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Research Article

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DNA Cleavage and Antimicrobial Activity Studies of Some New Sulfadoxine Complexes

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ABSTRACT

4-Amino-N (5,6-dimethoxy-4-pyrimidinyl)benzene sulfonamide, (SDX) sulfadoxine was reacted with Ag and Pt. The products were characterized by elemental (CHN) analysis, FTIR, conductance measurements, electronic spectra, NMR (^{1}H and ^{13}C) spectra. All complexes were investigated for their antibacterial activities against Gram-positive and Gram-negative bacteria and were also screened for DNA cleavage. Most complexes have higher antimicrobial activity than Sulfadoxine and complete cleavage of CT-DNA.

Keywords: Sulfadoxine; Complexes; Antibacterial activity; CT- DNA cleavages.

INTRODUCTION

A great deal of interest has been directed to pyrimidines due to their biological importance as Components of nucleic acid. Many compounds of therapeutic importance contain the pyrimidine ring system. Many studies have recently stressed the role of metal ions in important biological processes [1-5], whereas the inorganic pharmacology started to be an important field with more than 25 inorganic compounds being used in therapy as anti-bacterial, anti-viral and anticancer drugs [6-10]. This study aims at complexing the famous antimicrobial drug sulfadoxine , with Ag and Pt metals to study their anti-bacterial and DNA cleavage properties.

EXPERIMENTAL SECTION

Chemicals

The drugs, chemicals and solvents used in this study were of analytical grade and used as obtained from Aldrich without further purification. Sulfadoxine (4-Amino-N-(5, 6-dimethoxy-4-pyrimidinyl)benzenesulfonamide), Silver nitrate (AgNO3), Dipotassium Tetra Chloro Platinate (K2PtCl4) Methanol (CH3OH), deionized water.

Instrumental

The melting points were measured on an electro thermal melting point apparatus and were not corrected. Fourier-transform infrared spectra were recorded using the KBr disc technique on a JASCO 410 FTIR spectrophotometer. Elemental (CHN) analysis was performed using an Exeter CE-440 elemental analyzer. UV-visible absorption spectra were measured in DMF ($\approx 10^{-5}$ mole^{-1}) using a Pye–Unicam 8800a UV-visible automatic scanning spectrophotometer. Molar Conductivity was measured on a systronic conductivity bridge with a dip-type cell, using 1×10^{-3} M solution of complexes in DMF. ^{1}HNMR spectra of the ligands and their complexes were recorded on a Varian Gemini-200 spectrometer (200 MHZ) and (300MHZ) using DMSO-d_6 as solvent and TMS as internal reference. ^{13}C NMR spectra of the two complexes in DMSO were obtained using a Bruker 600MHZ using TMS as an internal standard. Microbiological analysis was carried out by the Micro analytical Center, Faculty of pharmacy, University of Science and Technology Sana'a.

Synthesis of silver nitrate ($AgNo_3$) with sulfadoxine complex

The compound was prepared by following procedure: 0.169 gm (0.001 mol) of ($AgNO_3$) was dissolved in 20 ml of methanol and 0.31 gm of sulfadoxine in 10 ml of methanol.

Synthesis of Di potassium Tetra Chloro Platinate (K_2PtCl_4) with sulfadoxine complex
The compound was prepared by following procedure: 0.035 gm (0.001 mol) of (K_2PtCl_4) was dissolved in 20 ml of methanol with some amount of deionized water and 0.31 gm of sulfadoxine in 10 ml of methanol.

Gel electrophoresis
The DNA cleavage experiment was conducted using CT-DNA by gel electrophoresis with the ligand and metal complex in the absence and presence of H_2O_2 as an oxidant. The reaction mixture was incubated before electrophoresis experiment at 37°C for 2 h as follows: CT-DNA 30 μM, 50 μM, each complex and 500 μM H_2O_2 in 50 mM Tris- HCl buffer (7.1). The samples were electrophoresed for 2 h at 50 V on1% agarose gel using tris-acetic acid-EDTA buffer at pH = 8.3. After electrophoresis, the gel was stained using 3 μL ethidium bromide (EB) and photographed under UV light using a digital camera.

