Med Chem Res (2012) 21:1341–1346
DOI 10.1007/s00044-011-9648-7

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antioxidant activities and transition metal ion chelating studies of some hydroxyl Schiff base derivatives

Ye Zhang · Biqun Zou · Kai Wang · Yingming Pan · Hong Liang · Xianghui Yi · Hengshan Wang

Received: 9 December 2010 / Accepted: 16 April 2011 / Published online: 2 May 2011

Abstract Several hydroxyl Schiff base (HSB) compounds (**1**–**10**) with good radical scavenging activity (RSA) were designed. Compounds **6**, **7**, and **10** showed better RSAs than the common synthetic antioxidant 2,6-diter-butyl-4-methylphenol (BHT) in DPPH$^{\bullet}$ and ABTS$^{\bullet}$ assays. To probe whether these HSB compounds may exert their antioxidant effect through transition metal ion chelation, the copper and ferrous chelating abilities of them were investigated. It was found by fluorescence quenching spectra that the binding constants K_a were in the range of 0.85×10^3–7.30×10^4 M^{-1}. Further study was carried out by the complexation of a representative compound **5** with ferrous ion in mass spectrum. A 2:1 **5**-ferrous complex was readily formed in a methanol–water solution (v:v, 8:2), which confirmed that the chelation happened when the HSB compounds were treated with transition metal ions. The above results indicated that the transition metal ion chelation play an important role in their antioxidant abilities.

Y. Zhang
School of Chemistry and Chemical Engineering, South Central University, Hunan 410083, People's Republic of China

Y. Zhang · B. Zou · Y. Pan · H. Liang (✉) · H. Wang (✉)
Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, College of Chemistry & Chemical Engineering, Guangxi Normal University, Guilin 541004, People's Republic of China
e-mail: hliang@gxnu.edu.cn

H. Wang
e-mail: wang_hengshan@yahoo.com.cn

Y. Zhang · K. Wang · X. Yi (✉)
Department of Chemistry and Engineering Technology, Guilin Normal College, Guilin 541001, People's Republic of China
e-mail: yixianghui2008@yahoo.com.cn

Keywords Hydroxyl Schiff base derivatives · Antioxidants activity · Transition metal ion chelation · Radical scavenging activity

Introduction

It is well known that the homeostasis of free radical and transition metal ion and Fenton reaction play an important part in the course of aging (Hipkiss, 2005; Halliwell and Gutteridge, 1999; Perez *et al*., 2009). Some age-related diseases such as atherosclerosis, Alzheimer's, and Parkinson diseases, are characteristically associated with free radical and transition metal ion catalyzed oxidative damage to carbohydrates, lipids, proteins, and nucleic acids (Hipkiss, 2005; Halliwell and Gutteridge, 1999). The homeostasis modulation of free radicals and transition metal ion, as well as Fenton reaction, is generally maintained by the balanced system of antioxidant defences (Hipkiss, 2005; Halliwell and Gutteridge, 1999; Perez *et al*., 2009). However, only endogenous antioxidant defenses are not entirely efficient, exogenous antioxidants are also required. The exogenous antioxidants, which have good RSAs and transition metal ion chelation ability, could not only diminish the cumulative effects of free radicals and oxidative damages, but also maintain the transition metal ion homeostasis modulation and Fenton reaction (Hipkiss, 2005; Halliwell and Gutteridge, 1999; Perez *et al*., 2009).

Some HSB derivatives, such as compound **1** and **4** (Fig. 1), have been showed to have good RSAs (Tang and Liu, 2007), it would be very interesting to explore if, transition metal ion chelation play an important role in their antioxidant abilities. In addition, a look at the structures of these HSB derivatives shows that they contain "metal ion-binding motifs" and thus are expected to bind transition

