Molecular docking, dynamics, and drug-likeness studies of alprazolam derivatives as a potent anxiolytic drug against GABAA receptors

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Abstract

GABAA receptors exhibit permeability to the chloride ion-gated channel, and an increase in excitability disrupts the ion gradients, hence contributing to the development of anxiety-related disorders. This study aims to repurpose potent inhibitors of alprazolam analogs, which were obtained from the PubChem database. These ligands are being investigated for their binding ability to the GABAA receptor. We employed molecular docking through Autodock vina V.4.2 software. The Swiss ADME server was utilized to assess the drug-likeness of the ligands. MDS was conducted using the iMODS platform. Compounds A3, A8, and A9 exhibited a notable binding score range of -8.0 to -8.4 kcal/mol^{-1} with GABAA protein. The drug-likeness analysis revealed that 3 ligands had compliance with Lipinski's RO5. Moreover, the A8 compound can traverse the BBB. In contrast, the A3 and A9 ligands remain localized in the GI region. The MDS of the GABAA receptor with the A8 molecule exhibited higher stability than alprazolam. The current investigation unveiled a highly effective antagonist of GABAA receptors, specifically A8 - 8-chloro-1,4-dimethyl-6-phenyl-4H- [1,2,4] triazolo [4,3-a] [1,4] benzodiazepine. This compound holds potential for future wet-lab experiments, perhaps leading to its utilization in therapeutic applications as an anxiolytic medication.

Keywords: GABAA antagonist, alprazolam, molecular docking, drug-likeness.

Docking molecular, dinâmica e estudos de semelhança com medicamentos de derivados de Alprazolam como um potente ansiolítico contra os receptores GABAA

Resumo

Os receptores GABAA exibem permeabilidade ao canal iônico regulado por cloreto, e um aumento na excitabilidade pode interromper os gradientes iônicos, contribuindo para o desenvolvimento de distúrbios relacionados à ansiedade. Este estudo busca repropor inibidores potentes de análogos do alprazolam, obtidos a partir do banco de dados PubChem. Esses ligantes estão sendo investigados quanto à sua capacidade de se ligar ao receptor GABAA. Utilizamos a docagem molecular por meio do software Autodock Vina V.4.2. O servidor Swiss ADME foi utilizado para avaliar a semelhança com medicamentos dos ligantes. As Dinâmicas de Simulação Molecular (MDS) foram realizadas na plataforma iMODS. Os compostos A3, A8 e A9 apresentaram uma pontuação de ligação notável na faixa de -8,0 a -8,4 kcal/mol^{-1} com a proteína GABAA. A análise de semelhança com medicamentos revelou que esses 3 ligantes estavam em conformidade com a Regra dos 5 de Lipinski (RO5). Além disso, o composto A8 demonstrou capacidade de atravessar a barreira hematoencefálica (BBB), enquanto os ligantes A3 e A9 tendem a permanecer localizados na região gastrointestinal. A MDS do receptor GABAA com a molécula A8 mostrou um nível de estabilidade superior ao do alprazolam. A investigação atual revelou um antagonista altamente eficaz dos receptores GABAA, especificamente o A8 - 8-cloro-1,4-dimetil-6-fenil-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepina. Este composto apresenta potencial para futuros experimentos laboratoriais e possivelmente para sua utilização em aplicações terapêuticas como medicamento ansiolítico.

Palavras-chave: antagonista GABAA, Alprazolam, *docking* molecular, semelhança com medicamentos.

1. Introduction

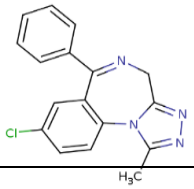
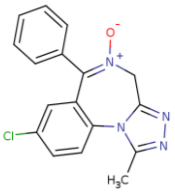
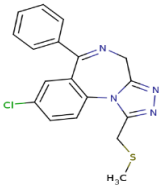
Anxiety frequently manifests itself in response to traumatic experiences or circumstances (Reiman et al., 1979). GABA receptors are the major regulatory inhibitory neurotransmitters in the central nervous system (CNS). They play a crucial role in the neurobiology of anxiety and are molecular targets in the therapy of a variety of anxiety disorders (Granger et al., 2005; Kent et al., 2002). GABA receptors play an essential role in the treatment of numerous anxiety disorders. Anywhere from 20 to 50 percent of synapses in the CNS are made up of GABAergic receptors (Bloom et al., 1971). The processes of GABA release are the same in both peripheral and central regions (Erdo et al., 1985). GABA receptors are ionotropic, GABAA, and GABAC receptors, whereas GABA B receptors are metabotropic (Nayeem et al., 1994). GABA A receptors can be found in both pre-and post-synaptic locations. These regions function as target sites lined up with the opening of the chloride ion channel (Gee et al., 1987; Azad et al., 2024a). Increased subjective anxiety and heart rate both have the effect of changing the blood flow and electrical activity linked with anxiety in the cortical areas (Chua et al., 1999).

