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EXPLORING THE EFFECTIVENESS OF GRAMINE FOR TREATING LIVER DISEASES BY APPROACHING MOLECULAR DOCKING.

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ABSTRACT

The aim of the present is to investigate the therapeutic capabilities of gramine in treating liver diseases by utilizing Insilco molecular docking technique and evaluating ADMET (absorption, distribution, metabolism, elimination and toxicity) parameters also. The study further assesses the binding effectiveness through docking studies against 10 target proteins (TNF α, IL6, PIK3, GSK3B, LPL, PPARG, AKTI, PPARA, MAPK 8 and NF-κβ1)associated with liver diseases. Ursodeoxycholic acid, silymarin and L Ornithine L Aspartate served as reference drugs. Notably gramine exhibited good binding affinity for PIK3, GSK3B, PPARA, PPARG, AKTI, MAPK8, TNFα, LPL, IL 6. The binding affinity was high for silymarin, ursodeoxycholic followed by gramine and L ornithine L aspartate. Gramine also showsgood intestinal absorption rate, plasma protein binding as well as non-carcinogenic in nature. However, further validating all the results in vitro and in vivo would be the best way to characterize the property of gramine as therapeutic liver drug.

Keywords: Liver diseases, gramine, silymarin, ursodeoxycholic acid, L ornithine L aspartate, molecular docking, ADMET.

INTRODUCTION

Liver, the largest gland in the body, is involved in many biochemical functions because of the fact that mitochondria are maximally present in them. It plays a crucial role in various metabolic functions (carbohydrate metabolism, protein metabolism, fat metabolism), secretary function, detoxicating and protective functions, storage functions, excretory functions, synthesis functions (albumin, SGOT,SGPT etc), hormone metabolism, and erythropoiesis.[1]The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory, and neoplastic insults. The major primary diseases of the liver are viral hepatitis, non-alcoholic fatty liver disease(NAFLD), alcoholic liver disease, and hepatocellular carcinoma (HCC). Hepatic damage also occurs secondary to some of the most common diseases in humans, such as heart failure, disseminated cancer, and extrahepatic infections.[2]
The rapid deterioration of normal liver function following injury is known as acute liver failure (AFL)[3], associated with infiltration of inflammatory cells and severe organ failure [4][5]and accumulation of excessive fat in the liver (Non-alcoholic fatty liver disease) is increasing rapidly and become a public health problem in world wide. [6] NAFL (Non-alcoholic fatty liver) with isolated steatosis and NASH (non-alcoholic steatohepatitis) characterized by hepatocyte ballooning, inflammatory injury that even progress to fibrosis

and cirrhosis, are the two phenotypes of NAFLD.[7]More than half of the NAFLD patients may develop to NASH and cirrhosis.[8][9]Acute liver failure exhibits high incidence of mortality rate.[10] To the best of our knowledge, presently there is no effective treatment for Acute liver failure and NAFLD, in case of acute liver failure liver transplantation is most widely followed where as in case of NAFLD it may be managed by administration of probiotic, weight loss, reduced intake of fat and sugar.[11]

Gramine, an indole alkaloid initially isolates from Arundo donax.[12]and according Chinese medicine it was widely used to control bad urination, heart diseases and tooth ache. [13] much attention has been drawn to it due to its anti-viral[14],anti bacterial [15], anti inflammatory (by inhibiting pro inflammatory mediators like interlukin 6 , TNF α)[16],anti tumour [17], serotonin receptor related activity [18] and against Alzheimer disease.[19]

Ligand protein interactions can be studied by molecular docking. It is widely applied on structural activity relationship studies, drug discovery, characterization of ligand -target interactions, drug.[20] Furthermore, ADMET score can also be determined using admetSAR online tool to predict properties such as human intestinal absorption, blood brain barrier permeability, acute oral toxicityand carcinogenicity.[21]

Therefore, the present study aimed to explore the ability of gramineto bind different proteins that are involved in progression of liver diseases through molecular docking and comparing against standard drugs like silymarin, ursodeoxycholic acid and L ornithine L aspartate.

METHOD

ADMET PREDICTION:

The absorption, distribution, metabolism, excretion and toxicity (ADMET) profiles of gramine, ursodeoxycholic acid, silymarin and Lornithine L aspartate were determined by admetSAR webserver (http://lmmd.ecust.edu.cn/admetsar2).[22] Properties like human intestinal absorption, brain penetration, protein binding, carcinogenicity and toxicity were established.

MOLECULAR DOCKING

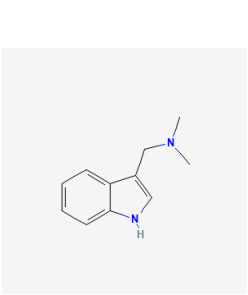
Preparation of the target protein

This study has focussed on proteins likeTumour necrosis factor (TNF), Interleukin 6 (IL 6), Phosphatidylinositol 4,5-biphosphate 3-kinase (PIK3), Glycogen synthase kinase- 3 beta (GSK 3B), Lipoprotein lipase (LPL), Peroxisome proliferator-activated receptor gamma (PPARG), RAC-alpha serine/threonine-protein kinase (AKTI),Nuclear factor kappa-B(NF-κβ), Peroxisome proliferator-activated receptor alpha(PPAR A),Mitogen activated protein kinase 8 (MAPK8), in order to assess the efficacy of the drug in preventing the progression of liver diseases. The 3D structure of these proteins was obtained from RCSB protein data bank (PDB)(https://www. rcsb.org) [23] and saved as PDB format. and then prepared with Biovia drug studio 2024. The water molecules and heteroatoms have been removed, and the hydrogen atoms added.

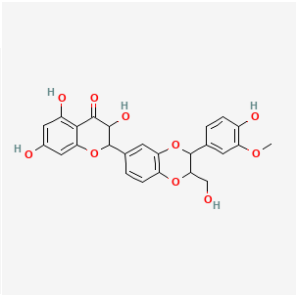
Preparation of ligands

Gramine and reference drugs (Ursodeoxycholic acid, Silymarin and L-Ornithine L-Aspartate) were obtained in SDF format from Pubchem (https:// pubchem.ncbi.nlm.nih.gov).[24-27] The structures were thensubjected to energy minimization and converted to PDBQT format using open Babel plugin of PyRX software. (https://pyrx.sourceforge.io/).[28]

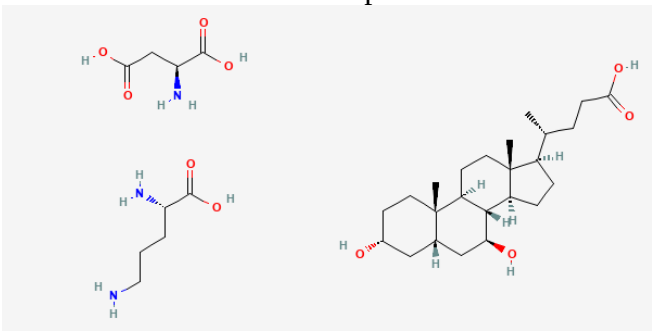
Structure of Gramine Structure of Silymarin



Structure of L ornithine L aspartate



Structure of Ursodeoxycholic acid



Protein-ligand docking

The minimized protein and ligand converted to PDBQT, selected maximized GRID parameter then performed docking studies using VINA WIZARD. Highest negative binding energy indicates good stability at binding site. The model with highest negative binding energy was preferred and visualized protein and ligand type of interaction using biovia discovery studio, 2024 tool.

Auto dock vina docking score below -7kcal/mol indicate strong binding energy, score between -5 kcal/mol and -7 kcal/mol suggest good binding ability. Whereas, score ranging from – 5kcal/mol to -4.25kcal/mol indicate that a certain binding ability exist between the ligand and the protein.[29][30]

RESULTS

Table 1: ADMET PROFILE OF Silymarin, Ursodeoxycholic acid, Gramine and L Ornithine L Aspartate

	HIA	BBB	Acute oral toxicity	Plasma protein binding	Carcinogenicity
Ursodeoxycholic acid	0.9878	0.5750	2.86	0.612	Non carcinogenic
Silymarin	0.9045	0.8000	2.358	0.888	Non carcinogenic
Gramine	0.9858	0.9000	2.412	0.587	Non carcinogenic
L Ornithine L Aspartate	0.7007	0.5750	1.346	0.144	Non carcinogenic

Molecular docking analysis

Interaction with TNF

Silymarin forms hydrogen bond with GLU A: 116, SER B:99, Pi alkyl bond with ARG A:103, Pi-Anion bond with GLU C:116, GLU B:104 and exhibited binding energy of -10.3kcal/mol.

Ursodeoxycholic acid form hydrogen bond with GLN C:102, ARG B:103 and exhibited a binding energy of -8.3kcal/mol. Gramine form hydrogen bond with GLN C:102, Pi-anion bond with GLUC:102 and Pi-alkyl bond with ARG B:103 and exhibited a binding energy of -5.8 kcal/mol. L Ornithine L aspartate form hydrogen bond PRO C:117, TYR C:119, PRO A:117, TYR A:119 and PRO B:117, exhibited binding energy of -5 kcal/mol.

Figure 1: Interaction of TNF with silymarin

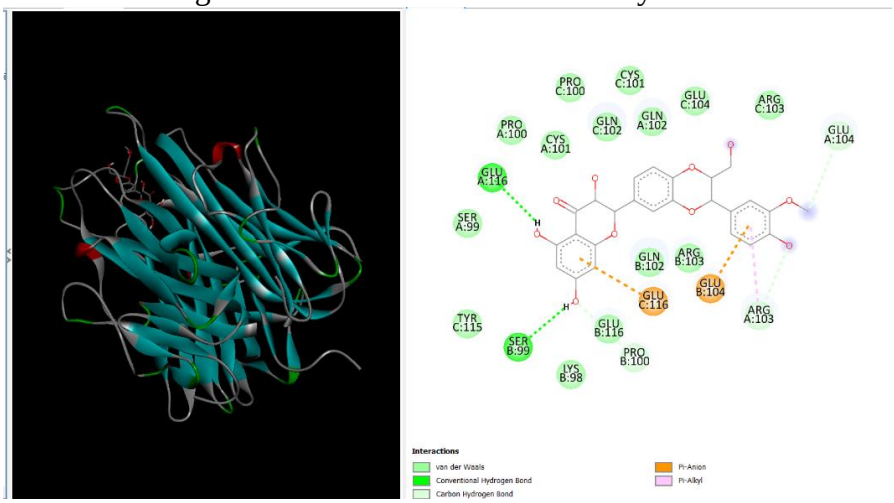


Figure 2: Interaction of TNF with Ursodeoxycholic acid

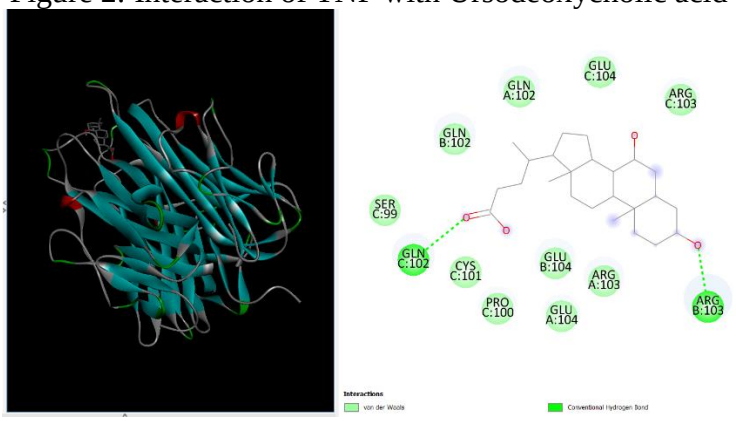


Figure 3: Interaction of TNF with Gramine.

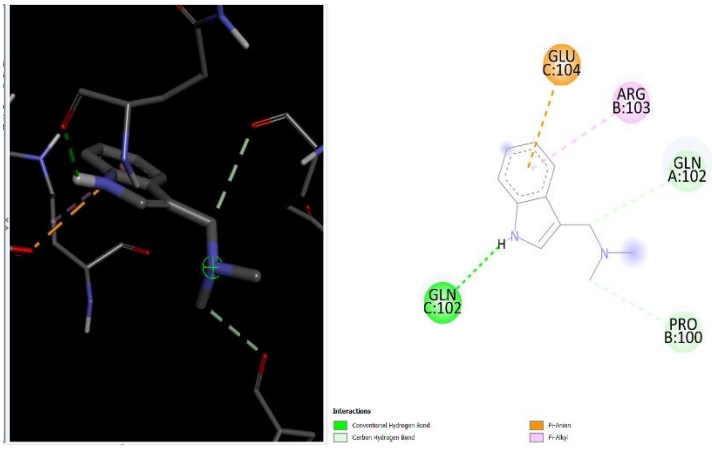
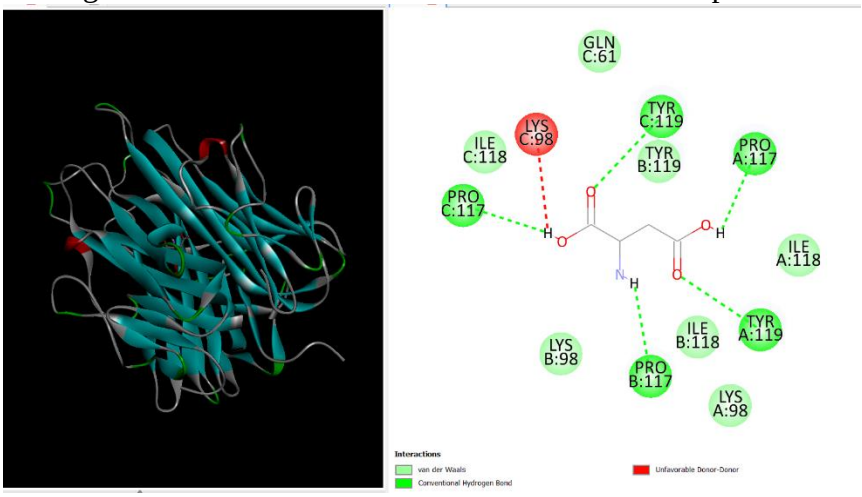


Figure 4: Interaction of TNF with L Ornithine L Aspartate.



