Structure−Activity Relationships for The Anti-HIV Activity of Flavonoids

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Quantitative structure−activity relationship (QSAR) models are useful in providing a biochemical understanding of the biological activity of natural and synthetic chemicals based solely on molecular structure. One- to three-parameter multiregression equations were generated for three different groups of flavonoids to model experimental flavonoid-induced cytotoxicity and inhibition of human immunodeficiency virus I replication in lymphocyte-infected cells, using quantum chemical and geometrical parameters derived from optimized molecular structures. Both biological properties were basically dependent on electronic parameters describing charge distribution on the two fused rings of the flavonoid molecule. Atomic charges in C3 and the carbonyl carbon as well as the dipolar moment were important electronic descriptors to define the studied biological properties of flavonoids.

INTRODUCTION

Flavonoids are natural products derived from plants that are found in many genus such as *Citrus*,[1] *Calluna*,[2] and *Erythrina*.[3] These compounds have been reported to display a variety of biochemical properties including antioxidant activity,[4] inhibition of tyrosine kinases[5] and cAMP phosphodiesterase,[6] and induction of phase II metabolizing enzymes both in vivo and in vitro.[7] These biochemical interferences elicited by flavonoids in some cell systems have been associated with their capacity to control cell growth or destroy pathogen organisms such as fungi[3,8] and viruses.[9] One of the most interesting biological properties of flavonoids is their ability to inhibit human immunodeficiency virus (HIV) transcriptase and HIV replication.[10]

The diversity in molecular architecture for flavonoid-like compounds has made possible the development of different quantitative structure−activity relationships (QSAR), allowing the identification of those molecular parameters responsible for their biological and physicochemical properties.[5,11−13] The goal of this paper is to present statistical relationships between molecular structure-derived parameters and the anti-HIV-I properties of flavonoids, to understand the chemical mechanisms associated with this biochemical effect.

METHODS

Data Set. Experimental data used in this study were taken from literature[14] and consisted of both pharmacological and cytotoxicity activities. Pharmacological data accounted for the ability of flavonoids to inhibit HIV replication in infected human lymphocytes (H9), measured as the flavonoid concentration that inhibits virus replication by 50% (EC_{50}). The cytotoxic potencies of the compounds were measured as the concentration of flavonoid required to decrease uninfected lymphocyte cell growth to 50% of the untreated cell culture (IC_{50}). Bioactivity data were given in μM concentrations for three different groups of flavonoids: group 1 consisted of flavonoids isolated from *Chrysanthemum morifolium*, group 2 included known natural flavonoids, and group 3 comprised synthetic flavonoids. Molecular structures and both IC_{50} and EC_{50} data for all three groups are shown in Tables 1−3, respectively. Those IC_{50} and EC_{50} values reported as "greater than" were not included in the data set, and for QSAR model construction the values were transformed to Log(IC_{50}) and Log(EC_{50}), respectively.

Descriptor Generation. A molecular descriptor is a numerical representation of the structure which describes a motif within the structure or the structure itself as a whole. They can be obtained in either empirical or nonempirical ways. In this study, molecular descriptors were calculated from computational calculations on the 3D molecular structure. Preliminary atomic coordinates for flavonoids were calculated using the program Hyperchem 5.1 (Hypercube, Inc., 1998). Structures were drawn as 2D structures and three-dimensional geometries were obtained after full optimization using molecular mechanics. Atomic coordinates were then transferred to Gaussian 94,[15] and all structures were submitted to a final geometry optimization and charge calculation using the AM1 Hamiltonian method.[16] Descriptor values were generated by single point calculations. The following computed quantum chemical descriptors were calculated: total energy, energy of the highest occupied molecular orbital (E_{HOMO}), energy of the lowest unoccupied molecular orbital (E_{LUMO}), the difference between E_{HOMO} and E_{LUMO} (GAP), dipolar moment (dipole), net atomic charges such as the most negative (MNC) and most positive charge (MPC), atomic charges for specific atoms (QA1, QA2, ..., QAn) within the flavonoid skeleton (Figure 1), the sum of more than one atomic charge belonging to an specific moiety, and the heat of formation (ΔH), among others. Geometrical descriptors comprise moments of inertia 1 (MI-1), 2 (MI-2), and 3 (MI-

