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Structure–activity relationship study of flavone compounds with anti-HIV-1 integrase activity: A density functional theory study

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Abstract—Human immunodeficiency virus type-1 integrase (HIV-1 IN) is an essential enzyme for effective viral replication. Flavone compounds have been very much studied due to their activity during the inhibition process of HIV-1 IN. In this study, we employed density functional theory (DFT) using the B3LYP hybrid functional to calculate a set of molecular properties for 32 flavonoid compounds with anti-HIV-1 IN activity. The stepwise discriminant analysis (SDA), principal component analysis (PCA) and hierarchical cluster analysis (HCA) methods were employed to reduce dimensionality and investigate possible relationship between the calculated properties and the anti-HIV-1 IN activity. These analyses showed that the molecular hydrophobicity ($C\log P$), charge on atom 11 and electrophilic index (ω) are responsible for the separation between anti-HIV-1 IN active and inactive compounds.

1. Introduction

The global immunodeficiency syndrome (AIDS) epidemic killed 3.1 million people in 2005. AIDS is caused by human immunodeficiency virus (HIV). The estimated number of people living with HIV in the world is 39.4 million. The HIV has the capability of selectively infecting and ultimately incapacitating the immune system whose function is to protect the body against such invaders.[1–3]

Three enzymes are essential for the replication cycle of human immunodeficiency virus type 1 (HIV-1): reverse transcriptase (RT), protease (PR) and integrase (IN), these enzymes are important targets for the development of anti-AIDS drugs.[4] HIV-1 IN has been an attractive and a validated target for anti-AIDS drug design because of its crucial role in the viral life cycle and the fact that there is no cellular homologue in humans.[5–7] IN catalyzes the integration of viral cDNA into the human genome, which facilitates stable viral replication and sustained infection.[4] Structure–activity relationships (SAR) for some inhibitors have been discerned, and it was proposed that the aromatic moiety is common to interact with the divalent cation in a cation–π type interaction.[8,9] There is also a possibility of a typical charge–charge interaction between the metal ions and ionic or partial charges of the ligands.[10] It has been shown that both types of interactions can co-exist in a binding site.[10] Other work has also suggested a binding mode in which inhibitor binds through bidentate chelation of this ion,[11,12] and recently SAR and QSAR studies showed that hydrophobic and electronic characteristics of the substituents have a main role in the anti-HIV-1 activity.[13–19]

Among several compounds that present anti-HIV-1 IN activity, flavone compounds have been very much studied due to their activity during the inhibition process of HIV-1 IN.[20] The flavones are classified as flavonoid compounds. Flavonoids are benzo-γ-pyrone derivatives that can be grouped according to the presence of different substituents on the rings and to the degree of benzo-γ-pyrone ring saturation, consisting of three aromatic rings with polar groups appended at various positions.[21,22] Flavones would be capable of chelating a divalent metal ion bound to the enzyme and of reacting chemically, perhaps by way of a free radical mechanism with essential groups at the enzyme site and then reacting chemically or by chelation with the enzyme.[20]

Recently, Souza et al.[13] have reported SAR study for 22 flavone compounds with anti-HIV IN activity but

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Keywords: Flavones; HIV-1 integrase; B3LYP; SAR; PCA; HCA; Electrophilic index.

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employing descriptors calculated by the semiempirical method AM1. In this work, we employed density functional theory (DFT) using the B3LYP hybrid functional to explore and determine various electronic descriptors, with better accuracy, to make the necessary improvement in the SAR models. Ionization potential (IP), electron affinity (EA), χ (electronegativity), hardness (η), softness (S), electrophilicity index (ω), charges and other properties were obtained for 32 flavonoid compounds as anti-HIV-1 IN (Fig. 1).[20] The DFT-based reactivity descriptors such as χ, η, S and ω, that have an important role in many areas of research,[23] were obtained through exact descriptions (for IP and EA). The principal component analysis (PCA), hierarchical cluster analysis (HCA) and stepwise discriminant analysis (SDA) methods were employed with the aim to obtain a relation between these descriptors and the anti-HIV-1 integrase activity of these compounds.

2. Results and discussion

2.1. Stepwise discriminant analysis (SDA)

The main objective of SDA is to determine discriminant functions using the measured variables that separate the groupings as distinctly as possible. In this work, we considered two groups: active molecules (numbered **1–15**) and inactive molecules (numbered **16–32**) against HIV-1 IN (Fig. 2).

