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RESEARCH ARTICLE

IN SILICO STUDIES ON BIOCHEMICAL MODULATION OF DGAT ANALOGS AND ANTIOBESITY BIOMARKERS

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ABSTRACT

Obesity is a pathological condition, in which, excess body fat accumulates to the extent, probably, having an adverse effect on health leading to reduced life expectancy and/or increased health problems. Some dietary components show promise in the treatment of obesity, one of which is oil rich in Diacylglycerols (DAGs). Excess body weight is the consequence of an imbalance between energy intake and energy expenditure that is stored as triacylglycerol (TAG) in adipose tissue. Triglyceride synthesis has been assumed to occur primarily through acyl CoA: Diacylglycerol transferase (DGAT), a microsomal enzyme that catalyses the final and only committed step in the glycerol phosphate pathway. Therefore, DGAT has been considered indispensable for adipose tissue formation and essential for survival. The genes encoding two DGAT enzymes, DGAT 1 and DGAT 2 were identified in past decade. Both enzymes may be useful as therapeutic targets for disease and controlling the over expression of triacylglycerides. The present study was undertaken to monitor the interaction between DGAT and previously discovered ligands by using Vlife MDS. Besides, the active sites of the enzyme and the residues involved were also validated through *in silico* experimentation. The data obtained from this study provide new insights into expression of high affinity of the ligands towards the receptor i.e. the enzyme and thus leading towards the possibility of developing a compatible biomolecule as confirmatory drug to treat obesity.

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INTRODUCTION

Obesity has become a major pandemic (Hedley *et al*., 2004; Akoh, 1995). Globally, the incidence and prevalence of obesity continue to increase at an alarming rates (Rosalyn, 2001). Excess body weight has being stored in the body in form of triacylglycerides (Kennedy, 1975). So, treating high triglyceride level is most promising approach for controlling the obesity (Arend Bonen *et al*., 2004; Gary F Lewis *et al*., 1993; Kanji Yamaguchi, 2007; Do, 1995). Diacylglycerol acyltransferase (EC 2.3.1.20) catalyses the final and the only committed step in the biosynthesis of TG's (Charles A. Harris *et al*., 2011; Kamisaka *et al*., 1997). DGAT occurs as two isomers namely: DGAT1 and DGAT 2 (Farese, Robert V. Jr *et al*., 2000). It is a microsomal enzyme that joins Acyl CoA to 1, 2 diacylglycerol and, as such, constitutes the final step in triglyceride biosynthesis (Chi-Liang Eric Yen *et al*., 2008). Increased DGAT2 activity has a role in steatosis, while DGAT1 plays a role in VLDL synthesis; increased plasma VLDL concentrations may promote obesity (Steven J. Smith *et al*., 2000; Hubert C. Chen *et al*., 2000). Thus DGAT1 is considered a potential therapeutic target of inhibition for obesity control (Claudio *et al*., 2009). Although both isozyme catalyze similar reactions, they have no sequence homology to each other. DGAT-1 inhibitors have potential for the treatment of obesity and a number of DGAT-1 inhibitors are in clinical trials (Claudio *et al*., 2009). Certain specific purification and biochemical characterization studies have been well documented reflecting interactions between lipid biosynthesis enzymes and putative inhibitors/activators (Mishra and Kamisaka, 2001). From the various previous work several ligands were reported (Matsuda *et al*., 2007; Tomoda, Minako *et al*., 1995; Won Keun *et al*., 2009; Brian *et al*., 2010; Nina Mayorek and Jacob Bar, 1985). Some of them are Triterpenoid betulinic acid, Emindole SB, Paspaline, Amidepsines D, Beauveriolide III etc. Through various researches it has been concluded that DGAT can function as a potential target for the treatment of obesity and hence the present study was

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undertaken in view of using molecular docking to design the potent inhibitor for DGAT (Sweta Sharma *et al*., 2015).

MATERIALS AND METHODS

Preparation of the Enzyme/Receptor

The 3D coordinates of the crystal structure of the DGAT enzyme was retrieved from Protein data bank (PDB) (www.rcsb.org)in dot (.pdb) format and taken as the receptor model in flexible dicking program. Before docking, the dot (.pdb) structure was converted into mol2 using VlifeMDS. After removing the water molecules, polar hydrogen atom were added to enzyme molecule.

Selection and Screening of the ligands

The ligand molecules, which have potentially used in treatment of obesity were retrieved from different databases (Mishra and Kamisaka, 2001). From the various previous work several ligands were reported (Matsuda *et al*., 2007; Tomoda, Minako *et al*., 1995; Won Keun *et al*., 2009; Brian *et al*., 2010; Nina Mayorek and Jacob Bar, 1985). The 3D structures of known inhibitors were downloaded from the PubChem compound database (htpp://www.ncbi.nlm.nih.gov/ pccompound) in (dot .sdf) format. They were later converted in mol2 format with the assistance of open babel software. The converted mol2 ligands were finally optimized by minimizing the energy.

Energy minimization

The energy of both the receptor and the ligand was minimized in order to achieve lowest free energy. The receptor was made free from all other molecules like water and ligand molecules. Energy minimization was done using VLifeMDS software (Sneha Manaswita *et al*., 2014; Tamanna Narsinghani *et al*., 2013). Various parameters for energy minimization were defined which includes force field (MMFF), charge (Gasteiger Marsili), and maximum number of cycles (10000). Both the receptor and ligand were minimized separately. After energy minimization local geometry check was performed to ensure whether the receptor model was free of any error and suitable for further use.

Cavity detection and selection

BioPredicta provides a utility to find cavities in enzyme and thereby identify the active site .i.e. the pocket in which ligand is likely to bind .It also provides a utility to find channels surrounding the cavity in enzyme and thereby identify the active site. The software also displays the Ramachandran plot showing distribution of residues in allowed and disallowed space. A 2D scatter plot was produced showing the backbone conformational angles (phi and psi) with the residue's name.

Molecular docking

Docking of ligands screened from literature against DGAT was done using genetic algorithm (GA) based docking Vlife MDS (Mamta Thakur *et al*., 2015). The docking method can be used to dock single ligand, which may be treated as flexible with a given receptor. This algorithm offers a strategy for globally searching the docked conformers' space. It follows Darwinian evolution and allows selected population of solution to exist in the next generation. Docking was accomplished at convergence factor of 0.001,1000 number of generations with dock score as the fitness function criteria and at default values of other docking parameters. Along with the isomers of DGAT (DGAT 1 & DGAT 2), the original substrate for DGAT was also docked with the ligands. The ligand and receptor were merged after obtaining the best ligand pose having minimum dock score. The energy of the docked complex was optimized to allow the relaxation of the protein to certain extent, which can account for the conformational changes, happening in the protein structure on binding of the ligand. In addition, the calculated energies were used to estimate the binding energy that helps in quantifying the binding process and to get a better understanding of the molecular recognition and interaction.

