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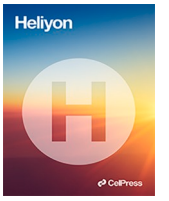

Research article

Site specific binding pattern of benzodiazepines with $GABA_A$ receptor and related impact on their activities in the human body: An in silico method based study

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ABSTRACT

Despite of being the drugs of the same therapeutic class (Benzodiazepines), each of them shows different actions prominently. It is commonly seen that Bromazepam, Clonazepam, and, Alprazolam are prescribed for the treatment of anxiety disorders, panic disorders, and phobias. On the other hand, Midazolam, Temazepam, Flurazepam, and Nitrazepam are indicated for the treatment of insomnia and Lorazepam is considered as a drug having anticonvulsant effects. As the mechanism of action is the same, there should be some differences in the binding patterns with the proteins that create differences in their impacts on the body. A deep screening of the binding patterns of the available Benzodiazepines in the market to the $GABA_A$ receptor will be beneficial to find out the responsible amino acids for being accountable for showing any specific action. This reveal will help design new molecules with the highest beneficial effect and lowest toxicity in the body. The *in silico* method provides the initial level of understanding regarding the binding patterns, performing in vitro and in vivo experiments will be more specific to claim the benefits of newly designed drugs.

1. Introduction

Benzodiazepines (BZDs) are one of the most commonly used CNS medications, known for their inhibitory effect to calm the nerves down. Gamma-aminobutyric acid (GABA) is a naturally occurring inhibitory neurotransmitter of the Central Nervous System (CNS) that reduces the excitability of neurons [1–3]. GABA binds to GABA receptors of a neuron, it makes the neuron less likely to fire an action potential or release neurotransmitters [4,5]. There are two types of GABA receptors with which GABA can interact, $GABA_A$ and $GABA_B$. $GABA_A$ is an ionotropic receptor and upon binding with GABA, it causes the opening of an associated ion channel that is permeable to negatively charged chloride ions [6–9]. As Cl^- ions flow into the neurons, they hyperpolarize the membrane potential of the neuron and makes the neurons less likely to fire an action potential. BZDs binds to $GABA_A$ receptors causing the positive modulation of the receptor and enhances the activity of GABA. Thus, a sedative or anxiolytic effect is observed [4,10–12].

$GABA_A$ receptors are pentameric transmembrane receptors containing five proteins or subunits, two alpha (α_1, α_2) subunits, two beta (β_1, β_2) subunits and one gamma (γ_2) subunit (Fig. 1). GABA binding points lie between α & β subunits (orthosteric site) and BZDs

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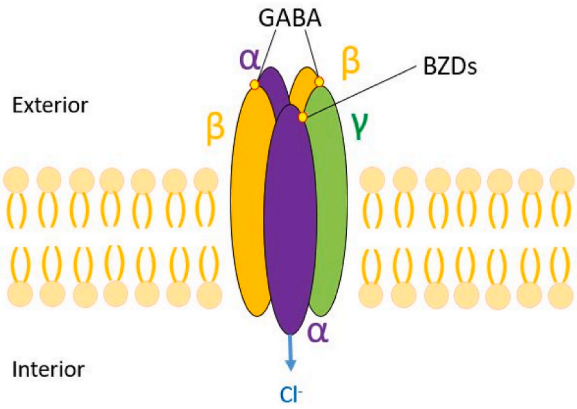


Fig. 1. Pentameric structure of GABA receptor[16].

binding site exists between α & γ [13–16].

Some commonly prescribed BZDs are Alprazolam, Bromazepam, Clonazepam, Flurazepam, Lorazepam, Midazolam, Nitrazepam, Temazepam and these are indicated for different indications. For example, Bromazepam/Clonazepam/Flurazepam/Alprazolam [Fig. 2 (A-D)] is prescribed for treatment of anxiety, panic disorder, Generalized Anxiety Disorder (GAD) or phobias [1]. Whereas, Midazolam/Temazepam/Flurazepam [Fig. 2(F–H)] is indicated for insomnia and Lorazepam [Fig. 2(E)] is considered to have an anticonvulsant effect [1,12,17,18]. Structure-activity relationship (SAR) studies are done to minimize the side effects shown by these drugs with little modification in their structures [19,20]. These modifications or alterations lead to a change in the activities of the drugs, for example, the anxiolytic property of a BZD drug may increase than others. Similarly, the other properties like sedative, anticonvulsant or muscle relaxant properties may also be increased or decreased for a BZD. The objective of the study is to determine that binding in a site-specific manner with $GABA_A$ receptors, which particular property a BZD shows more prominently than others.

In this study, we have performed molecular docking of considered BZDs with $GABA_A$ receptor to identify and visualize the specific site of the receptor that is responsible for the BZD's specific prominent action. Molecular docking is a basic tool of Computer-Aided Drug Design (CADD) that is used to predict the possible interaction between a ligand and a protein/a receptor to understand the ligand's efficacy to treat a particular disease [22,23]. Docking score analysis provides the opportunity to find the best conformation of ligand-protein complex and 3D visualization clarifies the orientation, binding and position of a ligand molecule into a protein or receptor structure [23].

2. Materials and methods

2.1. Ligand preparation

For the experiment, we had used PubChem [21] to download the structures of BZDs considered here for docking purposes. All the 3D structures had been downloaded as SDFs. Then these conformations had been opened in PyMol and saved as PDB files for further use.

