Study of the Action of Flavonoids on Xanthine-Oxidase by Molecular Topology

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A study was performed on xanthine-oxidase inhibition by 22 flavonoids, including flavones, flavonols, flavanones, and chalcones, using UV spectroscopy for experimental data and molecular topology to establish the structure−activity relationship (SAR) model. The flavonoids were classified into four groups according to their activity on xanthine-oxidase (inactive, low, significant, or high), and linear discriminant analysis was used to classify each compound within a group. The results led to a very good model, which was able to classify correctly as xanthine oxidase inhibitors, along with a test set of molecules including a variety of different compounds such as allopurinol, caffeic acid, esculetin, and alloxantin.

INTRODUCTION

Flavonoids are the subject of continuing biological studies due to their bactericidal,[1,2] spasmolytic,[3] hepatoprotecting,[4] and enzyme inhibiting (xanthine-oxidase, NADH-oxidase)[5−10] activities, among others. A further issue of multidisciplinary investigation is concerned with the reactions and action mechanisms of xanthine-oxidase (*XO*) and other enzymes present in biological fluids and tissues.[11−15]

Since 1968, when McCord and Fridovich[16] demonstrated the participation of *XO* in the generation of free radicals, interest in the new properties of this enzyme has considerably increased. Its role in the generation of oxygen active species, in viral infections[17] and in the process of ischemia/reperfusion,[18,19] has been determined. The inhibition of this enzyme as well as the antioxidant properties of flavonoids showing either known or potential pharmaceutical applications have also been studied.[10,20] Several authors have investigated the influence of substituent nature and position on the degree of *XO* inhibition by some flavonoids.[10,21,22] Most of these studies, however, are of a qualitative nature. Furthermore, the type of inhibition exerted by chalcones, flavanones, and flavones on this enzyme has not yet been completely elucidated.

Molecular topology has widely demonstrated its ability for an easy and efficient characterization of molecular structure, through the so-called "topological indices".[23] When these indices are selected adequately it is possible to have a very specific characterization of each chemical compound.[24] Moreover, these descriptors allow the obtention of SAR and QSAR relations able to select or design new drugs with a high level of accuracy, for instance, new antivirals,[25,26] hypoglycemic,[27] antibacterials,[28] betablockers,[29] analgesics,[30,31] bronchodilators,[32] sedatives,[33] antifungals,[34] antimalarials,[35] antitoxoplasmatics,[36] and also cytostatics,[37] most of which may be considered as new leads.

The purpose of the present paper is to establish the relationships between the structure and the *XO* inhibiting activity of these compounds by means of an experimental and theoretical study using UV spectroscopy as well as molecular topology, respectively.

MATERIALS AND METHODS

The structures of the flavones, flavanones, and chalcones studied are shown in Figures 1 and 2.

The formalism used includes the following steps.

Calculation of Topological Descriptors. In this work we have used Kier and Hall[38,39] connectivity indices up to the fourth order, ${}^m\chi_t$, as well as the topological charge indices (TCI) introduced by us.[40]

It is well-known that the connectivity indices may be derived from the adjacency matrix and they are defined as

$$ {}^m\chi_t = \sum_{j=1}^{N_m} {}^mS_j \tag{1}$$

where "*m*" is the subgraph order, i.e., the number of edges in the subgraph; N_m is the number of type "*t*" and order "*m*" subgraphs within the whole graph; and ${}^mS_j$ is a factor defined for each subgraph as

$$ {}^mS_j = \prod_{j-1}^{m+1} (\delta_i)_j^{-1/2} \tag{2}$$

where "*j*" denotes the particular set of edges that constitutes the subgraph and δi = vertices valence.

Topological charge indices G_k and J_k of a given order *k* into a graph representing a molecule are defined as

$$ G_k = \sum_{i=1}^{N-1} \sum_{j=i+1}^{N} |c_{ij}| \delta(k, d_{ij}) \tag{3}$$

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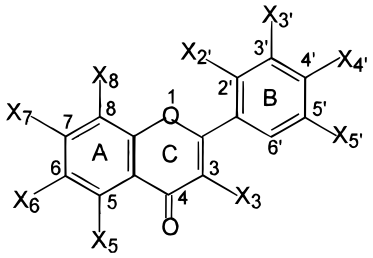
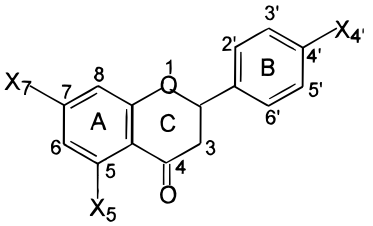
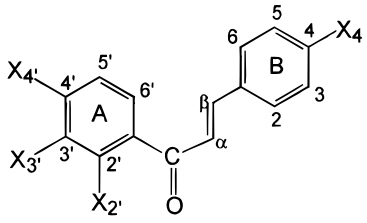


