Pseudoreceptor Models and 3D-QSAR for Imidazobenzodiazepines at $GABA_A$/BzR Subtypes $\alpha_x\beta_3\gamma_2$ [$x = 1-3$, 5, and 6] via Flexible Atom Receptor Model

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Since benzodiazepines have been used widely in the treatment of anxiety, sleeplessness, and epilepsy, the receptor sites for the benzodiazepine are of prime importance. Quantitative structure−activity relationship (QSAR) studies and receptor modeling via Flexible Atom Receptor Model(FLARM) for the binding affinities of a series of imidazobenzodiazepines at five recombinant receptor subtypes were carried out successfully. The 3D-QSAR models for all five receptor subtypes were examined by a set of test set and demonstrated their high predictability for affinities of imidazobenzodiazepines at five receptor subtypes. The pseudoreceptors yielded by FLARM were compared to the united pharmacophore/receptor model. The result shows that two hydrogen bonds and other regions in the united pharmacophore/receptor model are presented in the pseudoreceptors, which demonstrates the receptor modeling capability of FLARM. The models and pseudoreceptors can help design high affinity ligands on the $GABA_A$/BZ receptor and understand the $GABA_A$ receptor.

INTRODUCTION

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), operates through three different classes of receptors consisting of the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors.[1] Besides playing an important role in central transmission processes, these receptors, especially the $GABA_A$ receptors, have been associated with certain neurological and psychiatric disorders and become therapeutic targets in certain diseases.[2]

Benzodiazepine receptor (BzR) is coupled to the GABA receptor and is a part of the GABAA-controlled chloride ion channel (BzR/GABAA/Cl) complex. The GABAA/BzR complex is a membrane-bound heteropentameric protein constituted by different subunits. Molecular cloning has identified a total of 21 subunits ($\alpha_{1\_6}-\beta_{1\_4}-\gamma_{1\_4}-\theta-\pi-\epsilon-\rho_{1-3}$, δ), and 16 of them have been found in the mammalian CNS.[3,4] Advances in molecular cloning techniques have led to the characterization of more Bz/$GABA_A$ receptor subtypes, but molecular biological and physiological studies of recombinant $GABA_A$/BzR have shown that the presence of α, β, and γ subunits is necessary to constitute a fully functional, benzodiazepine receptor-mediated recombinant receptor/chloride ion channel which mimics the pharmacological, biochemical, and electrophysiological properties of a native receptor.[4] Among these, α_1 together with the β_2, γ_2 subunits combination can reconstitute and mimic many of the pharmacological properties of the classical type-I BzR,[5] while α_x ($x = 2$, 3, or 5) together with the β_2, γ_2 subunits combination can reconstitute and mimic the activity of the one termed type-II BzR.[6,5] Furthermore, these two types are diazepam-sensitive BzRs. There is another termed "diazepam-insensitive" (DI) subtype of BzR, which was initially described in rodent cerebellar membranes and cerebellar granule cell cultures.[7,8] DI are characterized by the low affinity ($> 1\ \mu$M) of prototypical l,4-benzodiazepines (e.g., diazepam, flunitrazepam), some β-carbolines (e.g., 3-carbomethoxy-β-carboline), triazolobenzodiazepines (e.g., triazolam), and triazolopyridazines (e.g., CL 218872) that are high affinity ligands at DS BzR isoforrns.[9−11] A pharmacological profile similar to that of the native DI BzR can be reconstituted in cell cultures transfected with α_6, β_2, and γ_2 subunits configuration.[12] Previous results also suggest that the biological activity of recombinant receptors composed of α_x, β_3, and γ_2 subunits is very close to that of recombinant receptors composed of α_x, β_2, and γ_2 subunits.[13]

Ligands of benzodiazepine receptors (BzR) elicit a wide range of pharmacological effects including sedation, muscle relaxation, anxiolytic, and anticonvulsant activities. The ligands modulate $GABA_A$ receptors to increase or decrease the action of inhibitory GABA neurotransmitter by binding to its receptor followed by the opening of an intrinsic chloride channel. According to the efficacy, the ligands have been classified into agonist, inverse agonist, and antagonist corresponding to different pharmacological activity. The pharmacological differences between ligands may be partially attributed to the extensive molecular diversity of $GABA_A$/BzR. Furthermore, the regional heterogeneity of the $GABA_A$/BzR complex has been suggested as another basis for the multiplicity of pharmacological properties of BzR ligands.[14−16] So a better understanding of the $GABA_A$/BzR should aid in the design and synthesis of more selective and higher affinity ligands that display some particular pharmacological activities and in turn serve to further elucidate the physiological and pharmacological function of $GABA_A$/BzR. Also this may result in new subtype-selective agents for the treatment of anxiety, sleep disorders, convulsions, or memory deficits as well as decreasing the potential for side effects.