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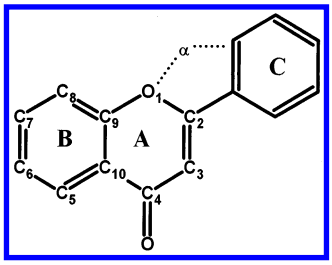


Figure 1. Flavonoid skeleton showing the atom numbers for which atomic charges were calculated.

3).[17] MI is the moment due to the various nuclei about each axis. Mathematically, MI is defined as $\Sigma m_i r_i^2$, where r_i is the perpendicular distance of the nucleus of mass m_i from the axis. Molecular lipophilicity (LogP) and solvent accessible surface area (SASA) were calculated using the program ChemPlus integrated in Hyperchem.

Statistical Analysis. QSAR models were developed by multivariate regression. This procedure estimates the value of a dependent variable (predicted biological property) from independent variables represented by the different molecular descriptors. These mathematical relationships were computationally generated by Forward Stepwise Multiple Regression techniques, resulting in the generation of an equation of the form

$$\text{biological property} = (a_0 \pm \Delta a_0) + (a_1 \pm \Delta a_1)D_1 + (a_2 \pm \Delta a_2)D_2 + ... + (a_i \pm \Delta a_i)D_i$$

where D_1, D_2, and D_i are the molecular descriptors, a_0, a_1, a_2, and a_i, the multiregression coefficients, and Δa_0, Δa_1, Δa_2, Δa_i the coefficient errors.[18] Best models were selected based on the multiple correlation coefficient (R), that is the correlation coefficient of the regression equation with all variables entered, the standard error of estimation (SE), and the value of F-ratio.[19]

RESULTS AND DISCUSSION

An attempt to develop a QSAR model including all the compounds belonging to the three flavonoid groups was not successful, probably due to the fact that the mechanisms of flavonoid interaction with subcellular components might differ between groups of molecules. To establish how the molecular descriptors and the anti-HIV activity could be globally interrelated, a hierarchical cluster analysis including nonredundant descriptors and EC_{50} data was performed. Nonredundant descriptors were generated calculating pairwise correlations between all calculated descriptors, and from each pair with R greater than 0.9, the descriptor with the lower R when correlating with the EC_{50} was removed from cluster analysis.[19] The resulting dendrogram is shown in Figure 2. This graph reveals molecular descriptors for flavonoids can be grouped in two main clusters. The first cluster suggests that the bioactivity of flavonoids against HIV-1 replication depends on a complex combination of multiple molecular parameters mostly describing several particular atomic charges on the flavonoid skeleton, the dipolar moment, and the moment of inertia I. On the other hand, the second cluster of variables shows that atomic charges, defined by group substitutions, determine the energy of the frontier orbitals and the (LogP). The electrostatic