Interaction with PIK3

Silymarin forms hydrogen bond with CYS A: 869, Pi-alkyl bond with LEU A:865, Pi-cation bond with GLU A:880, ARG A: 849 and exhibited a binding energy of -9.3 kcal/mol. Ursodeoxycholic acid forms hydrogen bond with GLU A:856, LYS A:298, TYR A:787 and exhibited a binding energy of -8.1 kcal/mol. Gramine forms hydrogen bond with TYR A:787, Pi sigma bond with PHE A: 694, Pi-cation bond with ARG A:849 and exhibited a binding energy of -6.7kcal/mol. L Ornithine L Aspartate forms hydrogen bond with ARG A:839, LYS A:668, ASN A:498, MET A:1036, MET A:1039, THR A:1037 and exhibited a binding energy of -5.1 kcal/mol.

Figure 5: Interaction of PIK3 with Silymarin

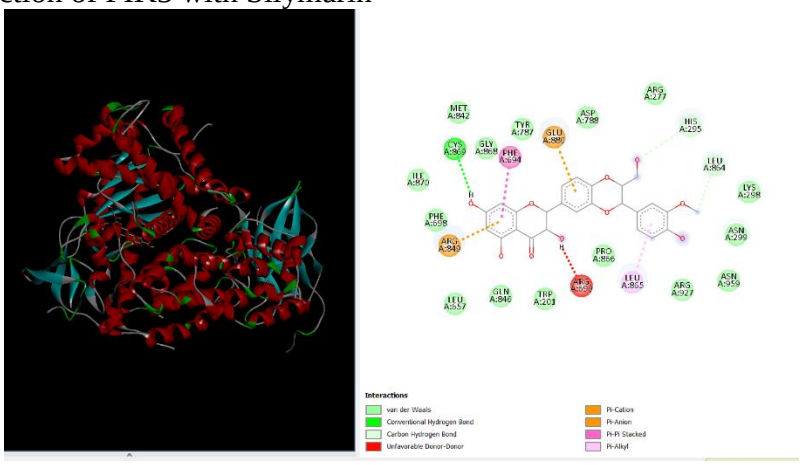


Figure 6: Interaction of PIK3 with Ursodeoxycholic acid

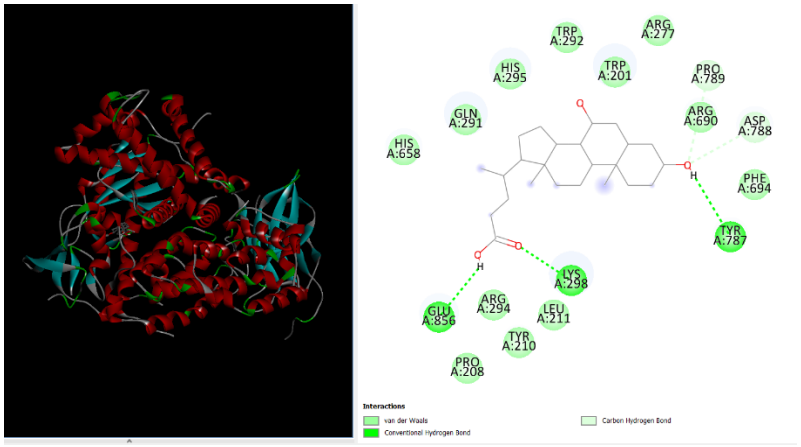


Figure 7: Interaction of PIK3 with Gramine

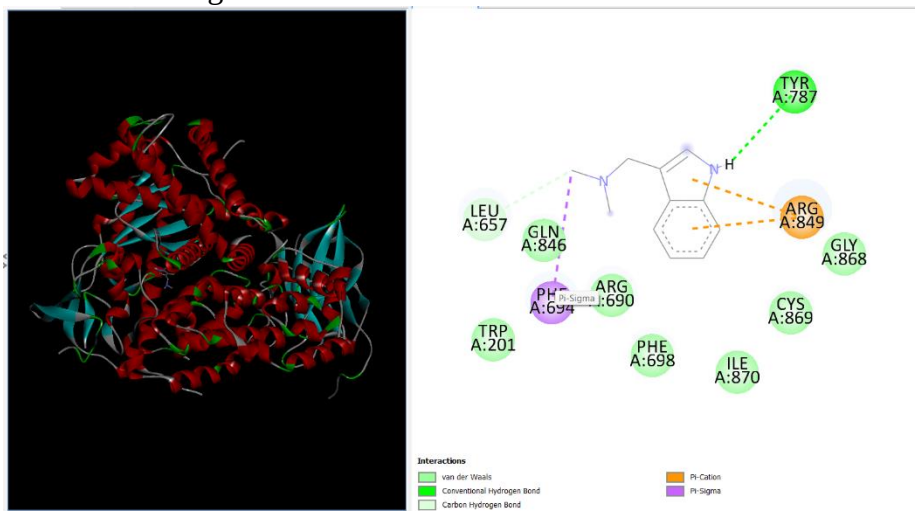
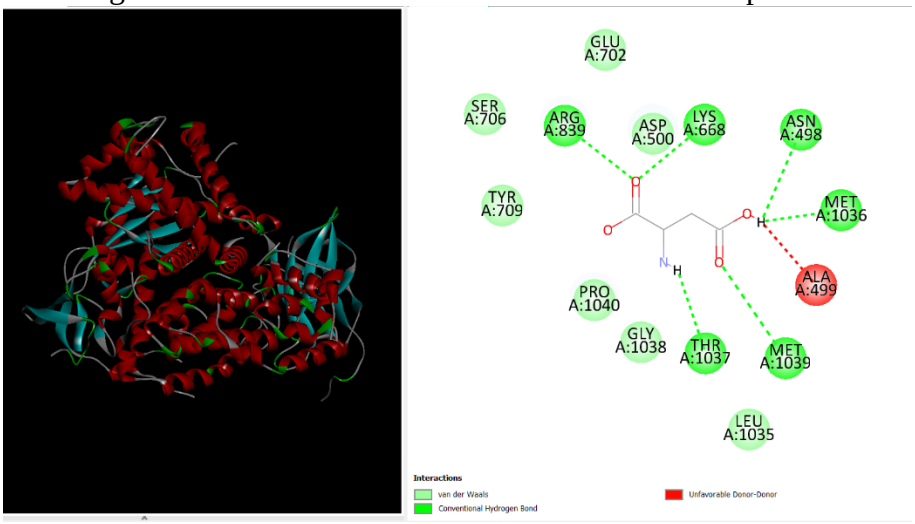


Figure 8: Interaction of PIK3 with L Ornithine L Aspartate



Interaction with GSK3B

Silymarin forms hydrogen bond with LEU A:266, Alkyl and Pi alkyl bond with PHE A:229, PHE A:293, PHE A 291, VAL B:214, VAL A:267, ILE A:270. And exhibited a binding energy of -9.6kcal/mol. Ursodeoxycholic acid form hydrogen bond with ASP B:200 and exhibited a binding energy of -9.1 kcal/mol. Gramine forms hydrogen bond with ASP B:133, Pi-sigma bond with LEU B:188, Pi-alkyl bond with ALA B:83, CYS B:199, LEU B:132, VAL B:110, VAL B:70 and exhibited binding energy of -6.4kcal/mol. L Ornithine L Aspartate form

hydrogen bond with ARG A:223, SER A:215, GLY A:230 and exhibited a binding energy of -4.6kcal/mol.

Figure 9: Interaction of GSK3B with Silymarin

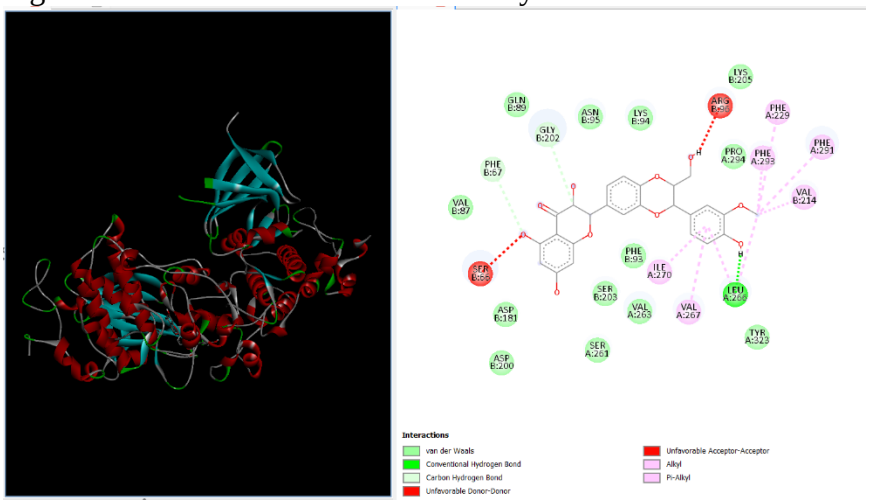


Figure 10: Interaction of GSK3B with Ursodeoxycholic acid

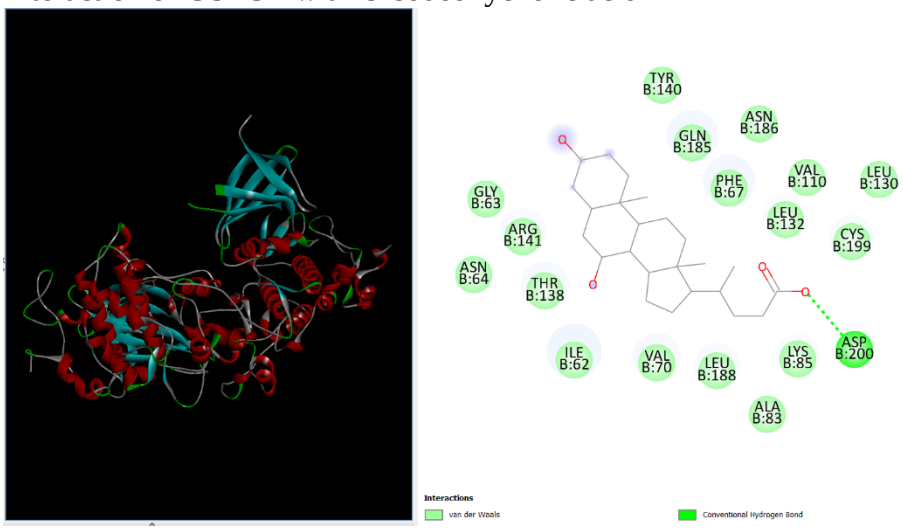


Figure 11: Interaction of GSK3B with Gramine

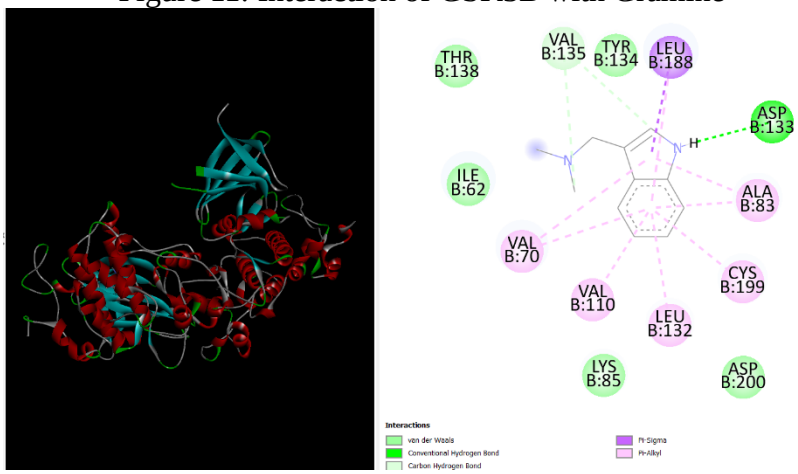
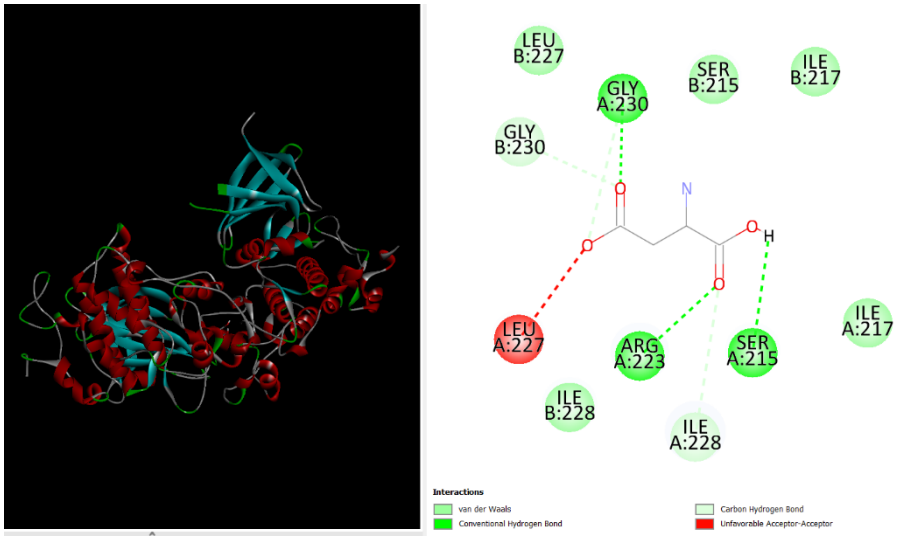


Figure 12: Interaction of GSK3B with L Ornithine L Aspartate.



Interaction with LPL

Silymarin form hydrogen bond with LYS A:236, THR A:219, Pi-sigma bond with TRP L:184, Pi-alkyl bond with LEU H:197, Pi-anion bond with GLU A:218 and exhibited a binding energy of -8.5kcal/mol. Ursodeoxycholic acid form hydrogen bond with SER L:63, GLN H:58, GLY H:61, alkyl bond with ARG L:62, VAL H:112 and exhibited a binding energy of -8.1kcal/mol. Gramine form hydrogen bond with GLN B:187, Pi-sigma and Pi-alkyl bond with LYS B:124 and exhibited a binding energy of -5.5kcal/mol. L Ornithine L Aspartate form hydrogen bond with GLN H:58, LYS L:60, GLN L:59 and exhibited a binding energy of -4.8kcal/mol.

