

MOLECULAR DOCKING STUDIES OF SOME OBESITY REDUCING DRUGS

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Abstract

Now a day's obesity is a major problem in worldwide, so many drugs are used to treating obesity or over weight. Rimonabant, a cannabinoid-1 receptor antagonist, Fenfluramine are withdrawn from the market. Obesity is not merely a cosmetic issue because it is causal factor in many serious diseases including dyslipidemia, hypertension, stroke, myocardial infarction, Type 2 diabetes and some cancers, high blood pressure (hypertension), cardiovascular diseases and stroke. α -phenoxy isobutyric acid and its derivative, we can see that Ala 376, Pro 246, Val 307 form hydrophobic interaction with all ligands, thus contributing to the stable inhibition of the protein. Similarly, LYS 265, Ser 428, Lys 434, Lys 438, Lys 336, Lys 458, Lys 457, Thr 461, GLN 345 Form Hydrogen bonding interaction with ligands that contribute to the stability of α -phenoxy isobutyric acid its derivatives.

Introduction

Obesity can be defined as a disease in which excess body fat has accumulated such that health may be adversely affected [1]. CPHA (Canadian Public Health Association) told Obesity can only occur when the energy value of food eaten exceeds energy expended. This situation is known as "a positive energy balance". In this situation the excess intake of energy inevitably appears as deposits of fat. Obesity is a worldwide communal health problem. The problem does not only affect developed countries, as there is now a significant increase in overweight and obesity throughout the developing world [2, 3]. The rapidly increasing level of obesity is likely to result in substantial health financial and social burdens across the world unless effective interventions are employed. For many obese subjects, attempts to adhere to a healthy diet and lifestyle not addressed this problem. In this case supplementation with pharmacotherapy or bariatric surgery will be required [4]. Obesity is a multifactorial disease characterized by an excessive weight to height proportion owing to enlarged fat deposition that is attributed to a higher calorie intake as compared to the energy expenditure [5]. Pancreatic lipase (PL) plays a key role in the efficient digestion of dietary fats by hydrolyzing them into mono – acylglycerol and fatty acids, thus minimizing the intestinal absorption of triglycerides [6]. This enzyme has been widely used for determination of the potential efficacy of natural products as anti-obesity agents [7].

A person is obese when they have a Body Mass Index (BMI) of 30 kg/m² or more. A person is considered 'overweight' when they have a BMI of 25 - 30 kg/m². BMI is calculated by dividing the person's weight in kg by the square of their height in meters (Kg /m²). E.g. Take your weight and convert it to kilograms (1stone = 14lbs & 2.2lb=1kg), Now take your height and convert it to meters (1 inch=0.025m) and multiply your height in meters by itself; then divide your weight in kilograms by this number. E.g. weight 76kg, height 1.70m BMI = 76/ (1.7x1.7) = 26.3 kg/m².

A BMI of 25 is regarded as the upper limit of normality, a BMI of 18-20 as the lower limit. A BMI between 25 and 30 is considered overweight. Unless an overweight person is also at extra risk for other life-threatening diseases, due to smoking habits, high serum cholesterol levels etc., this condition does not require urgent treatment. A BMI above 30 is considered obese. This condition enquires treatment quite urgently. Assessing obesity in children is more difficult than in adults. Application of the BMI to children is generally considered to be inappropriate [8]. The number of people in the population considered obese is increasing year-on-year [9]. In this project aimed to study the sum of molecular docking of reducing obesity drugs. The project involved α – phenoxy – isobutyric acid and its derivatives docked with 2PRG (PPAR gamma) Protein. The general objective of the present research programmed is to study the molecular docking of anti – obesity drug. Now a day's many

people are affected by obesity. Higher body weight is associated with an increased incidence of number of conditions including diabetes mellitus, cardiovascular disease and non alcoholic fatty liver disease and increased risk of disability. Obesity is associated with a modestly increased risk of all – cause mortality. Some weight loss drugs are associated with dangerous heart and lung side effects. Many of the weight loss drugs known as sympathomimetic amines can stimulate the heart and lead to high blood pressure and tachycardia (fast heart beat). These drugs may be associated with constipation, dry mouth, restlessness, withdrawal effects, or insomnia (difficulty falling asleep)

The recent years have witnessed an increased activity in the area of synthesis α – phenoxy isobutyric acid and derivatives. They are persistent free radicals that exhibit remarkable stability primarily due to the absence of dimerisation and disproportionation.

