Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 1064–1068

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Differential effects of Cu(II) and Fe(III) on the binding of omeprazole and pantoprazole to bovine serum albumin: Toxic effect of metal ions on drugs

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ARTICLE INFO

Article history:
Received 13 May 2011
Received in revised form 1 August 2011
Accepted 4 August 2011
Available online 11 August 2011

Keywords:
Omeprazole
Pantoprazole
Bovine serum albumin
Interaction
Metal ion

ABSTRACT

The interaction between omeprazole or pantoprazole and bovine serum albumin (BSA) has been investigated in the absence and presence of Cu(II) or Fe(III) by means of fluorescence spectroscopy. The fluorescence intensity of BSA decreased remarkably with slight blue shifts by adding omeprazole or pantoprazole. Similar blue shifts and fluorescence shape with larger quenching extent of BSA were observed with increasing concentrations of omeprazole or pantoprazole in the presence of Cu(II) or Fe(III). The presence of Cu(II) and Fe(III) increased the affinities of omeprazole with BSA about 12.0% and 3.9%, while the presence of Cu(II) decreased the affinity of pantoprazole with BSA about 25.7%, and the presence of Fe(III) improved the affinity of pantoprazole with BSA about 16.3%. The changeable affinity and increased binding distance in the presence of metal ions may result from a noncompetitive binding in different albumin sites. The results indicated that the structures of omeprazole or pantoprazole and kinds of metal ions together affected the binding interaction with BSA, which may have relevant consequence in rationalizing dosage for patients with gastric ulcer.

1. Introduction

Serum albumin is the major soluble protein in circulatory system, which has many physiological functions, such as maintaining the osmotic pressure and pH of blood and as carriers transporting a great number of endogenous and exogenous compounds such as fatty acids, amino acids, drugs and pharmaceuticals [1]. The drug–serum albumin interaction plays a dominant role in drug disposition and efficacy, and it is also useful to explain the relationship between the structures and functions of drugs. The bound drug acts as a depot while unbound drug produces the desired pharmacological effect. Up to now, many manuscripts have reported the interaction between drugs and serum albumin [2–6]. Moreover, drug–serum albumin interaction always causes interference of the binding of other drugs as the result of overlap of binding sites or conformational change [7,8]. Therefore, the detailed investigation of drug–serum albumin interactions in the presence of other compounds plays a dominant role for interpretation of toxic interactions.

Proton pump inhibitors (PPIs) of the substituted benzimidazole class are widely utilized for the treatment of gastric acid related disorders for about 25 years [9]. Omeprazole is the first of the PPIs marketed in 1987 [10], while pantoprazole is the third PPIs marketed in 1995 [11] (Fig. 1). Both of the drugs are effective and can be used safely, and the research has revealed that no significant differences have been observed between these two PPIs in the rate of endoscopic healing of reflux esophagitis at week 8 [12], in upper gastrointestinal hemorrhage induced by hypertensive encephalorrhagia [13], and in regard to transfusion units, death, surgery and rebleeding [14]. However, there is difference in pharmacokinetic and pharmacodynamic profiles that might influence their clinical utility. Besancon et al. compared the PPIs for *in vitro* inhibition of H^+/K^+-ATPase, and the results indicated that omeprazole had more rapid inhibition than pantoprazole [15]. Therefore, research on the interactions between PPIs and serum albumin can provide general information about the difference in free concentration, metabolism, efficacy and pharmacology of PPIs. The interaction of omeprazole with bovine serum albumin (BSA) has been investigated [16], and the results indicated that the fluorescence quenching of BSA by omeprazole was a static quenching, while hydrophobic force and electrostatic force played major roles for omeprazole–BSA association. However, there are no reports associated with the binding of pantoprazole on BSA up to now. And the effect of coexisting compounds on the binding affinities of omeprazole or pantoprazole to BSA was seldom studied, which

