Effect of branching in 4-alkylpyrazoles on liver alcohol dehydrogenase inhibition: A shape analysis study

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Shape analysis methodology is applied to the study of 4-alkylpyrazoles which are known inhibitors of liver alcohol dehydrogenase. Elongation of the alkyl chain increases the inhibitory power, whereas branching of the chain diminishes the activity. These two counterpoised effects are studied simultaneously in a selected set of 4-alkylpyrazoles. A systematic conformational analysis followed by topological characterization of the van der Waals surfaces of all the local minima restricts the conformational space to potential bioactive structures. The analysis of the interrelation between the molecular electrostatic potential and van der Waals surfaces provides certain shape codes characteristic of each 4-alkylpyrazole. In both topological analyses (van der Waals surfaces and molecular electrostatic potential–van der Waals surface interrelations) graphical representations and analytical methods were used. A good correlation between the shape codes and the inhibitory activity is found for the linear derivatives. For branched pyrazoles, a tendency in their inhibitory power is predicted. Isopentylpyrazole is suggested to have the same inhibitory profile as 4-butylpyrazole, the linear derivative with one less carbon atom.

Keywords: shape analysis, pyrazole, LADH inhibitor

INTRODUCTION

Continuing with our interest in 4-substituted pyrazoles as inhibitors of the enzyme liver alcohol dehydrogenase (LADH),[1–3] we deal with the branching in the alkyl chains of 4-alkylpyrazoles and how this affects the inhibition of the mentioned enzyme.

The enzyme LADH is known to be involved in the metabolism of alcohol. Its inhibitors have therapeutic application in the treatment of methanol and ethylene glycol poisoning, and of the ill effects of ethanol abuse.[4] It is well known that pyrazole and its 4-alkyl derivatives are potent inhibitors of this enzyme.[5] Substitution at any other position in the pyrazole ring cancels the activity, and the introduction of polarity in the 4-position diminishes the inhibitory power.[6]

Regarding the 4-alkylpyrazoles, it has been shown that by increasing the length of this alkyl chain the inhibitory power increases.[5] On the contrary, the branching or the cyclization of these alkyl chains lowers the inhibitory activity.[7] Taking into account and combining these two opposite effects, we have studied the effect that a methyl group has on the activity when it is introduced as a branch in the alkyl chain of some 4-alkylpyrazoles. Thus, the pyrazoles chosen for this study were the following (Figure 1): 4-methyl- (**1**), 4-ethyl- (**2**), 4-propyl- (**3**), 4-(1-methyl)ethyl- (**4**), 4-butyl- (**5**), 4-(1-methyl)propyl- (**6**), 4-(2-methyl)propyl- (**7**), 4-pen-

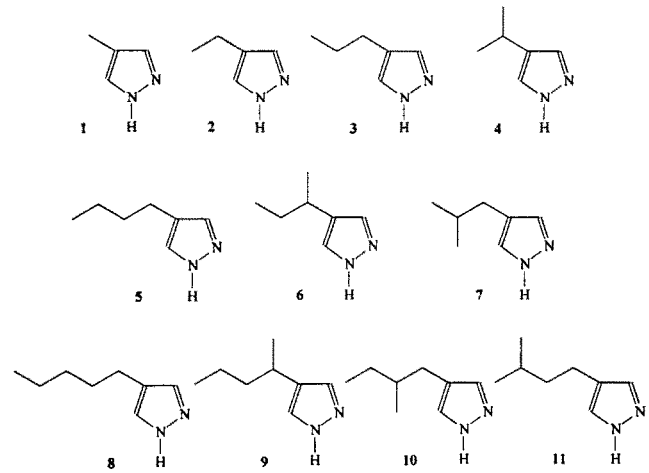

Figure 1. 4-Alkylpyrazoles studied in this work. One methyl group is introduced as a branch and moved along linear chains of different length.

Color Plates for this article are on pages 290–291.