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As computational approaches have become an important part of drug design and drug discovery, many modeling methods have been developed, such as DOCK, quantitative structure−activity relationships (QSAR). The pseudoreceptor modeling method is also a ligand-based drug design method for QSAR. The pseudoreceptor modeling method was proposed by Jain,[17] Snyder,[18] Walters,[19] Hahn,[20,21] and many other research groups.[22,23,18,24] Among them, Flexible Atom Receptor Model (FLARM) is based on an improvement of Walters' approach[19] and previous works[25,22] in our group. It allows the construction of a binding-site model for a structurally uncharacterized macromolecular based on the structures of its ligands (known bioactive compounds) with pseudoatoms at the topological level.

FLARM combines the present techniques but significantly extends their possibilities by the generation of an explicit receptor model. This model can also be used to perform 3D-QSAR and then to predict the activity of novel drugs or ligands. The explicit atom models can help to understand the mechanism of the interaction between ligands and receptors and even to preliminarily predict the active sites of the real receptors. The open receptor model can provide a better simulation of the real receptor and may rationally correspond to the pharmacophore model.

Previously, imidazobenzodiazepines were reported to bind to the DI site of the BzR with high affinity. Then binding studies of this series of ligands on recombinant BzR subtypes demonstrated that imidazobenzodiazepines are a class of compounds which bind to all five receptor subtypes with better selectivity at α5-containing receptors (a classical type-II BzR).[26] It was felt that affinities from studies on recombinant receptor subtypes mentioned above provided a great deal of information on the structure−activity relationship between the receptor subtype and the ligand in this series. Consequently, the 3D-QSAR studies via FLARM were conducted. In addition to some 3D-QSAR models obtained, the possible interactions between the receptor and the ligand are more interesting. These possible interactions can be seen from the pseudoreceptor model given by the FLARM method. Then the pseudoreceptor models were compared to the comprehensive pharmacophore/receptor model[27] for the BzR.

MATERIALS AND METHODS

The 28 compounds[28,29] for this study and their binding affinities at five recombinant $GABA_A$/BzR receptor subtypes $\alpha_x\beta_3\gamma_2$ ($x = 1-3$, 5, and 6) are listed in Table 1. For the FLARM calculation, the ligand bioactivities were from the transform of 9-lgk_i, where k_i (k_i in nM) is the affinity of the ligand in Table 1 at five recombinant BzR subtypes.

Structure Preparation. All structures are built and prepared on a SGI Octane-2 workstation using the molecular modeling package SYBYL6.5.[30] Ligand 1(Ro15-1788) was selected as the starting structure, and its starting geometry was taken from an available X-ray crystal structure[31,32] for which a Cambridge Crystallographic Database search was carried out. It was minimized with the TRIPOS force field. The atomic charges calculation and geometry optimization of the molecule was performed by a semiempirical AM1 method using the MOPAC module embedded in SYBYL6.5.[30] Then the prepared ligand 1 has been used as a template

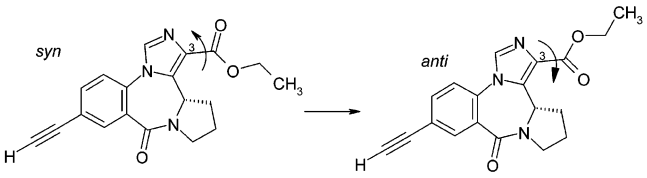


Figure 1. Syn and anti conformational of the 3-ester substituent of imidazobendiazepine.

