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Original article

A quantum chemical and statistical study of flavonoid compounds (flavones) with anti-HIV activity

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Abstract

The molecular orbital semi-empirical method AM1 was employed to calculate a set of molecular properties (variables) of 22 flavonoid compounds (flavones) with anti-HIV-1 activity and nine new compounds were proposed for anti-HIV-1 activity prediction. Pattern recognition techniques, principal component analysis (PCA), hierarchical cluster analysis (HCA), stepwise discriminant analysis (SDA) and K-nearest neighbor (KNN), were employed in order to reduce dimensionality and investigate which subset of variables could be more effective for classifying the flavones according to their degree of anti-HIV-1 activity. The PCA, HCA, SDA and KNN studies showed that the variables log *P* (partition coefficient), molecular volume (VOL) and electron affinity (EA) are responsible for the separation between anti-HIV-1 active and inactive compounds. The prediction study was done with a new set of nine analog compounds by using the PCA, HCA, SDA and KNN methods and only one of them was predicted as active against HIV-1.

Keywords: Flavones; Anti-HIV-1 activity; AM1; Principal component analysis; Hierarchical cluster analysis; Stepwise discriminant analysis; K-nearest neighbor

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immunodeficiency syndrome (AIDS) which is characterized as a systemic and fatal disorder [1,2]. Since the start of global HIV/AIDS epidemic, more than 30 million people can be infected with HIV [3]. After viral RNA is processed by the reverse transcriptase, the life cycle of the virus proceeds with the integration of the double-stranded DNA transcript into the host genome, a process that is mediated by the retroviral integrase [4]. Integrase presents an attractive possibility as an antiviral target because host cells do not make or require such enzymes. Although some HIV gene products may be expressed in the absence of integrase [5] and the possibility of HIV replication in some cell types in the absence of integrase has not been eliminated, recent evidences show that HIV replication in T-lymphoid cells requires integrase functions [6,7].

Among several compounds that present anti-HIV-1 activity, flavone compounds have been very studied due to their activity during the inhibition process of HIV-1 integrase [4]. A plausible view of the inhibition of integrase by flavones would be that these compounds bind to enzyme sites that normally interact with DNA bases blocking the action of HIV integrase [4].

The flavones are classified as flavonoid compounds and consist of three aromatic rings with polar groups appended at various positions [8]. The planarity, aromaticity and polarity may allow these compounds to bind by stacking with adenine or guanine, or to compete

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with purine moieties for binding to enzyme sites. Many of these molecules also have oxidation–reduction and metal chelation capabilities [4].

As the importance of three-dimensional microscopic interaction and binding between a substrate and a receptor increases, the importance of quantum chemical quantities in SAR analyses also increases. The quantum chemical parameters of molecules and even of interacting molecular systems can, in principle, express all electronic properties related to the molecular interactions. Thus, SAR studies using quantum chemical parameters have become important in qualitative and quantitative analyses of three-dimensional molecular interactions [9–12].

The present work employs the semi-empirical AM1 method [13] to calculate some atomic and molecular descriptors of 22 flavones compounds reported in the literature as potent and selective anti-HIV-1 agents [4] and nine new analogue compounds proposed as prediction set. Some structure–activity studies have been carried out with other flavonoid compounds [14–17] and these studies show that hydrophobic and electronic characteristics of the substituents have a predominant role in the anti-HIV-1 activity of those compounds.

The descriptors (variables) in this work were chosen taking into account three classes of variables: electronic, steric and hydrophobic, as they represent the possible molecular interactions between the flavone compounds and the biological receptor. The principal component analysis (PCA), hierarchical cluster analysis (HCA), stepwise discriminant analysis (SDA) and K-nearest neighbor (KNN), which were employed in this work to analyse the data set, are extremely useful to classify the molecules into groups that can be correlated to their anti-HIV-1 activity.

2. Methodology

2.1. Compounds

The molecular structure of each compound of the training set used in the present study is showed in Fig. 1. In Table 1, the chemical name, abbreviation and the activity indication used for the 22 compounds of the training set are presented. The compounds listed in Table 1 can be divided into two groups: group A, which contains the active compounds (labelled from **1** to **9** in Fig. 1) and group B, which contains the inactive compounds (labelled from **10** to **22** in Fig. 1). The molecular structures of the nine new compounds selected for the anti-HIV-1 prediction study are presented in Fig. 2 and the chemical name and the abbreviation used for these new compounds are also presented in Table 1. The biological evaluation of the compounds studied in this work was determined by using the numerical indicator for activity IC_{50}. The IC_{50} indicates the pharmacological potency and it represents the concentration that inhibits the virus replication by 50% [18].

