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Molecular docking study ON NS5B polymerase of hepatitis c virus by screening of volatile compounds from *Acacia concinna* and ADMET prediction

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Abstract

Hepatitis C virus (HCV) is one of the major causes of chronic liver diseases such as cirrhosis apparently leading to liver failure or liver cancer. HCV is the leading cause of liver transplantation and there is no unique effective drug for all hepatitis genotypes. The protein NS5B polymerase is a RNA-dependent RNA polymerase that contributes to the replication of viral RNA. By inhibiting the function of non structural protein 5B (NS5B) polymerase, the viral RNA of HCV fails to replicate. The present study deals with the development of HCV replication inhibitors by the volatile compounds extracted from the plant *Acacia concinna* belonging to family Acaciaceae which is followed by molecular docking against NS5B polymerase (PDB ID:- 4EO6). Molecular docking studies were performed using iGEMDOCK module and the Absorption Distribution Metabolism Excretion Toxicity (ADMET) properties of the best molecule that fits with NS5B polymerase was predicted using admetSAR database. The interaction of the extracted compounds showed their antiviral properties against NS5B polymerase which could be used for further analysis to inhibit HCV replication.

Key-Words: Hepatitis C virus, *Acacia concinna*, iGEMDOCK, ADMET, NS5B polymerase

Introduction

Hepatitis means inflammation of the liver. It is caused by several mechanisms, including certain infectious agents. Viral hepatitis is caused by different type of viruses such as hepatitis A, B, C, D and E. Jaundice is one of the characteristic features of liver disease and proper diagnosis could be made by testing the patient's sera for the deduction of antiviral antibodies [1]. More than 60% of acutely infected patients turned out to be chronically infected [2].

HCV belongs to family flaviridae and genus hepacivirus [3]. The genome of HCV is about 9.6 kilobase pairs in length encoding a polyprotein of over 3000 aminoacids which is cleaved by HCV genome proteases to 10 structural and non-structural proteins [4]. The protein NS5B polymerase is an RNA dependent RNA polymerase [5]. The antiviral drug development and the mechanism for RNA synthesis and replication for HCV have been facilitated by biochemical tests for NS5B [4]. It has been clinically validated that NS5B is essential for HCV replication [5]. A combination of two types of treatments are preferred than treating separately.

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The two commonly used vaccines, namely pegylated interferon and ribavirin are now being used in combination than separately which shows higher rate of response. But these treatments shows side effects such as hemolytic anemia, renal failure etc. [1]. Hence the inhibition of NS5B polymerase function becomes essential and the drug that inhibits the function of NS5B polymerase is said to be an effective inhibitor. This work was carried out by molecular docking studies to determine whether two molecules (the protein and the drug) interact and to find the orientation that enhances this interaction as well as minimizing the total energy of the interaction complex. Also, the present study aims to investigate the binding efficiency of the Acacia concinna plant [6] against the non structural 5B polymerase by extracting the volatile compounds from the novel plant using iGEMDOCK module. However, no such reports were found in literature *insilico* studies on plant active compounds of *Acacia concinna* which act against the protein from Hepatitis-C-virus.

Importance of *Acacia concinna*

Acacia concinna is a medicinal plant that belongs to the Acaciaceae family [6]. It is present in most parts of Asia. It is a common, prickly, scandent shrub occurring in tropical regions throughout India, especially in the

Deccan region [7]. Parts of *Acacia concinna* such as bark, root and leaves are used in a powdered form [6].

Material and Methods

Docking studies were performed for natural compounds (ligands) from the volatile compounds obtained from the plant *Acacia concinna* with NS5B polymerase of HCV which is RNA dependent RNA polymerase by using iGEM DOCK suite.

Preparation of the protein structure

The protein required for the docking studies has been retrieved from the Protein Data Bank at 1.3 Å root mean square deviations (RMSD) resolution which represents a three dimensional structure of target HCV polymerase enzyme NS5B (PDB : ID 4EO6) which is a RNA dependent RNA polymerase.