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tyl- (**8**), 4-(1-methyl)butyl- (**9**), 4-(2-methyl)butyl- (**10**), and 4-(3-methyl)butylpyrazole (**11**). Although there is no possibility for branching in compounds **1** and **2**, they have been studied to consider the effect of elongation of the alkyl chain and for the sake of comparison. Compounds **6**, **9**, and **10** have a chiral C atom in the alkyl chain, and only the S enantiomer has been studied because the activity values found for some of them correspond to that enantiomer.

By studying this series of compounds we can determine whether the negative effect of introducing a side chain in the 4-substituent can be canceled by elongating the alkyl chain. As well, we can determine whether the position of this branch in relation to the pyrazole ring affects the inhibitory power when the main alkyl chain has the same number of C atoms.

The activity parameter used for this study has been the inhibition constant of the enzyme (K_I, in μM), which is known for the most of the compounds.[8]

By the nature of the derivatives investigated here, this study presents two main problems. On the one hand, all the molecules exhibit flexible chains, and therefore, many possible conformers. It is known that the lowest energy conformation of a compound is not necessarily the one responsible for its interaction with the receptor. For this reason, all the conformational space should be analyzed and all the local minima within a general energy range of 5.0–20.0 kcal. mol^{-1} from the lowest one[9] should be considered. This problem has been approached[3] by combining the search for all local minima within a systematic sampling of an *N*-dimensional space with the shape group method (SGM) analysis,[10] which allows one to identify the molecular shape similarities between different conformers. In this way, the number of possible structures of interest from the whole conformational space is restricted.

On the other hand, enzyme-inhibitor interactions involve both electrostatic and steric effects. By combining molecular electrostatic potential (MEP) maps and molecular surfaces, such as van der Waals surfaces (VDWSs), we can describe simultaneously the reactivity of a molecule together with its size and shape. The interrelation between both of these properties can be characterized by shape codes that can be correlated with the activity parameters.

Hence, the aim of this paper is to rationalize the combined effects of elongating the chain and introducing a branch on the alkyl chain of 4-alkylpyrazoles on the inhibition of LADH by using shape analysis methodology. Furthermore, for compounds with unknown experimental K_I values, an approximated inhibitory behaviour is predicted. This article is organized as follows: First, the conformational analysis, minima search, and SGM application to a series of 4-alkylpyrazoles are described. Next, the interrelations between the MEPs and the VDWSs for this family of compounds are characterized, and the results correlated with the corresponding inhibitory power. Finally, conclusions and some final remarks are presented.

CONFORMATIONAL AND SHAPE ANALYSIS OF 4-ALKYLPYRAZOLES

The inhibitory power of 4-alkylpyrazoles increases when increasing the length of the alkyl chain, i.e., when increasing the degree of conformational freedom. For this reason, a systematic conformational search has been carried out for compounds **1–11** in order to localize all the possible low-energy conformers that can be involved in the interaction with the enzyme.

The conformational analysis of these compounds was carried out using the facilities within the CHEMX molecular modeling program.[11] The structures were built up using the standard bond lengths and angles from this program, and were optimized with the molecular mechanic force field implemented in this package.

For all the compounds studied, the conformational space was scanned by rotating all the C—C bonds of the alkyl chains in steps of 30°, from 0° to 180° because the pyrazoles have a plane of symmetry, giving a total of 7 increments. In the case of 4-methylpyrazole (**1**), due to the small size of the molecule, the C—C bond was rotated from 0° to 360°. The energy obtained for each conformer was provided by punctual molecular mechanic calculations.

Table 1 shows the number of bonds that has been rotated for each compound and also the number of conformers that has been generated.

A conformational space can be easily analyzed when the

Table 1. Number of rotated bonds, generated conformations, minima localized, and conformers with different shapes found for the 4-alkylpyrazoles studied (1 to 11).