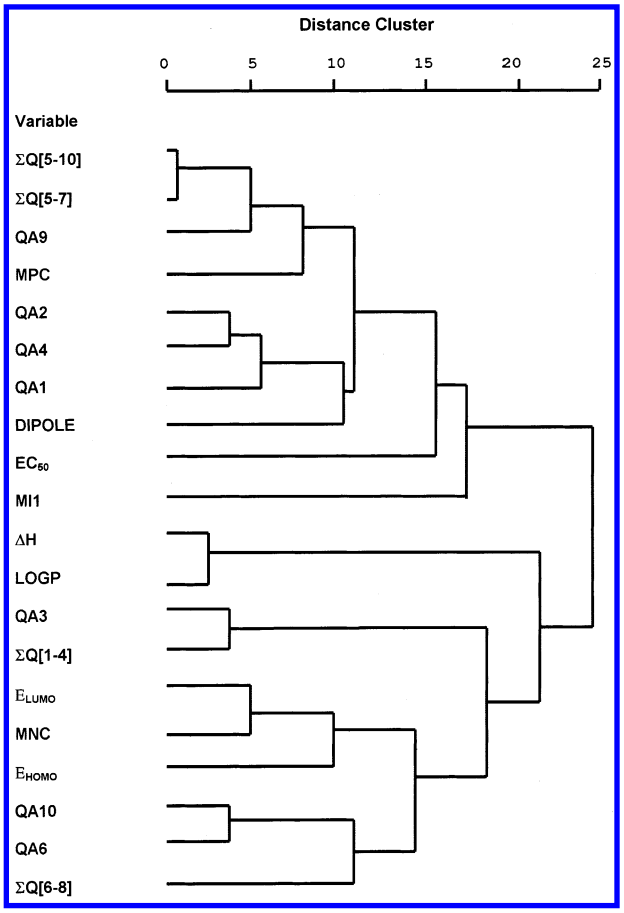
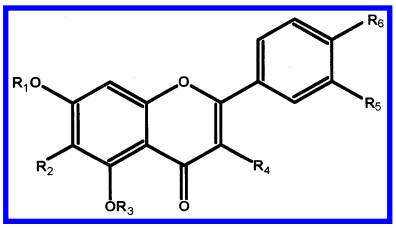


Figure 2. Dendogram from correlation cluster analysis obtained using average linkage between groups for nonredundant descriptors calculated for all the flavonoids in the data set and the bioactivity against HIV-1 (EC_{50}).

nature of atomic charges and the geometrical nature of dipolar moment suggest that flavonoid anti-HIV activity results from a specific electrogeometrical arrangement of atoms within the flavonoid, that interacts tridimensionally with a receptor-like structure directly in the HIV or in the host cell, the lymphocyte.

QSAR models and their statistical parameters, including the internal validation coefficient (R_{cross}), for the biological activities of each one of the three groups are shown in Table 4. All the descriptors included in the models showed low multicollinearity according to the correlation matrix presented in Table 5. The observed vs predicted values for Log(IC_{50}) and Log(EC_{50}) of flavonoids are plotted in Figure 3. Multiregression equations for the flavonoids included in group 1 showed that both Log(IC_{50}) and Log(EC_{50}) are a function of MPC. Most of the flavonoids in the data set have the MPC in the carbonyl carbon present in ring A, with the exception of some flavonoid-containing acetyl groups and the electron withdrawing group $-NO_2$. The atomic charge in the carbonyl carbon is important because although it is not connected to any hydrogen, it might be a valid measure of intramolecular hydrogen bonding ability on the carbonyl oxygen.[20] On the other hand, this positive charge in the carbon invokes the mechanism of an electrophilic interaction on a nucleophilic center at the active site. The analysis of variance revealed a statistical significance at $P = 0.006$ for the Log(IC_{50}) and 0.05 for Log(EC_{50}). Although the signifi-

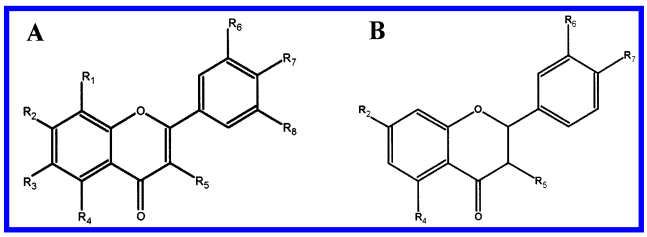
Table 1. Molecular Structure and Bioactivity of Flavonoids[a]



molecules	R_1	R_2	R_3	R_4	R_5	R_6	IC_{50}	EC_{50}
acacetin-7-*O*-β-D-galactopyranoside	gal	H	H	H	H	OMe	37	8
apigenin-7-*O*-β-D- galactopyranoside	gal	H	H	H	H	OH	115	61
luteolin	H	H	H	H	OH	OH	16	10
quercetin	H	H	H	OH	OH	OH	132	132
luteolin-7-*O*-β-D-glucopyranoside	glu	H	H	H	OH	OH	25	7
acetate of luteolin-7-*O*-β-D-glucopyranoside	tetraAc-glu	H	Ac	H	OAc	OAc	6	6
baicalin	glucosiduronyl	OH	H	H	H	H	72	112

