Predictive Models for $GABA_A$/Benzodiazepine Receptor Subtypes: Studies of Quantitative Structure−Activity Relationships for Imidazobenzodiazepines at Five Recombinant $GABA_A$/Benzodiazepine Receptor Subtypes [$\alpha x\beta 3\gamma 2$ ($x = 1-3$, 5, and 6)] via Comparative Molecular Field Analysis

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Affinities of a series of substituted imidazobenzodiazepines at recombinant $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 5\beta 3\gamma 2$, and $\alpha 6\beta 3\gamma 2$ $GABA_A$/benzodiazepine receptor subtypes are reported. Many of these ligands displayed high affinities (low-nanomolar to subnanomolar scale) at all five receptor subtypes. Furthermore, a number of imidazobenzodiazepines exhibited relatively good selectivity at the α5-containing receptor isoform. For example, ligand **27** (RY-023) demonstrated a 55-fold higher selectivity at $\alpha 5\beta 3\gamma 2$ isoforms in comparison to other receptor subtypes. The affinity ratio of α1 (the most prevalent subtype in the brain) to α5 of this series of ligands ranged from 60- to 75-fold for the most selective ligands. Studies of quantitative structure−activity relationships (QSAR) by means of comparative molecular field analysis (CoMFA) were carried out. As a result, examination of CoMFA models for all five receptor subtypes demonstrated their predictability for affinities of imidazobenzodiazepines at the five receptor subtypes. Regions of molecular fields which would favor or disfavor the binding affinity of a ligand at a specific receptor subtype were examined via CoMFA for α1, α2, α3, α5, and α6 subtypes. A CoMFA regression analysis was applied to predict the ratio of K_i α1/K_i α5, an index for the selectivity of a ligand at the α5 subtype. All of the CoMFA models offered good cross-validated correlations for the ligands in the test set as well as the ratios of K_i α1/K_i α5, which demonstrated their potential for prediction.

Introduction

$GABA_A$/benzodiazepine receptor/chloride ion channels comprise the major inhibitory neurotransmitter system in the central nervous system (CNS).[1,2] In addition to γ-aminobutyric acid (GABA), a number of classes of compounds such as benzodiazepines, barbiturates, neuronal steroids, anesthetics, and alcohol bind to this receptor complex. The latter compounds affect the GABA system allosterically and elicit a wide range of neuronal pharmacological activities.[3,4] Among these, receptor sites for the benzodiazepines (Bz) are of prime importance since these ligands have been employed widely in the clinic as anxiolytic/anticonvulsant agents since the 1960s.[5−7] Studies of molecular biology have suggested that the $GABA_A$/BzR complex is a heteropentameric protein polymer constituted principally from α, β, and γ subunits.[8,9] At present, a total of 16 subunits (6α, 4β, 3γ, 1δ, and 2ρ) have been isolated and identified from the CNS. Among these, 15 subunits have been found in the mammaliam CNS.[8,9] Recent 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.[8,9] Recombinant receptors constructed from αx, $\beta 2$, and $\gamma 2$ subunits most closely resemble the pharmacological profile of a native BzR obtained from mammalian brain cells. Receptor subtypes comprised of $\alpha 1\beta 2\gamma 2$ subunits resemble the activity of the classical Bz-I receptor subtype.[10,11] Receptors which contain α2, α3, or α5 subunits mimic the activity of the previously defined type-II BzR.[10] Recombinant receptors constituted from both $\alpha 4\beta 2\gamma 2$ and $\alpha 6\beta 2\gamma 2$ subunits represent "diazepam-insensitive" (DI) sites.[12,13] The extensive molecular diversity of $GABA_A$/BzRs which results from these subunits has been implicated in the multiple pharmacological properties elicited by ligands which lack subtype selectivity, such as diazepam.[14] 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.[14−17] Recent results also suggest that the biological activity of recombinant receptors composed of αx, $\beta 3$, and $\gamma 2$ subunits is very close to the activity of recombinant receptors composed of αx, $\beta 2$, and $\gamma 2$ subunits.[18] The identification of BzR subtype-selective ligands will provide agents with which to determine the relationship between physiological activity and BzR subtype. This may result in new subtype-selective agents for the treatment of anxiety, sleep disorders, convulsions, or memory deficits as well as decrease the potential for side effects.

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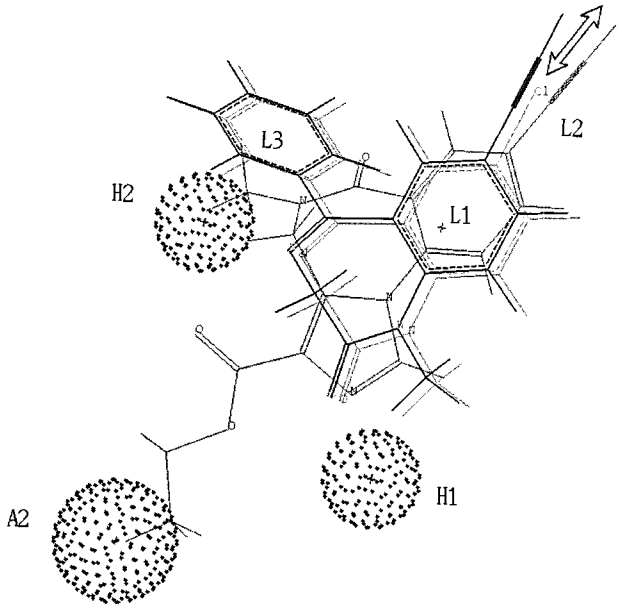
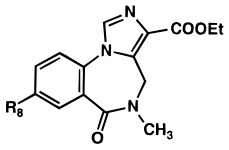


Figure 1. Overlap of RY-80 (**10**) (in gray) with an acetylene moiety at position 8 of the imidazobenzodiazepine nucleus with diazepam (in gray, identified by the double arrow with a chlorine at position 7 of the 1,4-benzodiazepine) illustrates the difference in the occupation of region L_2 in comparison to RY-80 (**10**). The ligand QH-II-66, a 7-acetylenyl-1,4-benzodiazepine (in black), was designed to offer better occupation of this region and exhibit α5 selectivity. Note that diazepam as shown was intentionally offset from its original position, which had overlapped completely with QH-II-66, solely for the sake of visualization.

Previously, imidazobenzodiazepines were reported to bind to the DI site of the BzR with high affinity.[19−27] Recent 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).[28] Among these ligands, RY-80 (**10**), RY-23 (**26**), and RY-24 (**27**) displayed 58−75-fold selectivity at the α5β3γ2 receptor subtype in comparison to the α1β3γ2 receptor subtype. These ligands are among the most selective compounds to bind at the α5-containing receptor subtype reported to date.[18,28−30] Extensive in vivo pharmacological studies carried out on these ligands suggest that convulsions effected by these compounds were induced by interaction at α5-containing receptors found in the brains of mice.[18,30] Results of a molecular modeling study of RY-80 (**10**) and related ligands suggested that occupation of region L_2 within the pharmacophore/receptor model might lead to a ligand with selectivity at α5-containing receptors.[18,28] In this regard, 7-acetylenyl-1,4-benzodiazepine, a diazepam congener, was synthesized and found to exhibit modest α5-selectivity, the first α5 selective agonist reported to date (Figure 1).[30,31] On the other hand, studies of a series of framework-constrained 4,5-substituted pyrroloimidazobenzodiazepines and azetidinylimidazobenzodiazepines (both *R* and *S* stereoisomers), demonstrated that only the *S* enantiomers of this series of ligands bound to BzR subtypes with high affinity.[29] This is in agreement with the earlier work of Haefely and Fryer on wild-type receptor populations (synaptosomal membranes).[24,32] The stereopreferences of recombinant receptors for the *S* enantiomers of these ligands suggested the conformational topography at the five recombinant receptor subtypes was well-conserved. McKernan et al. reported the activity of a ligand which was 55 times more selective at α5-containing receptors and was converted into a tritiated ligand.[33] Recent results on [^{3}H]RY-80 further demonstrated that this ligand (**10**) could also be employed to label specific populations of GABA$_A$ receptor isoforms which contained an α5 subunit.[30] The tritiated RY-80 (**10**) may be used in regard to α5 sites in much the same manner as [^{3}H]zolpidem is employed to study receptor populations bearing α1 subunits.[34] As such, [^{3}H]RY-80 may be employed to evaluate the potency and efficacy of ligands at wild-type GABA$_A$ receptors containing α5 subunits, as a radioligand for autoradiographic studies, and as a probe for examining these receptors after physiological and pharmacological manipulation.[30]

