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Electrochemical and Spectroscopic Study on the Interaction Between G-Quadruplex DNA and (R)-/(S)-2-(5-Fluorouracil-1-Acetyl) Amido-1, 5-Dimethyl Glutarate

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The cyclic voltammetry and ultraviolet-visible absorption spectrometry have proved to be powerful electrochemical means for the research of interactions between G-quadruplex DNA (G4-DNA) and chemical anti-carcinogens. In this study, the electrochemical behavior of (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate (i.e., (R)-5FUGlu and (S)-5FUGlu) and the interaction of (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate with DNA using $Fe(CN)_6^{3-/4-}$ as electroactive indicator were investigated, and the compounds were assayed for anticancer activities in HL-60 cells. The experiment showed that the interaction between (R)-/(S)-2-(5-fluorouracil-1-acetyl)amido-1,5-dimethyl glutarate and DNA were electrostatic, and the binding equilibrium constant $K_{G4\text{-}DNA}$ of the compounds is closely related to the configuration. It was observed that the binding equilibrium constant $K_{G4\text{-}DNA}$ of (R)-5FUGlu was about 2 times larger than that of (S)-5FUGlu. The inhibition rates of the different concentration of (R)-5FUGlu in tumor cell line HL-60 cells were larger than those of (S)-5FUGlu, which gave a good agreement with the above-mentioned electrochemical results.

Keywords: G-quadruplex DNA; Gold Electrode; (R)-5FUGlu; (S)-5FUGlu; Cyclic Voltammetry

1. INTRODUCTION

G-rich DNA sequences can adopt a special structure called G-quadruplex which comprises a stack of G-tetrads and the planar association of four guanines in cyclic Hoogsteen hydrogen bonds. Recently, G-quadruplex DNA (G4-DNA) ligands have been proposed to be selective anticancer agents by acting as telomerase inhibitors [1–3] and/or transcriptional repressors of oncogene c-MYC [4–7]. However, one problem that faces many G-quadruplex DNA ligands is the non-specific cytotoxicity

which is believed to arise from their interaction with duplex DNA [8–10]. Ideal G-quadruplex DNA ligands, therefore, should bind selectively to their target and have few interactions with duplex DNA. Therefore, studies regarding G-quadruplex DNA binding selectivity are essential to the development of G-quadruplex DNA ligands for therapeutic use.

In recent years, 5-Fluorouracil (5-FU) has been increasingly employed alone or in combination with various cytotoxic drugs and hormones in the treatment of tumours, such as breast, colorectal and gastric cancers [11–16]. Particularly, many derivatives of 5-FU have been developed to improve the topical delivery efficiency and to reduce the side effects of 5-FU [17–20] due to its poor tumor selectivity and high incidence of toxicity in the bone marrow, gastrointestinal tract, central nervous system and skin. On the other hand, aminophenol plays important roles in life status of human beings and other organisms, such as hormone, enzyme inhibitor/substrate, growth promoter, inhibitor, neurotransmitter, immunomodulating agents as well as antibiotics, and drives considerable pharmacological interest in the design and application of new drugs [21, 29]. To extend the interest in searching for new aminophenol derivatives of 5-FU with higher bioactivity and verifying the pharmaceutical concept of bioisosterism, we designed and synthesized and electrochemically characterized two 5-FU derivatives, i.e., (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate (the abbreviation for (R)-5FUGlu and (S)-5FUGlu).

2. EXPERIMENTAL DESIGN

2.1. Instrumentation

Electrochemical measurements were carried out with a model AUTOLAB PGSTAT30 electrochemical workstation (Metrohm AG) controlled by a personal computer. A conventional three-electrode system was used in the measurements at room temperature (25°C) with a bare or modified gold electrode (d = 2 mm) as the working electrode, an Ag/AgCl electrode (Ag/AgCl) as the reference electrode and a Pt plate as the counter electrode. The setup of all potentials was referred to the Ag/AgCl electrode. Unless specially stated, the electrolyte solutions were thoroughly degassed with N_2 and kept under a N_2 blanket.