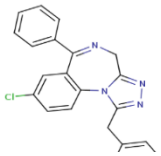
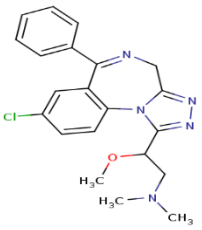
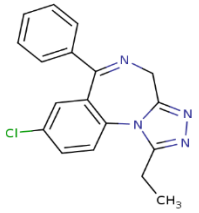
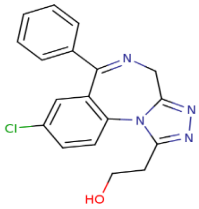
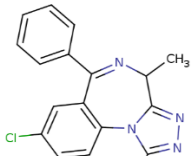
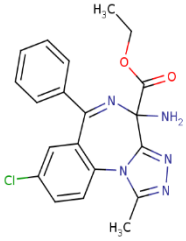
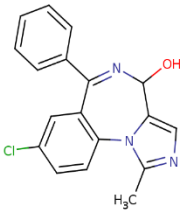
A connection between the GABAA receptor and benzodiazepines has been found (Vijayan et al., 2012; Praveen et al., 2024). This contact is part of a macromolecular complex related to binding sites for barbiturates, benzodiazepines, and steroids to the chloride ionophore (Brioni et al., 1986; Saano, 1987; Schwartz, 1998). Researchers demonstrated that benzodiazepines are powerful anxiety-relieving drugs that also engage in a wide variety of processes connected to the central nervous system (CNS) (Riba et al., 2001; Griffin et al., 2013; Praveen, 2024b; Praveen, 2024c).

Alprazolam (ALP) is a benzodiazepine derivative that has a 1,2,4-triazole fused to it. It is utilized as an antidepressant and an anxiolytic medication, and it binds with a high affinity to the benzodiazepine site (Renard et al., 1999). According to research conducted in clinical settings, ALP may be useful in the treatment of conditions such as agoraphobia, depression, and pain disorders (Junior et al., 2015). Poor compliance with the anxiety therapy and unpleasant side effects are the issues linked with it. ALP demands a dosage of three to four times daily in consideration to maintain reconcilable therapeutic help.

Considering the abovementioned statements, we utilized Insilco methodologies such as molecular docking, dynamics, and drug-likeness using ALP and its analogs (Table 1) with the GABAA receptor. Because each functional group has its applications, this study elucidates the potential ligand for adding side chains to the previously existing drug ALP without changing the prescribed drug ALP. First, the better binding affinity of the receptor-ligand complexes and dynamic simulations are defined. Then, the rule of five (RO5) is used to validate the drug-likeness of the ligands. These studies shall help to evaluate the therapeutic potential of ALP with modifications.

Table 1. Accession ID, IUPAC name, chemical formula of alprazolam, and respective anal

Compound	PubChem ID	IUPAC name	SMILES	
Alprazolam	2118	8-chloro-1-methyl-6-phenyl-4H- [1,2,4] triazolo [4,3-a] [1,4] benzodiazepine	CC1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4	
A1	327554	8-chloro-1-methyl-5-oxide-6-phenyl-4H- [1,2,4] triazolo[4,3-a] [1,4] benzodiazepine-5-ium	CC1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=[N+](C2)[O-])C4=CC=CC=C4	
A2	1032811	8-chloro-1-(methylsulfanylmethyl)-6-phenyl-4H-[1,2,4] triazolo[4,3-a] [1,4] benzodiazepine	CSCC1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4	

