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Synthesis and characterization some complexes of azo dye of pyrimidyniyl and evaluating their biological activity

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ABSTRACT:
The present study involves fabrication a heterocyclic azo dye: 2-[4-diamino-3-benzaldehyde diazenyl]pyrimidine (L). The dye was synthesized by reacting 2-aminopyrimidine with 4-diaminobenzaldehyde (*in situ*). The complexes of ([Ag(L)(H_2O)]NO_3, [Cd(L)$_2$]Cl_2 and [Hg(L)2]Cl_2) dyes were prepared and characterized by elemental analysis; mass spectra, molar conductivity and spectroscopies of infrared and UV-Vis. The L dye ligand is tridentate. The Cd(II) and Hg(II) are octahedral with mole ratio Metal:Ligand equal to 1:2 while Ag(I) complex is tetrahedral with mole ratio Metal:Ligand equal to 1:1. The molar conductivity indicates that Ag(I) complex is electrolyte (1:1) and Cd(II) and (II) are electrolytes with ratio 2:1. The synthesized ligand (L) and its complexes showed interest biological activity against *E-coli* and *Staph-aureus* bacteria and *Aspergillus Niger* and *Pinicillium.sp* fungi. The anti-activity of [Ag(L)(H_2O)]NO_3 complex against MCF-7 cell line and its cytotoxicity against WRL cell line were interesting in vitro.

KEYWORDS: Biological activity, azo dye, pyrimidinyl complexes, cytotoxicity

1. Introduction

The Azo dyes are important for many applications such as sensors, antibacterial, photochromosim(Cojocariu & Rochon 2004, Hunger et al. 2004, Hunger 2004, Paulo et al 2009). The chromophoric azo group –N=N- gives amazing properties for azo dyes such as the absorption in visible region, chromic and photochromic properties(Yuki 1998, Jaume 2012) bond to ion metals, binding to DNA(Al-Noaimi et al. 2016, Mohammed 2011). Azo dyes and their complexes play important role in the dye-sensitized solar cells (DSSCs) as sensitizers(Bagheri Novir & Hashemianzadeh 2015, Mahmood et al. 2015, Toor et al. 2018).
The complexes Cu(II), Ni(II), Co(II), Mn(II), Zn(II), and Pd(II) derived from pyrimidine ring azo Schiff-Base ligand exhibit good antimicrobial activities against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-positive bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*)(Gulcan et al. 2014).
The azo dye of 3,5-Bis(alkyl-1,3,4-oxadiazole-2-yl exhibits interesting properties such as biologically active molecules(Shridhar et al. 2016).
Pyrimidine and its derivatives show large application such as antibacterial(Bentley 2009), anticancer, and liver disorder(Yazdanbakhsh et al. 2012). There are many marketed drugs for pyrimidine and its derivatives such as trimethoprim, piromidic acid, tetroxoprim, metioprim for antibacterial, flucytosine for antifungal, broxuridine and idoxuridine for antivirals, pyrantel embonate for anthelmintic, dipyridamole for vasodilators, uramustine, tegafur, methotrexate for antineoplastic(Ghasemi 2018). Therefore we are interested in synthesized the azo compound of pyrimidine and its complexes.

2. EXPERIMENTAL PART

Infrared spectra of L dye and its complexes were done by FT-IR 8400S Spectrophotometer, Shimadzu. The UV-Visible spectra were done by UV -1650 PC UV –Visible Spectrophotometer. Mass spectra were measured by Agilent Technologies 5973C-Shimadzu. Element analysis of C.H.N was measured by EURO EA 3000.
Materials: para-dimethylaminobenzaldehyde was purchased from Sigma-Eldrich. Hydrochloric acid and 2-aminopyrimidone were purchased from B.D.H company. Silver nitrate, cadmium(II) chloride and mercury(II) chloride were purchased from Merck company.
Cytotoxicity assays: MCF-7 cancer cell line of breast women and normal cells (non cancer cells) WRL were used in this study. We used MTT assay to measure cytotoxicity of silver (I) complex of L dye(Ghasemi et al. 2018). We used different concentrations of silver (I) complex which were 12.5, 25, 50, 100, 200, 400 µg/mL.
The antimicrobiology activity of synthesized compounds in this study was done by diffusion method. We used Muler-Hinton agar and ethanol as solvent. We did four holes in each petri dish by crok porer. We used 50, 75 and 100 mg/mL concentrations of each synthesized compound.
Synthesis L azo dye(Cheon et al. 1998): 2-aminopyrimidine (0.63 g; 0.006 mol) was added to 10 mL distilled water. 5 mL of HCl acid (12 molary) was added to solution of 2-aminopyrimidine. The acid solution of 2-aminopyrimidine was cooled to 0 °C. Para-dimethylaminobenzaldeheyde (1g, 0.006 mol) was dissolved in 20 ml of ethanol. Sodium acetate (3g) was dissolved in 8 mL distilled water. Sodium acetate solution was added to solution of para-dimethylaminobenzaldehyde and the new solution was put under cooling. Sodium nitrate (0.5 g, 0.006 mol) was dissolved in 10 mL cold distilated water. The cold solution of $NaNO_2$ was added to solution of para-dimethylaminobenzaldehyde.

