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***Research Article***

# INTERACTION OF LEPTIN AND LEPTIN RECEPTORS WITH MAHANIMBINE: A DOCKING STUDY

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## ABSTRACT
Obesity is one of the silent killer worlds over. A variety of medicines in the name of obesity is available in the market. Leptin is an obesity hormone promised a lot recently. But scientists recently explored the fact that leptin is resistant in obese condition of the body and not respond to the receptors in the brain. Mahanimbine is recently in focus to reduce the extra fat due to its lipolytic properties. We proposed a model to explore the fact underlying the action of Mahanimbine. We proposed that Mahanimbine combine with leptin and induce leptin to promote the association of leptin to the brain receptors leads to the cascades of reaction culminating the loose of fats and obesity cure. The energy difference in the docking study by separate batch wise docking of leptin with receptor, leptin-receptor complex with Mahanimbine shows that Mahanimbine is more strongly associated with leptin in complex form rather than isolated form. The validity of the drug function of Mahanimbine is proved by ADMET study and study of five rules of Lipinski filter. A further study is needed to prove the fact.

***Key Words**: Mahanimbine, Leptin, ADMET, Lipinski Filter, Docking, Receptors, Ligands*

## INTRODUCTION
Leptin is a peptide hormone derived from adipocyte. It is circulated in the serum in the free and bound forms. The amount of energy stored in the adipose tissue can be deduced from the level of leptin in the serum. Leptin acts by binding to specific receptors in the hypothalamus to alter the expression of several neuropeptides that regulate neuroendocrine function and energy intake and expenditure. Thus, leptin plays an important role in the pathogenesis of obesity and eating disorders and is thought to mediate the neuroendocrine response to food deprivation. Research on pathophysiology of obesity revealed the role leptin in obesity (Zhang, *et al* 1994). It is observed that leptin level increase correspondingly with increase in fat mass (Lonnqvist *et al*, 1995, Considine *et al*, 1996). This observation is substantiated by the fact that leptin production is higher in the subcutaneous than in the visceral fat depots (Montague *et al*, 1997, Lonnqvist *et al*, 1997, Lonnqvist *et al*, 1995, Ronnemaa *et al*, 1995). It is also observed that prolonged fasting decrease the leptin level but overfeeding increase the level considerable (Tritos and Mantzoros, 1997, Flier, 1997, Kolaczynski, 1996). Another interesting observation is that, even after controlling of fat mass, women shows higher leptin level than men (Lonnqvist *et al*, 1995, Schrauwen *et al*, 1997, Hickey *et al*, 1997, Saad *et al*, 1997, Ostlund *et al*, 1996), this discrepancy may be due to the effect of androgen on leptin (Rosenbaum *et al*, 1996, Shimizu *et al*, 1997). Now days it is observed that Obesity increases the risk of diabetes, coronary artery disease, fatty liver, gall stones, sleep apnea, arthritis, and cancer and may shorten the lifespan (Ogden *et al*, 2007). Leptin induce various effects on human physiology and is regulated genetical factor sand environmental factors (Millington, 2011, Ribeiro, 2011, GonzÃlez, 2011, Bender, 2011, Deswal, 2011). Leptin receptor also known as LEP-R is a protein that in humans is encoded by the *LEPR* gene (Tartaglia *et al*, 1996, Winick *et al*, 1997) LEP-R functions as a receptor for the fat cell-specific hormone leptin. LEP-R has also been designated as CD295. The leptin hormone regulates adipose-tissue mass through hypothalamus effects on fullness and energy use, acts through the leptin receptor (LEP-R), a single-trans membrane-domain receptor of the cytokine receptor family(Tutino *et al*, 2011). Although leptin is a circulating signal that reduces appetite, obese individuals generally exhibit an unusually high circulating concentration of leptin (Considine *et al*, 1996). These people are said to be resistant to the effects of leptin, in much the same way that people with type 2

