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Antimicrobial and cytotoxic activities of 2-aminobenzoic acid and 2-aminophenol and their coordination complexes with Magnesium (Mg-II)

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Abstract: Owing to the biological importance of metalloelements in many biological processes, especially metabolic processes, magnesium(II) complexes were synthesized and examined for their antimicrobial and cytotoxic activities. Among the two synthesized organometalic complexes [$Mg(2\text{-ap})_2$, A] and [$Mg(2\text{-ab})_2$, C], the maximum antibacterial and antifungal activities were shown by the compound A. Among the ligands, 2-aminophenol (B) showed more antibacterial activity than 2-aminobenzoic acid (D). The minimum inhibitory concentration for the complex A against five pathogenic bacteria *Streptococcus-β-haemolyticus*, *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* were found in the range of 32-64 μg ml^{-1}. The complexes were also tested for their cytotoxicity using brine shrimp lethality bioassay method and the LC_{50} values of the complexes A and C were found to be 5.7 and 24.3μg ml^{-1} respectively. Antibacterial, antifungal and cytotoxic studies undertaken for the above compounds indicated structure-activity relationships. These metalloderivatives were more active than the parent compounds (ligands).

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

INTRODUCTION

Metal coordination complexes have been reported in cancer therapy for their cytotoxic activities. Platinum coordination complexes form a new class of active anticancer agents in animals and man (Rosenberg, 1977). Various tumor cell lines are now growing resistance to conventional antitumor metal coordination complexes e.g acquired cisplatin (platinum complex) resistance in some (mainly murine) preclinical tumor models (Kelland, 1993). Thinking of tumor resistance and its circumvention there remains scope for substantial improvement in the clinical utility of metal coordination complexes through the discovery of additional platinum-based complexes (or possibly alternative metals). In the continuation of this strategy of new drug discovery we have studied two new coordination complexes for their antimicrobial and cytotoxic activities.

MATERIALS AND METHODS

Sources of compounds: In the present study the compounds were synthesized according to the following procedure:

Preparation of [$Mg(2\text{-ap})_2$, A] about 0.3764 gm of magnesium chloride (MgCl) was dissolved in 5 ml methanol in a 50 ml beaker. A solution of 1.080 gm of 2-aminobenzoic acid was made in 5 ml methanol in a 50 ml beaker. A solution of 0.5342 gm of ethylene diamine was also made in 5 ml methanol in an another 50 ml beaker. Three solutions were mixed, stirred for half an hour at room temperature and allowed to stand for half an hour. A gray white colored precipitate was observed. The precipitate was filtered and dried in vacuum desiccators over anhydrous $CaCl_2$.

Preparation of [$Mg(2\text{-ab})_2$, C] About 0.3764 gm of magnesium chloride (MgCl) was dissolved in 5 ml methanol in a 50 ml beaker. A solution of 0.87304 gm of 2-amino phenol was made in 5 ml methanol in a 50 ml beaker. A solution of 0.5342 gm of ethylene diamine was also made in 5 ml methanol in an another 50 ml beaker. Three solutions were mixed, stirred for half an hour at room temperature and allowed to stand for half an hour. A light yellow colored precipitate was observed. The precipitate was filtered and dried in vacuum desiccators over anhydrous $CaCl_2$. The prepared complexes were characterized by IR, UV, magnetic moment, melting point, conductivity measurements and literature review.

Where, 2-ap = 2-amino phenol
2-ab = 2-aminobenzoic acid

Antimicrobial and Cytotoxicity Activity Studies: *In vitro* Antimicrobial screening is generally performed by disc diffusion method (Bauer *et al*., 1966; Rios *et al*., 1988) for primary selection of the compounds as therapeutic agent.

Disc diffusion method is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. Generally, the more susceptible the organism the bigger is the zone of inhibition. The method is essentially a qualitative or semi quantitative 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 Microbiology Laboratory of the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. The diameters of zones of inhibition produced by the compounds were compared with the standard antibiotic (Kanamycin 30 μg $disc^{-1}$) and antifungal (Nystatin 50 μg $disc^{-1}$) agents for their antibacterial and antifungal activities. 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.

Brine shrimp lethality bioassay (Persoone *et al*., 1980; Meyer *et al*., 1982; Mclaughlin *et al*., 1988; Mclaughlin, 1990) is a recent development in the assay procedure of bioactive compounds which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds. Here *in vivo* lethality, a simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening.

RESULTS AND DISCUSSION

Antibacterial activity: The complexes A [Mg $(2\text{-ap})_2$] and C [$Mg(2\text{-ab})_2$] and the ligands 2-aminobenzoic acid (2-ab) and 2-amino phenol (2-ap) at a concentration of 30 μg $disc^{-1}$ and 100 μg $disc^{-1}$ showed antibacterial activity against four Gram positive (*Sarcina lutea, Bacillus subtilis, Streptococcus-β-haemolyticus and Staphylococcus aureus*) and five Gram negative (*Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Escherichia coli and Shigella flexneri*) bacteria. The results are given in Table 1. From this present investigation we can conclude that when ligands bind with metals then the formed complexes show more activity than the ligands themselves. 2-amino phenol shows more activity than 2-aminobenzoic acid and this is probably due to phenolic hydroxyl group. To investigate the exact mechanism of higher antibacterial activity of 2-amino phenol than 2-aminobenzoic acid further study will be needed.

