NMR Data of Flavone Derivatives and Their Anti-oxidative Activities

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The ^{1}H and ^{13}C chemical shifts of eleven flavone derivatives were completely determined by basic 1D and 2D NMR experiments. Nineteen flavone derivatives including the above eleven derivatives were examined for anti-oxidative effects using the 1,1-diphenyl-2-picryl-hydrazyl assay and *Caenorhabditis elegans*. In order to understand the relationships between the structures of flavone derivatives and their anti-oxidative activities, a Comparative Molecular Field Analysis was performed.

Key Words : NMR, Flavone derivatives, Anti-oxidative activity

Introduction

Flavonoids, secondary metabolites produced by plants, can be classified as anthocyanins, flavans, proanthocyanidins, C-glycosylflavonoids, biflavonoids, triflavonoids, isoflavonoids, neoflavonoids, flavones, flavonols, flavone glycosides, and flavonol glycosides according to the oxidation state of a pyran ring.[1,2] Because of their well-known anti-oxidative activities, they have been used to protect against many diseases.[3] Although more than several thousands of flavonoids have been found, surprisingly novel compounds are still being discovered. In order to identify them, NMR spectroscopy can be applied. However, NMR data of many flavonoids have not been reported yet. In the present study, we present ^{1}H and ^{13}C NMR data of 11 flavone derivatives according to the results of our experiment on the based of the fact that flavones include a ketone group in a pyran ring.

It is well known that flavonoids can protect against many diseases due to their anti-oxidative activities. Therefore, we examined anti-oxidative activities of 19 flavone derivatives in this study. Accumulation of potentially harmful oxygen radicals increases with age in a number of species. In addition, this can cause cellular changes that could result in the loss of homeostatic control and organ function.[4] *In vivo*, a balance normally exists between the production of reactive oxygen species and the capacity of the endogenous anti-oxidant defense. Disturbance of this balance is termed oxidative stress, and results in cellular damage to cell constituents such as DNA, lipids and proteins and is implicated in over a hundred disease conditions in human.[5,6]

In order to evaluate anti-oxidative effects, the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and *Caenorhabditis elegans* (*C. elegans*) were used. *C. elegans* is an ideal model organism with convenient features for examining anti-oxidative activities by measuring survival rates after induction of oxidative damage. Because the DPPH assay provided the information about quantitative anti-oxidative effects of flavones, their structures could be elucidated. Among the 19 flavone derivatives tested, 3,6,4'-trihydroxyflavone showed good anti-oxidative activity, therefore its activity could be compared with the activity of vitamin C. Since an indirect ligand-based approach can help to understand the structure-activity relationships, a Comparative Molecular Field Analysis was performed to study quantitative structure activity relationships (QSAR) of the 19 flavone derivatives.

Materials and Methods

Materials. Nineteen flavone derivatives, 3-hydroxyflavone (**1**), 3,6-dihydroxyflavone (**2**), 3,7-dihydroxyflavone (**3**), 3,3'-dihydroxyflavone (**4**), 6-hydroxy-3'-methoxyflavone (**5**), 7,3'-dihydroxyflavone (**6**), 7,4'-dihydroxyflavone (**7**), 3,6,3'-trihydroxyflavone (**8**), 3,6,4'-trihydroxyflavone (**9**), 3,7,3'-trihydroxyflavone (**10**), 3,7,4'-trihydroxyflavone (**11**), 6,3'-dimethoxy-3-hydroxyflavone (**12**), 7,3'-dimethoxy-3-hydroxyflavone (**13**), 7,2',3'-trimethoxy-3-hydroxyflavone (**14**), 7,3'-dimethoxyflavone (**15**), 3,7,3'-trimethoxyflavone (**16**), 6,2',3'-trimethoxyflavone (**17**), 7,2',3'-trimethoxyflavone (**18**), 7,2',4'-trimethoxyflavone (**19**), were purchased from INDOFINE chemical company, Inc. (Hillsborough, NJ). Their structures and nomenclatures are shown in Figure 1. The chemicals were used for the experiments without further purification, which were supplied from the company at the purity of 98%.

