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Interaction of flavones with DNA *in vitro*: structure-activity relationships

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Abstract

Flavones are polyphenolic compounds endowed with diverse pharmacological and biological activities with which have many derivatives with different structures. In this work, UV−vis spectroscopy, fluorescence spectroscopy and molecular docking have been used to investigate the structure-activity relationship of the interaction between different flavones and DNA *in vitro*. From it we could know the studied flavones are all intercalate into the double helix of DNA, suggest that the same skeleton structure in flavones may the main reason for the interaction mode. At the same time, we could find that different flavones lead to different results in the interaction which results from different structure between them, such as the number of hydroxyl groups, the location of hydroxyl groups, and steric hindrance *et al*. These findings are important in the research about structure-activity relationships, which may meaningful for drug discovery, drug design, novel medicines design and help us understand the structure-activity relationship between flavones at the molecular level.

Keywords:

Flavones, structure-activity relationship, DNA, fluorescence spectroscopy, molecular docking.

1. Introduction

As a traditional chinese medicine, flavonoids are low-molecular-mass and polyphenolic compounds with widespread occurrence in the plant kingdom.[1-3] Over 5000 flavonoids have been isolated from plants, most of which are divided into subclasses, including anthocyanidins, flavanones, flavonols, flavones and isoflavones.[4] Between them, flavones are less common than flavonols, including the well-known flavones chrysin, baicalein and wogonin.[5] Chrysin (Figure 1B), is a naturally occurring flavones extracted from blue passion flower and honeycomb.[6,7] Baicalein (Figure 1A) and wogonin (Figure 1C) are flavones originally isolated from *Scutellaria baicalensis*, a chinese medicinal plant with various biological properties.[8,9] These polyphenolic benzo-γ-pyrone compounds have attracted great interest since the 1990s due to growing evidence of their pharmacological effects, including antiallergenic, antiviral, antiinflammatory, antitumor and vasodilating activity. [10-13]

Deoxyribonucleic acid (DNA) has long stood as a unique polymer due to its role in biology and biochemistry,[14] contains the genetic instructions for the development and functioning of living organismsis, the target molecule for many drugs, especially antitumor therapies,[15,16] always to be the hot topic of research.[17-19] In recent years, promoting a research focus many investigators focus on the interaction between small molecules and DNA,[20-22] where characterizing polyphenol–DNA interactions has been integral to understanding the bioactivity of said polyphenol micronutrient DNA ligands and presents opportunities for the design of new and effective drugs against a variety of diseases,[23] but rarely consideration about structure-activity relationship between small molecules was taken. Therefore, comparing the interactions between DNA and different flavones, a polyphenol subclass, can shed light on which aspects of flavones which are required for DNA binding and which parts are expendable.

Herein, the structure-activity relationship for the DNA interactions of flavones, baicalein, wogonin and chrysin, were characterized by UV−vis absorbance spectroscopy, fluorescence spectroscopy and *in silico* molecular docking. Results from this study provide insights into the structure-activity relationship at the molecular level which may be valuable in providing a theoretical basis for the subsequent development of flavones as potential therapeutics, and offers a promising approach to investigate structure-activity relationships.

2. Experimental materials and methods

2.1. Materials.

Calf thymus DNA was obtained from Sigma−Aldrich (St. Louis, MO); baicalein and wogonin were obtained from National Institutes for Food and Drug Control (Beijing, China); chrysin was obtained from Chengdu MUST Bio-Technology Co., Ltd. (Chengdu, China); Na_2HPO_4−NaH_2PO_4 (PBS) contain KCl and NaCl had a purity of no less than 99.5% and all other chemicals used were analytical–purity grade and used as purchased without further purification, except for the DNA solution, which was dissolved in buffer solution for one day in advance of use. The purity of DNA was verified by monitoring the ratio of absorbance at 260/280 nm (A_{260}/A_{280}). The concentration of DNA stock solution was determined according to the absorbance at 260 nm by using an extinction coefficient of 6600 $mol^{-1}\cdot cm^{-1}$.[24] Appropriate blanks were run under the same conditions and subtracted from the sample spectra.

2.2. Measurement of UV-vis Spectra.

The UV−vis absorbance spectrum was recorded at room temperature on a UV9000 spectrophotometer (Shanghai, China) equipped with 1.0 cm quartz cells. During this experiment, the concentration of DNA was kept at 5.0×10^{-5} $mol\cdot L^{-1}$ and

flavones was increased from 0 to 5.0×10^{-5}mol·L^{-1} at increments of 0.5. Appropriate blanks corresponding to the PBS buffer were subtracted to correct for background.

2.3. Measurement of Fluorescence Spectra.

All fluorescence spectra of the DNA-baicalein\wogonin\chrysin systems were recorded on a LS-55 spectrofluorimeter (Perkin Elmer, USA) equipped with 1.0 cm quartz cells and a thermostat bath. The width of the excitation slit and the emission slit was set to 4 nm. The excitation wavelength was set to 495 nm, and the emission wavelength was set from 505 nm to 600 nm. During this experiment, the concentration of DNA was kept at 5.0×10^{-5} mol·L^{-1} and flavones was increased from 0 to 5.0×10^{-5} mol·L^{-1} at increments of 0.5. Appropriate blanks corresponding to the buffer were subtracted to correct the fluorescence background.

