Protein binding of 4-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzoic acid to human serum albumin and their anti-proliferation on doxorubicin-sensitive and doxorubicin-resistant leukemia cells

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ABSTRACT

4-Hydroxybenzoic acids (4-HBA) and 4-hydroxy-3-methoxybenzoic acid (Vanillic acid, VA) have exhibited several pharmacological activities. Generally, the biological activities of compounds are highly involved in the interaction between protein and compounds in blood plasma. The objective was to investigate the interaction of 4-HBA or VA with human serum albumin (HSA) and their anti-proliferation properties on doxorubicin-sensitive K562 and doxorubicin-resistant K562/Dox leukemia cells. The protein binding of 4-HBA or VA to HSA was investigated using fluorescence quenching at temperatures of 298 and 310 Kelvin (K) under the pH of 6.0, 7.4, and 8.0 conditions. The effect of 4-HBA and VA on anti-proliferation was also studied on doxorubicin-sensitive K562 and doxorubicin-resistant K562/Dox leukemia cells using resazurin assay. The results showed that 4-HBA and VA could interact with HSA. The fluorescence quenching process in HSA-4-HBA system might be attributed to static quenching mechanism. In contrast, a dynamic quenching mechanism might be mainly involved in the fluorescence quenching process in the HSA-VA system. Thermodynamic data suggested that the spontaneous interaction between HSA and 4-HBA or VA had occurred in the system and it also indicated that hydrogen bonds and Van der Waals forces contributed to the binding of HSA to 4-HBA or VA. In addition, 4-HBA and VA decreased K562 and K562/Dox cells viability in a dose- and time-dependence manner. In conclusions, the 4-HBA and VA could interact with HSA. In addition, the 4-HBA and VA decreased in cell viability for both doxorubicin-sensitive K562 and doxorubicin-resistant K562/Dox leukemia cells in a dose- and time-dependence manner. Therefore, these current studies could provide useful information about the nature of 4-HBA or VA binding to protein HSA and their anticancer activities in both of these types of leukemia cells. The cell death mechanisms should be investigated through future study.

1. Introduction

Phenolic compounds are widely distributed in most plant tissues. The chemical structure of these compounds is composed of an aromatic ring which contains one hydroxyl group as a phenol or other hydroxyl groups as polyphenol. Phenolic compounds are very interesting due to their beneficial effects on health. However, their absorption, transportation, metabolism in *in vivo* level still remains uncertain [1–4].

Human serum albumin (HSA) is an abundant protein found in blood plasma. HSA is generally a protein transporter that exhibits reversibility binding to a variety of compounds such as metabolites, metal ions, fatty acids, and hormones [5]. The study of the interaction of HSA to phenolic compounds can contributes to understand absorption, transportation, and metabolism. Moreover, it is known that the biological activities of compounds are highly involved in the interaction between protein and compounds in blood plasma. Hence, any study on the binding of plasma

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protein to compounds can provide useful data on the chemical structure that is attributed to the biological effect of compounds. In addition, there are several studies that have described the interaction of HSA to some phenolic compounds [6–8]. Therefore, it is very importance to investigation the binding of HSA to phenolic compounds.

4-hydroxybenzoic acids (4-HBA), and 4-hydroxy-3-methoxybenzoic acid (Vanillic acid, VA). They are the phenolic compounds found in a variety of natural plants including carrots, oil palm, prickly ash, and oriental ginseng. [9,10] They have also exhibited several pharmacological activities such as anti-inflammatory [11], anti-oxidant [12], anti-cancer [13,14], and anti-microbial activities [15–17]. Due to the biological activities of 4-HBA and VA, the properties of 4-HBA and VA on transportation and distribution in the circulation system should be studied further. These properties can be beneficial through plasma protein binding.

Zhang et al. studied interaction of 111 phenolic acids and their derivatives included 4-HBA and VA, to HSA and effects on their antioxidant activity. The authors found the relationship between the structure of the phenolic acids and their derivatives and the affinity for HSA. The HSA–phenolic acids interaction had influence on their antioxidant activity [18]. These current studies was to investigate the interaction of HSA to 4-HBA or VA under pHs of 6.0, 7.4, and 8.0 conditions, by using fluorescence spectroscopy. The mechanisms of that interaction were also investigated. These current studies also determined the effect of 4-HBA and VA on anti-proliferation in doxorubicin-sensitive and doxorubicin-resistant leukemia cells. The authors believed that these current studies can provide useful information about the nature of HSA and 4-HBA or VA binding, and the contribution of binding values to anticancer activities, as well.

2. Material and methods

2.1. Chemicals

4-Hydroxybenzoic acid (4-HBA), 4-hydroxy-3-methoxybenzoic acid (Vanillic acid, VA), human serum albumin (HSA), and resazurin were obtained from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Capricorn Scientific. RPMI-1640 medium was bought from Caisson Labs.

