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Selection of Leucine as a Potential Antagonist from *In Silico* Analysis of μ-Opioid Receptor in the Treatment of Subjects with Herion and Opiate Addiction

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Abstract

The aim of this study was to analyze the antagonistic potential of leucine on μ-opioid receptor by molecular docking studies. Studies has shown that drug addiction has reached epidemic levels across the globe with approximately 247 million drug users worldwide. Heroin binds to and activates μ-opioid receptor thereby stimulating the release of neurotransmitter dopamine, causing reinforcement of drug taking behavior. The life-threatening side effects of the current μ-opioid receptor drugs (suboxone and naloxone) such as asthenia, insomnia, rhinitis, infections, pain, headache etc. Necessitate the discovery of novel potent and safe compounds as a therapeutic approach in the treatment of drug addiction. In view of this, computational tools were adopted to out-source for better antagonist for this drug-gable target. The leucine chemical compound was retrieved from pubchem data base and was screened for its inhibitory potential on μ-opioid receptor which was retrieved from protein data bank repository. Computational docking analysis was performed using PyRx AutoDock Vina option based on scoring functions and the target was validated so as to ensure that the right target and appropriate docking protocol was used for this study. Leucine was found to have a better binding affinity with the target (-4.7 kcal/mol) when compared with the co-crystallized molecule (-2.5 kcal/mol). Leucine has a Molecular Weight (MW) of 131.174 g/mol, number of hydrogen bond donor is 2, number of hydrogen bond acceptor is 3, LogP is -1.864 and number of rotatable bond is 3. Docking studies and ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties evaluation of leucin on μ-opioid showed that this ligand is a druggable molecule when docked well with the molecule. Therefore, Leucine plays an inhibitory role on μ-opioid receptor and thus should be implicated as a potential agent in drug addiction.

Keywords: μ-Opioid receptor; Suboxone and Naloxone; Leucine; PyRx AutoDock Vina

Introduction

Opioid addiction is a chronic mental illness that causes the addicted individuals to experience many relapses and remissions throughout their life, and they suffer from many uncomfortable symptoms, including tolerance development and withdrawal. Opioid drugs are widely used as analgesic to induce antinociception and to treat pain disorders. The over prescription of opioids for pain relief has led to a rapid surge in the non-medical use of prescribed opioids which has ensued as a major public health challenge over the past two decades with deaths by overdose and transition to heroin abuse rising at alarming rates. The increasing availability of low-cost synthetic opioids, such as non-pharmaceutical fentanyl's has played a significant role in fostering this endemic crisis [1].

The early 1970 s saw the game-changing discovery that opiate drugs bind to receptors in the brain and hijack a complex endogenous neuro modulatory system to exert their pharmacologic effects. The opioid system comprises three homologous G Protein-Coupled Receptors (GPCRs) known as mu-, kappa-opioid and delta receptors MORs, KORs and DORs respectively. This subtypes of the opioid receptor, share a common analgesic effect in brain, and each of them has their unique effects such as euphoria and respiratory depression for the MOR, dysphoria for the KOR, and anxiolysis for the DOR opioid receptor. Under physiological conditions, opioid receptors are stimulated by endogenous opioid peptides, forming a peptide family that includes β- endorphin, enkephalins and dynorphins. The MOR was the first discovered opioid receptor and its agonist action can trigger euphoria; therefore, it is essential for brain reward circuits which are highly dynamic, and it also plays an important role in goal-directed behavior such as drug-seeking behavior for pleasure [2]. In the brain, these receptors are highly concentrated in regions that are part of the pain and reward networks (ventral tegmental area, nucleus accumbens, and cortex) which accounts for its strong reinforcing effects, euphoria and the incentive properties of rewarding stimuli, playing an important role in goal-directed behavior respectively. In addition, MORs are located in brainstem regions that regulate breathing; there, agonists inhibit neuronal firing, which results in respiratory depression, which is the main cause of death and binds to the MOR at neighboring terminals, to send signals to the dopamine terminal, leading to a large increase in the release of dopamine by dopaminergic neurons in the tergmental area (by inhibiting γ-Aminobutyric Acid (GABA)). Dopamine increase in this circuit reinforces the behavior of taking the drug essentially teaching the brain to repeat the action. It is expected that reduction in the release of dopamine through MOR inhibition could beneficial in the treatment of opioid drug reinforcement [3].

