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In vitro Antimicrobial Screening of Three Cadmium Coordination Complexes and Two Addition Compounds of Antimony and Arsenic

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Abstract: Three cadmium coordination complexes (cadmium deprotonated phthalyl pyridine [Cd(DPH)$(Py)_2$, C_1], cadmium deprotonated phthalyl 8-hydroxy quinoline [Cd(DPH)-8-HQ, C_2] & cadmium deprotonated phthalyl isoquinoline [Cd(DPH)IQ, C_3] and two addition compounds 1:1 antimony(III) chloride with acetophenone [$SbCl_3.C_6H_5COCH_3$, C_4] & 1:1 arsenic(III)bromide with benzamide [$AsBr_3.C_6H_5CONH_2$, C_5] were tested for their antimicrobial activity by disc diffusion and serial dilution methods. All the compounds were active against various test pathogenic organisms. The maximum antibacterial and antifungal activities were shown by the compound C_4. The minimum inhibitory concentration (MIC) of the compound C_4 was determined against two Gram positive (*Bacillus subtilis* and *Streptococcus* β-*haemolyticus*) and two Gram negative (*Shilgella dysenteriae*, *Salmonella typhi*) bacteria and the values were found between 4 and 16 μg ml^{-1}.

Key words: Coordination complexes, antimicrobial activity, antifungal activity, pathogens

Introduction

The frequency of life threatening infections such as tuberculosis, cancer, AIDS etc caused by pathogenic microorganisms is increasing worldwide and becoming an important cause of morbidity and mortality in immuno-compromised patients. Synthetic compounds constitute an important source of various bioactive compounds such as antibacterial (Zakaria, 2000; Biswas *et al*., 2002) antifungal and anticancer (Pratt, 1979) compounds. The synthesized compounds which are used for the treatment of infectious diseases are known as chemotherapeutic agents. Every year thousands of compounds are synthesized with an aim to find a potential chemotherapeutic agent to combat pathogenic microorganisms. But very few compounds are withstood as therapeutic agent for various methodological tests. Antimicrobial screening is one of these tests required to perform for primary selection of compounds as the therapeutic agents.

The antimicrobial screening is necessary to find out the suitable candidate of therapeutic agent among the synthesized compounds. Usually many compounds possess antimicrobial properties but have serious toxic effects to the host, therefore in the ideal cases, the drug should be highly toxic to the parasite and completely atoxic for the host. In the continuation of our ongoing efforts aimed to find new compounds for chemotherapy (Islam *et al.,* 2001; Biswas *et al*., 2002), five new synthesized compounds are selected for antimicrobial screening.

Materials and Methods

Source of Compounds: The compounds used in the present study were synthesized according to the following general procedure:

Preparation of cadmium coordination complexes [Cd(DPH)$(LG^*)_x$]

An ethanolic solution of cadmium (II) chloride (1.833g; 1.0 mmol) and deprotonated Phthalic acid (DPH, 1.661g; 1.0 mmol) were mixed in the 1:1 ratio with constant stirring. Then an ethanolic ligand solution $[LG^*]_x$ (a variable ligand) was added to the resulting mixture and heated gently on a magnetic regulator hot plate with constant stirring. As a result the volume of the solution was reduced to one half and then allowed to cool. The precipitates formed were filtered, washed several times with ethanol and then dried in a vacuum desiccators charged with anhydrous $CaCl_2$.

When

$[LG]_x$ = (Pyridine)$_2$,

the compound is [Cd(DPH)$(Py)_2$, C_1],

when

$[LG]_x$ = (8-Hydroxy quinoline),

the compound is [Cd(DPH)(8-HQ), C_2] and

when

$[LG]_x$ = (Isoquinoline)$_2$

the compound is [Cd(DPH)(IQ), C_3].

Preparation of addition compound 1:1 antimony (III) chloride with acetophenone [$SbCl_3.C_6H_5COCH_3$, C_4]

A solution of acetophenone (1.201g, 1.0 mmol) in absolute alcohol was added drop wise with gently stirring to an ethanolic solution o f antimony (III) chloride (2.281g, 1.0 mmol) in warm condition .The resulting mixture

was refluxed for seven hours and allowed to cool . Then the volume of this solution was reduced to one half by heating on a hot plate and cooled in a freeze for two days. The precipitate obtained was filtered, washed with ethanol and dried in a vacuum desiccator charged with $CaCl_2$.

