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Biological Screening of Some Ferrocene Derivative Metal Complexes

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Abstract: The aim of the present study was to investigate the antimicrobial and cytotoxic activities of five newly synthesized ferrocene based complexes [Mn(Fcd(COO)$_2$), A], [Co(Fcd(COO)$_2$), B], [Ni(Fcd(COO)$_2$), C], [Cu(Fcd(COO)$_2$), D] and [Zn(Fcd(COO)$_2$), 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 cobult complexe B. The minimum activities were shown by zinc complexe 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 shirmp bioassay lethality was carried out for cytotoxicity measurements of the complexes and the LC_{50} values were calculated after probit transformation of the resulting mortality data. Among the five complexes manganese complex A was showed highest cytotoxic effect which is indicative of its probable effect on cancer cell lines.

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

INTRODUCTION

Cancer is caused when genetic damage to the cells prevents them being responsible to normal tissue controls. The cancer spreads when affected cells multiply rapidly, forming tumours of varying degrees. Different therapies can be used, depending on how far the cancer has spread. Anticancer drugs have originated from a variety of sources, including dyestuffs and chemical warfare agents and from natural products such as plants, microbes and fungi. One of the most potent and effective antitumour agents was discovered in the last century serendipitously by Rosenberg *et al.*[1]. Transition metal co-ordination complexes have now been widely studied for their antimicrobial[2] and anticancer[3,4] properties. Cisplatin is one of the most potent and effective antitumor agent but it lacks selectivity for tumour tissue and many tumours are growing resistance to this platinum complex. To address this problem modified versions of cisplatin, leading to second and third generation platinum-based drugs have been synthesized over the past 30 years and have got their less toxic effect to the host tissue[5]. The scientists are now engaged to explore other transition based complexes[6,7] and other complexes[8-13]. In the continuation of this discovery present studies synthesized five new ferrocene-derivative metal complexes and have studied their cytotoxicity, antibacterial and antifungal activities.

MATERIALS AND METHODS

Source of the complexes

Preparation of [Mn(Fcd(COO)$_2$)], A): The 5 mL aqueous solution of Fcd (COONa)$_2$ 0.0319 g (0.1 mM) was dropped slowly into the 4 mL CH_3OH solution of $MnCl_2.4H_2O$ 0.0198 g (0.1 mM) contained in a 50 mL round bottom flusk, the mixture was stirred and then was put in the dark. About 10 days later, the orange 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 TLC). Yield: 0.0297 g, 91%. Melting point: >300°C (decomp).

Preparation of [Co(Fcd(COO)$_2$)], B): The 5 mL aqueous solution of Fcd (COONa)$_2$ 0.0319 g (0.1 mM) was dropped slowly into the 4 mL CH_3OH solution of $(CH_3COO)_2Co.4H_2O$ 0.0249 g (0.1 mM) 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

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(CH_3OH) and dried under vacuum. The product was free from starting materials (Checked by TLC). Yield: 0.0275 g, 83%. Melting point: >300°C (decomp).

Preparation of [Ni(Fcd(COO)$_2$)], C: The 5 mL aqueous solution of Fcd ($COONa)_2$ 0.0319 g (0.1 mM) was dropped slowly into the 6 mL CH_3OH solution of $(CH_3COO)_2Ni.4H_2O$ 0.0248 g (0.1 mM) 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 TLC). Yield: 0.0261 g, 79%. Melting point: >300°C (decomp).

Preparation of [Cu(Fcd(COO)$_2$)], D: The 5 mL aqueous solution of Fcd ($COONa)_2$ 0.0319 g (0.1 mM) was dropped slowly into the 5 mL CH_3OH solution of $(CH_3COO)_2Cu.H_2O$ 0.0171 g (0.1 mM) 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 TLC). Yield: 0.0251 g, 75%. Melting point: >300°C (decomp).

Preparation of [Zn(Fcd(COO)$_2$)], E: The 5 mL aqueous solution of Fcd ($COONa)_2$ 0.0319 g (0.1 mM) was dropped slowly into the 5 mL CH_3OH solution of $(CH_3COO)_2Zn.2H_2O$ 0.0199 g (0.1 mM) 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 TLC) Yield: 0.0259 g, 77%. Melting point: >300°C (decomp).