2.2. Calculation of the atomic and molecular descriptors

All the molecular structures of the training set (numbered from **1** to **22** in Fig. 1) were fully optimized by using the semi-empirical AM1 method [13] with the eigenvector following (EF) keyword. When the gradient norm did not converge to a value below the standard limit, the optimization was restarted with the additional keyword NLLSQ. Thus, it was guaranteed that the optimized geometry obtained for each compound studied represents the equilibrium conformation assessed theoretically. Only these final structures, which represent the most stable ones for a given compound, have been used to obtain the molecular descriptors.

For some compounds of the prediction set (numbered from **I** to **IX** in Fig. 2), the initial structure used in the calculations was based on their X-ray crystal structures, i.e. for the 3-methoxyflavone (compound **I**) and 6,2′,3′,4′-tetramethoxyflavone (compound **IX**), the initial geometry used in the calculations was that determined by Wallet et al. [19,20]; for the compounds **II**, **III** and **VII**, the initial structure used in the calculations was that determined by Souza et al. [21]. For the other compounds (**IV**, **V**, **VI** and **VIII**), the initial structure was obtained using the methodology described for the training set.

The following descriptors were calculated in this work:

- The energy of the HOMO (highest occupied molecular orbital energy) and LUMO (lowest unoccupied molecular orbital energy);
- Mulliken electronegativity (χ): obtained from the following equation: $\chi = (E_{HOMO} + E_{LUMO})/2$;
- Partition coefficient ($\log P$): the values of this property were obtained from the hydrophobic parameters of the substituents [22];
- Dipole moment (μ), molecular polarizability (POL), heat of formation (ΔH_f), total energy (E_T), electronic energy (E_{el});
- Electron affinity (EA): obtained as ($-E_{LUMO}$)
- Superficial area (A) and molecular volume (VOL);
- Net atomic charge on atom N (Q_n)
- Hydration energy (HE) and molecular refractivity (MR).

The calculated descriptors were selected so that they could represent electronic (HOMO, LUMO,χ, μ, POL, Q_n, ΔH_f, MR, EA, E_T, E_{el} and HE), steric (A and VOL) and hydrophobic ($\log P$) features of the compounds studied. These features are supposed to be important for

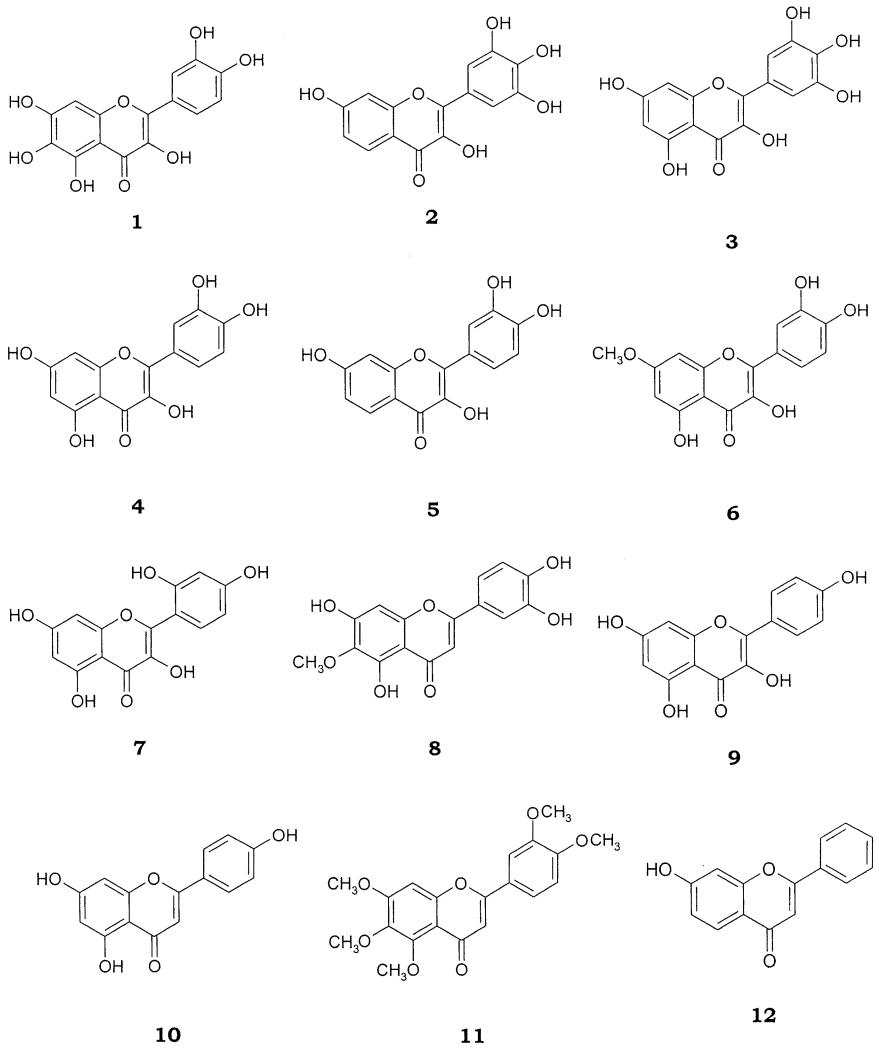

Fig. 1. Molecular structure of the 22 flavonoid compounds (flavones) studied.

the anti-HIV-1 activity presented by the flavones [14–17]. The statistical analysis (PCA, HCA, SDA and KNN) was performed using the MATLAB 6.0 program [23].

