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Study on the interaction of three classical drugs used in psychiatry in albumin through spectrofluorimetric modeling

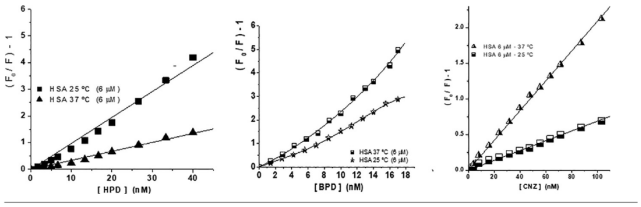
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HIGHLIGHTS

- Drugs interactions with albumin were studied through Spectrofluorimetric Modeling.
- Haloperidol, clonazepam and biperiden have high affinity for albumin.
- Binding sites in albumin for HPD, CNP and BPD are located at subdomain IB and IIA.

GRAPHICAL ABSTRACT



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ABSTRACT

Comparative study of haloperidol (HPD), biperiden (BPD) and clonazepam (CNZ) interactions with human and bovine serum albumin was performed based on fluorescence quenching analysis. We used mathematical modeling comparing spectrofluorimetric data to obtain information on the possibility of competition among three drugs by sites binding. Results showed that the three drugs studied have high affinity for albumin and suggest the existence of two site classes in HSA for HPD and only one class for BPD and CNZ, in the range of concentrations tested for each drug. Among them, only HPD forms complex with HSA. Comparing normalized quenching plots suggested that the primary sites in HSA and BSA for HPD and CNZ are located at subdomain IB, whereas BPD would bind in the subdomain IIA. Considering the competition for binding sites in HSA, titrations of HPD-HSA complex by BPD and CNZ, as well as the titration of HSA solution containing CNZ titrated by BPD, show that although the three drugs do not compete with each other for binding sites, their interaction with HSA can cause conformational change in the protein, and to increase or decrease the accessibility to binding sites for other drug. This may mean alteration in the free plasma drug concentrations.
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1. Introduction

Haloperidol (HPD), biperiden (BPD) and clonazepam (CNZ) are drugs frequently used in combination to treat mental health conditions with symptoms of psychosis. About 3.5% of the population experiences psychosis, and about 1% is diagnosed with Schizophrenia, with a high prevalence (16%) among people with a family history of schizophrenia [1–3].

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HPD is a typical or first-generation antipsychotic widely used in psychiatry in the treatment of various psychiatric disorders, such as schizophrenia, schizoaffective disorder, posttraumatic stress disorder, bipolar disorder and in dementia [4–6].

HPD is a neuroleptic of the butyrophenones (phenyl-piperidinyl-butyrophenone) group, which preferentially binds and blocks D_2 and α_1 receptors at low doses (0.13 and 0.42 mg/kg, respectively), and 5-HT2, in higher doses (2.6 mg/kg). It presents efficacy firmly established, however, it can as possible adverse effects extrapyramidal syndromes (dystonia, akathisia and parkinsonism) and tardive dyskinesia as a long-term sequela of use [4,5,7–10].

In this context, BPD may be prescribed associated with HPD to prevent or treat extrapyramidal symptoms, which accompany the use of this neuroleptic and other drugs that block dopamine receptors in the basal ganglia and cause functional dopamine deficiency [10–13]. BPD (3-piperidine-1-phenyl-1-bicycloheptenyl-1-propanol) is a muscarinic anticholinergic agent that acts centrally and peripherally, thus having some antisecretory, antispasmodic and mydriatic effects [14].

CNZ belongs to the benzodiazepine group (BDZ) and was initially introduced as an antiepileptic agent; however, is a therapeutic adjuvant useful in psychiatric disorders [15,16]. CNZ has been prescribed in medical treatment of anxiety disorders, insomnia, stress disorder, seizures etc., and also act as a muscle relaxant [17,18]. In psychotic disorders such as bipolar disorder and schizophrenia, CNZ decreases the level of agitation, anxiety, logorrhea, impulsivity, tension and aggressiveness [19].

Because of their therapeutic characteristics, these three drugs have been associated in treatment psychiatric disorders. But it is known that, when given simultaneously, drugs may compete with one another for binding sites in plasma proteins. Displacement of a drug bound to the plasma protein by another drug can increase its free concentration, compromise its therapeutic effects and even cause toxic effects. Albumin is the serum protein that has an effective role in the balance of plasma concentrations of drugs and their metabolites, contributing significantly to the transport, distribution and storage of various endogenous and exogenous substances [9,17,20,21].