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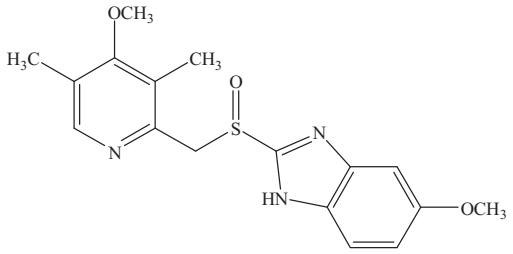
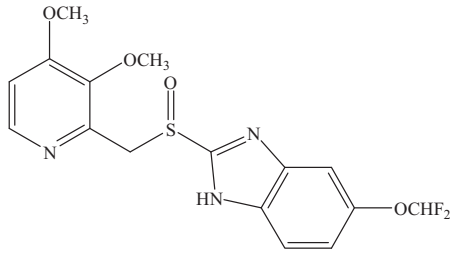
doi:10.1016/j.jpba.2011.08.012



Omeprazole



Pantoprazole

Fig. 1. Molecular structures of omeprazole and pantoprazole.

will directly correlate with the *in vivo* efficiency of omeprazole and pantoprazole.

Some metal ions are essential in the human body as well as an essential nutrient. Cu(II) is the third most abundant trace mineral in the human body. Fe(III) is one of the most essential trace elements in human body. It has reported that Cu(II), Fe(III) and other metal ions or metal ion complexes can react with serum albumins [17–21], which then affected the reactions of drugs, such as flavonoids [22–24], vitamins [25] and dexamethasone [7], with serum albumin. The results indicated that the presence of metal ions could change the binding constants between drugs and BSA. Herein, in view of the biological importance, it was worthwhile to study the effects of Cu(II) and Fe(III) on the binding of omeprazole and pantoprazole with BSA.

2. Materials and methods

2.1. Chemicals and reagents

Omeprazole and pantoprazole with purity over 99.5% were obtained commercially from Sigma Chemical Co. (St. Louis, MO, USA). Bovine serum albumin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals such as buffer Tris with the purity more than 99.5%, $CuCl_2$, $FeCl_3$, NaCl, HCl, and ethanol were all of analytical purity and used without further purification. Water used in all experiments was doubly distilled water.

2.2. Instrumentations

All fluorescence spectra were recorded on an F-2000 spectrofluorimeter equipped with 1.0 cm quartz cells and a 150 W xenon lamp (Hitachi, Tokyo, Japan). An excitation wavelength of 280 nm was used. The excitation and emission slit width were both set at 2.5 nm. The UV spectra were obtained on a Perkin-Elmer Lambda 17 UV spectrophotometer with the wavelength range of 200–450 nm (Perkin Elmer Corp., Edison, NJ, USA). The weight measurements were performed on an AY-120 electronic analytic weighing scale with a resolution of 0.1 mg (Shimadzu, Japan). The pH value was measured in a pHS-3 digital pH meter (Shanghai, China).

2.3. Preparation of solutions

Tris–HCl buffer solution (0.1 mol L^{-1} Tris, pH 7.4) containing 0.1 mol L^{-1} NaCl was prepared to keep the pH value and maintain the ionic strength of the solution. The working solutions of BSA (1×10^{-4} mol L^{-1}), omeprazole and pantoprazole (4×10^{-4} mol L^{-1}), Cu(II) and Fe(III) (2×10^{-4} mol L^{-1}) were prepared by dissolving them in Tris–HCl buffer solution, respectively, and stored in refrigerator at 4 °C prior to use.

2.4. Fluorescence spectra of omeprazole and pantoprazole binding with BSA in the absence or presence of Cu(II) and Fe(III)

300 μL of BSA solution (or 300 μL of BSA solution and 150 μL of Cu(II) or Fe(III)) were added to eleven 5 mL flasks, respectively. After reaction for 1 h, appropriate amounts of 4.0×10^{-4} mol L^{-1} omeprazole or pantoprazole were added, and diluted to 5 mL with Tris–HCl buffer. The final concentrations of omeprazole or pantoprazole were 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 μmol L^{-1}, and the concentration of Cu(II) or Fe(III) was 6 μmol L^{-1}, which was as the same as that for BSA. The resultant mixtures were then incubated at 298.15 K for 1.0 h. After 1.0 h incubation, the fluorescence emissions spectra were scanned in the range of 290–450 nm and the fluorescence intensity at 340 nm was measured. All the experiments were repeated in triplicate and found to be reproducible with the experimental error (<1%).