diabetes are resistant to the effects of insulin. The high sustained concentrations of leptin from the enlarged adipose stores result in leptin desensitization. The pathway of leptin control in obese people might be flawed at some point so the body does not adequately receive the satiety feeling subsequent to eating. A signal-to-noise ratio theory has been proposed to explain the phenomenon of leptin resistance (Pilon, 2011). Leptin interacts with six types of receptors (Ob-Ra–Ob-Rf, or LepRa-LepRf) that in turn are encoded by a single gene, LEPR. Ob-Rb is the only receptor isoform that can signal intracellular via the Jak-Stat and MAPK signal transduction pathways (Malendowicz *et al*, 2006), and is present in hypothalamic nuclei. It is unknown as to whether leptin can cross the blood-brain barrier to access receptor neurons, because the blood-brain barrier is attenuated in the area of the median eminence, close to where the NPY neurons of the accurate nucleus are. It is generally thought that leptin might enter the brain at the choroid plexus, where there is intense expression of a form of leptin receptor molecule that could act as a transport mechanism. Once leptin has bound to the Ob-Rb receptor, it activates the stat3, which is phosphorylated and travels to the nucleus to, it is presumed, effect changes in gene expression. One of the main effects on gene expression is the down-regulation of the expression of endocannabinoids, responsible for increasing appetite[]. There are other intracellular pathways activated by leptin, but less is known about how they function in this system. In response to leptin, receptor neurons have been shown to remodel themselves, changing the number and types of synapses that fire onto them. There is some recognition that leptin action is more decentralized than previously assumed. In addition to its endocrine action at a distance (from adipose tissue to brain), leptin also acts as a paracrine mediator (Margetic *et al*, 2002). Thus the survey of the available literature on leptin and its role in human body it is observed that the protein functions variedly and alter its effects as per the genetic constitution and environmental impact. To reach a conclusion, the available data is inadequate at present. In this study we performed a docking approach to find out the validity of Mahanimbine as a ligand to modify the leptin and some common leptin protein receptors separately so that to elucidate a modified pathway necessary to control many disease like obesity, diabetes type-II neuronal degenerative disease including Alzheimer, parkinsonism and many heart ailment. Antiobesity and antihyper**l**ipidemic activities of these extract are correlated with the carbazole alkaloids present in them, mahanimbine when given orally (30 mg/kg/day) also significantly lowered the body weight gain as well as plasma TC and TG levels. These findings demonstrate the excellent pharmacological potential of mahanimbine to prevent obesity **(**Rahul Birari, *et al* 2010**).**

## MATERIALS AND METHODS

### *Leptin and leptin receptor*

The PDB file of leptin and leptin molecule is manually extracted from PDB databank. The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. (See also crystallographic database). The data typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world (Berman, 2008). The search of the word leptin result only two items. Out of the two items the most probable one human obesity protein leptin (PDB ID: 1AX8) is downloaded in both pdb file format and FASTA sequence format (Zhang.1997). The receptor for leptin also extracted from the PDB databank as Tyrosine Phosphatase 1b C215a, S216a Mutant, Ptp1b-Inhibitor Complex and Cocaine and Amphetamine Regulated Transcript (1HY9-Signaling Protein)

### *Selection of leptin model*

The model was selected from structural biology knowledge database. We extracted model of 1xa8 pdb structure from the protein model portal. Out of the 76 model we selected a target protein P41159 having % seq id 99% secondary type, provided by MODBASE. The sequence lies between 24-167 amino acids. We selected P41159 protein as target. The model obtained against the query protein was 1xa8 and 1ax8A having seq id 99% each with sequence ranging from 24-167. Then the selected these two model and

analyzed structure variability. The model structure was then deduced with total RMSD and RMSD of final subset Table 1. This model 1x8a was used for the further docking purpose and receptor proper.

***Mahanimbine ligand and generation of its pdb structure***

Mahanimbine was retrieved from chemindustry powered by PubChem. The PubChem database is designed to provide and encourage access within the scientific community to the most up to date and comprehensive source of chemical structures of small organic molecules and their biological activities. PubChem houses both compound information from the scientific literature as well as screening and probe data from the Molecular Libraries Program. Citing the original generators of the data sets (see below for how to cite) will ensure that the corresponding scientists will get credit and allow readers to locate the source (Bolton *et al*, 2008). The pdb structure of the Mahanimbine was generated manually with the help of Marvin Sketch. We added suitable number of explicit hydrogen, clean in 3D structure and saved the generated 3D pdb structure in our departmental server.

***Validation of Mahanimbine***

Lipinski rule was used to validate drug like quality of Mahanimbine. Lipinski rule of 5 helps to distinguish between drug like and non-drug like molecules. If predicted high probability of success or failure due to drug likeness for molecules complying with 2 or more of the five rule (Table 2).

