
Molecular Determinants of Recognition and Activation at GABA_A/Benzodiazepine Receptors

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ABSTRACT: Benzodiazepines (BDZ) are among the most widely prescribed drugs known to date. However, their broad spectrum of behavioral activity and lack of specificity circumscribe their effectiveness as drugs. With the goal of designing more selective benzodiazepine receptor ligands, Dr. Loew's research group has contributed to literature over the years with both experimental and computational studies. We report here a summary of the most recent computational work carried out in our laboratory on 21 structurally diverse benzodiazepine receptor ligands, using as a data set their in-house experimental results of activity at the anxiolytic, sedative, and hyperphagic endpoints. The chemical and geometric determinants common to the overlapping binding regions of benzodiazepine agonists, antagonists, and inverse agonists were identified at each of these three behavioral endpoints. The three resulting 3D pharmacophores did not encompass all the molecular requirements for recognition and activation of GABA_A/BDZ receptors. They provided instead a means of obtaining accurate bioactive conformations for use in statistical studies, such as quantitative structure-activity relationship and multivariate discriminant analyses. These studies can serve as a guide to predict both ligand affinities and activities. In the present work, multivariate discriminant analysis of global physicochemical properties is performed to provide new hypotheses for activation selectivity at the sedation endpoint. Although this analysis relies on a relatively small set of compounds, its results can still aim at compound screening and future identification of novel selective benzodiazepine receptor ligands. © 2002 Wiley Periodicals, Inc. *Int J Quantum Chem* 88: 56–64, 2002

Key words: benzodiazepines; GABA receptors; 3D pharmacophore; multivariate discriminant analysis

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Introduction

GABA_A/benzodiazepine (BDZ) receptors are composed of a combination of transmembrane protein subunits, most of which exist in multiple polypeptide isoforms. Currently there are 21 subunit isoforms (α_{1-6} , β_{1-4} , γ_{1-4} , δ , ρ_{1-3} , ϵ , π , θ) [1, 2] known in the literature as belonging to the GABA_A/BDZ receptor family. These subunits assemble to form heteropentameric ion channel structures with chloride ion (Cl⁻) selectivity.

The function of GABA_A/Cl⁻ channel complexes is initiated by the binding of γ -aminobutyric acid (GABA), the principal inhibitory neurotransmitter of the central nervous system. A large number of ligands from diverse chemical families can enhance, diminish, or block the effect of GABA on the Cl⁻ ion channel. These structurally diverse compounds are classified as GABA_A/BDZ receptor agonists, inverse agonists, or antagonists, respectively.

Binding of these agonists, antagonists, and inverse agonists to GABA_A receptors results in multiple behavioral activities. Previous studies in our laboratory assessed the effect of 21 structurally diverse BDZ receptor ligands at different behavioral endpoints, including anxiolysis, sedation, and hyperphagia. The results clearly indicated behavioral heterogeneity [3–6] for many of the compounds evaluated. In other words, the same compound could have qualitatively different effects, i.e., agonist, antagonist, inverse agonist, or no effect, at different behavioral endpoints. Moreover, the pattern of heterogeneity was not the same for all compounds. For example, the subset of compounds that were agonists at the anxiolytic endpoint or had no effect at that endpoint was different from those that were agonists or had no effect at the other behavioral endpoints. This observation suggested that not all types of GABA_A/BDZ receptors are necessarily linked to each behavioral response.

Because of their important physiological effects, many BDZ receptor ligands are widely used as therapeutics. However, their broad spectrum of activity and lack of specificity hinder the effectiveness of these compounds as drugs. With the ultimate goal of designing more behaviorally selective BDZ receptor ligands, several different pharmacophore models for the benzodiazepine receptors were developed in our own laboratory [7–11] as well as from other research groups [12–26] during the past 15 years. Most of these models use a large number of structurally diverse BDZ receptor ligands to explain

ligand efficacy as a function of ligand–receptor interaction at the molecular level.