2.4. Molecular Docking Study.

Molecular docking was conducted by a Surflex Dock program in the Sybyl–X 2.1.1 package. The crystal structure of DNA was obtained from the Protein Data Bank (http://www.pdb.org/, PDB ID: 1Z3F). The crystal structure of DNA was only prepared with all H added and charge added by AMBER7 FF99 method. The structures of baicalein, wogonin, chrysin were drawn in the Sybyl–X 2.1.1 package, polar H added and being energy optimized with a tripos force field and charged optimized with Gasteiger–Huckel method. The protomol was generated in ligand mode with the threshold kept at 0.50 and the bloat 0. Ring flexibility was considered and other parameters during the docking program were determined through a number of attempts.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra.

To understand the mode of interaction between flavones and ctDNA, absorption titration studies were performed by monitoring the changes in absorption intensity with aliquot addition of one of the three flavones (baicalein, wogonin or chrysin). In general, hypochromism and hyperchromism are important spectral features to distinguish changes in the double–helical structure of DNA,[25] where intercalation interactions results in hypochromic effect with or without a red/blue shift and non-intercalative electrostatic interactions result in hyperchromism.[26,27]

As shown in Figure 2A-C, the maximum absorption peak of DNA at 260 nm exhibited a proportional increase with increasing concentrations of each of the three flavones along with a non-ignorable red shift，related results was shown in Table 1. Meanwhile, from the comparison of absorbance at 260 nm between the DNA−flavones complex and the sum values of them (Figure 2A-C insets) the absorption value of the simple sum of free DNA and free flavones were all slightly greater than the measured value of flavones–DNA complex, indicative of a weak hypochromic effect existing between DNA and the three flavones, pointing to a DNA intercalation binding mechanism for all three flavones examined. But turn to Table 1, we could find that the interactions between the three flavones and DNA have clear differences, among them, wogonin's redshift (258nm-279nm) and reduction in absorbance was most pronounced, which may due to the presence of a methoxyl group causing electron accumulation, making the π–π transition energy decrease, then the red shift phenomenon obvious, while opposite to hydroxyl. At the same time, coupled π orbitals partly filled with electrons also reduce π–π transition energy, resulting in a stronger hypochromic effect.

3.2. Fluorescence Characteristics of the DNA–AO–flavones.

Acridine orange (AO) is a classic intercalating dye, and the structure of the

DNA−AO intercalation complex has been solved by X–ray crystallography.[28,29] Because of the weak endogenous fluorescence of DNA complexed with baicalein, wogonin or chrysin, AO was used as the fluorescence probe. The fluorescence of AO increases after intercalating with DNA, hence, if any flavone intercalates into the helix of DNA, it would compete with AO for the DNA intercalation sites, leading to a significant decrease in fluorescence intensity.[15]

The emission spectra of the DNA–AO system decreased after separate addition of each of the three flavones (Figure 3A-C), which means that baicalein, wogonin and chrysin all intercalate into the helix of DNA *in vitro*. Moreover, there was an obvious change in the degree of quenching, this may due to the presences and positioning of different functional groups. For baicalein, an additional hydroxyl group may be conducive to the formation of an additional hydrogen bond, enhancing AO inhibition and the associated reduction of fluorescence. While wogonin's unique methoxyl group could predictably result in greater steric hindrance, inhibiting AO-DNA binding less readily (Table 1).

3.3. Quenching Mechanism.

The main fluorescence quenching mechanisms are usually classified as dynamic quenching and static quenching. Dynamic quenching results from collisional encounters between the fluorophore and the quencher, and static quenching is caused by the formation of a non–fluorescent ground state fluorophore–quencher complex.[30] Higher temperatures results in faster diffusion, leading to larger amounts of dynamic quenching, but also results in the dissociation of weakly bound complexes, hence, leading to smaller amounts of static quenching. In this work, we distinguished the quenching types on the basis of the calculation calculated K_{SV} received at different temperature.

For a fluorescence quenching process, the decrease in intensity is usually described by the well–known Stern–Volmer equation:[31]

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F denote the steady–state fluorescence intensity in the absence and presence of quencher (flavone) respectively, K_{SV} is the Stern–Volmer quenching constant, and [Q] is the concentration of the quencher. Hence, equation (1) is applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q].

The Stern–Volmer quenching constants were all inversely correlated with temperature (Table 2), which indicates that the fluorescence quenching mechanism of the three systems are all initiated by compound formation rather than by dynamic collision.

For static quenching, the data was analyzed using the modified Stern–Volmer equation:[15]

$$\frac{F_0}{\Delta F} = \frac{1}{f_a K_a}\frac{1}{[Q]} + \frac{1}{f_a} \quad (2)$$

Where F_0 and F denote the steady–state fluorescence intensity in the absence and presence of quencher (flavone) respectively, K_a is the associative binding constant, [Q] is the concentration of the quencher, and f_a is the fraction of accessible fluorescence.

Modified Stern–Volmer plots for the three systems at different temperatures are all shown in Figure 4A-C. Table 2 summarizes the calculated K_a from modified Stern−Volmer plots at each studied temperature (292, 298, 304, and 310 K). For all three systems, the decreasing trend of K_a with increasing temperature was in accordance with K_{SV}'s dependence on temperature as mentioned above, which may result from the reduction in stability of the DNA−flavone complexes. Comparing the results for each of the flavones (Table 2), the DNA–baicalein system was influenced

by temperature most greatly, which may due to the presence of an additional hydroxyl group, the more hydrogen bond may generate, more likely to form complex, then greater influenced by temperature.

