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Design, Molecular Docking, Molecular Dynamics, and Evaluation of Novel Ligands Targeting Beta-2 Adrenergic Receptor for Asthma Therapeutics

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Abstract: This study investigates the design and molecular docking of novel ligands targeting the beta-2 adrenergic receptor (β2AR), a critical protein involved in bronchoconstriction and asthma regulation. Utilizing molecular docking simulations, evaluated the binding affinities of proposed compounds, including the eight compounds, and the reference drug salbutamol, against β2AR. The docking studies were conducted using Glide software (Maestro 13.5) within the Schrodinger suite (Schrodinger, 2023), and binding interactions were analyzed to identify key residues responsible for ligand binding and receptor activation The MM-GBSA results indicate that all tested compounds exhibit favorable binding affinities with 7DHI, suggesting strong potential as ligands. Among them, two compounds demonstrated particularly strong binding to the β2-adrenergic receptor (β2AR), with MM-GBSA calculated binding free energies of -57.71 and -58.95 kcal/mol, closely comparable to that of salbutamol (-59.74 kcal/mol). These compounds exhibited the best stability and interaction with β2AR, underscoring their suitability for further development. The binding affinity is primarily driven by Van der Waals interactions and non-polar solvation, highlighting their strong receptor interaction and potential for optimization. Key residues such as SER 207, PHE 289, LYS 305, and ASP 192 played significant roles in stabilizing the receptor-ligand interactions. The inclusion of functional groups like NO_2 and NC was based on their demonstrated favorable interactions with the binding site, which enhanced affinity. While these groups contributed positively to binding, these findings indicate that further structural modifications—beyond these specific functionalities—may optimize β2AR binding even further. These insights into the molecular mechanisms underlying β2AR-ligand interactions highlight the potential of compounds as promising candidates for further development into β2 agonists for asthma treatment. Salbutamol, as a well-established β2 agonist, served as a benchmark for evaluating the efficacy of the novel ligands, confirming the feasibility of designing β2AR-targeting therapeutics with improved potency and selectivity.

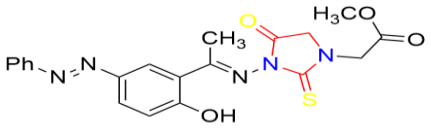
Keywords: Beta-2 adrenergic receptor (β2AR), salbutamol, Molecular docking, Molecular Dynamics, and Ligand design.

INTRODUCTION

Beta-adrenergic receptors are a subfamily of G-protein coupled receptors (GPCRs) that play pivotal roles in mediating physiological responses to catecholamines[1]. These receptors are classified into three main subtypes: β_1, β_2, and β_3. In particular, the β_2-adrenergic receptor (β_2AR) is predominantly expressed in the smooth muscle of the airways, uterus, and vasculature[2], where its activation leads to smooth muscle relaxation and bronchodilation[3, 4]. The mechanism of action involves receptor-mediated activation of adenylate cyclase[5], resulting in increased intracellular cyclic AMP levels, which ultimately relax airway smooth muscles. This pharmacological response underlies the therapeutic efficacy of β_2AR agonists in respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD) [6].

Salbutamol[7], a well-established short-acting β_2AR agonist, exemplifies the clinical utility of these agents. Its high selectivity for β_2 receptors facilitates rapid bronchodilation, making it the drug of choice for the immediate relief of acute bronchospasm in asthma and COPD patients [8]. Despite its effectiveness, prolonged or inappropriate use of salbutamol may lead to the development of tolerance and undesirable side effects[7], underscoring the need for novel therapeutics with enhanced efficacy and safety profiles [9].

Thioxoimidazolidinone derivatives represent a promising class of heterocyclic compounds with diverse pharmacological activities, including notable anti-inflammatory effects[10]. Unlike general imidazolidinone motifs, the thioxoimidazolidinone scaffold incorporates a sulfur atom, which can significantly influence its biological interactions[11]. Recent studies have demonstrated that thioxoimidazolidinone derivatives may modulate key inflammatory pathways and inhibit the synthesis of pro-inflammatory mediators[12], suggesting their potential utility in reducing airway inflammation associated with bronchoconstriction. However, further research is required to fully elucidate their role and optimize their pharmacological properties. Salma M. Khirallah et al. synthesized novel 2-thiohydantoin derivatives with anti-inflammatory properties. Inflammation is the main cause of several autoimmune diseases, including asthma, type I diabetes, rheumatoid arthritis, and multiple sclerosis. Compound (A) showed the most significant inhibited pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, making it a promising anti-inflammatory candidate[10].



Compound (A)

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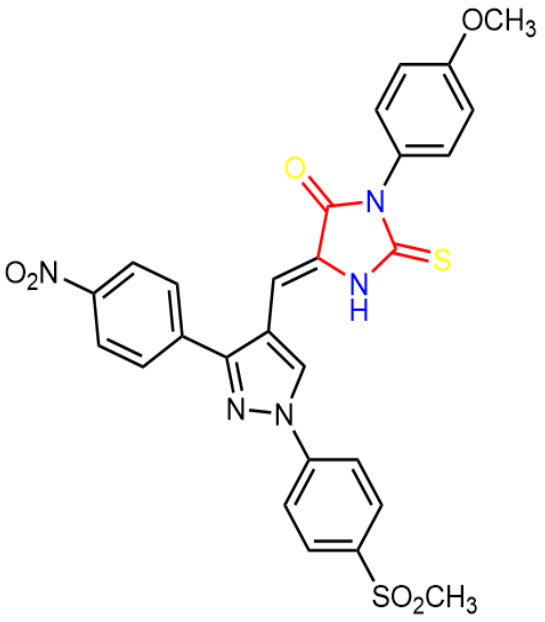
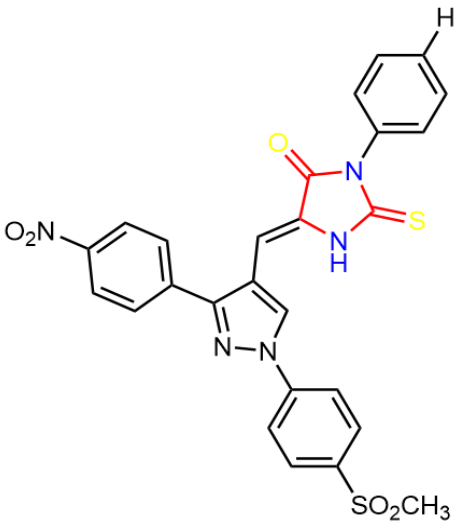
Khaled R. et al. developed a new series of hybrid thiohydantoin-pyrazole derivatives were synthesized for anti-inflammatory. These compounds selectively inhibited COX-2, showed good anti-inflammatory effects. Compounds (B), (C), and (D) showing potential function agents[13].



Compounds (B)



Compounds (C) **Compounds (D)**

There is a strong need for novel β_2AR agonists due to the limitations of current treatments and the emerging potential of thioxoimidazolidinone-based compounds. This study focuses on designing and evaluating new thioxoimidazolidinone derivatives as β_2AR agonists using computational approaches. Molecular docking simulations[14], were employed to assess the binding affinities and interaction profiles of eight designed compounds with β_2AR. Additionally, in silico "ADME analyses were conducted to evaluate their pharmacokinetic properties ", and molecular dynamics simulations were performed to confirm the stability of the most promising ligand-receptor complexes. This work provides valuable insights into the structure–activity relationships of thioxoimidazolidinone-based β_2AR ligands and identifies potential candidates for the development of safer and more effective therapies for managing bronchoconstriction in asthma.