(**1**) Flavone	X3=X5=X6= X7=X8=X2'=X3'=X4'=H		
(**2**) 7(OH)-flavone	X7=OH	X3=X5=X6=X8=X2'=X3'=X4'=H	
(**3**) 5,7(OH)$_2$-flavone	X5=X7=OH	X3=X6=X8=X2'=X3'=X4'=H	
(**4**) 3,7,3',4'(OH)$_4$-flavone	X3=X7=X3'=X4'=OH	X5=X6=X8=X3'=X4'=H	
(**5**) 3,5,7,3',4'(OH)$_5$-flavone	X3=X5=X7=X3'=X4'=OH	X6=X8=X2'=X4'=H	
(**6**) 3,5,7,2',4'(OH)$_5$-flavone	X3=X5=X7=X2'=X4'=OH	X6=X8=X3'=H	
(**7**) 5,7(OH)$_2$-6,4'(MeO)$_2$-flavone	X5=X7=OH; X6=X4'=MeO;	X3=X8=X2'=X3'=H	
(**8**) 5,7(OH)$_2$-6,8,4'(MeO)$_3$-flavone	X5=X7=OH; X6=X8=X4'=MeO;	X3=X2'=X3'=H	
(**9**) 5,7,4'(OH)$_3$-6,8(MeO)$_2$-flavone	X5=X7=X4'=OH	X6=X8=MeO	X3=X2'=X3'=H



(**10**) Flavanone	X5=X7=X4'=H		
(**11**) 5,7(OH)$_2$-flavanone	X5=X7=OH	X4'=H	
(**12**) 5,7,4'(OH)$_3$-flavanone	X5=X7=X4'=OH		

Figure 1. Structures of the flavones and flavanones studied.



(**13**) Chalcone	X2'=X3'=X4'=X4=H			
(**14**) 2'(OH)-chalcone	X2'=OH	X3'=X4'=X4=H		
(**15**) 4(OH)-chalcone	X4=OH	X2'=X3'=X4'=H		
(**16**) 4(F)-chalcone	X4=F	X2'=X3'=X4'=H		
(**17**) 4(MeO)-chalcone	X4=MeO	X2'=X3'=X4'=H		
(**18**) 2',4'(OH)$_2$-chalcone	X2'=X4'=OH	X3'=X4=H		
(**19**) 2',4'(OH)$_2$-3'MeO-chalcone	X2'=X4'=OH	X3'=MeO	X4=H	
(**20**) 2'(OH),4'(MeO)-chalcone	X2'=OH	X4'=MeO	X3'=X4=H	
(**21**) 2'(OH),4(MeO)-chalcone	X2'=OH	X3'=X4'=H	X4=MeO	
(**22**) 2'(OH),4F-chalcone	X2'=OH	X3'=X4'=H	X4=F	

Figure 2. Structures of the chalcones studied.

and

$$J_k = \frac{G_k}{N-1} \tag{4}$$

where N represents the number of vertices in the chemical graph representing the molecular structure. This is the number of atoms different from hydrogen in the molecule. c_{ij} is the charge term between i and j. It is defined as $c_{ij} = m_{ij} - m_{ji}$ being m_{ij} and m_{ji} elements of the $N \times N$ matrix **M**, obtained as product of two matrices: $\mathbf{M} = \mathbf{A}\cdot\mathbf{Q}$. Thus

$$m_{ij} = \sum_{h=1}^{N} a_{ih} q_{hj} \tag{5}$$

Matrix **A** is called the adjacency matrix. Its elements a_{ih} represent the bonds between the atoms corresponding to vertices i and h in the graph. The element a_{ih} takes value 0 whether $i = h$ or it is not bonded to h and it takes the value 1 if i is bonded to h by a simple bond; the value of 1.5 if the bond is aromatic; 2 for double bonds; and 3 for triple ones. Matrix **Q** is called the "coulombian matrix". Its elements q_{hj} take value 0 for $h = j$. If $h \neq j$, then $q_{hj} = 1/d^2_{hj}$, where d_{hj} is the topological distance between vertices h and j. δ represents the Kronecker delta symbol: $\delta(\alpha,\beta) = 1$, if $\alpha = \beta$; $\delta(\alpha,\beta) = 0$, if $\alpha \neq \beta$. d_{ij} is the topological distance between the vertices i and j. The topological distance between two vertices i and j is defined as the number of edges between the two vertices by the shortest path.

Thus, G_k represents the sum of all the c_{ij} charge terms for every pair of vertices i and j, at topological distance k.

Valence topological charge indices, G_k^v and J_k^v, are defined in a similar way, substituting the matrix **A** by $\mathbf{A}^v$. The elements of both matrices are identical except for the main diagonal of $\mathbf{A}^v$, which is obtained by Pauling electronegativity difference with respect to the carbon atom:

$$a_{ij} = \mathrm{EN}(i) - \mathrm{EN}(\mathrm{C}) \tag{6}$$

Actually, we use k values ranging from 1 to 5. Thus, a total of 20 TCI are used in the correlation analysis.

Linear Discriminant Analysis. Linear discriminant analysis[41] (LDA) was used to obtain the discriminant function able to differentiate the inhibiting activity of *XO* by the 22 flavonoids analyzed. The selection of the optimal discriminant function was carried out using the BMDP 7M package.[42] The method used for the selection of the descriptors was the F-Snedecor, and the classification criteria was the shortest Mahalanobis distance (distance of each case to the mean of all cases used in the regression equation). The quality of the discriminant function is evaluated by the parameter Wilk's lambda or *U*-statistic.

The analyzed compounds were classified into four groups, according to the following intervals of percent inhibition degree, PIG, values:

group 1. inactive	values of % inhibition lower than 5%
group 2. low activity	values of % inhibition between 5% and 25%
group 3. significant activity	values of % inhibition between 25% and 70%
group 4. high activity	values of % inhibition higher than 70%

Since LDA gives only percentages of probability for inclusion of a given compound within a certain category, a simple way to quantify the probability for a chosen molecule to be included within a group is

$$P = \sum_{i=1}^{G} X_i P_i \tag{7}$$

where the X_i values are either 0, 1 ,2, or 3, for inactive, low active, significant active, and high active drugs, respectively. Obviously, the maximum value for P would be 3 and the minimum is 0. Thus a compound showing the value 3 would

have virtually 100% probability to be a potent inhibitor, while another one showing $P = 0$ should be completely inactive.