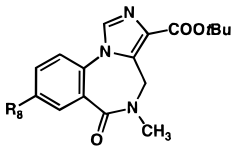
Table 1. Affinities of 5,6-Dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic Acid Ethyl Esters for α$x\beta$3γ2 (x = 1−3, 5, 6) Benzodiazepine Receptor Isoforms



		K_i (nM)[a]					
ligand	R_8	α1	α2	α3	α5	α6	α1/α5
1 (Ro 15-1788)	F	0.8	0.9	1.05	0.6	148	1.33
2 (Ro 15-1310)	Cl	6.8	16.3	9.2	0.85	54.6	8.00
3	Br	26.0	27.0	13.0	0.7	22.0	3.71
4	CN	10.0	45.0	19.0	6.0	>1000	1.67
5	$CH{=}CH_2$	8.3	10.2	6.9	0.4	NA[b]	20.75
6	Et	20.4	27	26.1	1.50	176	13.6
7	OEt	11.2	36	16.9	1.07	51.5	12.4
8 (Ro15-4513)	$N{=}N^+{=}N^-$	3.3	2.6	2.5	0.27	3.8	10.5
9	$CH{=}C{=}CH_2$	3.75	7.2	4.14	1.11	44.3	3.4
10 (RY-80)	$C{\equiv}C{-}H$	28.4	21.4	25.8	0.49	28.8	58.0
11	$C{\equiv}C{-}CH_3$	10.1	22.2	16.5	1.68	>100	6.0
12	$C{\equiv}C{-}Si(CH_3)_3$	121.1	141.9	198.4	5.0	113.7	24.2
13	$C{\equiv}C{-}CH_2Si(CH_3)_3$	>300	>300	>300	>300	>300	NA

[a] Data shown here are the means of two determinations which differed by less than 10%. [b] NA stands for data not available.

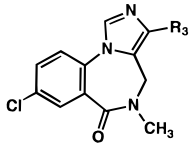
Table 2. Affinities of 5,6-Dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic Acid *tert*-Butyl Esters for α*x*β3γ2 (*x* = 1−3, 5, 6) Benzodiazepine Receptor Isoforms



		K_i (nM)[a]					
ligand	R_8	α1	α2	α3	α5	α6	α1/α5
14	Cl	17.3	21.6	29.1	0.65	4	26.6
15	Br	11.4	10.7	9.2	0.47	9.4	24.3
16	I	9.7	11.2	10.9	0.38	4.6	25.5
17	OH	1.50	NA[d]	0.53	0.14	6.89	10.7
18	OCH_3	6.74	NA	7.42	0.293	8.28	23.0
19	$N(CH_3)_2$	13.1	NA	38.1	0.78	118	16.8
20	X[b]	5.8	NA	169	9.25	325	0.63
21	Y[c]	6.44	NA	148	4.23	247	1.5
22	$N{=}N^+{=}N^-$	7.25	NA	5.66	0.3	5.25	24.3
23	NCS	17.1	33.7	50	2.5	30.7	6.8
24	NO_2	12.8	49.8	30.2	3.5	22.5	3.7
25	Et	14.8	56	25.3	1.72	22.9	8.6
26	C≡C−H	26.9	26.3	18.7	0.4	5.1	67.3
27	C≡C−$Si(CH_3)_3$	197	143	255	2.61	58.6	75.5
28	C≡C$CH_2Si(CH_3)_3$	275.0	387.0	337.0	23.0	301.0	12.0

[a] Data shown here are the means of two determinations which differed by less than 10%. [b] X, *N*-tetrahydropyrrole. [c] Y, *N*-hexahydropyridine. [d] NA, data not available.

Table 3. Affinities of 3-Substituted 5,6-Dihydro-5-methyl-6-oxo-8-chloro-4*H*-imidazo[1,5-*a*][1,4]benzodiazepines for α*x*β3γ2 (*x* = 1−3, 5, 6) Benzodiazepine Receptor Isoforms



		K_i (nM)[a]					
compd	R_3	α1	α2	α3	α5	α6	α1/α5
2 (Ro 15-1310)	$C_{O2}Et$	6.8	16.3	9.2	0.85	54.6	8.0
14	CO_2t-Bu	17.3	21.6	29.1	0.65	4	26.6
29	CO_2CH_2-c-Pro	16.4	48.2	42.5	9.8	168	1.7
30	C(=O)CH_3	17535	33834	22125	2612	29500	6.7
31	C(=O)*n*-Bu	1581	2865	2739	166	2930	9.6
32	CH_2OH	>300	>300	>300	>300	>300	
33	CH_2OCH_3	>300	>300	>300	38.8	>300	>7.7
34	CH_2Cl	>300	>300	>300	28.5	>300	>10.5
35	CH_2OEt	>300	>300	>300	82.7	>300	>3.6
36	$CH_2N(Et)_2$	9483	30000	15409	2583	30160	3.2
37	$CH_2N(i\text{-}Pr)_2$	4201	12590	6266	1346	8600	3.2
38	Et	408	1527	1125	182	3648	2.2
39	*n*-C_5H_{11}	2050	2900	2907	369	960	5.6

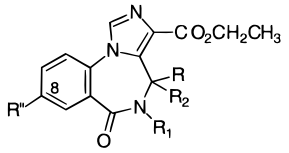
[a] Data shown here are the means of two determinations which differed by less than 10%.

It was felt that affinities from studies on recombinant receptor subtypes mentioned above provided a great deal of information on the structure−activity relationships of ligands and receptor subtypes in this series. Consequently, a quantitative structure−activity relationship (QSAR) study via a comparative molecular field analysis (CoMFA)[35] was carried out on the series of imidazobenzodiazepines listed in Tables 1−5. Here, we report QSAR models derived from CoMFA analyses for the affinities of this series of ligands at all five recombinant subtypes. All of the QSAR models have been evaluated by means of a set of test ligands. Results obtained from the test ligands indicate that all of the models are highly predictive.

Results and Discussion

Affinities of a series of imidazobenzodiazepines at recombinant α1β3γ2, α2β3γ2, α3β3γ2, α5β3γ2, and α6β3γ2 $GABA_A$/BzR subtypes are listed in Tables 1−5. From analysis of the in vitro affinities of ligands listed in Tables 1−5, it appears that an ester or an ester bioisostere group at position 3 of the imidazobenzodiazepine nucleus is of prime importance for high affinity binding at all five receptor subtypes. In the α5 subtype some flexibility in this regard was observed. This was demonstrated by ligands **33**−**35**, all of which displayed moderately high affinity (K_i = 29−83 nM) at the α5 subtype. Almost all of the imidazobenzodiazepine-3-carboxylates displayed very potent affinity at the α5

Table 4. In Vitro Affinities of Framework-Constrained Imidazobenzodiazepines at Recombinant α*x*β3γ2 (*x* = 1−3, 5, 6) GABA$_A$/BzR Subtypes

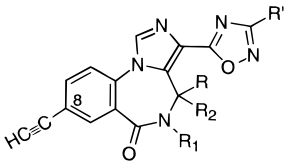


imidazobenzodiazepines

				K_i (nM)[a]					
compd	R″	R	R$_1$ R$_2$	α1	α2	α3	α5	α6	α1/α5
40	Br	(*S*)H	−CH$_2$CH$_2$CH$_2$−	49	29	15	1	46	49
41	Br	(*R*)H	−CH$_2$CH$_2$CH$_2$−	>1000	>1000	>1000	>1000	>1000	NA[b]
42	C≡CSi(CH$_3$)$_3$	(*S*)H	−CH$_2$CH$_2$CH$_2$−	200	124	79	4	340	50
43	C≡CSi(CH$_3$)$_3$	(*R*)H	−CH$_2$CH$_2$CH$_2$−	>1000	>1000	>1000	>1000	>1000	NA
44	C≡CH	(*S*)H	−CH$_2$CH$_2$CH$_2$−	59	44	27	1.3	126	45
45	C≡CH	(*R*)H	−CH$_2$CH$_2$CH$_2$−	283	318	102	7.2	61	39
46	Br	(*S*)H	−CH$_2$CH$_2$−	17	13	6.7	0.3	31	56
47	C≡CSi(CH$_3$)$_3$	(*S*)H	−CH$_2$CH$_2$−	83	60	48	2.6	180	32
48	C≡CH	(*S*)H	−CH$_2$CH$_2$−	21	12	10	0.37	42	57
58	OMe	(*S*)H	−CH$_2$CH$_2$CH$_2$−	48.5	27.4	24.5	0.45	83.2	108
10 (RY-80)	C≡CH	H	−CH$_3$	28.4	21.4	25.8	0.5	28.8	57

[a] Data shown here are the means of two determinations which differed by less than 10%. [b] NA stands for not applicable.