The average binding ratios for HPD, BPD and CNZ to plasma proteins in adults, when given alone, is approximately 88–92%; 94%; 82 to 86%, respectively [22,23]. Thus, the study of the interaction of these drugs to albumin to verify the competitive potential among them by binding sites is of great importance for understanding the pharmacokinetics and biodistribution of these drugs in the human body.

The fluorescence quenching technique has been widely applied for interaction studies for albumin with several ligands [24–30], by analyzing of natural fluorescent emission decrease of tryptophan (Trp) residues, when albumin solution is excited at 290–295 nm [31,32].

In present work, we performed a comparative study of HPD, BPD and CNZ interactions with human (HSA) and bovine serum albumin (BSA) based on fluorescence quenching analysis. Because the similarities and differences in the molecular structures of these two albumins are well known, we used mathematical modeling comparing spectrofluorimetric data to obtain information on the interaction of the drugs with HSA and also on the possibility of competition among three drugs by sites binding.

2. Material and methods

In the spectrophotometric measurements were used a Shimatzu UV-1800 spectrophotometer, and Hitachi F-3010 and Agilent Cary Eclipse spectrofluorimeters. Table 1 summarizes data on albumins and drugs (code, stock solution concentrations, solvents, pH) used in present study, including the concentrations of stock solutions and respective solvents, as well as concentrations of drugs used to titrate the albumins (6.0 μM).

Each drug was dissolved in a specific solvent to prepare stock-solutions. Stock solution (0.26 mM) of HPD was prepared by dissolving this drug in 10% ethanol/H_2O. DMSO was used to prepare 0.29 mM BPD solution, and acetic acid was used to dissolve CNZ powder (0.32 mM). Adequate aliquots of Stock-solutions were then diluted in 10 mM phosphate buffer and pH adjusted (pH 7.4) to get final concentration to titrate albumins.

In spectrofluorimetry, 2 ml of 6 μM albumin solution (in 10 mM phosphate buffer, pH 7.4) were titrated by the drug at 25 and 37 °C, with initial increments of 1 μl of drug at each addition. The incremental volume was increased according to the behavior of the fluorescence spectra. The excitation and emission slits were 3 nm and the fluorescence emission records covered the range of 300 to 450 nm, which is the typical region of the albumin emission, with the excitation wavelength of 290 nm [27,28]. The existence of an internal filter effect was verified through UV–Vis spectrophotometry, having been negative for the concentrations of drugs used here.

To verify the competition between the drugs for binding sites in albumin, a volume of drug was added to 2 ml of 6 μM HSA solution so that its concentration in the cuvette was equal to its equilibrium plasma concentration. After 3 min resting at 37 °C, the HSA + drug solution was titrated with another drug at this temperature, following the addition protocol described above. Thus, 6 μM HSA + 52 nM HPD solution (in 10 mM phosphate buffer, pH 7.4) was titrated by BPD and CNZ, and 6 μM HSA + 165 nM CNZ solution (in 10 mM phosphate buffer, pH 7.4) was titration by BPD.

From the fluorescence spectra obtained, a data analysis was performed to obtain the Stern-Volmer graph, F_o/F *versus* drug concentration (Q), according to the Stern-Volmer equation, $F_0 = F(1 + K_{SV}Q)$, where F_0 and F are the fluorescence intensities emitted by albumin, respectively, in the absence and presence of the drug, and K_{SV} is Stern-Volmer constant. This is related to the bimolecular collision process and corresponds to the slope of the Stern-Volmer graph [31].

Graphs were plotted using the arithmetic mean of 5 experiments performed for each type of titration and temperature, considering standard deviations less than 10%. In Stern-Volmer plot analysis, linear approach was considered for regression coefficients (r) higher than 0.9980 ($P \leq 0.0001$) [27,28]. For $r \leq 0.9980$ ($P > 0.0001$), the logarithmic equation $\log\left(\frac{F_0}{F} - 1\right) = \log K + n\log[Q]$ was used to adjust the plot. K and n coefficients give, respectively, the apparent binding constant and number of binding site classes.