Before developing the LDA analysis, a correlation study between PIG and TIs was performed in order to select the best descriptors possible to distinguish the inhibitory activity. The regression equations were obtained by multilinear regression between both PIG and the whole set of TIs.

The presence of possible "outliers" is easily detected through a cross-validation test, in which each case is eliminated from the data set and then the regression analysis is carried out again with the $N-1$ remaining cases, being predicted after the activity value for the deleted case. The procedure is repeated so many times as there are cases in the data. From the residuals obtained, the standard error of estimate SE(CV) is determined for the cross-validation.

Experimental Procedure. The flavones (**7**−**9**), flavanone (**11**), and chalcones (**18**, **19**) were obtained from natural products.[43] Chalcones **13**−**17** and **20**−**22** were synthesized using the Claisen-Schmidt reaction[44] and unsubstituted flavanone (**10**) according to the method developed by Geissman and Clinton.[45] Compounds **1**−**6** and **12** were purchased from Sigma Chemical Co. The purification and characterization of the compounds was performed by previously reported crystallization, chromatographic, and spectroscopic procedures.[46] The solvents used in this process were methanol (spectroscopic grade), cyclohexane, and ethyl acetate, all from Merck and without further purification.

Preliminary experiments of *XO* inhibition were carried out in order to select the best working conditions. The used concentration of xanthine (*X*) was 4.9×10^{-5} M in TRIS buffer (5 mM pH 8.1). The flavonoids were added to the reaction mixture dissolved in DMSO. The final concentration of DMSO was 0.34% (v/v). The *XO* (EC 1.1.3. 22, grade IV from milk) was obtained from Sigma Chemical Co. The inhibition of *XO* was determined by monitoring the formation of urate at 290 nm on a Shimadzu UV 160A double beam spectrophotometer with 1 cm thermostatically controlled cells, at 30 °C. In the procedure followed for quantitative determinations, 2.9 mL reaction mixtures were prepared containing *X* (4.9×10^{-5} M) and flavonoids (5.0×10^{-5} M). The reaction was initiated by adding 3 μL of *XO* solution containing 4.59 UI/mL, and the absorbances were recorded for 10 min. The percent inhibition degree was calculated by eq 8. In all experiments, the substrate ([So]) and flavonoid ([Fo]) concentrations indicated above were used.

XO is a molybdenum-containing oxido-reductase, which catalyzes the oxidation of *X* to uric acid.[47] To quantitatively analyze *XO* inhibition by flavonoids, the percent inhibition degree (PIG) was defined as

$$\mathrm{PIG} = \frac{100(\mathrm{VoC} - \mathrm{Vo})}{\mathrm{VoC}} \qquad (8)$$

where VoC is the initial rate (mol L^{-1} s^{-1}) of the control enzymatic reaction ([Fo] = 0) and Vo is the initial rate of the enzymatic reaction in the presence of the inhibitor.

RESULTS AND DISCUSSION

Biological Properties. Values of PIG of the analyzed flavonoids, at 30 °C, are listed in Table 1.

To allow for an easier discussion of results, we have divided the flavonoids included in this study into four groups.

Table 1. Percent Inhibiting Degree, PIG, of Xanthine Oxidase by Flavonoids, at 30 °C

compound	chemical name	PIG
1	flavone	3.0
2	7(OH)-flavone	49.7
3	5,7$(OH)_2$-flavone	91.1
4	3,7,3′,4′$(OH)_4$-flavone	91.8
5	3,5,7,3′,4′$(OH)_5$-flavone	91.4
6	3,5,7,2′,4′$(OH)_5$-flavone	70.1
7	5,7$(OH)_2$−6,4′$(OCH_3)_2$-flavone	77.8
8	5,7$(OH)_2$−6,8,4′$(OCH_3)_3$-flavone	61.4
9	5,7,4′$(OH)_3$−6,8$(OCH_3)_2$-flavone	72.7
10	flavanone	3.6
11	5,7$(OH)_2$-flavanone	20.1
12	5,7,4′$(OH)_3$-flavanone	28.7
13	chalcone	0.0
14	2′(OH)-chalcone	21.5
15	4(OH)-chalcone	45.5
16	4F-chalcone	0.0
17	4(OCH_3)-chalcone	4.8
18	2′,4′$(OH)_2$-chalcone	39.8
19	2′,4′$(OH)_2$−3′(OCH_3)-chalcone	17.1
20	2′(OH),4′(OCH_3)-chalcone	17.2
21	2′(OH),4(OCH_3)-chalcone	16.2
22	2′(OH),4F-chalcone	30.3

Group 1. Flavonoid Inactive. These flavonoids are either unsubstituted (compounds **1**, **10**, and **13**) or they do not have a hydroxyl group (compounds **16** and **17**). Thus, it can be inferred that the presence of hydroxyl groups in the flavonoid structures is essential for inducing the inhibition activity on *XO*.

Group 2. Flavonoids with Low Activity. Group 2 comprises flavonoids with PIG values in the 16−22% interval. It comprises five compounds belonging to two well-differentiated structural patterns: flavanones (**11**) and chalcones (**14** and **19**−**21**). These compounds have only an OH group in common at equivalent positions (C5 of flavanone and C2′ of chalcone). In chalcones, it is observed that the introduction of OCH_3 groups in the rings A or B of **14** lowers their enzymatic inhibition activity (compounds **19**−**21**).

