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Molecular Modelling of Cytochrome P450 2A5 [Mus musculus] and Molecular Docking Studies Involved in Non-Alcoholic Fatty Liver Disease

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ABSTRACT: Non-alcoholic fatty liver disease (NAFLD) affects 25% of the world's population and is the principal cause of cirrhosis and hepatocellular carcinoma. NAFLD is a disease spectrum that includes steatosis with or without moderate inflammation (non-alcoholic fatty liver) and non-alcoholic steatohepatitis (NASH), which is characterised by necroinflammation and a faster fibrosis progression than NAFLD. The pathophysiology of various liver illnesses also involves the hepatic CYPs. By controlling lipid metabolism-related pathways, such as oxidative stress, arachidonic acid metabolism, steroid hormone metabolism, and fatty acid metabolism, the CYP450 family plays an important role in the pathophysiology of fatty liver. Hepatotoxicity is brought on by medicines that are activated by CYP to produce harmful metabolites. Drugs, substances, and endogenous substrates are all metabolized by cytochrome P-450 (CYPs). Since the enzyme's crystal structure has not yet been made public, we created a homology model of Cytochrome P450 2A5 using a modeller. The optimized homology model was evaluated as a trustworthy structure by PROCHECK, ERRAT, WHAT-IF, PROSA2003, and VERIFY-3D after molecular dynamic refinement. NASH pathogenesis is complex, with multiple hits such as oxidative stress, free fatty acid-induced lipotoxicity, endoplasmic reticulum stress, cytokines, and gut flora all contributing to steatosis, liver injury, and disease progression (the integrated response hypothesis). Because these pathways contribute to the insulin resistance phenotype of NAFLD, treatments that improve it, such as thiazolidinediones (TZDs), may be beneficial. Hence Furan thiazolidinedione's physical and chemical properties are studied and protein Cytochrome P-450 2A5 is docked with Furan thiazolidinedione using docking tools.

Keywords - Non-alcoholic fatty liver disease, Cytochrome P450 2A5, Homology Model, thiazolidinediones, Molecular Docking.

Introduction

Similar to obesity, which has progressively increased over the world over the past thirty years, non-alcoholic fatty liver disease (NAFLD) is becoming more and more common (Calzadilla Bertot and Adams, 2016) [1]. The most frequent cause of fatty liver disease is non-alcoholic fatty liver disease (NAFLD) (Tushuizen *et al*., 2020) [2]. It is anticipated that the prevalence of NAFLD will continue to rise as a result of the increased incidence of obesity in both the adult and pediatric populations. It is a key contributor to chronic liver diseases, such as cirrhosis and hepatocellular cancer, across the world. The term "NAFLD" refers to a wide range of conditions, including simple steatosis, steatohepatitis (also known as "non-alcoholic steatohepatitis," or NASH), fibrosis, and - in the long run - cirrhosis and hepatocellular cancer (Tushuizen *et al*., 2020; Han *et al*., 2023) [2,3]. Nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver (NAFL) are two kinds of NAFLD. Most people get one type of NAFLD or the other, and those who have one form are occasionally found to have the other. A variant of NAFLD called NAFL is characterized by liver fat but little to no liver damage or inflammation. In addition to liver fat, NASH is a variant of NAFLD in which the liver is also inflamed and damaged [4].

Influence of Sex in NAFLD: In men of reproductive age, NAFLD is more common and more severe than in women. However, NAFLD in women is more common after menopause, indicating that estrogen may be protective. Menopausal status may have an impact on the relationship between sex and fibrosis. According to cross-sectional studies, men and post-menopausal women have a higher risk of fibrosis than pre-menopausal women do, and early menopause and longer menopause are linked to a higher risk of fibrosis (Calzadilla Bertot and Adams, 2016; Lonardo *et al*., 2016) [1,5].

Genetics of NAFLD: The primary genetic predictor of hepatic fat level is the ubiquitous I148M mutation of PNPLA3, which impairs the remodeling of hepatocellular lipid droplets. The I148M mutation significantly affects the complete range of fatty liver-related liver disease (Dongiovanni Valenti, 2016) [6]. Minor allele frequencies of 20%–50% and 10% are prevalent in the general population for polymorphisms in the PNPLA3 and TM6SF2 genes (Calzadilla Bertot and Adams, 2016) [1]. Common GCKR mutations increase de novo hepatic lipogenesis in response to inflammatory liver conditions and glucose. Additionally,

uncommon mutations in APOB and the low-frequency E167K variation of TM6SF2, which impede very low-density lipoproteins secretion, predispose to the progressive fatty liver (Dongiovanni Valenti, 2016) [6].
Age: Given the epidemics of obesity and type 2 diabetes mellitus, an increase in the prevalence of NAFLD/NASH is predicted. Older patients are disproportionately affected by NASH and associated complications like progressive fibrosis, cirrhosis, and hepatocellular carcinoma; however, due to their frailty and comorbidities, they are frequently ineligible for liver transplantation, and there is still a lack of effective medical treatments (Li *et al*, 2022) [7].

Lifestyle and Diet: Dietary patterns that are high in dietary fiber, monounsaturated fatty acids, and polyunsaturated fatty acids have a good impact on gut microbiota, intestinal barrier function, and intrahepatic triglyceride (IHTG) accumulation. These diets are low in high glycemic index foods and saturated fats (Riazi *et al*, 2022; Fernández *et al*., 2022) [8,9]. The therapy of NAFLD is effective with lifestyle changes. Exercise, a healthy diet, or a combination of the two helps treat NAFLD. Combining dietary and activity adjustments improves metabolic markers and liver function tests better than similar therapies done alone. Exercise may be more suitable for NAFLD patients since it improves cardiorespiratory function, which may act as a barrier against cardiovascular diseases (Fernández *et al*., 2022) [9].

Subtypes: Due to the intricacy of its pathophysiology and the clinical factors that cause it to arise, NAFLD has a diversity of clinical phenotypes and heterogeneity, resulting in various clinical prognosis (Han *et al*., 2023) [10].

Nonalcoholic fatty Liver Disease (simple steatosis): Several earlier research findings have claimed that NAFL is a benign condition. Several investigations using paired or repeat liver biopsies revealed that NAFL had a significantly better overall prognosis than NASH, including the progress of cirrhosis. Macro vesicular and microvesicular steatosis are the two subtypes. Although the steatosis in NAFLD is often macrovesicular, 10% of patients may also have microvesicular steatosis (Han *et al*., 2023) [10].

