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Studies on the Antibacterial and Cytotoxic Activities of Three Cobalt μ-Peroxo Complexes and Two Zirconium μ-peroxo Complexes

[1]M. Ashraful Alam, [2]Kudrat-e-zahan, [3]M. Shamim Hossain and [4]Fahmida Zerin Seema
[1]Rajshahi University of Engineering and Technology, Rajshahi-6204, Bangladesh
[21]Department of Chemistry, [3]Microbiology and Biotechnology Laboratory, Department of Pharmacy,
[4]Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: Three new cobalt μ-peroxo complexes [Co(2-apy)(O_2)(OX), C_1], [Co(2-apy)(O_2)(2-ap), C_2] and [Co(2-apy)(O_2)(2-ab), C_3] and two new zirconium μ-peroxo complexes [Zr(2-na)(O_2)(OX), C_5] and [Zr(2-ap)(O_2)(OX), C_5] were tested for their antibacterial activity by disc diffusion and serial dilution methods. All the coordination complexes were active against various test pathogenic organisms. The minimum inhibitory concentration (MIC) of the complexes was estimated between 32-64 μg ml^{-1} against *Bacillus subtilis* and *Eschericha coli*. The cytotoxicity of the five newly synthesized complexes was screened using brine shrimp lethality bioassay method and the LC_{50} values were calculated using probit analysis. LC_{50} values of the complexes C_1–C_5 were found 14.15, 9.12, 14.15, 29.51 and 5.75 μg ml^{-1}, respectively.

Key words: μ-peroxo complexes, antibacterial activity, cytotoxic activity, pathogens

INTRODUCTION

The frequency of life threaten infectious such as tuberculosis, cancer, AIDS etc. caused by pathogenic microorganisms is increasing worldwide and becoming an important cause of morbidity and mortality in immunocompromised patients. Synthetic compounds constitutes an important source of various bioactive compounds such as antibacterial[1,2] and anti cancer[3] compounds. The synthesized compounds which are used for treatment of infections diseases are known as chemotherapeutic. Every year thousands of compounds are synthesized with an aim to find potential chemotherapeutic agents to combat pathogenic microorganisms. But very few compounds are withstood as therapeutic agent for various methodological tests. Antibacterial is one of those tests required to perform for primary selection of compounds as the therapeutic agents.

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activity e.g. anticancer, antifungal, pesticidal, etc. Bioactive compounds are almost always toxic in high dose. Pharmacology is simply toxicology at lower dose or toxicology is simply pharmacology at a higher dose. *In vivo* lethality bioassay is simple conducted by a zoological organism (*Artemia salina*) for the convenient monitoring of the screening of bioactive synthetic compounds.

MATERIALS AND METHODS

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

Preparation of cobalt coordination complexes [Co(2-apy)(O_2)(LG*)]: A methanolic solution of Cobalt(II) chloride hexahydrate ($CoCl_2.6H_2O$) (3.09 g) and methanolic solution of 2-aminopyridine (1.22 g) were mixed in the 1:1 ratio with constant strring. Then a methanolic solution of second ligand (variable ligand) was added to the mixture. The resulting mixture were stirred for an hour at room temperature and allowed to stand for several minutes. Then 0.39g of H_2O_2 was poured down to the solution. The mixture was stirred for 22 h at room temperature and allowed to stand for several minutes. The precipitates formed were filtered, washed several times with mithanol and then dried in a vacuum desiccators charged with anhydrous $CaCl_2$.

When = (LG^*) = Deprotonated oxalic acid the compound is [Co(2-apy)(O_2)(OX)]
When = (LG^*) = 2-aminophenol the compound is [Co(2-apy)(O_2)(2-ap)]

Corresponding Author: Dr. Md. Ashraful Alam, Head, Department of Chemistry, Rajshahi University of Engineering and Technology, Rajshahi-6204, Bangladesh Tel: +88-0721-750924 E-mail: asraful_ruet@yahoo.com

When = (LG^*) = 2-aminobenzoic acid the compound is [Co(2-apy)(O_2)(2-ab)]
Where 2-apy = 2-aminopyridine

All the compounds were characterised by IR, UV, elementary analysis, magnetic moment, coductivity measurements, metal estimation, melting point measurements and literature review.

Preparation of [Zr(LG^*)(O_2)(OX)]: About 2.03 g of zirconium nitrate was dissolved in 5 mL methanol in a 50 mL beaker. A solution of 2.76 g of oxalic acid was also made in 5 mL methanol in a 50 mL beaker. Then a methanolic solution of second ligand (variable ligand) was added to the mixture. The three solutions were mixed, stirred for half an hour at room temperature and allowed to stand for several minutes. Then about 0.18 g of 30% H_2O_2 was poured down to the beaker. The mixture was stirred for 22 h at room temperature.

The precipitates formed were filtered, washed several times with methanol and then dried in a vacuum desiccators charged with anhydrous $CaCl_2$.

When = (LG^*) = 2-nitroaniline the compound is [Zr(2-na)(O_2)(OX)]
When = (LG^*) = 2-aminophenol the compound is [Zr(2-ap)(O_2)(OX)]
Where OX = deprotonated oxalic acid.

