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Synthesis, Characterization, Antimicrobial Activities and Cytotoxic Properties of Ferrocenedicarboxylate Ligand and Their Metal Complexes

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Abstract: Mixed ligand metal complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) with ferrocenedicarboxylates have been synthesized and characterized on the basis of elemental analysis, conductance, magnetic moment, infrared and electronic spectral studies. The complexes have been obtained to be $[Mn(Fcd(COO)_2)]$; $[Co(Fcd(COO)_2)]$; $[Ni(Fcd(COO)_2)]$; $[Cu(Fcd(COO)_2)]$ and $[Zn(Fcd(COO)_2)]$ where Fcd = $-H_4C_5FeC_5H_4-$ the compounds (T_1), (T_2), (T_3), (T_4) and (T_5) respectively. Antimicrobial activity of the complexes have been examined against eight Gram positive and Gram negative pathogenic bacteria and four pathogenic fungi by disc diffusion method and compared with that of standard antibiotics (Kanamycin for antibacterial activity and Fluconazole for antifungal activity). These complexes have been found to be moderate to strong antimicrobial activity against the tested microbes. Brine shrimp eggs were hatched in artificial sea water and exposed to the complexes. Median lethal concentration (LC_{50}) values were calculated. The complexes showed toxicity against brine shrimp but complex T_5 explored its potent cytotoxicity having LC_{50} values at 1.59 µg/ml.

Keywords: Ferrocenedicarboxylate Ligand, antimicrobial activity, cytotoxic properties

INTRODUCTION

Coordination complexes of transition metal had been widely studied for their antimicrobial[1,2] and anticancer properties[3,8]. One of the most potent and effective antitumour agents was discovered in the last century serendipitously by Rosenberg[9]. Rosenberg and his coworkers synthesized several simple platinum complexes, among which cisplatin - $Pt(II)(NH_3)_2Cl_2$ - showed remarkable efficacy in inhibiting the growth of tumours in mice[10]. McGowan[11] reported the first clinical trials of cisplatin in 1971, with official approval being granted in the US in 1978. Despite the success of cisplatin, however, it lacks selectivity for tumour tissue, which leads to severe side effects including renal impairment, neurotoxicity and ototoxicity. Various tumor cell lines are now growing resistance to cisplatin e.g., acquired cisplatin resistance in some preclinical tumor models[12].

The scientists are now engaged to explore other transition metal complexes as antitumour agents and considerable results have brought through the discovery of titanium based complexes[13,14] and other transition metal based complexes[15,21]. Among the other transition metal complexes the titanium complex, titanocene dichloride ($TiCp_2Cl_2$) is the only metallocene-based compound to have entered clinical trials for its potent and broad spectrum activity in mammalian tumors[11]. Compared to standard antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblastine, titanocenedichloride was found to exhibit higher cytotoxicity in renal cell carcinoma[13]. The titanocenedichloride was found to exhibit more effective in human ovarian cancer xenograft model than cysplatin[14]. Recently some derivatives of titanocenedichlorides showed enhanced anti-cancer activity[22]. Therefore, it is of our interest to study the cytotoxic and antimicrobial properties of some novel coordination complexes of different transition metals to assess their biological potency. We have found promising cytotoxic and antimicrobial activities of these novel complexes and further studies on mammalian cancer cell lines may explore their valuable cytotoxicity which may come as potent anticancer agent(s) in the modern clinical trials.

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MATERIALS AND METHODS

Reagents and Chemicals: All chemicals were analytical reagent grade and were used as supplied by BDH (England), E-merck (Germany), Fluka (Switzerland) and Carew and Co. (Bangladesh). Methanol was purified (99%) by refluxing the crude with iodine and magnesium turnings.

