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Investigation on the Interaction between Ilaprazole and Bovine Serum Albumin without or with Different C-Ring Flavonoids from the Viewpoint of Food−Drug Interference

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ABSTRACT: The interaction between ilaprazole and bovine serum albumin (BSA) has been investigated in the absence and presence of four popular flavonoids with different C-ring structures, quercetin, luteolin, taxifolin, and (+)-catechin, by means of fluorescence spectroscopy. The results indicated that ilaprazole had a strong ability to quench the intrinsic fluorescence of BSA, and site marker competitive experiments indicated that the binding of ilaprazole to BSA primarily took place in subdomain IIA. The quenching process of ilaprazole with BSA was easily affected by flavonoids,; however, they did not change the quchenching mechanism of ilaprazole with BSA, whereas all of the fluorescence quenching was initiated by a static quenching procedure combining with nonradiative energy transfer. The presence of flavonoids decreased the quenching constants of ilaprazole with BSA from 2.2 to 23.7% and decreased the binding constants from 73.7 to 98.3%, which depended on the different flavonoids' structures. The decreased binding constants and unchangeable spatial distance of ilaprazole with BSA by the introduction of quercetin, luteolin, and taxifolin may result from the competition of flavonoids and ilaprazole binding to BSA, whereas in the presence of (+)-catichin, decreased binding constants and increased spatial distance possibly resulted from the formation of a ternary ilaprazole−BSA−(+)-catechin complex. All of these results may have relevant consequences in rationalizing the interferences of common food to gastric ulcer treatments.

KEYWORDS: bovine serum albumin, flavonoids, fluorescence quenching, ilaprazole, interference

INTRODUCTION

Serum albumin is the major soluble protein in the circulatory system of a wide variety of organisms and has many physiological functions, such as maintaining the osmotic pressure and pH of blood and functioning as carriers transporting a great number of endogenous and exogenous compounds including fatty acids, amino acids, drugs, and pharmaceuticals.[1] The drug−serum albumin interaction plays a dominant role in drug disposition and efficiency because the bound fraction of drug is a depot, whereas the free fraction of drug shows pharmacological effects. Thus, it is important to study the interactions of drugs with serum albumin, which determines the pharmacology and pharmacodynamics of drugs. Up to now, many paper have reported the interaction between drugs and serum albumin.[2−6] Bovine serum albumin (BSA) is composed of three structurally homologous, predominantly helical domains (I, II, and III), each containing two subdomains (A and B), and the principal regions of drug-binding sites on albumin are located in hydrophobic cavities in subdomains IIA and IIIA, which exhibit similar chemical properties.[7] Therefore, drug−serum albumin interaction always causes interference with the binding of other drugs as the result of overlap of binding sites or conformational changes.[5] Detailed investigations of drug−serum albumin interactions play a dominant role in the interpretation of drug−drug interactions.

Flavonoids with the diphenylpropane ($C_6C_3C_6$) skeleton are important phytonutrient components widely distributed in the human diets with many biological activities, such as antioxidant activity,[8] anti-inflammatory activity,[9] antifungal activity,[10] and antitumor activity,[11] et al. Therefore, flavonoids have been linked with many health benefits, and so foods rich in flavonoids have attracted interest in recent years. The structural differences of flavonoids such as the hydroxylation pattern and the resonance of electrons between the A- and B-rings affect their bioactivities and binding process to serum albumin significantly.[12,13] Flavonoids can be further subdivided into several subgroups including flavones, flavonols, flavanonols, and flavanes according to the presence of the different substituents on the C-ring and the degree of C-ring saturation.[14] Quercetin is a rich flavonol present in many common foods and drinks such as tea, onions, olives, beer, and red wine.[15] Flavone luteolin usually occurs in celery, green pepper, perilla leaf, and chamomile tea.[16] The flavanonol taxifolin is mainly found in many citrus fruits, especially grapefruit and orange.[17] The flavane (+)-catechin is among the most widely consumed flavonoids, as it exists in many fruits, but green tea and chocolate are by far the richest sources.[18] Because of the long history of the consumption of foods containing flavonoids, interactions of flavonoids with plasma components must be considered because of their relevance to the transport, biological activity, and clearance of flavonoids; moreover, there is a concern

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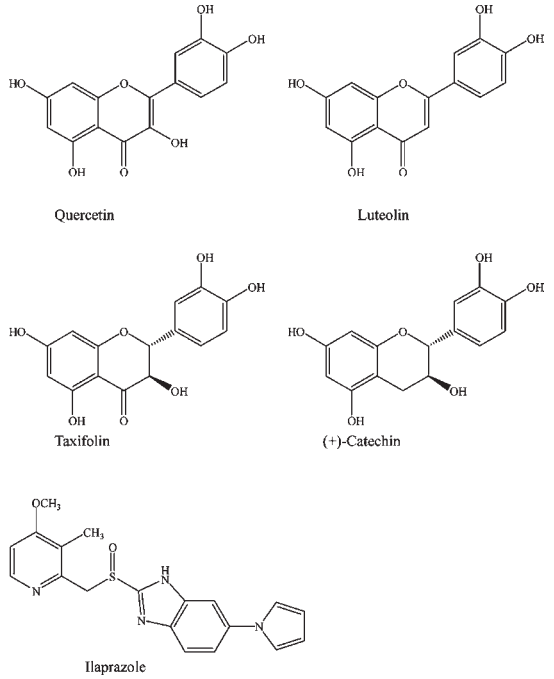

Figure 1. Molecular structures of quercetin, luteolin, taxifolin, (+)-catechin, and ilaprazole.

about the potential for clinically significant flavonoid−drug interactions with concomitantly administered medications. Up to now, most studies have focused on the binding process, including binding constants, binding sites, binding distances, and energy transfer between flavonoids and serum albumins.[19−22] However, few papers have focused on the influence of flavonoids on drug binding with serum albumin, especially for the effect of different substituents in the C-ring of flavonoids on drug binding with serum albumin.

Ilaprazole [2-[[(4-methoxy-3-methyl)-2-pyridinyl]methylsulfinyl]-5-(1*H*-pyrrole-1-yl)-1*H*-benzimidazole (previously named IY-81149)] is a newly developed proton pump inhibitor for the treatment of gastritic acid secretion. Up to now, most papers have focused on the pharmacology, pharmacokinetics, metabolism, and quantitative determination relevance about ilaprazole.[23−27] However, to our best knowledge, the interactions of ilaprazole with serum albumin have not been reported. The interactions of drug−serum albumin depend on surrounding circumstances such as pH, temperature, concentrations of serum albumin and drug, and the presence of other endogenous and exogenous compounds.[28] Therefore, it is necessary to understand the level of binding of ilaprazole for the serum albumin in the presence of different kinds of consuming phytonutrient flavonoids, which will directly correlate with the in vivo efficiency of ilaprazole.

