



Synthesis, characterization and DNA binding study of silver(I) complexes with 2'-thiazolylazo aminoanisole compounds (TAAA)

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Abstract

Bioinorganic chemistry has been attracted for many years on the biological studies of transition metal complexes, including those based on the silver(I) ion. Since azo compounds have significant biological activity, the development of new silver(I) complexes with such compounds might be of interest. Azo dyes 2-(2'-thiazolylazo)-5-aminoanisole (*p*-TAAA) and 4-(2'-thiazolylazo)-3-aminoanisole (*o*-TAAA) and their silver(I) complexes; [Ag-(*p*-TAAA)] and [Ag-(*o*-TAAA)] have been prepared and characterized by elemental analysis, mass spectrometry, IR spectroscopy, ¹H and ¹³C NMR spectroscopy. The results reveal structures of the synthesized complexes in which silver(I) ion have bound to two molecules of 2'-thiazolylazo aminoanisole compounds with nitrogen of azo group and sulfur of thiazole ring as donor atoms. The biological activity of such complexes was examined using calf thymus DNA (CT-DNA). The mode of DNA binding interaction was investigated by electronic absorption titration. The results indicate that the synthesized silver(I) complexes bind to CT-DNA *via* intercalative mode of binding with binding constants of $1.44 \times 10^5 \text{ M}^{-1}$ for [Ag-(*p*-TAAA)] and $9.77 \times 10^4 \text{ M}^{-1}$ for [Ag-(*o*-TAAA)], respectively.

Keywords: Thiazolylazo, Silver(I) complexes, DNA binding, Electronic absorption titration, Binding constants (K_b)

Introduction

Azo dyes are commonly recognized for their vivid colors, therefore, they have been utilized in several manufactures such as textiles, paints, medicines including analytical chemistry (Benkhaya, El Harfi, & El Harf, 2017; Mittal, Kurup, & Mittal, 2007). The compounds are generally synthesized by the reaction of primary aromatic amine *via* diazotization reaction followed by coupling reaction with phenol or aromatic amines (Robert, Robert, & Bhattacharjee, 2011; Zollinger, 2003). Hence, in order to generate a wide range of azo compounds, the derivatives of aromatic amines and phenols have been continuously developed. Recently, Sujatha et al. synthesized novel azo compounds using a bioactive 2-amino benzothiazole fused with synthetic phenolic antioxidants to study their versatile biological applications (Prakash et al., 2020). Moreover, a series of novel imidazole bound to azo-imidazole derivatives studied by Brahman and co-workers were carried out to determine the inhibitory action against the main protease (6LU7) of novel coronavirus (COVID-19) (Chhetri et al., 2021). Several studies on azo dyes and their applications could indicate the importance of structural modifying to improve properties of these compounds for the specific purposes. Apart from utilization of azo dyes, the compounds have also been employed in numerous studies in the interest of coordination chemistry. Metal complexes of azo dyes have been attracted in several research owing to the enhanced stability caused by the coordination of such azo ligands toward the metal centers (Clark, 2011; El-Wakiel, Rizk, & Ibrahim, 2017). One of the most attractive applications of azo dye complexes is associated



with biological studies such as antibacterial, anti-inflammatory, and antitumor activity (Mahmoud, Omar, & Sayed, 2016; Matada & Jathi, 2019) in which DNA is the primary target of these complexes. The interaction between DNA and these substrates cause DNA damage by the preventing of cell division and leading to cell death (Mathur & Tabassum, 2008; Nath, Singh, Eng, & Song, 2008). Amongst several transition metal complexes, silver(I) complexes were found to be the most effective substrate in antibacterial and antifungal studies for several years (Cavicchioli et al., 2010; Nawaz et al., 2011). In recent times, a new silver(I) pyridine-2-sulfonate was prepared and investigated in DNA binding properties and cytotoxic mechanism. The complex was discovered to perform higher antimicrobial activity than silver sulfadiazine which is a commercially used drug (Rendosova et al., 2018). Furthermore, the Bhatti group reported the synthesis of binuclear silver(I)-N-heterocyclic carbene complexes and examined their anticancer activity against HCT116 human colon cancer cell. The studied compounds were investigated in the interaction with DNA in which the results indicated that the silver(I) complexes could be a potential target for biological activities (Ashraf, Bhatti, Iqbal, & Jamil, 2020).

According to the ability of silver(I) complexes accompanied by the capability of azo dyes in biological applications, the combination of these moieties is supposed to provide favorable efficiency of the coordination compounds. Consequently, these results have encouraged to provide the silver(I) complexes of novel azo dyes 2-(2'-thiazolylazo)-5-aminoanisole (*p*-TAAA) and 4-(2'-thiazolylazo)-3-aminoanisole (*o*-TAAA) and investigate their interaction with CT-DNA. As this preliminary study could indicate the interaction capability of such complexes with biological molecules in which the acquired results could be expressed the further utilization of the complexes in several biological applications.

Methods and Materials

Materials

All chemicals used were of the analytical grade of highest purity available. Chemicals used in ligands and complexes synthesis were purchased from Aldrich, Fisher, Merck and RCI Labscan. Protein free calf thymus DNA (CT-DNA) from Aldrich was stored at 0–4 °C and its purity was checked by the measurement in its absorption at 260 nm. Tris-HCl buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.2) was used for the preparation of DNA stock solutions. Ethidium bromide (EB) was obtained from Loba Chemie and used without further purification.