A new generation of three-dimensional (3D) pharmacophores for ligand recognition of GABA_A/BDZ receptors initiating anxiolytic [27], sedative [6], and hyperphagic [28] responses were recently developed in our laboratory. Subsets of 21 diverse BDZ receptor ligands previously assessed in our laboratory at the anxiolytic, sedative, and hyperphagic endpoints were used as training sets for the development of these new 3D pharmacophores and an in-house software, MOLMOD [27], was developed *ad hoc* to generate them. Based on the direct evidence from competitive binding studies [29–31] that, with the exception of low affinity sites, the binding domains of BDZ receptor ligands with different activation properties are overlapping, these new 3D pharmacophores included the recognition elements common to the overlapping binding region of BDZ agonists, antagonists, and inverse agonists at each of the three different behavioral endpoints. Comparison of these three new 3D pharmacophores indicates that the chemical and geometric determinants common to the overlapping binding regions of the selected BDZ receptor ligands with different activation properties are similar at the three different behavioral endpoints. Thus, other factors outside the overlapping binding regions must be responsible of the binding specificity and receptor activation selectivity of the compounds included in the present work. As an example, in our previous work on sedation [6], different spatial properties of donors and acceptor groups were identified as elements of separation between binders and nonbinders.

The accurate bioactive conformations derived by definition of the three new 3D pharmacophores developed in our laboratory can be used in quantitative structure–activity relationship (QSAR) and multivariate discriminant analyses with the ultimate goal of identifying the determinants of specificity and selectivity at BDZ receptors. A 3D-QSAR analysis was recently carried out based upon the 21 ligands in the training set superimposed at their sedation pharmacophoric points [6]. This study demonstrated that the new 3D pharmacophore developed in our laboratory for the sedation endpoint is predictive of the selective binding to $\alpha 1$ containing GABA_A/BDZ receptor subtypes, according to the findings of a recent experimental work [32]. On the other hand, multivariate discriminant analysis of steric, topological, electrostatic, and thermodynamic properties calculated for all active (agonists) and inactive (antagonists) BDZ receptor ligands

at each behavioral endpoint may help to identify the molecular requirements for activation of receptors initiating different behavioral responses. In the present work, the extent to which such an approach may be used for pharmacophore refinement is shown for ligands of BDZ receptors initiating the sedative response. This is because of the larger number of antagonists determined at the sedation endpoint, as well as the evidence, from both experimental [29] and computational studies [6], of the association of a particular receptor subtype (α_1) with this behavioral endpoint.

Although our analysis relies on a relatively small set of compounds, the results reported in the present work provide new hypotheses into the only requirements that are necessary for BDZ agonistic activity at the sedation endpoint. In the future, this information may lead to the identification of novel behaviorally selective BDZ receptor ligands, which can be used as more effective therapeutics.

Methods

A NEW 3D PHARMACOPHORE-GENERATING SOFTWARE

The new software, MOLMOD, was developed in order to provide an accurate representation of 3D pharmacophores based upon large training sets with diverse templates. Details of this method have been previously described [27]. The MOLMOD program is a stand-alone program built on an extensible C++ molecule class library using dynamic memory allocation to allow for working with large numbers of compounds with extensive conformational variability. This new computer program refines on principles in the Distcomp software previously developed in our laboratory [33]. MOLMOD and the prior Distcomp programs systematically identify potential ligand recognition or activation pharmacophores by employing principles of conformational clustering and distance comparisons between user defined common pharmacophore points.

MOLMOD does not require a template, or any assumptions of a bioactive configuration. It only requires a database of known compounds with the desired recognition or activation properties (a training set) and as controls some compounds in which these properties are absent. Specifically, two types of input are required for the MOLMOD program: (i) conformational libraries for each of the ligands used that identify low energy conformers within

