# “Identification of key amino acid residues and structure-based computational design of a novel agonist for Farnesoid X Receptor (FXR): An In-Silico study”

A dissertation thesis submitted to the Institute of Science, Nirma University in partial fulfillment of the requirement for a degree of

**MASTER OF SCIENCE**

IN

BIOCHEMISTRY/BIOTECHNOLOGY/MICROBIOLOGY

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Under The Guidance of

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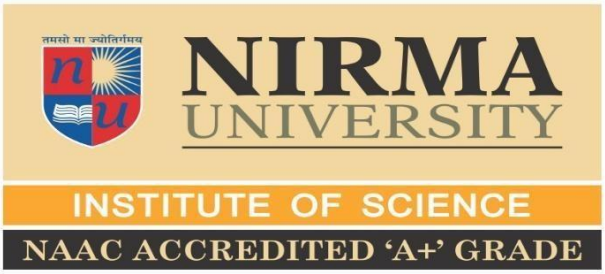


**Institute of Science, Nirma University**

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**Gujarat, India**

**May 2023**

NIRMA UNIVERSITY

INSTITUTE OF SCIENCE

NAAC ACCREDITED 'A' GRADE

# CERTIFICATE

This is to certify that the thesis entitled **"Identification Of Key Amino Acid Residues And Structure Based Computational Design Of A Novel Agonist For Farnesoid X Receptor (FXR): An In-Silico Study"** submitted to the Institute of Science, Nirma University in partial fulfillment of the requirement for the award of the degree of MSc. (Biochemistry/Biotechnology/Microbiology), is a record research work carried out by **Ms. Mahima Thakkar (21MBC006), Ms. Zeel Hirakani (21MBT009), Mr. Kuchhadiya Khimanand (21MMB012)** under the guidance of **Dr. Sriram Seshadri**. No part of the thesis has been submitted for any other degree or diploma.

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**Declaration**

The above Dissertation project was carried out jointly by **Ms. Mahima Thakkar (21MBC006), Ms. Zeel Hirakani (21MBT009), Mr. Kuchhadiya Khimanand (21MMB012)** under my guidance.

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## DECLARATION

We declare that the thesis **"Identification of key amino acid residues and structure-based computational design of a novel agonist for Farnesoid X Receptor (FXR): An In-silico study"** has been prepared by us under the guidance of Dr. Sriram Seshadri, Associate Professor of the Institute of Science, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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## ABBREVIATION

| | |
|---|---|
| **BA** | Bile Acid |
| **CDCA** | Chenodeoxycholic Acid |
| **CA** | Cholic Acid |
| **BSEP** | Bile Salt Export Pump |
| **BSH** | Bile Salt Hydrolase |
| **FXR** | Farnesoid X Receptor |
| **TGR5** | G-protein-coupled Bile Acid Receptor 1 |
| **DCA** | Deoxycholic Acid |
| **LA** | Lithocholic Acid |
| **GPBAR1** | G-protein-coupled Bile Acid Receptor 1 |
| **NAFLD** | Non-alcoholic fatty liver disease |
| **NASH** | Non-Alcoholic Steatohepatitis. |
| **TβMCA** | Tauro-β-muricholicAcid |
| **GβMCA** | Glycine- β- muricholic Acid |
| **XP** | Extra Precision |
| **EF** | Enrichment Factor |
| **GH** | Guner-Henry (Goodness of hit) score |
| **AUC** | Area under the ROC curve |
| **ROC** | Receiver Operating Characteristic curve |
| **BEDROC** | Boltzmann-enhanced Discrimination Receiver Operator Characteristic |

# ABSTRACT

Bile acid homeostasis is maintained by receptors and transporter present in the liver and the intestine. Farnesoid X receptor is one such nuclear receptor. It regulates the metabolism of biomolecules such as glucose and lipid. Farnesoid X receptor gets activated when excessive bile acid is produced by activating fibroblast like growth factor which further activates small heterodimer partner and this in turn will inhibit the transcription of CYP7A1 to maintain cholesterol homeostasis. Farnesoid X receptor turns out to be a demanding potent target for in-silico approaches. Agonist molecules of Farnesoid X receptor will bind to the allosteric sites of the receptor so that its activity gets enhanced. Comparative studies are done for Farnesoid X receptor agonist molecules where some of those have already been reported or are already in clinical trials. Amino acid interactions with receptor and ligands are studied to identify the key amino acid residues required for a ligand in order to bind specifically with Farnesoid X receptor. The newly developed pharmacophore has features that can help in designing of a novel drug which will act as an agonist molecule for Farnesoid X Receptor. Docking studies were carried out with libraries chosen from IBS Database and the best docked compounds were chosen. On the basis of the pharmacophore features and the top docked molecules, 55 novel quinoline-based derivatives were designed and only QC25 sufficed the docking parameters and the pharmacokinetic studies. Furthermore, the outcome of this study can be undertaken for MD simulation, synthesis, in-vitro and in-vivo analysis as well as nano-formulation studies for the potential activity.

# INTRODUCTION

Bile (from Latin *bilis*) is a chartreuse color fluid that is produced in the liver. Bile Acids (BA) are present in various forms such as primary, secondary, and conjugated. These are synthesized from cholesterol which acts as a precursor molecule. BA play a crucial role in the absorption of fat-soluble vitamins as well as in the hydrolysis of triglycerides during the digestion of fats (Griglione and Willingham, 2012). BA acts as a vital player in modulating glucose, lipid, and energy homeostatsis.

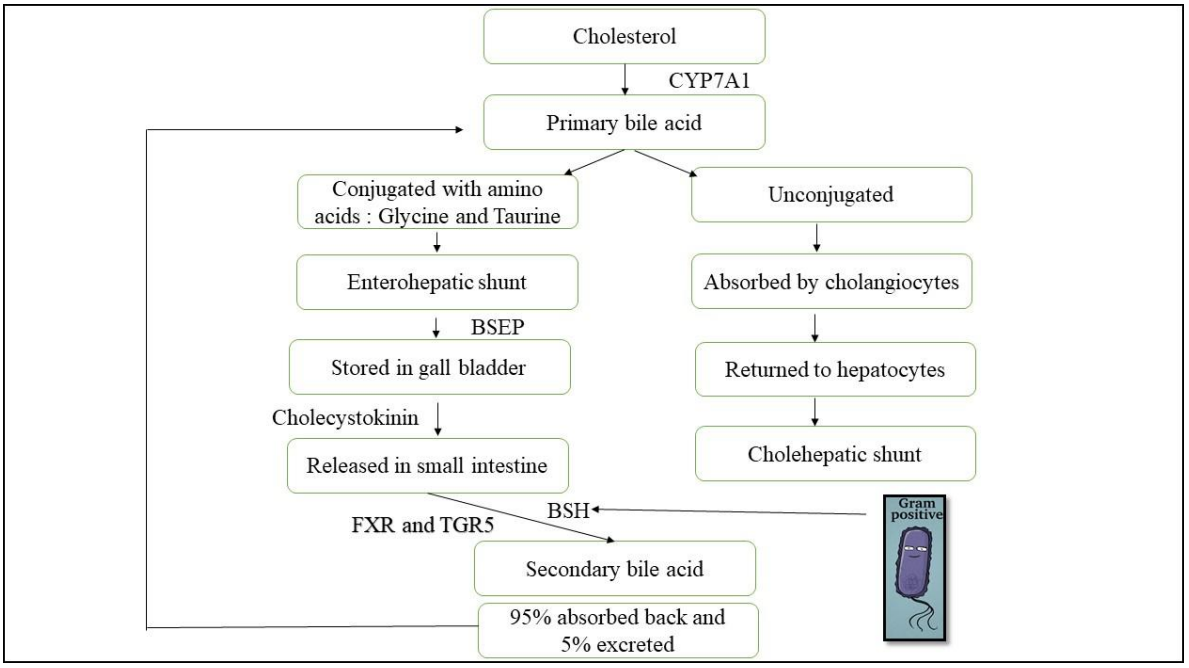



Figure 1: Bile Acid Biosynthesis Pathway (Jones, Alpini and Francis, 2015)(Deutschmann *et al.*, 2018)

There are multiple pathways for the biosynthesis of BA, which takes place in hepatocytes. One of these pathways is the classical pathway (Figure 1) or the neutral pathway that is majorly responsible for BA biosynthesis, where cholesterol in presence of enzyme CYP7A1 is converted into primary BA in the form of Cholic Acid (CA) and Chenodeoxycholic Acid (CDCA). These primary BA are then conjugate with glycine and taurine amino Acids. In mice, more than 95% of BAs are conjugated with taurine (Chiang,2013). Conjugated BAs enters into the entero-hepatic shunt via Bile Salt Export Pump (BSEP), where they are stored in the gallbladder. Unconjugated BAs are further absorbed by cholangiocytes and are returnedto hepatocytes. Cholecystokinin is a gastrointestinal hormone that is required for the contractionof the gallbladder, at the site where the BA is released into the small intestine (Otsuki, 2000). Primary BAs are converted into secondary BA in the presence of Bile Salt Hydrolase (BSH), which is produced by gram-positive bacteria. CA and CDCA are converted into DCA, and Lithocholic Acid (LA) respectively. Reabsorbed BA from the ileum is transported back into the liver via portal blood circulation so that the synthesis of BA can be inhibited (Chiang, 2013). Receptors which are involved in BA metabolism are Farnesoid X receptor (FXR), pregnane X receptor, constitutive androstane receptor, vitamin D receptor and G-protein-coupled Bile Acid Receptor 1(TGR5). Unabsorbed BA is excreted through feces. (add nuclear receptors in ba metabolism chiang)

**Key players involved in Bile Acid Metabolism**

1. **FXR**

   FXR is also known as NR1H4, which is a nuclear receptor encoded by the NR1H4 gene in humans (Gomez-ospina *et al*., 2016). It is involved in BA metabolism and other associated metabolic pathways. This receptor is mainly found in the liver and the intestine (Giancristofaro *et al*., 2018), but it is also known to play regulatory functions in **the** pancreas, brain, breast, kidney, and cardiovascular system (Zhang *et al*., 2020). CDCA and CA are ligands molecules for FXR, activation of FXR can be by both conjugated as well as unconjugated BAs. Activated FXR also suppresses one of the crucial enzymes cholesterol 7 alpha-hydroxylase (CYP7A1) in BA synthesis. Conjugated BAs are required to transfer from the liver to the intestine which is accomplished by the BSEP, and this BSEP is under the regulation of FXR (Grienke *et al*., 2011).

2. **TGR5**

   TGR5 is encoded by the GPBAR1 gene. TGR5 is a cell surface receptor. It is predominantly present in the gallbladder, placenta, spleen, and intestine (Cao *et al*., 2016). Activation of TGR5 triggers the release of the hormone glucagon-like peptide-1(GLP- 1) from the entero-endocrine cells. This hormone plays an important role in glucose metabolism and energy homeostasis. Stimulation of GLP-1 release by activation of TGR5, it's a novel concept for the treatment of type-II diabetes. Hence, TGR5 agonists can be used as a potent therapeutic agent for metabolic disorders (Sindhu and Srinivasan, 2017).

3. **BSEP**

   BSEP is present in the liver and plays an important role in bile salt transport. BSEP belongs to the ATP-binding cassette (ABC) family transporter, which is a superfamily of membrane proteins (Meier and Stieger, 2002). The export of bile salts by BSEP is a rate-limiting step of BA metabolism. FXR is a key regulator of BSEP in the biosynthesis of BA (Grienke et al., 2011).

4. **BSH**

   BSH is produced by gram-positive bacteria like *Lactobacillus, Bifidobacterium Enterococcus, and Bacteroides* of the intestinal flora (Song et al., 2019). BS converts the conjugated BAs (glyco-conjugated and tauro-conjugated) into free BAs by hydrolysis of the amide bond. This process is known as de-conjugation, and it is a key process that is required for further BA metabolism (Dong, 2018). BSH plays an important role in metabolic processes such as dietary lipid absorption, cholesterol metabolism, and the homeostasis of energy and inflammation (Bustos et al., 2018).

