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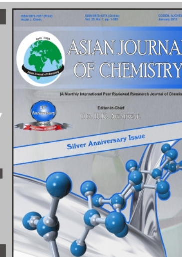

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Transition Metal Ion Chelating of Some Coumarin Antioxidants and the Interaction with Bovine Serum Albumin

YE ZHANG[1,2], BIQUN ZOU[2], YILIN FANG[2], ZHENGHONG PAN[3], XIANGHUI YI[2,*] and DIANPENG LI[3,*]

[1]School of Chemistry and Chemical Engineering, South Central University, Hunan 410083, P.R. China
[2]Department of Chemistry, Guilin Normal College, Guangxi 541001, P.R. China
[3]Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Guangxi Institute of Botany, Guilin 541006, P.R. China

*Corresponding authors: Fax: +86 77 32148118; Tel: +86 77 32148118; E-mail: yixianghui2008@yahoo.com.cn; ldp@xib.cn

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To investigate whether the coumarins antioxidants **1-14** may exert their antioxidant effect through transition metal ion chelation, the copper and ferrous chelating properties with coumarins were studied. It was found from fluorescence spectra that the binding constants K_a of coumarins **1-14** were in the range of 1.25×10^3 to 4.46×10^5 M^{-1}, which indicated that the transition ion chelation play an important role in their antioxidant abilities. The interaction of coumarins **1-14** with bovine serum albumin was also studied by fluorescence spectra, since that the interaction of drugs with bovine serum albumin may have important influence on the drugs' pharmacology and pharmacodynamics. The results showed that the probable mechanism of the coumarins **1-14** interaction with bovine serum albumin was a static process and the binding constants K_a were 5.96×10^4 to 5.22×10^6 M^{-1}, demonstrating a strong binding between coumarins **1-14** and bovine serum albumin. In addition, the binding numbers of coumarins **1-14** with transition metal ion and bovine serum albumin were determined to be 1, respectively, suggesting that each unit of coumarins **1-14** can bind with a unit of transition metal ion and a unit of bovine serum albumin. All the results may provide important information for the rational design of other analogous new antioxidants and drugs.

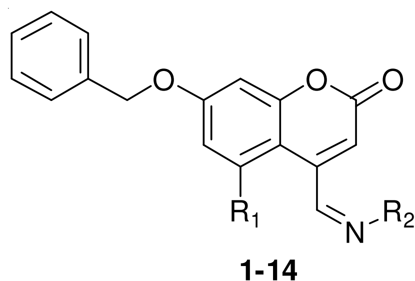
Key Words: Coumarin antioxidants, Transition metal ion chelation, Bovine serum albumin, Fluorescence spectra.

INTRODUCTION

The homeostasis of free radical and transition metal ion and Fenton reaction have important influence on aging[1-3]. Many age-related diseases such as atherosclerosis, Alzheimer's and Parkinson diseases, are diagnostically related with free radical and transition metal ion that would cause oxidative damage to lipids, proteins, nucleic acids and carbohydrates[1,2]. Generally, the dynamic equilibrium system of antioxidant defence is launched to maintain the homeostasis of free radicals and transition metal ion and Fenton reaction[1-3]. However, some exogenous antioxidants are also demanded to keep these homeostasis balance, since that only endogenous antioxidant defenses are not entirely efficient. The exogenous antioxidants, which possess of good antioxidant activities and transition metal ion chelation ability, could efficiently reduce the cumulative effects of free radicals and oxidative damages and maintain the transition metal ion homeostasis modulation and Fenton reaction[1-3]. Therefore, in the last years considerable attention has been devoted to design and synthesize many kinds of antioxidants. Antioxidants are currently fabricated as the drug candidates to counter the diseases of carcinogenesis, atherogenesis, drug-associated toxicity, *etc.*[4]

In our previous work, coumarin antioxidants **1-14** (Fig. 1) have been demonstrated to have good antioxidant activities[5], it would thus be very interesting to investigate if, transition metal ion chelation play an important role in their antioxidant abilities. In addition, previous study indicates that factors such as protein binding may weaken the antioxidants absorption and bio-availability and even mask their antioxidant activity[6,7]. So it is also important to study the interaction of coumarins **1-14** with protein. Serum albumin, one of the most abundant carrier proteins[8], plays a significant role in the transport and disposition of endogenous and exogenous drugs and ligands present in blood. Of many kinds of serum albumins, bovine serum albumin has been considered to be one of the most extensively studied of this group of proteins, particularly because of its structural homology with human serum albumin. Therefore, bovine serum albumin is herein chosen as our model protein to investigate the interaction of coumarins **1-14** with protein. The present work involves is to evaluate whether the coumarins antioxidants **1-14** may exert their antioxidant effect

through transition metal ion chelation and may the interaction of coumarins antioxidants **1-14** with bovine serum albumin have important influence on their pharmacology and pharmacodynamics.