Instruments

IR spectra were recorded on a Perkin-Elmer Spectrum GX spectrophotometer in wavenumber region 4000–400 cm⁻¹. The NMR spectra were analyzed using 400 MHz (Bruker). Mass spectrometric analysis was performed using the ESI technique (Agilent 6540 liquid chromatography/mass spectrometry (LC/MS) system. The binding study with CT-DNA was studied by absorption titration using UV-Vis spectrophotometer (Analytik Jena, SPECORD® 200 PLUS).

Synthesis of 2'-thiazolylazo aminoanisole (TAAA)

2-aminothiazole (1.00 g, 10.0 mmol) dissolved in 6 M HCl (16 mL) was cooled and slowly added by an aqueous solution of sodium nitrite (0.70 g, 10.2 mmol). The reaction was kept under low temperature (-5 °C to 0 °C) to give diazonium salt solution. The given solution was slowly poured into a cooled solution of



m-anisidine (1.00 g, 9.2 mmol) in 4 M HCl (40 mL). After an hour of stirring, 4 M NaOH was added until pH 6.0 was reached and red precipitate began to settle immediately. The solution was filtered and the precipitate was washed with water and dried at 80°C in an oven to give the mixture product consisted of 2-(2'-thiazolylazo)-5-aminoanisole (*p*-TAAA) and 4-(2'-thiazolylazo)-3-aminoanisole (*o*-TAAA). Each compound was separated by flash column chromatography using silica gel 60 (0.040–0.063 mm) as a stationary phase and a mixture of ethyl acetate: hexane (30:70) as a mobile phase. The products were purified by recrystallization with a mixture of ethanol–water (3:1).

2-(2'-thiazolylazo)-5-aminoanisole (*p*-TAAA)

Red powder. *Anal.* Cal. for C₁₀H₁₀N₄OS (%): C, 51.28; H, 4.29; N, 23.91; O, 6.83; S, 13.69. Found: C, 51.34; H, 4.20; N, 23.98; O, 7.31; S, 13.17. *m/z*⁺ Cal. 234.28. Found: 235.0629. Melting point: 170–173 °C. IR (ATR, cm⁻¹): ν(N-H) 3328, ν(C=N) 1615, ν(N=N) 1541, ν(C-N) 1190, ν(C-S) 1157, ν(C-O) 1017. ¹H NMR [400 MHz, DMSO-*d*₆, δ(ppm), *J*(Hz)]: 7.88 (1H, d, H_B, *J*_{AB} = 3.40 Hz), 7.70 (2H, s, H_F), 7.62 (1H, d, H_A, *J*_{AB} = 3.40 Hz), 7.56 (1H, d, H_C, *J*_{CD} = 8.78 Hz), 6.38 (1H, dd, H_D, *J*_{DE} = 2.60 Hz, *J*_{DC} = 8.78 Hz), 6.35 (1H, d, H_E, *J*_{ED} = 2.60 Hz), 3.80 (3H, s, H_G). ¹³C NMR [101 MHz, DMSO-*d*₆, δ(ppm)]: 176.82 (C3), 159.81 (C9), 151.78 (C7), 140.50 (C1), 130.68 (C5), 117.67 (C2), 115.92 (C6), 108.94 (C8), 99.35 (C4), 55.87 (C10). Yield: 42.93%

4-(2'-thiazolylazo)-3-aminoanisole (*o*-TAAA)

Green powder. *Anal.* Cal. for C₁₀H₁₀N₄OS (%): C, 51.28; H, 4.29; N, 23.91; O, 6.83; S, 13.69. Found: C, 49.34; H, 4.31; N, 23.90; O, 8.72; S, 13.73. *m/z*⁺ Cal. 234.28. Found: 235.0629. Melting point: 207–211 °C. IR (ATR, cm⁻¹): ν(N-H) 3343, ν(C=N) 1600, ν(N=N) 1541, ν(C-N) 1205, ν(C-S) 1130, ν(C-O) 1015. ¹H NMR [400 MHz, DMSO-*d*₆, δ(ppm), *J*(Hz)]: 7.83 (1H, d, H_B, *J*_{AB} = 3.40 Hz), 7.64 (1H, s, H_C, *J*_{CD} = 9.08 Hz), 7.53 (1H, d, H_A, *J*_{AB} = 3.40 Hz), 6.81 (1H, s, H_F), 6.30 (1H, dd, H_D, *J*_{DE} = 2.37 Hz, *J*_{DC} = 9.08 Hz), 6.28 (1H, d, H_E, *J*_{ED} = 2.37 Hz), 3.87 (3H, s, H_G). ¹³C NMR [101 MHz, DMSO-*d*₆, δ(ppm)]: 176.81 (C3), 161.40 (C7), 160.81 (C9), 145.78 (C1), 140.68 (C5), 127.60 (C2), 119.23 (C6), 105.27 (C4), 99.40 (C8), 55.40 (C10). Yield: 21.70%

Synthesis of silver(I) complexes

Silver(I) complexes were obtained by stirring a methanol solution (10 mL) of each 2'-thiazolylazo aminoanisole compound (0.47 g, 2.0 mmol) with 3 mL of an aqueous solution of AgNO₃ (0.17 g, 1.0 mmol). The reaction mixture was kept under stirring in the absence of light at room temperature for 72 h. The products were filtered *in vacuo*, washed with methanol, and dried under reduced pressure to obtain as fine solids.

