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Molecular Docking studies of Benzodiazepine Derivatives with GABA (B) Receptor

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Abstract

Benzodiazepines (BZDs) are pharmacologically significant compounds that act by binding to GABA A neurotransmitter receptors, subsequently augmenting GABA-induced chloride ion flux, consequently inducing neuronal hyperpolarization. Benzodiazepines are commonly used in the treatment of sleep disorders, anxiety, muscle spasms, seizure disorders, and some forms of depression. The primary inhibitory neurotransmitter, GABA, functions on two types of receptors: the ligand-gated GABA A/C receptors and the G protein-coupled GABA B receptors. These neurotransmitters play crucial roles in modulating numerous synapses, influencing both pre- and post-synaptic activity, and remain noteworthy targets for the treatment of various brain disorders, including addiction. Since both the GABA A and GABA B are subunits of the same receptor, binding affinity of BZDs to GABA B receptor have been assessed using docking approach in present work. Some of the most commonly used BZDs were preferred for docking and studies showed that they have got the affinity to bind with GABA B receptor too. This study opens a new path for repurposing BZDs for variety of pharmacological actions as well as for researchers to work in this new direction.

Keywords: Benzodiazepines, GABA A & B, Docking, Anxiety

INTRODUCTION

Gamma-aminobutyric acid (GABA) acts as the primary inhibitory neurotransmitter within the central nervous system (CNS). Derived from glutamate, it is distributed across approximately one-third of all synapses and is ubiquitous throughout the CNS. Acting as an inhibitory transmitter, GABA induces hyperpolarization of the neuronal cell membrane at the cell body level. Additionally, in certain circumstances, GABA exhibits presynaptic effects on synaptic boutons. Given its concentration, widespread distribution in the brain and spinal cord, and the fact that virtually all neurones can be hyperpolarised by GABA, it was believed initially that side-effects would preclude the development of GABAergic drugs because they would, presumably, modify virtually all central nervous system functions. GABA interacts with members of two families of GABA receptors: GABA A and GABA B.

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GABA interacts with members of two families of GABA receptors: GABA A and GABA B [1]. GABA-A receptors function as ligand-gated channels, facilitating the movement of chloride ions across the neuronal membrane in accordance with their electrochemical gradient, typically around -65 mV. Typically, this results in hyperpolarization of the cell membrane. However, there are instances where GABA A receptors induce neuronal depolarization, leading to excitation rather than inhibition. This occurs when there is an elevation in chloride concentration within the neuron, a phenomenon observed during CNS development. In adult neurons, intracellular chloride concentration is maintained at a lower level [2, 3].

GABA B receptors, classified as metabotropic receptors, elicit their effects through the activation of G-proteins, subsequently leading to the inhibition of neuronal activity. Due to the involvement of intricate signaling pathways mediated by G-proteins, the kinetics of G-protein-mediated events are comparatively slower than those mediated by ion channels. The discovery of GABAB sites advances have led to a better definition of the GABAB receptor and have helped define the therapeutic potential of compounds that interact with this site. The dysfunction of the GABA system has been linked to the underlying mechanisms of several neuropsychiatric disorders, particularly anxiety and depression. Nevertheless, the specific contribution of GABA B receptors to the behavioral processes associated with these disorders remains unresolved [4]. GABA B receptors are classified as G-protein-coupled receptors, which operate as heterodimers comprising GABA B(1) and GABA B(2) subunits. Alongside highly specific agonists and antagonists, recent advancements have led to the development of novel tools targeting GABA B receptors, facilitating a deeper understanding of their involvement in central nervous system (CNS) function [5].

The discovery of GABA B receptors has opened new possibilities for the development of chemically and mechanistically novel therapeutic agents for the treatment of conditions ranging from asthma to memory impairment. While significant progress has been made regarding the chemistry and biology of this system, much work remains to exploit these findings. Ionotropic (GABA A) and metabotropic (GABA B) receptors exhibit widespread expression throughout the central nervous system [6–9]. The GABA A receptor ($GABA_AR$) is recognized for its close involvement in the acute stress response, with clinically relevant anxiolytic drugs such as benzodiazepines exerting their effects through this receptor. Conversely, evidence supporting a role for the GABA B receptor in anxiety has emerged more recently. Compounds targeting both GABA A and GABA B receptors generally demonstrate efficacy in attenuating the stress-induced hyperthermia (SIH) response. SIH, characterized by its unconditioned, consistent, and robust nature, serves as a valuable paradigm for evaluating the effects of anxiolytic drugs on both the SIH response itself and basal body temperature [10–14].

