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Research Article

DOCKING STUDIES OF BENZODIAZEPINES AS A POSITIVE ALLOSTERIC MODULATOR OF GABA-A RECEPTOR

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ABSTRACT

Benzodiazepine is an antixylotic agent , induce and maintain sleep, reduce seizures, and induce conscious sedation. It acts as a positive allosteric modulator of GABAA receptor. The binding of benzodiazepine analogues to this allosteric modulatory site enhances the affinity of GABA (Gama amino butyric acid) for the agonist recognition site. In the present work, docking studies has been performed to understand the effect of substitution and structural features on the GABA agonist activity of Benzodiazepines and to study the interactions of benzodiazepine derivatives with the binding sites on GABAA receptor. In present study structure based drug design is applied to visualize the structural requirement of the compounds. Our previous QSAR model ($r = 0.995$, $r^2 = 0.990$, Std deviation $s = 0.0636$) reveled that the descriptors Surface Tension, Molar Volume and Parachor played an important role in binding affinity of Benzodiazepines derivatives to GABAA receptor . A docking study has been performed on the same set of compounds to re-examine our previous findings. The hypothesis has also been validated with an experimental data. The outcome of the present study may be useful in the designing of more potent Benzodiazepine analogue as an antixylotic agents.

Keywords: QSAR, Docking, Gama Amino Butyric Acid agonist, GABA receptor, Benzodiazepine derivatives.

INTRODUCTION

"Benzodiazepines (BZDs) are the chemicals having the versatile medicinal values as tranquillizers and were used therapeutically as anxiolytics and anticonvulsants in epilepsy. Benzodiazepines (BZDs) are the type of psychotropic drug, that is, they concern the mind and can amend frame of mind.[1,2].

BZDs bind with specific receptors in the nervous system that are the part of GABA neurotransmitter system. GABA (gamma-amino-butyric acid) is the major neurotransmitter for the maintenance of chloride channel which controls the anxiolytic activity[3]. BZDs are source for sedation, striated (skeletal) muscle relaxation, and have anxiolytic (anti-anxiety) and anticonvulsant properties along with some anti-HIV activities [2-6]. In terms of chemical structure, benzodiazepines exhibit the similar mechanism of biological action like flunitrazepam, temazepam, triazolam and diazepam[7] In reference to the psychotropic activity, after entering in the brain, benzodiazepines sprayed rapidly and work after the binding to a specific type of protein (GABAAreceptor) that is also widely disseminate in the groups of nerve cells involved in anxiety, memory, sedation and coordination[5]. Benzodiazepines bind tightly to a specific part of the GABA receptor, imaginatively called the benzodiazepine site, which is different from the GABA binding site. Binding of benzodiazepine derivatives to that particular site, enhance the effect of GABA to shut down brain activity more effectively [8],[9]

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"Molecular similarity approaches, quantitative structure-activity relationships (QSAR) and pharmacophore models are frequently used methods in the ligand-based drug design process [10]. By using the molecular fingerprints of known ligands, databases can be screened to find molecules with similar fingerprints [11]. To predict the activity of a novel molecule, models can be built with QSAR [12]. While a pharmacophore model may only indicate the activity-conferring features of an active ligand, the relationship between chemical or physical properties of ligand and biological activity can be more fully explored using the QSAR model.

If the drug design process can developed with a known target and if reliable information on the 3-D structure and active sites of the target protein can be obtained from X-ray crystallography, nuclear magnetic resonance, or 3-D structure databases, and incorporated into a computer model, compounds binding to the target can be designed [13]. This approach is known as "structure-based drug design". Frequently used techniques in this approach are docking and molecular dynamics simulation [14],[15]

Early SAR studies on benzodiazepines indicated that the seven- membered amino-ring was indispensable for its affinity towards the benzodiazepine binding site at GABA receptor[16]. Further quantitative structure activity relationship and structure property relationship studies[17] found that the molecular lipophilic properties of numerous BZs played a important role in their corresponding receptor affinity.

