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QSAR Analysis of the Lipid Peroxidation Inhibitory Activity with Structure and Energetics of 36 Flavonoids Derivatives

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The biological activity relationship of 36 flavonoid compounds was investigated using theoretical methods including quantitative structure activity relationships (QSAR) and quantum chemistry calculation. The results suggested that the 5 and/or 8 positions of the substituents of the hydroxyl group in the A ring and the 3' and 4' positions of substituents of the hydroxyl group in the B ring play an important role in flavonoid biological activity. This is probably due to the formation of an intra-molecular hydrogen bond. In addition, the electronic energy, electrostatic energy and bond energy may have an effect on the biological activity of flavonoids. Also, our analysis has shown that the presence of the 1,4 and 1,2-hydroquinone in the A ring and/or the B ring of flavonoids and the contribution of electronic energy, electrostatic energy and bond energy required consideration in the generation of the QSAR model and that the potential compounds will be predicted out of 36 flavonoids.

Keywords: QSAR; PM3; Flavonoids; Antioxidant activity; Lipid peroxidation.

INTRODUCTION

Over the last decade, the research into flavonoids has been of considerable interest to the chemist as these compounds have antioxidant activity.[1-3] Recently, a great number of papers have been presented relating to the pharmacological and biological effects of flavonoids. The anti-inflammatory, anti-osteoporotic, antiallergic, anti-hepatotoxic, anti-tumor, antimicrobial and antiviral activities of flavonoids have been described; these may also reduce the risk of cardiovascular diseases.[4-12] In particular, flavonoid antioxidant activity can protect against reactive oxygen species (ROS) generated in the human body causing lipid peroxidation (LPO) that leads to the development of degenerative diseases. The free radical scavenging ability of flavonoids is primarily attributed to the high reactivity of hydroxyl substituents that participate in the following reaction (FOH: flavonoids and R: alkyl group).[13]

$$\mathrm{FOH} + \mathrm{R}\cdot \rightarrow \mathrm{FO}\cdot + \mathrm{RH}$$

Here, the hydroxyl substituent of flavonoids donates a hydrogen atom to radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. Flavonoids can be found in vegetables, fruits, nuts, seeds, grains, spices, medicinal plants and beverages, like wine, tea and beer. As far as we know, the structures of over 9,000 flavonoids have been identified, and these are from the largest group of naturally occurring polyphenols. In particular, the flavonoid family members include isoflavones, flavanols, flavonols, anthocyanidines and flavones (Fig. 1).

For the LPO mechanism investigation, the free radical-mediate peroxidation of polyunsaturated fatty acids leads to the formation of lipid peroxidations through a chain reaction. Experimentally, flavonoids can inhibit lipid peroxidation and convert lipid peroxidation to stable products, thus preventing their breakdown to malondialdehyde (MDA). Miyahara et al. have studied the anti-MDA formation related to LPO.[15]

Through the process of organic synthesis, flavonoids are synthesized from two basic metabolites: malonyl-CoA

Dedicated to the memory of the late Professor Ho Tong-Ing.

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and *p*-coumaroyl-CoA, to make the fifteen-carbon skeleton parent compound of flavonoids. The crucial biosynthetic reaction is the condensation of three malonyl-CoAs and one *p*-coumaroyl-CoA producing a chalcone intermediate.[14] Chalcone and dihydrochalcone belong to the class of flavonoids which consists of two phenolic groups connected by an open three-carbon bridge. This is derived from the chalcone structure, a flavonoid-class containing three rings, from which flavonoids can be formed. Here, the three-carbon bridge is the part of an additional heterocyclic six-membered ring that includes one of the phenolic groups on the adjacent ring (Fig. 1).

Recently, quantitative structure-activity relationships (QSAR) have frequently been used to determine the correlations between the biological activities and the physicochemical properties of these compounds. Because of their great number and positive biological effects, flavonoids have become one of the most popular subjects of QSAR studies. Many structure-activity relationship (SAR) investigations were performed on the antioxidant activity of flavonoids; however, only a few were, in fact, quantitative. According to these studies, the antioxidant activity of flavonoids depends strongly on the number and the positions of the hydroxyl groups in the molecule.

In the present study, the density functional theory (DFT) B3LYP method with a 6-31G* basis set was employed for generating the global minimum structure of flavone, then the semi-empirical PM3 method was used to obtain the optimized structures and necessary quantum chemical parameters of flavonoids. The purpose of this paper was to generate the predictive QSAR model by analyzing a set of 36 flavonoids. This QSAR model describes and predicts the antioxidant activity of flavonoids based on anti-MDA formation and classifies them based on biological activities and structural encoding.

