



## **In-Silico Docking of Neuroactive Flavones on Benzodiazepine Binding Site of GABA<sub>A</sub> Receptor Homology Model**

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### **ABSTRACT**

Flavones share structural and mechanistic features of GABA<sub>A</sub> 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<sub>A</sub> 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<sup>1</sup>. An emerging area of interest is the direct activation of GABA(A) receptors by flavonoids<sup>2</sup>. 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<sup>3</sup>. 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<sup>4</sup>.

Zhang *et al.* (1995)<sup>5</sup> 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.<sup>6</sup> have described homology modeling and docking study on GABA<sub>A</sub>  $\alpha_1/\gamma_2$  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<sub>A</sub> subunits  $\alpha_1$  and  $\gamma_2$ .

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<sub>A</sub> receptors<sup>7,8</sup>, we report novel homology model development and active site prediction strategy for docking of flavone ligands on BZD binding site of GABA<sub>A</sub> 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<sub>A</sub> receptor BZD binding site was generated as described previously<sup>7</sup>. 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<sup>9-11</sup>. The mature protein sequences of the rat  $\alpha_1$  and  $\gamma_2$  subunits (accession numbers:  $\alpha_1$ , P62813;  $\gamma_2$ , P18508) were automatically aligned with sequences of two adjacent AChBP subunits (A and B, respectively) using ClustalW<sup>12</sup> 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<sup>13</sup>. The assumptions and calculations involved have been described elsewhere<sup>7</sup>. 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 surface 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 INMF, a downhill protein<sup>14</sup> and small peptides representing various functional groups<sup>15, 16</sup> 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<sup>TM</sup> microprocessor and

Windows7<sup>TM</sup> 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<sup>17</sup>. 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<sup>17</sup>, 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 22×22×22 Å<sup>3</sup> 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<sup>TM</sup> mode, it was docked with GADdock mode.

Concluding docking results were parameterized in terms of docking score in Kcal/mol. The docked GABA<sub>A</sub> receptor benzodiazepine site ligands **1a-l**, complexed with GABA<sub>A</sub> receptor  $\alpha_1/\gamma_2$  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<sub>A</sub> 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<sup>2</sup> 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 **1a-l** and Flavones **2a-2w**), the docking of diazepam into the active BZD binding site in

homology model of GABA<sub>A</sub> 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<sub>A</sub> 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<sub>A</sub> 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

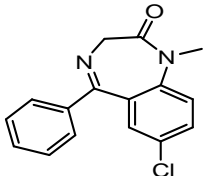
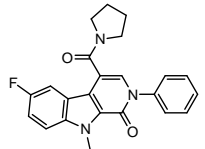
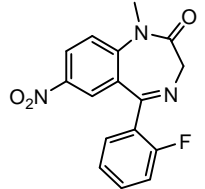
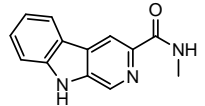
All the flavone ligands **2a-w** showed binding in the GABA<sub>A</sub> BZD binding site with the binding scores between -10.227 and -7.36141 kcal/mol, Table 2. These data clearly indicated their potency as ligands of the BZD binding site of GABA<sub>A</sub> receptor. Compound **2a** and **1b** showed the highest binding score with homology model of GABA<sub>A</sub> receptor active site

cavity with comparison to other ligands including well known ligands og BZD site of GABA<sub>A</sub> receptor. Further rationalization of mode of binding of these flavone molecules in active site of GABA<sub>A</sub> 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<sub>A</sub> 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)
<b>1a</b>	<b>Diazepam</b>		agonist	18;19	7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2(3H)-one	-10.6596
<b>1d</b>	<b>SL 651 498</b>		Agonist at $\alpha_2$ and $\alpha_3$ 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
<b>1i</b>	<b>Flunitrazepam</b>		Agonist, Non subtype selective	21	(E)-5-(2-fluorophenyl)-1-methyl-7-nitro-1H-benzo[e][1,4]diazepin-2(3H)-one	-10.0461
<b>1k</b>	<b>FG 7142</b>		Non subtype selective	22	N-methyl-9H-pyrido[3,4-b]indole-3-carboxamide	-9.59771