By using the quenching fluorescence methods, the aims of this study were to investigate in vitro the binding properties of ilaprazole, four kinds of flavonoids with different C-ring stuctures, quercetin, luteolin, taxifolin, and (+)-catechin, and then the influence of the four flavonoids on ilaprazole binding with BSA, which would be important to illustrate the interactions between foods and drugs.

MATERIALS AND METHODS

Chemicals and Reagents. Four flavonoids (Figure 1), including quercetin, luteolin, taxifolin, and (+)-catechin, with purities of >99% were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ilaprazole (Figure 1) with a purity of 99.1% was provided by Livzon Pharmaceutical Group Inc. (Zhuhai, China), which signed a license agreement and got the patent from ILYANG Pharmaceutical Co. Ltd. (Seoul, South Korea). Phenylbutazone was purchased from Sigma Chemical Co. (St. Louis, MO; purity of no less than 99.8%). Ibuprofen was obtained from Hubei Biocause Pharmaceutical Co., Ltd. (Hubei, China; purity of no less than 99.7%). Bovine serum albumin was purchased from Sigma Chemical Co. The other chemicals, such as Tris buffer with a purity of >99.5%, NaCl, HCl, and ethanol, were all of analytical purity and used without further purification. Water used in all experiments was doubly distilled water.

Instrumentations. All fluorescence spectra were recorded on an F-2000 spectrofluorometer 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 widths 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). 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).

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 solution of BSA (1×10^{-4} mol L^{-1}) was prepared by dissolving it in Tris-HCl buffer solution and stored in a refrigerator at 4 °C prior to use. The stock solutions of flavonoids, phenylbutazone, ibuprofen, and ilaprazole (2×10^{-4} mol L^{-1}) were prepared by dissolving them in a small amount of ethanol and then diluted by Tris-HCl buffer solution (the ethanol content was about 2%), respectively.

Fluorescence Spectra of Flavonoids Binding with BSA. Appropriate amounts of 2.0×10^{-4} mol L^{-1} flavonoids were added to 11 5 mL flasks, respectively, and then 300 μL of BSA solution was added and diluted to 5 mL with Tris-HCl buffer. The final concentrations of flavonoids in BSA solution were 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μmol L^{-1}, respectively. The resultant mixtures were then incubated at 298.15 K for 0.5 h. After 0.5 h of incubation, the fluorescence emissions spectra were scanned in the range of 290−450 nm and the fluorescence intensity at 340 nm was measured. All of the experiments were repeated in triplicate and found to be reproducible within experimental error (<1%).

Fluorescence Spectra of Ilaprazole Binding with BSA in the Absence or Presence of Flavonoids, Phenylbutazone, and Ibuprofen. Three hundred microliters of BSA solution (or 300 μL of BSA solution and 150 μL of flavonoids) was added to 11 5 mL flasks, respectively. After a while, appropriate amounts of 2.0×10^{-4} mol L^{-1} ilaprazole were added and then diluted to 5 mL with Tris-HCl buffer. The final concentrations of ilaprazole were 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μmol L^{-1}, and the concentration of flavonoids 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 of incubation, the fluorescence emissions spectra were scanned in the range of 290−450 nm and the fluorescence intensity at 340 nm was measured. All of the experiments were repeated in triplicate and found to be reproducible within experimental error (<1%).

Data Analysis. For the dynamic quenching, the fluorescence quenching data are described by the Stern−Volmer equation[29]

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \quad (1)$$

where F_0 and F denote the steady state fluorescence intensities of serum albumin with and without the existence of quencher, respectively; K_{SV} is the Stern−Volmer quenching constant with units of L mol^{-1}; and [Q]

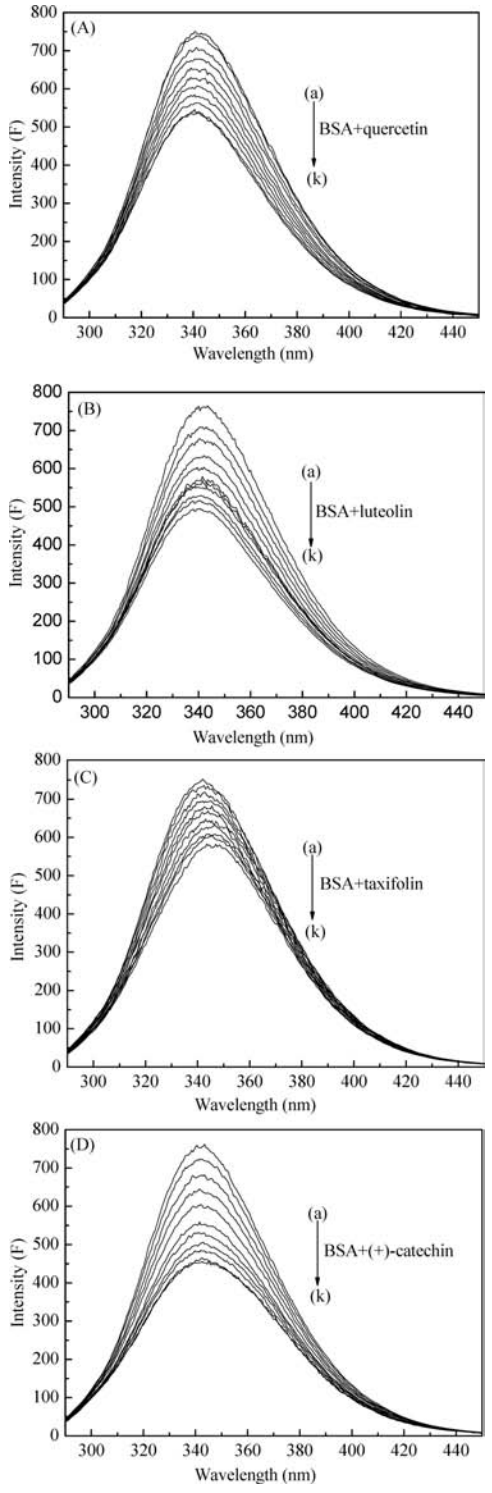

Figure 2. Fluorescence quenching spectra of BSA at various concentrations of flavonoids. λ_{ex}, 280 nm; C_{BSA}, 6.0 μmol L^{-1}; $C_{flavonoids}$ (a−k), 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μmol L^{-1} for quercetin (A), luteolin (B), taxifolin (C), and (+)-catechin (D), respectively; T, 298.15 K.

