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(Research Article)

In-Silico Docking of Neuroactive Flavones on Benzodiazepine Binding Site of $GABA_A$ Receptor Homology Model

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ABSTRACT

Flavones share structural and mechanistic features of $GABA_A$ ligands. The mode of binding and interactions of flavones was investigated in a homology model generated in an automated mode. The binding pocket was predicted with Fuzzy oil drop mathematical model. The ligands were docked using PM3 force field method and various ligand-protein docking interactions were calculated. The ligand poses with maximum negative docking scores were further observed for binding mode and the amino acid residues lining the 2-phenyl ring and flavone nucleus were noted. Flavones docked in the predicted binding pocket with energies comparable to $GABA_A$ benzodiazepine (BZD) site ligands diazepam and zolpidem. Two docking conformers were observed Flavones presented two binding conformers which bind head to tail with respect each other with respect to 114TYR residue. Flavones were classified into two conformers: Conformer A represented by 5'-Bromo-2'-hydroxy-6-methylflavone: in which 114 TYR surrounds the 2-phenyl group. Conformer B was represented by apigenin: in which 114 TYR surrounds the flavone nucleus.

Key Words: GABA-A receptors, Structure-Activity Relationship, Fuzzy oil drop model, Flavones, Flavonoids, Docking

INTRODUCTION

Flavonoids are being studied as modulators of GABA(A) receptor function influencing inhibition mediated by the major inhibitory neurotransmitter GABA in the brain. Flavonoids showing subtype selectivity in recombinant receptor studies in vitro consistent with their behavioural effects in vivo and the identification of the active site of flavonoids on GABA(A) receptor complexes [1]. An emerging area of interest is the direct activation of GABA(A) receptors by flavonoids [2]. The relatively rigid shape of flavonoids alongwith the classic planar nature of benzopyrone part makes it an attractive lead template for development of therapeutic agents. Flavonoids are active on numerous biological targets. The challenge is to understand the structural activity relationship of flavonoid effects on particular targets and to develop ligands for specific for these targets.

Different structural classes of flavonoids, share the main characteristics of the benzodiazepine (BDZ) nucleus, are active in the modulation of anxiety, sedation, convulsion, myorelaxation, hypnotic and amnesic states in mammals These compounds have high affinity for the benzodiazepine binding site (BDZ-bs) of the GABA(A) receptor complex [3]. Flavonoids and their glycosides can cross the blood brain barrier and bind to the benzodiazepine site on the GABA(A)-receptor resulting in sedation, anxiolytic or anti-convulsive effects [4].

Zhang *et al.* (1995)[5] have proposed a receptor and pharmacophore model of the benzodiazepine binding site that accounts for the general requirements that should be met by this receptor for ligand recognition. [6] have described homology modeling and docking study on $GABA_A$ α_1/γ_2 subunits for various ligands on benzodiazepine binding site. The study describe homology modeling approach for construction of binding site of the receptor by multiple sequence alignment and editing of amino acid sequence to match the template provided by conserved protein domains of murine $GABA_A$ subunits α_1 and γ_2.

The homology modeling and editing of multiple sequence alignments for determining 2D/ 3D coordinates of ligand binding site coordinates is complex and presents difficulties in large scale in silico molecular modeling and docking campaigns common in lead screening programs. In continuation of our work on automated *in-silico* homology modeling and docking of ligands on $GABA_A$ receptors[7,8], we report novel homology model development and active site prediction strategy for docking of flavone ligands on BZD binding site of $GABA_A$ receptors.

MATERIALS AND METHODS

Bioinformatics tools such as Swiss model server, Fuzzy oil drop server, Argus lab software, and Hex docking software's were used. PDB ID 1uw6 (X-ray structure of the AChBP complexed with nicotine) [Celie et al., 2004]. Sequences were retrieved from Uniprot website, after cross references with databases like NCBI, SWISS-PROT, TrEMBL and UNIPROT . The sequence length of 1uw6 is 211 residues.

A. Homology Modeling by Swiss 3D Modeler

Homology model of $GABA_A$ receptor BZD binding site was generated as described previously [7]. The SWISS-MODEL Repository is a database of annotated three-dimensional comparative protein structure models generated by the fully automated homology modeling pipeline SWISS-MODEL, run by the Swiss Institute of Bioinformatics [9-11]. The mature protein sequences of the rat α_1 and γ_2 subunits (accession numbers: α_1, P62813; γ_2, P18508) were automatically aligned with sequences of two adjacent AChBP subunits (A and B, respectively) using ClustalW [12] and submitted to the Swiss 3D modeling server for homology modeling and 3D structure generation. The server generated 1 structure for the sequence. For segment 1 modeled range is from 1 to 209 based on template 2zjuB with sequence identity of 98.565 % and Evalue = 0.00e-1. The 3D ribbon view of the generated homology model along with projecting amino acid residues was obtained (**Figure 2A)**.