Experiments at the two temperatures was compared to determinate the type of quenching in each interaction drug-HSA and confirmed by the analysis of the UV–Vis spectra. For statistical analysis and mathematical modeling, we used specific program in the C++ language.

3. Results

The fluorescence quenching plots, Stern-Volmer constants, binding constants and binding sites were plotted after titrating the BSA and HSA solutions by HPD, CNZ and BPD at 25 and 37 °C. Fig. 1 show results of experiments with HPD. Fig. 1a one can see normalized quenching plots for HSA and BSA titrated by HPD at 37 °C. Fig. 1b is the Stern-Volmer plot for HSA titrated by HPD at 25 °C and 37 °C. Note that plots become concave upwards with increasing HPD concentration for two temperatures. The detail in this figure shows the plot for small concentrations: [HPD] <8 nM. Values for the quenching ratios for 1/1000 [HPD]/[albumin] at 37 °C are shown in Table 1, as well as the Stern-Volmer constants

Table 1
Substance, code, molecular weight (MW) and stock-solution solvent.

Substance	Code Sigma-Aldrich	MW	Stock solution Conc./Solvent	Titration Sol. Conc. (PO_4-buffer)
HAS	A8763	69 kDa	10 mM/PO_4-buffer	6.0 μM
BSA	A0281			
HPD	H1512	375.9 g/mol	0.26 mM/ethanol-H_2O 10%	1.3 μM
BPD	B5311	311.5 g/mol	0.29 mM/DMSO	2.9 μM
CNZ	C1277	315.7 g/mol	0.32 mM/Acetic acid	15.8 μM

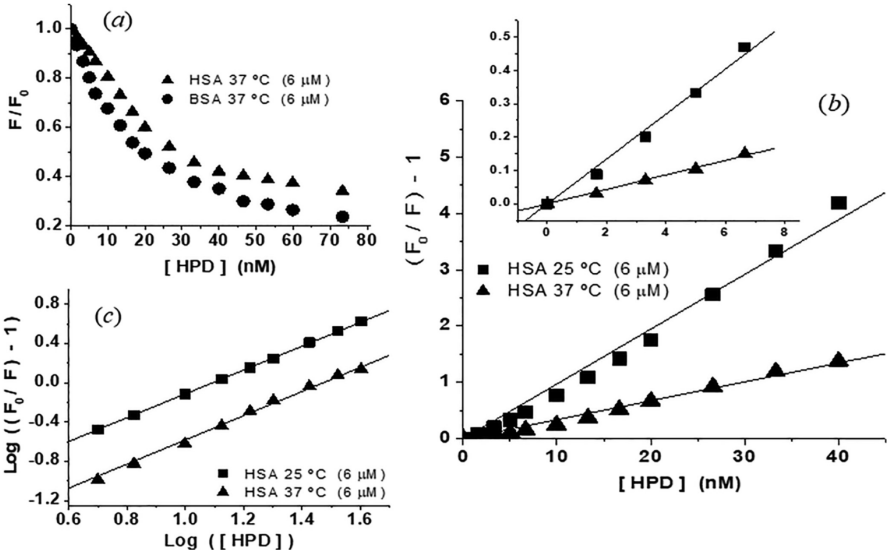


Fig. 1. (a) Normalized quenching plots of BSA e HSA titrated by HPD at 37 °C. (b) Stern-Volmer plots of HSA titrated by HPD at 25 °C e 37 °C, detail shows Stern-Vomer plot for [HPD] < 8 nM. (c) Linearized Stern-Volmer plots. [HSA] = [BSA] = 6 μM, 10 mM Phosphate buffer, pH 7.4. Excitation wavelength = 290 nm.

(K_{SV}) estimated for small concentration, referring to the occupation of primary sites. Fig. 1c shows the linearized Stern-Volmer plots, log ((F0/F) −1) *versus* log ([HPD]), for the same temperatures. The value of *n*, number of binding site classes, estimated from the linearized figure at 37 °C was about 2, and the values of *K* (apparent binding constant) are given in Table 2.

Fig. 2a show the normalized quenching plots for HSA and BSA titrated by BPD at 37 °C. In Fig. 2b are the Stern-Volmer plots for the HSA at 25 and 37 °C, which are also present concavity upward with the ligand increase. The detail in the figure shows the graph for small concentrations of the drug. Fig. 2c shows the plots linearized. The values of K_{SV} for small concentration and values of *K* are in Table 2. The value estimated for *n* was about 1.