2.2. Chemicals and reagents

G4-quadruplex DNA (G4-DNA) was purchased from Shanghai Chemical Reagents Company (China). Their base sequences are as follow:

G4-DNA: 5'SH-$(CH_2)_6$-$(TTA\ GGG)_4$

(R)-5FUGlu and (S)-5FUGlu were synthesized by our group (Fig. 1).

G4-DNA stock solutions (100μM /mL) were prepared with buffer solution (pH 6.8 Tris–HCl) and kept frozen. The DNA and 5-fluorouracil derivatives were dissolved in 5.0 mmol·L^{-1} Tris–HCl (pH 6.8) buffer solution containing mmol·L^{-1} NaCl, which was used as the supporting electrolyte. Other chemicals were at least of analytical reagent grades. The buffer solution referred to 5.0 mmol·L^{-1}

Tris–HCl buffer solution with pH 6.8 containing 5 mmol·L^{-1} NaCl supporting electrolyte. Ultra-pure water (18.22 MΩ / cm) was used for the preparation of all solutions.

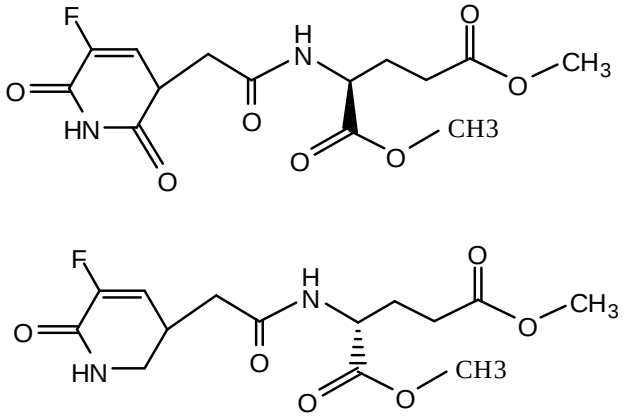

Figure 1. The structures of (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate

2.3. Preparation of DNA-modified gold electrodes

The gold electrodes were first polished carefully with 1.0, 0.3 and 0.05 μm alumina slurry groups, and then cleaned ultrasonically in acetone, ethanol and water, respectively, for 10 min. The real electrode area was estimated from cyclic voltammograms (CV) by integrating the cathodic peak for the reduction of the oxide layer in 0.5 M H_2SO_4. The freshly polished electrodes were scanned over the potential range of 0.0 to +1.5 V (vs. Ag/AgCl) in 0.5 mol·L^{-1} H_2SO_4 until a constant voltammogram was obtained. Afterward, they were polarized at 0 V for 3 min. Finally, the electrodes were rinsed with water and modified immediately by transferring a droplet of 20 μL of 0.5 mmol·L^{-1} DNA solution onto the surface, followed by air-drying overnight. The electrodes were then soaked in sterile water for at least 4 h before being rinsed with water to remove any unadsorbed DNA. The DNA-modified gold electrodes thus obtained are denoted as DNA/Au in the text.

3. RESULTS AND DISCUSSION

3.1. Electrochemical characterization of DNA-modified electrode

Cyclic voltammetry of electroactive species of $Fe(CN)_6^{3-/4-}$ has been used widely to test the kinetic of the barrier of the interface. The extent of kinetic hindrance to the electrontransfer process increased with increasing thickness and decreasing defect density of the barrier [30]. Fig. 2 shows the CV responses of 5.0 mM $Fe(CN)_6^{3-/4-}$ at bare Au and DNA/Au. $Fe(CN)_6^{3-/4-}$ produced a couple of well-defined redox waves at bare Au (Fig. 2a) with a peak-to-peak separation (ΔE_p) of 94 mV at 100 mV/s. After the electrode was modified with G4-DNA, an obvious decrease in redox peak current was observed (Fig. 2b), indicating that the DNA acted as the inert electron and mass transfer blocking layer

and thus hinders the diffusion of ferricyanide toward the electrode surface. This demonstrated that G4-DNA had been successfully assembled on Au surface. The cyclic voltammograms of the same G4-DNA electrode remained stable after 20 scans in the Tris-HCl buffer solution, suggesting the electrochemical stability of the DNA-coated film.