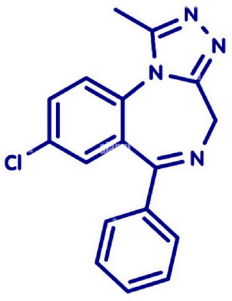
2.2. Receptor preparation

The receptor, $GABA_A$ had been downloaded from RCSB PDB (https://www.rcsb.org/) with the PDB ID 6 × 3T [24]. Using PyMol, the 3D structure of the receptor had been cleaned to remove the water molecules and unnecessary ligands bound to the receptor. Gamma-aminobutyric acid receptor subunit beta-2 (Chain A, C), Gamma-aminobutyric acid receptor subunit alpha-1 (Chain B, D), Gamma-aminobutyric acid type A receptor subunit gamma-2 (Chain E) had been kept and saved. To ensure proper docking it is necessary to set the receptor molecule at a lower energy state, that's why energy minimization is an important step. Energy minimization of $GABA_A$ receptor was done using Swiss PDB Viewer. Then the energy-minimized structure was saved for further use.

2.3. Docking

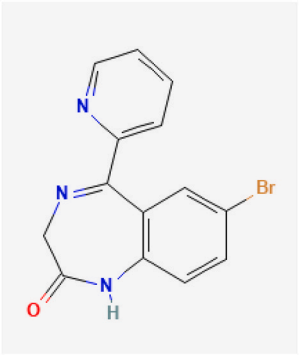
Using PyRx, each ligand had been docked with the 'Optimized $GABA_A$'. For each complex, a docking score had been obtained that will be analyzed in the following section. To visualize the complexes and to understand the binding site of the ligands, PyMol [25] & Discovery Studio [26] had been used. Ligand interaction with the receptor had been observed in 3D and 2D conformation. An overall comparison among the docking scores and binding patterns had been done to predict the drugs' action on the receptor.

A



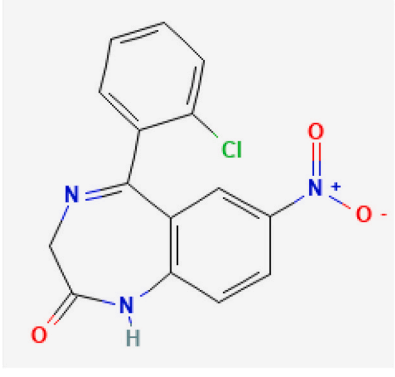
Alprazolam

B



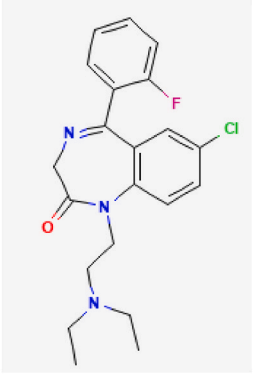
Bromazepam

C



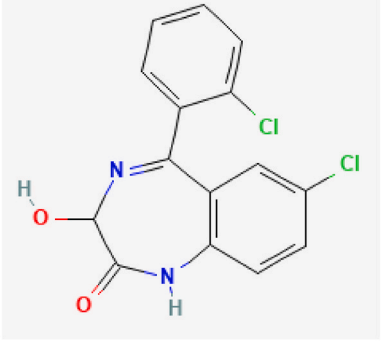
Clonazepam

D



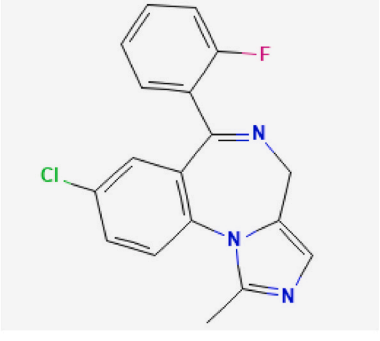
Flurazepam

E



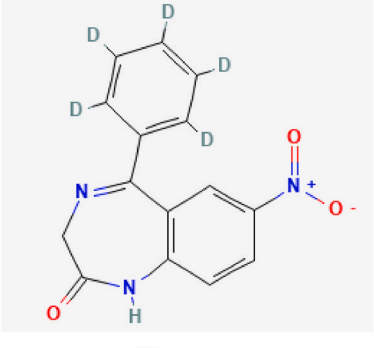
Lorazepam

F



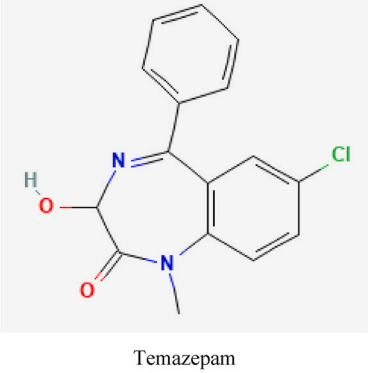
Midazolam

G



Nitrazepam

H



Temazepam

(caption on next page)

Fig. 2. Structures of BZNs considered in this study [21]. (A) is Alprazolam, (B) is Bromazepam, (C) is Clonazepam, (D) is Flurazepam, (E) is Lorazepam, (F) is Midazolam, (G) is Nitrazepam & (H) is Temazepam.

Table 1
Docking scores of ligand-receptor complexes.

Sl. No.	Ligand bound with $GABA_A$	Binding affinity
1	Alprazolam	−8.3
2	Midazolam	−7.9
3	Temazepam	−7.7
4	Clonazepam	−7.6
5	Lorazepam	−7.6
6	Bromazepam	−7.4
7	Nitrazepam	−7.3
8	Flurazepam	−6.8