2.2. Fluorescence quenching study

A 2 mL phosphate-buffered saline (PBS) solution containing 1 μM HSA was added by 4-HBA or VA solution with final concentration ranging from 0 to 10,000 μM. The additions were done manually by using a micropipette. The fluorescence emission spectra were recorded in the wavelengths ranging from 290 to 500 nm (excitation wavelength at 280 nm) by using a luminescence spectrometer (Perkin-Elmer LS-55) with water bath and 1.0 cm quartz cells. Excitation and emission slits were set at 5 nm. The fluorescence emission spectra were measured at temperature 298 and 310 Kelvin (K) and pH 6, 7.4, and 8 conditions.

2.2.1. Analysis of fluorescence quenching

In these current studies, the Stern-Volmer equation, Eq. (1) was used to analyze the fluorescence quenching of HSA [19–21] that is shown below.

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV} \left[Q \right] \tag{1}$$

Where; F_0 and F were the steady-state fluorescence intensities of HSA at 342 nm in the absence and in the presence of samples, respectively.

K_q and K_{sv} were the quenching rate constant and the Stern-Volmer quenching constant, respectively.

[Q] was the sample concentration.

τ_0 was the average life-time and its value was 5 ns [21].

2.2.2. Binding constants and number of binding sites

Binding constants and number of binding sites were calculated by using equation, Eq.(2) [19–21] that is shown here.

$$\log_{10} \frac{(F_0 - F)}{F} = \log_{10} K_b + n \log_{10} \left[Q \right] \tag{2}$$

Where; n was the number of binding sites per HSA.

K_b was the binding constant.

2.2.3. Thermodynamic parameters

The thermodynamic parameters, enthalpy change (ΔH), entropy change (ΔS), and Gibbs binding free energy change (ΔG) were calculated to determine the binding mode. These parameters were calculated using Eqs. (3)–(5) [22,23].

$$\ln \frac{K_{b2}}{K_{b1}} = \frac{\Delta H}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \tag{3}$$

$$\Delta G = -RT \ln K_b \tag{4}$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{5}$$

Where; T was temperatures (Kelvin).

R was the gas constant, 8.314×10^{-3} kJ mol^{-1} K^{-1}.

2.3. Cell lines and cell culture

The cancer cell lines used in these studies were human leukemia cell lines; doxorubicin-sensitive, (K562) and doxorubicin-resistant, K562/Dox (P-glycoprotein-overexpression) cell lines. The cells culture and cells preparation for performing the cell viability assay were performed according to directions provided by previous reports [24,25].

2.4. Cell viability assay

The cell viability assay was performed by using the resazurin assay according to directions provided by previous reports [24,25]. Briefly, a number of cells at 5×10^4 cell mL^{-1} were incubated with various concentrations of 4-HBA or VA. After incubation for 48, 72, and 96 h, 100 μL of resazurin (0.1 mg mL^{-1}) was added to the cells. After 4 h, the fluorescence emission intensity at 590 nm (excitation wavelength at 570 nm) was measured by a luminescence spectrometer (Perkin-Elmer LS-55). The cell viability percentage was calculated by using equation, Eq. (6) listed here.

$$\%Cell\ viability = \left(\frac{F_{treat}}{F_{control}} \right) \times 100 \tag{6}$$

Where; F_{treat} and $F_{control}$ were the fluorescence emission intensities in treated cells and control cells, respectively.

2.5. Cell morphological observation

A number of cells at 5×10^4 cell mL^{-1} were incubated with various concentrations of 4-HBA or VA for 48, 72, and 96 h. After incubation, the cells were observed under light microscope (20X magnification) without any cell disruption. The images of the cells were then acquired using light microscope.

2.6. Statistical analysis

The data was shown in mean ± standard error of the mean (S.E.). The student's *t*-test was independently used for evaluating statistical differences between each treated group and the non-treated control group. A p-value of less than 0.05 was considered as statistically significant. The data represented three independent experiments.
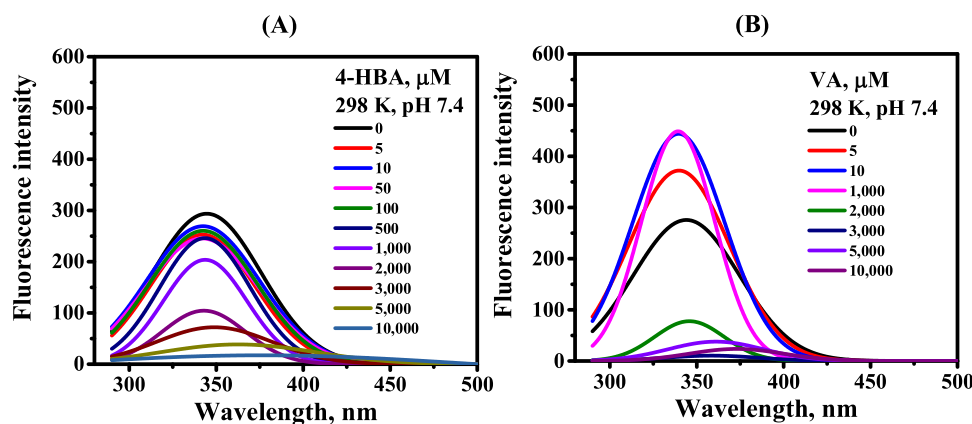
(A)