The descriptors HOMO, LUMO, ,χ, μ, POL, E_T, E_{el}, EA, ΔH_f and Q_n were calculated with the semi-empirical AM1 method [13] built-in the AMPAC 6.0 molecular package [24]. The atomic charges calculated in this work were derived from the electrostatic potential obtained with the AM1 method by using the routine developed by Connolly [25]. The electrostatic potential was obtained through the calculation of a set of punctual atomic charges so that it represents the possible best quantum molecular electrostatic potential for a set of points defined around the molecule [26,27]. This method uses a density of 1 point per Å in four layers placed at distances of 1.4, 1.6, 1.8 and 2.0 times the van der Waals radii. The charges derived from electrostatic potential have the advantage of being, in general, physically more satisfactory than the Mulliken's charges, especially when one is working with compounds that present biological activity [28]. The other descriptors were calculated by using the HYPERCHEM 5.0 molecular package [29].

3. Results and discussion

3.1. Principal component analysis (PCA)

The central idea of PCA is to reduce the dimensionality of a data set, explaining the variance-covariance structure [30]. This is achieved by linear transformation of the original data set of variables into a smaller number of uncorrelated significant principal components (PCs). Geometrically, this transformation represents the rotation of the original coordinate system and the direction of the maximum residual variance is given by the first principal component axis. The second principal component, orthogonal to the first one, has the second maximum variance and so on. In this way,

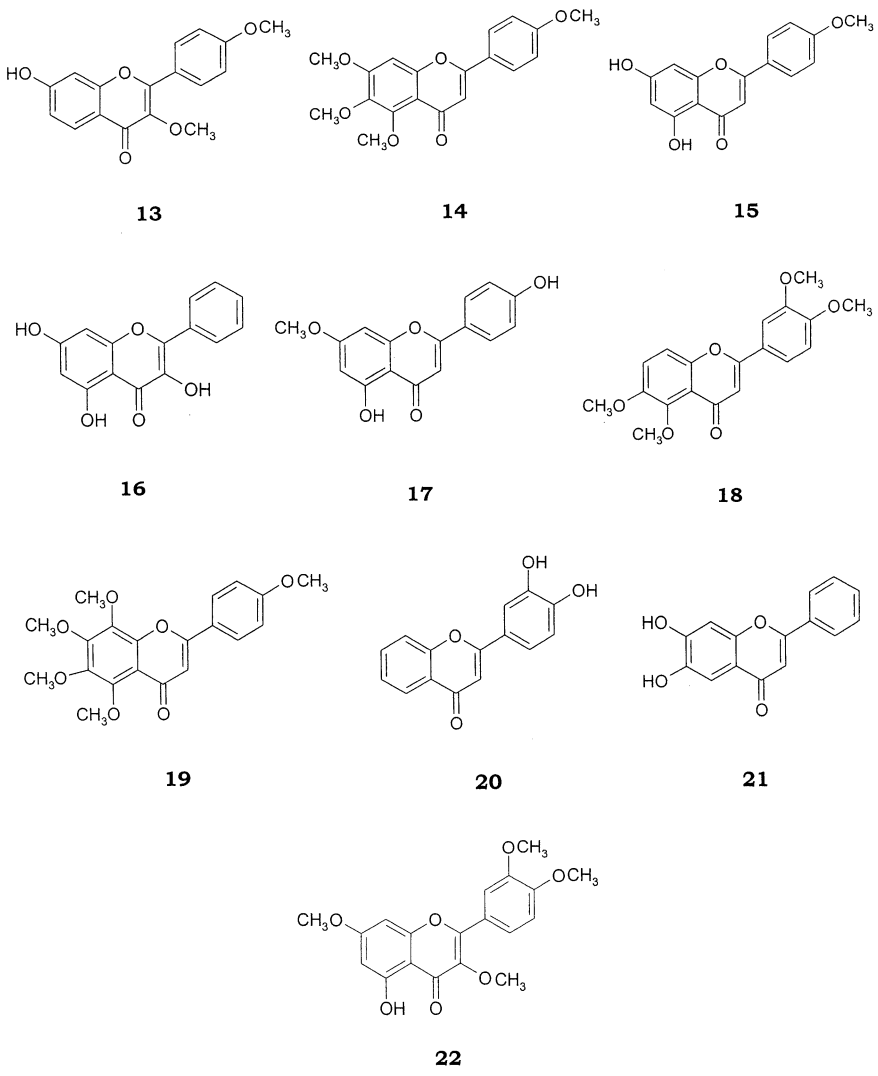

Fig. 1 (*Continued*)

projections preserving maximum amounts of statistical information can be visualized using microcomputers in order to display a more detailed study of the data structure [30,31].