A3	1032832	N-[(8-chloro-6-phenyl-4H-[1,2,4] triazolo[4,3-a] [1,4] benzodiazepin-1-yl) methyl] methanesulfonamide	CS(=O)(=O)NCC1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4	
A4	12560611	1-benzyl-8-chloro-6-phenyl-4H- [1,2,4] triazolo [4,3-a] [1,4] benzodiazepine	C1C2=NN=C(N2C3=C(C=C(C=C3)Cl)C(=N1)C4=CC=CC=C4)CC5=CC=CC=C5	
A5	12560615	2-(8-chloro-6-phenyl-4 H- [1,2,4] triazolo[4,3-a] [1,4] benzodiazepine-1-yl)-2-methoxy-N, N-dimethylethanolamine	CN(C)CC(C1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4)OC	
A6	12560621	8-chloro-1-ethyl-6-phenyl-4H- [1,2,4] triazolo [4,3-a] [1,4] benzodiazepine	CCC1=NN=C2N1C3=C(C=C(C=C3)Cl)C(=NC2)C4=CC=CC=C4	
A7	12562508	2-(8-chloro-6-phenyl-4 H- [1,2,4] triazolo[4,3-a] [1,4] benzodiazepin-1-yl) ethanol	C1C2=NN=C(N2C3=C(C=C(C=C3)Cl)C(=N1)C4=CC=CC=C4)CCO	
A8	12632256	8-chloro-1,4-dimethyl-6-phenyl-4H- [1,2,4] triazolo [4,3-a] [1,4] benzodiazepine	CC1C2=NN=C(N2C3=C(C=C(C=C3)Cl)C(=N1)C4=CC=CC=C4)C	
A9	12632257	ethyl 4-amino-8-chloro-1-methyl-6-phenyl- [1,2,4] triazolo[4,3-a] [1,4] benzodiazepine-4-carboxylate	CCOC(=O)C1(C2=NN=C(N2C3=C(C=C(C=C3)Cl)C(=N1)C4=CC=CC=C4)C)N	
A10	12802040	8-chloro-1-methyl-6-phenyl-4H-imidazo[1,5-a] [1,4] benzodiazepin-4-ol	CC1=NC=C2N1C3=C(C=C(C=C3)Cl)C(=NC2O)C4=CC=CC=C4	

Note: *A – Analogue, IUPAC – International Union of Pure and Applied Chemistry (https://iupac.org/).

2. Materials and Methods

2.1. Protein preparation

The crystal structure of the GABAA receptor complexed with a dibenzodiazepine (DZP) determined using electron microscopy with a resolution of 2.92 A^0 was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), PDB ID-6X3X (https://www.rcsb.org/). The protein that was obtained exhibits the presence of additional components. A drug discovery studio was used to remove any present ligands, water molecules, and heteroatoms to obtain the raw protein structure for drug discovery purposes.

The structure underwent repair by incorporating missing atoms, bonds, Kollman charges, and polar hydrogen atoms. This was achieved using the Autodock version 4.2 program (Goodsell et al., 1996). The restored structure was then saved in PDBQT format to facilitate additional docking experiments. The DZP ligand serves as the fundamental structure of alprazolam. Therefore, the grid layouts of the system were employed for docking. The grid dimensions of the Drug Discovery Studio were maintained at X = 36, Y = 36, and Z = 38, with the grid center located at X = 106.78, Y = 109.41, and Z = 23.75.

2.2. Ligand preparation

The 3D structures of the inhibitor Alprazolam (2118) and its analogs (327554, 1032811, 1032832, 12560611, 12560615, 12560621, 12562508, 12632256, 12632257, and 12802040) were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al., 2016). The structures were retrieved in SDF MOL format and subsequently converted into PDB format using Open Babel (O'Boyle et al., 2011) to facilitate the creation of ligand binding groups. Additionally, the characteristics of the ligand, including torsional bonds, steric hindrance, and appropriate bond ordering, were examined and stored in PDBQT format using AutoDock (Trott and Olson, 2009).

2.3. In-silico drug-likeness analysis

To estimate the drug-likeness of the ligand, various physicochemical parameters were calculated using SwissADME (http://www.swissadme.ch/) (Daina et al., 2017). These parameters included molecular weight (MW), the number of donor and acceptor hydrogen bonds (HA and HD), solubility (log S), topological polar surface area (TPSA), octanol-water partition coefficient (log Po/W), permeability in Madin-Darby Canine Kidney (PMDCK) cells, and blood-brain barrier (BBB) penetrability.

2.4. Molecular dynamics simulations (MDS)

Several studies have employed iMODS, a tool for conducting molecular dynamics simulations (Pokharkar et al., 2022; Zaib et al., 2022; Aldossari et al., 2023; Bhattacharya et al., 2022). The approaches employed in the present study for conducting molecular dynamics (MD) simulations are consistent with those referenced. The protein-ligand complexes underwent molecular dynamics simulations to conduct regular mode analysis of internal coordinates using iMODS (https://imods.iqfr.csic.es/). The validation process involves various methods, including deformability, B-factor, variance, and eigenvalue graph analysis. These methods were applied to the coarse-grained representation of the atomic model, specifically focusing on the protein backbone carbon alpha (CA) atoms.

The primary objective was to define the dihedral angles of the protein backbone. A harmonic potential energy model was employed specifically the standard mode analysis (NMA) approach. Additionally, clusters and deformities are defined at 40 modes for computational purposes.