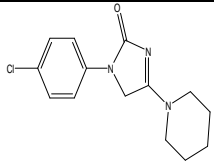
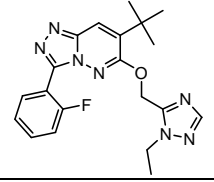
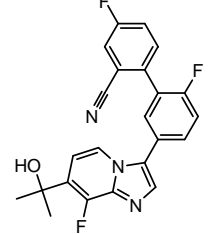
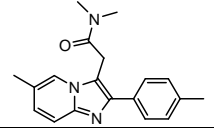
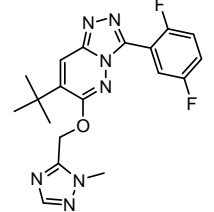
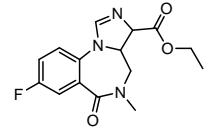
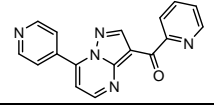
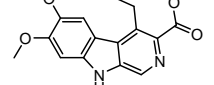
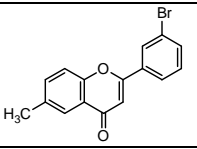
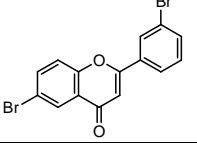
<b>Ig</b>	<b>ELB 139</b>		Partial agonist with highest potency at $\alpha_3$ subtype.	23	1-(4-chlorophenyl)-4-(piperidin-1-yl)-1H-imidazol-2(5H)-one	-9.402408
<b>Ie</b>	<b>TPA 023</b>		Partial agonist at $\alpha_2$ and $\alpha_3$ subtypes, antagonist at $\alpha_1$ and $\alpha_5$ subtypes.	24	6-((2-ethyl-2H-1,2,4-triazol-3-yl)methoxy)-7-tert-butyl-3-(2-fluorophenyl)-[1,2,4]triazolo[4,3-b]pyridazine	-9.2838
<b>If</b>	<b>TP003</b>		Selective agonist efficacy at $\alpha_3$ 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
<b>Ih</b>	<b>Zolpidem</b>		Partial affinity for $\alpha_1$	26;27	N,N-dimethyl-2-(6-methyl-2-p-tolylH-imidazo[1,2-a]pyridin-3-yl)acetamide	-8.36144
<b>Ib</b>	<b>L-838 417</b>		Partial agonist at $\alpha_2$ , $\alpha_3$ and $\alpha_5$ antagonist at $\alpha_1$ 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
<b>Ij</b>	<b>Flumazenil</b>		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
<b>Ic</b>	<b>Ocinaplon</b>		Partial agonist at $\alpha_2$ , $\alpha_3$ and $\alpha_5$ subtypes, nearly full agonist at $\alpha_1$ .	30	(pyridin-2-yl)(7-(pyridin-4-yl)pyrazolo[1,5-a]pyrimidin-3-yl)methanone	-5.44774
<b>Il</b>	<b>DMCM</b>		Inverse agonist at benzodiazepine site. $\alpha_1$ 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<sub>A</sub> receptors.

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

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

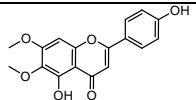
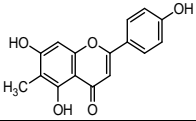
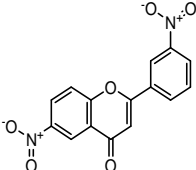
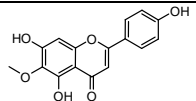
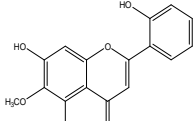
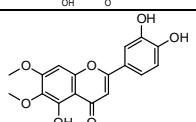
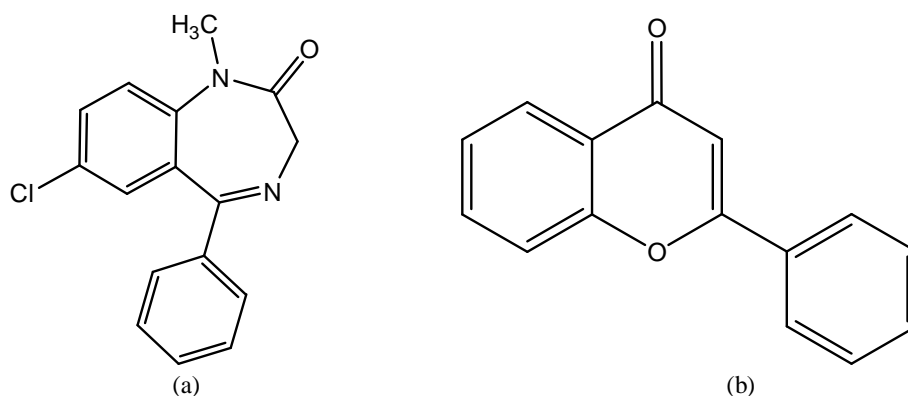
2r	Skrofullein		[Shen et al., 1994]	5-hydroxy-2-(4-hydroxyphenyl)-6,7-dimethoxy-4H-chromen-4-one	-8.38414
2s	6-methylapigenin		[Wasowski et al., 2002]	5,7-dihydroxy-2-(4-hydroxyphenyl)-6-methyl-4H-chromen-4-one	-8.21791
2t	6,3'-Dinitroflavone		[Wolfman et al., 1996]	6-nitro-2-(3-nitrophenyl)-4H-chromen-4-one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