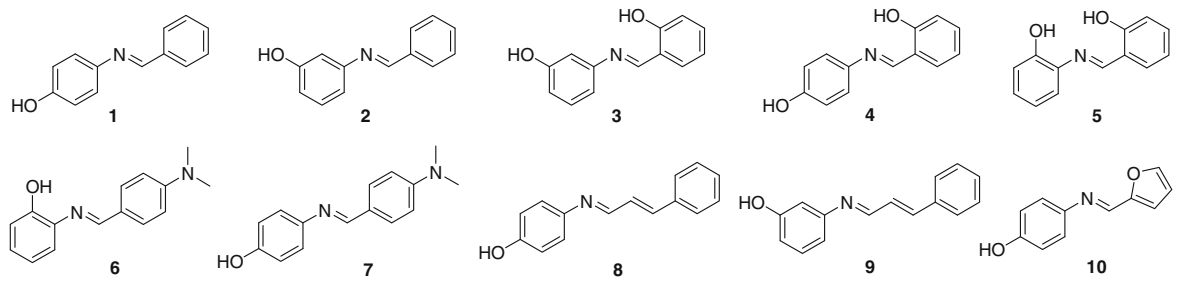

Fig. 1 Structure of the HSB compounds

metal ion. Therefore, other HSB derivatives were rationally designed (Fig. 1) and the antioxidant activities of all the HSB derivatives were evaluated, especially with regard to their transition metal ion chelation abilities.

Materials and methods

Reagents and apparatus

2,6-Diter-butyl-4-methylphenol (BHT), 2,2′-Azino-di(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS$^{\bullet}$) and 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents used were of analytical grade. The UV-1100 spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China) were used to evaluate the radical activity. The fluorescence spectroscopy was scanned by the RF-5301 spectrophotometer. The NMR study was carried out by the instrument NMR (BRUKER AVANCE 500, BRUKER company, Switzerland), and the mass spectral studies were done using BRUKER ESQUIRE HCT instrument (BRUKER DALTON company, USA).

Free radical scavenging activity

The experiments of HSB compounds to scavenge DPPH and ABTS free radical were performed following our previous reports (Pan *et al*., 2007, 2010). In brief, the stock solution of DPPH$^{\bullet}$ was prepared by dissolving DPPH$^{\bullet}$ in ethanol directly to make the absorbance $\sim$1.00 (Abs_{ref}) at 517 nm. ABTS$^{\bullet}$ aqueous (4.0 mM, 2.00 ml) was oxidized by 1.41 mM $K_2S_2O_8$ for 16 h, and then diluted with 100 ml of ethanol to make the absorbance $\sim$0.70 (Abs_{ref}) at 734 nm. The addition of various concentrations of HSB derivatives solution in DMF decreased Abs_{ref} to a stable value (Abs_{detect}). Thus, the scavenging percentage of DPPH$^{\bullet}$ or ABTS$^{\bullet}$ in the presence of different concentrations of HSB compounds can be calculated by Eq. 1. Then, the values of IC_{50} that the effective concentration at which 50% of DPPH$^{\bullet}$ or ABTS$^{\bullet}$ were scavenged, were calculated to evaluate the radical activity from Eq. 1:

$$\text{Scavenging DPPH}^{\bullet}(\text{or ABTS}^{\bullet})(\%) = \frac{Abs_{ref} - Abs_{detect}}{Abs_{ref}} \times 100\%. \quad (1)$$

The procedure of fluorescence spectrum studies on transition metal ion chelation

At room temperature, the ten compounds, ferrous nitrate and copper nitrate 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 Schiff bases solution, respectively, the mixture was allowed to stand for 5 min (until the stable) with intermittent shaking, and the fluorescence spectroscopy was then scanned.

The procedure of mass spectrum studies on transition metal ion chelation

A mixture of **5** (30 μM) and ferrous sulfate (10 μM) in methanol–water solution (v:v, 8:2) was allowed stand for 0.5 h with intermittent shaking, and the mass spectrum was then scanned.

Results and discussion

DPPH and ABTS RSAs evaluations were classical assays in antioxidant activity studies and offer rapid techniques for screening the RSAs of the compounds. The RSAs of the HSB compounds were estimated using these two methods (Pan *et al*., 2007, 2010). The values of IC_{50} for compounds **1–10** were tested to evaluate their radical activities (Table 1). The IC_{50} of BHT was also determined for comparison.