NMR spectra. All NMR measurements were carried out on a Bruker Avance 400 spectrometer system (9.4 T, Karlsruhe, Germany) at 298 K. The concentrations of the samples for ^{1}H NMR, ^{13}C NMR, distortionless enhancement by polarization transfer (DEPT), correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bonded connectivities (HMBC), and nuclear Overhauser and exchanged spectroscopy (NOESY) experiments were approximately 50 mM in DMSO-d_6. The number scans for the ^{1}H NMR were 16 and the 32K data points were acquired with a 1 sec relaxation delay. Its 90° pulse was 10.2 μsec with a spectral width of 12 ppm. The ^{13}C NMR and DEPT spectra were obtained with a spectral width of 210 ppm using 64 K data points. Their 90° pulses

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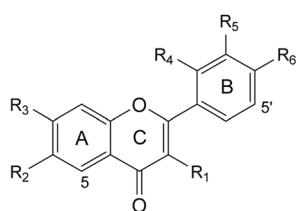
Flavone derivatives	Substituents	Name
1	$R_2=R_3=R_4=R_5=R_6=H$, $R_1=OH$	3-Hydroxyflavone
2	$R_3=R_4=R_5=R_6=H$, $R_1=R_2=OH$	3,6-Dihydroxyflavone
3	$R_2=R_4=R_5=R_6=H$, $R_1=R_3=OH$	3,7-Dihydroxyflavone
4	$R_2=R_3=R_4=R_6=H$, $R_1=R_5=OH$	3,3'-Dihydroxyflavone
5	$R_1=R_3=R_4=R_6=H$, $R_2=OH$, $R_5=OCH_3$	6-Hydroxy-3'-methoxyflavone
6	$R_1=R_2=R_4=R_6=H$, $R_3=R_5=OH$	7,3'-Dihydroxyflavone
7	$R_1=R_2=R_4=R_5=H$, $R_3=R_6=OH$	7,4'-Dihydroxyflavone
8	$R_3=R_4=R_6=H$, $R_1=R_2=R_5=OH$	3,6,3'-Trihydroxyflavone
9	$R_3=R_4=R_5=H$, $R_1=R_2=R_6=OH$	3,6,4'-Trihydroxyflavone
10	$R_2=R_4=R_6=H$, $R_1=R_3=R_5=OH$	3,7,3'-Trihydroxyflavone
11	$R_2=R_4=R_5=H$, $R_1=R_3=R_6=OH$	3,7,4'-Trihydroxyflavone
12	$R_3=R_4=R_6=H$, $R_1=OH$, $R_2=R_5=OCH_3$	6,3'-Dimethoxy-3-hydroxyflavone
13	$R_2=R_4=R_6=H$, $R_1=OH$, $R_3=R_5=OCH_3$	7,3'-Dimethoxy-3-hydroxyflavone
14	$R_2=R_6=H$, $R_1=OH$, $R_3=R_4=R_5=OCH_3$	7,2',3'-trimethoxy-3-hydroxyflavone
15	$R_1=R_2=R_4=R_6=H$, $R_3=R_5=OCH_3$	7,3'-Dimethoxyflavone
16	$R_2=R_4=R_6=H$, $R_1=R_3=R_5=OCH_3$	3,7,3'-Trimethoxyflavone
17	$R_1=R_3=R_6=H$, $R_2=R_4=R_5=OCH_3$	6,2',3'-Trimethoxyflavone
18	$R_1=R_2=R_6=H$, $R_3=R_4=R_5=OCH_3$	7,2',3'-Trimethoxyflavone
19	$R_1=R_2=R_5=H$, $R_3=R_4=R_6=OCH_3$	7,2',4'-Trimethoxyflavone

Figure 1. Structures and nomenclatures of 19 flavone derivatives.