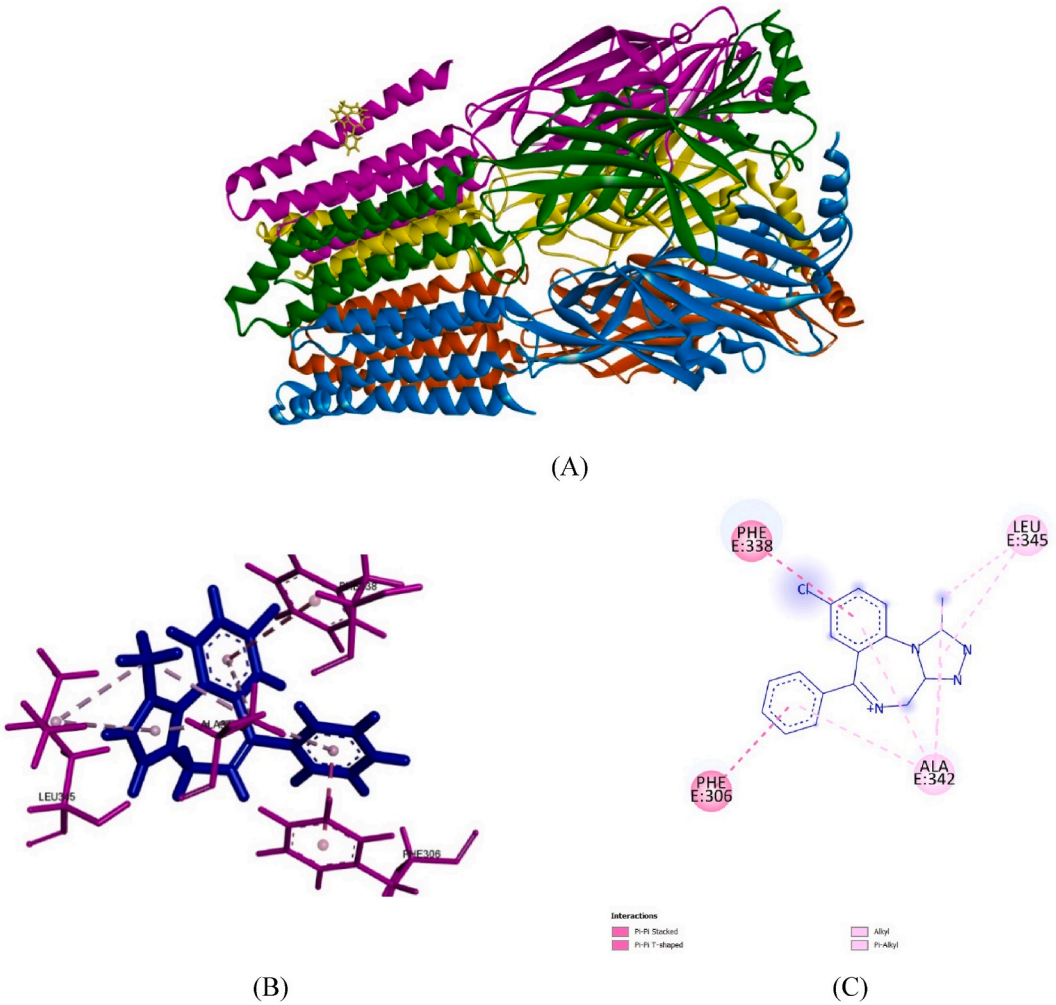


Fig. 3. Interactions between $GABA_A$ and Alprazolam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Alprazolam, (B) 3D binding sites of Alprazolam with $GABA_A$, (C) 2D view of interactions between Alprazolam and $GABA_A$.

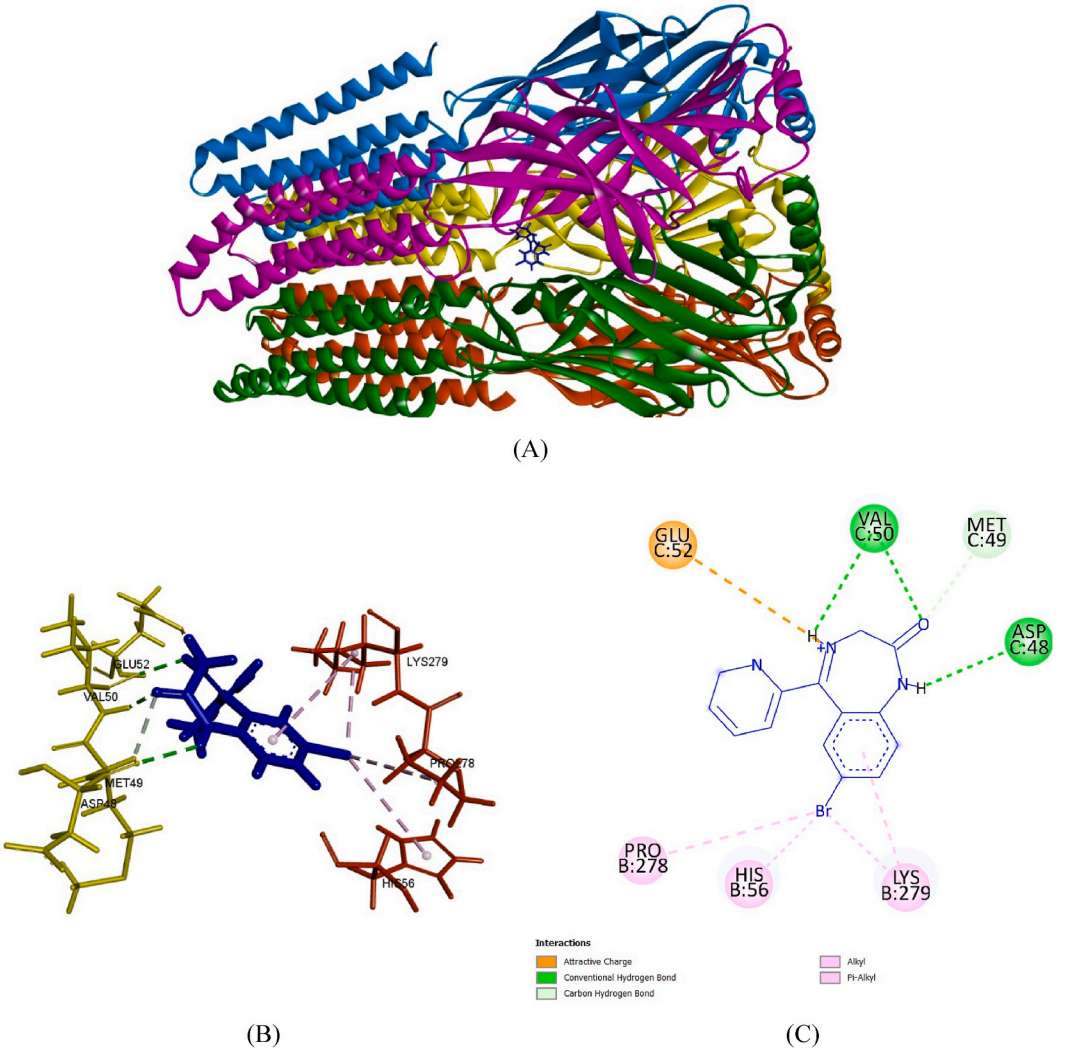
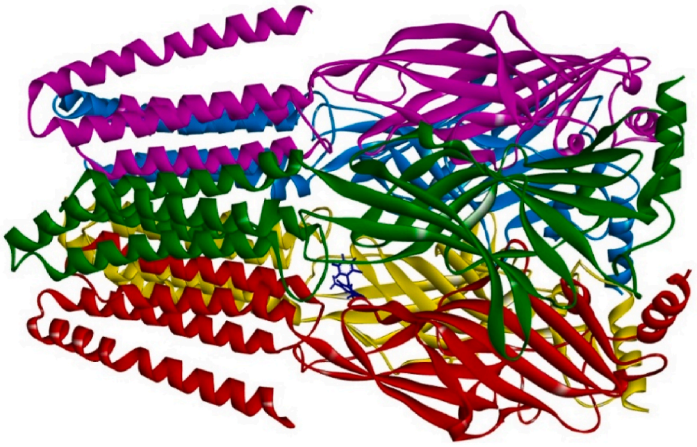
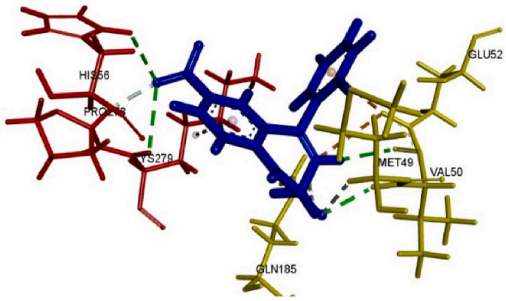