3. Results and Discussion

3.1. Molecular docking

We employed our previously established docking methodologies in this study (Praveen and Morales-Bayuelo, 2023). The compounds Alprazolam, A1, A2, A3, A4, A5, A6, A7, A8, A9, and A10, along with their interactions and the binding scores with 6X3X, are listed in Table 2. The binding values of ligands A3, A8, and A9 were higher than that of Alprazolam (-7.7 $kcal/mol^{-1}$). Their interactions with the receptors are depicted in (Figure 1). Hydrogen bonds are pivotal in drug binding, one of the most significant interactions required for strong binding, enhancing free energy values (Wade & Goodford, 1989).

Table 2. The docking scores of the compounds and their interacting amino acid residues of the GABAA receptor.

Compound	B.A. (k.cal/ mol)	Hydrogen Bonds		
		CHB	CaHB	Π-DHB
Alprazolam	-7.7	E THR 146	-	E THR 73
				E THR 146
A3	-8.1	D LYS 105	-	E THR 73
		E THR 146		E THR 146
		^{2}E ARG 197		
A8	-8.4	^{2}E THR 146	E THR 146	D LYS 105
				E THR 73
				E THR 146
A9	-8.0	B THR 268	-	^{2}C THR 263
		C THR 263		

Note: B.A.-Binding Affinity; K. Cal/mol^{-1}- Kilo Calorie per mole; CHB-Conventional Hydrogen Bond; CaHB-Carbon Hydrogen bond; Π- Cation- Pi Cation; Π-DHB- Pi Donor Hydrogen Bond; Π-Sigma-Pi Sigma; Π-Alkyl-Pi Alkyl. Source: Authors, 2024.

The drug alprazolam has a docking score of -7.7 kcal/mol^{-1} with the GABAA receptor. It had formed two types of hydrogen bonds with two residues. Among the hydrogen bond interactions, the residue E THR 146 formed a CHB by donating hydrogen atoms to the acceptor at the nitrogen atom of the ligand. A Π-DHB was formed between E THR 73 and E THR 146 by donating a hydrogen atom to the pi orbitals of the ligand.

Ligand A3 exhibited -8.1 kcal/mol of binding affinity. The complex has formed hydrogen bond interactions with six residues. Among these, four were CHBs and two Π-DHBs. Two amines end of E ARG 197 and one amine of D LYS 105 formed three CHBs with the oxygen atoms of the A3, and another CHB was formed between the E THR 146 hydrogen donating atom to the A3's hydrogen acceptor. E THR 73 and E THR 146 on donating the hydrogen to the A3 pi-orbitals formed two Π-DHBs.

A8 stood a higher docking score of -8.4 kcal/mol^{-1} compared to the alprazolam and the rest of the analogs with the GABAA receptor. E THR 146 is involved in forming two CHBs, a CaHB and a Π-DHB with the A8 ligand. E THR 73 and D LYS 105, by donating the hydrogen to the pi orbitals of the ligand, formed two Π-DHBs. A9 ligand exhibited -8.0 kcal/mol^{-1} of the binding score, and two amino acid residues, B THR 268 and C THR 263, were involved in hydrogen bond interactions, where two CHBs and two Π-DHBs interactions were observed.