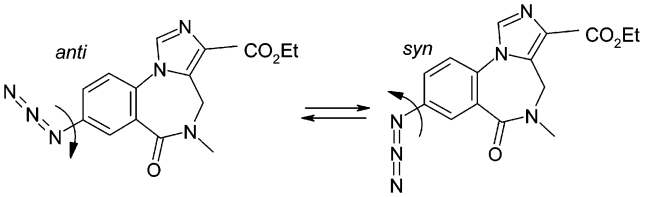
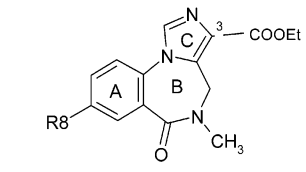


Figure 2. Syn and anti conformations of the 8-azido moiety of compound 4 (Ro 15-4513).

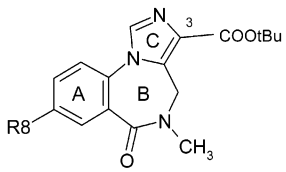
compound to construct other molecules by modifying and assembling fragments from the SYBYL standard library. Other molecules constructed have also been minimized by the minimize2 module in SYBYL6.5, then optimized, and charged by MOPAC model. For some conformation-required molecules, some adjustments have been done just as follows.

The active conformation of the ligands is important for the FLARM calculation, so it is determined as reported previously. During an earlier study of DI BzR ligands, the active conformations of imidazobenzodiazepines employed at DS and DI BzR subtypes were determined.[33] In short, the anti conformation of the ester functionality at position 3 was proposed as the active conformation for both the DS and the DI subtypes (Figure 1). In addition, in the case of 4,5-pyrroloimidazobenzodiazepines (**15**−**27**), the aliphatic ring plays an important role in the active conformation of the ester function. Ab initio calculations at the 6-31G* level revealed that energy of the syn conformer was 2.743 kcal/mol higher than that of the anti conformer of the 3-ethyl ester of pyrroloimidazobenzodiazepine (**17**).[28] Consequently, the equilibrium constant at the temperature under which the binding study was done (4 °C) between the two conformers of ligand 17 equals 144.9 ($\Delta G = -RT \ln K$) in favor of the anti conformation (Figure 2). Thus the anti conformation of the ester moiety was chosen as active conformation throughout this study. For the 3-oxadiazole analogues (**22**−**27**), an anti conformation was also chosen for this led to good overlap with the 3-ester functions, as expected. For the remainder of the ligands which contained a flexible side chain at position 3, a low-energy conformation was chosen which led to maximum overlap with the tert-butyl ester group of the high-affinity ligand 8. Previously, Fryer reported that only the S series of ring-constrained pyrroloimidazobenzodiazepines (**15**−**27**) bound to wild-type Bz receptors,[34] and then Liu[35] and Huang[28] drew the consistent conclusion according to the framework-constrained ligands, which were in agreement with the early work of Haefely.[36] This conformation was employed in the modeling of all benzodiazepine-related ligands in this study. Most of the substituents at position 8 were symmetrical or linear; therefore, conformational concerns were not an issue. In the case of compounds containing azido (**4**), or allene (**5**) groups, syn conformations were chosen for the DS subtype; on the contrary, anti conforma-

Table 1. Binding Affinities of Bz Ligands for $\alpha_x\beta_3\gamma_2$ ($x = 1-3$, 5, and 6) BzR Isoforms



1~7



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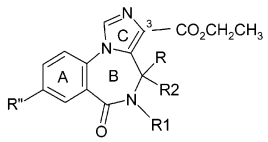

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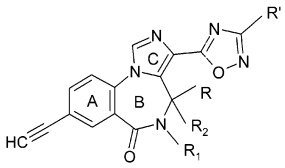
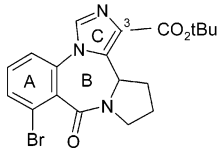


