



Targeting of Leptin Protein to Treat the Complications in Obesity, An Insilico Drug Designing

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Abstract:

The objective of the work is to analyze the diabetic protein Leptin, a major cause of several syndromes in the current era. The work involves the characterization of the Leptin protein and to establish its relation in Obesity. The protein is targeted for the treatment of obesity symptoms using several chemical ligands. The work included Drug Designing towards the obesity syndrome considering leptin as a receptor. The chemical compounds were collected from several data bases like ChEBI, NCBI etc. The collected chemicals were screened and the best compounds having good drug likeliness, non toxic and lowest ambiguity were finalized to dock with the selected receptor. The compounds were docked using the HEX software. The energy evaluation was performed and the best chemicals were selected to dock with the LEPTIN protein. Based on the docking results the best binding affinities were screened and the best drug was proposed.

Introduction:

Anti obesity diet and medications are the hot cakes in the market due to the ever increasing problem of obesity. The major reason for the outbreak of this problem is the sedentary life style of man. There is also a genetic factor that causes obesity in the normal individuals. The obese (ob) gene, also called as the leptin gene, the mutation of which results in severe hereditary obesity and diabetes in mice. It has recently been isolated through positional cloning. The nucleotide sequences of the human ob cDNA coding region were 83% identical to those of the mouse and rat ob cDNA coding regions. Analysis of the deduced amino acid sequences revealed that the human ob protein is a 166-amino acid polypeptide with a putative signal sequence and is 84 and 83% homologous to the mouse and rat ob proteins, respectively. Leptin is one of the genetic causes involved in Obesity and other diet related disorders. It is a hunger related gene that causes the feeling of hunger and maintains the diet. The improper balance in this gene expression or mutations would be one of

the causes of diabetes. LEPTIN (ACCESSION NO: NP_061764) NORMAL FUNCTION OF GENE:

Tools and database:

NCBI (URL: www.ncbi.nlm.nih.gov/)

The **National Center for Biotechnology Information (NCBI)** is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. It is a Public database that stores the data related to the genes, protein, their function, classification, related research etc. The NCBI houses a series of databases relevant to biotechnology and biomedicine. Major databases include GenBank for DNA sequences and PubMed.

GENECARDS (URL: <http://www.genecards.org/>)

Gene cards are a secondary database available free online with a direct access to all the human genes and the related annotation. Being a most user friendly database it has direct links to several other databases that can completely annotate the gene under consideration.

BLAST (URL: www.ncbi.nlm.nih.gov/BLAST/):

In bioinformatics, Basic Local Alignment Search Tool, or BLAST, is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold.

CLUSTAL-W (www.ebi.ac.uk/Tools/msa/clustalw)

Clustal is a widely used multiple sequence alignment computer program.^[3] This enables the multiple sequence alignment of a given set of proteins with a template sequence. It can perform the alignment both locally and globally. The tool is used for the identification of conserved regions on the given sequence.

SDSC BIOLOGY WORKBENCH (URL: <http://workbench.sdsc.edu/>)

The Biology Work Bench is a web-based toolkit for gene and protein characterization. The Work Bench allows biologists to search many popular protein and nucleic acid sequence databases. Database searching is integrated with access to a wide variety of analysis and modeling tools, all within a point and click interface that eliminates file format compatibility problems. The tools used from the workbench include Tex Shade and Box Shade for local and global alignment, Clustal Dist Matrix for the calculation of the evolutionary distances. The complete phylogenetic relationship among the sequences can be established using SDSC workbench.

SMART: (URL: <http://smart.embl-heidelberg.de/>)

SMART (a Simple Modular Architecture Research Tool) is used for the identification of the total domains present in the sequence along with their function and location. It uses internal databases of CDD that can be searched at the time of sequence SMART.

STRING (URL: <http://string-db.org/>):

STRING is a database of known and predicted protein interactions. The tool provides information related to the proteins that interact with the query protein and are related to it. It can provide an insight to the protein network.

PROTPARAM:

(URL: <http://web.expasy.org/protparam/>)

(PRIMARY STRUCTURE PREDICTION TOOL):

To analyze the protein physicochemical properties based upon its sequence composition, Prtparam tool can be used. This tool provides information like the molecular weight, hydrophobicity, instability index, half-life etc about the protein. All the values provided here are theoretical and are calculated based on the composition of the protein.