1: R_1=H, R_2=NH_2
2: R_1=OCH_2Ph, R_2=NH_2
3: R_1=OCH_2Ph, R_2=NHPh
4: R_1=H, R_2=*p*-NH-C_6H_4-NO_2
5: R_1=OCH_2Ph, R_2=*p*-NH-C_6H_4-NO_2
6: R_1=H, R_2=*o*-NO_2-*p*-NO_2-C_6H_3-NH
7: R_1=OCH_2Ph, R_2=*o*-NO_2-*p*-NO_2-C_6H_3-NH
8: R_1=H, R_2=*O*-$C_6H_4NH_2$
9: R_1=OCH_2Ph, R_2=*O*-$C_6H_4NH_2$
10: R_1=H, R_2=*p*-OH-Ph
11: R_1=H, R_2=*p*-OH-Ph
12: R_1=OCH_2Ph, R_2=*p*-OH-Ph
13: R_1=H, R_2=OH
14: R_1=OCH_2Ph, R_2=OH

Fig. 1. Synthetic route of coumarins **1-14**

EXPERIMENTAL

4-Schiff base-7-benzyloxy-coumarins **1-14** were synthesized according to the literature[5]. All chemicals and solvents used were of analytical grade. Methanol was distilled prior to use. The TU-1901 UV-VIS spectrophotometer was used to evaluate the the radical activity. The fluorescence spectroscopy was scanned by the RF-5301 spectrophotometer.

Fluorescence spectrum studies on transition metal ion chelation: At room temperature, the coumarins **1-14** and transition metal ions were dissolved in DMF-water (v:v = 7:3) to the proper concentration, respectively. After addition of Fe^{2+} or Cu^{2+} solution (in serial concentration) to the coumarins solution respectively, the reaction mixture was allowed to stand for 0.5 h (until the stable) with intermittent shaking and the fluorescence spectroscopy was scanned.

Fluorescence spectrum studies on interaction of coumarins 1-14 with bovine serum albumin: At room temperature, the coumarins **1-14** and bovine serum albumin were dissolved in DMF-water (v:v = 7:3) to the proper concentration, respectively. After addition of bovine serum albumin solution (in serial concentration) to the coumarins solution respectively, the reaction mixture was allowed to stand for 0.5 h (until the stable) with intermittent shaking and the fluorescence spectroscopy was then scanned.

RESULTS AND DISCUSSION

Chelating abilities to transition metal ions: To investigate whether compounds **1-14** may exert their antioxidant effect through transition metal ion chelation, their chelating properties with Cu^{2+} and Fe^{2+} were studied by fluorescence quenching/enhancement spectroscopy. Upon addition of Cu^{2+} or Fe^{2+} into the solution of compounds 1-14, the fluorescence intensity decreased or increased gradually (Figs. 2 and 3).