(B)

Fig. 1. Fluorescence emission spectra of HSA, excitation wavelength at 280 nm with various concentrations of 4-HBA (A) and VA (B) in PBS solution pH 7.4 at 298 K. 4-HBA: 4-hydroxybenzoic acid, VA: Vanillic acid, HSA: Human serum albumin, PBS: Phosphate-buffered saline, K: Kelvin.
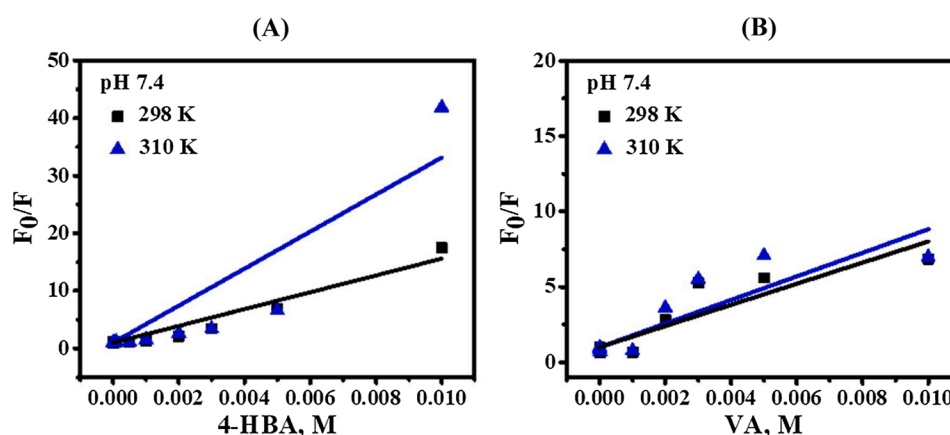
(A)

(B)

Fig. 2. Stern–Volmer plots of HSA (1 μM) quenched by 4-HBA (A) and VA (B) in PBS pH 7.4 at 298 and 310 K. 4-HBA: 4-hydroxybenzoic acid, VA: Vanillic acid, HSA: Human serum albumin, PBS: Phosphate-buffered saline, K: Kelvin.

3. Results

3.1. Fluorescence quenching of HSA by 4-HBA and VA

Fig. 1. shows the fluorescence emission spectra at excitation wavelength of 280 nm with various concentrations of 4-HBA and VA in PBS solution pH 7.4 at 298 K. The HSA had a fluorescence emission peak at 342 nm after being excited. The results showed that the fluorescence intensity of HSA decreased consistently with increases in 4-HBA concentrations (5–10,000 μM), while the fluorescence intensity of HSA decreased consistently with the increases in VA at concentrations ranging from 2,000 to 10,000 μM.

The corresponding results at temperatures 298 and 310 K and pHs 7.4, 6.0 and 8.0 were found whereby the fluorescence intensity of HSA had decreased with increased 4-HBA concentrations (5–10,000 μM), while concentration ranging from 2,000 to 10,000 μM of VA had decreased the fluorescence intensity of HSA. These results indicated that 4-HBA and VA could interact with the HSA.

3.2. Analysis of fluorescence quenching of HSA by 4-HBA and VA

The fluorescence quenching data was analyzed by the Stern–Volmer equation, Eq.(1). The Stern–Volmer plots, based on Eq. (1) of the quenching of HSA fluorescence by 4-HBA and VA in PBS pH 7.4 at 298 and 310 K are shown in Fig. 2. The corresponding correlation coefficients, at two different temperatures and pHs, were calculated and are shown in Table 1.

3.3. Binding constants and the number of binding sites

The binding constants and the number of binding sites were analyzed by using Eq. (2). The double-logarithm curve ($\log_{10}$ [(F_0-F)/F] versus $\log_{10}$ [4-HBA] and [VA]) in PBS pH 7.4 at 298 and 310 K are shown in Fig. 3. The intercept and the slope represented the values of binding constant (K_b) and number of binding sites per HSA (n), respectively. Of note; the double-logarithm curve for HSA-VA system could be plotted by 2,000, 3,000, 5,000, and 10,000 μM. The values of K_b and n at two different temperatures and pHs, were calculated and are shown in Table 2.

3.4. Thermodynamic parameters

Using the K_b values at 298 and 310 K, the thermodynamic parameters included the enthalpy changes (ΔH), Gibbs binding free energy changes (ΔG), and entropy changes (ΔS) could be calculated from Eqs. (3)–(5) respectively. These values are shown in Table 3.

3.5. Effect of 4-HBA and VA on cell viability

Fig. 4. shows the effects of 4-HBA on K562 and K562/Dox cancer cells viability at 48, 72, and 96 h. The cell viability percentage of cancer cells treated with concentrations of 4-HBA (0.01, 0.05, 0.1, 0.5, 1, and 5 mM) did not significantly change at all harvest time points, as compared with the control group.

In K562 cancer cells, the concentration of 4-HBA (10 mM) cell

Table 1

Stern-Volmer quenching constant (K_{SV}) and quenching rate constant (K_q) of HSA-4-HBA and HSA-VA.

