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Synthesis and Antimicrobial Studies of Copper (II) Metal Complexes that Specifically Recognize Nucleic Acid Bases

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Abstract

Antimicrobial activities of metal complexes have been investigated due to their potential biological activity. S1 and S2 have been synthesized and their antibacterial and antifungal activities have been studied against some pathogenic bacterial and fungal strains by using disc diffusion method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were studied by serial dilution method against some gram positive and gram negative pathogenic bacteria. The compounds showed significant antimicrobial activities against all tested organisms, which are quite comparable to standard antibiotic kanamycin. The MIC values of the compounds were found to be in the range of 32~256 μg/mL. The low MIC and MBC values and high sensitivity of pathogenic microorganisms to the synthesized compounds led to conclude that the complexes of have potential antibiotic properties.

Key words: Metal complex, Antibiotic, Antimicrobial agents, MIC and MBC.

Introduction

The discovery and development of antibiotics are among the most powerful and successful achievement of modern science and technology for the control of infectious diseases. However, the increasing microbial resistance to antibiotics in use now-a-days necessitates the search for new compounds with potential effects against pathogenic bacteria. Extensive investigations in the field of metal complexes and their Schiff bases have been reported. Their preparation, chemical and physical properties have been described by various workers. For developing potential antibiotic, metal complexes have been drawn attention of many investigators (Çukurovali *et al.*, 2010; Carcelli *et al.*, 1995; Anacona and Silva, 2005; Srinivasan, *et al.*, 2005; Hossain, *et al.*, 2008; Ajani, 2008; Nagar, 1990; Anacona and Patricia, 2010; Nagwa and Nassir, 2010) owing to their potential applications such as antibacterial, antifungal, antiviral, anti-inflammatory (Demertzi *et al.*, 2009; Chohan *et al.*, 2002; Hunoor *et al.*, 2011), anti-tubercular (William and Ronald 1958; Qin-xi *et al.*, 2000; Sandbhor *et al.*, 2002), anti-HIV, herbicidal (Matsuzawa *et al.*, 1987), etc. activities. Nickel (ìì) complexes of semicarbazone derivatives showed potent anticancer activity against MCF–7 cell lines (Afrasiabi *et al.*, 2005). Two new alkylamine–substituted nickel (II)-salphen complexes have been prepared and their interactions with DNA were investigated and they showed that these complexes had a remarkable ability to stabilize G–quadruplex DNA and was inhibited telomerase activity (Julie *et al.*, 2006). Mixed-Ligand Copper (II)–phenolate complexes showed potent anticancer activity against human cervical epidermoid carcinoma cell line (ME180) (Rajendiran *et al.*, 2007). Antimicrobial and antifungal activities of various metal complexes have also been reported (Zahid *et al.*, 2006). In this work, the synthesis and characterization of some metal complexes for pharmacological studies are reported.

Materials and Methods

Bacterial and fungal stains: The microorganisms used in the work were collected from the Microbiology Laboratory of the Institute of Nutrition and Food Sciences (INFS), Dhaka University.

Chemicals and reagents: All the chemicals and reagents used throughout the investigation were of reagent grade.

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Preparation of Cu^{2+}-tren-nucleobase complexe [Cu(tren)(adeninato].(ClO_4): An aqueous solution (5 ml) dissolving adenine (0.1 mmol), and acetonitrile solution (5 ml) dissolving tren (0.1 mmol) and $Cu(ClO_4).6H_2O$ (0.1 mmol) were mixed and the solution (pH ca. 6-7) were allowed to stand at room temperature and greenish-blue plates formed after two weeks. Yield: 20 mg (45 %), (Analytical calculation for $C_{11}H_{22}ClN_9O_4Cu$; C=29.82 %; H=4.96 %; N=28.44 % and found value C=29.66 %; H=4.89 %; N=28.23 %). The structure was given in Figure 1 (S1).

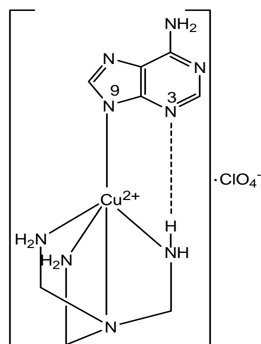

Figure 1. Molecular structure of [Cu(tren)(adeninato)].ClO_4 [S1]

Complex [{Cu(tren)}$_2$(hypoxanthinato)].(ClO_4)$_3$.: An acetonitrilic solution (5 mL) containing $Cu(ClO_4)_2.6H_2O$ (0.1 mmol) was mixed with tren and aqueous solution (5 ml) of hypoxanthine (0.1 mmol). The resultant solution (pH ca. 6-7) was allowed to stand at ambient temperature for reaction, and deep blue plates formed after six weeks. Yield: 25 mg (59 %), (Analytical calculation for $C_{17}H_{39}Cl_3N_{12}O_{13}Cu_2$; C=23.95%; H=4.57%; N=19.70%, and found value C=23.86 %; H=4.55 %; N=19.65 %). The structure was given in Figure 2 (S2).