## Reasons to target FXR

CYP7A1 enzyme converts cholesterol to primary BA. With the intake of food, primary BA is transported to the small intestine. BSH of the gram-positive gut microbiota converts primary BA to secondary BA. Where BA binds to FXR which activates the fibroblast growth factor complex (FGF-19 and FGF-4). This complex gets transported to the liver where FGF-19 activates FGF-4. This activated form of FGF-4 stimulates a small heterodimer partner (SHP) enzyme, which inhibits the CYP7A1 enzyme. This limits the conversion of cholesterol to primary BA and thus, stops the excess accumulation of the primary BA in the liver (Staels and Fonseca, 2009; Figure 2).

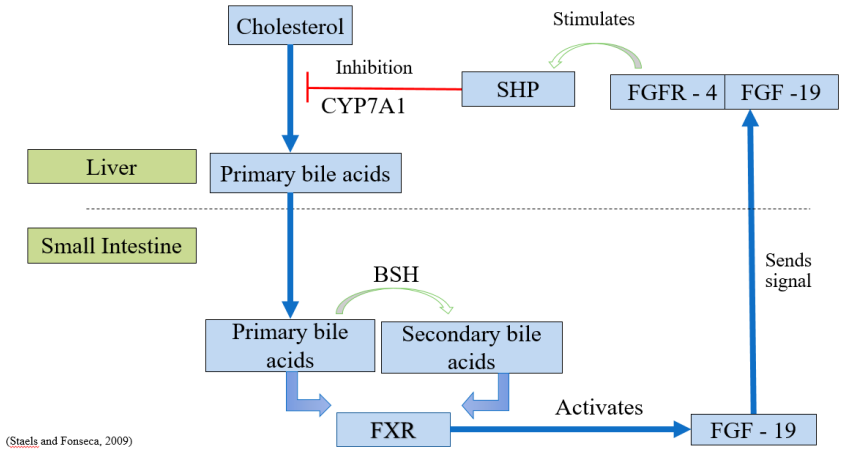



Figure 2: FXR signaling

FXR is a key regulator of BSEP that prevents BA accumulation in the liver (Grienke *et al.*, 2011). A major role of FXR is reported in metabolic regulation and homeostasis. Imbalance in the BA homeostasis has been linked to multiple metabolic diseases like inflammatory bowel disease, obesity, type 2 diabetes, colorectal cancer, hepatocellular carcinoma, non-alcoholic fatty liver disease, cholestasis, and primary biliary cholangitis. Currently, the use of FXR agonists and antagonists as therapeutic agents for treating these conditions has become a leading area of research.

## Significance of In-Silico Study

In-silico study is a very intricate study that is safe, cost-effective, and efficient. It scales down the need for funding and high-technology labs. It is a vital part of scientific research, especially drug discovery. This study helps make faster predictions of structures that are yet to be synthesized and unavailable in the market. As we discussed, an imbalance in the BA composition in the intestine leads to metabolic diseases. Our study was focused on finding an agonist molecule (a molecule that binds to a target receptor and stimulates a response) that will bind to the FXR receptor and restore BA homeostasis.

# REVIEW OF LITERATURE

FXR is a crucial nuclear receptor involved in regulating various metabolic processes, including Bile Acid and lipid metabolism (sun, 2021). Activated FXRhas been proven to improve type 2 diabetes induced by obesity through the regulation of glucose homeostasis and lipid metabolism (Ding *et al*., 2015; table1). As such, FXR has become a promising therapeutic target for several metabolic disorders. Recently, researchers haveincreasingly turned to computational methods to investigate the structure, function, andmolecular interactions of FXR.

Phospholipid transfer protein, Syndecan-1, the very low-density lipoprotein receptor (VLDLR), apolipoprotein C- II, and apolipoprotein E genes are regulated by FXR which are important regulators of lipoprotein metabolism. FXR knockout animals are used to analyses involvement of FXR in lipid metabolism (Wang *et al.*, 2008).

Recently, studies proved that activation of FXR via its ligands had an impact on immune cells as well as intestinal epithelium that contributed to intestinal immunomodulation. This provided a reason for FXR ligands to go under clinical trials for patients suffering from IBD (Ding *et al.*, 2015).

FXR is present in both the liver and the intestine thus making it very important to know the role of each tissue in metabolic disorders. In a research study, genetically modified mice were used to study the role of FXR in metabolic disease. Compared to the regular mice, the mice with knockout-FXR gene have been known to exhibit elevated levels of plasma triglycerides. Yet, its absence has not been shown to provide protection against obesity and insulin resistance induced by a high-fat diet (Sun, 2021).

Activated FXR inhibits Bile Acid synthesis, therefore, FXR activators are used to check effectiveness against Bile Acid-induced liver disease like Primary biliary cholangitis and Non-Alcoholic Steatohepatitis (NASH) (Sun, 2021).

Increased level of ceramide in serum and intestinal tissues is due to the very high level of activation of FXR by Bile Acid agonists which can cause metabolic disease in animal models and may be in humans (Sun, 2021).

Table 1: The role of FXR in systemic metabolism (Sun, 2021)

| **ORGAN** | **ASSOCIATED SIGNS** | **RELATED INDICATIONS** |
|---|---|---|
| Liver | Bile Acid Metabolism<br>Lipid Metabolism<br>Glucose Metabolism<br>Fibrosis<br>Inflammation<br>Cholestasis | NASH/ Non-Alcoholic Fatty Liver Disease (NAFLD)<br>Liver injury and fibrosis Alcohol-associated liver disease<br>Drug-induced liver injury<br>Liver regeneration |

| | | |
|---|---|---|
| Intestine | Bile Acid Transport<br>Inflammation<br>Glucose homeostasis<br>Antibacterial activity | Inflammatory bowel disease<br>Obesity<br>Insulin resistance<br>NAFLD<br>Mucosal injury |
| Kidney | Bile Acid Transport<br>Lipid metabolism<br>Fibrosis | Diabetic nephrotoxicity<br>Ischemia-reperfusion damage<br>Renal fibrosis |
| White adipose tissues | Adipogenesis<br>Insulin sensitivity | Obesity and insulin resistance |
| Pancreas | Lipid metabolism<br>β Cell function | Acute pancreatitis<br>Pancreatic lipid toxicity |
| Cardiovascular system | Lipid metabolism<br>Platelet | Atherosclerosis |

Studies show that certain key amino acid residues play important role in the receptor-ligand interaction. The common key amino acids with its features are as follows: Arg331 (HBA, H, NI), Met290 (H), Tyr369 (HBA, H), His294 (HBA), Leu287 (H), and Met328 (H) (Schuster *et al*., 2011). These key amino acid residues interact with the ligand to give features like HBA: Hydrogen bond Acceptor, H: Hydrophobic bond interactions, and NI: Negatively Ionizable groups.

Bile Acids have a different affinity with FXR, CDCA has a higher affinity as compared to the DCA, LCA, and CA. CA has the lowest affinity among these Bile Acids (Panzitt *et al*., 2022).

GW4064, Obeticholic Acid and Fexaramine have a higher agonist binding affinity with FXR than natural BAs. Bile salt hydrolase (BSH) can rapidly hydrolyze Tauro-β-muricholic Acid (TβMCA). Glycine- β- muricholic Acid (GβMCA) is designed by replacing taurinein TβMCA with glycine. GβMCA is less sensitive to microbial BSH-mediated hydrolysisand stability after oral administration is higher than TβMCA into mice (Sun, 2021).

FDA approved Obeticholic Acid for treatment of PBC31. Clinical trials for use of Obeticholic Acid in treatment of NASH is undergoing but changes in serum cholesterol pool level indicate risk of antherogenesis. For treatment of NASH clinical trials on Nidufexor (LMB763), Tropifexor (LJN452), Px-102/Px-104 and Cilofexor are undergoing (Sun, 2021).

FXR2 specific FXR agonists are more advantageous over currently available pan-FXR agonists in pathological circumstances like NAFLD (Panzitt *et al*., 2022).

To sum it up, in-silico research has proven to be a valuable tool for investigating the mechanisms and functions of FXR in metabolic regulation. By utilizing computational methods, researchers have been able to gain insights into the structural characteristics of FXR, decipher its molecular interactions, and develop novel compounds for potential therapeutic use. However, as with any complex biological system, there are still many unanswered questions surrounding the regulation and function of FXR. Future research efforts should aim to address these gaps and limitations, including the need for more sophisticated modelling and simulation techniques, the development of advanced molecular tools, and the integration of multiple computational and experimental approaches. By advancing our understanding of FXR and its involvement in metabolic disorders, we can make significant strides toward improving the treatment and management of these conditions, leading to better health outcomes for patients.

# HYPOTHESIS AND OBJECTIVE

# HYPOTHESIS

Interaction between the receptor and the ligand plays an important role in the inhibition or activation of any molecule. If the ligand binds at the catalytic site of the molecule, inhibition of the molecule occurs. This ligand is known as an antagonist. And if the ligand binds at the allosteric site of the molecule, the activation occurs. This ligand is known as an agonist. Both, agonists and antagonists require a connecting bridge to interact with the receptor.

We hypothesized that certain receptor-ligand interacting amino acid residues might provide specific features to the ligands.

We also hypothesized that the special pharmacophore features might help find a novel agonist for FXR as our target receptor and it might play a therapeutic aid for liver inflammation and its associated complications.

# OBJECTIVE

- To identify and compare the amino acid residues in differentmolecules
- To design a novel agonist for FXR – Structure based drug discovery

# MATERIALS AND METHODS

### Protein Structure Identification and Validation

Windows 10 Pro platform with a core i7 processor was used to perform all the computational work. FXR with bound agonists were downloaded from PDB at https://www.rcsb.org/. Available structures of FXR receptors with known agonists were downloaded from PDB (PDB IDs: 3FLI, 7VUE, 1OTJ, 6HL1, 3BEJ, 7D42, and 3DCT). These structures were validated using ERRAT plot and PROCHECK analysis (Ramachandran Plot) and the quality was checked at https://services.mbi.ucla.edu/SAVES/ (Vyas *et al*., 2022).

### Protein Preparation, Optimization & Minimization

The protein structure with PDB ID: 6HL1 was pre-processed and prepared by removing chain B and water molecules using the protein preparation wizard (Maestro 11.2). The structure was thenundergone through optimization and the energy was minimized.

### Protein Grid Generation

A grid was generated using a receptor grid generation module in GLIDE (Maestro 11.2). A grid of (xyz) 11.44 * -13.9 * 12.17 co-ordinates was generated with selected amino acid residues (metA:265, hisA:294, ileA:335, metA:290, leuA:348, pheA:336, metA:328, tyrA:369, sera:332, trpA:454, tyrA:361, pheA:329, hisA:447, metA:450, ileA:357, ileA:352, trpA:469, leuA:287, alaA:291, argA:331).

### Ligand Preparation

Three ligand libraries were prepared with reported FXR agonist molecules i.e., Endogenous, Synthetic, and Natural compounds. The endogenous compounds are present in the body. The synthetic compounds in the library are under trial or already available in the market. The natural compounds are the naturally available phytochemicals of the plants used for the treatment of metabolic disorders and some of them possess antioxidant properties. Ligand preparation was done by using Ligprep of the PHASE module (Maestro 11.2).

### Ligand Docking

The Ligands after preparation were docked with the grid-generated receptor. For this, Ligand Docking in PHASE (Maestro 11.2) was used and Extra Precision (XP) Docking was done for virtualscreening.

### Interaction Visualization

Key amino acid residues were identified from the screened molecules and visualized using Discovery Studio software.

### Generation and validation of Structure-based Pharmacophore

A manual pharmacophore was generated from the validated 6HL1 protein structure using the PHASE module of Maestro 11.2 Schrodinger. 6HL1 and its co-crystallized ligand CDCA were usedto create a pharmacophore using the PHASE module of Maestro 11.2. EF was calculated to evaluate the performance of the pharmacophore hypothesis. For this purpose, a set of 20 actives (agonists of FXR) and a set of 701 decoys were used. To validate pharmacophores, enrichment report, and ROC plot analysis were performed using hypothesis validation and enrichment viewer option of Phase module (Maestro 11.2) (Vyas *et al*., 2022).