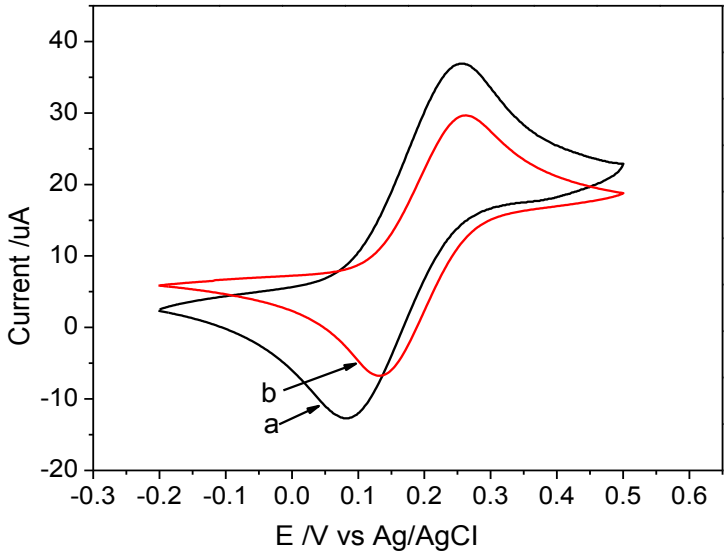

Figure 2. Cyclic voltammograms of 5 mmol·L^{-1} $K_2Fe(CN)_6$ in Tris-HC1 buffer solution at (a) Au and (b) Au/G4-DNA electrodes. The scan rate was 0.1 V·S^{-1}.

3.2. Interaction of G-quadruplex DNA with (R)-5FUGlu and (S)-5FUGlu

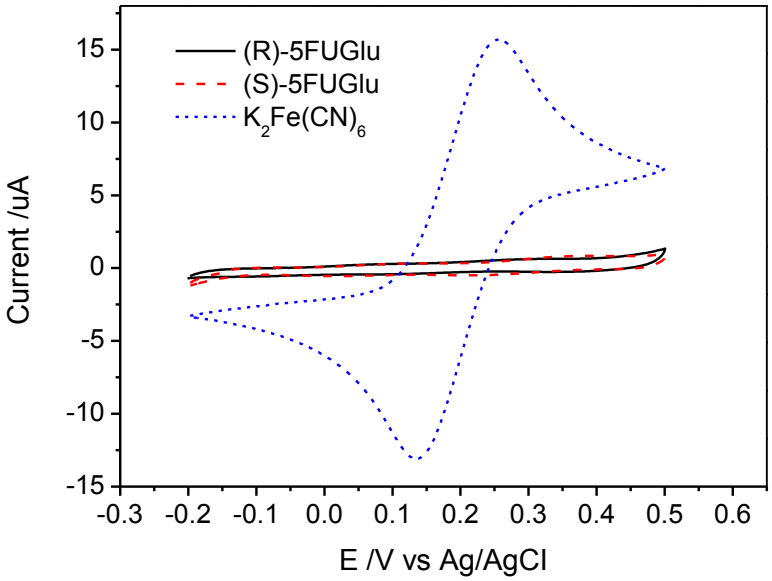

Figure 3. Cyclic voltammograms of Au electrode at 4.3 mmol·L^{-1} of (R)-5FUGlu, (S)-5FUGlu, $K_2Fe(CN)_6$.

