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Synthesis, Characterization, Antibacterial, Antifungal and Antituberculous Activities of Some Macrocyclic Coordination Compounds of Zn(II), Cd(II) & Hg(II)

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The increasing clinical importance of drug-resistant mycobacterial pathogens has lent additional urgency to microbiological research and new antimycobacterial compound development. For this purpose, in this study, Zn(II), Cd(II) and Hg(II) complexes of a heptadentate N_5S_2 donor Schiff base ligands including 2,6-*bis*(2-aminothiophenoxymethyl) pyridine and 2,2'-bipyridine-6,6'-dicarboxaldehyde were obtained by using the literature methods and evaluated for antituberculosis activity. Antituberculosis activities of the compounds were determined by broth microdilution assay, the microplate alamar blue assay, in BACTEC12B medium and results were screened *in vitro*, using BACTEC 460 radiometric system against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 µg/mL and tested compounds showed important inhibition ranging from 84 to 58 %. Antimicrobial activities of these complexes have also been searched. All complexes showed the same activity against *Pseudomonas aeruginosa* and moderate activities against *Proteus vulgaris*, but they didn't give significant antibacterial activity against other bacterial strains. On the other hand they showed moderate activities when compared with antifungal agent ketoconazole against *Candida albicans* and *Candida globrata*.

Key Words: Macrocyclic compounds, Bipyridine, Antituberculosis activity, Antimicrobial activity.

INTRODUCTION

Currently there has been an increasing attention to transition metal complexes containing sulphur and nitrogen donor atoms due to their significant anticancer[1], antifungal, antibacterial[2], herbicidal and catalytic activities[3,4]. Antimicrobial and antiviral activities of porphyrins are based on their ability to catalyze peroxidase and oxidase reactions, absorb photons and generate reactive oxygen species and partition into lipids of bacterial membranes. Natural and synthetic porphyrins have relatively low toxicity *in vitro* and *in vivo*. The ability for numerous chemical modifications and the large number of different mechanisms by which porphyrins affect microbial and viral pathogens place porphyrins into a group of compounds with an outstanding potential for discovery of novel agents, procedures and materials active against pathogenic microorganisms[5,6].

Tuberculosis is the leading infections cause of death in the world and also the number of diseases, caused by multidrug resistant gram-positive microorganism, has been continuously increasing. The ability of microorganism to become resistant to major therapies used against them has long been recognized and becomes increasing apparent. Increasing antimicrobial resistance presents major threats to public health[7,8]. The current rise in micobacterial-related infections and disease, coupled with drug resistance, has resulted in renewed interests in the identification of new classes of antimycobacterials. During recent years it has been reported that chelation is cause and cure of many diseases including cancer. In view of the scanty informations available on the substitution complexes of transition metals and promoted from the biological activities of such complexes are reported. A number of Schiff base complexes such as Co(II), Ag(I) have been found antibacterial[9,10].

One of the most attractive features of the ligands in the held of biological investigation has been their structural similarities with the common pyrimidine and purine type nucleobases. The most successful compounds seem to be those that interfere with the construction of the bacterial cell wall, the process of protein synthesis and replication or transcription of DNA. The metal oxidation state, the type and the number of donor atoms, as well as their relative disposition within the ligand and also the binding properties of metal complexes, with biomolecules such as nucleic acids, proteins and lipids are major factors determining structure-activity relationship of metal complexes[11,12]. For instance the iron(II) and zinc(II) are very abundant ubiquitous and essential trace elements in biological system and their binding

properties with the biomolecules, particularly to the oxygen and nitrogen donor centres, serve their biofunctionality. On the other hand, non-essential cadmium(II) and mercury(II) are soft acids with high toxicity. Their salts have a long history of use as antibacterial agents[13,14].

Here we describe the complexes of a N_5S_2 donor ligand which were obtained from the reaction of the Schiff base, resulting from the condensations of 2,6-*bis*(2-aminothiophenoxy methyl)pyridine and 2,2'-bipyridine-6,6'-dicarboxaldehyde with Zn(II), Cd(II) and Hg(II) perchlorate in methanol. The complexes were characterized by IR, ^{1}H NMR, FAB mass spectrometry and elemental analyses. Antituberculosis activities of the synthesized compounds were determined by broth microdilution assay, the microplate alamar blue assay, in BACTEC12B medium and results were screened *in vitro*, using BACTEC 460 radiometric system against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 µg/mL and tested compounds showed inhibition ranging from 74 to 49 %. Antibacterial and antifungal activity of these complexes are also elucidated.

EXPERIMENTAL

Melting points were determined using an Gallenkamp MPD350.BM2.5 digital melting point apparatus and were uncorrected. The compounds were checked for purity by TLC on silica gel 60 F_{254} (Merck). Elemental analyses were performed on a CHNS-O Carlo Erba EA 1108 elemental analyzer; IR spectra were obtained with a Shimadzu 470 IR spectrophotometer using nujol mulls or KBr disc; ^{1}H NMR spectra were recorded with a Varian (300 MHz) or Bruker spectrometer (250 MHz) in $CDCl_3$ or DMF-d_7 as solvent. MS-FAB$^+$ spectra were obtained with a Finnigan Mat 95 mass spectrometer.

