Direct Prediction of Dissociation Constants (pK_a's) of Clonidine-like Imidazolines, 2-Substituted Imidazoles, and 1-Methyl-2-substituted-imidazoles from 3D Structures Using a Comparative Molecular Field Analysis (CoMFA) Approach

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The applicability of a comparative molecular field analysis (CoMFA) method to reproduce and predict the pK_a values of 28 clonidine-like imidazoline analogues and 16 2-substituted imidazoles has been investigated with the GRID force field. Molecular fields calculated with an H^+ probe and AM1 partial atomic charges produced a correlation with a small standard deviation and a high correlation coefficient with cross validation. It was concluded that the CoMFA treatment of electrostatic effects is suitable for predicting pK_a values and thus for the examination of the electronic effects in 3D quantitative structure-activity relationships.

Introduction

The importance of electrostatic effects in drug-receptor interactions has long been recognized.[1,2] The well-known titrimetric methods[3] of determining pK_a values are cumbersome and often time-consuming and cannot be applied until a compound is synthesized. Thus, it is useful to be able to predict the pK_a values of the compounds of interest. Many approaches have been investigated to estimate the pK_a values of various compounds.[4-16] We report our experiences on the prediction of pK_a values, directly from 3D structures, using a comparative molecular field analysis (CoMFA) approach.[17] Previously, we showed that CoMFA predicts the pK_a's of substituted benzoic acids well, where AM1 charges and an H^+ probe were used.[18] A CoMFA analysis consists of the following steps: (1) establishing the conformation of each molecule, (2) superimposing the molecules, (3) calculating interaction energies with suitable probes at many points on a lattice, and (4) performing a statistical analysis of the relationship between the interaction energies and the property of interest. In order to establish proper conformation, it is often required to sample a large conformational space and examine if low-energy conformers can be superimposed in a consistent manner. The complexity of the conformation search depends on the flexibility of the molecules involved.

The CoMFA method not only is a convenient way to predict the pK_a values of compounds but also provides essential descriptors in 3D-QSAR for describing the electrostatic effects between a ligand and a receptor interaction. The advantage of the CoMFA over the traditional linear free energy relationship analysis is that substituent constants are not needed.

In this study we applied a CoMFA method to analyze the pK_a values of clonidine-like imidazoline analogues and 2-substituted imidazoles. Clonidine (I) is a very active antihypertensive drug.[19,20] It is considered that its overall action on arterial blood pressure is due to the vasoconstrictive peripheral and hypotensive central effects. A large number of studies have been devoted toward understanding its mode of interactions, including structure-activity relationship studies.[21-26] It was shown that the

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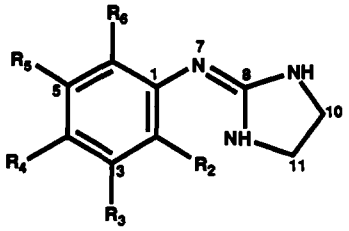
dissociation constant of clonidine analogues plays an important role for the activity.[21-24]



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Methods

Molecular Modeling. The starting coordinates were generated by adding appropriate substituents to the crystal structure of clonidine[27] or imidazole.[28] All geometric variables were optimized, and the partial atomic charges were calculated with AM1 of MOPAC.[29] Only the neutral molecules were studied. The tautomer shown was considered for clonidine analogues because it would not be correct to calculate the partial charges on the unsolvated cation. The molecules were aligned by superimposing the phenyl and imidazole rings (using C1, C3, C5, N7, C8, C10, and C11) for clonidine analogues and imidazole ring for imidazoles.

CoMFA Interaction Energy Calculation. The steric and electrostatic potential energy fields of each molecule were calculated at various lattice points surrounding the molecule using methyl and H^+ probe groups with the program GRID.[30] A zero van der Waals radius and a charge of 1.0 were used for the H^+ probe, and a radius of 1.95 Å and a charge of 0.0 were used for the methyl probe. The energies at a total of 1331 grid points were calculated for each molecule with 2-Å spacing in a lattice of 20 × 20 × 20 Å.

All steric energies with a value greater than 4.0 kcal/mol were truncated to 4.0. Any lattice point for which the standard deviation of the energies is less than 0.05 was discarded. In order to select only the electrostatic energies calculated outside the union volume of the molecules in the dataset, the points for which the steric energy for any molecule is 4.0 kcal/mol or greater were also discarded. These procedures reduced the number of lattice points to 1044 for the clonidines and 1125 for imidazolines.