Ag(I)- 2-(2'-thiazolylazo)-5-aminoanisole [Ag-(*p*-TAAA)]

Dark red powder. *Anal.* Cal. for C₂₀H₂₀AgN₈O₂S₂ (%): C, 41.67; H, 3.50; Ag, 18.11; N, 19.44; O, 5.55; S, 11.12. Found: C, 42.34; H, 4.20; Ag, 17.51; N, 20.98; O, 4.42; S, 10.55. *m/z*⁺ Cal. 576.42. Found: 577.0200. Melting point: 182–185 °C. IR (ATR, cm⁻¹): ν(N-H) 3320, ν(C=N) 1610, ν(N=N) 1490, ν(C-N) 1190, ν(C-S) 1205, ν(C-O) 1013. ¹H NMR [400 MHz, DMSO-*d*₆, δ(ppm), *J*(Hz)]: 7.88 (1H, d, H_B, *J*_{AB} = 3.40 Hz), 7.75 (2H, s, H_F), 7.64 (1H, d, H_A, *J*_{AB} = 3.40 Hz), 7.55 (1H, d, H_C, *J*_{CD} = 8.78 Hz), 6.38 (1H, dd, H_D, *J*_{DE} = 2.60 Hz, *J*_{DC} = 8.78 Hz), 6.35 (1H, d, H_E, *J*_{ED} = 2.60 Hz), 3.81 (3H, s, H_G). ¹³C NMR [101 MHz, DMSO-*d*₆, δ(ppm)]: 179.01 (C3), 161.01 (C9), 157.85



(C7), 142.39 (C1), 131.88 (C5), 119.71 (C2), 119.22 (C6), 108.45 (C8), 95.43 (C4), 55.70 (C10). Yield: 72.68%

Ag(I)- 4-(2'-thiazolylazo)-3-aminoanisole [Ag-(*o*-TAAA)]

Dark red powder. *Anal.* Cal. for $C_{20}H_{20}AgN_8O_2S_2$ (%): C, 41.67; H, 3.50; Ag, 18.11; N, 19.44; O, 5.55; S, 11.12. Found: C, 41.98; H, 3.62; Ag, 18.23; N, 18.90; O, 6.51; S, 10.76. m/z^+ Cal. 576.42. Found: 577.0198. Melting point: 218–223 °C. IR (ATR, cm^{-1}): $\nu(N-H)$ 3330, $\nu(C=N)$ 1625, $\nu(N=N)$ 1575, $\nu(C-N)$ 1208, $\nu(C-S)$ 1154, $\nu(C-O)$ 1012. 1H NMR [400 MHz, DMSO- d_6 , δ (ppm), J (Hz)]: 7.84 (1H, d, H_B , $J_{AB} = 3.40$ Hz), 7.67 (1H, s, H_C , $J_{CD} = 9.08$ Hz), 7.57 (1H, d, H_A , $J_{AB} = 3.40$ Hz), 6.93 (1H, s, H_F), 6.30 (1H, dd, H_D , $J_{DE} = 2.37$ Hz, $J_{DC} = 9.08$ Hz), 6.28 (1H, d, H_E , $J_{ED} = 2.37$ Hz), 3.87 (3H, s, H_G). ^{13}C NMR [101 MHz, DMSO- d_6 , δ (ppm)]: 178.39 (C3), 164.77 (C7), 162.31 (C9), 147.82 (C1), 142.66 (C5), 130.52 (C2), 119.64 (C6), 107.52 (C4), 97.89 (C8), 55.56 (C10). Yield: 50.91%

DNA binding study

Stock solution of calf thymus DNA (CT-DNA) was prepared in Tris-HCl buffer (NaCl 50 mM/Tris-HCl 5 mM, pH 7.2). The ratio of UV absorbance's at 260 and 280 nm (A_{260}/A_{280}) was measured to determine whether CT-DNA is adequately free of protein (El-Sonbati et al., 2017). In addition, the concentration of CT-DNA was calculated using molar absorption coefficient (ϵ) $6600 M^{-1} cm^{-1}$ with the absorption intensity at 260 nm (Reichmann, Rice, Thomas, & Doty, 1954). The electronic absorption spectra at the wavelength range of 250–650 nm were examined by fixing the concentration of silver(I) complexes at $1.00 \times 10^{-3} M$ with the increasing concentration of CT-DNA from 0.25 to 2.00 μM by successive addition. The binding constant (K_b) of the complexes with CT-DNA was determined using the following equation (Wolfe, Shimer Jr., & Meehan, 1987)

$$[DNA]/\epsilon_a - \epsilon_f = [DNA]/\epsilon_b - \epsilon_f + 1/K_b (\epsilon_b - \epsilon_f)$$

where [DNA] is the concentration of CT-DNA in base pairs, ϵ_a is the extinction coefficient for the $A_{obs}/[complex]$ at the given CT-DNA concentration, ϵ_f is the extinction coefficient of the free complex in solution and ϵ_b is the extinction coefficient of the complex fully bound to CT-DNA. The plots of $[DNA]/(\epsilon_a - \epsilon_f)$ versus [DNA] gives K_b by the ratio of the slope to the intercept.