Fig. 3(a) show the normalized quenching plots for HSA and BSA titrated by CNZ at 37 °C, and the Stern-Volmer plots for HSA at 25 °C and 37 °C are in Fig. 3b. Unlike HPD and BPD, the Stern-Volmer plots for the CNZ are linear (r > 0.9980, P < 0.0001) and the values of $K_{SV}$are also in Table 2.

Fig. 4 allow to compare the normalized quenching plots (*a*) pure 6 μM HSA solution and with the normalized quenching plots of 6 μM HSA + 52 nM HPD titrated by BPD at 37 °C (Fig. 4a); (*b*) pure 6 μM HSA solution and the normalized quenching plots of 6 μM HSA + 165 nM CNZ also titrated by BPD at 37 °C (Fig. 4b); (*c*) pure 6 μM HSA solution and the normalized quenching plots of 6 μM HSA + 52 nM HPD titrated by CNZ at 37 °C (Fig. 4c).

Fig. 5 show the Stern-Volmer plots for these quenching curves, and the values of K_{SV} are in Table 3. Note that, like the pure HSA titrated by BPD (Fig. 2b), the Stern-Volmer plots in Fig. 5a and b for 6 μM HSA + 52 nM HPD and 6 μM HSA + 165 nM CNZ solutions, respectively, titrated by BPD are concave upwards. Details of Fig. 5 presents plots for [BPD] <8 nM.

The respective Stern-Volmer constants are shown in Table 2, and their values estimated were about 65% and 74% of the respective values found for pure HSA (Table 1). Fig. 4c shows that CNZ quenched fluorescence of 6 μM HSA + 52 nM HPD solution more strongly than pure HSA, and the K_{SV} (Table 2) was 67% higher than for pure HSA (Table 1). It is important to note that the presence of HPD did not alter the linearity of the Stern-Volmer plot also observed in the pure HSA titration but increased the quenching constant.

Table 2
Quenching ratios, Stern-Volmer constants (K_{SV}) and apparent binding constants (K) for interactions of HPD, BPD and CNZ with HSA at 25 °C and 37 °C.

Drug	Quenching ratio for [Drug]/[HAS] = 1/1000–37 °C	37 °C		25 °C	
		K_{SV} ($\times 10^7$ M^{-1})	K ($\times 10^7$ M^{-1})/n	K_{SV} ($\times 10^7$ M^{-1})	K ($\times 10^7$ M^{-1})
HPD	12.2 (±0.6)%	2.17(±0.05)	1.34(±0.11)/2	6.74 (±0.29)	4.70 (±0.04)
BPD	49.6 (±3.1) %	20.1(±0.03)	15.5(±0.19)/1	12.5(±0.02)	11.4(±0.13)
CZP	12.8 (±2.0) %	2.10(±0.04)	—————	0.69(±0.01)	—————

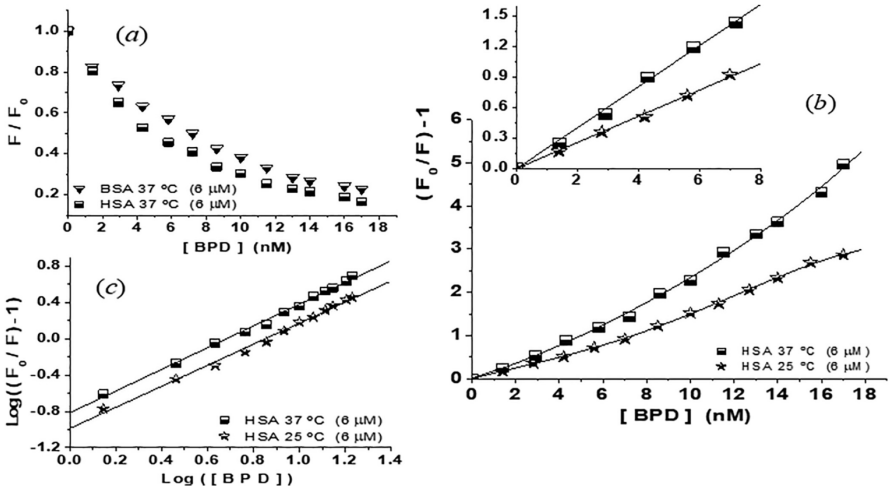


Fig. 2. (a) Normalized quenching plots of BSA e HSA titrated by BPD at 37 °C. (b) Stern-Volmer plots of HSA titrated by BPD at 25 °C e 37 °C, detail shows Stern-Vomer plot for [BPD] < 8 nM. (c) Linearized Stern-Volmer plots. [HSA] = [BSA] = 6 μM, 10 mM Phosphate buffer, pH 7.4. Excitation wavelength = 290 nm.