Table I. Observed and Calculated pK_a Values of Clonidine Analogues

entry no.	substituents	energy[a]	pK_a obsd[b]	pK_a calcd[c]	dev
1	2,3-Cl_2	52.55	8.55 (±0.02)	8.46	0.09
2	2,4,6-Br_3	81.07	7.46 (±0.03)	7.59	−0.13
3	2,4,6-Cl_3	44.90	7.75 (±0.02)	7.82	−0.07
4	2,4,6-Me_3	43.54	10.78 (±0.03)	10.74	0.04
5	2,4-Cl_2	51.16	8.73 (±0.01)	9.03	−0.30
6	2,4-Me_2	50.22	10.56 (±0.01)	10.13	0.43
7	2,5-Cl_2	51.07	8.50 (±0.03)	8.43	0.07
8	2,6-Br_2	75.64	7.80 (±0.04)	8.06	−0.26
9	2,6-Cl_2-4-Br	56.68	7.72 (±0.44)[e]	7.52	0.20
10	2,6-Cl_2-4-Me	43.54	8.29 (±0.03)	8.18	0.11
11	2,6-Cl_2-4-NO_2	55.92	6.86 (±0.44)[e]	6.74	0.12
12	2,6-Cl_2-4-OMe	14.36	8.57 (±0.04)	8.69	−0.12
13	2,6-Cl_2	51.18	8.05 (±0.04)	7.94	0.11
14	2,6-Et_2	40.87	10.61 (±0.04)	10.74	−0.13
15	2,6-F_2	−25.83	8.18 (±0.04)	8.35	−0.17
16	2,6-Me_2-4-Br	55.87	10.21 (±0.01)	10.11	0.10
17	2,6-Me_2-4-Cl	43.73	10.25 (±0.02)	10.39	−0.14
18	2,6-Me_2	51.46	10.53 (±0.01)	10.58	−0.05
19	2-Cl-4-Me	50.45	9.41 (±0.01)	9.21	0.20
20	2-Cl	57.99	9.15 (±0.04)	9.24	−0.09
21	2-Me-4-Cl	50.39	9.99 (±0.03)	10.05	−0.06
22	2-Me	57.83	10.23 (±0.01)	10.16	0.07
23	H	64.59	10.05 (±0.02)	10.07	−0.02

[a] AM1 energy in kilocalories/mole. [b] Timmermans, P. B. M. W. M.; Zwieten, P. A. *Drug Res.* **1978**, *28*, 1676. [c] Calculated from eq 1. [d] Standard deviation of eq 4 in ref 25.

Partial Least Squares (PLS) Calculations. Ten orthogonal latent variables were first extracted by the standard PLS algorithm.[31] These latent variables were subjected to the PLS cross-validation test in the order of their correlation with the dependent variable. The "best" correlation model was chosen as that which minimized the sum of squares of the difference in pK_a between the observed and predicted values made from a leave-one-out jackknife validation method.

Results and Discussion

Correlation of pK_a of Clonidine Analogues. Twenty-three compounds, listed in Table I, were used to derive the "best-fit" correlation described in eq 1. The measured pK_a values were taken from the report of Timmermans and Zwieten.[25]

$$pK_a = 0.33\ (\pm 0.02)Z_1 + 0.26\ (\pm 0.01)Z_2 + 0.25\ (\pm 0.03)Z_3 + 0.39\ (\pm 0.04)Z_4 + 0.14\ (\pm 0.06)Z_5 + 0.25\ (\pm 0.04) \quad (1)$$

$$n = 23,\ s = 0.19,\ r = 0.990,\ \text{press s} = 0.27$$

Since the electrostatic interaction energy was calculated at over 1000 points, the initial CoMFA interaction energy data matrix was analyzed with the statistical technique of partial least squares (PLS). Only the lattice points outside the union surface of the compounds were used for the PLS analysis, unlike Cramer et al.'s[17] method of using the mean value of the other molecules' electrostatic interactions at the same location for such grid points. These locations are sensible for quantitative correlation of biological activity of molecules, since some of these lattice points are assumed to be the locations of atoms of the receptor macromolecule.

In the correlation, Z_1–Z_5 are the latent variables obtained from the PLS analysis; n is the number of compounds used in the correlation; s is the residual standard deviation; r is the correlation coefficient; and press s is the standard deviation from leave-one-out jackknife cross validation.