	Temperature (K)	$K_{SV} \pm SE \times 10^3$ (L mole^{-1})	$K_q \pm SE \times 10^{12}$ (L. mole^{-1} s^{-1})	$R^2 \pm SE$
HSA-4-HBA				
pH 6.0	298	4.4982 ± 2.10	0.8996 ± 0.42	0.7815 ± 0.02
	310	4.2857 ± 0.93	0.8572 ± 0.19	0.8935 ± 0.03
pH 7.4	298	3.0657 ± 1.18	0.6131 ± 0.24	0.8812 ± 0.04
	310	2.7164 ± 0.27	0.5433 ± 0.05	0.8532 ± 0.02
pH 8.0	298	1.8046 ± 0.51	0.3609 ± 0.10	0.8865 ± 0.04
	310	1.4572 ± 0.25	0.2914 ± 0.05	0.9559 ± 0.01
HSA-VA				
pH 6.0	298	1.2621 ± 0.19	0.2524 ± 0.04	0.8875 ± 0.04
	310	1.4092 ± 0.09	0.2818 ± 0.02	0.8056 ± 0.05
pH 7.4	298	0.7564 ± 0.03	0.1513 ± 0.01	0.7906 ± 0.04
	310	0.8312 ± 0.09	0.1662 ± 0.02	0.6153 ± 0.06
pH 8.0	298	0.7592 ± 0.06	0.1518 ± 0.01	0.6685 ± 0.09
	310	1.0384 ± 0.07	0.2077 ± 0.01	0.7366 ± 0.06

R is the linear correlation quotient.

viability percentages were significantly changed to 90.31 ± 0.96 % at 72 h and 87.56 ± 1.01 % at 96 h, but did not significantly change at 48 h.

In K562/Dox cancer cells, the concentration of 4-HBA (10 mM) and cell viability percentages had significantly changed to 75.41 ± 8.61 % at 48 h, to 70.36 ± 5.21 % at 72 h, and 66.32 ± 7.67 % at 96 h.

Fig. 5. shows the effects of VA on K562 and K562/Dox cancer cells viability at 48, 72, and 96 h. The cell viability percentage of cancer cells treated with concentrations of VA (0.01, 0.05, 0.1, 0.5, and 1 mM) did not significantly change at all harvest time points, as compared with the control group.

In K562 cancer cells, 5 and 10 mM of VA led to cell viability percentages being significantly changed to 79.75 ± 3.98 % and 70.58 ± 2.93 % at 48 h, to 81.28 ± 6.51 % and 57.98 ± 5.46 % at 72 h, to 82.87 ± 5.89 %, and 56.73 ± 18.23 % at 96 h.

In K562/Dox cancer cells, 5 and 10 mM of VA led to cell viability

percentage being significantly changed to 75.30 ± 6.23 % and 53.49 ± 10.68 % at 48 h, to 70.74 ± 11.07 % and 41.00 ± 5.78 % at 72 h, 65.46 ± 2.36 %, and 35.94 ± 3.61 % at 96 h.

These results suggested that 4-HBA and VA decreased K562 cancer cells and K562/Dox cancer cells viability in a dose- and time-dependence manner.

3.6. Cell morphological observation in treated cells

The images of control and treated cancer cells were acquired at 48, 72, and 96 h after treatment with 4-HBA and VA. Fig. 6. shows the images of control and treated cancer cells with 10 mM 4-HBA and VA at 72 h. For comparison with corresponding control cells at each time points, the morphological changes such as rough cells, shrinking cells, irregular shape cells, and membrane blebbing were shown in the treated cancer cells with high concentrations 4-HBA and VA. Similarly, at 72 h,

Table 2

Binding constant (K_b) and number of binding site (n) for HSA-4-HBA and HSA-VA.

	Temperature (K)	$K_b \pm SE \times 10^3$ (L. mole^{-1})	$n \pm SE$	$R^2 \pm SE$
HSA-4-HBA				
pH 6.0	298	1.1585 ± 0.32	0.8623 ± 0.05	0.9342 ± 0.01
	310	0.4549 ± 0.10	0.9016 ± 0.06	0.7628 ± 0.05
pH 7.4	298	0.1045 ± 0.03	0.6130 ± 0.05	0.7653 ± 0.01
	310	0.0464 ± 0.002	0.7235 ± 0.05	0.7939 ± 0.03
pH 8.0	298	0.4549 ± 0.10	0.8005 ± 0.11	0.8214 ± 0.07
	310	0.2419 ± 0.05	1.0784 ± 0.29	0.8379 ± 0.07
HSA-VA				
pH 6.0	298	0.3174 ± 0.07	0.7071 ± 0.08	0.9248 ± 0.05
	310	0.1557 ± 0.03	0.6174 ± 0.08	0.8031 ± 0.07
pH 7.4	298	0.1018 ± 0.01	0.5786 ± 0.03	0.8033 ± 0.07
	310	0.0451 ± 0.01	0.3816 ± 0.09	0.5263 ± 0.10
pH 8.0	298	0.1782 ± 0.04	0.6340 ± 0.04	0.9144 ± 0.04
	310	0.1280 ± 0.05	0.5257 ± 0.08	0.7837 ± 0.11