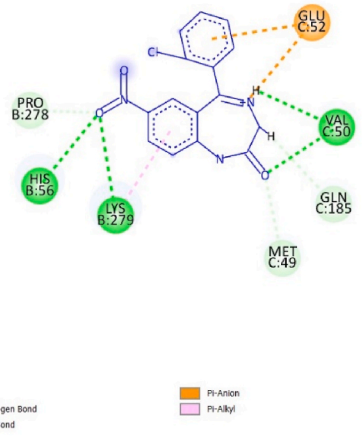
Fig. 4. Interactions between $GABA_A$ and Bromazepam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Bromazepam, (B) 3D binding sites of Bromazepam with $GABA_A$, (C) 2D view of interactions between Bromazepam and $GABA_A$.



(A)



(B)



(C)

Fig. 5. Interactions between $GABA_A$ and Clonazepam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Clonazepam, (B) 3D binding sites of Clonazepam with $GABA_{A,}$ (C) 2D view of interactions between Clonazepam and $GABA_A$.

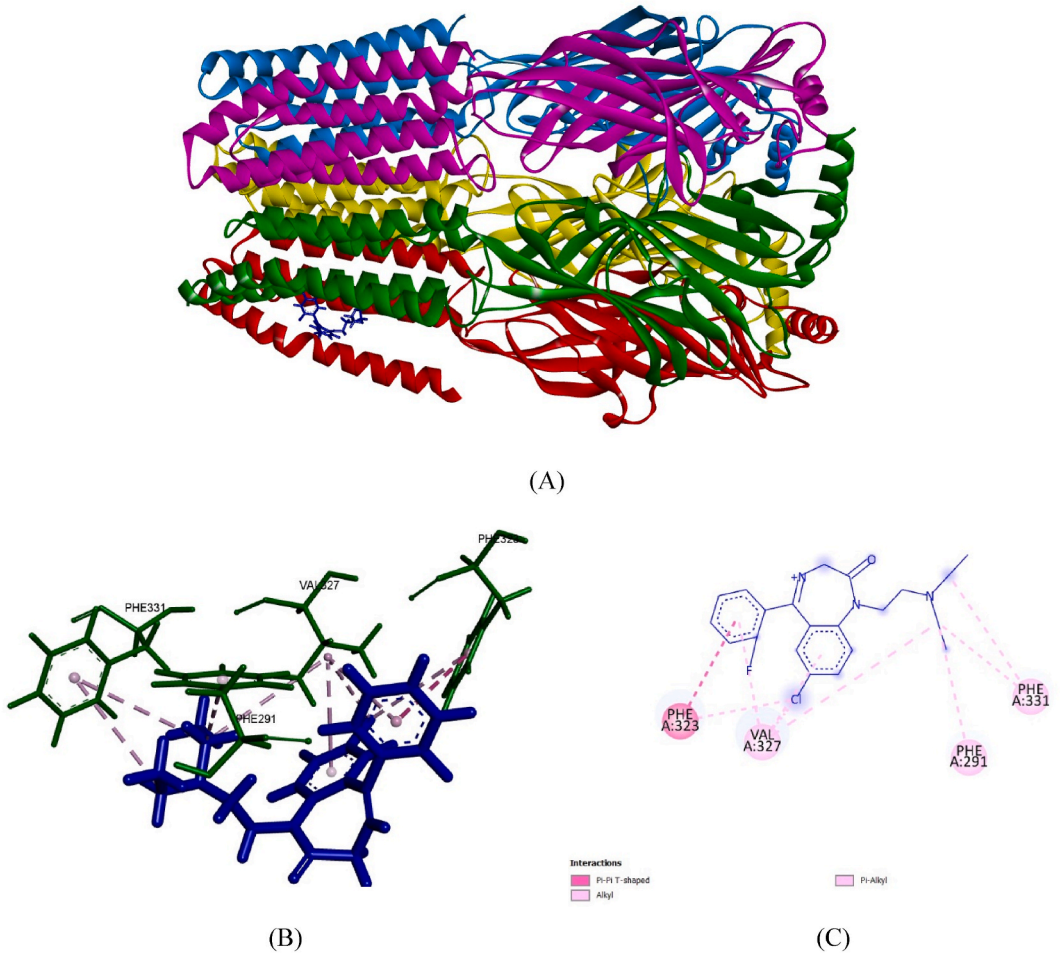


Fig. 6. Interactions between $GABA_A$ and Flurazepam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Flurazepam, (B) 3D binding sites of Flurazepam with $GABA_A$, (C) 2D view of interactions between Flurazepam and $GABA_A$.