RESULTS AND DISCUSSION

Molecular Docking

The ligands docked with isomers DGAT 1 & DGAT 2 were listed in the Table 1 along with their docking score. It was observed that the ligands NEM, Niacin, Triterpenoid betulinic acid, 2-phenyl oxazole, betulinic acid,4,4'-diisothiocyanostilbene-2,2'-disulfonatehad the dock score nearby to the original substrates and hence reflecting the highest affinity towards the substrate i.e Diacylglycerol (DGAT).

Active site identification

Active site of DGAT isomers, namely, DGAT 1 & DGAT 2were detected by identifying the amino acids residues interacting with ligands, Docking of DGAT isomers DGAT 1 & DGAT 2 with diacylglycerol, a natural substrate in Kennedy pathway are shown in Figure 3. It was found the amino acids residues LYS119, ASN120, THR184, CYS186, ILE189, LEU190, GLY193, ARG197, SER198, ILEU201, and PHE227 & GLN233 of DGAT 1 were interacting with the ligands. Similarly when the interaction of isomer DGAT 2 was studied, it was found that the amino acids LEU10, ASP11, VAL16, HIS17, TRY18, ALA19, PRO25, LEU26,ARG28 & ARG29 were interacting with the ligands.

Study the cavity

Cavities generated by Biopredicta module of VlifeMDS represented by green color (Fig 4). In first part of figure (4a,4b&4c) cavities of isomer DGAT 1 were represented, it was found that the presence of three cavities, which populate the residues list with names of residues that are in the vicinity of the selected cavity (cavity1concludes16196, cavity 2 concludes 5933 points &cavity 3 concludes 1664). The second part of figure (4d,4e,4f,4g,4h,4i) represent isomer DGAT 2 with six cavitiessix cavities are cavity1 concludes 9680, cavity 2 concludes 3695 points, cavity 3 concludes 1389 points, cavity 4 concludes 1253 points, cavity 5 concludes 1074points, cavity 6 concludes 1017 points.

Table 1. Docking scores of DGAT isomers DGAT 1 & DGAT 2 with ligands

LIGANDS	DOCKING SCORE (Kcal/mol)	LIGANDS	DOCKING SCORE (Kcal/mol)
Triterpenoidbetulinic acid	-4.4518	Xanthohumol	-1.1626
Emindole SB	-2.2600	Honokiol	-3.2347
Paspaline	-2.7918	Octanoate	-3.7027
Amidepsines D	-2.5601	Bisvertinol	-1.3789
Bauveriolide III	-0.1411	LCQ-908 (Novartis AG)	-0.7131
Beauveriolide I	3.8145	Ethylenediaminetetraacetate	-2.8656
Roselipins 2A	2.5347	Sigmoidin A	-0.8134
Roselipins 2B	3.3821	Sigmoidin B	-3.7002
NEM, *N*-ethylmaleimide	-4.0585	Erysenegalensein H	-2.3828
A-922500	-1.9938	Erysenegalensein I	-1.5338
Auriculatin	-3.2360	Senegalensin	-1.4462
Eysenegalensein O	-1.6112	Lupinifolin	-1.8961
Erysene-galensein D	-2.7872	Auriculatin	-3.236
Eysenegalensein N	-1.0903	T863	-1.9256
Derrone	-3.5454	2 phenyl oxazole	-4.4325
Alpinumisoflavone	-2.8929	Roselipin1A/1B	2.5347
Betulinic acid	-4.1237	Rselipin 2A/2B	3.3821
2-bromooctanoyl-CoA	5.8286	Gyroporic acid	0.7403
Amidepsines A	0.0868	Compound 23n	-3.0182
4,4'-diisothiocyanostilbene-2,2'-disulfonate	-4.4538	Compound 10j	-3.4722
NaF	-3.8955	Compound 7s	-3.1469
Phenylmethylsulfonyl fluoride,	-4.1669	BAY	-2.4006
Diethyl p-nitro phenyl phosphate	-3.4264	Auriculatin 2	-3.5068
Niacin	-4.4353	AZD7687	-2.0798
Vermisporin	-1.6382	DGAT-1	-1.6525
CI-976	0.2342	Rugosin B	-1.9379
PF-04620110	-2.3923	Rugosin D	16.915