Figure 13: Interaction of LPL with Silymarin

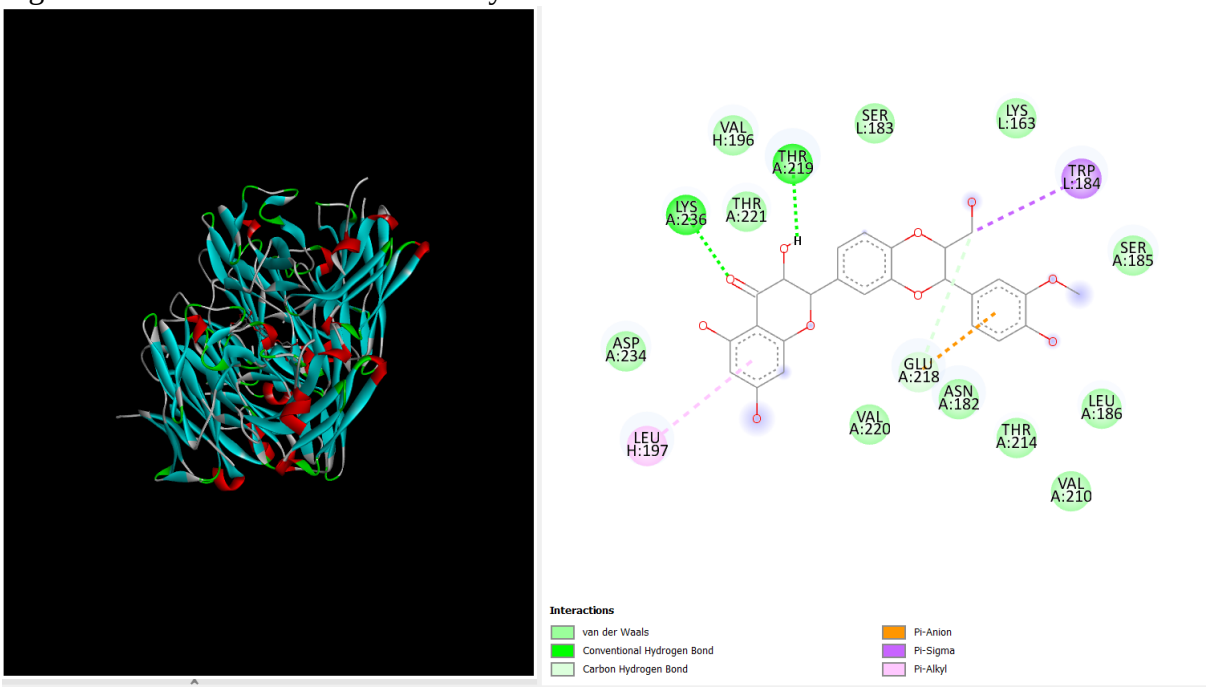


Figure 14: Interaction of LPL with Ursodeoxycholic acid

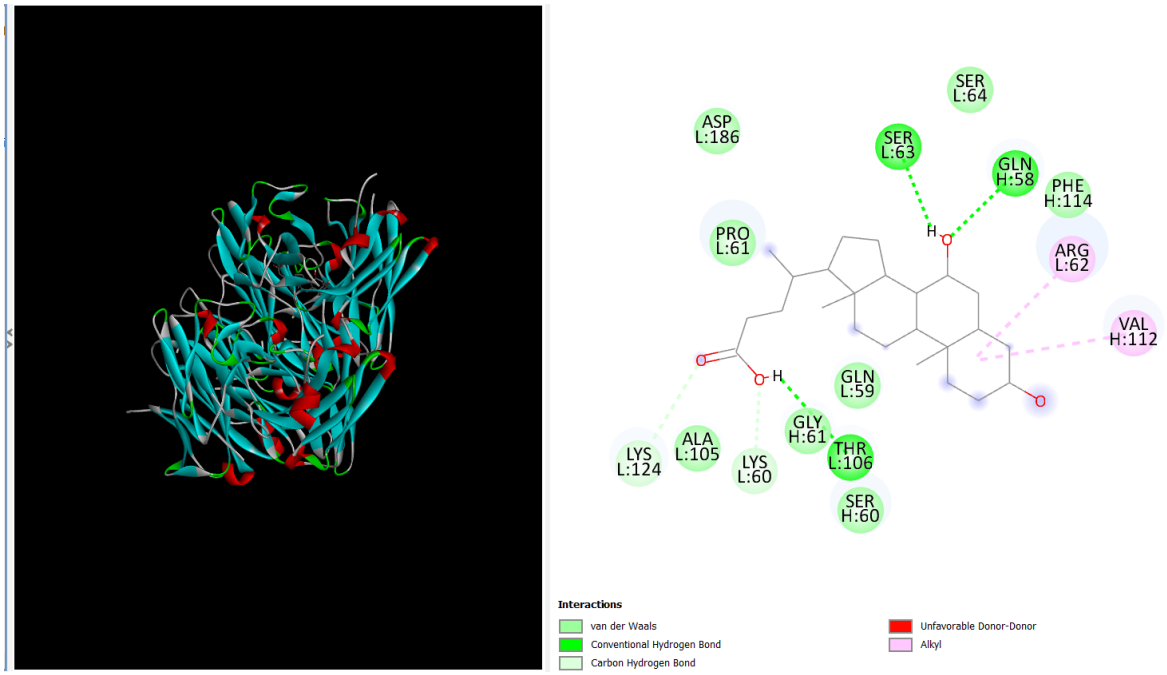


Figure 15: Interaction of LPL with Gramine

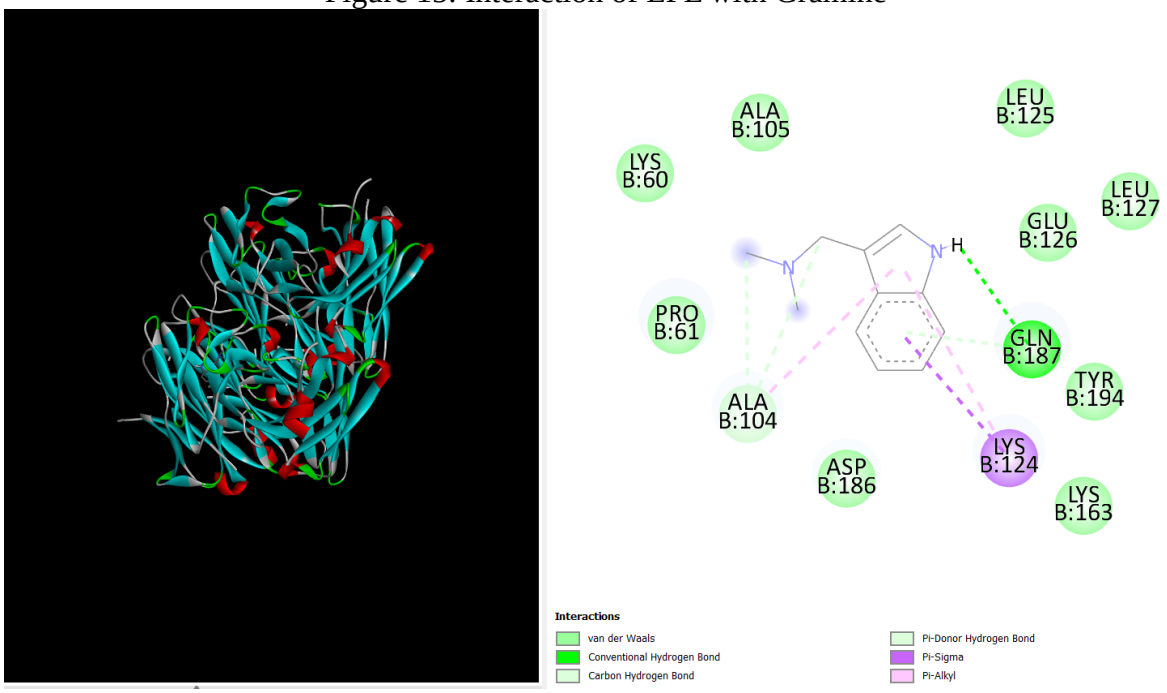
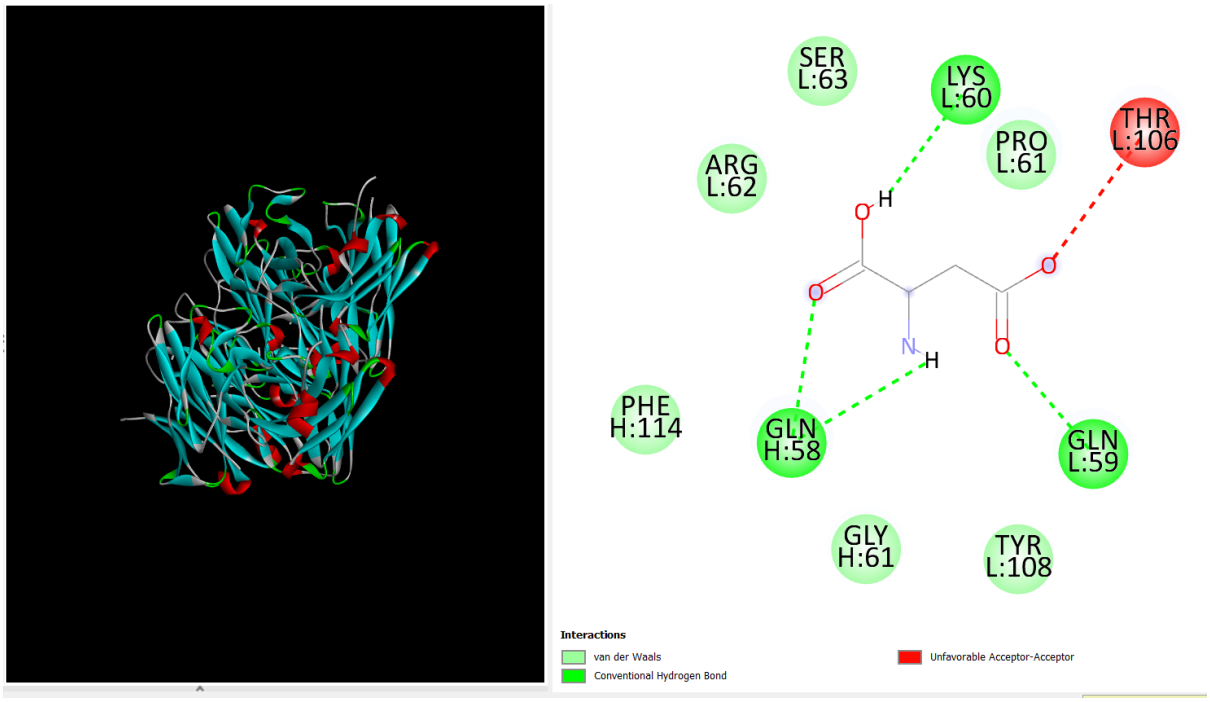


Figure 16: Interaction of LPL with L Ornithine L Aspartate



Interaction with PPARG

Silymarin form hydrogen bond with ILE A:262, GLY A:258, Pi- sigma bond with ILE A:341, LEU A:330, Pi-sulfur bond with CYS A:285, Alkyl and Pi-alkyl bond with ILE A:326 and exhibited a binding energy of -8.7kcal/mol. Ursodeoxycholic acid form hydrogen bond with ASP B:396 and exhibited a binding energy of -7.8kcal/mol. Gramine form hydrogen bond with LEU B:237, Pi-alkyl bond with PRO B:246 and exhibited a binding energy of -6.2 kcal/mol. L Ornithine L Aspartate form hydrogen bond with GLN A:444, THR B:440,THR B:447, ARG B:443, THR A:440 and exhibited a binding energy of -4.5 kcal/mol.

