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IN SILICO DOCKING ANALYSIS OF CURCUMIN – AN INHIBITOR FOR OBESITY

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ABSTRACT

Obesity refers to abnormal or excessive fat accumulation in the human body which results in health risk. Obesity leads to several risk factors for a number of chronic diseases such as diabetes, cardiovascular diseases, cancer and osteoarthritis. Curcumin, the major polyphenol of turmeric spice, is a member of the ginger family (Zingiberaceae). Growth of adipose tissue is enhanced by the process of angiogenesis. Curcumin, by its antiangiogenic activity may suppress the growth of adipose tissue. This study attempts to curtail the action of fat mass and obesity protein (FTO) by docking with Curcumin. Curcumin acts as an inhibitor and suppresses the action of obesity protein and hence may contribute to lower body fat and body weight gain. Some of the commonly used drugs to treat obesity includes Orlistat, Sibutramine and Rimonabant. These drugs were subjected to docking analysis for comparative studies.

KEYWORDS

Curcumin, BMI, Angiogenesis, Polyphenol, FTO, Antiobesity drugs

INTRODUCTION

Obesity is now commonly defined in adults as a Body mass index [BMI > 30 kg/m^2] [1]. Weight gain and obesity are major risk factors for conditions and diseases ranging from insulin resistance and type 2 diabetes mellitus to atherosclerosis and the sequelae of nonalcoholic fatty liver disease[2]. A higher body weight is associated with an increased incidence of a number of conditions, including diabetes mellitus, cardiovascular disease, and nonalcoholic fatty liver disease, and with an increased risk of disability. Obesity is associated with a modestly increased risk of all-cause mortality[3]. Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and is commonly used as a spice and food-coloring agent. Dietary curcumin is the major polyphenol found in turmeric with no known toxicity [4]. Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions. Safety evaluation studies indicate that curcumin is well tolerated at a very high dose without any toxic effects. Thus, curcumin has the potential for the development of modern medicine for the treatment of various diseases[5]. The desirable preventive or putative therapeutic properties of curcumin have also been considered to be associated with its

antioxidant and anti-inflammatory properties[6]. Epidemiological observations, though inconclusive, are suggestive that turmeric consumption may reduce the risk of some form of cancers and render other protective biological effects in humans. These biological effects of turmeric have been attributed to its constituent curcumin that has been widely studied for its anti-inflammatory, anti-angiogenic, anti-oxidant, wound healing and anti-cancer effects[7].

Computational (*In silico*) methods have been developed and widely applied to pharmacology hypothesis development and testing. These *in silico* methods include database searching, quantitative structure-activity relationships, similarity searching, pharmacophore identification, computational modeling and docking. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization[8]. The growth of blood vessels (a process known as angiogenesis) is essential for organ growth and repair[9]. The inhibition of angiogenesis in adipose tissue can be a strategy to prevent adipose tissue growth and obesity. For this effect, an antiangiogenic drug, TNP-470, has recently been shown to suppress obesity through suppression of angiogenesis in the adipose tissue of mice[10]. Pharmacological manipulation of adipose tissue neovascularization by angiogenic stimulators and inhibitors might therefore offer a novel therapeutic option for the treatment of obesity and related metabolic disorders[11]. Angiogenesis is necessary for the growth of adipose tissue. Dietary polyphenols may suppress growth of adipose tissue through their antiangiogenic activity and by modulating adipocyte metabolism. Curcumin has the ability to inhibit angiogenesis in adipose tissues and hence aid in lowering body weight and obesity. Curcumin at cellular and whole organism levels shows remarkable health benefits for prevention of obesity and associated metabolic disorders by supressing angiogenesis in adipose tissues[12]. Antiobesity drugs such as Orlistat (a gastrointestinal lipase inhibitor) and Sibutramine (a monoamine-reuptake inhibitor) are commonly used drugs in obesity treatment. Rimonabant, the first of the endocannabinoid receptor antagonists is also used as antiobesity drug. Antiobesity treatment is suggested for patients in whom lifestyle modification is unsuccessful[13]. We performed *in silico* docking study to prevent obesity in human using Curcumin as inhibitor against fat mass and obesity associated protein. Antiobesity drugs were also included in the docking study to perform comparative study and to prove that curcumin could be a potent inhibitor for obesity.