Synthesis of the Complexes: Ferrocene-1,1¢-dicarboxylic acid, $Fcd(COOH)_2$ (2)

Part-1: About 0.2 mole of 2-chlorobenzoyl chloride and 0.2 mole $AlCl_3$ in 600 ml CH_2Cl_2 were added the ferrocene (0.05mole) drop-wise over 35 minutes. The mixture was stirred for ca. 24 hours and then poured into ice/water (1:1 in 400 ml) and then stirred for ca. 30 minutes. The layers were separated by separating funnel and the aqueous extracted with CH_2Cl_2 (2 ´ 50 ml) and the combined organic washed with water (3 ´ 100 ml) and NaOH (10%, 50 ml) before drying and evaporating to a small volume. This was poured into swirling light petroleum which caused the formation of small red crystals on the flask wall on standing overnight and deposition of drown product on the flask base. The red crystals (4.2g) were separately removed and dried *in vacuo*.

Part-2: To a stirred solution of potassium tertbutoxide (0.41 mole) and water (2.2 ml) in 1, 2-dimethoxyethane (250 ml) was added the bisacylferrocene (0.04 mole) to give a red solution. This was stirred and heated at reflux for ca. 1.5 hour and then poured into water (1000 ml) and extracted with ether (3 ´ 150 ml). The combined ether extracts were back-extracted with NaOH (10%, 60 ml) and the combined aqueous extracts acidified with conc. HCl (pH 1). The yellow precipitate so formed was filtered and dried at 80°C (2 days).

Sodium ferrocene 1,1¢-dicarboxylate, $Fcd(COONa)_2$ (4): The 4 ml CH_3OH solution of NaOH (2m mol) was dropped slowly into the 13 ml THF (Tetrahydrofuran) solution of ferrocenedi-carboxylic acid 0.2748 g (1m mol) contained in a 250 ml round bottom flask. Then the mixture was stirred for ca. 6 hours and put in the room temperature. About 24 hours later, the yellow crystals were obtained. Then it was filtered and dried under vacuum.

The 5 ml aqueous solution of Fcd $(COONa)_2$ (0.1 m mol) was dropped slowly into the 5 ml CH_3OH solution of $MnCl_2.4H_2O$, $(CH_3COO)_2Co.4H_2O$, $(CH_3COO)_2Ni.4H_2O$, $(CH_3COO)_2Cu.H_2O$ and $(CH_3COO)_2Zn.2H_2O$ in 0.1 m mol respectively contained in a 50 ml round bottom flask, the mixture was stirred and then was put in the dark. About 10 days later, the orange, yellow, yellow, gray and reddish yellow crystals respectively were obtained. Then it was individually filtered and washed with methanol (CH_3OH) and dried under vacuum to yield $[X(Fcd(COO)_2)]$ where X= Mn, Co, Ni, Cu, and Zn the compounds T_1, T_2, T_3, T_4 and T_5 respectively.

Antibacterial Screening: *In vitro* antibacterial screening is generally performed by disc diffusion method[23,25] for primary selection of the compounds as therapeutic agent. Disc diffusion method is equally suited to screening of antibiotics or the products of plant evaluation[26] and 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. In this method the compounds are applied to the agar medium by using paper discs[27,28]. The method is essentially a qualitative or semi quantitative test which allows classification of microorganisms as susceptible, intermediate or resistance to the test materials as well as bacteriostatic or bactericidal activity of a compound[29].

The antibacterial activity of the complexes T_1, T_2, T_3, T_4 and T_5 was determined at a concentration of 30 µg/disc and 200 µg/disc against four Gram-positive and four Gram-negative bacteria. The diameters of the zone of inhibition produced by the compounds were compared with the standard antibiotic (Kanamycin, 30 µg/disc). The experiments were performed at four times to minimize the error.

Growth Media and Conditions: Nutrient broth was used as liquid culture of all the tested bacteria and is used in the minimum inhibitory concentration determining experiments. Antifungal activity of the complexes was done of PDA (Potato dextrose agar) media spreading with fungal spores and kept at 28 ºC for about 72 hours. For PDA preparation 20 gm Potato was extracted with distilled water 100 ml at 100 ºC for 1 hour and then mixed with 2 gm Dextrose and 1.5 gm agar and finally the pH of the prepared media (PDA) was adjusted at 7.00.