Table 5. Affinities of 3-Alkyl-1,2,4-oxadiazole 4,5-Substituted Imidazobenzodiazepines at Recombinant α*x*β3γ2 (*x* = 1−3, 5, 6) GABA$_A$/BzR Subtypes



				K_i (nM)[a]					
ligand	R′	R	R$_1$ R$_2$	α1	α2	α3	α5	α6	α1/α5
49	CH$_3$	(*S*)H	−CH$_2$CH$_2$CH$_2$−	89	70	91	3.7	301	24
50	CH$_3$	(*R*)H	−CH$_2$CH$_2$CH$_2$−	>1000	>1000	>1000	157	>1000	NA[b]
51	CH$_2$CH$_3$	(*S*)H	−CH$_2$CH$_2$CH$_2$−	86	40	85	2.4	150	36
52	CH(CH$_3$)$_2$	(*S*)H	−CH$_2$CH$_2$CH$_2$−	73	85	97	4.8	333	15
53	C$_6$H$_5$	(*S*)H	−CH$_2$CH$_2$CH$_2$−	33%[c]	26%	19%	455	4%	NA
54	CH$_3$	(*S*)H	−CH$_2$CH$_2$−	19	56	91	7.2	266	2.6
55	CH$_2$CH$_3$	(*S*)H	−CH$_2$CH$_2$−	220	150	184	12.7	361	17
56	CH(CH$_3$)$_2$	(*S*)H	−CH$_2$CH$_2$−	156	88	122	8.5	267	18
57	C$_6$H$_5$	(*S*)H	−CH$_2$CH$_2$−	10%	29%	15%	374	14%	NA

[a] Data shown here are the means of two determinations which differed by less than 10%. [b] NA stands for not applicable. [c] Percentage inhibition at a concentration of 1 μm.

subtype with a binding constant (K_i) in the low-nanomolar to subnanomolar range. Variation of the substituent at position 8 of the imidazobenzodiazepine nucleus did not alter the binding affinity of a ligand at the α5β3γ2 subtype when the ester group was held constant. However, this alteration did change the selectivity of a ligand at the α5β3γ2 receptor subtype, since the affinities of ligands toward the other subtypes were more sensitive to variations in the C-8 substitution pattern (e.g., the K_i α1/K_i α5 ratio ranges from 0.63 for **20** to 75.5 for **27**). Their affinities for α5β3γ2 subtypes remained potent, while those for the other receptor isoforms decreased. Most of the 3-substituted esters bound to the α1-containing receptor subtype with an affinity of about 1 order of magnitude lower than they bound to the α5 subtype.

Ligands with an acetyleno group at position 8, in particular, displayed high K_i α1/K_i α5 ratios (39−65-fold) at the α5β3γ2 subtype. In cases where the (trimethylsilyl)acetylene moiety was substituted at position 8, comparable K_i α1/K_i α5 ratios were observed for ligands **12**, **27**, and **47**. Examination of these results suggests that region L$_2$ within the binding pharmacophore/receptor model of the α1β3γ2 receptor subtype, which corresponds to the region occupied by a C-8 substituent in the unified pharmacophore/receptor model (Figure 1),[36] is not as deep nor as large as the analogous lipophilic pocket within the α5 subtype.

At present, analysis of data from binding affinities suggests that affinities of imidazobenzodiazepine-3-carboxylates at the α2- and α3-containing isoforms follow a similar pattern. It appears that region L$_2$ in the latter two receptor subtypes is not as deep nor as large as that of the α5 subtype, a result similar to that observed for the α1 receptor isoform. Low affinities observed for ligands **20** and **21** at the α3-containing receptor subtype suggest that region L$_2$ of the α3 subtype is not as wide as the analogous region in the α1 and α5 subtypes. Much more work will be required to determine if this supposition is correct.

Table 6. Bond Angles[a] of Aryl Isothiocyanates from the Cambridge Crystallographic Database

compd code	bond angle (deg)
BRPITC01	154.02
BUFJAN01	169.69
DAPMEM	168.70
HAMCAZ	143.91

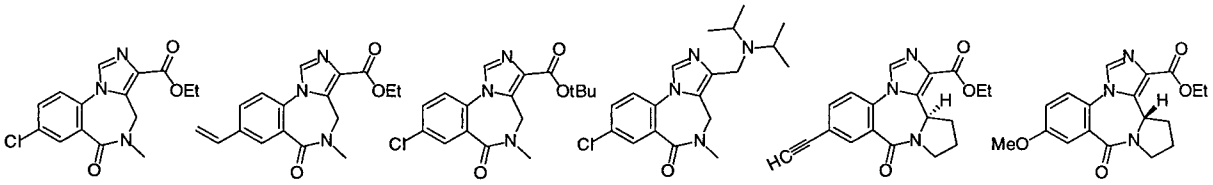
[a] C(aryl)−N−C bond angle of aryl isothiocyanates; $n = 4$, mean = 159.08, deviation = 12.39.

Previously, Fryer reported that only the *S* series of ring-constrained pyrroloimidazobenzodiazepines bound to wild-type Bz receptors.[24] On the basis of the α5 selectivity of imidazobenzodiazepine **10**, a number of ring-constrained pyrrolo- and azetidinylimidazobenzodiazepines of both the *R* and *S* stereochemical configurations were synthesized and evaluated in vitro on the series of recombinant BzR subtypes. Analysis of the affinities of ligands listed in Tables 4 and 5 demonstrated that only the *S* series of imidazobenzodiazepines bound tightly to all five recombinant receptor subtypes, which is in agreement with the earlier work of Fryer and Haefely.[24,32] This also suggests that the conformational topography as well as molecular descriptors (H_1, H_2, and L_1) within the recombinant receptors has been well-conserved in comparison to the native receptors. Consequently, molecular descriptors (H_1, H_2, and L_1) earlier defined for the unified pharmacophore/receptor model[36] could be applied to the pharmacophore/receptor models for the recombinant BzR subtypes.

Conversion of the ester function into its bioisostere, an oxadiazole congener (**49**−**57**), decreased the affinity of the ligand but generally maintained the α5 selectivity. This is important with respect to the α6 subtype, a DI site, in which alteration of an ester moiety to an oxadiazole group diminished the affinity of a ligand at this receptor subtype. In the case of the pyrroloimidazobenzodiazepines, the affinities of ligands remained the same within the receptor subtype when altering the alkyl substituent at position 3 of the oxadiazole moiety at all five receptor subtypes [from **49** (methyloxadiazole) through **51** (ethyloxadiazole) to **52** (isopropyloxadiazole)]. However, in the case of azetidinylimidazobenzodiazepines, the smaller the size of the alkyl substituent at position 3 of the oxadiazole, the better the affinity at α1, α2, and α3 receptor subtypes (see **54**−**56**). The affinities of ligands at the α5-containing receptors, however, experienced no significant changes. A phenyl substituent (see **53** and **57**) placed on the oxadiazole moiety, on the other hand, inhibited the binding of the ligand at all five receptor subtypes.

To obtain more information on the steric and electrostatic requirements for selective binding at all five receptor subtypes, a 3D-QSAR study was carried out by means of the CoMFA developed by Cramer et al.[35] The alignment rule and active conformations of the ligands were first determined as required before performing the 3D-QSAR analysis. Since all of the ligands used in this study contain the same imidazobenzodiazepine framework, these molecules were aligned via the common core as reported previously.[23] During an earlier study on diazepam-insensitive BzR ligands, the active conformations of imidazobenzodiazepines employed for CoMFA studies on DS and DI BzR subtypes were determined.[23] In short, the anti conformation of

Table 7. Statistics of CoMFA Models for the Five Bz Receptor Subtypes and Predicted Affinity versus Experimental Affinity of the Ligands in the Test Set