a user selected energy range of the lowest energy conformer found and (ii) user selection of candidate moieties in each compound that could be considered as possible components of the 3D pharmacophore such as hydrophobic groups and proton accepting and donating groups. The conformational library for each compound in the training set is generated for compounds with 5 or less rotatable bonds by a nested grid method and for compounds with >5 rotatable bonds by a hybrid genetic algorithm (GA) minimization protocol embedded in the program CCEMD (Sandia, CA) [34–36]. Several studies in the literature [35, 37–40] pointed out the efficiency of explorations by a GA based algorithm and its utility in the identification of low energy conformers of flexible molecules involving many torsional degrees of freedom. Conformational libraries for all molecules in the training set obtained by either method are generated with an assumed dielectric constant of 80 and a long potential function truncated at 90 Å in order to minimize truncation effects. The value of $\epsilon = 80$ is chosen based on the assumption that the accessible BDZ receptor ligand conformational pool is developed in a polar environment prior to binding to the GABA_A/BDZ receptors or that GABA_A/BDZ binding sites are themselves partially exposed to a polar environment. The second type of input required by MOLMOD is the selection for each compound of appropriate chemical moieties for consideration as pharmacophoric elements. To aid in this selection quantum chemical calculations of selected chemical properties such as relative proton donating and accepting abilities of candidate ligand moieties, group hydrophobicities [41] and electron distributions in the highest occupied (HOMO) and lowest empty (LUMO) molecular orbitals can be calculated. Distinct types of quantum chemical methods can be used for these calculations. These are the AM1 semiempirical method in MOPAC [42], the DFT program present in the Oxford Molecular DGauss/Unichem program, and standard ab initio Hartree–Fock methods in Gaussian 98 [43]. No significant differences in results between these different methods were noted in test cases run in the past in our laboratory. Using these two types of input the MOLMOD program then systematically searches for the common spatial arrangement of the chemical moieties selected for each binder or active compound and that are absent in the compounds with no effect. These chemical moieties in the specific spatial arrangement are candidate ligand moieties interacting with residues of the specific receptor under consideration.

It is possible that more than one pharmacophore will be determined by MOLMOD. The preferred 3D pharmacophore selected as the most reliable will be the one that (i) contains the maximum number of pharmacophore components and (ii) has the smallest total variance, calculated for each one found by MOLMOD in the distance matrix comprising the 3D pharmacophore.

GLOBAL MOLECULAR PROPERTIES AND DISCRIMINANT ANALYSIS

Diverse global molecular properties were computed for each of the selected BDZ receptor ligands with effect as agonist or antagonist at the sedation endpoint [6]. In particular, the bioactive conformation of each compound was employed as a starting point for semiempirical calculations using the AM1 Hamiltonian embedded in the MOPAC 7.0 software [42]. Energy minimization of these initial structures was carried out until gradient changes were less than 0.1 Å. The resulting conformations were found within the domain of the original conformations generated by conformational searches.

All properties were expressed in terms of computable molecular descriptors accounting for the steric, topological, electrostatic, and thermodynamic information contained in each selected BDZ agonist and antagonist at the sedation endpoint. Specifically, the molecular descriptors computed for describing the steric aspect of each molecule were total polar and nonpolar area, total polar and nonpolar volume, as well as total solvent-accessible area. The "sterimol" parameters [44] L, B1, B2, B3, and B4 were used to describe topological aspects of the selected BDZ ligands. Briefly, these parameters define the largest dimensions that can be computed for a molecule. Both steric and topological parameters were extracted using MOPAC results as inputs for the in-house software GRAPHIA, which is a FORTRAN77 program written for generating stereochemical and physicochemical indices for organic molecules. In particular, polar and nonpolar areas as well as polar and nonpolar volumes were computed using atomic contributions to the total area and volume. The contribution of nitrogen and oxygen atoms was considered as polar; all other atoms were considered nonpolar.

The semiempirical calculations carried out for each BDZ receptor ligand using the AM1 Hamiltonian embedded in the MOPAC7 package [42] also provided numerical values of electrostatic descriptors such as the LUMO and the HOMO, as well

as values of thermodynamic properties such as rotational and total entropy, total enthalpy, and heat capacity. Additional hydrophobic index values were calculated using the in-house software GRAPHIA and an AM1 three-parameter method developed in our laboratory [41]. Finally, values of free energy of solvation were calculated for all BDZ agonists and antagonists at the sedation endpoint, using the AMSOL 6.5.3/AM1 package [45] and water as solvent at the geometries determined in vacuo. Analysis of these molecular descriptors was performed using the S-PLUS 2000 package (StatSci Division, MathSoft Inc., Seattle, WA).

Results

A NEW GENERATION OF PHARMACOPHORES FOR LIGAND RECOGNITION OF BDZ RECEPTORS INITIATING ANXIOLYTIC, SEDATIVE, AND HYPERPHAGIC RESPONSE

Three new 3D pharmacophores for ligand recognition of the BDZ receptors initiating activity at the anxiolytic, sedative, and hyperphagic endpoints were recently developed [6, 27, 28] using subsets of a training set of 21 structurally diverse BDZ receptor ligands previously assessed by experiments in our laboratory [4–6, 46]. The chemical structures of these 21 BDZ receptor ligands are shown in Figure 1. Also indicated in this figure is the name of each compound as well as its anxiolytic (underlined), sedation (in bold), and hyperphagic (in italic) response.