3.4. Thermodynamic Analysis and the Nature Forces of the Interactions.

The interaction forces between small molecules and DNA may include electrostatic interactions, multiple hydrogen bonds, van der Waals interactions, hydrophobic and steric contacts within the antibody–binding site, etc.[32] When the enthalpy change (ΔH) does not change significantly within the temperature range studied, its value and entropy change (ΔS) could be evaluated from the van't Hoff equation:[32]

$$\ln K_a = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \qquad (3)$$

Where K_a is analogous to the associative binding constant at the corresponding temperature and R is the gas constant.

The free energy change (ΔG) was evaluated from the following equation:[32]

$$\Delta G = \Delta H - T\Delta S \qquad (4)$$

Deal the related experimental data above by these equations, Table 3 summarizes the values of enthalpy change (ΔH) and entropy change (ΔS) from the slopes and ordinates at the origin of the fitted lines. A linear relationship between $\ln K_a$ and T in all three systems, indicating that the enthalpy change does not change significantly within the studied range. The negative sign for ΔG means that the three binding processes are all spontaneous, while the ΔH and ΔS values of the interaction indicate that hydrogen bonds and van der Waals' interactions played an major role in these reactions. Comparing the results for the different flavones (Table 3), the enthalpy change and entropy change are related to the flavones' functional groups. For baicalein, an additional hydroxyl leads to a greater change in enthalpy and entropy.

For wogonin, its methoxyl group with greater steric hindrance of DNA binding leads to a smaller change in entropy. Considering the enthalpy change (ΔH) and entropy change (ΔS) obtained in this work, there is a linear regression between ΔH and ΔS (Figure 5), which is called the "enthalpy–entropy compensation" and expressed as the following equation:

$$\Delta H\ (\mathrm{kJ \cdot mol^{-1}}) = 93.52 + 3.43\ \Delta S\ (\mathrm{J \cdot mol^{-1} \cdot K^{-1}})$$

3.5. Effect of Different Degeneration Conditions.

Denaturation process often causes changes in the structure of the biomacromolecules. For DNA used in fluorescence experiments, denaturation may result in releasing of entrapped probe molecules, leading to a detectable modification of the fluorescence behavior of the probe. Several studies have reported denatured DNA could be detected after treated with urea, guanidine hydrochloride (GuHCl) or high temperature.[33] In this study, the effects of chemical and thermal denaturation on the binding of flavones to DNA are respectively discussed.

3.5.1. Effect of Chemical Denaturation.

In comparison with either acid or thermal unfolding, urea and GuHCl are more effective in disturbing non-covalent interactions and the extent of unfolding is generally greatest.[34] Urea and GuHCl were introduced to the baicalein\wogonin\chrysin−DNA (AO) system to investigate their effects on flavones-DNA interactions. Prior to the experiments, DNA solution was added to PBS buffer solution containing one of the two chemical denaturants for one day, and the amount of them are consistent with DNA. The results in Table 4 show that the Stern−Volmer parameters and modified Stern−Volmer parameters are all decreased in the presence of urea and GuHCl for each of the three systems. This is likely attributable to both urea and GuHCl unfolding the double helix of DNA to some

extent, and in doing so, disturbs the noncovalent interactions between the flavones and DNA. Moreover, the decrease in fluorescence was less intense for GuHCl than for urea, which may stem from for the amount of GuHCl added being insufficient to change the structure DNA's phosphoric acid backbone.

3.5.2. Effect of thermal denaturation.

High temperatures may cause local openings of the double helix that extend over the full molecule, resulting in a complete separation of the two strands.[35] In our work, native ctDNA solution divided into four parts, was incubated at different temperatures (25, 60, 80, and 100 ^{o}C) respectively for 30 minute in a water bath to produce varying degrees of DNA unwinding, before this experiment all resolution natural cooling to room temperature. The results in Table 5 show that the quenching constant and binding constant decreased with increasing temperature for all three flavones, which may due to higher temperatures converting dsDNA to be ssDNA, thus preventing the flavones intercalating with dsDNA. Results are supportive of being the predominant DNA binding mode.

Comparing the difference of these system at different states of DNA unwinding, the order of the effects for all three flavones are, wogonin>baicalein>chrysin, suggesting that in the denaturation experiment, space steric hin-drance is the most important factor, as for wogonin, connect with a methoxyl group, it's space hindrance is biggest, through related results above, we could know that under normal circumstances, wogonin intercalated into DNA weakest, turn to denaturation circumstances, partly dsDNA converting to be ssDNA, the proportion of double-stranded DNA decreased, then against its reaction mostly.

3.6. Effect of pH.

The stability of the dsDNA structure is closely related to environmental pH.[36] In

order to investigate and compare the effects of pH on the interaction between flavones and DNA, different ratio of Na_2HPO_4, NaH_2PO_4, H_3PO_4, KCl and NaCl were made up for PBS buffer with different pH values. DNA were dissolved in the corresponding pH buffer solution for one day before experiment. The results (Table 6) show that, both the quenching constants and binding constants are changed with different pH, and all got maximum at pH 7.2, the condition closest to physiological pH, probably due to the higher stability of the DNA double helix and flavones at pH 7.2, overly acidic or alkaline may lead to structure changed.