MATERIALS AND METHODS

Target Identification:

The three dimensional structure of fat mass and obesity associated (FTO) protein [PDB: 3LFM] [14] was obtained from Protein Data Bank (PDB) [15, 16]. This structure was determined using X-ray Diffraction.

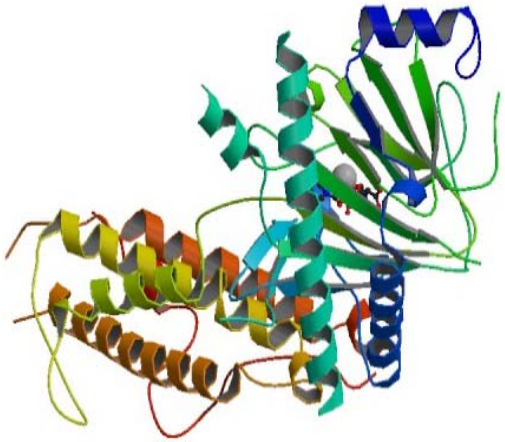

Figure 1
3D Structure of fat mass and obesity associated (FTO) protein [3LFM]

Ligand Identification:

Curcumin, the principal curcuminoid of the popular Indian spice turmeric is used as the ligand. (PubChem Compound ID: 969516), was retrieved from NCBI PubChem Compound database [17, 18]. The 2D and 3D structure of the ligand was shown in figure 2.1 and 2.2 respectively. The structure was downloaded in SDF format and was then converted to PDB format using OPEN BABEL 2.2.1[19] and further used for docking studies.

