Molecular Factors Influencing the Affinity of Flavonoid Compounds on P-Glycoprotein Efflux Transporter

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Abstract: The most common mechanism of the so-called multidrug resistance (MDR), is mainly associated with an over expression of P-glycoprotein (Pgp). It is an ATP-dependent transport protein that limits the intracellular accumulation of a variety of structurally unrelated compounds within various organs and normal tissues such as kidney, small intestine and the blood brain barrier. Thus, the expression of Pgp has a major impact on the pharmacokinetic profile of many therapeutic agents and therefore, overcoming Pgp-mediated efflux constitutes an attractive means of potentially enhancing their therapeutic efficacy.

The flavonoids comprise a large group of polyphenolic compounds that occur in plants and vegetables, and they have been shown to display a wide variety of biological activities. For example, anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities.

The interactions between flavonoids and Pgp have also been extensively studied and some quantitative structure-activity relationships (QSAR) have been reported. In the present work, we have employed 2D-QSAR analysis to evaluate the interactions between Pgp and several flavonoid compounds with the aim of identifying the molecular factors responsible for the Pgp-binding affinity evidenced by these compounds. Thus, the reported data for dissociation constants (*KD*) between Pgp and 62 flavonoid compounds were modeled by mean of multiple regression analysis (MLR), and structures of the compounds under study were characterized by means of calculated physicochemical properties and several topological and constitutional descriptors, as well as geometrical and quantum chemical indexes. The obtained results suggest that the hydrophobic and especially geometric factors are of prime importance for binding, whereas in the case of flavonoid derivatives with flavone (flavonols), flavanone and isoflavone nuclei, the electronic factors are also involved in electron donor/acceptor interactions. In addition, in the case of chalcones, the results suggest that the affinity toward P-gp of such compounds is mainly governed by intermolecular dispersive interactions at the binding site.

Keywords: Flavonoids, multivariate quantitative structure-activity relationships (QSARs), nonempirical descriptors, P-glycoprotein (Pgp).

INTRODUCTION

The frequent failure of chemotherapy in cancer patients is often due to the occurrence of the so-called multidrug resistance (MDR), particularly in the case of recurrent or metastatic neoplasms. The most common mechanism of MDR is mainly due to the over expression of P-glycoprotein (Pgp), a member of the ATP binding cassette (ABC) super-family of transporters, coded by the MDR1 and MDR3 members of gene in humans [1, 2]. This xenobiotic efflux protein is expressed and distributed in various organs and normal tissues such as kidney, small intestine and blood brain barrier where it plays a detoxification function. Pgp, as with many ABC transporters, possesses an architecture of a transmembrane domain of six putative α-helices and an intracellular nucleotide-binding domain (NBD) for fixing ATP [3-5].

A large variety of hydrophobic or amphiphilic drugs, such as HIV inhibitors, calcium channel blockers, antibiotics and cancer chemotherapeutics, are transported by Pgp and their activity is responsible for limiting their oral bioavailability as well as tissues and organs permeation [6]. The principal characteristics shared by these substrates are hydrophobicity, positive charge and a planar structure for neutral compounds; however, negatively charged compounds have also been reported bind to Pgp [7-9]. Thus, the expression of Pgp has a major impact on the

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pharmacokinetic profile of many therapeutic agents and therefore, overcoming Pgp-mediated efflux constitutes an attractive means of potentially enhancing their therapeutic efficacy [10].

Flavonoids are a large class of naturally occurring compounds in practically all plants, like fruits and vegetables. Furthermore, they are widely used in beverages such as wine and tea, and in many dietary supplements or herbal remedies such as *Ginkgo biloba*. They are also components of citrus fruits [11] and other food sources [12] which are consumed regularly with the human diet.

The flavonoids have long been recognized to display a wide variety of biological activities, such as anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities [13-18].