MIC Measurements: A current definition of the Minimum Inhibitory Concentration, MIC, is ``the lowest concentration which resulted in maintenance or reduction of inoculum viability"[30]. Serial dilution technique[29] was applied for the determination of minimum inhibitory concentration of complexes. The end result of the test was the minimum concentration of antimicrobial (test materials) which gave a clear

solution, i.e., no visual growth[31,32]. Four bacterial species and three fungal species were used. DMSO was used for our experiments made dilutions of the coordination complexes under test in MIC determination. Bacterial and fungal inocula were prepared at 5×10^6 - 5×10^7 cfu/ml. Final adjustment were made using optical density measurement for bacteria (absorbance 0.05 at a wavelength of 660 nm).

Collection of the Bacterial and Fungal Species: The bacterial species used in this experiment were *Bacillus subtilis* (QL-40), *Streptococcus b-haemolyticus* (ATCC-12873), *Staphylococcus aureus* (ATCC-25933), *Bacillus megaterium* QL-38), *Escherichia coli* (ATCC-25922), *Shigella sonnei* (AJ-8992), *Shigella dysenteriae* (AL-35587) and *Shigella shiga* (ATCC-26107) all of which were collected from the Institute of Nutrition and Food Sciences (INFS), Dhaka University, Bangladesh.

Tested fungi *Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (ATCC 1028), *Aspergillus niger* (CCRC 31494) and *Penicillium* species were collected from the Institute of Biological Sciences (IBSc), Rajshahi University, Bangladesh, from their stock culture.

Antifungal Screening: The antifungal activity of the complexes were tested by disc diffusion method[23-25] against the three pathogenic fungi *Candida albicans*, *Aspergillus niger Aspergillus fumigatus* and *Penicillium* species at a concentration of 200 µg/disc for each. The media used in this respect was potato dextrose agar (PDA). The activity was determined after 72 hours of incubation at room temperature (30 ºC).

Cytotoxicity Bioassay: Brine shrimp lethality bioassay[33,37] 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. The brine shrimp assay was proposed by Michael[38] and later developed by Vanhaecke[39] and Sleet[40]. It is based on the ability to kill laboratory-cultured brine shrimp (*Artemia* nauplii). The assay is considered a useful tool for preliminary assessment of toxicity[41] and it has been used for the detection of fungal toxins[42], plant extract toxicity[36], heavy metals[43], cyanobacterial toxins[37], pesticides[44], and cytotoxicity testing of dental materials[45].

In the present study, in-vivo lethality test was carried out using brine shrimp nauplii eggs (*A. 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. Three mg of the complexes were accurately measured and dissolved in 600 µL of DMSO to get a concentration of 5 mg/ml. From the stock solutions 1, 2, 5, 10, 20, 40 and 80 µL were placed in 7 different vials making the volume up to 5 ml by NaCl solution. The final concentration of the samples, in the vials became 1, 2, 5, 10, 20, 40 and 80 µg/mL respectively.

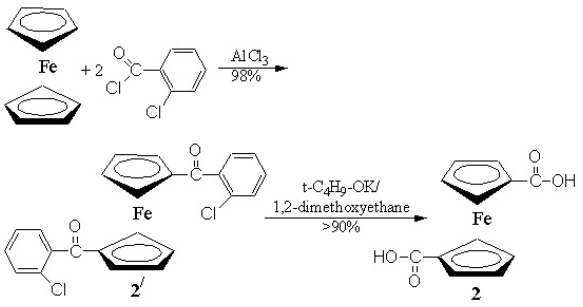
Ten brine shrimp nauplii were then placed in each vial. For the control test of each vial, one vial containing the same volume of DMSO plus water up to 5 ml was used. After 24 hours of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. The resulting data were transformed to the probit analysis[46] for the determination of LC_{50} values for the complexes.

Statistical Analysis: Statistical analyses of the antibacterial and antifungal activities of seven novel thiocyanato complexes with different concentrations of each (30 and 200 µg/disc) was performed using Kruskal-Wallis test[47]. Individual antibacterial and antifungal activity differences of the tested complexes (T_1-T_5) was examined using post hoc Nemenyi's test following Kruskal-Wallis test. A significance level of 5 % was considered as significance ($P < 0.05$) in all cases. Probit analysis[46] was used to determine the LD_{50} values from the mortality data using Probit software. The cytotoxicity of the novel thiocyanato coordination complexes was compared with the standard gallic acid and also with the anticancer agent bleomycin.