Benzodiazepines are a group of psychoactive substances distinguished by a fundamental chemical structure that combines a benzene ring with a diazepine ring. These compounds primarily exert their pharmacological effects by modulating gamma-aminobutyric acid (GABA), which serves as the principal inhibitory neurotransmitter in the central nervous system [15]. Among the most prominent agents in this pharmacotherapeutic class is diazepam, renowned for its anxiolytic, sedative-hypnotic, and anticonvulsant properties. Additionally, midazolam finds utility in preoperative settings for its sedative-anxiolytic effects coupled with anterograde amnesic properties. Benzodiazepines are frequently prescribed to alleviate symptoms associated with insomnia or anxiety, with insomnia being the most prevalent indication, particularly among older individuals. Some benzodiazepines can be used to treat health conditions such as epilepsy or for sedation before a procedure or operation. The most common benzodiazepines prescribed are nitrazepam, diazepam, oxazepam and alprazolam. Other benzodiazepines available are bromazepam, clobazam, clonazepam, flunitrazepam, lorazepam, and triazolam [16, 17].

Substantial evidence from electrophysiological, biochemical and behavioural studies suggests that many clinically relevant effects of benzodiazepines are mediated by their binding to a discrete high-affinity binding site located on the GABAA receptor, ultimately leading to an enhanced GABAergic inhibition in the central nervous system. While this allosteric modulation by BDZ of the GABAA receptor function has extensively been studied in the last few years, much less is known about the effects of BDZ which may be unrelated to GABA and about the relevance of these effects for the therapeutic actions of BDZ. Some factors those are probably responsible for this apparent discrepancy may include the rationale of a framework hypothesis (GABA enhancement) which explains the manifold therapeutic effects of BDZ by a unitary mechanism; pharmacological means which might be used to relate GABA-independent effects of BDZ to their diverse therapeutic actions are very limited; The absence of a congruent hypothesis summarizing and elucidating the distinct therapeutic actions of benzodiazepines (BDZ) in terms of their GABA-unrelated effects presents a notable challenge. While the primary

mechanism of action of BDZ involves potentiation of GABAergic neurotransmission via allosteric modulation of GABA A receptors, emerging evidence suggests the existence of GABA-independent pathways contributing to their therapeutic effects [18–20].

There is a mounting body of evidence underscoring the critical involvement of GABA B receptors in anxiety regulation. Baclofen, the prototypical GABA B receptor agonist, has exhibited anxiolytic efficacy across various clinical contexts. Studies have reported reductions in anxiety among individuals with post-traumatic stress disorder (PTSD), recovering alcoholics undergoing alcohol withdrawal, panic disorder patients, and those with acute spinal trauma following administration of baclofen. Moreover, preclinical investigations have corroborated these findings, demonstrating anxiolytic effects of baclofen in diverse paradigms such as ultrasonic vocalization in rat pups, decreased punished drinking behavior, and altered performance in the elevated plus maze and social interaction tests, particularly upon withdrawal from either diazepam or alcohol dependence. Notably, compelling evidence supporting the involvement of GABA B receptors in anxiety stems from studies utilizing GABA B receptor-deficient mice, further emphasizing the significance of these receptors in anxiety-related processes [21, 22]. Therefore keeping in mind the previous findings of researchers and through literature, it has been thought to assess the binding affinity of reported marketed drug with GABA B receptor through docking study. In this present work binding interactions between some commonly prescribed BDZs and GABA B receptors have been analysed and reported in terms of type of interaction and dock score.

EXPERIMENTAL WORK

Grid based docking study has been done in present work to find out the binding affinity of BZDs with GABA B receptor using VLife MDS software Biopredicta tools. GABA B receptor (Heterodimeric coiled-coil structure of human GABA(B) receptor, PDB ID: 4PAS) was downloaded from https://www.rcsb.org in PDB file format (Figure 1) and the PDB validation file for the same is shown in Figure 2.

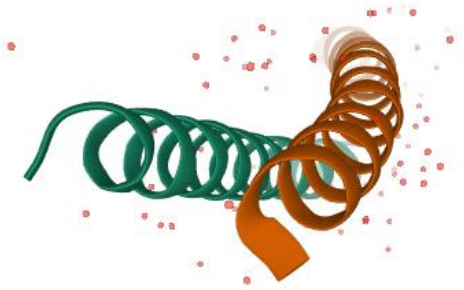


Figure 1.