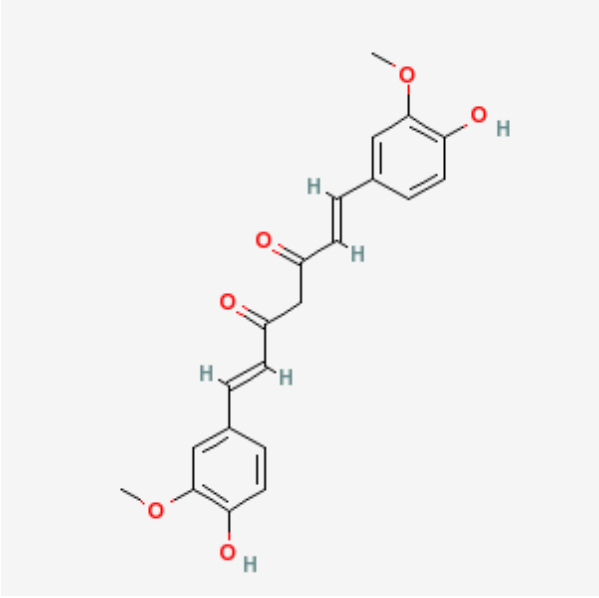

Figure 2.1
2D Structure of Curcumin

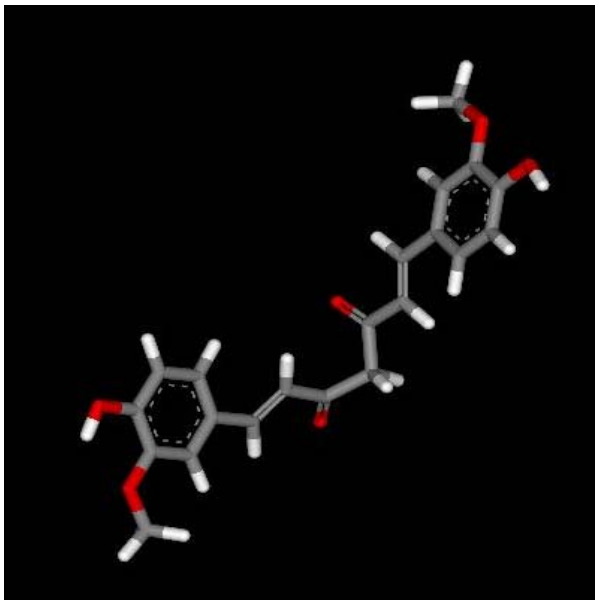

Figure 2.2
3D Structure of Curcumin

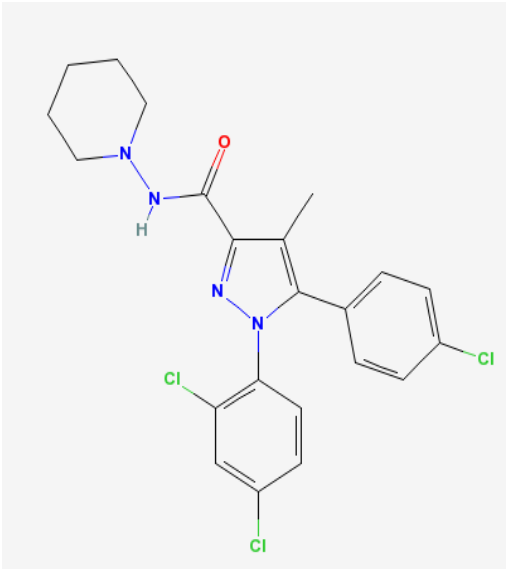

Figure 3.1
2D Structure of Rimonabant

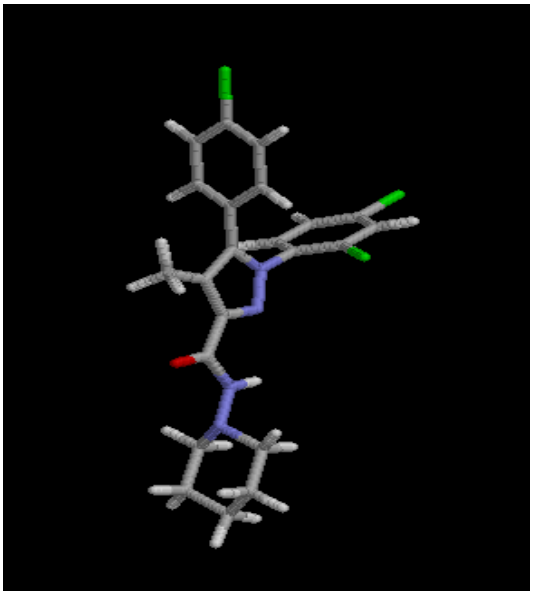

Figure 3.2
3D Structure of Rimonabant

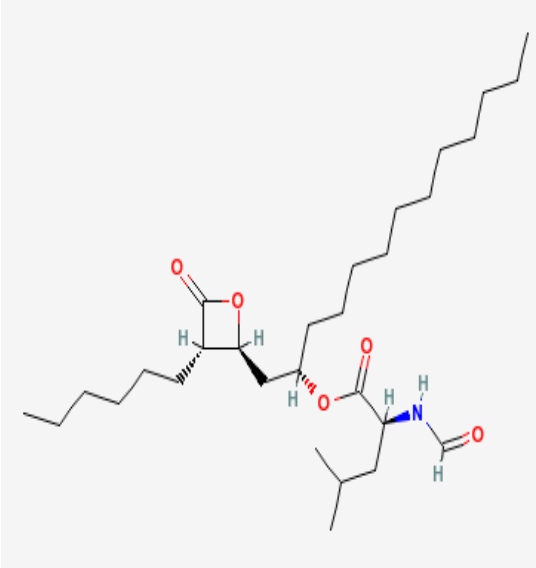

Figure 4.1
2D Structure of Orlistat

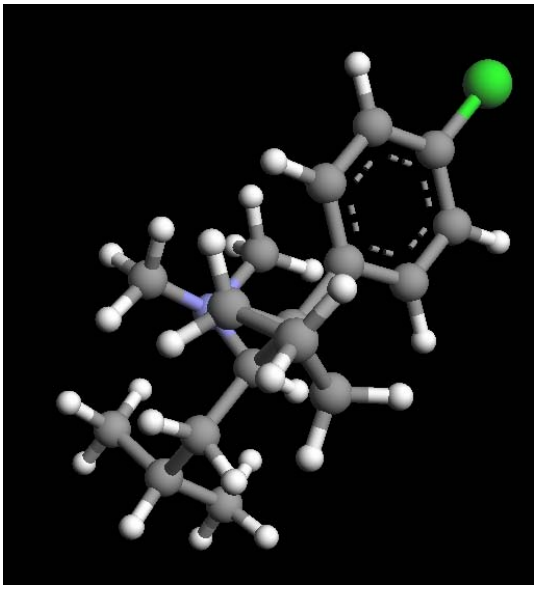

Figure 4.2
3D Structure of Orlistat

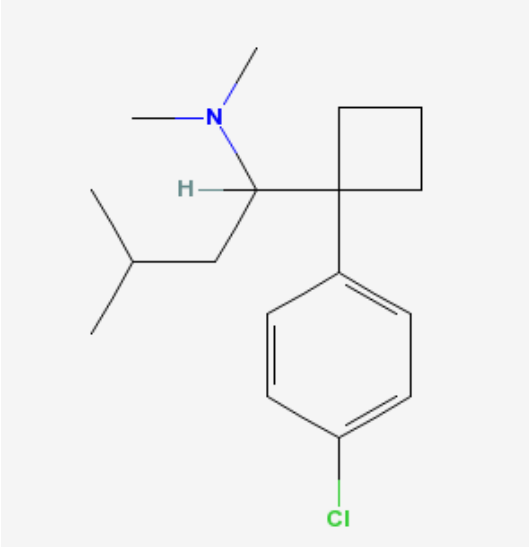

Figure 5.1
2D Structure of Sibutramine

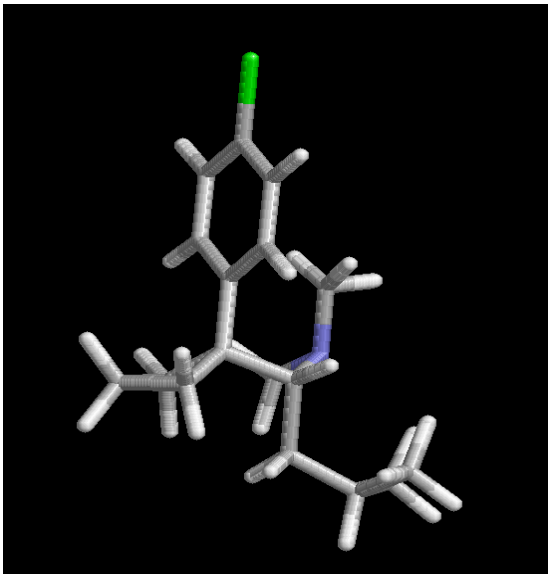

Figure 5.2
3D Structure of Sibutramine

Antiobesity Drugs:

Antiobesity drugs such as Orlistat, Sibutramine and Rimonabant were included in the docking analysis for comparative studies. Orlistat (PubChem Compound ID: 3034010), Sibutramine (PubChem Compound ID: 5210) and Rimonabant (PubChem Compound ID: 104850) was retrieved from