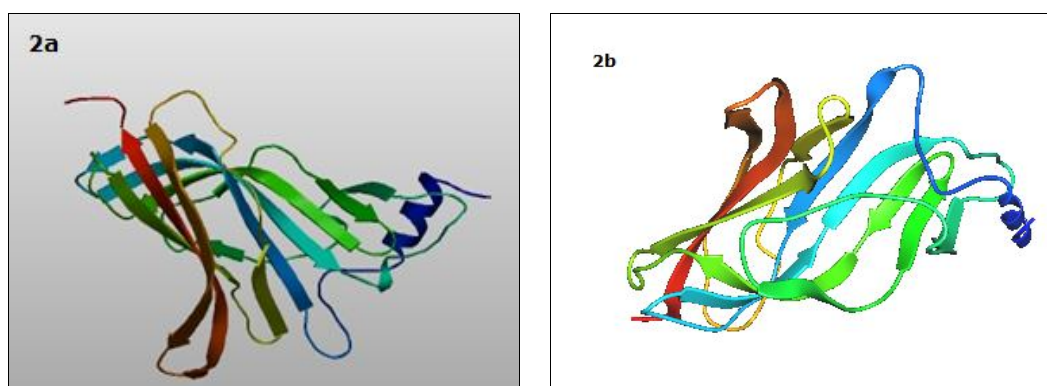
Table 3: Amino acid residues defining binding site of flavone ligands (2a-2w) on BZD binding

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

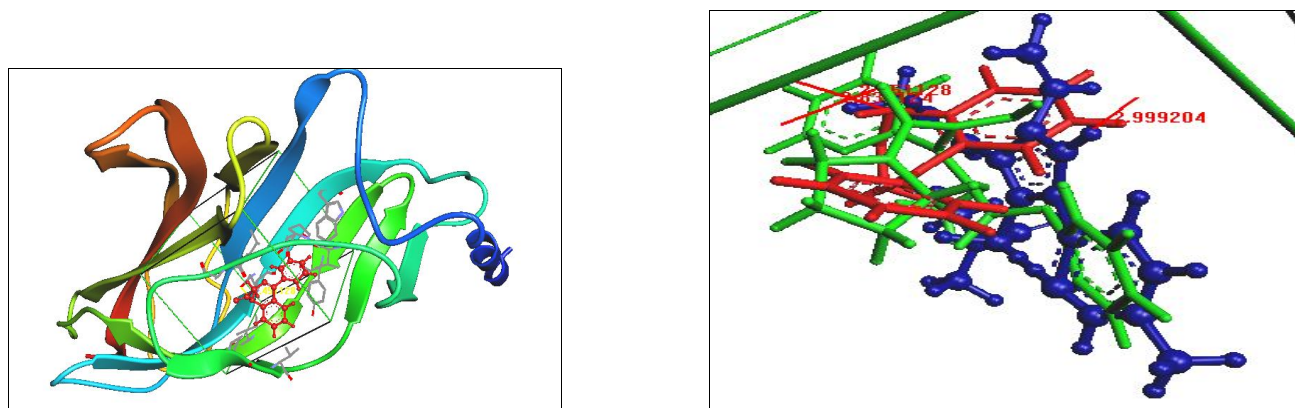
2u	88ALA, 89ALA, <b>55GLN</b> , 120GLN, 122PHE, 96PRO,	86ASP, 79ILE, 118ILE, 141ILE, 87LEU, 53PHE, 85PRO, 84VAL
2v	88ALA, 89ALA, <b>55GLN</b> , 120GLN, 118ILE, 139ILE, 141ILE,	86ASP, 87LEU, 53PHE, 96PRO, 116PRO, <b>114TYR</b>
2w	88ALA, 89ALA, <b>55GLN</b> , 120GLN, 87LEU, 96PRO,	86ASP, 79ILE, 141ILE,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