The three new pharmacophores developed in our laboratory [6, 27, 28] with the aid of MOLMOD show a similar chemical composition. Specifically, they all include two proton acceptor atoms (pharmacophoric components 1 and 2), a variable hydrophobic group (pharmacophoric component 3), and an aromatic electron-accepting ring (pharmacophoric component 4). The pharmacophores developed for ligand recognition of BDZ receptors initiating anxiolytic [27] and sedative [6] responses possess also a fifth pharmacophoric component corresponding to a ring that contains polar moieties. However, this moiety was only included into the original pharmacophores for the purpose of providing information of ring topology to facilitate database searching.

The in-house software MOLMOD allowed for identification of a common geometric arrangement of the pharmacophoric components found in all BDZ receptor ligands with an agonistic, antagonis-

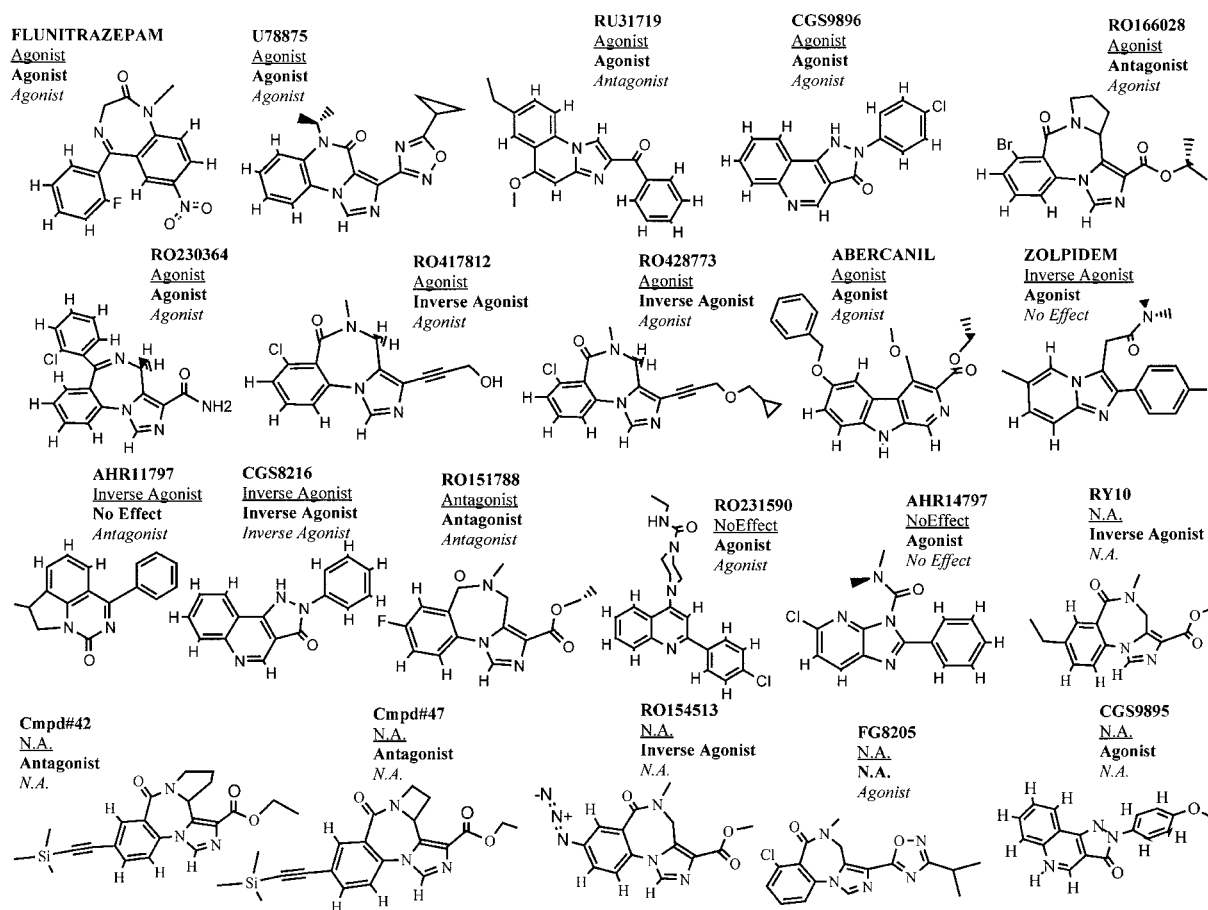


FIGURE 1. Chemical structures of the BDZ receptor ligands included in the present work. The name of each compound as well as its anxiolytic (underlined), sedation (in bold), and hyperphagic (in italic) response are also indicated.

tic, and inverse agonistic effect at each behavioral endpoint and absent in the inactive compounds. Details of the computational methodology applied to develop the three pharmacophores for ligand recognition of BDZ receptors initiating the anxiolytic, sedative, and hyperphagic response are reported elsewhere [6, 27, 28]. Table I shows a comparison of the pharmacophoric distances identified at each behavioral endpoint. The results reported in this table indicate the presence of both chemical and geometric commonalities into the three pharmacophores developed for recognition of agonists, antagonists, and inverse agonists at each behavioral endpoint. While the analysis of the distances between the overlapping binding region of the selected BDZ agonists, inverse agonist, and antagonists alone does not reveal the requirements for their behavioral selectivity, the computational approach employed provides accurate bioactive conformations for all the compounds included in the present work. This