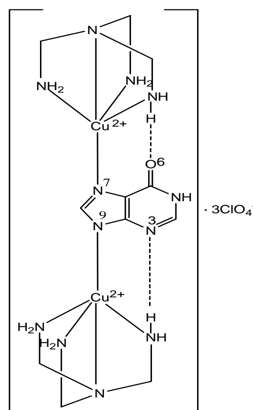

Figure 2. Molecular structure of [{Cu(tren)}$_2$(hypoxanthinato)}].(ClO_4)$_3$ [S2]

Characterization of the compounds

Preparation of inoculums: Suspension of organism was prepared as per McFarland nephelometer standard. A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted such that it contained 1.5×10^8 cells/mL approximately. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05 ml of 1.175% of barium chloride and 9.95 ml of 1% sulfuric acid.

Antibacterial screening: The synthesized compounds were screened *in vitro* for their antibacterial activity against four gram-negative *(Escheriachi coli, Shigella dysenteriae, Shigella shiga* and *Shigella sonnei*) and four gram-positive *(Staphylococcus aureus, Bacilus subtilis, Scrcina lutea* and *Bacillus megaterium*) bacterial strains using disc diffusion method (Bauer *et al*., 1966; Rois *et al*., 1988). Briefly, three calculated amount (5 mg, 10 mg and 20 mg) of S1 and S2 were dissolved in 1ml of distilled in three different vials for getting solutions having concentration 50 μg/disc, 100 μg/disc and 200 μg/disc respectively and then they were applied on filter paper disc. Standard kanamycin (30 μg/disc) was used as positive control. Both experimental and control discs were then placed in petridishes seeded with organism in nutrient agar medium. The petridishes were kept in a refrigerator at 4°C for 24 hours to ensure diffusion of the test materials. Finally they were incubated at 37 ± 1°C for 24 hours. Each experiment was carried out in triplicates, and the antibacterial activity was determined by measuring the diameter of zone of inhibition in mm.

Determination of MIC and MBC: The MIC and MBC of S1 and S2 were determined by serial dilution technique (Jawetz *et al*., 1980) against four pathogenic bacteria. For this test, S1 and S2 were used from a concentration of 1 μg/ml to 512 μg/ml. A control test-tube containing only medium (nutrient broth medium) was used to confirm the sterility of the medium. Bacterial suspension (10 μl) containing 107 cells/ml was inoculated into all tubes. All of the test tubes were incubated at 37 ± 1°C and observed for bacterial growth at 24 hours for MIC and 96 hours (4 days) for MBC determinations.

After inoculation for 24 hours, the test tube with no visible growth of the microorganism was taken to

represent the MIC value of the sample in µg/ml. MBC is that concentration in which no viable organism will present. It was determined by keeping the test tubes which was used for MIC determination for four days. After four days bacterial growth was observed and MBC was determined at lowest concentrations where no bacterial growth was observed.

Antifungal activity: For antifungal screening, each sample was tested at concentrations of 100, 200, 400 µg/disc. The experimental protocol same as antibacterial screening except the plates were incubated at 37 ± 1°C for 48 h and *Nystatin* disc were used as positive control. The experimental results have been expressed as the mean ± SEM (Standard Error of Mean). Statistical analysis was performing with SPSS software of 10 versions.

Results and Discussion

The results for the antimicrobial activity of S1 and S2 have been presented in Tables 1-5. The compounds showed a significant antibacterial activity against all test organisms. The results obtained with the standard drug kanamycin have also been presented here for the comparison.

The diameter of zone of inhibition of S1 at the dose of 50 µg/disc against *Shigella dysenteriae, Shigella sonnei, Escherichia coli, Shigella shiga, Staphylococcus aureus, Bacillus subtilis, Sarcina lutea* and *Bacillus megaterium* were found to be 17.4 ± 2.1, 14.2 ± 1.5, 13.3 ± 1.1, 17.1 ± 1.8, 14.0 ± 1.6, 17.0 ± 1.4, 15.5 ± 1.0 and 23.0 ± 1.5 mm respectively. These values were quite comparable with those obtained with Kanamycin at dose 30µg/disc (Tables 1-2). Zone of inhibition values of S2 at 50µg/disc are also quite comparable with kanamycin at dose 30µg/disc. Better results were obtained when the similar experiments were performed with higher doses (100 µg/disc and 200 µg/disc) of the compounds.

MIC values of S1 and S2 were determined against *Shigella dysenteriae, Shigella sonnei, Escherichia coli, Shigella shiga, Staphylococcus aureus, Bacillus subtilis, Sarcina lutea* and *Bacillus megaterium*. The results are shown in Table 3. The MIC values of S1 were found to be 128, 64, 64, 32, 64, 128, 64 and 32 µg/ml, respectively and MBC values for S1 were 512, 256, 128, 128, 256, 256, 128 and 128 µg/ml, respectively. These values are quite remarkable from the point of view of microbial activities. The MIC and MBC values of S2 are also very much interesting which are shown in Table 3.