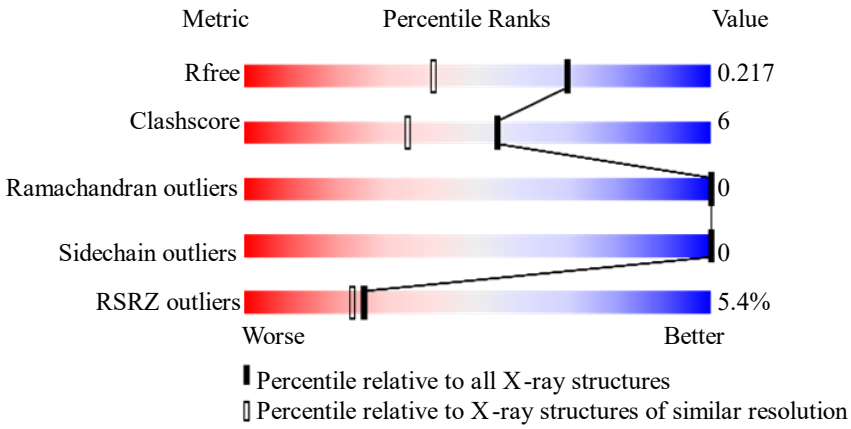
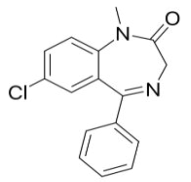
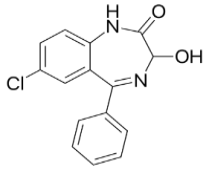
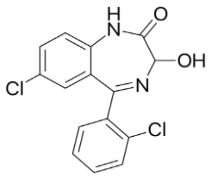
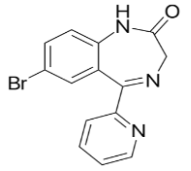
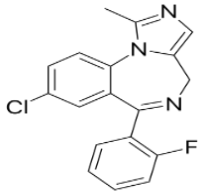
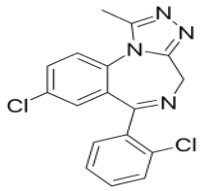
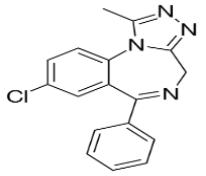


Figure 2.

Seven commonly prescribed drugs which belong to benzodiazepines category were randomly chosen and their structure were drawn using ChemSketch software (ACD Labs) and for present study those drugs were given code as SS01 to SS07 as shown in Table 1. Energy of all drawn structures were minimised by batch minimization using Monte Carlo method.

Table 1. Commonly prescribed benzodiazepines selected for docking study.

S.N.	Drug Name	Code	Structure of Drug
1	Diazepam	SS01	
2	Oxazepam	SS02	
3	Lorazepam	SS03	
4	Bromazepam	SS04	
5	Midazolam	SS05	
6	Triazolam	SS06	
7	Alprazolam	SS07	

In the Batch GA Docking, the receptor molecule was chosen as target molecule. Similarly ligand molecules (SS01-SS07) were also opened. Docking studies were conducted with specific parameters configured, including the designation of a specific cavity (cavity no 1), a total of 400 generations, utilization of the dock score as the scoring function, translation of the ligand within the receptor cavity with a value of 2 Angstroms, and a rotation step size of 100.0, which determines the degree to which

the ligand is rotated inside the receptor cavity to generate various ligand poses. The translation and rotation of the ligand within the receptor cavity were executed using the Genetic Algorithm (GA) algorithm. This process aimed to attain the optimal ligand pose, resulting in the lowest possible score, which represents the interaction or docking energy between the receptor and ligand molecules. Batch GA Docking process was started and the final minimum score for the best ligand pose for each of the ligand(s) was obtained. The best docked complex of the ligand(s)with GABA B receptor were saved and further analysed.

RESULTS AND DISCUSSION

The present docking studies revealed that benzodiazepines have good binding affinity for GABA B receptor. The docking score obtained between the range of -4.066818 and -4.620797 for selected commonly prescribed BDZs proves that these molecules binds effectively with GABA B receptor. The dock score obtained in present study are listed in Table 2 where Bromazepam showed maximum score of -4.066818 and Alprazolam showed least score of -4.620797 among the selected seven drug molecules. Dock score obtained from rest of the drugs are -4.514815, -4.269231, -4.543857, -4.148857 and -4.566512 for Diazepam, Oxazepam, Lorazepam, Midazolam and Triazolam respectively.

Binding affinity had been confirmed by hydrophobic, hydrogen bond and Van der Waals type of interaction between ligand and receptor molecules. Detailed data about total number and types of interaction varied with BZDs molecules and a list of the same has been shown in (Table 3). Diazepam showed only Van der Waals type of interaction and hydrophobic as well as Van der Waals interaction were shown by Oxazepam and Lorazepam. Hydrogen bond interactions including hydrophobic and Van der Waals type of interaction were shown by Bromazepam. Midazolam, Triazolam and Alprazolam showed hydrophobic as well as Van der Waals interaction. Amino acid residues which were actively involved in binding with ligand atoms were, GLU (Glutamic acid), LEU (Leucine), LYS (Lysine),ASP (Aspartic acid) and ARG (Arginine), and these all showed different type of interactions.

Table 2. Dock score of selected benzodiazepines.

S.N..	Ligand	Ligand Code	Dock Score
1	Diazepam	SS01	-4.514815
2	Oxazepam	SS02	-4.269231
3	Lorazepam	SS03	-4.543857
4	Bromazepam	SS04	-4.066818
5	Midazolam	SS05	-4.148857
6	Triazolam	SS06	-4.566512
7	Alprazolam	SS07	-4.620797

Table 3. Detailed data about the interaction between ligand and receptor.