Figure 17: Interaction of PPARG with Silymarin

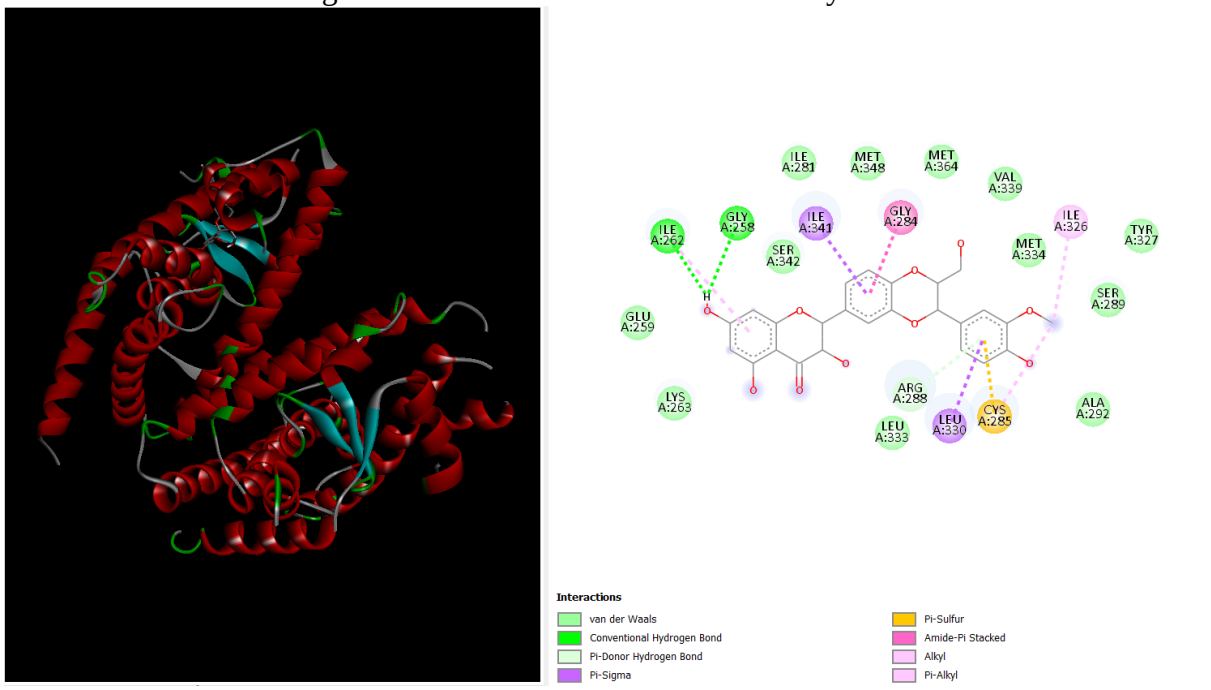


Figure 18: Interaction of PPARG with Ursodeoxycholic acid

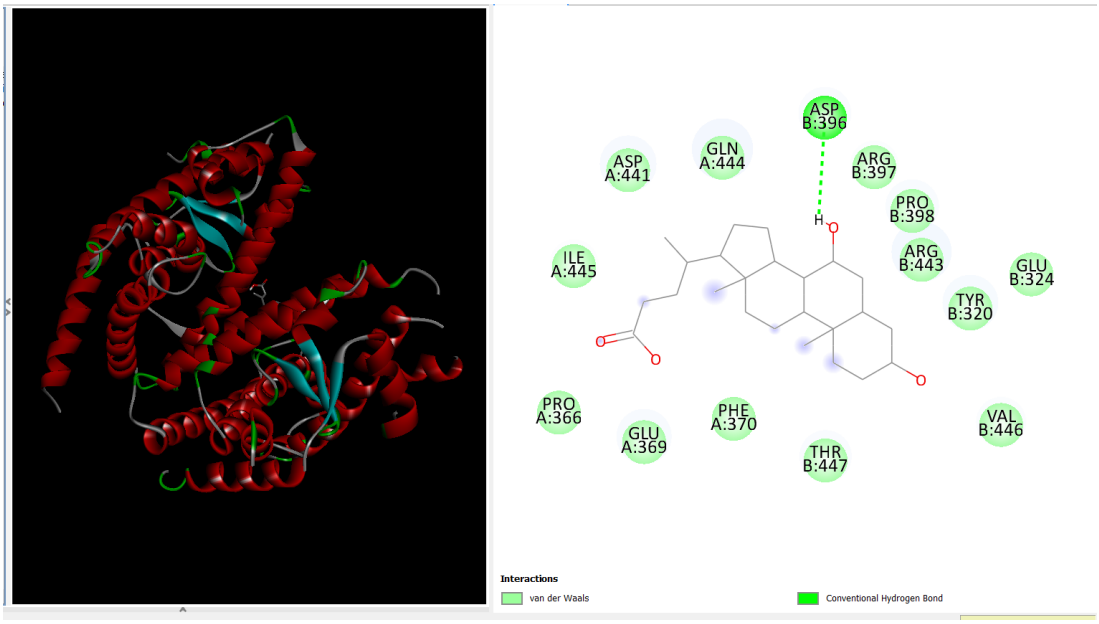


Figure 19: Interaction of PPARG with Gramine

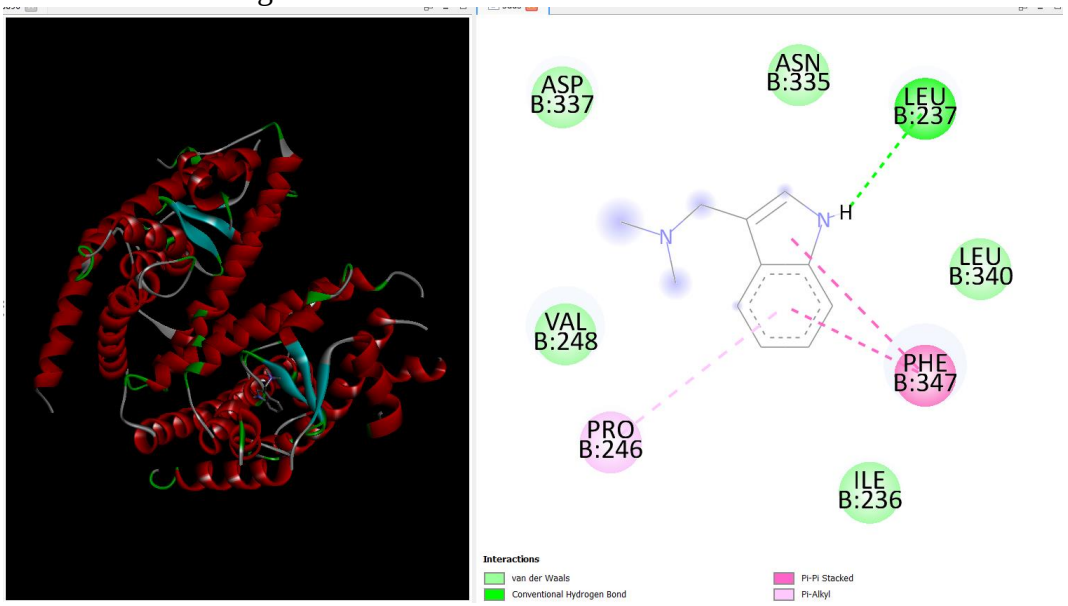
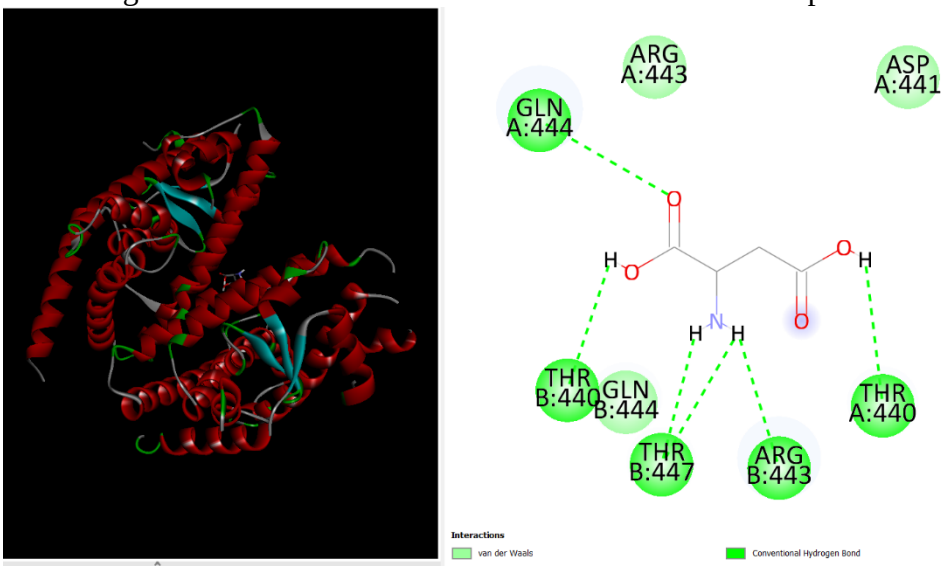


Figure 20: Interaction of PPARG with L Ornithine L Aspartate



Interaction with AKTI

Silymarin form hydrogen bond with ALA A:329, GLY A:395, ARG A:328, LYS A:389, ASN A:324 and exhibited a binding energy of -8.5kcal/mol. Ursodeoxycholic acid form hydrogen bond with GLU A:191, THR A:195,alkyl bond with VAL A:164 and exhibited a binding energy of -8.2kcal/mol. Gramine form hydrogen bond with LEU A:156, Pi-sigma bond with VAL A:164, MET A:28, Pi-alkyl bond with ALA A:177, ALA A:230 and exhibited a binding energy of -5.9kcal/mol. L Ornithine L Aspartate form hydrogen bond with CYS A:310, LEU A:275, TYR A:315 and exhibited a binding energy of -5.1kcal/mol.

Figure 21: Interaction of AKTI with silymarin

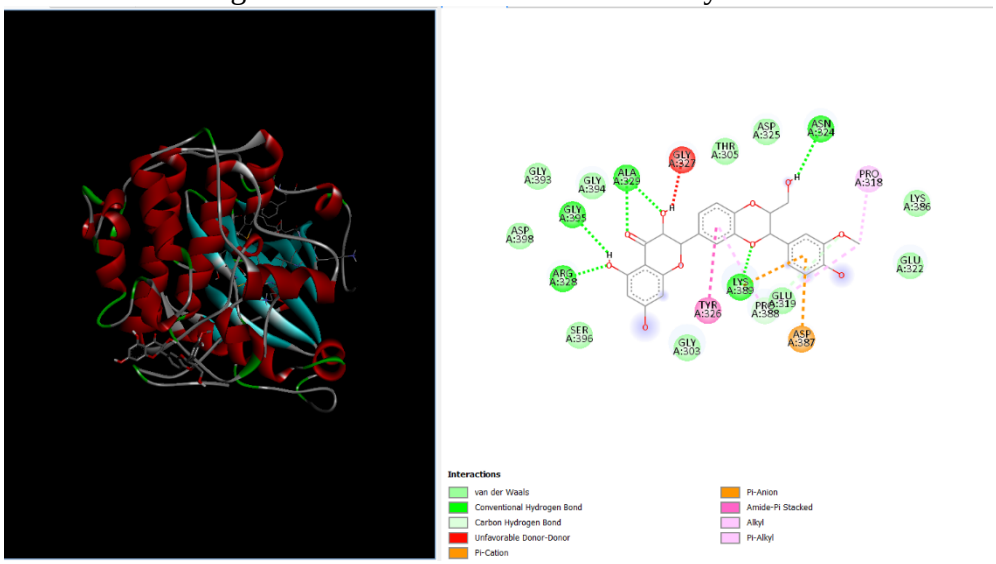


Figure 22: Interaction of AKTI with Ursodeoxycholic acid

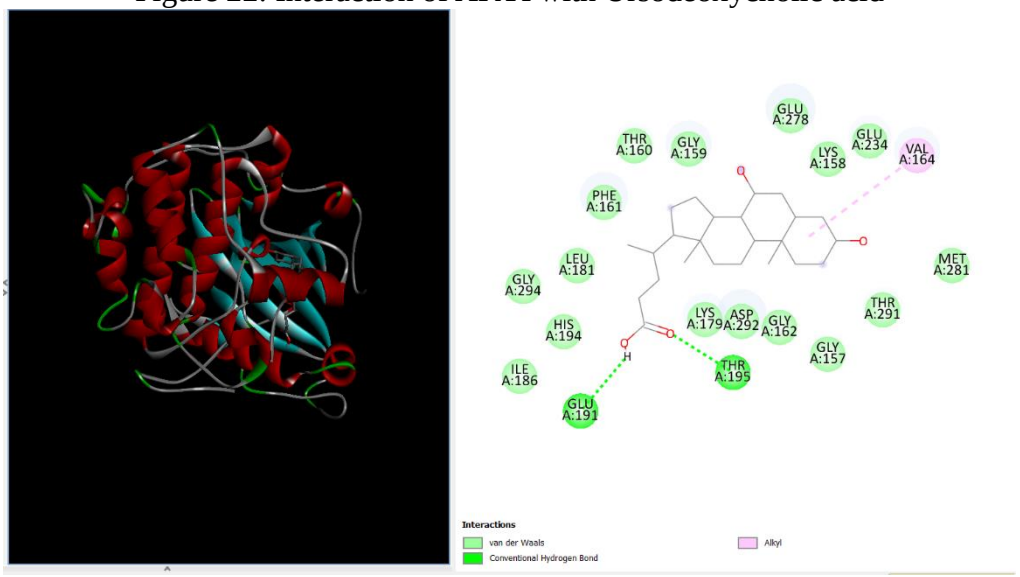


Figure 23: Interaction of AKTI with Gramine

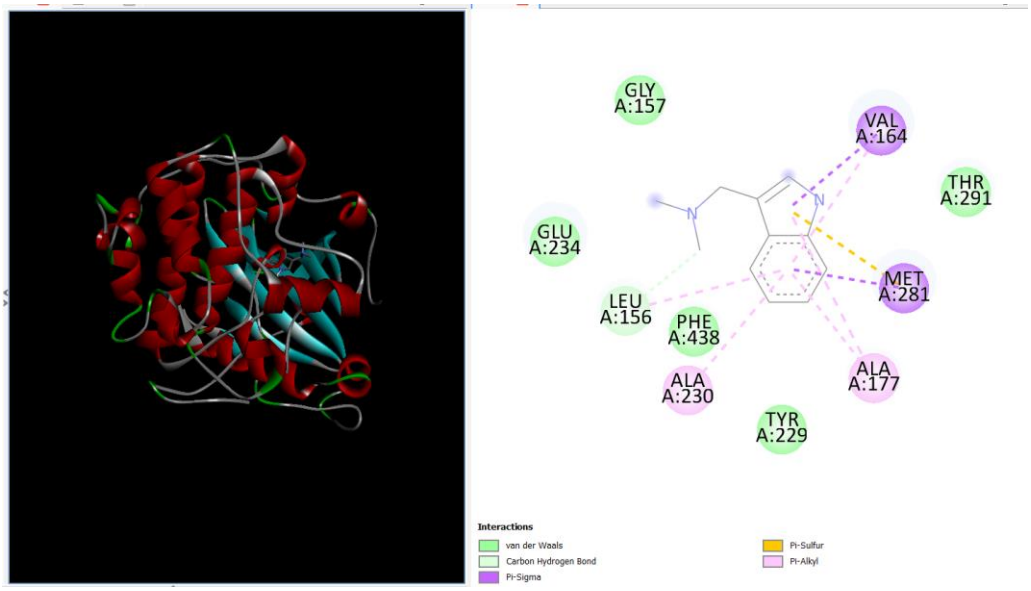
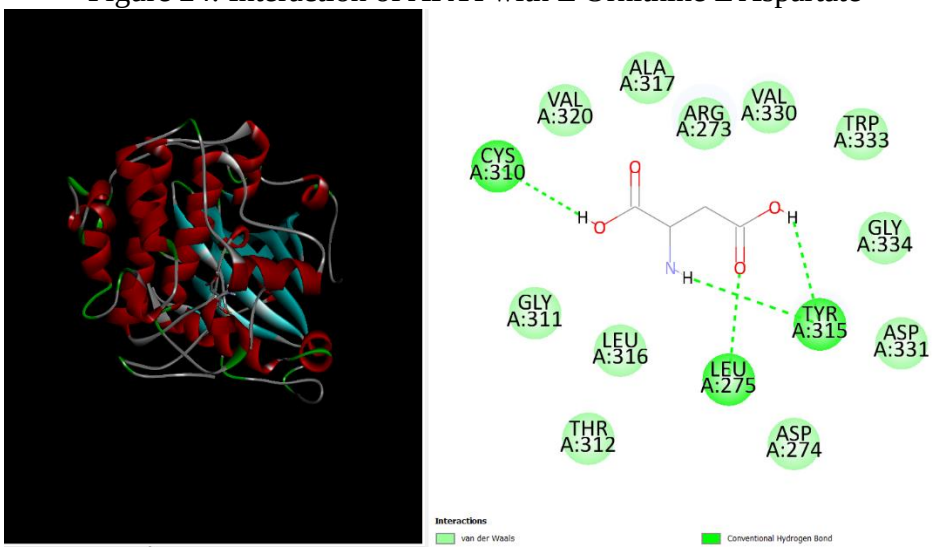


Figure 24: Interaction of AKTI with L Ornithine L Aspartate



Interaction with NF-κβ1

Silymarin form hydrogen bond with ASN P:250, SER P:243,Pi-alkyl bond with LYS P:275,LEU A:272, VAL P:254 and exhibited a binding energy of -7.3kcal/mol. Ursodeoxycholic acid form hydrogen bond with SER P:74, SER P:81and exhibited a binding energy of -6.8kcal/mol. Gramine form Pi-alkyl bond VAL P:61, LYS P:149,Pi-sigma bond with THR P:153, VAL P:145 and exhibited a binding energy of -4.6kcal/mol. L Ornithine L Aspartate form hydrogen bond with ILE P:142, ARG P:59. PRO P:65,TYR P:60 and exhibited a binding energy of -4.6 kcal/mol.