were 10.3 μsec. All two-dimensional spectra except NOESY were acquired with 2,048 data points for t_2 and 256 for t_1 increments using magnitude mode. The NOESY spectra were collected with 2,048 data points for t_2 and 256 for t_1 using time proportional phase increments at the mixing time of 1 sec. The long-ranged coupling time for HMBC was 70 msec. Prior to fourier transformation, zero filling of 2 K and sine squared bell window function were applied using XWIN-NMR (Bruker) and all NMR data were analyzed in Sparky.[7-10]

***In vitro* anti-oxidative activity.** In order to test DPPH radical-scavenging effects, 100 mL of the sample was adjusted to 0.1% methanol solution. One mL of 100 mM Tris-HCl buffer (pH 7.4) was added, and the mixture was added to 1 mL of 0.5 mM methanol solution of DPPH radical. After 15 min at 37 °C, absorbance was measured with a spectrophotometer at 517 nm (Shimadzu, Tokyo, Japan). The scavenging effects were calculated using the equation as follows:

scavenging effects (%)
= [1 − (absorbance of sample/absorbance of control)] × 100.

***In vivo* anti-oxidative activity.** Methods for maintenance

and handling *C. elegans* were followed to Brenner's method.[11] Strain N2 was used as a wild-type strain for most analyses. They were provided by the *Caenorhabditis* Genetics Center. Wild-type N2 worms were grown at 20 °C on nematode growth medium (NGM) agar plates containing *E. coli* (OP50). Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride hydrate, Sigma-Aldrich, St. Louis, MO) was used for generating internal reactive oxygen species in *C. elegans*. Wild type N2 worms were synchronized at L1 stage by hatching embryos in M9 buffer (42 mM of KH_2PO_4, 2 mM of Na_2HPO_4, 0.86 M of NaCl, 1 mM of $MgSO_4$) and 50 worms were transferred on to NGM agar plate containing OP50 *E. coli* and 1 mM of each flavone. Worms were grown at 20 °C until they reached at a gravid adult stage and they were then treated with 80 mM paraquat in M9 buffer for 20 h at 20 °C. Since flavone was dissolved in DMSO, worms were also grown on plates containing 0.5% DMSO. The treated worms were transferred onto the fresh NGM agar plate without OP50 and recovered for 1 hr and scored for survival. They were counted as dead if they did not respond to a touch on its head.

Structure-activity relationships (SARs). In order to identify the relationships between the structures of flavone derivatives and their anti-oxidative activities, a three-dimensional (3D) quantitative structure activity relationship study (QSAR) was conducted based on a Comparative Molecular Field Analysis (CoMFA).[12] They were carried out using SYBYL 7.2 (Tripos, St. Louis, MO) on a Pentium 3.2 GHz PC with a Linux OS (Red Hat Enterprise WS). The structures of flavone derivatives were built with the Sketcher module and the energy minimized by the conjugate gradient method using a Tripos force field. The steric and electrostatic fields in CoMFA were calculated at each lattice intersection of a regularly spaced grid of 0.25 Å in all three dimensions within the defined region, and the van der Waals potential and Coulombic energy between the probe and the molecule were calculated using a standard Tripos force field. The final CoMFA model exhibited a cross-validated correlation coefficient (q2) of 0.65 and conventional correlation coefficient (r2) of 0.84.[13]

Statistical analysis. The data are reported as the mean of three independent experiments, and were evaluated by Student's *t*-test. Values of $P < 0.05$ were considered to be statistically significant.

Results and Discussion

Of 19 flavone derivatives, NMR data of 8 derivatives (**1**, **4**, **8-13**) were reported previously.[14-16] Because 7,2',3'-trimethoxy-3-hydroxyflavone (**14**) is the most substituted compound among 11 flavone derivatives whose NMR data are not known, its complete assignments of ^1H and ^{13}C NMR data are explained in detail here. Eighteen peaks were observed in the ^{13}C NMR spectrum of derivative **14**. Its DEPT experiments gave nine singlets, six doublets, and three quartets. In A and C rings, the most downfield shifted peak was 172.2 ppm which was assigned ketone group (C-