is the concentration of the quencher with units of mol L^{-1}. The Stern−Volmer equation was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q]. k_q is the quenching rate constant with units of L mol^{-1} s^{-1}, whereas τ_0 is the average lifetime of the serum albumin without any quencher and is generally equal to 10^{-8} s.[30]

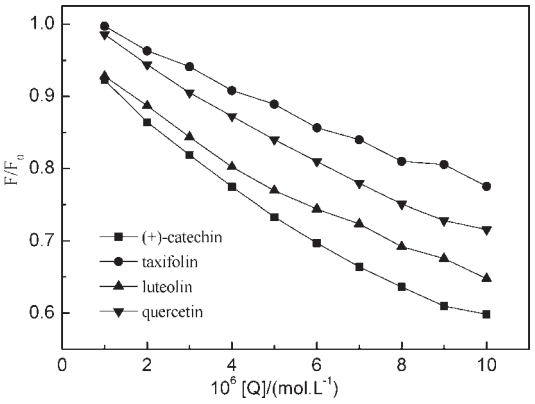

Figure 3. Quenching ratio (F/F_0) of the BSA fluorescence with the addition of quercetin, luteolin, taxifolin, and (+)-catechin at 298.15 K, respectively.

The binding constant (K) and binding sites (n) are calculated by the double-logarithm equation for static quenching:[31]

$$\log[(F_0 - F)/F] = \log K + n \log[Q] \quad (2)$$

The efficiency energy tranfer (E) was determined by Förster's energy transfer theory[32]

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (3)$$

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

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

In eq 4 k^2 is the orientation factor, N is 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 $\Phi = 0.14$.[33] J is the overlap integral of the fluorescence emission spectrum of donor and absorption spectrum of the acceptor, which is proximately given by the equation

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\sum F(\lambda)\Delta\lambda} \quad (5)$$

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

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

RESULTS AND DISCUSSION

Fluorescence Quenching of BSA by Quercetin, Luteolin, Taxifolin, and (+)-Catechin. 5,7,3′,4′-Hydroxy-substituted flavonoids are considered to be very efficient antioxidants. Quercetin, luteolin, taxifolin, and (+)-catechin are four characteristic 5,7,3′,4′-hydroxy-substituted flavonoids with different saturations in the C-ring. The fluorescence of BSA was quenched by various concentrations of quercetin, luteolin, taxifolin, and (+)-catechin as shown in Figure 2. It was observed that the fluorescence intensity of BSA dropped regularly with increasing concentrations of flavonoids, which indicated that the interaction had happened between flavonoids and BSA. Slight blue shifts of the maximum emission wavelength (λ_{em} = 2−3 nm) of fluorescence of BSA occurred for quercetin, luteolin, and (+)-catechin,

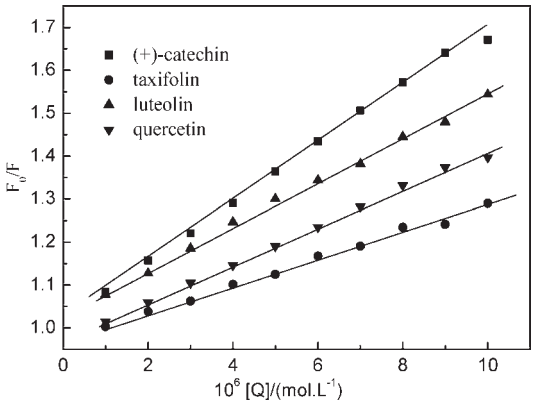

Figure 4. Stern−Volmer plots for BSA fluorescence quenching by quercetin, luteolin, taxifolin, and (+)-catechin at 298.15 K.

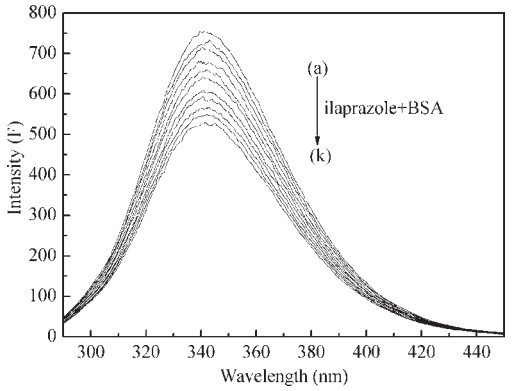

Figure 5. Fluorescence quenching spectra of BSA at various concentrations of ilaprazole. λ_{ex}, 280 nm; C_{BSA}, 6.0 μmol L^{-1}; $C_{ilaprazole}$ (a−k), 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μmol L^{-1}, respectively; T, 298.15 K.

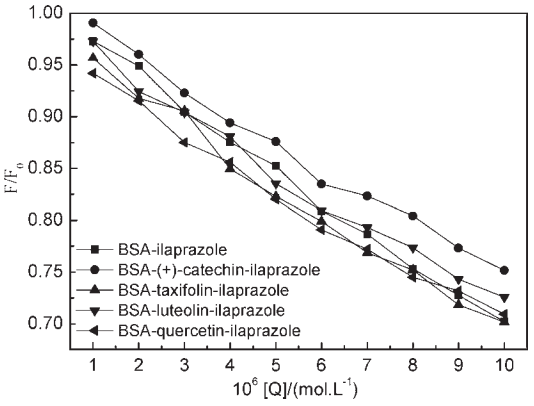

Figure 6. Quenching ratio (F/F_0) of the BSA fluorescence with the addition of ilaprazole in the absence and presence of quercetin, luteolin, taxifolin, and (+)-catechin at 298.15 K.

which suggested that the fluorescence chromophore of BSA was placed in a more hydrophobic environment by hydrophobic interactions of flavonoids bound with BSA via hydrogen bonds. However, a slight red shift of λ_{em} (3 nm) was observed for taxifolin, implying that the polarity of the protein environment has increased and the protein was unfolded.[33]