3.7. Molecular Docking Study.

Ligand binding to double stranded DNA can occur via three main modes, namely groove binding, intercalation between two base pairs, and covalent binding/metal coordination to the bases.[37] Molecular modeling can offer a visual representation of the binding characteristics of small ligands to DNA, which useful in complementing and substantiating experimental results.[38] Here, DNA with PDB ID: 1Z3F was employed to confirm the interaction mode between three flavones and DNA,[39] and the predicted binding model with the lowest docking energy was then selected for binding orientation analysis.

Three flavones are all intercalated into DNA and hydrogen bonds were formed which consistent with the aforementioned spectrophometry experiments (Figure 6). At the same time we could find that the numbers and location of hydrogen bond were different from each other. From detailed results in Figure 6A, five hydrogen bonds were formed between baicalein and DNA, four hydrogen bonds were built by the hydrogen atom H linked C-5, C-5, C-6, C-7 of baicalein with oxygen atom O, nitrogen atom N, nitrogen atom N and oxygen atom O of guanine, the other one was formed between oxygen atom O linked C-4 of baicalein and hydrogen atom H of

cytosine, their lengths were 2.475, 2.661, 2.696, 2.039 and 2.433 Å, respectively. When turn to Figure 6B, we could find that there are three hydrogen bonds formed between DNA and chrysin, two hydrogen bonds were built by hydrogen atom H linked C-5 with nitrogen atom N and oxygen atom O of guanine, and one formed between hydrogen H linked 7-C and oxygen O on phosphoric acid skeleton, their lengths were 2.299, 2.507 and 2.181 Å, respectively. But for wogonin (Figure 6C), there was only one hydrogen bond built by hydrogen H linked 5-C and oxygen on phosphoric acid skeleton with 2.012 Å. The difference in results may differ from the difference in structure, for baicalein, three phenolic hydroxyl group followed, conductive to the formation of hydrogen bonds, but turn to wogonin, with a methoxy on C-8, larger space steric hindrance, more difficult for it intercalated into the DNA base. The docking score which stands for pKd is 5.63 for baicalein, 4.88 for chrysin and 4.47 for wogonin, since higher score reflects greater binding constant between flavones and DNA, the docking results predict that the ability about intercalated into DNA is baicalein>chrysin>wogonin. These docking results have shown that three flavones were prone to bind to the base of DNA and implied that hydrogen bond forces play an important role in flavones interaction with ctDNA.

4. Conclusions

This study provides a comparison of the structure-activity relationships of three flavones, baiclein, chrysin and wogonin interactions with DNA. Results revealed that while all three flavones bound DNA by intercalation, there are some marked differences in the interactions and their affinities due to differences in structure. For the flavones examined, the number and location of hydroxyl groups, as well as steric hindrance from methoxy groups were the main factors influencing binding to DNA. Based on our investigations, the introduction of hydroxyl group may be conducive to

the interaction, whlie methoxy on the contrary. These results provide a promising foundation for the design of novel flavones-based medicines and will be advantageous to the development of pharmacologial effects about flavones.

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Figure Captions:

Figure 1. Molecular structures of baicalein (A), chrysin (B) and wogonin (C).

Figure 2. UV absorption spectra of DNA with various concentrations of baicalein (A), chrysin (B) and wogonin (C). The insets correspond to the comparison of absorption at 260 nm between the DNA−flavone complexs and the sum values of them. *c* (DNA) = 5.0×10^{-5} mol·L^{-1}; *c* (flavone)/(10^{-5}mol·L^{-1}), *a*–*k*: from 0.0 to 5.0 at increments of 0.5.

Figure 3. Fluorescence spectra of DNA−AO with various concentrations of baicalein (A), chrysin (B) and wogonin (C). *c* (DNA) = 1.0×10^{-5} mol·L^{-1}; *c* (AO) = 1.0×10^{-6} mol·L^{-1}; *c* (flavone)/(10^{-5} mol·L^{-1}), *a*–*k*: from 0.0 to 2.0 at increments of 0.2.

Figure 4. Modified Stern–Volmer plots of three systems at different temperatures.

Figure 5. Enthalpy–entropy compensation plots of three systems.

Figure 6. Energy-minimized structure of baicalein (A), chrysin (B) and wogonin (C) binding DNA. The white dashed lines stand for hydrogen bonds.

Table 1. The comparison of the difference in three interaction systems

Flavone	n (-OH)	n (-OCH_3)	Δλ (nm)	Decrease degree (%)	Quench degree (%)
Baicalein	3	0	8	3.01	77.86
Chrysin	2	0	10	2.33	29.46
Wogonin	2	1	21	6.86	18.68

Table 2. Constants for the interaction of DNA with flavones at different temperatures and the comparison about the effects of the temperature on the three systems

System	T/(K)	$10^{-4}K_{SV}$/ (L/mol)	R^a	$10^{-4}K_a$/ (L/mol)	R^a	n (−OH)	n (−OCH_3)	$K_{310/292}$
Baicalein	292	31.24	0.9932	6.19	0.9990	3	0	15.48
	298	23.80	0.9982	3.17	0.9999			
	304	19.23	0.9974	1.25	0.9994			
	310	12.75	0.9985	0.40	0.9991			
Chrysin	292	2.71	0.9708	5.45	0.9973	2	0	3.76
	298	2.04	0.9971	3.49	0.9987			
	304	1.33	0.9992	2.21	0.9972			
	310	0.94	0.9954	1.45	0.9978			
Wogonin	292	1.21	0.9977	8.62	0.9977	2	1	2.94
	298	0.91	0.9989	6.64	0.9990			
	304	0.76	0.9965	5.00	0.9995			
	310	0.49	0.9974	2.93	0.9997			

[a] ***R*** is the correlation coefficient.