As showed in Table 1, all the compounds exhibited good potent inhibition of DPPH and ABTS radical, since that the

Table 1 IC_{50} of the HSB compounds in scavenging of $ABTS^{\bullet}$ and $DPPH^{\bullet}$

Compounds	$DPPH^{\bullet}$ IC_{50} (μM)	$ABTS^{\bullet}$ IC_{50} (μM)	Compounds	$DPPH^{\bullet}$ IC_{50} (μM)	$ABTS^{\bullet}$ IC_{50} (μM)
1	76.05 (71.50[a])	6.03 (10.30[a])	**7**	20.82 (20.77[a])	4.16 (8.21[a])
2	466.46 (0.092[b]mg/ml)	33.46	**8**	398.62	4.93
3	406.93	3.68	**9**	143.32	3.99
4	117.44 (107.00[a])	4.68 (8.76[a])	**10**	59.41 (50.24[a])	6.14 (9.97[a])
5	98.49 (25.00[a])	5.16 (10.20[a])	BHT	65.80	36.98
6	10.83 (20.00[a])	7.07 (10.19[a])			

[a] The values in parenthesis were from reference (Tang and Liu 2007; Wang *et al*., 2007)

[b] The IC_{50} of the lowest RSA compound **2** was also equal to 0.092 mg/ml

IC_{50} of the lowest RSA compound **2** (0.092 mg/ml) was further lower than the standard value 10 mg/ml (Lee *et al*. 2007). Compounds **6**, **7**, and **10** were shown to be more potent inhibition of DPPH radical than the common synthetic antioxidant BHT (IC_{50} 65.80 μM), with IC_{50} of 10.83, 20.82, and 59.41 μM, respectively, while compound **6** displayed the best potent activity. The order of their RSAs was: **6 > 7 > 10 > BHT > 1 > 5 > 4 > 9 > 8 > 3 > 2**. On the basis of this observation, it can be suggested that the presence of electron-donating groups in the para-position of benzene moiety have an important influence on their DPPH RSAs.

In addition, all the compounds displayed stronger ABTS RSAs than that of BHT (IC_{50} 36.98 μM), while compound **3** showed the best activity (IC_{50} 3.68 μM) and compound **2** displayed the lowest (IC_{50} 33.46 μM). The order was listed as follow: **3 > 9 > 7 > 4 > 8 > 5 > 1 > 10 > 6 > 2 > BHT**. From this observation, it could be concluded that the position and number of hydroxyl groups, as well as electron-donating groups was very important to their ABTS RSAs.

To probe whether they may exert their antioxidant effect through transition metal ion chelation, the chelating abilities with Fe^{2+} and Cu^{2+} were investigated by fluorescence quenching spectroscopy. Upon addition of transition metal ions (Cu^{2+} and Fe^{2+}) into the HSB compounds solution, the fluorescence intensity decreased gradually as in Fig. 2.

Fluorescence quenching can be dynamic, confirmed by Stern–Volmer equation (1) (Eftink, 1991; Lakowica and Weber, 1973; Lakowicz, 1999), or static, confirmed by the modified Stern–Volmer equation (2) (Eftink, 1991; Lakowica and Weber, 1973; Lakowicz, 1999), and that would give the information about the binding to transition metal ions.

For dynamic quenching, the fluorescence data can be described by the Stern–Volmer equation (2) to confirm the mechanism.

$$\frac{F_0}{F} = 1 + K_q \tau_0 c(Q) = 1 + K_{SV} c(Q) \quad (2)$$

where F_0 and F are the steady-state fluorescence intensities in the absence and presence of quencher (transition metal ions), respectively, $c(Q)$ the concentration of quencher (Cu^{2+} or Fe^{2+}), τ_0 the average lifetime of the molecule without any quencher and the fluorescence lifetime of the biomolecule is 10^{-8} s (Eftink, 1991; Lakowica and Weber, 1973). From the Stern–Volmer plot (Fig. 3a), the values of Stern–Volmer quenching constant (K_{SV}) and bimolecular quenching constant (k_q) for the quenchers can be determined.