Figure 25: Interaction of NF-κβ1 with Silymarin

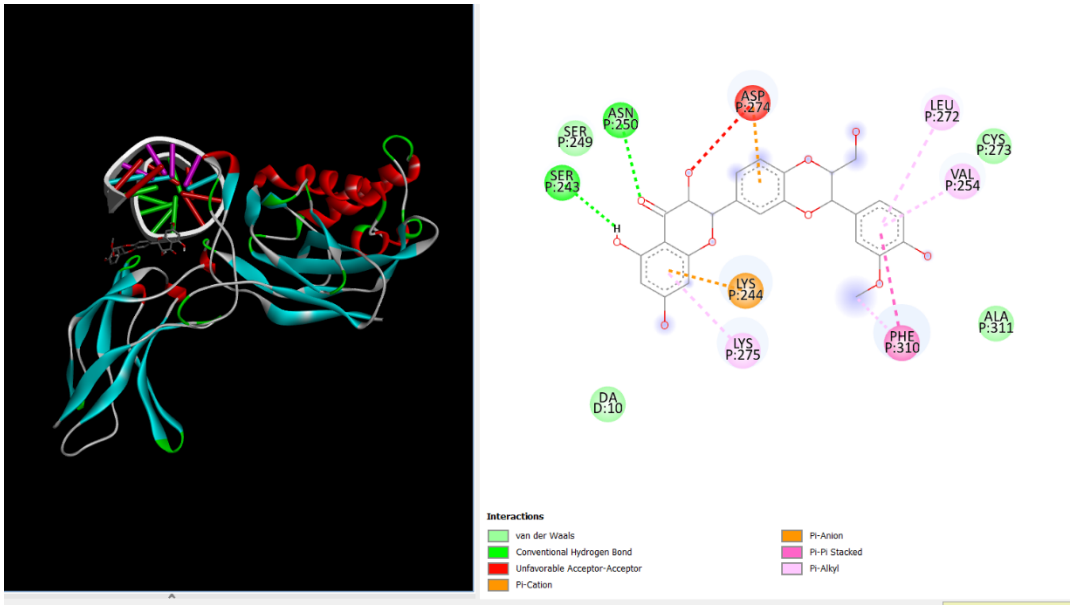


Figure 26: Interaction of NF-κβ1 with Ursodeoxycholic acid

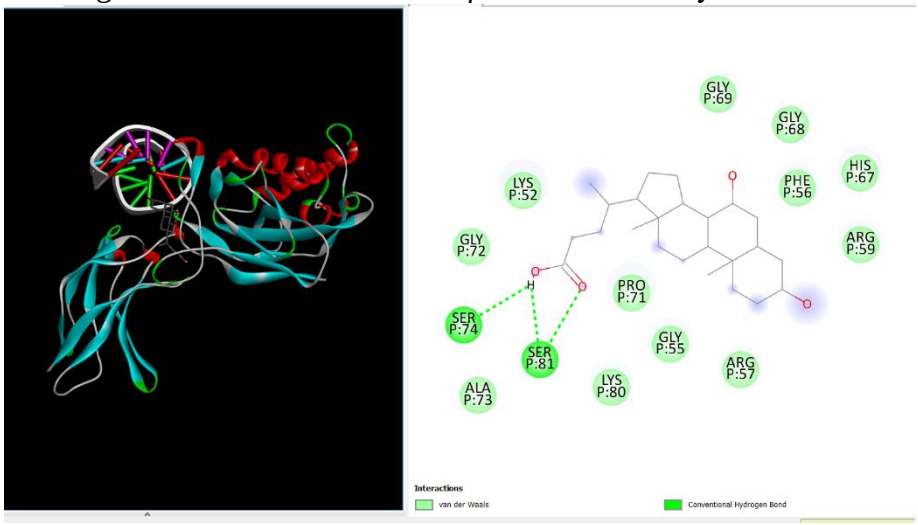


Figure 27: Interaction of NF-κβ1 with Gramine

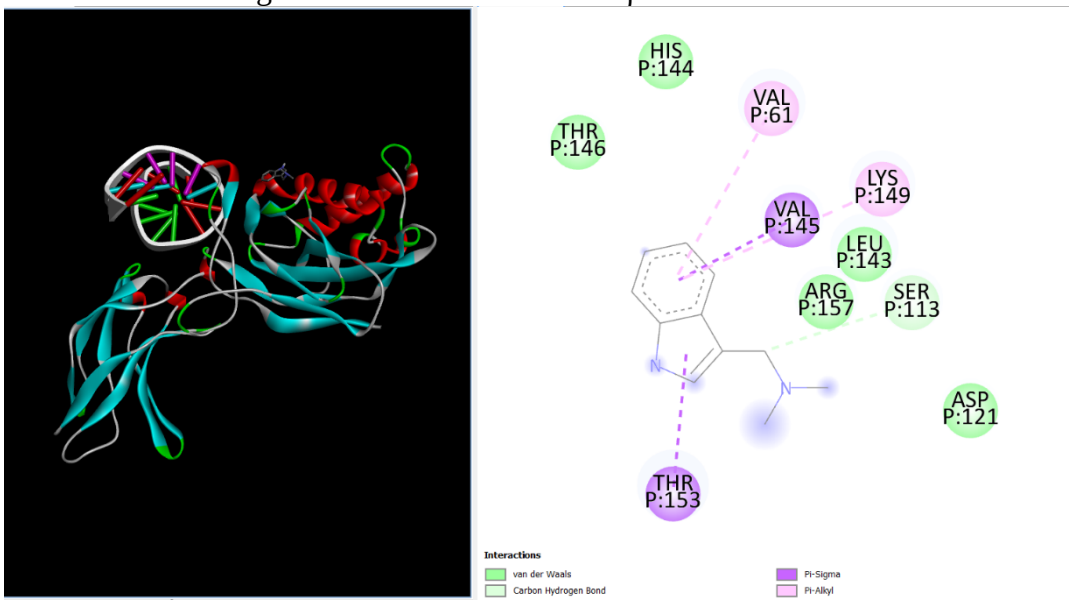
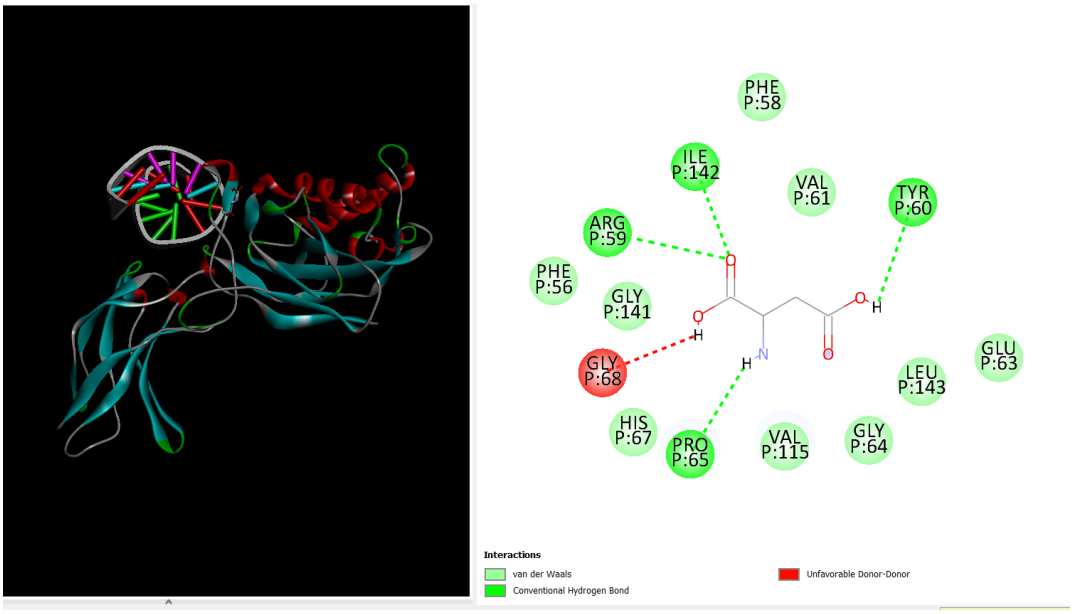


Figure 28: Interaction of NF-κβ1 with L Ornithine L Aspartate



Interaction with PPAR A

Silymarin form hydrogen bond with THR A:288, Alkyl and Pi alkyl bond with ILE A:463, LEU A:309, LYS A:310, VAL A:306 and exhibited a binding energy of -8.6 kcal/mol. Ursodeoxycholic acid form hydrogen bond with SER A:373 and exhibited binding energy of -7.5kcal/mol. Gramine form Pi-sigma bond with VAL A:444,Pi-alkyl bond with ILE A:354, ILE A:447 and exhibited a binding energy of -6.3kcal/mol. L Ornithine L Aspartate form hydrogen bond with PHE A:273 and exhibited a binding energy of -4.4kcal/mol.