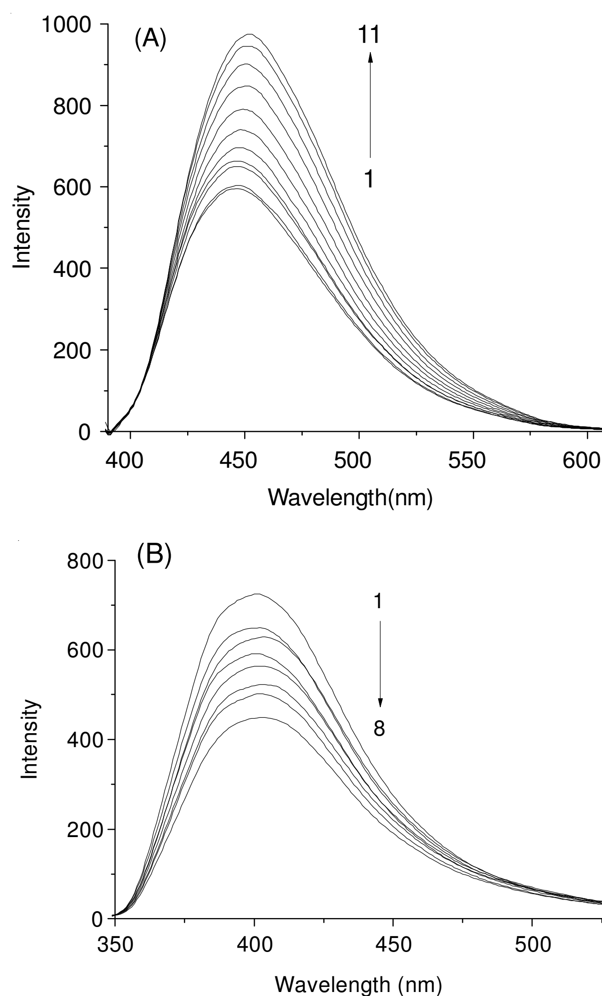
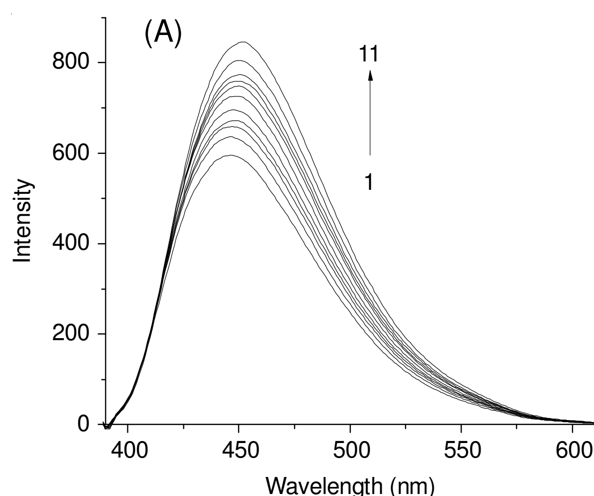


Fig. 2. Emission spectra of the representative compounds **1(A)** and **11(B)** in the presence of various concentrations of transition metal ion Cu^{2+}. The concentration of **1** and **11** were 5.0×10^{-4} and 5×10^{-5} mol L^{-1}, respectively. The concentrations of Cu^{2+} **1→11** (**1→8**) were: 0, 1, 2, 4, 8, 12, 16, 20, 24, 28, 30×10^{-5} (0, 3, 4, 5, 6, 8, 9, 11×10^{-5}) mol L^{-1}, respectively



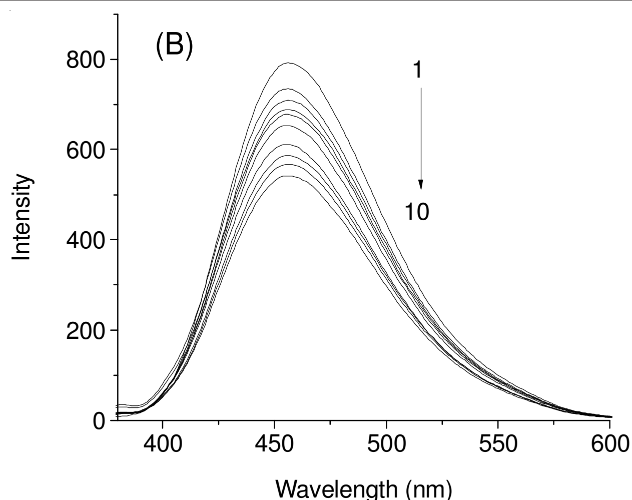


Fig. 3. Emission spectra of the representative compounds **1(A)** and **13(B)** in the presence of various concentrations of transition metal ion Fe^{2+}. The concentration of **1** and **13** were 5.0×10^{-4} and 5×10^{-5} mol L^{-1}, respectively. The concentrations of Fe^{2+} **1**→**11** (**1**→**10**) were: 0, 2, 3, 6, 8, 10, 13, 15, 17, 20, 25 × 10^{-5} (0, 2, 3, 4, 5, 6, 7, 8, 9, 10 × 10^{-4}) mol L^{-1}, respectively