In this work, before applying the PCA method, each variable was auto-scaled so that they could be compared to each other on the same scale. After several attempts to obtain a good classification of the compounds, the best separation was obtained with three variables (see Table 2).

The first two principal components explain 86% of the total variance in the data set as follows: $PC_1 = 56\%$ and $PC_2 = 30\%$. A number of score plots were examined and the most informative one is presented in Fig. 3. This projection has 86% of the total variance of the original data set and provides a reasonably accurate representation of the higher order space. Table 3 shows the loading vectors for PC_1 and PC_2.

From Fig. 3 we can see that the compounds studied are separated into two groups: group A (active compounds) and group B (inactive compounds). Also from Fig. 3, we can see that PC_1 alone is responsible for the separation between active and inactive compounds. Fig. 4 displays the plot of the loading vectors for these first two principal components (PC_1 and PC_2).

According to Table 3, PC_1 can be expressed through the following equation:

$$PC_1 = 0.610[\log P] + 0.403[\mathrm{VOL}] - 0.683[\mathrm{EA}] \quad (1)$$

From Eq. (1) we can notice that for a given flavone to become active it must have smaller values for log P and volume while the electron affinity must have larger values. Considering the interactions between active compounds and the biological receptor, we can say that the compounds studied need to present three main characteristics: (a) they must have small volume for casing suitably with the biological receptor; (b) they must have small values of log P; (c) they must have large values for the electron affinity (this indicates the compound has a great probability of interacting with

Table 1
Chemical name, activity indication and abbreviation used for the compounds (training and prediction sets) studied in this work

Number	Chemical name	Activity	Compound (abbreviation)
Training set			
1	3,3′,4′,5,6,7-hexahydroxy flavone	active	Quercetagetin
2	3,3′,4′,5′,7-pentahydroxy flavone	active	Robinetin
3	3,3′,4′,5,5′,7-hexahydroxy flavone	active	Myricetin
4	3,3′,4′,5,7-pentahydroxy flavone	active	Quercetin
5	3,3′,4′,7-tetrahydroxy flavone	active	Fisetin
6	3,3′,4′,5-tetrahydroxy-7-methoxy flavone	active	Rhamnetin
7	2′,3,4′,5,7-pentahydroxy flavone	active	Morin
8	3′,4′,5,7-tetrahydroxy-6-methoxy flavone	active	6-MeO-Luteolin
9	3,4′,5,7-tetrahydroxy flavone	active	Kaempferol
10	4′,5,7-trihydroxy flavone	inactive	Apigenin
11	3′,4′,5,6,7-pentamethoxy flavone	inactive	Sinensetin
12	7-hydroxy flavone	inactive	7-OH flavone
13	7-hydroxy-3,4′-dimethoxy flavone	inactive	7-OH-3,4′-MeO flavone
14	4′,5,6,7-tetramethoxy flavone	inactive	4′,5,6,7-MeO flavone
15	5,7-dihydroxy-4′-methoxy flavone	inactive	Acacetin
16	5,7-dihydroxy flavone	inactive	5,7-OH flavone
17	4′,5-dihydroxy-7-methoxy flavone	inactive	Genkwanin
18	3′,4′,5,6-tetramethoxy flavone	inactive	3′,4′,5,6′-MeO flavone
19	4′,5,6,7,8-pentamethoxy flavone	inactive	Tangeritin
20	3′,4′-dihydroxy flavone	inactive	3′,4′-OH flavone
21	6,7-dihydroxy flavone	inactive	6,7-OH flavone
22	5-hydroxy-5,7,3′,5′-tetramethoxy flavone	inactive	Quercetin tetramethyl ether
Prediction set			
I	3-methoxy flavone		
II	4′,5-dihydroxy-3′,5′-dimethoxy-6,7(2″,2″-dimethylpyran flavone)		
III	3′-acetoxi-4′,7-dimethoxy flavone		
IV	4′,5,6-trihydroxyflavone-3′,5′,7-trimethoxy flavone		LS4
V	3′,4′,5′,6,7-pentamethoxy flavone		LS11
VI	5-hydroxy-3′,4′,5-trimethoxy-6,7(2″,2″dimethylpyran) flavone		LS99
VII	4′,5-diacetate-3′,5′,6,7-tetramethoxy-flavone		
VIII	4′,5′,3,5,6,7-hexahydroxy flavone		
IX	6,2′,3′,4′-tetramethoxy flavone		

the biological receptor through a charge transfer mechanism). These characteristics can be useful in the design of new flavones with high anti-HIV-1 activity and it is interesting to notice that the selected variables represent three distinct classes of interactions between the compounds and the biological receptor: hydrophobic (log P), steric (VOL) and electronic (EA) interactions.