METHODS

In this study, the DFT B3LYP/6-31G* calculation was performed for the conformation search of flavones, and the semi-empirical PM3 method was used on a series of flavonoids. The QSAR analyses were then generated based on the quantum chemical descriptor and anti-MDA forma-

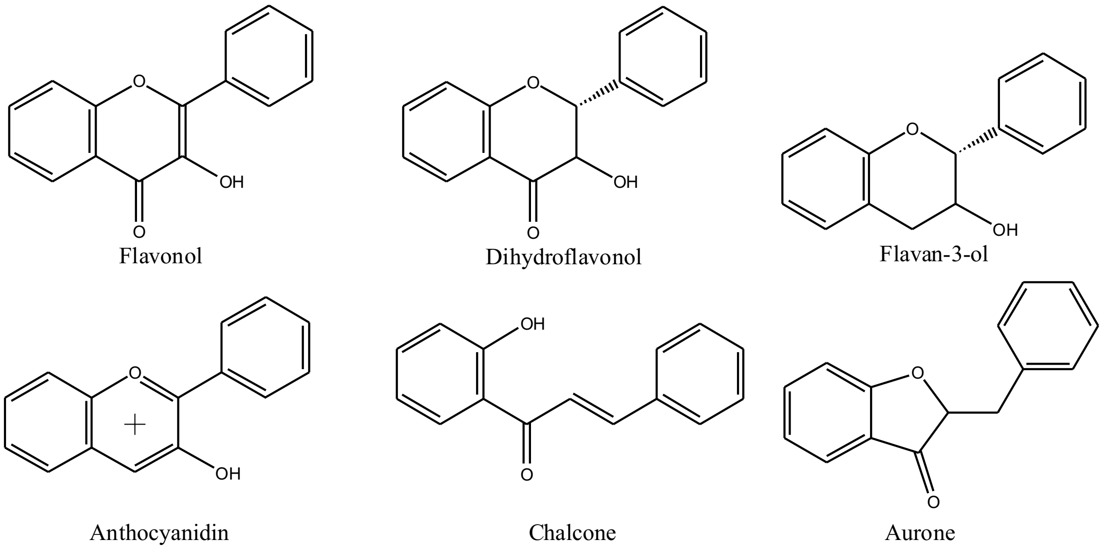

Flavone
Isoflavone
Flavonone
Flavonol
Dihydroflavonol
Flavan-3-ol
Anthocyanidin
Chalcone
Aurone

Fig. 1. Classification of flavonoids (isoflavones, flavanols, flavonols, anthocyanidines and flavones).

tion.

To build the molecular structures of flavonoids, we used the HyperChem7.0 software package to derive 36 flavonoids with minimal energy as initial structures.[16] Then their structures were optimized progressively by the semi-empirical PM3 method (which is implemented in Gaussian 03 package).[17] To generate the global minimum structure, a systematic conformational search for flavone molecules was performed with the DFT B3LYP method and the 6-31G* basis set; the torsion angle (between B ring and C ring) increment during the conformational search was set to 1° in the range 0°-180°, and the flexibility of all the torsion angles was considered. The global minimum-energy conformations, including the lowest-energy conformation, of each molecule was obtained in this way.

Miyahara et al. used 118 flavonoids to study the relationships between the substituents of flavonoids and antioxidant activity.[15] In this study, we selected 36 flavonoids, which were considered to have a higher antioxidant activity than the others, from 118 flavonoids. Their structures and relative bioactivities are given in Table 1; the inhibitory activities were given as the ability of the anti-MDA formation

Table 1. Substitution pattern of a series of flavonoids examined for their antioxidant activity* (degree of activity)

Compd. No.	Substituents of flavone										Degree of Activity
	3	5	6	7	8	2’	3’	4’	5’	6’	
1		OH	OH	OGlc-Glc							2
2		OH	OH	OGlc							2
3		OH	OH	OH							2
4		OH	OH	OGA							2
5		OH	OH	OGA-ME							2
6		OH	OH	OH				OH			1
7		OH	OH	OGA				OH			1
8		OH	OH	OPr				OPr			3
9		OH		OH			OH	OH			2
10		OH		OGlc			OH	OH			1
11		OGlc		OH			OH	OH			1
12		OH	OH	OH			OH	OH			1
13		OH	OMe	OH			OH	OH			1
14		OH	OH	OMe			OH	OH			1
15		OH	OMe	OMe			OH	OH			2
16		OH	OMe	OPr			OH	OH			2
17		OH	Glc	OH			OH	OH			1
18		OH	Glc	OMe			OH	OH			1
19	Fig. 4										1
20	Fig. 4										2
21		OH		OH	OH						3
22		OH		OPh	OH						4
23		OH		OH	OH			OH			3
24		OH		OH	OH			OMe			3
25		OH		OPr	OH			OPr			4
26		OH		OMe	OH			OMe			3
27	Fig. 4										2
28		OH		OGA	OH	OH					2
29		OH		OH	OH		OH	OH			1
30		OH		OH	OMe	OH			OH	OMe	2
31		OH	OH	OPr	OH			OPr			3
32	Fig. 4										2
33		OH	OH	OH	OH			OMe			2
34		OH	OH	OH	OPh			OPr			2
35		OH	OH	OMe	OMe	OH				OMe	3
36	ORha	OH		OH			OH	OH			1

* Flavonoid is defined in Fig. 2.