Results and Discussion

Synthesis of 2'-thiazolylazo aminoanisole (TAAA) and silver(I) complexes

2'-thiazolylazo aminoanisole compounds was synthesized *via* diazotization of 2-aminothiazole, then coupling with *m*-anisidine. The crude of a mixture product was separated by flash column chromatography to give 2-(2'-thiazolylazo)-5-aminoanisole (*p*-TAAA) and 4-(2'-thiazolylazo)-3-aminoanisole (*o*-TAAA). Synthesis pathway and chemical structures of both compounds are presented in Figure 1.

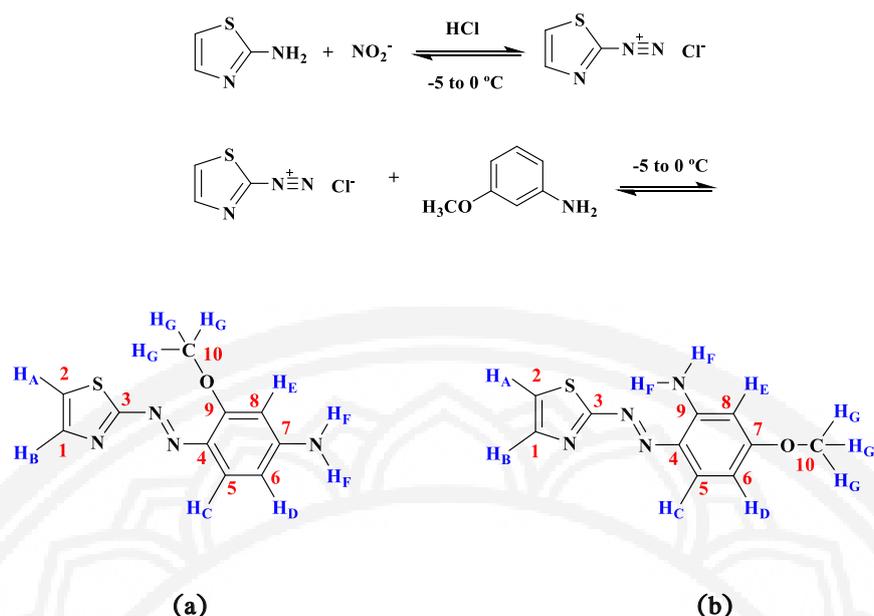


Figure 1 Synthesis pathway and chemical structures of (a) 2-(2'-thiazolylazo)-5-aminoanisole (*p*-TAAA) and (b) 4-(2'-thiazolylazo)-3-aminoanisole (*o*-TAAA) with hydrogen and carbon atoms numbering

The silver(I) complexes were prepared by the reaction of ligands TAAA with AgNO_3 in methanol solution. Structures of the synthesized complexes $\text{Ag(I)}-2-(2'-\text{thiazolylazo})-5\text{-aminoanisole}$ [$\text{Ag}-(p\text{-TAAA})$] and $\text{Ag(I)}-4-(2'-\text{thiazolylazo})-3\text{-aminoanisole}$ [$\text{Ag}-(o\text{-TAAA})$] are shown in Figure 2.

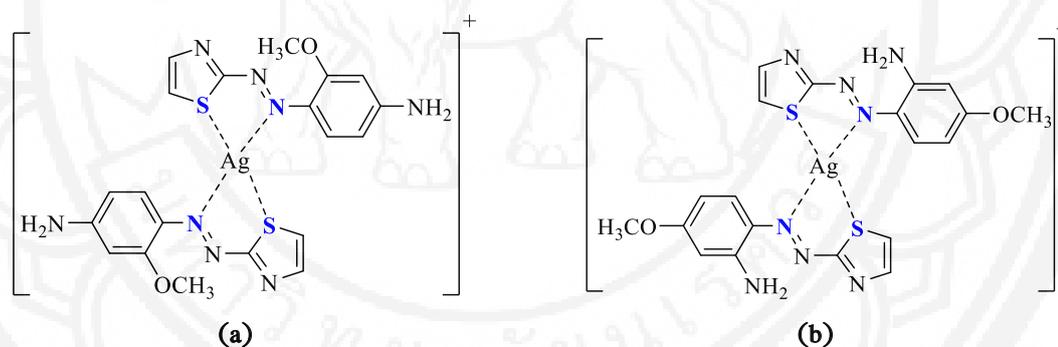


Figure 2 Chemical structures of (a) $\text{Ag(I)}-2-(2'-\text{thiazolylazo})-5\text{-aminoanisole}$ [$\text{Ag}-(p\text{-TAAA})$] and (b) $\text{Ag(I)}-4-(2'-\text{thiazolylazo})-3\text{-aminoanisole}$ [$\text{Ag}-(o\text{-TAAA})$]

Characterization of 2'-thiazolylazo aminoanisole (TAAA) and silver(I) complexes

Infrared spectra

Infrared spectroscopy could be employed to identify the donor atoms of ligands TAAA in silver(I) complexes. For ligand *p*-TAAA and [$\text{Ag}-(p\text{-TAAA})$] complex, the minor differences in wavenumbers could be noticed from the peaks of C=N, C-N and C-O stretching. The large changes in these numbers were observed from two peaks, the peaks assigned for N=N stretching at 1541 cm^{-1} and C-S stretching at 1157 cm^{-1} in which these peaks were shifted to 1590 cm^{-1} and 1205 cm^{-1} after the complex formation (Figure 3). The changes in IR spectra could indicate that nitrogen atom of N=N azo functional group of ligand *p*-TAAA



performs as a donor atom in [Ag-(*p*-TAAA)] complex and this result is comparable to the literature (Mahmoud et al., 2016). In addition, nitrogen and sulfur atoms of thiazole ring and oxygen atom of methoxy group (-OCH₃) can also act as donor atoms in the forming complex. However, the shifts in wavenumbers could specify that sulfur atom of thiazole ring displays as a coordinating atom. This is the results of more extensive change in C-S bond frequency of vibration (48 cm⁻¹) observed from IR spectra in comparison to the change in C-O of -OCH₃ (4 cm⁻¹) and no change in C-N of thiazole ring stretching.