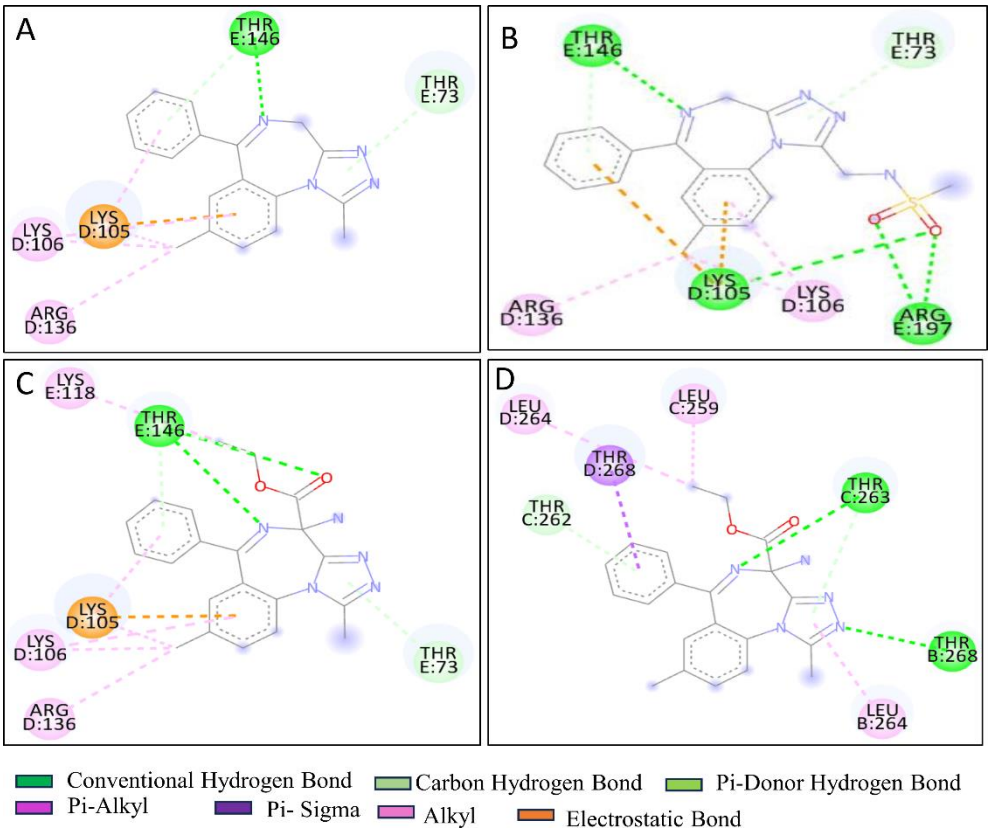


Figure 1. 2D interaction images of the ligands A - Alprazolam, B - A3, C - A8, and D - A9 with GABAA receptor residues. Source: Authors, 2024.

3.2. In-silico drug-likeness analysis

A list of nine characteristics linked with drug-likeness qualities of the ligands that were analyzed using SWISS ADME (http://www.swiss-adme.ch/) is mentioned in Table 3. According to Lipinski's rule of five (RO5), a drug molecular weight of less than 500 g/mol^{-1}, a hydrogen bond acceptor count of less than five, a hydrogen bond donor number of less than ten, and an octane/water partition ratio that falls within the range of -2 to 5 (Lipinski, 2004). Compounds with TPSA of less than 120[A^O]^2/mol^{-1} indicate the orally active drug transport root, whereas a value of less than 100[A^O]^2/mol suggests that there is good brain penetration of the CNS drug (Ertl et al., 2000). A PMDCK value of less than 25 indicates that the cell permeability is low, whereas a value of more than 500 shows that the cell permeability is high (Irvine et al., 1999). The apparent solubility might range from -6.0 to -0.5 (Lipinski, 2000).

In addition, the MW of every compound fell somewhere in the range of 401.87 (A3) to 395.89 (A5). Both A1-10 (analogs) and alprazolam have MW values of less than 500, which means that Lipinski's RO5 is satisfied. The compound alprazolam, which serves as a reference, had three HBAs. Compounds A1, A2, A4, A6, A8, and A10 that include three HBA, including HBA's A7 (4), A3 and A4 (5). Only the HBDs of A3, A7, A9, and A10 have one hydrogen donor each. The HBDs of A1, A2, A4, A5, A6, and A8 do not have any hydrogen donors. There is no hydrogen donor present in alprazolam. HBA and HDB of all the analogs and Alprazolam obey Lipinski's RO5.

Log P_0/W value ranges from 1.56 (A1) to 3.68 (A4). None of the compounds met the criterion for drug-likeness and did not even come close to exceeding or falling below the thresholds. The TPSA values that have been deduced fall within a range that extends from 97.62[A^O]^2/mol (A3) to 43.07[A^O]^2/mol (A4, A6, and A8). The amounts of AO found in compounds A1, A2, A5, A7, A9, and A10 were 59.46 [A^O]^2/mol, 68.37 [A^O]^2/mol, 55.54 [A^O]^2/mol, 63.30 [A^O]^2/mol, 95.39 [A^O]^2/mol, and 50.41 [A^O]^2/mol, respectively. The TPSA value for alprazolam is 43.07 [A^O]^2/mol. The log S values for the compounds were from -4.27 (A4) to -2.25 (A5). In addition, the value of the alprazolam was -3.60. All the compounds had a solubility and PMDCK range in the same ballpark as the drug-likeness values.

The BOILED-Egg model, developed by Dania and Zoete (2016) and displayed in (Figure 2), demonstrated that the ligands were passively absorbed via the gastrointestinal tract in the white region. This process is known as

human intestinal absorption (HIA). Molecule A3 and molecule 10 (A9) are compounds that cannot pass the BBB. The ability to penetrate the blood-brain barrier was demonstrated by the remaining ligands A1, A2, A4-8, A10, and alprazolam. Every ligand exhibited some level of permeability to the glycoproteins evaluated from the CNS (shown by the blue dots). Analogs of ligands A3 and A9 do not appear to be able to cross the blood-brain barrier. The remaining substances, including alprazolam, showed the ability to penetrate the blood-brain barrier (BBB). As shown in Figure 2, the BOILED-Egg model does not account for eliminating any ligands alprazolam (A1-A10) from the CNS.

