

Potential anti-obesity/inflammatory flavonoid-derived biomolecules against Obesity to prevent WAT differentiation by targeting a DNA-binding protein inhibitor, ID-1

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ABSTRACT

A concoction of unhealthy eating, inactivity, and the adverse effects of specific drugs brings on obesity. The primary cause of Obesity is the storage of too much energy and triglycerides in adipocytes, mainly white adipose tissue (WAT). In addition to modifying one's lifestyle, anti-obesity medicines are increasingly used as adjuvant therapy. Flavonoids are the primary class of compounds having significant biological impacts and health-improving properties. To find novel flavonoid compounds that fight obesity using computational drug design techniques. This work targets ID-1 protein to predict new flavonoid compounds against obesity. The study uses computational approaches to anticipate potential anti-obesity/inflammatory flavonoid compounds against obesity to prevent WAT differentiation by targeting ID-1, a DNA-binding protein inhibitor. Our investigation led to identifying the protein target inhibitor lead CID: 5280443, which was found to be a potent inhibitor of the receptor. According to the findings of this study, this bioactive molecule may be used as a lead for developing drugs that preferentially fight obesity without interfering with the functions of the human proteasome. The scientific community will benefit from these discoveries, which could aid in the creation of new medications that treat obesity more successfully.

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Introduction

Obesity is the accumulation of excessive fat with insufficient energy expenditure that is detrimental to health and may become a serious global health issue as the cases increase yearly (1,2). Obesity is a significant and dominant component of metabolic syndrome that leads to several life-threatening problems and is a crucial risk factor for diabetes, atherosclerosis, cardiovascular diseases, hypertension, and cancer (3-6). Obesity, with all related conditions, leads to a dramatic increase in morbidity and mortality. According to WHO 2016 report, the chronic medical disease obesity affects all age groups but is on the rise among children and young adults (13%) aged 18, and the prevalence rate in women (15%) is found to be higher than that of men (11%). Obesity is among Western countries' top ten health problems (7). The population in both developed and developing nations is affected by obesity (8,9). According to Genome-Wide Association Studies (GWAS), FTO genes on chromosome 16, salivary, and pancreatic amylase genes carry the highest risk for obesity. All these factors increase healthcare expenses that exceed 990\$ billion yearly (10).

Adipocytes act as reservoirs for the storage and

consumption of energy and can sense energy demands and secrete paracrine factors to regulate metabolic tissues. WAT increases in size and number in prolonged favourable energy balance conditions to balance the need for increased lipid storage. These cells will reach a threshold where they cannot handle extra pressure due to expansion limitations, which causes free fatty acids to be released into circulation. Reaching this threshold induces adipocyte stress, starting a provocative, inflammatory response. However, in obesity, WAT may become substantially dysfunctional and stimulate excessive fat accumulation in other tissues, contributing to the onset of cardiovascular disease, type 2 diabetes, and renal diseases.

Currently, there are five classes of pharmacotherapy for obesity. However, current pharmacological treatments have limited utility due to severe effects with minimal effectiveness (11). So, the aim of conducting this study is to find out natural compounds used to inhibit and overcome obesity and related diseases. Natural products have long been an important source of lead mixtures for medicinal purposes. Polyphenols are the most significant phytochemicals in fruits and vegetables (12). Their long-term intake benefits humans in overcoming obesity, CVD, type 2 diabetes, and cancer (13,14). Polyphenols are divided into

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two main groups, i.e., flavonoids and non-flavonoids. The natural bioactive compound flavonoid is derived from the Latin word "flavus," which means yellow and accounts for the colour, aroma, and taste of foods (15). They contain a specific C6-C3-C6 structure (two benzene rings joined by a chain of 3 carbon known as heterocyclic pyrone ring and mainly responsible for oxidation), which play a critical defensive role against oxidative damage phenomena. They are widely present in various fruits, vegetables, seeds, flowers, and nuts (16). Flavonoids have anti-obesity, antiviral, and antihypertensive properties, acting as anti-obesity drugs or foods with no side effects (17).