Tetrahydrofuran (THF) was distilled from sodium metal in the presence of benzophenone immediately prior to use. 1,4-Dioxane was distilled prior to use. Tetraethyl ammonium iodide (Et_4NI) was dried at 100 ºC under reduced pressure. All other reagents were used as purchased from commercial suppliers without further purification. The purification of Zn powder was both followed to literature method[14], 6-bromo-2-methylpyridine[15], 6,6'-dimethyl-2,2'-bipyridine[16], 2,6-*bis*-(bromomethyl)pyridine[16], 2,2'-bipyridine-6,6'-dicarboxaldehyde[17], 2,6-*bis*(2-aminothiophenoxymethyl)pyridine[18] and metal-ion controlled synthesis of L complexes in the presence of Zn(II), Cd(II), Hg(II) perchlorate salts[19] also synthesized by using literature methods. 2,2'-Bipyridine-6,6'-dicarboxaldehyde (0.18 g, 1 mmol) and the appropriate metal perchlorate salt (1 mmol) were dissolved in hot MeOH (25 mL) and 2,6-*bis*(2-aminothiophenoxymethyl)pyridine (0.36 g, 1 mmol) in methanol (25 mL) added dropwise with stirring. The mixture were refluxed for 3-4 h and filtered hot. The solvent of the reaction mixture was reduced to half its original volume and then the mixture was placed in a refrigerator to induce crystallisation. Desired product was filtered and dried.

[ZnL](ClO$_4$)$_2$: Pale yellow solid, yield: 0.32 g (40 %). IR (KBr, ν_{max}, cm^{-1}): 1628 (C=N), 1587 (pyridine), 1094 and 624 (perchlorate anion). m/z: 594 $[ZnL(ClO_4)]^+$, 693 $[ZnL]^{2+}$. (Found. (%): C, 46.6; H, 3.0; N, 8.5; S, 8.5. Calcd. (%) for $C_{31}H_{23}N_5S_2O_8Cl_2Zn$: C, 46.9; H, 2.9; N, 8.8; S, 8.1.

[CdL](ClO$_4$)$_2$: Yellow solid, yield: 0.67 g (80 %). IR (KBr, ν_{max}, cm^{-1}): 1638 (C=N), 1590 (pyridine), 1091 and 624 (perchlorate anion). m/z: 742 $[CdL(ClO_4)]^+$, 642 $[CdL]^{2+}$. (Found. (%): C, 44.2; H, 2.8; N, 8.0; S, 7.5. Calcd. (%) for $C_{31}H_{23}N_5S_2O_8Cl_2Cd$: C, 44.3; H, 2.7; N, 8.3; S, 7.6).

[HgL](ClO$_4$)$_2$: Yellow solid, yield: 0.69 g (74 %). IR (KBr, ν_{max}, cm^{-1}): 1628 (C=N), 1587 (pyridine), 1094 and 624 (perchlorate anion). m/z: 830 $[HgL(ClO_4)]^+$, 731 $[HgL]^{2+}$. (Found. (%): C, 39.1; H, 2.5; N, 7.1; S, 7.5. Calcd. (%) for $C_{31}H_{23}N_5S_2O_8Cl_2Hg\cdot H_2O$: C, 39.3; H, 2.6; N, 7.4; S, 6.8).

Antituberculosis activity: *In vitro* evaluation of anti-mycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ antituberculotic activities of the compounds were tested at the center of tuberculosis antimicrobial acquisiton and coordinating facility (TAACF). Compounds were tested for *in vitro* antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 µg/mL, in BACTEC 12B medium using a broth microdilution assay, the microplate alamar blue assay (MABA). Compounds exhibiting fluorescence are tested in the BACTEC 460 radiometric system[19]. Compounds effecting < 90 % inhibition in the primary screen (*i.e.*, MIC > 6.25 µg/mL) are not generally evaluated further.

BACTEC Radiometric method of susceptibility testing: Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 more, or suspension of organism isolated earlier on conventional medium. The culture was well mixed with a syringe and 0.1 mL of a positive BACTEC culture was added to each of the vials containing the test drugs. The drug vials contained rifampicin (0.25 µg/mL). A control vial was inoculated with a 1:100 microdilution of the culture. A suspension equivalent to a Mc Farland No. 1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used. Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 ºC and tested daily with a BACTEC instrument. When the growth index in the control read at least 30, the increase in growth index (ΔGI) from the previous day in the control was compare with that in the drug vial. The following formula was used to interpret results:

$$\Delta GI \text{ control} > \Delta GI \text{ drug} = \text{Susceptible} \quad (1)$$
$$\Delta GI \text{ control} < \Delta GI \text{ drug} = \text{Resistant}$$

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time the control ΔGI is 30, the vials were read for 1 or 2 additional days to establish a definite pattern of ΔGI differences.