4. Discussion

The drugs studied here have high affinity with albumin, considering the high values of the ratios and quenching constants (Table 2). Comparison of the normalized quenching plots of both albumins for the three drugs (Figs. 1a, 2a, 3a) suggests that the primary sites for HPD and CNZ in albumin would be located at subdomain IB, whereas BPD would bind in the subdomain IIA. It is known that two Trp residues of BSA are at positions 212, Trp-212, and 134, Trp-134, in molecular chain, and the single Trp residue of HSA is at position 214, Trp-214, in subdomain IIA [27,28]. The intensity of the BSA fluorescence emission is stronger than the fluorescence emitted by HSA due to its two tryptophan residues, but the quenching of the BSA fluorescence by HPD and CNZ was more intense than that of HSA at 37 °C. That suggests that HPD and CNZ quench more intensively the emission of the tryptophan residue 134 than the emission of tryptophan residue 212, indicating that the affinity sites for both drugs in albumins are closer to sub domain IB (or within this) than the sub domain IIA.

The results given by Figs. 1, 2 and 3 are already suggestive that BPD should not compete with the other two drugs for binding sites, at least for primary sites, but there may be competition between HPD and CNZ. On the other hand, the quenching capacity of BPD on HSA fluorescence was higher than that of the other two drugs (Table 2). For the drug/HSA ratio of 1/1000 at 37 °C, the quenching effect of BPD was about 4-fold higher than that caused by HPD and CNZ.

In previous work [33], the analysis of the fluorescence quenching of the two albumins by CNZ at 25 °C had already suggested the presence of a single binding site for this anxiolytic drug in subdomain IB. For the [CNZ]/[albumin] ratio of 1/100, the anxiolytic quenched about 25% and 50% of the fluorescence of the HSA and BSA, respectively. Following the method used by Carqueja and Cortez [2014] [34] to study the Trp accessibility, Valdez et al. [2016] [33] found that the residue of Trp-134 would be more accessible to the CNZ than the Trp-212. They concluded that the accessibility to the Trp residue was 91%, in which almost 66% was due to Trp-134. These results corroborate with the present observation that the primary site for CNZ is in subdomain IB. In the literature there is report on the high affinity of the CNZ with plasma proteins (82–88%) and its long plasma half-life (between 19 and 60 h) [35].

The values of the apparent binding constant, K, (Table 2) of the interaction of HPD and BPD with HSA are in the same order of magnitude of the respective values of the Stern-Volmer constants, K_{SV}. However, the K values are slightly lower than the values estimated for the first drug additions (details in Fig. 1b, 2b). This was to be expected since values of K_{SV} refer to small concentrations or to higher affinity binding sites.

The value of n estimated based on Fig. 1c indicates the existence of two classes of sites available for HPD in the HSA at 37 °C. Accord-