Since the early 1990s, several studies have investigated the interactions of flavonoids with Pgp efflux system. A comprehensive review on this topic corresponds to a work published by Morris and Zhang [19]. Because Pgp can interact with a large number of structurally diverse compounds, which suggests the existence of multiples drug-binding sites, the group of Di Pietro *et al*. [20, 21] demonstrated that certain flavonoids bind to the C-terminal nucleotide-binding domain of Pgp (NBD2), and the binding may partly overlap the ATP binding site and a vicinal hydrophobic region (steroid binding site) within the cytosolic domain of Pgp. Some years later, the same group (Boumendjel *et al*. [22]) studied the inhibitory effects of several flavonoid derivatives on Pgp-mediated drug transport by using an *in vitro* test for measuring their binding affinity toward the purified recombinant NBD2 protein of mouse Pgp.

The quantitative structure-activity relationships (QSAR) have found wide utility and acceptance in the drug design process, since they help to identify the physicochemical properties which govern the biological activities.

Some QSAR studies for flavonoid–Pgp interaction have been reported [23-26]. An interesting 3D-QSAR study has been recently conducted by Boccard *et al*. [27]. They derived a 3D linear solvation energy model for the set of flavonoids studied by Boumendjel and coworkers, and from the obtained results, the authors concluded that the size and molecular shape play a predominant role in the Pgp affinity exhibited for these compounds. A similar conclusion has also been drawn by Kothandan *et al*. [28] by using molecular docking and 3D-QSAR to evaluate the Pgp affinity of 42 flavones studied by Boumendjel *et al*. [22].

Among different methods for quantifying the size and shape characteristics of molecules, those based on the chemical graph theory have been found useful in establishing QSAR and QSPR models [29-31].

In the present work, we have employed quantitative structure-activity relationships (QSAR) analysis to more critically evaluate the interactions between Pgp and several flavonoid compounds with the aim of identifying the molecular factors responsible for the Pgp-binding affinity evidenced by these compounds; and also, provide additional guidance to directions of future molecular design for this class of compounds. The reported data for dissociation constants (*KD*) between Pgp and 62 flavonoid compounds were modeled by means of multiple regression analysis (MLR), and structures of the compounds under study were characterized by means of calculated physicochemical properties and several topological and constitutional descriptors, as well as geometrical and quantum chemical indexes.

MATERIALS AND METHODS

Biological Data

Data on Pgp binding affinity, which are expressed as dissociation constant KD, were taken from Boumendjel and coworkers [22]. The log 1/KD values expressed in molar concentrations were used as dependent variable for the development of QSAR models.

Structural Descriptors

The following descriptors were calculated to characterize the compounds under study: molar volume (Vm), molecular weight (MW), and molar refractivity (MR). To explain lipophilicity effects, the octanol-water partition coefficient, as MLOGP, was calculated by using the atomic parameters method such as implemented in the Dragon software, and also by using the Interactive Analysis LogP and LogW (water solubility) predictor website [32]. The other group of structural descriptors included several quantum chemical indexes. The compound starting geometries were built in a fully extended conformation within HyperChem package (release 7.5 for Windows). The 3D molecular structures were obtained by energy minimization using the MM+ molecular mechanics potential-energy function. In a follow-up procedure, a complete optimization of the geometrical parameters was carried out by using the AM1 method implemented in the standard version of MOPAC 7.0. The following indexes were used: total energy (*Etotal*), heat of formation (ΔHf), energy of highest occupied molecular orbital (*HOMO*), energy of lowest unoccupied molecular orbital (*LUMO*), dipole moment (μ), the absolute total charges (Qtotal), the most positive and the most negative absolute charges (qpmax, qnmax), and the positive and negative relative charge (RNCG, RPCG). Finally, several geometrical and topological indexes were used, such as, the Wiener index, the valence and connectivity molecular indexes, the kappa shape indexes and several geometrical indexes calculated from the optimized distance matrix AM1 by using the Dragon software.

Statistical Methods

Multiple regression analysis (MLR) is the method used here to search for relationships between the biological activity data and the structural descriptors. MLR analysis was performed by using the 15.1 version of Minitab software.

RESULTS AND DISCUSSION

Several subgroups were analyzed before the construction and derivation of a general QSAR model for the 62

flavonoids under study. The objective of constructing MLR models for the members of each group was to determine if additional information is captured by the subset models, as compared to the general model.