As shown in Figs. 2 and 3, the fluorescence intensity of compounds **1-5**, **8** and **14** gradually increased with the consecutive addition of Cu^{2+} [as that shown in Fig. 2(A)], respectively, while the fluorescence intensity of compounds **6**, **7** and **10-13** decreased with the addition of Cu^{2+} [as that shown in Fig. 2(B)]. In addition, the fluorescence spectroscopy that compounds **1-4**, **7**, **8** and **12** chelating with Fe^{2+} were similar with the phenomenon shown in Fig. 3(A), while the fluorescence spectroscopy that compounds **5, 6, 10, 11, 13** and **14** chelating to Fe^{2+} was similar with that shown in Fig. 3(B). Also, compound **9** displayed important chelation with Cu^{2+} and Fe^{2+}, though their fluorescence intensities did not regularly alter as that shown in Figs. 2 and 3. The above results implied that compounds **1-14** have important chelating abilities with transition metal ions.

The fluorescence spectroscopy was then analyzed and described by the Stern-Volmer eqn. (1) and the modified Stern-Volmer eqn. (2)[9-11].

$$\frac{F_0}{F} = 1 + k_q \tau_0 c(Q) = 1 + K_{sv} c(Q) \qquad (1)$$

$$\frac{F_0}{F_0 - F} = \frac{F_0}{\Delta F} = \frac{1}{f_a K_a c(Q)} + \frac{1}{f_a} \qquad (2)$$

where, F_0 and F were the steady-state fluorescence intensities in the absence and presence of transition metal ions, respectively, c(Q) the concentration of Cu^{2+} or Fe^{2+}, ΔF the difference of fluorescence intensity in absence and presence of transition metal ions at the concentration c(Q), f_a was the fraction of accessible fluorescence, τ_0 the average lifetime of the molecule without any additives and the fluorescence lifetime of the biomolecule is 10^{-8} s.

From the Stern-Volmer plots (Fig. 4), the values of bimolecular quenching/enhancement constant $K_{q/e}$, Stern-Volmer quenching/enhancement constant (K_{SV}) and binding constants (K_a) can be determined[9-11] (Table-1). In addition, it was important to note that the values of $K_{q/e}$, K_{SV} and K_a of compound **9** could not be calculated due to its irregular altering fluorescence spectroscopy.

As can be seen in Table-1, $K_{q/e}$ were in the range of 1.42×10^{11}-3.920×10^{13} $M^{-1}.s^{-1}$, while K_{SV} and K_a were within the ranges of 1.42×10^3-3.920×10^5 M^{-1} and 1.25×10^3-4.46×10^5 M^{-1}, respectively. Obviously, compound **4** showed the best chelating abilities to Cu^{2+} and Fe^{2+}, with the binding constants K_a 4.46×10^5 and 3.85×10^5 M^{-1}, while compound **6** and **3** demonstrated the lowest chelating to Cu^{2+} and Fe^{2+}, respectively, with the binding constants K_a 1.25×10^3 and 1.59×10^3 M^{-1}. The order of chelating to Cu^{2+} was listed as follow: **4 > 10 > 12 > 13 > 2 > 8 > 14 > 5> 11 > 3 > 1 > 7 > 6**, while the order of chelating to Fe^{2+} was: **4 > 10 > 6 > 2 > 11 > 5 > 8 > 7 > 13 > 14 > 12 > 1 > 3**. Based on the above observation, it could be summarized that the benzyloxy group in **7** position of coumarin and the hydroxyl and hydrazino groups in benzene had important influence on their transition metal ion chelation ability. The above results suggested that compounds **1-14** have important chelating abilities with transition metal ions and the transition metal ion chelation may play an important role in their antioxidant abilities. In addition, the binding numbers of coumarins **1-14** with Cu^{2+} and Fe^{2+} was found approximately to be 1 (Table-2), respectively, according to the eqn. (3)[9-11]. It suggested that each unit of coumarins **1-14** should bond with a unit of transition metal ions Cu^{2+} and Fe^{2+}.

$$\log\left(\frac{F_0 - F}{F}\right) = \log K_A + n \log c(Q) \qquad (3)$$

TABLE-1
VALUES OF K_{sv}(M^{-1}), $K_{q/e}$($M^{-1}.s^{-1}$) AND K_a(M^{-1}) OF COUMARINS **1-14** BINDING WITH TRANSITION METAL IONS