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Table II. Prediction of pK_a for Other Cloninide Analogues Using Eq 1

entry no.	substituents	energy[a]	pK_a obsd[b]	pK_a calcd	dev	av	dev
24a	2,4-Cl_2-6-Me	44.58	9.03 (±0.03)	9.62	−0.59		
24b	2-Me-4,6-Cl_2	43.92	9.03 (±0.03)	8.81	0.22	9.22	−0.19
25a	2,4-Me_2-6-Cl	42.80	9.59 (±0.03)	9.12	0.47		
25b	2-Cl-4,6-Me_2	43.66	9.59 (±0.03)	9.78	−0.19	9.45	0.14
26a	2-Br-6-Cl	63.18	7.93 (±0.02)	8.03	−0.10		
26b	2-Cl-6-Br	63.51	7.93 (±0.02)	7.94	−0.01	7.98	−0.05
27a	2-Cl-6-F	12.21	8.01 (±0.04)	8.31	−0.30		
27b	2-F-6-Cl	13.08	8.01 (±0.04)	8.02	−0.01	8.17	−0.16
28a	2-Me-6-Cl	50.54	9.40 (±0.01)	9.15	0.25		
28b	2-Cl-6-Me	51.18	9.40 (±0.01)	9.63	−0.23	9.39	0.01

[a] AM1 energy in kilocalories/mole. [b] Timmermans, P. B. M. W. M.; Zwieten, P. A. *Drug Res.* 1978, *28*, 1676.

Each coefficient's standard error of estimation is given in parentheses.

Equation 1 shows that the pK_a values of the clonidine-like imidazoline analogues are correlated well with the CoMFA electrostatic descriptors calculated with an H^+ probe atom. The equation accounts for 98% of the variance in the data. Table I lists the observed and calculated (from eq 1) pK_a values of the 23 compounds.

Mitchell et al.[11] recently reported, from the same dataset, a correlation between the pK_a and the HOMO energies or charges on the bridge nitrogen atom (N7) calculated by an ab initio method using a STO-3G basis set (eqs 2 and 3).

$$pK_a = 67.1E_{HOMO} + 26.9 \quad (2)$$

$$n = 20,\ s = 0.27,\ r = 0.969$$

$$pK_a = -311.6\ Chge_{N7} - 61.5 \quad (3)$$

$$n = 20,\ s = 0.30,\ r = 0.962$$

Two poorly fitted derivatives, 2,6-difluoro and 2-chloro-6-fluoro, were omitted in eqs 2 and 3. These compounds were included in eq 1. Both the standard deviations and the correlation coefficients of eqs 1–3 indicate that eq 1 is superior to eq 2 or 3.

The quality of eq 1 is in between those of the two traditional Hammett σ correlations shown in eqs 4 and 5. It is better than the correlation obtained with $\mathcal{F}$ and $\mathcal{R}$ (eq 6) reported by Timmermans and Zwieten.[24] Separate σ

$$pK_a = -1.567\sum\sigma_{o,m,p} + 10.139 \quad (4)$$

$$n = 28,\ s = 0.103,\ r = 0.996$$

$$pK_a = -2.602\sum\sigma_{m,o=p} + 9.556 \quad (5)$$

$$n = 28,\ s = 0.442,\ r = 0.923$$

$$pK_a = -1.286\mathcal{F} - 1.333\mathcal{R} + 10.106 \quad (6)$$

$$n = 28,\ s = 0.236,\ r = 0.980$$

values were used for the ortho, meta, and para substituents in eq 4, while the values for the ortho substituents were taken as identical with the one for the para position in eq 5.

Prediction of pK_a of Other Clonidine Analogues. In order to test the predictability of eq 1, we examined the pK_a's of five additional compounds. In deriving eq 1, these five compounds were not included because there are two ways to orient the substituents of these analogues in a three-dimensional space; the planes of the phenyl ring and the imidazoline ring form an angle of 75°.[27,32] For example, the positions of the two substituents in the 2-Br-6-Cl analogue can be switched to give a 2-Cl-6-Br arrangement.