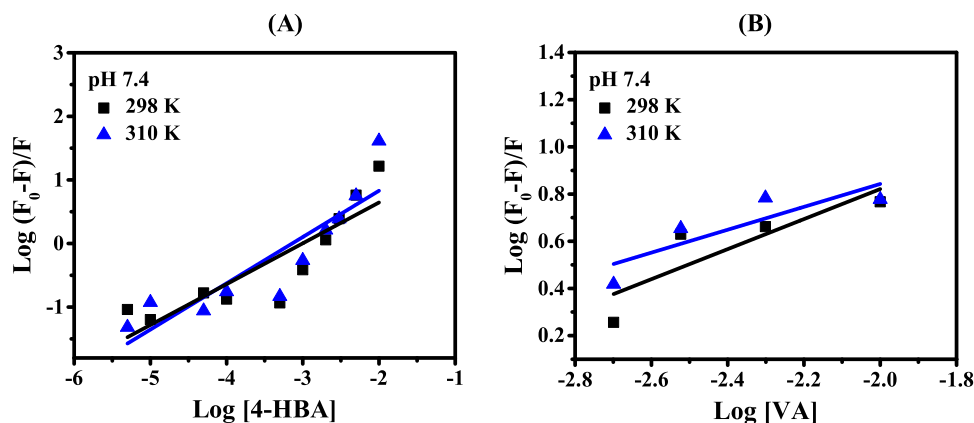R is the linear correlation quotient.

Fig. 3. Plot of $\log_{10}[(F_0-F)/F]$ versus $\log_{10}[4\text{-HBA}]$ (A) and [VA] (B) in PBS pH 7.4 at 298 and 310 K. 4-HBA: 4-hydroxybenzoic acid, VA: Vanillic acid, HSA: Human serum albumin, PBS: Phosphate-buffered saline, K: Kelvin.

Table 3
Thermodynamic parameters for the HSA-4-HBA and HSA-VA.

	Temperature (K)	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J. mol^{-1} K^{-1})
HSA-4-HBA				
pH 6.0	298	−17.48	−59.83	−142.12
	310	−15.77		
pH 7.4	298	−11.52	−51.96	−135.72
	310	−9.89		
pH 8.0	298	−15.16	−40.42	−84.76
	310	−14.15		
HSA-VA				
pH 6.0	298	−14.27	−45.59	−105.08
	310	−13.01		
pH 7.4	298	−11.45	−52.11	−136.42
	310	−9.82		
pH 8.0	298	−12.84	−21.18	−27.97
	310	−12.51		

the morphological changes in treated cells occurred at 48 and 96 h. These morphological changes (shrinkage and membrane blebbing) in treated cancer cells showed the typical signs of being apoptotic cells.

4. Discussion

The chemical structure of 4-HBA and VA is shown in Fig. 7.

For the fluorescence quenching and analysis section: The fluorescence emission intensity of HSA decreased when increasing the concentration of 4-HBA and VA (Fig. 1.). The results suggested that 4-HBA and VA could interact with HSA. 4-HBA and VA could act as a quencher in the intrinsic fluorescence of HSA. The quenching mechanism of the HSA-4-HBA and HSA-VA system was investigated by fluorescence data analysis. The Stern-Volmer equation, Eq. (1) was used to analyze that data.