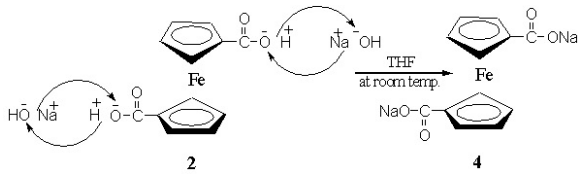
RESULTS AND DISCUSSIONS

Results:

Synthetic Pathways: During the course of reactions ferrocene first reacted with 2-chlorobenzoyl chloride in presence of $AlCl_3$ to form bisacylferrocene which in turn reacted with potassium tertbutoxide and water (2.2 ml) in 1, 2-dimethoxyethane to make Ferrocene-1,1¢-dicarboxylic acid, Fcd $(COOH)_2$, (2). The scheme of the synthesis, $Fcd(COOH)_2$, where Fcd = -$H_4C_5FeC_5H_4$- is given below:



Sodium ferrocene 1,1¢-dicarboxylate, $Fcd(COONa)_2$, (4) was formed when tetrahydrofuran, THF solution of ferrocenedi-carboxylic acid was dropped slowly into CH_3OH solution of NaOH in a round bottom flask. The scheme of the mechanism is given below:



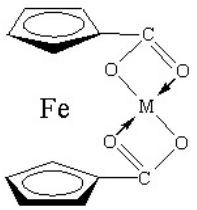
Characterization: From the ^{1}H-NMR spectra, it showed the broad multiplet at d 7.25 – 7.45 (8H, m) for the C_6H_4 protons and the multiplets at d 4.80 (4H, m) and 4.70 (4H, m) for the C_5H_4 protons. The ^{13}C-NMR spectra of the compound showed a singlet at d 197.4(s) for the C=O carbon. The bands at d 138.5(s), 131.1(d), 130.9(s), 130.3(d), 128.7(d), 126.4(d) suggest for the phenyl carbons[48]**.** The peaks at d 79.7(s), 74.7(d), 72.4(d) suggest for the C_5H_4 carbons. The above data reveal that the intermediate is (2¢). The IR spectrum of the compound (2) showed an absorption band at 3015 cm^{-1} which is assigned to the n(O-H) stretching of the acid (COOH) groups. A strong band at 1730 cm^{-1} is clearly indicated the presence of n(C=O) stretching of acid group of the compound (2)[49]. A strong band at 1490 cm^{-1} is due to the n(C-O) stretching of the acid (COOH) group.

The ^{1}H-NMR spectrum of the compound (2) showed the multiplets at d 4.85 (4H, m) and d 4.75 (4H, m) for the C_5H_4 protons. The UV-visible spectra of the compound (2) (Fig-1) are showed the absorption bands at 240 to 440 nm which are assigned to the p-p* and n-p* of C=O and ferrocenyl bonds respectively. The conductance (Table 2) of the compound (2) is 87.35 ohm^{-1} cm^2 mol^{-1} which reveals that the compound (2) is electrolytic in nature. On the basis of the IR data (Table 1), ^{1}H-NMR, UV-visible data (Table 4) and other analytical data (Table 3) and physical studies (Table 2) reveals that the proposed structure of the compound is (2).

The ligand (4) was prepared by the reaction of (2) with sodium hydroxide. The IR spectra (Table 1) of the ligand (4) showed that the absence of n(O-H) stretching frequency suggested that the formation of the ligand (4) from (2) having a strong band at 1685 cm^{-1} due to the presence of n(C=O) stretching frequency and a strong band at 1425 cm^{-1} which is assigned to the n(C-O) stretching frequency of the ligand (4). The UV-visible spectra of the ligand (4) (Table 4) showed the absorption bands at 240-440 nm which are assigned to the p-p* and n-p* of C=O and ferrocenyl bonds respectively. The conductance (Table 2) of the ligand (4) is 85.65 ohm^{-1} cm^2 mol^{-1} which suggests that the ligand (4) is electrolytic in nature. On the basis of the above assignment, the proposed structure of the ligand is (4).