3.2. Hierarchical cluster analysis (HCA)

The HCA method is an excellent tool for preliminary data analysis and it is very useful for examining data sets for expected or unexpected clusters, including the presence of outliers. This technique examines the distances between the samples in a data set and represents this information as a two-dimensional plot called dendrogram. It is informative to examine the dendrogram in conjunction with PCA results as they give similar information in different forms.

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 [32].

The results obtained with the HCA analysis were similar to those obtained with PCA and are displayed in the dendrogram showed in Fig. 5. In fact, the dendrogram can be used to provide information on chemical behavior and verify the results obtained by PCA. In the dendrogram of Fig. 5, 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 of compounds.

From Fig. 5 we can see that the two groups, group A and group B, present values of similarity equal to zero. The groups A and B in Fig. 5 correspond to the same groups A and B in Fig. 3 (PCA analysis). Both PCA and HCA methods classified the 22 compounds studied into two groups exactly in the same manner. Based on the

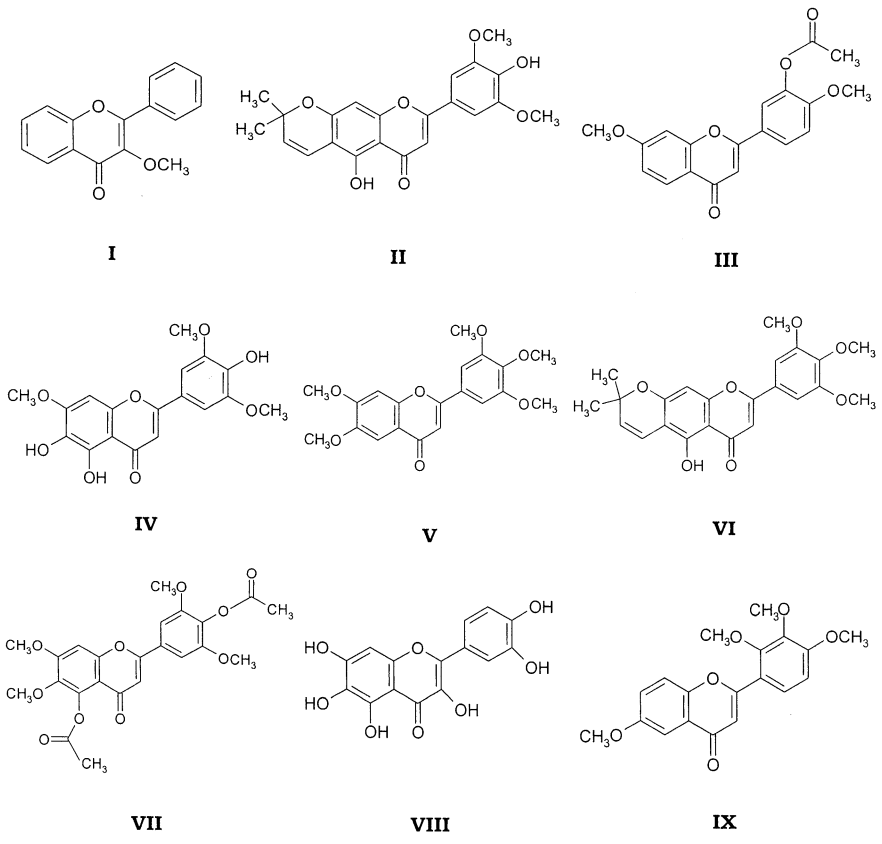

Fig. 2. Molecular structure of the nine new flavones used for the prediction study.

classification obtained with the PCA and HCA we can say that log P, EA and VOL are the descriptors responsible for the separation between the active and inactive compounds against HIV-1.

Table 2
Values of the three most important properties (variables) that classify the 22 flavones studied and the activity indication

Compound	Activity	VOL (Å^3)	log P	EA (kcal mol^{-1})
1	active	777.90	0.00	1.18
2	active	765.70	0.28	0.99
3	active	796.50	0.00	0.89
4	active	762.10	0.28	1.05
5	active	747.09	0.56	0.93
6	active	817.35	0.31	0.99
7	active	770.74	0.28	0.80
8	active	821.97	0.93	1.01
9	active	742.69	0.56	1.01
10	inactive	727.14	1.46	0.92
11	inactive	1039.70	1.05	0.74
12	inactive	690.77	2.03	0.80
13	inactive	838.15	0.91	0.76
14	inactive	957.11	1.31	0.74
15	inactive	782.15	1.78	0.82
16	inactive	706.05	1.75	0.70
17	inactive	777.75	1.50	0.87
18	inactive	969.01	1.31	0.59
19	inactive	1022.40	1.95	0.78
20	inactive	710.25	1.75	0.86
21	inactive	710.80	1.75	0.84
22	inactive	985.54	1.46	0.99

3.3. 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: group A which contains the active compounds (numbered **1** to **9**) and Group B that contains the inactive compounds (numbered **10** to **22**)