The SDA is a linear discriminant method based on the Fisher's test (F test) for the significance of the variables.[24] In each step one variable is selected based on its significance and after several steps, the more significant variables are extracted from the whole data set under investigation. The properties most significant selected by this analysis were: $C\log P$, Vol, charge on the oxygen atom 11 (O11) and electrophilic index.

The classification percent correct for inactive group was 100% and percent correct for active group was 93.33%, percent correct total was 96.88% resulting in a satisfactory separation of the two groups (Table 2). The compound **2** was classified incorrectly in the inactive group. The discriminant functions obtained with 32 compounds in this work are given as follows:

$$\begin{aligned}\text{Active} = {} & -2.57 - 3.53\ \mathrm{C}\log P - 1.69\ \mathrm{Vol} \\ & - 1.77\ \mathrm{O11} - 0.55\omega,\end{aligned} \tag{1}$$

$$\begin{aligned}\text{Inactive} = {} & -2.04 + 3.11\ \mathrm{C}\log P + 1.49\ \mathrm{Vol} \\ & + 1.56\ \mathrm{O11} + 0.49\omega.\end{aligned} \tag{2}$$

Through the discriminant functions above (Eqs. 1 and 2) and the values of each variable for the compounds studied (Table 1), we obtain the classification matrix by using all compounds in the analysis. The allocation rule derived from the SDA results, when the anti-HIV activity of a new flavone is investigated, is: (a) initially one calculates, for the new flavone compounds, the value of the more important variable obtained with the SDA methodology, that is, $C\log P$, Vol, O11 and ω; (b) substitutes these values in the two discriminant functions above (Eqs. 1 and 2); (c) checks which discriminant function (group active compounds or group inactive compounds) presents the higher value. The new molecule is active if the higher value is related to the discriminant function of group active or vice versa.

2.2. Principal component analysis (PCA)

A PCA extracts independent factors from a number of highly correlated variables by decomposing a matrix of data values into matrices: a loadings matrix, which contains the independent factors (principal components, PCs), and scores matrix.[25] In this analysis was made a combination between variables, the combination that presented larger variance was going to be selected.

The first principal components explain 48.79% of variance (PC1 = 48.79%). The second principal components explain 36.32% of variance (PC2 = 36.32%). PC1 and PC2 explain 85.11% of total variance in the data set. The scores of the PC1 and PC2 are shown in Figure 2, in this figure we can observe that, with exception of compounds **2** and **19**, the compounds are separated into two groups: active compounds and inactive compounds according to their degree of anti-HIV IN activity. Also from Figure 2 we can see that PC1 alone is responsible for this separation. The loading vectors for PC1 can be expressed through the following equation:

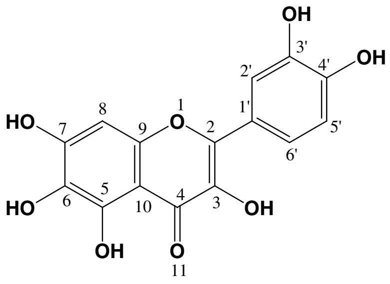
$$\mathrm{PC1} = -0.88\ \mathrm{C}\log P - 0.77\ \mathrm{O11} - 0.28\omega. \tag{3}$$

From Eq. 3, we can say that for flavones to become active they must have lower values for $C\log P$ and electrophilic index (ω) combined with more negative charge on oxygen atom 11.

2.3. Hierarchical cluster analysis (HCA)

In HCA, each point forms an only cluster initially and then the similarity matrix is analyzed. The most similar points are grouped forming one cluster and the process is repeated until all the points belong to an only group.[26]

The properties most significant selected by this analysis were: $C\log P$ and charge in the oxygen atom 11 (O11). In the dendrogram (Fig. 3), the vertical lines represent the compounds and the horizontal lines represent the similarity values between pair of compounds, a compound and a group of compounds and between groups. From this dendrogram we can notice that the similarity observed between the group of the active and inactive molecules is next to zero. In this dendrogram, with the exception of compounds **2** and **19**, we can observe that the $C\log P$ and O11 are responsible for the separation between active and inactive according to their degree of anti-HIV IN activity. Therefore, the $C\log P$ and O11 properties were very impotents in all three methodologies for the separation between the anti-HIV IN active and inactive flavone compounds. The results obtained with the HCA method were exactly the same to those obtained with PCA method.



