Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Investigation Of In-Silico Studies Of Cytochrome P450 Inhibitors

Jyoti Pandey [1]*, Niharika Gokhale [2], Poonam Sharma [3], Himani Hirvey [4], Sanjay Jain [5]

1. Assistant Professor, Faculty of Pharmacy, Medi-Caps University, Pigdamber, Rau, AB Road, Indore-453331
2. Assistant Professor, Faculty of Pharmacy, Medi-Caps University, AB Road, Pigdambar,Rau, Indore,453331,(M.P.)
3. Lecturer, Faculty of Pharmacy,Medi-caps University, A.B. Road, Pigdambar, Rau Indore-453331
4. Assistant Professor, Faculty of Pharmacy, Medi-Caps University, Pigdamber Rau, Indore 453331
5. Dean & Professor, Faculty of Pharmacy, Medi-Caps University, Pigdamber, A. B. Road Rau, Indore- 453331

Corresponding Author: Dr. Jyoti Pandey
Designation and Affiliation: Assistant Professor, Faculty of Pharmacy, Medi-Caps University, Pigdamber, Rau, AB Road, Indore-453331
Email id: jyoti.pandey@medicaps.ac.in

Cite this paper as: Jyoti Pandey, Niharika Gokhale, Poonam Sharma, Himani Hirvey, Sanjay Jain (2024) Investigation Of In-Silico Studies Of Cytochrome P450 Inhibitors. *Frontiers in Health Informatics, 13* (3),9848-9856

ABSTRACT:
The current paper describes computational techniques for designing the three-dimensional structure of the human enzyme "Cytochrome P450 4A11" using the Uniprot sequence (UniProtKB – Q02928). To create a three-dimensional model of the cytochrome P450 protein, a homology modelling study was conducted. The Modeler9.17 software application was used to create the model. The AUTODOCK4.2 program was used to further dock the generated model with medications that were already on the market. Molecular docking tests were conducted using Autodock4.2 with four medicines after the model was designed in order to determine the functional effect of the protein. Over 89% of the amino acids in the most preferred region are displayed in the created model. Every medication exhibits strong interactions and binding energy. When interacting with Gly453, the molecule telenzepine exhibits the greatest binding energy of -9.69 kCal/mol. various studies offer insight and interpretation into the data generated by various techniques. It clarifies how molecules interact in the area of the active site.

Keywords: *Timolol, Telenzepine, Benzylpyridine, Celecoxib, Molecular Docking*

INTRODUCTION

There are different classifications for the superfamily of membrane-bound hemoprotein isozymes known as cytochrome P450. [1] Although they are found in the majority of bodily tissues, CYP enzymes are mostly found in the liver, kidneys, and intestines, with the liver having the largest concentration. Six of the 57 isozymes that have been identified so far account for 90% of drug metabolism. CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 are among these six isozymes. [2, 3, 4] Determining which isozymes are impacted is crucial in drug development because different medications act on different isozymes. [5]

Most importantly, it is essential to determine which medications are inhibitors or inducers of these enzymes. The majority of medicines are deactivated by metabolism, either directly or through assisted excretion. [6] Drugs that target the same CYP isozyme and are taken concurrently are usually metabolised and eliminated more quickly if the drug works as a CYP inducer. In these situations, if a medicine is eliminated by the body more quickly, its plasma levels might not exceed a threshold value of benefit, which could result in treatment failure. Conversely, if a medication functions as a CYP inhibitor, it can lead to the accumulation of other medications to hazardous levels, which can result in overdoses and other effects. When it comes to prodrugs, these circumstances are not true. [7, 8, 9, 10, 11]

Timolol, (2S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol, Molecular formula $C_{13}H_{24}N_4O_3S$. [12] A nonselective beta-adrenergic antagonist called timolol is used as an eye drop solution to lower intraocular pressure, or eye pressure. It is also used as a medication to treat hypertension in tablet form. [13] The FDA initially authorised timolol in 1978. Numerous manufacturers sell this medication, which is useful in treating disorders like hypertension and open-angle glaucoma. [14] Timolol can be simultaneously estimated using a variety of HPLC techniques, including FTIR, UV, and others. Timolol was determined using a number of analytical techniques based on UV, FTIR, RP-HPLC, and HPTLC. [15, 16]

