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Novel Bioactive Fe (III) Complexes Derived From Succinimide and Amino acids

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Abstract: The aim of the present study was to investigate the antimicrobial and cytotoxic activities of four newly synthesized iron (III) based complexes [Fe(Suc)$_2$(Phenylamine)$_2$ F$_1$], [Fe(Suc)$_2$(Serine)$_2$ F$_2$], [Fe(Suc)$_2$(Leucine)$_2$, F$_3$] and [Fe(Suc)$_2$(Cystein)$_2$, F$_4$]. The complexes F$_1$, F$_2$, F$_3$, F$_4$ showed modest antibacterial and antifungal activities at the concentration of 200 μg disc^{-1} and gave MIC values between 16-64 μg ml^{-1} against the tested bacteria. Brine shrimp lethality bioassay was carried out for cytotoxicity measurements of the complexes and the LC$_{50}$ values were calculated after probit transformation of the resulting mortality data. All the complexes showed lower cytotoxic properties compared with the reference standard gallic acid (4.53 μg ml^{-1}) and bleomycin (0.41 μg ml^{-1}).

Key words: Iron coordination complexes, antibacterial, antifungal, cytotoxicity

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]. Rosenberg and his coworkers synthesized several simple platinum complexes, among them cisplatin-Pt(II)(NH$_3$)$_2$Cl$_2$-showed remarkable efficacy in inhibiting the growth of tumours in mice[2]. Cisplatin is one of the most potent and effective antitumor agent but it lacks selectivity for tumor tissue and many tumors 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[3]. 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[4,5] and other complexes[6-11].

In the continuation of this search for bioactive coordination complexes, we have synthesized four new Fe (III) based complexes and have studied their antibacterial, antifungal and cytotoxic properties.

MATERIALS AND METHODS

Preparation of Fe (III) complexes: The alcoholic solutions of ferric chloride and of potassium succinimide were mixed in the respective molar ratio and refluxed about fifteen minutes with gentle heat. Then aqueous solution of amino acids containing minimum amount of KOH (to make soluble) were mixed in a molar ratio with the previous mixture. To get the precipitate of the complexes, the mixture was then heated at 80°C for 40 min. and then allowed to stand for 10 min. The precipitates formed were removed by filtration, washed several times with distilled water and then with alcohol and dried in a vacuum desiccator over anhydrous CaCl$_2$.

The complexes were formed according to following reactions-

$$\text{H-Sim} + \text{KOH} \rightarrow \text{K-Sim} + \text{H}_2\text{O}$$
$$\text{FeCl}_3 + \text{A-H} + \text{K-sim} \rightarrow \text{K[Fe(Sim)}_2\text{(A)}_2\text{]}$$

Where,
Sim = anions of succinimide
A= amino acids e.g., phenylalanine, cystein, serine and leucine

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Antibacterial screening: *In vitro* Antibacterial screening is generally performed by disc diffusion method[12,13] 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[14]. The antibacterial activity of the complexes F_1, F_2, F_3 and F_4 was determined at a concentration of 30 and 200 μg disc^{-1} against two gram-positive (*Bacillus subtilis* and *Streptococcus β-haemolyticus*) and three gram-negative (*Shigella dysenteriae, Pseudomonas aeruginonosa and Escherichia coli*) bacteria. The diameters of the zone of inhibition produced by the complexes were compared with the standard antibiotic (Ciprofloxacin 30 μg disc^{-1}). The experiment was performed in triplicate to minimize errors.

Minimum inhibitory concentration (MIC) determination: MIC of a compound is defined as the lowest concentration of that compound in a medium without visible growth of the test organisms. The minimum inhibitory concentration of the complexes was determined against four pathogenic bacteria *Bacillus subtilis*, *Streptococcus β-haemolyticus*, *Escherichia coli* and *Salmonella typhi* by serial dilution technique[14]. The results were compared with the standard antibiotic, ciprofloxacin. The media used in this respect was nutrient broth (DIFCO).

Antifungal assay: The antifungal activity of the complexes were tested against the three pathogenic fungi *Candida albicans, Aspergillus niger* and *Aspergillus fumigatus* at a concentration of 200 μg disc^{-1} 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°C).

Cytotoxicity bioassay: Brine shrimp lethality bioassay[15-18] 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. Three 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 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[19] for the determination of LC_{50} values for the complexes.

RESULTS AND DISCUSSION

Antibacterial activity: The iron (III) complexes did not show remarkable antibacterial activity at the concentration of 30 μg disc^{-1} with respect to the standard antibiotic ciprofloxacin but showed modest activity at the high concentration of 200 μg disc^{-1}. Among the iron complexes, the cystein based complex F_4 showed comparatively better activity against *Bacillus subtilis*, *Streptococcus β-haemolyticus*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* (Table 1). The more antibacterial activity of the complex F_4 may be due to the amino acid cystein as the other group (succinimide) is common to all the complexes. Further studies are needed to explore the mechanism of antibacterial activity of these iron based complexes. Iron complexes had been reported for their antimicrobial activity[8,9]. Many authors also reported antibacterial activity of other transition metal complexes[20-22] and our present findings supported the previous results of antibacterial activity for both iron and other metal coordination complexes.