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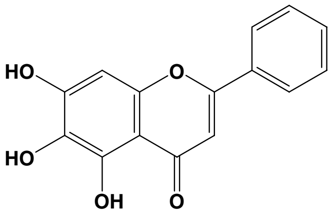

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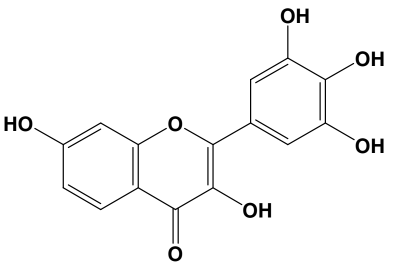

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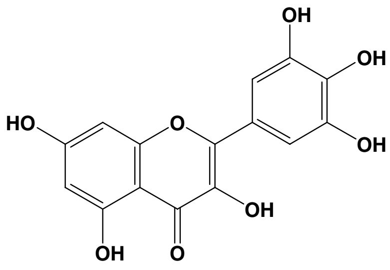

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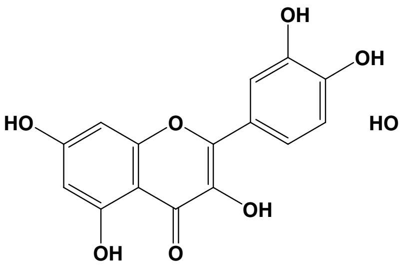

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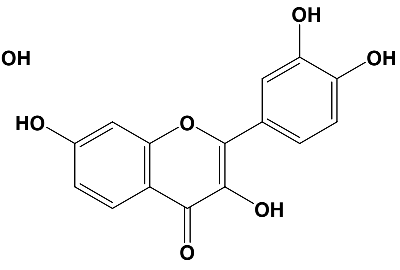

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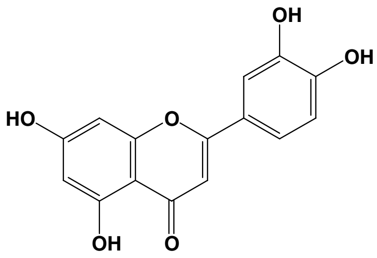

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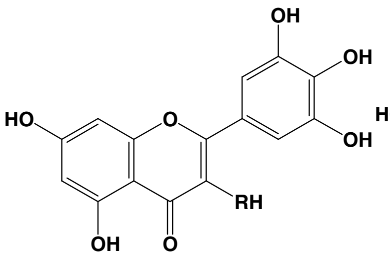

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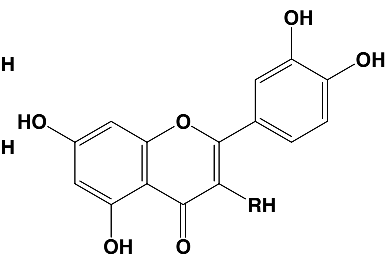

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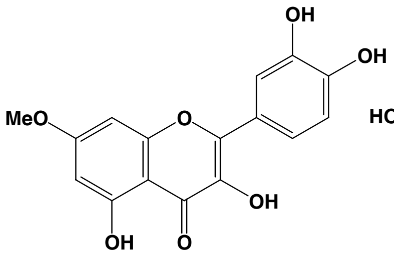

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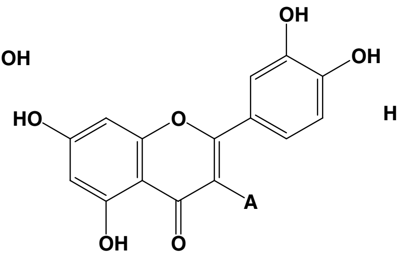

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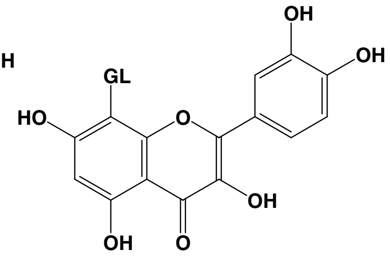