Group 3. Flavonoids with Significant Activity. Two subgroups can be distinguished within Group 3.

Subgroup 3.A. This subgroup comprises the compounds **2**, **8**, **15**, and **18**, which have PIGs between 40% and 70%. Structurally, all these flavonoids exhibit an α,β-unsaturated carbonyl group. Also, compounds **2** and **18** have a hydroxyl group in equivalent positions (C7 of flavone and C4′ of chalcone), while **15** possesses a hydroxyl bound to C4 (Figure 2). Considering flavone (**1**) and chalcone (**13**) are inactive, it is evident that the introduction of hydroxyl groups in their structures induces the inhibiting activity on *XO*.

Subgroup 3.B. The two flavonoids (**12** and **22**) of this subgroup have a hydroxyl group at equivalent positions (C5 of flavanone and C2′ of chalcone) and exhibit very similar PIG values (about 30%).

Group 4. Flavonoids with High Activity. This group is formed by six flavonoids (compounds **3**−**7** and **9**), which show the highest PIG values (PIG > 70%). All the compounds in this group exhibit the following: (a) the structure of benzo-γ-pyrone, which acts as antioxidant in various biological systems;[48,49] (b) the α,β-unsaturated carbonyl group, that favors the π-electronic delocalization of the phenyl ring B; and (c) OH groups at positions 7, 3, and/or 5.

The obtained PIG values (Table 1) indicate that the presence of a hydroxyl bound to C7 is fundamental. In Group 4 flavonoids, it is observed that the presence of OCH_3 groups (compounds **7** to **9**) decreases the inhibiting activity of 5,7-(OH)2-flavone (compound **3**).

Structure−Activity Relationship. The structure of drugs is closely related to their biological properties. As was stated above, all flavonoids with high inhibiting activity on *XO* (Group 4) present structural characteristics similar to those exhibited by other antioxidant flavonoids.[50,51]

It is known that the high chemical reactivity of carbonyl compounds is closely related to their acid and basic properties.[21] Pharmacological carbonyl compounds usually exert their action through direct interaction with the enzyme. In particular, the interaction of the molybdenum atom of *XO* with the oxygen atoms of the *X* carbonyl groups determines the oxidation of *X* to uric acid.[5]

Since flavonoids compete with *X* when interacting with the enzyme, it is reasonable to assume that such interactions are determined by the reactivity of the carbonyl and hydroxyl groups at position 7 of the flavones[21] as well as by structural characteristics such as planarity. The hydrogen atoms of the OH groups at positions 3 and 5 of the flavones can form intramolecular hydrogen bonds with the oxygen atom of the carbonyl, favoring the molecule planarity and, therefore, the delocalization of the π electrons. It is clear that this planarity also depends on the substituents in the phenyl ring B.

The inhibiting activity of *XO* by flavonoids is determined to a large extent by their structural properties. The flavonoids analyzed in this paper belong to four well differentiated structural patterns: flavones, flavonols, flavanones, and chalcones. Therefore, it is not at all an easy task to establish a simple SAR relationship for an efficient prediction by using only a few structural parameters.

The intercorrelation study (not shown here), which was performed including all the variables, demonstrated that the J_2 charge index was the best one to predict the inhibitory activity, showing the following statistics: $r = 0.8495$, $F = 51.6$, SE $= 0.617$, $p < 0.0001$ for $N = 22$ compounds. The cross-validation study revealed that no outliers were found, and, therefore, all the set of compounds can be input in the LDA. Figure 3 shows the results obtained from the plot of residuals versus deleted residuals. It is noteworthy that all the points are very close to the bisector of the coordinate axes. Moreover, the value of SE(CV) $= 0.638$ is similar to the one obtained using the whole set (SE $= 0.617$).

The classification functions obtained from LDA are shown in the Table 2. The statistical parameters are $N = 22$; U (statistics, Wilk's lambda) $= 0.2273$; and $F = 20.391$.

The topological charge index, J_2, takes into account the mean value of the charge transferred between atoms placed at a topological distance $= 2$. This means that the intramolecular charge transfers between atoms placed at a topological distance $= 2$ plays an important role in this property. A possible explanation is related to the above expressed influence of the presence of hydroxyl groups in the positions 5, 7, and 4′. Also the presence of the methoxy groups clearly decreases the XO inhibitor effect. The methoxy groups have a positive electronic inductive effect, while the hydroxyl ones have a negative inductive effect. It is to be expected that the presence of hydroxyl groups in the A ring increases the XO inhibition effect through an increment in the stabilization of the aromatic ring due to an inductive effect. Thus, compare the values for the flavones 5 and 6: While the PIG value is 91.4 for the first one, it lowers to 70.1 for the second one. The only structural difference is the presence of OH substituents in ortho against meta positions, respectively.

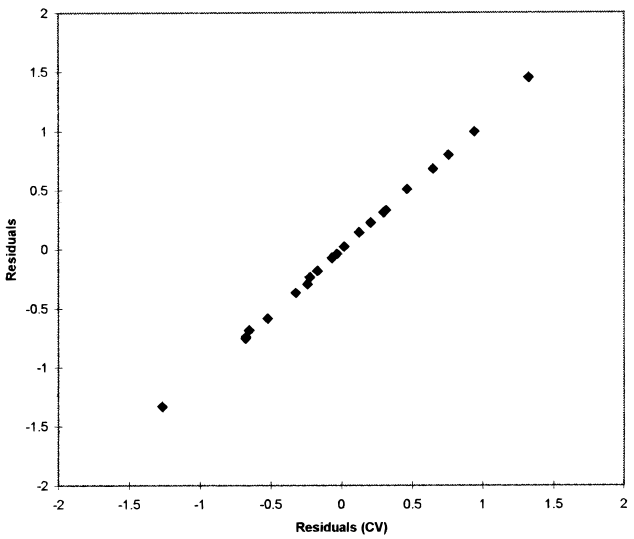


Figure 3. Plot of the residuals obtained in the cross-validation versus the residuals obtained with J_2 and XO inhibitory activity.