Antimicrobial activity
For antimicrobial activity, a filter paper sterilized disk saturated with a measured quantity of the sample is placed on the plate containing solid bacterial medium (nutrient agar broth) which has been heavily seeded with spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism [11-12].

RESULT AND DISCUSSION

Synthesis and characterization
Synthesis of silver nitrite ($AgNO_3$) with sulfadoxine compound 1:
The compound 1 was prepared by following procedure: 0.169 gm (0.001 mol) of ($AgNO_3$) was dissolved in 20 ml of methanol and 0.31 gm of sulfadoxine in 10 ml of methanol. (Figure 1) Table -3.1 summarizes the physical properties (melting point, color, percentage yield, and elements analysis) of the complexes, and Molar conductance.

Synthesis of Di potassium Tetra Chloro Platinate (K_2PtCl_4) with sulfadoxine compound 2:
The compound 2 was prepared by the following procedure: 0.035 gm (0.001 mol) of (K_2PtCl_4) was dissolved in 20 ml of methanol with some amount of deionized water and 0.31 gm of sulfadoxine in 10 ml of methanol. (Figure 2)

Conductance measurement
The ($AgNO_3$) and (K_2PtCl_2) complex (1 and 2) showed to be weak electrolytes as form their molar conductivity (^M) measurement in DMF, which were 10 and 14Scm^2 mol^{-1} respectively.

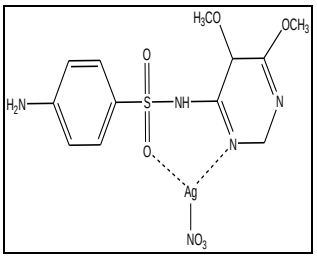

Figure 1: The proposed of sulfadoxine and ($AgNO_3$) Complex

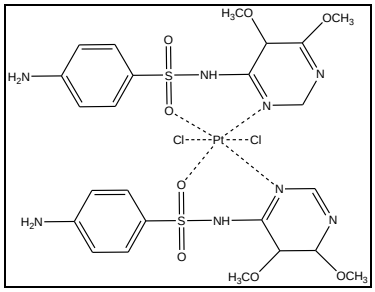

Figure 2: The proposed of Sulfadoxine and (K_2PtCl_2) Complex

Table 1: Physical properties of Sulfadoxine complexes

No	Unit formula	F. wt.	Color	Yield	M. p.	CHN cal/(f)		
						C	H	N
1	S. Ag 1:1 $C_{12}H_{16}AgN_5O_7S$	482	Gray	60%	225-228	29.88 (30.74)	3.08 (3.32)	14.5 (13.85)
2	S. Pt 1:2 $C_{24}H_{32}Cl_2N_8O_8PtS_2 .2H_2O$	926	Black	50%	>250	31.1 (30.9)	3.88 (4.21)	13.1 (12.82)

IR spectra

IR spectra of the ligand (sulfadoxine) and its complexes

The main IR Spectra of compound are summarized inTable (3.2) Sulfadoxin has potential binding sites for transition metal-ions Sulfadoxin has tow -nitrogen atoms , which can donate electron pairs nitrogen of pyrimidine ring. The IR Spectral data of sulfadoxine and its complexes showed intense absorption band in 3467cm-1. The range 3523cm-1 , 3470cm-1,3377cm-1, 3366cm-1 due to asymmetric and symmetric NH_2 stretching vibration of the amino groups and its Ag(I) and pt(II) complex displays the frequency shifts and intensity changes of the C=N group on complexation suggested that the C=N group is involved in coordination in Ag and Pt complexes the strong band at 1652 cm -1 assigned to C=N in free ligand was shifted to lower wave number in the complexes. Indicating participation of the C=N group in coordination.