Table 1: *In vitro* antibacterial activity of the compounds A, B, C, D and Standard Kanamycin

	Diameter of zone of inhibition (in mm)								
	A		B		C		D		Kanamycin
(μg $disc^{-1}$)	30	100	30	100	30	100	30	100	30
Gram positive bacteria									
Sarcina lutea	14	20	11	14	12	18	10	13	26
Bacillus subtilis	17	19	10	13	13	17	08	11	25
Streptococcus-β-haemolyticus	19	23	12	15	16	19	09	13	27
Staphylococcus aureus	16	21	09	13	11	15	08	12	26
Gram negative bacteria									
Salmonella typhi	16	19	11	13	14	16	09	11	27
Shigella dysenteriae	16	20	12	14	14	17	08	12	29
Pseudomonas aeruginosa	15	19	10	13	13	16	10	12	26
Shigella flexneri	14	19	11	13	13	15	09	11	25
Escherichia coli	16	21	10	12	12	15	08	10	27

Table 2: Minimum Inhibitory Concentration (MIC) values of the compounds A, B, C and D

	Minimum inhibitory concentration (μg ml^{-1})			
Test organisms	A	B	C	D
Bacillus subtilis	32	64	64	128
Streptococcus-β-haemolyticus	32	64	32	64
Escherichia coli	64	64	64	64
Salmonella typhi	32	128	64	64

Table 3: *In vitro* antifungal activity of the compounds A, B, C, D and standard Nystatin

	Diameter of zone of inhibition (in mm)				
	A	B	C	D	Nystatin
Test fungus	50 μg/disc	50 μg/disc	50 μg/disc	50 μg/disc	50 μg/disc
Candida albicans	09	00	00	00	20
Aspergillus niger	10	00	00	00	22
Aspergillus flavus	11	00	00	00	19

Table 4: The results of cytotoxic effect of the compounds A, B, C and D

Test samples	Medium lethal concentration (LC_{50}) values in μg ml^{-1}
A	5.7
B	56
C	24.3
D	63

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentrations (MIC) of the compounds A, B, C and D were determined against four pathogenic bacteria by serial dilution technique (Reiner,1982) and the values were given in the Table 2. The MIC values of the compound A against *Bacillus subtilis, Streptococcus-β-haemolyticus, Salmonella typhi* and *Escherichia coli* were found to be 32, 32, 64 and 32 μg ml^{-1} respectively, for compound B 64, 64, 64 and 128 μg ml^{-1} respectively, for compound C 64, 32, 64 and 64 μg ml^{-1} respectively, for compound D 128, 64, 64 and 64 μg ml^{-1} respectively. From the MIC values it was found that the complex A [$Mg(2\text{-ap})_2$] was more potent than the complex C [$Mg(2\text{-ab})_2$].

Antifungal activity: The compound A, B, C and D were tested against the pathogenic fungi *Candida albicans, Aspergillus niger* and *Aspergillus flavus* at a concentration of 50 μg $disc^{-1}$ for each and the result was compared with standard Nystatin 50 μg $disc^{-1}$. The activity was determined after 72 h of incubation at room temperature (30°C) and the obtained result are cited in Table 3. It was observed that only the complex A [$Mg(2\text{-}ap)_2$] is little active against the tested fungi in comparison with the standard Nystatin. Complex C [$Mg(2\text{-}ab)_2$] and both the ligands 2-amino phenol and 2-aminobenzoic acid were inactive in the concentration of 50 μg $disc^{-1}$ against the three pathogenic fungi.

These results (antibacterial and antifungal) suggest that the complex [$Mg(2\text{-}ap)_2$] is more active than others compounds (C, B and D) which is an interesting finding.

Cytotoxicity activity: The mortality rate of brine shrimp napulii was found to increase with concentration of sample and a plot concentration versus percent mortality on graph paper gave an almost linear correlation. The median lethal concentration (LC_{50}) of the complexes A and C was found to be 5.7 μg ml^{-1} and 24.3 μg ml^{-1} respectively (Table 4). The lowest LC_{50} found for the complex A, indicating that the cytotoxicity of the magnesium chelate with 2-amino phenol [$Mg(2\text{-}ap)_2$, A] is greater than the magnesium chelate with 2-aminobenzoic acid [$Mg(2\text{-}ab)_2$, C]. The cytotoxicity activity for the ligands 2-amino phenol (2-ap, B) and 2-aminobenzoic acid (2-ab, D) were little with high LC_{50} values 56μg ml^{-1} and 63μg ml^{-1} respectively but comparatively 2-amino phenol showed more cytotoxicity than 2-aminobenzoic acid. In the present investigation we can conclude that the complex [$Mg(2\text{-}ap)_2$] may have anticancer activity as it showed more cytotoxic activity and further investigations are needed to establish it as an anticancer agent.

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