Ligand preparation

The ligand molecules for the docking process are prepared from the volatile compounds obtained from the pods of *Acacia concinna* using Gas chromatography-Mass Spectroscopy [8]. The extracted compounds were then analyzed and the 3-dimensional structure views of the volatile compounds were obtained from the PubChem and Chemspider databases (Table 1). The structure of the compounds was downloaded in (.sdf) format and they were converted into (.mol) format by using openbabel software searching for tautomers and steric isomers and geometry minimization of ligands [9].

Binding site prediction

It is well known that viral RNA dependent RNA polymerase possesses catalytic and allosteric sites for its enzymatic activity. QSiteFinder was employed to identify ligand binding sites which utilize an interaction energy scheme to locate energetically favorable binding sites between the protein and a simple Van der waal's probe [10]. The site of amino acids (residues) of the respective protein was analyzed and downloaded from QSiteFinder [11] (Table 2).

After evaluating a number of geometries from protein data bank, (PDB ID: 4EO6) was selected for docking studies. However, to validate the docking procedure, the same procedures to dock a well known NS5B polymerase inhibitor against volatile compounds from *Acacia concinna* have been applied. In the present study, the top 5 compounds exhibiting larger binding energy have been mentioned.

Docking module

Docking software iGEM dock was used to dock the protein of the HCV with the drug molecule. iGEMDOCK is an integrated virtual screening (VS) environment from preparations through post-screening analysis with pharmacological interactions.

iGEMDOCK provides interactive interfaces to prepare both the binding site of the target protein and the screening compound library. Each compound in the library is then docked into the binding site by using the in-house docking tool iGEMDOCK. Subsequently, iGEMDOCK generates protein-compound interaction profiles of electrostatic (E), hydrogen-bonding (H), and Van der Waal's (V) interactions. Based on these profiles and compound structures, iGEMDOCK infers the pharmacological interactions and clusters the screening compounds for the post-screening analysis. Finally, iGEMDOCK ranks and visualizes the screening compounds by combining the pharmacological interactions and energy-based scoring function of iGEMDOCK [12].

ADMET tools

The adverse properties such as Absorption, Distribution, Metabolism, Excretion and the Toxicity of the volatile compounds are predicted using admetSAR database. They provide the latest and most comprehensive manually curated data for diverse chemicals associated with known ADMET profiles [13].

Mechanism of Docking

The ligands and crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. Following the above steps of preparation, the protein was subjected to energy minimization using the Universal Force Field (UFF). Q-SiteFinder server was used to detect the active sites and docking was performed by iGEMDOCK molecular docking software. Docking was performed with all the potential active sites detected on NS5B-polymerase enzyme. During Docking, at first the molecules were prepared and bonds, bond orders, explicit hydrogen's, charges, flexible torsions were assigned to both the protein and ligands. From the Docking, wizard ligands were selected and the scoring function used was iGEMDOCK score. If hydrogen bonding is possible, the hydrogen bond energy contribution to the Docking score is assigned a penalty based on the deviations from the ideal bonding angle. This option can significantly reduce the number of unlikely hydrogen bonds and also internal electrostatic interaction; internal hydrogen bond sp2-sp2 torsions are calculated from the pose by enabling the ligand evaluation terms. The search algorithm is taken as iGEMDOCK and numbers of runs taken are 10 and max interactions were 2000 with population size 200 and with an energy threshold of 100 also at each step least 'min' torsions/translations/rotations are tested and the one giving lowest energy is chosen. If the energy is positive

(i.e. because of a clash or an unfavorable electrostatic interaction), then additional 'max' positions will be tested. If the pose being docked is closer to one of the ligands in the list than specified by the Root Mean Square Deviation (RMSD) threshold, an extra penalty term (the Energy penalty) is added to the scoring function. This ensures a greater diversity of the returned solutions since the docking engine will focus its search on poses different from earlier poses found. The energy penalty was set to 100, RMSD threshold was 2.00 and RMSD calculation by atom ID (fast) were set. Docking was conducted between Protein and Inhibitor which results in binding affinities in kcal/mol and docking run time. The compound which gives lowest binding energy is chosen as the best inhibitor. iGEMDOCK showed better overall performance in docking simulations when compared with other software.