Initially, only a small group of 12 flavonoids was analyzed, with the particularity that such a group included the 4 basic nuclei of the studied compounds, namely: flavone, chalcone, flavanone and isoflavanone. Table **1** shows the analyzed structures and the corresponding KD values. Considering previous studies showing that lipophilicity is an important factor in determining the binding affinity of these compounds [27, 28], the following quadratic equation has been found between log 1/KD and MLOGP:

$$\log 1/KD = 6.290 - 1.852\ MLOGP + 0.534\ (MLOGP)^2$$
$$(0.000)\quad (0.008)\qquad (0.008)$$

$$R^2 = 0.596\ \ r = 0.772\ \ rcv = 0.490\ \ s = 0.295\ \ n = 12\ \ F = 5.95 \quad (1)$$

In these and the following equations, n is the number of compounds, s is the standard deviation, R^2 is the squared correlation coefficient, r is the correlation coefficient, F is the Fisher F-statistic, and the figures in parentheses are the P-values of coefficients. The rcv is the cross-validation coefficient which describes the predictive power of derived models.

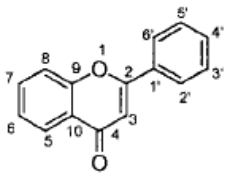
Though significant from a statistical point of view, the obtained equation shows a really modest adjustment quality, since it only accounts for 59.6% of the log 1/KD variance. However, as it is widely known, the molecular size is a prevailing contribution to molecular hydrophobicity. Therefore, we searched for a model with better adjustment for this data set by using descriptors related to the molecular size. Because of the large number of descriptors considered, a stepwise multiple regression procedure based on the forward-selection and backward-elimination methods was used for inclusion or rejection of descriptors in the screened models. In order to avoid overestimations or difficulties in interpretation of the resulting models, pairs of variables with an $r \geq 0.75$ were classified as intercorrelating ones, and only one of these was included in the screened model. Besides, considering the limited quantity of data analyzed in this subgroup (n =12), we searched for correlations including only a maximum of two molecular descriptors. After some consideration, the following equation was found:

$$\log 1/KD = -0.301 + 0.0544\ MR - 2.1319\ ELUMO$$
$$(0.548)\quad (0.000)\qquad (0.000)$$

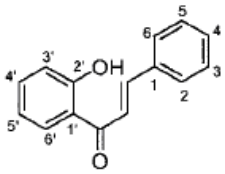
$$R^2 = 0.933\ \ r = 0.967\ \ rcv = 0.935\ \ s = 0.116\ \ n = 12\ \ F = 62.95 \quad (2)$$

To note that MR and ELUMO descriptors are not intercorrelated (r = 0.043), and even though this equation was based on a very small number of data points, both terms are highly significant statistically. This equation clearly shows the positive dependence of log 1/KD on the molecular size as reflected by molar refractivity (MR). On the other

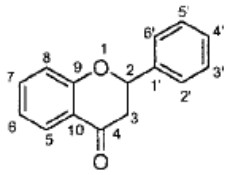
Table 1. First set of flavonoids under study.



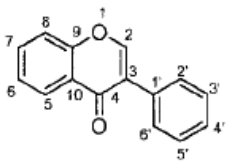
Flavone



Chalcone



Flavanone



Isoflavone

Number	Flavonoid	Substituent					logKD	logKD
		3	4	5	7	4'	(Obs.)	(Calc.)[a]
1	Flavone						4.467	4.491
2	3-Hidroxyflavone	OH					4.995	4.808
3	7-Hidroxyflavone				OH		4.457	4.588
4	Chrysin			OH	OH		5.050	4.917
5	Apigenin			OH	OH	OH	4.995	5.019
6	Galangin	OH		OH	OH		5.275	5.226
7	Kaempferol	OH		OH	OH	OH	5.174	5.309
8	Kaempferide	OH		OH	OH	OCH3	5.347	5.418
9	Chalcone		OH			OH	5.337	5.415
10	Chalcone		OCH3			OH	5.638	5.511
11	Flavanone			OH	OH	OH	4.437	4.422
12	Isoflavone			OH	OH	OH	4.576	4.624

(a) Calculated values by using equation 2.

hand, the ELUMO index (unoccupied molecular orbital energy) was the other parameter necessary to achieve a clear distinction between active and inactive compounds. The Fig. (**1**) shows the relation between log 1/KD and both parameters used in this equation. The numbering of data shown in that figure corresponds to the lists on Table **1**. The relation between 1/KD and ELUMO shows that those compounds presenting a lower energy in the LUMO orbital will act as strong electron acceptors, and thus, they will display a strong Pgp binding affinity. This is particularly observable in the case of flavonoids with a flavone nucleus, in contrast to isoflavone and flavanone nuclei. In the case of chalcones, the high inhibitory activity observed is explained by the high polarizability they present in relation to other flavonoid nuclei.