2 **5** **14** **37** **45** **58**

	α1β3γ2			α2β3γ2			α3β3γ2			α5β3γ2			α6β3γ2			p(K_iα5/K_iα1)		
NC[a]	42			36			42			47			40			42		
r^2[b]	0.988			0.826			0.985			0.957			0.803			0.848		
OC[c]	8			2			6			5			2			4		
SE[d]	0.112			0.415			0.130			0.236			0.413			0.206		
F[e]	339.77			78.38			379.07			184.05			75.28			51.77		
RC-S[f]	0.589			0.644			0.592			0.588			0.653			0.710		
RC-E[f]	0.411			0.356			0.408			0.412			0.347			0.290		
	pK_i[g]			pK_i			pK_i			pK_i			pK_i			p(K_iα5/K_iα1)[k]		
	Exp[h]	Pre[i]	Res[j]	Exp	Pre	Res	Exp	Pre	Res	Exp	Pre	Res	Exp	Pre	Res	Exp	Pre	Res
2	8.17	8.13	-0.04	7.79	7.81	0.02	8.04	8.32	0.28	9.07	9.11	0.04	7.26	7.26	0.00	0.90	0.90	0.00
5	8.08	7.88	-0.20	7.99	7.77	-0.22	8.16	7.91	-0.25	9.40	9.23	-0.17	NA	7.19	NA	1.32	1.08	-0.22
14	7.76	8.02	0.26	7.67	7.56	-0.11	7.54	7.71	0.18	9.19	9.29	0.10	8.40	7.89	-0.51	1.43	1.41	-0.02
37	5.38	5.25	-0.13	4.90	5.15	0.25	5.20	4.87	-0.33	5.87	6.21	0.34	5.07	5.40	0.33	0.49	0.55	0.06
45	6.55	7.70	1.16	6.50	6.71	0.21	6.99	7.11	0.12	8.14	7.84	-0.30	7.21	6.31	-0.90	1.59	0.76	-0.84
58	7.31	7.78	0.46	7.56	7.61	0.05	7.61	7.65	0.04	9.35	9.57	0.22	7.08	7.20	0.12	2.03	1.95	-0.08

[a] Number of the ligands used for CoMFA models. [b] r^2 non-cross-validated. [c] Number of optimal components. [d] Standard error of estimate. [e] *F*-value. [f] RC-S, relative contributions (steric); RC−E, relative contributions (electrostatic). [g] p$K_i = 9 - \log K_i$, K_i in nM. [h] Exp, experimental data. [i] Pre, predicted data by CoMFA models. [j] Res, Pre − Exp. [k] p(K_i α5/K_i α1) = log K_i α1 − log K_i α5.

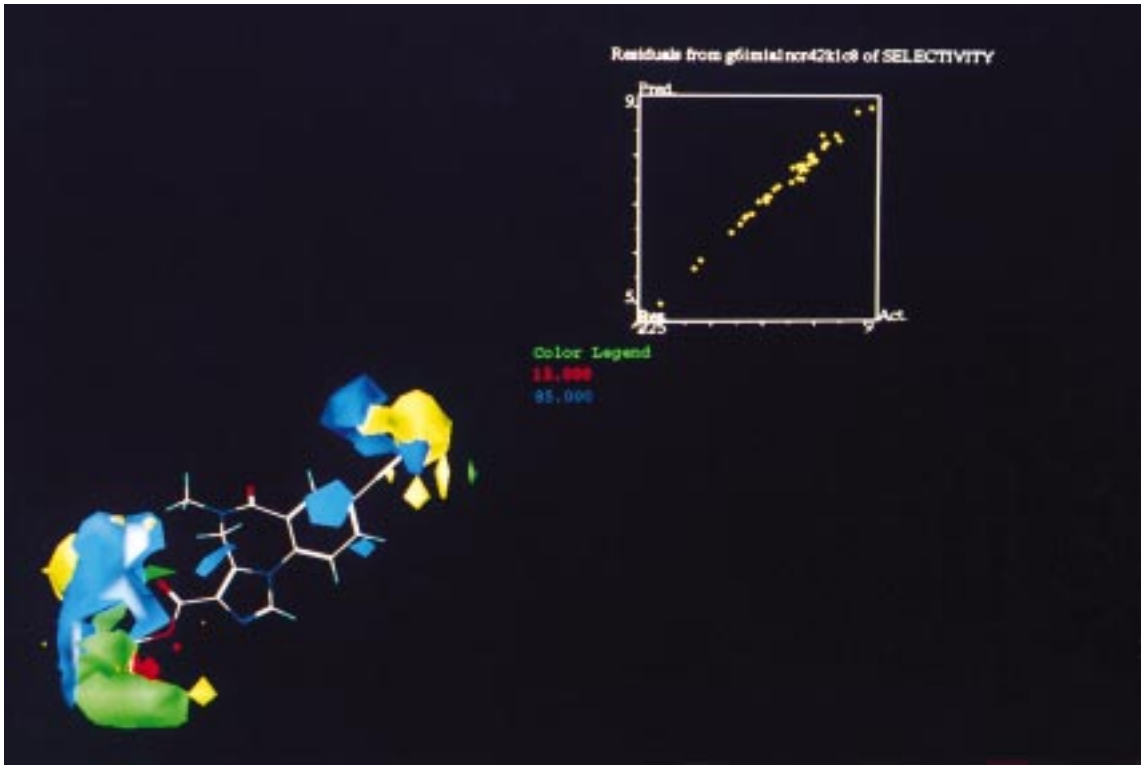


Figure 2. Non-cross-validated CoMFA steric and electrostatic STDEV*COEFF contour plots from the analysis of affinities of imidazobenzodiazepines at the $\alpha1\beta3\gamma2$ receptor subtype superimposed on ligand **10** (RY-80). Green contours represent regions of positive steric interaction at a contribution level of 85%, while yellow contours represent regions of negative steric interaction at a contribution level of 15%. Blue contours represent areas of positive charge interaction at a level of 85%, while red contours represent areas of negative charge interaction which would enhance the affinity of a ligand at a contribution level of 15%. The plot shown on the upper right-hand portion represents the predicted value of affinities of the ligands versus their actual affinity values.

the ester functionality at position 3 was proposed as the active conformation for both the DS and DI subtypes. In addition, in the case of 4,5-pyrroloimidazobenzodiazepines, 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 **44**. Consequently, the equilibrium constant between the two conformers of **44** at 4 °C equals 144.9 ($\Delta G = -RT \ln K$), at the temperature under which the binding study was done. Thus the anti conformation of the ester moiety was chosen as an active conformation throughout this study. For the 3-oxadiazole analogues, an anti conformation was also chosen for this led to good overlap with the 3-ester functions, as expected. In the case of ligands **30** and **31**, the anti conformer of the carbonyl function at the 3-position was employed, which is also a low-energy conformation. 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 **14**. Most of the substituents at position 8 were symmetrical or linear; therefore, conformational concerns were not an issue. In the case of compounds containing vinyl (**5**), azido (**8** and **22**), and allene (**9**) groups, syn conformations were chosen.[23] For 8-isothiocyano-imidazobenzodiazepine (**23**), ab initio calculations revealed that the bond angle C(8)−N−C was close to 180° (179.80°). To ensure the credibility of the calculated results, a Cambridge Crystallographic Database search for the crystallographic data of aryl isocyanates was carried out. Four entries of aryl isothiocyanates were obtained from the search. The bond angles of C(aryl)−N−C from the search ranged from 143.91° to 169.69° (see Table 6) which were close to the calculated results. To permit conjugation of the p-electrons of the nitrogen atom with the aryl system, the *N*-tetrahydropyrrole (**20**) and *N*-hexahydropyridine (**21**) moieties were located nearly in the same plane as ring A of the imidazobenzodiazepine. Conformations generated in this fashion were then subjected to a full geometry optimization for all bond lengths and bond angles with torsional angles fixed at the 3-21G basis set via Gaussian 92 or Gaussian 94, followed by a single-point calculation at a 6-31G* basis set with an "SCF=TIGHT" convergence criteria to obtain reliable electrostatic potentials.

Illustrated in Table 7 are CoMFA statistics which demonstrate a good correlation for the negative logarithms of affinities of this entire series of imidazobenzodiazepines at the five recombinant BzR subtypes. Steric and electrostatic contour maps derived from these CoMFA are presented in Figures 2−7. Since differences among most of the ligands only appeared at positions 3 and 8 of the imidazobenzodiazepine nucleus, analysis of contour maps from CoMFA not surprisingly revealed that regions occupied by substituents at positions 3 and 8 were the most sensitive in regard to the affinity and selectivity of the ligands.