22~27



28

						*K*i (nM)				
no.	R8	R″	R′	R	R_1,R_2	α_1	α_2	α_3	α_5	α_6
1 (Ro15−1788)	F					0.8	0.9	1.05	0.6	148
2 (Ro 15−1310)[a]	Cl					6.8	16.3	9.2	0.85	54.6
3	Et					20.4	27	26.1	1.5	176
4 (Ro15−4513)	N═N═N					3.3	2.6	2.5	0.27	3.8
5	CH═C═CH_2					3.75	7.2	4.14	1.11	44.3
6 (RY-80)	C≡C−H					28.4	21.4	25.8	0.49	28.8
7	C≡C−$Si(CH_3)_3$					121.1	142.9	198.4	5.0	113.7
8	Cl					17.3	21.6	29.1	0.65	4
9[a]	Br					11.4	10.7	9.2	0.47	9.4
10	I					9.7	11.2	10.9	0.38	4.6
11	Et					14.8	56	25.3	1.72	22.9
12	C≡C−H					26.9	26.3	18.7	0.4	5.1
13	C≡C−$Si(CH_3)_3$					197	143	255	2.61	58.6
14	C≡CCH_2−$Si(CH_3)_3$					275.0	387.0	337.0	23.0	301.0
15		Br		H	-$CH_2CH_2CH_2$-	49	29	15	1	46
16		C≡C−$Si(CH_3)_3$		H	-$CH_2CH_2CH_2$-	200	124	79	4	340
17[a]		C≡CH		H	-$CH_2CH_2CH_2$-	59	44	27	1.3	126
18		Br		H	-CH_2CH_2-	17	13	6.7	0.3	31
19		C≡C−$Si(CH_3)_3$		H	-CH_2CH_2-	83	60	48	2.6	180
20		C≡CH		H	-CH_2CH_2-	21	12	10	0.37	42
21		OMe		H	-$CH_2CH_2CH_2$-	48.5	27.4	24.5	0.45	83.2
22[a]			CH_3	H	-$CH_2CH_2CH_2$-	89	70	91	3.7	301
23			CH_2CH_3	H	-$CH_2CH_2CH_2$-	86	40	85	2.4	150
24			$CH(CH_3)_2$	H	-$CH_2CH_2CH_2$-	73	85	97	4.8	333
25			CH_3	H	-CH_2CH_2-	19	56	91	7.2	266
26[a]			CH_2CH_3	H	-CH_2CH_2-	220	150	184	12.7	361
27			CH(CH_3)	H	-CH_2CH_2-	156	88	122	8.5	267
28(Ro166028)						0.3	0.6	0.2	0.5	12.7

[a] Test set compounds.

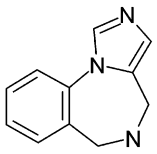


Figure 3. The common substructure of imidazobenzodiazepines for alignment.

tions were chosen for the DI subtype,[33,27] which is agreement with the previous study.[33,27]

Alignment Rules. The molecular alignment is another critical element in the FLARM calculation. Since every analogue examined in this study shares a common imidazo-1,4-diazepine substructure (Figure 3), these molecules were aligned via the common core as reported previously.[33] The molecules were aligned using the "Align Database" dialogue in SYBYL6.5 where ligand 1 was selected as the template molecule, and the structure illustrated in Figure 3 was used as the common structure, the imidazobenzodiazepine database was selected as the database to align, and other settings used the default values.

FLARM Computation. Like other pseudoreceptor methods, the FLARM method defines 15 kinds of pseudoreceptor atoms likely to be encountered in proteins. After a set of grid points is generated around the common surface of the superimposed training set molecules, receptor models are made by placing atoms which form the pseudoreceptor at these points in 3D space to simulate a receptor site and its interactions with the ligand. Then a genetic algorithm is used to optimize these models and a pseudoreceptor and select models with high cross-validated R^2. For each model, a linear

Table 2. Computation Results of 3D-QSAR Models for the Recombinant $\alpha_x\beta_3\gamma_2$ and Predicted Affinity versus Experimental Affinity of the Ligands in the Test Set

	$\alpha_1\beta_3\gamma_2$			$\alpha_2\beta_3\gamma_2$			$\alpha_3\beta_3\gamma_2$			$\alpha_5\beta_3\gamma_2$			$\alpha_6\beta_3\gamma_2$		
R_{cv}^2	0.862			0.855			0.904			0.851			0.855		
R^2	0.894			0.879			0.919			0.875			0.879		
SD[b]	0.251			0.245			0.228			0.197			0.233		
	pK_i [a]			pK_i			pK_i			pK_i			pK_i		
	exp	pre	res	exp	pre	res	exp	pre	res	exp	pre	res	exp	pre	res
9	7.94	8.03	−0.09	7.97	7.85	0.12	8.04	8.23	−0.19	9.33	9.37	0.04	8.03	8.35	−0.32
17	7.23	7.10	0.13	7.36	7.48	−0.12	7.57	7.51	0.06	8.89	8.94	0.05	6.9	7.13	−0.23
22	7.05	7.16	−0.11	7.15	7.31	−0.16	7.04	7.48	−0.44	8.43	8.58	0.15	6.52	6.41	0.11
26	6.66	6.78	−0.12	6.82	6.87	−0.05	6.74	6.62	0.12	7.9	8.20	0.30	6.44	6.68	−0.24
2	8.17	8.55	−0.38	7.79	8.16	−0.37	8.04	8.12	−0.08	9.1	9.12	0.02	7.26	7.23	0.03
predicted R^2		0.879			0.781			0.810			0.912			0.864	

