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A structure–activity relationship study of HEPT-analog compounds with anti-HIV activity

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Abstract

The molecular orbital method PM3 is employed to calculate a set of molecular descriptors (variables) for 36 deoxy analogs of 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) with anti-HIV-1 activity. Pattern recognition methods, principal component analysis (PCA) and stepwise discriminant analysis (SDA) were employed in order to reduce dimensionality and investigate which subset of variables should be more effective for classifying the HEPT-analog compounds according to their degree of anti-HIV-1 activity. The PCA showed that the variables log P (partition coefficient), MR (molecular refractivity), ΔH_f (heat of formation), Q_N (net atomic charge on atoms 2 and 3), and χ (Mulliken's electronegativity) are responsible for the separation between compounds with higher and lower anti-HIV-1 activity. By using the SDA we have found the following descriptors as responsible for the separation between the active and less active compounds: log P (partition coefficient), χ (Mulliken's electronegativity), μ (dipole moment), Q_4 (net atomic charge on atom 4), and t_2 (torsional angle). From the SDA we present a prediction rule for classifying new HEPT-analog compounds with anti-HIV-1 activity.

Keywords: Structure–activity relationship; PM3; Principal component analysis; Stepwise discriminant analysis

1. Introduction

AZT is a thymidine analog that suppresses HIV-1 replication and is currently a licensed compound available for the treatment of patients with AIDS [1,2]. Despite its clinical efficacy, long term administration of AZT often leads to toxic side effects, such as bone marrow suppression [3]. A purine dideoxynucleoside, 2′,3′-dideoxyinosine (DDI) [4], has recently been approved as an alternative drug for the patients who do not tolerate AZT, although it also has unfavorable side effects [5]. AZT and DDI act as inhibitors of viral reverse transcriptase after being phosphorylated to their 5′-triphosphates [6,7], and such 5′-triphosphates may also interact with the host cellular DNA polymerases [8]. This non-specific action appears to contribute to the toxic side effects for this class of compounds. Therefore, it is still necessary to find new compounds having low toxicity and, preferably, a different mode of inhibition of viral replication.

The quantitative structure–activity relationship (QSAR) is still a basic method in molecular modeling. As the importance of three-dimensional (3D) microscopic interaction and binding between a substrate

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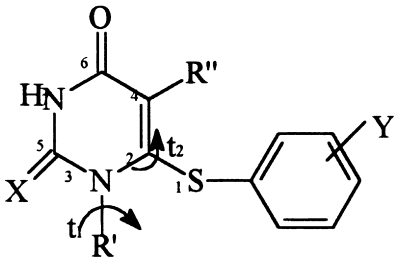
Table 1
Structure and numbering of the HEPT-analog compounds studied



Compounds	X	R′	R″	Y	EC_{50}
1	O	$CH_2OCH_2CH_2OMe$	Me	H	8.7
2	O	$CH_2OCH_2CH_2OC_5H_{11}$-*n*	Me	H	55
3	O	$CH_2OCH_2CH_2OCH_2Ph$	Me	H	20
4	O	CH_2OMe	Me	H	2.1
5	O	CH_2Pr	Me	H	3.6
6	O	$CH_2OCH_2CH_2SiMe_3$	Me	H	32
7	O	CH_2OCH_2Ph	Me	H	0.088
8	S	CH_2OEt	Et	H	0.026
9	S	CH_2OEt	Et	3,5-Me_2	0.0044
10	S	CH_2OEt	Et	3,5-Cl_2	0.013
11	S	CH_2-*i*-Pr	Et	H	0.22
12	S	CH_2OCH_2-c-Hex	Et	H	0.35
13	S	CH_2OCH_2Ph	Et	H	0.0078
14	S	CH_2OCH_2Ph	Et	3,5-Me_2	0.0069
15	S	$CH_2OCH_2C_6H_4$(4-Me)	Et	H	0.078
16	S	CH_2OCH_2(4-Cl)	Et	H	0.012
17	S	$CH_2OCH_2CH_2Ph$	Et	H	0.091
18	S	CH_2OEt	*i*-Pr	H	0.014
19	S	CH_2OCH_2Ph	*i*-Pr	H	0.0068
20	S	CH_2OEt	c-Pr	H	0.095
21	O	CH_2OEt	Et	H	0.019
22	O	CH_2OEt	Et	3,5-Me_2	0.0054
23	O	CH_2OEt	Et	3,5-Cl_2	0.0074
24	O	CH_2O-*i*-Pr	Et	H	0.34
25	O	CH_2OCH_2-c-Hex	Et	H	0.45
26	O	CH_2OCH_2Ph	Et	H	0.0059
27	O	CH_2OCH_2Ph	Et	3,5-Me_2	0.0032
28	O	$CH_2OCH_2CH_2Ph$	Et	H	0.096
29	O	CH_2OEt	*i*-Pr	H	0.012
30	O	CH_2OCH_2Ph	*i*-Pr	H	0.0027
31	O	CH_2OEt	c-Pr	H	0.1
32	O	H	Me	H	250
33	O	Me	Me	H	150
34	O	Et	Me	H	2.2
35	O	Bu	Me	H	1.2
36	O	$CH_2OCH_2CH_2OH$	Me	H	7.0

