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MOLECULAR DYNAMICS SIMULATION OF MITRAGYNINE INTERACTIONS WITH DOPAMINE D1 AND D2 RECEPTORS: ASSESSING PSYCHOACTIVE EFFECTS AND THERAPEUTIC POTENTIAL

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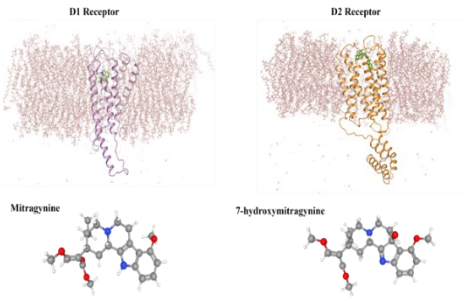
Graphical abstract



Abstract

The therapeutic and psychoactive properties of mitragynine, the primary alkaloid in Mitragyna speciosa (kratom), are well documented, particularly in pain therapeutics. However, the molecular interactions between mitragynine and the D1 and D2 dopamine receptors—essential targets for pain modulation and therapeutic interventions in conditions like schizophrenia and depression—remain poorly understood. We employed molecular dynamics simulations to investigate the binding interactions of mitragynine and 7-hydroxymitragynine at D1 and D2 dopamine receptors. Root mean square deviation (RMSD) analysis demonstrated that mitragynine stabilized at approximately 0.3 nm for D1 and 0.4 nm for D2, indicating substantial structural stability. In contrast, 7-hydroxymitragynine exhibited increased RMSD values of roughly 0.45 nm for D1 and 0.50 nm for D2, indicating greater structural flexibility and receptor activation potential. Furthermore, MM-PBSA analyses evaluating binding free energies revealed that mitragynine possessed thermodynamically favorable interactions at D1 (-6.46 kcal/mol) and D2 (-6.10 kcal/mol). Conversely, 7-hydroxymitragynine had a destabilizing positive total energy change at D1 (0.81 kcal/mol) despite demonstrating a comparable binding affinity at D2 (-6.02 kcal/mol). These quantitative findings demonstrate that mitragynine exhibits a significantly higher stabilizing affinity for both D1 and D2 receptors compared to 7-hydroxymitragynine. This structural and thermodynamic energy landscape highlights how mitragynine may be therapeutically beneficial for disorders associated with dopamine dysregulation, providing a foundation for future in vitro cellular assays and in vivo models.

Keywords: Dopamine receptors, mitragynine, 7-hydroxymitragynine, molecular dynamic simulation, MM-PBSA

Abstrak

Sifat terapeutik dan psikoaktif untuk mitragynine, alkaloid utama dalam Mitragyna speciosa (kratom), didokumenkan dengan baik terutamanya dalam terapi kesakitan. Walau bagaimanapun, interaksi molekul antara mitragynine dan reseptor dopamin D1 dan D2—sasaran penting untuk

modulasi kesakitan dan campur tangan terapeutik dalam keadaan seperti skizofrenia dan kemurungan—masih tidak difahami dengan baik. Kami menggunakan simulasi dinamik molekul untuk menyiasat interaksi pengikatan mitragynine dan 7-hydroxymitragynine pada reseptor dopamin D1 dan D2. Analisis Sisihan Punca Min Kuasa Dua (RMSD) menunjukkan bahawa mitragynine stabil pada kira-kira 0.3 nm untuk D1 dan 0.4 nm untuk D2, menunjukkan kestabilan struktur yang ketara. Sebaliknya, 7-hydroxymitragynine mempamerkan peningkatan nilai RMSD sekitar 0.45 nm untuk D1 dan 0.50 nm untuk D2, menunjukkan fleksibiliti struktur yang lebih besar dan potensi pengaktifan reseptor. Tambahan pula, analisis MM-PBSA yang menilai tenaga bebas pengikatan mendedahkan bahawa mitragynine mempunyai interaksi termodinamik yang menggalakkan pada D1 (-6.46 kcal/mol) dan D2 (-6.10 kcal/mol). Sebaliknya, 7-hydroxymitragynine mempunyai tenaga ATOTAL positif yang tidak stabil pada D1 (0.81 kcal/mol) walaupun menunjukkan pertalian pengikatan yang setanding pada D2 (-6.02 kcal/mol). Penemuan kuantitatif ini menunjukkan bahawa mitragynine mempamerkan pertalian penstabilan yang jauh lebih tinggi untuk kedua-dua reseptor D1 dan D2 berbanding 7-hydroxymitragynine. Landskap tenaga struktur dan termodinamik ini menyerlahkan bagaimana mitragynine boleh memberi manfaat secara terapeutik untuk gangguan yang berkaitan dengan disregulasi dopamin, menyediakan asas untuk ujian selular in vitro dan model in vivo pada masa hadapan.