SOPMA:

(URL: http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html)

SOPMA is a tool for the Secondary Structure Prediction of a given protein sequence. It detects the total concentration of secondary structural confirmations in a protein like the alpha chains, sheets, loops etc. It provides the composition with

respect to each of the amino acid present in the sequence.

CPH (URL: <https://www.cph.org/>):

CPH is a tertiary structure prediction tool that is used for the identification of the PDB ID for the given protein sequence whose structure can be obtained from the RCSB Protein Data Bank. It is a protein homology modeling server. The template recognition is based on profile-profile alignment guided by secondary structure and exposure predictions.

Rasmol Visualization:

The PDB ID obtained in the CPH results is used to download the structure from the RCSB PDB database. The structure thus obtained is visualized in RASMOL software.

HEX- Docking Software:

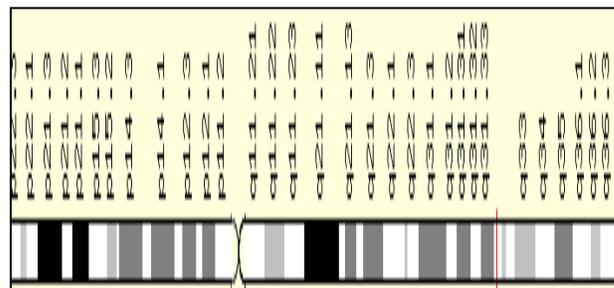
Hex is an interactive **protein docking** and **molecular superposition** program. It is used for both protein docking and protein ligand interaction. Based on the energy values obtained after docking the docking quality can be analyzed.

Results and discussion:

Sequence retrieval from Gene Cards

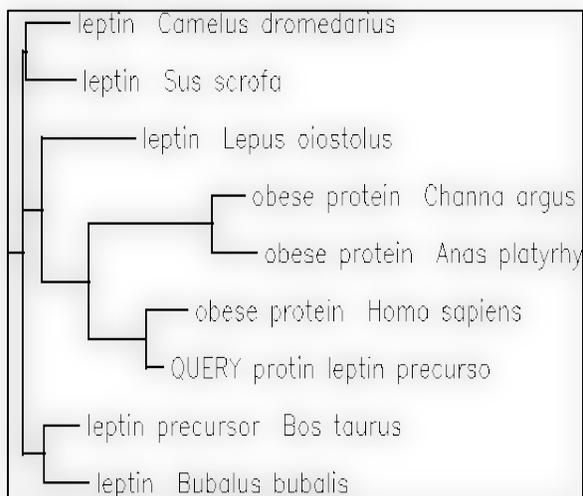
Leptin Protein is coded by LEP gene located on the chromosome 7 at the position q31.33 on the q arm of the chromosome. It starts from the position 127,881,331 bp from *pter* and ends at the position 127,897,682 bp. The size of the gene is found to be 16,352 bases.

Fig 1: The chromosomal location of the LEP gene as shown by gene cards:



Phylogenetic Analysis (USING CLUSTAL-W):

Fig 2: Rooted tree (generated by Phylip's Drawgram) showing the evolutionary relation among the selected organisms



From the above Phylogenetic tree it can be conformed that the query sequence selected is the obese protein of *homo sapiens*. All the other selected sequences share a common evolutionary branch to the query sequence.

Domain Analysis (USING SMART)

DOMAIN SEQUENCE:

SMART tool provides information regarding the domains present in the given sequence. The domain is shown below:

(PFAM)VPIQKVQDDTKLIKTIVTRINDISHTQS
VSSKQKVTGLDFIPGLHPILTLKMDQTLAVYQ
QILTSMPSRNVIQISNDLENLRDLLHVLAFSKSC
HLPWASGLETLDSLGGVLEASGYSTEVALSR
LQGS LQDMLWQLDLSPGC

Screening of the chemicals for the selection of the ligand:

Several chemicals are selected from different chemical databases and sources like ChEBI, PubChem, ChEMBL etc. All the chemicals that can act against the complications of obesity are selected and are screened at various stages like the screen for druglikeness using lipinsky's rule of five, screening for toxicity using mobile ADME server, screening for ambiguity using Dundee pro drg server. Only those chemicals that passed all the above screening stages are finalized to dock with the Leptin receptor.