2.5. Data analysis

The binding constant (K) and binding sites (n) are calculated by the double-logarithm equation for static quenching [26]:

$$lg\left[\frac{F_0 - F}{F}\right] = lg\,K + n\,lg[Q] \quad (1)$$

The efficiency energy transfer E was determined by Förster's energy transfer theory [27]:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (2)$$

where F and F_0 are the fluorescence intensities of BSA with or without drug, r is the distance between acceptor and donor and R_0 is the critical distance, which is evaluated as following when the transfer efficiency is 50%:

$$R_0^6 = 8.8 \times 10^{-25} k^2 N^{-4} \Phi J \quad (3)$$

where k^2 is the orientation factor, N the refractive index of the medium, and Φ is the fluorescence quantum yield of the donor. For BSA, $k^2 = 2/3$, $N = 1.36$ and $\Phi = 0.14$ [23]. J is overlap integral of the fluorescence emission spectrum of donor and absorption spectrum of the acceptor, which is proximately given by the following equation:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4\ \Delta\lambda}{\sum F(\lambda)\,\Delta\lambda} \quad (4)$$

where $F(\lambda)$ is the fluorescence intensity of the donor, while $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor.

All the above data points were fit to curves by means of OriginPro 7.5.

3. Results and discussion

3.1. Fluorescence quenching of BSA induced by omeprazole and pantoprazole

The fluorescence quenching has been characterized as a very sensitive way with potentiality to analyze the interactions between

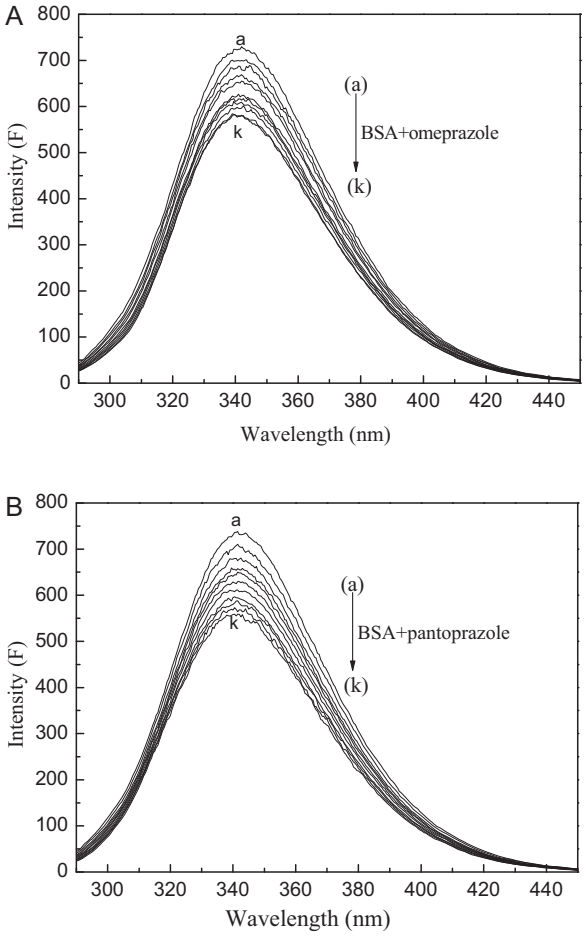


Fig. 2. The fluorescence quenching spectrum of BSA at various concentrations of omeprazole and pantoprazole. λ_{ex}, 280 nm; c_{BSA}, 6.0 μmol L^{-1}; c_{PPIs} (a → k), 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 μmol L^{-1} for omeprazole (A) and pantoprazole (B), respectively; *T*, 298.15 K.