Kata kunci: Reseptor dopamin, mitragynine, 7-hydroxymitragynine, simulasi dinamik molekul, MM-PBSA

1.0 INTRODUCTION

Dopamine and its receptors present as promising targets for the treatment of multiple neurological conditions, including depression, Parkinson's disease, schizophrenia, and substance addictions [1]. An understanding of these hormones is crucial in comprehending pain, reward, movement, and emotional reactions. The human body is equipped with five dopamine receptors: D1, D2, D3, D4, and D5. These receptors can be classified into two distinct groups. The interaction between these receptors plays a crucial role in neuronal growth, cognitive processes, and the functioning of the extrapyramidal system by enhancing the activity of postsynaptic receptors. The presence of D2-D4 receptors plays a crucial role in the viability and growth of dopamine neurons. [2, 3].

Kratom, also known as *Mitragyna speciosa*, is a plant that naturally grows in Southeast Asia, particularly in Malaysia and Thailand. Kratom is a member of the Rubiaceae family, also referred to as the coffee family [4]. Phsyiologically, in the human body, kratom interacts with serotonin and dopamine receptors, alongside its well-documented interaction with opioid receptors [5, 6]. Based on these studies, kratom may have potential benefits in addressing conditions such as anxiety, psychosis, and depression. Previous pharmacokinetic studies have demonstrated the pharmacological properties of *Mitragyna speciosa*, possess antinociceptive and sedative stimulating effects [7, 8], thus presents itself as a potential therapeutic drug for nociceptive pain relief.

Mitragynine and 7-hydroxymitragynine, both alkaloids found in the leaves of *Mitragyna speciosa*, are renowned for their positive effects due to their interaction with various brain receptors. Studies have shown that mitragynine has a subtle effect on dopamine receptors. It acts as an agonist, mimicking the effects of dopamine by binding to and activating these receptors. The mentioned stimulation leads to a positive sensation, as indicated by Amrianto et al. (2021) [9]. Additionally, it has a counteractive impact on dopamine receptors through the inhibition of the central D2 receptor. Pharmaceutical compounds known as dopamine antagonists have the ability to hinder the binding of dopamine-to-dopamine receptors. They have been proven to be highly effective in the treatment of disorders that are characterized by an overabundance of dopamine activity, such as schizophrenia.

The pharmacological potential of mitragynine and 7-hydroxymitragynine is currently an issue of ongoing debate, as there are concerns regarding the risks of dependence and addiction associated with the use of kratom. In 2019, a study conducted by White revealed that the US Drug Enforcement Administration (DEA) has categorized kratom and its alkaloids as a "drug of concern" due to their lack of acknowledged medical advantages and their potential to develop dependency [10]. A study conducted in 2013 by Sabetghadam et al. found that the use of mitragynine is generally deemed safe for a short period, if the dosage does not exceed 10 mg per kilogram [11]. Nevertheless, beyond a dosage of 100 mg per

kilogram can result in atypical alterations in the liver, kidneys, and brain at a histological, haematological, and biochemical level.

A complete comprehension of the long-term implications of kratom usage is still lacking, and conventional approaches that involve directly manipulating dopamine receptors have been deemed unsuitable for clinical application due to their adverse effects on arterial pressure [12, 13].This project uses molecular docking and simulation to analyze potential protein targets of mitragynine, particularly dopamine receptors, to evaluate their binding interactions. Based on our simulation, we show that mitragynine targets various dopamine receptor-binding sites, and thus can be a potential alternative to commercial medications. The result from our simulation may serve as a template for further in vitro and in vivo experiments to prove the potential for mitragynine as a natural nociceptive therapeutic.

2.0 METHODOLOGY

2.1 Docking Simulation

Molecular docking was employed to explore the interaction between dopamine receptors (D1 and D2) and the ligands mitragynine and 7-hydroxymitragynine. Dopamine D1 and D2 receptors were selected because they are the most prevalent subtypes involved in pain modulation, reward, and neuropsychiatric disorders. D1 is linked to excitatory G-protein signaling (Gs), while D2 is associated with inhibitory (Gi/o) pathways. The differential behavior of mitragynine at these two receptors provides mechanistic insights into its therapeutic and psychoactive roles. Protein structures of dopamine receptor 1 with code 7jvq and dopamine receptor 2 with code 7dfp were retrieved from the Protein Data Bank (PDB) and preprocessed using UCSF Chimera to remove extraneous entities such as water molecules, ions, and heteroatoms. Mitragynine (PubChem CID:3034396) and 7-hydroxymitragynine (PubChem CID: 44301524) structures were retrieved in SDF format from the PubChem database in compatible formats and prepared for docking simulations. AutoDock Vina docking was performed using an exhaustiveness of 8. The grid box was centered on the co-crystallized ligand coordinates with dimensions of 30 × 30 × 30 Å, ensuring full coverage of the orthosteric binding site. The top-ranked docking pose based on binding affinity (lowest predicted ΔG) was selected for each ligand. Pose selection was further validated by visual inspection to ensure correct positioning within the orthosteric site and to avoid steric clashes.