[a] pK_i = 9-lgK_i, K_i in nM/L. [b] Standard deviation of cross-validated fitting.

relation between the log of the bioactivity and the interaction energy is established, consistent with the assumed free energy relationships in ligand binding. Last, using this relation, the bioactivity of unknown compounds can be predicted. Using a robust evolutionary optimization process in most cases FLARM can find a reliable model that can be used to predict bioactivity of unknown compounds. In short, the procedure of FLARM includes two steps: first constituting the model and the pseudoreceptor with molecules in a training set and second validating the model with molecules in the test set. In the first step, there are several parameters set by the user. In this case, the parameter was set as follows. The cushion between the receptor and the ligand was 0.5. It is noted that the location of the receptor can be shifted, so this parameter is not insensitive. The atom number of the receptor was 105. The genetic population size was 100, and the genetic evolvement was completed when the cross-validated correlation coefficient reached 0.88 or the default maximum genetic generations reached 150.

To start the FLARM computation, two text input files are created in which the names and bioactivities of aligned molecules in the mol2 file format are included in the training set or the test set, respectively. Then the FLARM program was run and the file of the training set and the parameters listed above were input to constitute a model and a pseudoreceptor in the first step. After that, the file of the test set was input to predict the bioactivities of the test set in the second step. The whole computation took about 5 min on a Pentium II 350 MHz PC.

It is noted that the distances between the atoms of the pseudoreceptor and the ligand in FLARM are model distances, not actual ones, which are larger than actual ones. But the interaction between receptor and ligand can be modeled and visualized.

RESULTS AND DISCUSSION

3D-QSAR Models. A series of 3D-QSAR models will be obtained by using the FLARM method. The program lists the top 15 models, which express the log of the bioactivity as a linear relation of the interaction energy. Then these models can be validated by the test set. The final model will be selected according to the performance of the test set. In this study, the result of the best model for every recombinant subtype is presented in Table 2. As shown in Table 2, R_{cv}^2 for every recombinant subtype was 0.862, 0.855, 0.904, 0.851, and 0.855, and R^2 was 0.894, 0.879, 0.919, 0.875, and 0.879, respectively. The result of R^2 and R_{cv}^2 shows the least overfitting, which often exists in other QSAR methods. At the same time, the standard error of estimate was 0.251, 0.245, 0.228, 0.197, and 0.233, respectively. All models give high R_{cv}^2 and good results for other statistical parameters in training sets and low residuals for test sets, so all models obtained are highly predictive. As illustrated in Table 2, the predicted values are very close to the observed values from binding experiments on recombinant receptors in vitro. For example, the residuals of the model in the test set for each recombinant receptor range from −0.38 to 0.13, −0.37 to 0.12, −0.44 to 0.12, 0.02 to 0.30, and −0.32 to 0.11, respectively, which are of considerable low value. The predicted R^2 are from 0.781 to 0.912, which shows good correlation. So the 3D-QSAR models are high quality and predictability.

United Pharmacophore/Receptor Model. Since the 1980s, several different models of the pharmacophore for benzodiazepine receptor binding have been put forth by Loew, Crippen,[37,38] Codding,[32] Fryer,[39] Gilli and Borea,[40] Gardner [41] et al., which tried to explain ligand efficacy as a function of ligand−receptor interactions at the molecular level. Then a comprehensive pharmacophore/receptor model for the BzR was developed which united previous models by Zhang.[27] This unified pharmacophore/receptor model[27] was employed to include agonist, antagonist, and inverse agonist ligands, which encompassed 12 families of structurally diverse compounds (Figure 4[27]). Four basic anchor points, H1, H2, A2, and L1, were assigned, and three additional lipophilic regions were defined as L2, L3, and LDi (see captions in Figure 4 for details). Qi Huang et al.[42] also contributed to the unified pharmacophore/receptor model and described the imidazobenzodiazepines in the model in detail[42] (Figure 5). As for 6(RY80), the ring A, the lone pair of electrons of N2, and the lone pair of electrons of the oxygen atom of the carbonyl group at the C3 position interact with L1, H1, and H2 of the pharmacophore/receptor model, respectively.