Nonalcoholic steatohepatitis: Non-alcoholic fatty liver disease (NAFLD) gives rise to non-alcoholic steatohepatitis (NASH). It is believed that 25% of people have NAFLD and that 25% of people with NAFLD also have NASH. Typical NASH symptoms include liver steatosis, inflammation, and fibrosis that are caused by metabolic disturbances such as obesity, diabetes, and dyslipidemia (Peng *et al*., 2020) [11]. Approximately 82% of NASH patients are obese, 83% have hyperlipidemia, and 48% have type 2 diabetes, according to epidemiological research [12]. Progressive non-alcoholic steatohepatitis (NASH), which is a leading cause of cirrhosis, hepatocellular cancer, and liver transplant indications, is on the rise (Younossi *et al*., 2018) [12].

Non Alcoholic fatty liver disease and Type 2 Diabetes: Nonalcoholic fatty liver disease (NAFLD) and type 2 diabetic mellitus (T2DM) are frequently found together (Dharmalingam *et al*., 2018) [14]. Along with the obesity pandemic, non-alcoholic fatty liver disease (NAFLD) is becoming more commonplace worldwide. More than 70% of people with type 2 diabetes have NAFLD. Numerous recent research has further demonstrated that type 2 diabetes is directly related to the development of cirrhosis, hepatocellular carcinoma, liver-related morbidity, and mortality, despite the well-established mutually harmful association between NAFLD and type 2 diabetes (Lee *et al*., 2022) [13]. Cardiovascular events in NAFLD are 1.87-fold more likely in the context of T2DM (Dharmalingam *et al*., 2018) [14]. It is not surprising that persons with established diabetes have significantly higher liver fat when compared to controls of the same age, BMI, and gender given the sneaky nature of type 2 diabetes (Hazlehurst *et al*., 2016) [15]. The major ways to reduce NAFLD symptoms and indicators include weight loss and lifestyle modifications, however, antihyperglycemic medications can be used to treat diseases that are related to NAFLD (Tamaki *et al*., 2022) [16].

Non-Alcoholic Fatty Liver Disease and Obesity: Nonalcoholic steatohepatitis (NASH), NASH-related cirrhosis, and hepatocellular carcinoma have all been linked to obesity in addition to simple steatosis (SS), which is a milder form of the disease. These advanced diseases include hepatocellular carcinoma and nonalcoholic steatohepatitis (NASH), which are all closely related to the obesity epidemic (Polyzos *et al*., 2019) [17]. Due to an increase in the incidence of obesity, nonalcoholic fatty liver disease (NAFLD) is quickly taking over as the most common cause of chronic liver disease. Morbidity and mortality rise as a result of the emergence of NASH. While lifestyle improvements, such as dietary adjustments and increased physical activity, constitute the primary line of treatment for this condition (Sarwar *et al*., 2018) [18].

CytochromeP450 2A5: The CYPs are hemoproteins with a single heme prosthetic group in the active site and 400–500 amino acids in their amino acid sequences. The Protein Data Bank (PDB) presently contains 104 distinct CYP structures, and the mounting evidence implies that the overall CYP folds are relatively conservative (Zhao *et al*., 2021) [20]. The membrane-bound haemoproteins identified as cytochrome P450 (CYP) enzymes are essential for cellular metabolism, homeostasis, and the detoxification of xenobiotics. The activation or inhibition of CYP enzymes is an essential mechanism that underpins drug-drug interactions. Through receptor-dependent pathways, a variety of xenobiotics and endogenous substrates can transcriptionally activate CYP enzymes (Manikandan and Nagini, 2018) [19]. The majority of pharmaceuticals undergo oxidative biotransformation mediated by the cytochrome P450 (CYP) enzymes, which also play a significant role in determining the duration of pharmacological action (Murray and Petrovic, 2006) [21].

Cytochrome P450 2A5 and Nonalcoholic liver disease: The liver is a crucial organ for the metabolism of drugs, and cytochrome P450 (CYP) is known as the liver's primary drug-metabolizing enzyme. To present, 57 CYP genes, 20 subfamilies, and about 21 families have been found in humans (Chiba *et al*., 2016) [22]. According to the two-hit theory, which is the suggested mechanism for how NAFLD progresses, hepatocyte lipid buildup, which is considered the "first hit," is followed by a "second hit" that includes insulin resistance, oxidative stress, and cytokine production (Cui *et al*., 2013) [23]. Antioxidant response elements,

also known as electrophile response elements (AREs), play a key role in a cell's ability to cope with oxidative stress and are predominantly regulated by the transcription factor nuclear factor erythroid 2-like 2 (Nrf2) (Cui *et al*., 2013) [23]. By regulating pathways related to lipid metabolism, such as oxidative stress, arachidonic acid metabolism, steroid hormone metabolism, and fatty acid metabolism, the CYP450 family plays a crucial role in the pathophysiology of fatty liver (Liu *et al*., 2017) [24].

Treatment: There is currently no FDA-approved treatment for this illness, so the need for acceptable therapeutic targets is critical. It was observed that the function of pharmacological drugs, surgical techniques, and gut microbiomes in the treatment of NASH (Raza *et al*., 2021) [30]. Every patient who is at risk for or who has NAFLD should be advised that leading a healthy lifestyle is the cornerstone for preventing and managing the condition. Hepatic steatosis and insulin resistance have been demonstrated to decrease with calorie intake reductions of at least 500-1000 kcal (Paternostro and Trauner, 2022; Wong, 2018) [28,29]. Pharmacological treatments specially designed to reduce hepatic inflammation, fibrosis, and steatohepatitis are required for patients who do not respond well to lifestyle changes or have an advanced illness (significant fibrosis). We specifically concentrate on the function of antioxidants, cholesterol-lowering medications, incretins, thyroid hormone mimics, and cytokines as therapeutic targets for NASH (Raza *et al*., 2021) [30]. Thiazolidinediones (TZDs) may be useful in treating certain conditions. The symptoms of TZDs include improved insulin sensitivity, elevated levels of adiponectin, redistribution of adipose tissue to the periphery, anti-atherosclerotic effects, anti-inflammatory effects, and activation of the nuclear receptor, peroxisome proliferator-activated receptor (NPAR) (He *et al*., 2016) [31].

Thiazolidinediones: Thiazolidinediones (TZDs), often known as "glitazones," are a novel class of oral anti-diabetic medications that enhance metabolic management in type 2 diabetes patients by increasing insulin sensitivity (Hauner, 2002) [25]. Type 2 (noninsulin-dependent) diabetic patients, slowly produce an antihyperglycaemic effect by selectively enhancing or partially mimicking specific activities of insulin (Day, 1999) [26]. Thiazolidinediones like rosiglitazone and pioglitazone are used to treat non-insulin-dependent diabetic mellitus. Troglitazone and these substances were assessed for their capacity to activate P450 cytochrome P450 enzymes in primary human hepatocyte cultures (Sahi *et al*., 2003) [27]. Thiazolidinediones are peroxisome proliferator-activated receptor gamma (PPAR-γ) ligands that can reverse insulin resistance and adipose tissue dysfunction, which have been examined in NASH (Sarwar *et al*., 2018) [18]. Patients should diagnose liver function tests (LFT) assessed before starting TZD therapy as well as periodically during treatment. LFTs should not be initiated or should be stopped with TZD if they are three times the upper limit or more (Eggleton and Jialal, 2023) [32].

