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In vitro Antimicrobial and *in vivo* Cytotoxic Activity of Some Ferrocene Derivative Bioactive Metal Complexes of First Transition Series

[1]M.E. Haque, [1]M.Z. Rahman, [2]M. Faruk Hossen, [1]M.M. Pervin, [1]M.H. Kabir, [1]K.M.K.B. Ferdaus, [1]Luthfunnesa Bari, [2]C.M. Zakaria, [1]Pervez Hassan and [3]M. Khalekuzzaman
[1]Institute of Biological Sciences, [2]Department of Chemistry,
[3]Department of Genetics and Breeding, University of Rajshahi,
Rajshahi-6205, Bangladesh

Abstract: The aim of the present study was to investigate the antimicrobial and cytotoxic activities of five newly synthesized ferrocene based complexes [$Mn(FcCOO)_2$, A], [$Co(FcCOO)_2$, B], [$Ni(FcCOO)_2$, C], [$Cu(FcCOO)_2$, D] and [$Zn(FcCOO)_2$, E]. The maximum antibacterial (at the concentration 100 μg $disc^{-1}$) and antifungal (at the concentration 200 μg $disc^{-1}$) activities were shown by the manganese complex A followed by cobalt complex B. The minimum activities were shown by Zink complex E. The minimum inhibitory concentration of the complexes was determined against four pathogenic bacteria *Bacillus subtilis, Bacillus megaterinum, Escherichia coli* and *Shigella shiga* and the values of complex A were found between 16-32 μg mL^{-1}. Brine shrimp lethality bioassay was carried out and all the complexes also showed cytotoxic effect compared with the standard bleomycin (0.41 μg mL^{-1}).

Key words: Coordination complexes, antibacterial activity, antifungal activity, cytotoxicity, pathogens

INTRODUCTION

In the old age, millions of people were died in the epidemic form of infectious diseases like plaque, cholera etc. Due to indiscriminate use of some antibiotics, the pathogenic organisms are gaining resistance to the existing antimicrobial and chemotherapeutic agents. But after the introduction of newer antibiotics and tremendous advancement of medical sciences and technology, these infectious diseases could be managed successfully. Transition metal coordination complexes have been widely studied for their antimicrobial properties (Kannalakannan and Venkappayya, 2002).

Every year thousands of compounds are synthesized with an aim to find potential chemotherapeutic agents to combat pathogenic microorganisms. But very few compounds withstand as therapeutic agent for various methodical tests. Antimicrobial screening is one of these tests. However, though these compounds possess antimicrobial properties but have serious toxic effects to the host, therefore in the ideal case, the drugs should be highly toxic to the parasite completely atoxic for the host. Hence, chemists are looking to other transition metal complexes as potential antimicrobial agents. Zakaria *et al.* (2000) synthesized several ferrocene derivative compounds, which were active against pathogenic microorganism's. In the continuation of our ongoing efforts aimed at finding new ferrocene derivative bioactive compounds for chemotherapy. We have synthesized five new coordination complexes of first transition series and have studied their antimicrobial and cytotoxic properties.

MATERIALS AND METHODS

Source of the complexes

Preparation of [$Mn(FcCOO)_2$], A: The 4 mL aqueous solution of Fc COONa (0.0504 g, 0.2 m mol) was dropped slowly into the 4 mL CH_3OH solution of $MnCl_2.4H_2O$ 0.0198 g (0.1 m mol) contained in a 50 mL round bottom flusk, the mixture was stirred and then was put in the dark. About 10 days later, the reddish yellow crystals were obtained. Then it was filtered and washed with methanol (CH_3OH) and dried under vacuum. The product was free from starting materials (Checked by T.L.C.). Yield: 0.0445 g, 87%. Melting point: >300°C (decomp.).

Preparation of [$Co(FcCOO)_2$], B: The 4 mL aqueous solution of Fc COONa (0.0504 g, 0.2 m mol) was dropped slowly into the 4 mL CH_3OH solution of $(CH_3COO)_2Co.4H_2O$ (0.0249 g, 0.1 m mol) contained in a 50 mL round bottom flusk, the mixture was stirred and then was put in the dark. About 10 days later, the reddish

Corresponding Author: Md. Emdadul Haque, M.Phil Fellow, Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

brown crystals were obtained. Then it was filtered and washed with methanol (CH_3OH) and dried under vacuum. The product was free from starting materials (Checked by T.L.C.) Yield: 0.0387 g, 75%. Melting point: >300°C (decomp.).