Pyrazole derivatives	Rotated bonds	Generated conformers	Local minima	Different shape
4-methyl- (**1**)	1	12	3	1
4-ethyl- (**2**)	2	49	2	1
4-propyl- (**3**)	3	343	10	5
4-(1-methylethyl)- (**4**)	3	343	8	2
4-butyl- (**5**)	4	2401	20	10
4-(1-methylpropyl)- (**6**)	4	2401	20	5
4-(2-methylpropyl)- (**7**)	4	2401	20	5
4-pentyl- (**8**)	5	16807	40	20
4-(1-methylbutyl)- (**9**)	5	16807	28	7
4-(2-methylbutyl)- (**10**)	5	16807	43	12
4-(3-methylbutyl)- (**11**)	5	16807	40	8

number of rotated bonds is one (by two-dimensional plots) or two (by three-dimensional (3D) maps). But when the degree of rotational freedom is higher than 2, the search for all the conformers of minimum energy in a N-dimensional space becomes a difficult task.

To solve this problem, after the systematic conformational search, a simple program[3] that identifies as minima those conformers with lower energy than their "first" and "second" neighbors in the N-dimensional conformational space, has been utilized. By using this program, all the local minima for compounds **1–11** were localized. The most of these local minima for all the pyrazoles were found within a range of 10 kcal mol^{-1} from the lowest one in each case (see Table 1), and were considered for the shape analysis study.

As previously explained,[12] nuclear configuration and molecular shape are two different concepts. A conformation represents the geometry of the nuclear framework, whereas the shape of the conformer can be understood as an envelope surface enclosing the nuclei. In general, a conformational rearrangement of the nuclei disposition may or may not produce an essential change in the shape of the molecule. Thus, in the case of the 4-alkylpyrazoles studied, two different conformations can have similar shape characteristics and, therefore, interact in a similar way with LADH.

For this reason, and in order to simplify the number of structures of interest that can interact with the enzyme, a shape characterization of the VDWSs of the minima previously obtained has been carried out. This analysis has been performed by using SGM, which has been extensively explained in the literature.[10,12] But for the sake of clarity, we will explain the meaning of the two topological characteristics used in the analysis.

In order to build the van der Waals fused-sphere model of a molecule with N nuclei in a spatial configuration K we need a set of atomic radii $(\rho_1, \rho_2, \ldots, \rho_N)$[13]

$$\rho = (\rho_1, \rho_2, \ldots, \rho_N) \qquad \rho_i > 0$$

Superposition of these atomic spheres affords an envelope surface $G(K,\rho)$. To characterize $G(K,\rho)$, we truncate from this surface all spherical n-type faces (n-edged spherical polygons) for some fixed n.[14] Hence, if there is a number ϕ_n of n-type faces, we will have a series of truncated surfaces $G_n(K,\rho)$:

$$G_n(K,\rho) = G(K,\rho) \setminus \bigcup_{i=1}^{\phi_n} D_n^{(i)}$$

where $D_n^{(i)}$ is the ith n-type face, and $n = 1,2, \ldots, m$, where m is the largest number of spherical arcs that a spherical face presents.

Then, the characterization of the original surface $G(K,\rho)$ is given by two different descriptors:

$$\Phi(G(K,\rho)) = \{\phi_1, \phi_2, \ldots, \phi_m\}$$

where ϕ_n is the number of n-type faces that present the original surface $G(K,\rho)$, and

$$\chi(G(K,\rho)) = \{\chi_1, \chi_2, \ldots, \chi_m\}$$

where χ_n is the Euler–Poincaré characteristic[15] of each truncated surface $G_n(K,\rho)$. If G_n is a set of n_d disjoint surfaces, then $\chi_n = (\chi_{n1}, \chi_{n2}, \ldots, \chi_{nd})$.

In the case of conformer 12 of 4-methylpyrazole (**1**), the corresponding $\Phi(G(K,\rho))$ and $\chi(G(K,\rho))$ descriptors would be:

$$\Phi(G(K,\rho)) = \{6, 1, 2, 2, 0, 1, 0, 1\}$$
$$\chi(G(K,\rho)) = \{-4, 1, 0, (1,1,1,1,0), 2, (1,1)\,2, (1,1)\}$$

In Color Plates 1 and 2, the different types of faces and the truncated surfaces obtained for the 4-methylpyrazole are represented, respectively. The graphical representations were performed using the InsightII molecular modeling package[16] on a Personal Iris workstation.[17] This package allowed for a good visual analysis of the different types of faces on the VDWSs and of the truncated surfaces.