S.N.	Ligand	Residue	Atom	LigandAtom	Distance	Interaction Type
1	SS01	ARG796A	257C	22C	1 3.773	VDW_INTERACTION
2		ARG796A	258N	22C	1 3.327	VDW_INTERACTION
3	SS02	GLU804B	501C	17C	3.57	VDW_INTERACTION
4		GLU804B	506O	8C	3.652	VDW_INTERACTION
5		GLU804B	507O	8C	3.345	VDW_INTERACTION
6		LEU805B	508N	17C	3.737	VDW_INTERACTION
14		LEU805B	515C	17C	3.789	VDW_INTERACTION
15		LYS807B	532N	7N	3.121	VDW_INTERACTION
16		LYS807B	532N	12O	3.405	VDW_INTERACTION
17		ASP808B	538C	7N	3.609	VDW_INTERACTION
18		ASP808B	538C	13O	3.488	VDW_INTERACTION
19		ASP808B	538C	17C	3.687	VDW_INTERACTION
20		ASP808B	539O	6C	3.277	VDW_INTERACTION

21		ASP808B	539O	7N	3.17	VDW_INTERACTION
25		LYS807B	531C	6C	4.179	HYDROPHOBIC_INTERACTION
	SS03	GLU804B	505C	17C	3.63	VDW_INTERACTION
		GLU804B	506O	17C	3.487	VDW_INTERACTION
		LYS807B	531C	13O	3.249	VDW_INTERACTION
		LYS807B	532N	5C	3.56	VDW_INTERACTION
		LYS807B	532N	12O	3.14	VDW_INTERACTION
		ASP808B	538C	4N	3.336	VDW_INTERACTION
		ASP808B	538C	13O	3.431	VDW_INTERACTION
		ASP808B	538C	14H	3.376	VDW_INTERACTION
		ASP808B	539O	4N	3.237	VDW_INTERACTION
		ASP808B	540O	3C	3.381	VDW_INTERACTION
		ASP808B	540O	13O	3.469	VDW_INTERACTION
		ASP808B	540O	14H	3.167	VDW_INTERACTION
		LYS807B	531C	6C	4.307	HYDROPHOBIC_INTERACTION
		ASP808B	537C	6C	4.877	HYDROPHOBIC_INTERACTION
	SS04	LYS801B	476C	9C	3.736	VDW_INTERACTION
		LYS801B	478O	9C	3.437	VDW_INTERACTION
		LYS801B	481C	20Br	3.738	VDW_INTERACTION
		GLU804B	503C	1C	3.636	VDW_INTERACTION
		GLU804B	1C	11C	3.768	VDW_INTERACTION
		GLU804B	504C	2C	3.844	VDW_INTERACTION
		GLU804B	505C	7N	3.33	VDW_INTERACTION
		GLU804B	505C	9C	3.872	VDW_INTERACTION
		GLU804B	506O	3C	3.43	VDW_INTERACTION
		GLU804B	506O	7N	3.231	VDW_INTERACTION
		GLU804B	506O	10C	3.673	VDW_INTERACTION
		GLU804B	507O	1C	3.238	VDW_INTERACTION
		GLU804B	507O	7N	3.44	VDW_INTERACTION
		LEU805B	514C	10C	3.815	VDW_INTERACTION
		ASP808B	538C	12O	3.252	VDW_INTERACTION
		ASP808B	538C	13H	3.023	VDW_INTERACTION
		ASP808B	539O	12O	3.195	VDW_INTERACTION
		ASP808B	540O	3C	3.634	VDW_INTERACTION
		GLU804B	503C	6C	4.307	HYDROPHOBIC_INTERACTION
		GLU804B	504C	6C	4.666	HYDROPHOBIC_INTERACTION
		ASP808B	540O	13H	1.838	HYDROGENBOND_INTERACTION
	SS05	GLU789A	198O	9N	3.568	VDW_INTERACTION
		GLU789A	198O	11C	3.637	VDW_INTERACTION
		GLU804B	505C	4N	3.724	VDW_INTERACTION
		GLU804B	505C	5C	3.703	VDW_INTERACTION
		GLU804B	506O	6C	3.275	VDW_INTERACTION
		LEU805B	513C	11C	3.566	VDW_INTERACTION
		LEU805B	515C	8C	3.832	VDW_INTERACTION
		LYS807B	532N	12C	3.322	VDW_INTERACTION
		ASP808B	537C	14C	3.729	VDW_INTERACTION
		ASP808B	539O	12C	3.601	VDW_INTERACTION
		GLU804B	503C	5C	4.692	HYDROPHOBIC_INTERACTION
		GLU804B	504C	5C	4.774	HYDROPHOBIC_INTERACTION
		LEU805B	509C	11C	4.329	HYDROPHOBIC_INTERACTION
		LEU805B	512C	11C	4.576	HYDROPHOBIC_INTERACTION
		ASP808B	537C	11C	4.917	HYDROPHOBIC_INTERACTION