Table 2: Main IR absorption bands sulfadoxine and its complexes.

No	C=C[a]	o=s=o	NH_2	N-H	C= N Ring	Molar conductivity Λ_M [$Smol^{-1}cm^2$]
1 sulfadoxine	1599, 1589	1158	3467	3377 3239	1652	
2 Sulfa:Ag	1597, 1570	1127	3470	3366	1619	10
3 Sulfa:Pt	1592, 1578	1160 1109	3523		1620	14

[a] Aromatic ring stretch

^{1}H NMR study of Sulfadoxine and its complexes

The ^{1}HNMR spectrum of the sulfadoxine showed NH proton of sulfonamide at 10.6 ppm (S,H,D_2O exchangeable); H –pyrimidine ring at 8.1 ppm (S,1H); the multiple single around 7.63 -7.64 ppm (m,4H) Were ascribed to aromatic protons. The signal observed at 6.02 ppm (S,1H) is due to Amino proton.

In the ^{1}HNMR spectra of the complexes 2 and 3 an electron density shift from the ligand to the metal was observed .The signals of H- pyrimidine protons appeared at 7.9 and 8.59, 8.62 ppm in the Ag(I) and pt(II) complexes , respectively , as compared to 8.1 ppm in the sulfadoxine ligend ,inferring coordination through the pyrimidine ring of the ligand .The OCH_3 protons appeared at 3.71 ppm (S,3H) and at 3.93 ppm (S,3H).

Electronic spectra

The main electronic absorptions are summarized in Table .3 and shown in Fig 3 UV- Vis spectra of the ligands and its complexes were measured in the range 200-800 nm. The UV-visible absorption spectra were record in methanol solution.

The spectra of complexes generally showed the characteristic bond of the free ligands with some changes both in wave lengths .(λmax) and intensity together with appearances of a new bonds at longer wavelengths .The spectra of the ligand and its complexes exhibit bonds in the regions of 206-275 , 206, 2.77, 508, 206-530 nm , which may be due to the transition of π-π*, n- π* and d-d respectively.

Table 3: Main electronic absorptions of the Sulfadoxine and its complexes

	Λ max nm (cm^{-1})		
NO	d-d	π-π*	n-π*
sulfadoxine	-	206	275
Sulfadoxine:Ag	508	206	277
Sulfadoxine;Pt	510 530	206	241

Antimicrobial activity

For in vitro antimicrobial activity, the investigated compound was tested against the bacteria. *S-aureus, p.aeruginosa* and *E-coli* and fungi candidate; the values indicated that the complexs have higher antimicrobial activity than the free ligand (sulfdoxine).Table 4 shows the results of the bioassay.

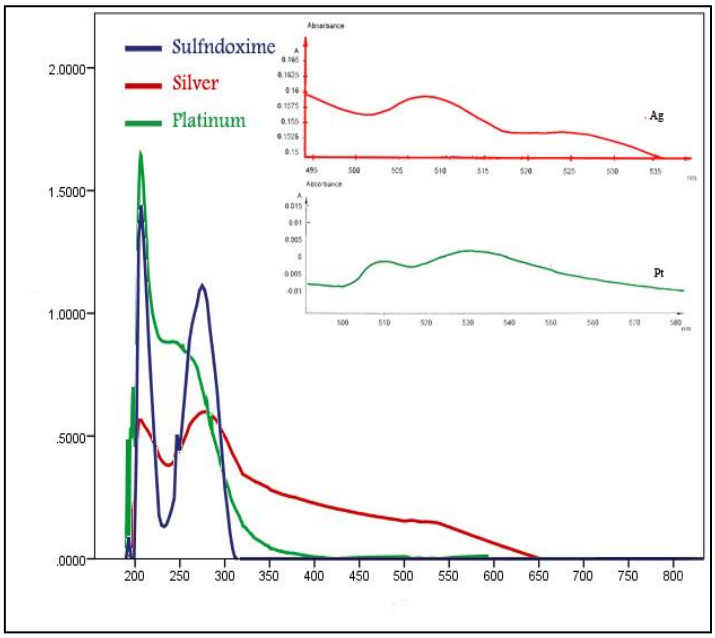

Figure 3: UV-visible spectra of sulfadoxine and ($AgNO_3$) complex and (K_2PtCl_2) complex