Table 3. Chemical compounds physiochemical properties by SWISS ADME.

Ligands	Mol. Wt (gm/mol^{-1})	HBA	HBD	Log P_O/W	TPSA	Log S	PMDCK
Alprazolam	308.76	3	0	2.12	43.07	-3.60	High
A1	324.76	3	0	1.56	59.46	-2.42	High
A2	354.86	3	0	3.32	68.37	-3.27	High
A3	401.87	5	1	1.93	97.62	-2.29	High
A4	384.86	3	0	3.68	43.07	-4.27	High
A5	395.89	5	0	2.65	55.54	-2.25	High
A6	322.79	3	0	3.31	43.07	-3.10	High
A7	338.79	4	1	2.49	63.30	-2.26	High
A8	322.79	3	0	3.41	43.07	-3.07	High
A9	395.84	5	1	2.59	95.39	-3.20	High
A10	323.78	3	1	3.10	50.41	-4.00	High

Note: Mol. Wt-Molecular weight (gm/mol)-gram per molecule; HBA-Hydrogen Bond Acceptors; HBD–Hydrogen Bond Donors; TPSA-Topological Polar Surface Area; Log S-Logarithm of Solubility; Log Po/W–Logarithm of Octane/Water partition; PMDCK-Permeability Maden Darby Canine Kidney; BBB- Blood Brain Barrier. Source: Authors, 2024.

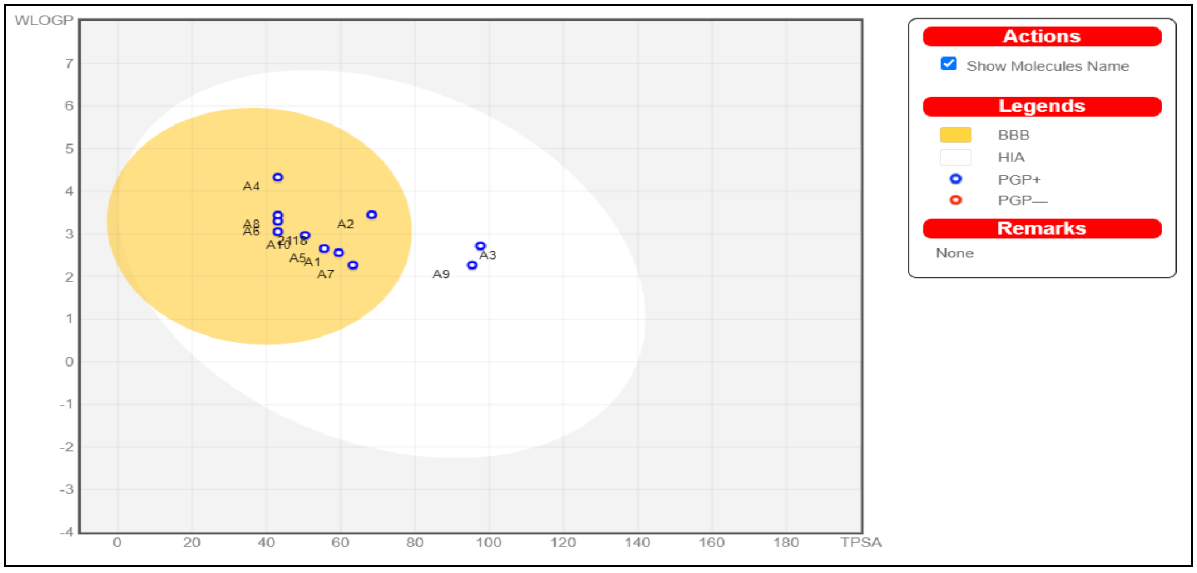


Figure 2. BOILED-Egg model representing ligands capable of crossing the Blood blood-brain barrier validated by Swiss ADME (http://www.swissadme.ch/). BBB-Blood Brain Barrier; HIA- Human Intestinal Absorption; PGP^+ - Permeability through Glycoprotein; PGP^- - No Permeability through Glycoprotein. Molecule 1-Alprazolam, Molecule 2- A1, Molecule 3- A2, Molecule 4- A3, Molecule 5- A4, Molecule 6- A5, Molecule 7-A6, Molecule 8- A7, Molecule 9- A8, Molecule 10- A9, Molecule 11- A10

3.3. Molecular dynamic simulations

Two complexes, namely the GABAA receptor with alprazolam and the GABAA receptor with A8 compounds, were selected to perform MD simulations using iMODS to evaluate the internal coordinates of the complexes through normal mode analysis. The main chain's deformability indicates each residue's deform capability value, which is the occurrence of the chain 'hinges' locations pointing to high deformability regions. The experimental B-factor is considered from the PDB, and the NMA is calculated by multiplying the NMA mobility, which denotes the average root mean square. The eigenvalue is related to individual normal mode motion stiffness representation, and its value is directly related to the structure deformation energy required. A lower value signifies the deformation is simpler. The variance is inversely associated with the eigenvalue of every normal mode. Colored bars illustrate the individual (red) and cumulative (green) variances.