NCBI PubChem Compound database [17, 18]. The 2D structure of these drugs was shown in figure 3.1, 4.1 and 5.1 respectively. Similarly 3D structure of these drugs was shown in figure 3.2, 4.2 and 5.2 respectively. The structures downloaded in SDF format were then converted to PDB format using OPEN BABEL 2.2.1 and were used for docking analysis [19].

Table 1
Ligand Molecules - IUPAC Name and SMILES

Ligand	IUPAC Name	SMILES
Curcumin	(1E,6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O
Orlistat	[(2S)-1-[(2S,3S)-3-hexyl-4-oxooxetan-2-yl]tridecan-2-yl](2S)-2-formamido-4-methylpentanoate	CCCCCCCCCCCC(CC1C(C(=O)O1)CCCCCC)OC(=O)C(CC(C)C)NC=O
Sibutramine	1-[1-(4-chlorophenyl) cyclobutyl]-N,N,3-trimethylbutan-1-amine	CC(C)CC(C1(CCC1)C2=CC=C(C=C2)Cl)N(C)C
Rimonabant	5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-piperidin-1-ylpyrazole-3-carboxamide	CC1=C(N(N=C1C(=O)NN2CCCCC2)C3=C(C=C(C=C3)Cl)Cl)C4=CC=C(C=C4)Cl