Entry	K_{sv}/Cu^{2+}	K_{sv}/Fe^{2+}	$K_{q/e}/Cu^{2+}$	$K_{q/e}/Fe^{2+}$	K_a/Cu^{2+}	K_a/ Fe^{2+}
1	2.03×10^3	1.40×10^3	2.03×10^{11}	1.40×10^{11}	1.36×10^3	2.44×10^3
2	7.25×10^3	8.45×10^3	7.25×10^{11}	8.45×10^{11}	5.45×10^3	1.25×10^4
3	2.69×10^3	3.01×10^3	2.69×10^{11}	3.01×10^{11}	3.04×10^3	1.59×10^3
4	3.92×10^5	3.82×10^5	3.92×10^{13}	3.82×10^{13}	4.46×10^5	3.85×10^5
5	2.52×10^3	2.39×10^3	2.52×10^{11}	2.39×10^{11}	3.57×10^3	1.19×10^4
6	4.61×10^3	2.1×10^4	4.61×10^{11}	2.1×10^{12}	1.25×10^3	3.53×10^4
7	4.72×10^3	4.11×10^3	4.72×10^{11}	4.11×10^{11}	1.37×10^3	7.30×10^3
8	1.25×10^4	1.79×10^4	1.25×10^{12}	1.79×10^{12}	4.28×10^3	1.10×10^4
9	-	-	-	-	-	-
10	4.29×10^4	3.75×10^5	4.29×10^{12}	3.75×10^{13}	1.51×10^5	2.67×10^5
11	6.09×10^3	3.49×10^3	6.09×10^{11}	3.49×10^{11}	3.43×10^3	1.23×10^4
12	1.09×10^4	2.39×10^3	1.09×10^{12}	2.39×10^{11}	1.63×10^4	2.47×10^3
13	4.24×10^3	4.84×10^3	4.24×10^{11}	4.84×10^{11}	9.62×10^3	3.78×10^3
14	2.18×10^3	2.96×10^3	$+2.18 \times 10^{11}$	2.96×10^{11}	3.78×10^3	2.81×10^3

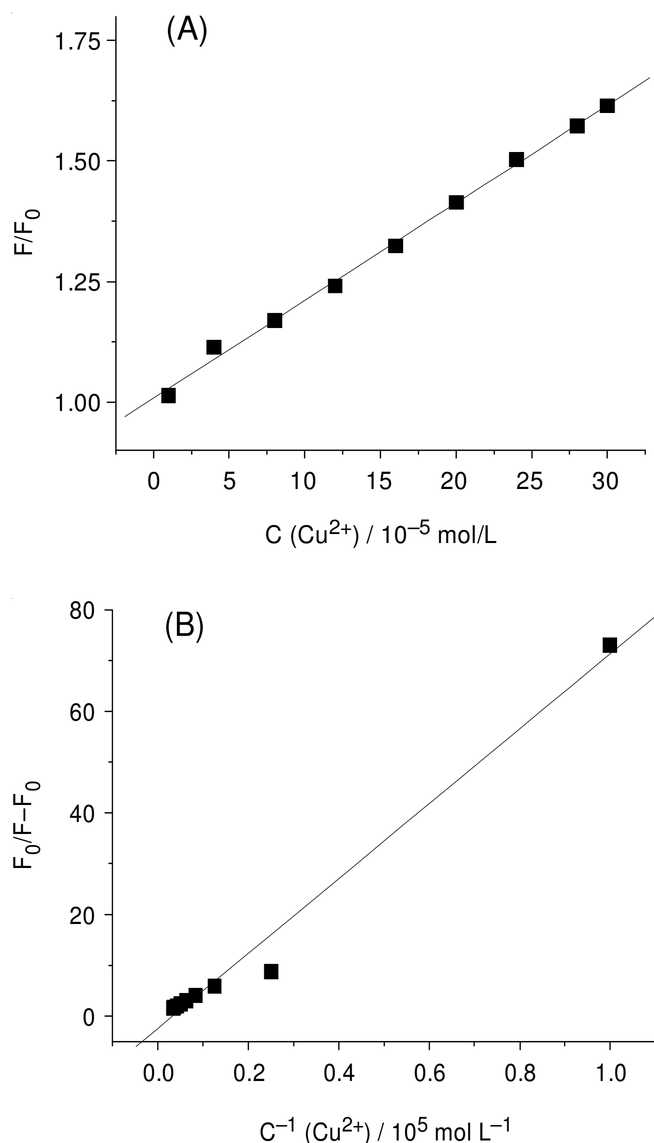


Fig. 4. Stern-Volmer plots (A) and modified Stern-Volmer plots (B) for the fluorescence spectra of **1** by transition metal ion Cu^{2+}

