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Original article

A quantum chemical and statistical study of ganoderic acids with cytotoxicity against tumor cell

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Abstract

A set of molecular properties (variables) of 24 ganoderic acids with cytotoxicities against Meth-A tumor cells was calculated by the molecular orbital semi-empirical method AM1 and ChemPropStd. Pattern recognition techniques, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to reduce dimensionality and investigate which subset of variables could be more effective for classifying the ganoderic acids according to their degree of cytotoxicities against tumor cells. The PCA and HCA studies showed that E_{HOMO} (highest occupied molecular orbital energy), Mulliken electronegativity (χ), electronic energy (E_{el}), log P (octanol/water partition coefficient), and Connolly molecular area (MA) are the most important variables for the classification between the ganoderic acids with higher and lower cytotoxicities against tumor cells.

Keywords: Ganoderic acid; Cytotoxicity; AM1; Principal component analysis; Hierarchical cluster analysis

1. Induction

Ganoderma lucidum is one of the most famous traditional Chinese medicine. In southeast Asian region, such as China, Korea, and Japan, it has been a popular folk or oriental medicine used to treat various human diseases, including hepatitis, hypertension, and gastric cancer [1]. Modern researches [2–5] showed the main bioactive compounds in *G. lucidum* are polysaccharides and ganoderic acids, a group of triterpenes. Recently, a series of ganoderic acids with cytotoxicity against Meth-A (sarcoma, murine) tumor cell have been isolated from the fruit bodies and spores of *G. lucidum* [6,7]. Previous works have also reported a series of other activities related to ganoderic acids, such as anti-HIV-1 and anti-HIV-protease [8–11].

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 [12–14].

In the present work, we employ the semi-empirical AM1 method [15] to calculate selected quantum chemical molecular variables of 24 ganoderic acids reported in the literature as presenting a certain degree of cytotoxicity against Meth-A tumor cell [6,7]. The pattern recognition methods, principal component analysis (PCA) [16] and hierarchical cluster analysis (HCA) [17], have been employed to obtain a relationship between the calculated variables and cytotoxicity against Meth-A tumor cell.

2. Methodology

2.1. Compounds

The main structure and numbering we have adopted to study the ganoderic acids are shown in Fig. 1. The structure,

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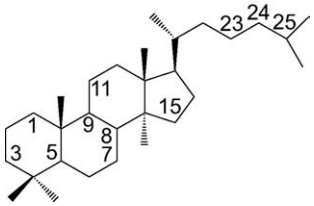

Fig. 1. Structural skeleton and numbering of the ganoderic acids studied.

the chemical name and the number of the 24 compounds are shown in Fig. 2. The biological evaluation of the ganoderic acids was done by using the logarithm of the numerical indicator for activity, ED50, which indicates 50% growth inhibition of Meth-A tumor cells [6,7]. The respective log ED50 of all the 24 ganoderic acids studied is shown in Table 1.

2.2. Calculation of the atomic and molecular descriptors

All the molecular structures of tested ganoderic acids (numbered from 1 to 24 in Fig. 2) were optimized by using the molecular mechanics method MM2 [18] and the semi-empirical method AM1 [15]. Then the following descriptors were calculated in this work:

- The energy of the highest occupied molecular orbital (HOMO energy) and lowest unoccupied molecular orbital (LUMO energy);
- Mulliken electronegativity (χ): obtained from the following equation: $\chi=(E_{HOMO}+E_{LUMO})/2$.
- Electron affinity (EA): obtained as ($-E_{LUMO}$);
- Dipole moment (m), heat of formation (ΔH_f), total energy (E_T), electronic energy (E_{el});
- Partition coefficient (log P);
- Connolly molecular area (MA) and Connolly solvent-excluded volume (SEV);
- Net atomic charge on atom N (Q_n), where n = 3, 7, 8, 9, 11, 15, 23, 24, 25.

The calculated descriptors selected could represent electronic (E_{HOMO}, E_{LUMO}, χ, EA, m, ΔH_f, E_T, E_{el} and Q_n), steric (MA and SEV) and hydrophobic (log P) features of the compounds studied. The variables were calculated with the semi-empirical AM1 method or ChemPropStd combined in CS Chem3D Ultra 6.0 program [19]. The statistical analysis (PCA and HCA) was performed using the MATLAB 6.0 program [20]. Before applying the PCA and HCA method, each variable was standardized so that they could be compared to each other on the same scale.

3. Results and discussion

3.1. PCA

The main purpose of employing the PCA method is to reduce the number of variables used in the analysis. The method creates new variables as linear combinations of all the initial variables so that the first new variable contains the largest variance, the second new variable contains the second largest variance, and so on, until the last variable can be truncated. The PCA method also allows us to diminish the number of total variables in a data set.