### Pharmacophore-based database screening

Validated pharmacophore was used for virtual screening of the IBS (InterBioscreen) database (https://www.ibscreen.com/). 3 libraries were taken: bioactive compounds, natural compounds and synthetic compounds. Molecular docking study was done for the retrieved hits by the Glide module of Maestro (Schrodinger) after performing Phase screening of the 3 libraries. For phase screening, four out of seven features were selected.

### Molecular docking of molecular hits

Retrieved compounds from database screening were taken for molecular docking. Virtual screening workflow was set (HTVS, SP and XP). Lipinski rule of 5 filter was set along with the workflow. Docking was carried out with the FXR receptor (PDB ID: 6HL1) using the Glide module of Maestro (Schrodinger). Previously prepared receptor grid was used. Further, the docked poses were visualized for the protein-ligand visualization using the software Discovery Studio.

### Design of quinolone-based derivatives as FXR agonist

HTVS, SP and XP docking studies of the retrieved hits from the IBS database and their receptor-ligand interactions helped design novel quinolone-based derivatives as FXR agonist.

### Docking study of the designed FXR agonist

A molecular docking study of the newly designed quinolone compounds was performed using Maestro 11.2. XP ligand docking for these compounds was carried out using the GLIDE module of Maestro. The above generated grid of the receptor was used for this purpose.

### In-Silico ADMET prediction

For the calculation of the physicochemical properties and the synthetic accessibility of the designed quionoline derivatives, ADMETlab (https://admet.scbdd.com/calcpre/index/) and SwissADME (http://www.swissadme.ch/index.php) was used. Smiles notation was required for the SwissADME platform and properties such as permeability, intestinal absorption, protein binding, volume of distribution, CYP450 enzyme substrate, clearance, and toxicity properties such as hepatotoxicity, mutagenicity was checked on the ADMETlab platform.

# RESULTS AND DISCUSSION

**Protein structure study and its validation**

Structural validation of different proteins was done by using ERRAT and PROCHECK analysis (Table 2), where identification of the best protein structure of FXR receptor for structure-based drug discovery. PROCHECK analysis is done to check the stereochemistry and quality of protein. For analyzing various frequencies of non-covalent interactions of different types of atoms ERRAT is used. Ramachandran plot analysis was also performed to check that more than 90% of residues fall under favored regions. This plot also states the phi and psi torsion angles for each polypeptide residue. The ERRAT and PROCHECK analysis confirmed that PDB ID:6HL1 showed the highest quality factor of more than 90% in the Ramachandran plot (Figure 3) and more than 95 score in ERRAT. Based on protein validation, PDB ID: 6HL1 (Figure 4) was considered for further computational studies.

Table 2: Screening and validation of proteins for further computational studies.

| PDB ID | PROCHECK | | ERRAT |
|---|---|---|---|
| | Ramachandran plot | Disallowed regions | |
| 3FLI | 95.50% | 0.00% | 99.5305 |
| 7VUE | 90.30% | 0.00% | 85.9649 |
| 1OT7 | 79.20% | 2.20% | 90.4444 |
| 6HL1 | 92.70% | 0.00% | 100 |
| 3BEJ | 90.30% | 0.90% | 98.441 |
| 7D42 | 90.20% | 0.00% | 97.807 |
| 3DCT | 89.10% | 0.00% | 100 |

Range: - Ramachandran plot >90%, Disallowed regions <2%, ERRAT >95

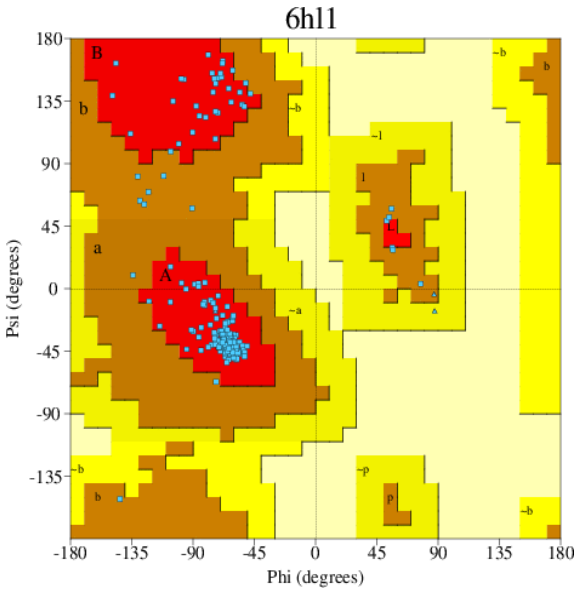



Figure 3: Ramachandran plot of PDBID: 6HL1 for protein validation.

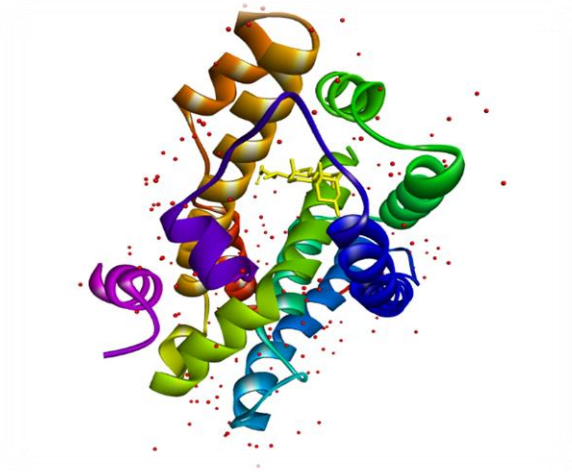


Figure 4: - Structure of protein fromPDB ID: 6HL1

**Comparative docking analysis: -**

Molecular docking is considered an approach to observe the characterization, orientation, and conformation of the interactions between a small molecule and a protein at the atomic level. Docking experimentally states the binding affinity with the targeted molecule. Three different libraries endogenous library (Table 3), synthetic library (Table 4) and phytochemical library (Table 5) were created and Extra Precision (XP) dockingwas carried out to compare the Docking scores and their binding efficiencies with other molecules.

Table 3: Library of endogenous molecules

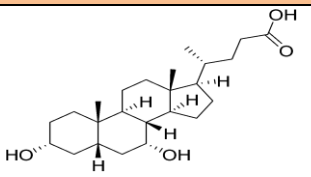
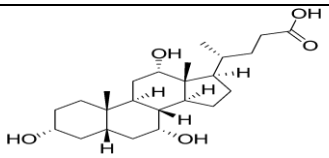
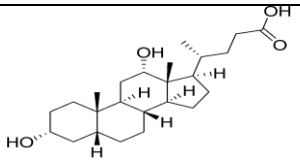
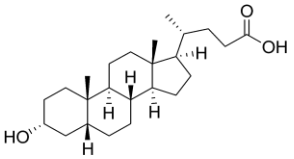
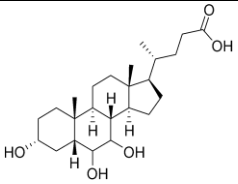
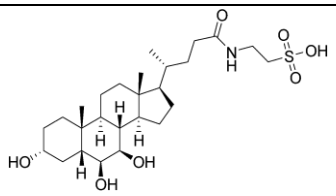
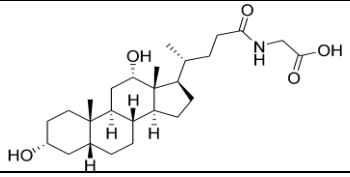
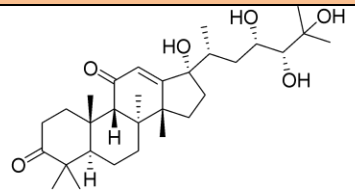
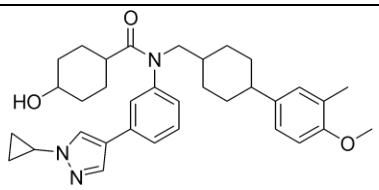
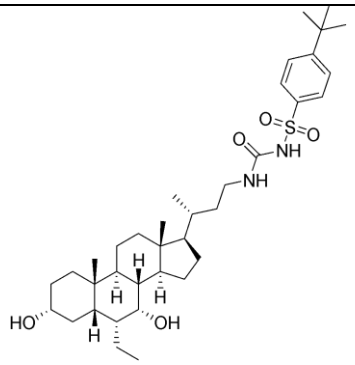
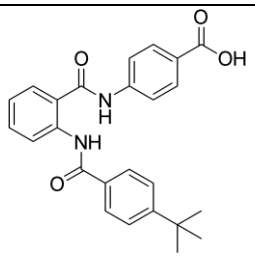
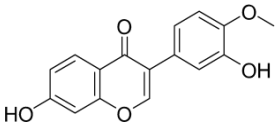
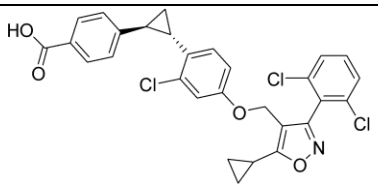
| Sr. No | Compounds | Structures |
|---|---|---|
| 1 | Chenodeoxycholic Acid |  |
| 2 | Cholic Acid |  |
| 3 | Deoxycholic Acid |  |
| 4 | Lithocholic Acid |  |
| 5 | Muricholic Acid |  |
| 6 | Tauro-β-Muricholic Acid |  |
| 7 | Glyco-deoxycholic Acid |  |

Table 4: Library of synthetic molecules

| Sr No | Compounds | Structures |
|---|---|---|
| 1 | Alismanol M |  |
| 2 | Omesdafexor |  |
| 3 | EDP-305 |  |
| 4 | DM175 |  |
| 5 | Calycosin |  |
| 6 | PX20606 trans-isomer |  |

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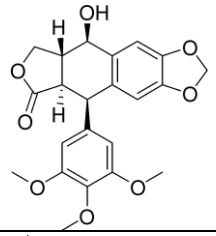
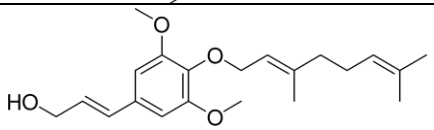
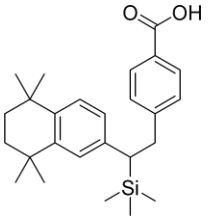
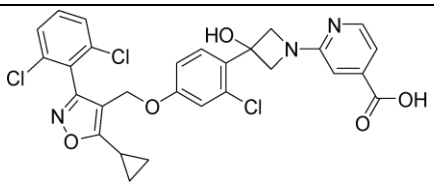
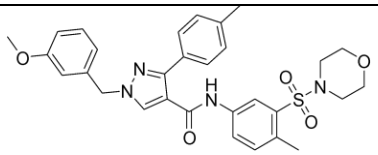
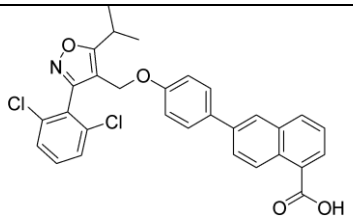
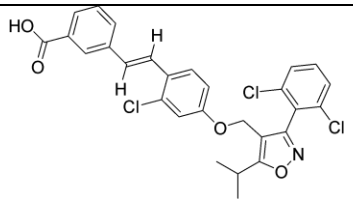
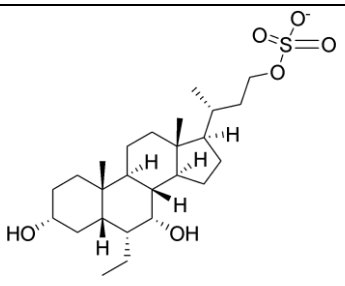
|---|---|---|
| 7 | Podophyllotoxin |  |
| 8 | Nelumol A |  |
| 9 | AGN-29 |  |
| 10 | Cilofexor |  |
| 11 | DY268 |  |
| 12 | GSK-8062 |  |
| 13 | GW4064 |  |
| 14 | INT767 |  |