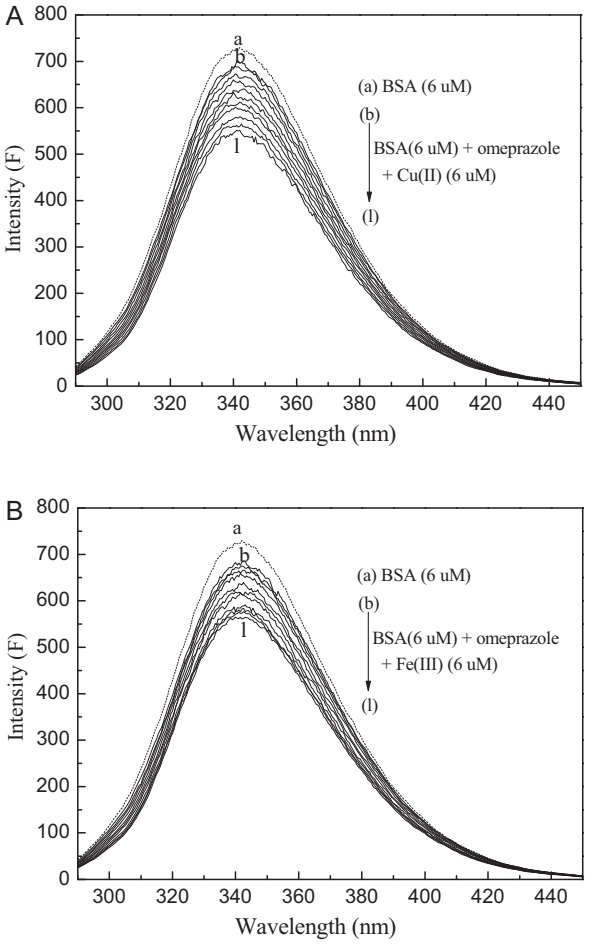


Fig. 3. The fluorescence quenching spectrum of BSA at various concentrations of omeprazole in the presence of Cu(II) and Fe(III). λ_{ex}, 280 nm; $c_{BSA} = c_{metal\ ions}$ = 6.0 μmol L^{-1}; $c_{omeprazole}$ (a → k), 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 μmol L^{-1}; *T*, 298.15 K.

drugs and proteins. When adding omeprazole or pantoprazole to BSA solution, the fluorescence of BSA was quenched as shown in Fig. 2. BSA exhibits a strong fluorescence emission band at 342 nm at pH 7.40, and the fluorescence intensity of BSA dropped regularly with the increasing concentrations of PPIs, which indicated that the interaction had been happened between PPIs and BSA. The weak blue shifts of the maximum emission wavelength (λ_{em}) of fluorescence of BSA induced by omeprazole and pantoprazole were 3 and 2 nm, respectively, and the results suggested that the fluorescence chromophore of BSA was placed in a more hydrophobic environment by hydrophobic interactions of PPIs with BSA via hydrogen bonds between the oxygen in PPIs and hydroxyl or amino groups in BSA.

About 20.2% and 23.4% of the fluorescence intensities of BSA were quenched by adding 20 μmol L^{-1} of omeprazole and pantoprazole, respectively (calculated from Fig. 2). The extent of the fluorescence attenuation was in the order: pantoprazole > omeprazole. The results indicated that the quenching effect of PPIs on BSA fluorescence highly depended on their structures.

3.2. Fluorescence quenching of BSA induced by omeprazole and pantoprazole in the presence of Cu(II) or Fe(III)

Fig. 3 shows the fluorescence spectra of BSA with various amount of omeprazole in the presence of Cu(II) or Fe(III) (the spectra for pantoprazole were not shown here). It was obvious that when omeprazole was added into BSA solution containing equimolar metal ions, further attenuation in the fluorescence of BSA was observed. The λ_{em} and shape at different concentrations of omeprazole in the presence of Cu(II) or Fe(III) were similar to those in the absence of Cu(II) or Fe(III), while the fluorescence quenching extent was larger than those without metal ions or metal ions alone. The weak blue shifts of the λ_{em} (2–3 nm) of BSA with the addition of omeprazole were also observed in the presence of Cu(II) or Fe(III). When the concentration of omeprazole reached 20 μmol L^{-1}, the fluorescence intensities of BSA decreased 21.4% and 17.8% in the presence of Cu(II) and Fe(III), respectively (calculated from Fig. 3), compared with 20.2% of omeprazole without metal ions. Therefore, Cu(II) enhanced the quenching effect of BSA fluorescence induced by omeprazole, and Fe(III) decreased the quenching effect. For pantoprazole, when the concentration reached 20 μmol L^{-1}, the fluorescence intensities of BSA decreased 20.7% and 24.1% in the presence of Cu(II) and Fe(III), respectively, from the original pantoprazole-resulted 23.4%. Therefore, the presence of Cu(II) decreased the quenching effect of BSA fluorescence induced by pantoprazole, and Fe(III) enhanced the quenching effect. All the fluorescence quenching extent of PPIs to BSA in the presence of metal ions are not the same as those resulted by either PPIs or metal ions alone. The resulted displayed that the structures of PPIs and kinds of metal ions together affected the binding interaction between PPIs and BSA.