In this work, after several attempts to obtain a good classification of the compounds, the best separation was obtained with five variables (see Table 1) out of the 20 we had initially. This suggests that the other 15 variables are not important for classifying these compounds.

The results of the PCA calculation show that the first three principal components (PC1, PC2, and PC3) describe 99.12% of the overall variance as follows (%): PC1 = 85.38, PC2 = 13.08, PC3 = 0.66 (see Table 2). The first two principal components (PC1 and PC2) describe 98.46% of the total variance. Table 3 shows the loading vectors for PC1, PC2, and PC3.

The plot of the score vectors of the two principal components (PC1 × PC2) is shown in Fig. 3. We can see from the Fig. 3 that the ganoderic acids studied are separated into two groups, A and B. Group A contains the ganoderic acids (compounds **1**–**12**, **18**, **19**—see Table 1) with lower degree of cytotoxicities against Meth-A tumor cells, i.e. the molecules with log ED50 > 1.05 except compounds **6** and **12**. Group B consists of the ganoderic acids (compounds **13**–**17**, **20**–**24**—see Table 1) with higher cytotoxicities against tumor cells, i.e. the molecules with log ED50 < 1.05 except compound **20**. Taking into account the experimental error, we think the classification of the 24 ganoderic acids by PCA is consistent with their cytotoxicities tested by experiment.

According to Table 3, PC1 can be expressed through the following equation:

$$PC1 = 0.4489[E_{HOMO}] + 0.4384[\chi] + 0.4730[E_{el}] + 0.4794[\log P] - 0.3909[MA].$$

From this equation, we can see that more active ganoderic acid can be obtained when we have higher values for the variables E_{HOMO}, χ, E_{el}, and log P combined with smaller values for MA. Considering the interactions between active compounds and the biological receptor, we can say that the ganoderic acids with higher activity present three main characteristics: (a) they must have large values for E_{HOMO}, χ and E_{el} (the variables E_{HOMO} and χ are related to the strength of the molecular association by charge transfer, and E_{el} is related to reactive behavior between the active compound and their biological receptor); (b) they must have small MA for casing suitably with the biological receptor; (c) they must have larger values of log P (this indicates higher hydrophobic force that facilitates the combination between the active compound and their biological receptor). These characteristics can be useful in the design of new ganoderic acid molecule with a high cytotoxicity against tumor cells.

3.2. HCA

The HCA method is an excellent tool for preliminary data analysis and it is very useful for examining data sets for