The stepwise discriminant analysis is a linear discriminant method based on the Fischer test (F-test) for the significance of the variables [31]. 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 discriminant functions obtained in this work are given as follows:

$$\text{Group A: } -94.11 + 0.10\text{VOL} + 11.98 \log P + 107.04\text{EA} \quad (2)$$

$$\text{Group B: } -105.65 + 0.12\text{VOL} + 26.26 \log P + 88.83\text{EA} \quad (3)$$

Through the discriminant functions above and the values of each variable for the compounds studied, we can obtain the classification matrix by using all com-

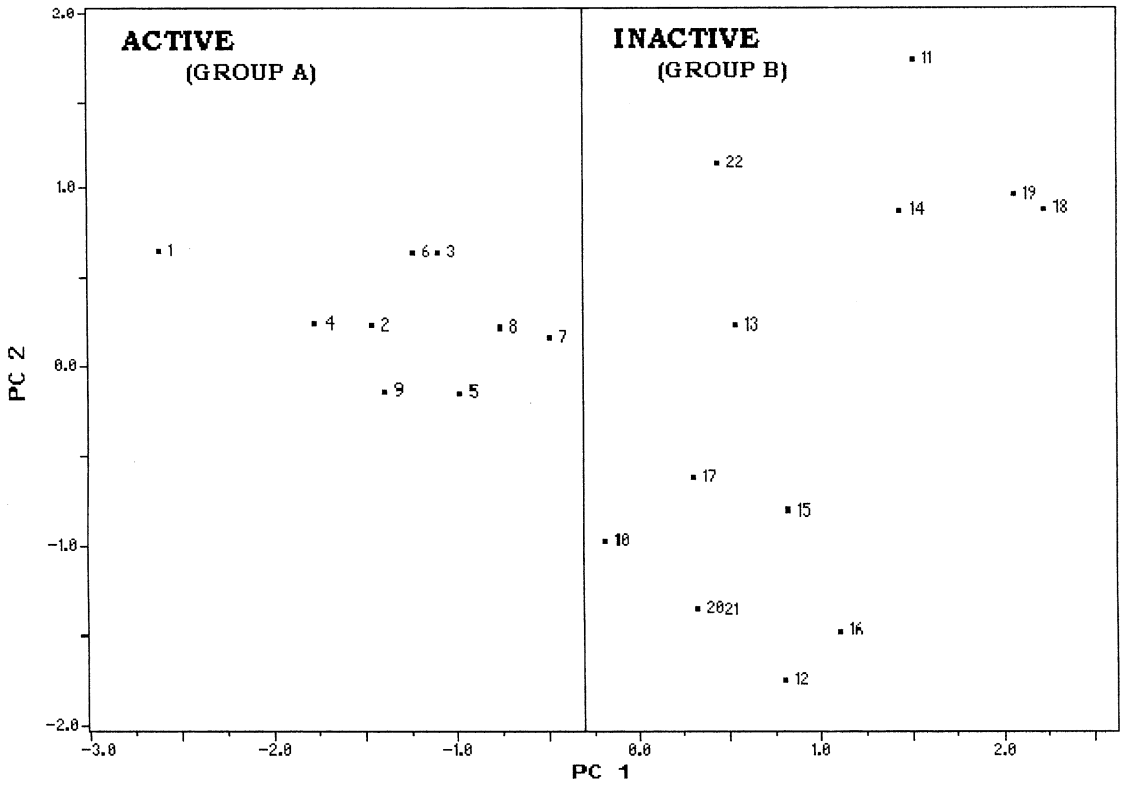

Fig. 3. Plot of the first two PC score vectors (PC_1 and PC_2) for the separation of the training set into two groups: active compounds (group A) and inactive compounds (group B).

pounds in the analysis (Table 4). The classification error rate was 0%, resulting in a satisfactory separation of the two groups. 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 compound, the value of the more important variables obtained with the SDA methodology (VOL, log *P* and EA); (b) substitute these values in the two discriminant functions obtained in this work; (c) check which discriminant function (group A—active compounds or group B—inactive compounds) presents the higher value. The new compound is active if the higher value is related to the discriminant function of group A and vice versa.

For determining if the model obtained is reliable we carried out a cross-validation test which uses the 'leave-one-out' methodology. In this procedure, one compound is omitted of the data set and the classification functions are build based on the remaining compounds.

Table 3
The loading vectors for PC_1 and PC_2

Variable	PC_1	PC_2
VOL	0.403	0.869
log *P*	0.610	−0.488
EA	−0.683	0.077

Afterwards, the omitted compound is classified according to the classification functions generated. In the next step, the omitted compound is included and a new compound is removed, and the procedure goes on until the last compound is removed. The results obtained with the cross-validation methodology are summarized in Table 5, and from these results we can verify that the model obtained with PCA, HCA and SDA is reliable once the cross-validation error is equal to 0%

Comparing the results obtained with the SDA, PCA and HCA methodologies, we can notice that the variables VOL, log *P* and EA are important in all three methodologies. Thus, combining the results obtained with PCA, HCA and SDA we can say that the three variables selected are important for explaining the anti-HIV activity of the flavone compounds studied.