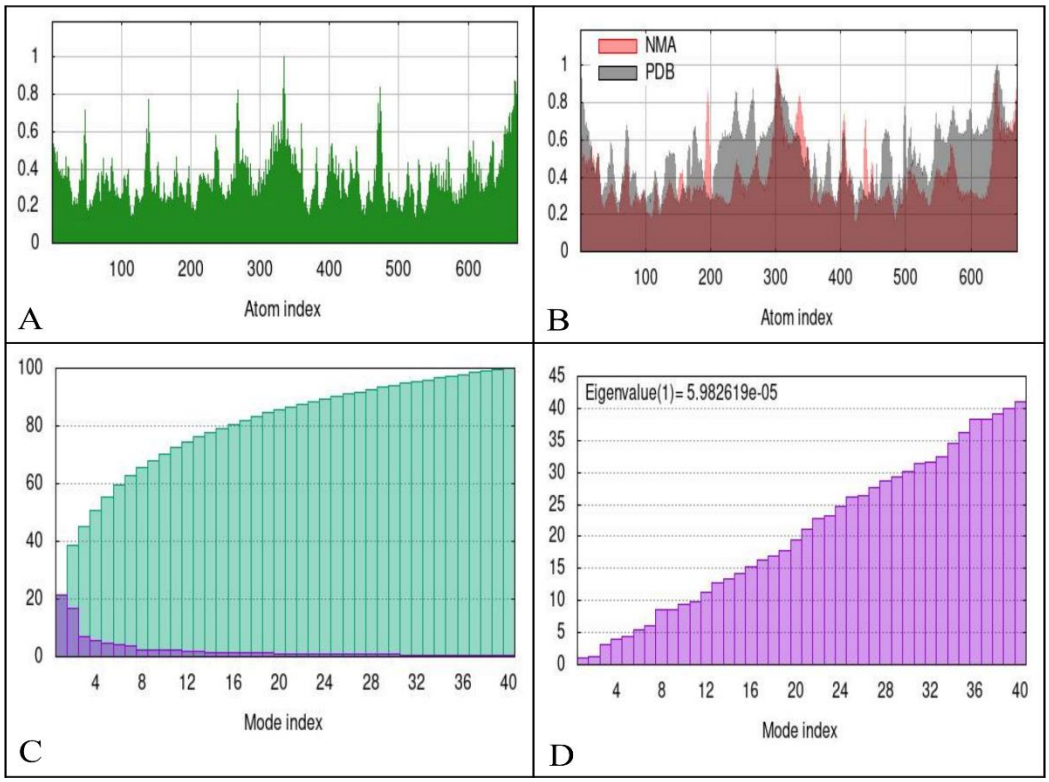


Figure 3. The Molecular Dynamics Simulations of the 6X3X with Alprazolam, A-Deformability; B-B-factor; C-Variance; D-Eigenvalue. Source: Authors, 2024.

The deformity of the main-chain graph in Figure 3A indicates the deformation of all residues from low to high. Hinges represent the GABAA receptor interacting regions with alprazolam. Peaks in B-factor graphs obtained values shown in Figure 3B are almost similar. The individual variance level is higher than 20% in Figure 3C, indirectly decreasing the eigenvalue. The obtained eigenvalue of this complex was 5.9*10-5 in Figure 3D, which indicates that the interaction between the protein and ligand was stable.

The main-chain deformity graph in Figure 4A indicates high deforming residues. Hinges are the interacting regions of the GABAA receptor with A8, B-factor graphs in (Figure 4B) obtained peaks were highly similar. 24% of the higher individual variance is observed in Figure 4C, which indirectly decreases the eigenvalue. The eigenvalue of the complex was $5.1*10^{-5}$ in (Figure 4D), suggesting the stable interactions in the complex.