(Effective Dosage 50%, ED_{50}). According to Table 1, the antioxidant activities of flavonoids are divided into four categories by their inhibitory activities (1) $ED_{50} \geq 5$ nmol/mg prot (2) $ED_{50} \geq 0.5$ nmol/mg prot (3) $ED_{50} \geq 0.05$ nmol/mg prot (4) $ED_{50} \leq 0.05$ nmol/mg prot, as described in detail by Miyahara et al.[15] In the present study, the antioxidant activity is based on these degrees of inhibitory activity. For the QSAR model analysis, the whole data set may be divided into the training and the testing sets. The training set consists of 30 compounds for QSAR analysis and 6 compounds for the testing set. Furthermore, we tested the correlation of substituents positions and activities from the various flavonoids with the QSAR model.

According to the optimized geometry of these 36 flavonoids found using the semi-empirical PM3 method, the highest-occupied molecular orbital (HOMO), and the lowest-unoccupied molecular orbital (LUMO) are calculated precisely. The relationships of the antioxidant activities with the calculated HOMO, LUMO and other quantum chemical descriptors were carried out.

A reliable QSAR model for the structure and activity relationship should have a high correction coefficient (R), low standard deviation (s) and the least variables. In order to generate a reliable model for the QSAR analysis, we used the Cerius^2 program with Multiple Linear Regression (MLR) method and Genetic Function Approximation (GFA).[18] Conventionally, the descriptors include the structural, the energetic and the topological descriptors, where the structural descriptors in each column correspond to its indicators. We employed indicator variables to mark the presence (I = 1) or absence (I = 0) of the hydroxyl substituent at any position. The energetic descriptors use the quantum chemical parameters, such as dipole moment, HOMO, LUMO, electronic energy, electrostatic energy and bond energy, etc. The topological descriptors help to differentiate the molecules mostly according to their size, degree of branching, flexibility, and overall shape. The stability and validity of the model has been tested by the leave-one-out (LOO) cross-validation technique. Further examination of the model applicability was undertaken by plotting the predicted *vs.* the experimental antioxidant activity data of flavonoids.

RESULTS AND DISCUSSIONS

In order to study the reliable structure of flavonoids, we generated the global minimum energy structure of flavone, which is the parent molecule of flavonoids. In particular, the torsion angle between the B-ring and the C-ring may play an important role in the flavone; the torsion angle of C_3-C_2-C_1'-C_2' is shown in Fig. 2. The DFT B3LYP method with the 6-31G* basis set was used to determine the global minimum energy structure of flavone. According to the calculation result (Fig. 3), there is a global minimum energy structure when the torsion C_3-C_2-C_1'-C_2' is 46°. In this study, the structures of 36 flavonoids were considered from the torsion C_3-C_2-C_1'-C_2' of 46° as an initial geometry and by subsequent optimization.

In previous structure-relationship studies of flavonoids, several papers selected the classes of flavonoids and indicated the importance of the hydroxyl group in flavonoids.[19-22] Theoretically, the bond dissociation energies and ionization potentials for phenolic compounds are related to phenolic antioxidants which were studied by Wright et al.[19] In the QSAR model, we generated a total of over 50 QSAR equations that each consist of three to five descriptors among the QSAR random model. The results of the best QSAR model using four descriptors are shown in Eq. (1),

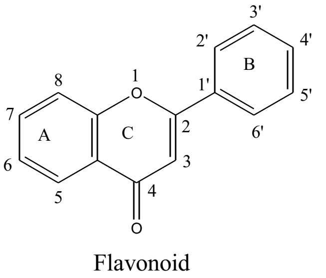


Fig. 2. General structure of a flavonoid.

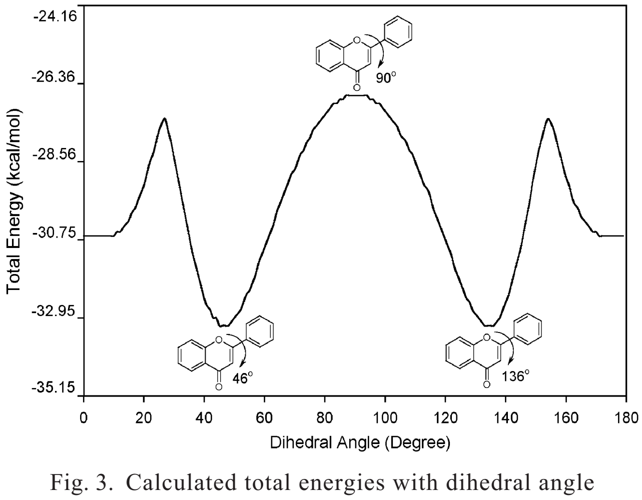


Fig. 3. Calculated total energies with dihedral angle scans.

which refer to the structural descriptor ($I_{A(5/8)}$, $I_{A(6/7)}$, $I_{B(3'+4')}$ and log$P$); $I_{A(5/8)}$ denotes that the hydroxyl groups are and/or at the positions 5, 8 in the A ring; $I_{A(6/7)}$ denotes that the hydroxyl groups are and/or at the positions 6, 7 in the A ring; and the $I_{B(3'+4')}$ denotes the hydroxyl group at 3 and 4 positions in the B ring. *logP* denotes the octanol/water partition coefficient, which is related to the hydrophobic character of the molecule, relating the chemical structure to the observed chemical behavior. These descriptors were also described by Miyahara et al.[15] The correlation matrix for these descriptors is given in Table 2. According to the MLR analysis, we determine Eq. (1) to be follows:

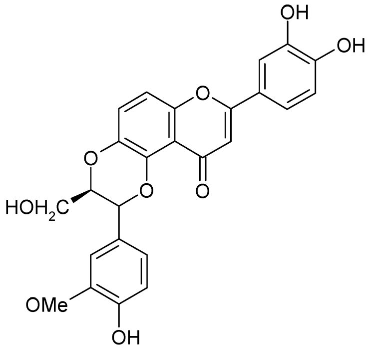
$$Activity = 0.472I_{A(5/8)} - 0.708I_{A(6/7)} - 0.709I_{B(3'+4')} + 0.319 \log P + 1.758 \quad (1)$$
$$R^2 = 0.8096,\ Q^2 = 0.7277,\ s = 0.4069,\ F = 32.96$$

The data sheet and the predicted activity for Eq. (1) are shown in Table 3. The plot of calculated *vs.* observed activities with Eq. (1) is shown in Fig.5. Eq. (1) provides a good correction for all the set of considered flavonoids except compounds 1, 15, 23, 29 and 35. These compounds do not generate the correct predicted activity using this QSAR equation.

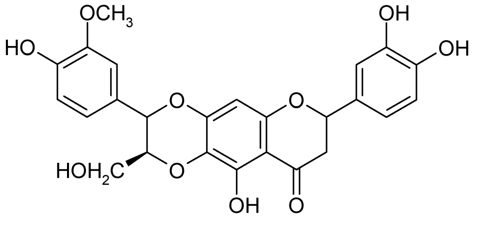
Table 2. Correlation matrix for the selected descriptors

	$log\ P$	$I_{A(5/8)}$	$I_{A(6/7)}$	$I_{B(3'+4')}$	E_{el}	E_{es}	E_b
$log\ P$	1.000						
$I_{A(5/8)}$	0.244	1.000					
$I_{A(6/7)}$	0.204	-0.249	1.000				
$I_{B(3'+4')}$	-0.350	-0.367	-0.156	1.000			
E_{el}	0.112	0.400	0.107	-0.141	1.000		
E_{es}	-0.392	-0.367	-0.164	0.257	-0.546	1.000	
E_b	-0.505	-0.356	-0.011	0.259	-0.790	0.516	1.000

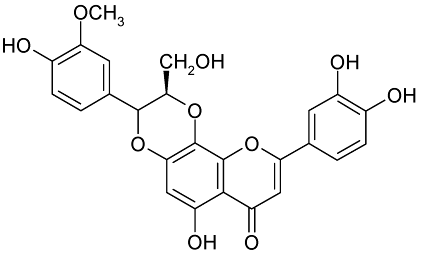
In order to modify Eq. (1), the energetic parameters were also considered in this QSAR model. Theoretically, the energetic parameters include HOMO, LUMO, dipole moment, electronic energy, electrostatic energy, bond energy, bond length, bond angle, torsion angle, total charge, total energy, molecular density, etc., for the QSAR modeling considerations. The energetic parameters could improve the prediction of antioxidant activity by using QSAR model analysis. Through the investigation of each of the descriptors of Eq. (1), we conclude that the descriptors log$P$ and $I_{A(6/7)}$ quite weakly describe the activity. Based on this conclusion, we have built a QSAR model, which excludes the descriptors *logP* and $I_{A(6/7)}$ and adds the energetic parameters. The most effective energetic parameters are



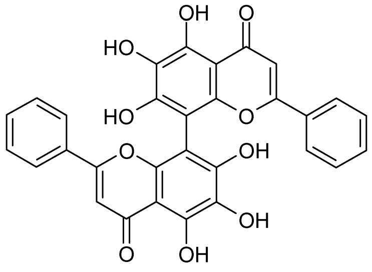
Compound 19



Compound 20



Compound 27



Compound 32

Fig. 4. Structures of compounds 19, 20, 27 and 32.

Table 3. Predicted activities of 36 flavonoids by structural parameters and *logP*

Compd. No.	$I_{A(5/8)}$	$I_{A(6/7)}$	$I_{B(3+4)}$	*log P**	Degree of Acivity	Pred.by Eq. 1**	Residual Value***
22	1	0	0	4.07	4	3.532	0.468
25	1	0	0	4.11	4	3.545	0.455
8	0	0	0	4.11	3	3.072	-0.08
21	1	0	0	1.74	3	2.787	0.213
23	1	0	0	1.07	3	2.573	0.427
24	1	0	0	1.72	3	2.780	0.220
26	1	0	0	2.37	3	2.989	0.011
31	1	0	0	3.44	3	3.331	-0.331
35	0	0	0	1.68	3	2.295	0.705
1	0	0	0	2.41	2	2.529	-0.529
2	0	0	0	0.08	2	1.783	0.217
3	0	1	0	1.74	2	1.606	0.394
4	0	0	0	0.07	2	1.780	0.220
5	0	0	0	0.46	2	1.905	0.095
9	0	0	0	1.07	2	2.099	-0.099
15	0	0	1	1.70	2	1.592	0.408
16	0	0	1	2.57	2	1.871	0.129
20	0	0	1	2.00	2	1.688	0.312
27	0	0	1	2.00	2	1.688	0.312
28	1	0	0	-0.60	2	2.039	-0.039
30	0	0	0	1.03	2	2.087	-0.087
32	0	1	0	3.02	2	2.015	-0.015
33	1	0	0	1.70	2	2.774	-0.774
34	0	1	0	4.60	2	2.520	-0.520
6	0	1	0	1.07	1	1.391	-0.391
7	0	0	0	-0.60	1	1.566	-0.566
10	0	0	1	-0.59	1	0.860	0.140
11	0	0	1	-0.59	1	0.860	0.140
12	0	1	1	0.40	1	0.468	0.532
13	0	0	1	1.05	1	1.385	-0.385
14	0	0	1	1.05	1	1.385	-0.385
17	0	0	1	-0.65	1	0.841	0.159
18	0	0	1	0.00	1	1.049	-0.049
19	0	0	1	2.00	1	1.688	-0.688
29	1	0	1	0.40	1	1.649	-0.649
36	0	0	1	-0.23	1	0.975	0.025