Table 4: Antimicrobial activity of the Sulfadoxine and its complexes. The inhibition zones (IZ) diameter is in mm

Compound	f.wt	Microorganisms			
		Fungus	Gram negative		Gram positive
		Candida albicans	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
sulfadoxine	310	11.5 (+1)	(-)	5	(-)
Sulfadoxine:Ag	482	13 (+1)	10(+1)	(-)	12 (+1)
Sulfadoxine;Pt	890	11 (+1)	(-)	10 (+1)	(-)

(+1) slightly sensitive (15-10mm), (-) no effect

DNA cleavage studies

The cleavage efficiency of the complexes compared to that of control is due to their efficient DNA-binding ability. The metal complexes were able to convert super coiled DNA into open circular DNA. The proposed general oxidative mechanisms and account of DNA cleavage is by hydroxyl radicals via abstraction of a hydrogen atom from sugar units that predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed [13]. Cleavage is inhibited by free radical scavengers. This implies that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by metallocomplexes bound hydroxyl radical or a peroxo species generated from the co-reactant H_2O_2. In the present study, CT-DNA gel electrophoresis experiment was conducted at 35°C using our synthesized complexes in the presence of H_2O_2 as an oxidant. As can be seen from the results illustrated in Figs 4 and 5, at very low concentrations, the ligand and its complexes exhibited nuclease activity in the presence of H_2O_2. Control-1 experiment, using DNA alone (line 1) did not show any significant cleavage of CT-DNA even on a longer exposure time. Control-2, using DNA+H_2O_2 (line 2) did not show any significant cleavage of CT-DNA. From the observed results, it can be concluded that the ligand (line3) and its complexes (lines 4 and 5) showed complete cleavage of CT-DNA.

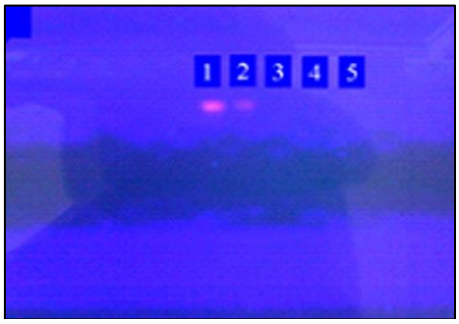

Figure 4: DNA with hydrogen peroxide and chemical compounds: 1- DNA alone; 2- DNA + H_2O_2; 3- DNA + H_2O_2 + Compound (1); 4- DNA + H_2O_2 + compound (2); 5- DNA + H_2O_2 + compound (3)

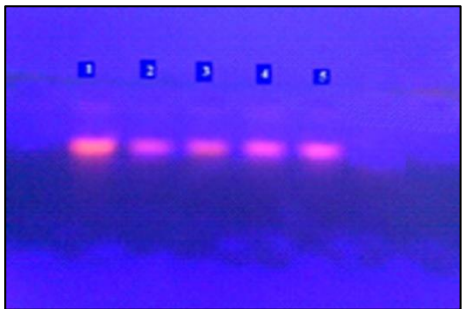

Figure 5: DNA without hydrogen peroxide: 1- DNA alone; 2- DNA + solvent (DMSO); 3- DNA + compound (1); 4- DNA+ compound (2); 5- DNA + compound (3)

CONCLUSION

The present work describes the synthesis and *in vitro* antimicrobial and DNA cleavage of sulfadoxine and its complexes (1-3). The complexes have higher antimicrobial activity than the free ligand (sulfdoxine) However all tested compounds showed complete cleavage of CT-DNA.

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