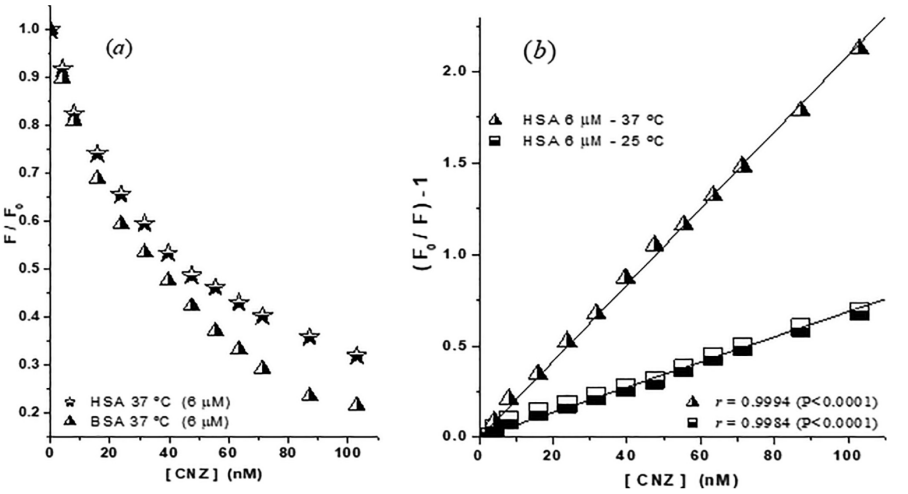


Fig. 3. (a) Normalized quenching plots of BSA e HSA titrated by CNZ at 37 °C. (b) Stern-Volmer plots of HSA titrated by CNZ at 25 °C e 37 °C. [HSA] = [BSA] = 6 μM, 10 mM Phosphate buffer, pH 7.4. Excitation wavelength = 290 nm.

ing to the above mentioned, these sites can be located at subdomain IB. It is possible found one or two high affinity sites in albumin for ligands and a larger number of low affinity sites [36]. But for BPD (Fig. 2c), the value of *n* suggests the existence of only one class of binding sites and, according to the analysis of Fig. 2a, in the subdomain IIA.

Contrary to HPD and BPD, the Stern-Volmer plots for CNZ at two temperatures (Fig. 3b) are linear. According to Lakowicz (2006) [31] it is compatible with the combination of three factors: (1) single residue of tryptophan in HSA; (2) a single class of CNZ binding site next to this tryptophan residue; and (3) occurrence of a single type of quenching.

In Table 2 it can be observed that the increase in temperature increased the value of K_{SV}, indicating dynamic quenching for the interaction CZP-HSA, ie, this drug does not form complex with albumin. But, in the case of the interaction HPD-HSA, Table 2 shows that the increase in temperature reduced the quenching constant, indicating static quenching for small HPD concentrations. However, for concentrations above 8 nM, the Stern-Volmer plots curves upward, suggesting that, in addition to the static process, dynamic quenching could also occur [30,31].

In a previous study [37], we had already shown that the values of K_{SV} for HPD quenched HSA at 25 °C were of the order of magnitude of 10^7 M^{-1}, being approximately 100-fold greater than for RPD [38], and about 1000 times higher than those estimated for chlorpromazine and sulpiride, two other typical antipsychotics [1,27]. The high values found for the fluorescence quenching ratios and Stern-Volmer constants (Table 2) attest to the already known high affinity of HPD with HSA. According to some authors [39–41], almost 90–92% of this antipsychotic are mainly bound to HSA and α1-acid glycoprotein at therapeutic concentrations. However, there is no information in the literature on binding sites.

Unlike HPD, the Stern-Volmer plots of the HSA-BPD (Fig. 2b) and HSA-CNZ (Fig. 3b) interaction at 25 °C and 37 °C suggest the occurrence of dynamic quenching. As is known, static quenching refers to the formation of a non-fluorescent complex between the quencher and the fluorophore. Thus, of the three drugs, the only one that forms complex with HSA is HPD. Complex HSA formation with some typical antipsychotic drugs derived from phenothiazine (such as thioridazine, triflupromazine) has been reported [42], as well as with the atypical antipsychotic drug clozapine [43].

The competition study for binding sites in HSA by titration of complex HPD-HSA confirmed that BPD does not compete with HPD by sites, and nor with CNZ. In fact, the literature shows that BPD does not affect plasma levels of HPD [12,13] and is not present in the list of drugs that interfere with the plasma pharmacokinetics of CNZ normally found in the package inserts.

Comparing results in Tables 2 and 3 show that K_{SV} for complex HPD-HSA titrated by BPD (Table 3) was 35% lower than for that to pure HSA. This difference can be attributed to some conformational change in the protein structure, which would difficult to access BPD to binding sites. Conformational changes resulting from the interaction of a low molecular weight substance with albumin is not uncommon, which this can facilitate or difficult internalization of ligands with increasing concentration [36].

As regards the interaction of BPD with the HSA in presence CNZ (Fig. 4b) we can observe that normalized quenching curve overlaps to plot for pure HSA at the highest concentrations [BPD > 8 nM]. For [BPD] < 8 nM, K_{SV} was 36% lower than that for pure HSA (Table 2). As discussed above, CNZ does not complex with albumin, and lower K_{SV} values to small concentrations may also result from decreased accessibility to the binding sites for BPD caused simply by the presence of the CNZ in the molecule or by conformational change of the protein [44].

