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A quantum chemical and chemometric study of sesquiterpene lactones with cytotoxicity against tumor cells

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The semi-empirical molecular orbital method PM6 was employed to calculate a set of molecular descriptors of 20 sesquiterpene lactones (SQLs) with cytotoxicity against HL-60 (leukemia) tumor cells. The principal component analysis (PCA) and hierarchical cluster analysis (HCA) methods were used to obtain possible relationships between the calculated descriptors and the biological activity of the lactones. Four descriptors were identified as responsible for the separation between the active and inactive compounds: E_{HOMO} (highest occupied molecular orbital energy); Q11 (net atomic charge on C11); Q12 (net atomic charge on C12) and Q13 (net atomic charge on C13). These results indicated that the presence of the α-methylene-γ-lactone group has a significant role in the mechanism by which SQLs exert their biological activities. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: sesquiterpene lactones; α-methylene-γ-lactone; cytotoxicity; quantum chemical; chemometrics

1. INTRODUCTION

Sesquiterpene lactones (SQLs) are an important class of natural products found in plants of the *Asteraceae* family, known for their various biological activities such as anti-inflammatory, phytotoxic, antiprotozoal and cytotoxicity against different tumor cell lines [1–9]. In most cases, the biological activity of SQLs is related to the α-methylene-γ-lactone functionality, which is prone to react with suitable nucleophiles as sulfhydryl groups of cysteine in a Michael addition type reaction [10–16].

Two different situations can be evaluated when a structure–activity relationship (SAR) study is performed: the active site of the receptor is known or unknown. For the first case, information about the receptor site can be obtained from molecular modeling, X-ray analysis or nuclear magnetic resonance (NMR) studies. When the active site is unknown, SAR or quantitative structure–activity relationship (QSAR) techniques can be applied to a series of similar compounds with known biological activity previously obtained [17–22].

SAR studies have been proven to be helpful in the understanding of the influence of molecular properties on the biological activity presented by several kinds of compounds. Quantum chemical parameters of molecules and even of the 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 [17–22].

Continuing our efforts to prepare compounds with high cytotoxic activity [16,18,23–27], we describe herein a study of the relationship between selected molecular parameters (descriptors) and cytotoxicity of a set of SQLs. The semi-empirical PM6 method was employed to calculate atomic and molecular descriptors of 20 SQLs reported in our previous works [16,28] as cytotoxic agents.

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 SQLs and the biological receptor. The principal component analysis (PCA) and the hierarchical cluster analysis (HCA) were employed to obtain a relationship between the calculated variables and the cytotoxicity against HL-60 (leukemia) tumor cells.

2. MATERIALS AND METHODS

2.1. Compounds

The chemical structures of the 20 SQLs studied in this work are presented in Scheme 1. The numbering adopted for the carbon