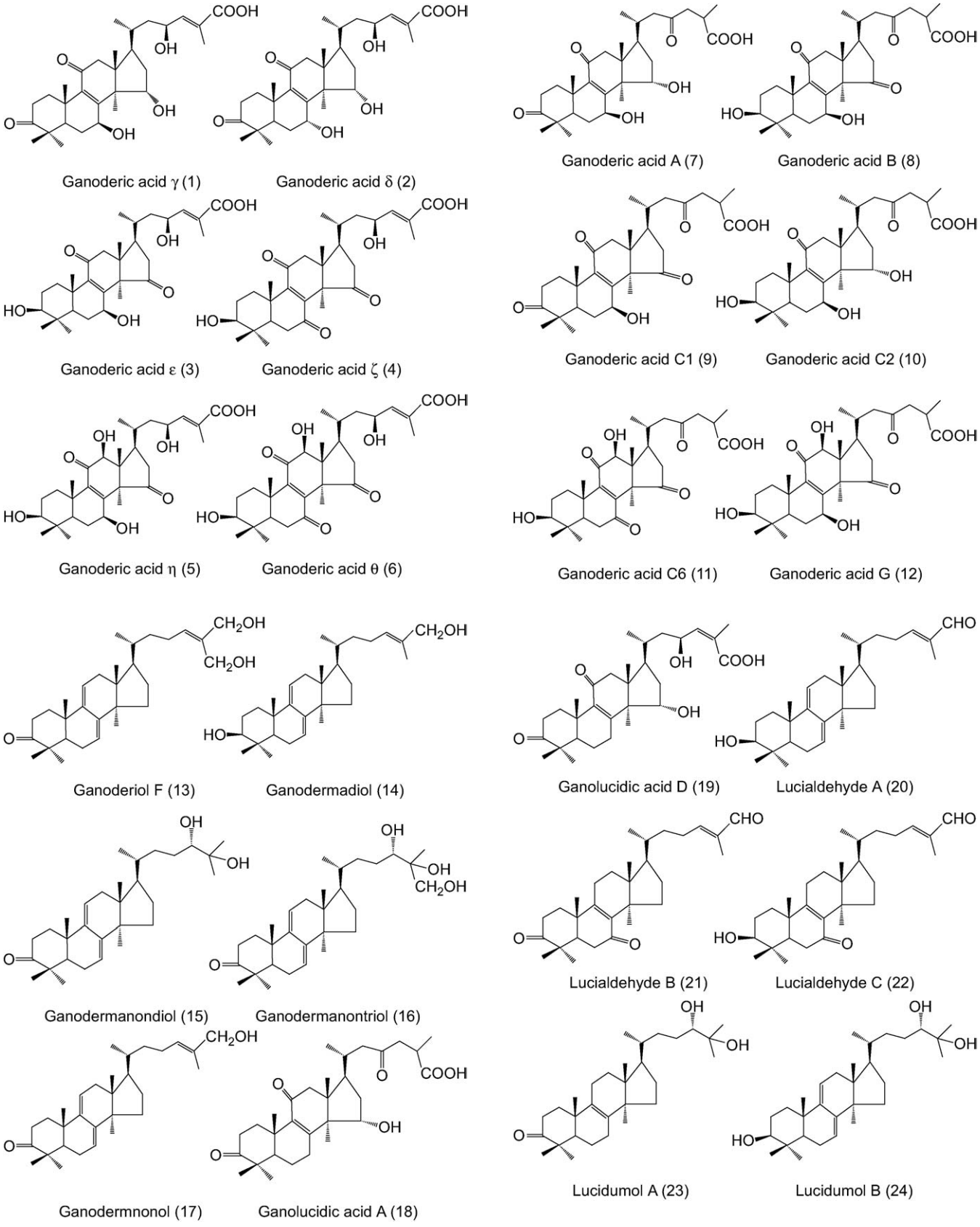

Fig. 2. Structure of the 24 ganoderic acids studied.

Table 1
Values of the five most important properties that classify the 24 ganoderic acids studied and their cytotoxicity $\log ED_{50}$

Compounds	E_{HOMO} (eV)	χ (eV)	E_{el} (eV)	log P	MA (Å^2)	$\log ED_{50}$ [6,7]
1	–9.790	–5.133	–66590.902	2.4365	426.598	1.193
2	–9.858	–5.148	–66842.659	2.4365	424.844	> 1.301
3	–9.958	–5.195	–66826.107	3.6645	423.727	1.086
4	–10.173	–5.455	–65747.448	2.8745	422.742	> 1.301
5	–10.050	–5.249	–70676.075	2.4566	425.907	> 1.301
6	–10.213	–5.572	–69382.526	1.6666	426.191	0.756
7	–9.743	–4.896	–67039.359	2.2531	428.426	> 1.301
8	–9.943	–5.056	–67079.176	3.4811	426.948	> 1.301
9	–10.022	–5.153	–66060.826	3.0791	426.433	> 1.301
10	–9.603	–4.768	–68143.004	2.6551	428.758	> 1.301
11	–10.227	–5.575	–70011.249	1.4832	427.156	> 1.301
12	–10.043	–5.249	–70831.508	2.2732	429.484	0.833
13	–8.803	–4.238	–53505.722	6.8490	421.145	0.643
14	–8.727	–4.162	–52111.720	8.3820	416.795	1.012
15	–8.752	–4.191	–55214.920	6.6898	420.546	0.531
16	–8.758	–4.194	–57812.030	6.2116	425.280	0.732
17	–8.833	–4.275	–51148.521	7.9800	416.400	0.447
18	–9.648	–4.880	–63136.952	3.6406	426.788	> 1.301
19	–9.669	–5.025	–62989.611	3.8240	422.010	> 1.301
20	–8.768	–4.429	–51153.420	8.2730	411.830	> 1.301
21	–9.759	–4.968	–53730.084	6.3090	413.532	0.602
22	–9.639	–4.849	–54692.203	6.3580	413.979	0.580
23	–9.039	–4.011	–56329.492	7.1138	419.997	0.623
24	–8.651	–4.086	–56131.363	7.0918	420.831	0.929

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 [21]. It is informative to examine the dendrogram in conjunction with PCA results as they give similar information in different forms.

In the HCA analysis, each object (the 24 ganoderic acids studied) is initially assumed to a lone cluster 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 [17].