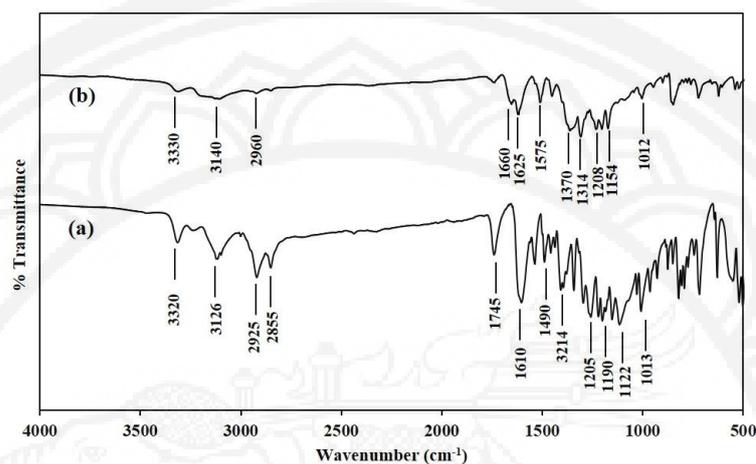


Figure 3 The FT-IR spectra of silver(I) complexes with ligands TAAA: (a) [Ag-(*p*-TAAA)] and (b) [Ag-(*o*-TAAA)]

The similar tendency in frequency changes were also observed for ligand *o*-TAAA and [Ag-(*o*-TAAA)] complex. The major differences in wavenumbers were detected from N=N and C-S stretching in which the peaks were shifted from 1541 cm⁻¹ to 1575 cm⁻¹ and from 1130 cm⁻¹ to 1154 cm⁻¹, respectively. Therefore, it can be concluded that nitrogen atom of N=N azo group and sulfur atom of thiazole ring perform as donor atoms in the synthesized silver(I) complex.

NMR spectra

The NMR spectra of ligands and silver(I) complexes were recorded in DMSO-*d*₆ at room temperature. In *p*-TAAA ligand, the distinct doublet peaks were observed at 7.88 ppm (H_B) and 7.62 ppm (H_A) assigned for the protons which are adjacent to nitrogen and sulfur atoms of thiazole ring. Protons of amine group (H_F) resonate at the chemical shift of 7.70 ppm as a singlet peak along with the protons of methoxy group (H_G) which present the chemical shift at 3.80 ppm. Other protons of *m*-anisidine display the resonances at the chemical shifts range of 7.56–6.35 ppm. These proton signals shift slightly after the complex formation (Figure 4). The shifts in NMR signals of thiazole ring protons must be extensively mentioned because the changes in chemical shifts at these positions could indicate the donor atoms of ligands *p*-TAAA in the synthesized complex. The ¹H NMR signal of H_A displays a downfield shift from 7.62 to 7.64 ppm whereas the signal of H_B remains consistent at 7.88 ppm. The result demonstrates that sulfur atom of thiazole ring performs as a donor atom in silver(I) complex as a consequence of the lower electron density after the donation of its electrons to the metal center and leading to the higher chemical shift in ¹H NMR spectrum. The similar observation was achieved from *o*-TAAA and its complex. The resonances at 7.83 ppm (H_B) and 7.53 ppm (H_A) in free *o*-TAAA appear at 7.84 and 7.57 ppm in silver(I) complex instead (Figure 5). More

significant difference in chemical shift arises from proton closed to sulfur atom of thiazole ring which can affirm that such atom evolves into the coordinating position in the synthesized complex. In addition, ^{13}C NMR results were also employed to verify the coordination of ligands toward the metal center in which the obtained results are corresponding to data collected from ^1H NMR studies.