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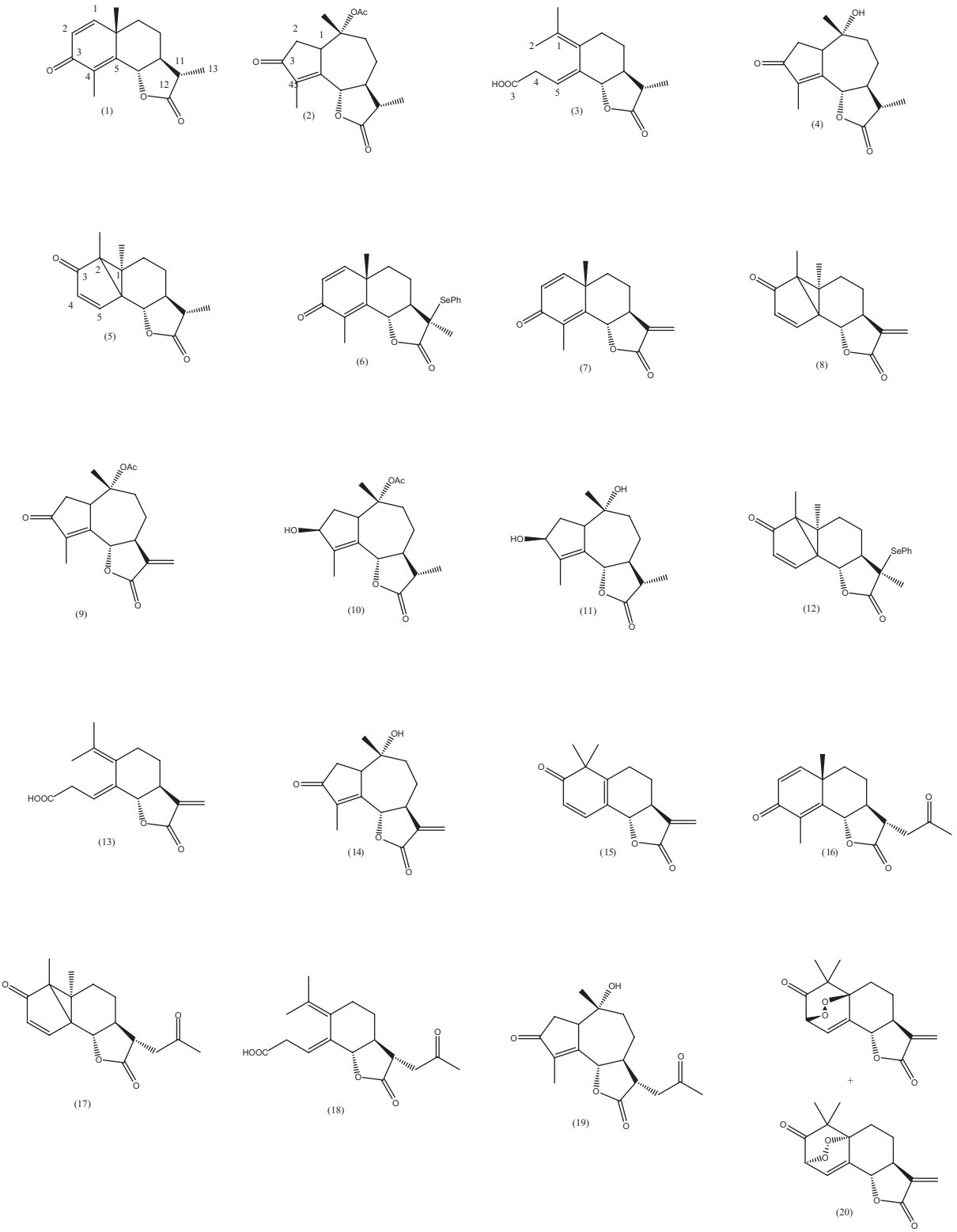
atoms is shown in the structure of compounds **1**, **2**, **3** and **5**. The respective IC_{50} values (concentration to exert 50% growth inhibition against HL-60 tumor cells) of all the SQLs studied are shown in Table I.

2.2. Calculation of the atomic and molecular descriptors

The geometries of the 20 SQLs were fully optimized using the molecular mechanics force field (MMFF) method [29]. When



Scheme 1. Structure of the 20 sesquiterpene lactones studied.

Table I. Values of the four most important properties that classify the 20 sesquiterpene lactones and their cytotoxicity (IC_{50}) against HL-60 (leukemia) tumor cells. E_{HOMO} is the energy of the highest occupied molecular orbital. Q11, Q12 and Q13 are the atomic charges on carbon atoms 11, 12 and 13, respectively