Minimum inhibitory concentration: The MIC values of the complex F_1 against *Bacillus subtilis*, *Streptococcus β-haemolyticus*, *Escherichia coli* and *Salmonella typhi* were 32, 32, 32 and 64 μg ml^{-1}, respectively (Table 2), for the complex F_2 32, 64, 32 and 64 μg ml^{-1}, respectively, for complex F_3 64, 64, 64 and 64 μg ml^{-1}, respectively and for complex F_4 16, 16, 16 and 32 μg ml^{-1}, respectively. From the MIC results we can conclude that the iron complexes are significantly less active compared with the standard ciprofloxacin which gave MIC values between 2 to 4 μg ml^{-1}. The cystein present in the complex F_4 lowered the MIC values and it is an interesting findings as it is evident that proper ligand may decrease the MIC values of iron complexes.

Table 1: *In vitro* antibacterial activity of the coordination complexes F_1, F_2, F_3, F_4 and standard Ciprofloxacin

	Diameter of zone of inhibition (in mm)								
	F_1		F_2		F_3		F_4		Ciprofloxacin
μg disc^{-1} →	30	200	30	200	30	200	30	200	30,
Gram positive bacteria									
Bacillus subtilis	09	14	10	16	00	13	10	20	31
Streptococcus β-haemolyticus	00	15	00	15	00	14	09	18	30
Gram negative bacteria									
Shigella dysenteriae	00	15	09	14	09	15	10	16	31
Pseudomonas aeruginosa	00	14	00	15	00	13	09	17	31
Escherichia coli	09	13	00	14	00	14	00	15	33

Table 2: Minimum Inhibitory Concentration (MIC) values of the compounds F_1, F_2, F_3, F_4 and standard Ciprofloxacin

	Minimum inhibitory concentration (μg ml^{-1})				
Test organisms	F_1	F_2	F_3	F_4	Ciprofloxacin
Bacillus subtilis	32	32	64	16	2
Streptococcus β-haemolyticus	32	64	64	32	2
Escherichia coli	32	32	64	32	4
Salmonella typhi	64	64	64	32	2

Table 3: *In vitro* antifungal activity of the complexes F_1, F_2, F_3, F_4 and standard Nystatin

	Diameter of zone of inhibition (in mm)				
	F_1	F_2	F_3	F_4	Nystatin
μg disc^{-1} →	200	200	200	200	30
Candida albicans	17	13	14	17	17
Aspergillus niger	17	15	16	18	16
Aspergillus fumigatus	16	14	15	16	16

Table 4: The results of cytotoxic effect of the complexes F_1, F_2, F_3, F_4 and standard Bleomycin and Gallic acid

		95% confidence limit (ppm)			
Test samples	LC_{50} (ppm)	lower	upper	Regression equation	x^2
[Fe(Suc)$_2$ (Phenylamine)$_2$, F_1]	116.73	29.21	466.52	Y = 1.47 + 1.71 X	0.02
[Fe(Suc)$_2$ (Serine)$_2$, F_2]	64.62	30.86	135.33	Y = 1.73 + 1.81 X	0.20
[Fe(Suc)$_2$ (Leucine)$_2$, F_3]	77.04	35.46	167.41	Y = 0.99 + 2.13 X	0.06
[Fe(Suc)$_2$ (Cystein)$_2$, F_4]	56.03	28.93	108.54	Y = 1.82 + 1.81 X	8.26
Standard bleomycin	0.41	0.276	0.62	Y = 3.16 + 2.99 X	0.62
Gallic acid	4.53	3.33	6.15	Y= 3.93 + 1.62 X	1.25

Antifungal activity: Cystein based iron complex F_4 showed comparatively better antifungal activity than the other iron complexes with comparison of the standard antifungal agent nystatin (Table 3). The phenylalanine based complex F_1 showed similar activity as the F_4 against *Candida albicans* and *Aspergillus fumigatus*. The antifungal activity of iron complexes at the present investigations suggested the previous results[8,9] for other iron based complexes.

Cytotoxic activity: The mortality rate of brine shrimp napulii was found to increase with concentration of the complexes. The LC_{50} values of the complexes F_1, F_2, F_3 and S_4 were found to be 116.73, 64.62, 77.04 and 56.03 μg ml^{-1}, respectively (Table 4). The standard anticancer drug bleomycin gave its LC_{50} value at 0.41 μg ml^{-1}. The lowest LC_{50} value was found in case of the cystein based complex F_4 (56.03 μg ml^{-1}) which is indicative of its higher cytotoxicity than other iron complexes. The cytotoxicity of the complex F_4 against the brine shrimp was half of the phenylalanine based complex F_1. These findings indicated that the different ligands attached with the metal iron (III) significantly changed the cytotoxicity of the resulting coordination complexes. These findings are interesting as it is clear from these evidences that, proper ligand selection may significantly increase the cytotoxicity of iron complexes. Many authors explored the cytotoxic properties of iron complexes[23,8] and our present results suggested the cytotoxicity of previously reported iron complexes.

In conclusion, we may say that, proper selection of ligands can significantly increase the cytotoxicity of iron complexes and these may be explored as potent cytotoxic agents with the hope of adding arsenal of weapons used against the fatal disease cancer.

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