Pseudoreceptor Model and Comparison with United Pharmacophore Model. The pseudoreceptor models given by FLARM are shown in Figures 6−10 where the ligand in the models is RY80 (**6**). The distances between the corresponding atoms (Å) are also illustrated in Figures 6−10. The atoms in the figures are based on a CPK color scheme. The interactions between the receptor and the ligand can be easily seen from the pseudoreceptor models in Figures 6−10.

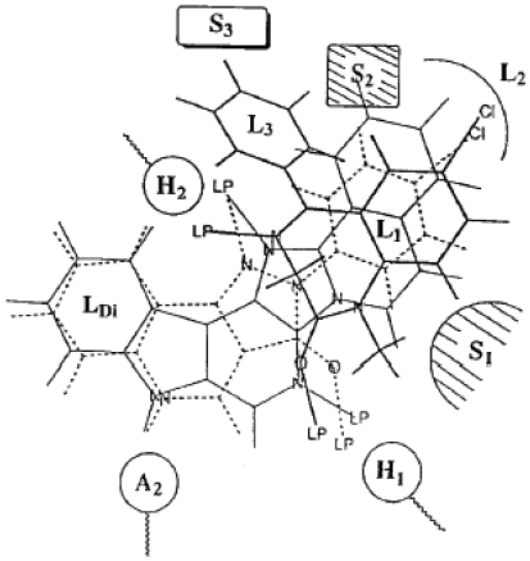


Figure 4. Pyrazolo[3,4-c]quinolin-3-one CGS-9896 (dottedline), a diazadiindole (thin line), and diazepam (thick line) fitted to the inclusive pharmacophore model for the BzR. Sites H1 and H2 represent hydrogen bond donor sites on the receptor protein complex, while A2 represents a hydrogen bond acceptor site necessary for potent inverse activity in vivo. L1, L2, and L3 are three lipophilic regions in the binding pharmacophore. Descriptors S1, S2, and S3 are regions of negative steric repulsion.

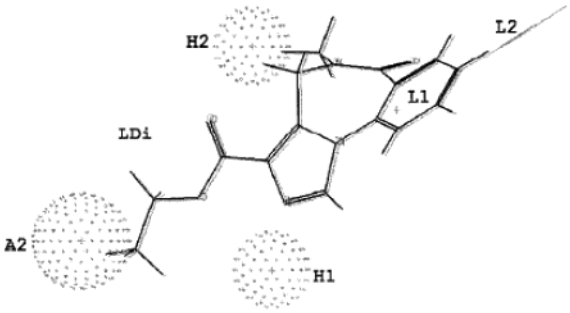


Figure 5. RY 80 (11) (gray) in the united pharmacophore/receptor model.

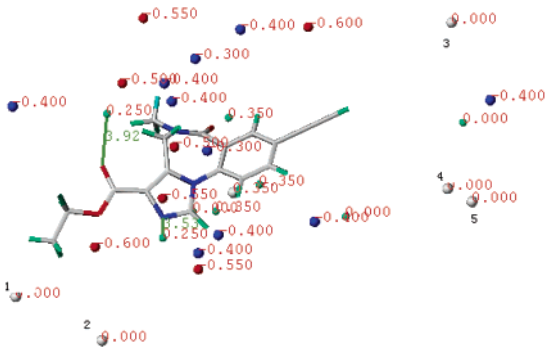


Figure 6. Pseudoreceptor model given by FLARM for $\alpha_1\beta_3\gamma_2$ recombinant $GABA_A/B_ZR$.

In the α_1-containing BzR pseudoreceptor, three possible interaction regions can be found. The first interaction is the hydrogen bond between N in the ligand and the hydrogen atom of the pseudoreceptor, which has a distance of 3.534 Å. The second one is the hydrogen bond between the carbonyl oxygen atom in the ligand and the hydrogen atom in the pseudoreceptor, which has a distance of 3.918 Å. The atoms labeled with numbers 1 and 2 in the pseudoreceptor are in the steric negative region S1 in the united pharmacophore/receptor model. The pseudoreceptor model is in accord with the united pharmacophore/receptor model which the two models both have a hydrogen bond in this position.