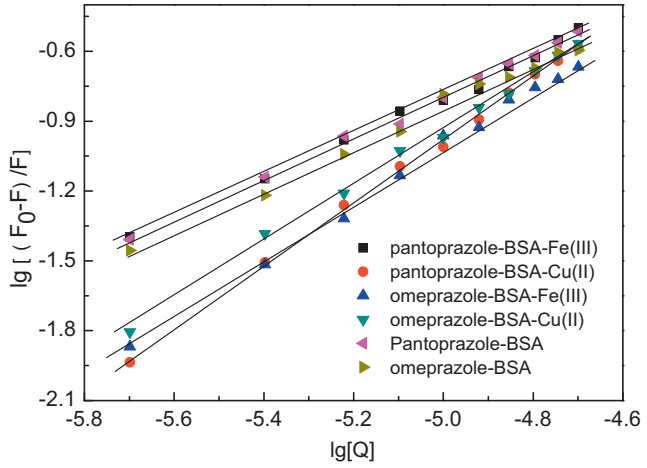


Fig. 4. Double-logarithm curves of omeprazole and pantoprazole quenching BSA fluorescence in the absence and presence of Cu(II) and Fe(III) at 298.15 K.

Table 1
The binding constants and binding sites for the interactions of omeprazole or pantoprazole with BSA with and without Cu(II) or Fe(III) at 298.15 K.

	Omeprazole				Pantoprazole			
	log K	n	R^a	S.D.[b]	log K	n	R^a	S.D.[b]
Free	4.58	1.12	0.9952	0.027	5.04	1.19	0.9931	0.016
Cu(II)	5.13	1.21	0.9984	0.023	4.01	0.97	0.9987	0.020
Fe(III)	4.76	1.15	0.9978	0.031	5.86	1.37	0.9976	0.024

[a] Correlation coefficient.
[b] Standard deviation.

3.3. Effect of Cu(II) or Fe(III) on the binding constants and the number of binding sites for omeprazole and pantoprazole binding to BSA

The value of K is significant to understand the distribution of drug in plasma since the weak binding can improve the concentrations of drug in plasma, and then increase its maximum effects, while strong binding can decrease the concentrations of free drug in plasma, and then lower its maximum effects [7,28]. The binding constants (K) and binding sites (n) can be calculated by the double-logarithm equation. Plots of $\lg[(F_0 - F)/F]$ versus lg[Q] for omeprazole–BSA and pantoprazole–BSA without and with Cu(II) or Fe(III) were shown in Fig. 4, and Table 1 lists the corresponding calculated results. The values of binding constants of PPIs to BSA in the presence of metal ions were in the range of 10^4–10^6 mol L^{-1}, which agreed with the common affinities of drugs for serum albumin [2–6]. The number of binding constants (n = 0.97–1.37) was about 1.0, which indicated that one binding site formed between PPIs and BSA, and the values corresponded with the binding sites with high affinity. The values of lg K are proportional to n with higher correlation coefficient (R = 0.9971), which confirmed that mathematical model used in the experiment was suitable to study the interaction between omeprazole or pantoprazole and BSA in the absence and presence of metal ions. The affinity of pantoprazole to BSA was about 1.1 times higher than that of omeprazole in the absence of metal ions. Compared with omeprazole, the fluorine in pantoprazole can form hydrogen bond with the hydroxyl groups in BSA, which improved the binding affinity between pantoprazole and BSA. Therefore, the concentration of free omeprazole was higher than that of free pantoprazole with the same initial concentration, and the lower free concentration of pantoprazole would weaken its maximum effects, which was consistent with Besancon's experiment that pantoprazole had less rapid inhibition than omeprazole [15]. The presence of Cu(II) and Fe(III) increased the affinities of omeprazole to BSA about 12.0% and 3.9%. However, the presence of Cu(II) decreased the affinity of pantoprazole to BSA about 25.7%, and the presence of Fe(III) improved the affinity about 16.3%. Then it is obvious that the presence of Cu(II) could reduce maximum effect of omeprazole and improve maximum effect of pantoprazole, and the presence of Fe(III) could reduce maximum effects of omeprazole and pantoprazole.