The authors have explained that the intrinsic fluorescence quenching mechanism can proceed by two mechanisms. First, there is a formation of a complex or static quenching. Second, there is a collisional process or
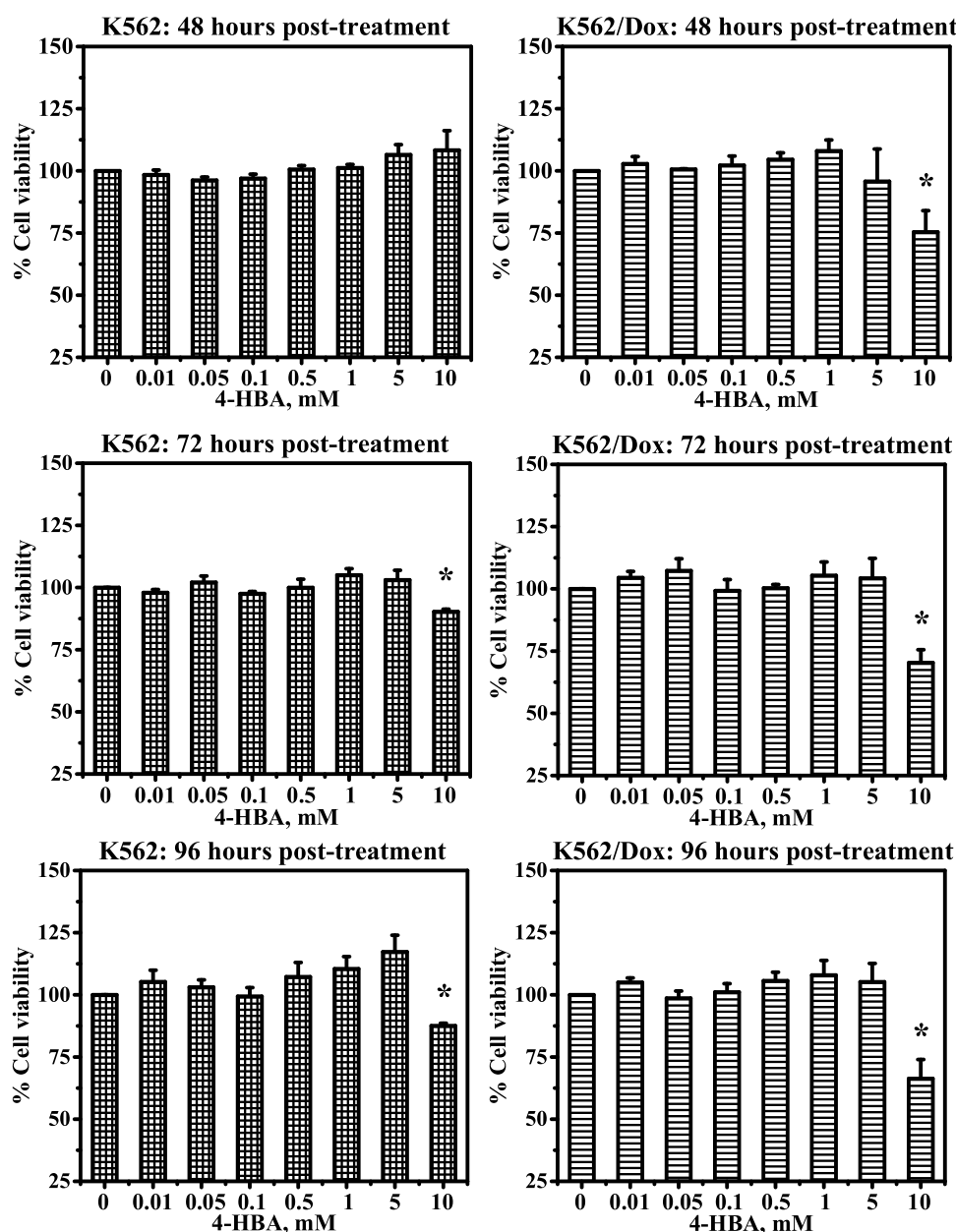

Fig. 4. Effects of 4-HBA on K562 and K562/Dox cancer cells viability percentage at 48, 72, and 96 h. 4-HBA: 4-hydroxybenzoic acid.
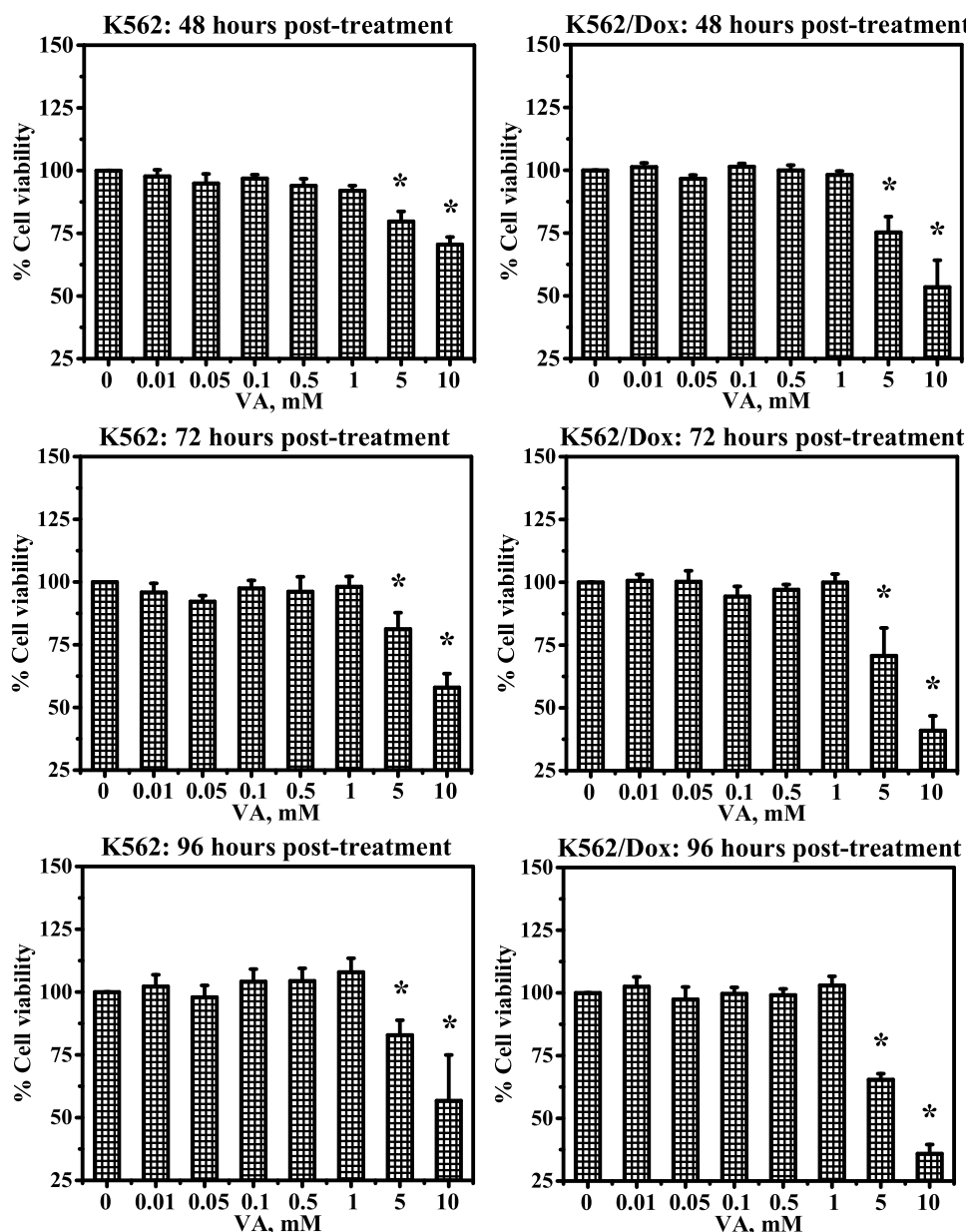
Fig. 5. Effects of VA on K562 and K562/Dox cancer cells viability percentage at 48, 72, and 96 h. VA: Vanillic acid.

