Original Research Article

~~Design~~ Molecular modelling of 1H-Benzo[b][1,5]diazepine-2(3H)-one Derivatives and ~~Molecular Docking~~ docking ~~Studies~~ studies against receptor associated protein ~~of~~ ~~1H-Benzo[b][1,5]diazepine-2(3H)-one Derivatives~~

ABSTRACT

In the present investigation, ~~of~~ some N^1-benzoyl/ N^1-chloroacetyl/ N^1-(1,3,4-thiadiazol-2-yl amino acetyl) -7-substituted- 4-methyl-1,5-benzodiazepine-2-one and 7-Substituted-4-methyl-1,5-benzodiazepin-2-one ~~are~~ were designed and docked at active site of cavity 1# of GABA-A receptor associated protein (1KJT) to distinguish from their hypothetical binding mode. The ~~the~~ X-ray crystal structure of mammalian GABA-A receptor associated protein (1KJT) ~~–~~obtained from protein data bank was used as target protein. In this investigation, the comparative analysis of the docking experiments of ~~designed~~ modelled compounds with known GABA agonists, Clobazam, Lofendazam were carried out. The dock scores calculated for Clobazam, Lofendazam were -5.2598,-4.7373 respectively. Among the ~~designed~~ modelled compounds, following conformation were found to have ~~lowest~~ lower dock scores in comparison to other conformers indicated in bracket;. N^1-benzoyl-7- methoxy- 4-methyl-1,5-benzodiazepine-2-one, conformer_C1 (-4.5991), N^1-chloroacetyl-7-methoxy-4-methyl-1,5-benzodiazepine-2-one, Conformer_C4 (-4.1805), N^1-(1,3,4-thiadiazol-2-yl amino acetyl) -7-methoxy- 4-methyl-1,5-benzodiazepine-2-one, Conformer_C4(-4.1628) and 4,7-dimethyl-1,5-benzodiazepin-2-one, Conformer_C5 (-3.1440). These conformers ~~and said to~~ have more affinity for active site of GABA-A receptor associated protein than other molecules.

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KEYWORDS

Docking, GABA-A receptor associated protein, 1,5- benzodiazepines, conformers

INTRODUCTION

1, 5 benziodiazepines have wide spectrum of biological activities including anticonvulsant activity.[1-2] In addition to presently available anticonvulsant drugs, there is a need to develop such new heterocycles with the expectation to have more anticonvulsant potential. There is an ever increasing need of research into newer molecules with lesser toxicities and side effects for treating epileptic seizures.Various docking studeis have reporetd ~~for~~ that benzodiazepine derivative ~~containg~~ contains heterocycles viz. traizole, pyrimidine, quinazoline.[3-4] Molecular docking helps in studying drug/ligand or receptor/protein interactions by identifying the suitable active sites in protein,obtaining the best geometry of ligand receptor complex and calculating the energy of interactions for different ligands to design more effective ligands. The interaction energy is calculated in terms of dock score. S~~,s~~coring functions are the fast approximate mathematical methods used to predict the strength of the noncovalent interaction between two molecules after they ~~have been~~ docked. Most scoring functions are physics-based molecular mechanics and the force fields that estimate the energy of the low (negative) energy indicates a

stable system and thus, alikely binding interaction.The options available forof docking are; rigid docking where a suitable position for the ligand in receptor environment is obtained, flexible docking where afavored geometry for receptor-ligand interactions is obtained, full flexible docking where the ligand is flexed via its torsion angles as well as the side chain of active site residues.[5,6]

MATERIALS AND METHODS

Hardware and Software

All Docking studies and conformational analysis were performed using the Molecular Design Suite (VLife MDS software package, version 4.3; from VLife Sciences, Pune, India) on Lenovo computer, i3 processor with XP operating system.

Structure Conformation Generation

Structures of compounds were sketched using the 2D structure draw application of Vlife2Ddraw and converted to 3D structures. All the structures were minimized and optimized with using the AMBER method taking the root mean square gradient (RMS) of 0.01 kcal/molA° and the iteration limit to 10,000. Conformers for each structure were generated using Monte Carlo be by applying AMBER force field method. The and least energy conformer was selected for further study.