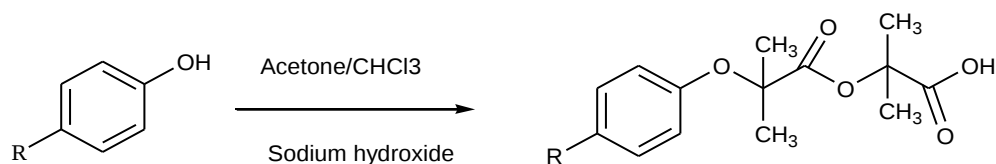
In the docking, protein-protein interaction, protein- ligand interaction, protein - molecular interaction are done. Mainly Hydrogen bonding interaction, hydro interaction, vander waals interaction, electrostatic interaction are carried out between protein and ligand. Here, docking studies the ligand interacts with active amino acid to exhibit binding energy and inhibition constant. The lower binding energy and inhibition constant with higher binding affinity contribute good obesity reducing drug.

Material And Methods

The ^1H NMR and ^{13}C NMR Spectra were measured on a Bruker Avance 400 (400) spectrometer using Tms as the internal standard. The Mass spectra were recorded on a JEOL JMS –D 300 Spectrometer operating at 70ev. The elemental analysis were measured on a (HERAEUS CHNO, Rapid) analyzer.

Preparation Of A- Phenoxy Isobutyric Acid Derivatives

Scheme 1



Phenol (21.2mmol), Acetone (20ml), Sodium hydroxide (215mmol) was mixed in an RB flask. The reaction mixture was stirred at room temperature for a period of 30 minutes. Subsequently a mixture chloroform (127mmol) and acetone 10ml were added in a time of 3 hrs. Due to exothermic nature, temperature of the reaction mixture was maintained below 55°C and continuous stirring for 48 hours below 55°C. After completion the reaction mixture was distilled under reduced pressure. The resulting suspension was quenched with 40ml water, and extracted with diethyl ether (3×10mL). After discarding organic layer, 2N hydrochloric acid was added to aqueous layer in order to adjusted the pH = 3 to 4. The solid thus obtained was filtered and the crude solid was separated by column chromatography using silica (200 – 300mesh), eluted with ethyl acetate and n-hexane.

Experimenatal

Step I – Building the Receptor:

This step the 3D structure of the receptor should be considered which can be downloaded from PDB; later the

available structure should be processed. This should include removal of the water molecules from the cavity, stabilizing the charges, filling the missing residues, generation the side chains etc according to the parameters available. The receptor should be biological active and stable state.

Step II – Identification of the Active Site:

After the receptor is built, the active site within the receptor should be identified. The receptor may have many active sites but the one of the interest should be selected.

Most of the water molecules and heteroatom if present should be removed.

Step III – Ligand Preparation:

Ligand 2D structures were drawn using ChemDraw Ultra 8.0 (ChemOffice 2002). Chem3D Ultra 8.0 was used to convert 2D structure into 3D and the energy minimised using semi-empirical AM1 method. Minimise energy to minimum RMS gradient of 0.100 was set in each iteration. All structures were saved as .pdb file format for input to ADT. All the ligand structures were then saved in PDBQT file format, to carry out docking in ADT.

Step IV -Target protein selection

The crystallographic three dimensional structure of selected target protein PPAR Gamma (PDB ID: 2PRG) was retrieved from the protein Data Bank (PDB) <http://www.pdb.org>. The apo protein molecule was selected for docking analysis. The probable binding sites of preferred target receptors were searched using Cast P server. It is important to keep the predicted ligand binding [10] site as small as possible without compromising accuracy for a range of application such as molecular docking, de nova drug design and structural identification and comparison of functional sites.

