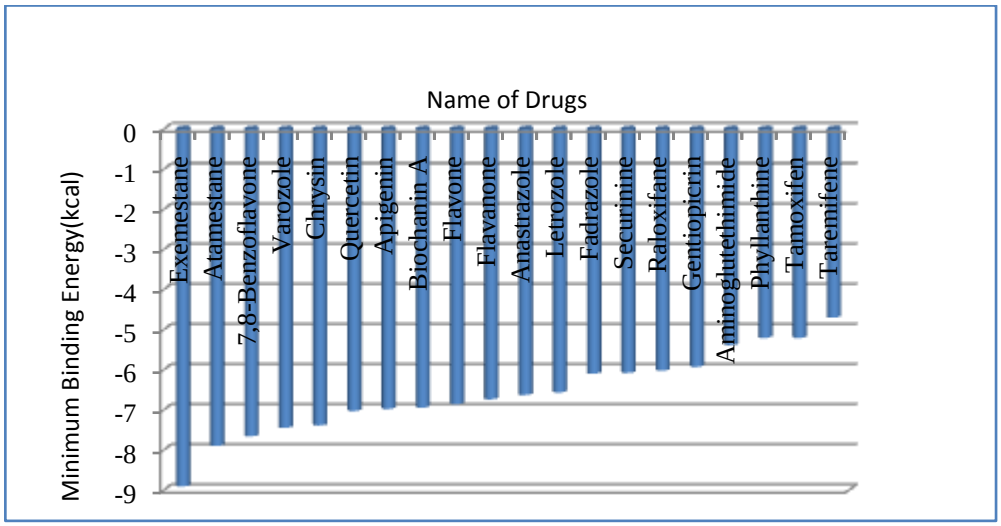


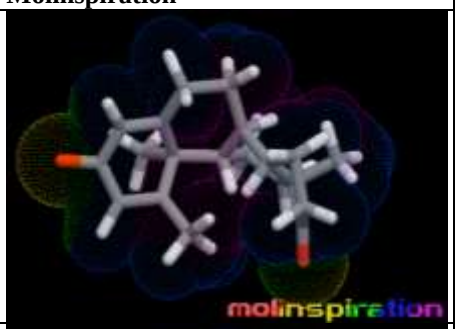
Figure 10-Graph on the basis of RMSD value.

As a result of docking we came to know about the result that complex of ligand (Exemestane) binds at the main active-site of cyp19a receptor with the lowest binding energy −8.91 kcal.

Molecular Analysis by Molinspiration-

Further analysis of top 5 inhibitors were carried out by Molinspiration and they are described as follows:-

Table 4-3D Structure obtained from Galaxy 3D Generator of Molinspiration.

S.No.	Inhibitor	SMILES	3D Structure obtained from Molinspiration
1	Exemestane	C[C@]12CC[C@H]3[C@H]([C@@H]1CCC2=O)CC(=C)C4=CC(=O)C=C[C@]34C	
2	Atamestane	CC1=CC(=O)C=C2[C@]1([C@H]3CC[C@]4([C@H]([C@@H]3CC2)CCC4=O)C)C	
3	7,8-Benzoflavone	c1ccc(cc1)c2cc(=O)c3ccc4ccccc4c3o2	

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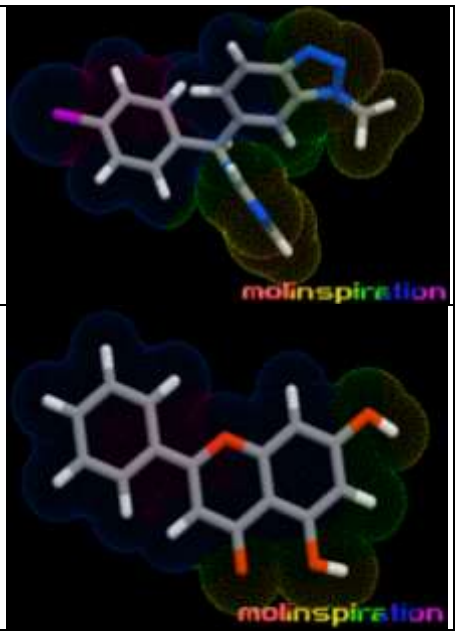
4	Varozole	CN1C2=C(C=CC(=C2)[C@H](C3=CC=C(C=C3)Cl)N4C=NC=N4)N=N1	
5	Chrysin	c1ccc(cc1)c2cc(=O)c3c(cc(cc3o2)O)O	