Table 2. Classification Functions Obtained from Linear Discriminant Analysis (LDA)

	groups			
	inactive	low	significant	high
J_2	116.31	137.37	150.10	193.20
constant	−24.85	−34.11	−40.46	−66.13

Table 3. Classification Matrix for the Set of Compounds Studied

			no. classified into group			
group	no. of cases	% correct	inactive	low	significant	high
inactive	5	80.0	4	1	0	0
low	5	60.0	1	3	1	0
significant	6	33.3	1	2	2	1
high	6	83.3	0	0	1	5

The mean charge transferred at a topological distance 2 in the aromatic rings would take into account the well-known ortho-para leading character of the OH which stabilizes the rings transferring charge at a distance 2 (ortho) and 4 (para). Obviously the higher the number of OH groups in the A ring (especially in the positions 5 and 7) the higher the PIG value is. However, the existence of such groups in the B ring (particularly in the positions 2′ and 4′) has a negative influence on the XO inhibition capability (compare flavones 5 and 6), probably through the impediment of the ring B for π electronic delocalization.

Tables 3 and 4 illustrate the results obtained for the global classification matrix as well as for one-to-one compound classification entries within a given category as well as their values of *P*.

The selected model is able to classify correctly over 80% of the inactive molecules and 83.3% of the active ones. Flavone (**1**) is included within the group "Low" with a 44.7% of probability and as "Significant" with a 38.1%. Since both values are rather close, an activity between low and

Table 4. Classification Matrix for Each One of the Compounds Analyzed[a]

compound	J_2	probability inact.	low	signif.	high	P	class.
Group Inactivity Activity							
flavone n1	0.486	0.169	0.447	0.381	0.003	1.218	low
flavanone n10	0.375	0.765	0.195	0.040	0.000	0.275	inact.
chalcone n13	0.341	0.877	0.108	0.015	0.000	0.138	inact.
chalcone n16	0.417	0.547	0.335	0.118	0.000	0.571	inact.
chalcone n17	0.399	0.651	0.273	0.077	0.000	0.427	inact.
Group Low Activity							
flavanone n11	0.481	0.187	0.449	0.361	0.003	1.180	low
chalcone n14	0.431	0.462	0.379	0.159	0.000	0.697	inact.
chalcone n19	0.532	0.052	0.360	0.552	0.036	1.572	signif.
chalcone n20	0.463	0.273	0.444	0.282	0.001	1.011	low
chalcone n21	0.475	0.214	0.450	0.335	0.002	1.126	low
Group Significant Activity							
flavone n2	0.536	0.046	0.349	0.562	0.043	1.602	signif.
flavone n8	0.653	0.000	0.011	0.077	0.912	2.901	high
flavanone n12	0.538	0.043	0.343	0.566	0.048	1.619	signif.
chalcone n15	0.417	0.547	0.335	0.118	0.000	0.571	inact.
chalcone n18	0.484	0.178	0.448	0.371	0.003	1.199	low
chalcone n22	0.497	0.132	0.436	0.426	0.006	1.306	low
Group High Activity							
flavone n3	0.580	0.010	0.187	0.528	0.275	1.881	signif.
flavone n4	0.689	0.000	0.002	0.017	0.981	2.979	high
flavone n5	0.720	0.000	0.000	0.005	0.995	2.995	high
flavone n6	0.720	0.000	0.000	0.005	0.995	2.995	high
flavone n7	0.636	0.000	0.025	0.142	0.833	2.783	high
flavone n9	0.676	0.000	0.003	0.030	0.967	2.964	high

[a] The last column illustrates the values of the probability of activity (P). The closer to 3 a given value, the higher the probability of activity is. The classification criterion is as follows: inactive: values between 0 and 1.0; low: values between 1 and 1.5; significant: values between 1.5 and 2.0; and high: values between 2.0 and 3.

significant for this compound is to be expected. Chalcone (**16**) is correctly classified as inactive.

Moreover, this discriminant model clearly outlines the decisive influence of the J_2 index. Indeed, just taking a look to Table 4, it is noteworthy that those compounds showing J_2 values lower than 0.48 are either inactive or low active. Most of the compounds with J_2 between 0.48 and 0.58 are significantly actives, while the compounds showing values higher than 0.60 are highly active.

To test the efficiency of the selected discriminant function, a validation test with a set of compounds was carried out, including both, highly heterogeneous structures and significant inhibition activity. All of them, namely allopurinol,[52] caffeic acid,[53] esculetin,[53] TEI-6720[52] (2-(3-cyano-4-isobutoxyphenyl)-4-methyl-5-thiazolecarboxylic acid), and alloxantin,[54] were correctly classified within the group of "high activity". On the contrary other uricosuric but noninhibitor compounds, such as probenecid[55] and sulfinpyrazone,[55] were correctly classified as such.

Table 5 shows the set of test compounds plus their J_2 values as well as their classification matrix. Their structures are illustrated in Figure 4.