CoMFA for Affinities at the $\alpha1\beta3\gamma2$ Receptor Subtype. A total of 42 ligands (**1**, **3**, **4**, **6**−**12**, **15**−**31**, **36**, **38**−**40**, **42**, **44**, **46**−**49**, **51**, **52**, and **54**−**56**) were included for a cross-validated partial least-squares

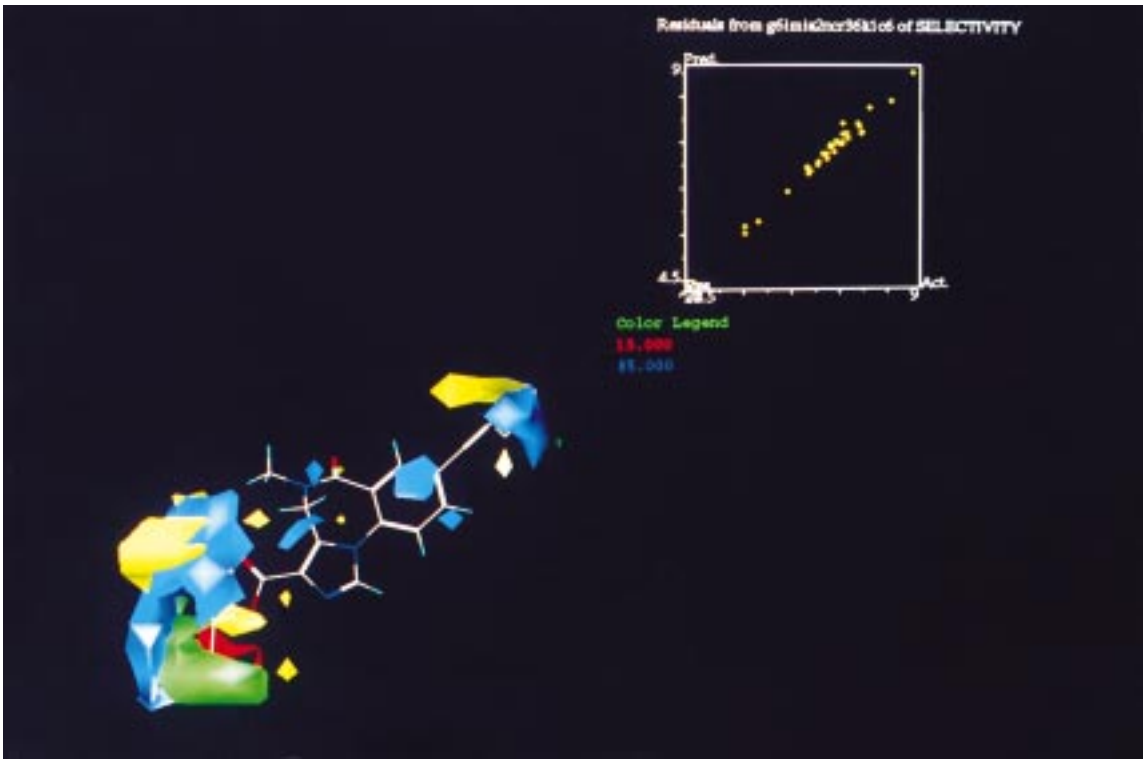


Figure 3. Non-cross-validated CoMFA steric and electrostatic STDEV*COEFF contour plots from the analysis of affinities of imidazobenzodiazepines at the α2β3γ2 receptor subtype with ligand **10** (RY-80). Green contours represent regions of positive steric interaction at a contribution level of 85%, while yellow contours represent areas of negative steric interaction at a contribution level of 15%. Blue contours represent areas of positive charge interaction at a level of 85%, while red contours represent areas of negative charge interaction which would enhance the affinity of a ligand at a contribution level of 15%. The plot shown on the upper right-hand portion is a representation of the predicted value of affinities of the ligands versus their actual affinity values.

analysis (PLS) of the affinities of ligands at the α1β3γ2 receptor subtype. The analysis resulted in a cross-validated r^2 value of 0.604 with an optimum number of components at 8. The non-cross-validated PLS analysis determined by using eight components yielded an r^2 of 0.988. The standard error of estimate was 0.112. The CoMFA-based QSAR for the affinities at the α1-containing receptors revealed a good cross-validated correlation. This indicated that the model would be highly predictive. This indication was realized when the model was employed to predict the affinities of the ligands of the test set listed in Table 7. These ligands encompassed structurally diverse imidazobenzodiazepines with various substituents at positions 3 and 8, and the affinities of these ligands at the α1-containing receptors were across a range of 2.79 log units. As illustrated in Table 7, the predicted values were very close to the observed values from in vitro binding experiments on recombinant receptors. Examination of the model also indicated that steric effects contributed more to the affinity at the α1-containing receptor isoform than the electronic effects. Displayed in Figure 2 is a CoMFA contour map for the ligands at the α1 subtype. The green area indicated a region of steric interaction which would enhance binding affinity. The yellow region is an area of steric interaction which would result in reduced binding affinity. On the other hand, the blue contour implies positively charged interactions in this region would increase the affinity of a ligand. Moreover, observation of the red region suggests that a negatively charged group from the ligand which interacts with this region of the receptor would enhance the affinity of a ligand. In particular, blue and green contours at the southwest region of the pharmacophore indicated a positive steric interaction in this area, which corresponds to the L_{Di} region of the unified pharmacophore receptor model, would lead to enhanced affinity at the α1-containing isoform. This result was in agreement with previous observations which indicated the size of the L_{Di} region in the α1-containing isoform was larger than the same region in the other subtypes. Analysis of the yellow contour in the northeast region of the pharmacophore/receptor model suggests that a negative steric interaction was observed in this region; consequently, ligands with a large trimethylsilyl substituent (**12**, **13**, **27**, and **28**) at position 8 would not bind to the α1 subtype with high affinity. At the upper right-hand corner of Figure 2 is a plot of predicted values versus actual values. The linearity of the plot demonstrates a very good correlation as well as high predictability for the CoMFA model developed here for affinities of imidazobenzodiazepines at the α1 receptor subtype.

CoMFA for the α2β3γ2 Receptor Subtype. The affinities of a total of 36 ligands (**1**, **3**, **4**, **6**−**12**, **15**, **16**, **23**−**31**, **36**, **38**−**40**, **42**, **44**, **46**−**49**, **51**, **52**, and **54**−**56**) were included in the CoMFA analysis for the α2-containing receptor isoform. The analysis yielded a good cross-validated r^2 value (0.607). When the number of optimal components was 2, the non-cross-validated PLS analysis resulted in a non-cross-validated r^2 value of 0.826 as well as a standard error of estimate of 0.415 and an F-value of 78.38. The non-cross-validated r^2 was relatively low due to the small number of components in comparison to the CoMFA analysis for the other

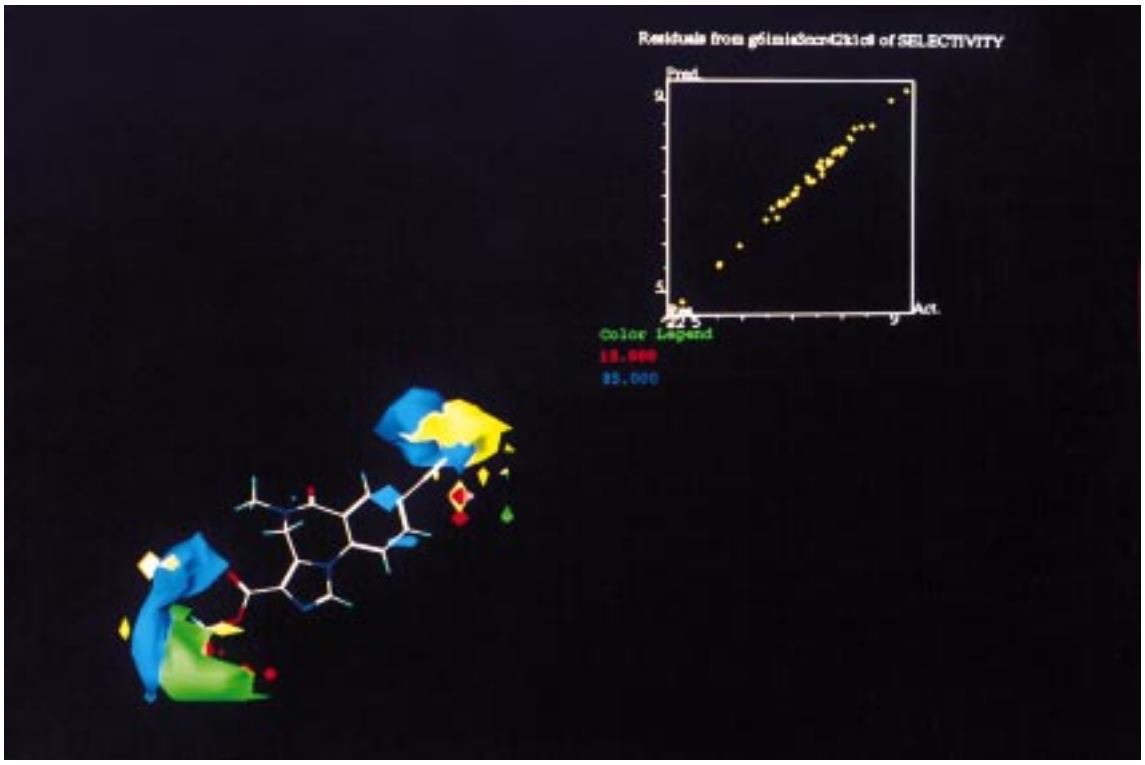


Figure 4. Non-cross-validated CoMFA steric and electrostatic STDEV*COEFF contour plots from the analysis of affinities of imidazobenzodiazepines at the α3β3γ2 receptor subtype with ligand **10** (RY-80). Green contours represent areas of positive steric interaction at a contribution level of 85%, while yellow contours represent areas of negative steric interaction at a contribution level of 15%. Blue contours represent areas of positive charge interaction at a level of 85%, while red contours represent areas of negative charge interaction which would enhance the affinity of a ligand at a contribution level of 15%. The plot shown on the upper right-hand portion is a representation of the predicted value of affinities of the ligands versus their actual affinity values.