[a] Group 1. Compounds isolated from *C. morifolium*. IC_{50} = concentration (μM) that inhibits uninfected growth of H9 lymphocytes by 50%. EC_{50} = concentration (μM) that inhibits HIV-1 replication in H9 lymphocytes by 50%.

Table 2. Molecular Structure and Bioactivity of Flavonoids[a]



molecules	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	IC_{50}	EC_{50}
				Structure A						
flavone	H	H	H	H	H	H	H	H	68	50
7,8-dihydroxyflavone	OH	OH	H	H	H	H	H	H	14	10
chrysin	H	OH	H	OH	H	H	H	H	47	5
apigenin	H	OH	H	OH	H	H	OH	H	35	9
3-hydroxyflavone	H	H	H	H	OH	H	H	H	17	13
fisetin	H	H	OH	H	OH	H	OH	H	157	122
myricetin	H	OH	H	OH	OH	OH	OH	OH	69	35
				Structure B						
flavanone		H		H	H	H	H		45	58
galangin		OH		OH	OH	H	H		44	28
4′,5,7-trihydroxyflavone		OH		OH	H	H	OH		294	92

[a] Group 2. Known flavonoids. IC_{50} = concentration (μM) that inhibits uninfected growth of H9 lymphocytes by 50%. EC_{50} = concentration (μM) that inhibits HIV-1 replication in H9 lymphocytes by 50%.

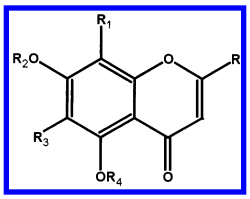
cance is good, the R_{cross} value for both models are below 0.8, which indicates that their prediction capability is modest.

QSAR equations for group 2 did not include a common descriptor for both Log(IC_{50}) and Log(EC_{50}) models. Descriptors in the Log(IC_{50}) model were both topological and geometrical, whereas in Log(EC_{50}) they were only electronic in nature. Both regressions were significant according to analysis of variance. However, when flavanone (lacking hydroxyl groups) is removed from the data set used to model EC_{50}, the *R* value increases from 0.847 to 0.974. Log(IC_{50}) QSAR model suggested that cytotoxicity is primarily a function of molecular bulk and shape encoded by the solvent accessible surface area (SASA) and the moment of inertia I (MI-1).[17] These terms might account for flavonoid transport into the cell and to the active site. The presence of dipole and the sum of charges in atoms 1−4 (ΣQ[1−4]) in the QSAR model for Log(EC_{50}) summarizes the importance of the stereoelectronic molecular architecture and the localized electronic nature on ring A, respectively, as determinant descriptors in the anti-HIV activity of the flavonoids in this group.

The best regression models were computed for flavonoids belonging to group 3, in which both Log(EC_{50}) and Log(IC_{50}) models gave statistical significance at $P = 0.001$. All the descriptors in both models were electronic, and the dipole and charge in atom 3 (QA3) were common to both of them. QA3 might be understood as a measure of electronic differences between substituents in C2 and consequently as an extension of the electron delocalization around the ring A. On the other hand, the electrogeometrical component encoded in the dipole could be related to the conformational preferences for the receptor site.[21]

As showed before, dipole was an important molecular descriptor both in groups 2 and 3 models. This observation would imply that the active conformer has or can obtain a conformation similar to that in the minimum energy stage. Due to the fact that the biological data were obtained from experiments using whole cells, and that the participation of