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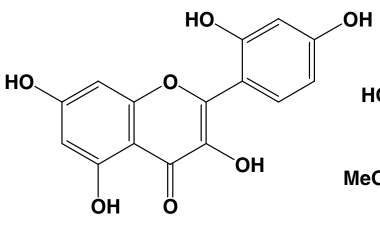

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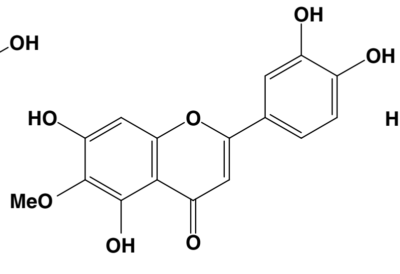

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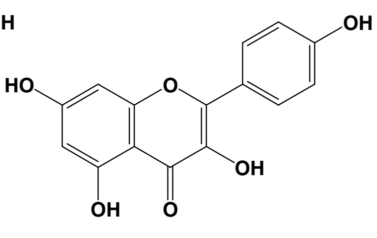

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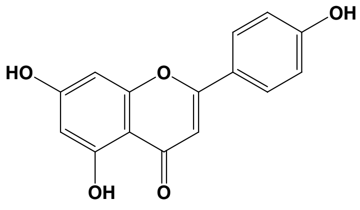
Figure 1. Molecular structure of the 32 flavone compounds studied. The first compound show the numbering of the flavones studied.



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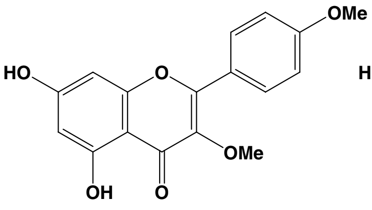

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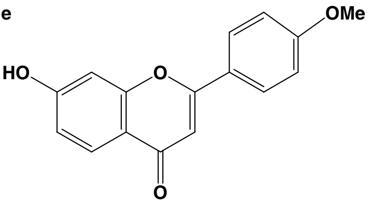

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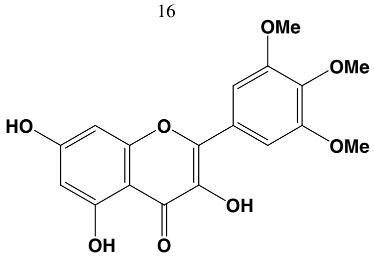

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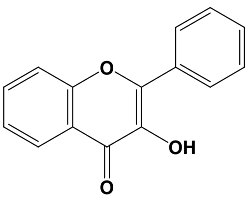

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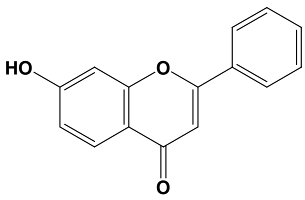

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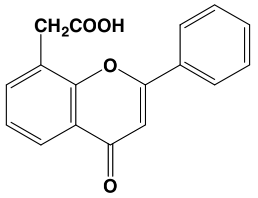

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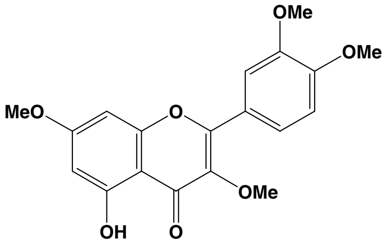

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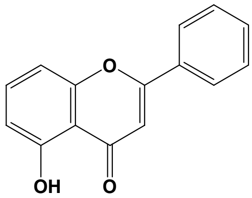

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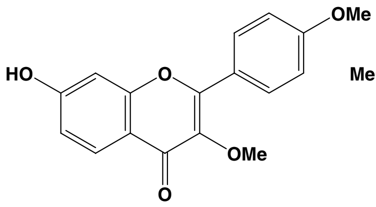

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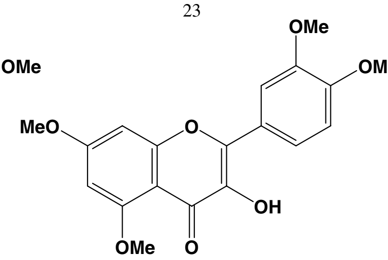

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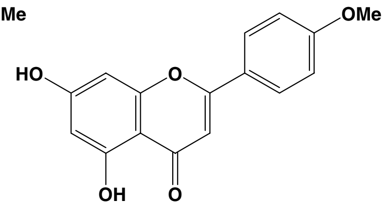