B. Active Site Identification by Fuzzy Oil Drop Calculation Server

The fuzzy oil drop is a gaussian model oriented on localization of area responsible for ligand binding or protein-protein complex creation is based on characteristics of spatial distribution of hydrophobicity in a protein molecule. It has long been used for recognition of ligand binding site in proteins [13]. The assumptions and calculations involved have been described elsewhere [7]. It is assumed that hydrophobicity changes from protein interior (maximal hydrophobicity) to exterior (close to zero level of hydrophobicity) according to the three-dimensional Gauss distribution. It is generally accepted that the core region is not well described by a spheroid of buried residues surrounded by surfaces residues due to hydrophobic channels that permeate the molecule. Therefore the simple comparison of theoretical (idealized according to Gauss function) and empirical spatial distribution of hydrophobicity in protein gives the opportunity to identify the regions with high deviation versus the ideal model. Those regions recognized by high hydrophobicity density differences seem to reveal functionally important sites in proteins. The model has been found to be verified positively for prediction of 3D coordinates of 1NMF, a downhill protein [14] and small peptides representing various functional groups [15]. [16] have described a method for prediction of ligand binding site based on location of a region of unusual hydrophobicity in a protein structure. The PDB file containing 3D co-ordinates of homology model obtained from SWISS MODEL workspace server was submitted to fuzzy oil drop model server at website (http://www.bioinformatics.cm-uj.krakow.pl/activesite/) for determination of ligand binding site. The calculated ligand binding site co-ordinates were saved on hard disk of a computer having Intel Core2Duo™ microprocessor and Windows7™ operating system as PDB file. The 3D ribbon structure of the binding site is shown in **Figure 2b**.

C. Construction of Ligands by Molecular Builder Tool of Argus Lab Software

The tool provided allows constructing new molecules and modifying existing molecules. Using its molecular formula, the ligands were constructed by Chemdraw4 software. Energy minimization was performed using molecular builder toolkit function of Arguslab 4.0.1 [17] The structures were manually checked for inconsistencies and corrected for hybridization states and bond orders. All the ligands were converted into PDB format for docking purpose.

D. Docking and Binding Evaluation

In the automated Argus Lab 4.0.1 system [17], using a generic algorithm with a fast-simplified Potential of Mean Force (PMF) carried docking of flavonoid ligands into 3D active site structure. It was assumed that the protein and the ligand docked non-covalently. The standard PMF implementation used UFF potential for this purpose. The docking was carried with flexible ligand into a rigid protein active site. The general procedure for the docking process started with the addition of energy minimized target ligand on the 3D coordinates of the predicted binding site on homology modeled protein obtained in earlier step. The predicted active site was defined by amino acid codes obtained from fuzzy oil drop calculations. The ligands were specified in the program. Using $22\times22\times22$ A^0 box located at the centre of the target active site optimized the different starting parameters. The whole procedure of docking was repeated until a constant value of docking score was achieved. If a ligand did not dock in Argusdock™ mode, it was docked with GADdock mode.

Concluding docking results were parameterized in terms of docking score in Kcal/mol. The docked $GABA_A$ receptor benzodiazepine site ligands **1a-*l*,** complexed with $GABA_A$ receptor α_1/γ_2 homology benzodiazepine model was interpreted by looking at the H-bonding or hydrophobic interactions of the ligand with the amino acid residues in the active site. The results obtained from the docking of these ligands of BZD site of $GABA_A$ receptor into the predicted target active site pocket are summarized in the **Table 1.**

Compounds ***2a-2w*** all have carbon atom of the central core (Flavone skeleton) as sp^2 hybridized while the C-2 carried differently substituted aryl group. These derivatives have some structural similarities with benzodiazepines. Due to decrease in steric repulsion between the C-2 phenyl ring and the flavone core ring provides a degree of freedom to overall structure. As all the ligands showed difference in their binding energies pointing towards the significant role of various substituents in their binding abilities.

The docked structures of ligands were overlaid and visually compared for characterization of their binding mode and a pharmacophore model was constructed based on observation of structures of ligands binding in similar conformation and docking scores.

RESULTS AND DISCUSSION

Validation of PMF Method

To validate the docking model, before docking the test ligands (BZD site ligands **1*a*-1*l*** and Flavones **2*a*-2*w***), the docking of diazepam into the active BZD binding site in

homology model of $GABA_A$ receptor was performed. Diazepam binds into the active site cavity with a binding score of -10.6596 kcal/mol and R.M.S.D in binding scores of two consecutive docking runs of the same ligand was observed to be 3.12 which was well within acceptance value of NMT 5%. The docked structure of diazepam in the active site of HMT enzyme is shown in Figure 3. The close overlapping of a docked structure with Zolpidem and SL 651 498 **(Figure 4)** demonstrates the validity of the model.