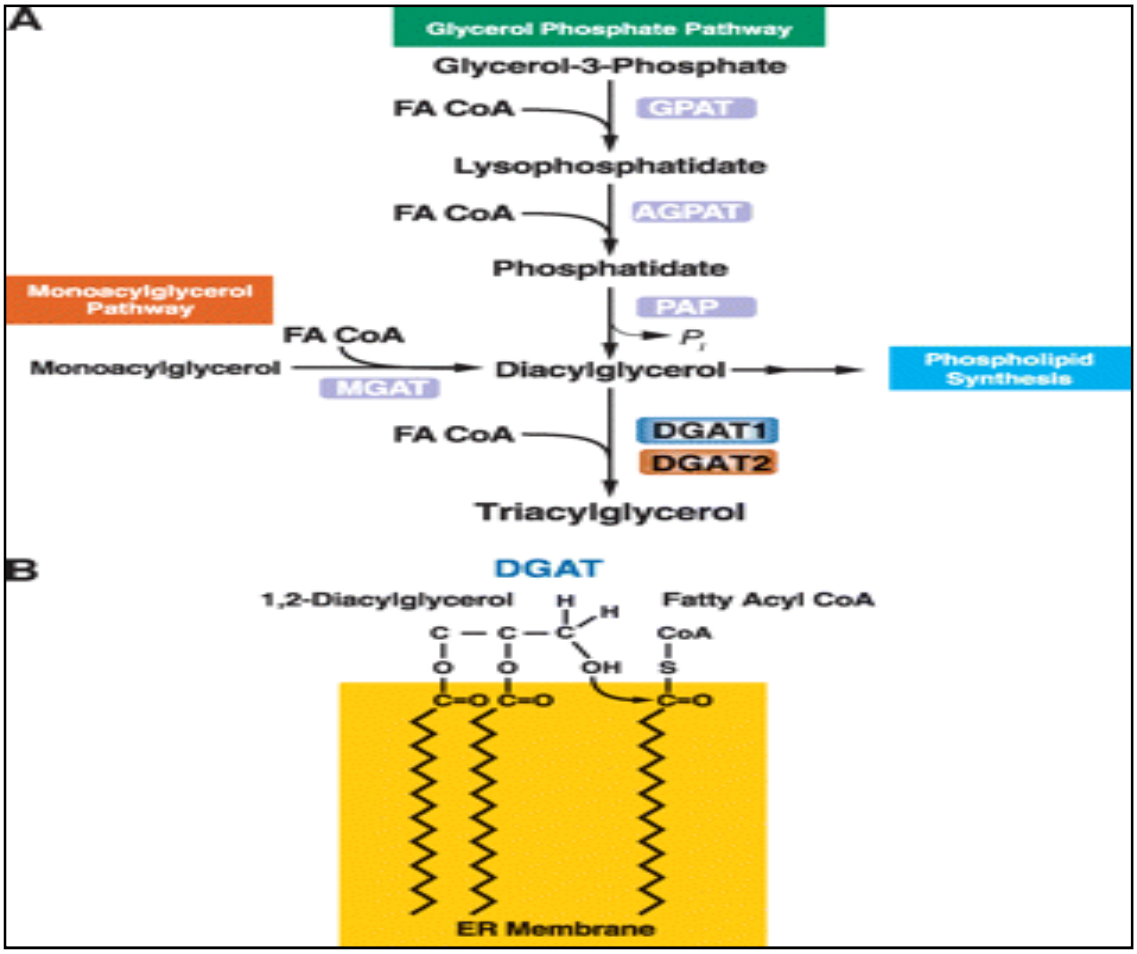


Fig. 1. Triacylglycerol synthesis and acyl-CoA:diacylglycerol acyltransferase (DGAT) enzymes. A: Triacylglycerols (triglycerides) are the end-product of a multi-step pathway. DGAT1 or DGAT2 catalyzes the final reaction. B: DGAT enzymes catalyze the formation of an ester linkage between a fatty acyl CoA and the free hydroxyl group of diacylglycerol. The model shows this reaction occurring at the surface of the endoplasmic reticulum (ER) bilayer membrane. GPAT, glycerol-phosphate acyltransferase; AGPAT, acylglycerol-phosphate acyltransferase; PAP, phosphatidic acid phosphohydrolase; MGAT, acyl CoA:monoacylglycerol acyltransferase (Chi-Liang Eric Yen *et al*., 2008)

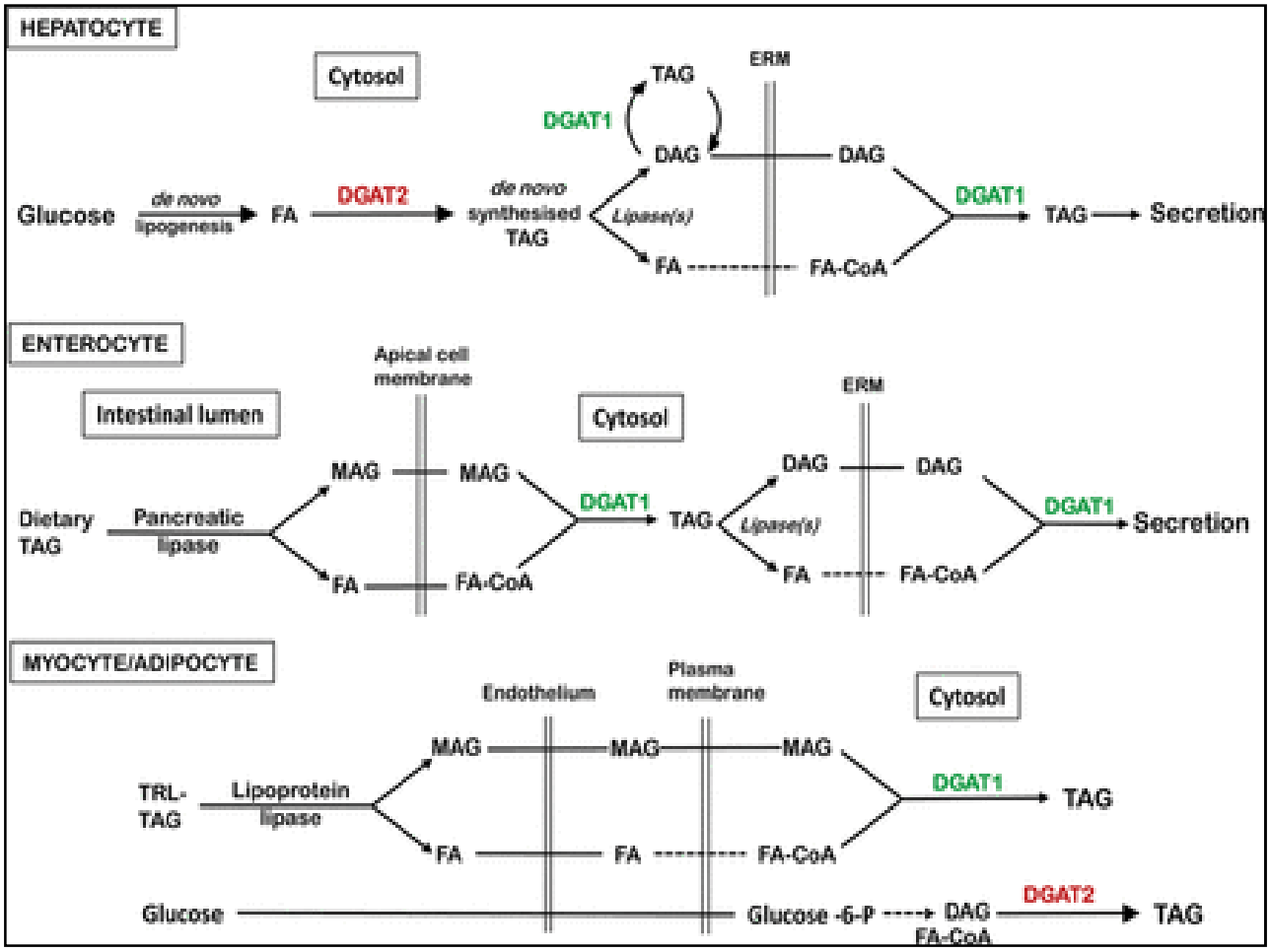


Fig. 2. Comparison of the possible routes to TAG synthesis in hepatocytes, enterocytes and myocytes (Chi-Liang Eric Yen et al., 2008)

In tissues in which, unlike the liver, *de novo* synthesis of fatty acids from carbohydrate sources is not a major process (intestine and muscle), DGAT2 may be bypassed through the formation of partial glycerides by lipase-mediated hydrolysis of TAG (e.g. by pancreatic lipase and LPL). These glycerides can then be used by DGAT1 to resynthesize TAG within the respective cell types (enterocytes and myocytes). When *de novo* fatty acid synthesis from glucose becomes more important (e.g. in hyperglycaemiac and hyperinsulinaemic states in muscle) it would provide substrates for DGAT2. In adipocytes, the involvement of DGAT2 may be more significant, as lipogenesis from glucose may be more prevalent. FA, fatty acid. (26).