The raw data of BSA fluorescence quenching by the addition of quercetin, luteolin, taxifolin, and (+)-catechin are shown in Figure 3. About 28, 35, 22, and 40% of the fluorescence intensities of BSA were quenched by adding 10 μmol L^{-1} of quercetin, luteolin, taxifolin, and (+)-catechin, respectively. The extent of the fluorescence attenuation was in the order (+)-catechin > luteolin > quercetin > taxifolin. The results indicated that the quenching effect of flavonoids on BSA fluorescence highly depended on the C-ring structures. Luteolin and quercetin with a double bond in the C-ring decreased the intensities of BSA fluorescence more highly than flavanonol taxifolin with a nonplanar C-ring, which may be explained by the noncoplanarity of taxifolin weakening its capacity to penetrate into hydrophobic regions of BSA. Compared with luteolin, quercetin with two hydroxyl groups at C3 and C5 positions could form two intramolecular hydrogen bonds, which weakened the formation of hydrogen bonds between quercetin and BSA and then led to its poorer quenching efficacy. However, (+)-catechin without the double bond and carbonyl group in the C-ring had the highest quenching efficiency. This may mean that (+)-catechin had a different binding site with a stronger binding capacity, which agreed with the studies showing that (+)-catechin bound to subdomain IIIA and that quercetin, luteolin, and taxifolin bound to a totally independent site located in subdomain IIA.[34,35]

As was seen, the plots of F_0/F for BSA versus the quencher concentrations exhibited good linearity (Figure 4). The quenching constants (K_{SV}) for quercetin, luteolin, taxifolin, and (+)-catechin were 4.38×10^4 ($R = 0.9989$), 4.99×10^4 ($R = 0.9926$), 2.93×10^4 ($R = 0.9963$), and 7.26×10^4 L mol^{-1} ($R = 0.9989$), respectively. The quenching rate constants (k_q) were 4.38×10^{12}, 4.99×10^{12}, 2.93×10^{12}, and 7.26×10^{12} L mol^{-1} s^{-1} for quercetin, luteolin, taxifolin, and (+)-catechin, respectively. Obviously, all of the values for k_q were far greater than the expected maximum dynamic quenching constant (2.0×10^{10} L mol^{-1} s^{-1});[36] therefore, this indicated that the fluorescence quenching process of BSA at the investigated concentrations of flavonoids might be mainly governed by a static quenching mechanism rather than a dynamic quenching process. For further discussion, the concentrations of flavonoids were fixed at 6 μmol L^{-1}, which is equal to that of BSA.

Fluorescence Quenching of BSA by Ilaprazole. The effect of ilaprazole on the fluorescence of BSA is shown in Figure 5. The results shown indicate that the increasing concentrations of ilaprazole could decrease the fluorescence intensity of BSA, and no obvious BSA maximum emission wavelength was observed. At the concentration of 10 μmol L^{-1}, ilaprazole had led to 29.8% quenching of BSA fluorescence (Figure 6). In the investigated ilaprazole concentrations (from 1 to 10 μmol L^{-1}), the Stern−Volmer equation exhibited good linearity, and the K_{SV} and k_q were 4.47×10^4 L mol^{-1} and 4.47×10^{12} L mol^{-1} ($R = 0.9976$), respectively (Table 1). The k_q was much greater than 2.0×10^{10} L mol^{-1} s^{-1} at 298.15 K, indicating that the probable quenching mechanism of ilaprazole with BSA was a static quenching for the formation of a ilaprazole−BSA complex.

Fluorescence Quenching of BSA Induced by Ilaprazole in the Presence of Quercetin, Luteolin, Taxifolin, and (+)-Catechin. As shown in Figure 7, when ilaprazole was added into a BSA solution (6 μmol L^{-1}) containing equimolar flavonoids, further attenuation in the fluorescence of BSA with a slight blue shift was observed. The λ_{em} and shape at different concentrations of pantoprazole in the presence of flavonoids were similar to those in the absence of flavonoids, whereas the fluorescence

Table 1. Stern−Volmer Quenching Constants (K_{SV}) for the Interactions of Ilaprazole with BSA with and without Quercetin, Luteolin, Taxifolin, and (+)-Catechin at 298.15 K

	K_{SV} ($\times 10^4$ L mol^{-1})	k_q ($\times 10^{12}$ L mol^{-1} s^{-1})	R^a	SD^b
ilaprazole−BSA	4.47	4.47	0.9976	0.008
ilaprazole−BSA−quercetin	3.92	3.92	0.9986	0.006
ilaprazole−BSA−luteolin	3.86	3.86	0.9966	0.009
ilaprazole−BSA−taxifolin	4.37	4.37	0.9953	0.010
ilaprazole−BSA−(+)-catechin	3.41	3.41	0.9975	0.007

[a] R is the correlation coefficient. [b] SD is the standard deviation.

quenching extent was larger than those without flavonoids or flavonoids alone. When the concentration of ilaprazole reached 10 μmol L^{-1}, the fluorescence intensities of BSA decreased about 29.1, 27.4, 29.9, and 24.8% in the presence of quercetin, luteolin, taxifolin, and (+)-catechin, respectively, compared with 29.8% in the absence of these four flavonoids (Figure 6). The presence of quercetin and taxifolin had little decreased effect on the ilaprazole quenching of BSA fluorescence, whereas the presence of luteolin and (+)-catechin reduced the quenching efficacy of ilaprazole with BSA remarkably. The results suggested that the different structures of flavonoids affected the ilaprazole quenching of BSA fluorescence.

The plots of F_0/F for BSA versus the quencher concentrations exhibited good linearity with high R values for ilaprazole with BSA in the presence of quercetin, luteolin, taxifolin, and (+)-catechin in the range of the investigated concentrations (Figure 8). As can be seen from Table 1, the magnitude of k_q was calculated to be 10^{12} greater than the value of the maximum scatter collision quenching constant (2.0×10^{10} L mol^{-1} s^{-1}), which indicated that all of the fluorescence quenching was not initiated by dynamic collision but originated from the formation of complex for the static quenching procedure. Therefore, the existence of quercetin, luteolin, taxifolin, and (+)-catechin could not change the quenching mechanism of ilaprazole with BSA. From the slopes of the curves for the quenching constants (K_{SV}), it could be concluded that the influence of the four investigated flavonoids was determined as follows: (+)-catechin > luteolin > quercetin > taxifolin. (+)-Catechin, luteolin, quercetin, and taxifolin decreased the quenching constants by 23.7, 13.6, 12.3, and 2.2%, respectively.