COMPUTATIONAL METHODS

In this study, a rational design approach was employed to develop novel β_2-adrenergic receptor agonists based on the thioxoimidazolidinone scaffold. this design choices were guided by previous evidence suggesting that thioxoimidazolidinone derivatives can effectively modulate inflammatory pathways and interact favorably with key receptor residues. Specific functional groups were selected to enhance binding interactions within the β_2AR binding pocket, drawing on insights from the binding profile of salbutamol. These modifications were expected to improve receptor affinity and selectivity, thereby providing a strong foundation for further drug optimization.

Ligand Preparation

The LigPrep tool transforms 2D structures into 3D models. These 3D structures, along with their activity values, are utilized to refine and generate conformers for each minimized ligand[15], using the OPLS (Optimized Potentials for Liquid Simulations) force field to prepare the ligands for molecular docking analysis[16].

Molecular Docking

Molecular docking evaluation study and molecular modeling drug design, were carried out by Glide software (Maestro 13.5) under Schrodinger software (Schrodinger, 2023) running on Windows 10 operating system on workstation (Intel(R) Core (TM) i7-10750 @ 2.60 GHz, 16.00 GB RAM)[16]. The crystal structure of the Cryo-EM structure of the partial agonist salbutamol-bound beta2 adrenergic receptor-Gs protein complex was taken from the Protein Data Bank under the PDB code 7DHI, with a 3.26 Å crystallographic resolution [17]. The Protein preparation steps occurred by using suitable program for preparation and optimization. Ligand structure preparation occurred by utilizing Ligprep program prior to docking to determine and add of hydrogens in order to obtain the optimal orientation and ionization position with low energy conformations of all ligands by OPLS4 force field[18]. The grid box was set by set an atom of the ligand with kept the default settings and best docking orientation was kept[19]. Then processing docking using glide and analysis the result depends on docking score and interaction between ligand and references drugs with amino acid residues[20]. The revised methodology by performing three independent docking attempts for each ligand-receptor pair. This adjustment is intended to minimize the impact of random variation and ensure more reliable and consistent results. This approach enhances the robustness of findings and strengthens the conclusions drawn from the study. To ensure the accuracy and reliability of the docking procedure, a superimposition test was conducted between the cocrystallized pose of the reference ligand and its docked pose, confirming the validity of the docking approach[21]. Additionally, the interaction profile of the docked ligand was compared with previously reported data, verifying that key interactions—such as those involving SER 207, PHE 289, LYS 305, and ASP 192—were consistent with literature findings. To further account for the intrinsic randomness of molecular docking calculations, three independent docking runs were performed for each ligand-receptor pair[22]. This approach ensured the consistency of docking scores and binding interactions, reinforcing the reliability of the results. The docking simulations were specifically designed to analyze how potential ligands interact with the beta-2 adrenergic receptor (PDB code: 7DHI) as agonists at a molecular level[23]. The results provided insights into the expected binding and activation of the receptor, highlighting the binding interactions and affinities of eight compounds alongside a reference molecule (salbutamol), as summarized in Table 2 and illustrated in Figures 1-6. Notably, the independent docking calculations demonstrated consistent

binding patterns, further strengthening the robustness of findings.

Molecular Dynamics Simulation

A 200-nanosecond simulation was done to check how stable the complex molecular dynamic simulation is and how the ligand-receptor binding mode works. The Desmond program in Schrodinger software was used on a Linux system for this experiment. First, the receptor and ligand were mixed in a simple point charge (SPC) water model[24]. They were put inside an orthorhombic box. To neutralize the system, sodium and chloride ions were added to a 50 mM solution. The simulation ran using the NPT ensemble, keeping the temperature steady at 300 K and pressure at 1.01325 bar. During this, an energy value of 1.2 was maintained, with results recorded every 200 picoseconds[25]. The OPLS3e force field was applied all through the molecular dynamic simulation[26]. Following the dynamic simulation analysis, the motion pathways of the ligand-receptor complex were generated using the Simulation Interaction Diagram (SID). That provided insights into the interactions, stability, and conformational changes occurring during the simulation, allowing for a comprehensive understanding of the molecular behavior over time. These trajectories, along with root-mean-square deviation (RMSD), root-mean- square fluctuation (RMSF), and protein-ligand contacts, were analyzed to interpret the stability and interactions of the protein- ligand complex[27].

Free Energy of Binding Calculations

MM/GBSA calculations were initially performed on the best-docked poses of all compounds using the Prime module within the Schrödinger suite[28]. These structures were optimized using the OPLS3 force field[29].

To complement the static results with dynamic information, MM/GBSA calculations were also carried out on single conformations extracted from MD simulations. Specifically, we used the representative conformation from the largest RMSD-based cluster for compounds 1, 5, and salbutamol. This method is supported in the literature as a viable approximation to full trajectory MM/GBSA [30], providing a balance between computational cost and accuracy[31]

ADMET prediction

To assess the safety of candidate compounds during drug development, preclinical research on safety and pharmacokinetics is essential[25]. The pharmacokinetic properties of eight imidazolidinone derivatives, including " (absorption, distribution, metabolism, and excretion) ADME", were analyzed using the freely accessible Swiss-ADME tool (http://www.swissadme.ch) [32]. This analysis helps determine the characteristics related to bioavailability and cellular permeability[26].

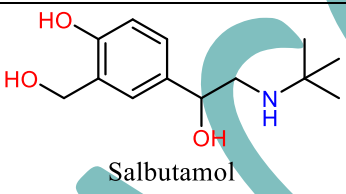
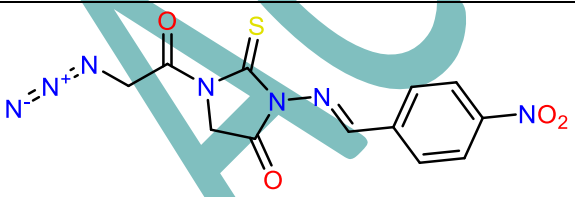
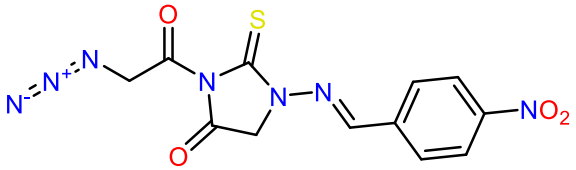
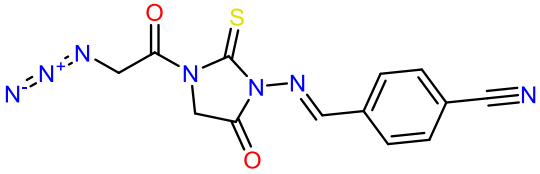
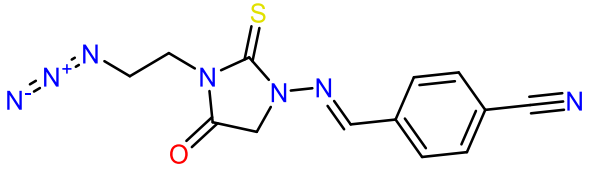
RESULTS AND DISCUSSIONS

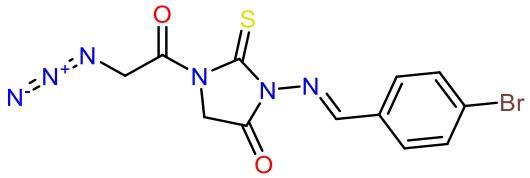
Molecular Docking Analysis

This study focuses on designing eight novel compounds as potential beta-2 adrenergic receptor (β2AR) agonists for treating respiratory conditions. Table (1), presents the eight designed compounds featuring a beta-2 adrenergic receptor agonist structure. Initially, computational

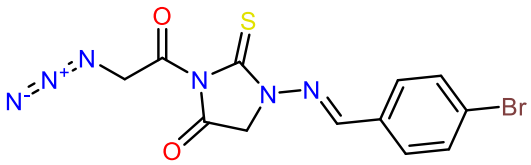
molecular docking was performed to examine how these newly developed derivatives interact with the beta-2 adrenergic receptor. The binding affinities of compounds (1, 2, 3, 4, 5, 6, 7 and 8) were evaluated in relation to their interaction with the beta-2 receptor to determine the strength and nature of their binding. In silico ADME (Absorption, Distribution, Metabolism, and Excretion, analysis was performed to assess the pharmacokinetic properties and drug-likeness of the compounds. Molecular dynamics simulations (MD), over 100 nanoseconds were carried out on the compound with the most favorable docking, further confirming the stability and binding interaction with the beta-2 receptor.