Preparation of protein

The PDB structures [1KJT] (PDB Nos.) were downloaded from www.rcsb.org and energy minimization of the protein complexes was done. During preprocessing, Aall the bound water molecules, ligands, and cofactors were removed (preprocess) from the proteins, which were taken in.pdb format. The tool neutralized the side chains that were not close to the binding cavity and did not participate in salt bridges. This step was then followed by restrained minimization of co-crystallized complex, which reoriented side-chain hydroxyl groups and alleviated potential steric clashes. The complex obtained was minimized using AMBER force field. The minimization was terminated after either completion of 5,000 steps or after convergence of the energy gradient converged below 0.05 kcal/mol.

Preparation of ligands

Structures of the1,5 benzodiazepaines derivatives ligands were sketched using builtin Vlife 2D draw taken in .mol2 format. Converted it into 3D structure and performed a geometry minimization of the ligands. AMBER Force Fields with default settings were used for the ligand minimization.

VlifeMDS software was used to prepare the ligand for docking. This procedure is outlined as follows.

- ✓ 2D structures of ligands were drawn in Chemdraw.
- ✓ 2D Structures were converted to 3D.
- ✓ Conformers were generated and optimized.
- ✓ Lowest energy conformer was selected and used for docking.
- ✓ Docking was done by GA based docking.
- ✓ Cavity 1 was selected for docking.
- ✓ Dock score was calculated.
- ✓ Docked Complex was optimized.

Docking methodology[7-9]

Docking study was performed on VlifeMDS version 4.3 on Lenovo computer, i3 processor with XP operating system. The GA-based ligand docking with genetic algorithm approximated a systematic search of positions, orientations, and conformations of the ligand in the enzyme binding pocket via a series of hierarchical filters. The minimum dock score of example may not be exactly reproducible because this is a Genetic Algorithm (GA) based run. However changing

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Comment [A4]: Mention PDB numbers.

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the different input parameters in GA Parameters dialog box (like No of Generations, Translation, Rotation limits etc.) can result in dock scoring energies within desired range and improvement in the orientation of docked ligand as close to that of co crystallized ligand as possible.
Genetic Algorithm implemented in Molecular design suite (MDS) has been successfully employed to dock inhibitors into catalytic site of the receptor and to well correlate the obtained binding score with inhibitory activities of compounds. In this docking studies carried out the comparative docking experiments of designed compounds with known calcium blockers Ethosuximide,gabapentine respectively. Obtained results were evaluated in terms of docking score in to the active site of 1KJT. During the docking process the system search of conformational, orientational and positional space of the docked ligand and eliminating the unwanted conformation using the scoring, the structure available on PDB, using AMBER force field then is optimized. Batch docking in MDS of designed ligands is performed with GABA-A receptor associated protein.

RESULTS AND DISCUSSION

Docking results

VLifeMDS provides a facility to dock different ligands in protein binding sites chosen by the user. VLifeMDS provides both rigid (no torsional flexibility for protein as well as ligand) and flexible (torsional flexibility to ligand with rigid protein) docking of the molecules. The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking. Here in this study the target protein was generated through knowledge based protein or homology modeling. VLifeMDS uses genetic algorithm, Piecewise Linear Pairwise Potential (PLP) and Grid algorithms to minimize the interaction energy between ligand and the receptor protein. The molecular docking scores identify the ligands that bind with similar orientation as observed with reference ligands. Most of the ligands make good docking poses in comparison to the reference ligand. Selective ligands docked deeply within the binding pocket region suggesting their shape complementarily with the reference ligands. ~~The vander walls , H bonding and hydrophobic interactions of the ligands with receptor proteins were analyzed which reveals novel set of information.~~

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The molecular docking studies of all possible three dimensional confirmations of N^1-benzoyl/ N^1-chloroacetyl/ N^1-(1,3,4-thiadiazol-2-yl amino acetyl) -7-substituted- 4-methyl-1,5-benzodiazepine-2-one and 7-Substituted-4-methyl-1,5-benzodiazepin-2-one were done using Vlife MDS Biopredict amodule using cavity#1 ~~of~~ against GABA-A receptor associated protein (1KJT) obtained from Protein Data Bank as target protein. The intermolecular interactions in between the ligand and the protein (receptor) were investigated. It ~~is~~ was processed by deleting the solvent molecule as well as correcting the structure with respect to bonds and the H-atoms.