Step V- Docking Analysis

This is the last step, where the ligand is docked onto the receptor and the interactions are checked. The scoring function generates score depending on which the best fit ligand is selected. To investigate the potential binding mode of inhibitors, all the compounds were subjected to molecular docking using the AUTODOCK 1.5.6 docking program. Because of the critical roles of aberrant Signaling in obesity, (2PRG) receptor is an attractive oncology target for therapeutic intervention. To this end, the X-ray crystal structure of 2PRG in complex was downloaded from the protein data bank 2PRG (PPAR gamma) and was used for the docking study. Ligand 2D structures were drawn using Chems Draw Ultra 8.0. Chem3D Ultra 8.0 was used to convert 2D structure into 3D and the energy minimized using semi-empirical MM2 method. Minimize energy to minimum RMS gradient of 0.0375 was set in each iteration. All structures were saved as pdp file format for input to Auto Dock – Tools (ADT) version 1.5.6. All the ligand structures were then saved in PDBQT file format, for input into AUTODOCK version 4.2.

For the molecular docking study, protein structure was obtained from the Protein Data Bank structure PDB ID was 2PRG. For the protein structure, all hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT version 1.5.6. Further ADT was used to remove crystal water, added Gasteiger charges to each atom, and merged the non – polar hydrogen atoms to the protein structure. The distance between donor and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor – hydrogen – donor angle was not less than 120°. The structures were then saved in PDBQT file format, for input into AUTODOCK version 1.5.4. A grid box with dimension of 60 X 60 X 60 Å³ with 0.886 spacing and centered on 38.083, 46.914, and 17.164 on 2PRG protein using Auto Dock Tools. The centre of the box was set at crizotinib and grid energy calculations were carried out. For the AUTODOCK docking calculation, default parameters were used and 10 docked conformations were generated for each compound, the energy calculations were done using genetic algorithms. Docking of different ligands to protein was performed using AUTODOCK, same protocols used in as that of validation study. All docking were

taken into 2.5 million energy evaluations were performed for each of the test molecules. Docked ligand conformations were analyzed in terms of energy, hydrogen bonding, and hydrophobic interaction between ligand and receptor protein 2PRG and also the inhibition constants (mM) were calculated. Detailed analyses of the ligand – receptor interactions were carried out, and final coordinates of the ligand and receptor were saved as pdb files. Docked structures were visualized using Discovery Studio Visualized 2.5 (Accelrys Software Inc.). The free energy of binding (FEB) of all compounds were calculated.

Result And Discussion

In the study, oriented towards the design and development of effective drug against obesity and diabetes, we determined the best components that could serve as lead molecules for drug design. The docking results are calculated according to binding energy and inhibitor constant. The docking score of 2PRG (PPAR gamma) were mention in the table. 2D structure of all new ligands N1 and N8 were converted into energy minimized 3D Structures and then used for protein – ligand docking. The protein with synthesized ligands N1 and N8 exhibited and established bonds with amino acids in the receptor active pocket.