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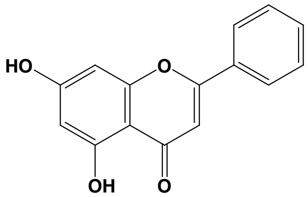

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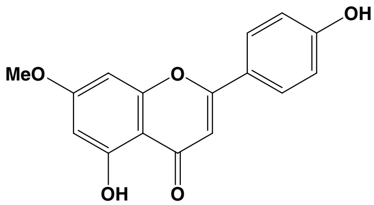

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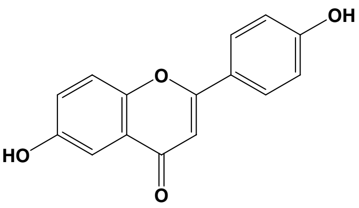

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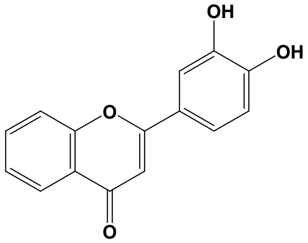

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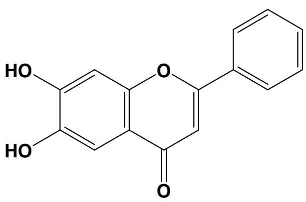

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Figure 1. (*continued*)

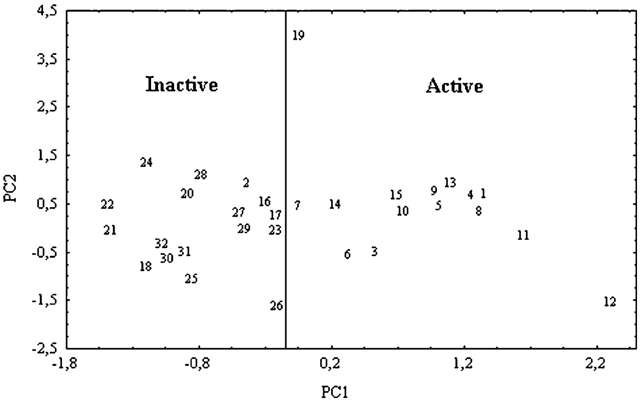

Figure 2. Plot of first two PC score vectors for the separation of the training set into two groups: Active and Inactive.

Table 1. Values of four most important properties (descriptors) that classify the 32 flavones studied and average values ($\overline{X}$) in bold font

Compound	$C\log P$	Vol (Å^3)	O11	ω (eV)	Activity
1	0.715	769.702	−0.636	2.071	Active
2	3.002	715.562	−0.595	2.220	Active
3	0.376	753.059	−0.566	1.997	Active
4	0.637	772.231	−0.632	2.099	Active
5	1.304	753.531	−0.631	2.086	Active
6	1.044	741.154	−0.571	1.957	Active
7	2.311	739.557	−0.594	2.117	Active
8	−0.451	1106.863	−0.603	2.119	Active
9	0.216	1087.287	−0.605	2.200	Active
10	1.948	808.795	−0.634	2.033	Active
11	−0.831	1045.559	−0.607	1.986	Active
12	−1.021	1095.399	−0.622	1.591	Active
13	0.734	747.453	−0.629	2.172	Active
14	1.820	811.611	−0.598	2.119	Active
15	1.900	734.262	−0.633	2.072	Active
16	2.905	720.404	−0.595	2.121	Inactive
17	2.933	847.075	−0.597	2.040	Inactive
18	3.144	764.787	−0.530	1.969	Inactive
19	1.866	940.087	−0.631	2.930	Inactive
20	2.843	680.054	−0.561	2.268	Inactive
21	3.211	688.006	−0.525	2.175	Inactive
22	2.752	778.728	−0.520	2.342	Inactive
23	3.233	976.637	−0.600	1.944	Inactive
24	4.111	678.837	−0.584	2.339	Inactive
25	2.623	834.794	−0.533	1.898	Inactive
26	2.495	982.101	−0.561	1.673	Inactive
27	3.486	776.045	−0.595	2.065	Inactive
28	3.563	699.867	−0.591	2.262	Inactive
29	3.492	775.751	−0.594	2.069	Inactive
30	2.575	709.177	−0.529	2.051	Inactive
31	2.354	706.998	−0.524	2.065	Inactive
32	2.733	705.996	−0.533	2.087	Inactive
$\overline{X}$	**0.914**	**845,468**	**−0.610**	**2.056**	Active
$\overline{X}$	**2.959**	**780,314**	**−0.565**	**2.135**	Inactive