Table (1): Structures of eight designed compounds.

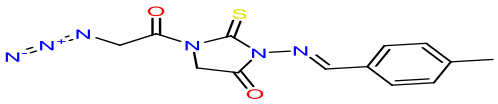
 Salbutamol	
 **1)** (E)-1-(2-azidoacetyl)-3-((4-nitrobenzylidene) amino)-2-thioxoimidazolidin-4-one	 **5)** (E)-3-(2-azidoacetyl)-1-((4-nitrobenzylidene) amino)-2-thioxoimidazolidin-4-one
 **2)** (E)-4-(((3-(2-azidoacetyl)-5-oxo-2-thioxoimidazolidin-1-yl) imino) methyl) benzonitrile	 **6)** (E)-4-(((3-(2-azidoacetyl)-4-oxo-2-thioxoimidazolidin-1-yl) imino) methyl) benzonitrile



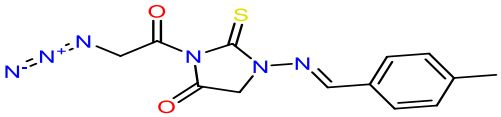
3) (E)-1-(2-azidoacetyl)-3-((4-bromobenzylidene) amino)-2-thioxoimidazolidin-4-one



7) (E)-3-(2-azidoacetyl)-1-((4-bromobenzylidene) amino)-2-thioxoimidazolidin-4-one



4) (E)-1-(2-azidoacetyl)-3-((4-methylbenzylidene) amino)-2-thioxoimidazolidin-4-one



8) (E)-3-(2-azidoacetyl)-1-((4-methylbenzylidene) amino)-2-thioxoimidazolidin-4-one

Performed molecular docking simulations to analyze how potential ligands interact with the beta-2 adrenergic receptor (PDB code: 7DHI) as agonists at a molecular level. The analysis of the docking results helped us understand the expected binding and activation of the receptor by these ligands[33]. The molecular docking results highlight the binding interactions and affinities of eight compounds and a reference molecule (salbutamol) with a target protein.As show in table (2) and Figure (1-6).

Compounds 1 and 5 demonstrated strong binding scores of -6.12 and -6.31, respectively, suggesting high affinity. Both compounds formed a single hydrogen bond with the residue **SER 207**, with the functional group **NO2** playing a critical role in the interaction. Additionally, these compounds engaged in three non-hydrogen interactions with the residues **PHE 289**, **LYS 305**, and **ASP 192**, indicating stable multi-residue interactions. This binding pattern aligns with the general trend of SER 207 being a key hydrogen bonding residue for strong binders.

Compound 2 showed moderate binding with a docking score of -5.71. Like compounds 1 and 5, it formed one hydrogen bond with **SER 207**, and the **NC** functional group contributed to this interaction. It also shared the same three non-hydrogen bonding residues (**PHE 289**, **LYS 305**, and **ASP 192**) as compounds 1 and 5, which might explain its relatively strong but slightly lower binding affinity.

Compound 3, with a docking score of -5.63, also exhibited moderate binding but did not form any hydrogen bonds. However, it engaged in fwe non-hydrogen interactions with residues **ASP 192**, **LYS 305**, **PHE 289**, and **SER 207**, suggesting that while hydrogen bonding was absent, other interactions compensated to stabilize its binding.

Compound 4 displayed the weakest binding among the higher-ranked compounds, with a docking score of -5.32. It formed one hydrogen bond with **PHE 193** and had the functional group **N=N=N** facilitating the interaction. Additionally, it only engaged in one non-hydrogen interaction with **LYS 305**, which might account for its lower binding affinity compared to other compounds.

Compound 6 exhibited strong binding, with a docking score of -6.05. It formed a hydrogen bond with **SER 207**, with the **NC** functional group playing a role. Furthermore, it showed robust non-hydrogen bonding interactions with four residues: **PHE 289**, **PHE 193**, **ASP 192**, and **LYS 305**, highlighting its ability to interact with multiple critical residues.

Compound 7 had the weakest binding affinity overall, with a docking score of -4.66. It did not form any hydrogen bonds and only engaged in two non-hydrogen interactions with **PHE 289** and **SER 207**, which likely explains its poor binding performance. **Compound 8**, with a score of -4.83, performed slightly better than compound 7. It formed a single hydrogen bond with **PHE 193**, with the **N=N=N** functional group facilitating the interaction, and engaged in two non-hydrogen bonds with **ASP 192** and **LYS 305**.

Salbutamol, the reference molecule, exhibited the best binding score of -7.89, underscoring its high affinity for the target protein. It formed six hydrogen bonds with key residues, including two bonds each with **ASN 312** and **ASP 113**, and one bond each with **SER 203** and **SER 207**. Its functional groups, **OH** and **NH2+**, were crucial in establishing these interactions. Additionally, salbutamol engaged in two non-hydrogen interactions with **ASP 113** and **PHE 193**, making it the most stable and versatile binder in this study.

The minimal difference in docking scores between Salbutamol and compounds 1 and 5 can be explained by the presence of additional stabilizing interactions in these compounds. Unlike Salbutamol, which primarily forms six hydrogen bonds, compounds 1 and 5 exhibit multiple key interactions, including **π-π stacking, salt bridges, and π-cation interactions**. These interactions play a crucial role in enhancing the binding affinity of the compounds. Specifically, **π-π stacking interactions** with **PHE289** help stabilize the ligand-receptor complex, while **strong salt bridge interactions** with **ASP192 and LYS305** further strengthen the binding. Additionally, both compounds engage in **extensive hydrophobic interactions** with multiple residues, including **TYR308, ILE309, PHE289, PHE290, VAL117, VAL114, TYR316, TRP109, PHE193, and CYS191**, which contribute significantly to their stability in the receptor pocket. These combined interactions compensate for the lower number of hydrogen bonds compared to Salbutamol, resulting in competitive docking scores and reinforcing the strong binding potential of compounds 1 and 5.