In the case of titration of complex HPD-HSA by the CNZ, the intensity of the fluorescence quenching (Fig. 4c) and consequently the Stern-Volmer constant (Table 3) were higher than for pure HSA (Table 2). K_{SV} was 67% higher than for pure HSA, suggesting facilitation perhaps caused by conformational change of HSA. Formation of the complex HPD-HSA may increase the accessibility to the CNZ

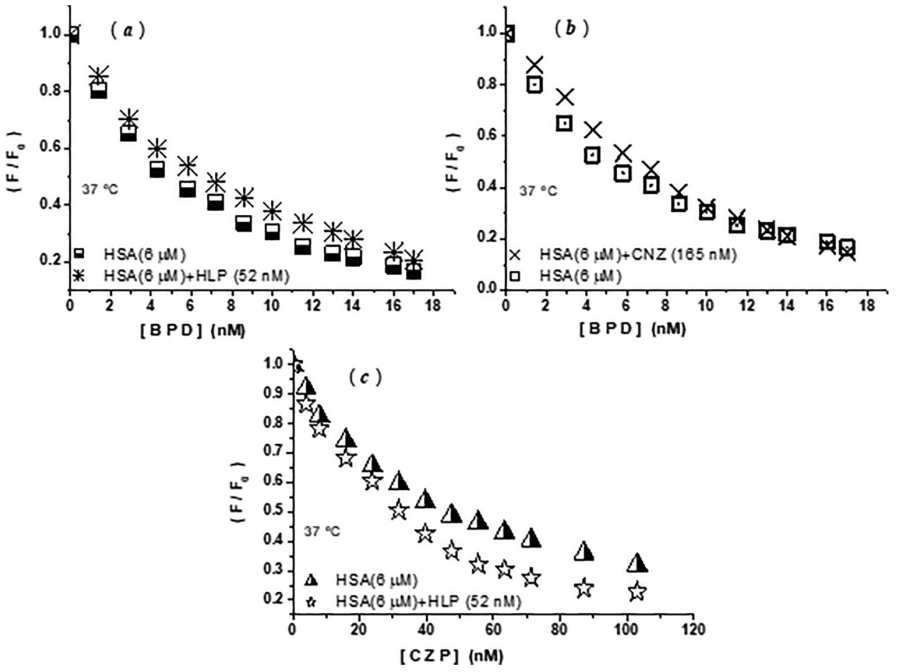


Fig. 4. Normalized quenching plots for solutions: (a) 6 μM HSA and 6 μM HSA + 52 nM HPD titrated by BPD; (b) 6 μM HSA and 6 μM HSA + 165 nM CNZ titrated by BPD; (c) 6 μM HSA and 6 μM HSA + 52 nM HPD titrated by CNZ. In 10 mM Phosphate buffer, pH 7.4, at 37 °C. Excitation wavelength = 290 nm.

molecules, facilitating the internalization [36]. This means that the concomitant use of CNZ and HPD can decrease the free plasmatic CNZ ratio at some level. As the Stern-Vomer plot for complex HPD-HSA (Fig. 5c) and pure HSA (Fig. 3b) are linear, the conformational change does not seem to expose other binding sites of different accessibilities.

In the literature there is information about interaction characteristics and binding sites of drugs used in psychiatric practice with albumin. We could not find no studies on competition between the three drugs here studied by binding sites in plasma proteins.

Some pharmacokinetic studies have reported that HPD levels may be moderately elevated when in combination with quinidine, buspirone and fluoxetine. On the other hand, prolonged use of some drugs such as carbamazepine, phenobarbital and rifampicin may reduce HPD levels. However, these changes were found in direct plasma measurements [45].

A limited number of studies show that CNZ does not alter the pharmacokinetics of other drugs, but some, such as phenobarbital, phenytoin and carbamazepine, may influence their plasma concentration, and that central anticholinergic syndrome may arise from the administration of BPD associated with certain phenothiazine antipsychotics, tricyclic antidepressants and antihistamines. However, these effects due to the interaction of CNZ with other drugs are due to metabolic alterations or their absorption and, in the case of biperiden, the effects are due to interactions in cholinergic receptors, and there is no report of any alterations in any of the three drugs plasma levels due to interactions in plasma [46,47].