4). In HMBC, C-4 was long-range coupled to the ^{1}H peak at 8.03 ppm which was directly attached to the ^{13}C peak at 126.3 ppm in HMQC, so that it should be assigned C-5. Because the ^{1}H at 8.03 ppm was long-range coupled to two other carbons at 163.5 ppm and 156.8 ppm in HMBC, they could be C-7 or/and C-9. Derivative **14** is methoxylated at C-7. Therefore, the former should be C-7 and the later is C-9. C-9 showed long-ranged coupling with the ^{1}H at 7.08 ppm which was attached to the ^{13}C at 100.2 ppm, so that it should be assigned C-8. Since H-8 was long-range coupled to two other carbons at 114.5 ppm and 115.8 ppm, they could be assigned C-6 or/and C-10. C-6 must be doublet carbon and the carbon peak at 114.5 ppm was determined doublet by DEPT. As a result, 114.5 ppm and 115.8 ppm could be assigned C-6 and C-10, respectively. Because the ^{1}H peak at 8.88 ppm was not attached directly to any carbon, it was assigned 3-OH. In HMBC, the ^{1}H peak at 8.88 ppm was long-range coupled to two carbons at 138.8 ppm and 146.0 ppm. Based on the chemical shift, the former should be C-3 and the later, C-2. In B ring, H-4', H-5', and H-6' could be assigned from the interpretation of COSY, which were 7.21 ppm, 7.12 ppm, and 7.80 ppm, respectively. Since the ^{13}C peak at 125.4 ppm showed a long-ranged coupling with H-5', it could be assigned C-1'. In order to distinguish C-2' and C-3', the NOESY experiment was carried out. Because H-4' showed an nOe cross peak with the ^{1}H at 3.87 ppm which was directly attached to 55.9 ppm in HMQC and was long-range coupled to the ^{13}C peak at 152.7 ppm, they should be assigned 3'-OCH$_3$ and C-3', respectively. The undetermined carbons were C-2' and 2'-OCH$_3$ which could be assigned easily 147.0 ppm and 60.6 ppm, respectively. The complete

assignments of the ^{1}H and ^{13}C chemical shifts of derivative **14** are listed in Tables 1 and 2, respectively.

The NMR data of the remained ten flavone derivatives (**2, 3, 5-7, 15-19**) were completely assigned based on the interpretation of 1D and 2D experiments as the same manner as derivative **14**. Their complete assignments of the ^{1}H and ^{13}C chemical shifts are listed in Tables 1 and 2, respectively.

Anti-oxidative effects of several flavone derivatives were tested in *C. elegans*. The activities were detected by examining survival rates of worms after treatment with paraquat to generate oxidative stress as described in Materials and Methods. While the survival rate of worms grown in normal NGM plates was 47.4%, the survival rates in addition of flavone derivatives were ranged between 59 and 81.6%. Hydroxylated flavonone derivatives with 3-hydroxy group (**8, 9, 10**) showed 1.62-fold increased survival rate, dimethoxyl-monohydroxylated flavonone derivatives with 3-hydroxy group (**12, 13**) showed 1.57-fold increased survival rate, and trimethoxylated flavonone derivatives without 3-hydroxy group (**17, 18, 19**) showed 1.38-fold increased survival rate.

In order to elucidate the relationships between the structures of flavone derivatives and their anti-oxidative effects quantitatively, the scavenging effects of flavone derivatives for DPPH radicals were tested, which were ranged between 9 and 86% as listed in Table 3. The structure-activity relationship study provides several interesting insights in understanding the specific binding mode of the flavone derivatives, **1-19**, to the target molecule. First, the 3-hydroxy group, which looks like to be working as a hydrogen bonding donor, is a prerequisite for a good binding affinity.