Prediction of bioactivity score-From the above analysis it has been explain thatExemestane is best ligand among all 20 ligands and in further analysis we will focus on Exemestaneonly.Bioactivity is an important parameter for the assessment of protein structure .In the Figure 11(a), 11(b), 11(c), 11(d), 11(e) and 11(f) graph shows about the comparative study of ideal model with that of selected ligands(Exemestane).

This gives us the overview of the some properties of selected ligand i.e, Exemestane.

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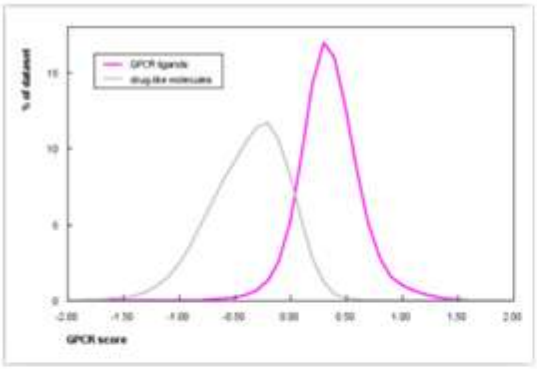
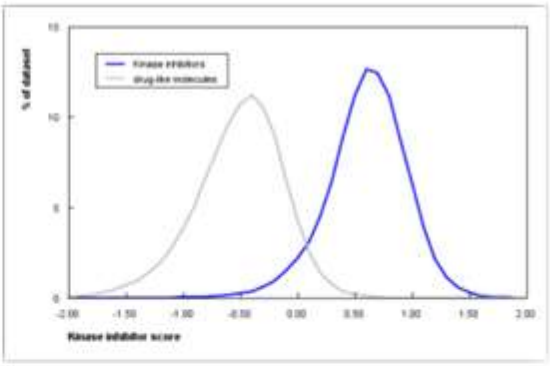
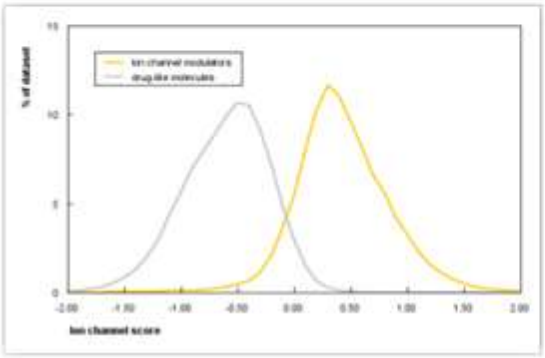


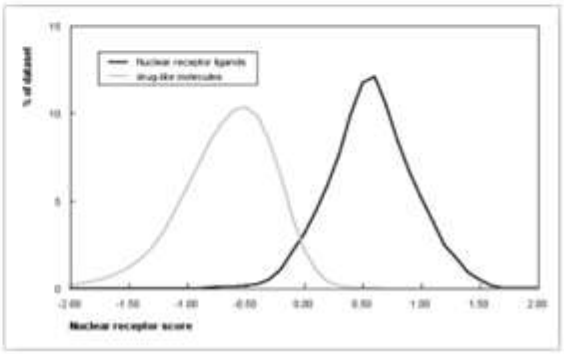
(a)



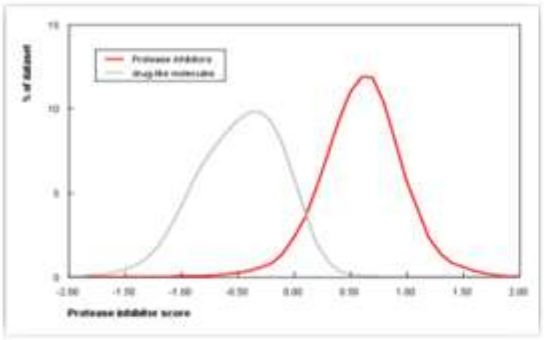
(b)