Table 1 shows Dock scores and binding energies of conformations of N^1-benzoyl-7-substituted-4-methyl-1,5 -benzodiazepine-2-ones. **Table 2** shows Dock scores and binding energies of conformations of N^1-chloroacetyl-7-substituted-4-methyl-1,5-benzodiazepin-2-ones. **Table 3** shows Dock scores and binding energies of conformations of N^1-(1,3,4-thiadiazol-2-yl amino acetyl)-7-substituted-1,5-benzodiazepin-2-ones. **Table 4** shows Dock scores and binding energies

of conformations of N^1-(1,3,4-thiadiazol-2-yl amino acetyl)-7-substituted-1,5-benzodiazepin-2-ones. Some of the molecules for which the confirmations shows lowest dock scores were selected to study their binding interaction with the cavity#1 of the receptor. The binding patteren of the docked molecules has been compared with few standard ligands like Clobazam and Lofendazam their intercations are also shown in **Figure 1**.

The Hydrophobic and Vander Waals interactions with residues at cavity#1 of 1KJT were studied for N^1-benzoyl-7-methyl-4-methyl-1,5-benzodiazepine-2-ones (Compound 4; Confirmor_C20)**;** the residues PHE77A, LEU76A, VAL114A, GLU112A, ASP111A, SER110A, TYR109A, VAL44A interact with the molecules during the binding as shown in **Figure 2** and for the N^1-(1,3,4-thiadiazol-2-yl amino acetyl)-7-methyl-1,5-benzodiazepin-2-one (Compound 14; Confirmor_C2); SER110A, ASP111A, GLU112A, VAL114A, ALA108A, TYR109A, PHE77A, LEU76A are the residues taking part in the interaction as shown in **Figure 3**.

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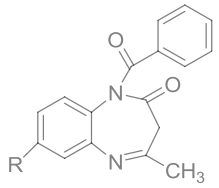
Table 1

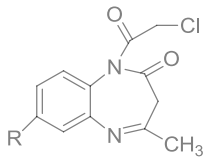
DOCK SCORES AND BINDING ENERGIES OF CONFORMATIONS OF N^1-BENZOYL-7-SUBSTITUTED- 4-METHYL-1,5 -BENZODIAZEPINE-2-ONE



Conformation of compounds	**R**	**Dock score**	**ΔG (Kcal/mol)**
1_C4	-Cl	-5.085262	-15.2341
2_C3	-Br	-5.091527	-16.1032
3_C8	-F	-5.064088	-16.3924
4_C20	$-CH_3$	-5.074595	-12.5565
5_C1	$-OCH_3$	-4.599131	-16.6361

Table 2

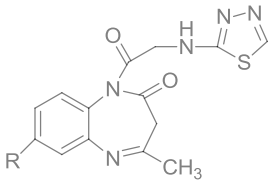
DOCK SCORES AND BINDING ENERGIES OF CONFORMATIONS OF N^1-CHLOROACETYL-7-SUBSTITUTED-4-METHYL-1,5-BENZODIAZEPIN-2-ONE



Conformation of compounds	R	Dock score	ΔG (Kcal/mol)
6_C12	-Cl	-4.769054	-16.1226
7_C5	-F	-4.451437	-17.6753
8_C4	-Br	-4.786992	-17.0146
9_C4	$-OCH_3$	-4.180516	-20.5896
10_C4	$-CH_3$	-4.598894	-16.4017

Table 3

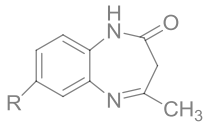
DOCK SCORES AND BINDING ENERGIES OF CONFORMATIONS OF N^1-(1,3,4-THIADIAZOL-2-YL AMINO ACETYL)-7-SUBSTITUTED-1,5-BENZODIAZEPIN-2-ONE