TABLE I
Comparison of the pharmacophoric distances identified at each behavioral endpoint.

| Distances between pharmacophoric components ^a | Anxiolysis | Sedation | Hyperphagia |
|----------------------------------------------------------|------------|-----------|-------------|
| 1-2 | 3.8 ± 1.2 | 4.9 ± 1.1 | 4.1 ± 1.0 |
| 1-3 | 5.6 ± 1.2 | 5.6 ± 1.8 | 6.2 ± 1.3 |
| 1-4 | 4.2 ± 1.0 | 4.4 ± 1.1 | 5.4 ± 2.1 |
| 1-5 | 1.8 ± 1.1 | 2.3 ± 0.9 | — |
| 2-3 | 4.0 ± 1.1 | 4.5 ± 1.2 | 4.3 ± 0.9 |
| 2-4 | 6.4 ± 1.9 | 4.7 ± 1.2 | 5.1 ± 1.5 |
| 2-5 | 3.3 ± 1.5 | 3.1 ± 1.3 | — |
| 3-4 | 5.2 ± 1.2 | 5.1 ± 1.1 | 5.8 ± 1.1 |
| 3-5 | 3.4 ± 1.7 | 3.0 ± 0.8 | — |
| 4-5 | 7.5 ± 2.1 | 7.8 ± 1.3 | — |

^a 1 and 2 are proton acceptor atoms, 3 corresponds to a variable hydrophobic group, 4 indicates an aromatic electron accepting ring, and 5 stands for a ring containing polar moieties.

structural information may be used in statistical studies, such as QSAR and multivariate discriminant analyses, to determine the association of the developed pharmacophores with particular receptor subtype binding [6] and, as shown in the present study, discovery of determinants of activation.

GLOBAL MOLECULAR PROPERTIES AS ACTIVITY DISCRIMINANTS

Since the three new 3D pharmacophores recently developed in our laboratory [6, 27, 28] only contain the minimal requirements for the recognition of the overlapping binding sites of agonists, antagonists, and inverse agonists acting at GABA_A/BDZ receptors initiating different behavioral responses, they cannot explain entirely behavioral selectivity. In order to discriminate among BDZ receptor ligands with different activation properties, other factors that cannot be identified by analysis of pharmacophoric points alone must play a significant role. These factors can be interpreted in terms of molecular descriptors accounting for all major aspects of activity at BDZ receptors, including steric, topological, electrostatic, and thermodynamic properties.