Table 3. Molecular Structure and Bioactivity of Flavonoids[a]



R	R_1	R_2	R_3	R_4	IC_{50}	EC_{50}
Me	H	H	H	H	214	49
n-propyl	H	H	H	H	73	27
cyclohexyl	H	H	H	H	42	19
4′-fluorophenyl	H	H	H	H	13	4
2′-chlorophenyl	H	H	H	H	17	5
3′-chlorophenyl	H	H	H	H	14	4
4′-chlorophenyl	H	H	H	H	16	4
4′-bromophenyl	H	H	H	H	21	5
phenyl	H	N-CBZ-glycine	H	H	30	9
phenyl	NO_2	H	H	H	12	12
phenyl	NO_2	H	NO_2	H	372	372
phenyl	NH_2	H	H	H	34	11

[a] Group 3. Synthetic flavonoids. IC_{50} = concentration (μM) that inhibits uninfected growth of H9 lymphocytes by 50%. EC_{50} = concentration (μM) that inhibits HIV-1 replication in H9 lymphocytes by 50%.

Table 4. Best Linear Multiregression Equations with One to Three Molecular Descriptors for the Bioactivity ($LogIC_{50}$ and $LogEC_{50}$) of Different Flavonoid Groups[a]

Group 1

$\text{Log}(IC_{50}) = -63.6 \pm 13.7(\text{MPC}) + 22.0 \pm 4.4$
($T = -4.6$, $P = 0.006$) ($T = 5.0$, $P = 0.004$)
$R = 0.901$, $R_{CROSS} = 0.762$, SE = 0.231, $N = 7$, $F = 21.5$, $P = 0.006$
$\text{Log}(EC_{50}) = -65.9 \pm 25.8(\text{MPC}) + 22.5 \pm 8.3$
($T = -2.6$, $P = 0.051$) ($T = 2.7$, $P = 0.042$)
$R = 0.753$, $R_{CROSS} = 0.667$, SE = 0.434. $N = 7$, $F = 6.6$, $P = 0.051$

Group 2

$\text{Log}(IC_{50}) = 0.028 \pm 0.009(\text{SASA}) - 0.002 \pm 0.001(\text{MI-1}) - 6.7 \pm 2.6$
($T = 3.2$, $P = 0.015$) ($T = -2.4$, $P = 0.049$) ($T = -2.6$, $P = 0.037$)
$R = 0.774$, $R_{CROSS} = 0.300$, SE = 0.286, $N = 10$, $F = 5.21$, $P = 0.041$
$\text{Log}(EC_{50}) = -0.31 \pm 0.07(\text{dipole}) - 2.8 \pm 1.1\ (\Sigma Q(1-4)) + 2.5 \pm 0.3$
($T = -4.19$, $P = 0.004$) ($T = -2.45$, $P = 0.044$) ($T = -8.98$, $P < 0.001$)
$R = 0.847$, $R_{CROSS} = 0.667$, SE = 0.281, $N = 10$, $F = 8.87$, $P = 0.012$

Group 3

$\text{Log}(IC_{50}) = 0.32 \pm 0.06(\text{dipole}) - 29.3 \pm 8.7(\text{QA3}) - 8.6 \pm 2.6$
($T = 5.19$, $P < 0.001$) ($T = -3.38$, $P = 0.008$) ($T = -3.28$, $P = 0.009$)
$R = 0.888$, $R_{CROSS} = 0.816$, SE = 0.247, $N = 12$, $F = 16.86$, $P = 0.001$
$\text{Log}(EC_{50}) = 0.29 \pm 0.05(\text{dipole}) - 28.3 \pm 7.5(\text{QA3}) + 173.0 \pm 46.1(\text{QA4}) - 65.3 \pm 14.5$
($T = 5.35$, $P < 0.001$) ($T = -3.79$, $P = 0.005$) ($T = 3.75$, $P = 0.006$)
($T = -4.5$, $P = 0.002$)
$R = 0.955$, $R_{CROSS} = 0.917$, SE = 0.203, $N = 12$, $F = 27.72$, $P < 0.001$