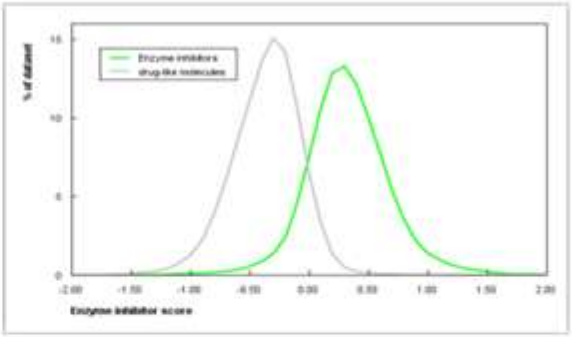
(c)



(d)



(e)



(f)

Figure 11(a)- Graph in between GPCR Ligands vs. Drug like molecules.

Figure 11(b)- Graph in between Kinase inhibitors vs. Drug like molecules.

Figure 11(c)- Graph in between Ion channel modulator vs. Drug like molecules.

Figure 11(d)- Graph in between Nuclear receptor ligands vs. Drug like molecules.

Figure 11(e)- Graph in between Protein inhibitors vs. Drug like molecules.

Figure 11(f)- Graph in between Enzyme inhibitors vs. Drug like molecules.

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Visualisation of Protein-Ligand Docked Structure in Chimera 1.8 -

The protein-ligand docked complex post docking analysis and amino acids found in binding pocket of protein were identified by Chimera 1.8.