	SS06	GLU804B	500C	20C	3.573	VDW_INTERACTION
		GLU804B	502O	20C	3.252	VDW_INTERACTION
		GLU804B	503C	17C	3.667	VDW_INTERACTION
		GLU804B	503C	18C	3.679	VDW_INTERACTION
		GLU804B	504C	17C	3.803	VDW_INTERACTION
		LYS807B	530C	19C	3.786	VDW_INTERACTION
		LYS807B	531C	18C	3.899	VDW_INTERACTION
		LYS807B	532N	6N	3.208	VDW_INTERACTION
		LYS807B	532N	20C	3.359	VDW_INTERACTION
		ASP808B	538C	20C	3.487	VDW_INTERACTION
		ASP808B	539O	21C	3.507	VDW_INTERACTION
		ASP808B	540O	18C	3.673	VDW_INTERACTION
		LYS807B	531C	5C	4.762	HYDROPHOBIC_INTERACTION
	SS07	GLU789A	196C	19C	3.452	VDW_INTERACTION
		GLU789A	197O	19C	3.566	VDW_INTERACTION
		GLU789A	197O	21C	3.244	VDW_INTERACTION
		GLU804B	505C	5C	3.87	VDW_INTERACTION
		GLU804B	506O	6N	3.479	VDW_INTERACTION
		LEU805B	513C	19C	3.555	VDW_INTERACTION
		LEU805B	515C	17C	3.724	VDW_INTERACTION
		ASP808B	538C	18C	3.865	VDW_INTERACTION
		ASP808B	540O	11C	3.668	VDW_INTERACTION
		GLU804B	503C	5C	4.989	HYDROPHOBIC_INTERACTION

The data given in the above table about the binding affinity between ligand and receptor molecules was validated by analysing the interactions. Individual interactions were analysed for each molecule and here in the results, all types of interactions have been shown for selected molecules. Following figures represents the interactions between selected benzodiazepines and GABA B receptors (Figures 3–16).

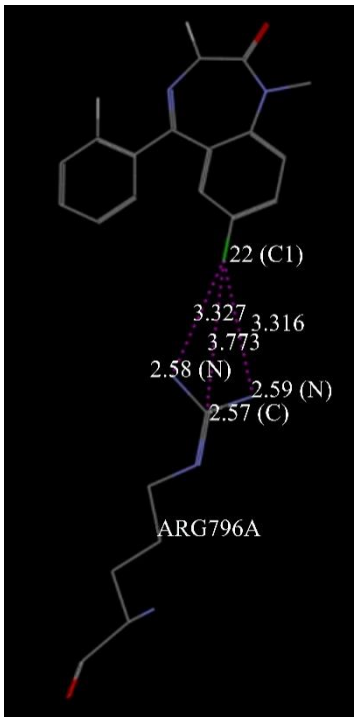


Figure 3. Diazepam and GABA B.

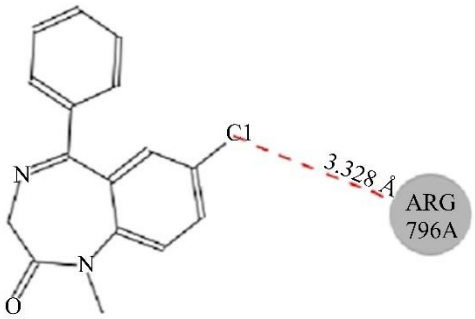


Figure 4. 2D representations of Diazepam and GABA B.