and a receptor increases, the employing of quantum chemical parameters in QSAR analysis becomes relevant [9]. The quantum chemical quantities of molecules and of interacting molecular systems can also, in principle, express all electronic properties relating to molecular interactions.

Traditional QSAR studies employ empirical physico-chemical (electronic, steric, hydrophobic

and topological) parameters related to a series of compounds. However, structural descriptors (variables) obtained from molecular orbital calculations [10–19] and topological indexes [20–22] have been successfully correlated with a variety of biological data in QSAR. In particular, topological descriptors are considered important in the design of new drugs and in the establishment of QSAR models. This kind of analysis, in contrast to the traditional methodology, is helpful to quantify different types of inter and intramolecular 3D interactions. These interactions are usually responsible for properties of biochemical systems, which justify the preference for theoretical methods in structure–activity studies.

The present work employs the semi-empirical PM3 [23] method to calculate selected molecular descriptors for 36 deoxy analogs of 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine (HEPT), reported in the literature as potent and selective anti-HIV-1 agents [24]. Some structure–activity studies have been carried out on HEPT compound derivatives [25–28]. The overall picture which emerges from these studies shows that the hydrophobic and principally the steric characteristics of substituents have a predominant role in the anti-HIV-1 activity of these compounds. The descriptors were chosen considering that electronic, steric and hydrophobic parameters are necessary for a good structure–activity study. The principal component analysis (PCA) and stepwise discriminant analysis (SDA), which were employed in this work to analyze 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 compounds used in the present study and their activity values are shown in Table 1. The compounds listed in Table 1 can be divided into two groups. Group A contains the compounds with higher degree of anti-HIV-1 activity, i.e. the molecules with $EC_{50} < 1$ (compounds 7–31). Group B contains the compounds that present lower anti-HIV-1 activity, i.e. the molecules with $EC_{50} > 1$ (compounds 1–6 and 32–36). The biological evaluation of these compounds was made by using one numerical indicator for activity, EC_{50}, and this indicates a pharmacological potency (concentration which inhibits virus replication by 50%) [24].

2.2. Calculation of the theoretical descriptors of molecular properties

Prior to any semiempirical calculation all structures were submitted to MM2 energy optimization followed by conformational search [29]. All the geometries were fully optimized by using the MNDO-PM3 Hamiltonian [23] with EF and PRECISE keywords. 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 obtained geometry represents the equilibrium conformation assessed theoretically. Only those conformations, which are most stable for a given compound, have been used to obtain the structural descriptors.

In this work the following descriptors were calculated:

HOMO	the highest occupied molecular orbital energy (eV)
χ	Mulliken's electronegativity (eV)
μ	dipole moment (a.u.)
POL	molecular polarizability (a.u.)
ΔH_f	heat of formation (kcal mol^{-1})
Q_N	net atomic charge on atom N
t_1 and t_2	torsional angles (in Table 1)
HE	hydration energy (kcal mol^{-1})
MR	molecular refractivity
A	surface area (Å^2)
VOL	molecular volume (Å^3)
log P	partition coefficient.

The calculated descriptors were selected so that they represent electronic (HOMO, χ, μ, POL, Q_2, Q_3, Q_4, ΔH_f, MR and HE), steric (t_1, t_2, A and VOL) and hydrophobic (log P) features of the compounds studied. These features are supposed to be important for their anti-HIV-1 activity [11]. The statistical analysis (PCA and SDA) has been done with the MINITAB 10.1 program [30].

The descriptors HOMO, χ, μ, POL, ΔH_f, Q_N, t_1 and t_2 were calculated with the semiempirical MNDO-PM3 method [23] in the AMPAC 5.0 program [31].