Results and Discussion

Docking results of NS5B polymerase protein with volatile compounds from *Acacia concinna*

Molecules selected from the plant source were docked using iGEMDOCK software and docked scores of those molecules were represented in (Table-3), with their binding energy, Vanderwaal energy, electrostatic and hydrogen bond profiles. Binding energies of the protein-ligand (drug) interactions are important to describe how fit the drug binds to the target macromolecule. Several ligands such as palmitic acid, methyl linoleate, methyl palmitate, linolenic acid, geranyl acetone, 5-methyl 2-furfural, trans-linalool oxide, cis-linalool oxide, phenylacetaldehyde, furfural, tetradecanoic acid, methyl salicylate, isopropyl palmitate and 6,10,14-trimethyl-2-pentadecannone were selected for docking studies against NS5B polymerase and docked separately as prepared.

The ligands viz., Palmitic acid (-103.88), methyl palmitate (-97.19), linolenic acid (-96.28), geranyl acetone (-93.15), methyl linoleate (-92.99), 6,10,14-trimethyl-2-pentadecannone (-86.23), isopropyl palmitate (-85.91), tetradecanoic acid (-84.81), trans linalool oxide (-77.42), methyl salicylate (-72.40), Phenylacetaldehyde (-64.04), cis linalool oxide (-60.60), furfural (-52.68),5-methyl-2-furfural (-51.5) docks into the binding pockets of NS5B polymerase. The docked poses of the molecules were represented in (Fig 2). From these results, the top 5 molecules showing their respective binding energies were evaluated based on the binding compatibility [docked energy (kcal/mol)] with the receptor. From the analysis of docking score and energy, the ligands Palmitic acid (-103.88), methyl palmitate (-97.19), linolenic acid (-96.28), Geranyl acetone (-93.15), methyl linoleate (-92.99) showed the best results than other ligands. The best molecule showing highest binding energy i.e., Palmitic acid (-103.88) is the effective inhibitor for the inhibition of HCV. Thus, it is evident that the plant *Acacia concinna* exhibits antihepatitis nature.

ADMET results for Palmitic acid

ADMET profile was evaluated using the admetSAR database for Palmitic acid shows the highest binding energy (Table 3). admetSAR predicted classification and regression values for Palmitic acid and the results seems to have been calculated for different types of models such as blood brain barrier, human intestinal absorption, $Caco_2$ permeability all of which showed positive results ensuring that the compound passes all the models and have no side effects on absorption. Similarly in case of metabolism, various Cytochrome P450 (CYP) substrate and inhibitor models were calculated and the results show that they are Non-substrate and Non-inhibitor except CYP450 1A2 Inhibitor. In terms of toxicity, it is found to be non-carcinogenic. Although some toxicity models show some negative results the regression profiles indicates that they have very low probability values.

Conclusion

The protein-ligand interaction plays a significant role in structural based drug designing. It has been clearly demonstrated that the approach utilized in this study is successful in finding novel anti-hepatitis inhibitors from *Acacia concinna*. The ligand Palmitic acid showed high binding affinity against NS5B polymerase (PDB ID: 4EO6). They exactly fit into the active site region and the ligand formed more number of H-bond interactions than the co-crystallized ligand. Therefore, this study states the importance of volatile compounds from *Acacia concinna* and their use to enhance protein-ligand interaction studies, *insilico*. From the docking results, it is possible to conclude that Palmitic acid could be a potential NS5B polymerase inhibitor. The ADMET properties have been predicted for Palmitic acid and it has been showed that it is non-carcinogenic and biodegradable; hence it is predicted to be fit for human consumption.