Fig. 3 shows no peaks that could be seen during electrochemical scanning in the presence of (R)-5FUGlu and (S)-5FUGlu in Tris-HC1 buffer containing 5 mmol·L^{-1} KCl. In order to investigate the interaction of G4-DNA with (R)-5FUGlu and (S)-5FUGlu, the G4-DNA -modified electrode was scanned in buffer solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$ probe molecule, then (R)-5FUGlu and (S)-5FUGlu were added into the test solution.

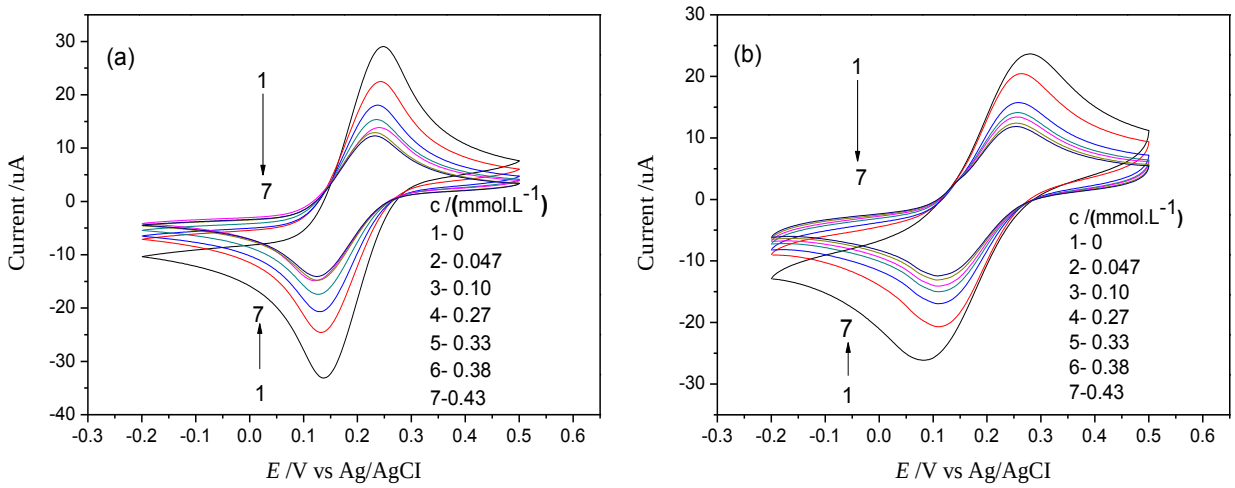

Figure 4. Cyclic voltammograms of 5 mmol·L^{-1} $K_2Fe(CN)_6$ in Tris-HC1 buffer solution containing different concentration of compound (a) (R)-5FUTyr, (b) (S)-5FUTyr at Au/G4-DNA electrodes. Scan rate was 0.1 V·S^{-1}.