Table 2
Values of the eight most important properties (variables) that classify the HEPT-analog compounds studied

Compounds	χ	μ	t_2	ΔH_f	Q_2	Q_4	MR	log P
1	4.9913	4.25	149.03	−55.51	0.4606	−0.4921	89.59	1.74
2	5.0239	4.83	−61.63	−104.42	0.3657	−0.4295	110.60	3.32
3	4.9597	4.16	83.05	−78.01	0.4182	−0.4610	113.90	2.33
4	5.1206	5.15	−64.35	−68.86	0.4576	−0.4874	78.54	1.90
5	4.8084	1.12	−89.79	−1.86	0.3604	−0.4386	87.82	2.71
6	4.9455	4.74	138.99	−114.86	0.4480	−0.4849	99.36	1.40
7	5.1190	4.73	117.36	−37.67	0.4238	−0.4782	107.29	2.91
8	5.1928	5.14	−102.24	−4.82	0.3901	−0.5121	95.88	3.29
9	5.1259	5.78	−129.81	27.19	0.5150	−0.6025	104.45	3.59
10	5.3370	4.79	137.65	−10.89	0.4069	−0.5212	105.32	2.84
11	5.2132	5.27	−100.21	22.30	0.4352	−0.5581	99.03	3.96
12	5.1551	5.46	−141.20	−19.34	0.4139	−0.4807	116.88	4.84
13	5.2174	5.27	58.80	28.53	0.4908	−0.5307	119.89	3.96
14	5.2226	5.20	64.08	12.08	0.3758	−0.4316	128.45	4.26
15	5.2086	5.65	61.99	20.71	0.3896	−0.4460	124.17	4.11
16	5.2902	4.52	62.50	23.91	0.4239	−0.4780	124.60	3.74
17	5.1996	5.29	−121.95	25.48	0.4392	−0.5349	124.64	4.21
18	5.2360	5.29	148.58	−1.54	0.3862	−0.4564	100.43	3.62
19	5.3587	5.74	−10.19	48.27	0.4206	−0.4761	124.44	4.29
20	5.2369	5.29	157.79	36.37	0.3596	−0.4433	103.15	3.58
21	5.0195	3.67	−86.53	−78.18	0.5093	−0.5970	92.42	3.11
22	5.0022	3.45	−83.64	−89.37	0.5374	−0.6257	100.98	3.41
23	5.0907	4.98	−89.60	−82.59	0.4637	−0.4855	92.31	3.05
24	5.1399	5.36	−124.31	−87.46	0.5020	−0.5363	104.16	3.88
25	5.0656	5.18	−154.93	−89.16	0.4806	−0.5732	108.89	4.20
26	5.0109	3.66	64.12	−40.86	0.5021	−0.5893	111.90	3.31
27	4.9395	4.27	65.17	−61.42	0.4955	−0.5956	120.46	3.62
28	5.0858	4.25	−126.06	−46.48	0.4964	−0.5897	116.65	3.56
29	5.1500	4.40	96.57	−78.95	0.4772	−0.5153	92.44	2.97
30	5.0139	5.22	32.35	38.84	0.5393	−0.6302	116.44	3.64
31	5.0085	4.93	149.33	−38.56	0.5294	−0.5675	95.16	2.93
32	5.2069	3.96	83.09	−36.74	0.2363	−0.4319	67.79	1.45
33	5.1702	4.66	103.51	−31.61	0.2944	−0.4319	72.69	1.70
34	5.1803	5.29	119.53	−35.02	0.4277	−0.4913	77.40	2.04
35	5.2244	4.02	−93.52	−47.83	0.4176	−0.4990	86.56	2.90
36	5.1399	5.08	−65.39	−80.00	0.4072	−0.4770	84.94	1.46

The atomic charges performed in this work were derived from the electrostatic potential obtained with the PM3 method. The electrostatic potential is 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 [32,33]. The routine developed by Connolly [34] was used. This method uses a density of 1 point per Å in four layers placed at distance of 1.4, 1.6, 1.8, and 2.0 times the van der Waals radii. The charges derived from electrostatic potential present the advantage of being, in general, physically more satisfactory than the Mulliken's charges [35], especially when working with biological activity. The rest of the descriptors were calculated with the HyperChem 4.5 program [36].