These results are important because they demonstrate that the topological model may be applied to rather different structures, thus opening doors to the future selection of new leads in this field. It must be also emphasized that all these results have been obtained with only a one-variable regression equation, using a topological index originally introduced by us, J_2 topological charge index, which also strengthens the importance of these indices.

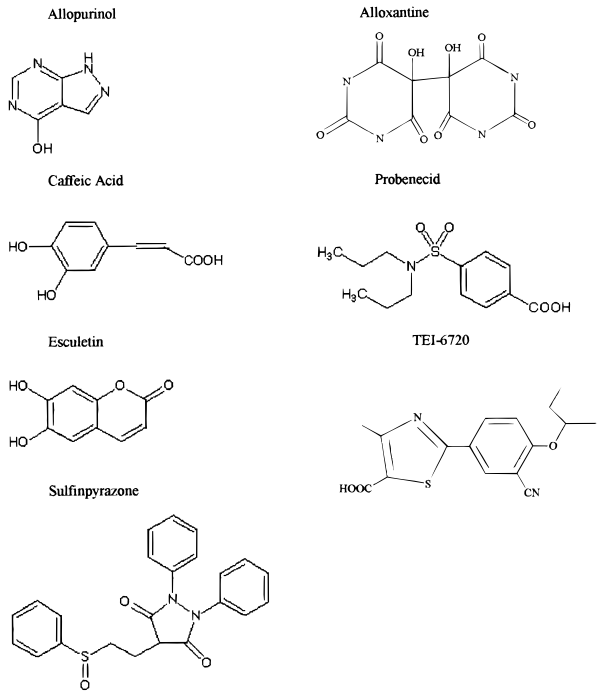


Figure 4. Structures of the compounds tested with the J_2 discriminant function.

Table 5. Classification Scheme for Each One of the Compounds of the Test Group

compound	J_2	probability inact.	low	signif.	high	P	class.	class (ref)
allopurinol	0.648	0.000	0.014	0.092	0.894	2.88	high	active[52]
probenecid	0.327	0.906	0.084	0.010	0.000	0.10	inact.	inact.[55]
sulfinpyrazone	0.294	0.953	0.044	0.003	0.000	0.05	inact.	inact.[55]
caffeic acid	0.648	0.000	0.014	0.092	0.894	2.88	high	active[53]
esculetin	0.741	0.000	0.000	0.002	0.998	3.00	high	active[53]
TEI-6720	0.696	0.000	0.001	0.013	0.986	2.98	high	active[52]
alloxantin	0.667	0.000	0.005	0.044	0.951	2.95	high	active[54]

CONCLUSIONS

The compounds investigated by UV spectroscopy and molecular topology, belong to the typical structural patterns of flavones, flavonols, flavanones, and chalcones. From the experimental and theoretical results obtained, it can be concluded that (a) the hydroxylation of flavone at position 3 substantially modifies its structure, while dihydroxylation of flavanone at positions 5 and 7 makes its behavior similar to that of 5,7(OH)2-flavones. (b) In all cases, the carbonyl region plays a major role in the *XO* inhibiting activity of these compounds. Likewise, the hydroxyl group at position 7 constitutes another important bioactive region. Both conclusions (a) and (b) are basically coincidental with other authors [10,21] for whom the presence of free hydroxyl groups at C5 and C7 as well as the existence of a double bond between C2 and C3 play an important role in the inhibition of *XO*. They also realized that the methylation of an hydroxyl group decreases the inhibition activity. The flavones most effective as *XO* inhibitors showed a 7-OH-substitution and a catechol or 3′,4′,5′-pyrogallol function on the B ring.[21] (c) By the LDA method, the studied flavonoids were classified

into four groups according to their activity on *XO* (inactive, low, significant or high). (d) A simple structure−activity relationship was proposed. The inhibiting effect grows with increasing values of the topological descriptor J_2. Furthermore, the SAR topological model used here allows not only a very significant level of correct prediction of flavonoids activity but also extrapolation of the results to other very different structures, for instance, allopurinol, caffeic acid, esculetin, or alloxantin, all of them potent *XO* inhibitors.

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REFERENCES AND NOTES

(1) Mori, A.; Nishino, C.; Enoki, N.; Tawata, S. Antibacterial activity and mode of action of plant flavonoids against Proteus vulgaris and Staphylococcus aureus. *Phytochemistry* **1987**, *26*, 2231−2234.

(2) Linuma, M.; Tsuchiya, H.; Sato, M.; Yokoyama, J.; Ohyama, M.; Ohkawa, Y.; Tanaka, T.; Fujuwara, S.; Fujii, T. Flavanones with potent antibacterial activity against methicillin-resistant Staphylococcus aureus. *J. Pharm. Pharmacol.* **1994**, *46*, 892−895.

(3) Ertan, M. Studies on the synthesis of some flavonoids derivatives possesing spasmolytic activity. *Arch. Pharm. (Weinheim)* **1987**, *320*, 1132−1138.

(4) Valenzuela, A.; Campos, R.; Garrido, A. Estudios sobre el mecanismo bioquímico de acción del flavonoide silybina: Relación con sus propiedades terapéuticas. *Arch. Biol. Med. Exp.* **1988**, *21*, 75−83.

(5) Rastelli, G.; Costantino, L.; Albasini, A. A model of the interaction of substrates and inhibitors with Xanthine Oxidase. *J. Am. Chem. Soc*. **1997**, *119*, 3007−3016.

(6) Shukla, M. K.; Mishra, P. C. Electronic spectra and structure of allopurinol: A xanthine oxidase inhibitor. *Spectrochim. Acta* **1996**, *52*, 1547−1557.