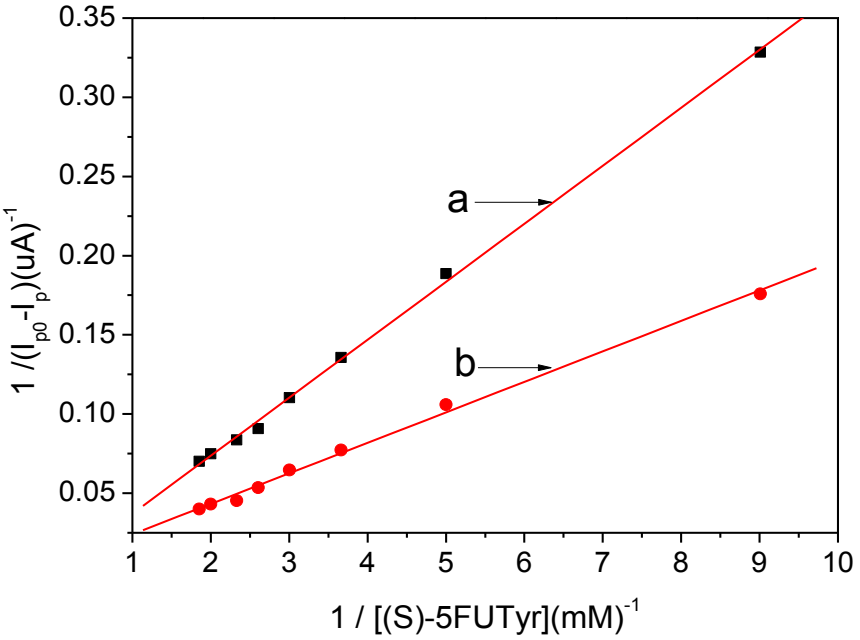

Figure 5. The relationship between $1/(I_{p0}-I_p)$ and 1/[*drug*] for (R)-5FUGlu, (S)-5FUGlu. Plots in each figure correspond to the measurement by G4-DNA modified Au electrode.

The experiment showed that the peak current of probe molecule decreased as (R)-5FUGlu and (S)-5FUGlu were added into the test solution. The more (R)-5FUGlu and (S)-5FUGlu were added, the more the peak current of probe molecule decreased. Thus, when the concentration of (R)-5FUGlu and (S)-5FUGlu were adjusted to 4.3×10^{-4} M, the peak current decreased with respect to original peak current, as showed in Fig. 4 and 5. The phenomenon confirmed that (R)-5FUGlu and (S)-5FUGlu interacted with G4-DNA under the three interaction models of DNA with target molecules [31]. With their concentration increasing, the peak potential shifted in a negative direction, confirming the dominance of electrostatic interaction between (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate and G4-DNA.

3.3. Binding constant

Cyclic voltammograms showed that DNA-modified electrode had a couple of stable voltammetric peaks from probe molecules when it was scanned in the probe solution. Further studies showed that the peak current had an obvious decreased when (R)-5FUGlu and (S)-5FUGlu were added into this solution.

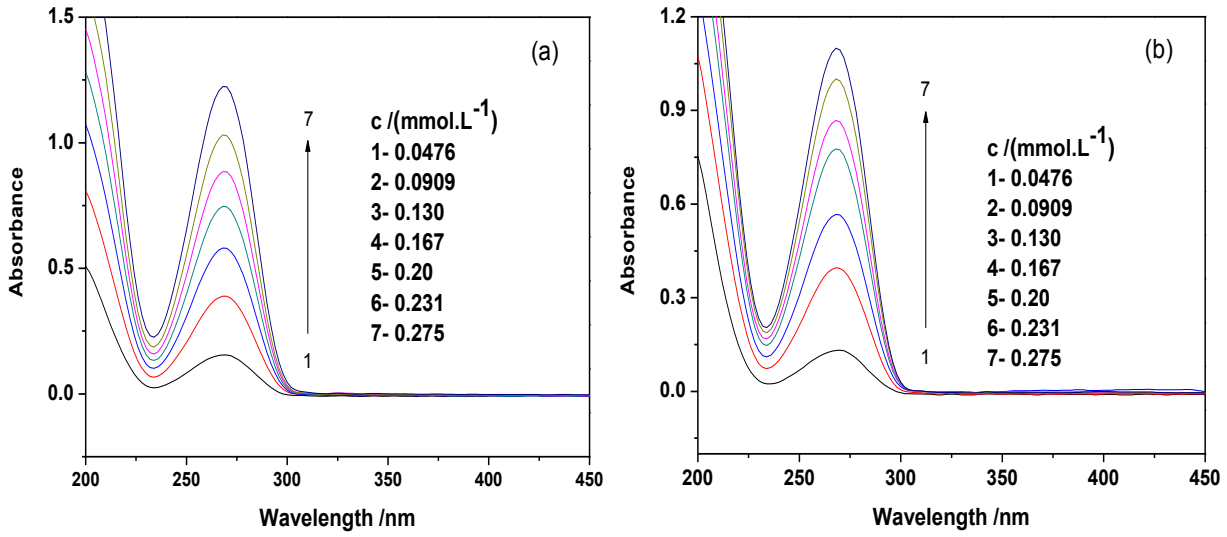

Figure 6. Ultraviolet–visible absorbance spectra of different concentration of (a) (R)-5FUGlu, (b) (S)-5FUGlu