Acknowledgement

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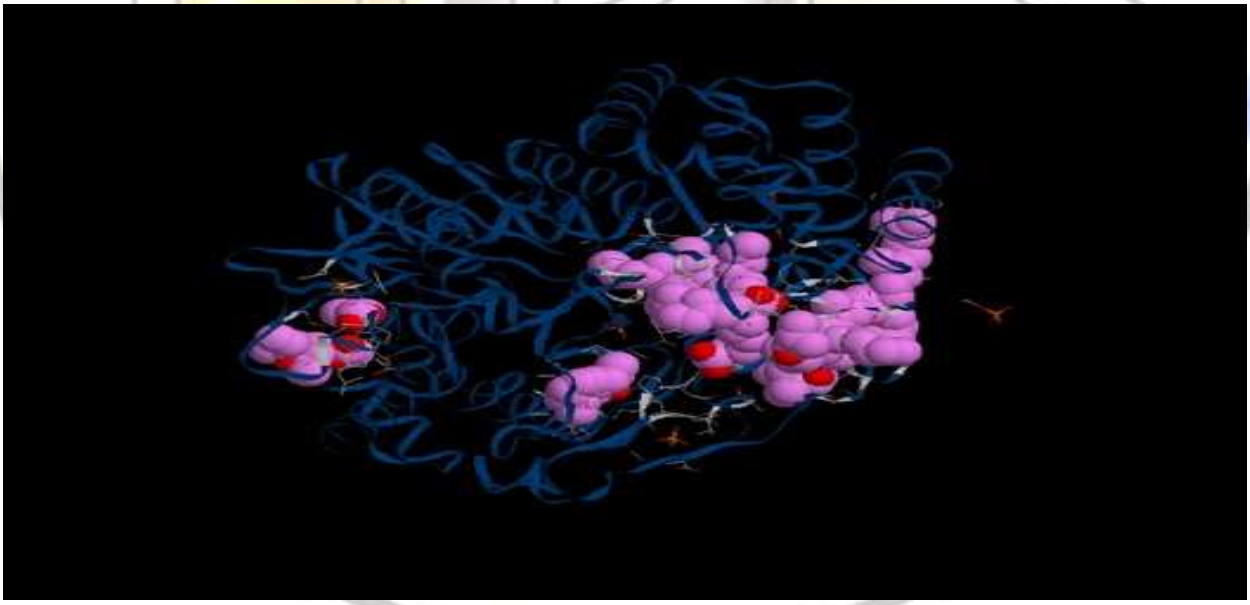


Fig. 1: Docking of NS5B polymerase with volatile compounds of *Acacia concinna*

Table 1: Name of the volatile compounds and the PubChem ID and Chemspider ID are given below

S.NO	Name of the Compound	PubChem ID
1	Furfural	7362
2	5-Methyl-2-furfural	12097
3	Phenylacetaldehyde	998
4	*cis*-Linalool oxide	6428574
5	*trans*-Linalool oxide	6427501
6	Methyl salicylate	4133
7	Geranyl acetone	1549778
8	Tetradecanoic acid	11005
9	Methyl palmitate	8181
10	Palmitic acid	985
11	Isopropyl palmitate	8907
12	Methyl linoleate	5284421
13	Linolenic acid	5280450
S.No	**Name of the compound**	**Chemspider ID**
14	6,10,14-Trimethyl-2-pentadecannone	18627