The results obtained with the HCA analysis were similar to those obtained with PCA and are displayed in the dendrogram showed in Fig. 4. The dendrogram can be used to provide information on chemical behavior and verify the results obtained by PCA. In Fig. 4, the vertical lines represent the compounds and the horizontal lines represent the distances between pair of compounds, a compound and a group of compounds and between groups of compounds. From Fig. 4 we can see that the 24 ganoderic acids are combined in two groups, group A and group B, according to the distance. The groups A and B in Fig. 4 correspond to the same groups A and B in Fig. 3 (PCA analysis). Both PCA and HCA methods classified the 24 ganoderic acids studied into two groups. Based on the classification obtained with the PCA and HCA, we can say that E_{HOMO} (highest occupied molecular orbital

Table 2
Variances (eigenvalues) obtained for the first three principal components

Component	Eigenvalue	Percentage (%)	Cumulative (%)
PC1	4.2692	85.38	85.38
PC2	0.6539	13.08	98.46
PC3	0.0330	0.66	99.12

Table 3
The loading vectors for the first three principal components

Variable	PC1	PC2	PC3
E_{HOMO}	0.4489	–0.4278	0.7555
χ	0.4384	–0.5027	–0.5266
E_{el}	0.4730	0.1960	0.0590
log P	0.4794	0.0793	–0.3704
MA	–0.3909	–0.7208	–0.1058

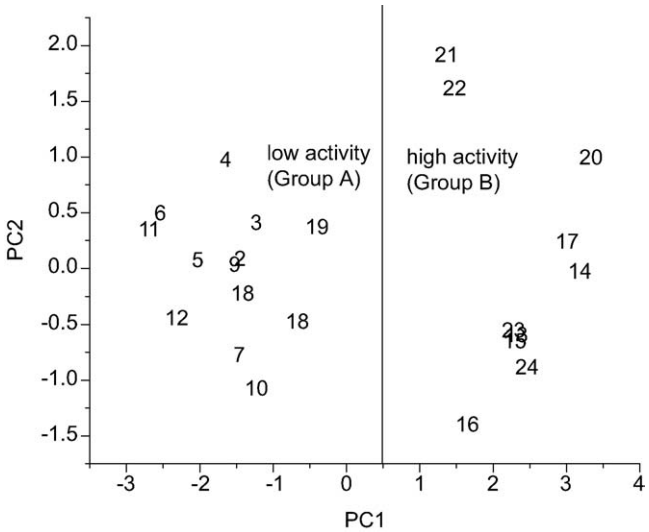

Fig. 3. The plot of the score vectors of the two principal components (PC1 × PC2) for the 24 ganoderic acids with cytotoxicities against Meth-A tumor cells. The PCA separates the compounds into two groups: lower activity (group A) and higher activity (group B).

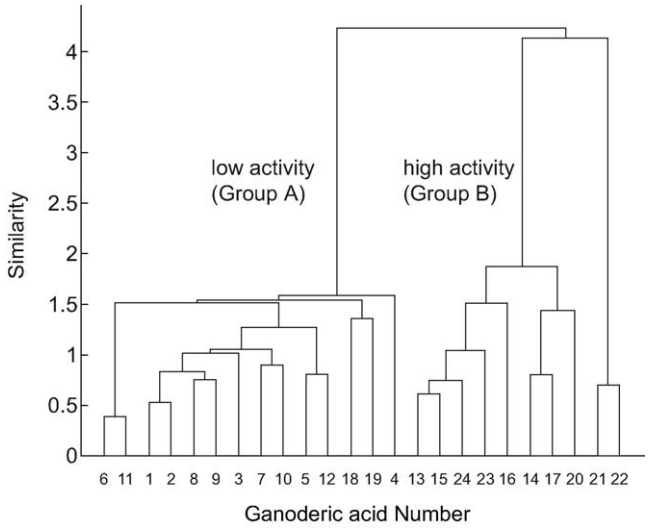

Fig. 4. Dendrogram obtained with HCA for the 24 ganoderic acids with cytotoxicity against Meth-A tumor cells. The HCA classifies the compounds into two groups: lower activity (group A) and higher activity (group B).

energy), Mulliken electronegativity (χ), electronic energy (E_{el}), log P (octanol/water partition coefficient), and Connolly MA are the most important variables for the classification between the 24 ganoderic acids with higher and lower cytotoxicities against Meth-A tumor cells.

4. Conclusion

PCA and HCA show that the 24 ganoderic acids studied here can be classified into two groups: lower active (group A) and higher active (group B) according to their degree of cytotoxicities against tumor cells. The variables, E_{HOMO}, χ, E_{el}, log P and MA, are the most important ones for the classification between the molecules with higher and lower cytotoxicities against tumor cells. The behavior of these five variables can be useful to design new ganoderic acid molecule with high cytotoxicities against tumor cells.

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