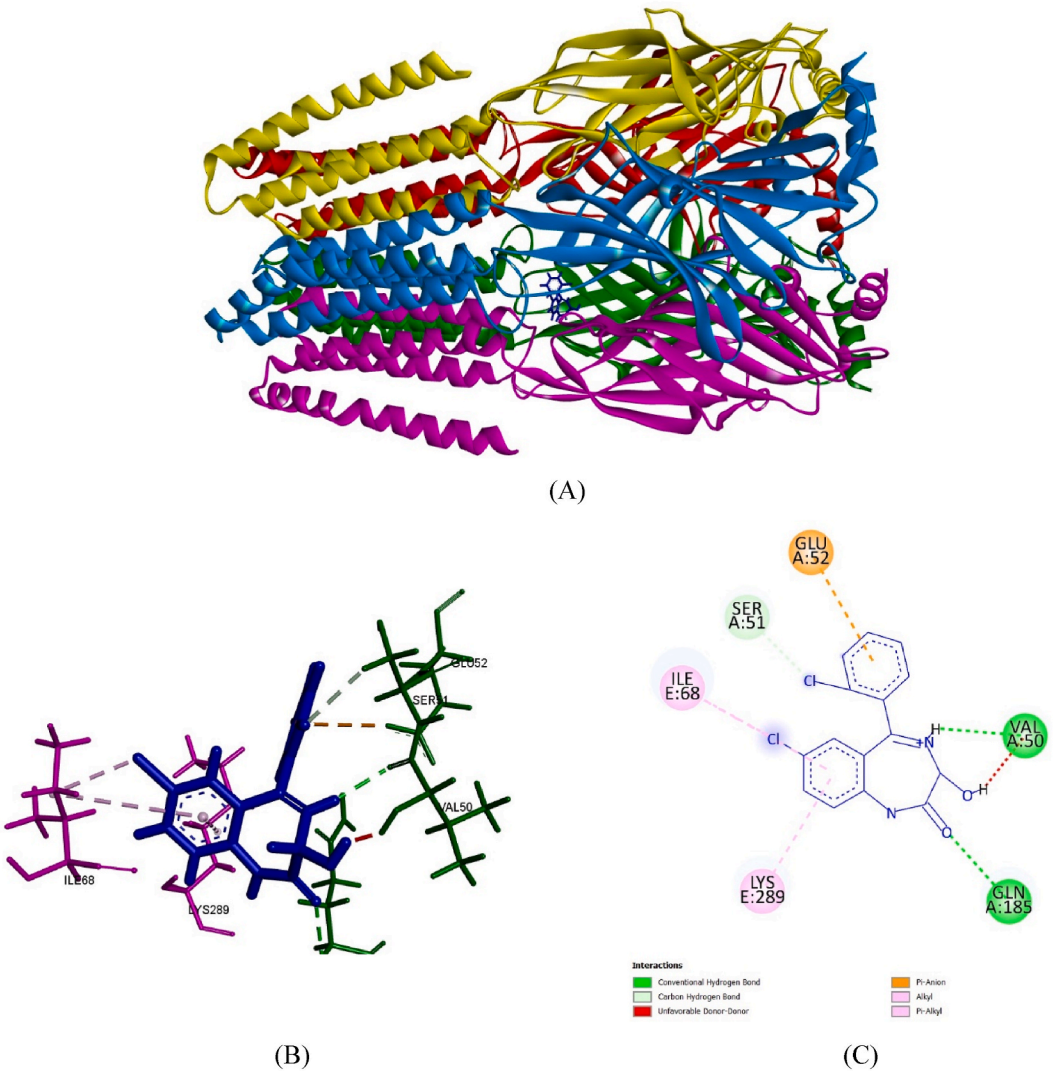


Fig. 7. Interactions between $GABA_A$ and Lorazepam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Lorazepam, (B) 3D binding sites of Lorazepam with $GABA_{A,}$ (C) 2D view of interactions between Lorazepam and $GABA_A$.

