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Molecular Docking of Apomorphine with Mu-Opioid Receptor: Understanding the Activity of a Novel Psychoactive Substance from *Nymphaea caerulea* (Blue Water Lily)

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Abstract: *Nymphaea caerulea*, commonly called the blue water lily, is studied to contain an alkaloid called Apomorphine, which has possible psychoactive activity. This study aims to correlate this activity by computationally studying its interaction at the molecular level. This study employs the use of molecular docking tools to study, analyze, and visualize the interaction of Apomorphine with the mu-opioid receptor in the human body. The binding affinity, various bond formations, and surface characteristics were studied computationally. This is the first study indicating the interaction of Apomorphine with the mu-opioid receptor, and it sheds light on the morphine-like activity of the drug along with the possibility of various toxicological effects.

Keywords: blue lily; Apomorphine; molecular docking; *in silico*; novel psychoactive substances.

1. Introduction

Drugs classified as narcotics provide pain alleviation and sleep-inducing effects yet are socially unacceptable. The Greek term "narkotikos," which refers to a state of sluggishness or lethargy, is where the word "narcotic" originates. Additionally, the Greek terms "psycho" (soul) and "trope" (turning) are where the term "psychotropic" originates [1,2]. Novel psychoactive substances (NPS) are a multifaceted class of compounds that are frequently referred to as designer or synthetic drugs or by the more prevalent but inaccurate colloquial term "legal highs". NPS drugs need not always be synthetic in nature. Even natural compounds with psychoactive properties that are not regulated by any governing bodies are also considered [3-5]. Natural products derived from plants have long been recognized as a valuable source of bioactive compounds with diverse therapeutic and addictive properties [6-8]. However, the conventional approaches used for natural product development are time-consuming, expensive, and frequently produce insufficient results. *In silico* analysis has been a potent method in recent

years for expediting the discovery and characterization of bioactive compounds derived from plant sources. This strategy makes use of computational techniques and algorithms. This is the main driving force behind the studies conducted using the *in silico* approach [9-12]. The *in silico* analysis of natural products offers several advantages, including reduced cost and time, increased efficiency, and the ability to explore a vast chemical space.

By providing preliminary insights into the biological activities, toxicity profiles, and potential mechanisms of action of natural products, *in silico* analysis guides experimental efforts, enabling the prioritization of promising leads for further investigation [13-16]. The United Nations Office on Drugs and Crime (UNODC) publishes reports annually where they mention the emergence of new psychoactive substances based on the reports they collect from various countries. In one of its reports published in 2013, the organization has brought the attention of the world to new emerging NPS substances and their challenges. The UNODC classified the substance of interest for this study as an NPS drug, and it was discovered that *Nymphea caerulea*, also known as the Egyptian blue lily, Blue Water Lilly, Egyptian Lotus, Sacred Lotus, Sacred Blue Lily of the Nile, Neela Aambal, etc., is the substance's biological source. According to the UNODC, aporphine alkaloids like Apomorphine, nuciferine, etc., are the chemicals of interest. Finland was the reporting nation for the same, although this plant material is widely used and very accessible almost all around the world [17-21]. The image depicting the morphology of the plant is presented in Figure 1.

Figure 1. *Nymphaea caerulea*, the blue water lily. (Original image credits are retained by the authors).

The plant material is rich in phytoactive constituents, and alkaloids, tannins, phenolic compounds, and flavonoids are only a few of this plant's different substances. The alkaloids like Apomorphine and nuciferine are the primary compounds responsible for the blue lily's psychoactive qualities [22-25]. Apomorphine is an alkaloid belonging to the aporphine family. The IUPAC name for Apomorphine is (6a*R*)-6-methyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinoline-10,11-diol and has a molecular formula of C17H17NO2. Apomorphine exists as a crystalline solid with a molecular weight of Apomorphine is 267.32 [26]. The research on this particular plant is very limited; however, in a 2017 study, Apomorphine in seized plant materials was analyzed using a Direct Analysis in Real-Time (DART) Mass spectrometer. The method was able to detect Apomorphine in 2 out of 5 samples at concentrations of 1 ng/mL and 130 ng/mL, respectively [27]. Apomorphine is a morphine analog D2 dopamine agonist used to treat hypomobile "off" episodes of progressive Parkinson's disease. The D2, D3, and D5 dopamine receptors have a high affinity for Apomorphine, a non-ergoline dopamine agonist. The stimulation of D2 receptors in the caudate-putamen, a region of the brain involved in locomotion control, may cause Apomorphine's activity [28-31]. However, the sedative and psychoactive effects of Apomorphine have not been explored in the

scientific community. The possibility of a favorable interaction of Apomorphine with the opioid receptors is a looming question, which the authors aim to tackle through this computational research.