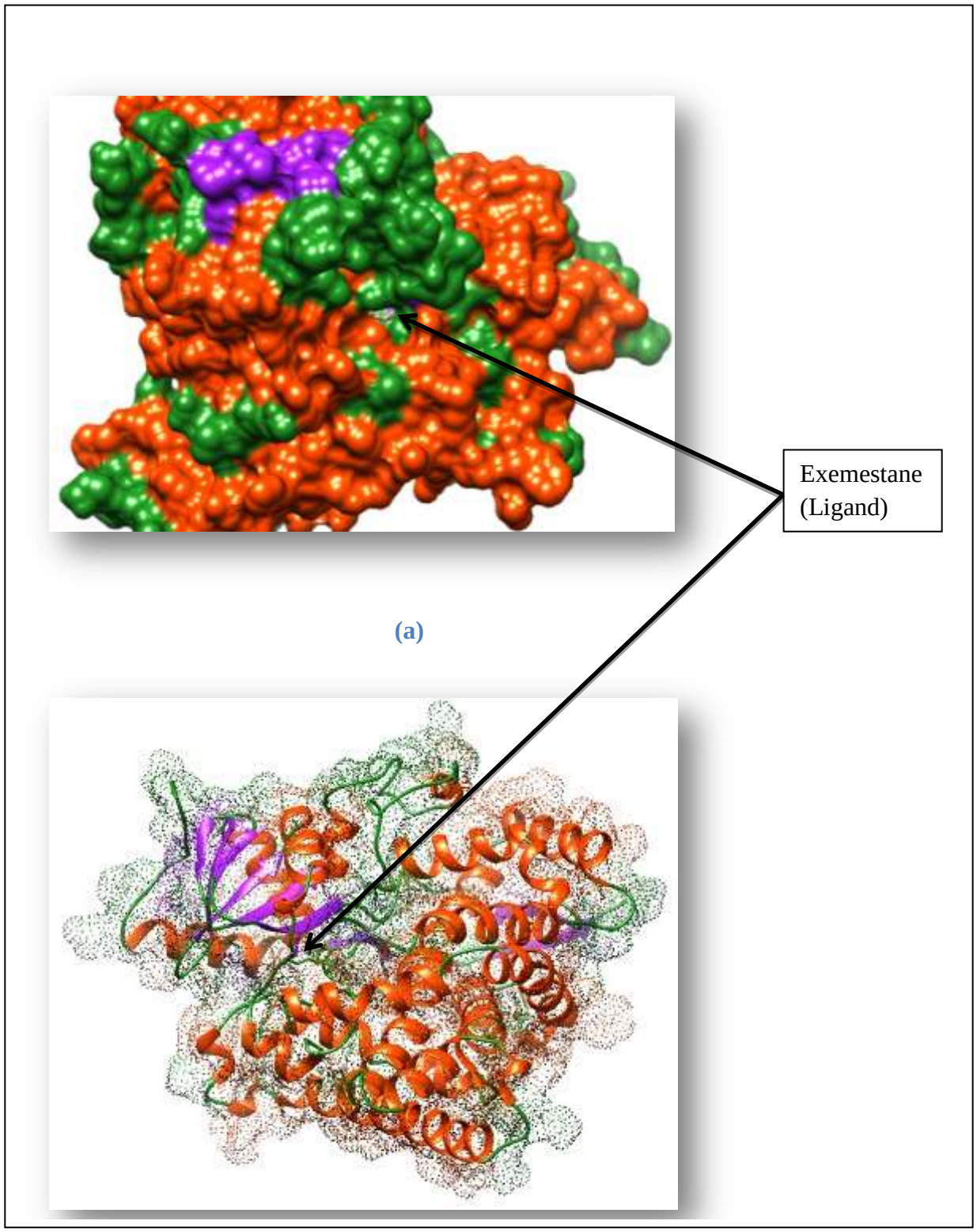

Figure 12-Visualisation of Docked structure of Exemestane with cyp19a.(a)Surface solid view,(b)Surface dot view.

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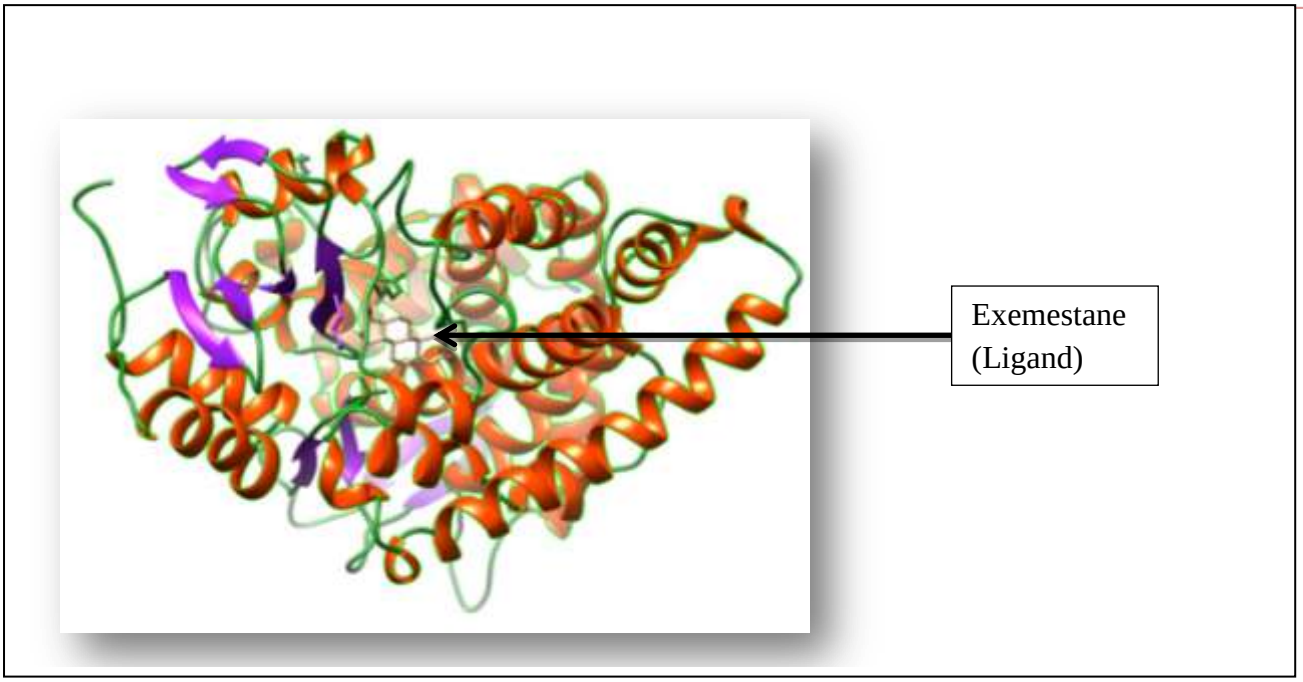