METERIALS AND METHODS

In the theoretical approach to get high frequency computational analysis for molecular modeling and protein ligand interaction, studies were performed on a workstation of AMD Ryzen 5 5500U with Radeon Graphics 2.10 GHz and 8 GB RAM.

Sequence Alignment and model building

Sequence alignment was performed with ClustalX 1.83 (Chenna *et al*., 2003) [33] and Clustal W. For this cytochrome, P450 2A5[Mus musculus] sequence was obtained from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/protein/NP_031838.2) in FASTA format. Homologous entries for cytochrome P450 2A5 sequence were obtained from the Protein Data Bank using BLASTp (Basic Local Alignment Search Tool) at NCBI (Altschul *et al*., 1997) for template search. In BLASTp, a closely related sequence cytochrome P450 2A5 was selected as a template. To identify the conserved sequences the cytochrome P450 2A5 and selected template sequences were subjected to the ClustalX 1.83 with default parameters (Chenna *et al*., 2003) [33]. The chosen sequence of cytochrome, P450 2A5[Mus musculus] model was built based on a template available in the Protein Data Bank (PDB) and a chain using SWISS MODELLER (https://swissmodel.expasy.org/), online software, The MODELLER program uses the spatial constraints determined from the crystal structures to build a 3D model of the target protein with unknown tertiary structure. The quality of the built model was assessed using the modeller objective function parameter. The main geometric parameters of the models were determined by PROCHECK (Laskowski *et al*., 1993) [34].

Model Assessment

In the final step of homology modelling, the chosen model was subjected to a battery of internal consistency and reliability tests. PROCHECK was used to assess the quality of the predicted cytochrome P450 2A5 model (Laskowski *et al*., 1993) [34]. The Ramachandran plot was used to provide the most powerful check for the stereochemical quality of a protein structure. PROCHECK was used to validate the stereochemical quality and structure analysis (backbone and dihedral angle values) for the model following its complete structural optimization (quality conformation) (Laskowski *et al*., 1993) [34]. To measure the packing quality of the homology model, the WHAT-IF Quality Control value was generated (Yu Yamamori *et al*., 2022) [35]. The PROSA [35] test was used to compare the potential of mean force produced from a broad group of known protein structures to energy criteria. The results were then compared to the corresponding analysis of the crystallographic structure, whose coordinates were acquired directly from the PDB, and the produced structure's Z-score was determined using WHAT-IF and ProSA online analysis. Pymol was an extremely versatile, extendable package for molecular visualization that was used to construct clear, informative, and appealing representations of atomic data. The constructed model of cytochrome P450 2A5 was uploaded to PDBSUM (Laskowski *et al*., 1993) [34], and the secondary structure was shown and analyzed.

Selection of Ligand

The substrate Furan thiazolidinedione was selected as a ligand molecule, it was already as part of continuing investigations that thiazolidinedione, had the ability to induce cytochrome P450 enzymes (P450) in primary human hepatocyte cultures. By

regulating lipid metabolism-related pathways such as oxidative stress, arachidonic acid metabolism, steroid hormone metabolism, and fatty acid metabolism, the CYP450 family plays an important role in the pathophysiology of fatty liver.

Docking

HDOCK is a brand-new web server for our hybrid docking technique that combines template-based modelling and free docking, allowing the free docking protocol to be used to save cases with misleading templates. The server accepts inputs for proteins' sequences and structures and facilitates docking between proteins and proteins and DNA/RNA. The docking procedure takes around 10 to 20 minutes per docking run. HDOCK server tool which has provided information that Furan Thiazolidinedione, is possessing the best lowest docking energy, and thus it has been selected for further analysis. Docking of the lead molecule for best interactions with cytochrome P450 2A5 has given an insight that the lead has shown interactions with active site amino acids of cytochrome P450 2A5.

RESULTS AND DISCUSSION

Sequence alignment and homology modelling

The target FASTA sequence of cytochrome P450 2A5 was retrieved from NCBI and was subjected initially to template search in BLASTp. Among the obtained proteins PDB ID-11z1 was selected as a template to model the structure of the cytochrome P450 2A5. The comparative homology score of the target protein was 85.74% shown in Fig.1. The high level sequence identity may ensure more precise alignment of the target sequence and template structure. No crystallographic data was observed when BLASTp analysis against Mus Musculus was performed for cytochrome P450 2A5.

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NP_031838.2        1 MLTSGLLLVAAVAFLSVLVLMSVWKQRKLSGKLPPGPTPLPFIGNFLQLN     50
                                          :.|:....|||||||||||||:||||
1Z11D              1 ----------------------MAKKTSSKGKLPPGPTPLPFIGNYLQLN     28

NP_031838.2       51 TEQMYNSLMKISQRYGPVFTIYLGPRRIVVLCGQEAVKEALVDQAEEFSG    100
                     ||||||||||||:||||||||:|||||:|||||.:||:||||||||||||
1Z11D             29 TEQMYNSLMKISERYGPVFTIHLGPRRVVVLCGHDAVREALVDQAEEFSG     78

NP_031838.2      101 RGEQATFDWLFKGYGVAFSSGERAKQLRRFSIATLRDFGVGKRGIEERIQ    150
                     |||||||||:|||||||.||:||||||||||||||||||||||||||||||
1Z11D             79 RGEQATFDWVFKGYGVVFSNGERAKQLRRFSIATLRDFGVGKRGIEERIQ    128

NP_031838.2      151 EEAGFLIDSFRKTNGAFIDPTFYLSRTVSNVISSIVFGDRFDYEDKEFLS    200
                     ||||||||:.|.|.||.|||||:|||||||||||||||||:|||||||
1Z11D            129 EEAGFLIDALRGTGGANIDPTFFLSRTVSNVISSIVFGDRFDYKDKEFLS    178

NP_031838.2      201 LLRMMLGSFQFTATSMGQLYEMFSSVMKHLPGPQQQAFKELQGLEDFITK    250
                     |||||||.||||:||.|||||||||||||||||||||:.||||||||.|
1Z11D            179 LLRMMLGIFQFTSTSTGQLYEMFSSVMKHLPGPQQQAFQLLQGLEDFIAK    228