DNA-binding protein inhibitor ID-1, the target protein, is determined by the ID1 protein sequence on human chromosome 20 and consists of 155 amino acids (18). The main helix-loop-helix (HLH) domain enables the formation of heterodimers with other essential HLH record factors. The encoded protein has no DNA binding domain, inhibiting the DNA binding and transcriptional activity. It regulates the differentiation of progenitor cells and cellular growth (19,20), increasing the WAT number and promoting obesity. Nowadays, obesity has become the most common disease worldwide. Due to the severe side effects of current anti-obesity medications, there is significant interest in predicting novel compounds to control obesity (21). Using natural compounds such as flavonoids is effective and gaining importance. Numerous research is being conducted these days applying *in silico* methods to predict novel bioactive compounds as these techniques save time and cost (22-25). Therefore, to overcome obesity and related diseases, flavonoids are investigated against DNA-binding protein inhibitor ID-1 using the *in silico* computational techniques.

Materials and Methods

Target sequence retrieval 3D structure prediction

The 3D construction of targeted proteins was not present in RCSB PDB for ID1. Protein sequence data and related information for ID1 were retrieved from UniProt. UniProt is a comprehensive database of information on protein sequence, function, and variation that is expert-led and open to the public (26). I-TASSER predicted the 3D structure of the protein. A hierarchical approach to protein structure prediction and structure-based function annotation is Iterative Threading ASSEMBLY Refinement (I-TASSER) (27)

Protein Optimization and Minimization

Swiss PDB Viewer (28) and RAMPAGE optimized and minimized the protein crystal structure. RAMPAGE created a Ramachandran Plot that revealed no protein conflicts. The plot also shows which residues are in the favoured, allowed, and outlier zones (29).

Binding Site Prediction

Computed Atlas of Surface Topography of Proteins (CASTp) predicted protein target binding locations. CASTp 3.0 identifies and quantifies protein topography reliably and thoroughly (30).

Molecular Docking

Thirty-seven flavonoids from different sources were chosen. These compounds were docked with receptors

using AutoDock Vina (31), and their binding affinities and "protein-ligand interactions" were analyzed. PyMOL (32) was used to create complex receptor and ligand files, whereas BIOVIA Discovery Studio (33) was applied to find interactions in two dimensions.

Validation of molecular docking

In virtual screening, scoring and rating the docked ligands are crucial. The target protein's optimum scoring function should be chosen to increase success rates. This investigation established the highest-scoring functions before a more thorough screening of unidentified compounds using a decoy dataset of inactive and active ligands. A Database of Useful Decoys Enhanced was used to create the decoy dataset (34). The SMILES of the decoys were converted to .sdf format using the DataWarrior software (35). The target was docked with active decoys. The ROC curves are employed in this study to assess how well scoring functions work when attributing higher points to active ligands vs inactive ligands (36).

Toxicity analysis

To determine compounds' drug-likeness and harmful effects, the pkCSM (37) and QikProp were developed by Professor William L. Jorgensen (38). These algorithms are reported as essential and valuable tools for evaluating critical druglike descriptors like adsorption, distribution, metabolism, excretion, and toxicity (ADMET). These tools are also valuable for predicting lead likeliness, mutagenicity, and carcinogenicity.

Lead Identification

Docking score, RMSD values, protein-ligand interactions, lead likeness, and drug-likeness analysis, as well as toxicity analysis studies such as Molecular Weight (MW), Hydrogen Bond Donor (donorHB), Hydrogen Bond Acceptor (acptHB), partial coefficient logP, rings, Polar Surface Area (PSA), rotatable bonds, Blood-Brain Barrier, and Ames Toxicity, were used to identify the most active inhibitors. Compounds with the lowest binding affinity, lower RMSD values, the highest lead likeness, and the best interactions were chosen as possible anti-aggregation inhibitors.

Molecular dynamics simulation

Schrodinger's Desmond program was employed for 200 nanoseconds molecular mechanics studies (39). Before MD simulation, docking was necessary to anticipate a static structure of the material's bonding location in the protein's active site (40). Using Newton's conventional theory of motion, MD models often anticipate ligand-binding status in the biological environment and model atom movements over the duration (41,42). The ligand-receptor combination was refined by Maestro's Protein Preparation Wizard, which optimized, minimized, and, if necessary, filled in empty acids. The system was developed using the Network Builder application. The TIP3P fluid model is built on an orthorhombic box at 300 K, 1 atm, and the OPLS 2005 force field (43). Employing counter ions and 0.15 M sodium chloride, the simulations were reduced to approximate physiological circumstances. During simulations, models were relaxed, and after every 100 PS, trajectories were saved for analysis.