Recent studies carried out in our laboratory [47, 48] demonstrated the efficacy of multivariate discriminant analysis of global molecular properties for the identification of the differences between agonists and antagonists, in cases in which a pure distance based pharmacophore representation cannot explain activation selectivity. In the present application to BDZ receptor ligands, the most robust statistical analysis can be obtained using the selected agonists and antagonists of BDZ receptors initiating sedation [6]. This is because (i) a statistically significant number of antagonists have been assessed in our laboratory only at the sedation endpoint as compared to the anxiolytic and hyperphagia endpoints, and (ii) both experimental [32] and computational [6] prior studies indicate a specific association of GABA_A receptor subtypes containing α 1 receptor subunits with the sedation endpoint. Therefore, we expect activation analysis at this endpoint to be the simplest in terms of underlying phenomenology and the most statistically robust.

Steric, topological, electrostatic, and thermodynamic descriptors were computed for the 10 BDZ receptor agonists and 4 BDZ receptor antagonists recently assessed in our laboratory at the sedation endpoint [6] in order to discriminate between compounds inducing sedation and inactive ones. Table II reports the numerical values for all the steric

TABLE II
Numerical values of the steric and topological molecular descriptors calculated for the BDZ receptor ligands acting as agonists or antagonists at the sedation endpoint.

| BDZR ligand | Molecular weight (g/mol) | Total volume (Å ³) | Polar volume (Å ³) | Nonpolar volume (Å ³) | Solvent acc area (Å ²) | Total area (Å ²) | Polar area (Å ²) | Nonpolar area (Å ²) | Globularity (Å) | L (Å) | B1 (Å) | B2 (Å) | B3 (Å) | B4 (Å) |
|---------------|--------------------------|--------------------------------|--------------------------------|-----------------------------------|------------------------------------|------------------------------|------------------------------|---------------------------------|-----------------|-------|--------|--------|--------|--------|
| Flunitrazepam | 313.29 | 226.92 | 34.32 | 192.6 | 500.38 | 296.6 | 61.81 | 234.79 | 0.61 | 12.28 | 7.23 | 4.82 | 2.65 | 2.76 |
| U78875 | 335.37 | 308.47 | 48.04 | 260.43 | 559.23 | 346.52 | 61.94 | 284.58 | 0.64 | 15.32 | 6.85 | 4.78 | 3.03 | 3.3 |
| Ru31719 | 330.39 | 263.73 | 21.48 | 242.25 | 595.39 | 352.13 | 34.03 | 318.1 | 0.56 | 15.88 | 8.93 | 3.93 | 3.08 | 2.25 |
| CGS9896 | 295.73 | 230.84 | 24.74 | 206.1 | 494.57 | 281.38 | 31.83 | 249.55 | 0.65 | 15.9 | 6.16 | 1.77 | 1.77 | 2.89 |
| Ro230364 | 336.78 | 286.59 | 33.12 | 253.47 | 533.92 | 323.88 | 44.7 | 279.18 | 0.65 | 14.08 | 8.55 | 3.95 | 3.71 | 1.61 |
| Abercarnil | 404.47 | 339.33 | 30.43 | 308.9 | 675.67 | 435.2 | 49.59 | 385.61 | 0.54 | 17.4 | 8.41 | 4.38 | 2.9 | 3 |
| Ro231590 | 394.9 | 371.31 | 29.16 | 342.15 | 676.18 | 409.04 | 33.52 | 375.52 | 0.61 | 18.95 | 10.11 | 4.27 | 2.5 | 2.79 |
| AHR14797 | 314.77 | 252.59 | 24.02 | 228.57 | 541.56 | 324.64 | 38.9 | 285.74 | 0.6 | 15.28 | 6.35 | 3.1 | 3.07 | 3.5 |
| CGS9895 | 291.31 | 253.55 | 29.18 | 224.37 | 516.48 | 295.5 | 39.62 | 255.88 | 0.66 | 16.56 | 6.45 | 2.56 | 2.37 | 2.37 |
| Zolpidem | 307.39 | 285.02 | 18.73 | 266.29 | 566.72 | 349.11 | 27.44 | 321.67 | 0.6 | 15.12 | 7.6 | 3.55 | 2.59 | 3.48 |
| Ro151788 | 303.29 | 247.97 | 34.18 | 213.79 | 507.03 | 300.51 | 48.37 | 252.14 | 0.63 | 15.46 | 5.05 | 3.28 | 3.07 | 3 |
| Cmpd #42 | 407.54 | 357.93 | 27.65 | 330.28 | 710.49 | 440.48 | 50.06 | 390.42 | 0.55 | 19.39 | 5.87 | 4.88 | 3.3 | 3.84 |
| Cmpd #47 | 393.52 | 362.75 | 34.16 | 328.59 | 696.05 | 421.68 | 51.43 | 370.25 | 0.58 | 20.37 | 5.37 | 4.78 | 3.29 | 3.9 |
| Ro166028 | 418.29 | 317.32 | 29.02 | 288.3 | 572.08 | 374.19 | 48.11 | 326.08 | 0.6 | 15.1 | 5.41 | 3.92 | 3.19 | 4.17 |