Table 1. The ^{1}H chemical shifts, δ ^{1}H (J, Hz), of flavone derivatives **2, 3, 5-7, 14-19**

position	2	3	5	6	7
3	–	–	6.95 (s)	6.76 (s)	6.71 (s)
5	7.37 (d, 3.0)	7.96 (d, 8.4)	7.33 (d, 3.0)	7.88 (d, 8.7)	7.86 (d, 8.7)
6	–	6.91 (dd, 8.4, 2.2)	–	6.92 (dd, 8.7, 2.1)	6.89 (dd, 8.7, 2.2)
7	7.25 (dd, 9.0, 3.0)	–	7.24 (dd, 3.0, 9.0)	–	–
8	7.62 (d, 9.0)	6.94 (d, 2.2)	7.62 (d, 9.0)	6.96 (d, 2.1)	6.96 (d, 2.2)
2'	8.20 (m)	8.16 (d, 7.3)	7.54 (d, 2.5)	7.38 (dd, 1.9, 2.2)	7.90 (d, 9.1)
3'	7.55 (m)	7.53 (dd, 7.3, 7.3)	–	–	6.92 (dd, 8.7, 2.2)
4'	7.48 (m)	7.46 (dd, 7.3, 7.3)	7.11 (dd, 2.5, 8.1)	7.37 (dd, 7.9, 2.2)	–
5'	7.48 (m)	7.53 (dd, 7.3, 7.3)	7.44 (dd, 8.1, 8.1)	7.34 (dd, 7.9, 7.9)	6.92 (dd, 8.7, 2.2)
6'	8.20 (m)	8.16 (d, 7.3)	7.60 (d, 8.1)	7.46 (d, 7.9)	7.90 (d, 9.1)
OH-2'	–	–	–	–	–
OH-3'	–	–	–	9.8 (s)	–
OH-4'	–	–	–	–	10.2 (s)
OH-3	9.40 (s)	9.32 (s)	–	–	–
OH-6	9.98 (s)	–	10.0 (bs)	–	–
OH-7	–	10.83 (s)	–	10.8 (s)	10.7 (s)
OCH$_3$-2'	–	–	–	–	–
OCH$_3$-3'	–	–	3.84 (s)	–	–
OCH$_3$-4'	–	–	–	–	–
OCH$_3$-3	–	–	–	–	–
OCH$_3$-6	–	–	–	–	–
OCH$_3$-7	–	–	–	–	–

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Table 1. Continued

position	14	15	16	17	18	19
3		6.86 (s)		6.74 (s)	6.69 (s)	6.83 (s)
5	8.03 (d, 8.9)	7.94 (d, 8.8)	7.98 (d, 8.9)	7.43 (d, 2.5)	7.94 (d, 8.0)	7.90 (d, 2.4)
6	7.05 (dd, 8.9, 2.2)	7.06 (dd, 8.8, 2.4)	7.06 (dd, 2.2, 8.9)		7.36 (dd, 2.4, 8.0)	7.02 (dd, 2.4, 8.8)
7				7.66 (dd, 0.6, 8.0)		
8	7.08 (d, 2.2)	7.25 (d, 2.4)	7.28 (d, 2.2)	7.38 (dd, 2.5, 8.0)	7.18 (d, 2.4)	7.21 (d, 2.4)
2'			7.59 (dd, 2.4, 1.6)			
3'		7.25 (d, 8.3)				6.70 (d, 2.4)
4'	7.21 (dd, 8.2, 2.0)	7.57 (ddd, 8.3, 7.4, 1.8)	7.15 (dd, 2.4, 8.0)	7.30 (dd, 1.9, 7.8)	7.27 (dd, 2.0, 8.0)	
5'	7.12 (dd, 7.3, 8.2)	7.15 (ddd, 7.8, 7.4, 0.9)	7.51 (dd, 8.0, 8.0)	7.22 (dd, 7.8, 7.6)	7.23 (dd, 7.4, 8.0)	6.73 (dd, 2.4, 4.0)
6'	7.80 (d, 7.3, 2.0)	7.93 (dd, 7.8, 1.8)	7.62 (dd, 1.6, 2.4, 8.0)	7.35 (dd, 1.9, 7.6)	7.36 (dd, 2.0, 8.0)	7.92 (d, 2.4)
OH-2'						
OH-3'						
OH-4'						
OH-3	8.88 (s)					
OH-6						
OH-7						
OCH$_3$-2'	3.80 (s)	3.93 (s)		3.81 (s)	3.76 (s)	3.86 (s)
OCH$_3$-3'	3.87 (s)		3.85 (s)	3.87 (s)	3.82 (s)	
OCH$_3$-4'						3.93 (s)
OCH$_3$-3			3.81 (s)			
OCH$_3$-6				3.86 (s)		
OCH$_3$-7	3.87 (s)	3.91 (s)	3.91 (s)		3.90 (s)	3.90 (s)