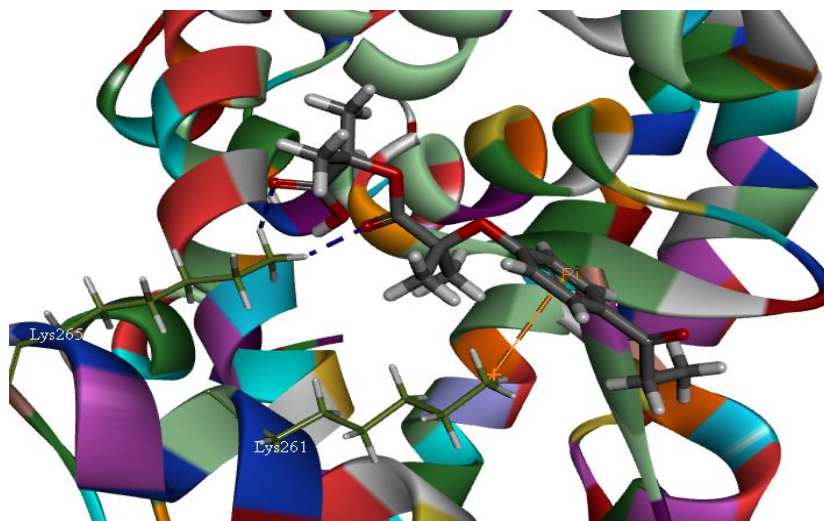


Fig 1 The molecular docking study of 2PRG (PPAR Gamma) protein complex with 2 – methyl – 2 – ((2 – methyl – 2 – (4 – propionylphenoxy)propanoyl)oxy) – propanoic acid. The blue color dashed line denotes H-bonds and red color dashed line denote electrostatic interaction.

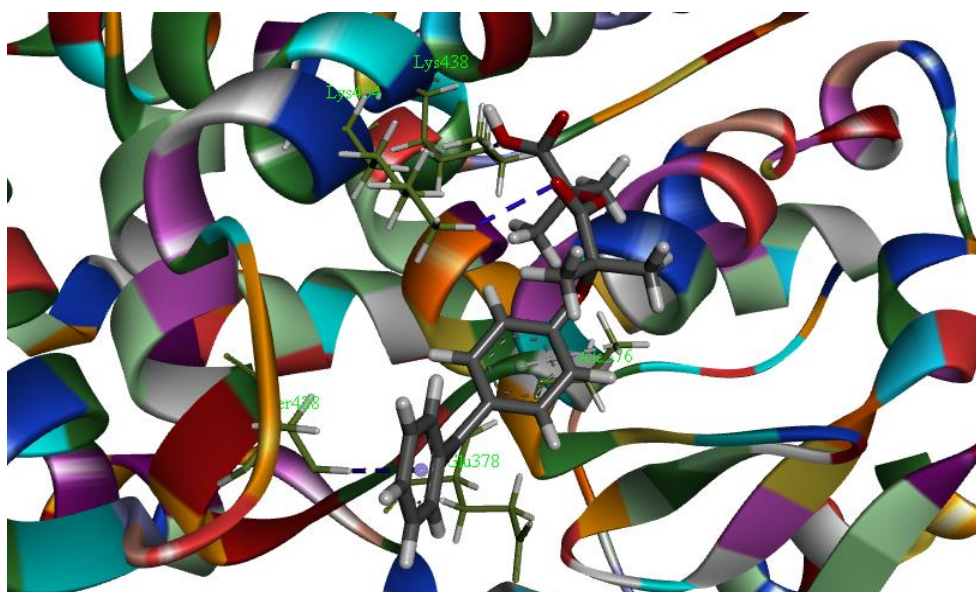


Fig 2 molecular docking study of 2PRG (PPAR Gamma) protein complex with 2 – ((2 – (4 – hexanoylphenoxy) – 2 – methylpropanoyl) oxy – 2 – methylpropanoic acid. The blue color dashed line denotes H-bonds.