Preparation of [Ni(FcCOO)$_2$], C: The 4 mL aqueous solution of Fc COONa (0.0504 g, 0.2 m mol) was dropped slowly into the 6 mL CH_3OH solution of $(CH_3COO)_2Ni.4H_2O$ (0.0248 g ,0.1 m mol) contained in a 50 mL round bottom flusk, the mixture was stirred and then was put in the dark. About 10 days later, the yellow crystals were obtained. Then it was filtered and washed with methanol (CH_3OH) and dried under vacuum. The product was free from starting materials (Checked by T.L.C.) Yield: 0.0398 g, 77%. Melting point: >300°C (decomp.).

Preparation of [Cu(FcCOO)$_2$] , D: The 4 mL aqueous solution of Fc COONa (0.0504 g, 0.2 m mol) was dropped slowly into the 5 mL CH_3OH solution of $(CH_3COO)_2Cu.H_2O$ (0.0171 g, 0.1 m mol) contained in a 50 mL round bottom flusk, the mixture was stirred and then was put in the dark. About 10 days later, the gray crystals were obtained. Then it was filtered and washed with methanol (CH_3OH) and dried under vacuum. The product was free from starting materials (Checked by T.L.C.). Yield: 0.0376 g, 72%. Melting point: >300°C (decomp.).

Preparation of [Zn(FcCOO)$_2$], E: The 4 mL aqueous solution of Fc COONa (0.0504 g, 0.2 m mol) was dropped slowly into the 5 mL CH_3OH solution of $(CH_3COO)_2Zn.2H_2O$ 0.0199 g (0.1 m mol) contained in a 50 mL round bottom flusk, the mixture was stirred and then was put in the dark. About 10 days later, the reddish yellow crystals were obtained. Then it was filtered and washed with methanol (CH_3OH) and dried under vacuum. The product was free from starting materials (Checked by T.L.C.). Yield: 0.0356 g, 68%. Melting point: >300°C (decomp.).

Antibacterial screening: *In vitro* antibacterial screening is generally performed by disc diffusion methods (Rios *et al*., 1988; Beur *et al*., 1966) for the primary selection of compounds as therapeutic agents. In this method activity of the test compounds are expressed by measuring the diameter of zone of inhibition. Generally, the more susceptible the organisms the bigger the zone of inhibition. The method essentially a qualitative or semi quantitative test indicating sensitivity or resistance of microorganisms to the test material as well as bacteriostatic or bactericidal activity of a compound (Relner, 1982). The antimicrobial activity of the complexes A [Mn(FcCOO)$_2$], B [Co(FcCOO)$_2$], C [Ni(FcCOO)$_2$], D [Cu(FcCOO)$_2$] and E [Zn(FcCOO)$_2$] was determined at a concentration of 30 and 200 μg disc^{-1} against six gram positive (*Staphylococcus aureus, Streptococcus-β-heamolyticus, Bacillus megaterium, Bacillus subtilis, Saracina lutea, Bacillus cereus)* and eight gram negative (*Salmonella typhi, Shigella dysenteriae, Shigella shiga, Shigalla flexneri, Shigella sonnei, Shigella boydii, Escherrichia coll* and,*Klebsiella species*) bacteria. The diameters of the zone of inhibition produced by the complexes were compared with the standard antibiotic (kanamycin 30 μg disc^{-1}). The experiment was performed three times to minimize the errors.

Minimum Inhibitory Concentration (MIC) determination: MIC of the compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. MIC of the complexes were determined against two pathogenic bacteria (*Bacilllus subtilis, Streptococcus -β-haemolyticus, Shigella flexneri* and *Escherichia coli*) by serial dilution technique (Relner, 1982). The results were compared with the standard antibiotic kanamycin. The media used in this respect was nutrient broth (DIFCO).