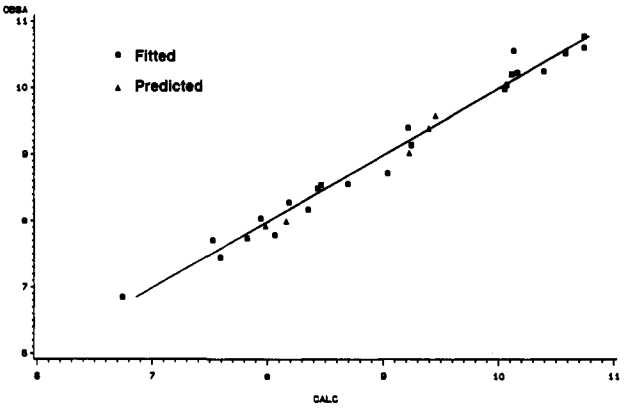

Figure 1. Plot of $pK_{a_{obsd}}$ versus $pK_{a_{calcd}}$ using eq 1.

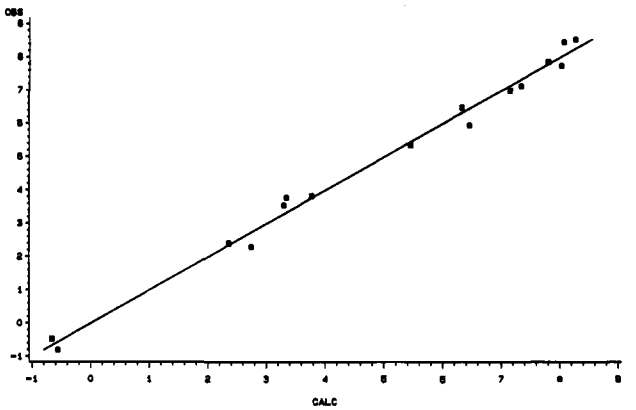

Figure 2. Plot of $pK_{a_{obsd}}$ versus $pK_{a_{calcd}}$ using eq 8.

For the purpose of predicting the pK_a of these molecules, the structures of both conformers were modeled. Their pK_a values were then predicted by using eq 1. Table II summarizes the results of the calculated pK_a values from the two individual conformations of these compounds and the average pK_a value of the two. The average pK_a values of all five compounds agreed well with the observed values, which had an average deviation of 0.11.

Equation 7 is the correlation between the measured pK_a ($pK_{a_{obsd}}$) and the calculated pK_a ($pK_{a_{calcd}}$) values of all 28 compounds included in this study. Figure 1 is the plot between $pK_{a_{obsd}}$ and $pK_{a_{calcd}}$ used in eq 7.

$$pK_{a_{obsd}} = 0.999\ (\pm 0.003)\ pK_{a_{calcd}} \quad (7)$$

$$n = 28,\ s = 0.159,\ r = 0.9995$$

Correlation of pK_a of Imidazoles. Sixteen compounds, listed in Table III, were used to develop the "best-fit" correlation described in eq 8. The measured pK_a

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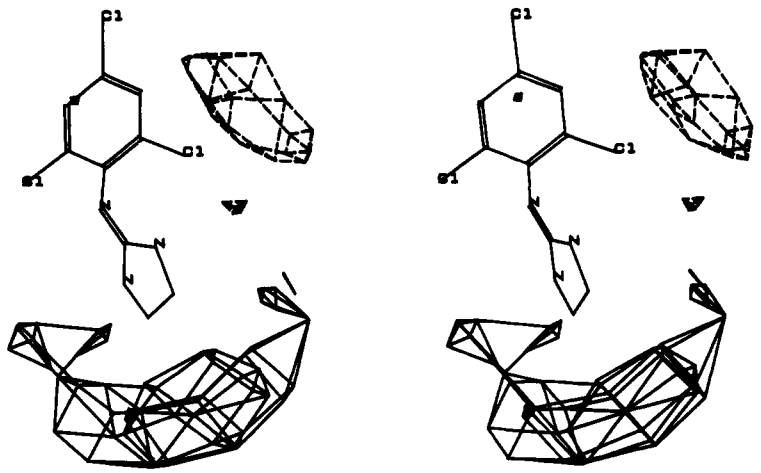

Figure 3. Stereoscopic view of the major electrostatic feature of eq 1. The negative contour regions (solid lines) decrease the pK_a values, while the positive contour regions (dashed lines) increase the pK_a values. (The contour is shown at the 0.1 coefficient level.)