In our previous work[9], Quantitative Strucutre Activity relationship (QSAR) study followed by the conformational analysis was performed with an objective to develop an efficient predictive QSAR model for the binding affinity of benzodiazepine derivatives and to determine the suitable conformers of the compounds. In continuation to our previous study, docking studies has been performed to reinvestigate the importance of structural descriptors viz., Surface Tension (ST), Molar Volume (MV) and Parachor(Pc). This leads to elaborate the effect of substitution and structural features, on the given set of Benzodiazepine for its binding affinity towards the GABAA receptor. The objective of the present study is to optimize the relative values of structural parameter viz., ST, MV and Pc, and to demonstrate the most effective substituent for the binding of benzodiazepine with GABAA receptor.

EXPERIMENTAL AND METHOD

Biological Activity The data sets used in the study includes a set of 27 substituted benzodiazepines. The biological activity for the set of 27 compounds used in the present study, analyzed as IC50 (primarily Ki, that is, binding affinity with receptor), were taken from the literature [18,9]. The parent compounds and substitution are presented in Figure 1 and Table 1

IC50 and affinity

"IC50 is not a direct indicator of affinity although the two can be related at least for competitive agonists and antagonists by the Cheng-Prusoff equation.[19]

Ki = IC50/(1+[s]/Km)

where Ki is the binding affinity of the inhibitor, IC50 is the functional strength of the inhibitor, [S] is fixed substrate concentration and Km is the concentration of substrate at which enzyme activity is at half maximal (but is frequently confused with substrate affinity for the enzyme, which it is not).[20]

Preparation of Ligands

The ligand preparation included few steps : (i) 2D–3D conversions, (ii) correcting structures, (iii) generating variations of these structures, (iv) validate and optimizing the structures. All these tasks were performed using Hyperchem. ACD Chemsketch was used for drawing, displaying and to characterize the chemical structures. Geometry optimization has been performed using MM+ force field and Polak Ribiere algorithm (Conjugate gradient).

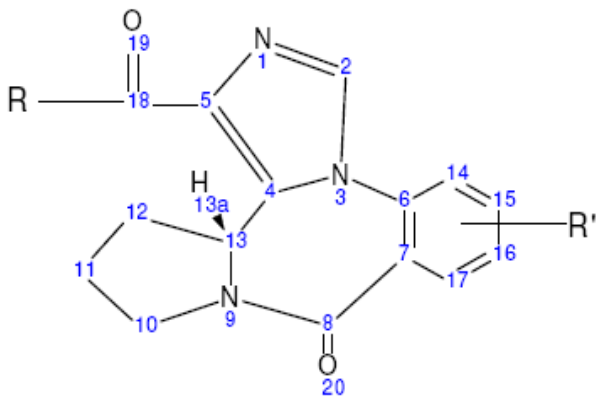

Figure 1. Basic structure of the benzodiazepine derivatives.

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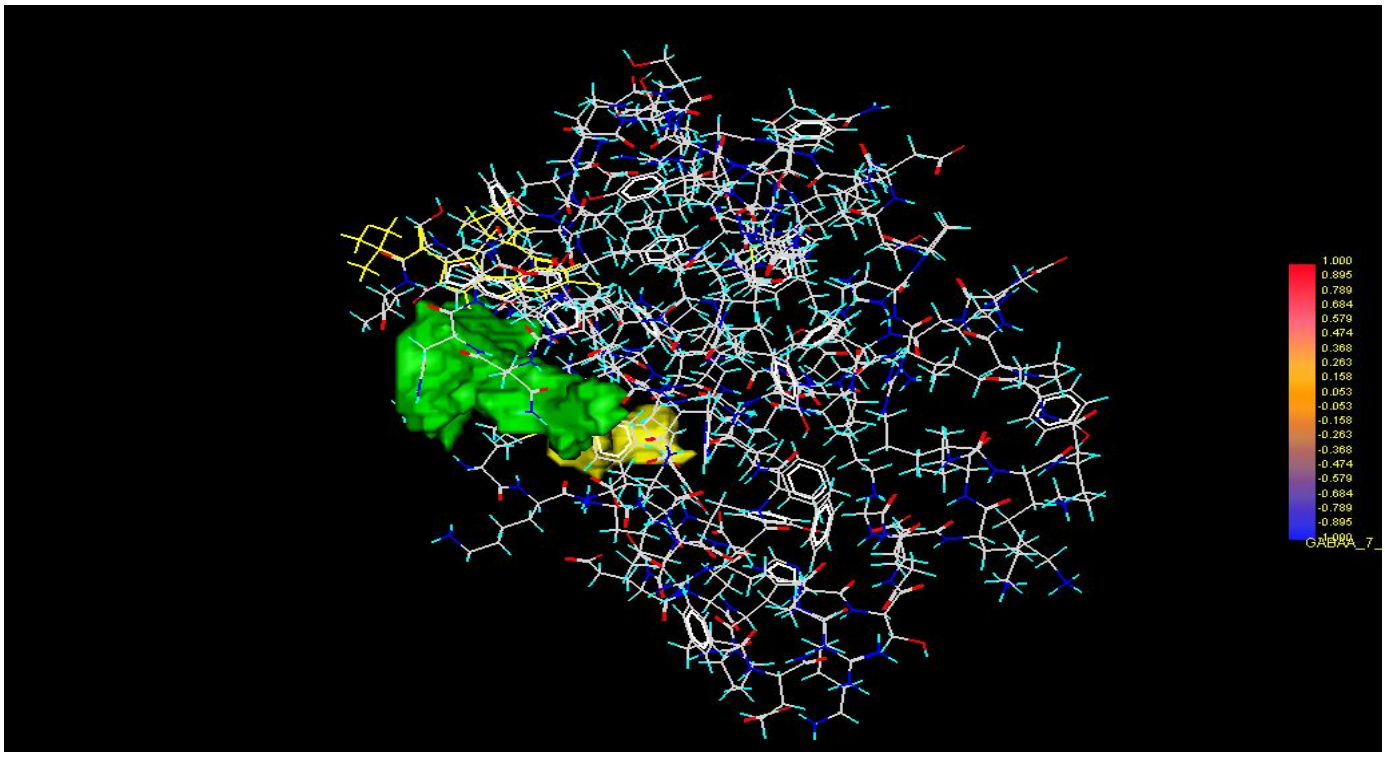