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



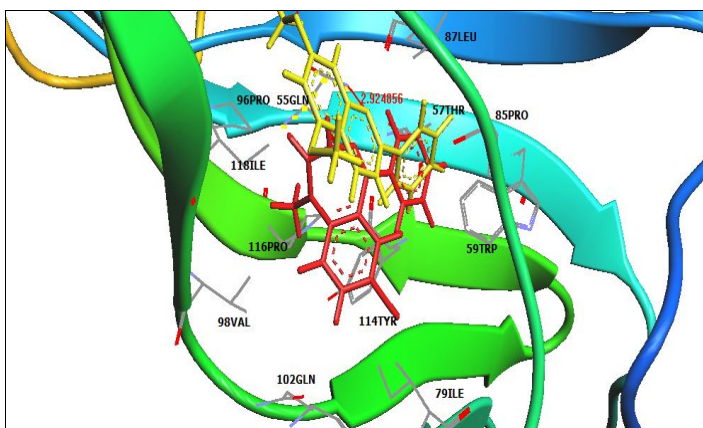
**Figure-2:** 3D ribbon views of Homology model generated from SWISS – MODEL workspace (2A) and binding site generated from fuzzy oil drop model server (2B).



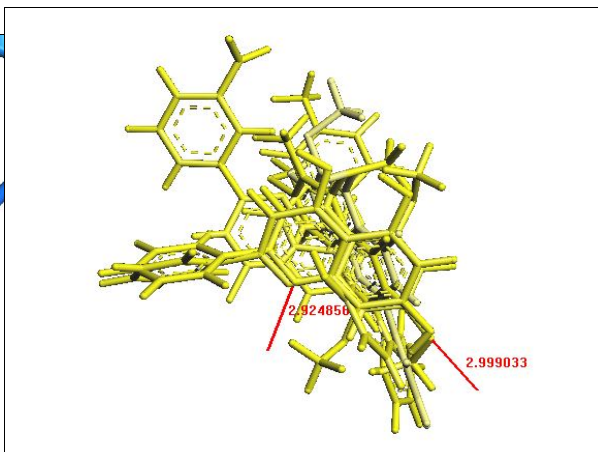
**Figure-3:** Docking of diazepam in predicted binding site homology model of GABA<sub>A</sub>  $\alpha_1/\gamma_2$  receptor subunits.

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

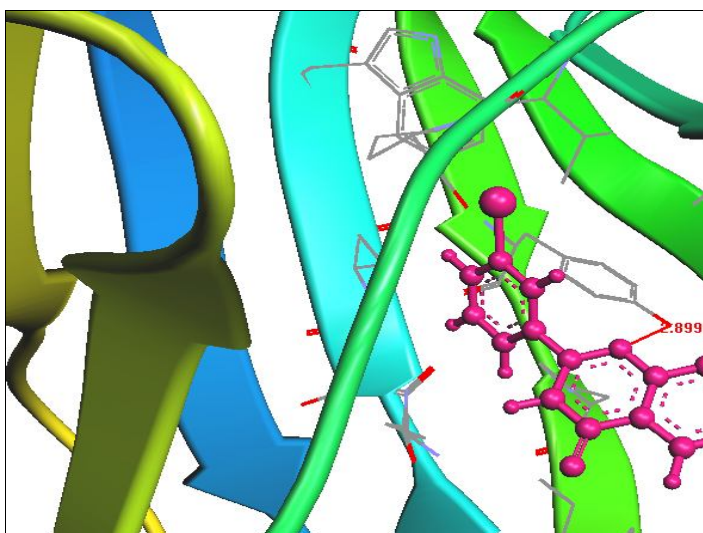




**Figure-5:** Structure overlay and binding site of diazepam and 5,7-dimethoxyflavone in the binding site of GABA<sub>A</sub> receptor. Notice close overlapping of 2-phenyl rings.