Docking of $GABA_A$ receptor ligands 1a-1l into active site

Known BZD site ligands ***1a-1l*** **(Figure 3)** were docked into the active site of the homology model. The ligands were selected on basis of differences their reported subtype selectivity and agonist properties reported in the literature. Diazepam, SL651498 and Flunitrazepam showed highest docking scores **(Refer table no. 1)** which was found to be in good agreement with the fact that the three ligands are known agonists at the $GABA_A$ receptor BZD binding site. Partial agonists such as ELB 139, TPA 023, TP 003 and Zolpidem showed binding scores less than that of the agonists but considerably more than the antagonists such as flumazenil (-6.39436 kcal/Mole).

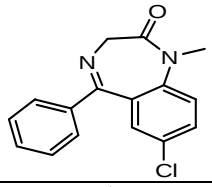
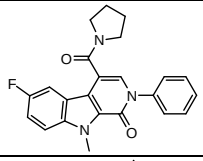
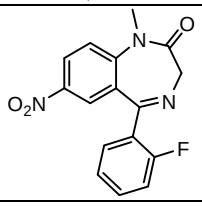
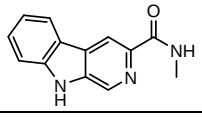
Docking of flavone ligands 2a-l in the active site

All the flavone ligands **2*a-w*** showed binding in the $GABA_A$ BZD binding site with the binding scores between -10.227 and -7.36141 kcal/mol, Table 2. These data clearly indicated their potency as ligands of the BZD binding site of $GABA_A$ receptor. Compound **2*a*** and **1*b*** showed the highest binding score with homology model of $GABA_A$ receptor active site cavity with comparison to other ligands including well known ligands og BZD site of $GABA_A$ receptor. Further rationalization of mode of binding of these flavone molecules in active site of $GABA_A$ BZD binding site has been based upon the amino acid residues present around the ligand. Depending upon the structural features essential for binding in the cavity, flavone molecules could be divided into two segments *viz*. C-2 phenyl and differently substituted main flavone central core structure.

The hydrogen bond formed by 4-Nitrogen of diazepine ring with 55 GLN otr 114 TYR residues was found to be shared by ring oxygen of the flavones. The 5-phenyl function of diazepam and 2-phenyl function of flavonols overlapped well indicating presence of a hydrophobic packet in the binding site **(Figure8).** For ligand **1c, (Figure 6)** the C-2 phenyl ring is surrounded by the amino acid residues like 56 GLN, 85PRO, 57THR, 59TRP, 84VAL and 114TYR while its central skeleton is being enveloped by amino acid residues like 55GLN, 102GLN, 97GLU, 79ILE, 118ILE, 96PRO, 116PRO and 98VAL. The oxygen attached to the C-4 of the central structure was found to have characteristic orientation with the active site. The binding score of ***2c*** to ***2l*** indicate their potential as $GABA_A$ receptor BZD site ligands.

Flavones presented two binding conformers which bind head to tail with respect each other with respect to 114TYR (green) residue of the receptor as seen in **Figure 8**. This is exemplified by 5'-Bromo-2'-hydroxy-6-methylflavone and apigenin. Thus flavones can be classified into two groups A: in which 114 TYR surrounds the 2-phenyl group. B: in which 114 TYR surrounds the flavone nucleus.

Table 1: Overview of known BZD site ligands with their agonist properties and binding scores.

S. No.	Ligand	Structure	Description	Reference	IUPAC Name	Docking score (kcal/ mole)
1a	**Diazepam**		agonist	18;19	7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2(3H)-one	-10.6596
1d	**SL 651 498**		Agonist at α_2 and α_3 subtypes.	20	6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyrido[3,4-b]indol-1-one	-10.593
1i	**Flunitrazepam**		Agonist, Non subtype selective	21	(E)-5-(2-fluorophenyl)-1-methyl-7-nitro-1H-benzo[e][1,4]diazepin-2(3H)-one	-10.0461
1k	**FG 7142**		Non subtype selective	22	N-methyl-9H-pyrido[3,4-b]indole-3-carboxamide	-9.59771