The IR spectra (Table 1) of the complexes (T_1 to T_5**)** showed the strong bands in the region (1610-1590) cm^{-1} suggest for the (C = O) stretching frequencies due to the coordination with metal, as a result the (C = O) stretching frequency decreases. The strong bands of the complexes (T_1 to T_5) in the region (1340-1310) cm^{-1} that attributed to the n(C-O) of (COOH) group. The UV-visible spectra of the complexes (T_1 to T_5) (Fig.1) showed the absorption bands in the region (480-580) nm due to the d-d transition. The d-d transition for the complexes (T_1 to T_5) is characteristics of square-planar geometry. It appears from the magnetic moment measurement data (Table 2) that the complexes (T_3) and (T_5) showed diamagnetic property of square-planar Ni(II), Zn(II) symmetry[50].

The magnetic moment of the complexes (T_1) is 2.92 B.M. corresponding to the presence of three unpaired electrons and the magnetic moment of the complexes (T_2) and (T_4) are 1.47 and 1.23 B.M. respectively corresponding to the presence of one unpaired electron. The conductance data (Table 2) of the complexes (T_1 to T_5) in DMSO reveal that these are non-electrolytic[51] in nature. On the basis of the above assessments, the proposed structure of the complexes (T_1 to T_5) is square planar as in below.



where, T_1, M = Mn(II); T_2, M = Co(II); T_3, M = Ni(II); T_4, M = Cu(II) & T_5, M = Zn(II)

Antibacterial Activity: At concentration of 100 µg/disc the complexes T_3 and T_5 did not show remarkable biocidal activity whereas the T_1, T_2 and T_4 showed modest antibacterial activity against the tested Gram positive and Gram negative bacteria (Table 5). It was found that the metal complexes T_1> T_2 were more active than others against all of the test bacteria. The metal complex T_4 also was shown substantial antibacterial activity. At concentration of 30 µg/disc the platinum and titanium based complexes T_1 and T_2 showed remarkable antibacterial activity against the tested bacteria in comparison with the standard kanamycin. In the present investigation we found that the complexes showed comparatively better antibacterial

Table 1: Major IR spectral data (cm^{-1}) for the complexes (T_1 –T_5) and their assignment

Complexes	n(C=O)	n(C-O)	n(=C-H)	n(C=C)	n(M-O)
2	-	-	-	-	-
4	1817	1629	3357	1681	-
T_1	1716	1592	3368	1680	491
T_2	1720	1591	3368	1679	489
T_3	1717	1593	3363	1684	490
T_4	1723	1595	3367	1675	488
T_5	1721	1593	3366	1675	494

Table 2: Physical properties of complexes (T_1 –T_5)

Complexes	Molar conductance ohm^{-1} cm^2 mol^{-1}	Magnetic moment (B.M.)	M.P.(±0.5°C)	Colour
T_1	9.31	2.92	> 300	Orange
T_2	15.16	1.47	> 300	Yellow
T_3	11.20	Dia	> 300	Yellow
T_4	8.35	1.23	> 300	Gray
T_5	9.37	Dia	> 300	Reddish Yellow

Table 3: Analytical and physical data of complexes (T_1 –T_5)

Complex	% Yield	% Metal	% Carbon	% Nitrogen	% Hydrogen	MW
T_1	91	16.8	43.9	-	2.33	326.9
T_2	83	17.8	43.4	-	4.37	330.9
T_3	79	17.7	43.5	-	2.39	330.7
T_4	75	18.9	42.8	-	2.28	335.6
T_5	77	19.4	42.5	-	2.30	337.4

Table 4: UV-Visible Spectral data and physical properties of the complexes (T_1 –T_5)

Complexes	Solubility	λ_{max} (nm)	Absorption (A)	Molar Conductance (ohm^{-1} cm^2 mol^{-1})
2	Dichloromethane	240	1.776	87.35
4	Water	240	1.642	85.65
T_1	DMSO	480	1.785	5.75
		540	1.335	
T_2	DMSO	480	1.598	
		550	1.301	4.65
T_3	DMSO	480	2.223	
		540	1.982	6.65
T_4	DMSO	480	2.193	
		530	1.772	4.35
T_5	DMSO	480	1.835	
		540	1.537	7.75

activity against the Gram positive bacteria than the Gram negative bacteria. Many authors reported antibacterial activity of different transition metal complexes[52-54] and our present findings supported the previous investigations.