Preparation of addition compound 1:1 Arsenic (III) bromide with benzamide, [$AsBr_3.C_6H_5CONH_2$, C_5]
The benzamide solution (1.21 g, 1.0 mmol) in absolute alcohol was mixed drop wise with gently stirring to an alcoholic solution of arsenic (III) bromide (3.14g, 1.0 mmol) in warm condition. The resulting mixture was refluxed for seven hours and allowed to cool. Then the volume of this solution was reduced one half by heating on a hot plate and cooled in a freeze for two days. The precipitate obtained was filtered, washed with absolute alcohol and dried in a vacuum desiccator charged with $CaCl_2$.

Antimicrobial screening: "Disc diffusion method" (Bauer *et al.*, 1966; Barry, 1980; Rios *et al.*, 1988), is a widely accepted procedure for the *in-vitro* investigation of the susceptibility of microorganisms to the compounds, so this method is adopted in this investigation. The method is essentially a qualitative or semiquantitative test indicating sensitivity or resistance of microorganisms to the test materials as well as bacteriostatic or bactericidal activity of a compound (Reiner, 1982).
The standard test microorganisms were collected from the Department of Microbiology, University of Dhaka and ICDDR'B, Dhaka, Bangladesh. The diameters of zones of inhibition produced by the compounds were compared with standard antibiotics (Kanamycin 30 μg $disc^{-1}$ and Nystatin 200 μg $disc^{-1}$). The experiment was performed in duplicate to minimize errors.
Minimum inhibitory concentration (MIC) of a compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. The basic principle is the dilution tests which comprises the serial dilution of the antimicrobial agent inoculated with the organism. For the test, standard serial dilution technique (Reiner 1982) was employed. The media used in this respect were nutrient agar & nutrient broth (DIFCO).

Results & Discussion

Antibacterial activity: The inhibitory activity of the compounds C_1, C_2, C_3, C_4 & C_5 was determined at a concentration of 100μg/disc against a series of Gram positive and Gram negative pathogenic bacteria. The results are shown in Table 1. Results revealed that all the tested compounds have strong activity against most of the Gram positive & Gram negative bacteria. Among the tested materials, the compound C_4 (i.e.$SbCl_3.C_6H_5COCH_3$) was found to be most active than others. When compared with standard antibiotic Kanamycin at the same concentration of 30 μg $disc^{-1}$, the compound C_4 also has shown substantial antimicrobial activity.
In Table 1, the antibacterial activity of the three synthetic cadmium coordination complexes against 14 pathogenic bacteria is presented. Among these three complexes the antibacterial activity of compound C_2 is greater than compound C_1 and compound C_3. So the structural activity correlation with antibacterial screening data reveal that the cadmium complexation with the ligand 8- hydroxy quinoline rather than pyridine and isoquinoline is necessary for greater antibacterial activity (activity of the compounds can be represented sequentially, $C_2 > C_1 > C_3$). On the other hand, among the two addition compounds C_4 and C_5, the antibacterial activity is approximately same in both compounds but in case of compound C_5 the antibacterial activity against *Sarcina lutea* and *Shigella dysenteriae* is so much lower than the compoundC_4. So we can select the compound C_4 for further tests such as subacute toxicity as for suitable therapeutic antibacterial agent.

Antifungal activity: The compounds C_1, C_2, C_3, C_4 & C_5 were tested against the pathogenic fungi at a concentration of 200 μg $disc^{-1}$ for each and the result was compared with standard antibiotic Nystatin 200 μg $disc^{-1}$. The antifungal activity was determined after 72 hours of incubation at room temperature (30°C) and the obtained results are cited in Table 2. It was observed that three compounds i.e. C_1, C_2, & C_4 showed more antifungal active than other two compounds C_3 and C_5.