All the compounds were characterised by IR, UV, elementary analysis, magnetic moment, coductivity measurements, metal estimation, melting point measurements and literature review.

Antibacterial screening: "Disc diffusion method"[4-6] 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 semi quantitative test indicating sensitivity or resistance of microorganisms to the test materials as well as bacteriostatic or bactericidal activity of a compound[7].

The standard test microorganisms were collected from the Department of Microbiology, University of Dhaka, Dhaka, Bangladesh. The diameters of zones of inhibition produced by the compounds were compared with standard antibiotics (Kanamycin 30 μg $disc^{-1}$). The experiment was performed in duplicate to minimize errors.

Minimum inhibitory concentration (MIC): 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 antibacterial agent inoculated with the organism. The minimum inhibitory concentration (MIC) of the compounds C_1, C_2, C_3, C_4 and C_5 was determined against *Bacillus subtilis* and *Escherichia coli*. For the test, standard serial dilution technique[7] was employed. The media used in this respect was nutrient agar.

Cytotoxicity bioassay: Brine shrimp lethality bioassay[8-11] 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 test were carried out using brine shrimp nauplii eggs (*Artemia salina* L.). Eggs were placed in one side of a small tank divided by a net containing 3.8% NaCl solution for hatching. In other side of the tank, a light source was placed in order to attrack the nauplii. After two days of hatching period the nauplii were ready for the experiment. 3 mg of the complexes were accurately measured and dissolved in 0.6 ml (600 μl) of DMSO to get a concentration of 5 mg ml^{-1}. From the stock solutions 5, 10, 20, 40 and 80 μl were placed in 6 different vials making the volume up to 5 ml by NaCl solution. The final concentration of the samples, in the vials became 5, 10, 20, 40 and 80 μg ml^{-1}, respectively.

Ten brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used. After 24 h of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. The resulting data were transformed to the probit analysis[12] for the determination of LC_{50} values for the complexes.

RESULTS AND DISCUSSION

Antibacterial activity: The complexes C_1, C_2, C_3, C_4 and C_5 showed moderate antibacterial activity at the concentration of 100 μg $disc^{-1}$ in comparison with the standard kanamycin against the tested bacteria (Table 1). Metal coordination complexes have been reported for their antibacterial activity[2,13,14] and present findings supported those previous results of antibacterial activity for metal coordination complexes. These findings of our present investigation are interesting as these are new complexes. Though it is our primary investigation, further investigation are required to explore the mechanism of action of these new coordination complexes.

Minimum inhibitory concentration (MIC): The MIC values of the compound C_1 against *Bacillus subtilis* and

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)										
	C_1		C_2		C_3		C_4		C_5		
Test organisms	100	200	100	200	100	200	100	200	100	200	Kanamycin 30
Gram positive bacteria											
Streptococcus á-haemolyticus	12	15	13	16	11	14	12	16	12	16	22
Bacillus subtilis	13	17	12	14	12	16	11	14	10	16	23
Staphylococcus aureus	12	16	13	15	11	15	12	15	11	15	21
Sarcina lutea	14	17	12	14	13	16	11	15	11	17	22
Gram negative bacteria											
Escherichia coli	10	14	12	15	11	14	10	15	11	14	22
Salmonella typhi	11	15	11	15	10	13	11	14	12	15	21
Shigella sonnei	12	16	12	14	11	14	11	16	10	16	23
Shigella flexneri	10	14	12	16	12	15	12	15	11	14	22
Listeria monocytogenes	11	15	13	16	12	16	11	15	13	17	23

Table 2: MIC values of the compounds C_1, C_2, C_3, C_4 and C_5 against two Gram positive and Gram negative bacteria

	Minimum inhibitory concentration (μg ml^{-1})				
Test organisms	C_1	C_2	C_3	C_4	C_5
Bacillus subtilis	64	32	64	64	32
Escherichia coli	32	64	32	32	64

Table 3: Results of the compound C_1, C_2, C_3, C_4 and C_5 on brine shrimp lethality bioassay

Complexes	LC_{50} values (after probit transformation) in ppm (μg ml^{-1})
C_1	14.15
C_2	9.12
C_3	14.15
C_4	29.51
C_5	5.75
Standard gallic acid	4.53

Escharichia coli were 64 and 32 μg ml^{-1}, respectively, for compound C_2, 32 and 64 μg ml^{-1}, respectively, for compound C_3, 64 and 32 μg ml^{-1}, respectively, for compound C_4, 64 and 32 μg ml^{-1}, respectively, for compound C_5, 32 and 64 μg ml^{-1}, respectively (Table 2).

Cytotoxicity assay: From the probit analysis, the LC_{50} values of the samples were estimated and were found 14.15, 9.12, 14.15, 29.51 and 5.75 μg ml^{-1}, respectively (Table 3). The complex C_5 showed significant cytotoxicity in comparison with the standard gallic acid. Previously, many authors explored the cytotoxic activity of metal coordination complexes[15-17]. At the present investigation we also found significant cytotoxic activity for the tested coordination complexes. These findings of cytotoxicity properties of the tested complexes may suggest the probable anticancer properties and further studies are required to establish the anticancer activity of these new coordination complexes.

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