Demonstrating that the MOR is the sole responsible receptor for both the therapeutic and the adverse actions of morphine. MOR is a key molecular target for development of novel therapy in the treatment of opioid addiction. Leucine belongs to the group of Branched Chain Amino Acids (BCAAs), (3 isoleucine (ILE), and Valine (VAL)) which participate directly and indirectly in a variety of important biochemical functions in the brain and has been examined as a treatment for several neurologic diseases. They can be predominantly found in animal foods: Eggs, dairy, meat (chicken and fish) and Plant foods: Fruits, vegetable and grains [4]. BCAAs plays an important role in brain function by influencing brain protein synthesis and production of energy and additionally, may influence synthesis of different

neurotransmitters, that is, serotonin, dopamine, norepinephrine, and so forth, directly or indirectly. Administration of competing neutral amino acid (for example, leucine) increases BCAAs plasma concentration and brain absorption of BCAAs. This ultimately, leads to decrease in the rate of conversion to Dihydroxyphenylalanine (DOPA) to Dopamine and synthesis of other related neurotransmitters [5]. This study aims at utilizing computational approach to predict the interaction between the ligand (Leucine) and the receptor (Mu-receptor). Lipinski rule of five on ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) properties was used to evaluate Leucine ligand was found to fulfill the rule of five on ADMET properties [6].

Materials and Methods

Ligand selection and preparation

The chemical structures of Leucine was obtained from PubChem compound database. The MOL SDF format of this ligands was converted to PDBQT file using PyRx tool to generate atomic coordinates and energy was minimized by optimization using the optimization algorithm at force field set at uff (required) on PyRx.

Accession and preparation of the target protein

The protein mu-receptor was prepared by retrieving the three dimension crystal structure of mu-receptor in complex with a co-crystallized ligand (PDB: 6DDE) from RCSB PDB. The protein was subsequently cleaned by removing the bound complex molecule, the non-essential water molecules and all the heteroatoms using Pymol tool. The co-crystallized ligand (PRD_002308) was extracted (not removed) from the active site so as to reveal the grid coordinate around the binding pocket when viewed on Pymol [7].

Molecular docking using PyRx

After the preparation of the receptor and ligands, molecular docking analysis was performed by PyRx, Auto dock vina option based on scoring functions. For our analysis we used the PyRx, Auto Dock Vina exhaustive search docking function.

After the minimization process, the grid box resolution was centered at 1.878, 15.7749, -48.3753 along the x, y and z axes respectively at grid dimension of 25 × 25 × 25 Å to define the binding site. The co-crystallized ligand which serves as the standard was first docked within the binding site of MOR and the resulting interaction was compared with that of lecuine into the similar active sites using the same grid box dimension (Tables 1-3 and Figures 1a and 1b) [8].

Results

In the present study, Leucine was docked into the binding pocket of MOR for its (antagonistic) properties. Leucine was discovered as the lead compound with the energy of -4.7 kcal/mol when compared with the co-crystalized ligand.

The drug-likeness of Leucine was assessed by subjecting it to the Lipinski's rule of five, afterwards the lead compound, Leucine violated none of the rules, and this describes its bioavailability and binding potential (Table 4).

Ligand	Binding Affinity (kcal/mol)	RMSD/UD	RMSD/LD
Buprenorphine	-4.9	0	0
Leucine	-4.7	0	0
Co-crystalized Ligand	-2.5	0	0

Table 1: Docking scores and RMSD values of ligands.

Molecular properties	Lipinski's rule of five	Leucine's drug-like properties
Molecular Mass g/Mol	<500	131.174
Hydrogen bond Acceptor	<10	3
Hydrogen bond donor	<5	2
LogP	<5	1.864
No of rotatable bond	<5	3

Table 2: Lipinski's drug-like properties of Leucine: The rule describes drug candidate's pharmacokinetics in the human body which also including their absorption, distribution, metabolism, and excretion.