[a] *T*- and *P*-values for regression coefficients are given in parentheses.

a specific biochemical target was not known, the role for dipole at a particular bioactive conformation cannot be ruled out from this QSAR. A search for flavonoid-protein crystal structures showed that for several molecules having a double bond between C2 and C3, the A- and C-ring planes prefer a quasi-planar conformation ($\approx -5°$). This has been shown

Table 5. Correlation Matrix for Molecular Descriptors Included in the Models

	group 2				group 3		
	SASA	MI-1	dipole	ΣQ(1−4)	dipole	QA3	QA4
SASA	1						
MI-1	−0.79	1					
dipole			1				
ΣQ(1−4)			0.51	1			
dipole					1		
QA3					−0.15	1	
QA4					−0.37	0.30	1

Table 6. Statistical Parameters for Regression Models and the Descriptor Dipole When This Is Calculated at the Angle (α) Obtained at the Minimum Energy Conformation and When It Is Set up at $-5°$

	group 2 Log (EC_{50})		group 3 Log (IC_{50})		group 3 Log (EC_{50})	
	min.	-5^0	min.	-5°	min.	−5°
			Model			
R(model)	0.847	0.830	0.888	0.732	0.955	0.879
P(model)	0.012	$p = 0.017$	0.001	0.032	<0.001	0.006
			Dipole			
T	−4.19	−3.93	5.19	2.70	5.35	2.47
(*P*)	(0.004)	(0.006)	(<0.001)	(0.02)	(<0.001)	(0.038)

for molecules in the Protein Data Bank such as 1E8W, 1E90,[22] and 2HCK.[23] However, in some cases, the torsional angle seems to have a larger deviation from planarity, such as in 1C8K.[24] This tendency to planarity has also been reported for the crystal structure of isolated 5-hydroxyflavone[25] and quercetin.[26,27]

To establish if dipole, calculated at a coplanar conformation for flavonoid molecules having a double bond between C2 and C3, was determinant for bioactivity, new QSAR models were generated using the dipole calculated at a torsional angle (α) of $-5°$. The results are presented in Table 6. Although based on the *R*-, *T*-, and *P*-values from analysis of variance, the model performance decreased for all the cases, the dipole as descriptor was still significant. This might suggest that flavonoids can undergo conformational changes depending on the type of interaction with the receptor and how they are approaching dynamically toward its surface.

In general, most of the descriptors selected in the models were electronic and included selected atomic charges. Similar results were obtained by Alves et al.[28] who used the molecules in groups 2 and 3 to develop QSAR models employing descriptors calculated by the semiempirical method PM3. The presence of these atomic properties in the model could be a measure of the capacity of the flavonoid molecule to access specific sites in the receptor based on the physicochemical properties of those particular atoms.[29] This suggests that the biological activity of flavonoids is mainly governed by electronic interactions with biomolecules within the cell.

There are several mechanisms by which flavonoids could induce inhibition of HIV in lymphocytes. For instance, they have been shown to inhibit key signal transduction pathways such as protein tyrosine kinase activation[30] and cAMP phosphodiesterase activity.[5] Although chemicobiological mechanisms can be addressed using QSAR models, it is quite difficult to overcome problems associated with heterogeneity of enzymes and receptors and possible metabolic side