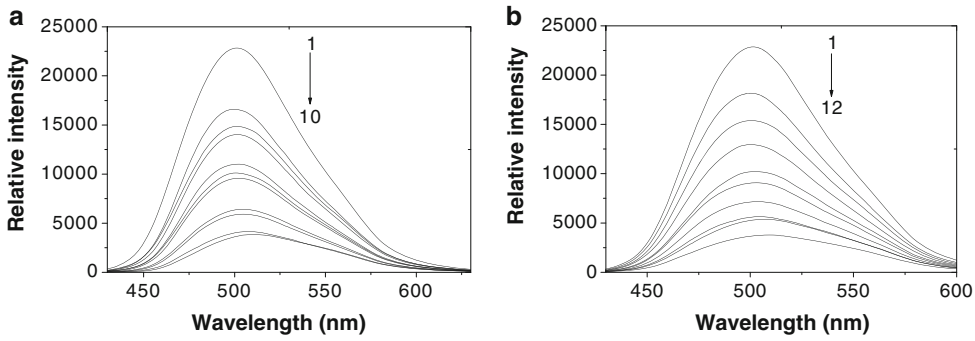

Fig. 2 Emission spectra of the representative compound **5** in the presence of various concentrations of transition metal ion Cu^{2+} (**a**) and Fe^{2+} (**b**). The concentrations of Cu^{2+}(Fe^{2+}) 1 → 10 (1 → 12) were: 0, 4.0, 6.0, 7.0, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0 × 10^{-5} (0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 15.0 × 10^{-5}) mol l^{-1}, respectively

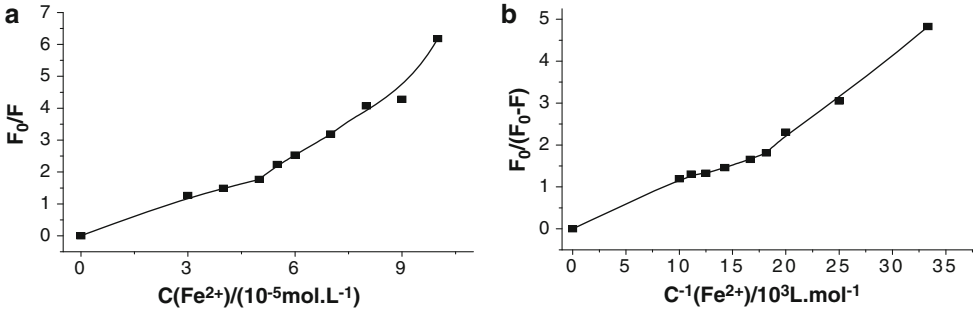

Fig. 3 Stern–Volmer plots (**a**) and Modified Stern–Volmer plots (**b**) for the quenching of **5** by transition metal ion Fe^{2+}

Table 2 Binding constants of the HSB compounds

Products	K_{sv}/Cu^{2+} (M^{-1})	k_q/Cu^{2+} (M^{-1} s^{-1})	K_{sv}/Fe^{2+} (M^{-1})	k_q/Fe^{2+} (M^{-1} s^{-1})	K_a/Cu^{2+} (M^{-1})	K_a/Fe^{2+} (M^{-1})
1	3.49×10^3	3.49×10^{11}	4.09×10^3	4.09×10^{11}	3.17×10^3	1.62×10^4
2	5.20×10^3	5.20×10^{11}	7.03×10^3	7.03×10^{11}	1.39×10^4	1.47×10^4
3	1.10×10^5	1.10×10^{13}	2.61×10^4	2.61×10^{12}	3.02×10^3	3.21×10^3
4	7.21×10^4	7.21×10^{12}	1.43×10^5	1.43×10^{13}	4.43×10^3	9.67×10^3
5	6.07×10^4	6.07×10^{12}	6.70×10^4	6.70×10^{12}	1.26×10^4	6.48×10^3
6	1.53×10^4	1.53×10^{12}	5.19×10^3	5.19×10^{11}	2.36×10^4	7.30×10^4
7	2.69×10^4	2.69×10^{12}	7.40×10^3	7.40×10^{11}	3.05×10^3	5.03×10^3
8	2.49×10^3	2.49×10^{11}	6.60×10^3	6.60×10^{11}	1.45×10^4	2.47×10^4
9	2.80×10^3	2.80×10^{11}	8.91×10^3	8.91×10^{11}	3.51×10^3	6.25×10^3
10	1.17×10^3	1.17×10^{11}	0.90×10^3	0.90×10^{11}	0.85×10^3	1.81×10^3