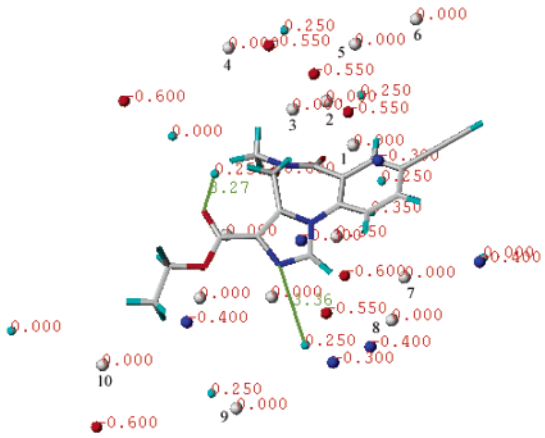


Figure 7. Pseudoreceptor model given by FLARM for $\alpha_2\beta_3\gamma_2$ recombinant $GABA_A/B_ZR$.

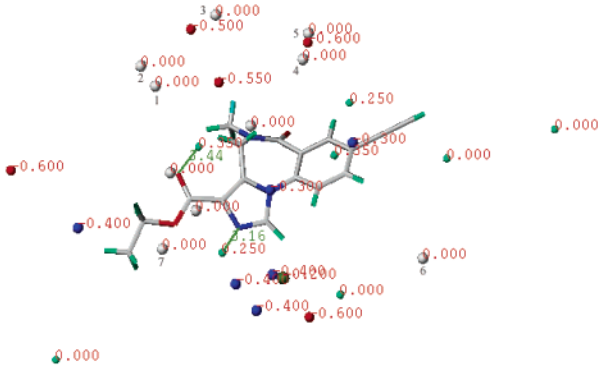


Figure 8. Pseudoreceptor model given by FLARM for $\alpha_3\beta_3\gamma_2$ recombinant $GABA_A/B_ZR$.

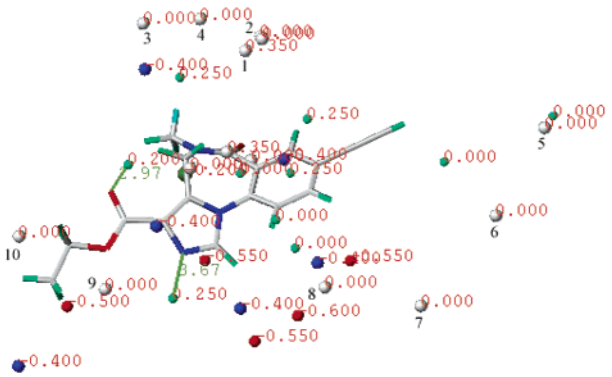


Figure 9. Peudoreceptor model given by FLARM for $\alpha_5\beta_3\gamma_2$ recombinant $GABA_A/B_ZR$.

In the α_2-containing BzR pseudoreceptor, five possible interaction regions can be seen. There are two hydrogen bond regions with N and a carbonyl oxygen atom in the ligand, which has distances of 3.359 Å and 3.272 Å, respectively. The third possible interaction region is a lipophilic region called L3 in the united model. Above the aromatic plane, there are two neutral carbon atoms labeled 1 and 2 corresponding to the L3 region in the united pharmacophore/receptor model. At the same time, the labeled atoms of 3 and 4 near the aromatic plane may constitute S3 steric repulsive region, while the other ones of 5 and 6 may be the S2 repulsive region in the united model. The pseudoreceptor

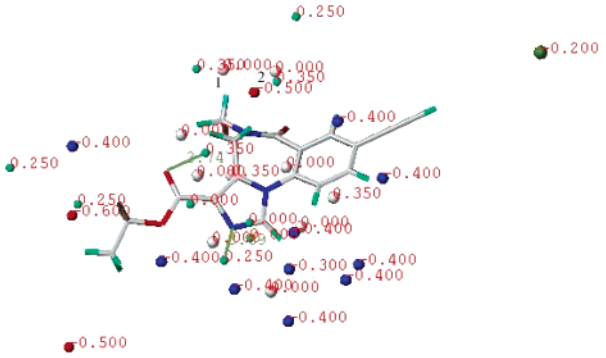


Figure 10. Pseudoreceptor model given by FLARM for $\alpha_5\beta_3\gamma_2$ recombinant $GABA_A/B_ZR$.