Figure 29: Interaction of PPAR A with Silymarin

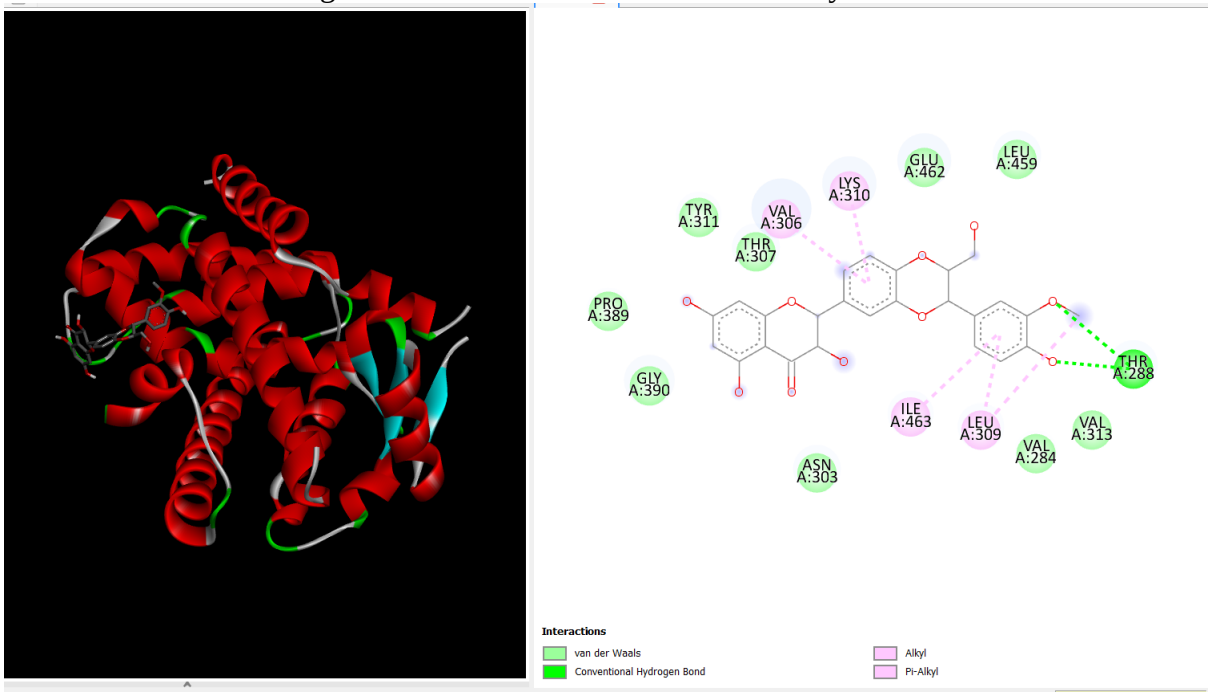


Figure 30: Interaction of PPAR A with Ursodeoxycholic acid

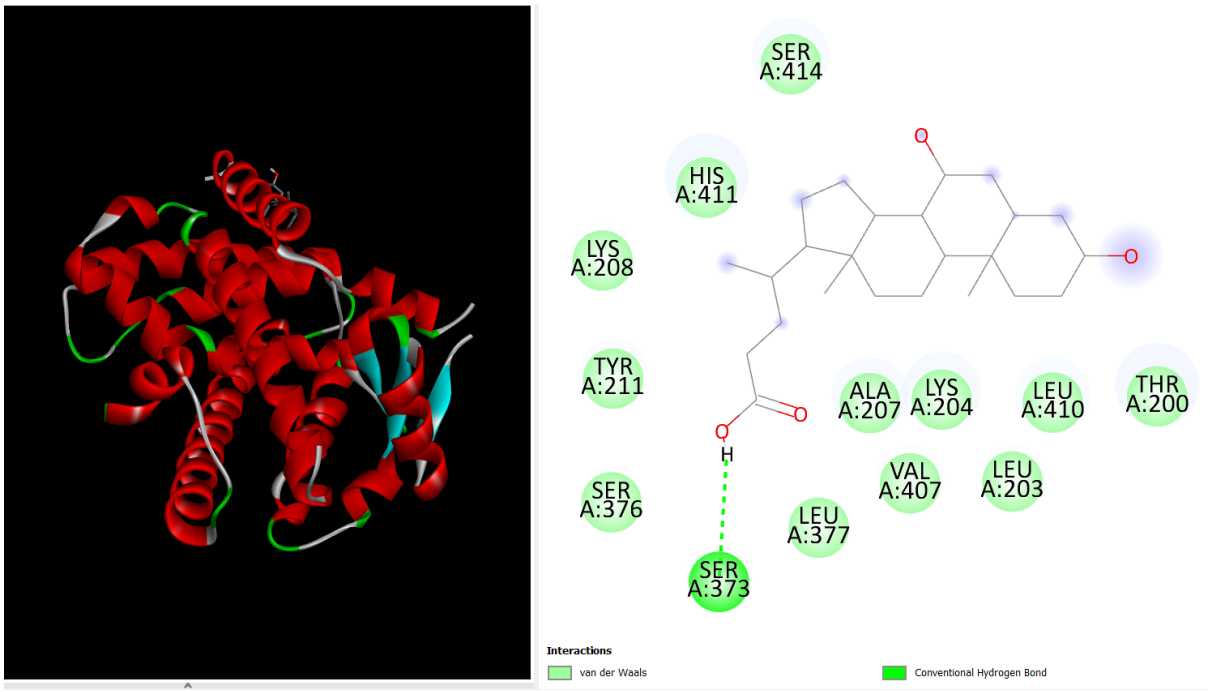


Figure 31: Interaction of PPAR A with Gramine

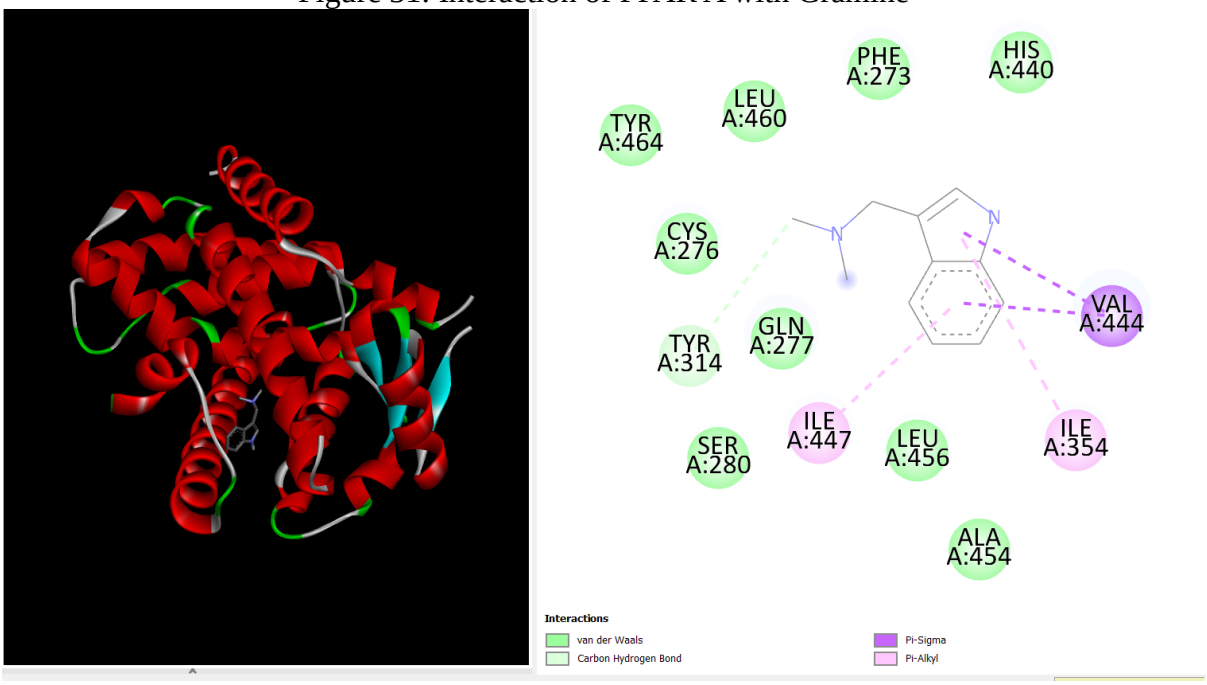
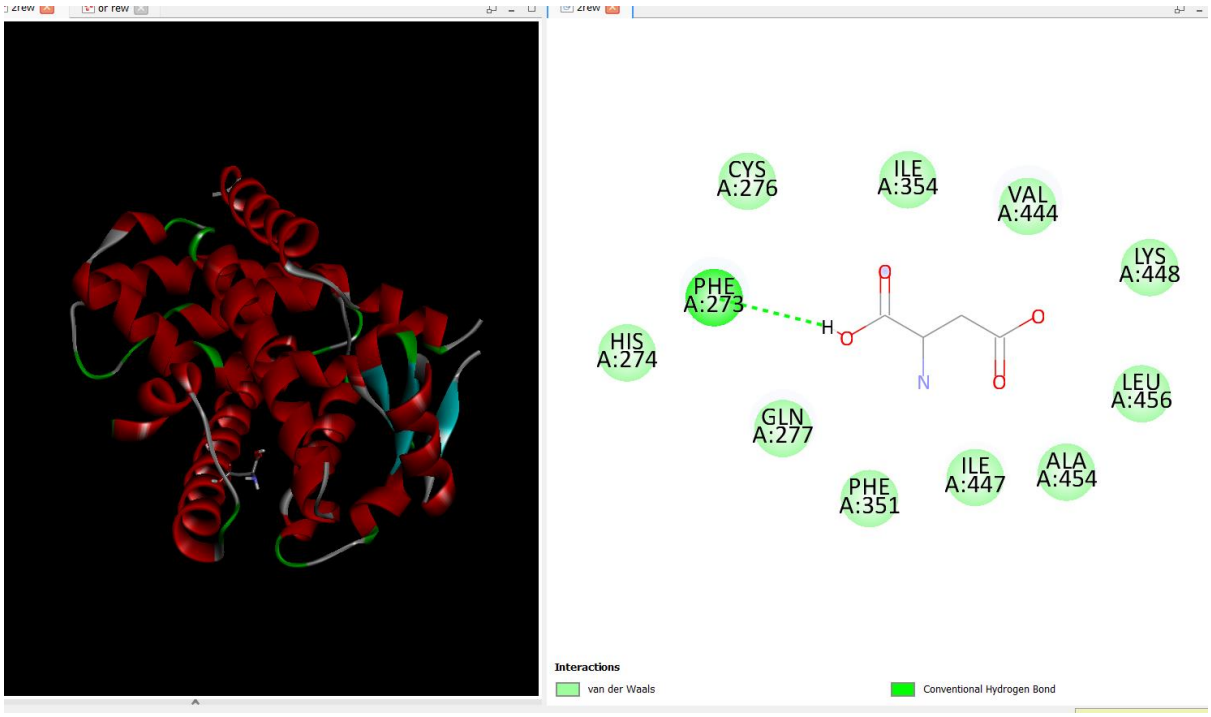


Figure 32: Interaction of PPAR A with L Ornithine L Aspartate



Interaction with MAPK 8

Silymarin form hydrogen bond ASP A:296, ILE A:304, Pi-anion bond with GLU A:272, Alkyl and Pi- alkyl bond with LYS A:308, VAL A:303, LEU A:241 and exhibited a binding energy of -7.9kcal/mol. Ursodeoxycholic acid form hydrogen bond with ILE A:310, LYS A:300, LEU A:302, ILE A:304 and alkyl bond with VAL A:303 and exhibited a binding energy of -7.7kcal/mol. Gramine form Pi-sigma bond with LEU A:168, VAL A:40, ILE A:32,VAL A:158, Pi-alkyl bond with ALA A:53, Pi-sulfur bond with MET A:108 and exhibited a binding energy of -5.9kcal/mol. L Ornithine L Aspartate form hydrogen bond with LEU A:241, THR A:243, ILE A:304, CYS A:245 and exhibited a binding energy of -4.3kcal/mol.

Figure 33: Interaction of MAPK 8 with Silymarin

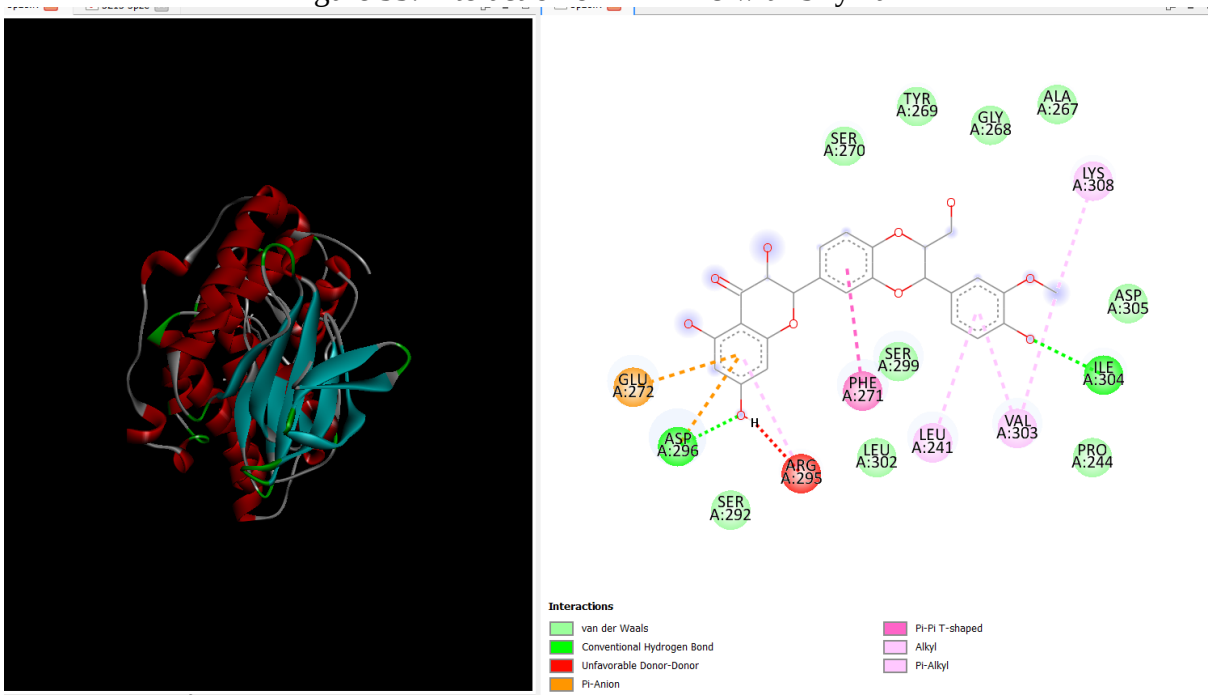


Figure 34: Interaction of MAPK 8 with Ursodeoxycholic acid

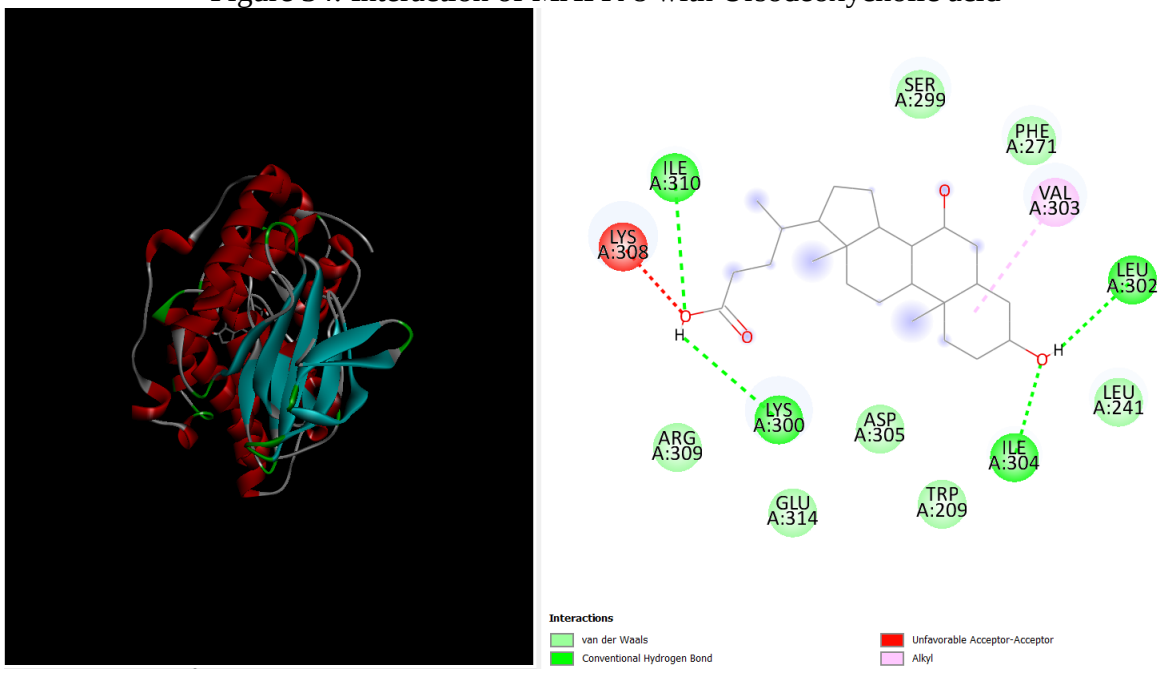


Figure 35: Interaction of MAPK 8 with Gramine

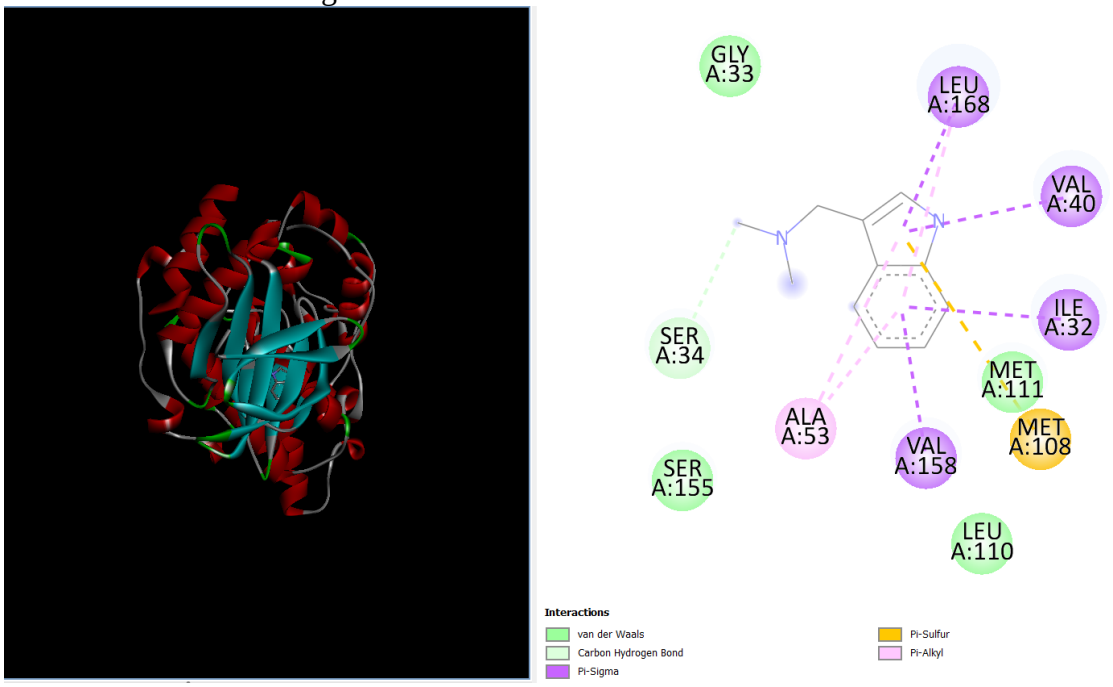
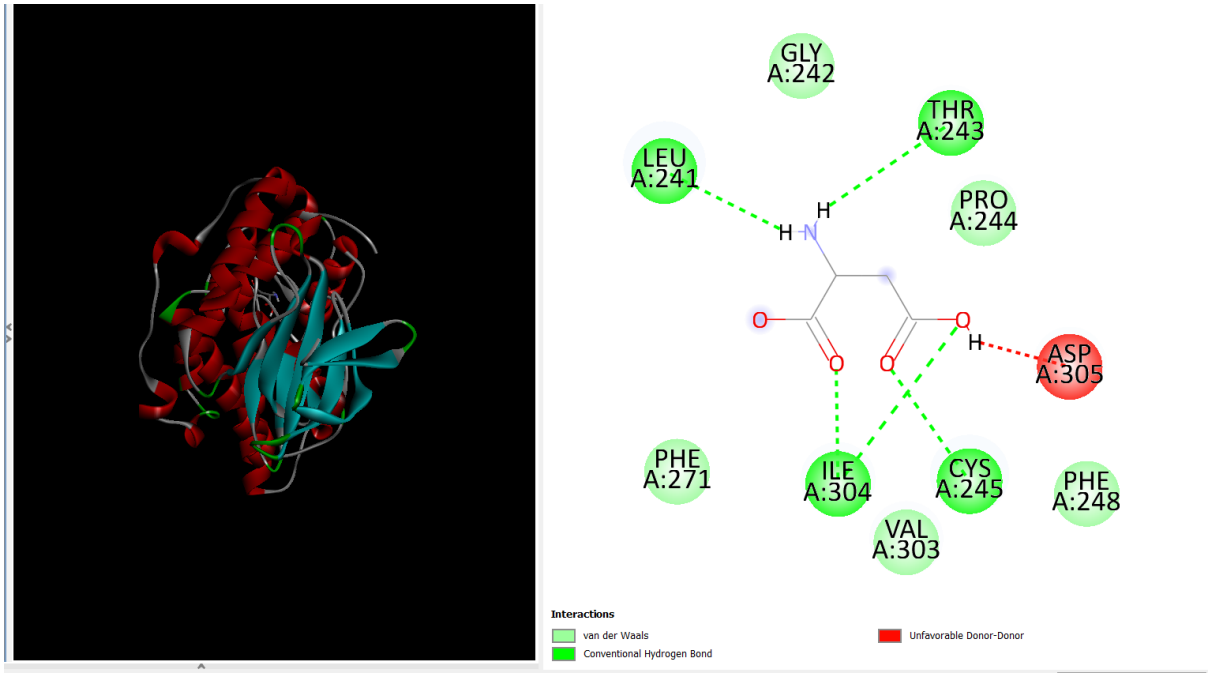