dynamic quenching. These mechanisms can be distinguished by their temperature-dependent behavior. In the static quenching process, the increase of temperature results in a decrease in the formation complex and the quenching constant. In contrast, there was dynamic quenching taking place which is the increase of temperature resulting in an increase in the collisions and the quenching constant [26,27]. Hence, the quenching constant can be used to indicate the quenching mechanism. In these current studies, the results (Table 1) shows K_{sv} and K_q values in HSA-4-HBA system had decreased with the temperature increases. This result suggested that the fluorescence quenching process in HSA-4-HBA system might have been the primary contributer to a static quenching mechanism. In contrast to the HSA-4-HBA system, the K_{sv} and K_q in HSA-VA system (Table 1) were correlated with temperature. This result indicated that a dynamic quenching mechanism might be the primary contributer involved in the fluorescence quenching process in the HSA-VA system.

For the binding constants and number of binding sites section: The double-logarithm curve ($\log_{10}$ [(F_0-F)/F] versus $\log_{10}$ [4-HBA] and [VA]) in PBS pH 7.4 at 298 and 310 K base on Eq. (2) were determined

for demonstrating the K_b and n values in the HSA-4-HBA and HSA-VA system. The results in Table 2 indicated that 4-HBA and VA could bind, and that there was approximately one binding site on the HSA. The results also indicated that the values of K_b and n decreased when there was increases in the temperature for both the HSA-4-HBA and HSA-VA system. This data suggested that the complex formation was not stable. The binding of HSA to 4-HBA or VA would be possibly partially dissociated with temperature increases.

For the thermodynamic parameters section: The binding of biomolecules to a ligand commonly involved four types of forces including hydrogen bonds, Van der Waals forces, hydrophobic forces, and electrostatic forces. The binding mode was described based on the thermodynamic parameters including enthalpy change (ΔH), entropy change (ΔS), and Gibbs binding free energy change (ΔG). The negative value of ΔG represented the spontaneous interaction [21,28]. The results in this current study showed that ΔG values were negative in the HSA-4-HBA and HSA-VA system. It could be suggested that the spontaneous interaction between HSA and 4-HBA or VA had occurred in the systems. The negative value of ΔH and ΔS represented the hydrogen bonding and Van
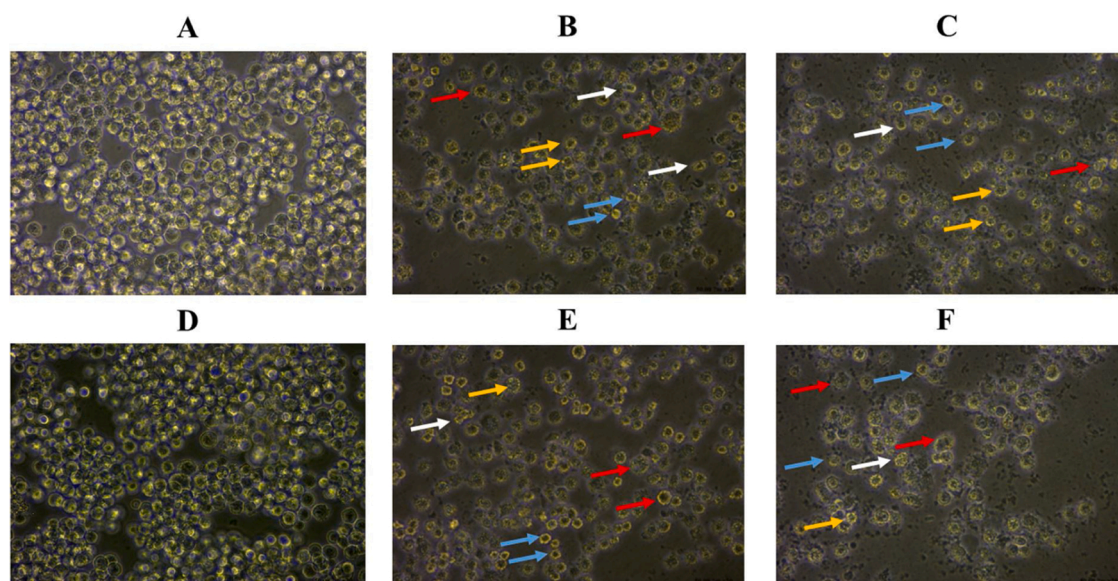
Fig. 6. The images of control and treated cancer cells with 10 mM 4-HBA and VA at 72 h K562 cancer cells (A), K562 cancer cells treated with 10 mM 4-HBA (B), K562 cancer cells treated with 10 mM VA (C), K562/Dox cancer cells (D), K562/Dox cancer cells treated with 10 mM 4-HBA (E), and K562/Dox cancer cells treated with 10 mM VA (F). Rough cells (red arrow), Shrinking cells (blue arrow), Irregular shape cells (white arrow), and Membrane blebbing (orange arrow). 4-HBA: 4-hydroxybenzoic acid, VA: Vanillic acid.
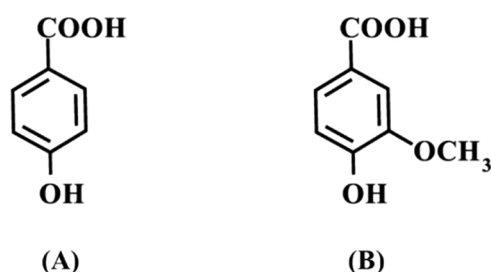