2. Materials and Methods

2.1. Computational hardware and software.

All the molecular docking analysis was performed using an Acer V Nitro laptop with an Intel i7 processor, 1 TB HDD, 16 GB RAM, and Nvidia GTX 960 M dedicated graphics. The software requirements included RCSB protein data bank [32], Autodock Tools (v4.2) [33], OpenBabel (v3.1.1) [34], and Discovery Studio (2021) [35].

2.2. Molecular docking.

2.2.1. Protein and ligand data.

The X-ray crystallography structure of the mu-opioid receptor-Gi protein complex (6DDF) was obtained from the RCSB Protein Data Bank, which was solved from Homo sapiens. The protein was obtained as a 5-chain protein bound to a peptide-like synthetic opioid, which was removed using autodock tools [33]. The structure of Apomorphine was obtained using the PubChem database (ID 6005). The three-dimensional structure was obtained as a spatial data file (sdf) format and further converted to an auto dock structure file (.pdbqt) using OpenBabel software (Version 3.1.1) to be used for docking [34]. The structures of the protein and the Ligand are presented in Figure 2.

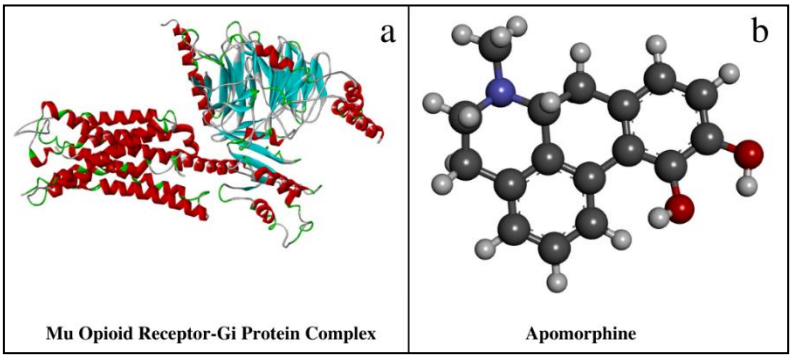

Figure 2. The ribbon and chemical structures of the protein and the Ligand, respectively **(a)** μ-opioid receptor; **(b)** apomorphine.

2.2.2. Protein and ligand preparation.

The protein data was loaded into the Autodock software for protein preparation. The water molecules from the protein were deleted to avoid interferences. The polar hydrogen atoms were then added to the protein structure, followed by the addition of Kollman charges, and the prepared protein structure was saved. The Ligand data was loaded into the Autodock software for the Ligand preparation. The Gasteiger charges were added, and the Ligand torsion settings were applied. The prepared Ligand was then saved as an autodock structure file (.pdbqt).

2.2.3. Docking of Apomorphine to the mu-opioid receptor.

Apomorphine was docked to 6DDF using Autodock tools (Version 4.2). The mu-opioid receptor protein contains 5 chains. Hence, the preliminary docking analysis was performed in a blind format by targeting the whole protein structure. Prior to docking, it was ensured that all water molecules were removed, polar hydrogens were added, and Gasteiger charge was computed using Autodock tools (Version 4.2). A grid box of 120 A x 120 A x 120 A was chosen to encapsulate the whole mu-opioid protein complex. The grid parameters were saved as a configuration text file and used for docking. A docking output file for the blind docking was obtained based on the affinity between the Ligand and the protein, which was expressed in kcal/mol. The best-suited conformer of the Ligand was selected based on the highest binding affinity, and site-specific docking was performed on the protein chain responsible for maximum binding to obtain an accurate result. A grid box of dimensions 45.5 A x 45.5 A x 45.5 A was chosen for the site-specific docking of the protein chain with the highest binding affinity. The docked structures with their amino acid interactions, hydrogen bonding, solvent accessible area, and ionization parameters were calculated using Discovery Studio (2021) [35].

3. Results and Discussion

3.1. Molecular docking.

The output of the docking analysis was observed in Discovery Studio (2021), and in the blind docking study, the cluster analysis was performed, and the binding affinity of Apomorphine with the mu-opioid receptor was the highest with the B chain of the polypeptide. The energy of binding in Kcal/mol was calculated to be -4.47, -5.86, -6.35, -6.14, -6.45, -6.36, -6.38, -6.92, -6.12, and -4.19 for the docking conformations (1-10) of Apomorphine with the receptor. In the blind docking study, it was observed that the least binding energy was observed for the 8th conformer of Apomorphine, with a binding energy of -6.92 Kcal/mol. The 8th conformer with the strongest affinity was present on the B chain of the mu-opioid receptor. Hence, the B chain was selectively isolated using autodock tools for the site-specific molecular docking of Apomorphine. The cluster analysis for binding energy and the affinity towards the B chain is represented in Figure 3.