Topological characterization of all the minima obtained for compounds **1–11** was carried out analytically.[18] For every minima of each pyrazole, two shape descriptors Φ and χ were obtained. When two minima showed equal topological descriptors, only the one with lower energy was considered for semiempirical optimization. Comparison of both shape descriptors Φ and χ for each set of conformers reduced the number of structures to be studied to those minima with different shape characteristics (see Table 1).

The geometry and energy of the 76 conformational minima with different shapes were then optimized using the semiempirical PM3 method[19] as implemented in the MOPAC 6.0 package.[20] For each calculation the gradient norm was optimized to values lower than 1.0, and the precision was incremented by a factor of 100. Every structure was characterized as a minimum by frequency calculations. Torsional angles and heats of formation of the final optimized minima obtained for all the compounds are given in Table 2.

SHAPE CHARACTERIZATION OF MOLECULAR ELECTROSTATIC POTENTIAL AND VAN DER WAALS SURFACE INTERRELATIONS FOR 4-ALKYLPYRAZOLES

Surfaces combining two properties as the MEP maps (electronic properties) and VDWSs (steric properties) allow a deeper understanding not only of the characteristics of biomolecules, but also of the nature of the interaction with the corresponding receptor.

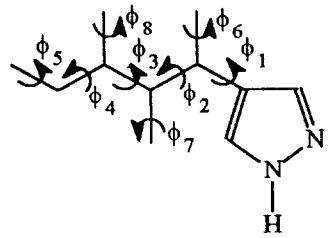
The method used to compute the MEP and characterize its interrelation with the VDWS has been described extensively in the literature.[1,18,21] However, included is a brief explanation of the main concepts and parameters that have been used in this work. The MEP at a point r in 3D-space produced by a molecule in a given nuclear configuration is expressed by:

$$V(r) = \sum_A Z_A / \|r - R_A\| - \int \{\rho(r') / \|r - r'\|\} dr'$$

where Z_A is the nuclear charge of nucleus A; R_A is the nuclear position vector of nucleus A; and $\rho(r')$ is the one-particle electron density function.

The calculation of the MEP for a relatively isotropic distribution of points on a VDWS (as given by the MS program[22]) was performed with a version of the program Gaussian80.[23] The visual analysis of MEP distributions on VDWS within desired ranges was performed with the mo-

Table 2. Dihedral angles (°) for all the local minima obtained for the 4-alkylpyrazoles 1 to 11 optimized at the PM3 level. Heats of formation are given in kcal. mol^{-1}.



Local Minima		ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_6	ϕ_7	ΔHf°
1:	12	179.5°						34.09
2:	28	−89.7°	−61.1°					34.70
3:	45	−0.6°	179.8°	58.9°				29.84
	66	−28.5°	−65.3°	61.0°				30.31
	164	−85.2°	−68.8°	55.3°				29.91
	241	−120.5°	−178.8°	59.6°				29.27
	311	179.9°	−76.3°	58.2°				30.41
4:	49	2.4°	176.0°			−177.0°		30.32
	245	−118.7°	179.3°			−179.2°		29.68
5:	315	3.8°	177.9°	−64.6°	169.3°			24.82
	343	−0.6°	179.8°	−179.5°	179.1°			24.42
	462	−32.2°	−59.9°	−70.9°	−179.1°			25.46
	490	−24.9°	−68.7°	−172.8°	−178.1°			24.91
	1148	−88.3°	−60.5°	−67.1°	−171.6°			25.09
	1176	−83.6°	−70.0°	179.8°	−179.3°			24.45
	1687	−115.1°	−174.5°	−73.5°	−177.5°			24.35
	1715	−120.8°	−177.3°	−179.8°	179.6°			23.85
	2177	178.2°	−64.6°	−63.8°	−174.1°			26.05
	2205	−176.8°	−72.7°	−176.1°	−176.9°			25.16
6:	119	0.4°	−70.7°	179.5°		59.0°		26.99
	147	6.2°	172.8°	170.6°		55.7°		25.48
	1491	−106.9°	−71.3°	177.9°		57.2°		26.10
	1862	−146.8°	−170.9°	171.6°		53.7°		25.01
	2177	−143.3°	−72.7°	−168.0°		76.2°		25.59
7:	311	9.4°	−165.2°	60.6°			−58.1°	24.79
	486	−46.7°	−71.8°	56.9°			−179.8°	24.26
	1172	−84.8°	−73.5°	54.3°			−178.9°	24.06
	1711	−117.5°	−166.3°	59.6°			−177.7°	24.09
	2201	173.0°	−72.8°	59.9°			179.8°	24.70
8:	2176	4.6°	174.6°	−65.0°	−79.9°	−179.5°		19.57
	2201	1.7°	175.9°	−65.4°	171.5°	61.9°		19.22
	2380	−2.5°	177.1°	178.9°	−77.0°	−177.8°		19.54
	2397	−0.4°	179.9°	−179.8°	180.0°	59.7°		18.99
	3205	−44.6°	−74.9°	−64.4°	−79.6°	−179.9°		19.70
	3234	−31.6°	−64.9°	−72.9°	178.0°	179.8°		19.94
	3409	−28.1°	−68.4°	−177.6°	−72.0°	−179.6°		19.89
	3430	−25.4°	−69.7°	−177.9°	−178.9°	−179.5°		19.44
	8007	−89.1°	−74.6°	−63.5°	−78.7°	179.4°		19.48
	8036	−83.5°	−74.0°	−65.0°	171.0°	179.1°		19.06
	8207	−83.0°	−73.2°	−175.8°	−71.5°	63.2°		19.57
	8228	−80.2°	−73.0°	176.4°	−179.7°	61.0°		18.98