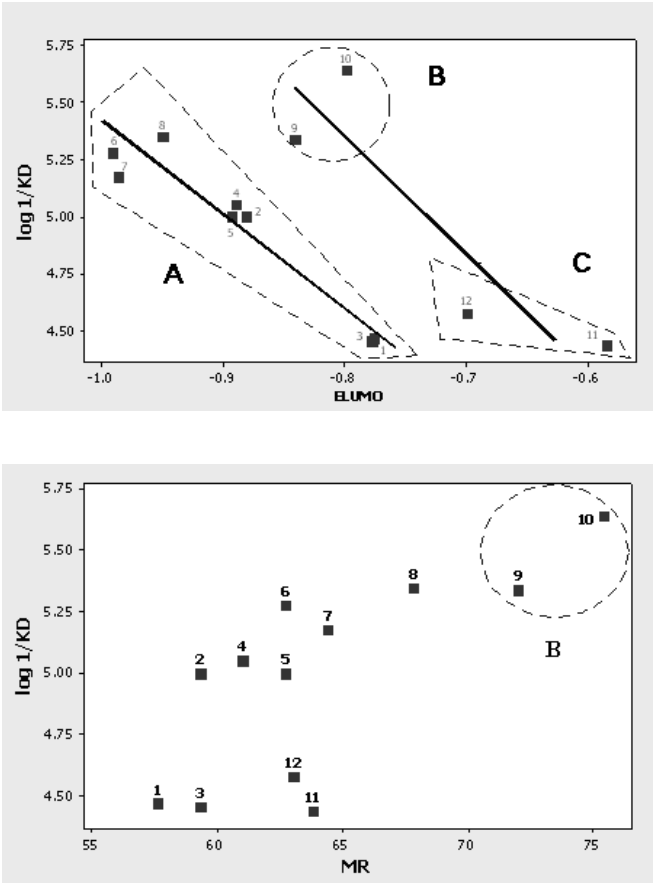
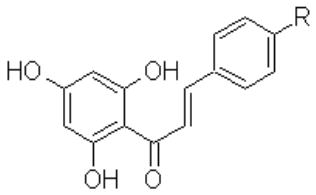


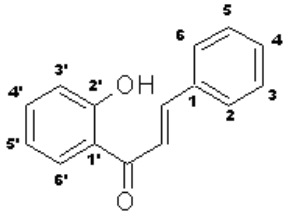
Fig. (1). Relationships between the experimental log 1/KD values and the molecular descriptors MR and ELUMO used in equation 2. **A**: Flavones, **B**: Chalcones, **C**: Isoflavone and Flavanone.

The second subgroup of analyzed flavonoids consisted of 20 chalcones whose structures and KD values are shown in the Table **2**. Following an analysis similar to that conducted for the previous subgroup, the following cubic equation was found between log 1/KD and MLOGP:

Table 2. Second set of flavonoids under study.



Number	R	logKD (obs.)	logKD (calc.)[a]
13	H	5.337	5.222
14	OH	5.319	5.441
15	OCH3	5.638	5.689
16	F	5.444	5.440
17	Cl	5.886	5.655
18	Br	6.244	6.078
19	I	6.602	6.392
20	O-n-C2H5	5.678	5.970
21	O-n-C4H9	6.000	6.550
22	O-n-C6H13	6.569	7.037
23	O-cyclohexyl	6.270	6.142
24	O-n-C8H17	7.699	7.283
25	O-n-C10H21	7.222	7.126
26	O-n-C14H29	4.848	4.918



Number	Substitution	logKD (obs.)	logKD (calc.)[a]
27	H	5.051	4.988
28	5'-(1,1-DMA)[b]	6.357	6.171
29	4-OH	4.936	5.219
30	3-Prenyl	6.276	6.309
31	4-OCH3	5.738	5.473
32	3-Prenyl-4-OCH3	6.569	6.576

(a) Calculated values by using equation 5. (b) DMA, dimethylallyl.