(7) Kopcikova, Y.; Rotrekl, V.; Suchy, V.; Brzobohaty, B. Flavonoids: novel beta-glucosidase inhibitors. *Chemicke Listy* **1996**, *90*, 599−599.

(8) Bezakova, L.; Kartnig, T.; Psenak, M. Effect of flavonoids of Hypericum perforatum L. on lipoxygenase activity. *Chemicke Listy* **1996**, *90*, 697−698.

(9) Bohmont, C.; Aaronson, L. M.; Mann, K.; Pardini, R. S. J. Inhibition of mitochondrial NADH oxidase, Succinoxidase, and Atpase by naturally occurring flavonoids. *J. Nat. Products* **1987**, *50*, 427−433.

(10) Hayashi, T.; Sawa, K.; Kawasaki, M.; Arisawa, M.; Shimizu, M.; Morita, N. Inhibition of cow's milk xanthine oxidase by flavonoids. *J. Nat. Products* **1988**, *51*, 345−348.

(11) Hille, R.; Sprecher, H. On the mechanism of action of xanthine oxidase. *J. Biol. Chem.* **1987**, *262*, 10914−10917.

(12) Marx, G.; Chevion, M. Site-especific modification of albumin by free radicals. Reaction with cooper (II) and ascorbate. *Biochem. J.* **1986**, *236*, 397−400.

(13) Craig, D. B.; Arriaga, E. A.; Wong, J. C. Y.; Lu, H.; Dovichi, N. J. Studies on single alkaline phosphatase molecules: reaction rate and activation energy of a reaction catalyzed by a single molecule and effect of thermal denaturation. The death of an enzyme. *J. Am. Chem. Soc*. **1996**, *118*, 5245−5253.

(14) Silverman, R. B. *Mechanism-Based Enzyme Inactivators. In Contemporary Enzyme Kinetics and Mechanism*, 2nd ed.; Purich, D. L., Ed.; Academic Press: New York, 1996; pp 291−334.

(15) Stutz, A. E. A novel approach for clarifying the reaction mechanism of retaining glycoside hydrolases. *Angew. Chem. Intl.* **1996**, *35*, 1926−1928.

(16) McCord, J. M.; Fridovich, I. The reduction of cytochrome *c* by milk xanthine oxidase. *J. Biol. Chem.* **1968**, *243*, 5753−5760.

(17) Akaike, T.; Ando, M.; Oda, T.; Doi, T.; Ijiri, S.; Araki, S.; Maeda, H. Dependence on O-2 generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. *J. Clin. Invest.* **1990**, *85*, 739−745.

(18) Granger, D. N. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *Am. J. Physiol.* **1988**, *255*, 1269−1275.

(19) Hamer, I.; Wattiaux, R.; Coninck, S. W. Deleterious effects of xanthine oxidase on rat liver endothelial cells after ischemia/reperfusion. *Biophys. Acta* **1995**, *1269*, 145−153.

(20) Costantino, L.; Albasini, A.; Rastelli, G.; Benvenuti, S. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta. Med.* **1992**, *58*, 342−344.

(21) Cotelle, N.; Bernier, J. L.; Catteau, J. P.; Pommery, J.; Wallet, J. C.; Gaydou, E. M. Antioxidant properties of hydroxy-flavones. *Free Radic. Biol. Med*. **1996**, *20*, 35−43.

(22) Costantino, L.; Rastelli, G.; Albasini, A. A rational approach to the design of flavones as xanthine oxidase inhibitors. *Eur. J. Med. Chem*. **1996**, *31*, 693−699.

(23) Randic, M. In Search of Structural Invariants. *J. Math. Chem.* **1992**, *9*, 97−146.

(24) Kier, L. B.; Hall, L. H. *Molecular connectivity in structure−activity analysis*; Research Studies Press: Letchworth, England, 1986.

(25) Muñoz, C.; de Julián-Ortiz, J. V.; Gimeno, C.; Catalán, V.; Gálvez, J. Detección y ensayos de nuevos compuestos activos frente a virus Herpes Simplex tipo 1. *Rev. Esp. Quimioter.* **1994**, *7*, 279−280.

(26) Gálvez, J.; García, R.; de Julián-Ortiz, J. V.; Soler, R. Topological Approach to Drug Design. *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 272−284.

(27) Antón-Fos, G. M.; García-Domenech, R.; Perez-Gimenez, F.; Peris-Ribera, J. E.; García-March, F. J.; Salabert-Salvador, M. T. Pharmacological studies of the two new hypoglycaemic compounds 4-(4-methyl-5-oxo-2-pyrazolin-1-yl) benzoic acid and 1-(mesitylen-2-sulfonyl)1-H-1,2,4-triazole. *Arzneim-Forsch/Drug Res.* **1994**, *44*, 821−826.

(28) Gálvez, J.; García-Domenech, R.; de Gregorio-Alapont, C.; de Julián-Ortiz, J. V.; Salabert-Salvador, M. T.; Soler, R. *Advances in molecular similarity*; JAI Press Inc.: 1996; Vol. 1, pp 267−280.

(29) García-Domenech, R.; de Gregorio-Alapont, C.; de Julián-Ortiz, J. V.; Gálvez, J.; Popa, L. Molecular Connectivity to Design β-Blockers with Low Toxicity. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 567−572.

(30) Gálvez, J.; García, R.; de Julián-Ortiz, J. V.; Soler, R. Topological Approach to Analgesia. *J. Chem. Inf. Comput. Sci*. **1994**, *34*, 1198−1203.