Table III. Observed and Calculated pK_a Values of Imidazole Analogues

entry no.	substituents	energy[a]	pK_a obsd	pK_a calcd[d]	dev
1	1-Me-2-Br	59.46	3.82[b]	3.76	0.06
2	1-Me-2-F	17.30	2.30[b]	2.74	−0.44
3	1-Me	55.10	7.12[b]	7.33	−0.21
4	1-Me-2-NH_2	57.21	8.54[b]	8.25	0.29
5	1-Me-2-NO_2	65.43	−0.48[b]	−0.67	0.19
6	2-Br	54.88	3.79[b]	3.34	0.46
7	2-Cl	44.59	3.55[c]	3.29	0.26
8	2-Et	36.50	7.73[b]	8.02	−0.29
9	2-F	12.83	2.40[b]	2.35	0.05
10	H	50.84	6.99[b]	7.14	−0.15
11	2-Me	42.83	7.86[b]	7.79	0.07
12	2-NH_2	52.59	8.46[c]	8.06	0.40
13	2-NO_2	61.02	−0.81[b]	−0.56	−0.25
14	2-Ph	76.42	6.48[b]	6.33	0.16
15	2-Pyr	85.56	5.36[b]	5.45	−0.09
16	2-SMe	47.76	5.95[b]	6.46	−0.51

[a] AM1 energy in kilocalories/mole. [b] Catalan, J.; Abboud, J. L. M.; Elguero, J. *Adv. Heterocycl. Chem.* **1987**, *41*, 187. [c] Ganellin, C. R. In *Molecular and Quantum Pharmacology*; Bergmann, E. D., Pullman, B., Eds.; D. Reidel Pub. Co.: Boston, MA, 1975; p 43. [d] Calculated from eq 8.

values were taken from the compilation of Catalan et al.[33] and Ganellin.[34]

$$pK_a = 0.45\ (\pm 0.02)Z_1 + 0.38\ (\pm 0.06)Z_2 + 0.24\ (\pm 0.05)Z_3 + 1.42\ (\pm 0.13)Z_4 + 1.02\ (\pm 0.24)Z_5 + 4.94(\pm 0.09) \quad (8)$$

$$n = 16,\ s = 0.35,\ r = 0.995,\ \text{press s} = 0.69$$

Equation 8 shows that the pK_a values of the imidazole analogues are reasonably well correlated with the CoMFA electrostatic descriptors, calculated with an H^+ probe atom, considering that the sources of pK_a values were numerous. The standard deviation of eq 8 is not as low as one would like it to be, but the correlation coefficient is very high. The equation accounts for 99% of the variance in the data. Table III lists the observed and calculated (from eq 8) pK_a values of the 16 compounds.

Charton[35] reported significant correlations between the pK_a values and Hammett σ_m constants of two sets of imidazoles: 2-substituted imidazoles and 2-substituted 1-methylimidazoles. However, only six and three corresponding analogues were included in his study (eqs 9 and 10).

$$pK_a = -10.9\sigma_m + 7.00 \quad (9)$$

$$n = 6,\ s = 0.198,\ r = 0.9987$$

$$pK_a = -10.6\sigma_m + 7.12 \quad (10)$$

$$n = 3,\ s = 0.270,\ r = 0.9992$$

Ganellin[34] observed a similar but statistically less significant correlation (eq 11) with eight 2-substituted imidazoline analogues.[36]

$$pK_a = -10.43\ (\pm 0.58)\sigma_m + 7.21 \quad (11)$$

$$n = 8,\ s = 0.443,\ r = 0.991$$

Compared to eqs 9–11, the correlation described in eq 8 is an excellent one considering the following: twice as many compounds were included, the experimental data were compiled from different sources, and mixed MNDO (for SMe) and AM1 parameters were used in the charge calculation.[37] The results are far superior to those using the method recently described by Rashin et al.[38] Figure 2 is the plot between $pK_{a_{obsd}}$ and $pK_{a_{calcd}}$ from eq 8.

3D Coefficient Contour Map. The quantitative correlations described in eqs 1 and 8 are represented by the 3D coefficient contour maps shown in Figures 3 and 4. The negative regions are visualized with solid lines and the positive regions with dashed lines.

Figure 3 shows that an important electrostatic region in space influencing the pK_a values of clonidine analogues is around the imidazoline ring. Electron-releasing substituents on the phenyl ring make the negative contour region more electronegative. More electronegative charge in this region increases the energy needed to remove the acidic H^+ and thus increases the pK_a value of clonidine analogues.