The above regression shows that there is a significant non-linear relationship between log 1/KD and MLOGP, which clearly shows the existence of an optimal lipophilicity

$$\log 1/KD = 10.352 - 4.461\ MLOGP + 1.239\ (MLOGP)^2 - 0.097\ (MLOGP)^3$$
$$(0.000) \quad (0.073) \quad (0.022) \quad (0.008)$$
$$R^2 = 0.769 \quad r = 0.877 \quad rcv = 0.719 \quad s = 0.391 \quad n = 20 \quad F = 17.79 \qquad (3)$$

range for Pgp-flavonoid binding affinity (between 5 and 6 MLOGP values). However, considering the structural variation for this group of chalcones (to see Table **2**), the lipophilicity of these compounds, which is expressed by MLOGP, strongly correlates with descriptors that codify the molecular size. Thus, the intercorrelation between MLOGP and SASA parameters (Solvent Accessible Surface Area) or the molecular size (Mw) was 0.902 y 0.942, respectively. Consequently, various QSAR models between log 1/KD and descriptors related to molecular size were investigated. The selected model included the Tm geometrical descriptor, which belongs to the group of so-called WHIMs (*Weighted Holistic Invariant Molecular Descriptors*) [33]. These descriptors are constructed in such a way they can collect relevant information at a 3D molecular level, considering aspects like molecular size, shape, symmetry and atom distribution in relation to invariant reference frameworks. The selected descriptor, Tm, which belongs to the group of Global WHIM indices, encodes on total molecular size. Therefore, the following quadratic equation, highly significant from a statistical point of view, was generated:

$$\log 1/KD = 3.220 + 0.168\ Tm - 0.002\ (Tm)^2$$
$$(0.000)\ \ (0.000)\qquad (0.000)$$
$$R^2 = 0.789\ \ r = 0.888\ \ rcv = 0.707\ \ s = 0.362\ \ n = 20\ \ F = 31.79 \quad (4)$$

On analyzing this quadratic equation, it can be seen that increasing Tm value (increasing size) the Pgp-flavonoid binding increases; however, there is a maximum binding encountered when an optimum size is reached (to see Table **2**). From a physicochemical point of view, dependence of log 1/KD on Tm clearly reflects the decisive role played by Van der Waals non-specific interactions in the Pgp affinity of the compounds under study.

On the other hand, comparison of the equations 3 and 4 leads to the conclusion that binding power will be stronger if hydrophobicity or molecular size increases. However, even when it is clear that both molecular factors are very important in the Pgp-flavonoid binding, they do not completely account for the variability shown by the chalcones under study. For this reason, and trying to avoid exceeding the maximum data/variables ratio allowed, models with three independent variables were investigated. After some consideration, the following equation was found:

$$\log 1/KD = -0.082 + 0.167\ Tm - 0.002\ (Tm)^2 + 1.423\ J3D$$
$$(0.928)\qquad (0.000)\qquad (0.000)\qquad (0.001)$$
$$R^2 = 0.891\ \ r = 0.944\ \ rcv = 0.843\ \ s = 0.268\ \ n = 20\ \ F = 43.59 \quad (5)$$

The statistical quality of the derived model is very good, and the presence of the geometric descriptor J3D suggests that the molecular shape also plays an important role in the affinity of chalcones over Pgp. The J3D descriptor (Balaban 3D index) is calculated from the geometrically optimized distance matrix, and it encodes the different aspects of the molecular shape. Thus, considering that the coefficient value for this parameter is positive, it is possible to infer that the more elongated molecules will have a higher J3D value and will consequently interact more strongly at the Pgp binding site. Another aspect worth mentioning is that the intercorrelation between Tm and J3D is low (r = 0.237), which allows a clear interpretation of the obtained model. The Fig. (**2**) shows the tridimensional relation of the variables used in the derived model.