Hester et al.,1980, conducted research on the QSAR of benzodiazepine compounds by varying the substituents present at the 4th carbon position. 8-chloro-1,4-dimethyl-6-phenyl-4H-[1,2,4] triazolo [4,3-a] is the chemical formula for A8 (PubChem ID: 12632256). [1,4] Benzodiazepine exhibited a greater binding affinity (Table 2), did not break Lipinski's rule of five (Table 3), and was able to pass the blood-brain barrier (Table 3, Figure 2). Therefore, the design of pharmaceuticals ought to focus on the 4th carbon site of the benzodiazepine compounds to become a powerful anxiolytic drug. This will guide better drugs toward suppressing the activity of the GABAA

receptor.

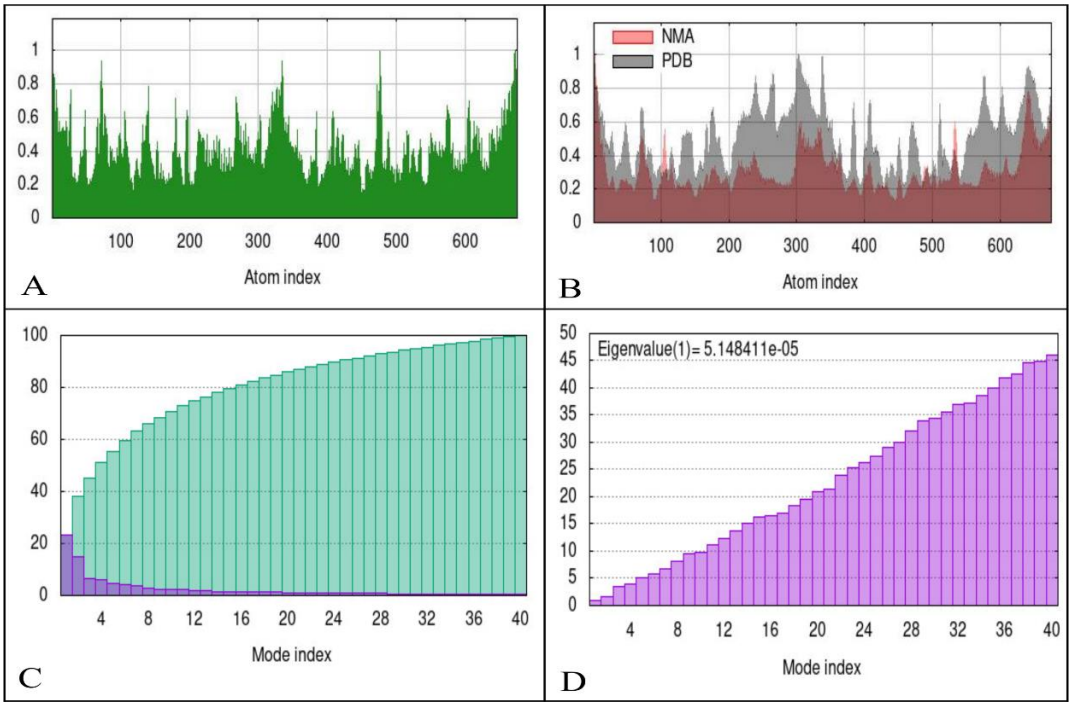


Figure 4. The Molecular Dynamics Simulations of the 6X3X with A8, A-Deformability; B-B-factor; C-Variance; D-Eigenval. Source: Authors, 2024.

4. Conclusions

The GABAA receptor is responsible for anxiolytic effects; the inhibitory drug alprazolam and its analogs' antagonist activity was discovered through computational tools. Ten different analogs of alprazolam were retrieved from PubChem. Among all the ligands, the docking scores of the three compounds A3 (-8.1 kcal/mol^{-1}), A8 (-8.2 kcal/mol^{-1}), and A9 (-8.0 kcal/mol^{-1}) were higher than that of the reference substance alprazolam (-7.7 kcal/mol^{-1}). All the ligands adhered to Lipinski's rule of five, an indicator of drug-likeness. While A8 can cross the blood-brain barrier (BBB), A3 and A9 cannot enter the central nervous system (CNS) due to efflux. Therefore, A8 (8-chloro-1,4-dimethyl-6-phenyl-4H-[1,2,4] triazolo [4,3-a] [1,4] benzodiazepine) demonstrated greater binding affinity, as well as drug-like properties that allow it to penetrate the BBB. The GABAA receptor complexed with the A8 molecule exhibited relatively more substantial stability in molecular dynamics (MD) simulations. Wet laboratory experiments are needed to validate the predictions made in this research.

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6. Authors' Contributions

Vijay Paramanik: designed the research, and wrote the final version of the manuscript. *Mallari Praveen*: designed the research, performed research, wrote the first draft, and wrote the final version of the manuscript.

7. Conflicts of Interest

The authors declare no conflict of interest.

8. Ethics Approval

Not applicable.

9. References

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