Antibacterial screening: *In vitro* antibacterial screening is generally performed by disc diffusion methods[14,15] 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 bacteriostetic or bactericidal activity of a compound[16]. The antimicrobial activity of the complexes [Mn(Fcd(COO)$_2$), A], [Co(Fcd(COO)$_2$), B], [Ni(Fcd(COO)$_2$), C], [Cu(Fcd(COO)$_2$), D] and [Zn(Fcd(COO)$_2$), E]. 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, Sarcina lutea* and *Bacillus cereus)* and eight gram negative (*Salmonella typhi, Shigella dysenteriae, Shigella shiga, Shigalla flexneri, Shigella sonnei, Shigella boydii, Escherrichia colli* and *Klebsiella* sp.) 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 four pathogenic bacteria (*Bacilllus subtilis, Staphylococcus aureus, Salmonella typhi* and *Shigella dysentariae*) by serial dilution technique[16]. 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[17-20] 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[18]. Standard solution of the complexes were prepared whose concentration was 5 μg mL^{-1} (3 mg of each complexes 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 μg 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 5mL. 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 LC_{50} values of the complexes were determined using probit analysis[21].

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[22-24]. Many authors also reported antibacterial activity of other transition metal complexes[25-27] and present results 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, Staphylococcus aureus, Salmonella typhi and Shigella dysenteriae* 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 complexe 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 reported by Mishra *et al.*[10] and Bacchi *et al.*[11]. Our present results 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.

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 $disc^{-1}$)		
Test organisms	30	100	30	100	30	100	30	100	30	100	Kanamycin 30 (μg $disc^{-1}$)
Gram positive bacteria											
Bacillus subtilis	20	35	13	23	00	7	00	9	00	7	30
Streptotococcus-β-haemolyticus	18	32	13	21	00	8	00	10	00	8	29
Bacillus megaterinun	22	32	13	25	00	8	00	11	00	7	30
Staphylococcus aureus	22	35	14	25	00	9	00	12	00	8	29
Sarcina lutea	20	29	12	20	00	10	00	8	00	7	30
Bacillus cereus	17	22	12	21	00	9	00	10	00	8	31
Gram negative bacteria											
Escherechia coli	23	32	12	24	00	10	00	9	00	8	30
Ssalmonella typhi	24	36	12	20	00	9	00	10	00	7	31
Shigega sonnei	21	30	11	21	00	8	00	9	00	8	30
Shigella dysenteriae	20	35	13	23	00	9	00	10	00	7	32
Shigella shiga	22	32	11	20	00	8	00	9	00	8	31

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

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
Staphylococcus aureus,	16	128	-	-	-	5
Salmonella typhi	16	128	-	-	-	4
Shigella dysenteriae	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 sp.	21	11	00	00	00	25
Aspergillus flavus	22	12	00	00	00	28
Human pathogen						
Candida sp.	21	13	00	00	00	24
Aspergillus niger	16	10	00	00	00	20

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

		90% confidence limit (ppm)			
Test samples	LC_{50} (ppm)	Lower	Upper	Regression equation	χ^2
A	8.14	5.02	13.19	Y=3.7959+1.3224X	0.3776
B	12.38	8.06	19.02	Y=3.650+1.2353X	0.1290
C	19.52	12.72	30.13	Y=3.542+1.052X	0.1651
D	17.95	11.50	28.00	Y=3.644+1.081X	0.0958
E	36.03	22.71	57.16	Y=3.142+1.19X	0.1992
Standard bleomycin	0.41	0.276	0.620	Y=3.16+2.99X	0.62
Galic acid	4.53	3.330	6.150	Y=3.93+1.62X	1.25

The LC_{50} values of the complexes A, B, C, D and E are 8.14, 12.38, 19.52, 17.95 and 36.03 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 (8.14 μg mL^{-1}) followed by B (12.38 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 derivatives compounds[28-32] and found higher activities in case of manganese complexes. Present results suggested the cytotoxicity of previously reported ferrocene based complexes.

It may be conclude that among the five complexes the tested manganese complexe A has strong cytotoxic activity but this investigation is a primary one and farther tests are required to investigate its actual mechanism of cytotoxicity and its probable effects on higher animal model and on cancer cell line. Then we may be explored it as potent cytotoxic agents with the hope of adding arsenal of weapons used against the fatal disease cancer.

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