In Table 2, k_q was much greater than the value of the maximum scatter collision quenching constant 2.0×10^{10} M^{-1} s^{-1} (Eftink, 1991; Lakowica and Weber, 1973), which indicated that the fluorescence quenching was caused by a specific interaction, and the quenching was mainly arisen by complex formation (Maurice and Camillo, 1981), while dynamic collision could be negligible in the concentration studied (Papadopoulou *et al.*, 2005). In addition, the UV–Vis absorption spectra of the compounds with transition metal ions system (Fig. 4) were also found to be obviously different, which confirmed that the quenching was mainly a static quenching process. Therefore, the mechanism of fluorescence quenching was a static quenching procedure and the quenching data must be analyzed by the modified Stern–Volmer equation (3) (Lakowica and Weber, 1973):

$$\frac{F_0}{F_0 - F} = \frac{F_0}{\Delta F} = \frac{1}{f_a K_a c(Q)} + \frac{1}{f_a}. \quad (3)$$

In this case, ΔF was the difference of fluorescence intensity in absence and presence of the quencher at the concentration $c(Q)$, f_a was the fraction of accessible fluorescence. From the modified Stern–Volmer plots (Fig. 3b), the corresponding binding constants (K_a) could be obtained (Table 2).

As can be seen in Table 2, the binding constants K_a were in the range of 0.85×10^3–7.30×10^4 M^{-1}. Their orders for the chelation with copper and ferrous ions were: **6** > **8** > **2** > **5** > **4** > **9** > **1** > **7** > **3** > **10** and **6** > **8** > **1** > **2** > **4** > **5** > **9** > **7** > **3** > **10**, respectively. Compound **6** showed the best transition metal ions chelating abilities with Fe^{2+} and Cu^{2+}, with binding constants of 2.36×10^4 and 7.30×10^4 M^{-1}, respectively. Based on the above observation, it could be summarized that the position of hydroxyl group and electron-donating atmosphere had important influence on their transition metal ion chelation ability. The above results suggested that the HSB compounds have good chelation abilities with transition metal ions and the transition metal ion chelation play an important role in their antioxidant abilities.

Further studies on the chelation of transition metal ions by HSB compounds were carried out by the complexation of the representative compound **5** with ferrous ion in a mass spectrometer, since that its salen structure may have good complexation ability. A 8:2 (v:v) mixture of methanol and water was used as the solvent.

Figure 5 showed the mass spectra for a mixture of 30 μM of **5** with 10 μM of freshly prepared ferrous solution. Species corresponding to the formation of a 2:1 complex between **5** and ferrous ion was observed in this case: a **5**-Fe^{2+} complex ($m/z = 480$, [**5**-Fe(II)-(**5**-H)]$^+$,

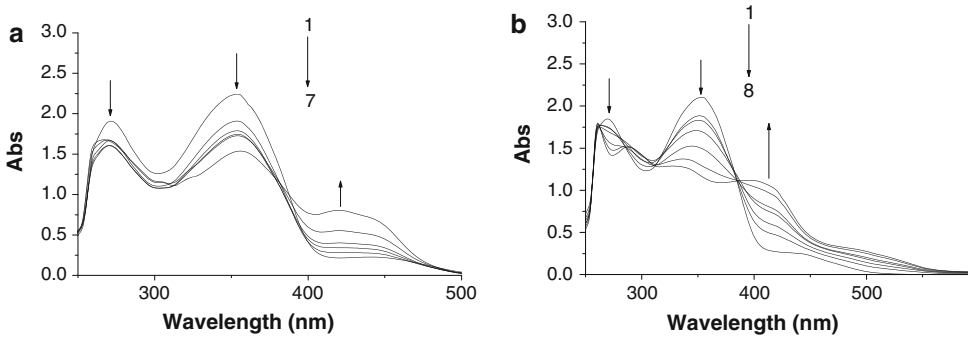

Fig. 4 The UV–Vis absorption spectra of compound **5** in the absence and presence of Cu^{2+} (**a**) and Fe^{2+} (**b**). The concentrations of Cu^{2+}(Fe^{2+}) 1 → 7 (1 → 8) were: 0, 2.0, 3.0, 4.0, 5.0, 9.0, 14.0 × 10^{-5} (0, 3.0, 4.0, 5.0, 7.0, 9.0, 11.0, 12.0 × 10^{-5}) M, respectively