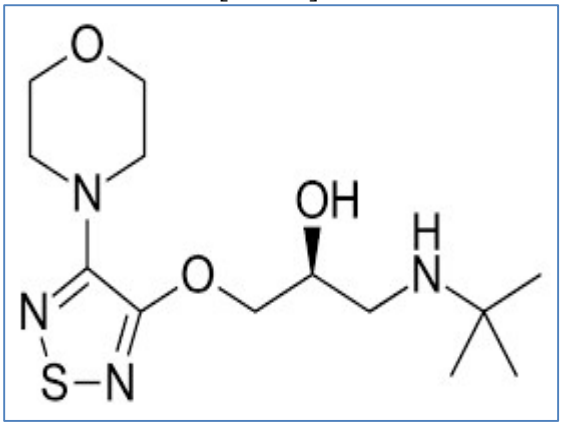


Figure 1: Structure of Timolol

Telenzepine, 1-methyl-10-[2-(4-methyl-1-piperazinyl)-1-oxoethyl]-5H-thieno[3,4-b][1,5] ben zodiazepine-4-one, Molecular formula- $C_{19}H_{22}N_4O_2S$. [17] A review of the literature revealed that HPTLC, UV, FTIR, and HPLC methods have been reported for the assay of Telenzepine in pharmaceuticals. [18] Telenzepine is a thienobenzodiazepine that acts as a selective M1 antimuscarinic and is used to treat peptic ulcers. [19]

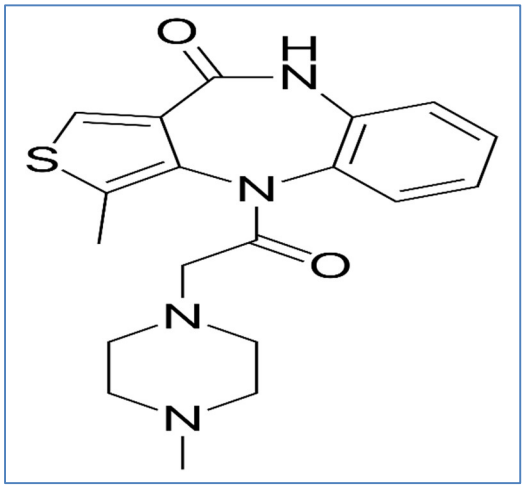


Figure 2: Structure of Telenzepine

Benzylpyridine, 4-benzylpyridine, Molecular Formula-$C_{12}H_{11}N$. [20] An analogue of haloperidol and a derivative of 4-benzylpiperidine, it was shown to exhibit NMDA antagonist pharmacology, which may help cure psychosis and prevent brain damage. [21] The estimate of benzoylpyridine in pharmaceutical formulations was demonstrated by a review of the literature using HPLC, FTIR, and UV techniques. [22, 23]

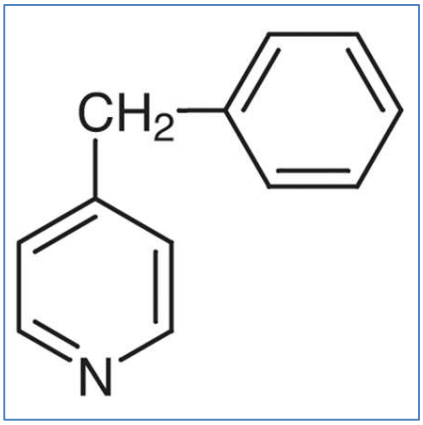


Figure 3: Structure of Benzylpyridine

Celecoxib is chemically 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzenes-ulfonamide and is a diaryl substituted pyrazole. [24] Compared to other nonsteroidal anti-inflammatory drugs (NSAIDS), celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor, is known to have a lower risk of gastrointestinal bleeding. [25, 26] It is used in familial adenomatous polyposis (FAP) to lessen colon precancerous polyps and to treat the symptoms of various forms of arthritis pain. According to a review of the literature, celecoxib in pharmaceutical formulations can be estimated using HPLC, FTIR, and UV techniques. [27]