2.2 Model Building

The receptor-ligand complexes derived from docking were further refined for molecular dynamics (MD) simulations using CHARMM-GUI. Receptor structures were incorporated into a membrane lipid bilayer composed of POPC, with a 70:70 ratio, mimicking the native cellular environment. Ligand parameters were generated with the CHARMM36m force field, and the system was solvated using the TIP3P water model. Sodium (Na^+) and chloride (Cl^-) ions were added to neutralize the system and simulate a physiological ionic strength of 0.15 M. Initial energy minimization resolved steric conflicts and prepared the system for equilibration.

2.3 Molecular Dynamics Simulation

MD simulations were conducted using GROMACS to evaluate the stability and dynamic behavior of the receptor-ligand complexes. The system underwent stepwise equilibration in NVT and NPT ensembles to stabilize temperature and pressure. A 200-nanosecond production run was performed at 310 K and 1 atm, with periodic boundary conditions applied. Key metrics such as root mean square deviation (RMSD) were calculated to assess conformational stability. Post-simulation analyses using VMD and PyMOL revealed detailed interaction dynamics and confirmed the stability of the receptor-ligand complexes. The native co-crystallized ligands from the receptor PDB structures were used as controls. Specifically, apomorphine from the D1 receptor structure (PDB ID: 7JVQ) and spiperone from the D2 receptor structure (PDB ID: 7DFP) were retained in the respective binding sites and included in MD simulations and MM-PBSA calculations to serve as biologically relevant reference ligands. For MM-PBSA, the gmx_MMPBSA tool was used to calculate binding free energies based on 200 snapshots extracted from the last 50 ns of the trajectory. The calculations included van der Waals, electrostatic, polar solvation, and non-polar solvation energy components. WhileFor Principal Component Analysis (PCA), the gmx covar and gmx anaeig tools in GROMACS were used. Covariance matrices were constructed based on Ca atomic fluctuations, and eigenvectors were extracted to project the trajectory onto the first two principal components (PC1 and PC2).

3.0 RESULTS AND DISCUSSION

3.1 Trajectory Analysis

Molecular docking was performed to estimate the binding affinities of mitragynine and 7-hydroxymitragynine toward dopamine D1 and D2 receptors. The top-ranked binding poses for each ligand were selected based on their lowest predicted binding free energies. Mitragynine exhibited strong docking scores of −9.1 kcal/mol for the D1 receptor and −8.7 kcal/mol for the D2 receptor. In comparison, 7-hydroxymitragynine showed slightly weaker affinities with docking scores of −8.3 kcal/mol and −8.0

kcal/mol for D1 and D2, respectively. These docking results suggest a higher initial binding preference of mitragynine for both receptor subtypes and provided the structural basis for subsequent molecular dynamics simulations.

MD simulations provided comprehensive insights into the stability, flexibility, and conformational dynamics of dopamine receptors D1 and D2 bound to mitragynine and 7-hydroxymitragynine. Key metrics such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and solvent accessible surface area (SASA) were employed to evaluate ligand-receptor interactions over a 200 ns simulation. These metrics highlighted differences in the structural and dynamic responses of the receptor-ligand systems compared to controls.

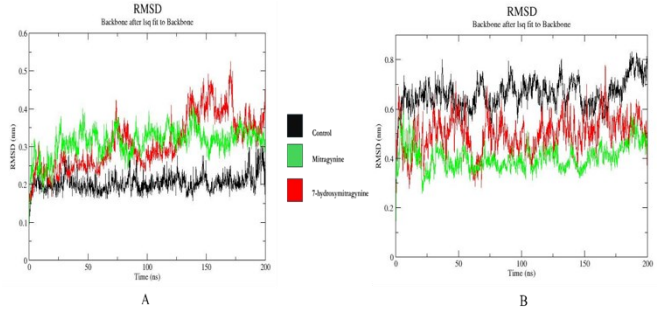


Figure 1 RMSD profile explaining the equilibrating nature of the dopamine receptors during the 200 nanosecond MD simulation for control, mitragynine and 7-hydroxymitragynine at (A) Dopamine receptor 1 and (B) Dopamine receptor 2

RMSD analysis assessed the overall stability and structural deviations of receptor-ligand complexes. For dopamine receptor 1, the control system exhibited minimal fluctuations, stabilizing at ~0.2 nm, as shown in Figure 1 (A), indicating of a highly stable conformation. Mitragynine caused a slight increase in RMSD (~0.3–0.35 nm), reflecting minor conformational adjustments while maintaining stability. In contrast, 7-hydroxymitragynine exhibited larger deviations (~0.4–0.45 nm) and increased oscillations after 150 ns, suggesting greater flexibility but reduced structural stability as noted by Liu et al. (2017), where high RMSD values are often associated with significant instability [14]. For dopamine receptor 2, shown in Figure 1 (B), mitragynine exhibited stable RMSD values (~0.4–0.5 nm) with minimal fluctuations, whereas 7-hydroxymitragynine displayed intermediate stability (~0.5–0.6 nm) with more pronounced variability. These findings indicate that mitragynine effectively stabilizes receptor structures while allowing functional flexibility as shown by the low RMSD values [15], whereas 7-hydroxymitragynine induces destabilization, particularly in receptor 1.