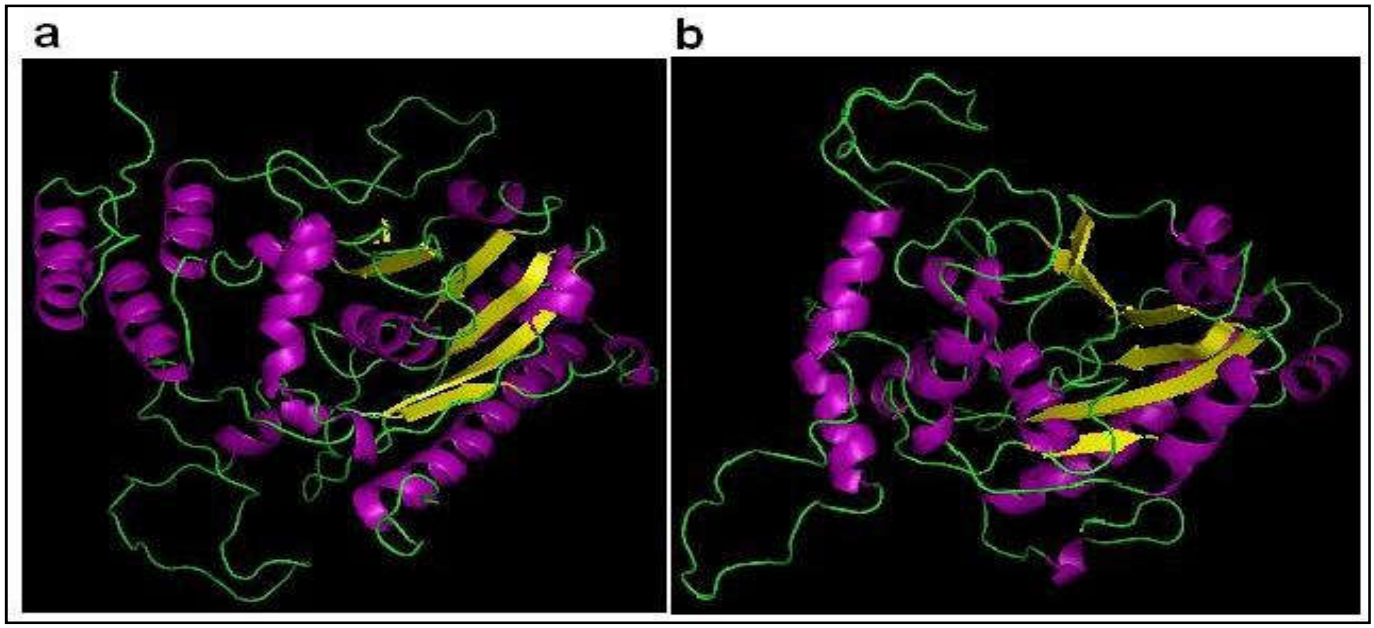


Fig. 3. DGAT 1(Sanjay Mishra *et al*., 2009) **DGAT 2 (Sanjay Mishra *et al*., 2009)**