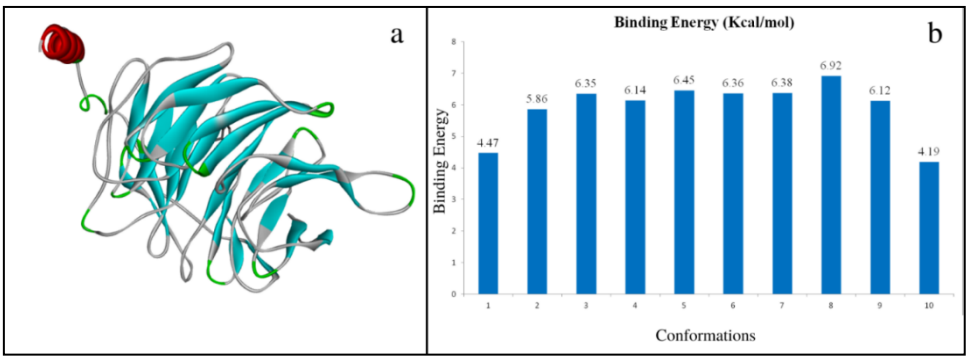

Figure 3. (a) The B chain of the μ-opioid protein; **(b)** The binding energy variation in 10 conformers of Apomorphine in the blind docking.

The binding energy results of Apomorphine with the B chain showed the energy in Kcal/mol as -6.4, -7.29, -6.8, -5.2, -7.5, -7.4, -6.64, -7.27, -7.81, and -7.3 respectively for the conformations (1-10) of Apomorphine. It was noted that at the B chain site, the 9th conformer had the least binding energy of -7.81, which indicated the strong binding potential of

Apomorphine at that site of the mu-opioid receptor. The docking is visualized and represented along with the site-specific binding energy cluster analysis at the B chain, as shown in Figure 4.

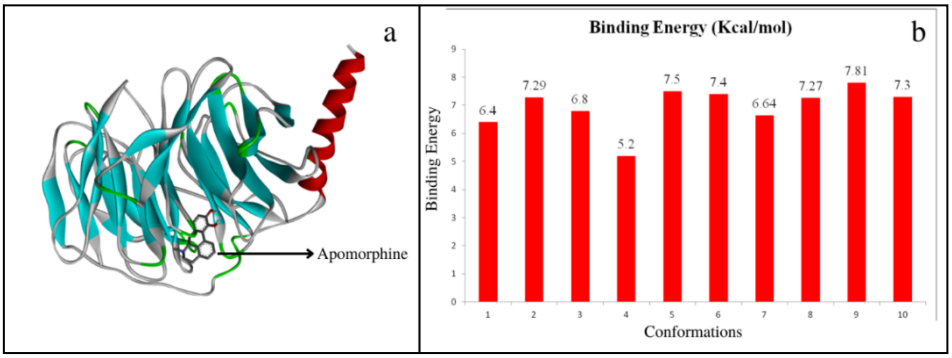

Figure 4. (a) The Apomorphine docked μ-opioid protein; **(b)** the binding energy variation in 10 conformers of Apomorphine in the site-specific docking.

The extensive analysis of the 9th conformer was performed, and the results showed the amino acid interactions of Apomorphine with the receptor. The amino acid interactions are the core of the binding of the Ligand with the receptor. The interactions observed were Pro236, Pro194, Thr321, Phe278, Phe234, Cys233, and Leu192 at the B chain, as presented in the 2D interaction diagram in Figure 5.

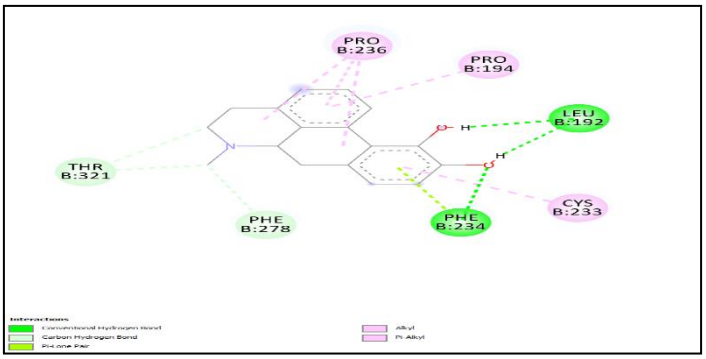

Figure 5. The 2D interaction diagram of the amino acid residues with Apomorphine at the binding site.