Effect of Quercetin, Luteolin, Taxifolin, and (+)-Catechin on the Binding Constants and the Number of Binding Sites for Ilaprazole−BSA. The value of K is significant in understanding the distribution of drug in plasma because the weak binding can improve the concentrations of drug in plasma and then lead to a short lifetime or poor distribution, whereas strong binding can decrease the concentrations of free drug in plasma and then improve the pharmacology effects.[5] The binding constants (K) and binding sites (n) can be calculated by the double-logarithm equation (eq 2). Plots of $\lg(F_0 - F)/F$ versus $\lg[Q]$ for ilaprazole−BSA without and with the existence of quercetin, luteolin, taxifolin, and (+)-catechin are shown in Figure 9, and Table 2 lists the corresponding calculated results with the correlation coefficients >0.99. It is obvious that the presence of flavonoids decreased the binding constants and binding sites of ilaprazole with BSA, which indicated that the presence of flavonoids could affect the binding ability of ilaprazole with BSA. It can be inferred from the values of n that there

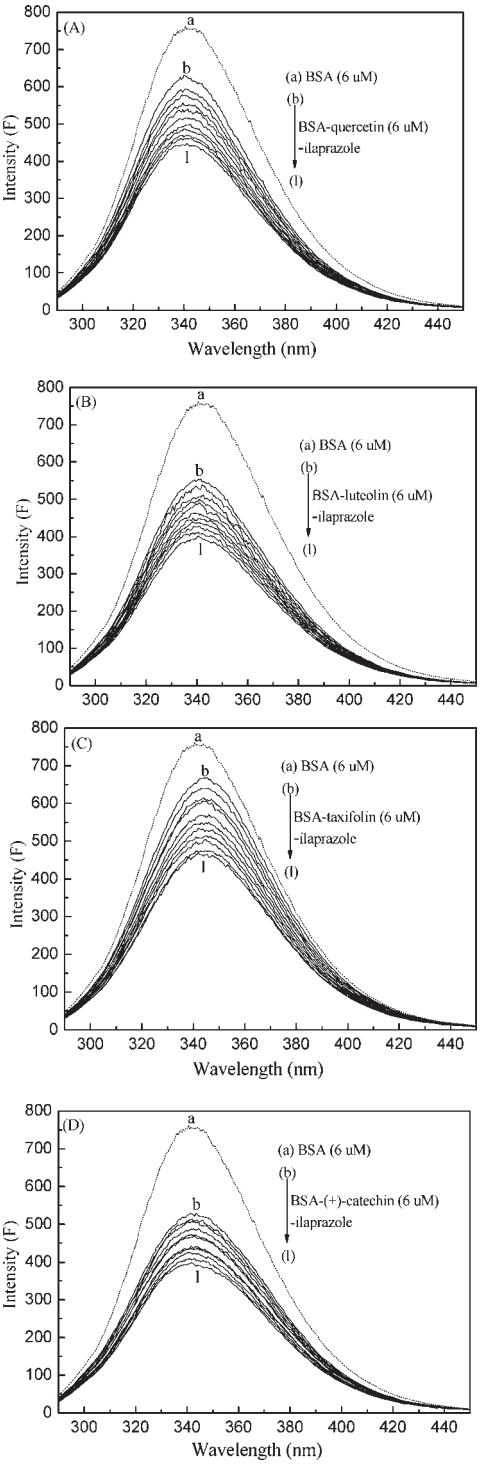

Figure 7. Fluorescence quenching spectra of BSA at various concentrations of ilaprazole in the presence of quercetin (A), luteolin (B), taxifolin (C), and (+)-catechin (D). λ_{ex}, 280 nm; $C_{BSA} = C_{flavonoids} = 6.0$ μmol L^{-1}; $C_{ilaprazole}$ (a−k), 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 μmol L^{-1}, respectively; T, 298.15 K.

was about one independent class of binding site on BSA in the absence and presence of flavonoinds for ilaprazole. However, the decreasing binding constants led to a decreasing quantity of ilaprazole binding to BSA and then a slight decreasing of the number of binding sites. Therefore, the presence of flavonoids

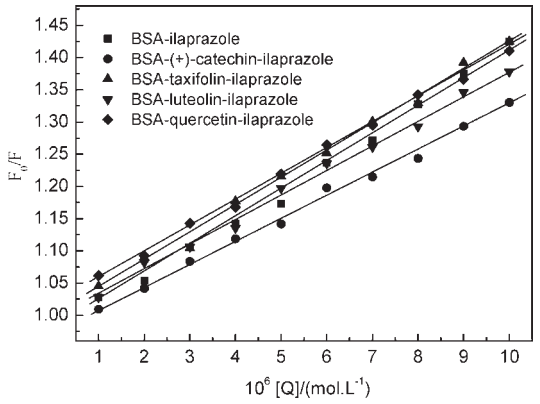

Figure 8. Stern−Volmer plots of ilaprazole quenching BSA fluorescence in the absence and presence of quercetin, luteolin, taxifolin, and (+)-catechin at 298.15 K.

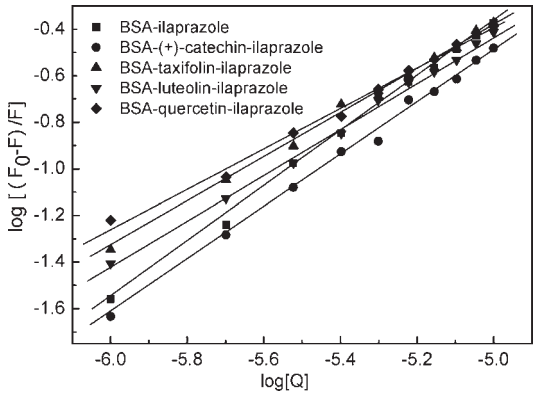

Figure 9. Double-logarithm curves of ilaprazole quenching BSA fluorescence in the absence and presence of quercetin, luteolin, taxifolin, and (+)-catechin at 298.15 K.