Figure 36: Interaction of MAPK 8 with L Ornithine L Aspartate



Interaction with IL6

Silymarin form hydrogen bond with ARG A:105, TRP A:158, Pi-cation bond ASP A:161 and exhibited a binding energy of -7.6kcal/mol. Ursodeoxycholic acid form hydrogen bond with GLU A:96 and exhibited a binding energy of –6.9 kcal/mol. Gramine form hydrogen bond with ASP A:161, Pi-cation bond with ARG A:105 and exhibited a binding energy of -5.4kcal/mol. L Ornithine L Aspartate form hydrogen bond with ARG A:105 and exhibited a binding energy of -4.1kacl/mol.

Figure 37: Interaction of IL6 with Silymarin

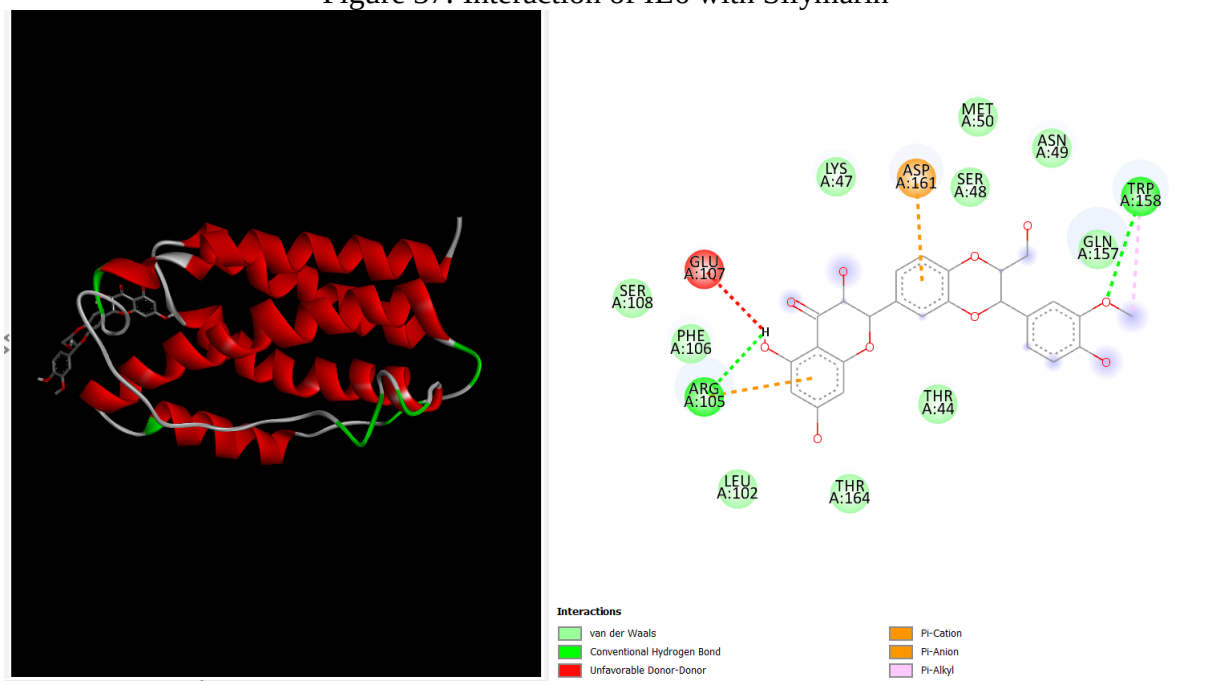


Figure 38: Interaction of IL6 with Ursodeoxycholic acid

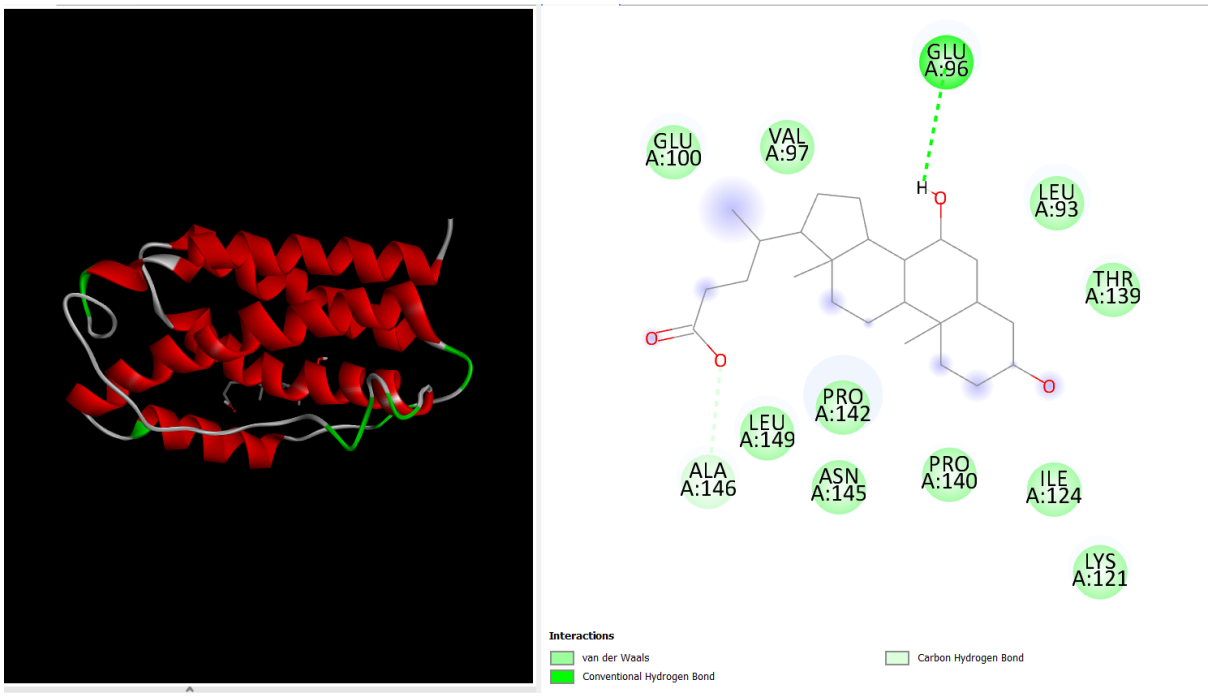


Figure 39: Interaction of IL6 with Gramine

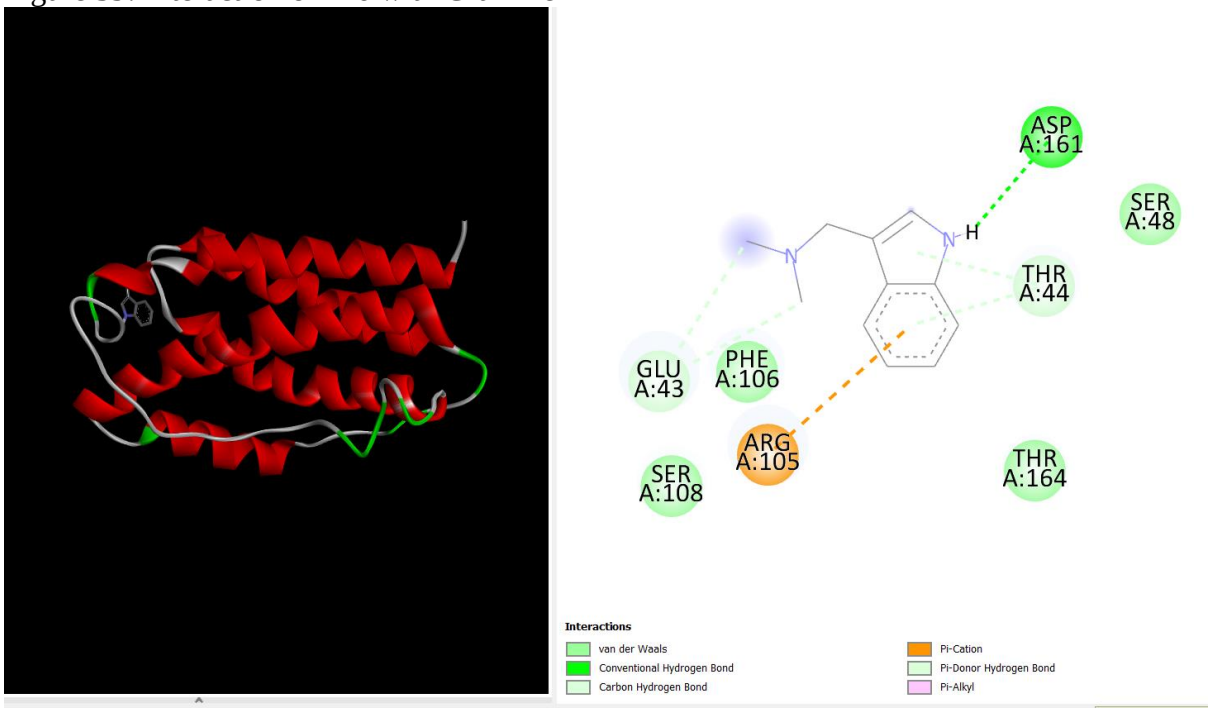


Figure 40: Interaction of IL6 with L Ornithine L Aspartate

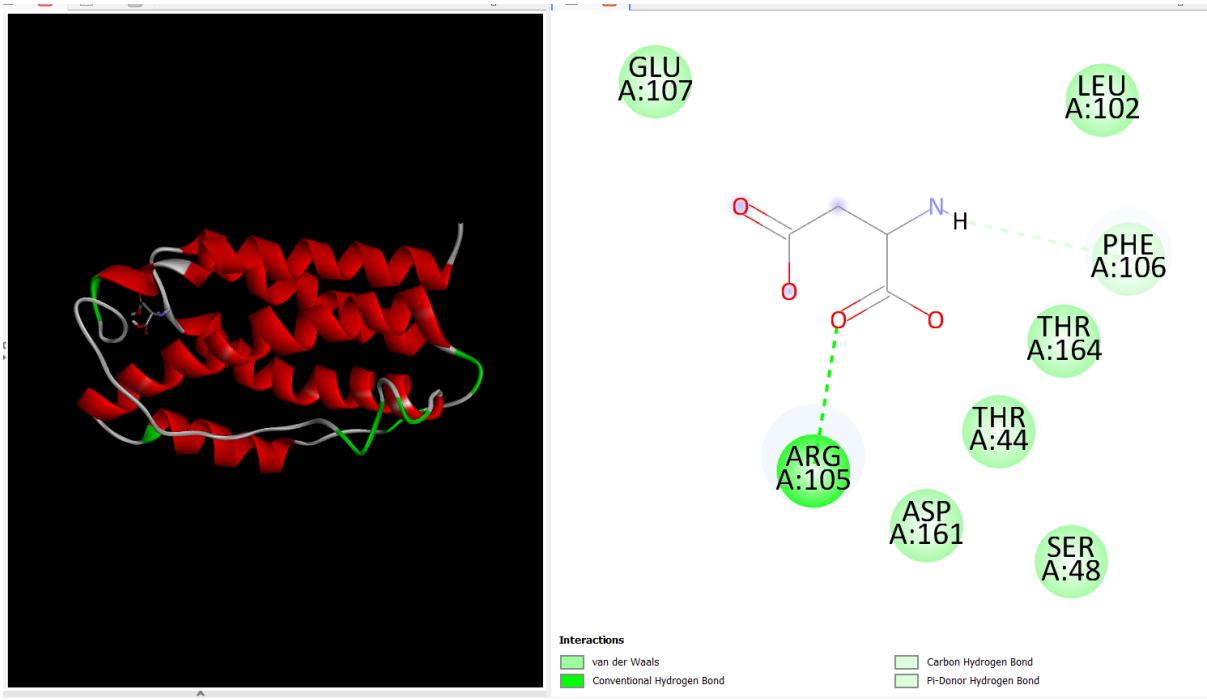


Table 2: Binding energy for different proteins

Protein(PDB ID)	Ursodeoxycholic acid	Silymarin	Gramine	L ornithine l aspartate
TNF α (1 TNF)	-8.3kcal/mol	-10.3kcal/mol	-5.8 kcal/mol	-5 kcal/mol
IL 6 (1 IL6)	-6.9kcal/mol	-7.6 kcal/mol	-5.4 kcal/mol	-4.1 kcal/mol
PIK3 (4wwp)	-8.1 kcal/mol	-9.3 kcal/mol	-6.7 kcal/mol	-5.1 kcal/mol
GSK3B(5HLN)	-9.1 kcal/mol	-9.6 kcal/mol	-6.4 kcal/mol	-4.6 kcal/mol
LPL(6WN4)	-8.1 kcal/mol	-8.5kcal/mol	-5.5 kcal/mol	-4.8 kcal/mol
PPARG(3ADS)	-7.8 kcal/mol	-8.7 kcal/mol	-6.2 kcal/mol	-4.5 kcal/mol
AKTI (4EKL)	-8.2 kcal/mol	-8.5 kcal/mol	-5.9 kcal/mol	-5.1 kcal/mol
NF-κβ1 (1 svc)	-6.8 kcal/mol	-7.3 kcal/mol	-4.6 kcal/mol	-4.6 kcal/mol
PPARA(2REW)	-7.5 kcal/mol	-8.6 kcal/mol	-6.3 kcal/mol	-4.4 kcal/mol
MAPK8(3PZE)	-7.7 kcal/mol	-7.9 kcal/mol	-5.9 kcal/mol	-4.3 kcal/mol

DISCUSSION

Liver plays a key role in metabolism of drugs, alcohol and foreign chemicals[31] and thus it is susceptible to damage from microorganism, metabolites, drugs. Thus, these factors contribute to various liver diseases. [32-35]Hence, the present employed molecular docking to investigate the potential targets in preventing and treating liver diseases.