Table (2): Docking scores for final compounds (1-7) and salbutamol docked with (7DHI).

compounds	salbutamol	1	2	3	4	5	6	7	8
Docking score Kcal/mol	- 7.89	-6.12	-5.71	-5.63	-5.32	-6.31	-6.05	-4.66	-4.83
H-bond	ASN 312 (2) ASP 113 (2) SER 203 SER 207	SER 207	SER 207		PHE 193	SER 207	SER 207		PHE 193

compounds	salbutamol	1	2	3	4	5	6	7	8
Hydrophobic	VAL114, TYR316, VAL117, PHE193	TYR308, ILE309, PHE289, PHE290, VAL117, VAL114, TYR316, TRP109, PHE193, CYS191	TYR308, ILE309, TRP313. TYR316, ILE121, ALA119, VAL117, CYS116, LEU115, TRP109	ILE309, PHE289, PHE290, VAL117, VAL114, TYR316, TRP109, PHE193, CYS191	ILE309, TRP313. TYR316, ILE121, ALA119, VAL117, CYS116, LEU115, TRP109	TYR308, ILE309, PHE289, PHE290, VAL117, VAL114, TYR316, TRP109, PHE193, CYS191	TYR308, ILE309, TRP313. TYR316, ILE121, ALA119, VAL117, CYS116, TRP109	ILE309, TRP313. TYR316, ILE121, ALA119, VAL117, CYS116, TRP109, TYR308	PHE289, PHE290, VAL117, VAL114, TYR316, TRP109, PHE193, CYS191
Salt bridge		ASP192, LYS305	ASP192	ASP192, LYS305		ASP192, LYS305	ASP192	LYS305	ASP192, LYS305
π cation	PHE193		HIS93				HIS93		
π-π stacking			PHE289	PHE193		PHE289	PHE289	PHE193	

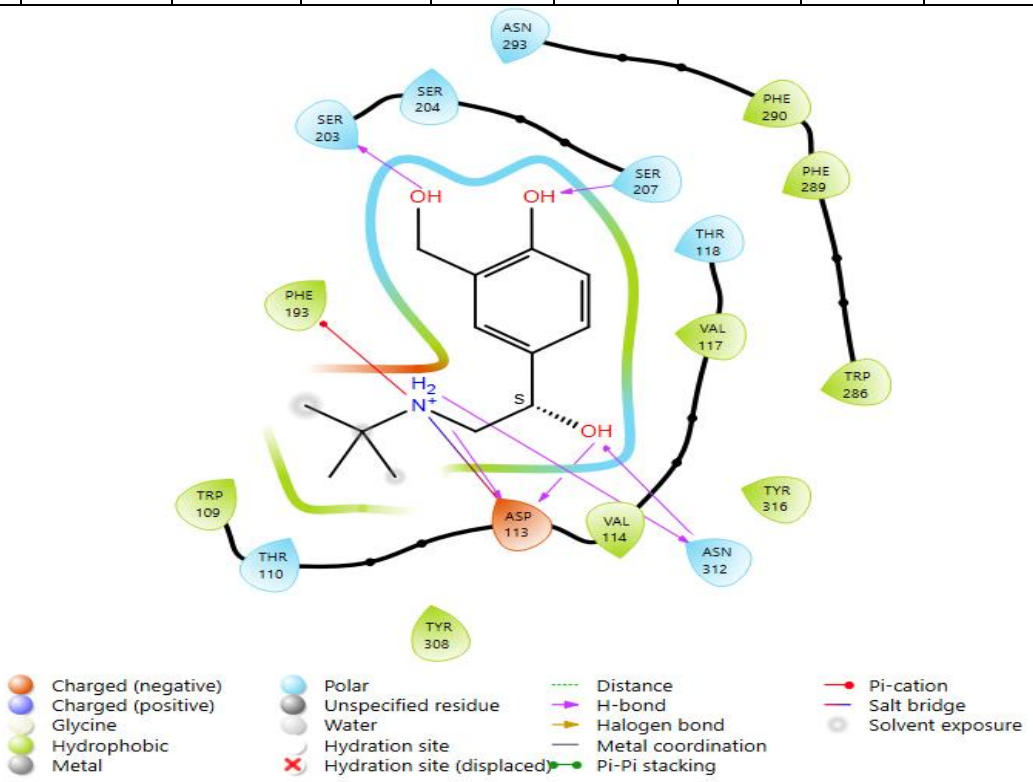


Figure (1): 2D shape of interaction mode of Salbutamol.

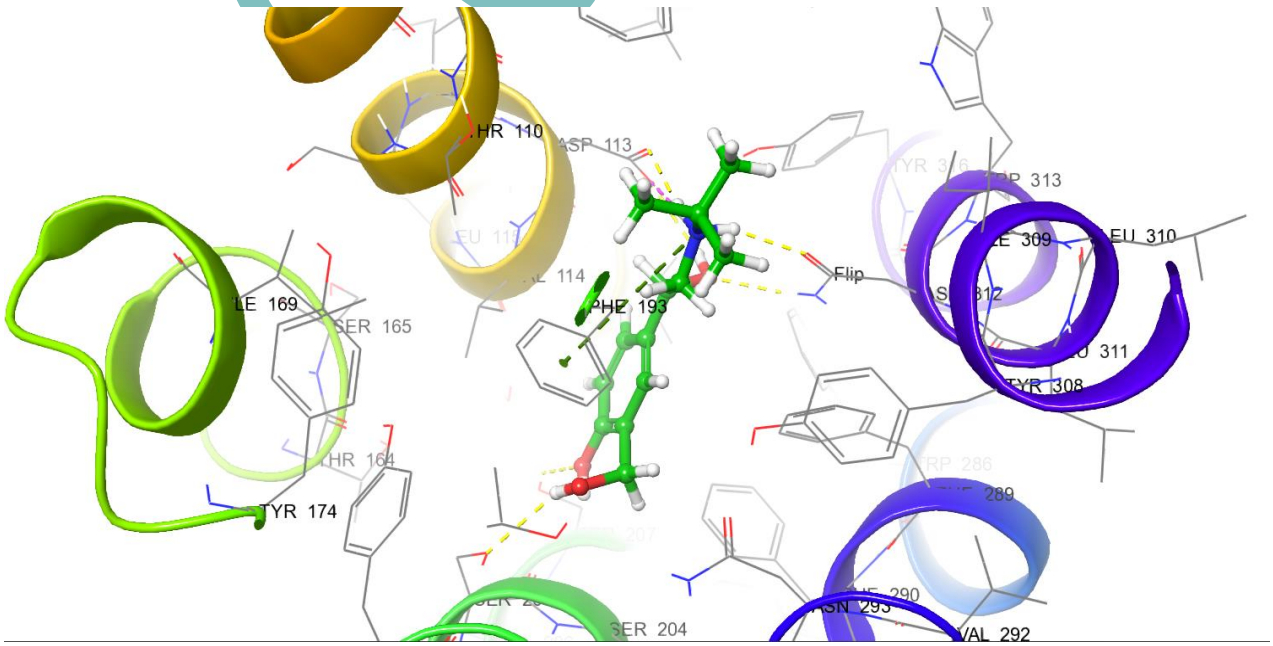


Figure (2): 3D shape of interaction mode of Salbutamol. (H bond: yellow, bad contact: Orange, Halogen bond: Purple, Green: pi-cation, Sky blue: Pi-Pi Stacking).

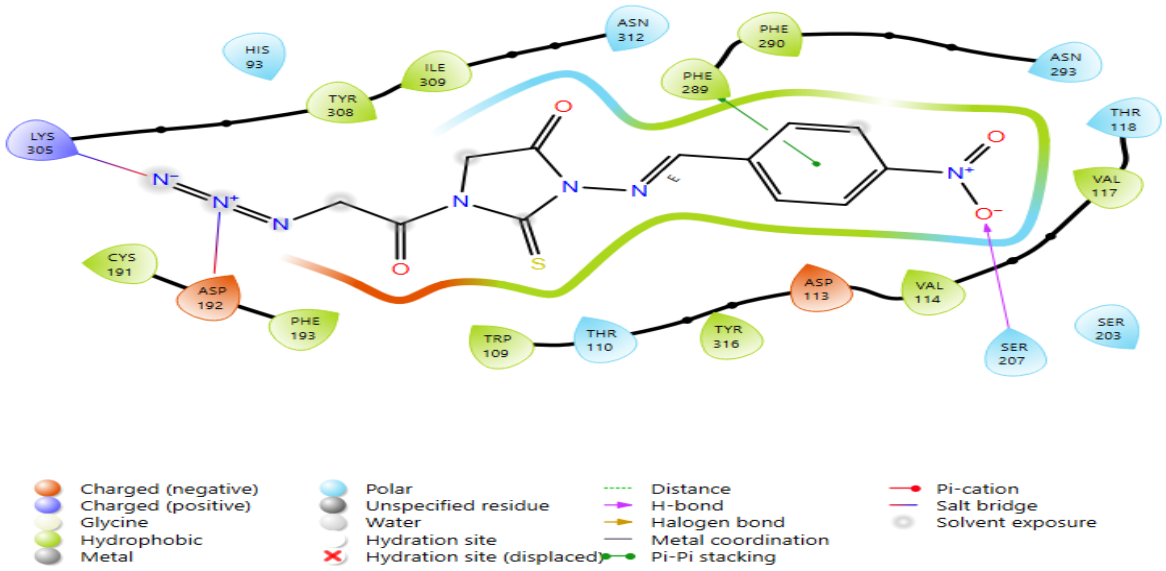


Figure (3): 2D shape of interaction mode of COMPOUND (1).