3. Results and discussion

3.1. Principal component analysis

The central idea of PCA is to reduce the dimensionality of the data set, explaining the variance–covariance

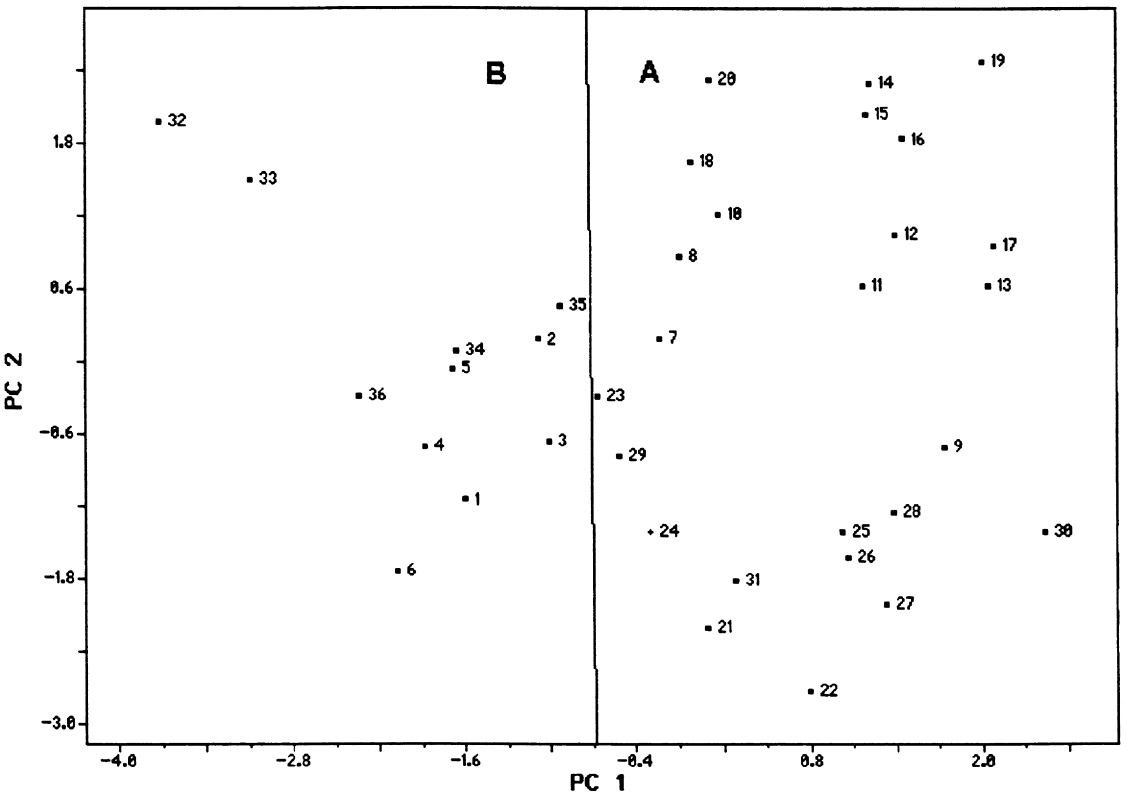


Fig. 1. PCA score (PC_1 and PC_2) for the 36 HEPT-analog compounds with anti-HIV-1 activity. The PC analysis leads to a separation into two groups: low activity (Group B) and high activity (Group A).

structure [37]. 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. 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, projections preserving maximum amounts of statistical information can be visualized using microcomputers in order to display a more detailed study of the data structure [37,38].

Before applying the PCA method, each one of the variables was autoscaled so that they could be compared to each other on the same scale. After several attempt to obtain a good classification of the compounds, the best separation was obtained with six variables (see Table 2) out of the 15 that we had initially. This suggests that the other nine variables are not so important for classifying these compounds.

The first three principal components explained 88% of total variance in the data as follows: $PC_1 = 41$, $PC_2 = 36$, $PC_3 = 11$. A number of score plots were examined and the most informative ones are presented in Fig. 1 which shows the first principal component against the second component. This projection

Table 3
Loadings of the first three principal components

	PC_1	PC_2	PC_3
χ	0.121	0.519	−0.576
ΔH_f	0.342	0.439	−0.281
Q_2	0.355	−0.518	−0.226
Q_4	−0.352	0.485	0.453
MR	0.537	0.099	0.541
log P	0.574	0.156	0.200