Table 2. Dihedral angles (°) for all the local minima obtained for the 4-alkylpyrazoles 1 to 11 optimized at the PM3 level. Heats of formation are given in kcal. mol^{-1} (continued)

Local Minima	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_6	ϕ_7	ΔHf°
11780	−117.3°	176.0°	−64.8°	−80.8°	−179.3°		19.05
11809	−119.1°	177.1°	−65.2°	171.4°	178.7°		18.67
11980	−122.4°	178.3°	178.4°	−76.0°	60.4°		18.96
12001	−120.6°	−177.2°	−179.9°	180.0°	60.12°		18.43
15210	−143.8°	−79.5°	−62.0°	−78.8°	179.3°		19.13
15235	−149.3°	−78.8°	−62.7°	170.1°	58.4°		18.86
15410	−179.0°	−79.3°	171.5°	−66.7°	49.2°		19.59
15431	−144.7°	−78.7°	−176.1°	−178.6°	60.4°		18.66
9: 833	6.8°	−67.0°	−164.0°	−178.9°		74.3°	20.59
1001	13.5°	171.6°	−80.7°	179.2°		56.6°	20.17
1029	9.0°	173.0°	171.0°	177.7°		56.1°	19.89
10437	−108.2°	−66.7°	−165.7°	−179.5°		72.4°	20.43
13006	−123.7°	173.2°	−79.6°	179.8°		54.5°	19.54
13034	−145.4°	170.9°	172.1°	178.8°		54.8°	19.42
15239	−168.1°	−68.7°	−166.6°	−179.5°		74.8°	20.29
	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_7	ϕ_8	ΔHf°
10: 2030	6.7°	−159.5°	75.5°	−179.7°	−178.3°		20.88
2352	12.2°	−166.8°	171.4°	172.4°	170.8°		19.94
3381	−25.4°	−66.6°	172.4°	172.3°	171.7°		19.64
3396	−47.0°	−74.3°	−59.7°	57.3°	167.7°		19.92
3402	−46.6°	−77.8°	−60.3°	176.1°	168.1°		19.94
8183	−80.1°	−71.9°	159.9°	179.6°	178.9°		19.22
8204	−85.6°	−64.8°	−63.2°	−168.5°	−164.5°		19.93
11956	−18.9°	−171.2°	170.9°	173.8°	169.8°		19.34
11971	−121.6°	−176.7°	−60.3°	59.3°	165.4°		20.28
11977	−120.2°	−174.7°	−60.9°	178.5°	164.7°		20.30
15386	−178.7°	−75.2°	162.4°	177.4°	179.3°		19.94
15407	−145.4°	−80.4°	−58.3°	178.4°	168.4°		19.41
11: 1344	3.4°	−100.1°	−166.3°	−177.7°		−60.5°	20.91
2177	3.4°	173.6°	−65.5°	179.2°		−69.0°	18.91
3206	−28.8°	−73.9°	−65.0°	179.4°		−69.2°	19.25
5799	−52.9°	−83.5°	−158.6°	63.2°		−58.9°	20.15
8008	−82.7°	−76.3°	−65.5°	179.8°		−68.3°	18.73
11977	−118.5°	−171.4°	−173.3°	−170.8°		−60.2°	18.30
15211	172.7°	−76.0°	−64.5°	179.5°		−69.0°	19.25
15746	−174.7°	−100.7°	−167.2°	62.8°		−60.2°	20.76