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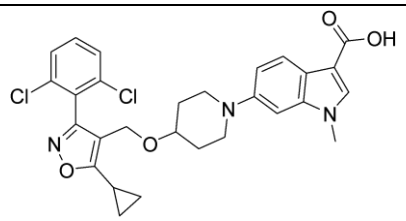
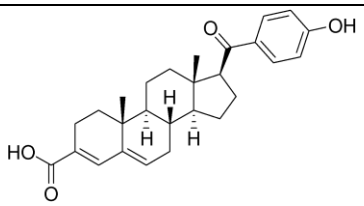
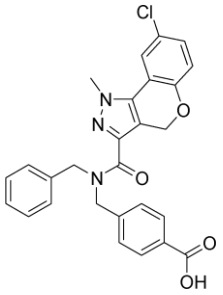
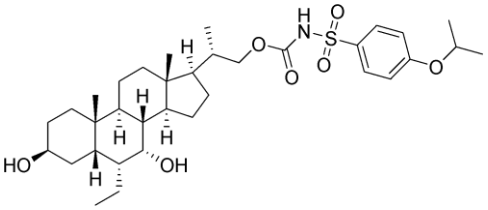
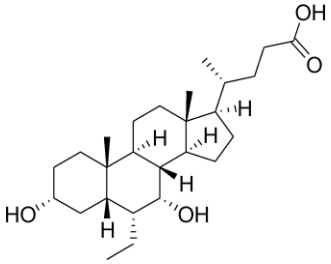
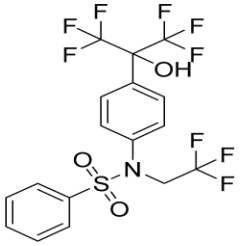
|---|---|---|
| 15 | LY2562175 |  |
| 16 | MFA-1 |  |
| 17 | Nidufexor |  |
| 18 | NR1H4 activator 1 |  |
| 19 | Obeticholic Acid |  |
| 20 | T0901317 |  |

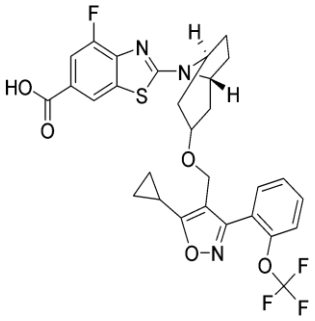
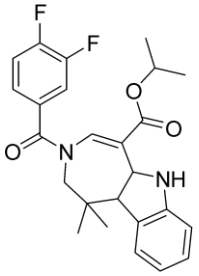
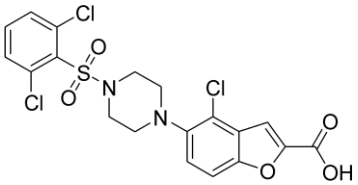
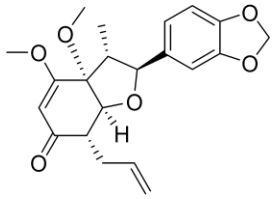
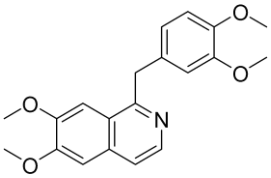
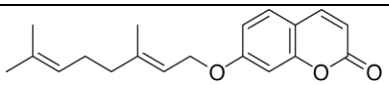
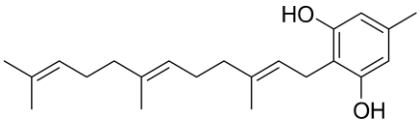
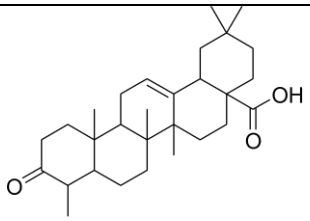
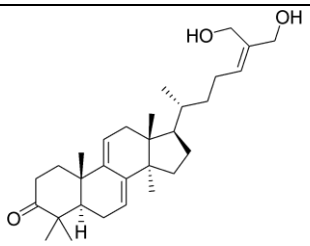
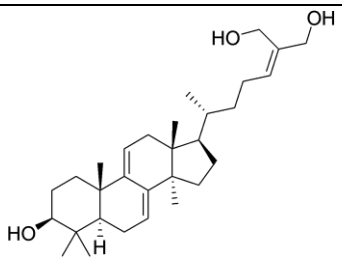
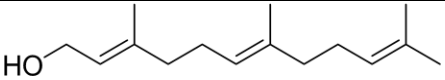
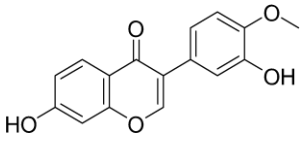
| | | |
|---|---|---|
| 21 | Tropifexor |  |
| 22 | Turofexorate isopropyl |  |
| 23 | Vonafexor |  |

Table 5: Library of phytochemical molecules

| Sr No | Compounds | Structures |
|---|---|---|
| 1 | Fargesone A |  |
| 2 | Papaverine |  |
| 3 | Auraptene |  |
| 4 | Grifolin |  |
| 5 | Hedragonic Acid |  |
| 6 | Ganoderiol F |  |
| 7 | Ganodermatriol |  |
| 8 | Farnesol |  |
| 9 | Calycosin |  |

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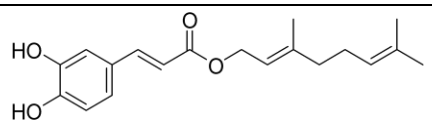
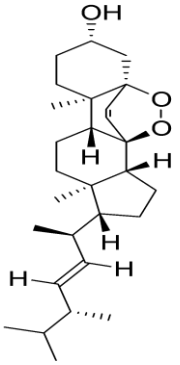
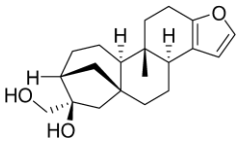
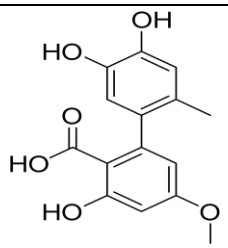
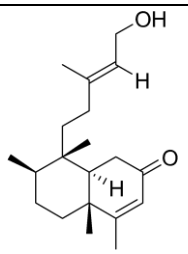
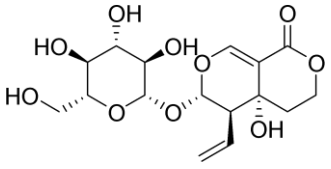
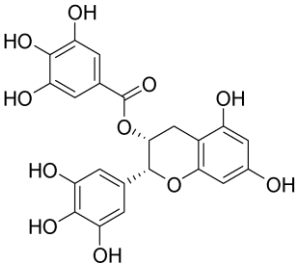
|---|---|---|
| 10 | Geranyl caffeate |  |
| 11 | Ergosterol peroxide |  |
| 12 | Cafestol |  |
| 13 | Altenusin |  |
| 14 | 2-Oxokolavenol |  |
| 15 | Swertiamarin |  |
| 16 | Epigallocatechin gallate |  |

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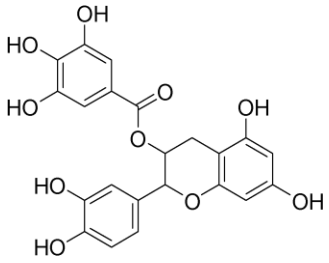
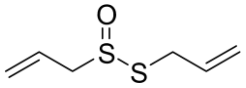
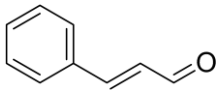
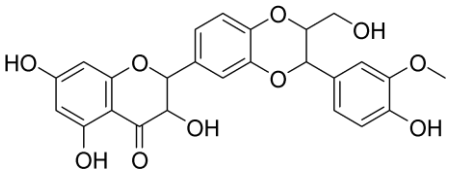
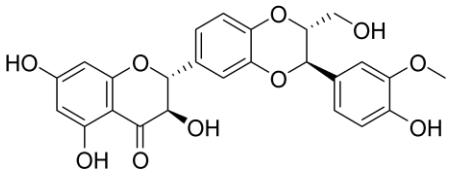
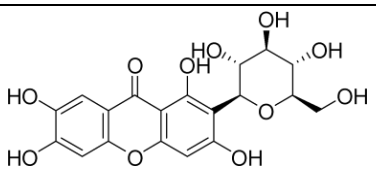
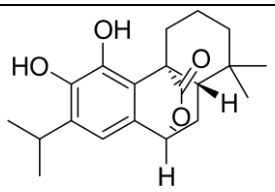
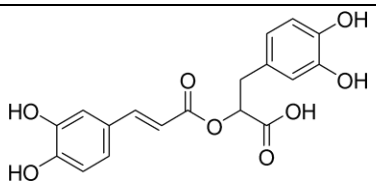
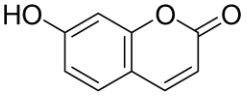
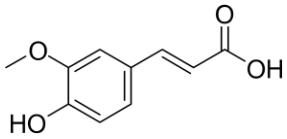
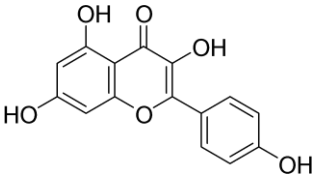
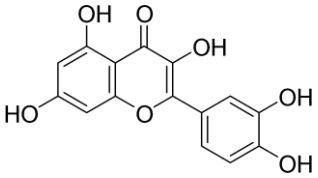
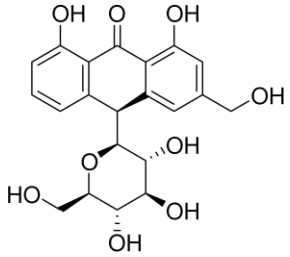
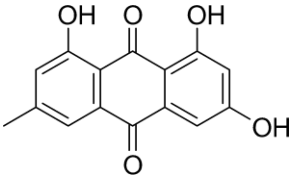
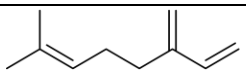
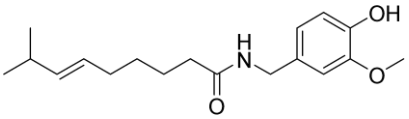
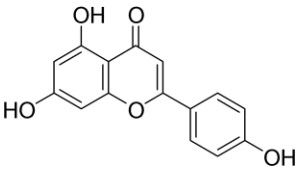
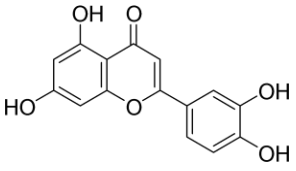
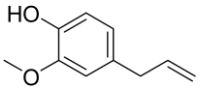
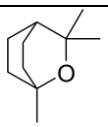
|---|---|---|
| 17 | Epicatechin gallate |  |
| 18 | Allicin |  |
| 19 | Cinnamaldehyde |  |
| 20 | Silymarin |  |
| 21 | Silybin |  |
| 22 | Mangiferin |  |
| 23 | Carnosol |  |
| 24 | Rosemarinic Acid |  |
| 25 | Umbelliferone |  |
| 26 | Ferulic Acid |  |

| | | |
|---|---|---|
| 27 | Kaempferol |  |
| 28 | Quercetin |  |
| 29 | Aloin |  |
| 30 | Emodin |  |
| 31 | Myrcene |  |
| 32 | Capsaicin |  |
| 33 | Apigenin |  |
| 34 | Luteolin |  |
| 35 | Eugenol |  |
| 36 | Eucalyptol |  |

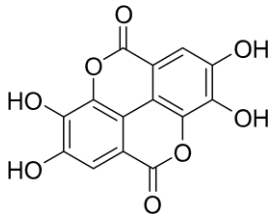
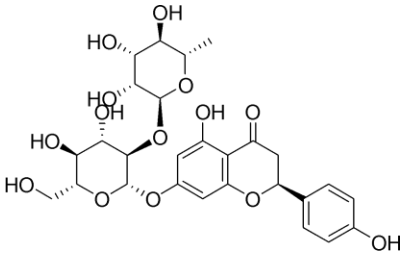
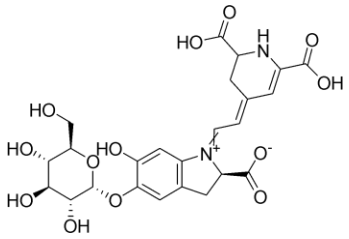
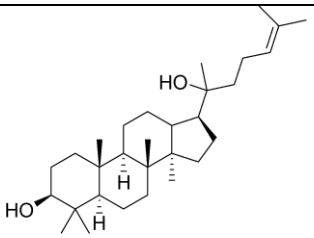
| | | |
|---|---|---|
| 37 | Ellagic Acid |  |
| 38 | Naringin |  |
| 39 | Betalains |  |
| 40 | Ginsenosides |  |