Results

The 3D structure of the receptor was predicted using I-TASSER. Figure 1 depicts the protein structure after optimization and minimization and the associated Ramachandran plot.

Thirty-seven compounds were chosen after sorting for docking. The ADMET analysis was performed with QikProp and pkCSM. The top 10 compounds are included in Table 1 based on ADMET and docking findings for the target protein.

ROC (receiver operating characteristic curves) depicts the relationship between a test's specificity and sensitivity. It is produced by graphing the percentage of genuine positives relative to the percentage of false positives relative to true negatives (Figure 2).

In Table 1, MW represents the Molecular weight of compounds with a recommended range of 130.0 – 725.0. donorHB is the expected number of hydrogen bonds the

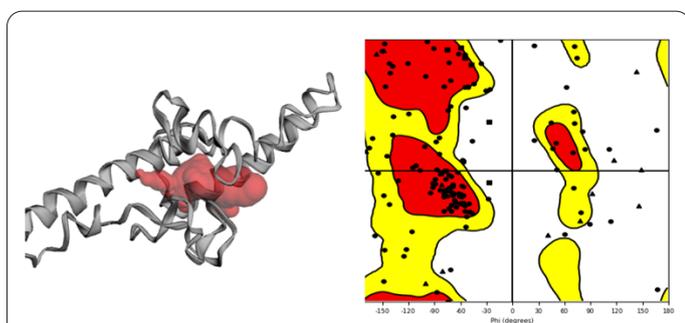


Figure 1. 3D structure of the protein (with predicted active site) and its Ramachandran plot shows distinct areas of protein structure.

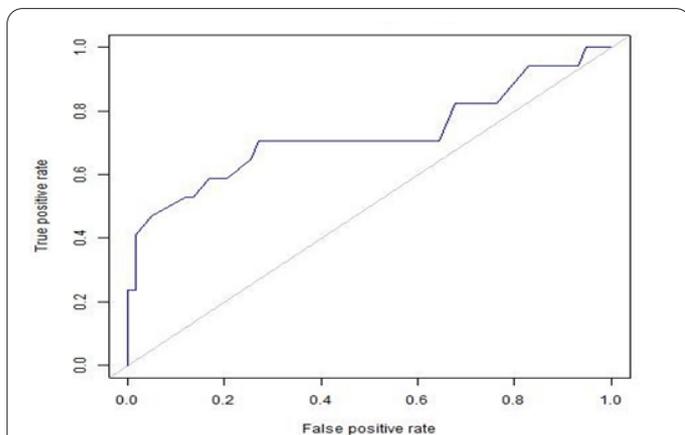


Figure 2. ROC curves of docking validation score.

solute would donate to water molecules. At the same time, acceptHB is the anticipated number of H-bonds accepted/absorbed from water molecules within an aqueous medium.

Following lead identification, one compound (CID: 5280443) was the most active. Figure 3 depicts the best one's 2D interactions. The properties of the best ones are shown in Table 1.

The protein of interest was simulated using MD simulations for 200 ns using the CID:5280443, after which the model path was analyzed. MD trajectory analysis calculated various parameters, including RMSD (Figure 4), RMSF (Figure 5), and protein-ligand interactions (Figure 6).

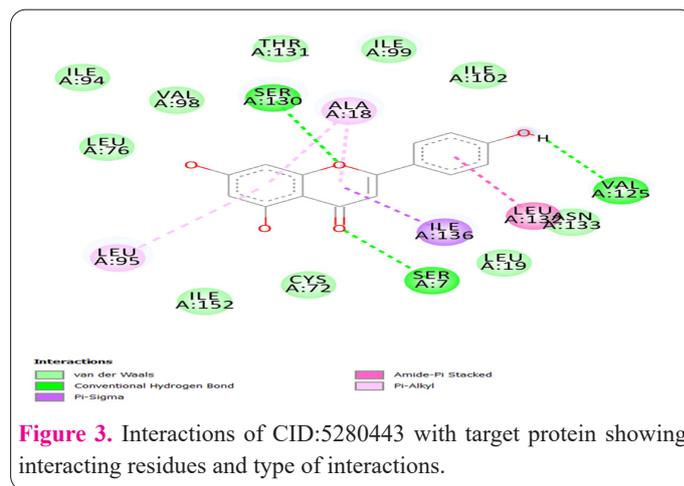


Figure 3. Interactions of CID:5280443 with target protein showing interacting residues and type of interactions.