The interactions favor efficient binding as all the bonds formed between the amino acid and Apomorphine are ideal. Conventional hydrogen bonds were formed between Leu192 with two hydroxyl groups present on the Apomorphine structure, followed by the hydrogen bond formed between Phe234 and one of the hydroxyl groups. Carbon-hydrogen bonds were observed in the aromatic amino acids, namely Thr321 and Phe278, with the Apomorphine structure at the alkyl group extending from the tertiary amine and the C5 carbon atom. Phe234 shared the Pi-lone pairs with the aromatic ring (D). Pro236 and Pro194 formed Alkyl and Pi-alkyl bonds with the structure's A, B, and C rings, followed by Cys233 at the D ring of Apomorphine. The hydrogen bonding surface area was also calculated and found to be favorable as acceptor and donor pockets are present in the interaction, which is essential for receptor binding and cell membrane transportation. It can also be noted that the mu-opioid receptor is a membrane receptor. The binding performed in this study shows the presence of the strongest binding near the receptor entrance and activation site. This information is crucial to understanding the possibility of effective cellular transport of the compound by strong receptor binding. The ionizability of Apomorphine in the binding pocket was visualized. It was observed that the alkaloid has ionization tendencies towards the extremes of acidic and basic pH. Still, it is also noted that the compound largely remains unionized in the protein pocket,

indicating a more non-polar nature of the compound as the LogP value of the compound is 2.3 and ensures a stronger protein binding with a 98-99% protein binding efficiency. The solvent-accessible surface for Apomorphine is excellent, with a greater part of the molecule available for interaction; the solvent-accessible surface area plays an important role in biological systems. The various properties of the Apomorphine docked structure at the binding site are illustrated in Figure 6.

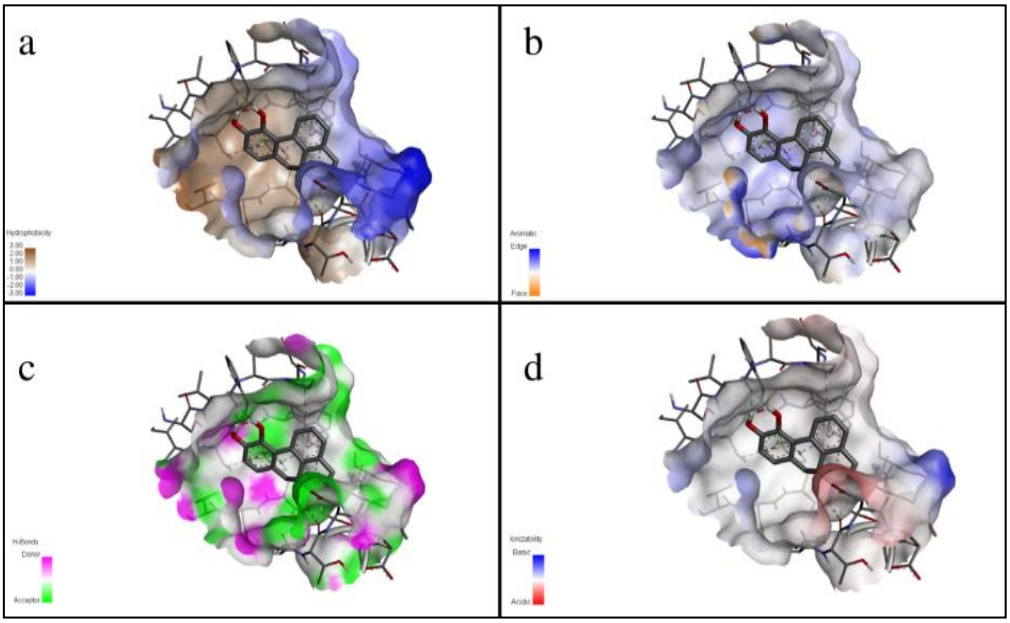

Figure 6. The various properties of Apomorphine were studied at the binding site. **(a)** hydrophobicity; **(b)** aromaticity; **(c)** hydrogen bonds; **(d)** ionizability.

This molecular docking study indicates the possibility of strong biological activity of Apomorphine. Since Apomorphine is considered a novel psychoactive substance, it is important to learn the effect of the same in the living system. This is the first computational work that has demonstrated that the molecule of Apomorphine can bind efficiently with the mu-opioid receptor at the activation site for effective cellular uptake, which further increases its morphine-like effect as well as the possibility for cytotoxicity in larger doses.

4. Conclusions

This *in silico* molecular docking experiment showcases a novel possibility in the interaction of Apomorphine with the mu-opioid receptor, which is a valuable indication of its role as a psychoactive substance. Blue Lilies and their products are still legal in many countries and are easily accessible; hence, it is important to assess their biological interaction and activity to ensure it does not cause a toxic effect on the living system or cause another drug pandemic worldwide. The experimental computation involved in the study is easy to follow and effective in determining the preliminary information regarding the analyte before proceeding with laboratory experiments. Theoretical modeling and experimentation are valuable resources that can also help reduce the world's carbon footprint associated with extensive laboratory workflow.

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Conflicts of Interest

The authors declare no conflict of interest.

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