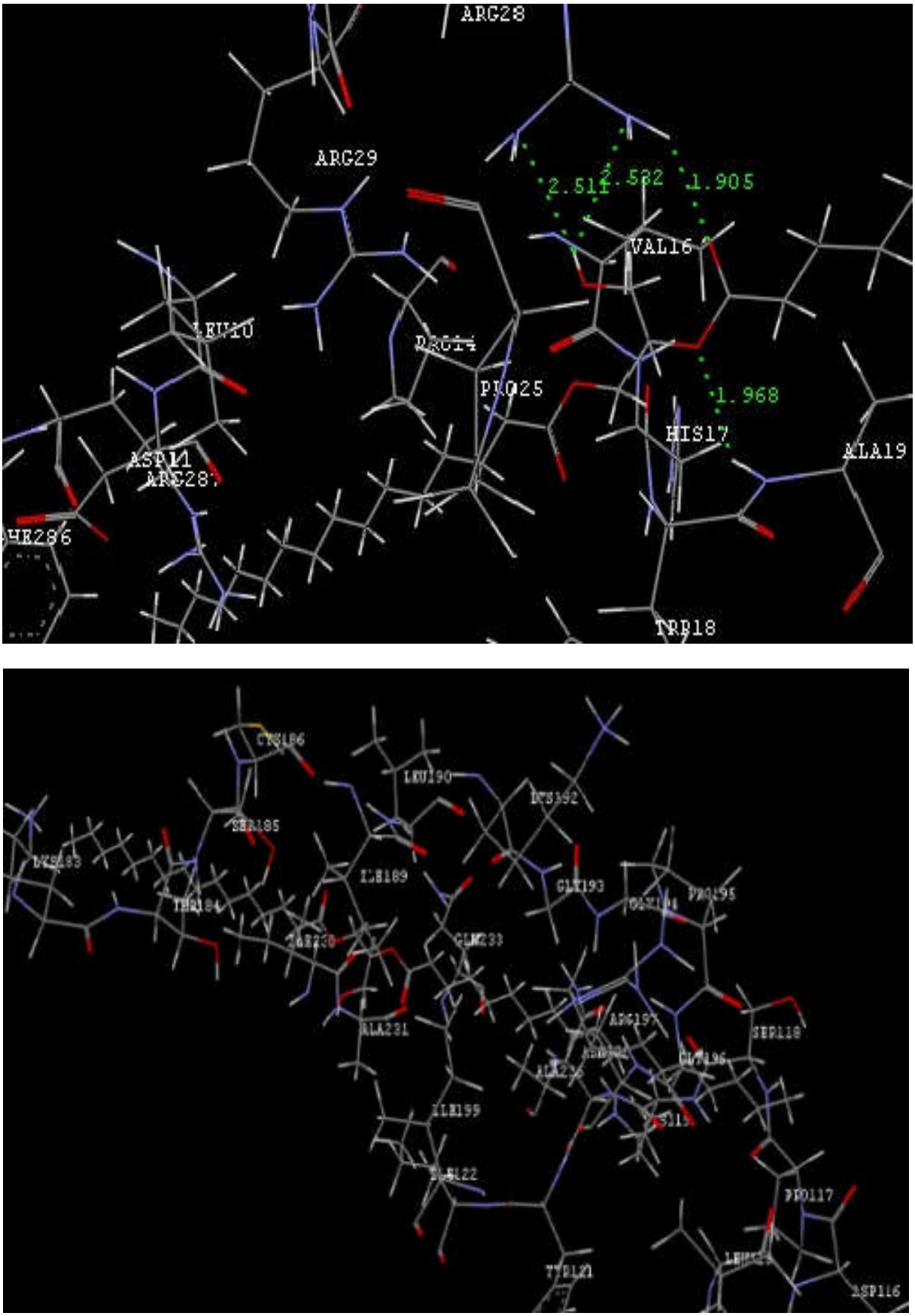
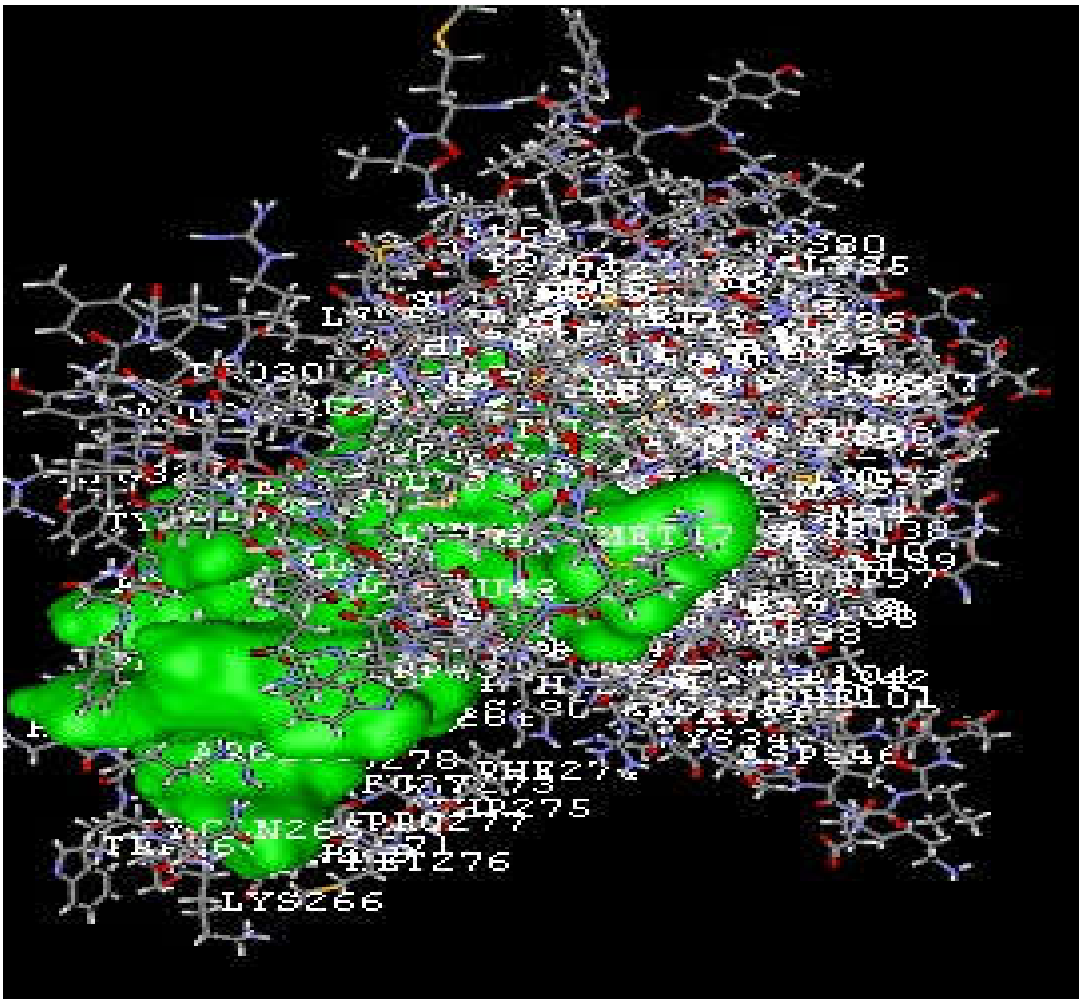
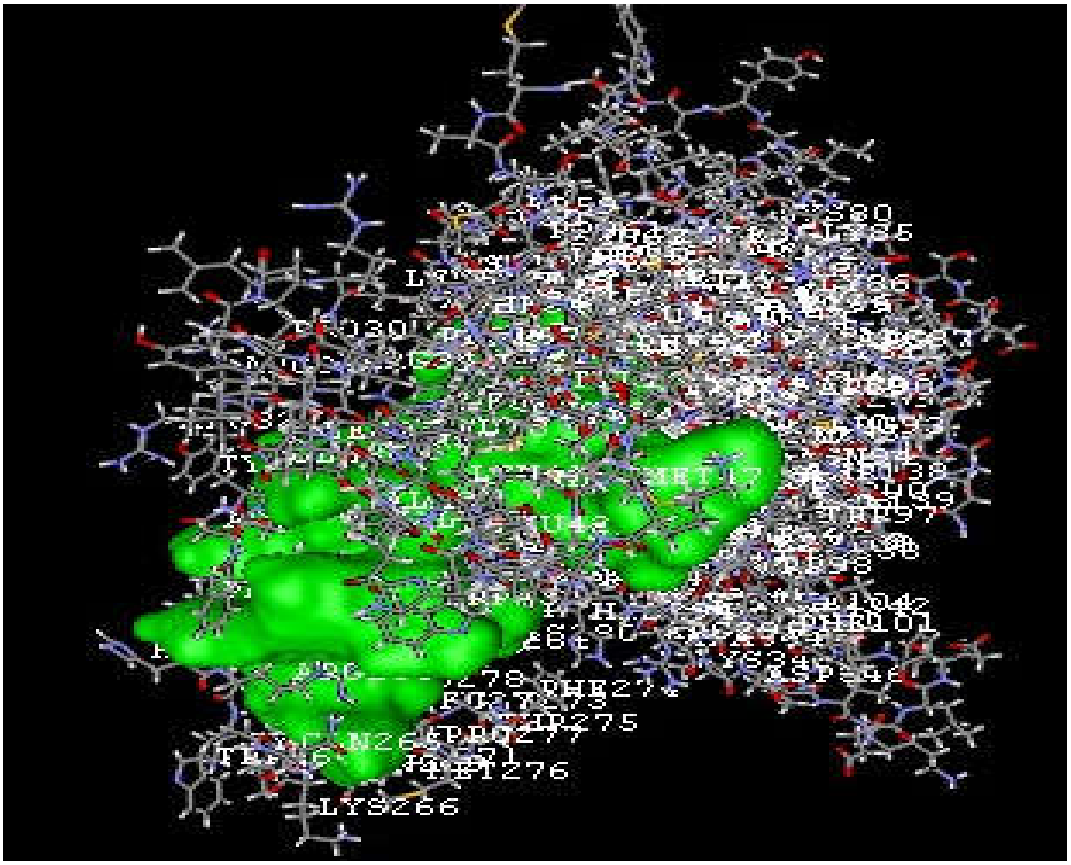