NP_031838.2      251 KVEHNQRTLDPNSPRDFIDSFLIRMLEEKKNPNTEFYMKNLVLTTLNLFF    300
                     ||||||||||||||||||||||||.||:||||||||:||||:||||||.
1Z11D            229 KVEHNQRTLDPNSPRDFIDSFLIRMQEEEKNPNTEFYLKNLVMTTLNLFI    278

NP_031838.2      301 AGTETVSTTLRYGFLLLMKHPDIEAKVHEEIDRVIGRNRQPKYEDRMKMP    350
                     .|||||||||||||||||::|||||||||||||:|||||:|||.|||
1Z11D            279 GGTETVSTTLRYGFLLLMKHPEVEAKVHEEIDRVIGKNRQPKFEDRAKMP    328

NP_031838.2      351 YTEAVIHEIQRFADMIPMGLARRVTKDTKFRDFLLPKGTEVFPMLGSVLK    400
                     |.||||||||||.|:|||.|||||.||||||||.|||||||:|||||||:
1Z11D            329 YMEAVIHEIQRFGDVIPMSLARRVKKDTKFRDFFLPKGTEVYPMLGSVLR    378

NP_031838.2      401 DPKFFSNPKDFNPKHFLDDKGQFKKNDAFVPFSIGKRYCFGEGLARMELF    450
                     ||.|||||:||||:|||::||||||:||||||||||.||||||||||||
1Z11D            379 DPSFFSNPQDFNPQHFLNEKGQFKKSDAFVPFSIGKRNCFGEGLARMELF    428
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Figure 1: Alignment of cytochrome P450 2A5(Mus musculus) with the experimental structure 11z1.1

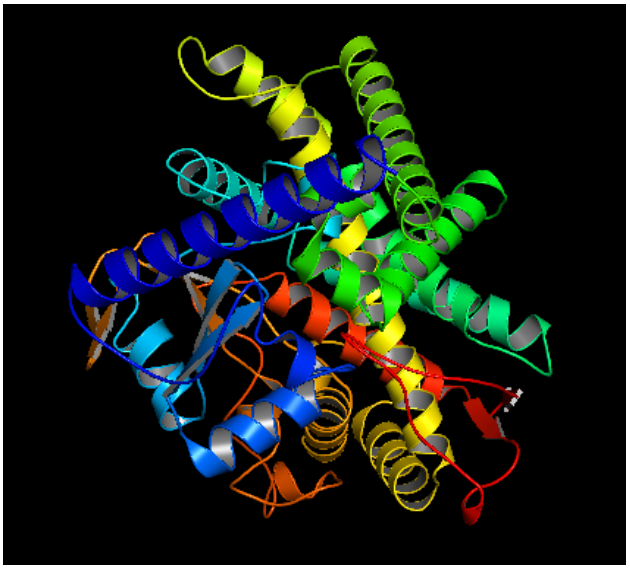
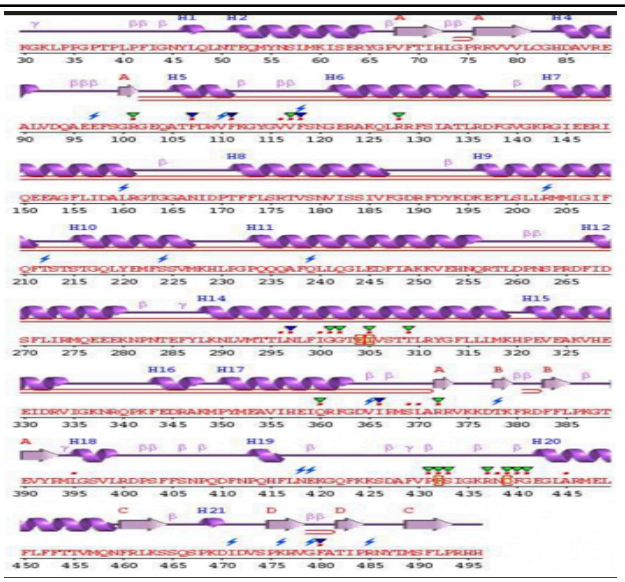
(a)  (b) 

Figure 2: (a) 3D model of Cytochrome P450 2A5(Mus musculus) after energy minimization. (b) Secondary structure of Cytochrome P450 2A5.

This allowed us to use this enzyme for molecular level structural studies for the development of potential drugs for NALFD. The Modeller program uses the spatial constraints determined from the crystal structure of a template protein to build a 3D model of the target protein with an unknown tertiary structure, on the basis of amino acid sequence homology to the sequence alignment. The coordinate of the crystal structure of cytochrome P450 2A5 (mus musculus) (PDB ID: 1lz1) with resolution **2.05** Å (http://www.pdb.org/pdb/explore/explore.do? structureId=1lz1) was used as a template to build the model of cytochrome P450 2A5 (Mus Musculus). The 3D structure of the cytochrome P450 2A5 (Fig. 2(a), was built by using a Swiss modeller (https://swissmodel.expasy.org/interactive/qsHc69/models/), online software. The secondary structure information of the predicted model by PDBSUM shows four beta sheets, four beta Hairpins, two beta bulges, eleven strands, twentyone helices, fouty one helix-helix interactions, thirty five beta turns and five gamma turns in Fig. 2(b).,

Validation of Homology Modelling

Energy minimized accurate structure obtained for cytochrome P450 2A5(Mus musculus) was subjected to structural evaluation in PROCHECK, Saves server, and ProsA-Web. The values obtained are within the values of determined homolog structures. The low overall RMS Z-score values for backbone superposition reflect the highly accurate structural conservation of this complex through evolution, making it a good system for homology modeling. Ramachandran plot (RP) calculations were computed with the PROCHECK program by checking the detailed residue-by-residue stereo-chemical quality of a protein structure (Pisabarro *et al*, 1974) [35] .The RP of the shade, according to the PROCHECK program, symbolizes the distinct sections of the plot; the darker the area, the more favourable combination. The RP depicted in Fig. 3 reveals that 92.8% of the amino acid residues are in the favourable regions and 6.5% of the amino acids are in additional allowed regions and 0.5% residues in generously allowed and 0.2% in disallowed regions of the plot for the whole established model of the enzyme.