Compounds	E_{HOMO} (eV)	Q11	Q12	Q13	IC_{50} (μM)
1	−10.008	−0.2033	0.6032	−0.4572	>100
2	−10.062	−0.2070	0.6075	−0.4574	>100
3	−9.229	−0.2119	0.6133	−0.4512	>100
4	−10.037	−0.2086	0.6072	−0.4570	>100
5	−10.013	−0.2022	0.6052	−0.4572	80.16
					65.68–91.31
6	−8.921	−0.0970	0.6023	−0.4757	>100
7	−10.045	−0.1537	0.6112	−0.2374	1.14
					0.23–2.77
8	−10.041	−0.1528	0.6133	−0.2377	2.30
					1.87–2.84
9	−10.089	−0.1574	0.6149	−0.2333	1.60
					1.09–2.35
10	−9.704	−0.2085	0.6094	−0.4565	>100
11	−9.688	−0.2102	0.6092	−0.4562	>100
12	−8.974	−0.0899	0.5953	−0.4752	8.73
					6.98–10.97
13	−9.255	−0.1570	0.6197	−0.2363	8.70
					6.87–11.07
14	−10.059	−0.1579	0.6144	−0.2346	5.70
					4.56–6.84
15	−9.683	−0.1509	0.6148	−0.2379	11.90
					8.62–16.41
16	−10.142	−0.2095	0.6049	−0.4453	>100
17	−9.981	−0.2032	0.6044	−0.4451	>100
18	−9.391	−0.2009	0.6067	−0.4570	>100
19	−10.155	−0.2148	0.6087	−0.4455	>100
20	−9.410	−0.1528	0.6144	−0.2343	1.45
					1.45–1.81

necessary, several conformations were calculated for a given compound and only the most stable one was considered further. The molecular descriptors were calculated using the semi-empirical PM6 method [30], based on the most stable conformation of each derivative. MMFF and PM6 calculations were done using the PC Spartan Pro [31] and MOPAC 2009 [32] software, respectively.

The following descriptors were calculated:

(1) Electronic descriptors

- The energy of the highest occupied molecular orbital (HOMO energy) and of the lowest unoccupied molecular orbital (LUMO energy);
- Mulliken electronegativity (X), obtained according to the following equation: $X = (E_{HOMO} + E_{LUMO})/2$;
- Electron affinity (EA), obtained as $(-E_{LUMO})$;
- Dipole moment (μ);
- Heat of formation (ΔH_f);
- Total energy (E_t);
- Electronic energy (E_{el});
- Net atomic charge on the carbon atoms C1 (Q1), C2 (Q2), C3 (Q3), C4 (Q4), C5 (Q5), C11 (Q11), C12 (Q12) and C13 (Q13).

(2) Steric descriptors

- Molecular area (MA);
- Molecular volume (MV).

(3) Hydrophobic descriptor

- Partition coefficient (log P).
- The partition coefficients were calculated using the PC Spartan Pro software. The statistical analysis (PCA and HCA) was performed using the MATLAB 6.0 program [33].

3. RESULTS AND DISCUSSION

3.1. PCA

PCA is frequently employed to reduce the dimensionality of a multidimensional system. The main objective of PCA is to compress data into a small group of new variables, which are linear combination of the original variables that maximize description of the total variance data. Geometrically, this transformation represents rotation of the original coordinate system, so that the direction of the maximum residual variance is given by the first principal component axis. The second principal component axis, orthogonal to the first one, has the second maximum variance and so on [34,35].

The PCA method was used in order to obtain a separation of the set of compounds in two groups (actives and inactives), according to the calculated molecular descriptors. In addition, and most importantly, following the classification PCA is able to indicate the relevance of the selected molecular descriptor to differentiate between the active and inactive compounds. Therefore, these results give indication of the molecular descriptors responsible for the activities. Before applying the PCA method, each variable was auto-scaled so that they could be compared to each other on the same scale. After scaling, several attempts were made to obtain a good classification of the set of compounds. The best separation was obtained with four variables (see Table I) out of the 19 we initially had: E_{HOMO} (highest occupied molecular orbital energy); Q11 (net atomic charge on C11); Q12 (net atomic charge on C12) and Q13 (net atomic charge on C13). This suggests that the other variables, including the charges on the other sp^2 carbon atoms, are not important for classifying these compounds according to their cytotoxicities. Further analysis was done using only this subset of molecular descriptors.

Using this reduced set of variables, the total variance of the original data set is represented by four PCs as follows: PC1 = 46.09%, PC2 = 39.89%, PC3 = 13.26% and PC4 = 0.76%. A number of score plots were examined and the most informative one (PC1 × PC2) is presented in Figure 1. This projection keeps 85.98% of the total variance of the original data set and can be expected to provide a reasonably accurate representation of the higher order space.