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2-aminopyrimidine solution was added drop by drop to para-dimethylaminobenzaldehyde solution at 0°C under stirring for one hour. The reaction mixture was kept for overnight then it was filtreted. The green powder of azo dye was recrystallized by ethanol solvent and dried in the desiccator.

The complex of Cd(II) was synthesized by raitio 1:2 metal:Ligand. 0.1 g, 0.42 mol of L azo dye was dissolved in 20 mL of ethanol. Cadmium(II) chloride (0.036 g, 0.0021 mol) was dissolved in 10 mL distileted water with pH=8. Hot solution of L ligand was added to solution of cadmium salt. The mixture of reaction was refluxed at 60 °C for 15 minutes. The solution of reaction was filtrated. The precipitate was washed with water. The powder of cadmium complex was greenish yellow.

The Hg(II) complex was synthesized by the same method accepting the period of reflux was 180 minutes. The Ag(I) complex was synthesized by the same method accepting the mole ratio of reaction was 1:1 metal to ligand.

The yield of L azo dye:51%, FTIR(KBr,cm^{-1}): 3186, 2908, 2792, 1700, 1666, 1610, 1590, 810 due to ν(C-H aromatic), ν(C-H aliphatic), ν(C-H aldehyde), ν(C=O), ν(C=N), ν(N=N), ν(C=C), ν(C-H bending of aromatic). Anal. Calc. for $C_{13}H_{13}N_5O$: C, 61.17; H, 5.13; N, 27.44%. Found C,61.24 ; H,5.22; N, 27.38%. *M/Z*: 256.8

Ag(I) complex: bright green powder, yield 73% FTIR(KBr,cm^{-1}): 3085 , 2903, 2707, 1705, 1656, 1600, 1590, 810 due to ν(C-H aromatic), ν(C-H aliphatic), ν(C-H aldehyde), ν(C=O), ν(C=N), ν(N=N), ν(C=C), ν(C-H bending of aromatic). Anal. Calc. for $C_{13}H_{15}N_6O_5Ag$: C, 35.23; H, 3.41; N, 18.96%. Found C, 35.30; H, 3.49; N, 19.02%. *M/Z*: 381,8.

Cd(II) complex, Greenish yellow powder, yield 73% FTIR(KBr,cm^{-1}): 3186, 2916, 2730, 1700, 1600, 1542, 1442, 810, 725 due to ν(C-H aromatic), ν(C-H aliphatic), ν(C-H aldehyde), ν(C=O), ν(C=N), ν(N=N), ν(C=C), ν(C-H bending of aromatic). Anal. Calc. for $C_{26}H_{26}N_{10}O_2Cl_2Cd$: C, 45.01; H 3.78; N, 20.19%. Found C, 44.95; H, 3.68; N, 20.21%. *M/Z*: 313.6.

Hg(II) complex, yellow powder, yield 69% FT-IR(KBr,cm^{-1}): 3110, 2912, 2750, 1680, 1600, 1535, 1460, 815 due to ν(C-H aromatic), ν(C-H aliphatic), ν(C-H aldehyde), ν(C=O), ν(C=N), ν(N=N), ν(C=C), ν(C-H bending of aromatic). Anal. Calc. for $C_{26}H_{26}N_{10}O_2Cl_2Hg$: C, 39.93; H 3.35; N, 17.91%. Found C, 39.90; H, 3.32; N, 17.92%. *M/Z*: 356.09

3. Results and Discussion

The synthesized azo dye L was prepared by coupling 4-diaminobenzaldehyde with diazotized of 2-aminopyrimidine. The diazonium salt of pyrimidine was prepared *in situ* with coupling compound to protect the diazonium salt from decomposition[18].