Docking FTO with Curcumin using Autodock

The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation. AutoDock[20, 21, 22] requires pre-calculated grid maps, one for each atom type present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. This grid must surround the region of interest in the rigid macromolecule. The grid box size was set at 70, 70 and 70 A° (x, y, and z) to include all the amino acid residues that present in rigid macromolecules. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.375 angstroms. The Lamarckian Genetic Algorithm (LGA) [23] was chosen search for the best conformers. During the docking process, a maximum of 10 conformers was considered. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 500000, maximum number of generations 1000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, Step sizes were 0.2 Å for translations, 5.0° for quaternions and 5.0° for torsions. Cluster tolerance 0.5Aº, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed. All the AutoDock docking runs were performed in Intel(R) Xeon(R) CPU 5150 @ 2.66GHz, 2GB RAM in Apple system. AutoDock was compiled and run under Windows XP operating system. Autodock results were analyzed to study the interactions and the binding energy of the docked structure.

Drug likeliness and Bioavailability:

Lipinski rule states, that most "drug-like" molecules have logP <= 5, molecular weight <= 500, number of hydrogen bond acceptors <= 10, and number of hydrogen bond donors <= 5. Molecules violating more than one of these rules may have problems with bioavailability[24, 25]. The rule is called "Rule of 5", because the border values are 5, 500, 2*5, and 5. Drug likeliness and bioavailability of the ligand is inspected using the Molinspiration tool [26].

RESULTS AND DISCUSSION

FTO, the Macromolecule and the ligands (Curcumin, Rimonabant, Orlistat, Sibutramine) were subjected to docking analysis using Autodock 4.0. Molecular docking simulations were conducted with this software suite. 10 docking runs were performed. Grid parameters were set as mentioned earlier and spacing between grid points was 0.375 Å. After the simulations were complete, the docked structures were analyzed and the interactions were seen. Hydrogen bond interactions and the binding distance between the donors and acceptors were measured for the best conformers. Distinct conformational clusters were formed at an RMSD-tolerance of 2.0 Å. Van der waals scaling factor was found to be 1.0 Å.

Docking Curcumin into FTO:

Docking simulation of Curcumin into FTO produced six clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -6.57 kcal/mol at 10th run has three hydrogen bond interactions at residues GLN 86, LYS 107 and GLU325 with cluster RMSD 0.00 and reference RMSD 38.15. Hydrogen bond distance between the donor and acceptor atoms was found to be 2.185, 2.234 and 2.128 respectively (shown in Table 2). Docking interaction between the ligand and the macromolecule is shown in Figure 6.

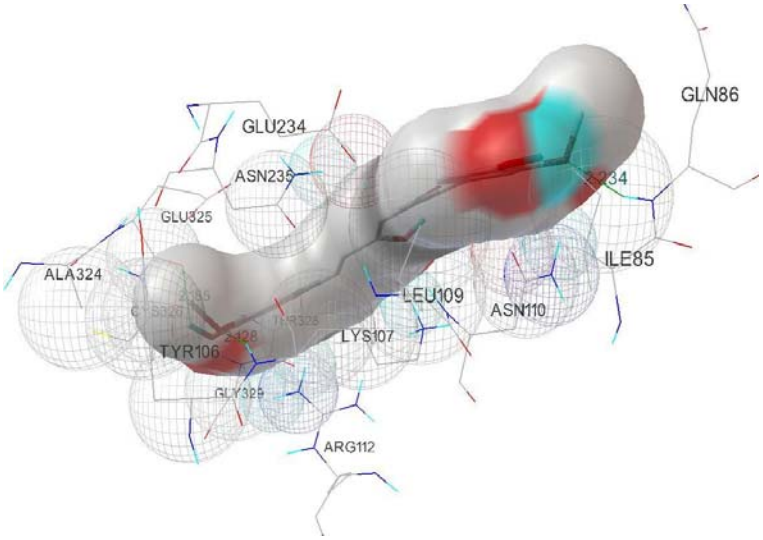

Figure 6
Docked Structure of the receptor (3LFM) and the ligand (Curcumin)