Fig. 7. Chemical structure of 4-hydroxybenzoic acid (4-HBA), (A) and 4-hydroxy-3-methoxybenzoic acid (Vanillic acid, VA), (B).

der Waals forces [28,29]. The results in Table 3 showed that the ΔH and ΔS values also were negative values in HSA-4-HBA and HSA-VA system, indicating that hydrogen bonds and Van der Waals forces contributed in the binding of HSA to 4-HBA or VA. The ΔH values less than 0 referred to the binding process that was an exothermic reaction and the binding constant decreased with increases in the temperature [30].

There have been studies that have suggested that polyphenols-protein interaction was stabilized by hydrogen bonding and hydrophobic forces [31,32]. Moreover, the chemical structure of polyphenols showed effects on the interaction with proteins such as rutin that was less hydrophobic than quercetin, and had less affinity to protein compared with quercetin [33]. Thus, the methoxyl group substitutions in VA might have contributed to the interaction with the lessened affinity to protein compared with 4-HBA. It is worthy to note that these current studies also found interesting information regarding pH in the system whereby the environmental pH played an important role in the parameters of the both HSA-4-HBA and HSA-VA systems.

For the cell viability section: The 4-HBA and VA induced cell death in K562 and K562/Dox cancer cells as a dose- and time-dependent manner. More interesting, the 4-HBA and VA appeared to have an effect on K562/Dox cancer cells that was higher than the effect on K562 cancer cells. This effect is promising to help overcome the drug resistance that takes place in cancer cells. However, both 4-HBA and VA were less toxic to cancer cells, compared to other polyphenols (i.e.; catechin, eriodictyol, apigenin, kaempferol, and quercetin) [34]. Previously studies suggested

that hydrogen atoms and/or hydroxyl groups substituted by a methoxyl group in the structure of polyphenol compounds could enhance anti-proliferation of cancer cells [34]. Consistent with previous a study [34], VA was seem to be more cytotoxic than 4-HBA in both K562 and K562/Dox cancer cells.

In terms of the the binding constants and cytotoxicity effects, it found that the binding constants in 4-HBA-HSA were higher than that of the VA-HSA system, while cytotoxicity to K562 and K562/Dox cancer cells was higher in VA than 4-HBA. We proposed that it might be suggested that 4-HBA was strongly bound to protein in a culture medium, resulting in less cytotoxicity than VA.

5. Conclusion

In this studies reported that the 4-HBA and VA could interact with HSA. The fluorescence quenching process in HSA-4-HBA and HSA-VA system might be attributed to static quenching and dynamic quenching mechanisms, respectively. Thermodynamic data suggested that the spontaneous interaction between HSA and 4-HBA or VA had occurred in the system and it also indicated that hydrogen bonds and Van der Waals forces contributed to the binding of HSA to 4-HBA or VA. In addition, the 4-HBA and VA decreased in cell viability for both doxorubicin-sensitive K562 and doxorubicin-resistant K562/Dox leukemia cells in a dose- and time-dependence manner. These current studies could provide useful information about the nature of how 4-HBA or VA binds to protein HSA and their anticancer activities in leukemia cells, as well. This cell death mechanism should be investigated in future studies.

Author statement

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

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