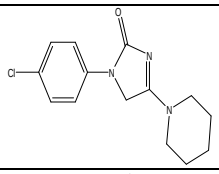
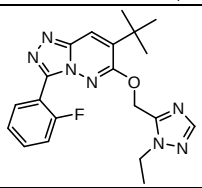
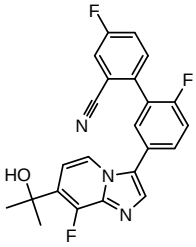
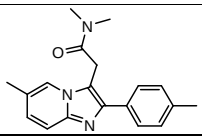
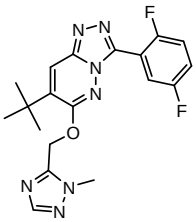
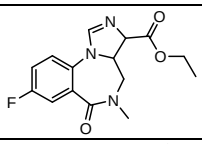
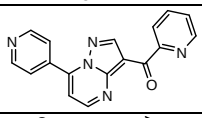
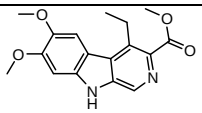
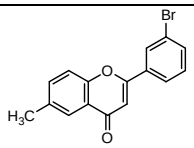
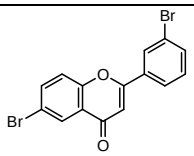
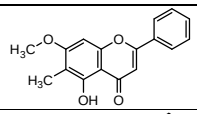
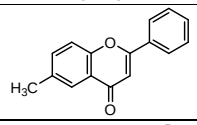
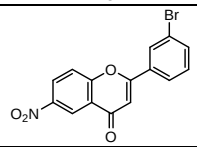
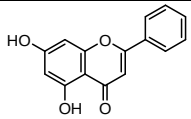
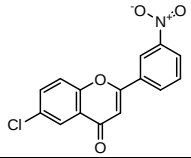
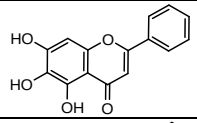
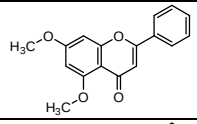
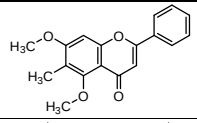
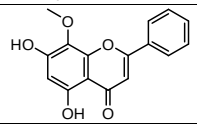
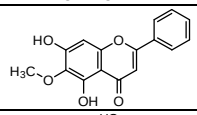
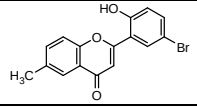
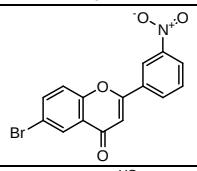
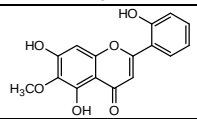
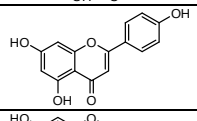
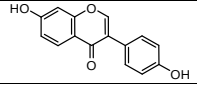
1g	**ELB 139**		Partial agonist with highest potency at α_3 subtype.	23	1-(4-chlorophenyl)-4-(piperidin-1-yl)-1H-imidazol-2(5H)-one	-9.402408
1e	**TPA 023**		Partial agonist at α_2 and α_3 subtypes, atagonist at α_1 and α_5 subtypes.	24	6-((2-ethyl-2H-1,2,4-triazol-3-yl)methoxy)-7-tert-butyl-3-(2-fluorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine	-9.2838
1f	**TP003**		Selective agonist efficacy at α_3 subtype	25	4,2'-Difluoro-5'-[8-fluoro-7-(1-hydroxy-1-methylethyl)imidazo[1,2-a]pyridin-3-yl]biphenyl-2-carbonitrile	-9.1692
1h	**Zolpidem**		Partial affinity for α_1	26;27	N,N-dimethyl-2-(6-methyl-2-p-tolylH-imidazo[1,2-a]pyridin-3-yl)acetamide	-8.36144
1b	**L-838 417**		Partial agonist at α_2, α_3 and α_5 antagonist at α_1 subtype.	28	6-((2-methyl-2H-1,2,4-triazol-3-yl)methoxy)-7-tert-butyl-3-(2,5-difluorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine	-6.99906
1j	**Flumazenil**		Antagonist	29	Ethyl-1,2-fluoro- 8-methyl- 9-oxo-2,4,8- triazatricyclo[8.4.0.$0^{2,6}$]tetradeca-1(10),3,5,11,13- pentaene-5-arboxylate	-6.39436
1c	**Ocinaplon**		Partial agonist at α_2, α_3 and α_5 subtypes, nearly full agonist at α_1.	30	(pyridin-2-yl)(7-(pyridin-4-yl)pyrazolo[1,5-a]pyrimidin-3-yl)methanone	-5.44774
1l	**DMCM**		Inverse agonist at benzodiazepine site. α_1 selective	31	methyl 4-ethyl-6,7-dimethoxy-9H-pyrido[3,4-b]indole-3-carboxylate	No suitable binding pose found

Table 2: Overview and docking scores of flavone ligands in binding site of $GABA_A$ receptors.