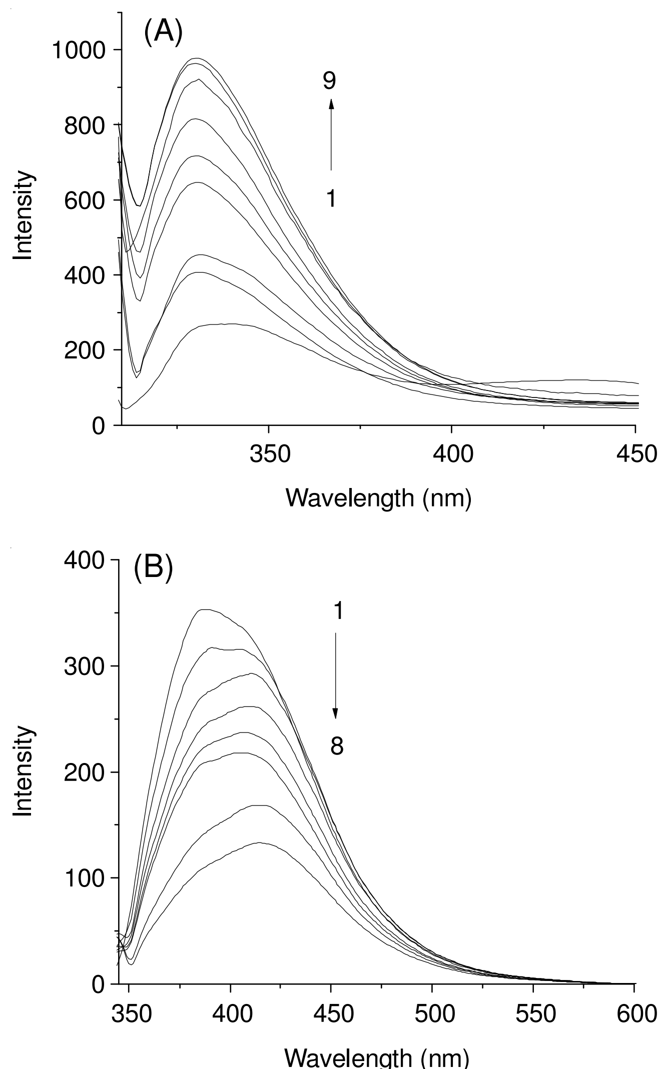


Fig. 5. Emission spectra of the representative compounds **3**(A) and **10**(B) in the presence of various concentrations of BSA. The concentration of **3** and **10** were 3×10^{-7} and 5×10^{-4} mol L^{-1}, respectively. The concentrations of BSA 1→11 (1→8) were: 0, 1, 2, 5, 7, 9, 11, 12, 13 × 10^{-7} (0, 2, 3, 4, 5, 6, 8, 9 × 10^{-7}) mol L^{-1}, respectively

Interaction of compounds 1-14 with bovine serum albumin: It is important to investigate the interaction of the drugs with bovine serum albumin. The interaction of drugs with bovine serum albumin may have important influence on the drugs' pharmacology and pharmacodynamics. Antioxidants are currently forged as the drug candidates to counter some diseases, such as carcinogenesis, drug-associated toxicity, inflammation and atherogenesis[12-14], so the determination and understanding of coumarins antioxidants **1-14** interacting with serum albumin are important for the rational design of this kind of new drugs. Fig. 5 showed that the fluorescence intensity of compounds **1-3, 5-8** and **12** gradually increased with the consecutive addition of bovine serum albumin [Fig. 5(A)], respectively, while that of compounds **4**, **9-11, 13** and **14** decreased with the addition of bovine serum albumin [Fig. 5(B)]. The fluorescence quenching/enhancement spectroscopy was also analyzed and described by the Stern-Volmer eqn. (1) and the modified Stern-Volmer eqn. (2) and the values of $K_{q/e}$, K_{SV} and K_a were determined (Table-2).