Figure 1 shows that the SQLs analyzed are separated into two groups, A and B. Group A contains the SQLs **1**–**6**, **10**–**12**, **16**–**19**, with lower degree of cytotoxicity ($IC_{50} > 80.16\ \mu M$) against HL-60 tumor cells, except compound **12** with IC_{50} of 8.73 μM (Table I). Group B consists of the SQLs (compounds **7**, **8**, **9**, **13**, **14**, **15**, **20**) with higher cytotoxicity ($1.14 < IC_{50} < 11.90\ \mu M$) against HL-60 tumor cells (Table I). Additionally, it can be seen that Groups A and B are separated mainly along PC1. The loading vectors for the selected variables in PC1, PC2, PC3 and PC4 are given in Table II. Figure 2 displays the plot of the loading vectors for the first two PCs (PC1 × PC2). According to Figure 2, the E_{HOMO} descriptor is responsible for describing the inactive compounds (PC1 < 0) and the Q11, Q12 and Q13 descriptors are responsible for describing the active ones (PC1 > 0).

Table II. Loading vectors for the first four principal components

Variable	PC1	PC2	PC3	PC4
E_{HOMO}	−0.1145	0.1904	0.6609	0.7169
Q11	0.6807	0.7080	−0.1710	0.0783
Q12	0.6642	−0.4881	0.5138	−0.2379
Q13	0.2871	−0.4735	−0.5197	0.6507

According to Table II, PC1 can be expressed through the following equation:

$$PC1 = 0.6807[Q11] + 0.6642[Q12] + 0.2871[Q13] - 0.1145[E_{HOMO}]$$

From this equation, it can be seen that for a given SQL to become active (PC1 > 0) it must have more negative values for E_{HOMO} (note that E_{HOMO} has negative values), less negative values of Q11 and Q13, together with more positive values of Q12.

The energy of the frontier orbitals is an important property in several chemical and pharmacological processes, and the reason for this is the fact that this property gives information on the electron-donating and electron-accepting character of a

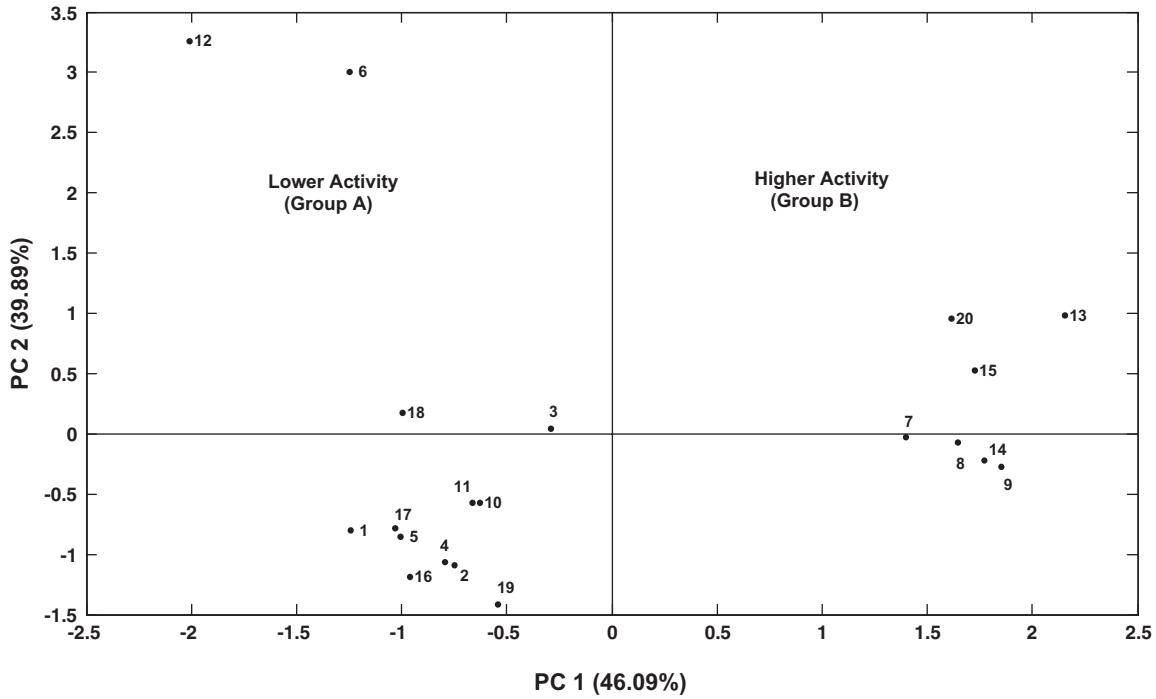


Figure 1. Plot of the score vectors of the principal components (PC1 × PC2) for the 20 sesquiterpene lactones with cytotoxicities against tumor cells. The PCA separates the compounds into two groups: lower activity (group A) and higher activity (group B).