S/N	Name	Category	Type
1	N:UNK1:H-A:ASP147:OD2	Hydrogen Bond	Conventional Hydrogen Bond
2	N:UNK1:C-A:ILE296	Hydrophobic	Alkyl
3	N:UNK1:C-A:ILE322	Hydrophobic	Alkyl
4	N:UNK1:C-A:ILE296	Hydrophobic	Alkyl

5	N:UNK1:C-A:ILE322	Hydrophobic	Alkyl
6	A:TRP293-N:UNK1:C	Hydrophobic	Pi-Alkyl
7	A:TYR326-N:UNK1:C	Hydrophobic	Pi-Alkyl

Table 3: Interaction table showing the various chemical interactions of Leucine within the binding pocket (Viewed on Discovery studio Visualizer).

S/N	Name	Category	Type
1	N: UNK1: C-A:ASP147:O	Hydrogen bond	Carbon hydrogen bond
2	A: HIS54: NE2-N: UNK1	Electrostatic	Pi-Cation
3	A: ASP147: OD2-N: UNK1	Electrostatic	Pi-Anion
4	N: UNK1:C-A: TYR148	Hydrophobic	Pi-Sigma
5	N: UNK1:C-A:TRP318	Hydrophobic	Pi-Sigma
6	A: HIS54-N:UNK1	Hydrophobic	Pi-Pi-T-shaped
7	A: HIS297-N:UNK1	Hydrophobic	Pi-Pi-T-shaped
8	A: MET151-N:UNK1	Hydrophobic	Alkyl
9	N: UNK1: C-A: ILE322	Hydrophobic	Alkyl
10	N: UNK1-A: ILE296	Hydrophobic	Alkyl
11	N: UNK1-A:ILE322	Hydrophobic	Alkyl
12	N: UNK1:C-A:MET151	Hydrophobic	Alkyl
13	A:TRP293-N:UNK1:C	Hydrophobic	Pi-Alkyl
14	A:TYR326-N:UNK1	Hydrophobic	Pi-Alkyl
15	N:UNK1-A:MET151	Hydrophobic	Pi-Alkyl
16	N:UNK1-A:ILE296	Hydrophobic	Pi-Alkyl
17	N:UNK1-A:VAL300	Hydrophobic	Pi-Alkyl
18	N:UNK1-A:ILE144	Hydrophobic	Pi-Alkyl

Table 4: Interaction table showing the chemical interactions of the co-crystalized ligand within the binding pocket (Viewed on Discovery studio Visualizer).

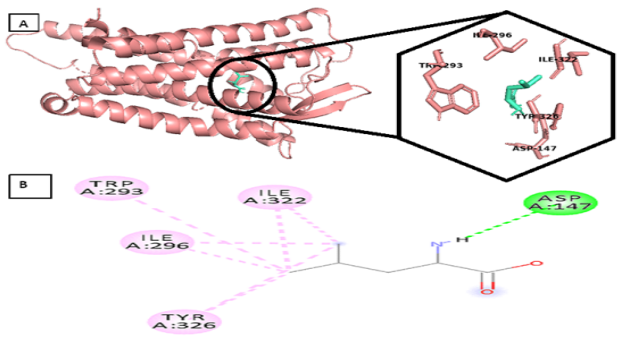

Figure 1: a) Pose view of leucine at optimum binding ; b) 2D interactions of the leucine within the binding pocket.
Note: Conventional hydrogen bond Pi-Alkyl Alkyl.

The high binding energy (-4.7 Kcal/Mol) attributed to Leucine when compared to the co crystalized ligand (-2.5 Kcal/Mol) in this regard is believed to be as a result of its chemical interaction at the receptor active site, which includes one conventional hydrogen bond involving A-147 residues; six hydrophobic interaction involving A-326, A-293 , A-296 and A-322 residues [9-11].

While that of the co-crystalized ligand which serves as standard presents with the following chemical interaction at the binding pocket.

One carbon hydrogen bond involving A-147; two electrostatic interaction involving A-54 and A-147 residue; and fifteen hydrophobic interactions (Figures 2a , 2b and 3) [12].