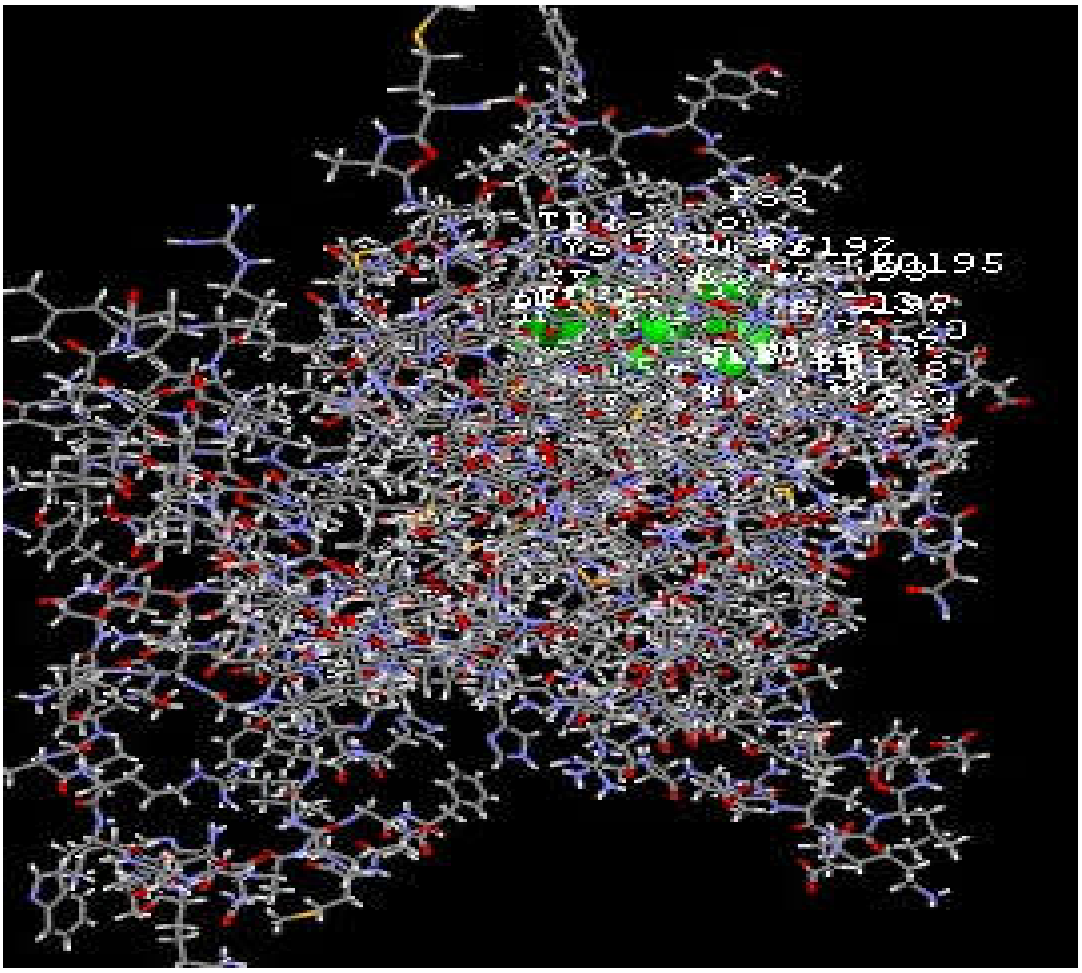
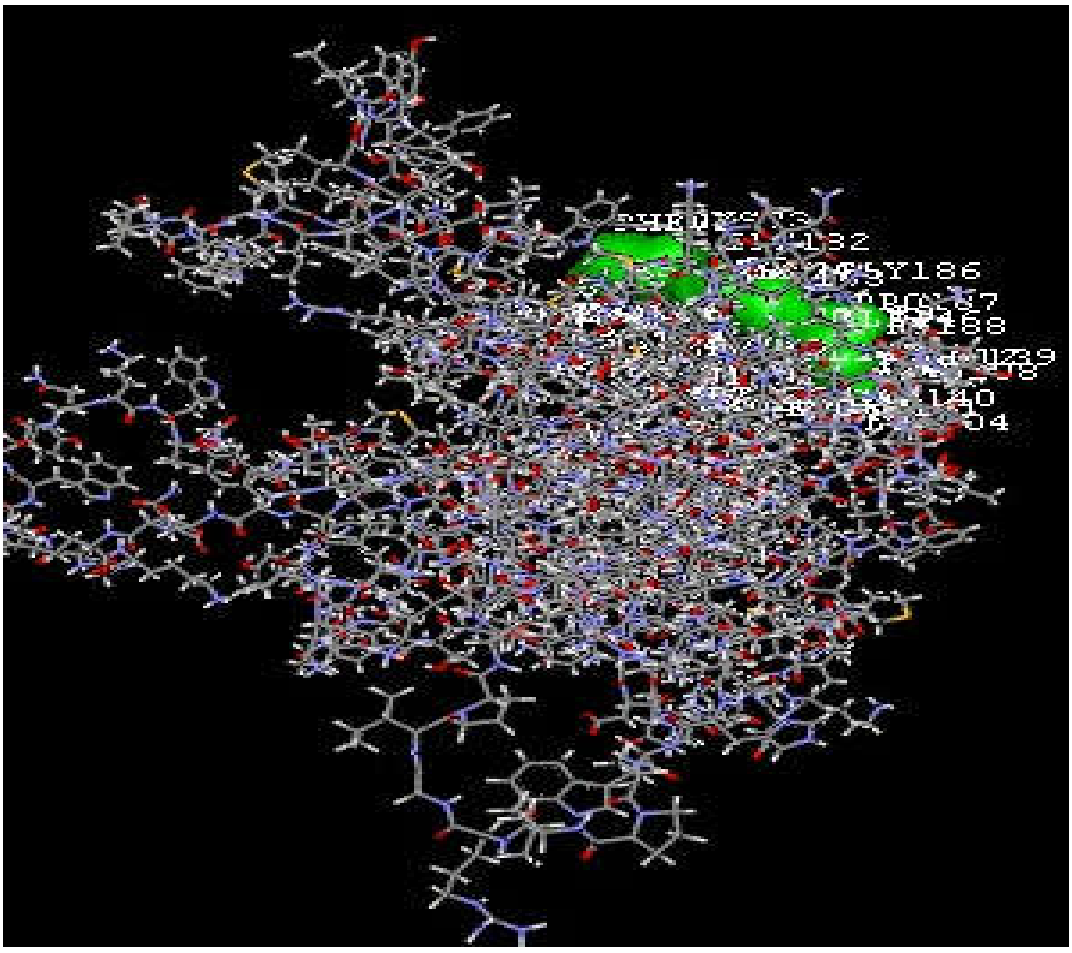