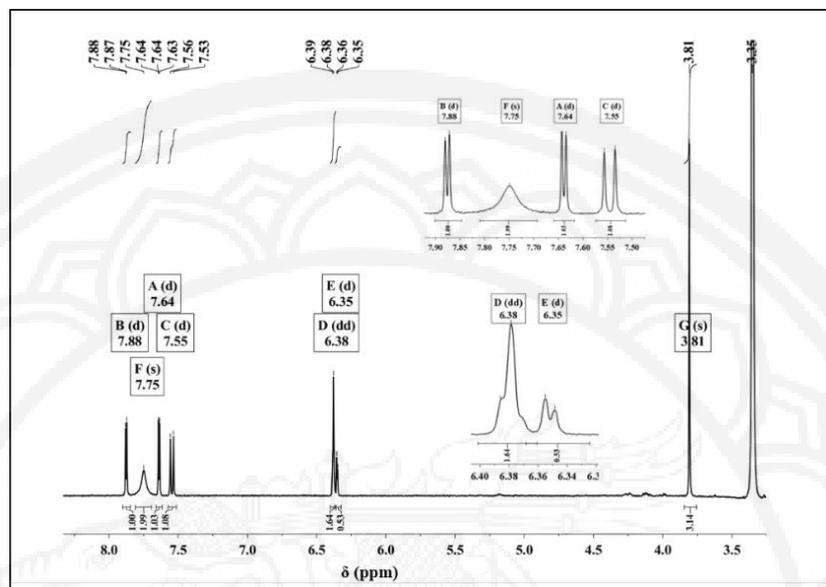


Figure 4 The ^1H NMR spectrum of $[\text{Ag}-(p\text{-TAAA})]$

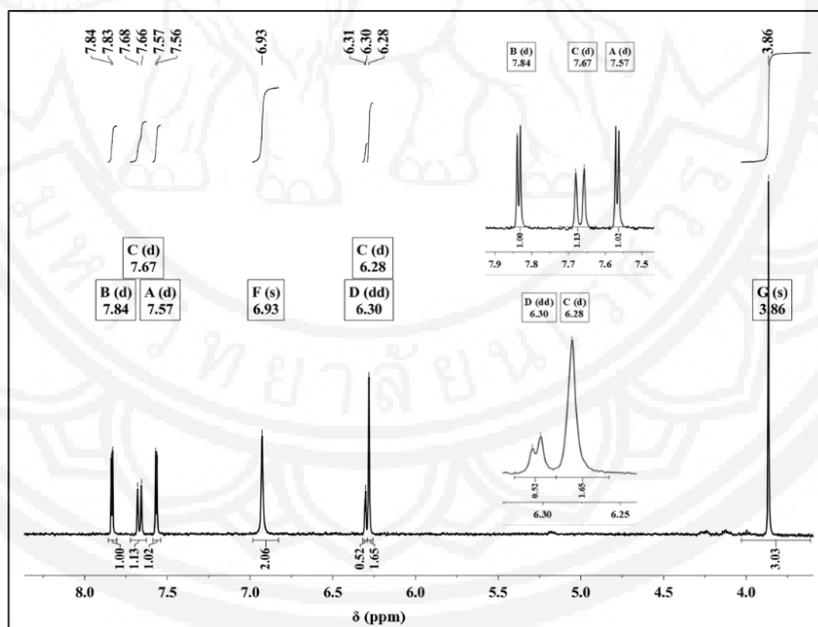


Figure 5 The ^1H NMR spectrum of $[\text{Ag}-(o\text{-TAAA})]$

Mass spectra

The mass spectra of the synthesized silver(I) complexes were determined by relative intensity molecular ion peaks at 100 eV. Mass spectra of both complexes provided good evidence for the molecular formula with



well-defined signals at m/z (cal.) = 577.0200 (576.42) $[M+H]^+$ for $[Ag-(p-TAAA)]$ and 577.0198 (576.42) $[M+H]^+$ for $[Ag-(o-TAAA)]$, respectively.

The results from all characterization techniques can verify the formation of silver(I) complexes with 2'-thiazolylazo aminoanisoole compounds. Mass spectra demonstrate the molecular mass of such complexes which reveal that silver(I) ion bound to two molecules of the synthesized ligands. IR and NMR spectroscopy can indicate donor atoms of such ligands in silver(I) complexes in which nitrogen of azo group and sulfur of thiazole ring donate their lone pairs of electrons toward the metal center to form entirely stable 5-membered ring chelate complexes as the structure shown in Figure 2.

DNA binding study

The mode of interaction between metal complexes and DNA depends on several factors including nature and geometry of the ligands and the molecular structure of the complexes (Zeglis, Pierre, & Barton, 2007). In order to examine the interaction of silver(I) complexes with CT-DNA, electronic absorption titration using UV-Vis spectroscopy has been employed. The changes in the absorption bands of CT-DNA in UV region and the bands of complexes in visible region could demonstrate the interaction and feasible mode of binding. The spectra of both complexes from electronic absorption titration are presented in Figure 6 and Figure 7.

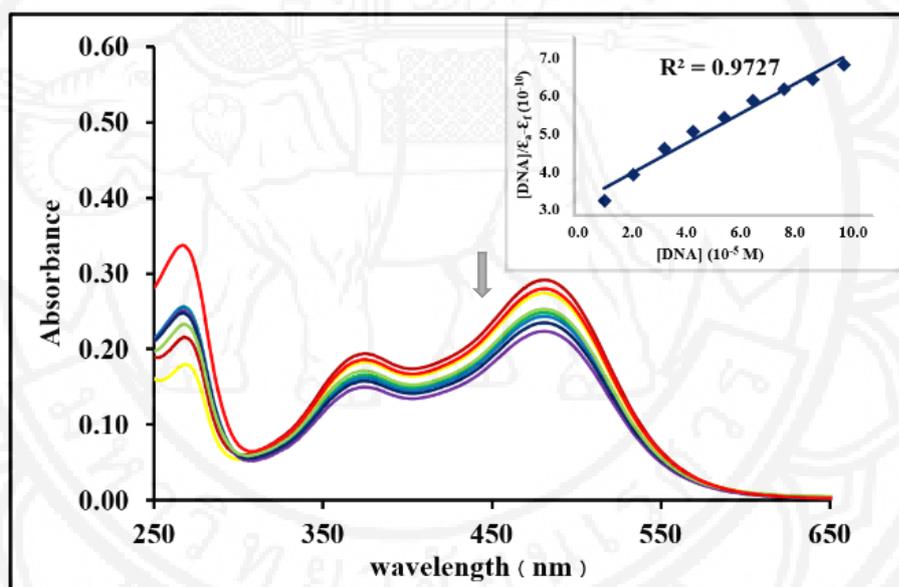


Figure 6 Electronic absorption spectra of $[Ag-(p-TAAA)]$ in the absence and presence of increasing amount of CT-DNA