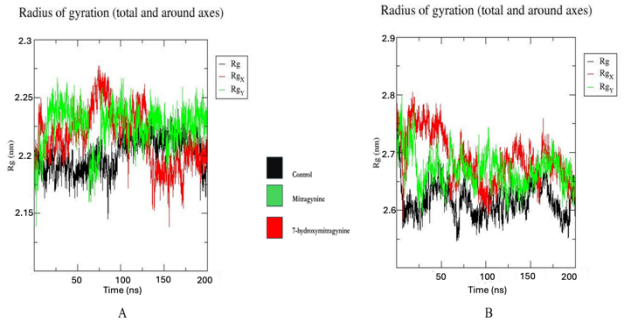


Figure 2 Rg profile explaining the equilibrating nature of the dopamine receptors during the 200 nanosecond MD simulation for control, mitragynine and 7-hydroxymitragynine at (A) Dopamine receptor 1 and (B) Dopamine receptor 2

Rg analysis provided insights into receptor compactness and conformational changes. Furthermore, Rg is a crucial parameter that assesses the protein's proper folding and compactness [16, 17]. Dopamine receptor 1 in the control system showed consistent Rg values (~2.15–2.2 nm), reflecting a stable and compact structure, as depicted in Figure 2 (A). Mitragynine induced minor increases in Rg (~2.2–2.25 nm), suggesting a balance between stability and functional adaptability. In contrast, 7-hydroxymitragynine caused substantial fluctuations (~2.15–2.3 nm), indicating pronounced conformational changes and reduced stability. For dopamine receptor 2, Figure 2 (B) illustrates that the control system demonstrated low variability (~2.6–2.7 nm), while mitragynine maintained moderate fluctuations (~2.6–2.8 nm), supporting receptor flexibility without compromising integrity. However, 7-hydroxymitragynine displayed broader Rg variations (~2.6–2.9 nm), suggesting destabilization. These results confirm mitragynine's ability to preserve receptor stability while accommodating necessary conformational dynamics.

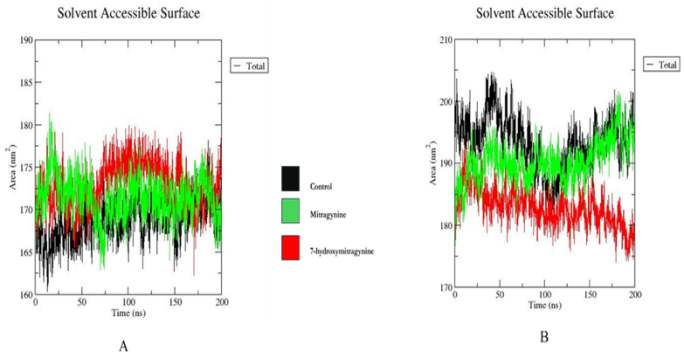


Figure 3 SASA profile explaining the equilibrating nature of the dopamine receptors during the 200 nanosecond MD simulation for control, mitragynine and 7-hydroxymitragynine at (A) Dopamine receptor and (B) Dopamine receptor 2

SASA analysis highlighted solvent exposure and conformational changes induced by ligand binding. For dopamine receptor 1, the control system maintained stable SASA values (~165–175 nm²), while mitragynine showed slightly increased SASA (~170–180 nm²), as shown in Figure 3 (A), reflecting ligand-induced flexibility and effective receptor modulation. Conversely, 7-hydroxymitragynine exhibited broader SASA variations (~165–185 nm²), indicative of reduced structural stability. In dopamine receptor 2, Figure 3 (B) illustrates that mitragynine achieved a favorable balance of increased SASA (~190–210 nm²) while maintaining receptor integrity, whereas 7-hydroxymitragynine induced compact receptor conformations (~175–190 nm²), potentially hindering dynamic interactions. These findings support mitragynine as a superior ligand for receptor modulation, achieving optimal stability and functional flexibility compared to 7-hydroxymitragynine. Across all metrics, mitragynine demonstrated superior stability and functional adaptability compared to 7-hydroxymitragynine, making it a promising therapeutic candidate for modulating dopamine receptors. While 7-hydroxymitragynine exhibited increased receptor activation potential, its reduced stability and pronounced flexibility may limit its suitability for long-term therapeutic applications.