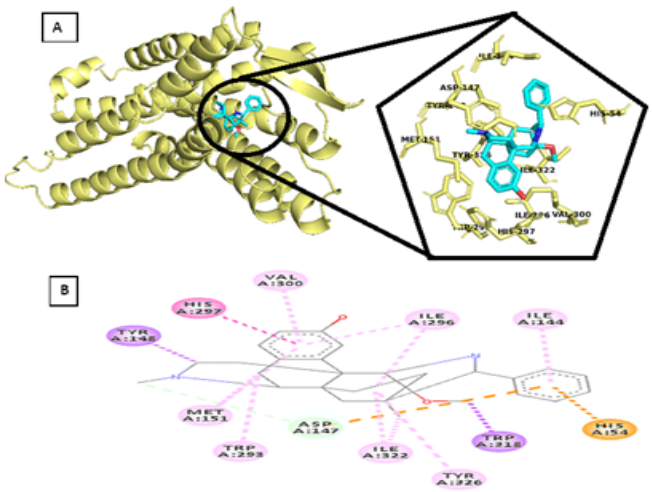

Figure 2: a) Pose view of Co-crystakized ligand at optimum binding; b) 2 D interactions of the co-crystalized ligand within the binding pocket.

Note: Carbon Hydrogen bond Pi- cation Pi-anion Pi-Sigma Pi-Pi T-shaped Alkyl Pi-alkyl.

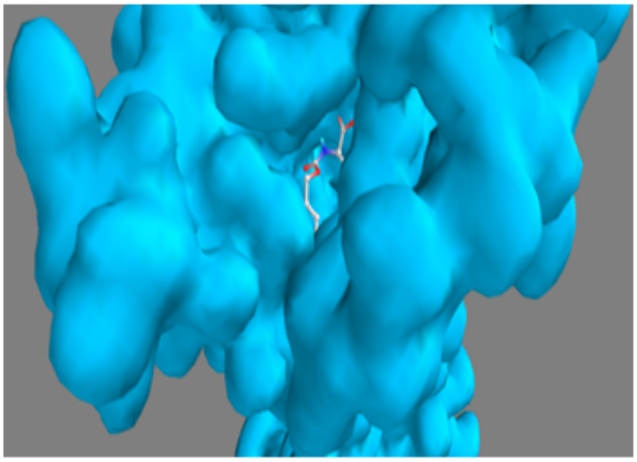

Figure 3: Leucine within the binding pocket.

Discussion

Hydrogen (H)-bonds potentiates diverse cellular cellular fucntions by facilitating molecular interactions. In order words, hydrogen bonds are considered to be facilitators of protein-ligand binding. Previous studies have shown that synergistics receptor-ligand H-bond pairing potentiate high-affinity binding which corresponds to an increase in binding affinity.

It is obvious that the higher binding affinity of leucine to the binding pocket of the MOR when compared to that of the co-crystalized ligand is attributed to the presence of "strong" conventional hydrogen bond present in Leucine when compared to the standard which has the "weak" carbon hydrogen bond [13].

We validated the accuracy of our docking protocol by redocking the c-crytslaized ligand back into the binding pocket of the MOR. As stated, the redocked pose overlapped almost totallly with the experiemental orientation, indicating that Auto dock vina on PyRx re-docked the co crystallized ligand, with a very high accuracy, back into the binding pocket of the MOR. This reveals that our docking methodology was reliable and the docking scores obtained are correct (Figure 4) [14].

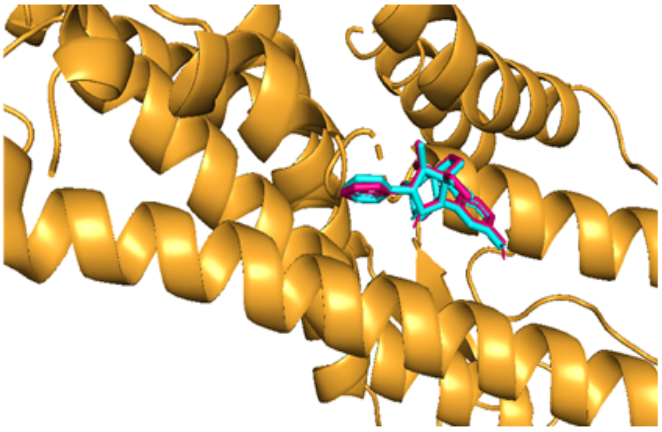

Figure 4: Validation of docking: Comparability of the re-docked binding mode of co-crystalized ligand within MOR binding pocket. A snapshot from PyRx.

Binding site prediction and binding mode analysis

Based on the MetaPocket 2.0 server, we were able to identify three potential binding sites capable of accommodating the ligands with varying binding affinities. This suggests that leucine binds to mu-receptor with varied binding affinities based on its binding modes or orientation of binding with respect to the respective binding sites. The three sites with their respective amino acids residues are located in the monomer (Figures 5-7) [15].