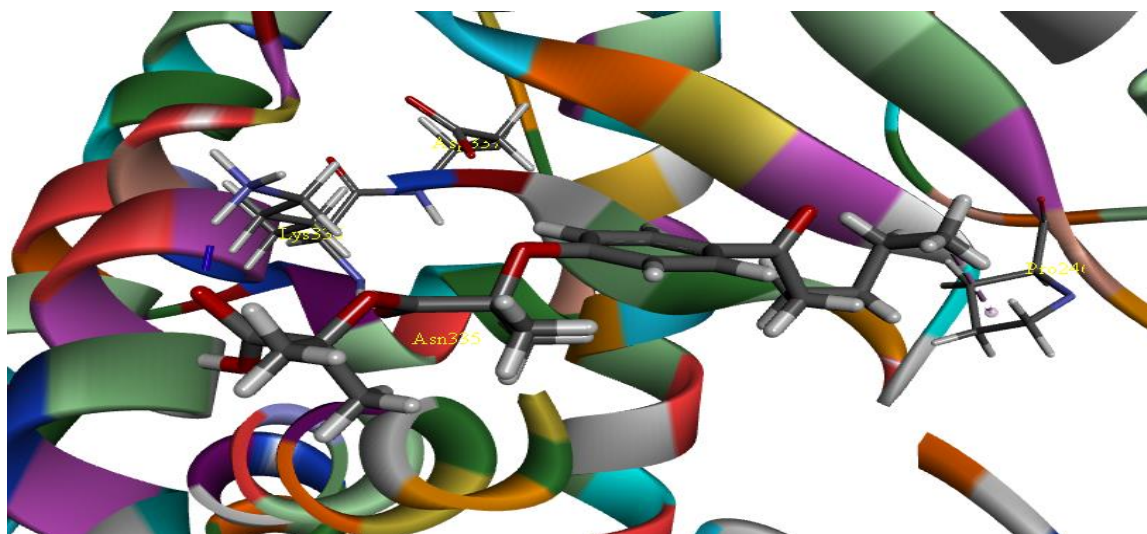


Fig 3 molecular docking study of 2PRG (PPAR Gamma) protein complex with 2 – ((2 – (4 – benzoylphenoxy) – 2 – methylpropanoyl)oxy) – 2 – methylpropanoic acid. The blue color dashed line denotes H-bonds.

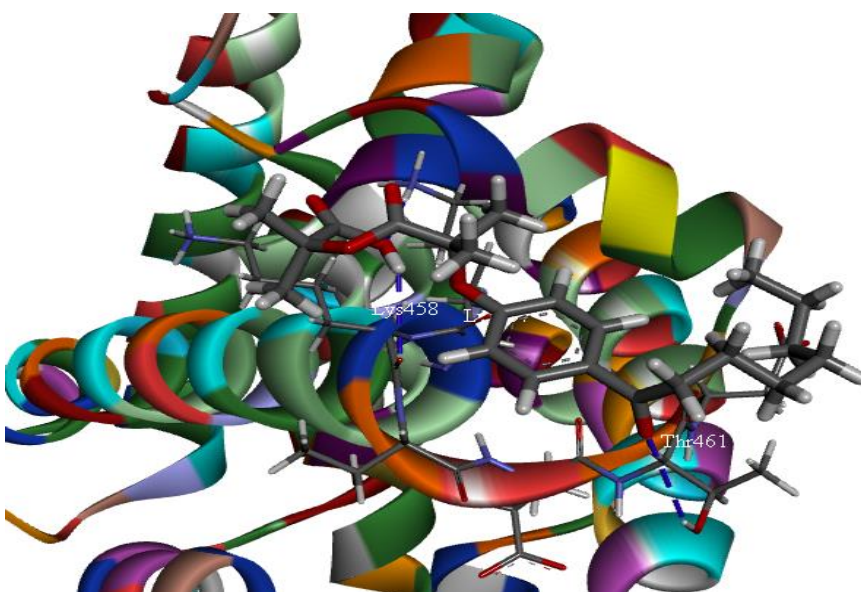


Fig 4 Molecular docking study of 2PRG (PPAR Gamma) protein complex with 2 – methy – 2 – ((2 – methyl – 2 – (4 – octanoylphenoxy)propanoyl)oxy)propanoic acid. The blue color dashed line denotes H-bonds.