is also in agreement with the region S3 located in the direction of area L3.[27]

With regard to the α_3-containing BzR pseudoreceptor, the two hydrogen bonds with the same atoms in the ligand can also be seen with distances of 3.155 Å and 3.444 Å, respectively. In addition, the neutral carbon atoms labeled with numbers 3, 4, and 5 are in the S3 region, and the neutral carbon atoms labeled with 6 are in the S1 region of the united pharmacophore/receptor model.

For the α_5-containing BzR pseudoreceptor, the two hydrogen bonds with the same atoms in the ligand can also be seen the same as the former pseudoreceptor which has distances of 3.668 Å and 2.970 Å, respectively. At the same time, the neutral carbon atoms labeled with 1, 2, 3, and 4 are in the S3 region, and the neutral carbon atoms labeled with 6 and 7 are near the S1 region, while the one labeled with 5 corresponds to the counterpart L2 in the united pharmacophore/receptor model.

In the α_6-containing BzR pseudoreceptor, the two hydrogen bonds with the same atoms in the ligand are also presented which has distances of 3.866 Å and 3.740 Å, respectively. The L3 lipophilic region is presented in the pseudoreceptor by the atoms labeled with 1 and 2 located above the core structure of the ligand.

Though the united pharmacophore/receptor model obtained via ligand-mapping was different from the receptor modeling used in this study, the models show consistency with each other. Because the united pharmacophore/receptor model for all five subtypes is deducted from 12 families while the pseudoreceptor for a particular subtype is derived from one family, it is rational that not all interaction regions in the united pharmacophore/receptor model are present in our particular pseudoreceptor. For example, S3, L2, L1, and S1 are absent in the α_2-containing BzR pseudoreceptor described above. But if five pseudoreceptors are integrated, there are only L1 and LDi absent compared with the united pharmacophore/receptor. Furthermore, FLARM yields a pseudoreceptor by 3D-QSAR studies, so the region less contributed to the bioactivities of the training set in this study will have less information in the pseudoreceptor. Among the five pseudoreceptors, only the α_5-containing BzR pseudoreceptor presents the L2 region, which shows the importance of the substitutent at position 8 for α_5 selectivity. The result also corresponds to the previous studies that the region L2 in the α_1-containing, α_2-containing, and α_3-containing isoforms is not deep nor as large as that of the α_5 subtype.[28]

The pseudoreceptors for five subtypes are all characterized with two hydrogen bonds, which shows the hydrogen is necessary for bioactivities of the analogues at five recombinant subtypes. Finally, the united pharmacophore/receptor model interprets the bioactivity only at a qualitative level, while our pseudoreceptor provided quantitative relationships between the structures of imidazobenzodiazepines and their binding affinities.

CONCLUSIONS

3D-QSAR studies and receptor modeling via the FLARM method for the binding affinities of a set of imidazobenzodiazepines at five recombinant receptor subtypes were performed successfully. Some 3D-QSAR models were built with high correlation coefficients and then predicted the binding affinities of ligands in the test set well. The FLARM method also yielded the pseudoreceptor models, which indicate the possible interactions between receptor and ligand. These possible interactions include two hydrogen bonds and other interaction regions as S1, S3, L2, and L3, which are included in the united pharmacophore/receptor model. In addition, the united pharmacophore/receptor model integrates five receptors' interaction, this is to say, not every particular receptor possesses all characteristics. The result also shows the importance of the substitutent at position 8 for α_5 selectivity. FLARM is developed from PARM[22,25] (Pseudo Atomic Receptor Model) in our research group, so FLARM has the characteristics of PARM. According to the previous research, PARM can gain an equivalent or even better result compared with COMFA.[22,25] In addition, FLARM is able to build models with high R_{cv}^2 using a small set of even less than 10 training molecules. At the same time, FLARM can furthest void the overfitting existing in most QSAR method. In short, it is a good choice to do the QSAR study. The FLARM method is a pseudoreceptor model method, which belongs to an alternative 3D-QSAR method. This also shows that the FLARM method can bridge 3D-QSAR and receptor modeling in computer-aided drug design.

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