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) of the compounds C_1, C_2, C_3, C_4 and C_5 was determined against *Shigella dysenteriae, Salmonella typhi, Bacillus subtilis and Streptococcus* β*-haemolyticus* by serial dilution technique and the values were shown in the Table 3. The MIC values of the compound C_1 against *Shigella dysenteriae, Salmonella typhi, Bacillus subtilis and Streptococcus* β*-haemolyticus* were 64, 32, 32 and 64 μg ml^{-1} respectively, for compound C_2, 64, 32, 16 and 32 μg ml^{-1} respectively, for compound C_3, 128, 64, 32 and 32 μg ml^{-1} respectively, for compound C_4, 16, 8, 8 and 4 gμ ml^{-1} respectively and that for compound C_5, 64, 16, 64 and 32 μg/ ml respectively. From the MIC values, it was found that the compounds C_2, C_4 and C_5 were more potent against *Bacillus subtilis* and *Salmonella typhi*.
It was concluded that among the tested compounds, the compound C_4 (i.e. $SbCl_3.C_6H_5COCH_3$) possesses substantial antimicrobial activity with a minimum inhibitory concentration. By comparing the results with previously published results (Biswas *et al.*, 2002) of antibacterial activity of benzene sulfonic acid derivative complex compounds, we can say that our

Table 1: *In vitro* antibacterial activity of the compound C_1, C_2, C_3, C_4, C_5 and Standard Kanamycin

	Diameter of zone of inhibition (in mm)						
Test organisms	C_1 100 μg disc^{-1}	C_2 100 μg disc^{-1}	C_3 100 μg disc^{-1}	C_4 100 μg disc^{-1}	C_4 30 μg disc^{-1}	C_5 100 μg disc^{-1}	Kanamycin 100 μg disc^{-1}
Gram positive bacteria							
Bacillus subtilis	17	25	19	38	27	40	24
Bacillus megaterius	18	28	18	30	22	28	28
Staphylococcus aureus	8	9	16	35	25	32	22
Streptococcus β haemolyticus	16	24	22	33	26	28	22
Sarcina lutea	19	21	17	34	26	12	23
Gram negative bacteria							
Salmonella typhi	25	18	15	30	25	29	19
Shigella dysenteriae	20	20	21	28	20	28	20
Shigella boydii	21	19	22	26	21	29	24
Shigella sonnei	20	21	18	25	16	24	23
Shigella flexneri	23	24	16	22	15	17	28
Shigella shiga	20	22	22	23	14	30	26
Escherichia coli	23	23	23	26	19	25	20
Klebsiella sp.	18	20	16	30	23	31	21
Pseudomonas aeruginosa	20	18	15	26	20	29	20

Table 2: *In vitro* antifungal activity of the compounds C_1, C_2, C_3, C_4, C_5 and Nystatin

	Diameter of zone of inhibition (in mm)					
Test fungus	C_1 200 μg disc^{-1}	C_2 200 μg disc^{-1}	C_3 200 μg disc^{-1}	C_4 00 μg disc^{-1}	C_5 200 μg disc^{-1}	Nystatin 200 μg disc^{-1}
Human pathogen						
Epidermophyton floccosum	10	9	10	18	0	22
Aspergillus niger	14	18	15	19	6	30
Candida albicans	20	14	18	13	10	20
Plant pathogen						
Aspergillus flavus	20	15	10	12	8	20
Fusarium species	7	25	7	10	6	15
Tricroderma species	15	20	9	10	8	18
Bipovis species	20	35	10	10	0	16
Mucor species	21	18	15	12	10	30

Table 3: MIC values of the compounds C_1, C_2, C_3, C_4 and C_5, against two Gram positive and two Gram negative bacteria

	Minimum inhibitory concentration (μ gm ml^{-1})				
Test organisms	C_1	C_2	C_3	C_4	C_5
Shigella dysenteriae	64	64	128	16	64
Salmonella typhi	32	32	64	8	16
Bacillus subtilis	32	16	32	8	64
Staphylococcus β *haemolyticus*	64	32	32	4	32

tested compounds are more superior for selection of a suitable chemotherapeutic agent. Further, acute toxicity and other pharmacological tests are necessary to utilize the compound C_4 as a potential therapeutic agent.

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