* reference 15; ** pred. means predicted; *** Difference of degree of activity and predicted activity.

electronic energy, electrostatic energy and bond energy in this QSAR model. The modified QSAR model of Eq. (2) was obtained:

$$Activity = 0.868\mathrm{I}_{A(5/8)} - 0.401\mathrm{I}_{B(3'+4')} - 0.471E_{el} - 0.486E_{es} - 0.206E_b + 3.286 \quad (2)$$

$$R^2 = 0.93775,\ Q^2 = 0.8124,\ s = 0.2437,\ F = 75.36$$

In Eq. (2), the quantum chemical descriptors E_{el}, E_{es} and E_b are denoted as the electronic energy, the electrostatic energy and the bond energy, respectively. A plot of the observed activities *vs.* the predicted activities is shown in Fig. 6. This equation has a higher correction coefficient (R) and lower standard deviation (s) than that of Eq. (1). We conclude that the QSAR model of Eq. (2) has a better correction than that of Eq. (1).

According to this QSAR model, the structural descriptor $I_{A(5/8)}$ is more effective in antioxidant activity than that of $I_{A(6/7)}$. And the $I_{B(3'+4')}$ is not the major effective factor in the antioxidant activity for this QSAR model. For the electronic parameter, the antioxidant activity increases due to the electronic energy and electrostatic energy, while the bond energy decreases. The predicted activity given by Eq. (2) is shown in Table 4. In order to clarify each parameter of

Eq. (2), we will discuss the effectiveness of each parameter as follows:

Geometric parameters

For the structure-activity relationship, Miyahara et al. generated the significance of the B ring and the influences from the rest of the flavonoids.[15] As has been observed, $I_{A(5/8)}$ and $I_{A(6/7)}$ were a multiple substitution on the A ring and they play an important role in the hydrogen atom transfer reaction. In the A ring of flavonoids, the positions 5- and 8- are shown to be dose dependent on the antioxidant activity. The hydroxyl group at position 5 in the A ring has an intra-molecular hydrogen bond with the ketone, which is located in the C ring. The hydroxyl group at the positions 3'- and 4'- of the B ring can easily form an intra-molecular hydrogen bond, which is shown in Fig. 7. On the other hand, it will stabilize the flavones radical as intermediate and the radical products given their capacity to scavenge free oxygen radicals.

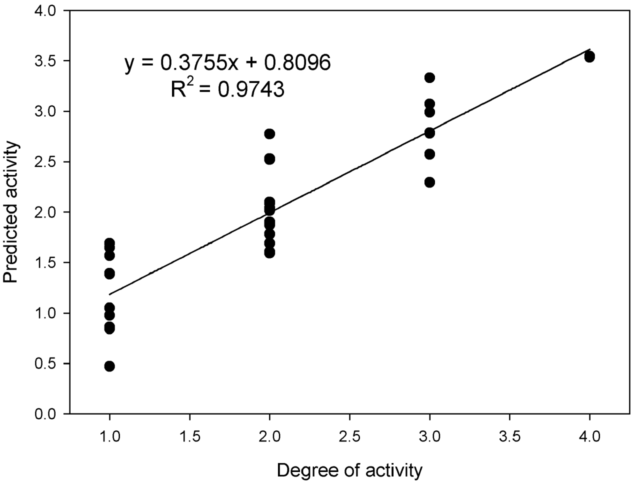


Fig. 5. A correlation plot of degree of activity and predicted activity values for a set of 36 flavonoids, Eq. (1) (linear correlation).

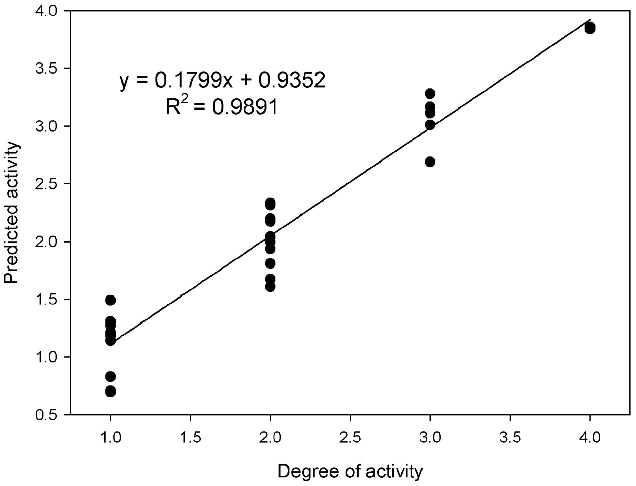


Fig. 6. A correlation plot of degree of activity and predicted activity values for a set of 36 flavonoids, Eq. (2) (linear correlation).