The higher the amount of (R)-5FUGlu and (S)-5FUGlu added, the lower the peak current was. When the concentration of (R)-5FUGlu and (S)-5FUGlu reached 4.3 mM, the peak current decreased no more. From Fig. 3, we can obtain an $1/\Delta I_P = 1/\Delta I_{Pmax} + 1/(\Delta I_{Pmax} kC)$ (Fig. 6). It is apparent that a good linear relationship existed between the reciprocal of the current drop and that of the (R)-5FUGlu, (S)-5FUGlu concentration. It is in good agreement with Langmuir equation[32] below:

$$1/\Delta I_P = 1/\Delta I_{p,max} + 1/(\Delta I_{p,max} kC)$$

Where k is equilibrium constant of the interaction between (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate and DNA, label C is the concentration of (R)-5FUGlu, (S)-5FUGlu, and ΔI_p is the current drop and $\Delta I_{p,max}$ stands for the maximum of the current drop. According to the equation above and the curve slope in Fig.5, the binding equilibrium constant can be estimated, as shown in Table 1.

Table 1. The association equilibrium constants between(R)-5FUGlu, (S)-5FUGlu and G4-DNA.

Compound	G4-DNA	
	(R)-5FUGlu	(S)-5FUGlu
$K_{G4\text{-}DNA}$ ($\times 10^4$)	6.29	2.56

From the analysis of the relationship between structure and selectivity, it was suggested that the identification of 5FUGlu on the role of double-stranded DNA, G4-DNA was closely related to its configuration space.

3.4 Anticancer activity test

(R)-5FUGlu and (S)-5FUGlu had been investigated for their anticancer activities against human HL-60 cell lines (Table 2).

Table 2. The inhibition rate of the chiral isomers 5-FUGlu to cell line HL-60.

Compound	Concentration (mol·L^{-1})		
	10^{-4} mol/L	10^{-6} mol/L	10^{-8} mol/L
(R)5-FUGlu	55.8	12.8	5.9
(S)5-FUGlu	2.8	1.5	0.6

The antitumor activities in vitro for these compounds were evaluated by SRB method for HL-60 cells. The result of these assays was shown in Tables 2. The result indicated that among the tested compounds, almost all of the newly prepared compounds showed a moderate to good inhibiting effect on the growth of the HL-60 cells at the concentrations of 10^{-4} mol/L, 10^{-5} mol/L, 10^{-6} mol/L, 10^{-7} mol/L, and 10^{-8} mol/L, which might indicated that the peptide derivatives could be more easily to release 5-fluorouracil [33, 34]. By inspection of the chemical structure of (R)-5FUGlu and (S)-5FUGlu, it was observed that the inhibition rate of 5FUGlu on the cytotoxic role of G-quadruplex DNA in cell line HL-60 cells was closely related to its configuration space.

3.5 UV–vis spectra studies

Ultraviolet–visible absorbance spectra of (R)-5FUGlu, (S)-5FUGlu,G4-DNA and their mixture were measured, respectively (Fig. 6,7).