Docking Rimonabant into FTO:

Docking simulation of Rimonabant into FTO produced six clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -5.46 kcal/mol at 2nd run has formed one hydrogen bond interaction at residue LYS 107 with cluster RMSD 0.00 and reference RMSD 35.30. Hydrogen bond distance between the donor and acceptor was found to be 2.179 (as shown in Table 2). Docking interaction between the ligand and the macromolecule is shown in Figure 7.

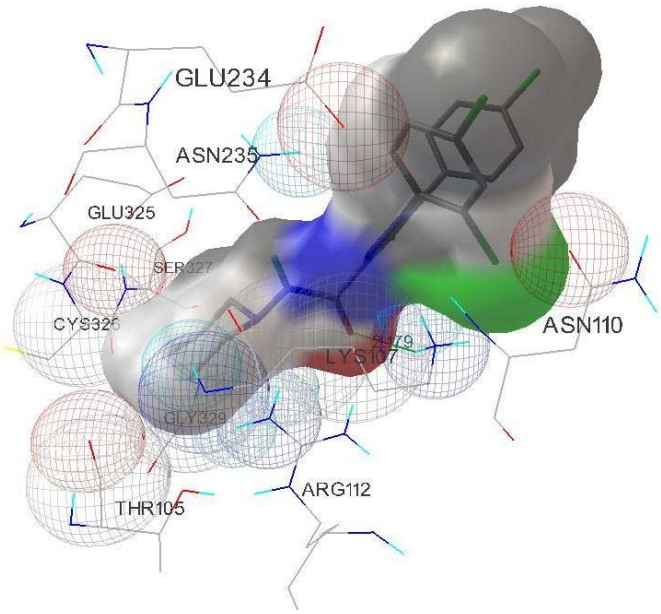

Figure 7
Docked Structure of the receptor (3LFM) and the ligand (Rimonabant)

Docking Orlistat into FTO:

Docking simulation of Orlistat into FTO produced seven clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -4.72 kcal/mol at 4th run has formed one hydrogen bond interaction at the residue THR328 with cluster RMSD 0.00 and reference RMSD 32.54. Hydrogen bond distance between the donor and acceptor was found to be 2.935 (as shown in Table 2). Docking interaction between the ligand and the macromolecule is shown in Figure 8.

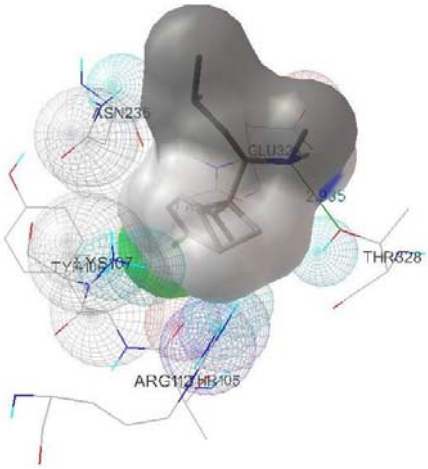

Figure 8
Docked Structure of the receptor (3LFM) and the ligand (Orlistat)

Docking Sibutramine into FTO:

Docking simulation of Sibutramine into FTO produced seven clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -4.92 kcal/mol at 1st run has formed one hydrogen bond interaction at the residue THR328 with cluster RMSD 0.00 and reference RMSD 32.48. Hydrogen bond distance between the donor and acceptor was found to be 2.704 (as shown in Table 2). Docking interaction between the ligand and the macromolecule is shown in Figure 9.