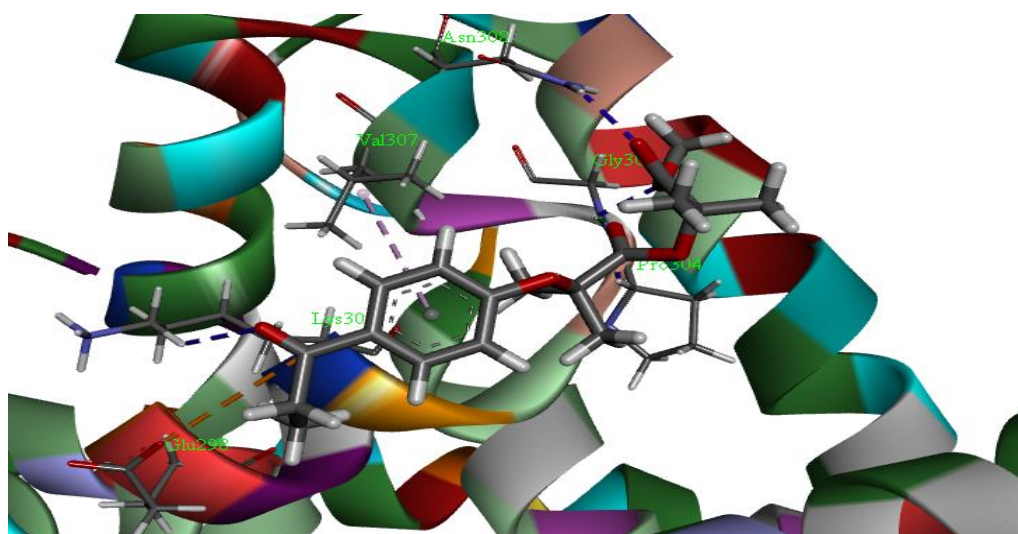


Fig 5 Molecular docking study of 2PRG (PPAR Gamma) protein complex with Methyl – 2 – methyl – 2 – ((2 – methyl – 2 – (4 – propionylphen) oxy)propanoyl)oxy – propionate . The blue color dashed line denotes H-bonds.

Figure Shows docked images of 2-methyl-2-((2-methyl-2-(4-propionylphenoxy) propanoyl) oxy) – propanoic acid and Fig 2 Shows docked images of 2-((2-(4-hexanoylphenoxy) –2-methylpropanoyl) oxy–2-methylpropanoic acid and fig 3 shows docked images of 2 – ((2 – (4-hexanoylphenoxy)–2-methylpropanoyl) oxy–2-methylpropanoic acid and Fig4 Shows docked images of 2-methyl-2-((2-methyl-2-(4-octanoylphenoxy) propanoyl) oxy) propanoic acid. And Fig5 shows docked images of Methyl-2-methyl-2-((2-methyl-2-(4-propionylphen) oxy)propanoyl)oxy–propaonate and Fig 6 shows docked images of 2-((2-(4-benzoylphenoxy– 2-methylpropanoyl)oxy) –methyl propaonate and Fig7 shows docked images of Isopropyl-2 –((2 –(4-hexanoylphenoxy)–2-methypropanoyl)oxy)–2-methylpropanoate and Fig8 shown docked images of Ethyl –2-methyl-2 –((2-methyl –2-(4 –octanoylphenoxy)propanoyl)oxy)propaonate.

Table 1: Shows Binding energy and Inhibition Constant against 2PRG Protein.

Ligand	Number of H-Bonds	Binding energy [kcal/mol]	Inhibitory Constant(mM)	Amino acids
N1	2	-3.64	19.57	Lys 265, Lys 261,
N2	3	-3.87	11.45	Ser 428, Lys 434, Lys 438
N3	1	-2.92	46.07	Lys 336
N4	4	-2.04	172.76	Ly458, Lys 457, Thr 426
N5	4	-3.14	27.07	Asn308, Gly305, Pro304, Lys301
N6	1	-2.72	55.0	Gln 345
N7	4	-2.69	58.0	Glu 378, Asn 378, Asn 335, Leu 237.
N8	1	-1.5	96.04	Asp 396.