Table 6: Docking scores of the top five compounds from all three libraries with their amino Acid interactions for the identification of key residues.

| Sr No. | Name of Compound | Key Residues | Docking Scores |
|---|---|---|---|
| **ENDOGENOUS** | | | |
| 1 | Cholic Acid | tyrA:361, his A:447, **tyrA:369**, serA:332, **hisA:294**, **argA:331**, **metA:290**, leuA:348, **leuA:287**, trpA:454, IleA:352 | -15.694 |
| s2 | Chenodeoxycholic Acid | tyrA:361, his A:447, **tyrA:369**, serA:332, **metA**:**290**, **hisA:294**, ArgA:33, alaA:291, **metA:328**, **leuA:287**, trpA:454, IleA:352 | -15.603 |
| 3 | Muricholic Acid | tyrA:361, his A:447, **tyrA:369**, serA:332, **hisA:294**, **argA:331**, **metA:290**, leuA:348, **leuA:287**, trpA:454, IleA:352 | -15.121 |
| 4 | Deoxycholic Acid | tyrA:361, his A:447, **tyrA:369**, serA:332, **hisA:294**, **argA:331**, **metA:290**, **leuA:287**, trpA:454, ileA:352 | -14.064 |
| 5 | Lithocholic Acid | tyrA:361, his A:447, ileA:352, **argA:331**, **hisA:294**, **metA:290**, alaA:291, **metA:328**, **leuA:287**, trpA:454 | -13.933 |
| **PHYTOCHEMICALS** | | | |
| 6 | Ganodermatriol | **argA:331**, valA:297, ileA: 335, leuA:348, alaA:291, **leuA:287**, ileA:352, **metA:328**, **metA:290**, argA:264 | -11.661 |
| 7 | Epigallatocatechin gallate | **argA:331**, **metA:328**, alaA:291, **leuA:287**, ileA:352, **tyrA:369**, serA:332, **metA:290**, ileA:335, metA:265 | -11.506 |
| 8 | Ellagic Acid | ileA:335, **metA:290**, **hisA:294,** **argA:331**, metA:265, argA:264 | -11.47 |
| 9 | Cafestol | LeuA:348, **metA:290**, trpA:454, ileA:352, hisA:447, alaA:291, **metA:328** | -11.156 |
| 10 | Mangiferin | **argA:331,** metA:265, **hisA:294,** **metA:328**, **leuA:287**, serA:332, **tyrA:369**, **metA:290**, ileA:335 | -11.11 |
| **SYNTHETIC** | | | |
| 11 | Obeticholic Acid | tyrA:361, hisA:447, ileA:352, ileA:362, pheA:366, **tyrA:369**, serA:332, **metA:290**, metA:265, ileA:335, **hisA:294**, **argA:331**, alaA:291, **metA:328**, trpA:454, **leuA:287** | -14.74 |
| 12 | INT767 | argA:264, **argA:331**, metA:265, ileA:335, **hisA:294**, **metA:328,** **tyrA:369**, ileA:352, tyrA:361 | -14.239 |
| 13 | NR1H4 activator 1 | trpA:454, **leuA:287**, **metA:328**, alaA:291, **metA:290**, leuA:348, **hisA:294**, metA:265, **argA:331**, serA:345, serA:332, ileA:335, **tyrA:369**, pheA:366, ileA:352, ileA:362 | -13.732 |

| | | | |
|---|---|---|---|
| 14 | MFA-1 | **argA:331,** metA:265, **hisA:294,** alaA:291, tyrA:361, **metA:328**, **leuA:287,** ileA:352, ileA:335, leuA:348, **metA:290** | -11.591 |
| 15 | Nidufexor | **metA:328,** alaA:291, **metA:290**, **hisA:294**, metA:265, tyrA:260, valA:297, argA:264, **argA:331**, pheA:336, ileA:335, leuA:348, ileA:352 | -11.154 |

Three libraries were created for the comparative docking studies where it was observed that CA which is an endogenous molecule has the highest docking score of -15.694. In the library of phytochemicals, Ganodermatriol has a -11.661-docking score that was found lowest in comparison to other synthetic and endogenous library molecules. Obeticholic acid is a synthetic molecule thatis a modification of CA and its docking score is -14.74. tyrA:369, metA:328, hisA:294, metA:290, argA:331, and leuA:287 are key residue aminoacids (Table 6) that are necessary for FXR-ligand interaction. Top docked molecules from all three libraries are bio-visualized which are shown in (figure5-7).

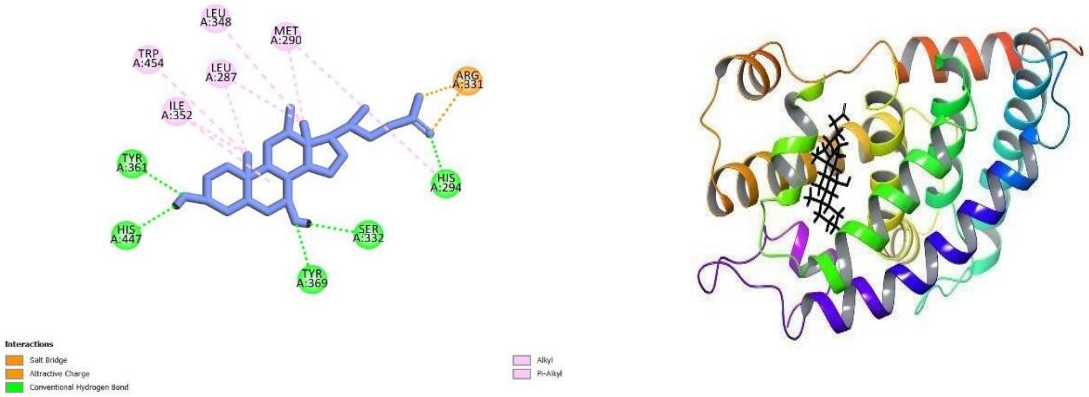



Figure 5: - Bio-visualization of 6HL1 with Cholic Acid (endogenous molecule)

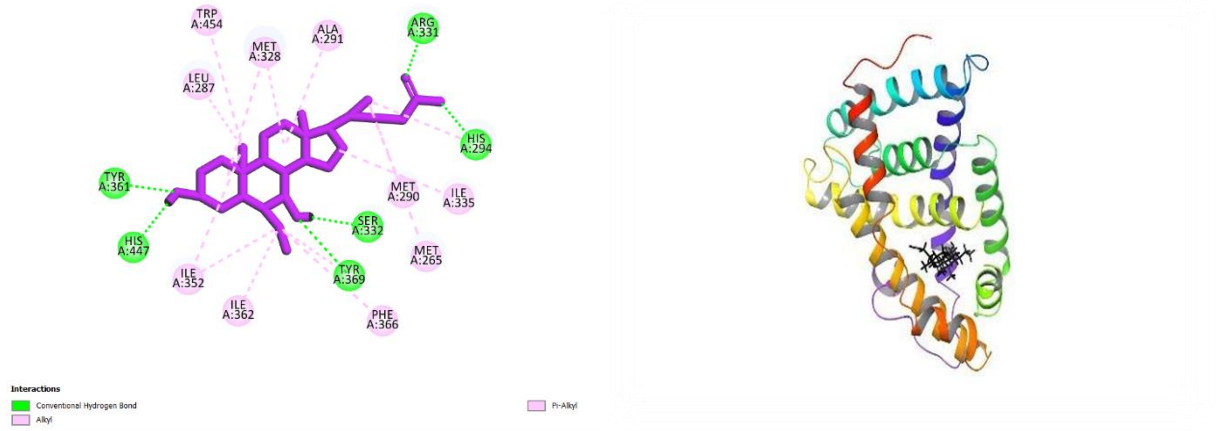



Figure 6: - Bio-visualization of 6HL1 with Obeticholic Acid synthetic (natural molecule)


Figure 7: - Bio-visualization of 6HL1 with Ganodermatriol (natural molecule)

**Energy optimized structure-based pharmacophore generation: -**

Receptor-ligand based E-pharmacophore was generated. The validated protein structure (PDB ID: 6HL1) with its ligand was chosen from PDB. The pharmacophore hypothesis was generated based on AADHHN (Figure 8) where (A) states thehydrogen bond acceptor, (D) stands for hydrogen bond donor, (H) is for hydrophobic, and (N)is for negative ionic features.

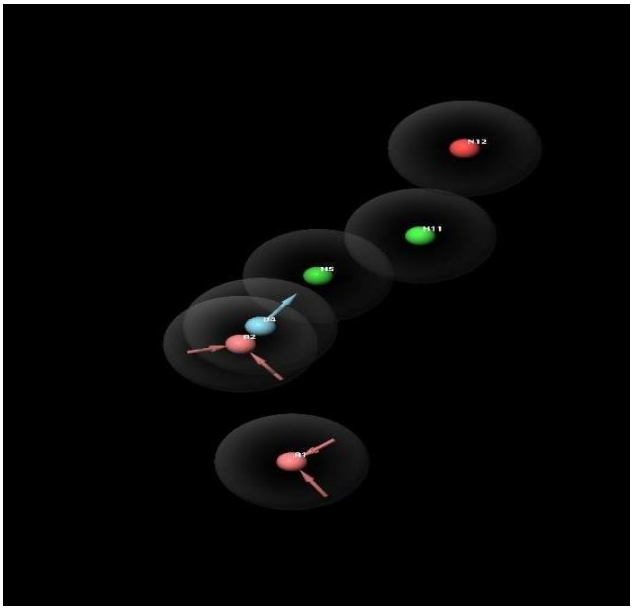


Figure 8: Structure-based E pharmacophore generation based on receptor-ligand interaction with (AADHHN) features.

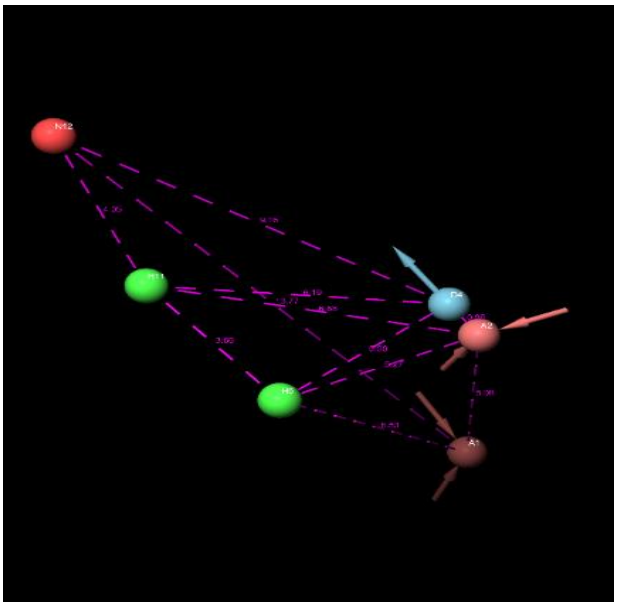


Figure 9: Distance between each 2 pharmacophore features.

**E-pharmacophore validation: -**

Pharmacophore validation was done by using hypothesis validation of the phase module. It was done by adding 28 active molecules from the literature review which were reported as FXR agonists in the internal library with 701 decoy molecules. For the validation, statistical analysis was performed through GH score, BEDROC, EF, False Negative, and False positive values (Table 7,8).