3.4. K-nearest neighbour (KNN)

The KNN method classifies a new object (compound) according to its distance to an object of the training set. The closest neighbors of the training set are found and the object will be assigned into the class that have the majority of its nearest neighbors. This method is self-validating because in the training set each sample (object) is compared with all of the others in the set but not with itself. The best value of K can be chosen based on the results from the training set alone [33].

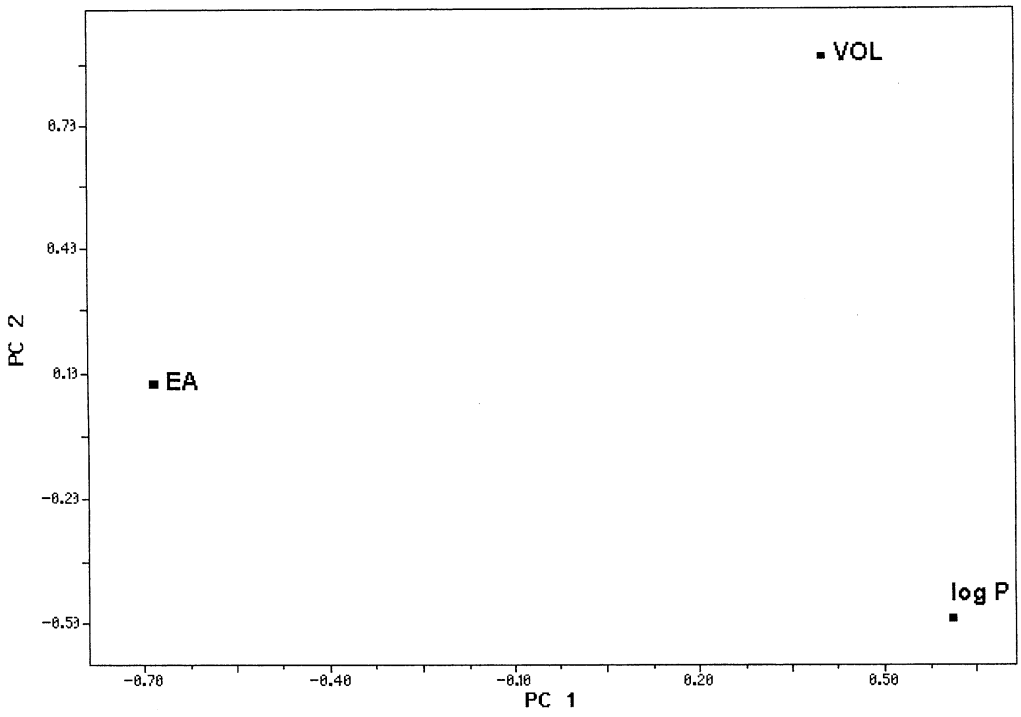

Fig. 4. Plot of the first two PC loading vectors (PC_1 and PC_2) for the three variables responsible for the separation of the training set (the 22 compounds) into two groups: active compounds (group A) and inactive compounds (group B).

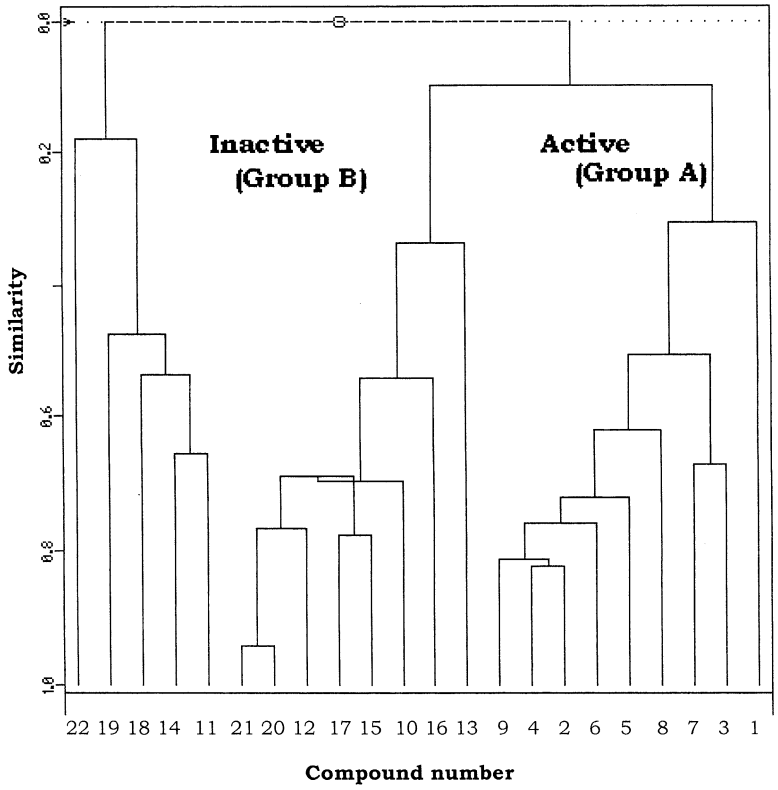

Fig. 5. Dendrogram obtained with the HCA for the training set (the 22 compounds). The HCA classifies the compounds into two groups: active compounds (group A) and inactive compounds (group B).