Sr. No.	Molecule	Structure	Reference	IUPAC Name	Dock score (kcal/Mole)
2a	6-Methyl-3'-bromoflavone		32	2-(3-bromophenyl)-6-methyl-4H-chromen-4-one	-10.227
2*b*	6,3'-Dibromoflavone		33	6-bromo-2-(3-bromophenyl)-4H-chromen-4-one	-10.0987

2*c*	5-Hydroxy-7-methoxy-6-methylflavone		34	5-hydroxy-7-methoxy-6-methyl-2-phenyl-4H-chromen-4-one	-9.74589
2*d*	6-Methylflavone		[Ai et al., 1997]	6-methyl-2-phenyl-4H-chromen-4-one	-9.73165
2*e*	6-Nitro-3'bromoflavone		[Viola et al., 2000b]	2-(3-bromophenyl)-6-nitro-4H-chromen-4-one	-9.63158
2*f*	Chrysin		[Wolfman et al. 1994, Medina et al. 1990]	5,7-dihydroxy-2-phenyl-4H-chromen-4-one	-9.48117
2*g*	6-Chloro-3'-nitroflavone		[Viola et al., 2000a]	6-chloro-2-(3-nitrophenyl)-4H-chromen-4-one	-9.41604
2*h*	Baicalein		[Liao et al., 2003, Xu et al., 2006]	5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one	-9.41595
2*i*	5,7-Dimethoxyflavone		[Haberlein et al., 1994]	5,7-dimethoxy-2-phenyl-4H-chromen-4-one	-9.21554
2*j*	5,7-Dimethoxy-6-methylflavone		[Haberlein et al., 1994]	5,7-dimethoxy-6-methyl-2-phenyl-4H-chromen-4-one	-9.18837
2*k*	Wogonin		[Hui et al., 2002]	5,7-dihydroxy-8-methoxy-2-phenyl-4H-chromen-4-one	-9.15894
2*l*	OroxylinA		[Huen et al. 2003b]	5,7-dihydroxy-6-methoxy-2-phenyl-4H-chromen-4-one	-9.15503
2*m*	5'-Bromo-2'-hydroxy-6-methylflavone		[Kahnbery et al., 2000]	2-(5-bromo-2-hydroxyphenyl)-6-methyl-4H-chromen-4-one	-8.52807
2*n*	6-Bromo-3'nitroflavone		[Wolfman et al. 1998]	6-bromo-2-(3-nitrophenyl)-4H-chromen-4-one	-8.52688
2*o*	K 38		[Huen et al., 2003b]	5,7-dihydroxy-2-(2-hydroxyphenyl)-6-methoxy-4H-chromen-4-one	-8.52207
2*p*	Apigenin		[Viola et al., 1995, Avallone et al. 2003]	5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one	-8.50487
2*q*	Daidzein		[Shen et al., 1996]	7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one	-8.40218

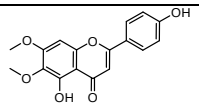
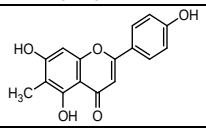
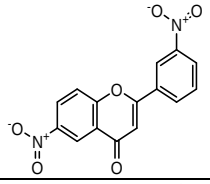
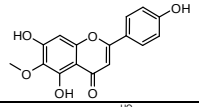
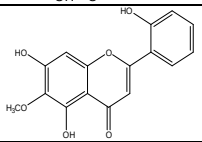
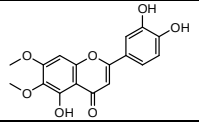
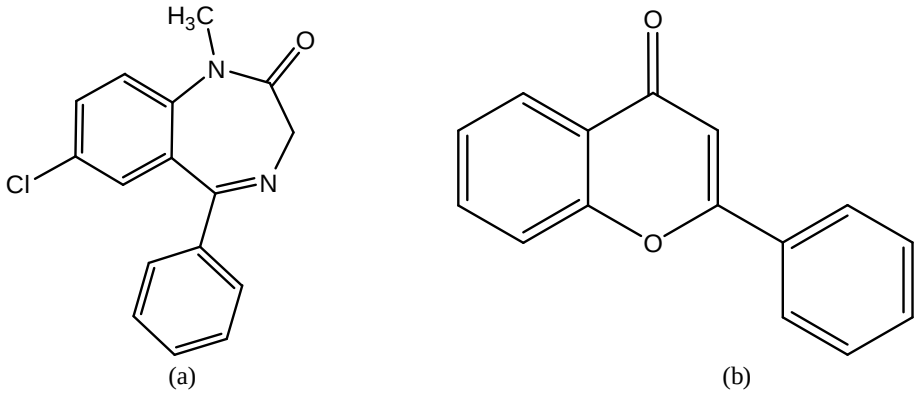
2*r*	Skrofulein		[Shen et al., 1994]	5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxy-4H-chromen-4-one	-8.38414
2*s*	6-methylapigenin		[Wasowski et al., 2002]	5,7-dihydroxy-2-(4-hydroxyphenyl)-6-methyl-4H-chromen-4-one	-8.21791
2*t*	6,3'-Dinitroflavone		[Wolfman et al., 1996]	6-nitro-2-(3-nitrophenyl)-4H-chromen-4-one	-8.05322
2*u*	Dinatin		[Shen et al., 1994]	5,7-dihydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-chromen-4-one	-7.94967
2*v*	K 36		[Huen et al., 2003a]	5,7-dihydroxy-2-(2-hydroxyphenyl)-6-methoxy-4H-chromen-4-one	-7.70639
2*w*	Cirsilol		[Marder et al., 1996]	5-hydroxy-2-(3,4-dihydroxyphenyl)-6,7-dimethoxy-4H-chromen-4-one	-7.36141