The synthesized complexes were prepared by mole ratio Metal-Ligand: 1:1 in the case of Ag(I) complex while 1:2 in the case of Cd(II) and Hg(II) complexes which are depicted in Figure 1.

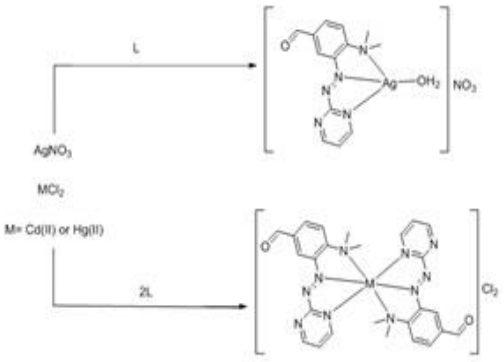

Figure 1 Synthesis the complexes of L azo dye ligand

The L dye and its complexes showed peaks for C-H aromatic, C-H aliphatic, C=O, C=N, N=N, C=C, C-H bending functional groups. The azo and C=N functional groups suffered changes in intensities and the frequency vibrations in the synthesized complexes with red shifts(Kılınçarslan et al. 2007). The molar conductivities of Cd(II) and Hg(II) complexes were 78 and 85 $S.cm^2.mol^{-1}$ respectively which means that the complexes are electrolytes with ratio 2:1. The molar conductivity of Ag(I) complex was 40 $S.cm^2.mol^{-1}$ which means that the complex is salt with ratio 1:1.

The elemental analysis (C.H.N) and mass spectral data were found in good agreement with the L dye and its synthesized complexes.

UV-Vis spectra of L ligand and its complexes (Figure 2) were done in ethanol at room temperature. The UV-Vis spectrum of L dye showed bands at 245 and 340 nm which are due to π→π* and other band at 420 nm which is due to n→π*(Melvin 2014 et al, Toro et al. 2011).

The Ag (I) complex spectrum showed band at 355 nm with red shift of 15 nm comparison to the L ligand(Shahriar 2012 et al). The spectrum of Cd(II) complex showed a band at 353 nm with red shift 13 nm comparison to the L ligand. The UV-Vis spectrum of Hg(II) complex showed bands at 423 and 355 nm with a red shift comparison to the L ligand. Additionally, the complex of Hg(II) showed bands at 320 and 240 nm which are due to charge transfer transition from Ligand to Metal(Mugesh et al. 1998).

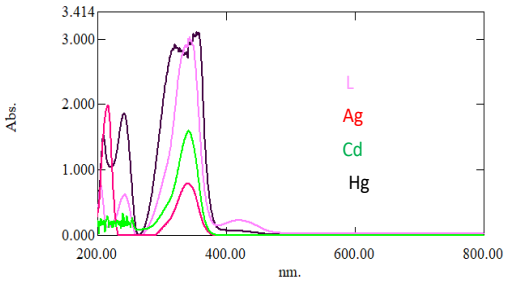

Figure 2 UV-Vis spectra of L dye and its complex at room temperature in ethanol

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The anti-activities of the L azo dye and its complexes of Ag(I), Cd(II) and Hg(II) against *E-coli* and *Staph-aureus* bacteria are depicted in Table 1. The data in table 1 showed that L dye and its complexes exhibit remarkable properties against both type of bacteria. The L dye and Ag(I) complex exhibited good activity against the bacteria while Cd(II) and Hg(II) complexes exhibited perfect activity against the bacteria. These results are possible due to lipophilic behaviour of the compounds. The lipid membrane of the cells prefers to pass liquid-soluble particles(Shridhar et al. 2016).

Table 1 Antibacterial activities of L azo dye and its complexes

Bacteria	Concentration mg/ml	The diameter inhibition zone (cm)			
		L dye	Ag(I)	Cd(II)	Hg(II)
E- coli (Gram- negative)	100	1	2.5	2.5	8
	75	0.5	1	0.5	8
	50	1	—	-	8
Staph- aureus (Gram- positive)	100	3	1	8	8
	75	1.5	1	8	8
	50	2	0.5	8	8

The L dye and complexes exhibited good activity against *Aspergillus Niger* and *Pinicillium.sp* fungi which listed in Table 2. The diameter inhibition zone of synthesized compounds were 8-3.5 cm. The L dye exhibited perfect antiacivitiy against fungi while was good against bacteria.