Quantum chemical parameters

The electronic energy parameter (E_{el}) determined a positive relation when the glucose groups were substituted, such as compounds 2, 4, 5 and 28. Unfortunately, it did not indicate a relation with the antioxidant activity directly. According to Eq. (2), the predicted antioxidant activity is proportional to the electronic energy, i.e., the higher electronic energy flavonoids have a higher antioxidant activity. We conclude that flavonoids with electronic energy over -4.0 eV have a good predicted antioxidant activity. This QSAR model also shows that flavonoids with lower electrostatic energy have higher antioxidant activity, especially compounds 8, 25, 32 and 34 due to the benzyl substituents at position 8 in the A ring. Figs. 8, 9, and 10 show the relationships between the predicted antioxidant activity and the electronic energy, the electrostatic energy, and the bond energy, respectively. The positive electrostatic energy contributes higher predicted antioxidant activity (Fig. 9). Fig. 10 shows that the bond energy affects the antioxidant activity, i.e., the bond energy increases as the antioxidant activity decreases. And bond energy lower than 15 kcal/mol will contribute to a higher antioxidant activity.

Prediction of antioxidant activity

We also tried to determine the structure activity relationship of compounds 22 and 25 and changed the hydroxyl, methoxyl, ethoxyl, iso-propoxyl and phenoxyl substituents at position 4' in the B ring of compound 22 to compounds A_1 - A_5 (Table 5). For compound 25, we changed the isopropoxyl group to hydrogen, hydroxyl, methoxyl

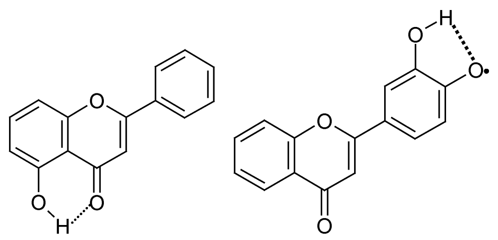


Fig. 7. Hydrogen bond in flavone.

Table 4. Predicted activities of 36 flavonoids by structural and quantum chemical parameters

Compd. No.	$I_{A(5/8)}$	$I_{B(3+4)}$	Electornic Energy (eV)	Electrostatic Energy (kcal/mol)	Bond Energy (kcal/mol)	Degree of Activity	Pred. Eq. 2*	Residual Value**
22	1	0	-3.1099	-0.1732	9.1509	4	3.856	0.144
25	1	0	-3.5626	-1.1749	11.9995	4	3.841	0.159
8	0	0	-3.5529	-1.0141	12.0431	3	3.010	-0.010
21	1	0	-2.1952	-0.1395	10.5784	3	3.110	-0.110
24	1	0	-2.5657	-0.0027	13.1350	3	2.689	0.311
26	1	0	-2.7685	0.0762	10.6713	3	3.279	-0.279
31	1	0	-3.8528	-0.4136	14.4573	3	3.164	-0.164
2	0	0	-4.7351	0.7598	15.3869	2	2.174	-0.174
3	0	0	-2.1786	-0.0928	10.6640	2	2.313	-0.313
4	0	0	-4.6921	0.2279	15.5068	2	2.331	-0.331
5	0	0	-4.9314	0.4583	17.2035	2	1.993	0.007
9	0	0	-2.3980	-0.5738	13.3029	2	2.036	-0.036
15	0	1	-2.9987	0.3993	11.9371	2	1.671	0.329
16	0	1	-3.4307	-0.1192	12.5612	2	1.936	0.064
20	0	1	-5.6141	0.7051	16.4719	2	1.808	0.192
28	1	0	-4.9015	-0.0358	20.6308	2	2.194	-0.194
30	0	0	-3.3898	0.0618	16.3469	2	1.608	0.392
32	0	0	-6.5174	-0.0107	21.4982	2	1.996	0.004
33	1	0	-2.7985	0.7047	15.4184	2	2.043	-0.043
34	0	0	-4.3337	0.0231	15.1259	2	2.323	-0.323
6	0	0	-2.3680	-0.1665	15.0781	1	1.489	-0.489
7	0	0	-4.7815	1.2236	18.8875	1	1.274	-0.274
10	0	1	-4.9224	0.3671	17.9105	1	1.307	-0.307
11	0	1	-5.0036	0.2707	18.7126	1	1.211	-0.211
12	0	1	-2.5812	0.0764	15.5568	1	0.828	0.172
13	0	1	-2.7862	0.2574	14.0068	1	1.185	-0.185
14	0	1	-2.7840	0.2685	13.5591	1	1.275	-0.275
17	0	1	-5.0721	0.1433	21.5115	1	0.695	0.305
18	0	1	-5.3788	0.3973	19.6368	1	1.141	-0.141
19	0	1	-5.2571	1.0709	17.9246	1	1.193	-0.193
36	0	1	-5.1371	0.4209	21.1026	1	0.707	0.293

* pred. means predicted; ** Difference of degree of activity and predicted activity.