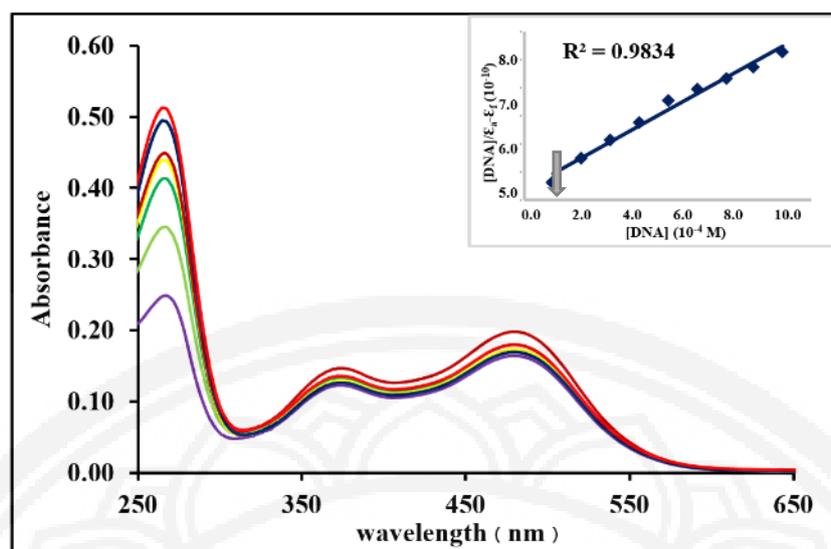


Figure 7 Electronic absorption spectra of [Ag-(*o*-TAAA)] in the absence and presence of increasing amount of CT-DNA

The spectra of [Ag-(*p*-TAAA)] complex display significant hypochromic shifts up to 19.90% at λ_{\max} 487 nm upon the increasing amount of CT-DNA. The results indicate that the added CT-DNA interact with the synthesized complex and leading to the lower intensity of its absorption band. Moreover, the spectra also present small red shift up to 490 nm which could affirm such the interaction. These changes may be attributed to π - π stacking interactions between the synthesized complex and DNA base pairs (Li et al., 2007) and related to the intercalative mode of binding (Pyle et al., 1989). Similar changes in the UV-Vis spectra of [Ag-(*o*-TAAA)] were also observed. The percentage of hypochromism observed for this complex is up to 8.83%. The DNA binding constants (K_b) of the complexes were calculated by the Wolfe-Shimer equation in which the K_b values of [Ag-(*p*-TAAA)] and [Ag-(*o*-TAAA)] are $1.44 \times 10^5 M^{-1}$ and $9.77 \times 10^4 M^{-1}$, respectively. K_b value of [Ag-(*p*-TAAA)] is higher than [Ag-(*o*-TAAA)] which suggests a stronger binding of such complex with CT-DNA. However, the obtained binding constants of the synthesized complexes are nearly the same as the classical intercalator EB ($1.74 \times 10^5 M^{-1}$) (Tang, 1999) which indicate favorable affinity of these silver(I) complexes with CT-DNA *via* the intercalative binding.

Conclusion and Suggestions

In this research, 2-(2'-thiazolylazo)-5-aminoanisole (*p*-TAAA) and 4-(2'-thiazolylazo)-3-aminoanisole (*o*-TAAA) and their silver(I) complexes; [Ag-(*p*-TAAA)] and [Ag-(*o*-TAAA)] have been prepared. The synthesized azo compounds and their complexes were fully characterized by spectroscopic techniques which all verified the formation of the desired products. The interaction of these complexes with calf thymus DNA (CT-DNA) was investigated and the results from electronic absorption titration indicated that the DNA binding mode of the complexes was intercalation. The study also revealed greater binding constant (K_b) of [Ag-(*p*-TAAA)] which suggest superior capability in the interaction with CT-DNA than [Ag-(*o*-TAAA)]. According to great ability of the synthesized silver(I) complexes with CT-DNA, these new complexes are promising for versatile biological studies such as antibacterial and anticancer activities.



However, the mode of DNA binding could be affirmed by luminescence titration and toxicity of the complexes against biological molecules should be further investigated.