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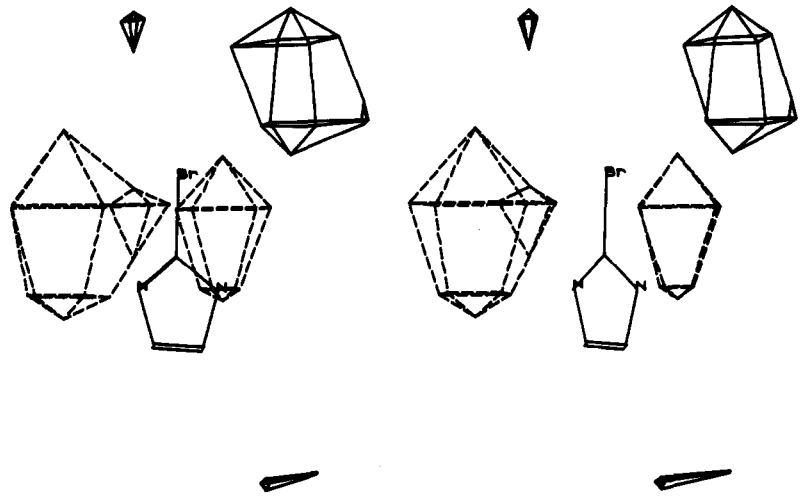

Figure 4. Stereoscopic view of the major electrostatic feature of eq 8. The negative contour regions (solid lines) decrease the pK_a values, while the positive contour regions (dashed lines) increase the pK_a values. (The contour is shown at the 0.35 coefficient level.)

Figure 4 shows important electrostatic regions in space that influence the pK_a values of imidazolines. Electron-withdrawing substituents at the 2-position make the positive contour region more electropositive and the negative contour region more electronegative. More electropositive charge in the positive contour region decreases the energy needed to remove the acidic H^+ and thus decreases the pK_a value of imidazoline analogues.

The contour regions show where strong electrostatic influences may be expected, if these compounds were placed in a biological receptor site.

We have oversimplified the description of the substituent effects on the pK_a values of the clonidine-like imidazolines and the substituted imidazoles by basing our calculation on the unsolvated, unprotonated species calculated in a vacuum, and only on one tautomer. This might explain why there are still some unaccounted-for deviations in the calculated pK_a values. Clearly, calculations considering other aspects would involve a lot more computer time and also present the ambiguity as to where to place the solvent molecules, how many solvent molecules to use, and the relative orientation of the solute and the solvent. For these reasons we chose to examine the correlations based only on the unsolvated neutral molecule. Changing any of these conditions and assumptions would likely affect the results. In ligand binding to a macromolecule, which is our primary interest, the macromolecular binding site is more fixed in space, since the side chains of a protein are not as free to move as are individual water molecules. Therefore, the substituent effect on pK_a is not a perfect model for the substituent effect on the electrostatic contribution to the binding affinity of a ligand for a macromolecule.

Conclusions

Molecular fields, calculated with an H^+ probe and AM1 partial atomic charges using a CoMFA method, reproduced and predicted the pK_a values of 28 clonidine-like imidazoline analogues and 16 imidazoles. The results of this study show that the CoMFA treatment of electrostatic effects is suitable to predict pK_a values and, along with our previous investigation[18] of this method, support its application in studies of 3D quantitative structure–activity relationships.

2-(Oxadiazolyl)- and 2-(Thiazolyl)imidazo[1,2-*a*]pyrimidines as Agonists and Inverse Agonists at Benzodiazepine Receptors

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Oxadiazoles, like the benzoyl group in a series of imidazo[1,2-*a*]pyrimidines, have been found to be metabolically stable alternatives to ester groups in benzodiazepine-receptor ligands. This change has lead to a number of compounds which bind to the receptors and which exhibit potent agonist activity in a food-motivated conflict test thought to predict anxiolytic properties. Compounds **4**, **5**, and **13** were equipotent with chlordiazepoxide but showed little or no myorelaxant effects. Replacing the oxadiazole group by thiazole gave compounds such as **23** which binds to the benzodiazepine receptor but exhibits the intrinsic activity of a partial inverse agonist in vivo.

A number of (imidazo[1,2-*a*]pyrimidin-2-yl)phenylmethanones and related compounds have been shown to specifically interact with benzodiazepine receptors and to possess partial agonist properties, resulting in differing degrees of separation of anxiolytic and sedative/myorelaxant effects in vivo.[1] Modification of the benzoyl group

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