Fig. 4. Interaction of DGAT 1 & DGAT 2 with the original substrates


LYS266


LYS266





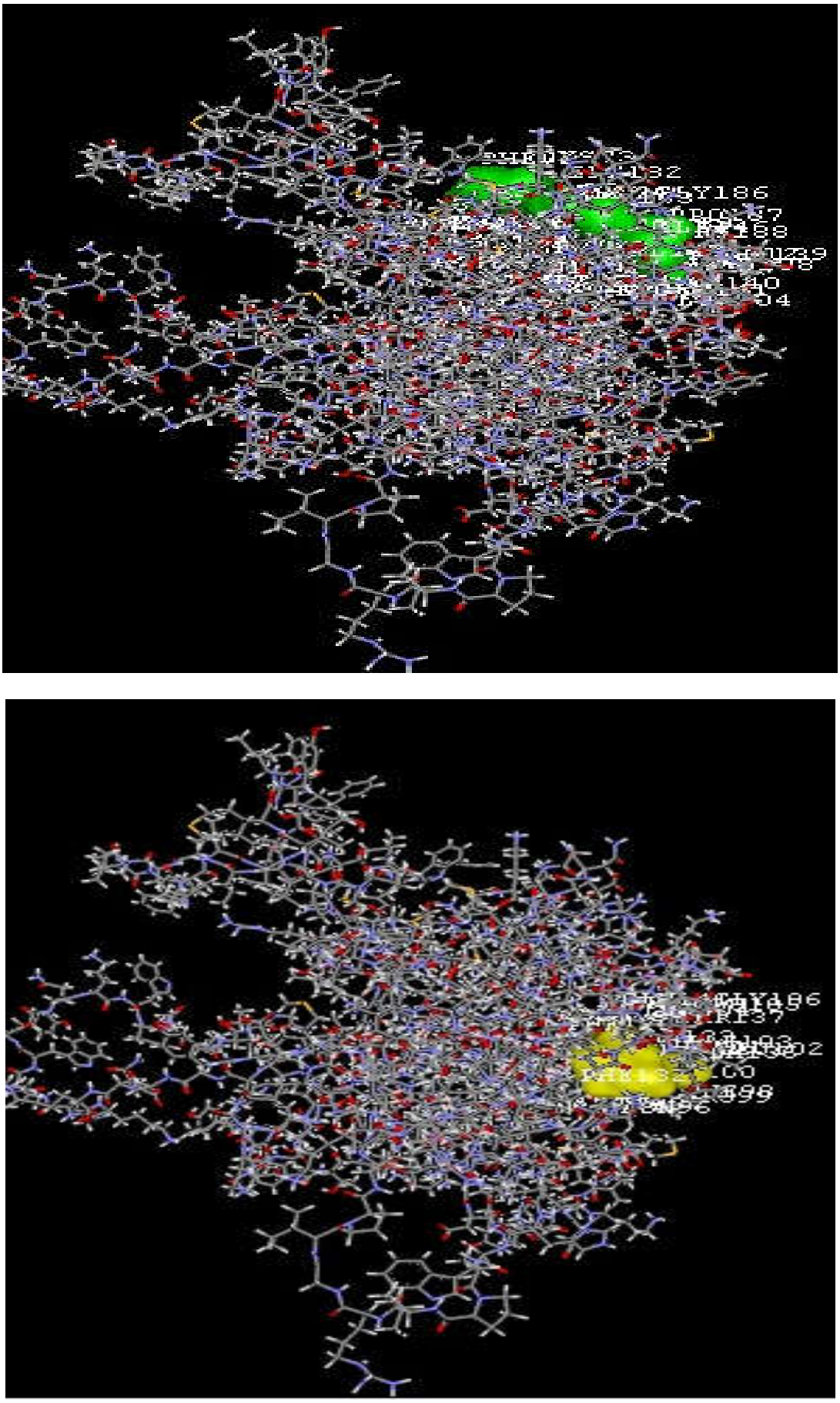
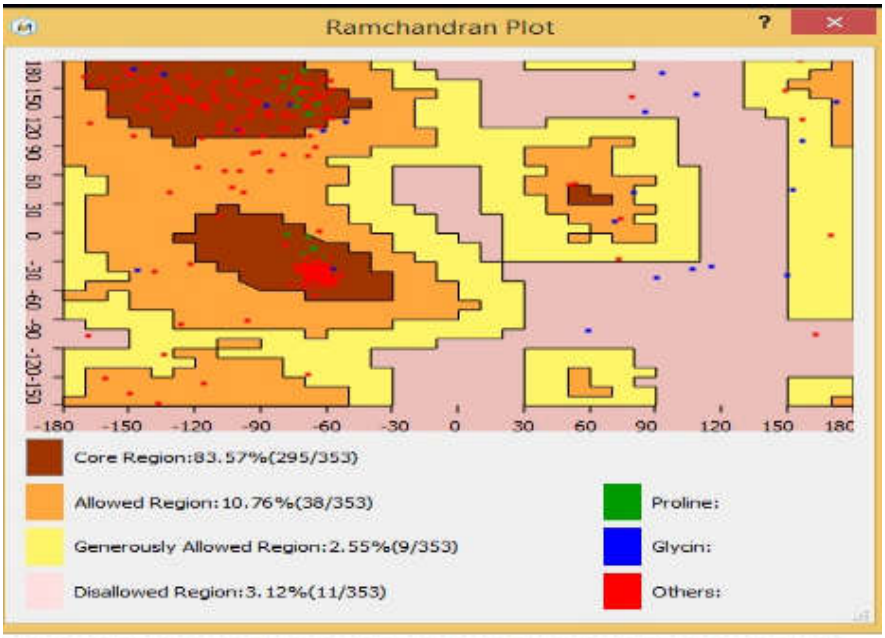


Fig. 5. Binding cavities of DGAT isomers DGAT1 (4a, 4b, 4c) & DGAT 2 (4d, 4e, 4f) respectively

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* Note - All counts are including GLY & PRO residues
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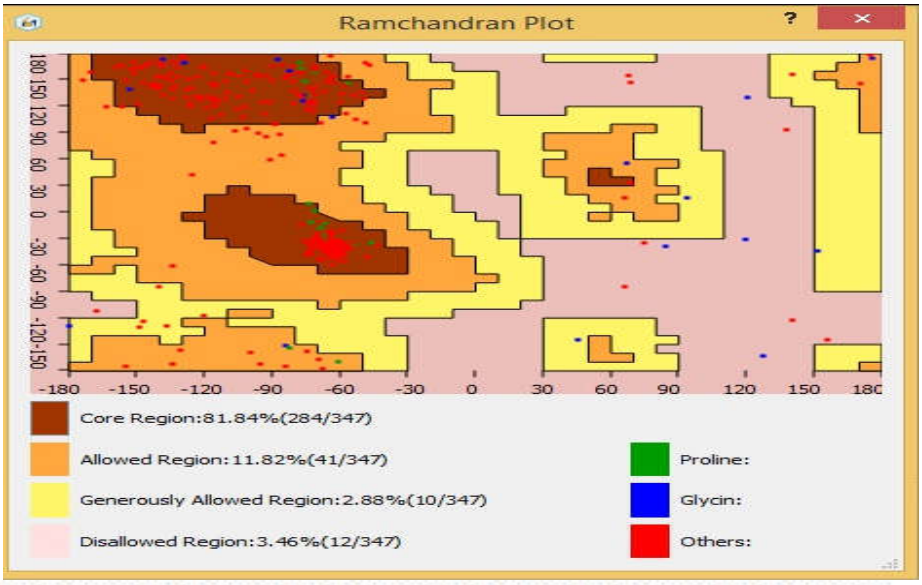


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* Note - All counts are including GLY & PRO residues
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Fig. 6. Ramachandran plot for DGAT1 & DGAT 2

Ramachandran plot

The Ramachandran plot of isomers 1IUQ & 1K30 were studied using Vlife MDS (Fig. 5).