***Docking studies***

For the docking purpose we used Hex software version6.3 with CUDA GPU support. Hex is an interactive protein docking and molecular superposition program, written by Dave Ritchie. Hex understands protein and DNA structures in PDB format, and it can also read small-molecule SDF files (Ritchie and Venkatraman, 2010).

**RESULTS AND DISCUSSION**

The model of leptin retrieved from PDB databank is 1Ax8. This pdb structure was refined by information from the Structural Biology Knowledgebase-Models from the Protein Model Portal. The Models from the Protein Model Portal present 14 models for the 1xA8 leptin molecule. Out of the 14 models the best one with respect to percent sequence ID was retrieved as 1ax8A (Fig3 and Table 3). It has 99% sequence similarity with 1Ax8 3D structure. The further study was performed with this homologous 3D structure. The homologous 3D structure was validated by model selection criteria (Table 1).

**Table 1: Model selection criteria**

| | Total RMSD | RMSD of final subset |
|---|---|---|
| RMSD of model 2 to model1 | 5.1Aº//144 residues | 0.3Aº //132/144 residue |

The total RMSD and RMSD of final state reveals that the model is best homologous with 1Ax8 structure (Table1). The variation of the homologous model from the 1Ax8 was also find out (Fig1 A, B, C). The fig 3 clearly indicates the homologous 3D structure shows least variability form the 1Ax8. The MODEBASE was further used to assess the quality criteria of the model (Table 2). The quality criteria shows that out of 14 nine values are in the acceptable region (green colour). The PDB file of the leptin homologue was retrieved and used RASMOL to generate the 3D structure of the molecule with ball and stick display. Rasmol information option proved that the structure contain 2 chain, 130 groups, 1003 atoms and 1015 bonds, and it classified the structure as cytokinin and obesity protein leptin.

Mahanimbine (Fig1 and 2) is found to be one of the potent anti-obesity agents Mahanimbine. When given orally significantly lowered the body weight gain as well as plasma TC and TG levels. These findings demonstrate the excellent pharmacological potential of Mahanimbine to prevent obesity (Birari, 2010). Mahanimbine combines with leptin and induce the antiobesity effect is reported. Leptin is observed to be resistant in many obese people. It cannot stimulate the brain receptor to induce lipoid lowering effect of