Table 2. Static Binding Constants and Binding Sites for the Interactions of Ilaprazole with BSA with and without the Existence of Quercetin, Luteolin, Taxifolin, (+)-Catechin, Phenylbutazone, and Ibuprofen at 298.15 K

system	log$K$	n	R^a	SD^b
BSA−ilaprazole	5.63	1.20	0.9985	0.021
BSA−quercetin−ilaprazole	3.86	0.85	0.9963	0.024
BSA−luteolin−ilaprazole	4.58	1.00	0.9984	0.019
BSA−taxifolin−ilaprazole	4.49	0.97	0.9986	0.017
BSA−(+)-catechin−ilaprazole	5.05	1.11	0.9962	0.023
BSA−phenylbutazone−ilaprazole	3.92	0.88	0.9964	0.012
BSA−ibuprofen−ilaprazole	5.52	1.18	0.9981	0.020

[a] R is the correlation coefficient. [b] SD is the standard deviation.

could improve the concentrations of ilaprazole in plasma and then enhance its maximum effects. The values of log$K$ are proportional to the binding sites (n) with a higher correlation coefficient (R = 0.9966) (Figure 10), which confirmed that the mathematical model (eq 2) used in the experiment was suitable to study the interaction between ilaprazole and BSA. Quercetin, luteolin, taxifolin, and (+)-catechin decreased the binding constants to 98.3, 91.1, 92.8, and 73.7%, respectively. It is obvious

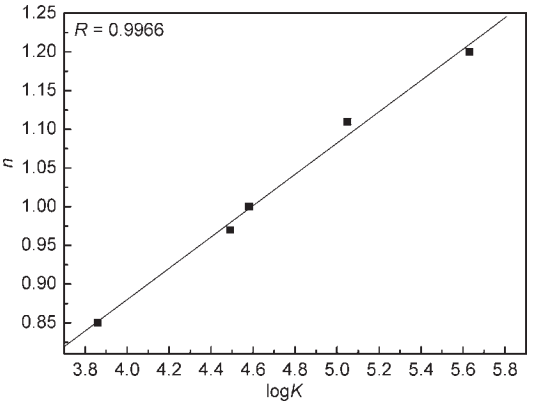

Figure 10. Relationship between the binding constants (logK_a) and the number of binding sites (n) between ilaprazole and BSA.

that the order of the influence of the four investigated flavonoids was determined as quercetin > taxifolin > luteolin > (+)-catechin.

From the above results, it could be concluded that the flavonoids with structural difference in the C-ring affected the binding constants of ilaprazole with BSA differently. (+)-Catechin, with the highest quenching efficiency to BSA, had the least influence, from which it may be deduced that ilaprazole and (+)-catechin had noncompetitive binding in a different albumin site, and a ternary nonfluorescent complex, ilaprazole−BSA−(+)-catechin was formed. In this situation, the first formation of the (+)-catechin−BSA complex changed the conformation of BSA and then made it more difficult for ilaprazole to bind with BSA. Quercetin, taxifolin, and luteolin may have the same binding sites as ilaprazole. Quercetin and taxifolin affected ilaprazole binding with BSA more than luteolin. The results may suggest that the added ilaprazole may compete for flavonoids with BSA, partially catching the BSA flavonoids and then complexing with ilaprazole by hydrogen bonds that act as the new species to quench the fluorescence of BSA, and more hydroxyl groups in quercetin and taxifolin combined with ilaprazole with more affinity, which weakened its capacity to penetrate into the tryptophan-rich hydrophobic regions of BSA because of the increased molecular size.

Site-Selective Binding of Ilaprazole on BSA. In the experiments, flavonoids were first added into the BSA solution for some time, and then ilaprazole was added to the equimolar solution of flavonoids and BSA; therefore, flavonoids first combined with BSA to form a flavonoid−BSA complex, and then ilaprazole reacted with the flavonoid−BSA complex. Overall, the smaller binding constants of ilaprazole in the flavonoid−BSA complexes were consistent with two distinct interpretations:[33,34] (1) a competitive binding in the flavonoids high-affinity site; (2) a noncompetitive binding in a different albumin site. It was a puzzle about whether the presence of flavonoids affected the binding mode of ilaprazole with BSA. To further ascertain the binding mode of ilaprazole with BSA in the presence of flavonoids, site marker competitive experiments were carried out. Phenylbutazone and ibuprofen were used as site marker fluorescence probes for monitoring subdomains IIA and IIIA of serum albumin, respectively.[37,38] The fluorescence quenching data of the ilaprazole−BSA system in the presence of site markers were calculated by eq 2. As shown in Table 2, the binding affinity of ilaprazole to BSA was remarkably decreased in the presence of phenylbutazone, whereas the addition of ibuprofen resulted in

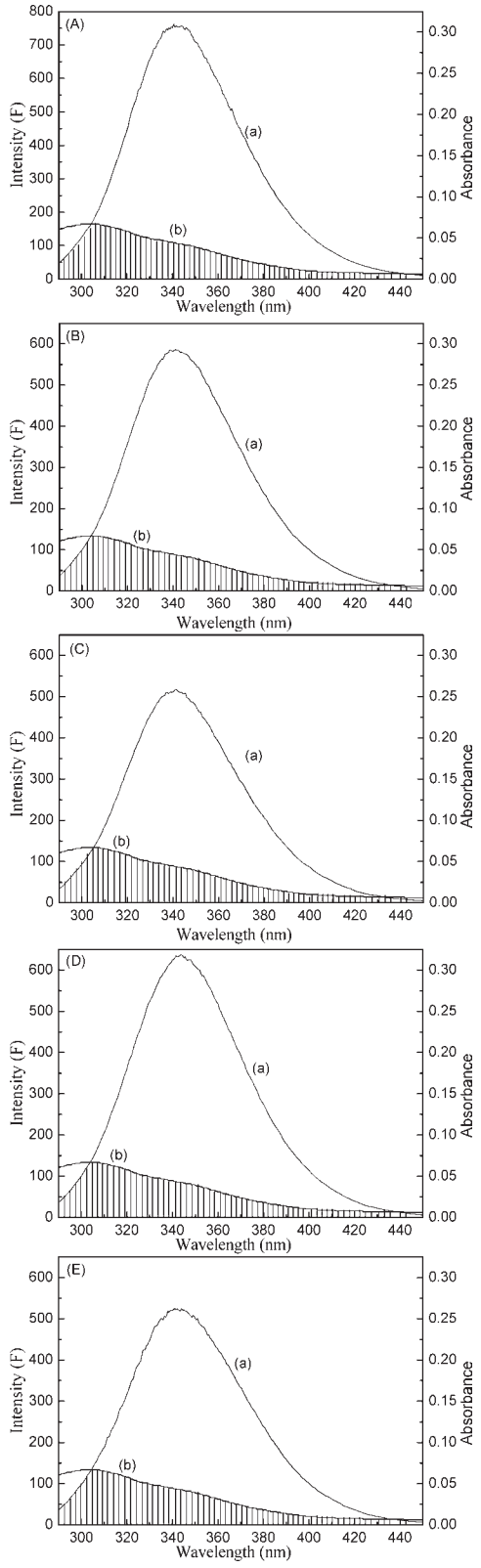

Figure 11. Overlaps of the fluorescence spectra (a) of BSA with the absorption spectra (b) of ilaprazole with or without the existence of quercetin, luteolin, taxifolin, and (+)-catechin: (A) ilaprazole−BSA system; (B) ilaprazole−BSA system in the presence of quercetin; (C) ilaprazole−BSA system in the presence of luteolin; (D) ilaprazole−BSA system in the presence of taxifolin; (E) ilaprazole−BSA system in the presence of (+)-catechin; $C_{BSA} = C_{flavonoids} = C_{ilaprazole} = 6.0\ \mu mol\ L^{-1}$; T, 298.15 K.