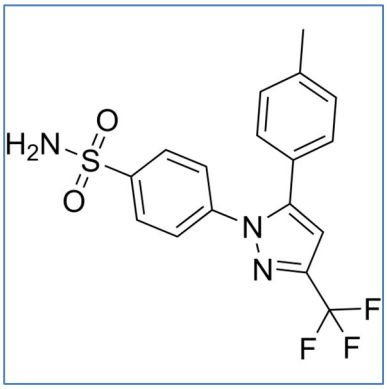


Figure 4: Structure of Celecoxib

The goal of the current study was to create the three-dimensional structure of the human cytochrome P450 4A11 (Uniprot accession number: Q02928). The homology modelling was conducted using Modeller 9.17. PROCHECK was used to validate the model. [28] The findings from the current investigation may be helpful in determining these enzymes' functional characteristics. Celecoxib, benzylpyridine, telenzepine, and timolol are among the medication compounds that were used in molecular docking investigations.

MATERIALS AND METHODS

Cytochrome P450 4A11's amino acid sequence was obtained from Uniprot. A three-dimensional model of "Cytochrome P450 4A11" was produced. Using the Protein BLAST program, a sequence similarity search was conducted to determine the structural similarity of the query sequence by choosing a database against the Protein Data Bank (PDB) to find a template for creating a homology model. The template was chosen based on the maximum score, greater than 30% identity, and reduced E-value. The protein 5T6Q was chosen as the model's template. Using the ClustalX and online ClustalW tools, comparative sequence alignment experiments were conducted with query and template structures. [29]

Molecular docking studies: Scientific literature was consulted to gather all of the molecules, which were then sketched in SYBYL6.7 and energetically minimised by adding Gasteiger Huckel charges. After that, the molecules were stored in the.mol2 format for molecular docking. To explain how proteins and medications bind, molecular docking experiments were conducted. [30] The Autodock 4.2 program was used to dock all of the current medications. In Autodock4.2, each molecule was docked separately. After being loaded into Autodock 4.2, the three-dimensional model of the Cytochrome P450 4A11 protein was structurally optimised by adding hydrogens to the protein that were assigned kollaman charges. [31, 32] The model was saved in PDBQT format after the hydrogens were added, and ligands were subsequently created by optimising the torsion angles and saved in the same format. [33] To determine the XYZ coordinates (X=71.434, Y=47.507, and Z=56.155) surrounding the Cytochrome P450 4A11 protein binding site, a grid was created. For autodock4.2's freezing, docking, and default parameters, the Lamarckian genetic algorithm (LGA) was chosen. [34]

RESULTS AND DISCUSSION

Following homology modelling and sequence alignment, Cytochrome P450 4A11 displays highly conserved amino acid sequence regions. Using the protein blast approach, the most homologous template for creating a homology model for Cytochrome P450 4A11 was found. The crystallographic

structure of cytochrome P450 4B1 (CYP4B1) complexed with octane was determined by the homology search. The chosen template was an n-Alkane and Fatty Acid Omega-hydroxylase with a covalently bound heme (PDB entry: 5T6Q). Modeller 9.17 was used to create twenty models. To achieve a perfect fit in the sequences, the alignment file was manually adjusted. The model with the least object function was chosen for additional protein stereochemistry analysis (phi and psi angles) using Procheck software after models for all primary sequences had been constructed. Cytochrome P450 4A11's homology-derived protein cartoon and super pose of model and template structures with backbone trace are displayed in Figures 5 and 6.