Table 2. Classification matrix obtained by using SDA for 32 compounds

Classified group	True group		
	Percent	Inactivity	Activity
Inactivity	100	17	0
Activity	93.33	1	14
Total	96.88	18	14

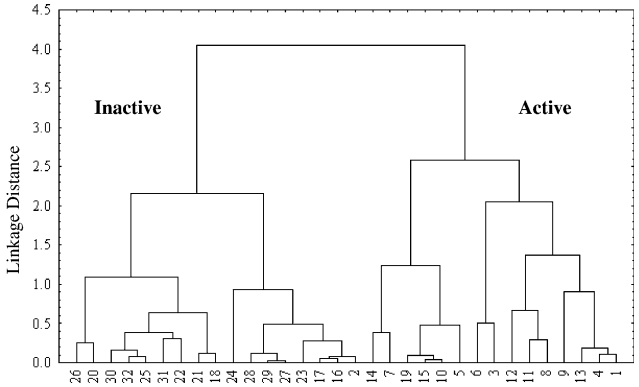

Figure 3. Dendrogram obtained with the HCA for training set (32 compounds). HCA classifies the compounds into two groups: Active and Inactive.

The $C\log P$ is a measure of hydrophobicity; molecules with large value of $C\log P$ have higher hydrophobicity and consequently better transport through membranes. In Figure 4 and Table 1, we can observe that in general molecules with lower values of $C\log P$ are most active, while the molecules with higher values of $C\log P$ are less active. The compounds that present more hydroxyl groups are what present lower values for $C\log P$, these compounds have a good solubility in water, consequently, they are compounds more hydrophilic, what can indicate that the compounds more active must interact with some enzyme polar region or with molecules of water in or out of the enzyme. The molecule 2 has an elevated value for this variable, while the molecule 19 has a very low value; this would explain the incorrect classification observed in SDA, PCA and HCA.

On the other hand, also two electronic properties are important: the charge on atom 11 and electrophilic index (ω) (Figs. 5 and 6). In Figure 5 and Table 1, we can observe that in general more active flavonoids have more negative charge on atom 11. Therefore, the carbonyl oxygen may act as an acceptor in a hydrogen bond formed with the side chains of residues containing a hydroxyl group or might be involved in the chelation with Mg^{2+} ion of cofactor. The charge is electronic

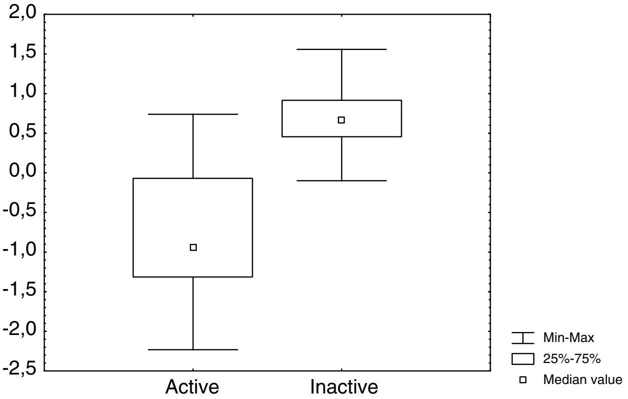

Figure 4. Box plot for $C\log P$ considering 32 flavone compounds.

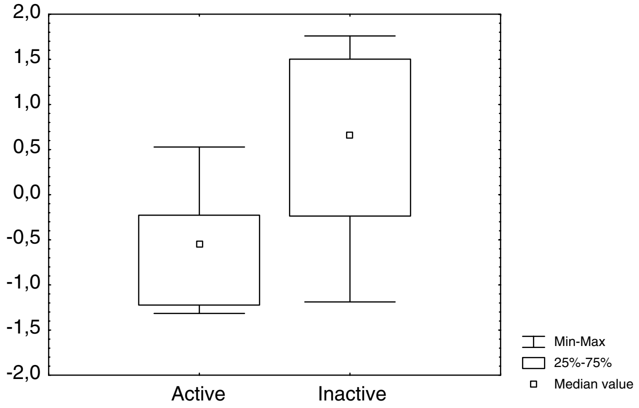

Figure 5. Box plot for O11 considering 32 flavone compounds.