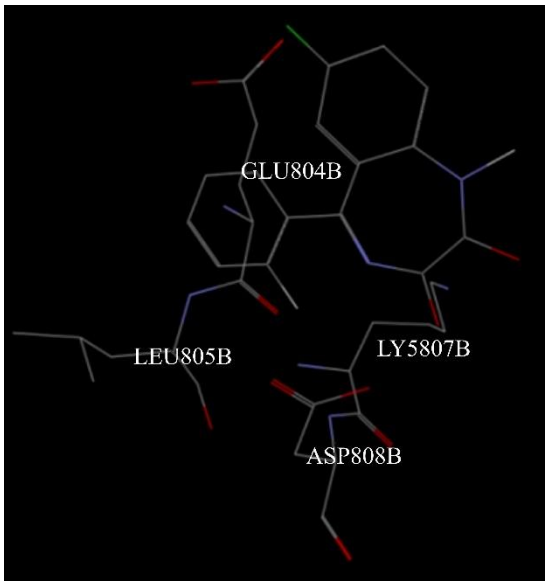


Figure 5. Oxazepam and GABA B**.**

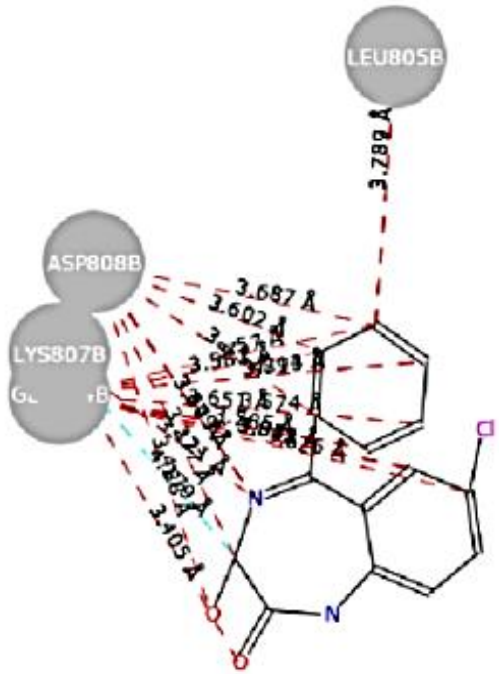


Figure: 6 2D representations of Oxazepam and GABA B.

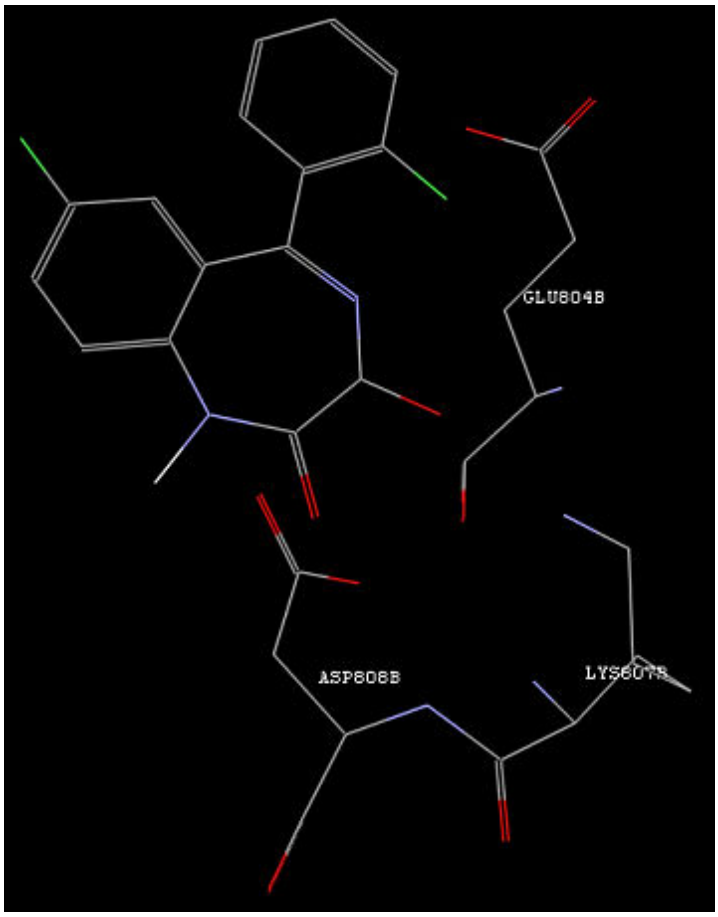


Figure 7. Lorazepam and GABA B.

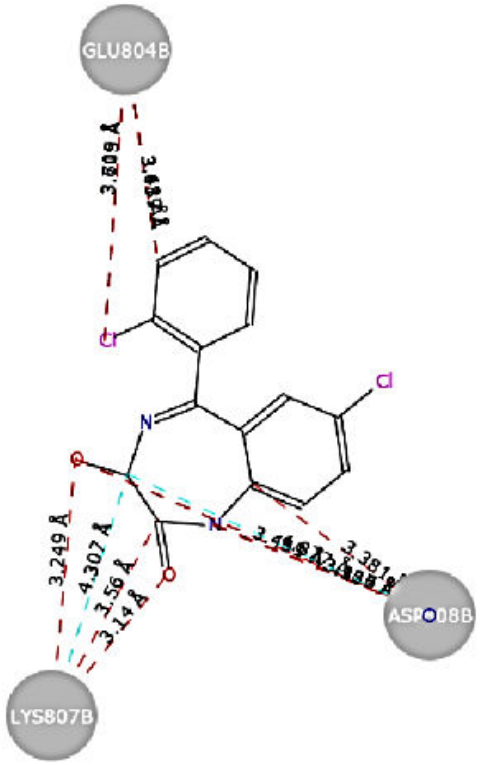


Figure 8. 2D representations of Lorazepam and GABA B.