Antifungal assay: The antifungal activities of the complexes were tested against four pathogenic fungi (*Candida albicans, Aspergillus niger, Aspergillus fumigatus and aaspergillus flavus*) at a concentration of 50 and 200 μg disc^{-1} for each. The media used in this respect was potato dextrose agar (PDA). The activity was determined after 72 h of incubation at room temperature. For a better correlation of the anti fungal activities Fluconazole 50 μg disc^{-1} was used as a standard.

Cytotoxicity bioassay : Brine shrimp lethality bioassay (Persoone, 1980; Mayer *et al*., 1982; Mclaughlin and Anderson, 1988; Mclaughlin, 1990) is a recent development in the assay procedure of bioactive compound which indicates cytotoxicity as well as a wide range of pharmacological activities (such as anticancer, antiviral, insecticidal, pesticidal, AIDS etc) of the compounds. Here *in vivo* lethality test was carried out by using brine shrimp nauplii eggs (*Artemia salina* L.). Eggs were hatching 48 h in 3.8% NaCl solution (Sea water) and after two days of hatching, the nauplii were ready for experiment as described previously (Mayer *et al*., 1982). Standard solutions of the complexes were prepared whose concentration was 5 μg mL^{-1} (3 mg of each complex was dissolved in 0.6 mL of DMSO). From the stock solution

5, 10, 20, 40 and 80 μL were placed in 5 different vials and the volume was made upto 5 mL with NaCl (3.8%) solution. Thus the final concentration of the sample in the vials became 5, 10, 20, 40 and 80 μm mL^{-1}, respectively. Then 10 brine shrimp nauplii were placed in each vial. For the control of each vial, one vial containing equal volume of DMSO and NaCl solution upto 5 mL. After 24 h of incubation, each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. From the data % of mortality was calculated and plotted against Log dose (logC). From the graph LC50 values of the complexes were determined using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Antibacterial activity: The metal complexe A show moderate antibacterial activities at the concentration of 30 μg $disc^{-1}$ with respect to the standard antibiotic Kanamycin but showed remarkable activities at the high concentration of 100 μg $disc^{-1}$ against both gram positive and gram negative bacteria. The other metal complexes (B-E) did not show remarkable activities at the concentration of 30 μg $disc^{-1}$ but show moderate activity at the concentration of 100 μg $disc^{-1}$ (Table 1). The more antibacterial activity of the complex A may be due to the metal manganese. Further studies were needed to explore the mechanism of antibacterial activity of these ferrocene derivative compounds. Manganese complexes have been reported for their antibacterial activity (Dendrinou-Samara *et al*., 2002; Chaudary *et al*., 2003; Saglam *et al*., 2002). Many authors also reported antibacterial activity of other transition metal complexes (Islam *et al*., 2002; Sultana *et al*., 2003; Biswas *et al*., 2002) and our present findings supported the previous results of antibacterial activity for both manganese and other metal coordination complexes.

Minimum Inhibitory Concentration (MIC): The MIC value of the complex A against *Bacillus subtilis, Bacillus megaterinum, Escherichia coli, and Shigella shiga* were 16 , 16 , 16 and 32 μg mL^{-1}, respectively (Table 2); for the complex B, 128, 128, 128, 128, respectively and for other two complexes D and E no remarkable MIC values can be found. From the MIC values it was found tat the ferrocene coordination complex A was more potent than the other complexes B, C, D and E of which E was least active.

Antifungal activity: The antifungal activities of the metal complexes (A-E) and standard Fluconazole (F-50 μg $disc^{-1}$) were determined at the concentration of 200 μg $disc^{-1}$ against four pathogenic fungi (Table 3). It was found that the metal complexes A was shown greater activity than others against all of the pathogenic fungi. The metal complex B was shown moderate activity. The antifungal activity of other transition metal complexes also

Table 1: *In vitro* antibacterial activities of the coordination complexes A, B, C, D, E and standard Kanamycin