Table 2 Antifungal activities of L azo dye and its complexes

Fungi	Concentration mg/ml	The diameter inhibition zone (cm)			
		L dye	Ag(I)	Cd(II)	Hg(II)
Aspergillus Niger	100	3.5	5	—	8
	75	7	—	3.5	8
	50	—	5	3.5	8
Pinicillium.sp	100	7	8	7.5	3.5
	75	8	2.5	—	7.5
	50	8	8	—	—

A B

Figure 3 Biological activity of Hg(II) complex: A-against *E-coli* B- against *Aspergillus Niger* at 50 mg/mL.

Anticancer activity evaluation of Ag(I) complex: We study the cytotoxicity of Ag(I) complex against the MCF-7 cell line and normal cell line (WRL) and the data of concentrations and viability of MCF-7 and WRL were depicted in Table 3 and 4 respectively. Figure 4 shows that cell viability of MCF-7 decreases with increasing the concentrations of Ag(I) complex while the cell viability of WRL does not decrease with increasing the concentrations of Ag(I) complex exceept at high concentrations of silver (I) complex. The concentration of Ag(I) complex which kills half cells line of WRL (IC50) is 292.3 µg/mL which means that the Ag(I) complex is not harmful for normal cell line(Jean-de-Dieu 2016 et al). On the other hand, the IC50 of Ag(I) complex against MCF-7 was 92.3 µg/mL which means that there is large difference between the IC50 of Ag(I) complex against MCF-7 and WRL cell line(Ghasemi et al. 2018).

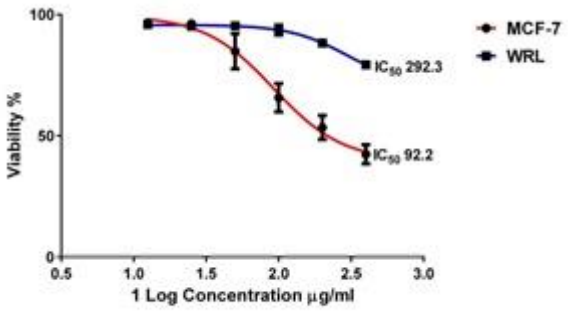

Figure 4 The cytotoxicity of Ag(I) complex on MCF-7 cell line and normal cells (WRL cell line)

Table 3 Data of antitumor activity of Ag(I) complex on MCF-7 cell line

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	Col. stats	A	B	C	D	E	F
		400	200	100	50	25	12.5
		Y	Y	Y	Y	Y	Y
1	Number of values	3	3	3	3	3	3
2							
3							
4	Mean	42.36	53.40	65.70	84.88	96.22	96.64
5	Std. Deviation	4.004	4.938	5.870	7.307	0.6782	1.365
6	Std. Error of Mean	2.312	2.851	3.389	4.219	0.3916	0.7878

Table 4 Data of the cytotoxicity of Ag(I) complex on cell line of WRL

	Col. stats	A	B	C	D	E	F
		400	200	100	50	25	12.5
		Y	Y	Y	Y	Y	Y
1	Number of values	3	3	3	3	3	3
2							
3							
4	Mean	79.28	88.27	93.60	95.33	95.22	95.95
5	Std. Deviation	0.6945	1.457	2.100	1.183	0.8209	1.028
6	Std. Error of Mean	0.4010	0.8410	1.212	0.6828	0.4739	0.5937

4. Conclusions

The azo dye 2-[4-diamino-3-benzaldehyde diazenyl]pyrimidine (L) and its complexes of Ag(I), Cd(II) and Hg(II) were synthesized and characterized. Depending on the data of molar conductivity, infrared spectra and elemental analysis, the Ag(I) complex has tetrahedral shape and the Cd(II) and Hg(II) complexes have octahedral environmental around the central metal. The azo ligand (L) acts as tridentate ligand in its synthesized complexes. L dye and its complexes possess interesting biological activities. In vitro, the Ag(I) complex exhibited interesting results which can be used as a drug to treat breast cancer.

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