Table 7: Enrichment report and ROC analysis for validation of AADHHN pharmacophore.

| Sr. No. | Parameters | Values |
|---|---|---|
| 1 | ROC | 0.95 |
| 2 | AUC | 0.96 |
| 3 | RIE | 14.55 |
| 4 | EF (%) | 36.05 |
| 5 | BEDROC (α= 8) | 0.949 |
| 6 | BEDROC (α=20) | 0.948 |
| 7 | BEDROC (α=160.9) | 0.986 |

Table 8: Validation of structure-based pharmacophore (AADHHN) based on Goodness of hit (GH) score and their parameters.

| Sr. No. | Parameters | Values |
|---|---|---|
| 1 | Total number of decoy molecules (D) | 701 |
| 2 | Total number of active molecules (A) | 20 |
| 3 | Total Hits (Ht) | 23 |
| 4 | Active hits (Ha) | 19 |
| 5 | % yield of actives [(Ha/Ht) *100] (%) | 82.60 |
| 6 | % Ratio of actives [(Ha/A*100] (%) | 95 |
| 7 | Enrichment factor (EF) | 28.95 |
| 8 | False positives, FP [Ht-Ha] | 4 |
| 9 | False negatives, FN [A-Ha] | 1 |
| 10 | Goodness of fit (GH) | 0.85 |

EF= [(Ha * D)/ (Ht * A)], GH= [Ha (3A+Ht)/(4HtA)] * [1-{(Ht-Ha)/(D-A)}]

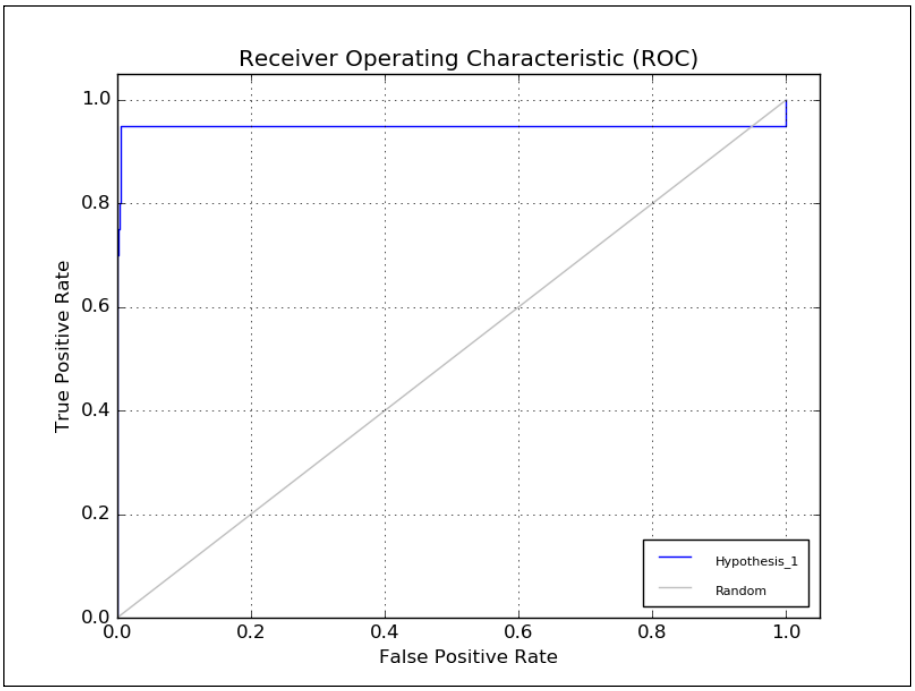



Figure 10: Generation of ROC plot for the validation of pharmacophore.

The purpose of enrichment metrices is to assess the enrichment of active compounds in the screening process which includes a set of actives and decoys.

(BEDROC)Boltzmann-enhanced Discrimination Receiver Operator Characteristic area under the curve. It tells about the ratio of total active to total ligands. The values of BEDROC shall range from 0 to 1. BEDROC (α = 20.0) exhibits 80% of the BEDROC results coming from the first 8% of ranked molecules

(ROC) Receiver Operator Characteristic area under curve. The value ranges between 0 and 1. Values greater than 0.7 are considered to be good (Figure 9). ROC is the probability that a randomly chosen active have a higher chance to bind than a randomly chosen decoy. (Maestro 11.2)
Potency validation was carried out to validate the features of pharmacophore where a set of decoys and actives are selected and also based on their binding activities. GH (Guner-Henry) Goodness of hit score was calculated. GH score >0.6 indicates the quality of pharmacophore which can be accepted.

The EF (36.05%), ROC (0.95), AUC (0.96), GH (0.85), and BEDROC (α= 20) (0.948) states that the hypothesis has been validated and it can be used for further virtual screening procedures.

Pharmacophore hypothesis was generated and validated which can be used for further screening and docking studies as well as for the synthesis of novel drug for FXR agonist.

Virtual screening of synthetic, natural and bioactive compounds was conducted, which were obtained from IBS database. High throughput virtual screening was performed followed by standard precision and extra precision respectively. After the above library screening, in synthetic section 9 molecules had the docking score < -11.00. Top five molecules out of those 9, were shortlisted on the basis of their docking score to meet the best binding affinity. While in case of natural molecules, total 87 molecules had the docking score < -11.00. Out of those 87 molecules, top 5 molecules were shortlisted on the basis of their docking score falling in the range of -14 to -15. And after screening of bioactive molecules, only one molecule is screened, with the docking score of -11.003.

These shortlisted 11 molecules from all the three libraries were undergone through bio-visualization to find the key amino acid residues playing part in the receptor-ligand interaction (Table )

The figures (Figure ) show the interaction between FXR and the screened ligands and the key amino acid residues playing a part in their interaction.

| Sr No. | Name of Compound | Key Residues | Docking Scores |
|---|---|---|---|
| **SYNTHETIC COMPOUNDS** | | | |
| 1 | STOCK4S-23098 | ileA:335, ileA:273, **metA:328**, trpA:454, **leuA:287**, alaA:291, **metA:290**, **hisA:294**, **argA:331**, metA:265 | -11.617 |
| 2 | STOCK4S-39430 | **hisA:294**, ileA:335, trpA:454, **leuA:287**, tyrA:361, **metA:328**, trpA:469, hisA:447, **tyrA:369**, metA:365, ileA:352, **argA:331** | -11.61 |
| 3 | STOCK5S-76631 | **metA:328**, alaA:291, serA:332, argA:331, **metA:290,** **hisA:294**, metA:265, pheA:336, leuA:348, ileA:335, tyrA352, ileA:357, ileA:362 | -11.366 |
| 4 | STOCK5S-82921 | metA:265, **metA:290**, **hisA:294**, **argA:331,** serA:332, **metA:328**, alaA:291, **tyrA:369**, ileA:362, ileA:352, leuA:348, ileA:335, metA:265 | -11.333 |
| 5 | STOCK4S-80889 | **tyrA:369**, tyrA:361, hisA:447, trpA:469, trpA:454, **metA:290**, leuA:348, ileA:335, **argA:331**, **metA:328** | -11.298 |
| **NATURAL COMPOUNDS** | | | |
| 6 | STOCK1N-78606 | valA:297, **hisA:294**, leuA:348, **metA:290**, ileA:352, **leuA:287**, **metA:328**, trpA:454, tyrA:361, hisA:447, **tyrA:369**, serA:332, **argA:331** | -15.295 |
| 7 | STOCK1N-78102 | **hisA:294**, leuA:348. **metA:290**, ileA:352, **leuA:287**, **metA:328**, trpA:454, tyrA:361, hisA:447, **tyrA:369**, serA:332, **argA:331**, valA:297, argA:264 | -15.232 |
| 8 | STOCK1N-78353 | hisA:447, **metA:328**, **tyrA:369**, serA:332, **argA:331**, ileA:273, metA:265, leuA:348, **metA:290**, **hisA:294**, **leuA:287**, trpA:454, ileA:352, tyrA:361 | -15.077 |
| 9 | STOCK1N-78303 | serA:345, metA:265, leuA:348, **hisA:294**, **metA:290**, ileA:352, **leuA:287**, **metA:328**, trpA:454, tyrA:361, hisA:447, **tyrA:369**, serA:332, **argA:331**, ileA:335 | -14.783 |

| | | | |
|---|---|---|---|
| 10 | STOCK1N-76776 | hisA:447, **metA:328**, **tyrA:369**, serA:332, **argA:331**, valA:297, **hisA:294**, **metA:290,** leuA:348, **leuA:287**, trpA:454, ileA:352, ileA:357, tyrA:361 | -14.736 |
| **BIOACTIVE COMPOUND** | | | |
| 11 | Bio-0850 | metA:265, valA:297, **argA:331**, **hisA:294**, **leuA:287**, tyrA:361, met:328, ileA:352 | -11.003 |

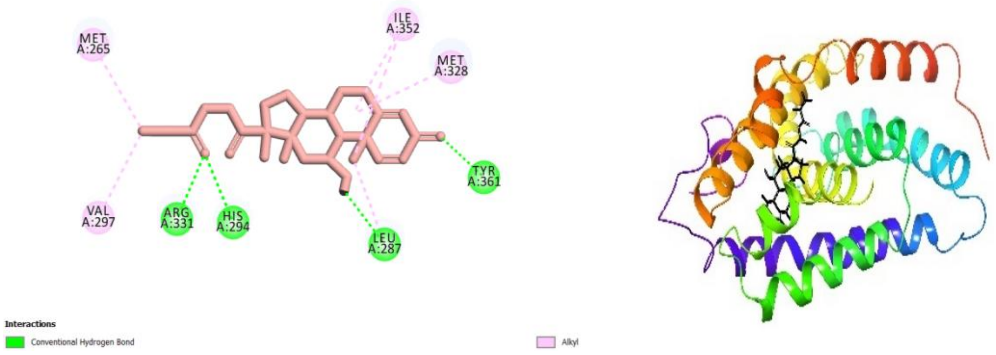



Figure: - Bio-visualization of 6HL1 with Bio-0850 (bioactive molecule)

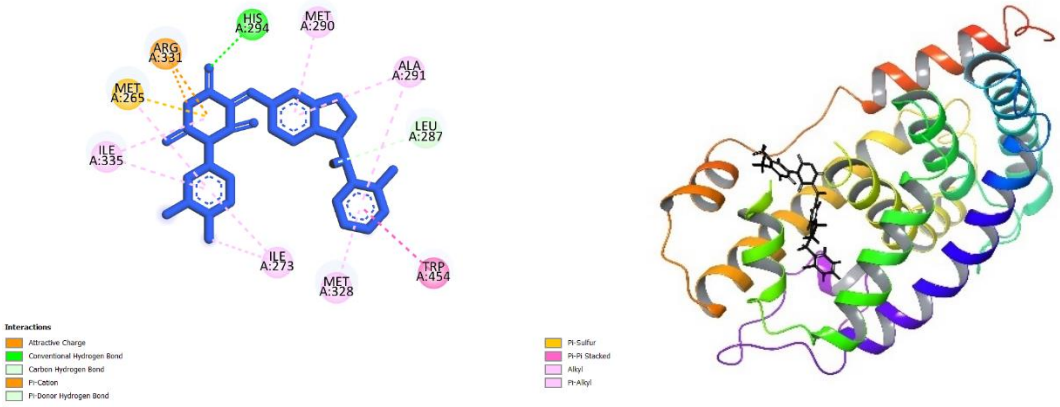



Figure: - Bio-visualization of 6HL1 with STOCK4S-23098 (synthetic molecule)

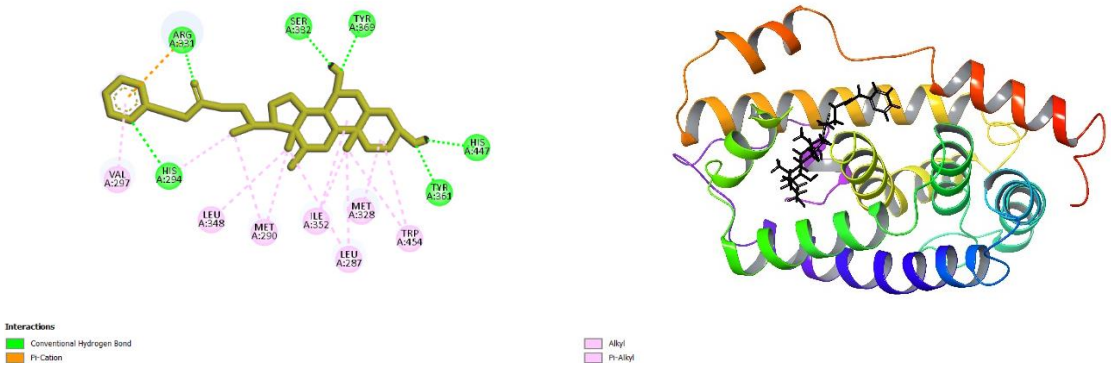
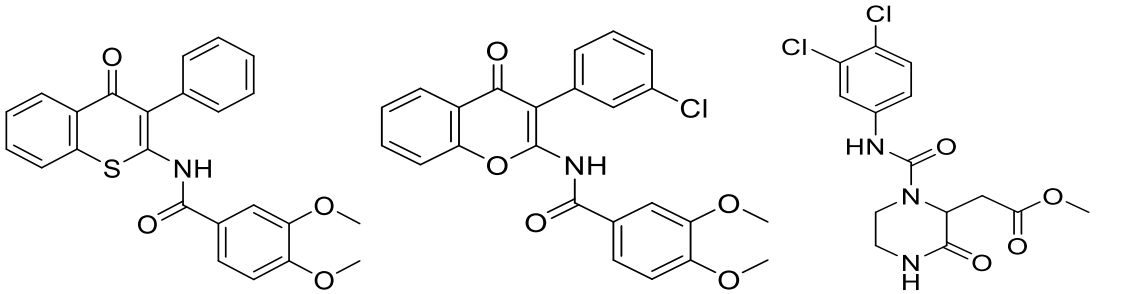



Figure: - Bio-visualization of 6HL1 with STOCK1N-78606 (natural molecule)

**Design of quinoline based derivatives**

STOCK4S-23098, STOCK4S-39430, STOCK5S-76631, STOCK5S-82921, STOCK4S-80889 are the molecules with top docking score. Based on STOCK5S-76631(Figure), STOCK5S-82921(Figure), STOCK4S-80889(Figure) compounds novel molecule is designed.