Table 3. Thermodynamic parameters of three systems

System	T (K)	n (–OH)	n ($–OCH_3$)	ΔH (kJ·mol^{-1})	ΔG (kJ·mol^{-1})	ΔS (J·mol^{-1}·K^{-1})	R[a]
Baicalein	292	3	0	–114.49	–27.12	–299.39	0.9739
	298				–25.32		
	304				–23.52		
	310				–21.73		
Chrysin	292	2	0	–53.07	–10.64	–90.95	0.9974
	298				–10.67		
	304				–10.71		
	310				–10.74		
Wogonin	292	2	1	–44.04	–27.74	–55.80	0.9960
	298				–27.41		
	304				–27.07		
	310				–26.74		

[a]***R*** is the correlation coefficient.

Table 4. Stern–Volmer quenching constants and binding constants for the interaction of DNA with flavones at different chemical denaturants

System		$10^{-4}K_{SV}$/ (L/mol)	R[a]	$10^{-4}K_a$/ (L/mol)	R[a]	K_{Urea}/K_{Blank}
Baicalein	Blank	19.73	0.9978	28.94	0.9989	0.42
	+GuHCl	15.34	0.9951	20.84	0.9995	
	+Urea	12.27	0.9985	12.07	0.9996	
Chrysin	Blank	5.97	0.9738	2.82	0.9987	0.39
	+GuHCl	4.44	0.9732	1.56	0.9998	
	+Urea	3.61	0.9747	0.30	0.9980	
Wogonin	Blank	2.14	0.9989	9.58	0.9997	0.89
	+GuHCl	1.79	0.9992	9.56	0.9982	
	+Urea	1.67	0.9995	8.49	0.9987	

[a]***R*** is the correlation coefficient.

Table 5. Stern–Volmer quenching constants and binding constants for the interaction of DNA with flavones at different denaturation temperatures

System	*T*/ (°C)	$10^{-4}K_{SV}$/ (L/mol)	R[a]	$10^{-4}K_a$/ (L/mol)	*R*[a]	$K_{100℃}/K_{25℃}$
Baicalein	25	12.67	0.9984	16.11	0.9972	0.55
	60	11.92	0.9994	11.15	0.9991	
	80	10.72	0.9995	9.33	0.9995	
	100	7.99	0.9981	8.88	0.9996	
Chrysin	25	7.53	0.9974	6.34	0.9971	0.11
	60	7.20	0.9953	4.88	0.9999	
	80	6.56	0.9868	4.52	0.9995	
	100	4.80	0.9782	2.45	0.9973	
Wogonin	25	1.27	0.9990	7.96	0.9977	0.77
	60	1.04	0.9992	6.50	0.9996	
	80	0.82	0.9989	6.44	0.9998	
	100	0.72	0.9967	6.11	0.9991	

[a]***R*** is the correlation coefficient.

Table 6. Stern−Volmer quenching constants and binding constants of three systems at different pH.

System	pH	$10^{-4}K_{SV}$ (L/mol)	R^a	$10^{-4}K_a$ (L/mol)	R^a
Baicalein	4.2	3.53	0.9973	6.16	0.9996
	6.2	13.17	0.9981	9.46	0.9930
	7.2	19.59	0.9987	9.48	0.9876
	8.2	18.82	0.9986	8.01	0.9827
	10.2	6.00	0.9984	9.47	0.9944
Chrysin	4.2	1.55	0.9975	5.07	0.9989
	6.2	3.28	0.9976	4.89	0.9974
	7.2	3.70	0.9992	5.24	0.9993
	8.2	1.49	0.9979	4.09	0.9986
	10.2	1.10	0.9983	4.09	0.9998
Wogonin	4.2	1.58	0.9938	5.23	0.9959
	6.2	1.92	0.9856	7.85	0.9938
	7.2	2.14	0.9975	9.15	0.9986
	8.2	1.62	0.9989	7.11	0.9999
	10.2	1.09	0.9959	6.33	0.9998

[a] ***R*** is the correlation coefficient.