Microbiology: The study was designed to compare MICs obtained by the CLSI reference M7-A7 and M100-S16 broth microdilution methods[20,21]. Twice MIC readings were performed by each chemical agent. For both the antibacterial and antimycotic assays the compounds were dissolved in DMSO. Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.40 µg/mL concentrations with Mueller-Hinton broth and Sabouroud dextrose broth. In order to ensure that the solvent per had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO

at the same dilutions used in our experiments and found inactive in culture medium.

All the compounds were tested for their *in vitro* growth inhibitory activity against human pathogenic as Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, as Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *E. coli* ATCC 35218, *Salmonella thyphimurium* NRRL B-4420, *Proteus vulgaris* NRLL B-123 and yeast *Candida albicans* (isolated from Faculty of Medicine Osmangazi University, Eskisehir, Turkey), *Candida globrata* ATCC 36583. Chloramphenicol and ketokanozole were used as control drugs.

Antibacterial and antifungal assay: The cultures were obtained from Mueller-Hinton broth (Difco) for the bacterial strains after overnight incubation at 35 ± 1 ºC. The yeasts were maintained in Sabouroud dextrose broth (Difco) after overnight incubation 35 ± 1 ºC. The inocula of test microorganisms adjusted to match the turbidity of a Mac Farland 0.5 standard tube as determined with a spectrophotometer and the final inoculum size was 0.5-2.5 × 10^5 cfu/mL for antibacterial and antifungal assays.

Testing was carried out in Mueller-Hinton broth and Sabouroud dextrose broth (Difco) at pH 7 and the two-fold serial dilutions technique was applied. The last well on the microplates was containing only inoculated broth was kept as controls and the last well with no growth of microorganism was recorded to represent the MIC expressed in µg/mL. Every experiment in the antimicrobial assays was replicated twice in order to define the MIC values.

RESULTS AND DISCUSSION

All the complexes were prepared by a template synthesis, in which the Schiff base macrocyclic ligand resulted from the condensation of 2,2'-bipyridine-6,6'-dicarboxaldehyde with 2,6-*bis*(2-aminothiophenoxymethyl)pyridine in the presence of Zn(II), Cd(II) and Hg(II) ions. For the synthesis of 2,2'-bipyridine-6,6'-dicarboxaldehyde and the precursor diamino compound, 2,6-*bis*(2-aminothiophenoxymethyl) pyridine was prepared by using literature method[14-19].

The complexes were obtained with 35-75 % yields. The infrared spectra of all metal complexes in the region 4000-400 cm^{-1} show a strong absorption band around 1636-1614 cm^{-1} which assigned to the C=N streching vibration. For the metal complexes absorbtions were assigned at 1094-1090 and 626 cm^{-1}. The proton NMR spectra for the complexes was consisted with the given formulation. The composition of the complexes was also confirmed by elemental analyses and by fast atom bombardment mass spectrometry.

Antituberculosis activity: The antituberculosis activities of the synthesized compounds were screened *in vitro* using BACTEC 460 radiometric system against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) at 6.25 µg/mL. Rifampicin was used as the standard in this test. All of the compounds tested showed significant antituberculosis activity as can be inferred from the Table-1. The Hg(II) complex showed the high inhibition with 74 %. Other compounds showed varying inhibition degrees between 59 to 49 %. We concluded from present investigations that new complexes may be considered promising for the development of new antituberculosis agents with the metals on 2B group.

TABLE-1
ANTITUBERCULOSIS ACTIVITY OF THE COMPOUNDS

Comp.	[ZnL] $(ClO_4)_2$	[CdL] $(ClO_4)_2$	[HgL] $(ClO_4)_2$	Rifampicin
MIC (µg/mL)	> 6.25	> 6.25	> 6.25	0.25
Inhibition (%)	49	59	74	98

Antibacterial and antifungal activities: The chemical agents were evaluated also for its antimicrobial properties. In comparison with the control antibacterial agent chloramphenicole, the chemical agents showed the same activity against *P. aeruginosa* and moderate activities against *P. vulgaris*, but they didn't give significant antibacterial activity against other bacterial strains. On the other hand they showed moderate activities when compared with antifungal agent ketoconazole against *C. albicans* and *C. globrata*. The observed data on the antibacterial and antifungal activity of the compounds and the control drugs are given in Table-2.

TABLE-2
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE COMPOUNDS

Chemical agent	MIC (µg/mL) of bacterial strains				
	EC	PA	PV	ST	KP
Chloramphenicol	12.5	50	50	12.5	12.5
$[ZnL](ClO_4)_2$	50.0	50	50	100.0	50.0
$[CdL](ClO_4)_2$	100	50	100	50.0	100.0
$[HgL](ClO_4)_2$	50.0	50	50	50.0	50.0
		SA	EF	CA	CG
Chloramphenicol		12.5	12.5	–	–
Ketocanosole		–	–	50	100
$[ZnL](ClO_4)_2$		100.0	100	50	100
$[CdL](ClO_4)_2$		100.0	100	100	100
$[HgL](ClO_4)_2$		50.0	100	100	100

EC: *E. coli*, PA: *P. aeruginosa*, PV: *P. vulgaris*, ST: *S. thphimurium*, KP: *K. pneumoniae*, SA: *S. aureus*, EF: *E. faecalis*, CA: *C. albicans*, CG: *C. globrata.*

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