Table 2. The ^{13}C chemical shifts (ppm) of flavone derivatives **2**, **3**, **5-7**, **14-19**

Position	2	3	5	6	7	14	15	16	17	18	19
2	145.0	144.2	162.0	162.1	162.5	146.0	160.2	154.1	161.3	161.0	159.9
3	138.5	138.5	106.2	106.6	104.5	138.8	111.5	140.7	110.4	153.0	110.0
4	172.7	172.4	177.1	176.4	176.3	172.2	176.4	173.2	176.6	176.3	176.5
5	106.9	126.6	107.5	126.6	126.5	126.3	126.1	126.2	104.6	126.2	126.0
6	154.2	115.0	154.9	115.1	114.8	114.5	114.6	114.7	156.6	120.5	114.3
7	123.4	162.6	123.1	162.8	162.5	163.5	163.8	163.8	120.1	163.9	163.7
8	119.9	102.1	119.9	102.5	102.5	100.2	100.8	100.5	123.3	100.8	100.7
9	148.7	156.6	149.4	157.5	157.4	156.8	157.7	156.5	150.6	157.7	157.5
10	122.2	114.3	124.3	116.2	116.1	115.8	116.9	117.3	123.8	117.0	116.8
1'	131.6	131.5	132.8	132.6	121.8	125.4	119.9	131.7	126.0	125.9	112.3
2'	127.7	127.4	111.4	112.6	128.1	147.0	157.5	113.7	147.1	147.1	163.0
3'	128.6	128.5	159.7	157.9	115.9	152.7	112.5	159.1	153.0	153.0	106.1
4'	129.8	129.6	117.4	118.6	160.7	114.8	132.7	116.1	115.8	115.8	159.3
5'	128.6	128.5	130.2	130.2	115.9	123.8	120.7	129.8	124.5	124.5	99.0
6'	127.7	127.4	118.5	117.0	128.1	122.4	129.0	120.4	120.6	120.5	130.1
OCH$_3$-2'	–	–	–	–	–	60.6	55.9	–	60.6	60.6	55.6
OCH$_3$-3'	–	–	55.4	–	–	55.9	–	56.1	56.0	56.0	–
OCH$_3$-4'	–	–	–	–	–	–	–	–	–	–	56.3
OCH$_3$-3	–	–	–	–	–	–	–	55.3	–	–	–
OCH$_3$-6	–	–	–	–	–	–	–	–	55.7	–	–
OCH$_3$-7	–	–	–	–	–	56.0	56.0	59.6	–	56.1	56.0

No flavonone analogues without 3-hydroxy group tested showed better activity than 3-hydroxyflavone (**1**). Second, substitution at the 2'-position of the B ring (**14**, **17**, **18**, **19**) always resulted in significant reduction of the biological activity, which implies that the B ring might be located in a narrow binding pocket. In contrast, 3-hydroxyflavone substituted with either methoxy or hydroxyl group at the 3'- or 4'-positions show increased activity. Thus, it is likely that the B ring binds at a specific pocket which has a narrow entry but enough room inside. Also, as both hydroxyl and methoxy group at the 3'- (**8**, **10**, **12**, **13**) or 4'- positions (**9**, **11**) increase the biological activity of 3-hydroxyflavone (**1**), it is apparent that the substituents at these positions can interact with the target molecule as both hydrogen bond donor and acceptor. However, A ring substituents at 6- or 7-positions are highly specific for hydrogen bonding donor