**Figure-7:** Structure overlay of flavones in the binding site of homology model of GABA<sub>A</sub> receptor.



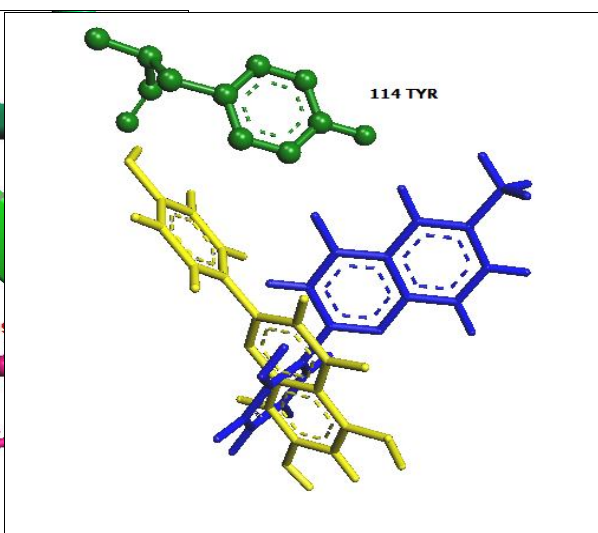
**Figure-6:** 6-Methyl-3'-bromoflavone in its binding site. Notice the involvement of flavonoid ring oxygen in hydrogen bonding.

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**REFERENCES**

- 1) Hanrahan JR, Chebib M, Johnston GA. Flavonoid modulation of GABA(A) receptors. Br J Pharmacol 2011; 163(2): 234 - 245.
- 2) Fernandez SP, Mewett KN, Hanrahan JR, Chebib M, Johnston GA. Flavan-3-ol derivatives are positive modulators of GABA(A) receptors with higher efficacy for the alpha(2) subtype and anxiolytic action in mice. Neuropharmacology 2008; 55(5):900-907.
- 3) Marder M, Estiu G, Blanch LB, Viola H, Wasowski C, Medina JH et al. Molecular modeling and QSAR analysis of the interaction of flavone derivatives with the benzodiazepine binding site of the GABA(A) receptor complex. Bioorg Med Chem 2001; 9(2):323-335.



**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.

- 4) Jager AK, Saaby L. Flavonoids and the CNS. Molecules 2011; 16(2):1471-1485.
- 5) Zhang W, Koehler KF, Zhang P, Cook JM. Development of a comprehensive pharmacophore model for the benzodiazepine receptor. Drug Des Discov 1995; 12(3):193-248.
- 6) Berezhnoy D, Gibbs TT, Farb DH. Docking of 1,4-benzodiazepines in the alpha1/gamma2 GABA(A) receptor modulator site. Mol Pharmacol 2009; 76(2):440-450.
- 7) Vaishnav GA, Kulkarni GK, Sankar S, Vaishnav S. Automated *in-silico* modelling and docking of ligands on benzodiazepine binding site of GABA<sub>A</sub> receptors. J. Pharm. Res. 2011; 4(9): 3162-3165.