Figure 2: Compound 13 Docked on the active site of GABA receptor. The ligand is highlighted by yellow

Table 1: The docking score and physicochemical properties of Benzodiazepine ligands

S.No	R	R'	logI C_{50}	D Score	MV	PC	ST
1.	NH2	17Cl	3.000	3.74	186.9	549.6	74.6
2.	O-Et	---	0.0064	3.62	223.9	607.0	53.9
3.	O-Et	17Cl	0.0017	4.33	233.2	635.8	55.2
4.	O-Et	16Cl	0.0620	3.88	233.2	635.8	55.2
5.	O-t-Bu	17Et	0.0460	4.98	281.4	734.3	46.3
6.	O-t-Bu	17Me	0.0032	3.70	274.9	715.1	45.7
7.	O-t-Bu	----	0.0320	3.15	259.7	684.0	48.1
8.	O-t-Bu	17-SMe	0.0034	4.57	289.4	764.6	48.7
9.	O-t-Bu	17-F	0.0064	3.32	262.6	684.2	46.0
10.	O-t-Bu	16-F	0.0077	2.86	262.6	684.2	46.0
11.	O-t-Bu	17-Cl	0.0025	3.71	269.0	712.9	49.3
12.	O-t-Bu	17-Cl, 16-F	0.0031	4.61	271.9	713.1	47.3
13.	O-t-Bu	16-Cl	0.0900	2.82	269.0	712.9	49.3
14.	O-t-Bu	17-Br	0.0022	4.48	272.2	727.6	50.9
15.	O-t-Bu	17-I	0.0021	4.15	277.3	746.8	52.5
16.	O-t-Bu	17-CF_3	0.0033	5.25	277.3	746.8	52.5
17.	O-t-Bu	17-NO_2	0.0028	4.01	265.0	729.5	57.4
18.	O-n-Pr	17-Cl	0.0014	4.14	244.0	669.9	56.8
19.	O-i-Pr	17-Cl	0.0025	3.84	243.1	662.4	55.0
20.	O-$CH_2CH{=}CH_2$	17-Cl	0.0017	4.29	249.3	674.4	53.5
21.	O-n-Bu	17-Cl	0.0026	4.07	265.4	713.0	52.1
22.	O-i-Bu	17-Cl	0.0063	4.28	264.5	705.5	50.6
23.	O-CH2-CyPr	17-Cl	0.0029	3.82	264.5	705.5	50.6
24.	O-n-Hexyl	17-Cl	0.0023	3.77	237.7	665.8	61.4