3.2 Hydrogen Bond Interaction

Hydrogen bonding plays a critical role in ligand-receptor interactions, influencing molecular recognition, stability, and specificity. The analysis of hydrogen bond interactions during molecular dynamics (MD) simulations provided insights into the binding affinity and stability of dopamine receptor complexes with mitragynine and 7-hydroxymitragynine.

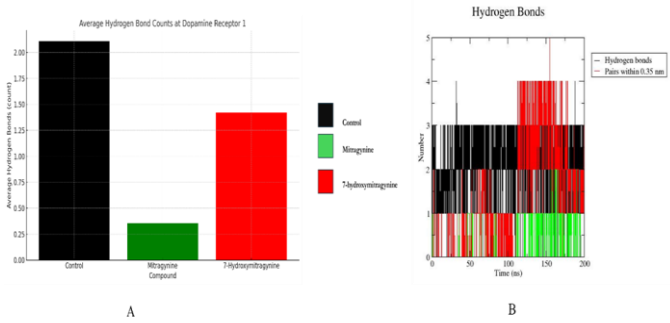


Figure 4 A comparison of hydrogen bond profile between control, mitragynine and 7-hydroxymitragynine explaining their equilibrating nature at dopamine receptor 1 during the 200 nanosecond MD simulation. (A) Average hydrogen bond counts for each compound and (B) Temporal hydrogen bond fluctuations over the simulation time

As shown in Figure 4 (A), the control system demonstrated the highest average hydrogen bond count (~2.0 bonds), reflecting a stable receptor environment. In contrast, mitragynine exhibited the lowest average count (~0.25 bonds), suggesting weak and transient interactions with the receptor. Conversely, 7-hydroxymitragynine showed improved compatibility, with an average hydrogen bond count of ~1.5 bonds, indicating stronger receptor interactions. The temporal fluctuations, illustrated in Figure 4 (B), revealed that the control maintained consistent hydrogen bonds throughout the simulation, stabilizing early at 20–50 ns. Mitragynine displayed the least stability, with counts fluctuating between 0 and 1 bond. In comparison, 7-hydroxymitragynine exhibited moderate stability, with counts fluctuating between 2 and 4 bonds, suggesting more robust receptor interactions than mitragynine.

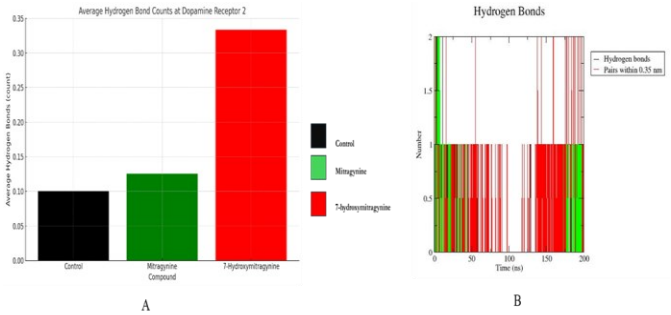


Figure 5 A comparison of hydrogen bond profile between control, mitragynine and 7-hydroxymitragynine explaining their equilibrating nature at dopamine receptor 2 during the 200 nanosecond MD simulation. (A) Average hydrogen bond counts for each compound and (B) Temporal hydrogen bond fluctuations over the simulation time

For dopamine receptor 2, Figure 5 (A) shows the control system had the lowest average hydrogen bond count (~0.1 bonds), while mitragynine achieved a modest count (~0.15 bonds). 7-hydroxymitragynine surpassed both, with an average count of ~0.3 bonds. Temporal variations in hydrogen bonds, as shown in Figure 5 (B), indicated that the control maintained consistent bonding, while mitragynine displayed reduced stability with counts ranging between 0.5 and 1 bond. In contrast, 7-hydroxymitragynine demonstrated intermediate stability, with counts fluctuating between 0.5 and 1.5 bonds and occasional peaks above 2 bonds, indicating dynamic interactions and repositioning within the binding site. The hydrogen bond interactions between dopamine receptors and ligands (7-hydroxymitragynine and mitragynine) were analyzed at 0 ns, 100 ns, and 200 ns to assess the stability, binding dynamics, and role of key amino acid residues in the receptor-ligand complex. The results highlight significant differences in binding stability and receptor adaptability.