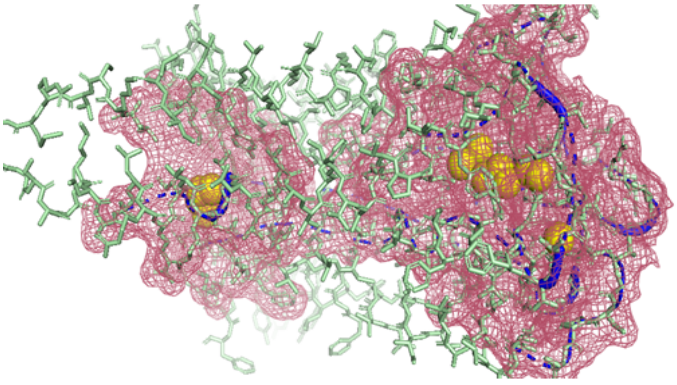

Figure 5: The result of residue mapping of mu-receptor. Image generated from PyMOL 1.2. PDBID: 6DDE. Ligand binding sites are illustracted in yellow ball, potential binding atoms are in blue cartoon, functional residues are in red mesh, other parts of the protein are in green sticks.

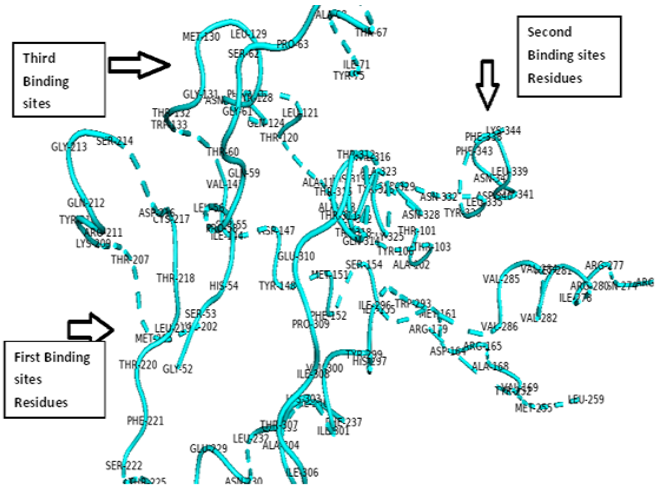

Figure 6: The functional amino acids residues for the three predicted binding sites

Leucine associate with the highest binding affinity with amino acids predicted in binding pocket 1 such as MET151, ILE296, ILE322, TRP293, ASP147 and TRP326 a lower binding affinity with binding pocket 2. This reveals that the activity of mu-receptor is better regulated at the predicted binding pocket 1 and not 2.

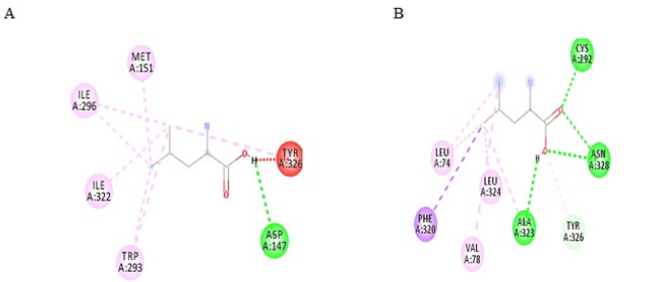

Figure 7: Predicted binding sites by MetaPocket 2.0 sever a) Pocket 1; Binding energy: -4.7Kcal/mol Predicted Binding sites: First; b) Pocket 2, Binding energy: - 4.5 Kcal/mol Predicted Binding sites: Second. Note: Conventional Hydrogen bond, Unfavorable donor-donor Pi- alkyl, Pi-Sigma, Carbon hydrogen bond, Alkyl.

Conclusion

Docking studies and ADMET evaluation of Leucine with MOR showed that this ligand is a drug-gable molecule, which docks well with MOR. Therefore, Leucine molecule plays an important role in inhibiting MOR and thus should be implicated as a potential agent in substance abuse disorder. So it is concluded that Molecular docking approach can be used in various steps of the drug design and identification for infectious diseases and cancer. It can be suggested to identify a lead molecule against a protein target or in contrary it can be employed to identify protein target against a query ligand.

Conflicts of Interest

None

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