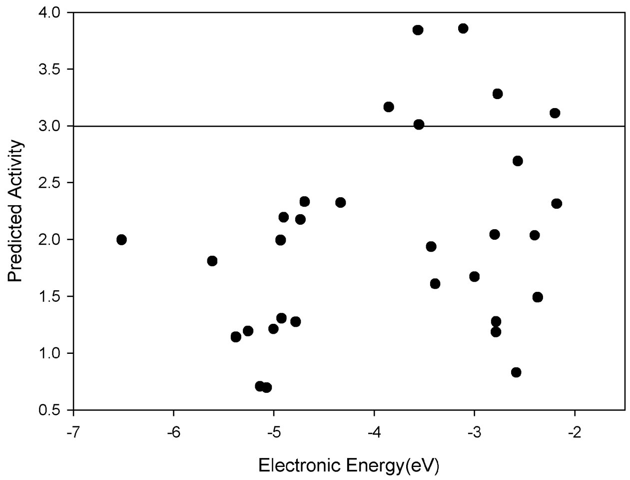


Fig. 8. Relationship between electronic energy and predicted activity.

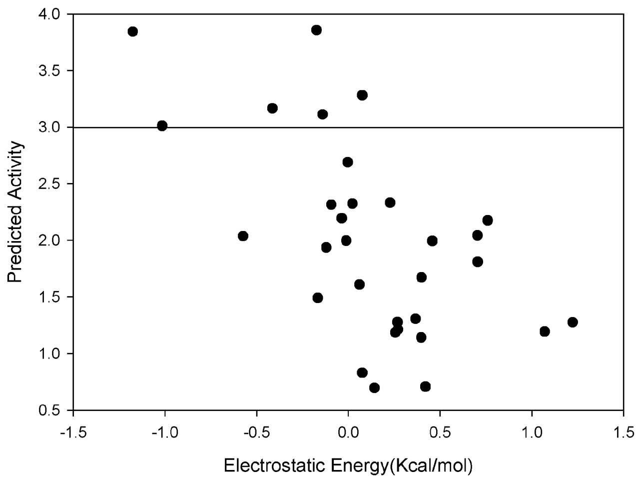


Fig. 9. Relationship between electrostatic energy and predicted activity.

Table 5. Predicted activities of compound 22 and test compounds A_1 – A_5

Compd. No.	Substituents of Flavone				E_{el}	E_{es}	E_b	Pred. Activity
	5	7	8	4’				
22	OH	OPh	OH	H	-3.1099	-0.1732	9.151	3.856
A_1	OH	OPh	OH	OH	-3.3147	-0.1969	13.901	2.937
A_2	OH	OPh	OH	OMe	-3.5206	0.0288	11.710	3.377
A_3	OH	OPh	OH	OEt	-3.7073	-0.2031	11.733	3.573
A_4	OH	OPh	OH	OPr	-3.9132	-0.6171	12.307	3.753
A_5	OH	OPh	OH	OPh	-4.2830	-0.2422	12.459	3.713

Table 6. Predicted activities of compounds 25 and test compounds A_6 – A_9

Compd. No.	Substituents of Flavone				E_{el}	E_{es}	E_b	Pred. Activity
	5	7	8	4’				
25	OH	OPr	OH	OPr	-3.5626	-1.1749	11.999	3.841
A_6	OH	H	OH	OPr	-2.7085	-0.7943	12.853	3.159
A_7	OH	OH	OH	OPr	-2.9362	-0.6197	13.637	3.019
A_8	OH	OMe	OH	OPr	-3.1406	-0.4810	11.623	3.464
A_9	OH	OEt	OH	OPr	-3.3353	-0.7133	11.654	3.662

Table 7. Predicted activities of compounds 22 and 25 and test compounds B_1 – B_8, A_4 and A_5

Compd. No.	Substituents of Flavone					E_{el}	E_{es}	E_b	Pred. Activity
	5	7	8	3’	4’				
22	OH	OPh	OH	H	H	-3.1099	-0.1732	9.151	3.856
B_1	OH	OPh	OH	OH	H	-3.3317	-0.3144	52.224	3.456
25	OH	OPr	OH	H	OPr	-3.5626	-1.1749	11.999	3.841
B_2	OH	OPr	OH	OH	OPr	-3.8191	-0.9200	48.700	3.786
A_4	OH	OPh	OH	H	OPr	-3.9132	-0.6171	12.307	3.573
B_3	OH	OPh	OH	OH	OPr	-4.1925	-0.4148	62.928	3.690
A_5	OH	OPh	OH	H	OPh	-4.2830	-0.2422	12.459	3.753
B_4	OH	OPh	OH	OH	OPh	-4.5684	0.0189	63.899	3.592
B_5	OH	OPh	OH	H	OH	-3.3241	-0.2032	13.848	2.955
B_6	OH	OPh	OH	OH	OH	-3.5582	0.05761	56.301	2.441
B_7	OH	OPr	OH	H	OH	-2.9749	-0.7137	13.734	3.063
B_8	OH	OPr	OH	OH	OH	-3.2019	-0.4643	43.071	2.550

and ethoxyl substituents at position 7 in the A ring as compounds A_6 - A_9 (Table 6). In compound 22, the antioxidant activity increases as the substituents functional group volume increases at position 4’ in the B ring. According to the quantum chemistry calculation results, compounds A_4 and A_5 have a lower calculated electronic and electrostatic energy than those of compound 22. In compound 25, we determined the same trend as in compound 22; the A_9 has a lower electronic energy and higher antioxidant activity than that of compound 25. These results match our previous results.