Minimum Inhibitory Concentration (MIC): The MIC values of the complexes against *Bacillus subtilis, Shigella dysenteriae*, *Salmonella typhi*, *Eschericia coli, Candida albicans, Aspergillus fumigatus and Aspergillus niger* were shown in Table 7. The MIC

Table 5: In vitro antibacterial activities of Complexes T_1 to T_5 and standard, Kanamycin. Diameter of zone of inhibition (in mm).

Compounds	T_1		T_2		T_3		T_4		T_5		Kanamycin
µg/disc	30	100	30	100	30	100	30	100	30	100	30
G. positive bacteria *Staphylococcus aureus*	14	29	9	20	7	16	6	19	7	15	24
Streptococcus-b-haemolyticus	14	33	9	19	7	16	9	19	00	16	28
Bacillus megaterium	15	34	10	22	8	17	8	20	6	15	27
Bacillus subtilis	13	32	10	20	6	16	8	19	7	15	27
G. negative bacteria *Shigella shiga*	12	31	8	19	00	17	8	19	00	17	22
Shigella sonnei	10	29	7	18	7	16	8	20	6	15	20
Escherichia coli	14	33	8	20	00	15	9	19	7	17	22
Shigella dysenteriae	12	31	10	20	7	16	7	20	8	18	26

Table 6: Antifungal activities of the complexes (T_1 –T_5) and standard Fluconazole.

	Diameter of zone of inhibition (in mm)					
	T_1	T_2	T_3	T_4	T_5	Fluconazole
µg/disc	200	200	200	200	200	200
Candida albicans	17	10	00	6	00	24
Aspergillus niger	16	10	00	7	00	20
Aspergillus fumigatus	21	12	00	6	00	28
Penicillium species	18	10	00	7	00	25

Table 7: The results of MIC values (in mg/ml) of Complexes (T_1 –T_5)

Test Organism	Minimum inhibitory concentration (µg/ ml)						
	T_1	T_2	T_3	T_4	T_5	Kanamycin	Fluconazole
Bacillus subtilis	16	8	128	64	128	2	-
Shigella dysenteriae	4	16	128	64	128	2	-
Salmonella typhi	16	64	32	4	128	2	-
Eschericia coli	32	64	16	2	64	4	-
Candida albicans	64	128	32	-	4	-	2
Aspergillus fumigatus	32	32	16	-	16	-	2
Aspergillus niger	32	64	32	-	4	-	2

values of the complexes T_3, T_4 and T_5 against the tested bacteria were maximum which indicated that the complexes were less active against the bacteria and T_4 was inactive against the fungus. For T_1 the MIC values were 16, 4, 16, 32, 64, 128 & 64 µg/ml, respectively against the organisms; whereas the complexes T_2, T_3 and T_5 showed MIC values between 4-128 µg/ml which was indicative of their potent antibacterial properties than the other complexes. From the MIC results it was indicated T_4 was more potent against fungi than that of bacteria.