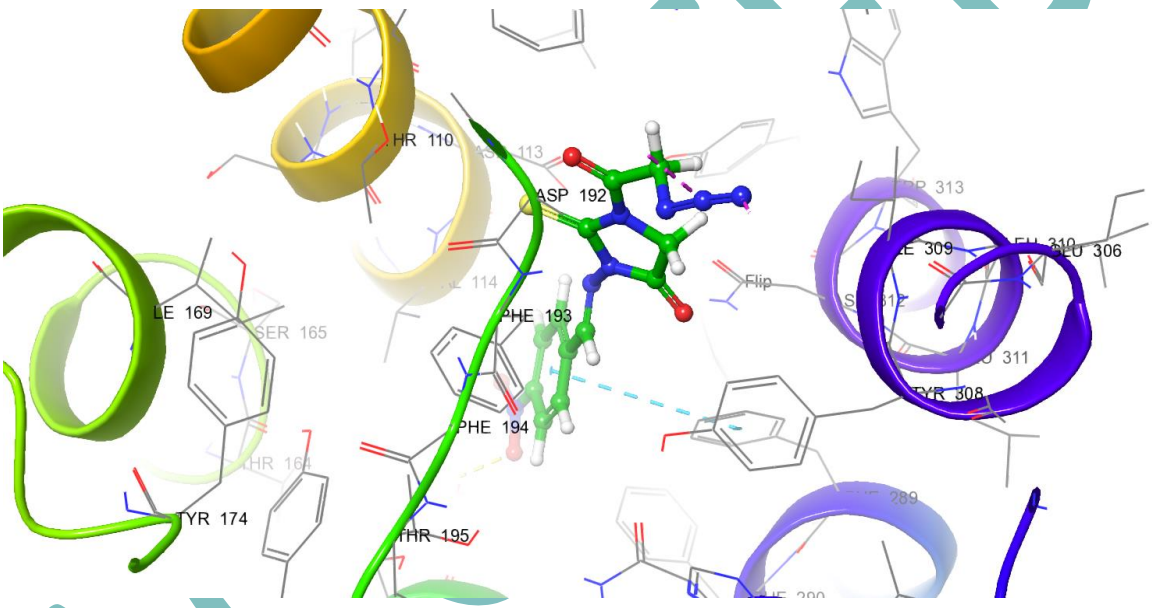


Figure (4): 3D shape of interaction mode of Compound [1]. (H bond: yellow, bad contact: Orange, Halogen bond: Purple, Green: pi-cation, Sky blue: Pi-Pi Stacking).

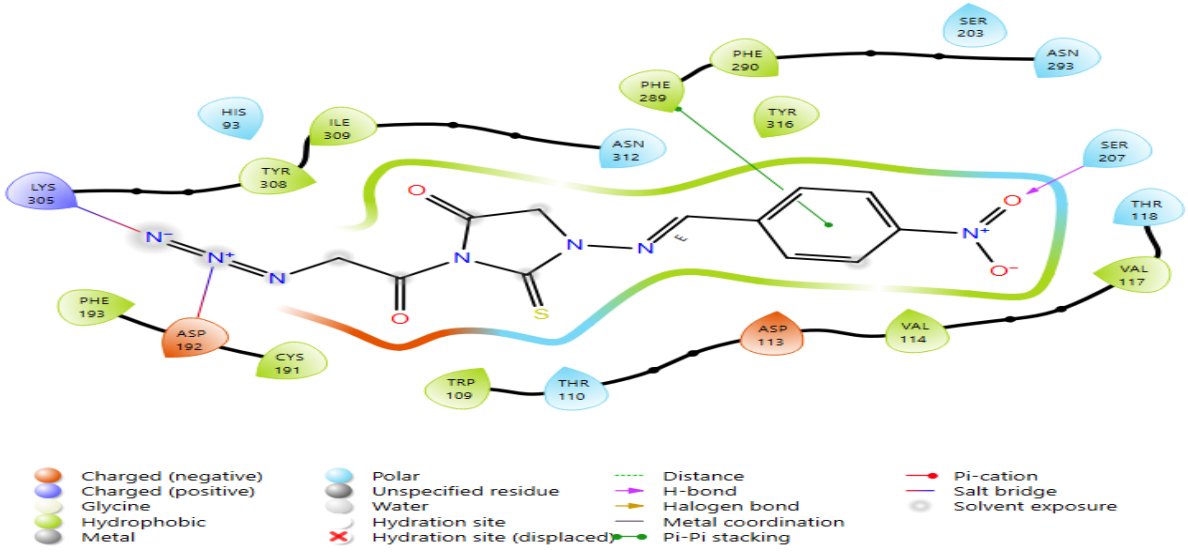


Figure (5): 2D shape of interaction mode of COMOUND 5.

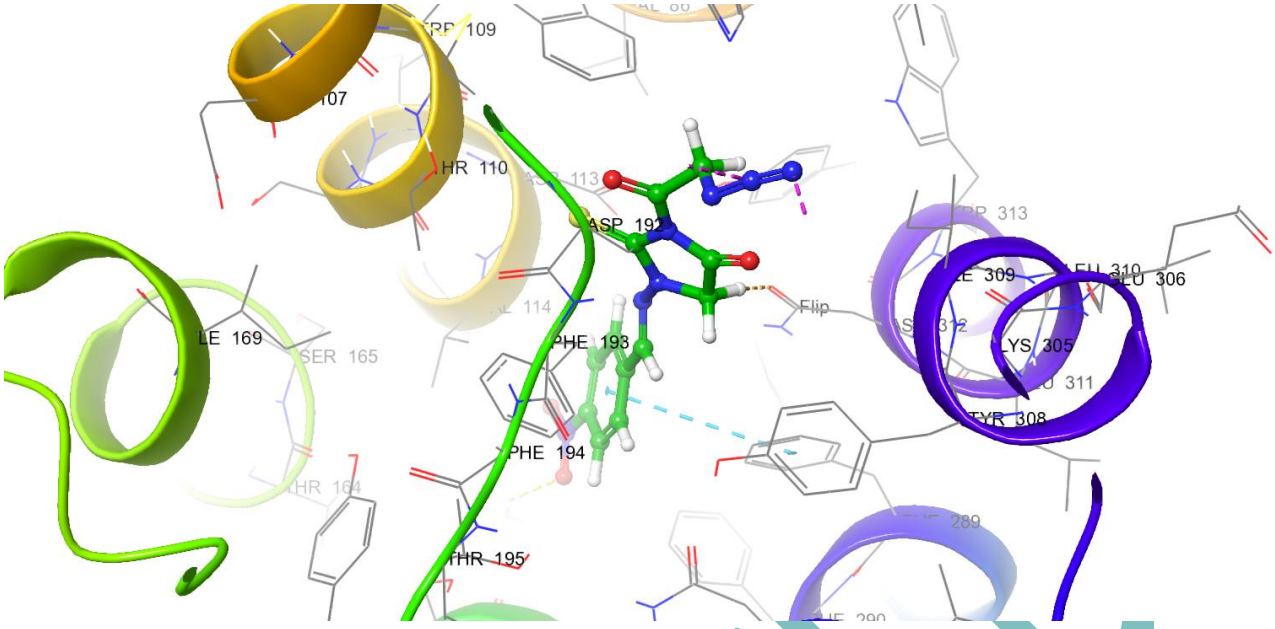


Figure (6): 3D shape of interaction mode of COMPOUND 5) H bond: yellow, bad contact: Orange, Halogen bond: Purple, Green: pi-cation, Sky blue: Pi-Pi Stacking).