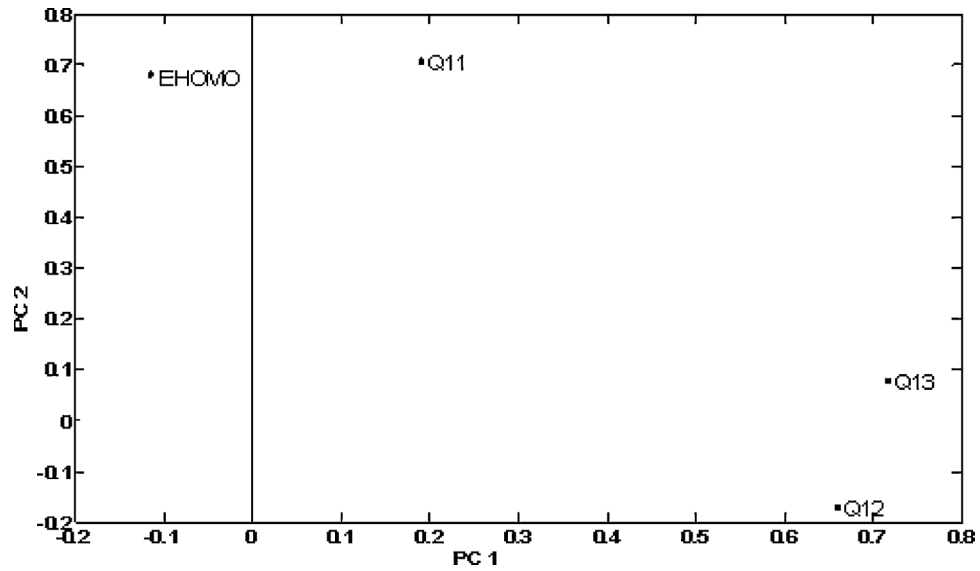


Figure 2. Plot of the first two loadings vectors (PC1 and PC2) of the variables responsible for the separation of the active and inactive compounds.

compound, i.e. on the formation of a charge transfer complex. The above equation indicates that the energy of HOMO (E_{HOMO}) for the active compounds must present lower (more negative) values than the inactive compounds. This means that the active compounds are not good electron donors when compared to the inactive ones, which is consistent with the fact that, in most cases, the biological activity of SQLs is related to reaction of the α-methylene-γ-lactone group with suitable nucleophiles, e.g. sulfhydryl groups of cysteine, in a Michael addition type reaction [36]. This is also a consequence of the higher positive charge on C12. Thus, bioactive SQLs are, generally, good electron acceptors.

3.2. HCA

In the HCA methodology, distances between pairs of samples are calculated and compared. Small distances between samples imply that they are similar. On the other hand, dissimilar samples will be separated by relatively large distances. HCA starts with