Table 3. The anti-oxidative effects of flavone derivatives in DPPH radical-scavenging

derivative	Name	anti-oxidative effects (%)
1	3-Hydroxyflavone	34.6
2	3,6-Dihydroxyflavone	70.4
3	3,7-Dihydroxyflavone	68.5
4	3,3'-Dihydroxyflavone	38.5
5	6-Hydroxy-3'-methoxyflavone	24.1
6	7,3'-Dihydroxyflavone	19.1
7	7,4'-Dihydroxyflavone	12.8
8	3,6,3'-Trihydroxyflavone	77.0
9	3,6,4'-Trihydroxyflavone	86.0
10	3,7,3'-Trihydroxyflavone	60.3
11	3,7,4'-Trihydroxyflavone	70.8
12	6,3'-Dimethoxy-3-hydroxyflavone	43.6
13	7,3'-Dimethoxy-3-hydroxyflavone	44.3
14	7,2',3'-trimethoxy-3-hydroxyflavone	27.8
15	7,3'-Dimethoxyflavone	17.6
16	3,7,3'-Trimethoxyflavone	10.3
17	6,2',3'-Trimethoxyflavone	9.8
18	7,2',3'-Trimethoxyflavone	21.5
19	7,2',4'-Trimethoxyflavone	12.1

Figure 2. CoMFA contour specification showing electrostatic sites (Blue: positive potential is favored, Red: negative potential is favored).

because 6-methoxy (**12**) or 7-methoxy (**13**) 3-hydroxyflavones are 43% and 27% less potent than the corresponding 6-hydroxy (**8**) or 7-hydroxy (**10**) analogues, respectively. Even though the difference is marginal, hydroxyl groups at the 6-position (**2**, **9**) is more beneficial than 7-position (**3**, **8**) for 3-hydroxyflavone to increase its biological activity. Taken together, the structure-activity relationship study reveals that the multiple hydrogen bonding interactions between the flavone derivatives and the target molecule are responsible for the binding affinity of the ligands and thereby the biological activity. The 3-hydroxy group at the C ring must be involved in the key hydrogen bonding interaction, and B ring as well as A ring substituents assist further stabilization of the ligand through additional hydrogen bonding with the target molecule.

The colored CoMFA contours in the map (Fig. 2) represent the areas in 3D space where the change in the electrostatic field values of a compound correlate strongly with a concomitant change in its activity. The analysis of the electrostatic contours revealed two red contour (electro-negative substituents favored) and two blue contours (electropositive substituents favored). The red contours were near the 3-hydroxyl group in C ring and C-6 in A ring, which mean that the molecules with electronegative substituent at the 3-hydroxyl group including **2**, **3**, **8**–**11** could exhibit good activity. The blue contours near C-4' position in B ring and C-7 in A ring indicated that electropositive substituents would be favored at this position. Of 19 flavone derivatives tested here, derivative **9**, 3,6,4'-trihydroxyflavone satisfying the conditions mentioned above showed the best scavenging effect for DPPH radicals, 86%. At the same experimental
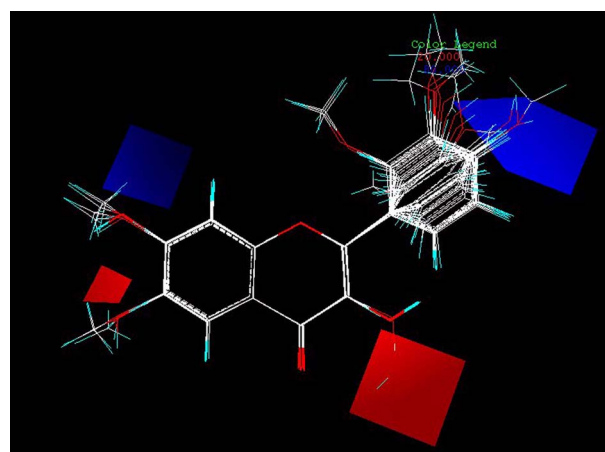
condition, vitamin C showed the value of 88.1%. Consequently, the CoMFA analysis provided meaningful structural insights into possible modifications of flavone derivatives that could improve anti-oxidative effects for the future work.

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