The docking results reveal several key insights. Compounds 1, 5, and salbutamol showed the strongest binding affinities, with salbutamol outperforming all other compounds. The hydrogen bonding residue **SER 207** was a common feature among the strongest binders, indicating its critical role in stabilizing ligand-receptor interactions. Non-hydrogen bonding residues like **PHE 289**, **LYS 305**, and **ASP 192** were also frequently involved, emphasizing their importance in overall binding stability.

The functional groups **NO2** (in compounds 1 and 5) and **NC** (in compounds 2 and 6) were effective in facilitating hydrogen bonding, suggesting that such groups could be key structural elements in designing potent ligands. Conversely, compounds like 3 and 7, which lacked sufficient hydrogen bonding interactions, exhibited weaker binding. The role of diverse non-hydrogen interactions was also highlighted, as compounds like 6 and 3, despite moderate binding scores, engaged multiple residues through non-hydrogen bonds.

Salbutamol's superior performance can be attributed to its high number of hydrogen bonds and its ability to interact with multiple residues, showcasing its optimal structure for binding. This makes it an excellent reference for designing new compounds with similar functional groups and interaction profiles. Overall, the results suggest that designing ligands with functional groups capable of forming strong hydrogen bonds with **SER 207** and engaging residues like **PHE 289**, **LYS 305**, and **ASP 192** could lead to potent inhibitors or activators for the target protein.

The binding of beta-2 adrenergic receptor agonists, including Salbutamol and other agonists, were examined. Salbutamol is an ideal reference for assessing the effectiveness and selectivity of other beta-2 receptor agonists due to its high selectivity for the beta-2 receptor over other adrenergic receptors. Research shows that salbutamol binds to the beta-2 receptor in a distinct way, making it crucial for developing specific agonists. The structure and binding features of salbutamol have been extensively studied, providing a solid reference point for comparison. Salbutamol forms precise hydrogen bonds and hydrophobic contacts with key residues in the beta-2 receptor's binding pocket, which are critical in determining the efficacy of new agonists. Additionally, salbutamol has been used in numerous molecular docking and simulation studies as a reference compound due to its well-documented pharmacological properties. Its crystal structure data is available in the Protein Data Bank, enhancing the reliability and stability of comparisons and allowing us to better understand the potential of novel beta-2 receptor agonists.

Molecular Dynamics Simulations

Studying how ligands affect specific proteins through molecular dynamics (MD) simulations is crucial due to the role of conformational stability in theoretical analyses. This research explores the conformational stability of Beta-2 adrenergic receptor agonists, including compound 5 and salbutamol, over a 200-nanosecond period. By evaluating the RMSD of the Beta-2 adrenergic receptor agonist backbone[34], examined the influence of compound 5 on the receptor's structure over time, focusing on changes in conformation and interactions with the ligands. The simulation results provide valuable structural insights into the physical alterations occurring within the protein[35].

The RMSD plot for the ligand showed fluctuations throughout the simulation, indicating conformational modifications occurring within the protein. The RMSD plot for the ligand demonstrated a consistent interaction with the protein, with the ligand's RMSD fluctuations stabilizing around 4.8 Å and the protein's RMSD remaining within 3.6 Å, achieving stability after 75 ns during the MD simulation, as shown in figure (7-A). The stable RMSD values suggest that the ligand retained its conformation throughout the simulation, indicating no major structural changes during this period. This stability reflects strong and reliable binding interactions between the ligand and protein. The stable arrangement of the protein backbone suggests that there were no significant conformational changes or denaturation events affecting the protein structure, this finding further highlights the stability of the ligand-protein complex. In contrast, the salbutamol RMSD plot showed that the system reached stability, with the ligand's RMSD values stabilizing around 2.0 Å and the protein RMSD stabilizing around 5.5 Å as shown in figure (7- B).

The Root Mean Square Fluctuation (RMSF) profile shown in the figure (8) offers a detailed assessment of the flexibility of individual residues in the 7DHI protein structure. The RMSF values, expressed in angstroms (Å), reflect the degree of atomic positional variations during the molecular dynamic simulation.

The majority of residues show RMSF values below 2.5 Å, indicating a generally stable protein structure. However, prominent peaks around residues 180–220 indicate increased flexibility, likely associated with loop regions or solvent-exposed domains, as show in figure (8-A), the green markers highlight particular residues with significantly low RMSF values, indicating their involvement in ligand binding or structural stabilization. These residues show minimal fluctuations, further supporting their role in preserving the integrity of the ligand-protein complex. Notably, residues within the active site remain below 1.5 Å, reinforcing the idea that the binding site maintains its structural rigidity, ensuring stable interactions with the ligand.

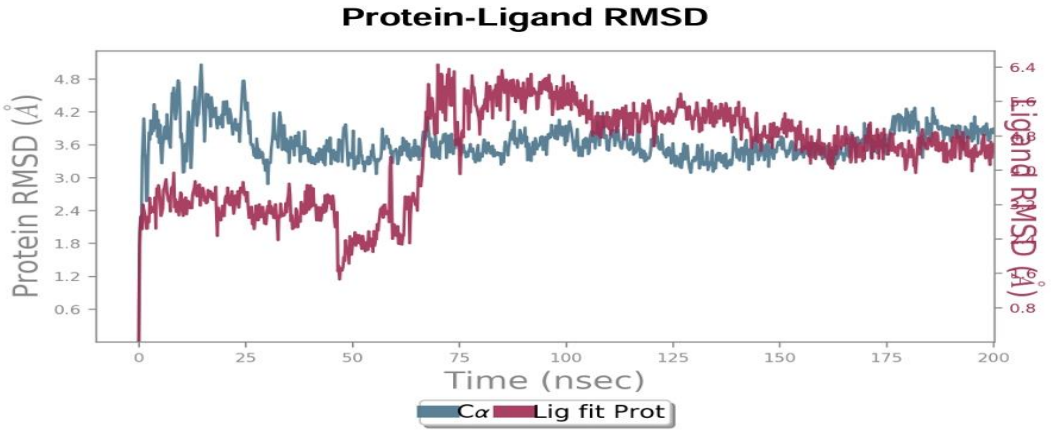
In figure (8-B) show the RMSF of salbutamol indicate Most residues values below 3 Å, indicating a generally stable protein conformation. However, notable peaks around residues 180–220 point to areas of increased flexibility, likely corresponding to loop regions or solvent-exposed segments. The sharp fluctuations in this region suggest dynamic movements that may affect ligand binding or allosteric regulation. In contrast, regions with low RMSF values (below 1 Å) are associated with structurally rigid segments, which could form stable ligand interaction domains.