Except for the positions 7 and 4’, the positions 5 and 7 of flavonoids may have a significant role in the structure activity relationship. We also consider the other substituents which cause the effect in the antioxidant activity. We chose the positions 3’ and 4’ in the B ring to predict the antioxidant activity and compare the quantum parameter results. Firstly, there are 12 compounds, including compounds 22 and 25 and ten testing compounds. We compare these to the substituents at position 3’ in the B ring, shown in Table 7. We found that when there is hydroxyl group substitution, the antioxidant activity decreases, as the bonding

Table 8. Predicted activities of compounds 22 and 25 and test compounds A_4, A_5, B_3 and C_1 – C_5

Compd. No.	Substituents of Flavone						E_{el}	E_{es}	E_b	Pred. Activity
	3	5	7	8	3’	4’				
22	H	OH	OPh	OH	H	H	-3.1099	-0.1732	9.151	3.856
A_4	H	OH	OPh	OH	H	OPr	-3.9132	-0.6171	12.307	3.573
A_5	H	OH	OPh	OH	H	OPh	-4.2830	-0.2422	12.459	3.753
25	H	OH	OPh	OH	H	OPr	-3.5626	-1.1749	11.999	3.841
B3	H	OH	OPh	OH	OH	OPr	-4.1925	-0.4148	62.928	3.690
C_1	OH	OH	OPh	OH	H	H	-3.3712	0.5895	12.017	2.970
C_2	OH	OH	OPh	OH	H	OPr	-4.2492	-0.0829	14.967	3.101
C_3	OH	OH	OPh	OH	H	OPh	-4.6187	0.3528	13.089	3.451
C_4	OH	OH	OPr	OH	H	OPr	-3.8590	-0.4098	12.608	3.564
C_5	OH	OH	OPh	OH	OH	OPr	-4.4988	0.2874	15.499	2.928

energy is increasing. For position 4’ of the B ring, it can be seen that the substitution is in contradiction to the antioxidant activity in the C test set as compounds C_1 - C_5, show in Table 8. According to the results, we know that the electronic energy, the electrostatic energy, the bonding energy and the 1,4 and 1,2-hydroquinone substituted in the A ring and/or the B ring are the most important parameters in the flavone’s structure activity relationship.

CONCLUSION

In light of these results, the torsion angle between the B-ring and the C-ring may play an important role in the flavone; it has a global minimum structure while the torsion angle C_3-C_2-C_1’-C_2’ is 46°. All of the flavonoids in this paper are based on this conformation. The hydroxyl group at position 5- in the A ring forms an intra-molecular hydrogen bond with the ketone, which is located in the C ring, and it should stabilize the flavones radical intermediate. Particularly, we used calculation methods including the QSAR model and quantum chemistry calculations to elucidate the relationships between the antioxidant abilities of the 36 flavonoids and the related descriptors in this study. Also, the 7-substituted in the A ring and 4’-substituted in the B ring show a higher antioxidant activity when the larger functional group acts as a substituent. The analysis has shown that the presence of $I_{A(5/8)}$, $I_{B(3'+4')}$, E_{el}, E_{es} and E_b in generating the QSAR model. The predicted activities were dependent on two structural parameters and three energy parameters. Using the QSAR model, we can provide calculation results for chemists and biochemists to design models and to predict their bioactivities.

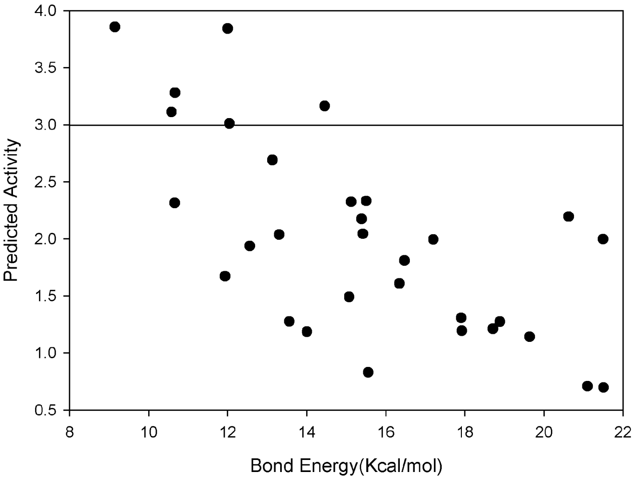


Fig. 10. Relationship between bond energy and predicted activity.

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