The selected chemicals for docking are

1. Vortioxetine hydrobromide; 1-(2-((2,4-Dimethylphenyl)sulfanyl)phenyl)piperazine monohydrobromide; 960203-27-4

2. FLUOXETINE HYDROCHLORIDE; Prozac; Sarafem

3. Z)-3-(4-Bromophenyl)-N,N-dimethyl-3-(pyridin-3-yl)prop-2-en-1-amine dihydrochloride; 60525-15-7; 56775-88-3

4. Zimeldine hydrochloride; Normud; Zelmid

5. Zimelidine dihydrochloride; Normud; Zelmid

Docking of the selected ligands with the target receptor Using HEX

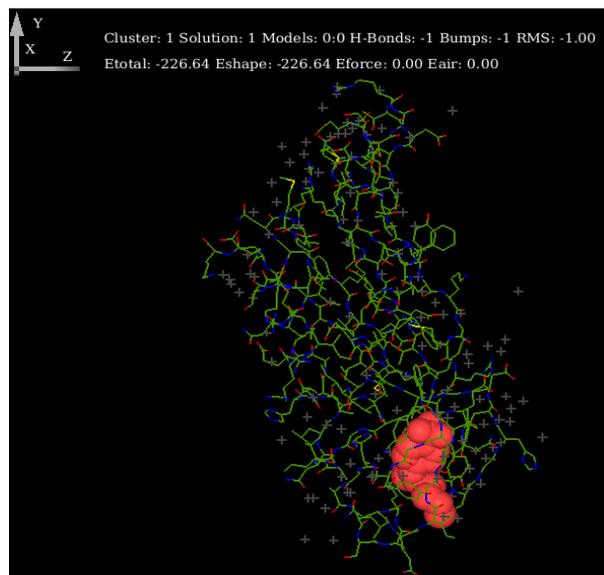
The above five ligands are used to dock with the selected receptor leptin. The docking results were evaluated and the docking energies were compared to propose the best ligand for docking with the leptin protein so as to act against the symptoms of diabetes.

Table 1: Docking of the five ligands with the selected receptor and evaluation of the docking energies

S.No	Chemical	Docking Energy
1	Vortioxetine Hydrobromide	-214.56
2	Fluoxetine Hydrochloride	-226.64
3	Z)-3-(4-Bromophenyl)-N,N-dimethyl-3-(pyridin-3-yl)prop-2-en-1-amine dihydrochloride	-198.22
4	Zimeldine hydrochloride	-198.09
5	Zimelidine dihydrochloride	-193.94

From the docking energies it can be inferred that the chemical 2, *Fluoxetine Hydrochloride*, is the best ligand to dock with the leptin receptor due to its lowest energy value that indicates highest stability.

Fig 3: Docking with *Fluoxetine Hydrochloride*



From the above docking results it can be inferred that the docking energy of the ligand – receptor pair is - 226.64 which the best pair among the screened ligands is. Thus, the compound can be selected as a best drug to fight against the obesity symptoms.

Conclusion:

Obesity is one of the common syndromes seen in the current era. The rate of epidemic is increasing day by day due to the sedentary life style. It is the high time to fight against the problem and eradicate the same. The current work aims to design a drug against the symptoms of Obesity using Leptin protein as the target receptor. Leptin is also known as obase protein due to its major role in diet and body mass. This is a protein that signals for hunger. Literature study reveals that the mutation at the protein and molecular level would lead to obesity complication. Thus, the current work involves targeting of the Leptin receptor with the suitable chemicals that has the property to block the effect of leptin mutations. The Chemicals were collected from the PubChem database and were subjected for intensive screening for the Druglikeness, Toxicity and Ambiguity. The best chemicals qualifying the required parameters were selected to dock with the same receptor. The energy evaluation was performed and the best chemicals were selected based on the lowest energy and highest

stability. The final chemical selected to target leptin was *Fluoxetine Hydrochloride* with the energy -226.64

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