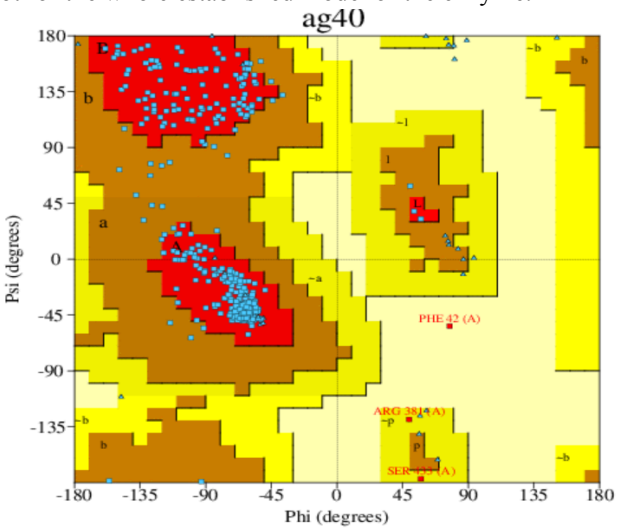


Figure 3: Predicted Ramachandran plot of cytochrome P450 2A5(Mus musculus)

(a) (b)

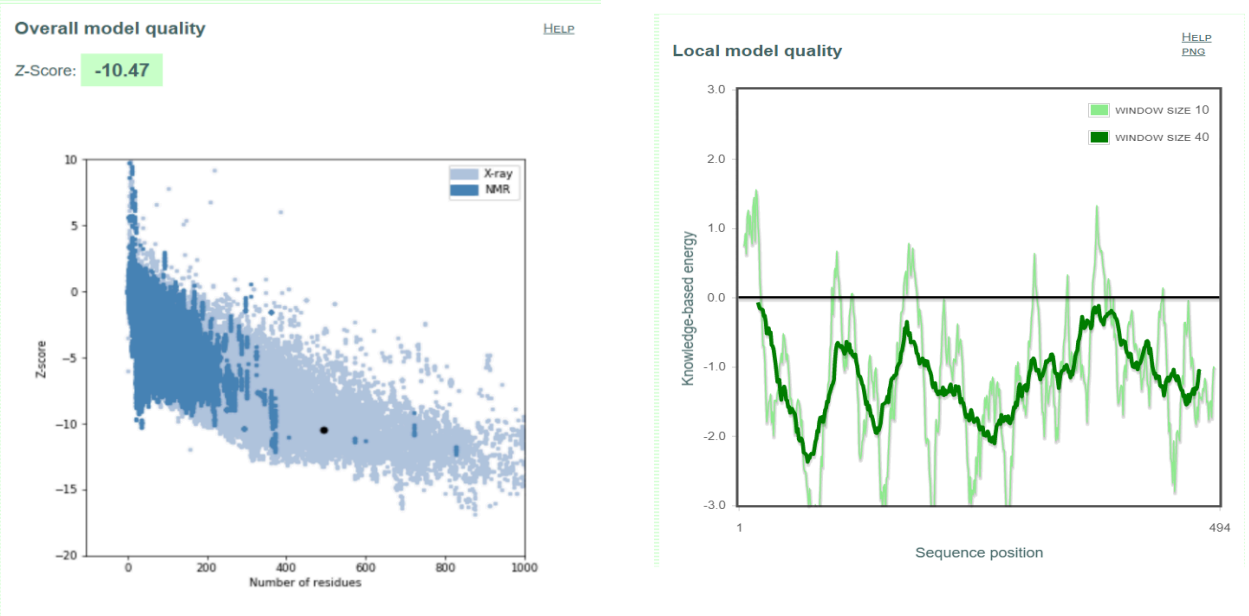


Figure 4: (a) Prosa energy plots showing that local model quality by plotting energies as a function of amino acid sequence position; positive values correspond to problematic or flawed parts of the input structure whereas the negative values correspond to good quality of the structure. (b) Complete model quality of cytochrome P450 2A5(Mus musculus)

The PROSA2003 program was used to determine the interaction energy per residue (Laskowski *et al*., 1993) [34]. The values are less than 0.5, representing that the model is of good quality. ProSA-Web analysis of a cytochrome P450 2A5(Mus musculus) structure shows the knowledge-based energy graphs having negative values corresponding to stable parts of the structure shown in Figs. 4(a) and 5(b). The complete model quality showed the Z scores −10.47 for cytochrome P450 2A5(Mus musculus). Saves is a protein structure validation server. The saves server runs 6 programs for checking and validating the protein structures. Each program can be run independently. It consists of ERRAT, VERIFY3D, PROCHECK, WHATCHECK, etc. ERRAT is a program that validates protein structures determined using crystallography. Shows overall quality factor for non-bonded atomic interactions. A higher score means better quality of models. Errat is used as the support for the statistical analysis of protein models. The ERRAT has produced an overall quality score of 96.2963%, indicating a good quality model shown in Fig. 5(a). Verify3D Determines the compatibility of an atomic model with its own amino acid sequence. To evaluate the quality of the homology protein structure presented in Fig. 5(b), provide a 3D-1D score for each residue. Protein structures where more than 80% of the residues had a score greater than 0.2 are of high quality. cytochrome P450 2A5(Mus musculus) is a reasonable homology model, it has been obtained as a good representation of the actual system, and it can be exposed for examination of protein-substrate and protein inhibitor interactions.

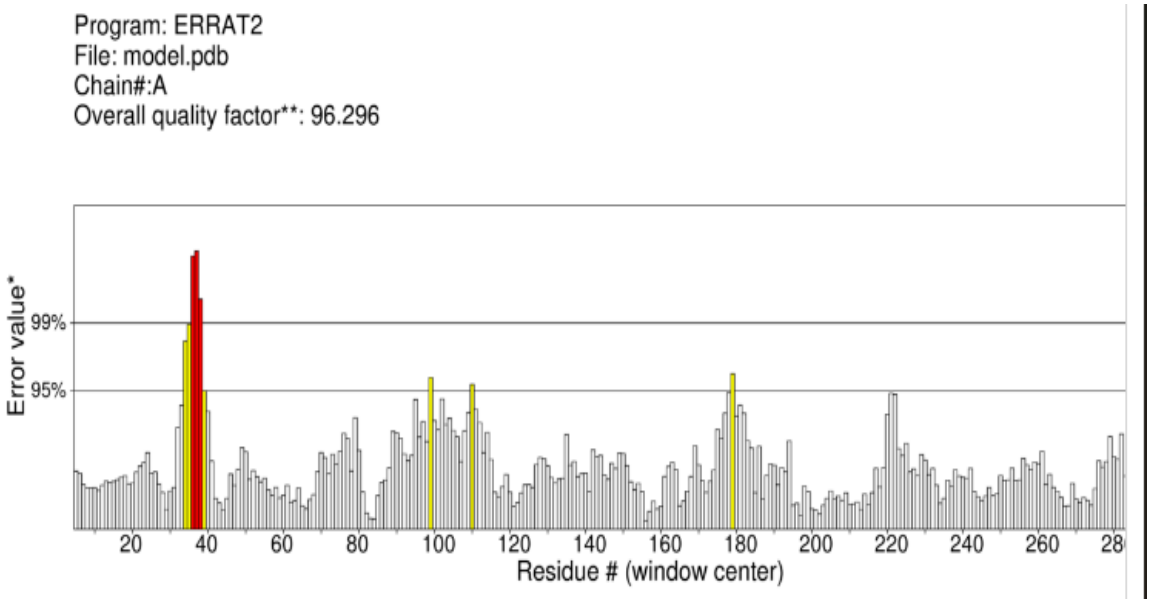


Figure 5: (a) ERRAT results showing overall quality factor of protein cytochrome P450 2A5(Mus musculus) as 96.296