In general, mitragynine's diminished and ephemeral binding indicates that it may function as an antagonist, fighting for the binding site without adequately activating the dopamine receptors. Conversely, 7-hydroxymitragynine is more inclined to function as an agonist, stabilising receptor activation, owing to its increased hydrogen bond counts and

enhanced interactions. This finding is supported by a few recent studies by Johnson et al. (2020) and Vijeepallam et al. (2016), which suggest that mitragynine may antagonise dopamine D2 receptors, given that most antipsychotics function as antagonists at post-synaptic dopamine D2 receptors [18,19]. Some antipsychotics have a dual mechanism of action on dopamine 2 receptor, functioning at presynaptic locations at low doses and at postsynaptic sites at high doses, proposed that mitragynine may have a similar dose-dependent effect on dopamine receptor 2 sites) [20, 21]. Consequently, mitragynine is suitable for drug development approaches that emphasise receptor selectivity and reduce off-target effects.

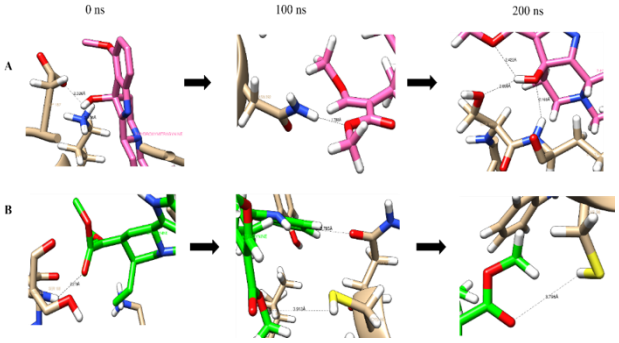


Figure 6 Hydrogen bond distance at different timeframe between amino acids and ligand at dopamine receptor 1. (A) 7-hydroxymitragynine and (B)Mitragynine

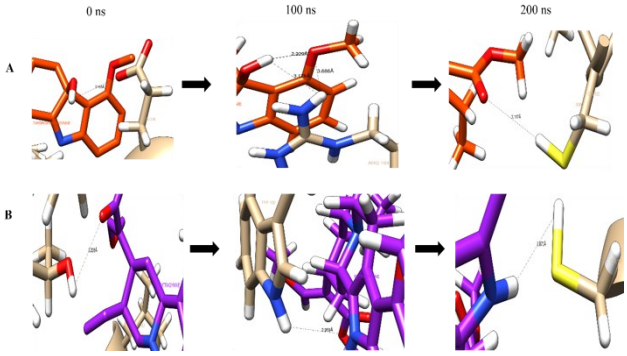


Figure 7 Hydrogen bond distance at different timeframe between amino acids and ligand at dopamine receptor 2. (A) 7-hydroxymitragynine and (B)Mitragynine

As shown in Figure 6 (A), 7-hydroxymitragynine initially formed hydrogen bonds with ASP 187 and LYS 81 at distances of 2.326 Å and 3.156 Å, respectively. At 100 ns, a strong interaction with ASN 292 was observed at 1.788 Å, indicating stable binding. By 200 ns, hydrogen bond distances with SER 189 ranged from 2.168 Å to 2.422 Å, suggesting dynamic but less rigid interactions, potentially enhancing receptor activation while compromising stability. Conversely, as illustrated In Figure 6 (B), mitragynine formed a bond with SER 188 at 2.270 Å at 0 ns, which persisted as a key interaction site. Over time, hydrogen bonds with CYS 96 and ASN 97 remained stable, with distances increasing slightly to 3.8 Å at 200 ns, indicating minimal receptor conformational changes and sustained binding stability. These findings suggest that mitragynine promotes receptor stability, whereas 7-hydroxymitragynine enhances dynamic receptor activation.

The interaction dynamics of dopamine receptor 2 are depicted in figure 7. For 7-hydroxymitragynine, Figure 7 (A) shows initial hydrogen bonds with GLU 95 at 3.166 Å at 0 ns. By 100 ns, additional interactions with ARG 104 (2.229 Å and 3.126 Å) and stabilization with CYS 182 at 3.117 Å at 200 ns were observed, indicating stable and consistent binding. In contrast, as shown in Figure 7 (B), mitragynine exhibited more variable interactions. It formed a bond with THR 412 at 3.228 Å at 0 ns, and a more robust connection with TRP 100 at 2.959 Å at 100 ns. By 200 ns, a stable interaction with CYS 107 was established at 2.870 Å, representing the shortest bond length for mitragynine. However, the variability in residue engagement suggests lower stability compared to 7-hydroxymitragynine.

3.3 Principal Component Analysis (PCA)

Principal component analysis is a widely used technique for analysing protein movements. It involves the comparison of the motions of two molecular dynamics trajectories using data obtained from molecular dynamics simulations. The dimensionality necessary to characterise protein dynamics was methodically reduced through the use of Principal Component Analysis (PCA). This process involves a decomposition that prioritises observed motions from the most significant to the least significant spatial atomic displacements in each conformation within a trajectory. The gmx anaeig instrument was employed to investigate the interaction of mitragynine and 7-hydroxymitragynine with dopamine receptors 1 and 2, respectively, by examining the first two principal components, PC1 (projection on eigenvector 1) and PC2 (projection on eigenvector 2). A scatter diagram was employed to illustrate the results of PCA (PC1 and PC2) for each interaction.