	Diameter of zone of inhibition (mm)										
	A (μg $disc^{-1}$)		B (μg $disc^{-1}$)		C (μg $disc^{-1}$)		D (μg $disc^{-1}$)		E (μg $disc^{-1}$)		Kanamycin 30
Test organisms	30	100	30	100	30	100	30	100	30	100	(μg $disc^{-1}$)
Gram positive bacteria											
Bacillus subtilis	20	32	14	22	00	8	00	10	00	8	30
Streptococcus-β-haemolyticus	19	32	13	20	00	8	00	11	00	8	29
Bacillus megaterinum	22	35	14	25	00	9	00	12	00	8	30
Staphylococcus aureus	22	32	13	23	00	8	00	12	00	8	29
Sarcina lutea	19	28	12	19	00	9	00	8	00	8	30
Bacillus cereus	17	23	12	22	00	8	00	10	00	8	31
Gram negative bacteria											
Escherechia coli	24	36	13	24	00	8	00	11	00	8	30
Salmonella typhi	23	31	12	19	00	8	00	10	00	8	31
Shigella sonuei	22	31	11	21	00	8	00	9	00	8	30
Shigella dysenteriae	22	33	11	19	00	8	00	10	00	8	32
Shigella shiga	23	35	13	23	00	8	00	10	00	8	31

Where: Complexes, A = $[Mn(FcCOO)_2]$, B = $[Co(FcCOO)_2]$, C = $[Ni(FcCOO)_2]$, D = $[Cu(FcCOO)_2]$, E = $[Zn(FcCOO)_2]$

Table 2: Minimum Inhibitory Concentration (MIC values) of the complexes (A-E) and Kanamycin

	Minimum inhibitory concentration (μg mL^{-1})					
Test organisms	A	B	C	D	E	Kanamycin
Bacillus subtilis	16	128	-	-	-	4
Bacillus megaterinum	16	128	-	-	-	5
Escherichia coli	16	128	-	-	-	4
Shigella shiga	32	128	-	-	-	4

Table 3: Antifungal activities of the complexes (A-E) and standard Fluconazole

	Diameter of zone of inhibition (in mm)					
Fungal strains	A (200 μg disc^{-1})	B (200 μg disc^{-1})	C (200 μg disc^{-1})	D (200 μg disc^{-1})	E (200 μg disc^{-1})	Fluconazole (50 μg disc^{-1})
Plant pathogen						
Penicillium species	20	11	00	00	00	25
Aspergillus flavus	22	13	00	00	00	28
Human pathogen						
Candida sp.	20	12	00	00	00	24
Aspergillus niger	15	10	00	00	00	20

Table 4: The results of cytoxic effect of complexes A, B, C, D, E and standard Bleomycin

Test samples	Medium lethal concentration (LC_{50}) values in μg mL^{-1}
A	10.60
B	11.75
C	27.60
D	20.20
E	31.35
Standard bleomycin	0.41

reported by Mishra *et al.* (1995), Bacchi *et al.* (1999). Our present findings supported the previous results.

Cytotoxic activity: In the brine shrimp lethality bioassay the synthetic complexes (A-E) showed positive results indicating that the complexes are biologically active. The mortality rate of brine shrimp nauplii was found to increase with the increase of concentration of the sample. The LC_{50} values of the complexes A, B, C, D and E are 10.60, 11.75, 27.60, 20.20 and 31.35 μg mL^{-1}, respectively (Table 4). The standard anticancer drug gave its LC_{50} value at 0.41 μg mL^{-1}. The lowest LC_{50} value was found in case of complex A (10.60 μg mL^{-1}) followed by complex B (11.75 μg mL^{-1}) which is indicative of its higher cytotoxicity and anticancer effect on cancer cell lines. Many authors explored the cytotoxic properties of ferrocene derivative compounds (Zakaria *et al.*, 2001; Islam, 2000; Reich and Gennaro, 1990) and our present results suggested the cytotoxicity of previously reported ferrocene based complexes.

In conclusion, we may say that various bioactive compounds can be explored from ferrocene derivative complexes. From this experiment we were observed that compound A (a compound with manganese metal) followed by compound B (a compound with Cobalt metal) shows substantial antimicrobial activity with a minimum concentration. Complex A followed by complex B also showed higher cytotoxic activity. The activity profile of these complexes, may be considered as potential lead compounds for the development of new bioactive compounds. But these investigation is a primary one and further tests for its actual mechanism of cytotoxicity and extensive investigation on higher animal model for its other biological effects is required to utilize this complexes as potential chemotherapeutic agents.

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