receptor isoforms. It is known that the use of a larger number of components than the optimal number will result in a less predictive model although a higher non-cross-validated r^2 value will be observed. The predictive power of this model was proven to be high by examination of the affinities of the ligands of the test set which are listed in Table 7. Predicted affinity values were within a quarter of a log unit of the actual values. Illustrated in Figure 3 are the CoMFA contour maps of the affinities at the α2β3γ2 subtype. Definitions of Figure 3 are comparable to those of Figure 2 for the α1β3γ2 receptor subtype.

CoMFA for the α3β3γ2 Receptor Subtype. The affinities of a total of 42 ligands (**1**, **3**, **4**, **6**−**12**, **15-31**, **36**, **38**−**40**, **42**, **44**, **46**−**49**, **51**, **52**, and **54**−**56**) were included in the CoMFA analysis for the α3β3γ2 receptor subtype. A cross-validated PLS analysis resulted in a satisfactory r^2 value of 0.691. Seven components, an optimal number, were applied for the non-cross-validated PLS analysis, which yielded a non-cross-validated r^2 of 0.991. The standard error of estimate was 0.101. At the top right region of Figure 4, analysis of the plot revealed a high linearity on examination of actual values versus predicted values, which indicated high predictability for the model. The power of prediction of the model was confirmed by the close margin between the predicted affinity values of the test ligands versus the actual affinities observed from the binding experiments (Table 7). The resulting CoMFA contour map displayed in Figure 4 demonstrated the most favored and disfavored regions of steric and electrostatic interactions similar to that described in the previous paragraphs.

CoMFA for the α5β3γ2 Receptor Subtype. A total of 47 ligands (**1**, **3**, **4**, **6**−**12**, **15**−**31**, **33**−**36**, **38**−**40**, **42**, **44**, **46**−**49**, and **51**−**57**) were included in the CoMFA analysis for the α5-containing receptor subtype. In addition to the ligands used in the test set, ligand **50**, a compound with the *R* configuration, was also excluded from the PLS analysis, because it would be the only *R*-configured ligand in the set. A cross-validated PLS analysis of the 47 ligands resulted in an r^2 value of 0.648 which indicated a high statistical correlation. By applying a component number of 5, the non-cross-validated PLS analysis furnished an r^2 value of 0.957, as well as an *F*-value of 184.05 and a standard error of estimate of 0.236. All of these numbers indicate the high potential of this QSAR model for prediction. Once again, the power of prediction was proven true by comparison of the affinities of ligands in the test set calculated via this model to the actual affinities of these ligands (see Table 7). Across a range of affinities of 3.53 log units, the root-mean-square of error of estimate for the test ligands was less than 20% of a log unit. Illustrated in Figure 5 is a CoMFA contour map in which regions of steric and electrostatic interaction are highlighted which would most favor or disfavor the binding affinities of a ligand at the α5-containing isoform. The color representations here are the same as those illustrated in the previous description for the α1β3γ2 receptor subtype. It is noteworthy to mention that the blue contour located at the northeast region of the pharmacophore (at the tip of the 8-acetyleno sub-

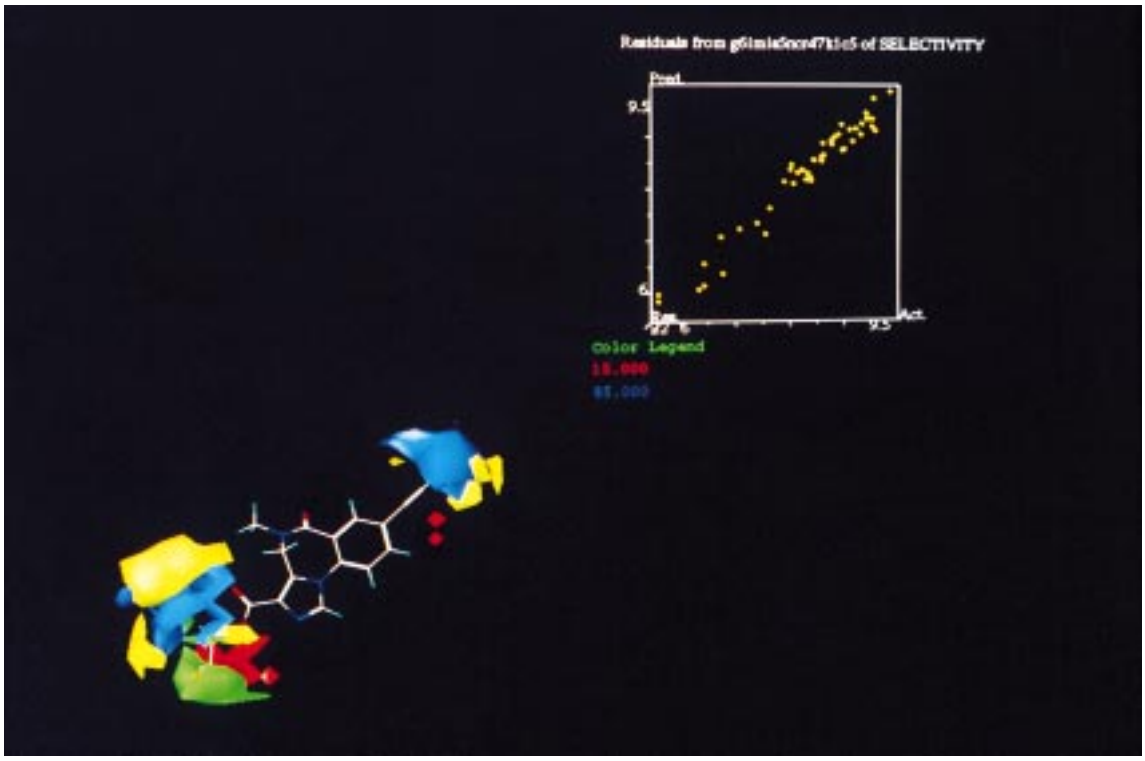


Figure 5. Non-cross-validated CoMFA steric and electrostatic STDEV*COEFF contour plots from the analysis of affinities of imidazobenzodiazepines at the α5β3γ2 receptor subtype with ligand **10** (RY-80). Green contours represent areas of positive steric interaction at a contribution level of 85%, while yellow contours represent areas of negative steric interaction at a contribution level of 15%. Blue contours represent areas of positive charge interaction at a level of 85%, while red contours represent areas of negative charge interaction which would enhance the affinity of a ligand at a contribution level of 15%. The plot shown on the upper right-hand portion is a representation of the predicted value of affinities of the ligands versus their actual affinity values.

stituent) represents a positive lipophilic interaction between a ligand and a receptor in this region. This indicates that this model, which is highly predictive, is also in agreement with our previous observations that region L_2 is larger in the α5 receptor subtype than in the α1-containing isoform.[18,28,29,31]

CoMFA for the α6β3γ2 Receptor Subtype. The affinities of a total of 40 ligands (**1**, **3**, **6**−**10**, **12**, **15**−**31**, **36**, **38**−**40**, **42**, **44**, **46**−**48**, **49**, **51**, **52**, and **54**−**56**) were included in the CoMFA analysis for the α6-containing receptor subtype, a DI subtype. A cross-validated PLS analysis resulted in a satisfactory r^2 value of 0.604, which implied a potentially highly predictive QSAR model. A non-cross-validated PLS analysis with the number of components as 5 furnished an r^2 value of 0.948, as well as a standard error of estimate of 0.232 and an F-value of 123.08. The power of this model was illustrated by the predictions observed on applying this QSAR model to the test ligands. With the exception of the affinity of ligand **45** (which is an *R*-configured pyrrazoloimidazobenzodiazepine), affinities of all the other test ligands were successfully predicted in close agreement with the observed affinity values from binding experiments on recombinant receptors. Illustrated in the CoMFA contour map (Figure 6) are regions of steric and electrostatic fields which would affect ligand binding, as previously described. The plot on the upper right-hand corner of Figure 6 demonstrates a good linear correlation between the actual affinity values and the predicted affinity values of these ligands.