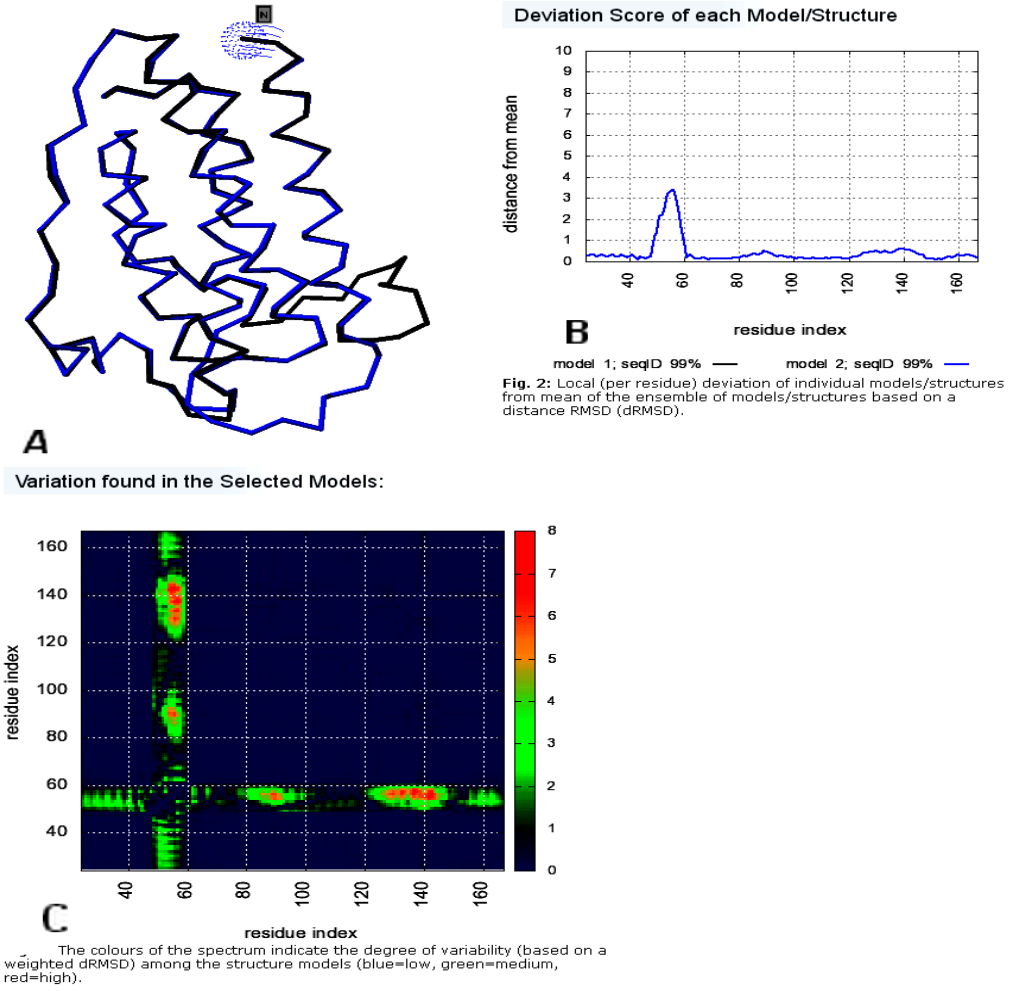


Fig. 2: Local (per residue) deviation of individual models/structures from mean of the ensemble of models/structures based on a distance RMSD (dRMSD).

The colours of the spectrum indicate the degree of variability (based on a weighted dRMSD) among the structure models (blue=low, green=medium, red=high).

**Figure 1: Model selection and its criteria deduced from structural biology knowledge database**

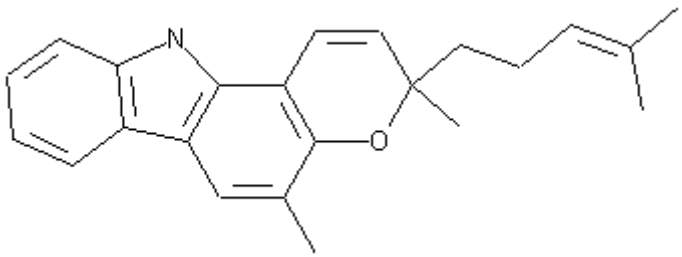


**Figure 2: Mahalnimbine structure generated from SMILE**

**Table 2: Quality criteria indicate whether the mode is considered Reliable (green) or unreliable (red)**

| Attributes | Explanation |
|---|---|
| Target | Region 24-167 |
| Protein Length | 167 |
| Template PDB Code | 1ax8A |
| Template region | Region3-146 |
| Sequence Identity | 99.00% |
| E-Value | 0 |
| GA | 3411.00 |
| MPQS | 2.00198 |
| z-DOPE | -1.58 |
| TSVMod Method | MSALL |
| TSVMod RMSD | 1.199 |
| TSVMod NO | 350.894 |
| Dataset | human_2010_A1 |
| ModPipe Version | SVN.r1188:1193 |

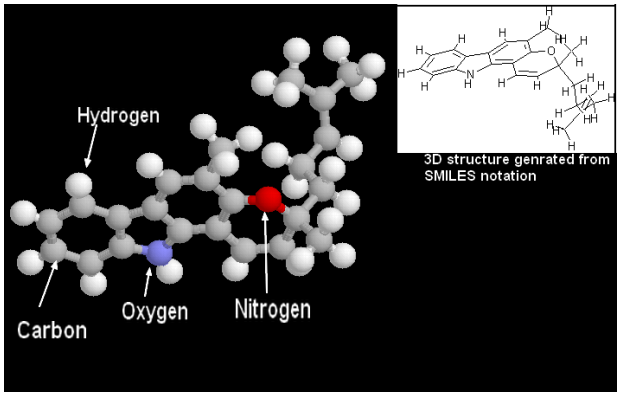


**Figure 3: PDB structure of Mahanimbine**

**Table 3: pdb structure validation of Mahanimbine with Marvin Sketch**

| Five rules of Lipinski filter | Lipinski range | Lipinski range for Mahanimbine | Remarks |
|---|---|---|---|
| Mol.wt. | 500 Dalton | 331.00 | Good |
| LogP | >5 | 1 | Good |
| Hydrogen bond donor | >5 | 2 | Good |
| Hydrogen bond acceptor | >10 | 6.444 | Good |
| Molar refractive index | 40-130 | 107-445 | Good |