Conclusion

In this molecular interaction study, we have docked various ligands with this receptor model after energy minimization. Dock score and the binding energy obtained after optimization of the docked complex indicated formation of a stable complex and strong affinity binding towards the enzyme isomers. It was found that amino acid residues lying in DGAT 1 & DGAT 2were mainly involved in the interaction and the most important residues predicted to be involved in these interactions are LEU10, ASP11, VAL16, HIS17, TRY18, ALA19, PRO25, LEU26, ARG28 & ARG29, LYS119, ASN120, THR184, CYS186, ILE189, LEU190, GLY193, ARG197, SER198, ILEU201, PHE227 & GLN233 respectively. Conclusively, the present study concerning with the molecular interaction between DGAT and its receptor provides new insights into the elucidation of structural domains and development of functional analogues with higher binding affinity and new drug combination therapies for the treatment of obesity.

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REFERENCES

Akoh CC. 1995. Lipid based fat substitutes. *Crit Rev Food Sci Nutr*., 35:405-30.

Arend Bonen *et al*. 2004. Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemma FAT/CD36. *The Faseb Journal*, July 18,1144-11464.

Brian M. *et al*. Discovery of pyrrolopyridazines as novel DGAT1 inhibitors.October 2010.Bioorganic & Medicinal Chemistry Letters Volume 20, Issue 20,Pages 6030–6033.

Charles A. Harris *et al*. DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. *J Lipid Res*., 2011 Apr; 52(4):657-67

Chi-Liang Eric Yen *et al*. 2008. Thematic Review Series: Glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *J Lipid Res*., Nov; 49(11):2283-301

Chi-Liang Eric Yen *et al*. DGAT enzymes and triacylglycerol biosynthesis.2008; *The Journal of Lipid Research*, 49, 2283-2301.

Claudio J *et al*. 2009. A Specific Role for *Dgat1* in Hepatic Steatosis Due to Exogenous Fatty Acids Hepatology., Aug; 50(2): 434–442.

Do, R. *et al*. 1995. "Common variants associated with plasma triglycerides and risk for coronary artery disease." Nature Genetics. DOI: 10.1038/ng.2795

Farese, Robert V. Jr. *et al*. 2000. Triglyceride synthesis: insights from the cloning of diacylglycerol acyltransferase. Current Opinion in Lipidology: June, Volume 11 - Issue 3:229-234.

Gary F Lewis *et al*. 1993. Effects of Acute Hyperinsulinemia on VLDL Triglyceride and VLDL Apo B Production in Normal Weight and Obese Individuals. *Diabetes,* June, 42, 833-842

Hedley AA. *et al*. 2004. Prevalence of overweight and obesity among US children, adolescent and adults, 1999-2002. *JAMA*, 2847-50.

Hubert C Chen *et al*. 2000. DGAT and Triglyceride Synthesis A New Target for Obesity Treatment. *Trends Cardiovascular Med*., Jul; 10(5):188-92.

Kamisaka, Y., Mishra, S. and Nakahara, T. 1997. Purification and characterization of diacylglycerol acyltransferase from the lipid body fraction of an oleaginous fungus. *Journal of Biochemistry*, 121: 1107-1114.

Kanji Yamaguchi. 2007. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 6 June, Volume 45, Pages 1366–1374.

Kennedy, E. 1957. Metabolism of lipids. *Annu.Rev.Biochem*., 26:119-148.

Mamta Thakur *et al*. 2015. Docking studies of benzodiazepines as a positive allosteric modulator of Gaba -a receptor. April – May. *International Journal of Research and Development in Pharmacy and Life Sciences*, Vol. 4, no.3, pp 1577-1581issn (p): 2393-932x, ISSN (e): 2278-0238

Matsuda D *et al*. 2007. DGAT inhibitors for obesity. *Curr Opin Investig Drugs*, Oct; 8(10):836-41.

Mishra, S. and Kamisaka, Y. 2001. Purification and characterization of thiol reagent- sensitive glycerol-3-phosphate acyltransferase from the membrane fraction of an oleaginous fungus. *Biochemical Journal*, 355: 315-322.

Nina Mayorek and Jacob Bar. 1995. Inhibition of Diacylglycerol Acyltransferase by 2-Bromooctanoate in Cultured Rat Hepatocytes. June 1985. *The Journal of Biological Chemistry*, Sep, Vol. 260, No. 11, pp. 6528-6532.

Rosalyn, Carson-Dewitt, MD. "Obesity." The Gale Encyclopedia of Medicine. 2001.

Sanjay Mishra, *et al*. 2009. A molecular model for diacylglycerol acyltransferase from *Mortierella ramanniana var. angulispora*. *Bioinformation*., 3(9): 394–398.

Sneha Manaswita *et al*. 2014. In silico study to evaluate the molecular interactions between somatostatin and its receptor sstr5. Mar, World Journal of Pharmacy and Pharmaceutical Sciences Volume 3, Issue 3, Pgs. 2278 – 4357.

Steven J. Smith *et al*. 2000. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat Nature Genetics, 25, 87-90.

Sweta Sharma *et al*. 2015. *In silico* Study on the Active Site Conformation and Structural Modulation of Glycerol-3-Phosphate Acyltransferase in Relevance to Medicinal

Significance. July, *International Journal of Multidisciplinary and Current Research*, Vol 3, pages 713-718.

Tamanna Narsinghani, *et al*. 2013. Synthesis, docking studies and antioxidant activity of some chalcone and aurone derivatives Sep; *Medicinal Chemistry Research*, Volume 22, Issue 9, pp 4059-4068.

Tomoda, Minako *et al*. Inhibitors of diacylglycerol acyltransferase produced by Humicola sp. fo-2942 I. Production, Isolation and biological properties the. *Journal of Antibiotics*, vol. 48 no.9

Won Keun Oh *et al*. 2009. Diacylglycerol Acyltransferase-Inhibitory Compounds from E.rythrinasenegalensis, *Arch Pharm Res*., Vol .32, No 1, 43-47.

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