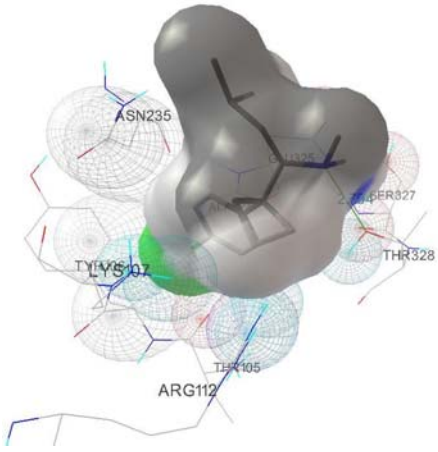

Figure 9
Docked Structure of the receptor (3LFM) and the ligand (Sibutramine)

Table 2
Molecular interactions of Curcumin and Anti-obesity drugs into FTO

Docked Molecule	No. of Hydrogen Bonds	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Length of hydrogen bond (Å)	Lowest Binding free energy (kcal /mol)	Cluster RMSD	Reference RMSD
Curcumin-FTO	3	Curcumin:1050:H12:	3LFM:A:GLU325:O	2.185	-6.57	0.00	38.15
		3LFM:A:GLN86:HN:	Curcumin:1050:O2	2.234			
		3LFM:A:LYS107:HN:	Curcumin:1050:O1	2.128			
Rimonabat-FTO	1	3LFM:A:LYS107:HZ3	Rimonabant:LIG1:O	2.179	-5.46	0.00	35.30
Orlistat-FTO	1	Orlistat:LIG1:N:	3LFM:A:THR328:OG1	2.935	-4.72	0.00	32.54
Sibutramine-FTO	1	Sibutramine:LIG1:N:	3LFM:A:THR328:OG1	2.704	-4.91	0.00	32.48

Drug Likeliness of ligand Molecules:

Molecules violating more than one of the Lipinski's rules may have problems with bioavailability. Lipinski's rule of five is calculated for the ligand molecule using Molinspiration tool. The molecular properties of Curcumin and antiobesity drugs were listed in Table 3. It was found that the ligand molecule (Curcumin) satisfies the 'rule-of-5' and could be a potent inhibitor. It was also found that antiobesity drugs such as Rimonabant and Orlistat violates one of the Lipinski rule (shown in table 3 - logP > 5) while Sibutramine satisfies the Lipinski rule. Drug likeliness score for the curcumin and antiobesity proteins are listed in Table 4.

Table 3
Molecular Properties of Curcumin

Property	Details	Value			
		Curcumin	Rimonabat	Orlistat	Sibutramine
logP	Octanol-water partition coefficient	2.303	5.217	9.014	4.999
TPSA	Polar surface area	93.066	50.162	81.708	3.238
Natoms	Number of nonhydrogen atoms	27	30	35	19
MW	Molecular weight	368.385	463.796	495.745	279.855
nON	Number of hydrogen-bond acceptors (O and N atoms)	6	5	6	1
nOHNH	Number of hydrogen-bond donors (OH and NH groups)	2	1	1	0
nviolations	Number of Rule of 5 violations	0	1	1	0
nrotb	Number of rotatable bonds	8	4	23	5
Volume	Molecular volume	332.182	382.577	525.129	283.132

Table 4
Drug-likeliness Score

Property	Score			
	Curcumin	Rimonabat	Orlistat	Sibutramine
GPCR ligand	-0.12	0.42	0.28	0.70
Ion channel modulator	-0.31	-0.71	-0.34	0.14
Kinase inhibitor	-0.42	-0.62	-0.26	-0.13
Nuclear receptor ligand	-0.01	-0.79	-0.54	-0.51

CONCLUSION

Obesity being one of the major health threat has increased dramatically worldwide and caused deadly health problems such as diabetes, heart disease and some forms of cancer. Curcumin with its anti angiogenic property, acts as an inhibitor against the fat mass and obesity protein and hence influences weight control in human body. Curcumin interacts with FTO protein at GLN 86, LYS 107 and GLU 325 forming three hydrogen bonds and has high binding affinity. These sites could be the best possible binding sites to inhibit the FTO protein. Comparative docking analysis of commonly used drugs for treatment of obesity also suggests that curcumin can be an

alternative source for obesity. Further, this work can be extended to experimental study on how curcumin inhibits obesity protein and hence reduces obesity in human.

ACKNOWLEDGEMENT

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