Figure 13-Visualisation of Docked structure of Exemestane with cyp19a (Surface hide).

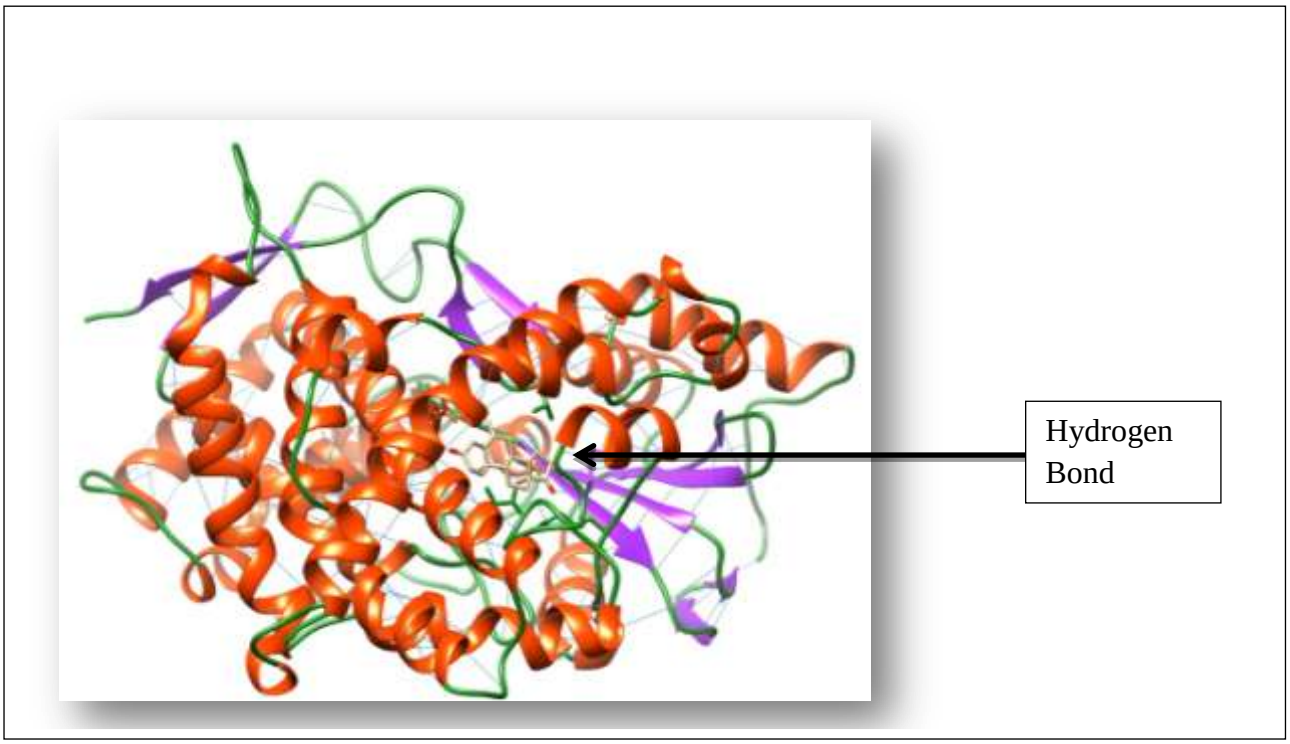

Figure 14-Visualisation Hydrogen Bond in Docked structure of Exemestane with cyp19a1a

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DISCUSSION

Analysis of Docked Structure with Protein-Ligand Interaction Profiler –

As a result of molecular docking which were conducted by automated docking Tools (AutoDock) that has allowed to conclude the ligand-receptor interactions of selected inhibitor. The analyses of the interactions between *cyp19a* and inhibitor(Exemestane) have pointed out the best complex as a result of Protein-Ligand Interaction Profiler. By analysing drug score and Lipinski's rule of five, it is suggested that inhibitor is proven as a potential inhibitor for *cyp19a*.