Table 3. J, E, R_0, and r Values of Ilaprazole with BSA in the Absence and Presence of Quercetin, Luteolin, Taxifolin, and (+)-Catechin

	J (cm^3 L mol^{-1})	E (%)	R_0 (nm)	r (nm)
BSA−ilaprazole	8.71×10^{-15}	19.1	2.44	3.10
BSA−quercetin−ilaprazole	9.72×10^{-15}	20.9	2.48	3.11
BSA−luteolin−ilaprazole	8.76×10^{-15}	19.1	2.44	3.10
BSA−taxifolin−ilaprazole	8.67×10^{-15}	20.1	2.43	3.06
BSA−(+)-catechin−ilaprazole	8.65×10^{-15}	16.5	2.43	3.19

only a small influence. These results demonstrated that the binding site of ilaprazole mainly located within subdomain IIA of BSA. This was also correlated with the above inference that quercetin, taxifolin, and luteolin shared a common binding site on the BSA molecule, whereas (+)-catechin had a different binding site on the BSA molecule.

Effect of Quercetin, Luteolin, Taxifolin, and (+)-Catechin on the Binding Mode and Binding Distance for Ilaprazole−BSA. The binding distances (r) between the donor and acceptor were calculated according to the Förster nonradiation energy transfer theory (eqs 3, 4, and 5). As shown in Figure 11 and Table 3, all of the values of r are much smaller than 7 nm, which suggests that the nonradiative energy transfer from BSA to ilaprazole may occur with high possibility in the presence or absence of flavonoids. Therefore, the quenching mechanism for ilaprazole−BSA was the static quenching combining with nonradiative energy transfer, whatever the presence or absence of flavonids. The values of r for distances between ilaprazole and BSA in the presence of quercetin, luteolin, and taxifolin were 3.11, 3.10, and 3.06 nm, respectively, which showed no major difference from that in the absence of flavonoids, which again verified the possible existence of competition of ilaprazole and flavonoid (quercetin, luteolin, and taxifolin) binding to BSA. Therefore, ilaprazole, quercetin, luteolin, and taxifolin had the same binding sites located in subdomain IIA, and the competitive binding decreased the binding capability of ilaprazole with BSA. The binding distance between ilaprazole and BSA in the presence of (+)-catechin increased, which further declared that the first formation of the (+)-catechin−BSA complex changed the conformation of BSA and then made it more difficult for ilaprazole to bind with BSA.

Our work brings forward a rational approach to illustrate the food−drug interaction. Rational dosage and diet regimens could be taken into account for patients with gastric ulcer.

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REFERENCES

(1) Huang, B. X.; Kim, H. Y.; Dass, C. Probing three-dimensional structure of bovine serum albumin by chemical cross-linking and mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1237–1247.

(2) Kanakis, C. D.; Tarantilis, P. A.; Tajmir-Riahi, H. A.; Polissiou, M. G. Crocetin, dimethylcrocetin, and safranal bind human serum albumin: stability and antioxidative properties. *J. Agric. Food. Chem.* **2007**, *55*, 970–977.

(3) Martini, S.; Bonechi, C.; Rossi, C. Interaction between vine pesticides and bovine serum albumin studied by nuclear spin relaxation data. *J. Agric. Food Chem.* **2010**, *58*, 10705–10709.

(4) Soares, S.; Mateus, N.; De Freitas, V. Interaction of different polyphenols with bovine serum albumin (BSA) and human salivary α-amylase (HSA) by fluorescence quenching. *J. Agric. Food Chem.* **2007**, *55*, 6726–6735.

(5) Qin, P. F.; Liu, R. T; Pan, X. R.; Fang, X. Y.; Mou, Y. Impact of carbon chain length on binding of perfluoroalkyl acids to bovine serum albumin determined by spectroscopic methods. *J. Agric. Food Chem.* **2010**, *58*, 5561–5567.

(6) Minoda, K.; Ichikawa, T.; Katsumata, T.; Onobori, K.; Mori, T.; Suzuki, Y.; Ishii, T.; Nakayama, T. Influence of the galloyl moiety in tea catechins on binding affinity for human serum albumin. *J. Nutr. Sci. Vitaminol.* **2010**, *56*, 331–334.

(7) Zhang, G.; Que, Q.; Pan, J.; Guo, J. Study of the interaction between icariin and human serum albumin by fluorescence spectroscopy. *J. Mol. Struct.* **2008**, *881*, 132–138.

(8) Rodrigues, A. S.; Perez-Gregorio, M. R.; Garcia-Falcon, M. S.; Simal-Gandara, J.; Almeida, D. P. F. Effect of meteorological conditions on antioxidant flavonoids in Portuguese cultivars of white and red onions. *Food Chem.* **2011**, *124*, 303–308.

(9) Kobori, M.; Masumoto, S.; Akimoto, Y.; Takahashi, Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. *Mol. Nutr. Food Res.* **2009**, *53*, 859–868.

(10) Salas, M. P.; Celiz, G.; Geronazzo, H.; Daz, M.; Resnik, S. L. Antifungal activity of natural and enzymatically-modified flavonoids isolated from citrus species. *Food Chem.* **2011**, *124*, 1411–1415.

(11) Huang, X. H.; Xiong, P. C.; Xiong, C. M.; Cai, Y. L.; Wei, A. H.; Wang, J. P.; Liang, X. F.; Ruan, J. L. *In vitro* and *in vivo* antitumor activity of *Macrothelypteris torresiana* and its acute/subacute oral toxicity. *Phytomedicine* **2010**, *17*, 930–934.

(12) Xiao, J. B.; Suzuki, M.; Jiang, X. Y.; Chen, X. Q.; Yamamoto, K.; Ren, F. L.; Xu, M. Influence of B-ring hydroxylation on interactions of flavonols with bovine serum albumin. *J. Agric. Food Chem.* **2008**, *56*, 2350–2356.

(13) Xiao, J. B.; Cao, H.; Wang, Y. F.; Yamamoto, K.; Wei, X. L. Structure−affinity relationship of flavones on binding to serum albumins: effect of hydroxyl groups on ring A. *Mol. Nutr. Food Res.* **2010**, *54*, S253–S260.