Figure 8 Scatter plot of PCA over 200 nanoseconds MD simulation at dopamine receptors. The blue plot represents 7-hydroxymitragynine meanwhile yellow plot shows mitragynine. (A) Protein-ligand interaction at dopamine receptor 1 and (B) Protein-ligand interaction at dopamine receptor 2

Figure 8 (A) disclosed that the yellow plots denoting mitragynine were more densely clustered in the middle compared to the blue plots indicating 7-hydroxymitragynine. The blue plots have a wider range with identifiable clusters at the dopamine 1 receptor, and their plots are more scattered along the Principal Component 1 axis, ranging about from -10 to 10 nm. This indicates a greater diversity in the results for 7-hydroxymitragynine. The yellow plots (mitragynine) exhibit greater density and clustering around the centre, extending approximately from -5 to 15 nm on the Principal Component 1 axis. Similarly, Principal Component 2 (PC2) demonstrates that mitragynine ranges from -6 to 4 nm, whereas 7-hydroxymitragynine extends from -6 to 6 nm, so highlighting its greater variability compared to mitragynine about dopamine receptor 1. This suggests that the data variability for mitragynine is more limited compared to that of 7-hydroxymitragynine.

Analogous to Figure 8 (A), the yellow plots in figure 8 (B) exhibit a dense concentration at the middle of the plot, indicating that the principal components for mitragynine are more closely clustered. Moreover, this indicates that the conformations of mitragynine exhibit greater similarity. In contrast, the blue plots of 7-hydroxymitragynine exhibit more dispersion, indicating increased variability in the major component. The range of Principal Component 1 (PC1) for mitragynine is around -10 to 15 nm, but 7-hydroxymitragynine spans a little larger range of -15 to 20 nm, indicating a greater conformational diversity along this axis. Mitragynine spans a range of -10 to 10 nm for Principal Component 2 (PC2), while 7-hydroxymitragynine extends from -15 to 20 nm, highlighting its superior variability relative to mitragynine at the dopamine receptor 2.

Previous research indicates that the core cluster, distinguished by a large concentration of data points, is likely the most energetically advantageous and often used configuration under the simulated conditions, consistent with the results of this work. The centre and density indicate a conformational state of significant stability, perhaps associated with the protein's functionally inactive or quiescent state [22]. The proximity of mitragynine clusters in both dopamine receptors may be associated with conformational stability throughout the simulation, resulting in the stabilisation of the dopamine receptor in a limited number of conformational states. This signifies a more constrained structural impact. This indicates that mitragynine may stabilise the receptor, perhaps leading to more selective or potent signalling. In contrast, 7-hydroxymitragynine enables a more comprehensive examination of receptor states, potentially supporting several signalling pathways. This arises from the distributed distribution of 7-hydroxymitragynine sites, signifying dynamic transitions between states. Mitragynine is a superior option for both dopamine receptor 1 and dopamine receptor 2 when stability and specific receptor conformations are necessary, as shown by the PCA projection map in Figure 8. The functional state of the receptor may indicate a significant agonistic or antagonistic effect, shown by its capacity to stabilise the receptor in fewer conformations.

3.4 Molecular Mechanics Poisson-Boltzmann Surface Area Analysis (MM-PBSA)

Table 1 Average MM-PBSA free energy of dopamine receptor 1 complex of protein-ligand interaction with mitragynine and 7-hydroxymitragynine

Component	Control	Mitragynine	7-hydroxymitragynine
Van der Waals energy change (ΔVDWAALS)	-31.66	-41.64	-41.35
Electrostatic energy change (ΔEEL)	-7.64	-9.07	-11.88
Polarization energy change (ΔEPB)	33.76	49.32	59.12
Non-polar solvation energy change (ΔENPOLAR)	-3.41	-5.07	-4.90
Gaseous phase energy change (ΔGGAS)	-39.31	-50.71	-53.41
Solvation phase energy change (ΔGSOLV)	30.35	44.25	54.22
Total energy change (ΔTOTAL)	-8.96	-6.46	0.81

Table 2 Average MM-PBSA free energy of dopamine receptor 2 complex of protein-ligand interaction with mitragynine and 7-hydroxymitragynine

Component	Control	Mitragynine	7-hydroxymitragynine
Van der Waals energy change (ΔVDWAALS)	-46.37	-31.45	-36.16
Electrostatic energy change (ΔEEL)	-15.21	-9.51	-3.60
Polarization energy change (ΔEPB)	48.78	39.41	38.50
Non-polar solvation energy change (ΔENPOLAR)	-4.86	-4.54	-4.76
Gaseous phase energy change (ΔGGAS)	-61.58	-40.96	-39.76
Solvation phase energy change (ΔGSOLV)	43.92	34.86	33.73
Total energy change (ΔTOTAL) (kcal/mol)	-17.66	-6.10	-6.02