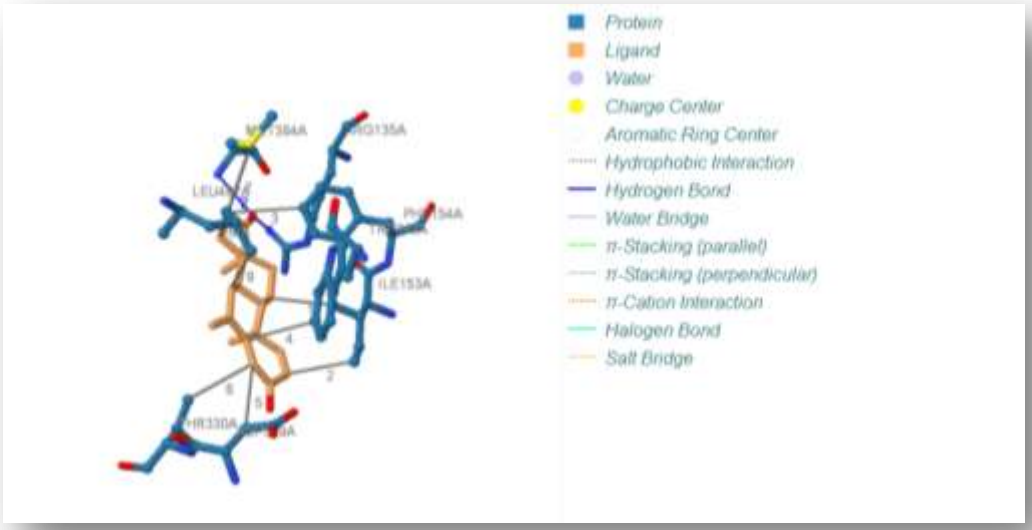

Figure 15-Visualisation of docked structure in Protein-Ligand Interaction Profiler

In the Figure 15 visualisation of docked structure and the interaction of ligand with the residue has been described and in Figure 16 the other factor like Hydrophobic interaction and Hydrogen bonding has been described.

Figure 16-Binding sites of the residues

In this *in silico*analysiswe concluded about the predicted structure of *cyp19a1a* has a good degree of accuracy.The final refined model was assessed by different evaluation programs.Ramachandran plot values indicated ideal results of predicted model as residues in favoured regions which are 92.9%,allowed region which are 6.4% while only 0.7% residue was in the outlier region. Overall model quality was measured by ERRAT showing reliable model having 84.49%, overall quality factor.Tools (AutoDock) that has allowed the ligand-receptor interactions of selected inhibitor and the analysis of the interactions between *cyp19a* and inhibitor have given result on the basis of least binding energy. Binding Energy is nothing but it is a mechanical work that must be done against the force which hold the molecule together.Among the rest 19 drugs,Exemestane is highly structurally similar to known activecompounds. In conclusion, this analysis suggests that the selected inhibitor is very affective to *cyp19a1a*.

Predicted protein model described in this work may be used for finding interactions with other ligands which are associated with certain type of diseases involving disruption of steroid pathway. In this way, the biochemical and pharmaceutical properties of Exemestane can bealtered to prepare more effective drug molecules in a desired chemical form and thus further studies and synthesis of novel compounds considering these findings can expect similar response rates and cure of various diseases. Further, this information can be exploited in selection of suitable inhibitor molecules and then these molecules can be tested with wet lab experiments in relation to various disorders.

ACKNOWLEDGEMENTS: Partial assistance by UGC-CAS is acknowledged.

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