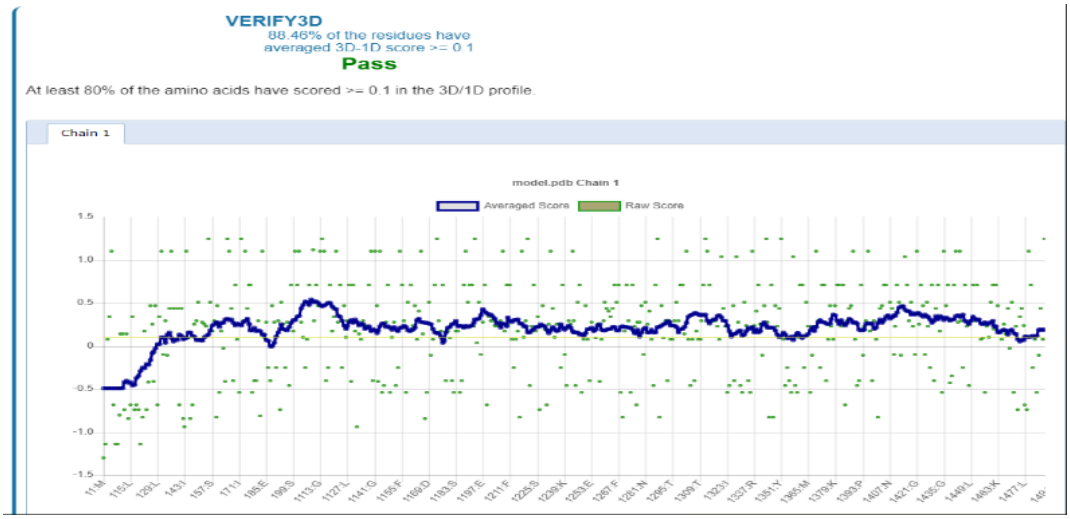


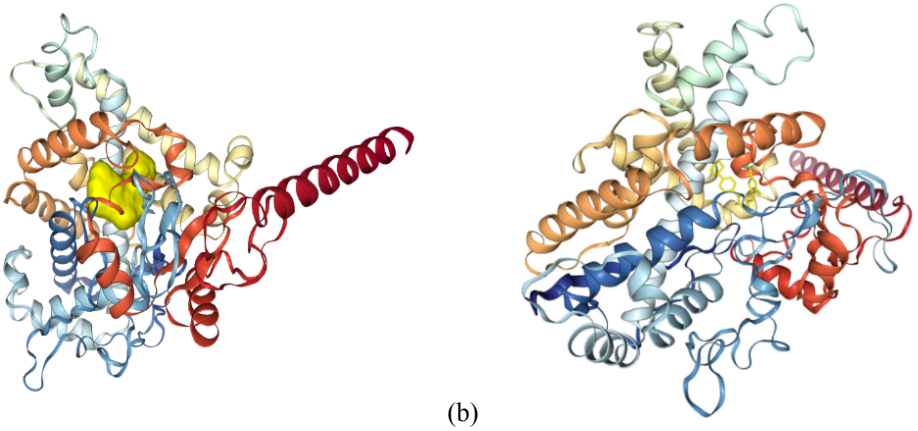
Figure 5: (b) Graph showing protein structures where more than 80% of the residues had a score more than 0.2 are of high quality

Docking

HDOCK is a brand-new web server for our hybrid docking technique that combines template-based modelling and free docking, allowing the free docking protocol to be used to save cases with misleading templates. The server accepts inputs for proteins' sequences and structures and facilitates docking between proteins and proteins and DNA/RNA. The docking procedure takes around 10 to 20 minutes per docking run. The Protein cytochrome P450 2A5 [Mus musculus] PDB file format as well as the drug Furan thiazolidinedione is submitted to the H Dock server and docking is been carried out. It produces the top 10 models showing the Docking score, Coincidence score, and ligand RMSD values as specified in Table 1.

Table 1: The Docking score, Coincidence score, and ligand rmsd values

Rank	1	2	3	4	5	6	7	8	9	10
Docking Score	-163,60	-154,87	-153,73	-152,84	-150,50	-145,98	-145,03	-144,53	-144,26	-142,41
Confidence Score	0.5676	0.5243	0.5186	0.5142	0.5025	0.4799	0.4752	0.4727	0.4713	0.4521
Ligand rmsd (Å)	7.92	7.27	10.85	9.88	8.54	29.37	17.78	19.70	18.13	10.79



(a) (b)

Figure 6: (a) & (b) Docking Results of Cytochrome P450 2A5(Mus musculus) with the ligand molecule Furan thiazolidinedione

Table 2: Showing Catalytic residues that exclusively contribute to the binding with the target ligand molecule