It can be seen from Table-2 that the values of $K_{q/e}$ were in the range of 2.27×10^{13}-2.16×10^{14} M^{-1} s^{-1}, which was much greater than the value of the maximum scatter collision quenching/enhancement constant[9,10] 2.0×10^{10} M^{-1} s^{-1}, indicating that the probable mechanism of compounds **1-14** interaction with bovine serum albumin was a static process. The Stern-Volmer quenching/enhancement constant K_{SV} were within the range of 2.27×10^{5}-2.16×10^{6} M^{-1} and binding constants K_a were in the scope of 5.96×10^{4}-5.22×10^{6} M^{-1}. The results implied that coumarins **1-14** have strong binding to bovine serum albumin. Since that strong binding can decrease the concentrations of free drugs in plasma, whereas weak binding can lead to a short lifetime or poor distribution, thus it can be concluded that the concentrations of coumarins **1-14** in plasma is low and they may have long duration of drug action. Evidently, compound **10** displayed the strong interaction with bovine serum albumin, with binding constants K_a 5.22×10^{6} M^{-1}, while compound **14** demonstrated the lowest, with binding constants K_a 5.96×10^{4} M^{-1}. The order was shown as follow: **10 > 3 > 11 > 8 > 7 > 12 > 6 > 1 > 4 > 9 > 13 > 2 > 14 > 5**. Therefore, it

could be concluded that the structure of the coumarins **1-14**, such as the benzyloxy group in 7 position of coumarin and the different sub-positions of hydroxyl and hydrazino groups in benzene had significant influence on the interaction of compounds **1-14** with bovine serum albumin. The binding numbers of coumarins **1-14** with bovine serum albumin was determined to be 1 (Table-2), according to the eqn. (3)[9-11]. It indicated that each unit of coumarins **1-14** should bind with a unit of bovine serum albumin.

TABLE-2
BINDING NUMBERS OF COUMARINS **1-14** AND THEIR VALUES OF K_{sv}(M^{-1}), $K_{q/e}$($M^{-1}.s^{-1}$) AND K_a (M^{-1}) THAT BINDING WITH BOVINE SERUM ALBUMIN (BSA)

Entry	K_{sv}/BSA	$K_{q/e}$/BSA	K_a/BSA	n/ Cu^{2+}	n/ Fe^{2+}	n/ BSA
1	3.39×10^5	3.39×10^{13}	3.80×10^5	1	1	1
2	7.12×10^5	7.12×10^{13}	2.51×10^5	1	1	1
3	1.60×10^6	1.60×10^{14}	4.34×10^6	1	1	1
4	4.48×10^5	4.48×10^{13}	3.51×10^5	1	1	1
5	5.22×10^5	5.22×10^{13}	5.96×10^4	1	1	1
6	6.85×10^5	6.85×10^{13}	5.44×10^5	1	1	1
7	4.97×10^5	4.97×10^{13}	6.44×10^5	1	1	1
8	2.31×10^5	2.31×10^{13}	7.14×10^5	1	1	1
9	2.56×10^5	2.56×10^{13}	3.18×10^5	-	-	1
10	2.16×10^6	2.16×10^{14}	5.22×10^6	1	1	1
11	3.12×10^5	3.12×10^{13}	7.42×10^5	1	1	1
12	3.26×10^5	3.26×10^{13}	5.62×10^5	1	1	1
13	$4. 60 \times 10^5$	$4. 60 \times 10^{13}$	2.87×10^5	1	1	1
14	2.27×10^5	2.27×10^{13}	2.42×10^5	1	1	1

Conclusion

In conclusion, transition metal ion chelation abilities of coumarins **1-14** have been studied by fluorescence spectroscopy. The results indicated that these coumarin derivatives may exert their antioxidant effect through transition metal ion chelation and the transition metal ion chelation play an important role in their antioxidant abilities. In addition, the interaction of these coumarins **1-14** with bovine serum albumin has been also investigated and the results show that the probable mechanism of the interaction of coumarins **1-14** with bovine serum albumin was a static process and coumarins **1-14** have strong binding ability to bovine serum albumin. All the above results may provide important information for the rational design of other analogous new antioxidants and drugs.

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