Table 3: Amino acid residues defining binding site of flavone ligands (*2a-2w*) on BZD binding

Sr. No.	2-Phenyl	Flavone nucleus
2a	88ALA, 89ALA, **55GLN**, 120GLN, 53PHE, 122PHE,	102GLN, 79ILE, 118ILE, 141ILE, 87LEU, 96PRO, 116PRO, 114TYR
2b	**55GLN**, 85PRO, 116PRO, 57THR, 59TRP, **114TYR**, 84VAL,	102GLN, 97GLU, 79ILE, 118ILE, 96PRO, 98VAL,
2c	89ALA, **55GLN**, 120GLN, 97GLU, 118ILE, 141ILE, 53PHE, 122PHE, 117SER, 100THR, **114TYR**	88ALA, 102GLN, 79ILE, 87LEU, 99LEU, 96PRO, 116PRO, 84VAL,
2d	88ALA, 89ALA, **55GLN**, 118ILE, 14ILE, 96PRO, 116PRO, **114TYR**, 98VAL	102GLN, 120 GLN, 139ILE, 34LEU, 87LEU, 140LYS, 53PHE, 122PHE
2e	104ALA, 116PRO, 84VAL, 98VAL	102GLN, 79ILE, 82LEU, 85PRO, 57THR, 114THR
2f	**55GLN**, 85PRO, 116PRO, 59TRP, **114TYR**, 84VAL	88ALA, 89ALA, 120GLN, 141ILE, 87LEU, 96PRO, 57THR, 98VAL
2g	**55GLN**, 118ILE, 87LEU, 85PRO, 116PRO, 57THR, **114TYR**	102GLN, 79ILE, 82LEU, 80SER, 84VAL
2h	56GLN, 165TYR	104ALA, 105ARG, 103LEU, 113LEU, 115MET, 116PRO, 54TRP, **114TYR**, 84VAL, 98VAL
2i	**55GLN**, 85PRO, 57THR, 59TRP, **114TYR**, 30VAL, 84VAL,	88ALA, 89ALA, 120GLN, 141ILE, 87LEU, 96PRO,
2j	89ALA, **55GLN**, 120GLN, 118ILE, 141ILE, 53PHE, 122PHE,	88ALA, 102GLN, 79ILE, 87LEU, 96PRO, 116PRO, **114TYR**, 84VAL
2k	86ASP, 85PRO, 116PRO, 57THR, 59TRP, **114TYR**,	88ALA, 89ALA, **55GLN**, 120GLN, 141ILE, 87LEU, 96PRO,
2l	88ALA, 89ALA, **55GLN**, 120GLN, 118ILE, 141ILE, 53PHE, 122PHE, 98VAL	102GLN, 87LEU, 96PRO, 116PRO
2m	88ALA, 89ALA, **55GLN**, 120GLN, 118ILE, 141ILE, 87LEU, 53PHE, 96PRO	102GLN, 79ILE, 116PRO, **114TYR**
2n	798ILE, 116PRO, 80SER, 57THR, 84VAL	**55GLN**, 120GLN, 118ILE, 96PRO, 98VAL
2o	104ALA, 56GLN, 115MET, 54TRP, 165TYR	105ARG, 103LEU, 113LEU, 116PRO, **114TYR**
2p	**55GLN**, 115MET, 85PRO, 116PRO, 57THR, 59TRP, **114TYR**, 84VAL,	88ALA, 89ALA, 120GLN, 118ILE, 87LEU, 96PRO
2q	79ILE, 82LEU, 80SER, 83TRP, 84VAL,	102GLN, 97GLU, 118ILE, 96PRO, 116PRO, **114TYR**, 98VAL,
2r	88ALA, 89ALA, **55GLN**, 120GLN, 139ILE, 141ILE, 53PHE, 122PHE,	86ASP, 102GLN, 79ILE, 87LEU, 85PRO, 96PRO, **114TYR**, 84VAL
2s	54TRP, 165TYR,	105ARG, 56GLN, 113LEU, 115MET, **114TYR**,
2t	102GLN, 97GLU, 118ILE, 96PRO, 116PRO, 117SER, 83TRP, **114TYR**, 98VAL,	86ASP, 79ILE, 82LEU, 87LEU, 85PRO, 84VAL,