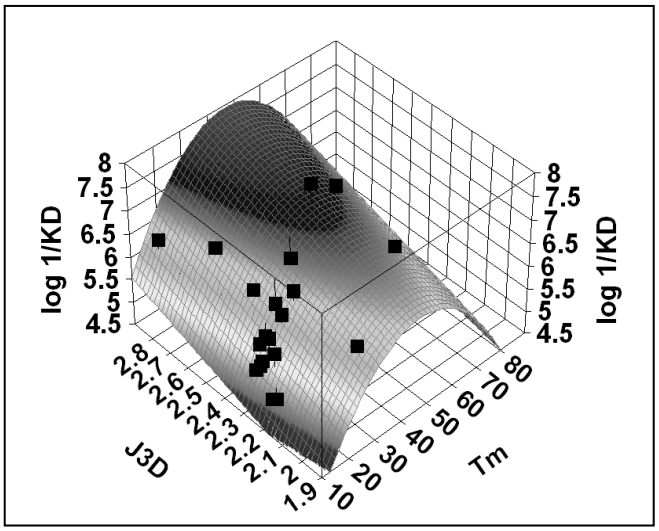


Fig. (2). Three-dimensional plot of experimental log 1/KD values and the parameters Tm and J3D calculated for the 20 analyzed Chalcones, and used in equation **5**.

A way of assessing the robustness of the obtained models is to construct a more general model including the analysis of the 12 previously studied flavonoids. Thus, the new set of 32 flavonoid compounds allowed deriving the following QSAR model:

$$\log 1/KD = 6.559 + 0.144\ Tm - 0.00156\ (Tm)^2$$
$$(0.007)\qquad (0.000)\qquad (0.000)$$
$$+ 0.798\ J3D - 0.586\ GAP$$
$$(0.004)\qquad (0.037)$$
$$R^2 = 0.884\ \ r = 0.940\ \ rcv = 0.750\ \ s = 0.293\ \ n = 32\ \ F = 51.56 \quad (6)$$

It should be noted that the molecular information contained in Eq. 5 is similar to that obtained from Eq. 6, both regarding statistical quality and signs of the coefficients. However, taking into account the presence of LUMO parameter in Eq. 2, which reflect the electronic factors involved in the interaction between the previously studied 12 flavonoids and Pgp, the inclusion of GAP term in Eq. 6 was required. This parameter, which corresponds to the difference between the HOMO and LUMO energies, gives information related to molecule reactivity/stability. Thus, it is apparent from the obtained equation that lower GAP energy of flavonoid compound will generally result in a stronger electron donor-acceptor interaction with Pgp and, therefore, in a higher affinity for Pgp.

The third subgroup of analyzed flavonoids consisted of 13 flavonols and 17 flavones, whose structures and KD values are shown in Tables **3** and **4**, respectively. Following an analysis similar to that conducted for the previously studied subgroups, we searched for a relation between log 1/KD and the octanol-water partition coefficient. In this case, a statistically accepted correlation was not found, since the group of complex flavonoids derived from sibilin (compounds 40-45) clearly forms a subgroup with respect to the rest of the analyzed flavonoids. In order to illustrate this

fact, the relationship between the log 1/KD and MLOGP values is depicted in Fig. (**3**). As shown in Table **3**, the sibilin-derived flavonols present a larger size and structural complexity in comparison with the rest of the analyzed flavonoids. Thus, with the aim of finding a model which accounts for these compounds, the following equation was derived:

$$\log 1/KD = 24.655 + 0.483\ MLOGP - 6.568\ (X3A/SASA)$$
$$(0.000) \quad (0.000) \quad . \quad 000)$$
$$- 1.931\ EHOMO$$
$$(0.004)$$
$$R^2 = 0.875\ \ r = 0.935\ \ rcv = 0.919\ \ s = 0.328\ \ n = 30\ \ F = 60.80 \quad (7)$$

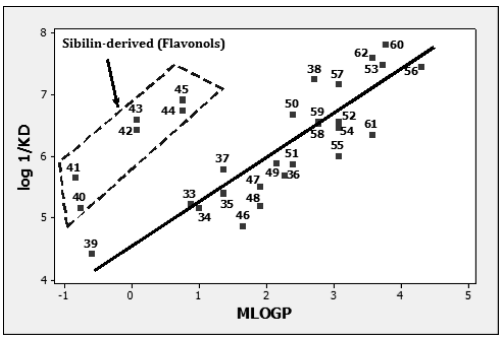
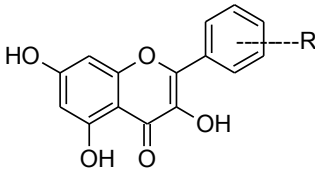
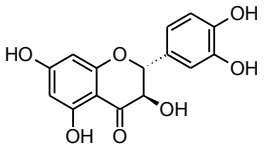


Fig. (3). Relationships between the experimental log 1/KD values and the MLOGP parameter calculated for the flavonoids shown in Tables **3** and **4**.