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Preparation of Receptor

The GABAA were chosen as the target receptor due to their vital role in anxiety, memory, sedation and coordination. The structure of GABAA receptor were retrieved from Protein Databank (PDB) (http://www.pdb.org/). The pdb file for receptor contain water molecule and three cofactors viz, Nitrogen and two Nickel atom. The 3D structure of GABAA receptor (PDB id 1KJT) has been prepared by removing water molecules and cofactors using VlifeMDS 3.1

Docking using VlifeMDS 3.1

The procedure of docking of ligands (Benzodiazepines derivatives) with the receptor (GABAA) has been performed using VLife MDS 3.1 Modules. Docking is virtual screening of a database of compounds and predicting the efficiently binding ligand(s) based on various scoring functions. The ligand library has been generated by gathering all the 27 Benzodiazepine derivatives in a Vlife folder. The preparation of the library helps in making an easy comparative study between ligands by performing simultaneous docking of multiple ligands against the receptor. The grid batch docking has been performed using Biopredicta module of Vlife MDS 3.1 with 10o rotation angle, Dock Score as fitness function and allowing 4 bumps. The result of each docked molecule appears in terms of final minimum score (Dock score interaction/ docking energy of receptor-ligand). The docking score of 27 benzodiazepine derivatives is presented in Table 1

QSAR Study of Benzodiazepine Derivatives:

In our previous QSAR study, the model obtained shows the participation of Surface Tension, Molar Volume and Parachor in modeling of binding affinity of Benzodiazepines[9]. The mathematical model obtained along with its statistical parameter is shown in Eq (1)

$\log IC_{50} = 0.3573 (\pm 0.0191)\ ST + 0.2817 (\pm 0.0172)\ MV - 0.1041 (\pm 0.0062)\ Pc - 19.1479$ .....(1)

n = 27, Se = 0.112, R = 0.9831, R2 = 0.9665, F = 221.118, Q = 8.78

According to Eq (1) surface tension (ST) plays the direct role in binding. This relationship shows that inter and intra molecular forces, helps the benzodiazepine ligand to bind with receptors in the combination with molecular size and steric effects in terms of molar volume (MV) in approximately same magnitude. On the other hand lowering of parachor (Pc) is favourable to increase binding affinity.[9] The values of ST, MV & Pc is given in Table 1.

RESULT AND DISCUSSION

In present docking study, we evaluate the structural features that are effectively participating in the binding of benzodiazepines and optimized the relative value of ST, MV and Pc for the binding of Benzodiazepines. Present Study is an extension of our previous ligand based study to the structure based study.

Docking scores revels that Compound 13 (D Score = 2.82) with substitutents O-t- Butyl with 16-Cl., is the compound showing efficient binding with GABA receptor. There are some other compounds present in data set, that demonstrating higher value of MV and ST but still showing less efficient docking, this is mainly because of their higher Pc value, which need to be lower for efficient binding. All the three parameters are relatively optimized in case of compound 13.

An additional important observation of the present docking study is the role of position 16 of the ligands. This can be clearly noticeable by comparing the parameters of Compound 11 & 13. These compounds are sharing identical values of MV, ST & Pc but exhibiting largely different D-Score. Merely changing the position of –Cl group from 17 to 16 position improves docking score.

Compound 13 showing minimum docking score, their docked complex structures with GABA receptor have been shown in Figure 2.

Validation of Hypothesis using Experimental Data:

Compound 1 showing highest experimental value of logIC50, (3.000) in comparison to other ligands in a series showing binding affinity in decimal values ranging from (0.0014 to0.0900). Compound 1 is excluded from the study, due to its abnormally high log IC50.

After excluding compound 1 from the study, next highest experimental logIC50 belongs to Compound 13, same ligands successfully demonstrating lowest docking score. This lead to the assumption that the corresponding values of MV, ST and Pc (269.0, 49.3 and 712.9 respectively) are the relative optimum values of QSAR parameters for the efficient binding.

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CONCLUSION

The present study supports our previous finding which illustrate the role of surface tension, molar volume and parachor in modeling binding affinity of benzodiazepine ligands. Also, the relative optimized value of these three descriptors has been determined.

On the basis of docking study, it has been reveled that substitutents O-t-Butyl at –R and Chloride group at 16th position i.e., at R' is the most appropriate arrangement for an efficient binding of the Benzodiazepine ligands.

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