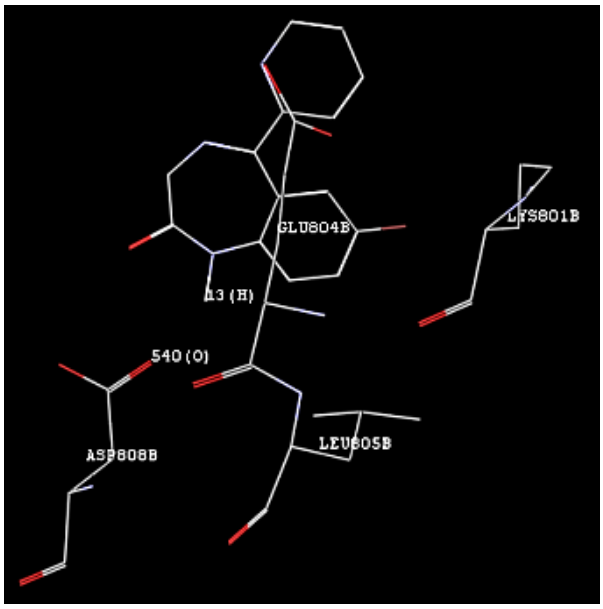


Figure 9. Bromazepam and GABA B.

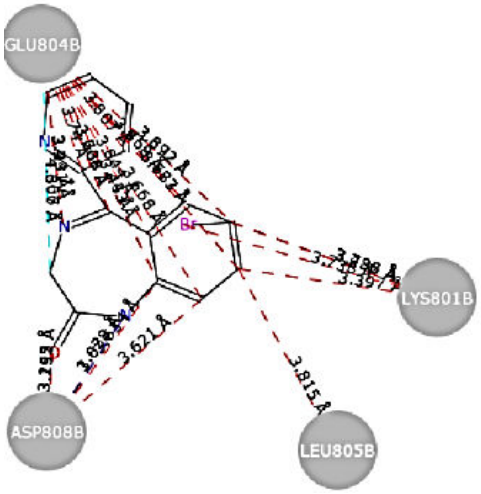


Figure 10. 2D representations of Bromazepam and GABA B.

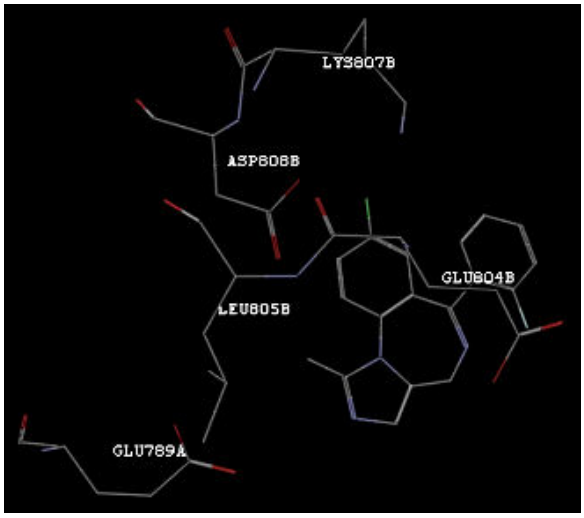


Figure 11. Midazolam and GABA B.

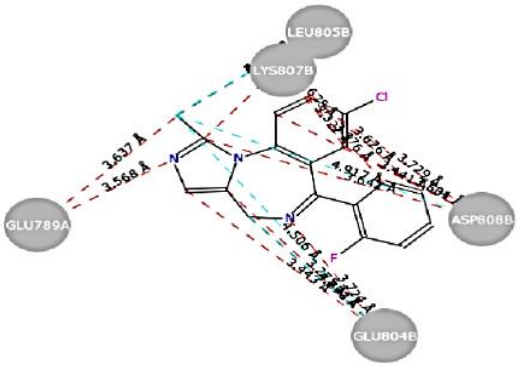


Figure 12. 2D representations of Midazolam and GABA B.

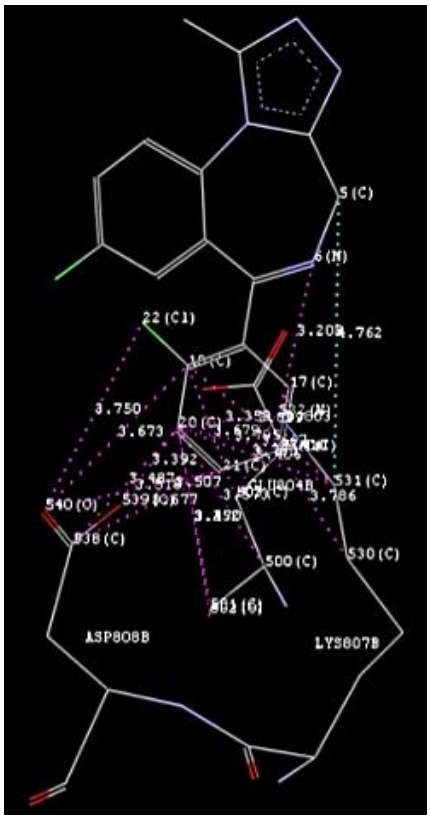


Figure 13. Triazolam and GABA B.