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# Receptor interface residue(s):
ARG     101A     3.850
PHE     107A     3.155
PHE     111A     2.435
VAL     116A     3.253
ALA     117A     3.398
PHE     118A     3.268
ARG     128A     3.111
LEU     241A     4.226
LEU     296A     4.452
ASN     297A     2.903
PHE     300A     2.525
ALA     301A     3.658
THR     305A     4.898
ILE     366A     3.163
GLY     369A     4.880
LEU     370A     3.178
ARG     437A     2.348
TYR     438A     2.064
CYS     439A     3.130
PHE     440A     2.873
PHE     480A     2.990
```

The root mean square deviation (RMSD) would be positive. The closer a structure is to a reference structure, as measured by the RMSD; the lower the RMSD, the better. Some docking methods, on the other hand, generate a docking score that is related to the free energy of binding a ligand to a receptor. The greater negative the score for this form of docking score, the better. Since model 1 is having a greater negative score of -163.60 and less RMSD value of 7.92 it is considered the best model as shown in Fig. 6 (a) & (b). Catalytic residues were identified that exclusively contribute to the binding with the target ligand molecule shown in Table 2. Pymol is an offline software maintained by Schrödinger. It is a molecular visualization system supported by users and built on an open-source model. The obtained docking results from H DOCK can be visualized using PyMol which gives a clear understanding of polar contacts, and amino acids in active sites shown in Fig. 7 (a), (b). From standard Protein Data Bank file input, the LIGPLOT program automatically creates schematic 2-D representations of protein-ligand interactions. The result is a color or black-and-white PostScript file that presents an easy-to-understand and instructive depiction of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic interactions, and atom accessibilities. The program can be used to demonstrate additional kinds of interactions in proteins and nucleic acids and is entirely general for any ligand. Even though it was created to make it easier to quickly evaluate several enzyme complexes, it has a wide range of uses. The Cytochrome P450 2A5(Mus musculus) interaction with the ligand molecule Furan thiazolidinedione is shown in Fig. 8.

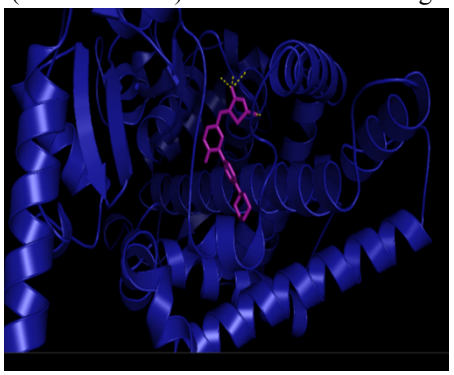
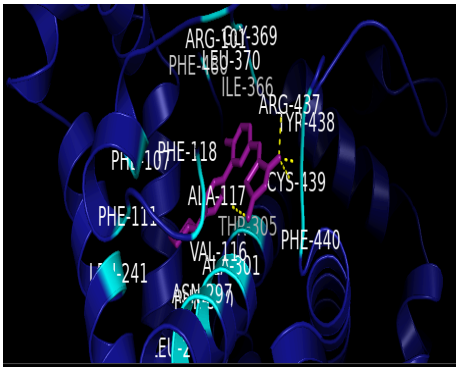
(a)  (b) 

Figure 7: (a) showing polar contacts between the protein Cytochrome P450 2A5(Mus musculus) with the ligand molecule Furan thiazolidinedione (b) Showing amino acids in the active site interacting with the ligand Furan thiazolidinedione

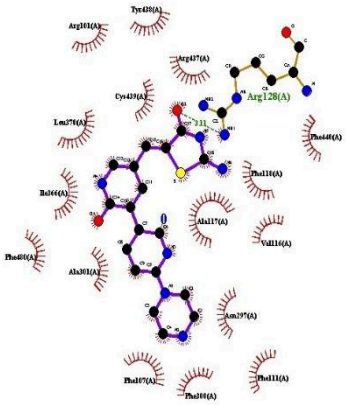


Figure 8: Cytochrome P450 2A5(Mus musculus) interaction with the ligand molecule Furan thiazolidinedione obtained from Ligplot

CONCLUSION

The present study restricted the homology structure of Cytochrome P450 2A5(Mus musculus) to a template crystal structure (PDB ID: 11z1). The favourable structural similarity recognized between the template and target (85.74) is more than that of the threshold value (30%) to get a better homology model. The Model was established using a Swiss Modeller. The developed model of Cytochrome P450 2A5(Mus musculus) was validated with PROCHECK, SAVES Server, and ProsA-Web analysis along with molecular dynamics.

The highly precise homology model was used to predict secondary structures through PDBSUM. The Model established is used for Docking studies. HDOCK server tool which has provided information that Furan Thiazolidinedione, is possessing the best lowest docking energy, and thus it has been selected for further analysis. The predicted structure of Cytochrome P450 2A5(Mus musculus) through *in silico* studies concludes and believes that these results may be helpful to understand the mechanism of action of the protein. Further, it will provide information for those who want to pursue their work in the design of selective Drugs for the treatment of Non-Alcoholic Fatty Liver Disease.

Conflict of interest declaration

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

1. Calzadilla Bertot L, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. International Journal of Molecular Sciences, 2016; (5):774. doi: 10.3390/ijms17050774.
2. Tushuizen ME, Holleboom AG, Koot BGP, Blokzijl H, van Mil SWC, Koek GH. Niet-alcoholische leververvetting [Non-alcoholic fatty liver disease; a full-bodied epidemic]. Ned Tijdschr Geneeskd, 2020; 27: 164: D4096. Dutch. PMID: 32267638.
3. Han SK, Baik SK, Kim MY. Non-alcoholic fatty liver disease: Definition and subtypes. Clinical and Molecular Hepatology, 2023; 29 (suppl): S5-S16. doi 10.3350/cmh.2022.0424.
4. https://www.niddk.nih.gov/health-information/liver-disease/nafld-nash/definition-facts
5. Lonardo A, Nascimbeni F, Ballestri S, Fairweather D, Win S, Than TA, Abdelmalek MF, Suzuki A. Sex Differences in Nonalcoholic Fatty Liver Disease: State of the Art and Identification of Research Gaps. Hepatology, 2019; 70(4):1457-1469. doi 10.1002/hep.30626..
6. Dongiovanni P, Valenti L. Genetics of nonalcoholic fatty liver disease. Metabolism, 2016; 65(8):1026-37. doi: 10.1016/j.metabol.2015.08.018.
7. Li Y, Adeniji NT, Fan W, Kunimoto K, Török NJ. Non-alcoholic Fatty Liver Disease and Liver Fibrosis during Aging. Aging Dis. 2022; 13(4):1239-1251. doi: 10.14336/AD.2022.0318.
8. Riazi K, Swain MG, Congly SE, Kaplan GG, Shaheen AA. Race and Ethnicity in Non-Alcoholic Fatty Liver Disease (NAFLD): A Narrative Review. Nutrients, 2022, 28;14(21):4556. doi: 10.3390/nu14214556.
9. Fernández T, Viñuela M, Vidal C, Barrera F. Lifestyle changes in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis. PLoS One, 2022; 17(2):e0263931. doi: 10.1371/journal.pone.0263931.