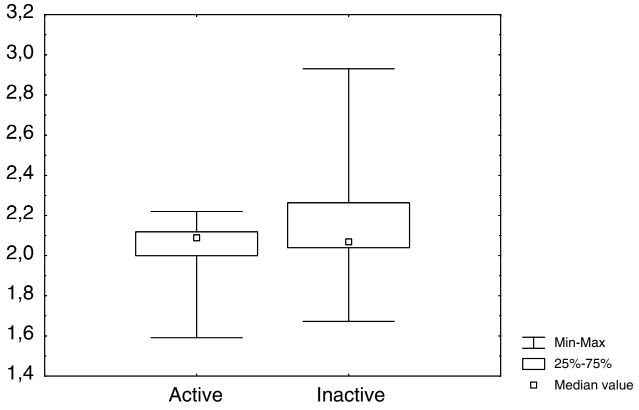

Figure 6. Box plot for ω considering 32 flavone compounds.

descriptor; therefore, we can conclude that electronic effects have a very important role when one is trying to understand the activity of flavonoid compounds with anti-HIV IN activity, therefore, can indicate the probability of an interaction between the flavones and the biological receptor through a charge transfer mechanism. Recently, we have found similar results for flavonoid compounds with anti-HIV-1 activity in lymphocyte.[14–16]

The index ω is interpreted as the capacity of an electrophile to promote (stabilize) a covalent (soft) reaction.[28] The concept of electrophilicity viewed as a reactivity index was introduced by Maynard et al.[28] to study the reaction of the human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein p7 (NCp7) with a variety of electrophilic agents. In Figure 6 and Table 1, we can observe that in general more active flavones have low values for ω. Then, we may suppose an interaction between flavone actives and electron-acceptor groups at the enzyme site. However, when comparing Figures 4 and 5 with Figure 6 it is evident that electrophilic index global plays a secondary role in the classification.

2.4. Electrostatic potential

Figure 7 displays the three-dimensional molecular electrostatic potential (MEP) surfaces, generated in Spartan program,[27] for the compounds **1** (active, it is the most potent compound) and **22** (inactive). These surfaces correspond to the isodensity value of 0.001 a.u., and it is interesting to highlight the noncovalent interactions occurring at the molecular surface. The most nucleophilic regions (negative electronic potential, in red) can be found around the oxygen atoms, while the most electrophilic regions (positive electrostatic potential, in blue) can be found around the hydrogen atoms. In Figure 7 we can observe that the compound **1** (active) provides a much more intense region of negative electrostatic potential than the compound **22** (inactive), and thus it constitutes a more attractive cation-binding site. In general, we observed that active compounds have more intense region of negative electrostatic potential than the inactive ones. These results are in agreement with the proposed that the aromatic moiety is common to interact with the divalent cation in a cation–π type interaction,[8,9] and also with our study of molecular dynamics.

3. Conclusions

The SDA, PCA and HCA methods showed to be quite efficient to classify the 32 flavone compounds studied in two groups (active and inactive) according to their degree of anti-HIV IN activity. Three descriptors were found to be important in the classification: C log *P*, charge on atom 11 and electrophilic index, with secondary role in the classification. The SDA, PCA and HCA methods showed that lower values for C log *P* and electrophilic index combined with more negative charge on oxygen atom 11 (O11) lead to an increase of the anti-

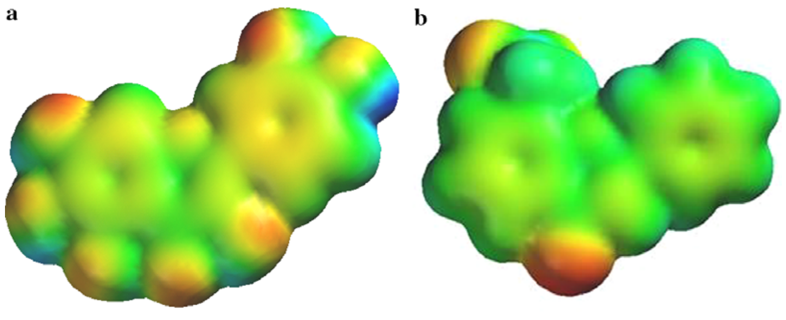

Figure 7. MEP electrostatic potential of (a) compound **1** and (b) compound **22** derived from ab initio HF/6-31G*. The increase of negative charges comes from the blue (positive) to red (negative).