2u	88ALA, 89ALA, **55GLN**, 120GLN, 122PHE, 96PRO,	86ASP, 79ILE, 118ILE, 141ILE, 87LEU, 53PHE, 85PRO, 84VAL
2v	88ALA, 89ALA, **55GLN**, 120GLN, 118ILE, 139ILE, 141ILE,	86ASP, 87LEU, 53PHE, 96PRO, 116PRO, **114TYR**
2w	88ALA, 89ALA, **55GLN**, 120GLN, 87LEU, 96PRO,	86ASP, 79ILE, 141ILE, 85PRO, 84VAL,



(a) (b)

Figure- 1: Structures of Diazepam (a) and neuroactive flavonoids (b).

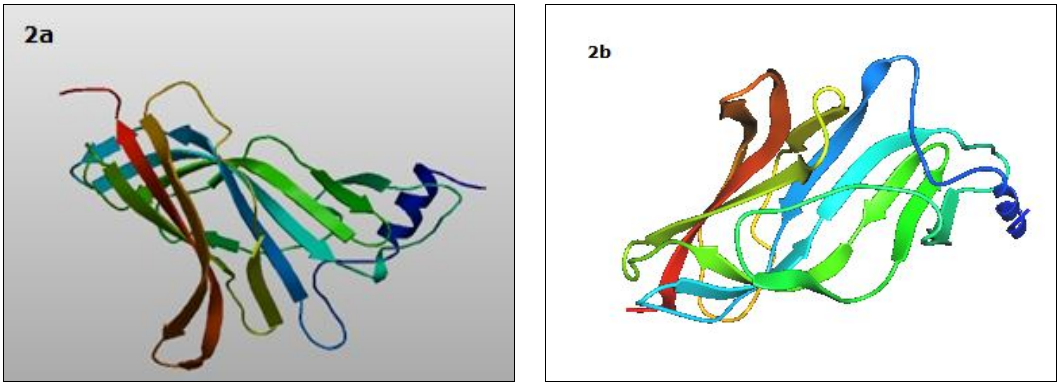


Figure-2: 3D ribbon views of Homology model generated from SWISS – MODEL workspace (2A) and binding site generated from fuzzy oil drop model server (2B).

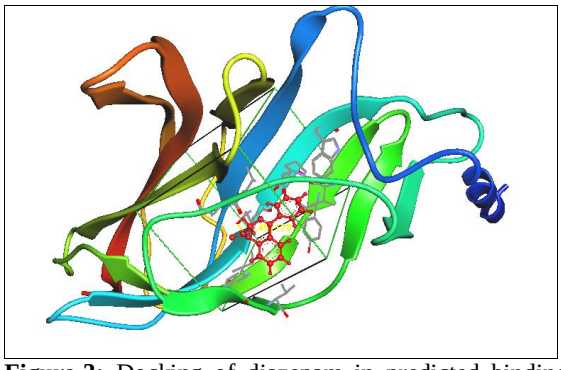


Figure-3: Docking of diazepam in predicted binding site homology model of $GABA_A$ α_1/γ_2 receptor subunits.

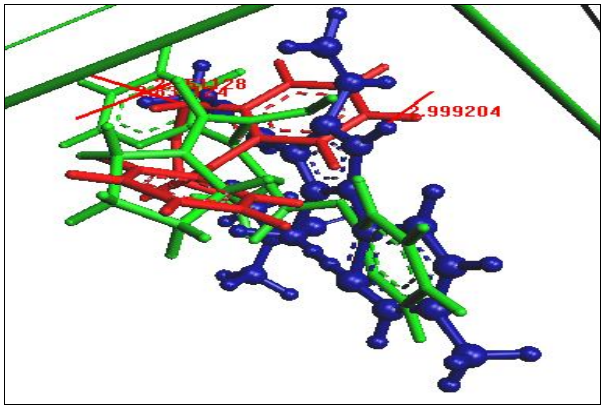


Figure-4: Structure overlay of Diazepam (red) and Zolpidem (blue) and SL 651 498 (green) in their best docking poses.