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References

- Ashraf, R., Bhatti, H. N., Iqbal, M. A., & Jamil, Y. (2020). Synthesis of aryl linked binuclear silver N-heterocyclic carbene complexes, DNA interaction study and biological potentials. *Inorganic Chemistry Communications*, *119*, 108077.
- Benkhaya, S., El Harfi, S., & El Harf, A. (2017). Classifications, properties and applications of textile dyes: A review. *Applied Journal of Environmental Engineering Science*, *3*, 311–320.
- Cavicchioli, M., Massabni, A. C., Heinrich, T. A., Costa-Neto, C. M., Abrão, E. P., Fonseca, B. A. L., ... Leite, C. Q. F. (2010). Pt(II) and Ag(I) complexes with acesulfame: Crystal structure and a study of their antitumoral, antimicrobial and antiviral activities. *Journal of Inorganic Biochemistry*, *104*(5), 533–540.
- Chhetri, A., Chettri, S., Rai, P., Mishra, D. K., Sinha, B., & Brahman, D. (2021). Synthesis, characterization and computational study on potential inhibitory action of novel azo imidazole derivatives against COVID-19 main protease (Mpro: 6LU7). *Journal of Molecular Structure*, *1225*, 129230.
- Clark, M. (2011). *Handbook of Textile and Industrial Dyeing: Volume 1 Principles, Processes and Types of Dyes*. Oxford: Woodhead Publishing.
- El-Sonbati, A. Z., Diab, M. A., El-Bindary, A. A., Shoair, A. F., Hussein, M. A., & El-Boz, R. A. (2017). Spectroscopic, thermal, catalytic and biological studies of Cu(II) azo dye complexes. *Journal of Molecular Structure*, *1141*, 186–203. doi: 10.1016/j.molstruc.2017.03.082
- El-Wakiel, N. A., Rizk, H. F., & Ibrahim, S. A. (2017). Synthesis and characterization of metal complexes of azo dye based on 5-nitro-8-hydroxyquinoline and their applications in dyeing polyester fabrics. *Applied Organometallic Chemistry*, *31*(10), 3723.
- Li, Y. T., Song, C. H., Wang, Y. Q., Wei, Y., Wei, Y. J., & Hu, Y. Z. (2007). High photoluminescence quantum yield of TiO₂ nanocrystals prepared using an alcohothermal method. *Luminescence*, *22*(6), 540–545. doi: 10.1002/bio.997
- Mahmoud, W. H., Omar, M. M., & Sayed, F. N. (2016). Synthesis, spectral characterization, thermal, anticancer and antimicrobial studies of bidentate azo dye metal complexes. *Journal of Thermal Analysis and Calorimetry*, *124*(2), 1071–1089. doi: 10.1007/s10973-015-5172-1
- Mathur, S., & Tabassum, S. (2008). Template synthesis of novel carboxamide dinuclear copper (II) complex: spectral characterization and reactivity towards calf-thymus DNA. *Biometals*, *21*(3), 299–310. doi: 10.1007/s10534-007-9119-2



- Mittal, A., Kurup, L., & Mittal, J. (2007). Freundlich and Langmuir adsorption isotherms and kinetics for the removal of Tartrazine from aqueous solutions using hen feathers. *Journal of Hazardous Materials*, *146*(1–2), 243–248. doi: 10.1016/j.jhazmat.2006.12.012
- Nath, M., Singh, H., Eng, G., & Song, X. Q. (2008). New di- and triorganotin(IV) derivatives of tyrosinylphenylalanine as models for metal-protein interactions: Synthesis and structural characterization. Crystal structure of Me₂Sn(Tyr-Phe)·MeOH. *Journal of Organometallic Chemistry*, *693*(15), 2541–2550. doi: 10.1016/j.jorgchem.2008.04.032
- Nawaz, S., Isab, A. A., Merz, K., Vasylyeva, V., Metzler-Nolte, N., Saleem, M., & Ahmad, S. (2011). Synthesis, characterization and antimicrobial studies of mixed ligand silver(I) complexes of triphenylphosphine and heterocyclic thiones: Crystal structure of bis[μ (2-diazinane-2-thione)(diazinane-2-thione)(triphenylphosphine)silver(I) nitrate]. *Polyhedron*, *30*(9), 1502–1506. doi: 10.1016/j.poly.2011.02.054
- Prakash, S., Somiya, G., Elavarasan, N., Subashini, K., Kanaga, S., Dhandapani, R., ... Sujatha, V. (2020). Synthesis and characterization of novel bioactive azo compounds fused with benzothiazole and their versatile biological applications. *Journal of Molecular Structure*, *1224*, 129016.
- Pyle, A. M., Rehmann, J. P., Meshoyrer, R., Kumar, C. V., Turro, N. J., & Barton J. K. (1989). Mixed-ligand complexes of ruthenium(II): factors governing binding to DNA. *Journal of the American Chemical Society*, *111* (8), 3051–3058.
- Reichmann, M. E., Rice, C. A., Thomas, C. A., & Doty, P. (1954). A Further Examination of the Molecular Weight and Size of Deoxypentose Nucleic Acid. *Journal of the American Chemical Society*, *76*, 3047–3053.
- Rendosova, M., Vargova, Z., Sabolova, D., Imrichova, N., Hudecova, D., Gyepes, R., & Elefantova, K. (2018). Silver pyridine-2-sulfonate complex-its characterization, DNA binding, topoisomerase I inhibition, antimicrobial and anticancer response. *Journal of Inorganic Biochemistry*, *186*, 206–216. doi: 10.1016/j.jinorgbio.2018.06.006
- Robert, T. M., Robert, N. B., & Bhattacharjee, S. K. (2011). *Organic Chem.* New Delhi: Prentice Hall.
- Tang, T. C., & Huang, H. J. (1999). Electrochemical Studies of the Intercalation of Ethidium Bromide to DNA. *Electroanalysis*, *11*(16), 1185–1190.
- Wolfe, A., Shimer Jr., G. H., & Meehan, T. (1987). Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA. *Biochemistry*, *26*(20), 6392–6396.
- Zeglis, B. M., Pierre, V. C., & Barton, J. K. (2007). Metallo-intercalators and metallo-insertors. *Chemical Communications*, *44*, 4565–4579. doi: 10.1039/b710949k
- Zollinger, H. (2003). *Color Chemistry Syntheses, Properties, Application of Organic Dyes and Pigments.* New York: Wiley-VCH.