The analytical evaluation of the RMSF profiles from both simulations reveals differences in protein flexibility and ligand-binding stability. While compound 5 and salbutamol both show overall structural stability, fluctuations are mostly concentrated in the loop regions around residues 180–220. Compound 5 in figure (8-A) shows slightly less flexibility, with lower RMSF values in critical regions and more residues displaying minimal fluctuations, indicating a more rigid and stable ligand-binding conformation. In contrast, the salbutamol in figure (8-B) shows higher peak fluctuations, particularly above 7 Å, suggesting a more dynamic protein structure. These variations may be due to differences in ligand interaction. The lower fluctuations in compound 5 simulation indicate stronger ligand-protein interactions, leading to improved complex stability during the MD simulation. These results highlight the importance of structural rigidity in preserving a well-defined binding conformation, which is essential for the development of effective therapeutic inhibitors.

The interaction between the ligand and MEK was evaluated in figure **14 (B)** with an emphasis on the primary binding forces involved. The findings revealed the existence of hydrogen bonding with the residues THR 110, PHE 139, SER 203, SER 204, and SER 207. Water bridge interaction, which play a crucial role in stabilizing the ligand within the active site. Furthermore, hydrophobic interactions were noted with the residues Leu118, Phe129, Ile141, Met143, Phe209, Ile216, and Met219, which enhanced the incorporation of the ligand. Additionally, Asp190 and Arg189 were recognized as significant contributors to ionic interactions, thereby improving the binding affinity of the complex. Water-mediated interactions were also observed with the residues Asn660, Asn661, Asp663, Gly79, Phe209, Val211, and Asp190.

During the MS simulation, compound 5 exhibited strong binding to the target protein, interacting with most of the key amino acid residues in its active site (figure 9-A). It shares interactions with salbutamol (figure 9-B), particularly with residues such as ASP113, VAL114, PHE193, PHE194, PHE289, PHE290, ASN295, ILE309, and ASN312. These interactions were mediated through hydrogen bonds, hydrophobic contacts, ionic interactions, and water bridges, all of which played a crucial role in maintaining the stability and structural integrity of the ligand-protein complex during the simulation.

A)



B)

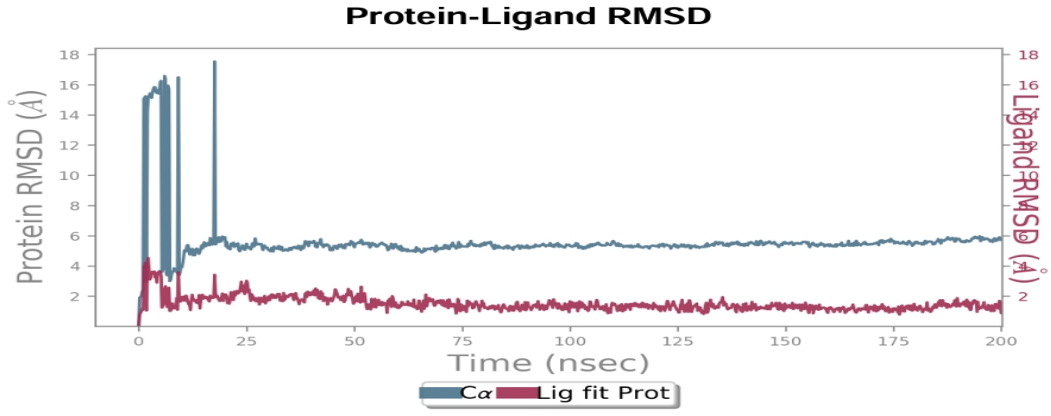
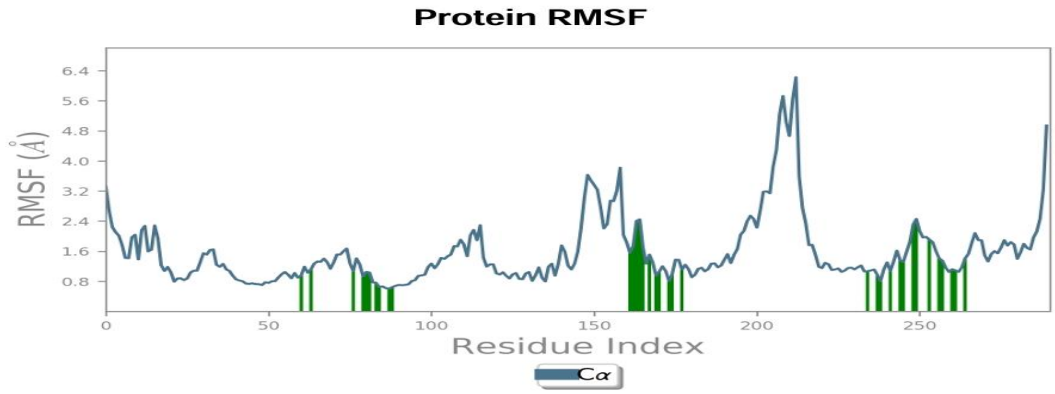


Figure (7): A) compound 5 RMSD plot, B), salbutamol RMSD plot.

A)



B)

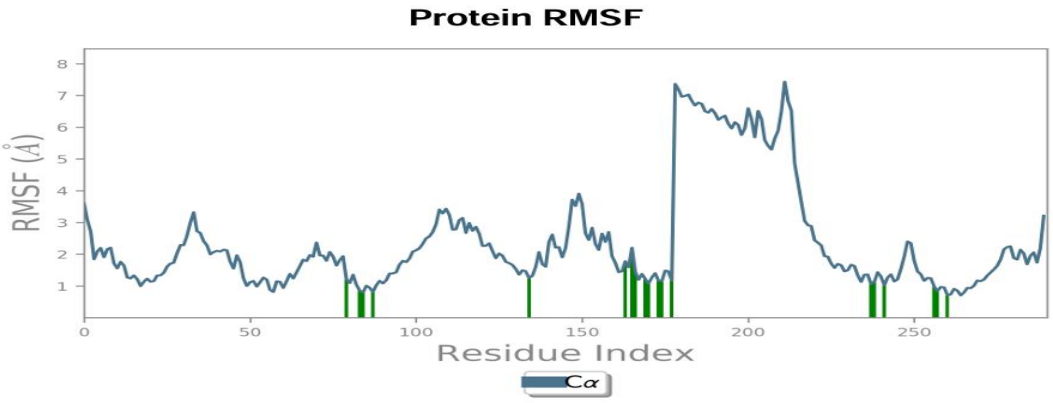
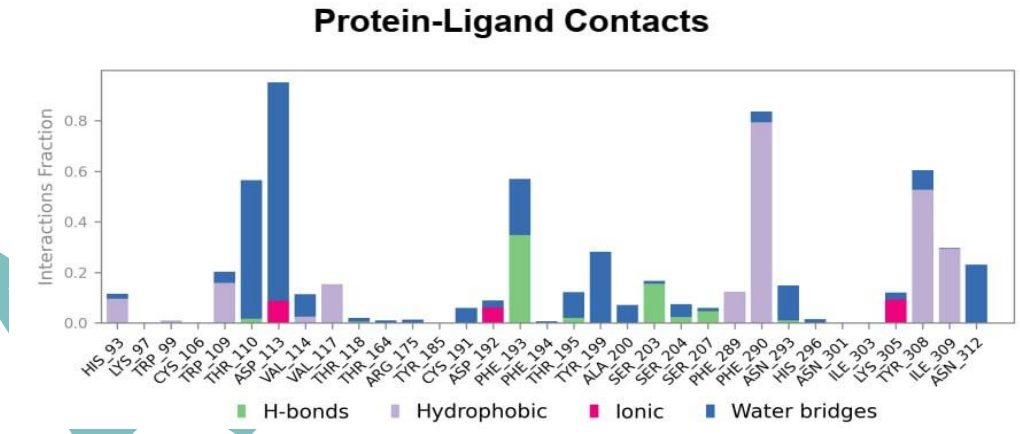


Figure (8): A) compound 5 RMSF, B) salbutamol RMSF.