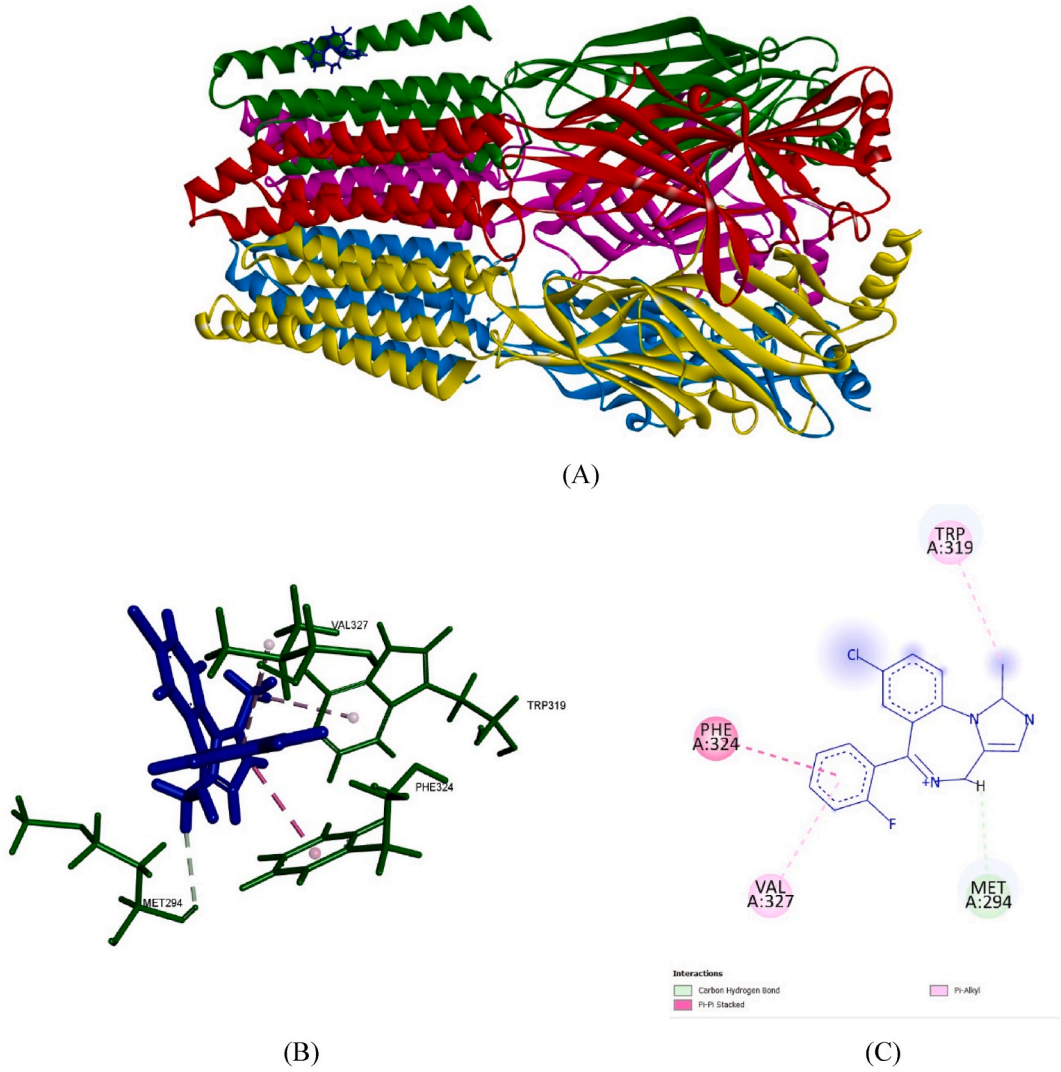


Fig. 8. Interactions between $GABA_A$ and Midazolam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Midazolam, (B) 3D binding sites of Midazolam with $GABA_A$, (C) 2D view of interactions between Midazolam and $GABA_A$.

3. Result & discussion

3.1. Docking score

After docking each drug molecules with the target receptor in AutoDock vina (PyRx) [27], a docking score had been obtained. Docking score predicts the binding affinity of a ligand and a protein after docking. The lower the docking score the better the docking is. We got eight docking scores for eight molecules while docked with $GABA_A$ receptor.

According to docking score from AutoDock Vina (Table 1), it had been predicted that the binding affinity of $GABA_A$ with Alprazolam is the strongest and with Flurazepam is the weakest among all the BZDs considered.

3.2. Visualization

The 2D and 3D binding conformations had been viewed in BIOVIA Discovery Studio to analyze the result and to predict the possible binding site of the BZDs to the receptor.

3.2.1. Alprazolam

In Fig. 3 (A), the green chain represents the A chain (beta-2 subunit), the blue chain represents the B chain (alpha-1 subunit), the red chain represents the C chain (beta-2 subunit), the yellow chain represents the D chain (alpha-1 subunit) and the pink chain