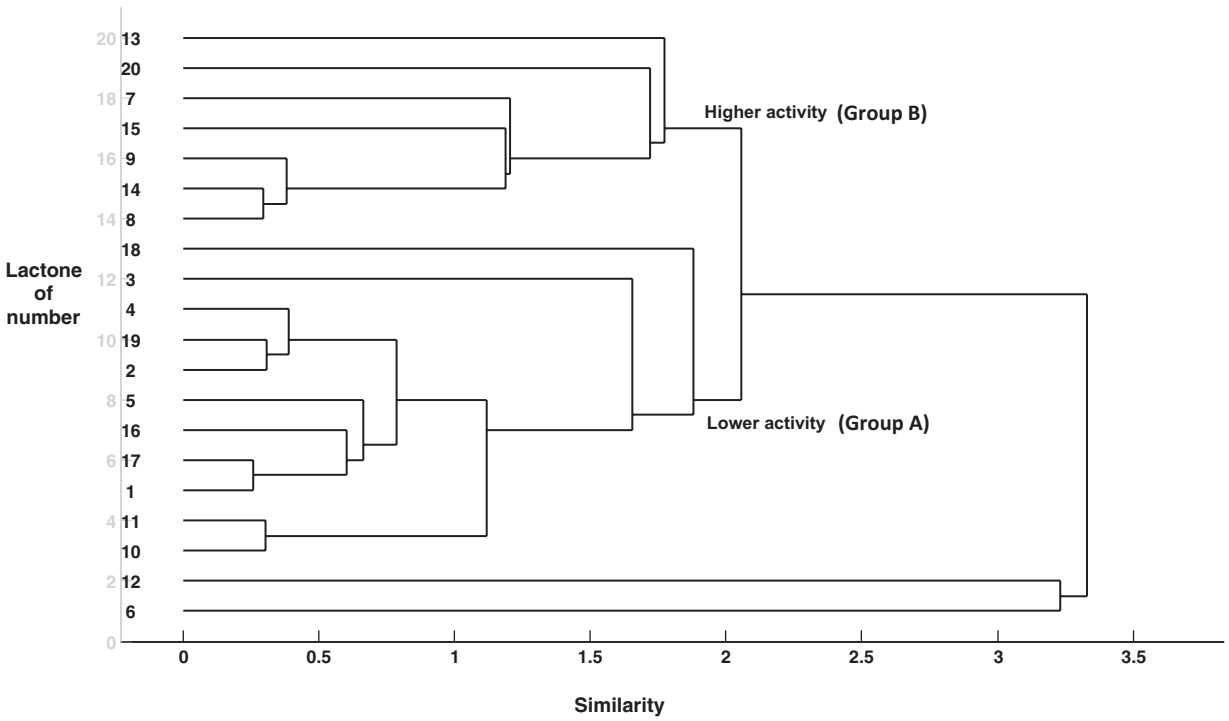


Figure 3. Dendrogram obtained with hierarchical cluster analysis for the 20 sesquiterpene lactones with cytotoxicities against tumor cells. The HCA classifies the compounds into two groups: lower activity (group A) and higher activity (group B).

each sample defined as its own cluster, then similar samples are grouped together to form new clusters until all samples are part of a single cluster. The main purpose of HCA is to represent data in a manner that emphasizes natural groupings assigning, thus, categories to which samples belong. The visualization of the groups corresponding to different classes is achieved in the form of dendrograms where the vertical lines represent the compounds and the horizontal lines represent the similarity between them [20].

The results obtained with the HCA analysis are displayed in the dendrogram shown in Figure 3. The same descriptors selected by PCA were used (E_{HOMO}, Q11, Q12, Q13). Figure 3 shows that the 20 SQLs are separated in the two groups A and B, exactly as observed in the PCA analysis. Thus, the E_{HOMO}, Q11, Q12 and Q13 descriptors are confirmed as the most important ones for classification of the SQLs in inactive or active compounds against HL-60 tumor cells.

4. CONCLUSIONS

PCA and HCA showed that the 20 SQLs studied can be classified into two groups: active (group A) and inactive (group B) against HL-60 tumor cells. The electronic descriptors E_{HOMO}, Q11, Q12 and Q13 are the most important for the separation between active and inactive molecules. This indicates that electronic effects play an important role in the understanding of cytotoxicity of the SQLs against tumor cells, while steric (MA and MV) and hydrophobic (Log P) descriptors are not important for classifying these compounds according to their cytotoxicities. The PCA analysis indicates that compounds with more negative E_{HOMO}, less negative Q11 and Q13 and more positive values of Q12 are the most active ones. These results reinforce the fact that the presence of the α-methylene-γ-lactone group, prone to react with nucleophiles in a Michael addition reaction, has a significant role in the mechanism by which SQLs exert their biological activities. On the basis of the results of this study, new SQLs can be designed which will probably show higher cytotoxicities against tumor cells.

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