3.4. Effect of Cu(II) or Fe(III) on the binding mode and binding distances for omeprazole and pantoprazole to BSA

To achieve more insight into the effect of Cu(II) or Fe(III) in the interaction between omeprazole or pantoprazole and BSA, the binding distance between omeprazole or pantoprazole and BSA in the presence and absence of Cu(II) or Fe(III) were investigated. In the experiments, Cu(II) or Fe(III) was first added into the BSA solution, and then omeprazole or pantoprazole was added, therefore, metal ion first combined with BSA to form metal ion–BSA complex, and then omeprazole or pantoprazole reacted with metal ion–BSA complex. Overall, the changeable binding affinities of PPIs–BSA complex in the presence of metal ions were consistent with two distinct interpretations [29]: (1) a competitive binding in the metal ion high-affinity site and (2) a noncompetitive binding in a different albumin site. It was a puzzle about whether the presence of metal ions affected the binding mode of omeprazole or pantoprazole with BSA. To further ascertain the binding mode of omeprazole or pantoprazole with BSA in the presence of Cu(II) or Fe(III), the binding distances (r) between the donor and acceptor were calculated according to the Förster non-radiation energy transfer theory. As shown in Table 2, all the values of r are much smaller than 7 nm, which suggested that the non-radiative energy transfer from BSA to omeprazole or pantoprazole may occur with high possibility regardless of the presence or absence of metal ions. The values of r for distances between omeprazole and BSA in the absence and presence of Cu(II) and Fe(III) were 2.69, 2.85 and 2.97 nm, respectively, and those between pantoprazole and BSA were 2.39, 3.79 and 3.41 nm, respectively. It is obvious that the binding distance of omeprazole or pantoprazole in the presence of Cu(II) and Fe(III) increased, which was reasonable to assume that omeprazole or pantoprazole had noncompetitive binding in a different albumin site with Cu(II) or Fe(III), which then led to the formation of a ternary nonfluorescent complex, PPIs–BSA–metal ion. In this situation, the first formation of metal ion–BSA complex changed the conformation of BSA, which then affected PPIs binding with BSA.

Table 2
J, E, R_0 and r values of omeprazole or pantoprazole with BSA in the absence and presence of Cu(II) or Fe (III).

	Omeprazole				Pantoprazole			
	J (cm^3 L mol^{-1})	E (%)	R_0 (nm)	r (nm)	J (cm^3 L mol^{-1})	E (%)	R_0 (nm)	r (nm)
Free	1.54×10^{-15}	8.91	1.82	2.69	5.81×10^{-16}	6.95	1.55	2.39
Cu(II)	1.37×10^{-15}	5.81	1.79	2.85	6.82×10^{-16}	5.21	2.34	3.79
Fe(III)	1.37×10^{-15}	4.59	1.79	2.97	6.79×10^{-16}	9.46	2.34	3.41

4. Conclusions

About 20–30 essential trace metal ions exist in human organism, which play important roles in good human health. The existence of metal ions may affect drugs binding with proteins. The work brings forward a rational approach to illustrate the metal ion/drug interaction. The results demonstrated that the presence of Cu(II) or Fe(III) could significantly change the binding constants of omeprazole or pantoprazole with BSA, which then could affect their maximum effects, and the structures of PPIs and kinds of metal ions together affected the binding interaction between PPIs and BSA. Therefore, rationalized dosage could be taken into account for patients with gastric ulcer.

Acknowledgements

This work was supported by the National Scientific Foundation of China (No. 21005089), Natural Science Foundation of Hunan Province of China (No. 10JJ4006), Science and Technology Program of Hunan Province of China (No. 2009RS3030), the freedom explore Program of Central South University (201012200015), the Shenghua Yuying Project of Central South University and aid program for Science and Technology Innovative Research Team (Chemicals of Forestry Resources and Development of Forest Products) in Higher Educational Institutions of Hunan Province.

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