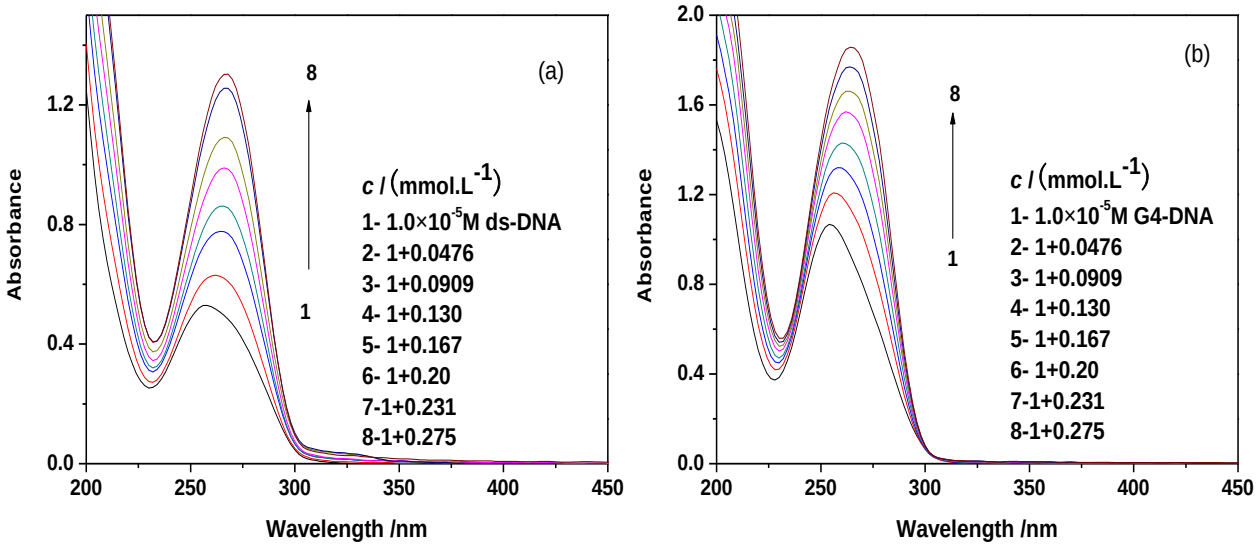

Figure 7. Ultraviolet–visible absorbance spectra of G4-DNA with different concentration of (a) (R)-5FUGlu, and (b) (S)-5FUGlu

(R)-5FUGlu, (S)-5FUGlu had an absorption peak at 268 nm, and G4-DNA has an absorption peak at 260 nm, which was from nucleotide base pairs on both the DNA strands. However, after the mixing of DNA and (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate, the spectrum was changed to spectra and the absorption peak moved to 274 nm, indicating the strong interaction between DNA and (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate. It was found that the absorption spectrum is significantly different from the sum of corresponding absorption spectrum of G4-DNA or (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate alone. In the experiment, the concentration of G4-DNA was always kept at 1.0×10^{-5} M, while the concentrations of (R)-5FUGlu, (S)-5FUGlu were increasing. Then, these solutions of mixtures were kept at 4 °C for 1h for full completion of binding between G4-DNA and (R)-/(S)-2-(5-fluorouracil-1-acetyl) amido-1,5-dimethyl glutarate. These results showed that the value of absorbance increased as the concentration of (R)-5FUGlu and (S)-5FUGlu increased, and a bathochromic shift of 6 nm was observed (Fig. 7). It could be attributed to the formation of new π-conjugated system. Bathochromic shift and hypochromic effect were also suggested due to a strong interaction between the electronic states of intercalative chromophores and those of DNA bases [35-39].

4. CONCLUSIONS

In this work, the electrochemical behavior of anticancer drug (R)-5FUGlu, (S)-5FUGlu and their interaction with G4-DNA were investigated by electrochemical and spectroscopic methods. The experimental result indicated that the principal interaction mode of (R)-5FUGlu and (S)-5FUGlu with G4-DNA was a kind of cooperative intercalative interaction. The interaction could be quantified in terms of the Hill model of cooperative interactions. The result also demonstrated that the electrochemistry was available and provided significant promise to study the mechanism of the interaction of G4-DNA with targeting compounds at both the macrocosmic and molecular levels. The result could serve as good references for the synthesis, structural characterization of new 5-FU derivatives.

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