Conformation of compounds	R	Dock score	ΔG (Kcal/mol)
11_C2	-Cl	-4.653290	-23.4524
12_C2	-Br	-4.535921	-19.5515
13_C5	-F	-4.358920	-24.5772
14_C2	$-CH_3$	-4.614051	-18.9478
15_C4	$-OCH_3$	-4.162892	-30.1304

Table 4

DOCK SCORES AND BINDING ENERGIES OF CONFORMATIONS OF N^1-(1,3,4-THIADIAZOL-2-YL AMINO ACETYL)-7-SUBSTITUTED-1,5-BENZODIAZEPIN-2-ONE



Conformation of compounds	R	Dock score	ΔG (Kcal/mol)
16_C13	-Cl	-5.048574	-17.3405
17_C10	-Br	-3.663538	-16.3842
18_C1	-F	-4.046493	-17.5037
19_C5	$-CH_3$	-3.144092	-13.7129
20_C5	$-OCH_3$	-3.958974	-19.0389

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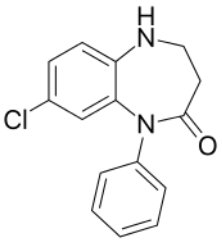
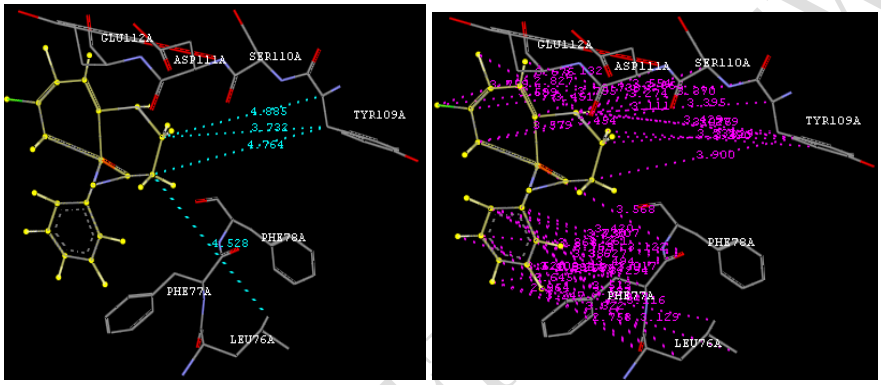
Standard : Lofendazam

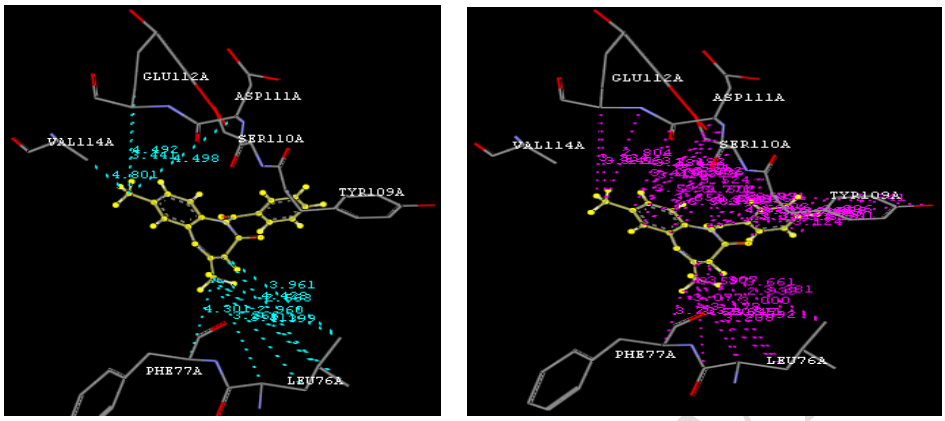




(A) (B)

Figure 1: BINDING INTERACTIONS OF LOFENDAZAM WITH CAVITY # 1 of 1KJT
(A) Blue colour dotted lines indicate hydrophobic interactions with the residues TYR109A and LEU76A
(B) Magenta colour dotted lines indicates Van der Waals interactions with the residues PHE77A, PHE78A, LEU76A, GLU112A, ASP111A, SER110A and TYR109A with cavity # 1 of Crystal structure of GABA-A receptor associated protein (1KJT).