Table 1. Antibacterial activity of S1 and Kanamycin

Name of bacteria	Diameter of zone of inhibition (mm) of compound S1			Standard (Kanamycin) 30 µg/disc
	50 µg/disc	100 µg/disc	200 µg/disc	
Gram negative bacteria				
Shigella dysenteriae	17.4 ± 2.1	19.7 ± 2.5	22 ± 1.4	24 ± 1.3
Shigella sonnei	14.2 ± 1.5	16 ± 1.2	19.4 ± 1.7	20.3 ± 2.4
Escherichia coli	13.3 ± 1.1	15.4 ± 1.6	21 ± 2.0	22.4 ± 1.0
Shigella shiga	17.1 ± 1.8	21 ± 1.1	23.5 ± 1.8	24.5 ± 1.6
Gram positive bacteria				
Staphylococcus aureus	14.0 ± 1.6	16 ± 0.8	19 ± 1.1	23 ± 0.5
Bacillus subtilis	17.0 ± 1.4	18.4 ± 1.7	20.4 ± 2.7	24 ± 2.1
Sarcina lutea	15.5 ± 1.0	18 ± 2.1	24.1 ± 3.5	29 ± 2.0
Bacillus megaterium	23.0 ± 1.5	22 ± 2.3	25 ± 4.1	31.4 ± 2.6

Table 2. Antibacterial activity of S2 and Kanamycin

Name of bacteria	Diameter of zone of inhibition (mm) of compound S2			Standard (Kanamycin) 30 µg/disc
	50 µg/disc	100 µg/disc	200 µg/disc	
Gram negative bacteria				
Shigella dysenteriae	R	10 ± 1.1	12 ± 1.0	21 ± 1.0
Shigella sonnei	11 ± 1.6	13 ± 0.8	18 ± 0.4	29 ± 0.9
Escherichia coli	12 ± 1.2	15 ± 0.7	19 ± 1.5	27 ± 0.3
Shigella shiga	09 ± 2.1	11 ± 1.4	14 ± 1.3	31 ± 0.1
Gram positive bacteria				
Staphylococcus aureus	10 ± 1.6	13 ± 1.0	17 ± 1.2	22 ± 2.1
Bacillus subtilis	R	08 ± 0.5	12 ± 0.5	27 ± 1.5
Sarcina lutea	R	07 ± 2.0	10 ± 1.0	29 ± 0.5
Bacillus megaterium	11±1.7	14 ± 2.1	18 ± 1.6	32 ± 1.1

Table 3. Minimum inhibitory concentration and minimum bactericidal concentration of S1 and S2.

Test organism	S1		S2	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
Shigella dysenteriae	128	512	256	512
Shigella sonnei	64	256	128	512
Escherichia coli	64	128	128	256
Shigella shiga	32	128	64	256
Staphylococcus aureus	64	256	64	128
Bacillus subtilis	128	256	128	512
Sarcina lutea	64	128	256	-
Bacillus megaterium	32	128	128	256

Table 4. Antifungal activity of S1 and *Nystatin*

Name of fungi	Diameter of zone of inhibition (mm) of the compound S1			Standard (*Nystatin*) 100 µg/disc
	100µg/disc	200µg/disc	400µg/disc	
Candida albicans	11.0 ± 1.5	13.0 ± 1.6	14.0 ± 2.1	21.4 ± 2.3
Aspergillus fumigatus	R	12.0 ± 1.2	14.0 ± 1.8	16.0 ± 1.1
Vasinfctum sp.	R	R	13.0 ± 1.2	21.2 ± 0.6
Human-3 sp.	R	R	14.0 ± 1.0	21.0 ± 1.5
Aspergillus flavus	R	15.0 ± 1.4	17.0 ± 0.4	24.3 ± 1.7
Aspergillus niger	R	R	11.0 ± 1.4	21.2 ± 1.3

Table 5. Antifungal activity of S2 and *Nystatin*

Name of fungi	Diameter of zone of inhibition (mm) of the compound S2			Standard (*Nystatin*) 100µg/disc
	100µg/disc	200µg/disc	400µg/disc	
Candida albicans	R	11.0 ± 1.0	12.8 ± 1.3	21.0 ± 2.2
Aspergillus fumigatus	R	R	09.0 ± 1.1	18.4 ± 3.5
Vasinfctum sp.	R	R	09.0 ± 1.4	17.0 ± 3.2
Human-3 sp.	R	R	11.0 ± 1.5	14.5 ± 1.0
Aspergillus flavus	R	R	11.0 ± 2.3	21.3 ± 1.5
Aspergillus niger	R	10.1 ± 1.0	14.5 ± 2.2	24.1 ± 2.5

Antifungal activity of the compounds were also determined at three different dose (100, 200, 400 µg/disc) against six pathogenic fungi such as *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans, Human-3 sp,* and *Vasinfctum sp.* At lower doses all three compounds were almost oblivious to organisms but at higher doses the compounds showed mild to moderate antifungal activities which are given in table 4.

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