HIV-1 IN activity. These variables allowed a physical explanation of electronic molecular properties contributing to HIV inhibitory potency as the electronic character relates directly to the electron distribution of interacting molecules at the active site. Also the biological activity is highly influenced not only by their inherent electronic properties but also by their transportability represented by C log *P*. In general active compounds have more intense region of negative electrostatic potential than the inactive compounds.

4. Methodology

Quantum chemical calculations at the DFT/RB3LYP (restricted B3LYP) level of theory, together with the 6-31G* basis set, were used to fulfil the geometry optimization of the selected neutral compounds. The geometries of the radicals studied were optimized independently from the neutral molecules prior to the calculations of energies, treated as open shell systems DFT/UB3LYP (unrestricted B3LYP). All calculations were performed by using the Gaussian 03 package of programs.[29] The nature of each stationary point was established by calculating analytically and diagonalizing the matrix of the energy second derivatives to determine the number of imaginary frequencies (zero for local minimum and one for TS). The calculated vertical ionization potential (IP) and vertical electron affinity (EA) were corrected for zero-point vibrational energy (ZPVE). The IP and EA were calculated through following equations:

$$\begin{aligned}\mathrm{IP} = {} & [(TE_{\mathrm{CATION}} + TCE_{\mathrm{CATION}}0.9806) \\ & - (TE_{\mathrm{NEUTRAL}} + TCE_{\mathrm{NEUTRAL}}0.9806)]27.2114, \quad (4)\end{aligned}$$

$$\begin{aligned}\mathrm{EA} = {} & [(TE_{\mathrm{NEUTRAL}} + TCE_{\mathrm{NEUTRAL}}0.9806) \\ & - (TE_{\mathrm{ANION}} + TCE_{\mathrm{ANION}}0.9806)]27.2114, \quad (5)\end{aligned}$$

where TE_{NEUTRAL} is the energy of the neutral molecule, TCE_{NEUTRAL} is the corrected for zero-point energy of the neutral molecule, TE_{CATION} is the energy of the radical cation, TCE_{CATION} is the corrected for zero-point energy of the radical cation, TE_{ANION} is the energy of the radical anion and TCE_{ANION} is the corrected for zero-point energy of the radical anion. The value 0.9806 is the scaling factor of corrected to ZPVE for theoretical B3LYP/6-31G*. The DFT-based reactivity descriptors were obtained from Eqs. 6–9.[30–33]

Electronegativity (χ):

$$\mu = -\chi \approx -(\mathrm{IP} + \mathrm{EA})/2, \quad (6)$$

Hardness (η):

$$\eta \approx (\mathrm{IP} - \mathrm{EA})/2, \quad (7)$$

Softness (S):

$$S = 1/(2\eta), \quad (8)$$

Electrophilic index:

$$\omega = \mu^2/2\eta. \quad (9)$$

Other calculated descriptors were: electronic (Mulliken's charges), hydrophobic (C log *P*) and steric (molecular volume, Vol). The C log *P* was calculated using Chem3D Ultra 9.0[34] and volume was calculated using HyperChem 7.5.[35] The C log *P* is based on Hansch–Leo's log *P* calculation method. It divides molecules into fragments and uses the constants of these fragments and correction factors taken from its database for log *P* calculation.[36,37]

In this work were employed the principal component analysis (PCA), hierarchical cluster analysis (HCA) and stepwise discriminant analysis (SDA) methods. The values of the variables were standardized in all statistical analyses. These statistical analyses were performed using Statistica[38] and Matlab[39] package of programs.

Pharmacological data were taken from the work of Fesen et al.[20a] The biological evaluation of the flavonoids was done by using the IC_{50}, that indicates pharmacological potency (concentration which inhibits the cleavage and integration reactions by 50%). The compounds listed in Figure 1 can be divided into two groups: which inhibited 3′-processing or strand transfer with an IC_{50} of less than 100 μM are considered active compounds (numbered **1–15**), and which produced weak or undetectable inhibitory activity at 100 μM are considered inactive (numbered **16–32**).[20a]

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