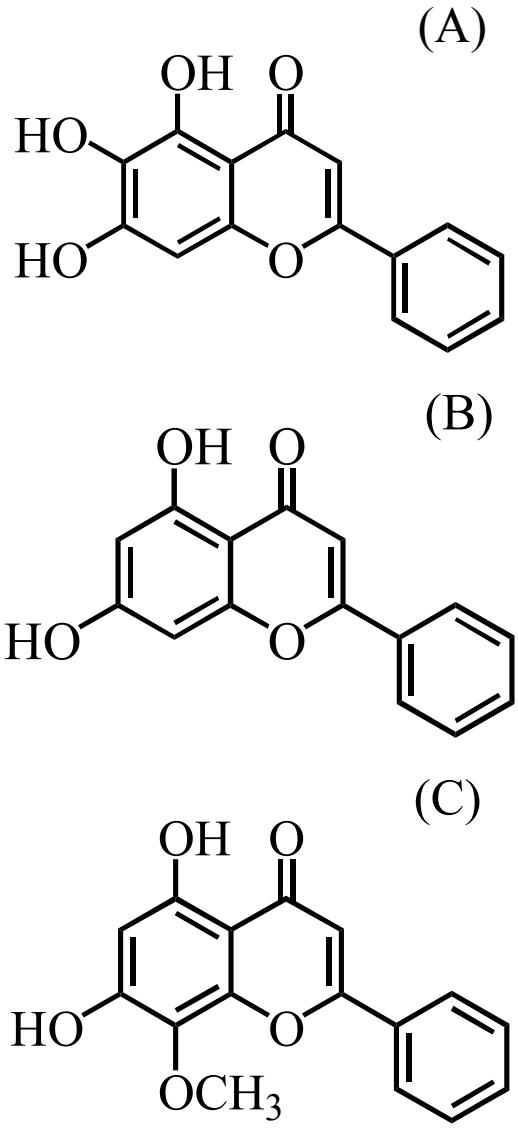


Figure 1

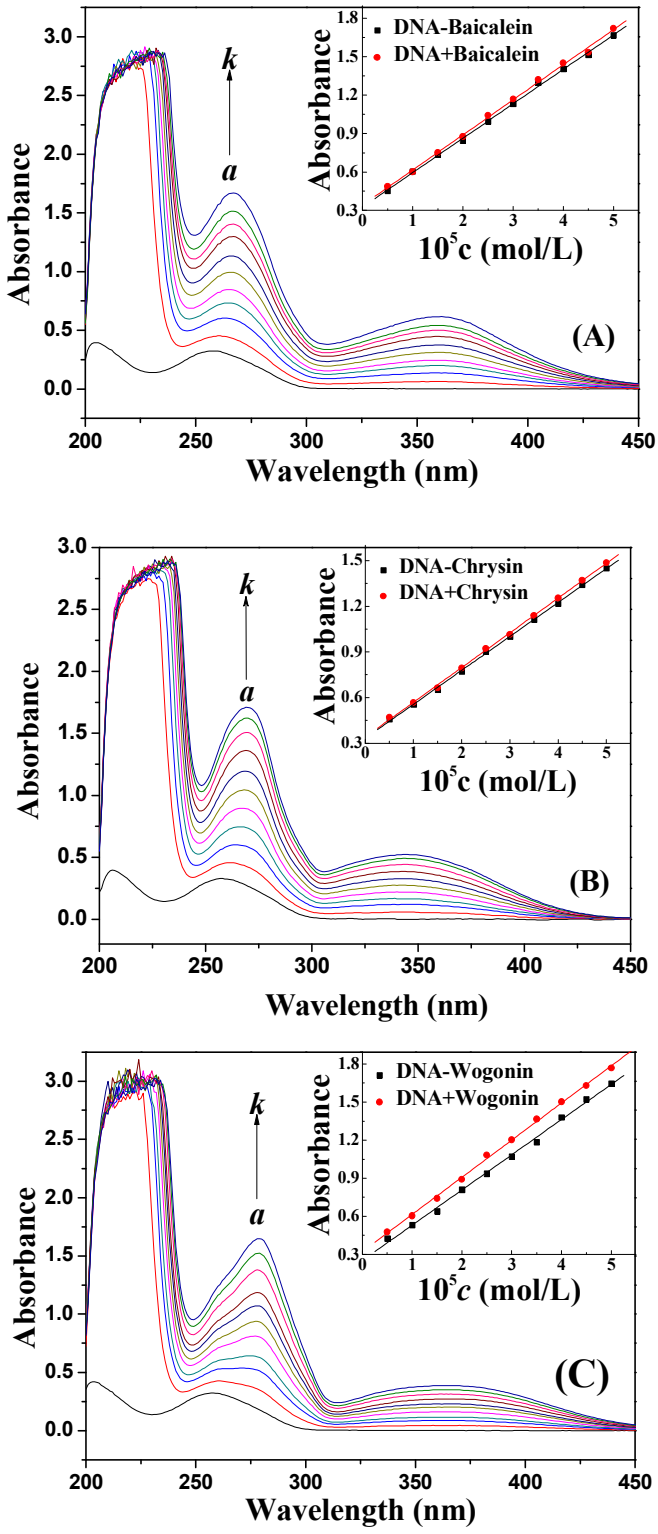
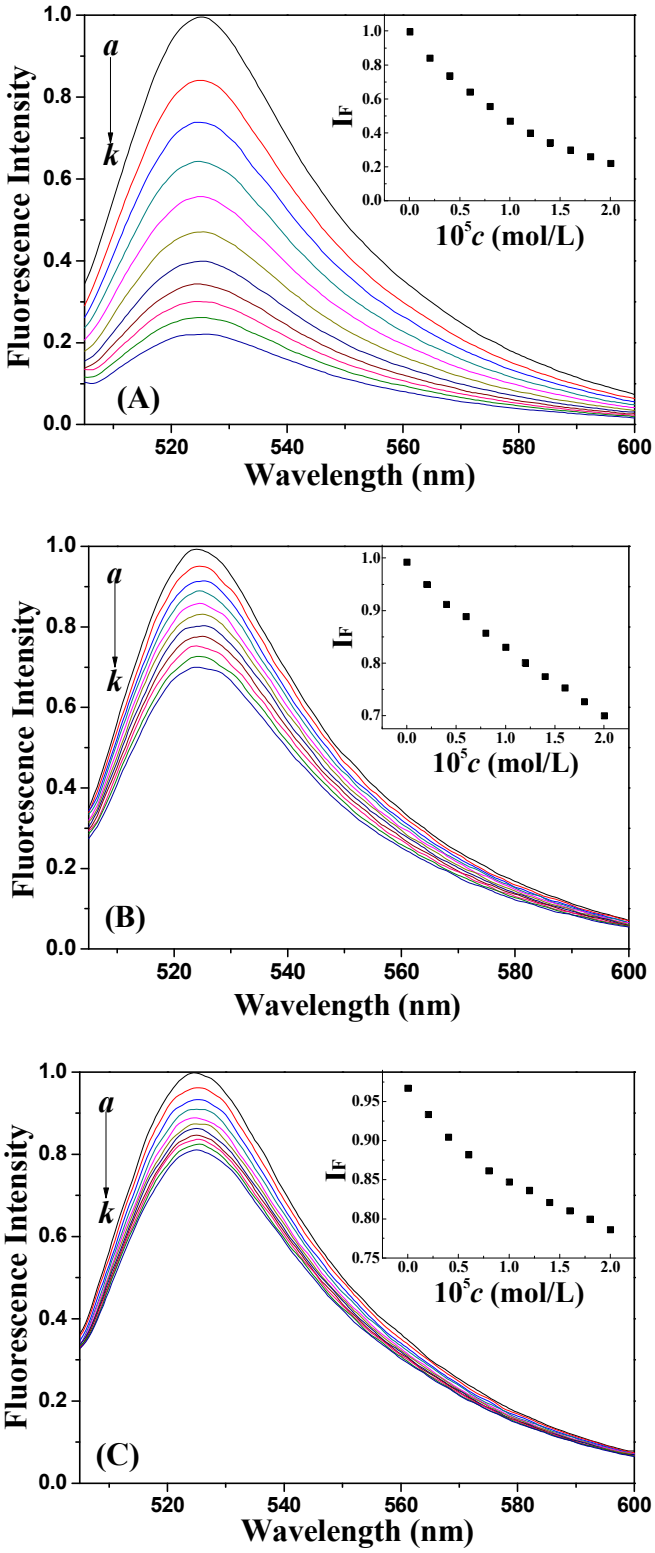