STOCK5S-76631 STOCK5S-82921 STOCK4S-80889

Figure: Chemical compounds used to design quinolone-based derivatives

STOCK5S-76631 contains a fused ring system which gives hydrophobic features. It also contains an amide linker that connects the fused ring and dimethoxyphenyl group and serves as an acceptor as well as a donor. The dimethoxyphenyl group possesses an electron donating group.

STOCK5S-82921 also contains fused ring system, amide linker and Dimethoxyphenyl group.it has 3 chlorophenyl group where chlorine will act as a negative ion.

STOCK4S-80889 Contains piperazine ring (heterocyclic ring with 2 nitrogen atoms). Contains an amide group and phenyl ring.

Based on these three compounds and features showed by pharmacophore we have designed novel quinoline based derivatives.in this compound quinoline gives hydrophobic feature. Amide linker serves as an acceptor as well as a donor. Oxygen group on the quinoline ring serve as negative ion.by permutation and combination total 55 compounds were designed with the selection of functional groups and substituents, all the features are properly satisfied in the designed compounds.

**Docking study of the designed FXR agonist**

Molecular docking study was carried out of designed compound. Out of 55 designed compounds, 20

compounds have docking score of -9 and from that top 10 molecules were selected. QC49 showed the highest docking score of -10.089(table…)

Table: Docking score of the top 10 designed molecules

| Name of Compounds | Docking score |
|---|---|
| QC49 | -10.089 |
| QC25 | -9.925 |
| QC34 | -9.793 |
| QC4 | -9.747 |
| QC28 | -9.726 |
| QC30 | -9.722 |
| QC29 | -9.716 |
| QC32 | -9.693 |
| QC51 | -9.534 |
| QC22 | -9.492 |

**ADMET prediction and synthetic accessibility**

In silico prediction of ADMET properties helps in optimization of designed compounds, selection of the compounds for synthesis and further in vitro and in vivo assays. For the SMILES notation of designed compounds, ADMET properties were predicted (Table 4). Two properties, namely human intestinal absorption (HIA) and Caco-2 permeability, were predicted for absorption of designed compounds. HIA probability values of more than 0.5% and Caco-2 permeability values in the log unit of − 4.70 or − 4.80 are considered best for drug absorption. It was observed that all the designed compounds were predicted to have HIA probability values in the range of 0.742% to 0.886, and showed Caco-2 permeability in the range of probability log unit − 4.684 to − 4.810, which indicated that all these compounds may possess very high absorption rate. Plasma protein binding (PPB) was predicted to know the distribution of designed compounds. PPB probability values near 90% indicate good protein binding and distribution of drug in the body. All the designed compounds were predicted with more than 90% probability value for PPB, which indicated that these compounds may have a good distribution profile in the body. Drug metabolism was predicted by knowing the ability of the designed compounds to get metabolized as substrates by CYP450 subtype 1A2, 2D6 and 3A4 enzymes. Based on the probability values of more than 0.5, all the designed compounds might get metabolized in vivo by CYP450 subtype 1A2 enzyme, and in case of subtype 3A4 and 2D6 mixed responses were observed. As an excretion parameter, clearance (CL) probability values were predicted for the designed compounds. A probability value of < 5 mL/min/kg indicates low clearance of drugs from the body. Designed compounds showed probability values in the range of 1.5–2.118 mL/min/kg, which indicated their low clearances and high retention in the body. Human hepatotoxicity and AMES mutagenicity were predicted as toxicity parameters for the designed compounds. Probability values near to 0 indicate that the compounds are non-toxic, and values close to 1 indicate that the compounds are toxic. Along with the probability values, ADMETlab also provides category values of 0 and 1. A probability value of < 0.5 is considered as a 0-category value, and compounds are considered as non-toxic. A probability value of > 0.5 is considered a 1 category value, and compounds are considered as toxic.

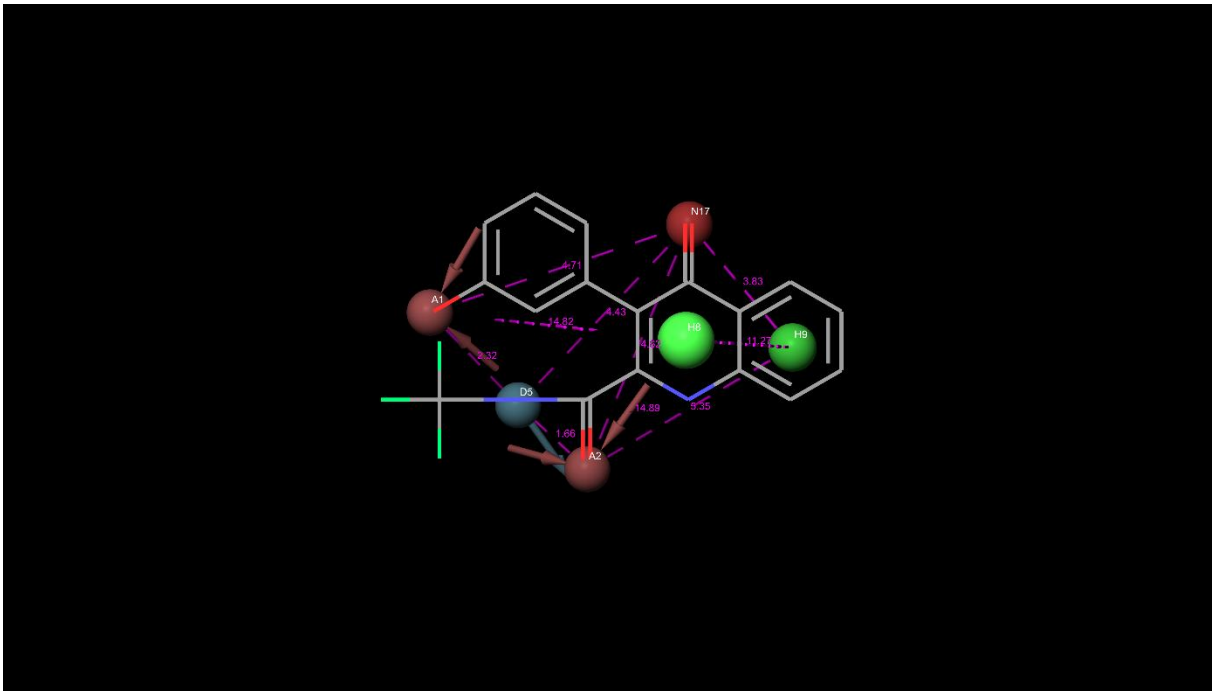
Amino acid residues play an important role in deciding the receptor-ligand interaction. Our study focused on finding the interacting amino acids of the FXR receptor. These receptors are known to give hydrophobic, hydrogen donor/acceptor or negative ionic features to the ligand. On the basis of the co-crystallized ligand CDCA interacting with our target receptor 6HL1, we prepared a grid and allowed re-docking of our set of ligands. We used 3 libraries and found few common amino acid residues of the FXR receptor, having the capability to interact with our set of ligands.

We used the same co-crystallized structure, 6HL1 to generate a pharmacophore hypothesis and received 6 features AADHHN where (A) is hydrogen bond acceptor, (D) is hydrogen bond donor, (H) is hydrophobic, and (N) is negative ionic feature. The pharmacophore was validated through ROC value and GH score.

This validated model can be further used to phase screen compounds from databases like ZINC or IBS for ligands with similar features as our pharmacophore. (Vyas *et al*., 2022). Lipinski rule of 5 is also used for screening of molecules. (Vyas *et al*., 2022). Lipinski rule of 5 are: molecular weight of molecule should not bemore than 500 daltons, hydrogen bond acceptor should not be more than 10, hydrogen bond donor should not be more than 5 and logP value should not be more than 5. Violation of morethan 1 rule is not acceptable so if any molecule is violating more than 1 rule it will be screenedout. These screened molecules can be further docked with our target receptor 6HL1 to find outdocking scores of the top ligands. Furthermore, these ligands with the top docking scores can be used to design new molecules with the same pharmacophoric features.

New molecules need to be synthesized in the lab but synthesis of molecules is not an easy task. Complex molecules need more time for synthesis and if a new synthesized molecule does not work appropriately then this molecule is not useful. Once synthesized, it is very important to check their toxic properties. ADMETis used to check absorption, distribution, metabolism, excretion, and toxicity of any molecule.This test gives us an idea if our drug/molecule crosses the blood brain barrier, or if it has skin permeability, Caco-2 permeability, CYP inhibition and metabolism, hepatotoxicity etc. and helps predict the nature of our molecule.

Molecular dynamics (MD) simulation is a computational method used to predict how protein and drug interact with each other. It also predicts how molecules will move over time.
Benzimidazole derivatives (Sindhu and Srinivasan, 2014) and Chembridge_9149693 (Sindhu and Srinivasan, 2015) compounds are identified as agonist molecules for FXR. Chembridge_9149693 is dual agonist of FXR and TGR5. These molecules are identified by the in-silico method. Research has been going on in this field to find novel molecules that can be used as FXR agonists.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A1
H8
H9
D5
A2
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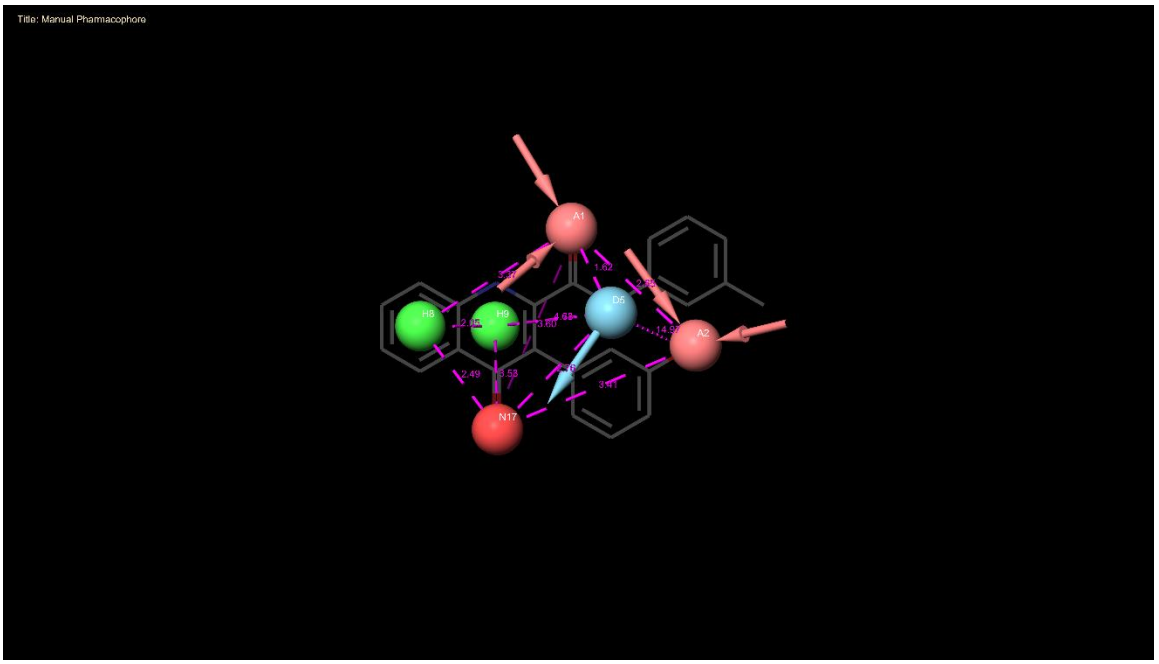
4.43
14.82
2.32
11.27
14.88
9.35
1.66