MM-PBSA analysis was conducted to estimate the binding free energy of ligand-receptor complexes involving mitragynine, 7-hydroxymitragynine, and dopamine receptors. Binding free energy was decomposed into van der Waals, electrostatic, solvation, and polarization components to evaluate ligand stability and therapeutic potential. Table 1 presents the average MM-PBSA free energy for dopamine receptor 1.The control ligands—apomorphine (D1 receptor, PDB ID: 7JVQ) and spiperone (D2 receptor, PDB ID: 7DFP)—provided baseline free energy values for comparison. These control simulations helped contextualize the binding profiles of mitragynine and 7-hydroxymitragynine in terms of native ligand affinity The control ligand exhibited a total energy change (ΔTOTAL) of -8.96 kcal/mol, indicating stable binding. Mitragynine showed a slightly less negative ΔTOTAL of -6.46 kcal/mol, suggesting thermodynamically favorable binding but reduced affinity compared to the control. In contrast, 7-hydroxymitragynine exhibited a positive ΔTOTAL of 0.81 kcal/mol, indicating unfavorable binding and instability. The binding affinity of mitragynine was driven by significant van der Waals (-41.64 kcal/mol) and electrostatic (-9.07 kcal/mol) contributions, counterbalanced by high polarization (49.32 kcal/mol) and solvation energies (44.25 kcal/mol). Similarly, 7-hydroxymitragynine demonstrated stronger electrostatic interactions (-11.88 kcal/mol) but was destabilized by even higher polarization (59.12 kcal/mol) and solvation (54.22 kcal/mol) penalties. These findings position mitragynine as a more stable ligand for dopamine receptor 1, closely aligning with the control.

As shown in Table 2 the control ligand displayed the most stable interaction with dopamine receptor 2, with a ΔTOTAL of -17.66 kcal/mol. Both mitragynine (-6.1 kcal/mol) and 7-hydroxymitragynine (-6.02 kcal/mol) showed comparable binding affinities, albeit significantly weaker than the control. Mitragynine's binding was supported by stronger electrostatic interactions (-9.51 kcal/mol), while 7-hydroxymitragynine benefitted from slightly more favorable van der Waals interactions (-36.16 kcal/mol vs. -31.45 kcal/mol for mitragynine). Although both ligands maintained thermodynamic stability, their lower ΔTOTAL values compared to the control highlight diminished binding efficiency. The minimal difference between the ligands suggests similar potential as modulators of dopamine receptor 2, with mitragynine having a slight edge due to enhanced electrostatic contributions. Mitragynine demonstrated a ΔTOTAL closer to the control for both receptors, highlighting its superior stability and binding efficiency compared to 7-hydroxymitragynine. While both ligands exhibited favorable van der Waals and electrostatic contributions, higher polarization and solvation penalties limited their overall binding affinity. These findings underscore the potential of mitragynine as a therapeutic candidate for dopamine receptor modulation, with 7-hydroxymitragynine providing dynamic but less stable interactions.

4.0 CONCLUSION

This study confirmed the initial hypotheses, demonstrating significant binding affinities between dopamine receptors D1 and D2 and the ligands, mitragynine and 7-hydroxymitragynine. Molecular docking provided preliminary binding affinity estimates that guided the selection of receptor-ligand complexes for molecular dynamics simulations, supported by stable root mean square deviation (RMSD) profiles from molecular dynamics simulations. MM-PBSA analysis further validated these findings, with negative binding free energies (ΔG) reflecting strong ligand-receptor interactions driven by van der Waals forces and solvation effects.

Mitragynine emerged as the most stable ligand, maintaining core receptor stability with moderate flexibility, making it well-suited for therapeutic applications requiring sustained and regulated dopamine receptor activation. Conversely, 7-hydroxymitragynine exhibited greater receptor activation potential but induced higher structural variability, potentially limiting its suitability for long-term therapeutic use. Both ligands demonstrated robust interaction potential, achieving the research objectives of assessing binding affinities, stability, and energy components. Mitragynine offers a balanced approach for therapeutic applications, while 7-hydroxymitragynine could serve in scenarios requiring aggressive receptor activation. The optimal ligand choice depends on the desired balance between receptor stability and activation intensity.

Further in vitro studies are recommended to validate the *in silico* findings, including cell-based assays to examine the pharmacological effects and safety profiles of mitragynine and 7-hydroxymitragynine. These experiments will provide empirical support for their therapeutic potential and psychoactive properties, advancing translational research. Collaboration with pharmacologists and clinical researchers could explore their application in treating neurological disorders, potentially leading to novel therapeutic strategies.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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