Antifungal Activity: The antifungal activities of the metal complexes and standard Fluconazole (F-50 mg/disc) were determined at the concentration of 200 mg/disc against four pathogenic fungi. It was found that the metal complexes $T_1 > T_2$ were shown greater activity than others against all of the pathogenic fungi. The metal complex T_4 was shown substantial antifungal activity. Table 6 showed that the complex T_1 was noticeable active against the tested fungi at concentration of 200µg/disc with comparing the standard fluconazole. The maximum zone of inhibition

Table 8: Cytotoxic effect of complexes (T_1 –T_5) and standard bleomycin and gallic acid

Test samples	LC_{50} (ppm)	Regression equation	c^2 (df)
T_1	6.49	Y = 3.174 + 2.278 X	0.35 (2)
T_2	2.28	Y = 4.263 + 2.076 X	0.15 (2)
T_3	5.56	Y = 3.298 + 2.285 X	0.15 (2)
T_4	3.01	Y = 3.878 + 2.368 X	3.30 (2)
T_5	1.59	Y = 1.921 + 2.569 X	1.94 (2)
Standard Bleomycin	0.41	Y= 3.163+ 2.989 X	0.62 (2)
Gallic acid	4.53	Y= 3.933 + 1.626 X	1.25 (2)

against *Aspergillus fumigatus* and *Penicillium* species were found to be 21 and 18 mm respectively, for the complex T_2 and T_4 which were near to the zone of inhibition of 10 mm, T_3 and T_5 were inactive against all the fungi. Different metal coordination complexes have been previously reported for their antifungal properties[52-53] which supports our present findings.

Cytotoxicity: The mortality rate of brine shrimp nauplii was found to increase with increasing the concentration of complexes. Table 8 summarizes that the LC_{50} values of the complexes T_1, T_2, T_3, T_4 and T_5 were found at 6.49, 2.28, 5.56, 3.01 and 1.59µg/ml (ppm), respectively. The standard anticancer drug bleomycin gave its LC_{50} value at 0.41 µg/ml. The lowest LC_{50} value at 1.59 ppm was found in case of complex T_5 which was indicative of its potent cytotoxicity than the other coordination complexes in this experiment. Ferrocene based complexes have been reported previously for their potent cytotoxic properties than platinum based complexes[13,14] and our present findings also support the previous investigations as the titanium based complex T_5 (LC_{50}= 1.59 ppm) showed more cytotoxicity than the platinum based complex T_4 (LC_{50}= 3.01 ppm).

Discussion: Compared to standard antineoplastic agents such as cisplatin, doxorubicin, mitoxantrone and vinblastine, titanocenedichloride (titanium complex) was found to exhibit higher cytotoxicity in renal cell carcinoma[13]. The titanium based complexes was also found to exhibit more effective in mammalian cancer model than cisplatin[14]. Therefore it is of our interest to explore some novel transition metal based complexes as potent cytotoxic agents which might come as potent anticancer agent in clinical trials. In the present investigations we found a novel Mn based complex T_1 with potent antimicrobial agent and had moderate cytotoxicity. Among the other four new ferrocene complexes only T_2 and T_4 showed promisng cytotoxic effect compared with the reference standard gallic acid. Cytotoxic properties of coordination complexes had been previously reported by many authors[3,8,55,57] and our present findings also displayed the similar type of properties for the newly synthesized ferrocene complexes. The different LC_{50} values for the ferrocene complexes indicated the different mode of actions of their cytotoxicity. Further investigations are required to explore the exact mechanism of their cytotoxic properties which may be helpful for to explore new type of potent cytotoxic agent(s) with the hope of adding new and alternative chemotherapeutic agent(s) in clinical implications.

The newly synthesized complex T_1 displayed poor antibacterial activity at the concentration of 30 µg/disc, but gave promising activity at concentrations of 100µg/disc. The MIC values of this complex against the tested organisms indicated their noticeable antibacterial and antifungal potencies compared with standard antibiotic, kanamycin and fluconazole respectively. The mechanism of biocidal activity of these coordination complexes may be due to oxidative DNA damage as the previous reports[19,20]. The different antibacterial activity of the complexes indicated their different mechanism of biocidal property and further studies are required to explore the exact mechanism of antibacterial potency[58].

It was concluded that among the tested complexes, the complex T_1 possesses substantial antimicrobial activity with a minimum inhibitory concentration and moderate cytotoxicity. By comparing the results with previously published results[54] of benzene sulfonic acid derivative complex compounds, we can say that our tested complexes are more superior for selection of a suitable chemotherapeutic agent. Further, acute toxicity and other pharmacological tests are necessary to utilize the complex T_1 as a potential therapeutic agent.

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