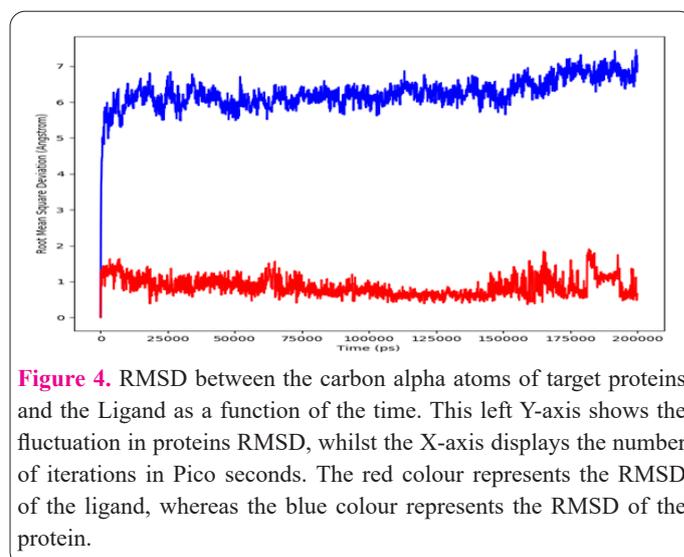


Figure 4. RMSD between the carbon alpha atoms of target proteins and the Ligand as a function of the time. This left Y-axis shows the fluctuation in proteins RMSD, whilst the X-axis displays the number of iterations in Pico seconds. The red colour represents the RMSD of the ligand, whereas the blue colour represents the RMSD of the protein.

Table 1. shows ADMET properties, binding affinity, and pharmacophore score of top compounds.

Molecule PubChem ID	MW	donorHB	acceptHB	QPlog Po/w	QPlog HERG	QPP Caco	QPlog BB	QPlog Khsa	Binding Affinity (Kcal/mol)
5280445	286.24	4	5.25	0.362	-5.035	20	-2.352	-0.354	-8.5
932	272.257	3	4.75	0.875	-4.632	50.276	-1.797	-0.218	-8.2
5280863	286.24	3	4.5	0.941	-5.022	45.023	-1.91	-0.205	-8.1
440735	288.256	3	4.5	1.036	-5.14	55.32	-1.843	-0.201	-8.1
5280343	302.24	3	5.25	1.236	-4.991	69.341	-1.83	-0.164	-8
5281654	316.267	2	4	1.792	-5.152	382.701	-0.923	-0.133	-8
1794427	354.313	2	4.75	1.975	-5.034	472.152	-0.918	-0.106	-7.9
5280443	270.241	2	3.75	1.694	-5.042	170.821	-1.314	-0.099	-8.6
5280961	270.241	2	4	1.549	-4.73	139.122	-1.311	-0.065	-8.6
5281708	254.242	2	3.75	1.624	-5.125	124.496	-1.411	-0.043	-8.2

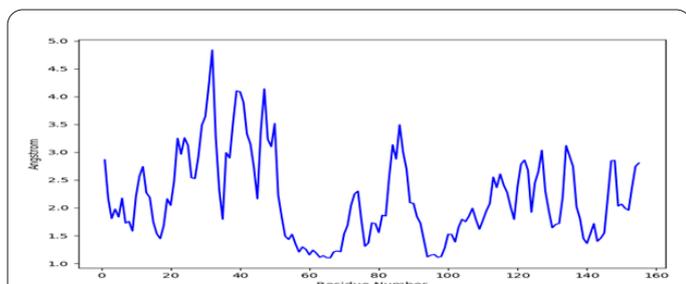


Figure 5. Residue-wise RMSF of protein complexed with Ligand.

Discussion

The Ramachandran plot (Figure 1) indicated that the 3D receptor 3D structure's overall quality was 98 percent, with highly preferred observations. All other residues are displayed as circles in the plot, triangles represent glycine, and prolines are depicted as squares. The orange areas were deemed as the "favoured" areas, the yellow spots as "allowed" areas, and the white regions represented "disallowed" sites.