**Table 4:** ADMET prediction of Mahanimbine

| Attributes | Values | Remarks |
|---|---|---|
| S+logP | 7.116 | Reliable |
| S+LogD | 7.116 | Reliable |
| MlogP | 4.259 | Reliable |
| M.Wt | 331.461 | Reliable |
| HBDH | 1.000 | Reliable |
| M_NO | 2.000 | Reliable |
| T_PSA | 25.020 | Reliable |
| RuleOoFive | 1.00 | Reliable |
| RuleOfFive code | <P | Reliable |

***S+LogP:*** *logP calculated using Simulations Plus' highly accurate internal model.* ***S+LogD:*** *logD at user-specified pH (default 7.4), based on S+logP.* ***MlogP:*** *Moriguchi estimation of logP.* ***HBDH:*** *Number of Hydrogen bond donor protons* ***M_NO:*** *Total number of Nitrogen and Oxygen atoms.* ***T_PSA:*** *Topological polar surface area in square angstroms* ***RuleOfFive:*** *Lipinski's Rule of Five* ***RuleOfFIve code:*** *Lipinski's Rule of Five codes: LP = logP; Hb = number of Hydrogen bond donor protons; Mw = molecular weight; NO = number of Nitrogen- and Oxygen-based Hydrogen bond acceptors. The presence of a code means that the corresponding Lipinski rule was violated.*

**Table 5: Docking result with Hex**

| Receptor | Ligand | Energy |
|---|---|---|
| *(R1)Tyrosine Phosphatase 1b C215a, S216a Mutant* | Mahanimbine | -422.51 |
| (R2)Ptp1b-Inhibitor Complex | Mahanimbine | -1753.6 |
| (R3)Cocaine And Amphetamine Regulated Transcript | Mahanimbine | -584.43 |
| Leptin | Mahanimbine | -146.60 |
| Leptin+R1 | Mahanimbine | -2121.02 |