A)



B)

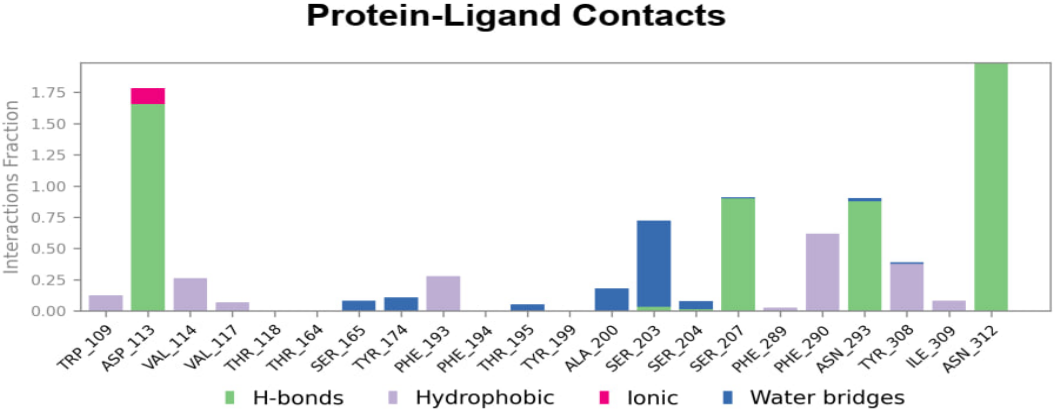


Figure (9): Beta-2 adrenergic receptor -compound 5 (A-B) contacts) explain the proportion of binding interactions through 200 ns of MD Stimulation.

Free Energy of Binding Results

Binding free energy analysis was performed using MM/GBSA to assess the binding affinity and stability of selected compounds with the beta-2 adrenergic receptor (7DHI)[36]. Initial calculations were carried out on the best-docked conformations of each ligand. All tested compounds exhibited favorable binding free energies, confirming their strong potential as effective ligands. Among them, salbutamol (reference), compound 1, and compound 5 showed the most negative binding energies, sugges...

To further validate the docking-based MM/GBSA estimates and incorporate dynamic insights, we extracted representative conformations from molecular dynamics (MD) simulations for compounds 1, 5, and salbutamol. This was achieved using RMSD-based clustering analysis, selecting the most populated cluster and the lowest RMSD conformation within it. MM/GBSA binding free energy was then computed for each extracted structure.

Notably, the MM/GBSA values from these MD-derived conformations closely matched those from the best-docked poses, reaffirming the stability of the complexes. Additionally, per-residue decomposition revealed important dynamic interactions not observed in static docking. For example, salbutamol showed strong interactions with ASP113 and ASN312, while compound 5 exhibited significant contributions from PHE289, ILE309, and LYS305. These residues were added in the revised Table 3.

The Van der Waals energy (ΔG_vdW) and lipophilic interactions (ΔG_Lipo) remained the primary contributors across all systems, consistent with the results from static docking. Hydrogen bonding (ΔG_HBond), while present, had a comparatively smaller role in the overall free energy. as show in table (3)

Table (3): MM-GBSA values of salbutamol and designed compounds (1,2, and 5) in Beta-2 adrenergic receptor (PDB code: **7DHI**).

Compound	ΔG_bind (kcal/mol)	ΔG_vdW	ΔG_Lipo	ΔG_HBond
Salbutamol	-58.45	-41.68	-21.39	-27.61
1	-59.50	-45.45	-23.27	-23.29
5	-57.51	-44.62	-24.92	-31.42
2	-46.23	-42.43	-20.48	-29.82

This observation concludes that (ΔGvdW) and (ΔGLipo) primarily influence the interaction within the compounds and the Beta-2 adrenergic receptor. Nevertheless, hydrogen bonding plays a relatively minor function in this context.

Drug-Likeness Evaluation

Computational prediction of pharmacokinetic properties plays a vital role in accelerating the discovery of promising compounds by enabling the efficient screening of those with suboptimal pharmacokinetic profiles in the early stages of drug design. Key properties such as molecular weight (MW), the number of hydrogen bond acceptors (nHBA), hydrogen bond donors (nHBD), molar refractivity (MR), gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, bioavailability score, and Lipinski's rule violations are crucial indicators for predicting a compound's pharmacokinetic behavior[37, 38].

For instance, compounds with a molecular weight (MW) under 500 g/mol, nHBA ≤ 10, nHBD ≤ 5, and a molar refractivity (MR) in the range of 40–130 are often considered favorable for drug-like properties. Additionally, a bioavailability score of ≥ 0.55 and minimal Lipinski violations (ideally between 0-1) are desired, while QPLOGHERG values below –5 may signal concerns. Moreover, compounds should exhibit appropriate P-glycoprotein (Pgp) affinity, which is an essential factor for their ability to cross biological membranes effectively. These predictive parameters are crucial for optimizing drug design and reducing the risk of failure in later drug development stages[39]. As show in table (4).

Table (4): Computational predictions of the pharmacokinetic properties of the designed compounds.

Compound name	M.wt (g/mole)	n-HBA	n HBD	MR (m3/mol)	GI absorption	BBB permeability	Bioavailability score	Lipinski violation	QPLOGHERG	Pgp
Ideal range	**MW < 500 g/mol**	**nHBA ≤ 10**	**nHBD ≤ 5**	**40 130**	**------**	**-------**	**≥ 0.55**	**0-1**	**concern below –5**	**---**
Salbutamol	239.31	4	4	67.60	High	No	0.55	0	-6.895	No
1	347.31	8	0	92.47	low	No	0.55	1	-5.303	No
2	327.32	7	0	88.36	low	No	0.55	0	-5.647	No
3	381.21	6	0	91.35	High	No	0.55	0	-5.362	No
4	316.34	6	0	88.61	High	No	0.55	0	-4.885	No
5	361.34	8	0	96.72	low	No	0.55	1	-6.084	No
6	327.32	7	0	88.36	low	No	0.55	0	-5.231	No
7	381.21	6	0	91.35	High	No	0.55	0	-4.816	No
8	316.34	6	0	88.61	High	No	0.55	0	-5.782	No

CONCLUSION

Molecular docking results confirm that compounds 1, 5, and salbutamol strongly bind to the beta-2 adrenergic receptor, interacting with key residues like SER207, PHE289, LYS305, and ASP192. Compounds 1 and 5 exhibit additional stability through π-π stacking, salt bridges, and hydrophobic interactions, which compensate for the fewer hydrogen bonds. Functional groups NO2 and NC enhance binding, suggesting their potential as beta-2 agonists. Compound 5 shows the lowest binding free energy, making it a strong candidate for further development, while salbutamol serves as a reference for ligand optimization. RMSD and RMSF analyses confirm complex stability, with transient flexibility in loop regions and a stable binding site. The additional MM/GBSA evaluations based on MD simulations reinforce the reliability of the lead compounds, confirming that the strong binding affinities observed in docking are sustained in dynamic contexts. The presence of additional contributing residues such as ASN312 and ASP113 in salbutamol, and PHE289 and ILE309 in compound 5, suggest favorable and stable interactions suitable for further development as beta-2 adrenergic receptor agonists.

DISCLOSURE STATEMENT

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- **Conflict of Interest:** The authors declare no conflict of interest.
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