(14) Ross, J. A.; Kasum, C. M. Dietary flavonoids: bioavailability, metabolic effects and safety. *Annu. Rev. Nutr.* **2002**, *22*, 19–34.

(15) Harborne, J. B. *The Flavonoids: Advances in Research since 1986*; Chapman and Hall: London, U.K., 1994; pp 620−645.

(16) Kim, S. H.; Shin, K. J.; Kim, D.; Kim, Y. H.; Han, M. S.; Lee, T. G.; Kim, E.; Ryu, S. H.; Suh, P. G. Luteolin inhibits the nuclear factor-κB transcriptional activity in Rat-1 fibroblasts. *Biochem. Pharmacol.* **2003**, *66*, 955–963.

(17) Wang, X. D.; Meng, M. X.; Gao, L. B.; Liu, T.; Xu, Q.; Zeng, S. Permeation of astilbin and taxifolin in Caco-2 cell and their effects on the P-gp. *Int. J. Pharm.* **2009**, *378*, 1–8.

(18) Manach, C.; Scalbet, A.; Morand, C. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.

(19) Kitson, T. M. Spectrophotometric and kinetic studies on the binding of the bioflavonoids quercetin to bovine serum albumin. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2165–2170.

(20) Papadopoulou, A.; Green, R. J.; Frazier, R. A. Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. *J. Agric. Food Chem.* **2005**, *53*, 158–163.

(21) Shi, X. L.; Li, X. W.; Sun, Y. T.; Wei, W.; Yang, R. J.; Zhang, H. Q.; Jin, Y. R. In vitro study of the binding of taxifolin to bovine serum albumin and the influence of common ions on the binding. *J. Solution Chem.* **2010**, *39*, 482–494.

(22) Yang, Y. M.; Hu, Q. L.; Fan, Y. L.; Shen, H. S. Study on the binding of luteolin to bovine serum albumin. *Spectrochim. Acta A* **2008**, *69A*, 432–436.

(23) Myung, S. W.; Min, H. K.; Jin, C.; Kim, M.; Lee, S. M.; Chung, G. J.; Park, S. J.; Kim, D. Y.; Cho, H. W. Identification of IY81149 and its metabolites in the rat plasma using the on-line HPLC/ESI mass spectrometry. *Arch. Pharm. Res.* **1999**, *22*, 189–193.

(24) Kwon, D.; Chae, J. B.; Park, C. W.; Kim, Y. S.; Lee, S. M.; Kim, E. J.; Huh, I. H.; Kim, D. Y.; Cho, K. D. Effects of IY-81149, a newly developed proton pump inhibitor, on gastric acid secretion *in vitro* and *in vivo*. *Araneimittel-Forschung* **2001**, *51*, 204–213.

(25) Li, Y. L.; Zhang, W.; Guo, D.; Zhou, G.; Zhou, H. H.; Xiao, Z. S. Pharmacokinetics of the new proton pump inhibitor ilaprazole in Chinese healthy subjects in relation to CYP3A5 and CYP2C19 genotypes. *Clin. Chim. Acta* **2008**, *391*, 60–67.

(26) Zhou, G.; Tan, Z. R.; Zhang, W.; Ou-Yang, D. S.; Chen, Y.; Guo, D.; Liu, Y. Z.; Fan, L.; Deng, H. W. An improved LC-MS/MS method for quantitative determination of ilaprazole and its metabolites in human plasma and its application to a pharmacokinetic study. *Acta Pharmacol. Sin.* **2009**, *30*, 1330–1336.

(27) Zhou, G.; Shi, S. Y.; Zhang, W.; Tan, Z. R.; Chen, Y.; Guo, D.; Zhou, H. H.; Hu, H. T.; Tan, J. Identification of ilaprazole metabolites in human urine by HPLC-ESI-MS/MS and HPLC-NMR experiments. *Biomed. Chromatogr.* **2010**, *24*, 1130–1135.

(28) Vial, Y.; Tod, M.; Hornecker, M.; Urien, S.; Conti, F.; Dauphin, A.; Calmus, Y.; Blanchet, B. *In vitro* influence of fatty acids and bilirubin on binding of mycophenolic acid to human serum albumin. *J. Pharm. Biomed. Anal.* **2011**, *54*, 607–609.

(29) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Springer: New York, 2006.

(30) Lakowicz, J. R.; Weber, G. Quenching of fluorescence by oxygen. Probe for structural functions in macromolecules. *Biochemistry* **1973**, *12*, 4161–4170.

(31) Mote, U. S.; Bhattar, S. L.; Patil, S. R.; Kolekar, G. B. Interaction between felodipine and bovine serum albumin: fluorescence quenching study. *Luminescence* **2010**, *25*, 1–8.

(32) Förster, T.; Sinanoglu, O. *Modern Quantum Chemistry*; Academic Press: New York, 1996; pp 93−94.

(33) Li, D. J.; Zhu, M.; Xu, C.; Chen, J. J.; Ji, B. M. The effect of Cu^{2+} or Fe^{3+} on the noncovalent binding of rutin with bovine serum albumin by spectroscopic analysis. *Spectrochim. Acta A* **2011**, *78*, 74–79.

(34) Dufour, C.; Dangles, O. Flavonoid−serum albumin complexation: determination of binding constants and binding sites by fluorescence spectroscopy. *Biochim. Biophys. Acta* **2005**, *1721*, 164–173.

(35) Jurasekova, Z.; Marconi, G.; Sanchez-Cortes, S.; Torreggiani, A. Spectroscopic and molecular modeling studies on the binding of the flavonoid luteilin and human serum albumin. *Biopolymers* **2009**, *91*, 917–927.

(36) Ware, W. R. Oxygen quenching of fluorescence in solution: an experimental study of the diffusion process. *J. Phys. Chem.* **1962**, *66*, 445–458.

(37) Ding, F.; Liu, W.; Zhang, X.; Wu, L. J.; Zhang, L.; Sun, Y. Identification of pyrazosulfuron-ethyl binding affinity and binding site subdomain IIA in human serum albumin by spectroscopic methods. *Spectrochim. Acta* **2010**, *75*, 1088–1094.

(38) Hu, Y. J.; Wang, Y.; Ou-Yang, Y.; Zhou, J.; Liu, Y. Characterize the interaction between naringenin and bovine serum albumin using spectroscopic approach. *J. Lumin.* **2010**, *130*, 1394–1399.