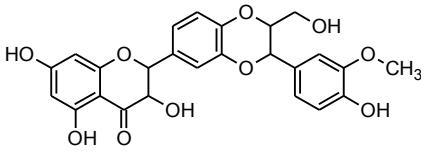
Table 3. Third set of studied flavonoids: 13 flavonols.



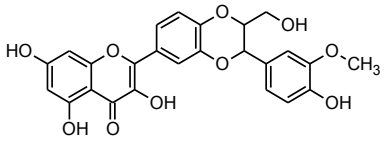
Number	R	logKD (obs.)	logKD (calc.)[a]
33	4'-H (galangin)	5.229	5.293
34	4'-F	5.167	5.281
35	2',4'-Cl	5.398	5.323
36	PhR= -CH(Ph)$_2$	5.699	6.205
37	4'-I	5.796	5.501
38	4'-n-C8H17	7.244	7.199



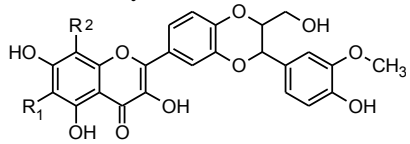
Taxifolin



Silybin



Dehydrosilybin



Number	Flavonoid	R1	R2	logKD (Obs.)	logKD (Calc.)[a]
39	Taxifolin			4.427	4.595
40	Silybin			5.167	5.248
41	Dehydrosilybin			5.657	5.673
42	Prenylated Dehydrosilybin	Prenyl	H	6.431	6.416
43	Prenylated Dehydrosilybin	H	Prenyl	6.602	6.349
44	Geranylated Dehydrosilybin	Geranyl	H	6.744	6.877
45	Geranylated Dehydrosilybin	H	Geranyl	6.920	6.823

(a) Calculated values by using equation 7.

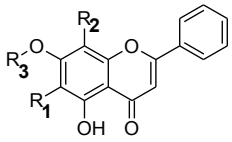
This equation is highly significant statistically and there are no strong intercorrelations among the parameters included, which is fundamental to reach a correct physicochemical interpretation. The correlation between the pairs of variables is the following: MLOGP/EHOMO -0.394; MLOGP/(X3A/SASA) 0.068; and (X3A/SASA)/EHOMO -0.632. As it can be observed, the third-order average molecular connectivity index (normalized to the compound's size and calculated by dividing the X3A value by the solvent-accessible surface area, SASA) is one of the important parameters used in this QSAR model. Molecular connectivity indices, in general, codify aspects of size and molecular shape. In this case, as the X3A index is normalized to the molecular size, the aspects of molecular shape become more important in the context of the information carried by this index. It is also interesting to note that the information codified by the Balaban index (J3D) used in the previous models is, in a way, part of the X3A/SASA index, since it codifies the molecular length (lowest values of X3A/SASA) and informs the type and the number of branches present in the molecule. Thus, considering the conducted analysis and the negative value of the regression coefficient obtained for X3A/SASA, it is possible to hypothesize that as the molecular length increases, there will be a lower degree of branching, less adjacent branching points, and the flavonoid will show a more intense Pgp binding affinity. The other significant parameter is EHOMO molecular orbital energy, which shows that the Pgp-flavonoid interaction is also ruled by electronic interactions, particularly by electron donor/acceptor interactions.