Table 2: Residues obtained from the QSiteFinder

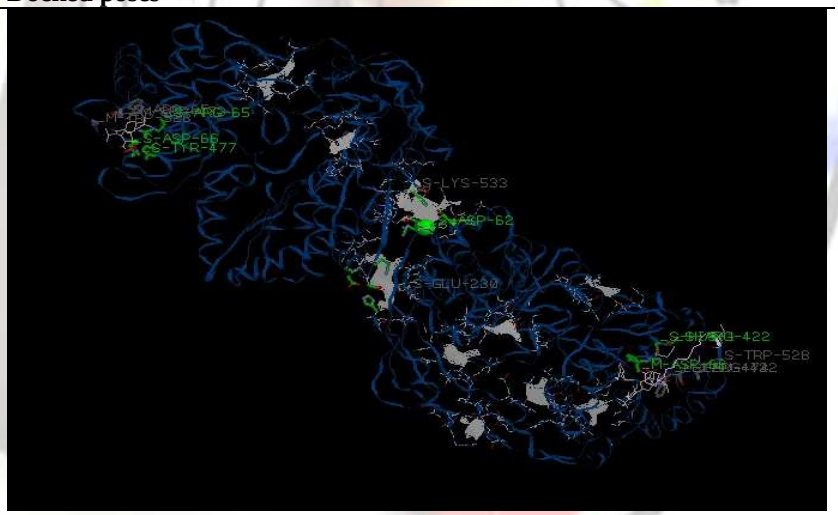
29 CE1 TYR A 4	1221 O ALA A 157	1254 CB VAL A161	1779 CA SER A 226
30 CE2 TYR A 4	1223 N ARG A 158	1256 CG2 VALA161	1782 CB SER A 226
31 CZ TYR A 4	1224 CA ARG A158	1750 N CYS A 223	1783 OG SER A 226
32 OH TYR A 4	1225 C ARG A 158	1751 C CYS A 223	
371 CG AARG A 48	1227 CB ARG A158	1752 O CYS A 223	
372 CD AARG A 48	1228 CG ARG A158	1753 CA ACYSA223	
373 NE AARG A 48	1229 CD ARG A158	1754 CB ACYSA223	
374 CZ AARG A 48	1230 NE ARG A158	1755 SG ACYSA223	
375 NH1AARG A 48	1231 CZ ARG A158	1756 CA BCYSA223	
376 NH2AARG A 48	1232 NH1 ARGA158	1757 CB BCYSA223	
379 CG BARG A 48	1233 NH2 ARGA158	1758 SG BCYSA223	
380 CD BARG A 48	1234 N LEU A 159	1759 N PHE A 224	
381 NE BARG A 48	1235 CA LEU A 159	1760 CA PHE A 224	
382 CZ BARG A 48	1236 C LEU A 159	1761 C PHE A 224	
383 NH1BARG A 48	1237 O LEU A 159	1763 CB PHE A 224	
384 NH2BARG A 48	1238 CB LEU A 159	1764 CG PHE A 224	
409 CA VAL A 52	1239 CG LEU A 159	1765 CD1 PHE A224	
411 O VAL A 52	1240 CD1 LEU A159	1770 N ASP A 225	
412 CB VAL A 52	1242 N ILE A 160	1771 CA ASP A 225	
413 CG1 VAL A 52	1243 CA ILE A 160	1772 C ASP A 225	
414 CG2 VAL A 52	1244 C ILE A 160	1774 CB ASP A 225	
1104 CG LYS A 141	1246 CB ILE A 160	1775 CG ASP A 225	
1105 CD LYS A 141	1247 CG1 ILE A 160	1776 OD1 ASP A225	
1106 CE LYS A 141	1249 CD1 ILE A 160	1777 OD2 ASP A225	
1107 NZ LYS A 141	1250 N VAL A 161	1778 N SER A 226	