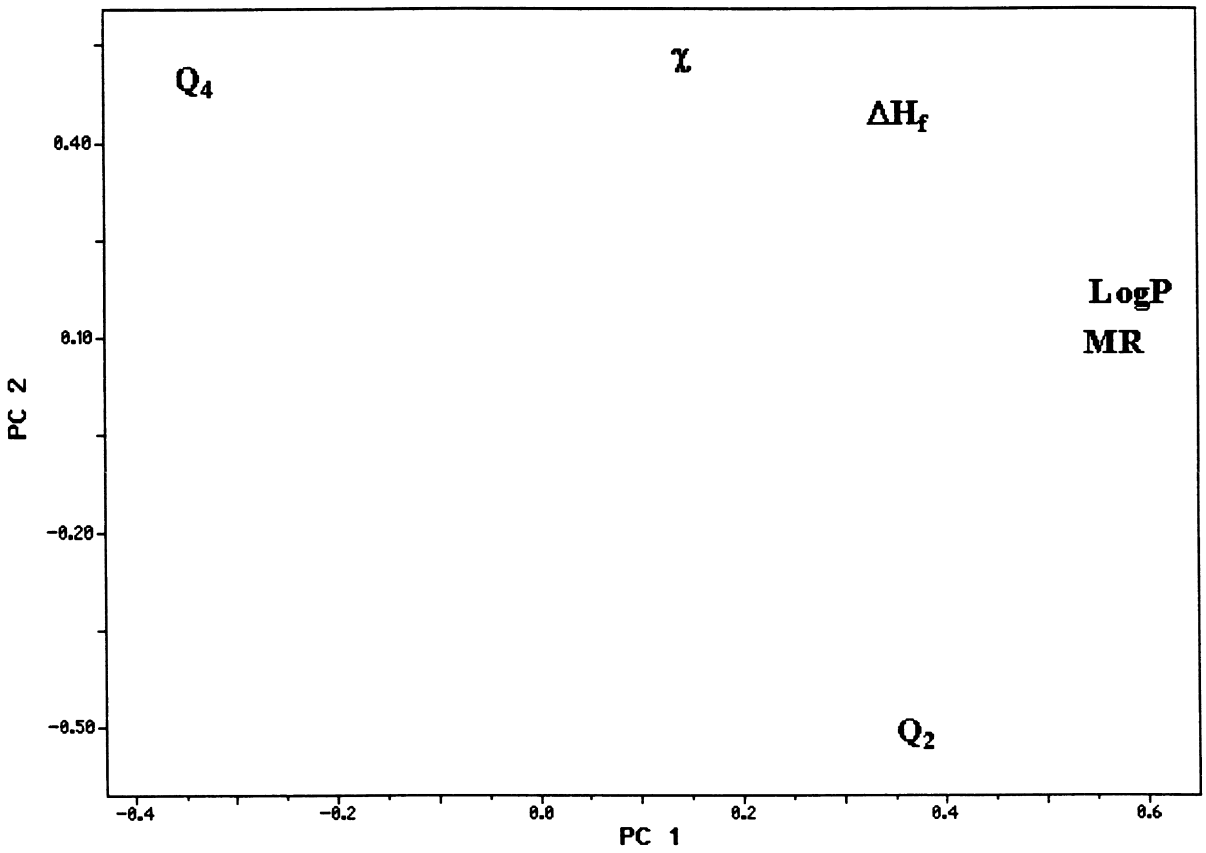


Fig. 2. PCA loading vectors (PC_1 and PC_2) for the six variables responsible for the separation of the 36 HEPT-analog compounds with low and high anti-HIV-1 activity.

conserves 77% of the total variance of the original data and can be expected to provide a reasonably accurate representation of the higher order space. Table 3 shows the loading vectors for PC1, PC2, and PC3.

The plot of the score vectors for the first two principal components (PC1 × PC2) is shown in Fig. 1. From Fig. 1, it can be seen that the HEPT-analog compounds studied are separated into two groups, A and B. Group A contains the compounds with higher degree of anti-HIV-1 activity, i.e. the molecules with $EC_{50} < 1$, and group B consists of the less active compounds, i.e. the molecules with $EC_{50} > 1$. Also from Fig. 1, it can be seen that PC1 alone is responsible for the separation between the compounds with higher and lower anti-HIV-1 activity. Fig. 2 displays the plot of the loading vectors for these first two principal components (PC1 and PC2).