Finally, with the purpose of obtaining a general QSAR model for the complete set of analyzed flavonoids, we studied models including the variables used for the subgroups which we have formerly examined. The equation of minimal complexity in which all terms are of high level of significance, is as follows:

$$\log 1/KD = 16.062 + 0.443\ MLOGP + 0.066\ Tm$$
$$(0.000) \quad (0.000) \quad (0.000)$$
$$- 0.00105\ (Tm)^2 - 6.931\ (X3A/SASA) - 1.169\ GAP$$
$$(0.000) \quad (0.000) \quad (0.000)$$
$$R^2 = 0.873\ \ r = 0.934\ \ rcv = 0.861\ \ s = 0.331\ \ n = 62\ \ F = 76.97 \quad (8)$$

The developed general model shows a very good data-fitting/predictive ability, which may also be demonstrated by the direct comparison between the experimental and calculated log 1/KD values given in Fig. (**4**). On the other hand, as can be observed in Table **5**, the parameters show an acceptable intercorrelation degree, lower than 0.70, a value which was taken as a threshold to include or reject the incorporation of a variable in the analyzed regression equation.

Table 4. Third set of studied flavonoids: 17 flavones.



Number	R1	R2	R3	logKD (Obs.)	logKD (Calc.)[a]
46	H	H	H	4.883	4.968
47	CH3	H	H	5.509	5.429
48	H	H	CH3	5.201	5.402
49	CH3	H	CH3	5.886	5.985
50	i-Pr	H	H	6.678	6.017
51	H	H	i-Pr	5.879	5.953
52	i-Pr	H	i-Pr	6.553	6.960
53	i-Pr	i-Pr	i-Pr	7.481	7.634
54	Bn	H	H	6.469	6.421
55	H	Bn	H	6.004	6.437
56	Bn	Bn	H	7.444	7.671
57	H	H	Bn	7.167	6.463
58	3,3-DMA[b]	H	H	6.523	6.346
59	H	3,3-DMA	H	6.553	6.383
60	3,3-DMA	3,3-DMA	H	7.824	7.534
61	geranyl	H	H	6.347	7.026
62	H	geranyl	H	7.602	7.071

(a) Calculated values by using equation 7. (b) DMA, dimethylallyl.

Finally, the fact that different subgroups of analyzed flavonoids yield equations with similar structural information provides further evidence that the results obtained are not artifactual.

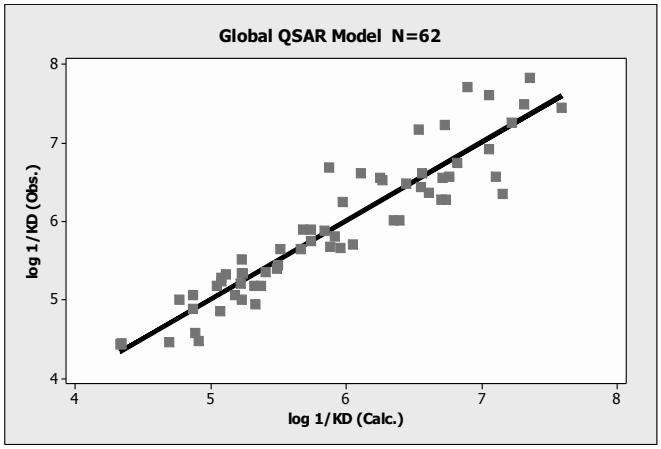


Fig. (4). Relationships between the experimental and calculated log 1/KD values obtained for the 62 flavonoids modeled by equation 8.

Table 5. Correlation matrix for descriptors used in equation 8 (n = 62).

	MLGOP	Tm	X3A//SASA
TM	0.170		
X3A/SASA	-0.046	-0.642	
GAP	0.333	-0.331	0.405

In summary, the developed QSAR models clearly indicate the main molecular factors that govern the flavonoid-Pgp binding. Thus, the obtained results suggest that the hydrophobic and especially geometric factors have prime importance in binding, whereas in the case of flavonoid derivatives with flavone (flavonols), flavanone and isoflavone nuclei, the electronic factors are also involved *via* electron donor/acceptor interactions. In addition, in the case of chalcones, the results suggest that the affinity towards P-gp of such compounds is mainly governed by intermolecular dispersive interactions at the binding site. To this end, another interesting point to highlight is that obtained results in this study are in agreement with those reported by Carrupt *et al*. [27] and by Kothandan *et al*. [28], but using a simpler and computationally, not a demanding semi-empirical method.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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