CoMFA for the Ratio of Affinities of a Ligand at the α1-Containing Receptor Subtype Versus that at the α5-Containing Receptor Subtype. A total of 42 ligands (**1**, **3**, **4**, **6**−**12**, **15**−**31**, **36**, **38**−**40**, **42**, **44**, **46**−**49**, **51, 52**, and **54**−**56**) were included in the CoMFA analysis for the ratio of affinities at the α5- vs α1-containing receptor subtype. A cross-validated PLS analysis resulted in a poor r^2 value of 0.289, which implied a weak correlation. Efforts were made to attempt to obtain a satisfatory cross-validated r^2 value by ruling out some of the ligands with high cross-validated residuals. Elimination of outliers did not lead to a significant improvement in the correlation. This may stem from the fact that the ratio itself is a deduced value, not an independent value from binding experiments. It does not reflect the potency of affinity of a ligand at either receptor subtype due to the cancellation of this factor via the division of the affinities of the ligands at one subtype by the other in the determination of the ratio. On the other hand, it is also possible that the good cross-validated correlations obtained for the separate receptor isoforms may in part be due to the large spread in affinities (greater than 3 orders of magnitude) and low estimated errors for the binding affinities (less than 10%). In contrast, the poor correlation for selectivity may be due to the smaller spread in the value of the ratios (only 2 orders of magnitude) and greater estimated errors in the ratio duo to propagation of errors (≈15%). However, this r^2 value of 0.289 indicated a 50% chance of a good correlation, which indicated a possible correlation between the ratio and the structure of the ligand. Thus, a non-cross-validated PLS analysis was carried out despite the poor preliminary results. The use of the resulting model appears

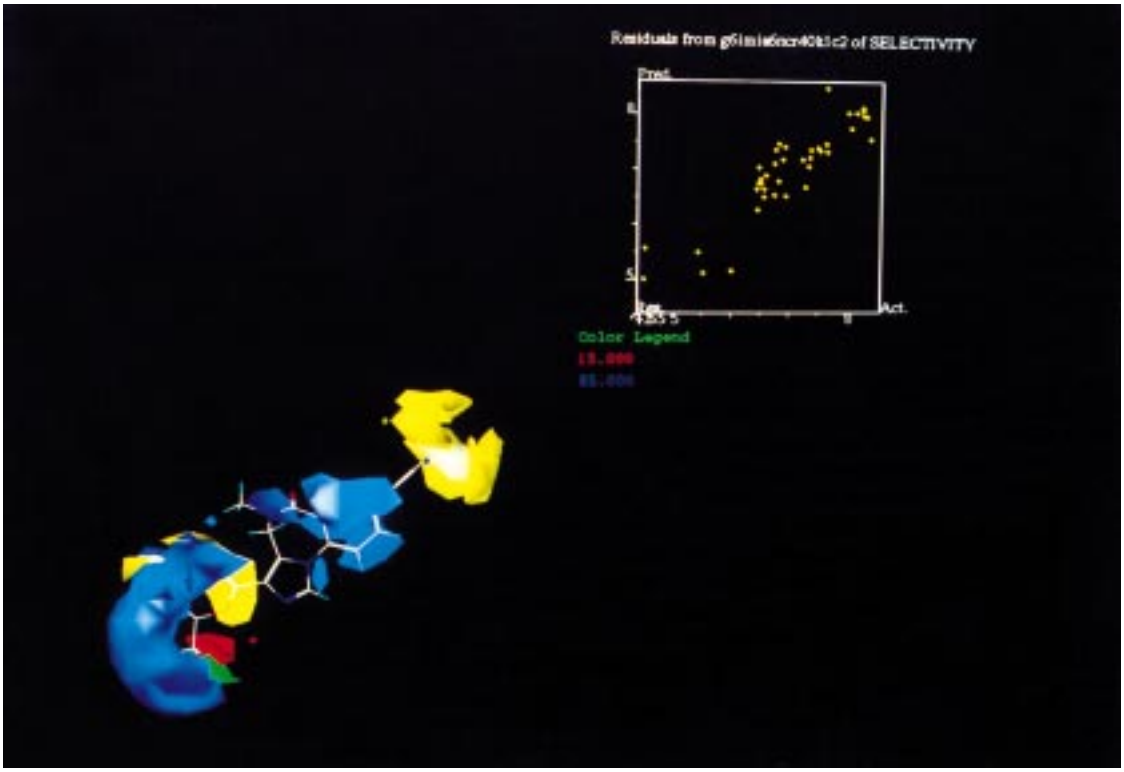


Figure 6. Non-cross-validated CoMFA steric and electrostatic STDEV*COEFF contour plots from the analysis of affinities of imidazobenzodiazepines at the $\alpha 6\beta 3\gamma 2$ receptor subtype with ligand **10** (RY-80). Green contours represent areas of positive steric interaction at a contribution level of 85%, while yellow contours represent areas of negative steric interaction at a contribution level of 15%. Blue contours represent areas of positive charge interaction at a level of 85%, while red contours represent areas of negative charge interaction which would enhance the affinity of a ligand at a contribution level of 15%. The plot shown on the upper right-hand portion is a representation of the predicted value of affinities of the ligands versus their actual affinity values.

to permit prediction of the ratios of affinities at α1- vs α5-containing receptor subtypes. The predicted values of ratios of affinities of the ligands at α1 vs α5 receptor subtypes were very close to the actual values with the exception of ligand **45**, which is the *R*-configured pyrrazoloimidazobenzodiazepine. The inability to employ this lone *R* isomer in the study was expected. Depicted in the CoMFA contour map (Figure 7) is a structural characteristic which might lead to high selectivity at the α5 receptor subtype. In brief, an increase in lipophilic interactions in the L_2 region (the upper right-hand region of the pharmacophore in Figure 7) should enhance the α5 selectivity of a ligand. However, lipophilic interactions at the L_{Di} region (the lower left-hand region of the pharmacophore in Figure 7) could effect a decrease in α5 selectivity, by enhancing the selectivity at the α1-containing receptor subtype. Both observations are in agreement with our previous results.[18,28−31,37,38]

Conclusion

Quantitative structure−activity relationship studies via CoMFA for the binding affinities of a series of imidazobenzodiazepines at five recombinant receptor subtypes, as well as the ratios for affinities at the α1-containing receptors versus the α5-containing receptors, were carried out successfully. In general, a good cross-validated correlation existed for each receptor subtype. Analysis of calibrated non-cross-validated PLS models permitted demonstration of high predictability for the affinities of the ligands in the test set at all five receptor subtypes as well as for the ratios of the affinities of these ligands at α1- vs α5-containing receptor subtypes. Examination of the CoMFA for p(K_i α5/K_i α1) also demonstrated a structural preference for α5 selectivity or α1 selectivity. The use of these models not only permitted the prediction of the binding affinities of in-house ligands but also permitted the prediction of the affinity of the MSD ligand L-655,708 (**58**).[33] Models were used to predict the affinty of this ligand at all five receptor subtypes as well as the ratio of affinities between α1 and α5 receptor subtypes. These results will permit the design of more selective ligands for α5-containing receptors, as well as ligands which are selective at other receptor subtypes. Radiolabeled potent selective ligands, such as tritiated RY-80 (**10**),[30] can be employed to investigate the populations of $GABA_A$ receptors bearing certain subunits. As such, [^{3}H]RY-80 (**10**) was used to evaluate the potency and efficacy of compounds at wild-type receptors containing α5 subunits.[30] It could also be used as a probe to examine these receptors after physiological and pharmacological manipulation.[30] Efforts to employ these CoMFA models for the development of more potent and more α5-selective ligands at BzR are underway.