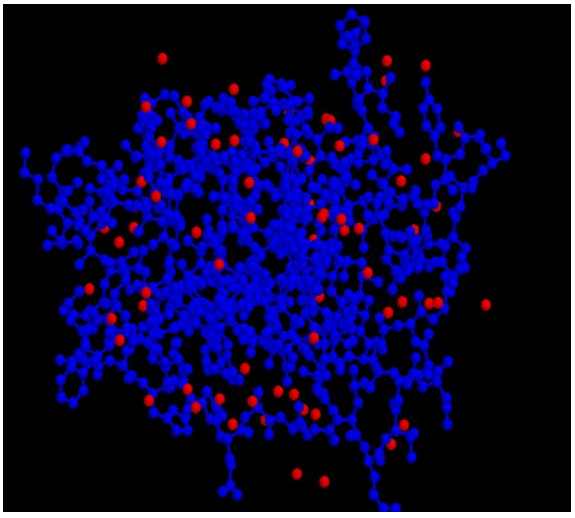

**Figure 4: PDB structure of Leptin**

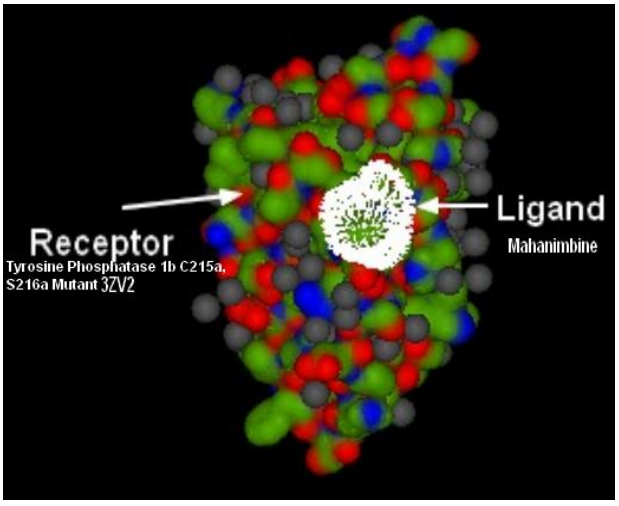


**figure 5: Receptor 1 docked with Mahanimbine**

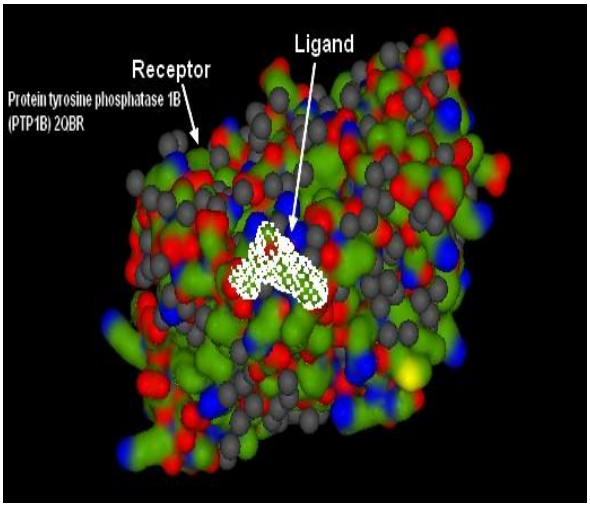


**Figure 6:Receptor2 docked with Mahanimbine**

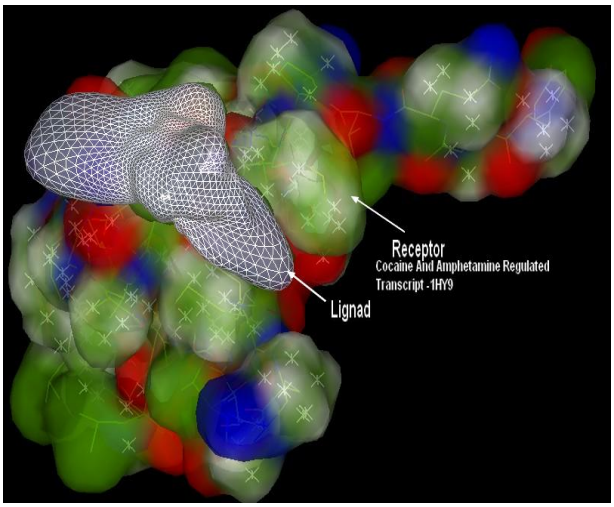


**Figure 7: Receptor3 docked with Mahanimbine**

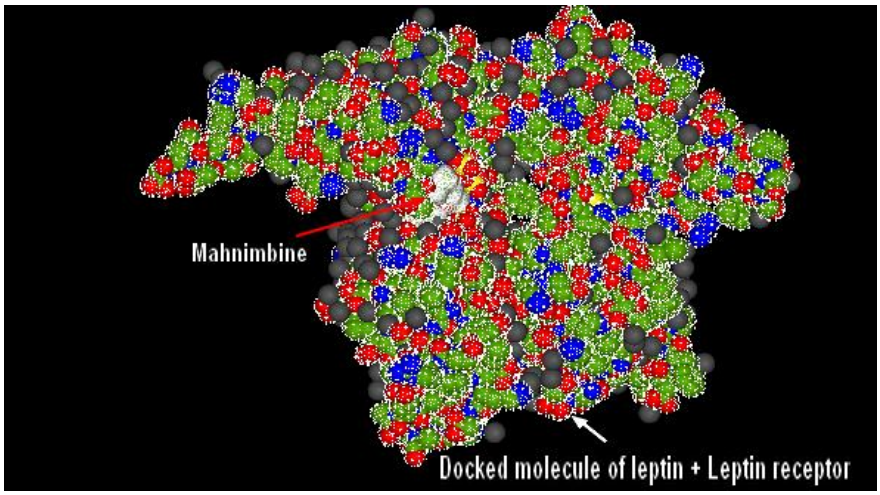


**Figure 8: Docking of Leptin-leptin Receptor Complex with Mahanimbine**

the hormone. In this contest we tried to remove the effect of the resistance of leptin by docking the Mahanimbine ligand to the leptin and study the energy level of both before and after the formation of leptin-receptor complex and leptin Mahanimbine combination. Drug properties of the Mahanimbine were assessed. It shoes that Mahanimbine can be safely used as drug. The AMET- Absorption, Distribution, Metabolism, Elimination, and Toxicity prediction (Table 4) shows that Mahanimbine is ideal drug. Thus the PDB structure validation Marvin Sketch (Table 3) and ADMET prediction strongly support that Mahanimbine can be used as drug for required target.

The docking study with Hex software shows that (Table 5, Fig 3,4,5,6 and 7) the energy is maximum and more than fourfold comparing to receptor 1 and 3, more than two fold comparing to receptor 2 and more than 20 folds comparing to leptin-Mahanimbine. This may indicates that Mahanimbine may be used as combination with leptin to treat obesity patient. The combination of leptin with Mahanimbine may be useful to remove the resistant effect of leptin in obese people. A further study regarding this effect is needed to substantiate these findings.

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