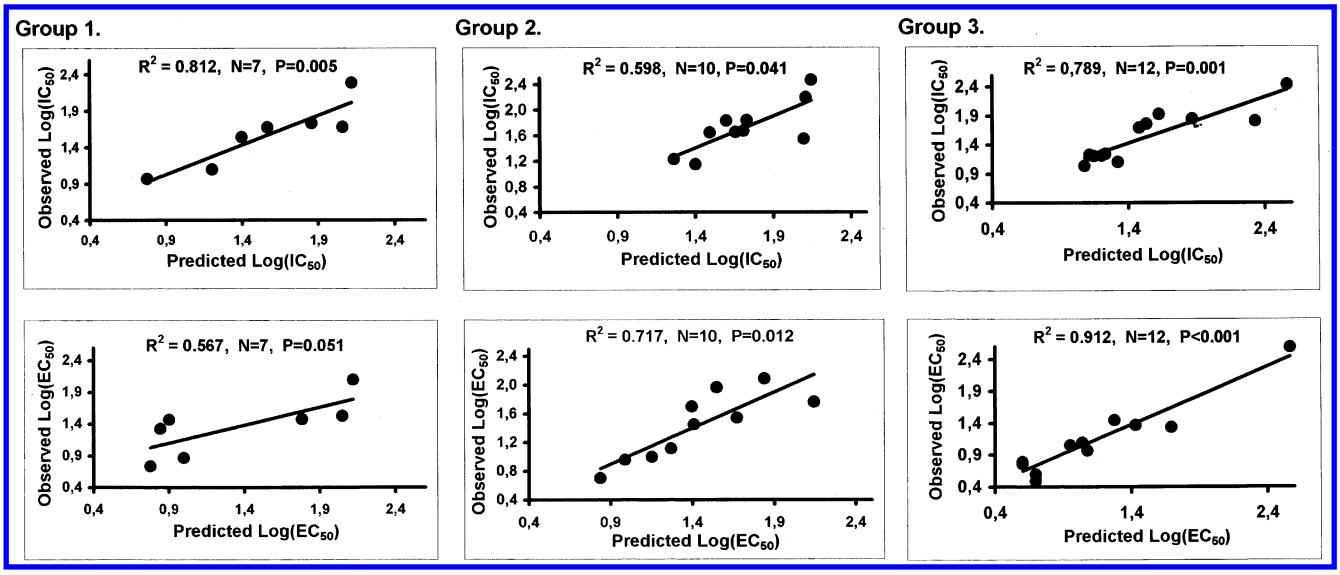


Figure 3. Plot of observed vs predicted activities (Log(IC_{50}) and Log (EC_{50})) derived from the best QSAR multiregression models for three different groups of flavonoids.

reactions.[31] Despite this, the mechanisms and molecular parameters involved in the interaction between flavonoids and some biochemical systems have been well documented. For instance, although some flavonoids have been reported to have prooxidants properties,[32] many of them have been associated with inhibition of lipid peroxidation.[33] Antioxidant mechanisms have been related to the intramolecular formation of an hydrogen bond between the carbonyl oxygen and a collateral hydroxyl.[34] The volume-to-surface ratio and the electron densities on the C3 are critical parameters for the inhibition of the metabolizing enzyme cytochrome P450.[35] The binding between flavonoids and the GABA(A) receptor channel depends on the interaction with the oxygen atom of the carbonyl group and the nature of the substituent in position 3′.[12] Dipole and the presence of hydroxyl groups in the phenyl ring have also been important in predicting flavonoid-induced inhibition of heterocyclic amines.[36]

According to the classical QSAR models presented in this paper, molecular parameters encoding the electronic architecture of flavonoids are important contributors to their biological properties. In addition, the differences in descriptors selected to build the models suggest that although specific atomic charge distribution around the molecule accounts for most of the bioactivity, there are other electronic or geometrical characteristics among groups that might explain the associated differences in targets or mechanisms.

It is concluded that the HIV-inhibitory properties of flavonoids are mainly the outcome of electronic interactions between atomic charges within these compounds in both rings A and B and possible receptor-like structures in the HIV or the lymphocyte itself. These agonist-receptor interactions are enhanced by hydrogen bonding contributions and by specific geometrical arrangements associated with each flavonoid. On the other hand, cytotoxicity not only requires electrostatic features on the flavonoid structure but also bulk and shape parameters which could be linked to cell penetration mechanisms.

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