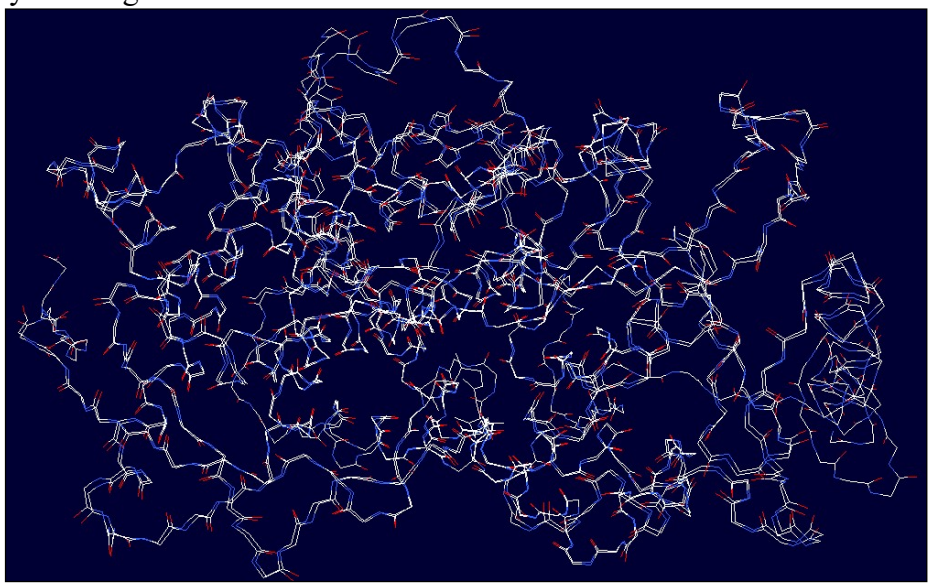


Figure 5: Super Pose Of Model And Template Structures With Backbone Trace. The Models Were Superimposed By Using Swiss PDB Viewer (SPDBV)

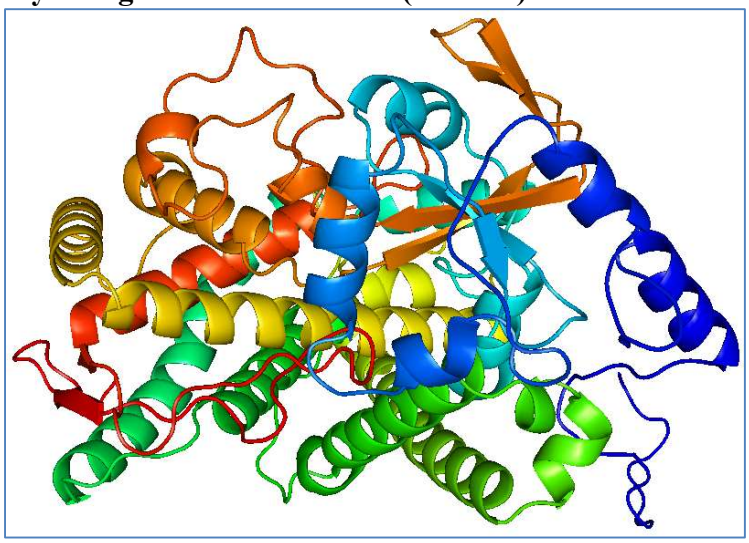


Figure 6: The cartoon of homology derived protein of cyp450 modelled protein.

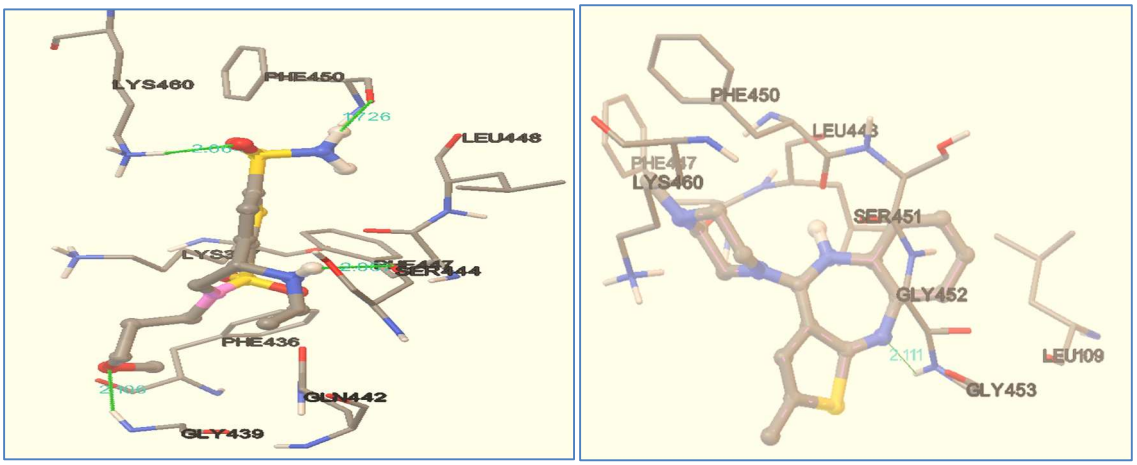
A crucial step in the structure-based drug design process is molecular docking of medicinal molecules into receptor binding sites and determining the ligand's binding affinity. All of the investigated alkaloid