Table 4
Classification matrix obtained by using SDA

Classified group	True group	
	A	B
A	9	0
B	0	13
Total	9	13
Percentage	100	100

Table 5
Cross-validation matrix obtained by using SDA

Classified group	True group	
	A	B
A	9	0
B	0	13
Total	9	13
Percentage	100	100

The KNN method was used for the validation of the initial data set and Table 6 presents the results obtained with one (1NN), three (3NN) and five (5NN) nearest neighbors. For all cases (1NN, 3NN and 5NN) the percentage of correct information was 100% and we decided to use 5NN because the greater the number of nearest neighbors, the better the reliability of the KNN method.

Knowing the performance of the four pattern recognition methods (PCA, HCA, SDA and KNN) used for the 22 compounds studied, we decided to apply them to a series of nine new flavone compounds (see Fig. 2) with similar chemical structure to the training set (the 22 compounds) and anti-HIV activity not yet known. The nine molecules proposed for the activity prediction study were supplied by the organic chemistry group of the Federal University of Pará, in Brazil, and the biological tests were not performed with them yet. In the future, the anti-HIV tests with these 9 flavone compounds can be used to validate our statistical models.

We applied the previous results obtained for the 22 compounds studied by using PCA, HCA, SDA and KNN to a new set of nine analogue compounds in order to verify which molecules of the new set would be predicted as active or inactive against HIV-1. The values of the properties selected (VOL, log P and EA) for the nine new compounds are presented in Table 7.

Table 7
Values of the properties selected for the nine new compounds

Compound	VOL (Å^3)	log P	EA (kcal mol^{-1})
I	736.21	1.45	0.84
II	1071.00	1.74	0.99
III	963.03	1.31	0.75
IV	950.85	0.71	0.72
V	1040.60	1.05	0.83
VI	1122.90	1.78	0.92
VII	1239.40	0.30	0.94
VIII	780.37	0.00	1.18
IX	762.54	1.31	0.74

The results (predictions) obtained with the PCA, HCA, SDA and KNN methods for the prediction set were similar and are summarized in Table 8. The compounds **I**, **II**, **III**, **IV**, **V**, **VI** and **IX** were predicted as inactive against HIV-1 with the four methods and only the compound **VIII** was predicted as active by using the four methods. On the other hand, only the PCA analysis classified the compound **VII** as active. In

Table 8
Prediction results obtained with four chemometric methods (PCA, HCA, SDA and KNN) for the nine new compounds: active compound (+) and inactive compound (−)

Compound	Anti-HIV-1 activity			
	PCA	HCA	SDA	KNN
I	−	−	−	−
II	−	−	−	−
III	−	−	−	−
IV	−	−	−	−
V	−	−	−	−
VI	−	−	−	−
VII	+	−	−	−
VIII	+	+	+	+
IX	−	−	−	−

Table 6
Classification obtained with the KNN method for the training set (the 22 compounds)

Category	Number of compounds	Compounds incorrectly classified		
		1NN	3NN	5NN
Active	9	0	0	0
Inactive	13	0	0	0
Total	22	0	0	0
Percentage of correct information		100	100	100

this way, we can consider only the compound **VIII** as potentially active in a future biological test.

According to the three variables (EA, VOL and log P) found here as having an important role in anti-HIV-1 activity, we can verify that they belong, respectively, to three distinct classes of variables: electronic, steric and hydrophobic. Therefore we can conclude that these properties have a very important role when one is trying to understand the activity of flavone compounds against HIV-1.

4. Conclusions

Principal component analysis (PCA), hierarchical cluster analysis (HCA), stepwise discriminant analysis (SDA) and K nearest neighbor method (KNN) showed that the 22 flavonoid compounds (flavones) studied can be classified into two groups: active (group A) and inactive (group B) according to their degree of anti-HIV-1 activity. The variables log P, EA, VOL are the ones responsible for the separation between active and inactive molecules and it is interesting to notice that these variables represent three distinct classes of interactions between the compounds and the biological receptor: hydrophobic (log P), electronic (EA) and steric (VOL) interactions. The behaviour of these three variables can be useful when one is trying to obtain flavones with high anti-HIV-1 activity.

The prediction study with nine new compounds employing the PCA, HCA, SDA and KNN analyses showed that the four methods predicted that only one compound would be active against HIV-1.

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