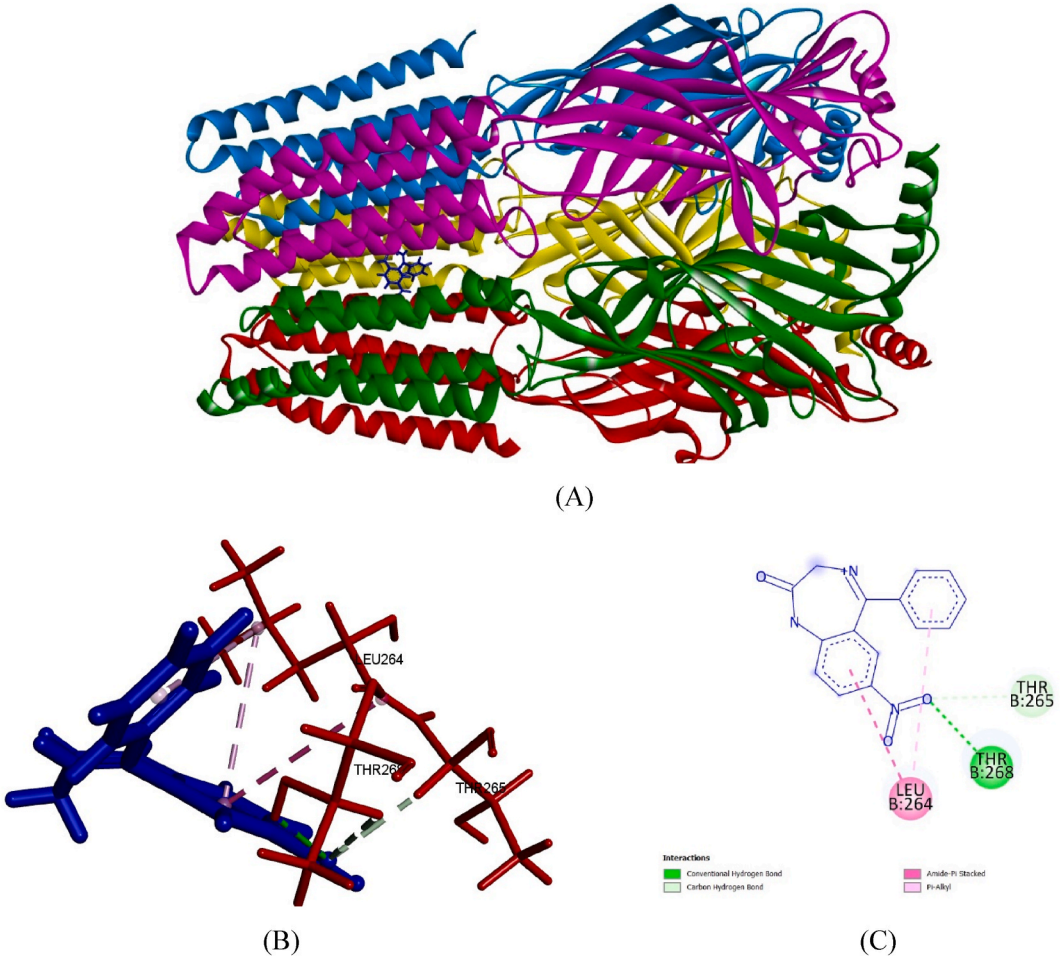


Fig. 9. Interactions between $GABA_A$ and Nitrazepam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Nitrazepam, (B) 3D binding sites of Nitrazepam with $GABA_A$, (C) 2D view of interactions between Nitrazepam and $GABA_A$.

represents the E chain (gamma-2 subunit) of $GABA_A$ receptor. This color code would be the same for all the structures used here. According to BIOVIA Discovery Studio, Alprazolam binds to the E chain (gamma-2 subunit) by pi bonds with PHE338, PHE306, ALA342 & LEU345 [Fig. 3(B–C)].

3.2.2. Bromazepam

Bromazepam binds to GLU52 of D chain (alpha subunit) with an attractive charge, with VAL50, ASP48 & MET49 of the same chain by H-bond, Bromine entity binds with PRO278, HIS56 & LYS279 of C chain (beta subunit) by pi-alkyl or alkyl bonds [Fig. 4(A-C)].

3.2.3. Clonazepam

Clonazepam binds strongly to $GABA_A$ receptor [Fig. 5(A)]. It binds to HIS56, LYS279 & PRO278 amino acids of C chain (beta chain) and to VAL50, MET49 & GLN185 amino acid of D chain (alpha subunit) by H-bonds. It also binds to GLU52 of D chain (alpha subunit) with an attractive charge, similar to Bromazepam [Fig. 5(B)]. In case of Clonazepam, it had been seen that there are 6 H-bonds with five amino acids of two chains, whereas Bromazepam has only 4 H-bonds with three amino acids of only one chain [Fig. 5(C)]. So, the binding of Clonazepam to the receptor is stronger than that of with Bromazepam.

3.2.4. Flurazepam

Flurazepam doesn't share any H-bond with any amino acid of the receptor, but shares some Pi bonds with PHE323, VAL327, PHE291 & PHE331 amino acids of A chain (beta subunit) [Fig. 6(A-C)].

3.2.5. Lorazepam

Lorazepam shares 3 H-bonds with GLN185, VSL50 & SER51 amino acids of A chain (beta subunit) and Pi bonds with GLU52, ILE68 & LYS289 amino acids of E chain (gamma subunit) [Fig. 7(A-C)].