0In docking studies revealed that all the synthesized molecules showed good binding energy towards the target protein ranging from –3.64 to –1.5 kcal/mol. The compound N1 shown 2 hydrogen bonding interaction with active site amino acids Lys 265, Lys 261 having binding energy –3.64 kcal/mol and inhibition constant is 19.57

mM. The other molecule such as N2 shown 3 hydrogen bonding interaction with active site amino acids Ser 428, Lys434, Lys438 having binding energy -3.87 kcal/mol and inhibition constant 11.45 mM, N3 shown 1 hydrogen bonding interaction with amino acid Lys336 having binding energy -2.92 kcal/mol and inhibition constant 46.07 mM, N4 and N5 shown 4 hydrogen bonding interaction with active site amino acid Lys 456, Lys457, Thr 461 and Asn 308, Gly 305, Pro 304, Lys 301 having binding energy -2.04 kcal/mol and -3.14 kcal/mol and inhibition constant 172.76 mM, 27.07 mM, structure N6 shown 1 hydrogen bonding interaction with active amino acids Gly 345 having binding energy -2.72 kcal/mol and inhibition constant 55.0 mM, structure N7 shown 4 hydrogen bonding interaction with amino acids Glu 378, Asn 378, Asn 335, Leu 237, electrostatic interaction with amino acids Arg 234, having binding energy -2.69 kcal/mol and inhibition constant 58.0 mM, Structure N8 shown 1 hydrogen bonding interaction with amino acids Asp 396 having binding energy -1.5 kcal/mol, inhibition constant 79.04 mM. Decreasing order of binding energy $N2 > N1 > N5 > N3 > N6 > N7 > N4 > N8$.

Lower binding energy of the ligands indicates better inhibition affinity and thus low K_i value. Comparing the molecular properties tabulated in table 1. It was observed that 2-((2-(4-hexanoylphenoxy)-2-methylpropanoyl) oxy-2-methylpropanoic acid has a low binding energy and low inhibition constant (K_i). From the docking analysis it is observed that 2-((2-(4-hexanoyl phenoxy)-2-methylpropanoyl) oxy-2-methylpropanoic acid could be used for the inhibition of obesity.

Conclusion

Various structurally distinct α -phenoxy isobutyric acid and its derivatives have been conveniently synthesized and characterized. The molecular docking study of α -phenoxy isobutyric acid and its derivatives shows different binding energy and inhibition constant. Figure shows the result of interaction. The blue dotted line represents the H-bond of the ligand with the amino acid of the protein represented in green. The hydrophobic interaction with the amino acids presented in white. For α -phenoxy isobutyric acid and its derivative, we can see that Ala 376, Pro 246, Val 307 form hydrophobic interaction with all ligands, thus contributing to the stable inhibition of the protein. Similarly, LYS 265, Ser 428, Lys 434, Lys 438, Lys336, Lys 458, Lys 457, Thr 461, GLN 345 form Hydrogen bonding interaction with ligands that contribute to the stability of α -phenoxy isobutyric acid and its derivatives.

Obesity being one of the major health threats has increased dramatically worldwide and caused deadly health property, act as an inhibitor against the fat mass and obesity protein and hence influences weight control in human body. α -phenoxy isobutyric acid and its derivatives interact with 2PRG (PPAR gamma) 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 2PRG (PPAR gamma) protein. Comparative docking analysis of commonly used drugs for treatment of obesity also suggests that α -phenoxy isobutyric acid and its derivatives can be an alternative source for obesity. Further, this work can be extended to experimental study on how α -phenoxy isobutyric acid and its derivatives inhibit obesity protein and hence reduce obesity in human.

Problems are traditionally tackled with single-objective, as well as with multi-objective approaches, to minimize the binding energy.

Out of these eight ligands 2-((2-(4-hexanoylphenoxy)-2-methylpropanoyl) oxy-2-methyl propanoic acid is high binding energy. Only two ligands were found to be most energetically stable on the basis of mol dock 1.5 and also found promising in protein-ligand interaction. So we may conclude that 2-((2-(4-hexanoylphenoxy)-2-methylpropanoyl) oxy-2-methylpropanoic acid ligand can work with 2 PRG (PPAR gamma) inhibitor and thus could be useful for controlling the obesity.

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