Thirty-seven compounds were chosen after sorting for docking. The ADMET analysis was performed with QikProp and pkCSM. The top 10 compounds are included in Table 1 based on ADMET and docking findings for the target protein.

We utilized ROC curves (Figure 2) to see if a docking software can choose active ligands over inactive ligands (decoys) and if it can choose these active ligands from the highest % of a classified database. The area under the curve was 0.7253, and the enrichment factor top 1% is 13.88, which is acceptable.

Suggested values for donorHB and acptHB are non-integer averages within the 0.0 – 6.0 and 2.0 – 20.0 range, respectively. With –2.0 to 6.5, QPlogPo/w represents the expected octanol/water partition coefficient. QPlogHER, determine IC_{50} value for the inhibition of HERG K^+ channels. Concerns below the number 5. QPPCaco calculates the apparent permeability of Caco-2 cell in nm/sec. The gut-blood barrier can be conceptualized using Caco2 cells as a model. QikProp provides predictions for passive transport with criteria <25: poor, >500: great. QPlogBB parameter predicts blood-brain barrier permeability while QikProp filter orally delivered drugs with unsuitable ADMET properties. Furthermore, QPlogKhsa Predicts binding to human serum albumin and shows values that range from 1.5 – to 1.5. The compound CID: 5280443 was the most active. Figure 3 depicts the best one's 2D interactions.

The RMSD pattern (Figure 4) shows the alpha carbon atoms in ligand-bound proteins throughout the simulation period. The proteins in the compound reached steadiness at 20 ns, according to the RMSD plot. After then, throughout the simulation, RMSD values oscillate within 1.0 Angstrom, which is appropriate and acceptable. Once the Ligand reached equilibrium, it remained stable throughout the simulation. Occasionally, the RMSD values changed abruptly, rising or falling. The fact that the ligand RMSD remained constant for the duration of the simulation after equilibrium was attained suggests that a ligand mode flip caused this.

According to MD trajectory findings, the loop, N, and C terminal areas pertain to higher points. Negative amounts for RMSF use within residue imply high binding

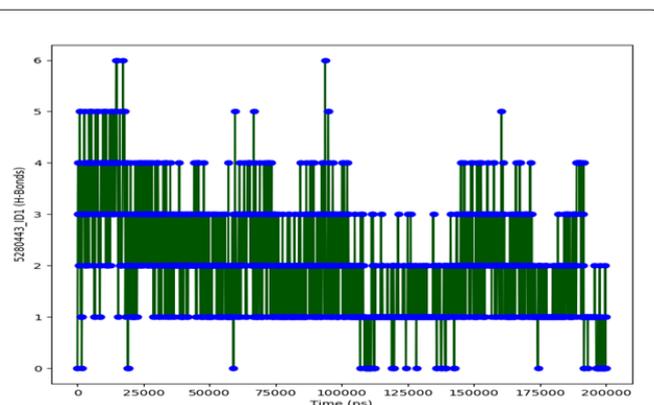


Figure 6. No. Of hydrogen links between protein and Ligand concerning time in Pico seconds.

stability. Because the secondary structural systems, chains, and strands are observed throughout the simulation. The RMSF (Figure 6) shows that hydrogen bonds are essential receptor-ligand connections determined with MD. We calculated the number of hydrogen links between the target protein and Ligand concerning time (Pico seconds). The highest number of H-Bonds was 6. We found a good number of H-Bonds throughout the time of the simulation.

Our research has focused on a few transdisciplinary tactics that can expedite and lower the cost of drug development. To select a lead medication to treat obesity, this study first set out to discover target proteins. We choose the flavonoids' chemical compounds that fight fat. The substances with the lowest binding energies to the target protein were isolated using docking analysis. The protein target inhibitor CID:5280443, which targets the receptor and works to reduce obesity, was chemically identified. This research has determined that this biochemical may be considered a lead in making drugs that preferentially combat obesity, lacking interference with the activities of the human proteasome. The scientific community will gain from these findings, which may help develop new drugs to treat obesity more effectively.

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Interest conflict

None

Consent for publications

The authors read and approved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

Authors' Contribution

All authors had equal roles in study design, work, statistical analysis and manuscript writing.

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Ethics approval and consent to participate

No human or animals were used in the present research.

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