Figure 2



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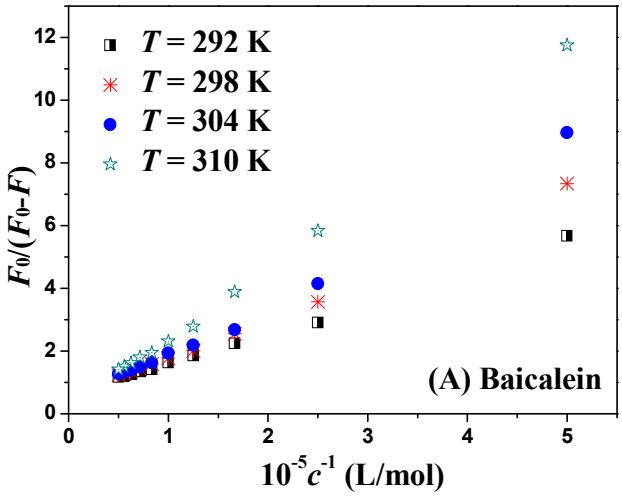
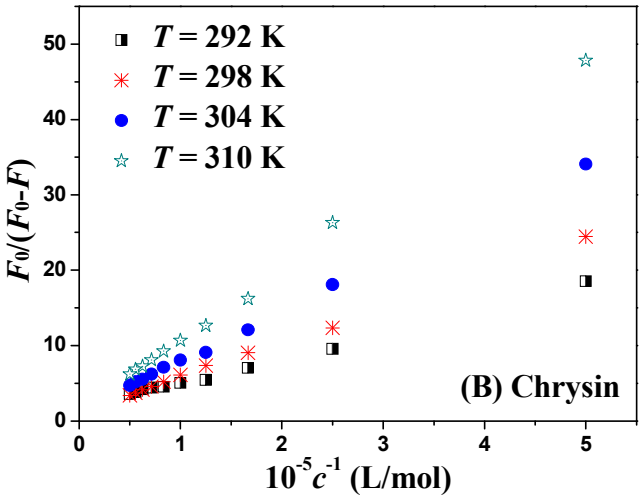