compounds occupy a nearly identical space in the binding site, according to the molecular docking studies. Figure 7 depicts the optimal binding mechanism of telenzepine against the simulated CYP450 protein. Only the protein's active site pocket that best fits the ligands is chosen by the software during the molecular docking process. The binding orientation of ligands at the active site region is indicated by AutoDock 4.2. The docking program place both ligand and protein in different orientations, conformational positions and the lowest energy confirmations which are energetically favorable are evaluated and analyzed for interactions. Free energies of binding (ΔGb) and dissociation constants (Ki) as calculated by AutoDock are summarized.

Table 1 lists the interacting amino acid residues and binding energy. The binding energy (ΔG) value was used to characterise the binding affinity of each molecule. When interacting with Gly453, ligand telenzepine exhibits the greatest binding energy of -9.69 kcal/mol. The binding energy of the ligand timolol with the interacting Gly439, Lys460, Phe450, and Phe447 is -11.56 kcal/mol. Figure 7 displayed all of the compounds' docking positions.

Table 1: Binding energy and interacting residues of drugs with modelled CYP450 protein

Name of the ligand	Interacting amino acids	Grid X-Y-Z coordinates	Binding Energy ΔG (Kcal/Mol)	Dissociation constant (kI) (µM)
Timolol	Gly439, Lys460, Phe450, Phe447	71.234, 48.112, 58.233	-11.56	3.98
Telenzepine	Gly453	71.234, 48.112, 58.233	-9.69	2.86
Benzylpyridine	Leu448, Lys460,	71.234, 48.112, 58.233	-8.57	3.64
Celecoxib	Lys460, Phe450	71.234, 48.112, 58.233	-9.87	4.54



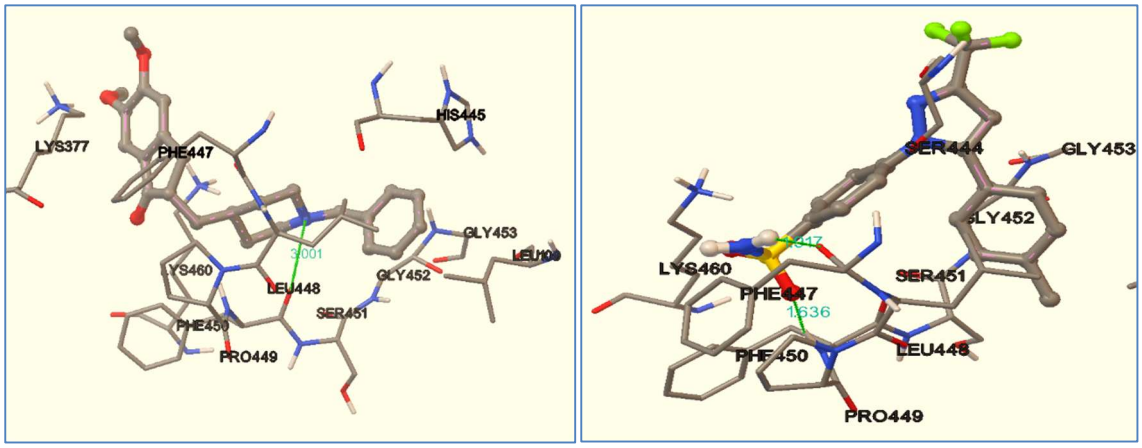


Figure 7: Docking interactions of CYP450 protein with drugs

CONCLUSION

In order to investigate the structural characteristics and binding mechanism of current medications as Cytochrome P450 4A11 inhibitors and to build a model for the design of novel Cytochrome P450 4A11 proteins, homology modelling and molecular docking experiments were conducted. The statistics of the homology-derived model resemble those of the template, or crystal structure. Drug docking of the model protein revealed information about the binding and interaction with the enzyme. Furthermore, we may gain a better knowledge of the mechanism underlying protein-ligand interactions and their binding patterns by combining the structure-based drug discovery process with protein information about therapeutic targets.

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