- 8) Vaishnav GA, Kulkarni GK, Sankar S, Vaishnav S. Homology model and *in-silico* docking of flavonols on benzodiazepine binding site of GABA<sub>A</sub> receptors. 2011. Unpublished work
- 9) Peitsch MC, Wells TN, Stampf DR, Sussman JL. The Swiss-3DImage collection and PDB-Browser on the World-Wide Web. Trends Biochem Sci 1995; 20(2):82-84.
- 10) Kiefer F, Arnold K, Kunzli M, Bordoli L, Schwede T. The SWISS-MODEL Repository and associated resources. Nucleic Acids Res 2009; 37(Database issue):D387-D392.
- 11) Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 2006; 22(2):195-201.
- 12) Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22(22):4673-4680.
- 13) Brylinski M, Konieczny L, Roterman I. Ligation site in proteins recognized in silico. Bioinformation 2006; 1(4):127-129.
- 14) Banach M, Prymula K, Konieczny L, Roterman I. "Fuzzy oil drop" model verified positively. Bioinformation 2011; 5(9):375-377.
- 15) Prymula K, Salapa K, Roterman I. "Fuzzy oil drop" model applied to individual small proteins built of 70 amino acids. J Mol Model 2010; 16(7):1269-1282.
- 16) Brylinski M, Prymula K, Jurkowski W, Kochanczyk M, Stawowczyk E, Konieczny L et al. Prediction of functional sites based on the fuzzy oil drop model. PLoS Comput Biol 2007; 3(5):e94.
- 17) Mark A. Thompson. ArgusLab 4.0.1. <http://www.arguslab.com>. 2011. Planaria Software LLC, Seattle, WA.
- 18) Huen MS, Leung JW, Ng W, Lui WS, Chan MN, Wong JT et al. 5,7-Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from *Scutellaria baicalensis* Georgi, with selective antagonistic properties. Biochem Pharmacol 2003; 66(1):125-132.
- 19) Huen MS, Hui KM, Leung JW, Sigel E, Baur R, Wong JT et al. Naturally occurring 2'-hydroxyl-substituted flavonoids as high-affinity benzodiazepine site ligands. Biochem Pharmacol 2003; 66(12):2397-2407.
- 20) Griebel G, Perrault G, Simiand J, Cohen C, Granger P, Depoortere H et al. SL651498, a GABAA receptor agonist with subtype-selective efficacy, as a potential treatment for generalized anxiety disorder and muscle spasms. CNS Drug Rev 2003; 9(1):3-20.
- 21) Strakhova MI, Harvey SC, Cook CM, Cook JM, Skolnick P. A single amino acid residue on the alpha(5) subunit (Ile215) is essential for ligand selectivity at alpha(5)beta(3)gamma(2) gamma-aminobutyric acid(A) receptors. Mol Pharmacol 2000; 58(6):1434-1440.
- 22) Mirza NR, Munro G. The role of GABA(A) receptor subtypes as analgesic targets. Drug News Perspect 2010; 23(6):351-360.
- 23) Rabe H, Kronbach C, Rundfeldt C, Luddens H. The novel anxiolytic ELB139 displays selectivity to recombinant GABA(A) receptors different from diazepam. Neuropharmacology 2007; 52(3):796-801.
- 24) Atack JR, Wafford KA, Tye SJ, Cook SM, Sohal B, Pike A et al. TPA023 [7-(1,1-dimethylethyl)-6-(2-ethyl-2H-1,2,4-triazolo-3-ylmethoxy)-3-(2-fluorophenyl)-1,2,4-triazolo[4,3-b]pyridazine], an agonist selective for alpha2- and alpha3-containing GABAA receptors, is a non-sedating anxiolytic in rodents and primates. J Pharmacol Exp Ther 2006; 316(1):410-422.
- 25) Dias R, Sheppard WF, Fradley RL, Garrett EM, Stanley JL, Tye SJ et al. Evidence for a significant role of alpha 3-containing GABAA receptors in mediating the anxiolytic effects of benzodiazepines. J Neurosci 2005; 25(46):10682-10688.
- 26) Crestani F, Martin JR, Mohler H, Rudolph U. Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol 2000; 131(7):1251-1254.
- 27) Langer SZ, Arbilla S. Imidazopyridines as a tool for the characterization of benzodiazepine receptors: a proposal for a pharmacological classification as omega receptor subtypes. Pharmacol Biochem Behav 1988; 29(4):763-766.
- 28) McKernan RM, Rosahl TW, Reynolds DS, Sur C, Wafford KA, Atack JR et al. Sedative but not anxiolytic properties of benzodiazepines are mediated by the GABA(A) receptor alpha1 subtype. Nat Neurosci 2000; 3(6):587-592.
- 29) Eng W, Atack JR, Bergstrom M, Sanabria S, Appel L, Dawson GR et al. Occupancy of human brain GABA(A) receptors by the novel alpha5 subtype-selective benzodiazepine site inverse agonist alpha5IA as measured using [(1)1C]flumazenil PET imaging. Neuropharmacology 2010; 59(7-8):635-639.
- 30) Lippa A, Czobor P, Stark J, Beer B, Kostakis E, Gravielle M et al. Selective anxiolysis produced by ocinaplon, a GABA(A) receptor modulator. Proc Natl Acad Sci U S A 2005; 102(20):7380-7385.
- 31) Sancar F, Czajkowski C. Allosteric modulators induce distinct movements at the GABA-binding site interface of the GABA-A receptor. Neuropharmacology 2011; 60(2-3):520-528.
- 32) Paladini AC, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. J Pharm Pharmacol 1999; 51(5):519-526.
- 33) Viola H, Marder M, Wasowski C, Giorgi O, Paladini AC, Medina JH. 6,3'-dibromoflavone and 6-nitro-3'-bromoflavone: new additions to the 6,3'-disubstituted flavone family of high-affinity ligands of the brain benzodiazepine binding site with agonistic properties. Biochem Biophys Res Commun 2000; 273(2):694-698.
- 34) Haberlein H, Tschiersch KP, Schafer HL. Flavonoids from *Leptospermum scoparium* with affinity to the benzodiazepine receptor characterized by structure activity relationships and in vivo studies of a plant extract. Pharmazie 1994; 49(12):912-922.

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