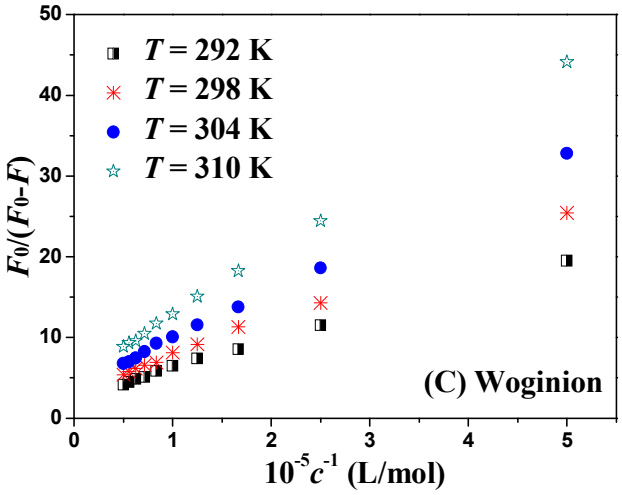


Figure 4

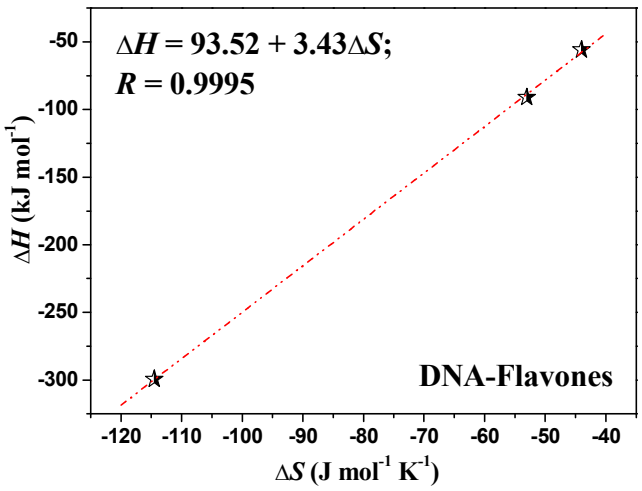


Figure 5

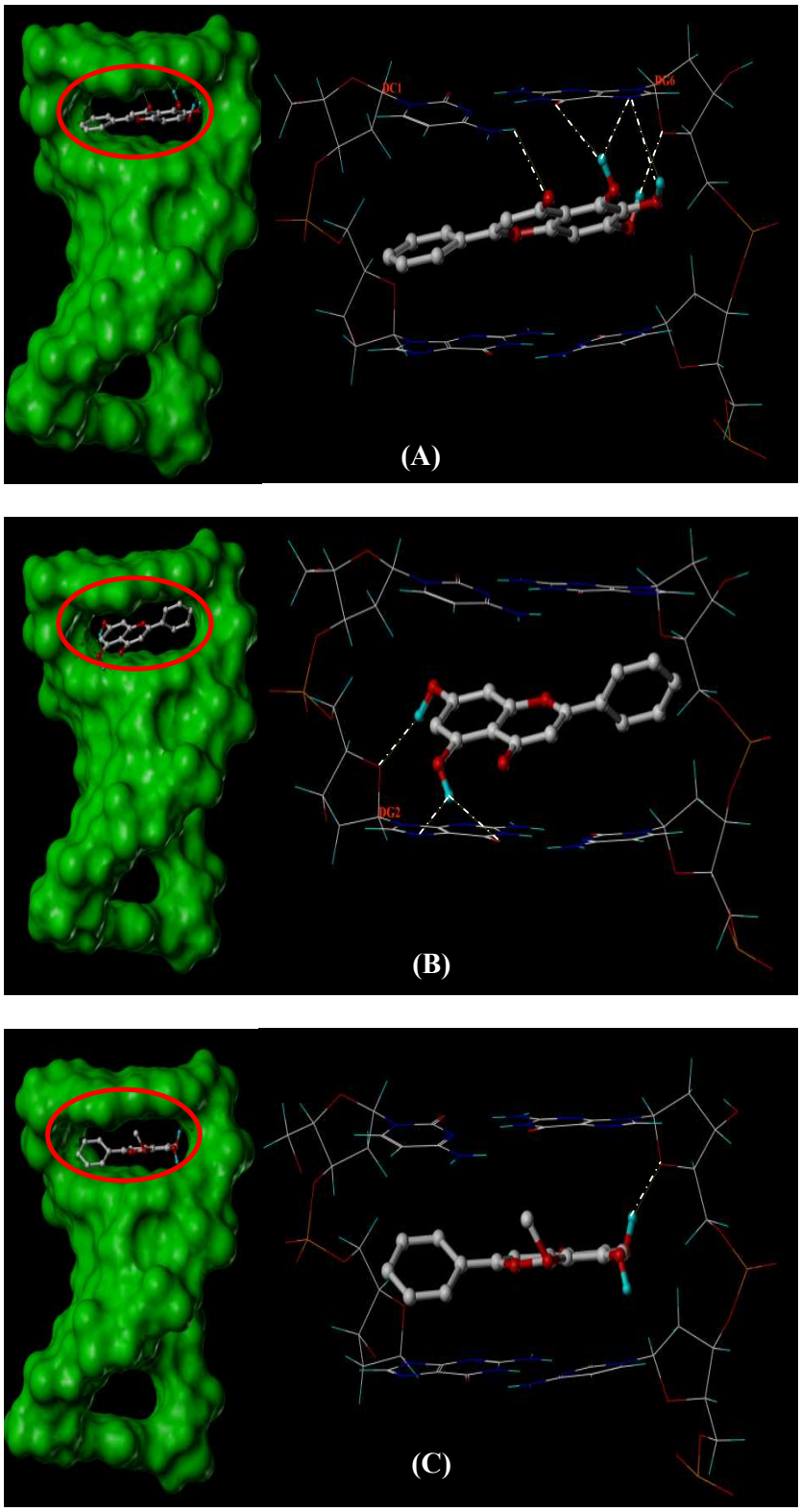
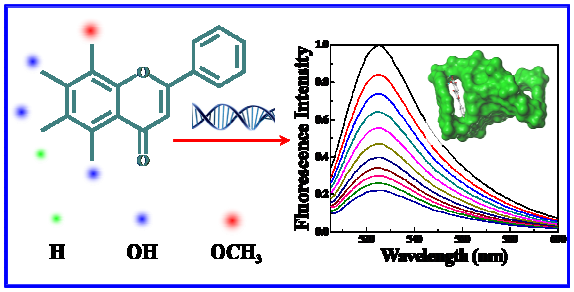


Figure 6

TOC Graphical Abstract



The structure-activity relationship of the different flavones has been investigated, which may meaningful for drug discovery, and novel drug design.