lecular modeling program InsightII,[16] and the analysis of VDWS–MEP interrelations was carried out analytically with a program developed by us.[24] This analysis provided the characterization of the MEP function on a VDWS by certain topological invariants.[15]

Considering a range of MEP above a given value V_j, a subset $D_j(V_j)$ of the total molecular surface $G(K,\rho)$ containing the points associated with that MEP range is defined.

$D_j(V_j)$ can be considered derived from the total surface $G(K,\rho)$ by a truncation (i.e., by separating all the points on the VDWS with MEP value below V_j), and can be characterized by different topological invariants.[15] In the present study these truncated surfaces were characterized by Euler–Poincaré characteristics $\chi_j(D_j)$. The MEP–VDWS interrelation can then be described by $\chi(K,d,\rho)$, which depends on the number K of intervals in which the MEP range is divided, the width d of those intervals, and the VDW radii ρ:

$$\chi(K,d,\rho) = (\chi_1, \chi_2, \ldots, \chi_j, \ldots, \chi_k)$$

These vectors $\chi(K,d,\rho)$ allow for the comparison between different conformations or different molecules with given configurations.

In the present work, the topological analysis of the different local minima obtained for each pyrazole was carried out using Gavezotti radii[14] and a width d of 0.0025 a.u. in all cases. The program that performs this analysis starts study-

ing the surface associated with the MEP interval 0.0025 a.u. below the maximum MEP value. Then, it analyzes topologically the surface associated with those points with a MEP value 0.0050 a.u. below the maximum MEP value. The last step is the analysis of the surface associated with a MEP interval between the maximum and minimum values of MEP (i.e., the total surface).

In the case of conformation 12 of 4-methylpyrazole (**1**), the total descriptor $\chi_j(Dj)$ for the truncated VDWS is given in Table 3. Color Plate 3 shows the surfaces associated with different MEP ranges. The red and yellow stars represent clusters of points on the Connolly surface within and out of the MEP interval considered, respectively. These graphical representations were carried out by using the InsightII package,[16] and aided in a better understanding of the analytical topological characterization.[24]

The topological analysis of the MEP–VDWS interrelations for the 76 different minima found for compounds **1–11** was carried out in the same analytical way. A width d of 0.0025 a.u. was used for all the local minima with different shapes.

Table 3. The χ_j shape descriptors of the interrelation between MEP and VDWS for 4-methylpyrazole (1) and the corresponding δ_j codes.

MEP	χ_j	δ_j
Maximum = 0.0667		
0.0505	(1)	1
0.0480	(1)	1
0.0380	(1,1)	2
0.0355	(1)	1
0.0180	(1,1,1)	3
0.0155	(1,1,1,1,1)	5
0.0130	(1,1,1,1)	4
0.0105	(1,1,1,1)	4
0.0080	(1,1,1,1)	4
0.0055	(1,1,1)	3
0.0030	(1,1)	2
0.0005	(0)	0
−0.0020	(1)	1
Minimum = −0.0884	(2)	

CORRELATION OF THE SHAPE CODES AND THE INHIBITORY ACTIVITY OF 4-ALKYLPYRAZOLES

In a previous work,[1] an almost linear relationship between maximum and minimum MEP values on the VDWSs of substituted pyrazoles was found. Two different clusters were formed. Those pyrazoles with high inhibitory power presented the most negative MEP minima and lower MEP maxima. On the contrary, low power inhibitors exhibited less negative MEP minima and higher MEP maxima. All the local minima studied for the eleven pyrazoles showed a relationship MEP minimum/MEP maximum similar to that of the high power inhibitors. This is in agreement with the K_I values known for these pyrazoles.