TABLE III

Numerical values of the electrostatic and thermodynamic molecular descriptors calculated for the BDZ receptor ligands acting as agonists or antagonists at the sedation endpoint.

| BDZR ligand | Hydrophobic index | ΔG solvation (kcal/mol) | HOMO | LUMO | Total enthalpy (cal/mol) | Rotational entropy (cal/K/mol) | Heat capacity (cal/K/mol) | Total entropy (cal/K/mol) |
|---------------|-------------------|---------------------------------|-------|-------|--------------------------|--------------------------------|---------------------------|---------------------------|
| Flunitrazepam | 0.52 | -13.65 | -9.81 | -1.42 | 14,424.71 | 34.93 | 77.38 | 150.12 |
| U78875 | 0.44 | -19.57 | -8.84 | -0.8 | 16,102.21 | 35.5 | 87.43 | 163.09 |
| Ru31719 | 3.58 | -13.94 | -8.59 | -0.69 | 16,361.44 | 35.99 | 88.84 | 164.27 |
| CGS9896 | 2.71 | -16.4 | -8.14 | -0.84 | 12,457.19 | 34.99 | 68.67 | 136.77 |
| Ro230364 | 1.88 | -19.85 | -9.32 | -0.71 | 14,570.92 | 35.27 | 80.6 | 150.38 |
| Abercanil | 2.79 | -14.21 | -8.54 | -0.66 | 21,147.66 | 37.26 | 112.22 | 199.49 |
| Ro231590 | 3.46 | -18.08 | -8.74 | -0.7 | 18,871 | 37.53 | 102.95 | 184.56 |
| AHR14797 | 2.2 | -12.8 | -8.98 | -0.8 | 15,492.57 | 35.11 | 80.55 | 159.7 |
| CGS9895 | 1.81 | -18.75 | -7.92 | -0.71 | 13,533.32 | 34.97 | 74.46 | 142.87 |
| Zolpidem | 2.84 | -12.69 | -8.34 | -0.18 | 16,958.89 | 35.26 | 89.29 | 168.64 |
| Ro151788 | 0.88 | -13.47 | -9.69 | -1.11 | 14,867.15 | 34.81 | 78.08 | 153.37 |
| Cmpd #42 | 6.49 | -15.88 | -9.5 | -1.19 | 22,792.4 | 37 | 117.89 | 216.8 |
| Cmpd #47 | 6.2 | -14.97 | -9.53 | -1.24 | 21,862.06 | 36.85 | 113.49 | 207.96 |
| Ro166028 | 1.7 | -17.04 | -9.64 | -0.89 | 18,084.92 | 36.21 | 97.08 | 174.69 |

and topological descriptors calculated for each BDZ receptor ligand acting as agonist or antagonist at the sedation endpoint. Similarly, Table III reports the numerical values for all their electrostatic and thermodynamic descriptors.

Multivariate discriminant analysis of the global molecular properties calculated for all BDZ receptor agonists and antagonists at the sedation endpoint shows that the "sterimol" parameter B1 is the best discriminant between compounds inducing or blocking sedation. Specifically, B1 defines the greatest perpendicular distance from any point in a molecule to the L axis, which corresponds to the vector between the pair of nuclei whose geometric separation is greatest.

As shown in Table I, values of B1 higher than 6 Å are required for agonism at the BDZ receptors initiating the sedative response. Figure 2 shows plots of the B1 descriptor calculated for each selected BDZ receptor agonist and antagonist versus other noncorrelated properties. A clear discrimination between the 10 agonists and 4 antagonists selected at the sedation endpoint is shown in these plots. This analysis provides an initial activation hypothesis which could be simply tested experimentally.