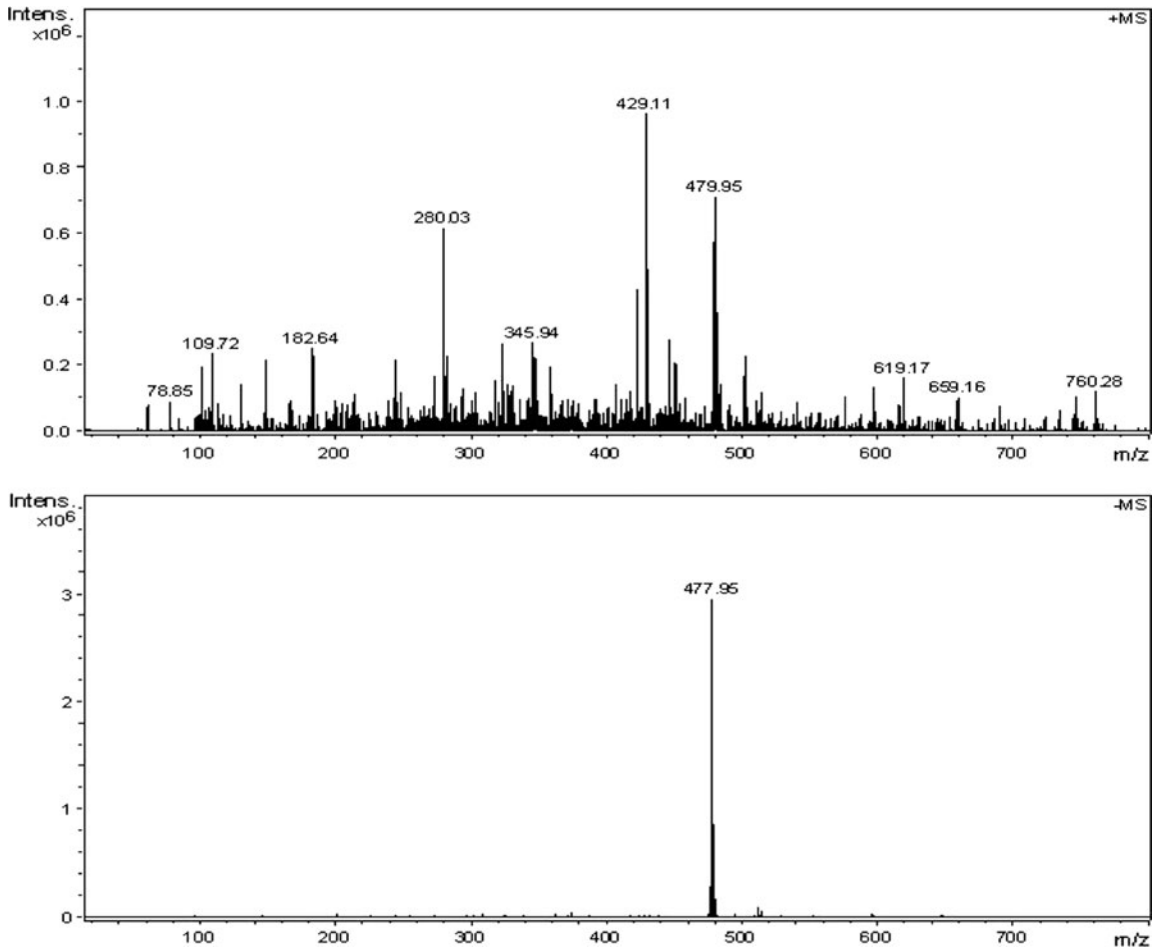

Fig. 5 Mass spectra of 30 μM **5** with 10 μM Fe^{2+} in a methanol–water solution (v:v, 8:2)

Fig. 5). A close look at both the +MS and –MS patterns of the **5**-Fe complex (Fig. 5) suggested that they fit well the above result. This observation confirmed that the chelation of transition metal ions happened when the HSB compounds were treated with transition metal ions.

Conclusion

In conclusion, we have shown that the rationally designed HSB derivatives have good RSAs and chelation with transition metal ions. The results indicated that the HSB 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.

Acknowledgments This study was supported by the National Natural Science Foundation of China (No. 20762001), the Project of the Key Laboratory of Medicinal Chemical Resources and Molecular Engineering, Guangxi Normal University, China (No.0630006-5D09), and the Guangxi Department of Education research project (No. 200807MS075, 200807MS076, 200911MS281, 200911MS282).