In the present study, the relationship between MEP minima and MEP maxima did not allow us to distinguish between the power of these inhibitors because all of them were in the range of high inhibitory power. For this reason, the shape codes obtained from the topological analysis of MEP–VDWS interrelations are more useful in finding a correlation with the inhibitory activity.

The parameter δj was previously defined[1] as the sum of Euler-Poincaré characteristics:

$$\delta_j = \Sigma \chi_{ji}$$

Table 4. Maximum δ_j values found for every minima of compounds 1 to 11. Underlined is shown the δ_{max} for each pyrazole derivative.

Compound	δ_j	Compound	δ_j	Compound	δ_j	Compound	δ_j
1:12	5	**6**:119	7	**8**:8036	9	**10**:2352	9
2:28	6	**6**:147	8	**8**:8207	10	**10**:3381	8
3:45	9	**6**:1491	7	**8**:8228	10	**10**:3396	8
3:66	6	**6**:1862	9	**8**:11780	10	**10**:3402	8
3:164	7	**6**:2177	8	**8**:11809	10	**10**:8183	8
3:241	8	**7**:311	9	**8**:11980	11	**10**:8204	8
3:311	7	**7**:486	8	**8**:12001	12	**10**:11956	10
4:24	7	**7**:1172	7	**8**:15210	10	**10**:11971	9
4:49	8	**7**:1711	8	**8**:15235	10	**10**:11977	10
5:315	10	**7**:2201	9	**8**:15410	9	**10**:15386	9
5:343	11	**8**:2176	11	**8**:15431	10	**10**:15407	10
5:462	7	**8**:2201	11	**9**:833	10	**11**:1344	9
5:490	7	**8**:2380	12	**9**:1001	9	**11**:2177	12
5:1148	8	**8**:2397	13	**9**:1029	11	**11**:3206	9
5:1176	9	**8**:3205	8	**9**:10437	9	**11**:5799	9
5:1687	8	**8**:3234	9	**9**:13006	8	**11**:8008	10
5:1715	10	**8**:3409	9	**9**:13034	11	**11**:11977	11
5:2177	8	**8**:3430	10	**9**:15239	10	**11**:15211	9
5:2205	8	**8**:8007	8	**10**:2030	9	**11**:15746	9

Thus, each conformer can be represented by the maximum value of δ_j. In the case of the minima of 4-methylpyrazole (**1**:12), the representative δ_j value will be $\delta_j = 5$ (see Table 3). In general, most of the 76 conformers studied here present their maximum δ_j value at approximately 0.013 a.u. of the MEP. For each pyrazole, δ_{max} is defined as the maximum value that δ_j can attain for all of its minima. The δ_j values obtained for every minima are gathered in Table 4, and the corresponding δ_{max} for each pyrazole appears underlined. Thus, comparing this parameter for each pyrazole, it is observed that δ_{max} increases as the length of the alkyl chain increases (see Table 5). On the contrary, branching of the chain decreases the value of δ_{max} compared to the linear chain derivative with the same number of C atoms.

For the linear derivatives (compounds **1**, **2**, **3**, **5**, and **8**) a good correlation was found between δ_{max} and the $\ln(K_I)$ as a measure of the activity ($\ln(K_I) = -2.795 - 0.325\ \delta_{max}$; $r^2 = 0.978$). Cross-validation is a recent statistical development[25] that helps on the computation of quantitative structure–activity relationships (QSAR) correlations. In cross-validation one pretends that one or more objects are unknown, and the entire QSAR is rederived with those objects omitted and then used to predict the dependent values of the omitted objects. Thus, cross-validated statistics have been carried out for the linear derivatives, by omitting one of them each time, and predicting its $\ln(K_I)$ from the equation obtained in each case. The most widely used metric for evaluating the QSAR is its r^2 value, defined as:

$$r^2 = (SD_{original} - SD_{remaining\ error})/SD_{original}$$

where $SD_{original}$ is the sum of the squared deviations of the original dependent values from their mean, and $SD_{remaining\ error}$ is the sum of squared differences between original and "predicted dependent values" after the QSAR has been derived.[25] The cross-validated r^2 (or q^2 as recommended by Clementi[25]) seems to be a quite good estimation of the accuracy of the prediction. In the case of the linear pyrazoles studied here $q^2 = 0.954$.

However, in the case of branched 4-alkylpyrazoles, there is no linear relationship with their activity (see Table 5). The activities of compounds **4**, **6**, and **7** can be explained only in relation to their isomers with linear chains. Thus, for a pyrazole with a three C-atom chain, the branched derivative (**4**) will be less active than the linear one (**3**) as is reflected in the smaller value found for δ_{max} (K_I(**3**): 0.004 versus K_I (**4**): 0.008; δ_{max} (**3**): 9 versus δ_{max} (**4**): 8). In the same way, one can expect a lower activity for the branched derivatives **6** and **7** than for the linear isomer **5** by looking at the smaller values of δ_{max} found for **6** and **7** than for **5**. A similar inhibitory activity should be expected for **6** and **7** since both exhibit similar δ_{max} values (see Table 5). That is, for branched chains the model is not quantitative, but it provides a general tendency of the activity that allows one to establish a relative order of K_I for 4-alkylpyrazoles. Our hypothesis is that for chains of five C atoms or more, when it starts being possible to have a branch at a distance of two —CH_2— groups from the ring, the activity of that branched derivative would be similar to that of the linear derivative with one fewer C atom.

Thus, according to Table 5, derivatives **9**, **10**, and **11**, for which K_I was unknown, were predicted to have an inhibitory power smaller than the linear isomer (**8**) and similar to the linear derivative with one less C atom, 4-butylpyrazole (**5**). This prediction was confirmed for the 4-isopentyl derivative **11**. This pyrazole **11** was synthesized in our laboratory and its inhibitory activity measured ($K_I = 0.002\ \mu M$).[26]

Table 5. Values of the shape code δ_{max} and the inhibition constant K_I for 4-alkylpyrazoles 1 to 11.

Pyrazole	δ_{max}	K_I
1	5	0.013
2	6	0.007
3	9	0.004
4	8	0.008
5	11	0.0018
6	9	0.014
7	9	0.013
8	13	0.0008
9	11	—
10	10	—
11	12	—

CONCLUSIONS

The combined use of systematic conformational analysis, localization of all the local minima in n-dimensional spaces, and shape analysis of the VDWSs of all local minima reduced the number of conformers to study for each pyrazole derivative.

Topological characterization of the MEP–VDWS interrelations of all these minima by analytical and graphical methods yielded certain shape codes which correlate well with the inhibitory activity. Nonbranched 4-alkyl derivatives showed a linear correlation between the δ_{max} code and the K_I against LADH. This fact was in agreement with the literature;[27] the inhibitory power increases by a factor of 2 for each methyl group added to a linear chain.

The δ_{max} code represents the maximum number of truncations that a 4-alkylpyrazole can show in a certain conformation. In some way, this parameter represents the conformer of each 4-alkylpyrazole which has more H atoms exposed, i.e., which has a more extended chain. Accordingly, the alkyl chain of any 4-alkylpyrazole should be as extended as possible for a better interaction with the enzyme, since this improves the inhibitory activity.

All these correlations allowed us to predict 4-isopentylpyrazole (**11**) a similar inhibitory power to that of the linear derivative with one less C atom, 4-butylpyrazole (**5**). From these results it could be postulated that the negative effect on the LADH inhibition caused by branching at the chain of 4-alkylpyrazoles is counterpoised if such branch is introduced far enough from the pyrazole ring. As a hypothesis, it is proposed that the minimum distance between the branch and the ring to compensate both effects would be two —CH_2— groups.

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