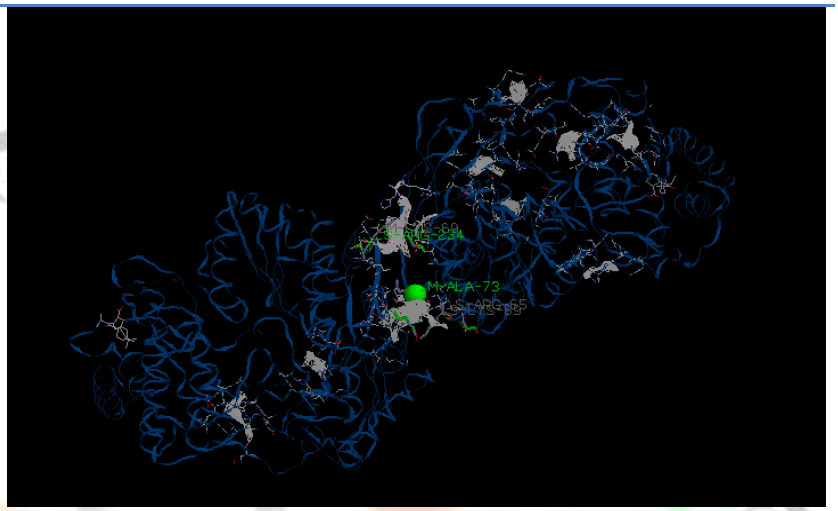
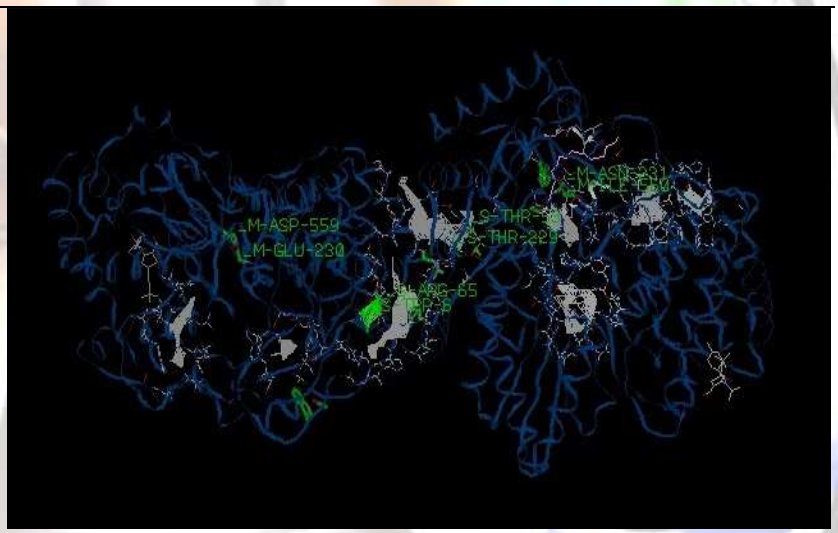
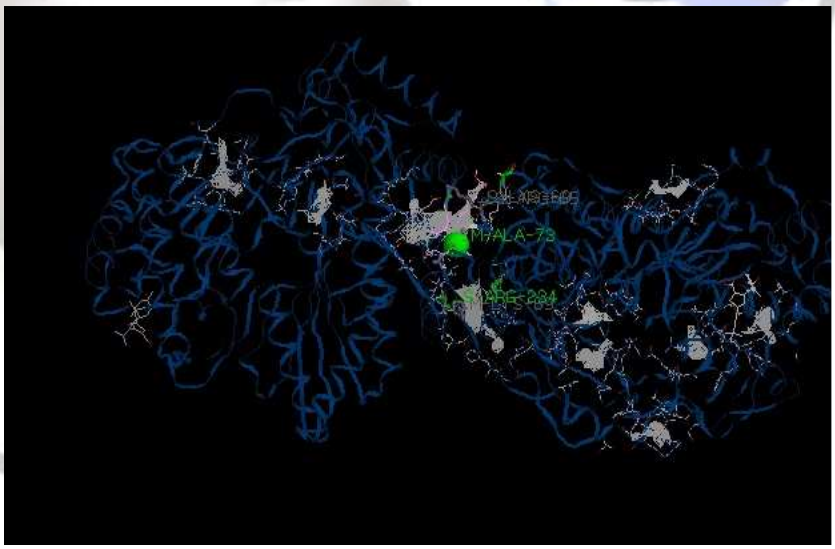
Table 3: Docking Results of NS5B against volatile compounds of *Acacia concinna*

S.No	Name of the compound	Energy	Van der waal	Hbond	Electrostatic
1	Palmitic acid	-103.88	-84.95	-15.88	-3.05
2	Methyl palmitate	-97.19	-85.85	-11.35	0
3	Linolenic acid	-96.28	-90.65	-3.5	-2.13
4	Geranyl acetone	-93.15	-86.15	-7.0	0
5	Methyl linoleate	-92.99	-89.49	-3.5	0

6	6,10,14-Trimethyl-2-pentadecannone	-86.23	-85.06	-1.17	0
7	Isopropyl palmitate	-85.91	-80.5	-5.41	0
8	Tetradecanoic acid	-84.81	-77.32	-7.49	0
9	Trans-linalool oxide	-77.42	-60.44	-16.97	0
10	Methyl salicylate	-72.4	-51.35	-21.05	0
11	Phenylacetaldehyde	-64.04	-55.35	-8.69	0
12	Cis-linalool oxide	-60.6	-54.65	-5.96	0
13	Furfural	-52.68	-35.85	-16.83	0
14	5-Methyl-2-furfural	-51.5	-41.01	-10.49	0