ADmetSAR server was used to evaluate absorption, distribution, metabolism excretion and toxicity of gramine, ursodeoxycholic acid, silymarin and L Ornithine L aspartate. Ursodeoxycholic acid and gramine showed close and high human intestinal absorption (HIA) values followed by silymarin and L ornithine L aspartate. Thus, it can be depicting that they are good for oral administration.[36] Gramine and silymarin showed better blood brain barrier values than ursodeoxycholic acid and L ornithine L aspartate. All four compounds showed non carcinogenic effect, thus their accumulation in the human body might not result in cancer

development. Silymarin showed high protein binding effect, whereas gramine and ursodeoxycholic acid showed almost similar values followed by L Ornithine L aspartate.

In pathogenesis of NAFLD,TNF and PPAR γ plays a crucial role.[37-38]Activated PPAR γ modulates the transcription of acyl-Co A oxidase and increases fatty acid β oxidation and thus alleviate hepatic steatosis.[39]PPAR belongs to nuclear receptor and classified into three isotypes α, β and γ. PPAR γ regulates TGF-b/Smad, JAK/STAT,MAPK and NF-κB signalling pathway to protect the liver from inflammation and fibrosis[40-42] PPAR γ is a well-known anti-inflammatory transcription factor that inhibits the expression of IL6,IL1 and TNF α[43]PPAR agonist are useful in various liver diseases by reducing inflammation, fibrosis[44]Consequently this mechanism may contribute to the reduction in the inflammatory response of liver fibrosis. From the table through docking score, it was clear that the binding energy to these proteins is highest in silymarin followed by ursodeoxycholic acid, gramine and L Ornithine L aspartate.

AKT 1 is widely expressed in liver and plays a crucial role in PI3K/AKT signalling pathway. Excessive AKT 1 inhibition can induce liver injury[45][46] IL-1 plays an important role as inflammatory regulator and helps in deposition of lipids in hepatocytes;thus, it helps in progression of acute liver failure[47-48]Based on previous studies it was identified that MAPK 8, IL 6, TNF, PIK3.LPLproteins play an important role in pathogenesis of NAFLD.[49]

CONCLUSION:

Liver diseases involve multiple targets like TNF α, IL6, PIK3, GSK3B, LPL, PPARG, AKTI, PPARA, MAPK 8 and NF-κβ1. This work includes molecular docking and ADMET properties of gramine and compared to silymarin, ursodeoxycholic acid, L ornithine L aspartate, to investigate the effect of gramine in liver diseases. These results indicated that gramine shows good binding ability with proteins like PIK3,GSK3B,PPARA,PPARG,AKTI,MAPK8, TNFα,LPL, IL6 and certain binding affinity for NF-κβ1.Gramine show excellent intestinal absorption rate, plasma protein binding as well as non-carcinogenic in nature. However, further validating all the results in vivo would be the best way to characterize the property of gramine as therapeutic liver drug.

REFERENCES

1. Indu Khurana, Arushi Khurana, Narayan Gurukripa Kowlgi, Textbook of medical physiology, 3 rd edition, Elsevier publication, Pg no 554-557.
2. Gu, Xinsheng, and Jose E. Manautou. "Molecular mechanisms underlying chemical liver injury." *Expert reviews in molecular medicine* 14 (2012): e4.
3. W. Bernal, J. Wendon, Acute liver failure, N Engl J Med 369 (26) (2013) 2525–2534.
4. W.M. Lee, R.H. Squires, S.L. Nyberg, E. Doo, J.H. Hoofnagle, Acute liver failure: summary of a workshop, Hepatology 47 (4) (2008) 1401–1415.
5. P.B. Watkins, Drug-induced liver injury network, Am. J. Gastroenterol. 103 (6) (2008) 1574–1575
6. Wruck, W., Graffmann, N., Kawala, M. A., & Adjaye, J. (2017). Concise review: Current status and future directions on research related to nonalcoholic fatty liver disease. Stem Cells, 35(1), 89–96.
7. Oseini, A. M., & Sanyal, A. J. (2017). Therapies in non-alcoholic steatohepatitis (NASH). Liver International, 37, 97–103.
8. Fazel, Y., Koenig, A. B., Sayiner, M., Goodman, Z. D., & Younossi, Z. M. (2016). Epidemiology and natural history of non-alcoholic fatty liver disease. Metabolism: Clinical and Experimental, 65(8), 1017–1025.
9. Rosmorduc, O., & Fartoux, L. (2012). Treatment of non-alcoholic fatty liver disease — Current perspectives. Clinics and Research in Hepatology and Gastroenterology, 36 (3), 202–208.

10. S.L. Flamm, Y.X. Yang, S. Singh, Y.T. Falck-Ytter, AGA institute clinical guidelines committee, American gastroenterological association institute guidelines for the diagnosis and management of acute liver failure, Gastroenterology 152 (2017) 644–647.
11. Schuppan, D., Schattenberg, J.M., 2013. Non-alcoholic steatohepatitis: Pathogenesis and novel therapeutic approaches. J. Gastroenterol. Hepatol. 28, 68–76.
12. Orechoff, A.; Norkina, S. Über die Alkaloide von *Arundo donax* L. *Ber. Dtsch. Chem. Ges.* **1935**, *68*, 436–437
13. Li, L.; Yang, P.; Li, X.; Pan, F.; Yan, H. Optimization of extracting process of gramine from *Arundo donax* by response surface method. *Food Ind.* **2020**, *41*, 47–51
14. Zhao, X.; Zhao, L.; Zhao, Y.; Huang, K.; Gong, W.; Yang, Y.; Zhao, L.; Xia, X.; Li, Z.; Sheng, F.; et al. 3-Indoleacetonitrile is highly effective in treating influenza a virus infection in vitro and in vivo. *Viruses* **2021**, *13*, 1433
15. Yang, C.; Yu, Y.; Sun, W.; Xia, C. Indole derivatives inhibited the formation of bacterial biofilm and modulated Ca^{2+} efflux in diatom. *Mar. Pollut. Bull.* **2014**, *88*, 62–69
16. Lu, A.; Wang, T.; Hui, H.; Wei, X.; Cui, W.; Zhou, C.; Li, H.; Wang, Z.; Guo, J.; Ma, D.; et al. Natural products for drug discovery: Discovery of gramines as novel agents against a plant virus. *J. Agric. Food Chem.* **2019**, *67*, 2148–2156
17. Alnaim, A.S. Formulation, characterization, and cytotoxic effect of PVA incorporated iron oxide nanoparticles of gramine using HCT-116 cell line in vitro. *Indian J. Pharm. Educ. Res.* **2023**, *57*, 1–9.
18. Orchard, I.; Lange, A.B. Pharmacological profile of octopamine receptors on the lateral oviducts of the locust, *Locusta migratoria*. *J. Insect Physiol.* **1986**, *32*, 741–745
19. Jadhav, G.B.; Sable, R.R. Neuroprotective impact of zingerone and gramine on scopolamine-induced amnesia model. *J. Pharm. Negat. Result.* **2023**, *14*, 775–784.
20. R. Jakhar, M. Dangi, A. Khichi, A.K. Chhillar, Relevance of molecular docking studies in drug designing, Curr. Bioinform. 15 (2020) 270–278,
21. Y. Chen, Q. Liu, H. Xie, S. Yin, L. Wu, X. Yu, L. Fan, L. Lin, Is Chinese medicine injection applicable for treating acute lung injury and acute respiratory distress syndrome? A systematic review and meta-analysis of randomized controlled trials, Chin. J. Integr. Med. 26 (2020) 857–866
22. F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee, Y. Tang, admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties, J. Chem. Inf. Model. 52 (2012) 3099–3105
23. H.M. Berman, The Protein Data Bank, Nucleic Acids Res. 28 (1) (2000) 235–242
24. M. Kitahara, Y. Hozumi, A. Nakamura, K. Tachi, H. Saitoh, T. Iijima, HER2-positive conversion in a metastatic liver focus in late recurrent breast cancer, Case Reports in Oncology 12 (2) (2019)
25. S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, PubChem in 2021: new data content and improved web interfaces, Nucleic. Acids. Res. (49) (2021) D1388–D1395
26. S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, Methods Mol. Biol. (1263) (2015) 243–250
27. J. Eberhardt, D. Santos-Martins, A.F. Tillack, S. Forli, AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings, J. Chem. Inf. Model. (61) (2021) 3891–3898.
28. S.K. Kondapuram, S. Sarvagalla, M.S. Coumar, Docking-based virtual screening using PyRx tool:autophagy target Vps34 as a case study. Molecular docking for computer-aided drug design, ScienceDirect (2021)

29. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings. J Chem Inf Model. 2021;61(8):3891e3898.
30. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455e461
31. Jahan A, Shams S, Ali S, et al. Govaniadine ameliorates oxidative stress, inflammation, and kupffer cell activation in carbon tetrachloride-induced hepatotoxicity in rats. ACS Omega. 2021;6(4):2462e2472
32. Rowland A, Miners JO, Mackenzie PI. The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. Int J Biochem Cell Biol. 2013;45(6): 1121e1132.
33. Mao S, Gao D, Liu W, Wei H, Lin JM. Imitation of drug metabolism in human liver and cytotoxicity assay using a microfluidic device coupled to mass spectrometric detection. Lab Chip. 2012;12(1):219e226.
34. Dawson S, Stahl S, Paul N, Barber J, Kenna JG. In vitro inhibition of the bile salt export pump correlates with risk of cholestatic drug-induced liver injury in humans. Drug Metab Dispos. 2012;40(1):130e138.
35. Luyendyk JP, Kassel KM, Allen K, et al. Fibrinogen deficiency increases liver injury and early growth response-1 (Egr-1) expression in a model of chronic xenobiotic-induced cholestasis. Am J Pathol. 2011;178(3):1117e1125
36. M. Rashid, Design, synthesis and ADMET prediction of bis-benzimidazole as anticancer agent, Bioorg. Chem. 96 (2020) 103576.
37. Abiru, S., Migita, K., Maeda, Y., Daikoku, M., Ito, M., Ohata, K., Nagaoka, S., Matsumoto, T., Takii, Y., Kusumoto, K., Nakamura, M., Komori, A., Yano, K., Yatsuhashi, H., Eguchi, K., Ishibashi, H., 2006. Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. Liver Int. 26, 39–45.
38. Alshawsh, M.A., Alsalahi, A., Alshehade, S.A., Saghir, S.A.M., Ahmeda, A.F., Zarzour, R. H.A., Mahmoud, A.M., 2022. A Comparison of the Gene Expression Profiles of Non-Alcoholic Fatty Liver Disease between Animal Models of a High-Fat Diet and Methionine-Choline-Deficient Diet. Molecules 27, 858.
39. Ables, G.P., 2012. Update on Pparc and nonalcoholic fatty liver disease. PPAR Res. 2012, 1–5
40. Han X, Wu Y, Yang Q, Cao G. Peroxisome proliferator-activated receptors in the pathogenesis and therapies of liver fibrosis. Pharmacol Ther. 2021;222: 107791. 54. Ni XX, Li XY, Wang Q, Hua J. Regulation of peroxisome proliferator-activated receptor-gamma activity affects the hepatic stellate cell activation and the progression of NASH via TGF-b1/Smad signaling pathway. J Physiol Biochem. 2021;77(1):35e45.
41. De Souza Basso B, Haute GV, Ortega-Ribera M, et al. Methoxyeugenol deactivates hepatic stellate cells and attenuates liver fibrosis and inflammation through a PPAR-g and NF-kB mechanism. J Ethnopharmacol. 2021;280:114433
42. Wu L, Guo C, Wu J. Therapeutic potential of PPAR-g natural agonists in liver diseases. J Cell Mol Med. 2020;24(5):2736e2748
43. Chang F, Wang C, Zheng P, et al. Malat1 promotes macrophage-associated inflammation by increasing PPAR-g methylation through binding to EZH2 in acute myocardial infarction. Int Immunopharm. 2023;123:110695
44. Wu L, Guo C, Wu J. Therapeutic potential of PPAR-g natural agonists in liver diseases. J Cell Mol Med. 2020;24(5):2736e2748.

45. Xu Zhong, et al., The mTORC2-Akt1 cascade is crucial for c-Myc to promote hepatocarcinogenesis in mice and humans, Hepatology 70 (5) (2019) 1600–1613 (Baltimore, Md.).
46. W. Qi, C. Xinyu, H. Nissim, Akt as a target for cancer therapy: more is not always better (lessons from studies in mice), Br. J. Cancer 117 (2) (2017) 159–163
47. H. Tilg, A.R. Moschen, IL-1 cytokine family members and NAFLD: neglected in metabolic liver inflammation, J. Hepatol. 55 (5) (2011) 960–962.
48. L. Barbier, M. Ferhat, E. Salam´e, et al., Interleukin-1 family cytokines: keystones in liver inflammatory diseases, Front. Immunol. 10 (2019) 2014.
49. Salah Abdulrazak Alshehade , Raghdaa Hamdan Al Zarzour, et al., Mechanism of action of Orthosiphon stamineus against non-alcoholic fatty liver disease: Insights from systems pharmacology and molecular docking approaches, Saudi Pharmaceutical Journal,2022