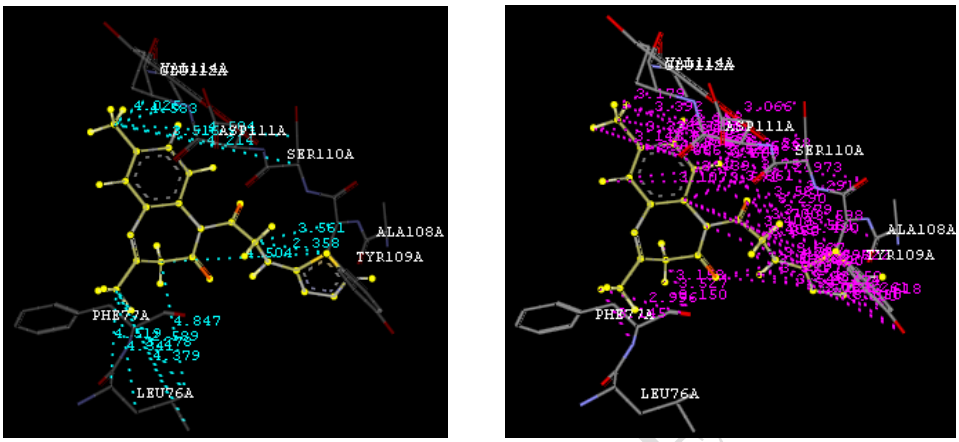
Compound 4



(A) (B)

Figure 2: BINDING INTERACTIONS OF COMPOUND 4_C20 WITH CAVITY # 1 of 1KJT
(A) Blue colour dotted lines indicate hydrophobic interactions with residues PHE77A, LEU76A, Val114A, GLU112A, and ASP111A
(B) Magenta colour dotted lines indicates Van der Waals interactions with the residues VAL44A, ASP111A, SER110A, TYR109A, LEU76A, PHE77A and GLU112A.

Compound 14



(A) (B)

Figure 3: BINDING INTERACTIONS OF COMPOUND 14_C2 WITH CAVITY # 1 of 1KJT
(A) Blue colour dotted lines indicate hydrophobic interactions with the residues SER110A, ASP111A, GLU112A, VAL114, ALA108A, TYR109A, PHE77A and LEU76A
(B) Magenta colour dotted lines indicates Van der Waals interactions with residues SER110A, ASP11A, GLU112A, VAL114, ALA108A, TYR109A, PHE77A and LEU76A.

The molecular docking scores ~~identify~~ identified the ligands that bind with similar orientation as observed with standard ligand. Most of the ligands make good docking poses in comparison to the standard ligand. Selective ligands docked deeply within the binding pocket region suggesting their shape complementarily with the standard ligands. The vander walls, H-bonding and hydrophobic interactions of the ligands with receptor proteins were analyzed which revealed novel set of information regrading the similarity of amino acid residues that are participating in the intercation of the standarad, Lofendazam and the designed compounds at the Cavity # 1 of 1KJT. It was fopund that amino acid residues viz. PHE77A, LEU76A, GLU112A, ASP111A, SER110A, TYR109A are the similar residues among those that participating in the intercation with 1KJT. Thus the docking simulation suggested that the modifications in the series of N^1-benzoyl/ N^1-chloroacetyl/ N^1-(1,3,4-thiadiazol-2-yl amino acetyl) -7-substituted- 4-methyl-1,5-benzodiazepine-2-one and 7-Substituted-4-methyl-1,5-benzodiazepin-2-one resulted in identification of ligands with better binding potential. The Vander walls, hydrophobic, hydrogen interactions are responsible for forming the stable complexes of the ligands with receptor. The studies also resulted in highlighting the ligands and their conformations which efficiently fit into the cavity of target protein. The newly designed molecules viz. N^1-benzoyl-7- methoxy- 4-methyl-1,5-benzodiazepine-2-one, N^1-chloroacetyl-7-methoxy-4-methyl-1,5-benzodiazepine-2-one, N^1-(1,3,4-thiadiazol-2-yl amino acetyl) -7-methoxy- 4-methyl-1,5-benzodiazepine-2-one and 4,7-dimethyl-1,5-benzodiazepin-2-one can be priritized for synthesis and can be studied for Pharmacological screeing.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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