Qc-25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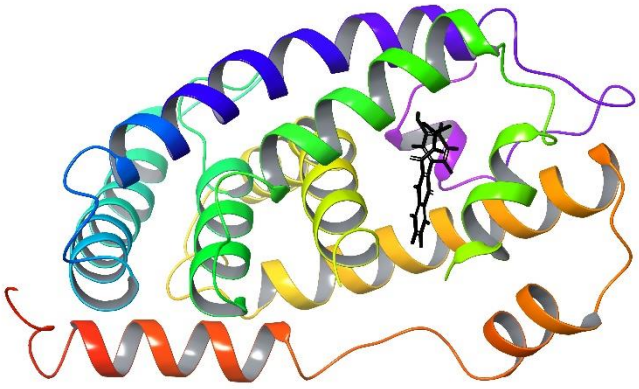
H8
H9
D5
A2
N17
1.82
2.49
3.53
3.41

Qc49



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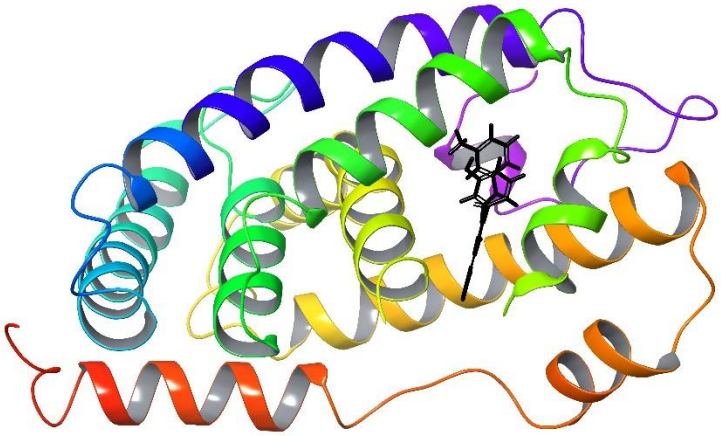

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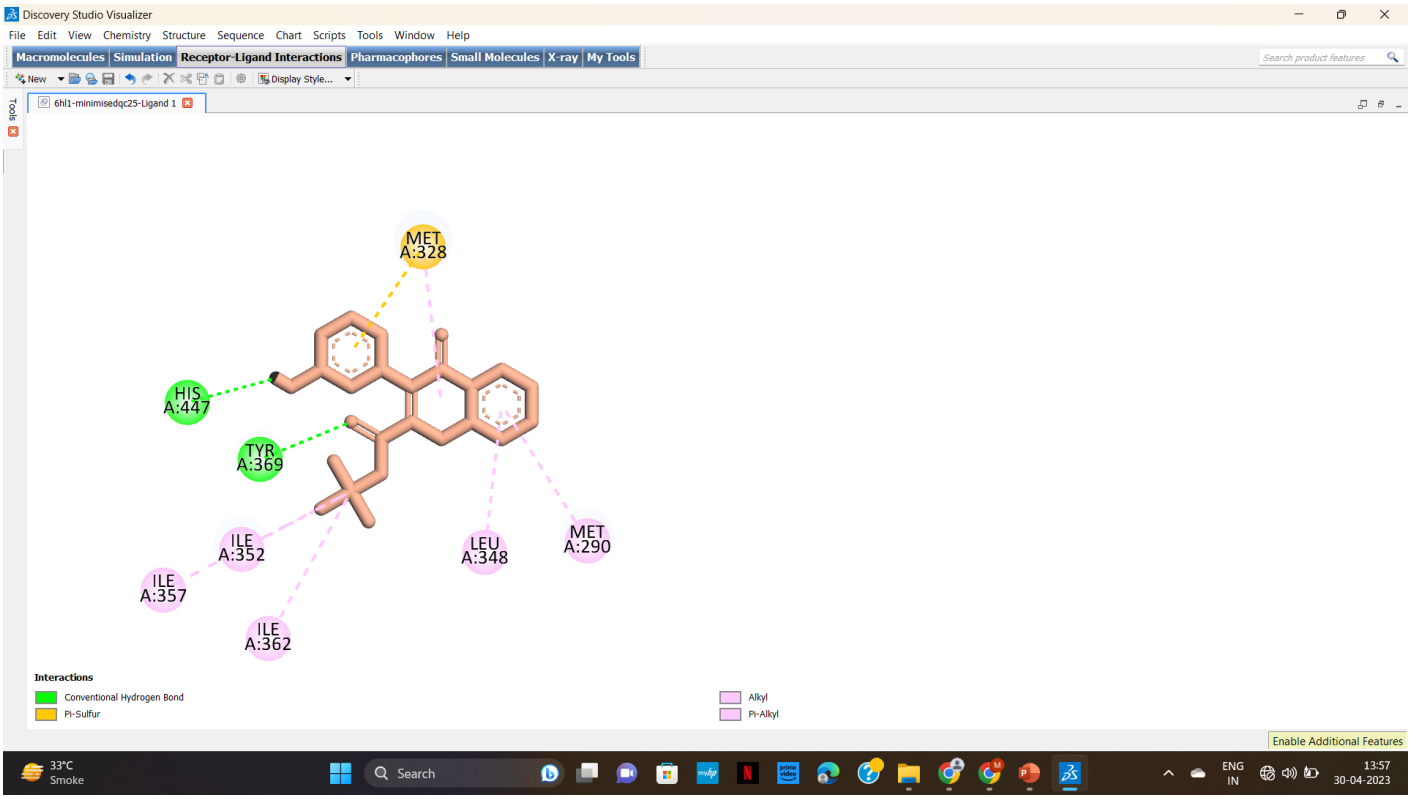
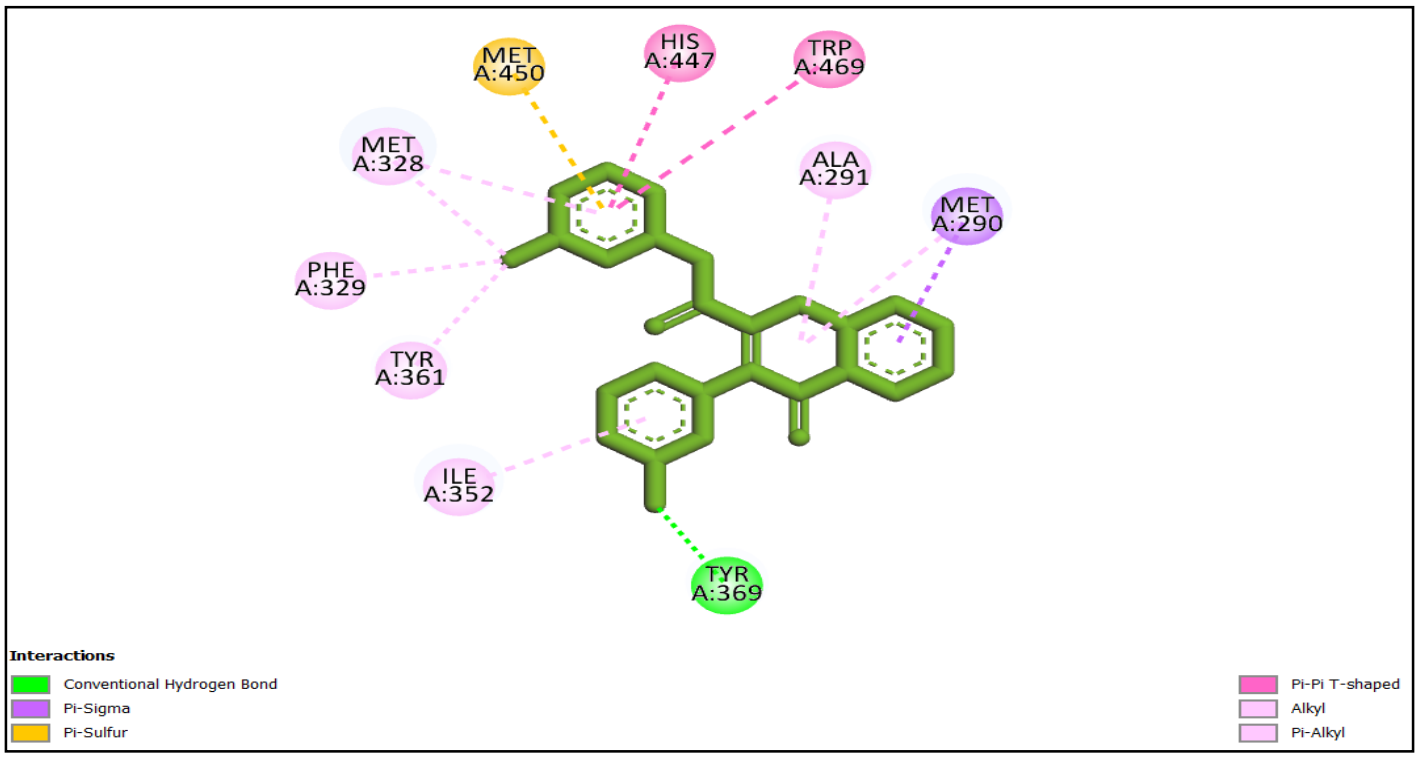

Discovery Studio Visualizer
File Edit View Chemistry Structure Sequence Chart Scripts Tools Window Help
Macromolecules Simulation Receptor-Ligand Interactions Pharmacophores Small Molecules X-ray My Tools
6hl1-minimisedqc25-Ligand 1
MET A:328
HIS A:447
TYR A:369
ILE A:352
ILE A:357
ILE A:362
LEU A:348
MET A:290
Interactions
Conventional Hydrogen Bond
Pi-Sulfur
Alkyl
Pi-Alkyl
Enable Additional Features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ALA A:291
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PHE A:329
TYR A:361
ILE A:352
TYR A:369
Interactions
Conventional Hydrogen Bond
Pi-Sigma
Pi-Sulfur
Pi-Pi T-shaped
Alkyl
Pi-Alkyl

Qc49

# CONCLUSION

In order to wrap our thesis, it was concluded that tyrA:369, metA:328, hisA:294, metA:290, argA:331, and leuA:287 are key residues which are required for binding of FXR with ligand. On the other hand, comparative studies of endogenous, phytochemical and synthetic libraries stated that out of all molecules CA which is an endogenous molecule has highest docking score of -15.694 which means that is has high binding efficiency with FXR receptor. Furthermore, a receptor-ligand pharmacophore hypothesis was generated with features having two acceptors, one donor, two hydrophobic and a negative ion (AADHHN). This pharmacophore was validated by actives and decoys and can be used for further studies.

# FUTURE SCOPES

- The validated pharmacophore can be used further for screening and docking studies to discover a novel drug which can have higher binding affinity in comparison to endogenous compounds. MD simulation and pharmacokinetic studies can also be carried out
- In-vitro and in-vivo studies of potent molecules can be done to check the activity of FXR and toxicological studies can also be done which will open the doors of combinational drug concept.
- Nano formulation of potent molecule can be done to have targeted drug delivery.

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