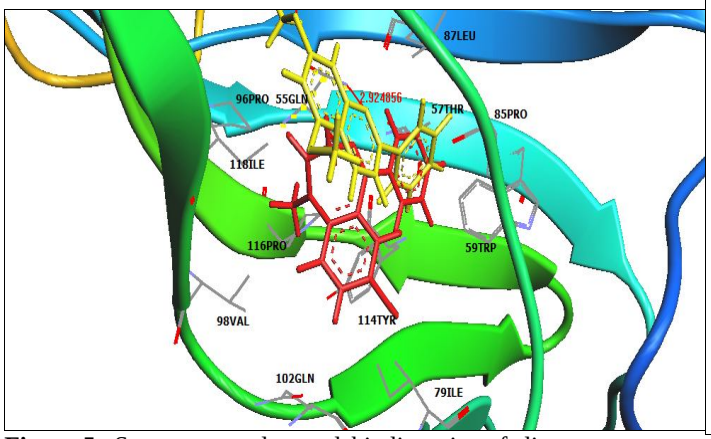


Figure-5: Structure overlay and binding site of diazepam and 5,7-dimethoxyflavone in the binding site of $GABA_A$ receptor. Notice close overlapping of 2-phenyl rings.

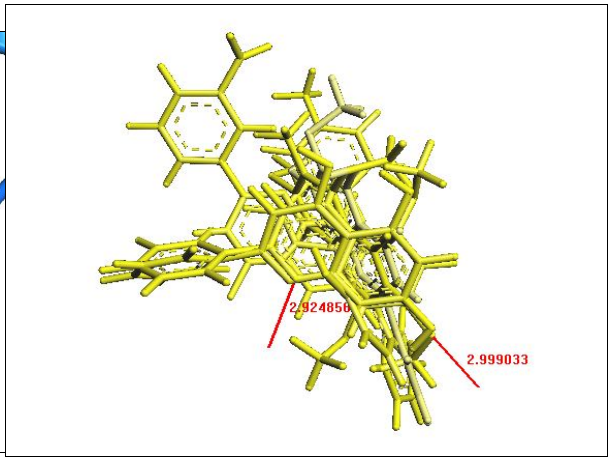


Figure-7: Structure overlay of flavones in the binding site of homology model of $GABA_A$ receptor.

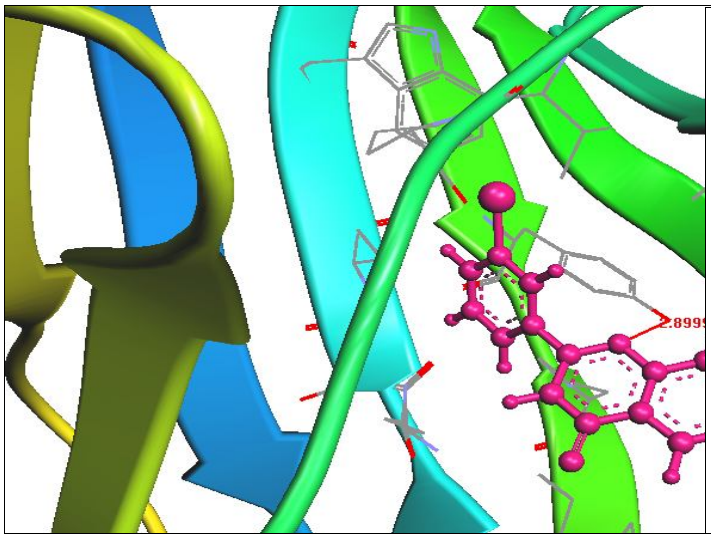


Figure-6: 6-Methyl-3'-bromoflavone in its binding site. Notice the involvement of flavonoid ring oxygen in hydrogen bonding.

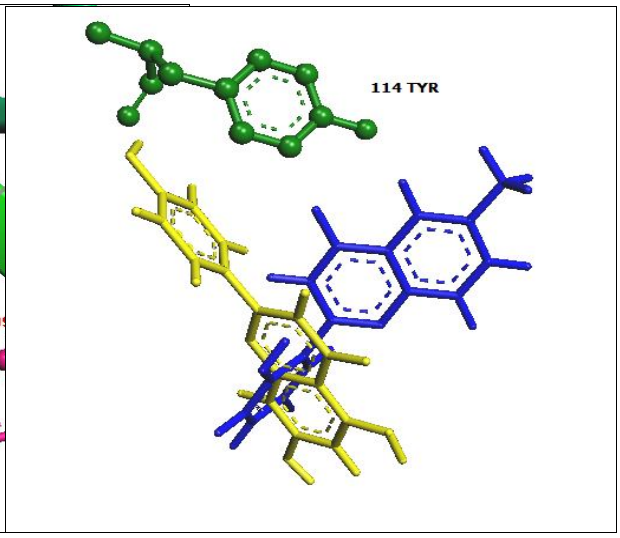


Figure-8: Flavones exist as at least two conformers which bind head to tail with respect each other with respect to 114TYR residue of the receptor as seen in this figure 5'-Bromo-2'-hydroxy-6-methylflavone and apigenin. Thus flavones can be classified into two groups A: in which 114 TYR surrounds the 2-phenyl group. B: in which 114 TYR surrounds the flavone nucleus.

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