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

$$PC_1 = 0.574[\log P] + 0.121\,[\chi] + 0.537[\mathrm{MR}] + 0.342[\Delta H_f] + 0.355[Q_2] - 0.352[Q_4]$$

From this equation, we can see that the more active molecules can be obtained when we have higher values for the variables $\log P$, MR, ΔH_f and, χ combined with more positive charges on atoms 2 and a more negative charge on atom 4. These characteristics can be useful in the design of new compounds with high anti-HIV-1 activity, as some of these six variables can be related to properties

Table 4
Classification matrix

	True group	
Group	A	B
A	10	0
B	1	25
Total	11	25
Percentage	91%	100%

such as strength of molecular association by charge transfer (χ), electrostatic interaction (Q_2, Q_4, and MR) and hydrophobicity (log P).

3.2. Stepwise discriminant analysis

Discriminant analysis is a multivariate technique that has two principal objectives: (1) to separate objects from distinct populations; and (2) to allocate new objects into populations previously defined [37,38]. In this section, we consider the two populations previously mentioned (groups A and B).

The SDA is a linear discrimination method based on F-test for the significance of the variables. In each step one variable will be selected on the basis of its significance. Five significant variables were extracted from the 15 variables investigated after five steps: log P, χ, μ, Q_4 and t_2. The discrimination function for groups A and B is given below:

$$\text{Group A}: \quad -2522.5 + 57.9 \log P + 842.4\chi - 48.3\mu - 1384.5Q_4 + 0.31t_2$$

$$\text{Group B}: \quad -2323.4 + 50.0 \log P + 814.3\chi - 46.4\mu - 1301.1Q_4 + 0.3t_2$$

where the two new variables represent the strength of molecular association by electrostatic interaction (μ) and steric interaction (t_2).

From the two discrimination functions obtained with the SDA study, one can see that the variables log P, χ, and Q_4 have the higher weights in this classification methodology. Comparing the results by using SDA and PCA methodologies, we can see also that log P, χ, and Q_4 are key properties for explaining the anti-HIV-1 activity of the HEPT-analog compounds studied here, but also the properties MR, ΔH_f, Q_2, μ, and t_2 are important when one is trying to design HEPT-analog compounds that present anti-HIV-1 activity. It is interesting to notice that the descriptors atomic charge, log P and MR were also important in other studies with anti-HIV-1 HEPT molecules [26].

One way to judge the performance of the classification rule (see discrimination function for groups A and B above) obtained with the SDA is to calculate the classification matrix or the cross-validation matrix (they show the actual versus the predicted group membership). The difference between them is that the procedure to calculate the classification matrix considers all information to develop the classification function and to classify the objects, and for the cross-validation matrix the procedure omits the first compound and develops a classification function using the remaining ones and finally classifies the omitted compound. In a second step, the first compound is included and now the second one is removed, and the procedure goes on until the last compound is removed.

Using the coefficients shown in the discrimination functions, the classification and cross-validation matrixes are given in Tables 4 and 5, respectively. The error obtained with the classification and cross-validation matrices were low, 2.8 and 5.6%, respectively. The separation of the two groups is quite good and the allocation rule derived from the SDA results, when the anti-HIV-1 activity of a new HEPT-analog compound is investigated, are: (a) initially one calculates, for the new HEPT-analog compound, the value of the five variables obtained here with the SDA methodology (log P, χ, μ, Q_4 and t_2); (b) substitute these values in the two discrimination functions obtained in this work; (c) check which discrimination function (Group A—compounds with higher anti-HIV-1 activity or Group B—compounds with lower

Table 5
Cross-validation matrix

	True group	
Group	A	B
A	10	1
B	1	24
Total	11	25
Percentage	91%	96%

anti-HIV-1 activity) presents the higher value. If the higher value is related to the discrimination function of Group A, the new HEPT-analog compound is active, and vice versa.

4. Conclusions

The method of principal component analysis shows that the 36 HEPT-analog compounds studied here can be classified into two groups (A and B) according to their degree of anti-HIV-1 activity. The variables log P, MR, χ, ΔH_f, Q_2, and Q_4 are those responsible for the separation between the molecules with higher (Group A) and lower (Group B) anti-HIV-1 activity. Five significant variables were extracted from the stepwise discriminant analysis method: log P, χ, μ, Q_4 and t_2. The low error in the discriminant analysis shows that the Groups A and B were well separated, and that this methodology provides a reliable rule for classifying new HEPT-analog compounds with anti-HIV-1 activity. The descriptors atomic charge, log P and MR that we have found here to be important in the HIV-1 activity were also important in other studies with anti-HIV-1 HEPT molecules.

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