Experimental Section

Computational Chemistry and Molecular Modeling. General: Molecular mechanics calculations with MMFF force fields[39−43] and Monte Carlo conformational searches were computed by using MacroModel 6.0.[44] Molecular graphics, RMS Fit, and CoMFA were carried out by means of SYBYL 6.4.[45] Both programs were carried out on a Silicon Graphics Octane SI2P175R10000 workstation. Molecular orbital calculations at the 3-21G basis set for geometry optimization and 6-31G* single-point calculations were carried out via Gaussian

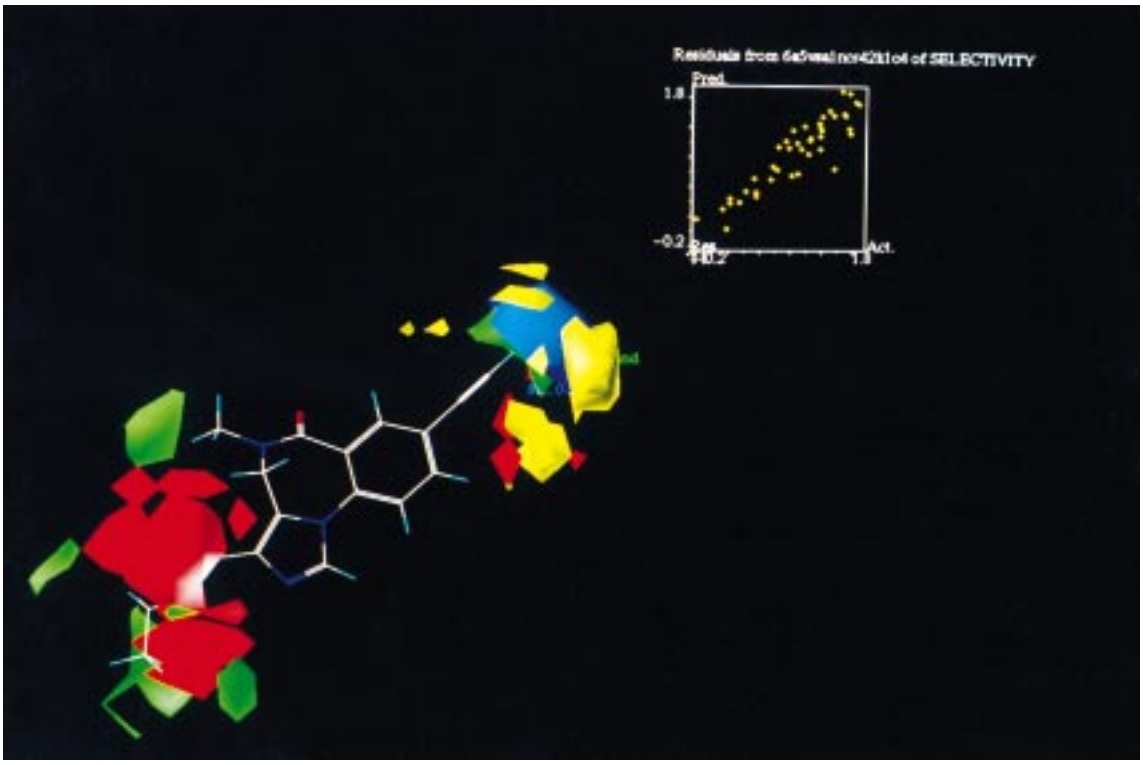


Figure 7. Non-cross-validated CoMFA steric and electrostatic STDEV*COEFF contour plots from the analysis of ratios of affinities of imidazobenzodiazepines at the $\alpha1\beta3\gamma2$ isoform versus the $\alpha5\beta3\gamma2$ receptor subtype with ligand **10** (RY-80). Green contours represent areas of positive steric interaction at a contribution level of 85%, while yellow contours represent areas of negative steric interaction at a contribution level of 15%. Blue contours represent areas of positive charge interaction at a level of 85%, while red contours represent areas of negative charge interaction which would enhance the affinity of a ligand at a contribution level of 15%. The plot shown on the upper right-hand portion is a representation of the predicted value of ratios of affinities of the ligands versus their actual values.

92[46] on a Silicon Graphics Indigo^2 R4000 workstation or Gaussian 94[47] on a Silicon Graphics Octane SI2P175R10000 workstation.

Molecular Structural Optimization: The starting geometries of the 6-oxoimidazo[1,5-*a*][1,4]benzodiazepines were constructed from the X-ray crystal structure of **1** (Ro 15-1788)[48,49] and modified where necessary in SYBYL. Structures which resulted were then minimized using MMFF94S force fields in MacroModel version 6.0 with a planar delocalized sp^2 nitrogen setting.[39−43] Low-energy conformations were obtained by a Monte Carlo conformational search in MacroModel. The conformations which resulted were subjected to ab initio 3-21G geometry optimization with both bond lengths and angles optimized while holding the torsional angles fixed. The ab initio 3-21G optimized geometries were then subjected to a single-point calculation using the 6-31G* basis set with an "SCF=TIGHT" convergence criteria to obtain reliable electrostatic potentials. To be sure of the consistency of the results from Gaussian 92 and Gaussian 94, molecule **49** was optimized via both Gaussian 92 and Gaussian 94 at the 3-21G basis set and then a single-point calculation was carried out at the 6-31G* level. The calculated geometries were identical to each other, and so were their electrostatic potentials and energies. In cases such as ligands **3**, **15**, **16**, **40**, **41**, and **46**, where the molecules contain heavy atoms (bromine or iodine), the necessary 6-31G* basis sets were not included in the commercially available Gaussian 92 and Gaussian 94 programs. These basis sets were taken from splitting the MP4 basis set reported by Andzelm et al. and the addition of *d* functions.[50−54]

3-D QSAR: The CoMFA study was performed within the QSAR module of SYBYL. Unless specified otherwise, default settings were used throughout. The centroid of ring A of **1** (Ro 15-1788) was placed at the origin. This molecule was then rotated about the A-ring centroid so that the A-ring is coplaner with the $x-y$ plane. The rest of the molecules were least-squares-fitted to the A-, B-, and C-rings of **1** by using the RMS Fit option in SYBYL. Steric and electrostatic potentials were generated using an sp^3 carbon probe with a +1 charge. The step size was 1.0 Å. Dimensions were −16 to 14 Å along the *x*-axis, −10 to 14 Å for the *y*-axis, and −10 to 8 Å on the *z*-axis. These dimensions ensured the grid extended beyond the largest molecular dimension by 4.0 Å in all directions. The "Minimum_Sigma" value was set to 1.00. The "QSAR Empty_Value", "CoMFA Switching", and "PLS Scaling" options were set to "Column_Mean", "No", and "CoMFA_Std", respectively. For cross-validated PLS calculations, "Column Filtering" and "Number of Components" was set to "1.0 kcal/mol" and "15", respectively.

Radioligand Binding.[17,55] In brief, the affinity of compounds at $GABA_A$/BzR subtypes was measured by competition for [^{3}H]Ro 15-1788 (83 Ci/mmol; NEN) binding to Ltk$^-$ cells expressing human receptors of composition $\alpha1\beta3\gamma2$, $\alpha2\beta3\gamma2$, $\alpha3\beta3\gamma2$, $\alpha5\beta3\gamma2$, and $\alpha6\beta3\gamma2$. Cells were removed from culture by scraping into phosphate-buffered saline, centrifuged at 3000*g*, and resuspended in 10 mL of phosphate buffer (10 mM KH_2PO_4, 100 mM KCl, pH 7.4 at 4 °C) for each tray (25 cm^2) of cells. Radioligand binding assays were carried out in a volume of 500 μL which contained 100 μL of cells, [^{3}H]Ro 15-1788 at a concentration of 1−2 nM, and the test compound in the range 10^{-9}−10^{-5} M. Nonspecific binding was defined by 10^{-5} M diazepam and typically represented less than 5% of the total binding. For cells expressing $\alpha6\beta3\gamma2$, [^{3}H]Ro 15-4513 (**8**) was used as the radioligand. Assays were incubated to equilibrium for 1 h at 4 °C and harvested onto GF/B filters (Brandel) by filtration using a Tomtec cell harvester and washing with ice-cold assay buffer. After drying, filter-retained radioactivity was detected by liquid scintillation counting. K_i values were calculated using the least-squares iterative fitting routine of RS/l analysis software (BBN Research System, Cambridge, MA) and are the means of two determinations which differed by less than 10%.

The synthesis of ligands found in Tables 1−5 has been previously reported.[56−58]

Acknowledgment. The authors wish to thank NIMH for generous financial support and Dr. Ashutosh Misra for helpful discussions on bromine- and iodine-related ab initio calculations as well as sources of 6-31G* basis sets for bromine and iodine. We also thank Dr. Konrad Koehler for many helpful discussions on computational chemistry and molecular modeling.

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