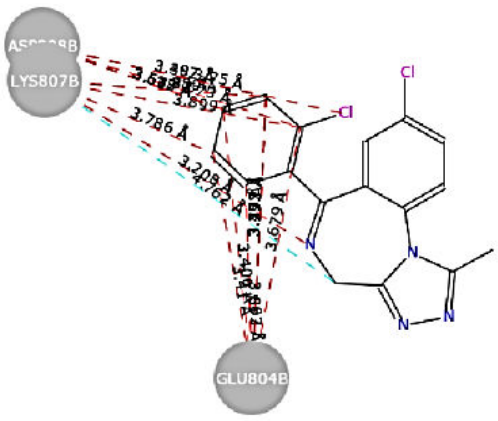


Figure 14. 2D representations of Triazolam and GABA B.

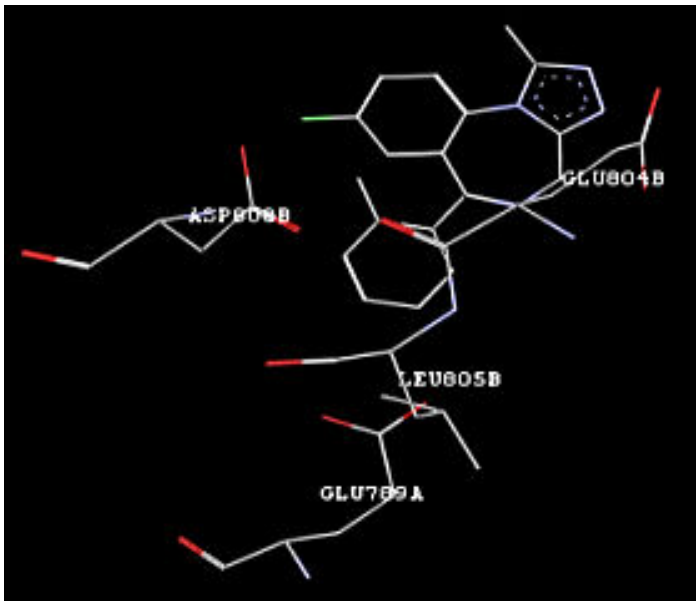


Figure 15. Alprazolam and GABA B.

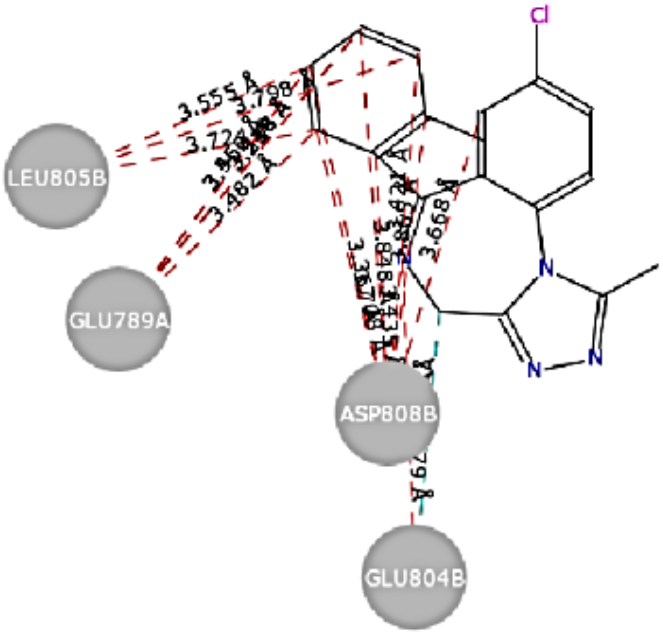


Figure 16. 2D representations of Alprazolam and GABA B.

CONCLUSION

Molecular docking emerges as a promising tool in the identification and optimization of ligands in modern drug discovery. By combining the chemical information derived from natural products with docking-based virtual screening, significant contributions are anticipated in drug discovery, particularly in the post-genomic era where an increasing number of potential targets emerge from functional genomics studies. In this docking study, all selected benzodiazepines (BZDs) demonstrated favorable binding affinity with the GABA B receptor, exhibiting dock scores ranging from -4.066818 to -4.620797. Interactions such as Van der Waals, hydrophobic, and hydrogen bond interactions were observed between the ligand and receptor molecules. Notably, Van der Waals interactions predominated in the docking study. Based on the dock scores and binding interactions, it can be inferred that all seven selected BZDs possess binding efficacy for the GABA B receptor, albeit with subtle differences in their binding patterns. These findings offer valuable insights that may pave the way for further exploration

of benzodiazepine pharmacology and mechanism of action, potentially leading to their repurposing beyond their current clinical uses. Overall, the results of this docking study provide a foundation for future investigations aimed at elucidating novel therapeutic avenues and optimizing the therapeutic potential of benzodiazepines.

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