Appendix: synthesis of the derivatives

Synthesis

General procedure for the preparation of **1–10**: the mixture of aminophenol (5 mmol), aromatic aldehydes (6 mmol), ethanol (10 ml), and triethylamine (0.1 mmol) was refluxed at 90°C for 2 h and then filtered to give crystal or powder of HSB derivatives.

Spectra data for compounds **1–10**

Compound **1**: ^{1}H NMR (DMSO, 500HZ) δ: 9.50 (s, 1H, OH), 8.62 (s, 1H, N=CH), 7.90 (d, $J = 7.67$ Hz, 2H, Ar-H), 7.49–7.51 (m, 3H, Ar-H), 7.21 (d, $J = 8.57$ Hz, 2H, Ar-H), 6.82 (d, $J = 8.57$ Hz, 2H, Ar-H). EMS: *m/z*: 198 $[M + H]^+$.

Compound **2:** ^{1}H NMR (DMSO, 500HZ) δ: 8.29 (d, $J = 7.20$ Hz, 1H, N=CH), 7.90 (d, $J = 8.50$ Hz, 1H, Ar-H), 7.68 (dd, $J = 8.79$ Hz, 5.5 Hz, 2H, Ar-H), 7.62 (t, $J = 7.26$ Hz, 1H, OH), 7.51 (d, $J = 7.38$ Hz, 1H, Ar-H), 7.37 (d, $J = 7.27$ Hz, 1H, Ar-H), 7.29 (s, 2H, Ar-H), 6.83 (t, $J = 7.30$ Hz, 1H, Ar-H), 6.65 (t, $J = 7.57$ Hz, 1H, Ar-H), 6.18 (d, $J =$ 7.5 Hz, 1H, Ar-H). EMS: *m/z*: 198 $[M + H]^+$

Compound **3**: ^{1}H NMR (DMSO,500HZ) δ: 13.12 (s, 1H, OH), 9.64 (s, 1H, OH), 8.91 (s, 1H, N=CH), 7.67 (d, $J = 7.53$ Hz, 1H, Ar-H), 7.42 (d, $J = 7.75$ Hz, 1H, Ar-H), 7.25 (d, $J = 7.86$ Hz, 1H, Ar-H), 6.96 (d, $J = 7.47$ Hz, 2H, Ar-H), 6.84 (d, $J = 7.65$ Hz, 1H, Ar-H), 6.78–6.74 (m, 2H, Ar-H). EMS: *m/z*: 214 $[M+H]^+$.

Compound **4**: ^{1}H NMR (DMSO,500HZ) δ: 13.41 (s, 1H, OH), 9.67 (s, 1H, OH), 8.91 (s, 1H, N=CH), 7.60 (d, $J = 8.11$ Hz, 1H, Ar-H), 7.32-7.39 (m, 3H, Ar-H), 6.93-6.98 (m, 2H, Ar-H), 6.85 (d, $J = 8.6$ Hz, 2H, Ar-H). EMS: *m/z*: 214 $[M + H]^+$.

Compound **5**: ^{1}H NMR (DMSO,500HZ) δ: 13.76 (s, 1H, OH), 9.71 (s, 1H, OH), 8.97 (s, 1H, N=CH), 7.62 (d, $J = 7.50$ Hz, 1H, Ar-H), 7.36-7.41 (m, 2H, Ar-H), 7.14 (t, $J = 7.50$ Hz, 1H, Ar-H), 6.87–6.98 (m, 4H, Ar-H). EMS: *m/z*: 214 $[M + H]^+$.

Compound **6**: ^{1}H NMR (DMSO,500HZ) δ: 9.42 (1s, 1H, OH), 8.58 (s, 1H, N=CH), 7.82 (d, 2H, $J = 8.84$Hz, Ar-H), 7.28 (d, $J = 7.87$Hz, 1H, Ar-H), 7.14 (d, $J = 7.94$Hz, 1H, Ar-H), 7.01 (d, $J = 8.16$Hz, 1H, Ar-H), 6.91 (d, $J = 7.5$Hz, 1H, Ar-H), 6.78 (d, $J = 8.9$Hz, 2H, Ar-H), 3.01 (s, 6H, CH_3). EMS: *m/z*: 241 $[M + H]^+$

Compound **7**: ^{1}H NMR (DMSO, 500HZ) δ: 9.31 (1s, 1H, OH), 8.39 (s, 1H, N=CH), 7.70 (d, $J = 8.67$ Hz, 2H, Ar-H), 7.10 (d, $J = 8.56$ Hz, 2H, Ar-H), 6.77 (m, 4H, Ar-H), 3.00 (s, 6H, CH_3). EMS: *m/z*: 241 $[M + H]^+$.