10. Han SK, Baik SK, Kim MY. Non-alcoholic fatty liver disease: Definition and subtypes. Clinical and Molecular Hepatology, 2023; 29(suppl): S5-S16. doi: 10.3350/cmh.2022.0424.
11. Peng C, Stewart AG, Woodman OL, Ritchie RH, Qin CX. Non-Alcoholic Steatohepatitis: A Review of Its Mechanism, Models and Medical Treatments. Front in Pharmacology, 2020; 3 (11): 603926. doi: 10.3389/fphar.2020.603926.
12. Younossi ZM, Loomba R, Rinella ME, Bugianesi E, Marchesini G, Neuschwander-Tetri BA, Serfaty L, Negro F, Caldwell SH, Ratziu V, Corey KE, Friedman SL, Abdelmalek MF, Harrison SA, Sanyal AJ, Lavine JE, Mathurin P, Charlton MR, Chalasani NP, Anstee QM, Kowdley KV, George J, Goodman ZD, Lindor K. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology, 2018; 68(1):361-371. doi: 10.1002/hep.29724.
13. Lee CH, Lui DT, Lam KS. Non-alcoholic fatty liver disease and type 2 diabetes: Journal of Diabetes Investigation. 2022;13(6):930-940. doi: 10.1111/jdi.13756..
14. Dharmalingam M, Yamasandhi PG. Nonalcoholic Fatty Liver Disease and Type 2 Diabetes Mellitus. Indian Journal pf Endocrinology Metabolism, 2018 May-Jun;22(3):421-428. doi: 10.4103/ijem.IJEM_585_17. PMID: 30090738; PMCID: PMC6063173.
15. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. Metabolism, 2016; 65(8):1096-108. doi: 10.1016/j.metabol.2016.01.001.
16. Tamaki N, Ahlholm N, Luukkonen PK, Porthan K, Sharpton SR, Ajmera V, Kono Y, Dave S, Ahmed A, Sundaram V, Wilkinson MJ, Patton H, Gupta H, Cervantes V, Hernandez C, Lopez SJ, Loomba R, Baumgartner A, Richards L, Arkkila PE, Nemes K, Isoniemi H, Yki-Järvinen H, Loomba R. Risk of advanced fibrosis in first-degree relatives of patients with nonalcoholic fatty liver disease. Journal of Clinical Investigation, 2022;132(21): e162513. doi: 10.1172/JCI162513.
17. Polyzos SA, Kountouras J, Mantzoros CS. Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. Metabolism, 2019; 92:82-97. doi: 10.1016/j.metabol.2018.11.014.
18. Sarwar R, Pierce N, Koppe S. Obesity and nonalcoholic fatty liver disease: current perspectives. Diabetes Metab Syndr Obes, 2018; 2511:533-542. doi: 10.2147/DMSO.S146339.

19. Manikandan P, Nagini S. Cytochrome P450 Structure, Function and Clinical Significance: A Review. Curr Drug Targets, 2018;19(1):38-54. doi: 10.2174/1389450118666170125144557.
20. Zhao M, Ma J, Li M, Zhang Y, Jiang B, Zhao X, Huai C, Shen L, Zhang N, He L, Qin S. Cytochrome P450 Enzymes and Drug Metabolism in Humans. International Journal of Molecular Sciences, 2021; 22(23):12808. doi: 10.3390/ijms222312808.
21. Murray M, Petrovic N. Cytochromes P450: decision-making tools for personalized therapeutics. Current opinion in molecular therapeutics, 2006; 8(6):480-6..
22. Chiba T, Noji K, Shinozaki S, Suzuki S, Umegaki K, Shimokado K. Diet-induced non-alcoholic fatty liver disease affects expression of major cytochrome P450 genes in a mouse model. Journal of Pharmacy and Pharmacology, 2016; 68(12):1567-1576. doi: 10.1111/jphp.12646..
23. Cui Y, Wang Q, Li X, Zhang X. Experimental nonalcoholic fatty liver disease in mice leads to cytochrome p450 2a5 upregulation through nuclear factor erythroid 2-like 2 translocation. Redox Biology, 2013; 24: 1(1): 433-40. doi: 10.1016/j.redox.2013.08.003.
24. Liu Y, Cheng F, Luo YX, Hu P, Ren H, Peng ML. [The role of cytochrome P450 in nonalcoholic fatty liver induced by high-fat diet: a gene expression profile analysis]. Zhonghua Gan Zang Bing Za Zhi. 2017: 20-25(4): 285-290. Chinese. doi: 10.3760/cma.j.issn.1007-3418.2017.04.010.
25. Hauner H. The mode of action of thiazolidinediones. Diabetes/Metabolism Research and Reviews, 2002;18(2): S10-5. doi: 10.1002/dmrr.249.
26. Day C. Thiazolidinediones: a new class of antidiabetic drugs. Diabetic Medicine, 1999; 16(3):179-92. doi: 10.1046/j.1464-5491.1999.00023.x.
27. Sahi J, Black CB, Hamilton GA, Zheng X, Jolley S, Rose KA, Gilbert D, LeCluyse EL, Sinz MW. Comparative effects of thiazolidinediones on in vitro P450 enzyme induction and inhibition. Drug Metabolism & Disposition, 2003;31(4):439-46. doi: 10.1124/dmd.31.4.439.
28. Paternostro R, Trauner M. Current treatment of non-alcoholic fatty liver disease. Journal of Internal Medicine, 2022; 292(2):190-204. doi: 10.1111/joim.13531.
29. Wong VW. Current Prevention and Treatment Options for NAFLD. Advances in Experimental Medicine and Biology, 2018;1061:149-157. doi: 10.1007/978-981-10-8684-7_12.
30. Raza S, Rajak S, Upadhyay A, Tewari A, Anthony Sinha R. Current treatment paradigms and emerging therapies for NAFLD/NASH. Frontiers in Bioscience-Landmark (Landmark Ed), 2021; 26(2): 206-237. doi: 10.2741/4892.
31. He L, Liu X, Wang L, Yang Z. Thiazolidinediones for nonalcoholic steatohepatitis: A meta-analysis of randomized clinical trials. Medicine (Baltimore), 2016; 95(42): e4947. doi: 10.1097/MD.0000000000004947.
32. Eggleton JS, Jialal I. Thiazolidinediones. [Updated 2023 Feb 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. https://www.ncbi.nlm.nih.gov/books/NBK551656/
33. Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research, 2003; 31(13):3497-500. doi: 10.1093/nar/gkg500.
34. Laskowski, R. A. MacArthur M. W., Moss D. S. Thornton J. M. PROCHECK: a program to check the stereochemical quality of protein structures Journal of Applied Crystallography, 1993; 26: 283-291. https://doi.org/10.1107/S0021889892009944
35. Pisabarro MT, Ortiz AR, Serrano, L, Wade RC. Homology modeling of the Abl-SH3 domain. Proteins, 1994; 20(3):203-15.
36. Yu Yamamori, Kentaro Tomii, Application of Homology Modeling by Enhanced Profile–Profile Alignment and Flexible-Fitting Simulation to Cryo-EM Based Structure Determination, International Journal of Molecular Sciences, 2022; 2 (4); 1977. 10.3390/ijms23041977.