(31) García-Domenech, R.; García-March, F. J.; Soler, R.; Gálvez, J.; Antón-Fos, G. M.; de Julián-Ortiz, J. V. New analgesics designed by Molecular Topology. *Quant. Struct.-Act. Relat*. **1996**, *15*, 201−207.

(32) Rios-Santamarina, I.; Gálvez, J.; García-Domenech, R.; Cortijo, J.; Santamaria, P.; Morcillo, E. New Bronchodilators Selected by Molecular Topology. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 477−482.

(33) Estrada, E.; Peña, P.; García-Domenech, R. Designing sedative/hypnotic compounds from a novel substructural graph-theoretical approach. *J. Comput. Aid. Mol. Des.* **1988**, *12*, 583−595.

(34) Pastor, L.; García-Domenech, R.; Wolski, S.; García, M. D.; Gálvez, J. New antifungals selected by molecular topology. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2577−2582.

(35) Gozalbes, R.; Gálvez, J.; Moreno, A.; García-Domenech, R. New Antimalarials Found by Molecular Connectivity. *J. Pharm. Pharmacol.* **1999**, *51*, 111−117.

(36) Gozalbes, R.; Gálvez, J.; García-Domenech, R.; Derouin, F. Molecular search of new active drugs against *Toxoplasma gondii*, SAR and QSAR. *Environ. Res.* **1999**, *10*, 47−60.

(37) Gálvez, J.; Gomez-Lechón, M. J.; García-Domenech, R.; Castell, J. V. New Cytostatic Agents Obtained by Molecular Topology. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2301−2306.

(38) Kier, L. B.; Murray, W. J.; Randic', M.; Hall, L. H. Connectivity Series Concept Applied to Density. *J. Pharm. Sci*. **1976**, *65*, 1226−1230.

(39) Kier, L. B.; Hall, L. H. *Molecular connectivity in chemistry and drug research*; Academic Press: London, 1976; pp 46−79.

(40) Gálvez, J.; García, R.; Salabert, M. T.; Soler, R. Charge Indexes. New Topological Descriptors. *J. Chem. Inf. Comput. Sci.* **1994**, *34*, 520−525.

(41) Collous, T. *Discriminant Analysis and Applications*; Academic Press: New York, 1973.

(42) Dixon, W. J. *Manual BMD 'Biomedical Computer Programs'*; University of California Press: Los Angeles, 1982.

(43) Pederiva, R.; Kavka, J.; D'Arcángelo, A. T. Chalconas y flavanonas aisladas de Zuccagnia punctata Cav. *Anal. Asociación Quím. Argentina* **1975**, *63*, 85−90.

(44) Wagner, H.; Farkas, L. *Synthesis the Flavonoids. In The Flavonoids, Part I*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds.; Academic Press: New York, 1975; p 131.

(45) Geissman, T. A.; Clinton, R. O. Flavanones and related compounds. I. The preparation of polyhydroxychalcones and flavanones. *J. Am. Chem. Soc*. **1946**, *68*, 697−700.

(46) Debattista, N. B.; Blanco, S. E.; Pappano, N. B.; Kavka, J.; Ferretti, F. H. Equilibrio químico y cinética de reacción: diseño de experiencias para su enseñanza simultánea. *Rev. Soc. Quim. Mex.* **1987**, *31*, 30−36.

(47) Holmer, S. A.; Houlton, C. L.; Westmoreland, T. D. A new irreversibly inhibited form of xanthine oxidase from ethylisonitrile. *J. Inorg. Biochem.* **1997**, *66*, 63−65.
(48) Morel, I.; Lescoat, G.; Cogrel, P.; Sergent, O.; Pazdecoup, N.; Brissot, P.; Cillard, P.; Cillard, J. Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. *Biochem. Pharmacol*. **1993**, *1*, 13−19.
(49) Salah, N.; Miller, N. J.; Paganga, G.; Tijburg, L.; Bolwell, G. P.; Evans, C. R. Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain-breaking antioxidants. *Arch. Biochem. Biophys*. **1995**, *2*, 339−346.
(50) Bors, W.; Hellers, W.; Michel, C.; Saran, M. *Radical chemistry of antioxidant flavonoids. In Antioxidants in therapy and preventive medicine*; Emerit, Ed.; Plenum Press: New York, 1990; Vol. 1, pp 165−170.
(51) Bors, W.; Hellers, W.; Michel, C.; Saran, M. Flavonoids as antioxidants. Determination of radical-scavenging efficiencies. In *Methods in enzymology*; Packer, L., Glazer, A. N., Eds.; Academic Press: San Diego, CA, 1990; Vol. 186, pp 343−355.
(52) Osada, Y.; Tsuchimoto, M.; Fukushima, H.; Takahashi, K.; Kondo, S.; Hasegawa, M.; Komoriya, K. Hypouricemic effect of the novel xanthine oxidase inhibitor, TEI-6720, in rodents. *Eur. J. Pharmacol*. **1993** Sep 14; *241*(2−3), 183−8.
(53) Chang, W. S.; Chang, Y. H.; Lu, F. J.; Chiang, H. C. Inhibitory effects of phenolics on xanthine oxidase. *Anticancer Res.* **1994** Mar-Apr; *14*(2A), 501−6.
(54) Foye, W. O. *Principios de Química Farmacéutica*; De. Reverté, SA: Barcelona, 1988; p 618.
(55) *The Merck Index*, 12th ed.; Budavari, S., Ed.; Merck: Rahway, NJ, 1996.

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