In summary, the requirements for ligand recognition of GABA_A/BDZ receptors initiating the anx-

olytic, sedative, and hyperphagic responses are similar and consist of the presence of two proton acceptor atoms, a variable hydrophobic group, and an aromatic electron accepting ring in a geometric arrangement common to all agonists, antagonists, and inverse agonists but not included in compounds with no effect. In order to provide new hypotheses for activation and behavioral selectivity of BDZ ligands, additional steric, topological, electrostatic, and thermodynamic properties that cannot be identified by analysis of pharmacophoric points alone must be considered. Results of a statistically significant multivariate discriminant analysis of global molecular properties were only obtained for agonists and antagonists at the sedation endpoint. These results suggested that values of the "sterimol" parameter B1 higher than 6 Å provide some discrimination between agonists and antagonists at BDZ receptors initiating sedation. Although this analysis relies on a relatively small set of compounds, its results can be used as a guide for compound screening, ultimately aiming at the identification of novel behaviorally selective BDZ receptor ligands.

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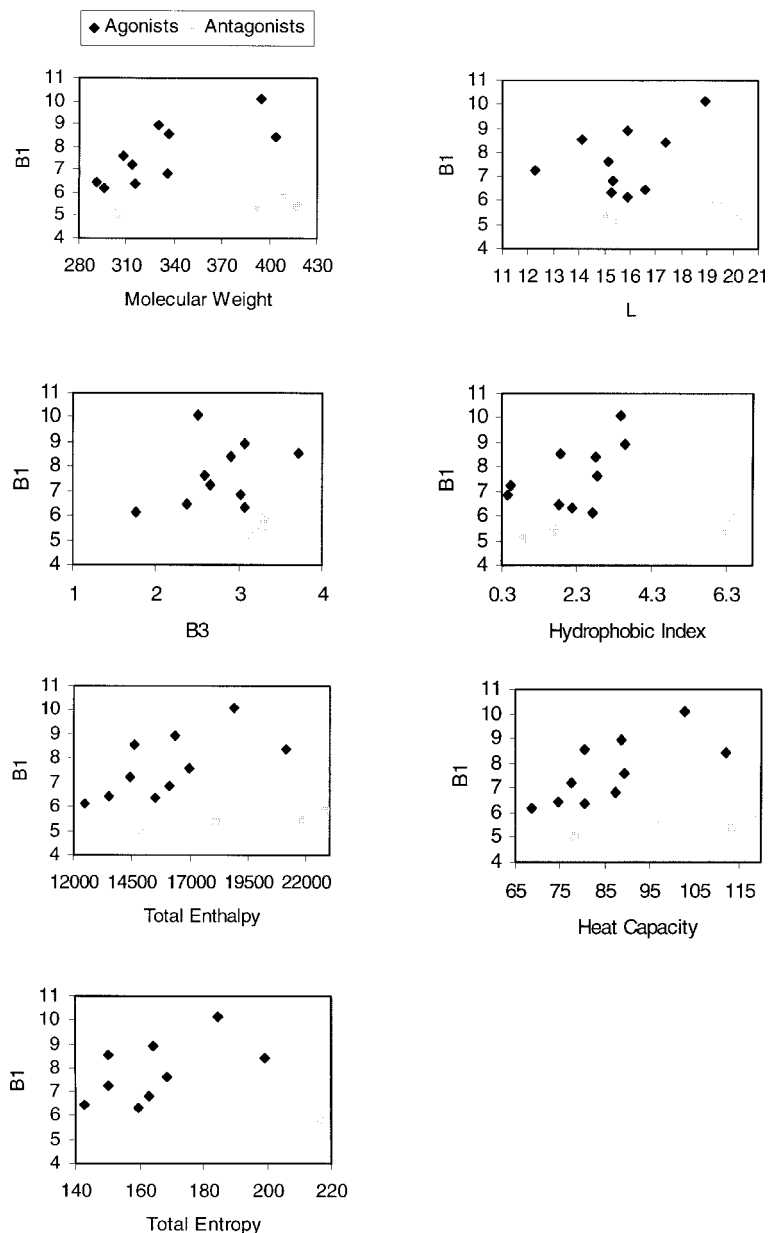


FIGURE 2. Plots of the B1 descriptor calculated for each selected BDZ receptor agonist and antagonist at the sedation endpoint versus other noncorrelated properties.

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