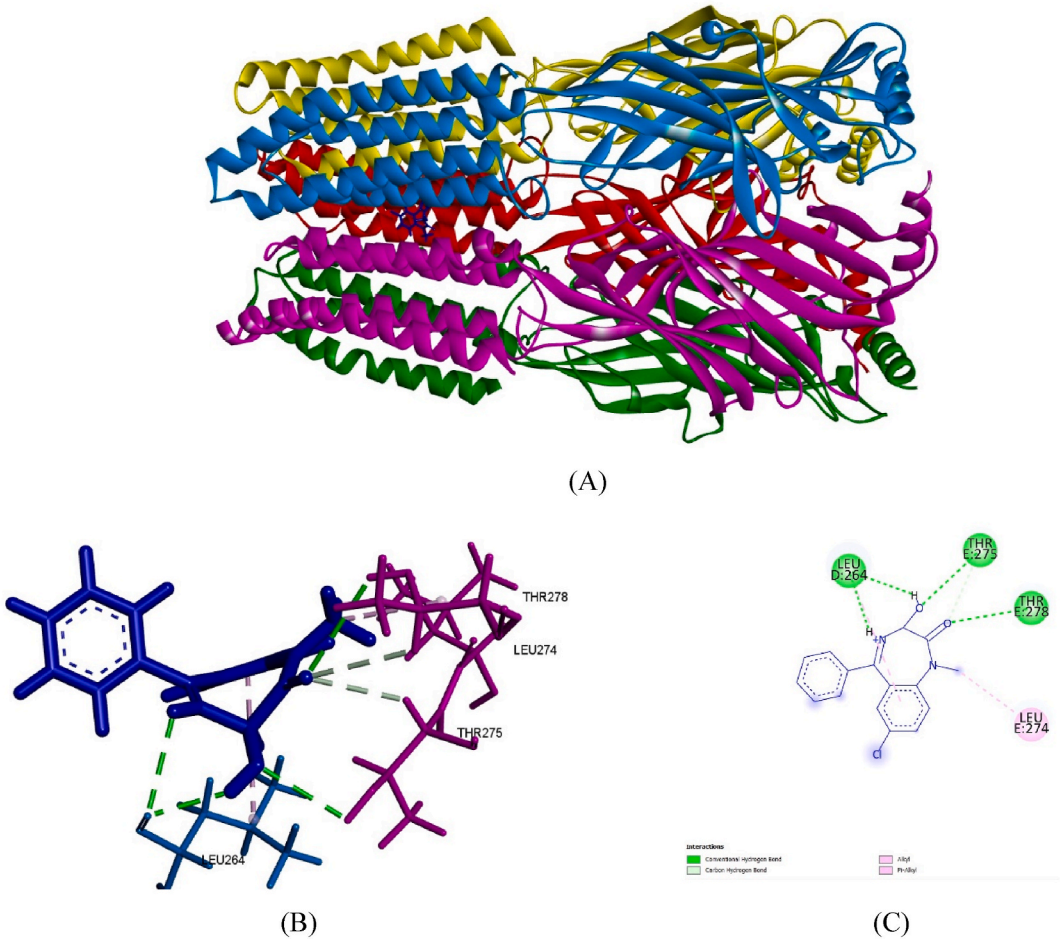


Fig. 10. Interactions between $GABA_A$ and Temazepam visualized in Biovia Discovery Studio. (A) 3D Docked complex of $GABA_A$ receptor and Temazepam, (B) 3D binding sites of Temazepam with $GABA_A$, (C) 2D view of interactions between Temazepam and $GABA_A$.

3.2.6. Midazolam

Midazolam shares 1 C–H bond with MET294 amino acid of A chain (beta subunit) and 3 pi bonds with Phe324, TRP319 and VAL327 amino acids of the same chain [Fig. 8(A-C)].

3.2.7. Nitrazepam

Nitrazepam binds through 2 H-bonds with THR268 and THR265 of Chain C (beta-2 subunit) and 2 pi bonds with LEU264 of the same chain [Fig. 9(A-C)].

3.2.8. Temazepam

Temazepam shares 4 H-bonds with LEU264, THR275, THR278 of chain E (gamma-2 subunit) and 1 pi bond with LEU274 of chain B (alpha-2 subunit) [Fig. 10(A-C)].

The interactions between the drugs and the GABAA receptor visualized in BIOVIA Discovery Studio provides the clear idea about the site-specific binding of the drugs to the receptor. Site specific binding plays important role to justify the action of the drug. Table 2 shows the specific amino acids of particular chains with which the BZDs interact through H-bond, pi-bond or other bonds and shows particular action. When BZDs bind to both alpha (D chain) and beta subunits (C chain), anxiolysis property becomes prominent (Bromazepam < Clonazepam). Specifically, binding to PRO278, HIS56, LYS279, GLU52, VAL50, MET49 amino acids are commonly found in the case of Bromazepam and Clonazepam, to be responsible for a drug to show anxiolytic effect. While binding to only the gamma subunit (E chain) also shows anxiolysis properties (Alprazolam).

The binding of BZDs with A chain of beta subunit or E chain of gamma subunit shows sedation property (Flurazepam, Midazolam, Temazepam). In case of Flurazepam and Midazolam, VAL327 amino acid plays a common role in showing a sedative effect. Nitrazepam shows improved sedation properties while binding to the C chain of the beta subunit. But it is seen that LEU and THR amino acids play a significant role in the case of sedative effects.

Lorazepam shows anticonvulsant properties by binding to A chain (beta subunit) and E chain (gamma subunit) of the receptor.

Table 2
Site specific binding of BZDs to the receptor (Green colored cells represent H-bonds, pink color cells represent pi-Alkyl bonds and orange cells represent pi-anion bond).

		B-2 Subunit		A-2 Subunit		γ-2 Subunit	
Sl. No.	Drug	A Chain	C Chain	B Chain	D Chain	E Chain	
1	Alprazolam	-----	-----	-----	-----	7 bonds (PHE338, PHE306, ALA342, LEU345)	Anxiolytic
2	Bromazepam	-----	------ 4 bonds (PRO278, HIS56, LYS279)	-----	1 bond (GLU52) 4 bonds (VAL50, ASP48, MET49)	-----	Anxiolytic
3	Clonazepam	-----	3 bonds (HIS56, LYS279, PRO278)	-----	2 bonds (GLU52) 4 bonds (VAL50, GLN185, MET49)	-----	Anxiolytic
4	Flurazepam	8 bonds (PHE323, VAL327, PHE291, PHE331)	-----	-----	-----	-----	Sedative
5	Lorazepam	3 bonds (GLN185, VAL50, SER51)	-----	-----	-----	1 bond (GLU52) 2 bonds (ILE68, LYS289)	Anticonvulsant
6	Midazolam	1 bond (MET294) 3 bonds (PHE324, TRP319, VAL327)	-----	-----	-----	-----	Sedative
7	Nitrazepam	-----	2 bonds (THR268, THR265) 1 bond (LEU264)	-----	-----	-----	Improved Sedative
8	Temazepam	-----	-----	-----	-----	5 bonds (LEU264, THR275, THR278) 1 bond (LEU274)	Sedative

4. Conclusion

BZDs are widely used drugs for the treatment of anxiety, insomnia, convulsions, phobias etc. The structures of BZDs and their way of binding to $GABA_A$ receptors justify the action of a specific BZD drug. The study here used *in silico* method to dock between BZDs and $GABA_A$ receptors to visualize their 2D and 3D interactions to find out the relation between binding pattern and action. The *in silico* method is a computer-based method, uses certain software to predict interactions between ligands and proteins within the shortest possible time. This study identifies the interactions between currently used BZDs with $GABA_A$ receptor to predict the relation between site-specific binding of drugs to amino acids of the receptor and it's impact on their action inside the body. Identifying the amino acids responsible for specific effects will help design new molecules in the future for treating these diseases with improved mechanisms of action and less side effects.

Data availability statement

The data that support the findings of this study are available on request.

CRediT authorship contribution statement

Matrika Saha Roy: Writing – review & editing, Writing – original draft, Visualization, Project administration, Formal analysis, Data curation, Conceptualization. **Bidduth Kumar Sarkar:** Writing – review & editing, Validation. **Sukalyan Kumar Kundu:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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