Fig. 2: Post screening analysis results after docking process

S.No	Name of the compound	Docked poses
1	Palmitic acid	

2	Methyl palmitate	
3	Geranyl acetone	
4	Methyl linoleate	

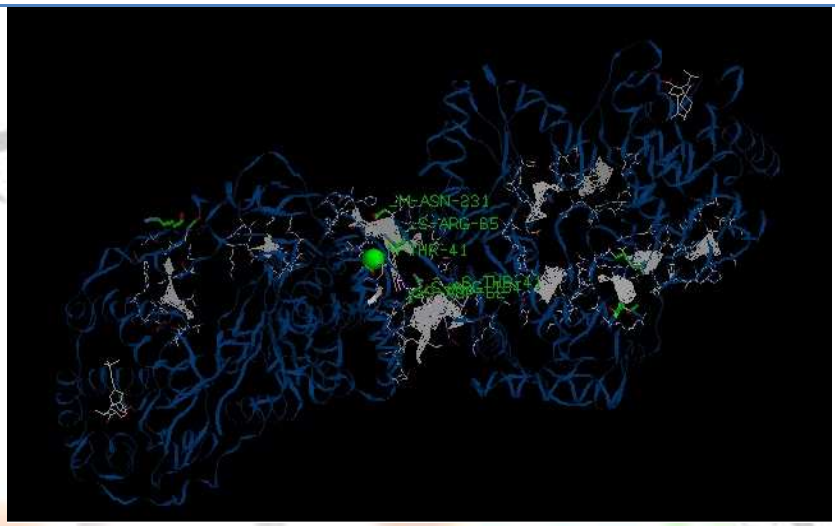
5	6,10,14-Trimethyl-2-pentadecannone	

Table 4: ADMET Predicted Profile - Classification for Palmitic acid

Model	Result	Probability
For Absorption		
Blood-Brain Barrier	BBB+	0.9488
Human Intestinal Absorption	HIA+	0.9888
Caco-2 Permeability	Caco2+	0.8326
P-glycoprotein Substrate	Non-substrate	0.6321
P-glycoprotein Inhibitor	Non-inhibitor	0.9598
	Non-inhibitor	0.9277
Renal Organic Cation Transporter	Non-inhibitor	0.9266
For Metabolism		
CYP450 2C9 Substrate	Non-substrate	0.7886
CYP450 2D6 Substrate	Non-substrate	0.8956
CYP450 3A4 Substrate	Non-substrate	0.6982
CYP450 1A2 Inhibitor	Inhibitor	0.8326
CYP450 2C9 Inhibitor	Non-inhibitor	0.8808
CYP450 2D6 Inhibitor	Non-inhibitor	0.9554
CYP450 2C19 Inhibitor	Non-inhibitor	0.9578
CYP450 3A4 Inhibitor	Non-inhibitor	0.9484
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9647
For Toxicity		
Human Ether-a-go-go-Related	Weak inhibitor	0.9322
Gene Inhibition	Non-inhibitor	0.8868
AMES Toxicity	Non AMES toxic	0.9865
Carcinogens	Non-carcinogens	0.6452
Fish Toxicity	High Fathead minnow	0.9144
Tetrahymena Pyriformis Toxicity	High TPT	0.9990
Honey Bee Toxicity	High HBT	0.6691
Biodegradation	Ready biodegradable	0.8795

Table 5: ADMET Predicted Profile - Regression for Palmitic acid

Model	Value	Unit
Predicted values for Absorption		
Caco-2 Permeability	1.3950	LogPapp, cm/s
Predicted values for Toxicity		
Rat Acute Toxicity	1.3275	LD50, mol/kg
Fish Toxicity	1.8920	pLC50, mg/L
Tetrahymena Pyriformis Toxicity	0.3852	pIGC50, ug/L