Compound **8**: ^{1}H NMR (DMSO,500HZ) δ: 9.48 (s, 1H, OH), 8.38 (d, $J = 8.78$ Hz, 1H, N=CH), 7.63 (d, $J = 7.39$ Hz, 2H, Ar-H), 7.33-7.42 (3H, m, Ar-H), 7.26 (d, $J = 15.96$ Hz, 1H, Ar-H), 7.07-7.12 (m, 3H, Ar-H), 6.76 (d, 2H, $J = 7.76$ Hz, =CH). EMS: *m/z*: 224 $[M + H]^+$.

Compound **9**: ^{1}H NMR (DMSO,500HZ) δ: 9.77 (s, 1H, OH), 8.39 (d, $J = 8.78$ Hz, 1H, N=CH), 7.77 (d, $J = 7.39$ Hz, 2H, Ar-H), 7.35–7.44 (3H, m, Ar-H), 7.28 (d, $J = 15.96$ Hz, 1H, Ar-H), 7.09–7.14 (m, 3H, Ar-H), 6.73 (d, 2H, $J = 7.76$ Hz, =CH). EMS: *m/z*: 224 $[M + H]^+$.

Compound **10**: ^{1}H NMR (DMSO, 500 Hz) δ: 9.90 (s, 1H, OH), 8.41 (s, 1H, N=CH), 7.83 (d, $J = 7.37$ Hz, 1H), 7.20 (d, $J = 8.73$ Hz, 2H), 7.07 (t, $J = 11.62$ Hz, 1H), 6.80 (d, $J = 8.32$, 2H), 6.67 (d, $J = 7.47$ Hz, 1H), 6.53-6.46 (m, 1H). EMS: *m/z*:188 $[M + H]^+$.

References

Eftink MR (1991) Fluorescence quenching reactions. In: Dewey TG (ed) Biophysical and biochemical aspects of fluorescence spectroscopy. Plenum Press, New York, pp 1–44

Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Oxford University Press, Oxford

Hipkiss AR (2005) Glycation, ageing and carnosine: are carnivorous diets beneficial. Mech Ageing Dev 126:1034–1039

Lakowica JR, Weber G (1973) Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules. Biochemistry 12:4161–4170

Lakowicz JR (1999) Principles of fluorescence spectroscopy. Plenum Press, New York

Lee YL, Yen MT, Mau JL (2007) Antioxidant properties of various extracts from *Hypizigus marmoreus*. Food Chem 104:1–9

Maurice RE, Camillo AG (1981) Fluorescence quenching studies with proteins. Anal Biochem 114:199–212

Pan YM, Zhu JC, Wang HS, Zhang XP, Zhang Y, He CH, Ji XW, Li HY (2007) Antioxidant activity of ethanolic extract of *Cortex fraxini* and use in peanut oil. Food Chem 103:913–918

Pan YM, He CH, Wang HS, Ji XW, Wang K, Liu PZ (2010) Antioxidant activity of microwave-assisted extract of *Buddleia officinalis* and its major active component. Food Chem 121:497–502

Papadopoulou A, Green RJ, Frazier RA (2005) Interaction of flavonoids with bovine serum albumin: a fluorescene quenching study. J Agric Food Chem 53:158–163

Perez CA, Wei Y, Guo M (2009) Iron-binding and anti-Fenton properties of baicalein and baicalin. J Inorg Biochem 103:326–332

Tang YZ, Liu ZQ (2007) Free-radical-scavenging effect of carbazole derivatives on DPPH and ABTS radicals. Cell Biochem Funct 25:149–158

Wang HX, Tang YZ, Liu ZQ (2007) Ability of Schiff bases with hydroxyl-substituent to scavenge radicals. Chin J Synth Chem 24:1105–1108