5. Conclusions

Results presented here show that the three drugs studied have a high affinity for albumin, since high values of quenching ratios and Stern-Volmer constants were observed.

Comparison of normalized quenching plots suggests that primary sites in HSA and BSA for HPD and CNZ are located at subdomain IB, whereas the BPD would bind in subdomain IIA. The results also suggest the existence of two classes of sites in HSA for HPD, and only one class for BPD and CNZ, within the range of concentrations tested for each drug. Among them, only HPD forms complex with HSA.

From the comparison between the quenching ratios, we can highlight the similarity between HPD and CNZ with respect to the fluorescence quenching capacity of HSA, and the superiority of the Stern-Volmer constant of BPD which is an order of greater magnitude than for the other two drugs.

Considering the competition for binding sites in HSA through the titrations of the HPD-HSA complex by BPD and CNZ, as well as the titration of HSA solution containing CNZ titrated by BPD, there was confirmation that the three drugs do not compete with each other for binding sites. However, the interaction of HSA with one of them (HPD or CNZ) can cause conformational change in the protein structure, and to increase or decrease the accessibility (or internalization) to binding sites for another drug (BPD or CNZ). This may mean alteration in the free plasma concentration of the drug.

The results presented here are of importance due to the lack of experimental results on the competition between these three

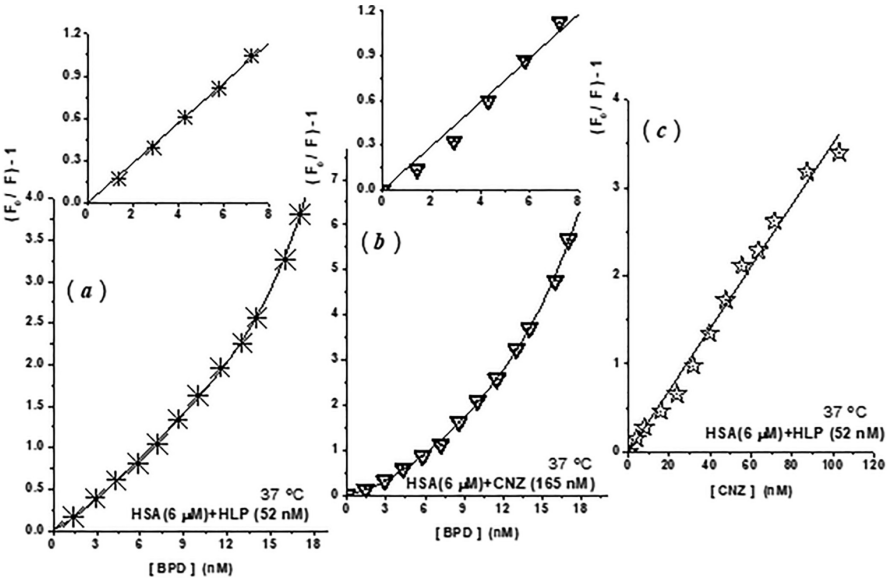


Fig. 5. Stern-Volmer plots for solutions: (a) 6 μM HSA + 52 nM HPD titrated by BPD; (b) 6 μM HSA + 165 nM CNZ titrated by BPD; (c) 6 μM HSA + 52 nM HPD titrated by CNZ, at 37 °C. In 10 mM Phosphate buffer, pH 7.4, at 37 °C. Excitation wavelength = 290 nm.

Table 3
Stern-Volmer constants (K_{SV}) for solution of HSA + HPD titrated by BPD/CNZ, and for solution of HSA + CNZ titrated by BPD.

Solution	K_{SV} (M^{-1})	
	BPD	CNZ
HSA + HPD	$1.42(\pm0.02) \times 10^8$	$3.50(\pm0.06) \times 10^7$
HSA + CNZ	$1.48(\pm0.06) \times 10^8$	–

drugs for plasma protein binding sites, despite the high applicability of this parameter in Computational Pharmacology.

CRediT authorship contribution statement

Carla Patrícia de Morais Coura: Investigation, Writing - original draft. **Viviane Muniz da Silva Fragoso:** Conceptualization, Resources, Writing - original draft, Writing - review & editing, Funding acquisition. **Ethel Celene Narvaez Valdez:** Writing - original draft, Investigation. **Érica Tex Paulino:** Investigation, Writing - original draft. **Dilson Silva:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Supervision. **Celia Martins Cortez:** Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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