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Antitumor and Antimicrobial Activities of Fe(II)/Fe(III) Complexes Derived from some Heterocyclic Compounds

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Abstract—The antitumor activities of some Fe(II)/Fe(III) complexes containing 1,3-diacetyl-2*H*-benzimidazole-2-thione along with a few derivatives of 1,2,4-triazol, 1,3,4-oxadiazole and 1,3,4-thiadiazole as co-ligands have been investigated. Antibacterial and antifungal activities of disulfido-/dichloro-bridged dinuclear Fe(III)/Fe(II) complexes containing similar heterocycles as terminal ligands have also been investigated.

Introduction

Among the five-membered heterocycles, the derivatives of oxadiazoles, triazoles, imidazoles and thiazoles are well reported as antimicrobial agents.[1–3] The antitumor and anticonvulsant activities of various drugs are also related to the relative accessibility of the two carbonyl groups[4] in their structures. Beside this, it is observed that antitumor drugs interfere with the metalloenzymes of the tumor cell, thus inhibiting the tumor growth.[5] Some of these antitumor drugs are also found to be metabolized to chelating agents *in vivo*.[5] In addition to the chelating abilities of these drugs, their non-polarity is also found to be essential in order to penetrate through intracellular sites. In this context, it is worthwhile mentioning that the presence of these heterocycles in some of the antibiotics, e.g. the presence of bithiazole unit in bleomycin, allowed early workers to isolate the drugs containing such heterocyclic moieties and their Fe(II) complexes to explore their antitumor activities.[6] Few reports on the Fe(II)/Fe(III) complexes of similar heterocyclic derivatives are also well documented in the literature.[6,7]

In view of the above facts, our current interest is focused in the new heterocycles and their Fe(II)/Fe(III) complexes.[8–12] We found it worthwhile to make investigations on our few newly synthesized heterocycles and their complexes for possible antitumor and antimicrobial activities.

Results and Discussion

The cytotoxic activity of the complexes (Fig. 2), along with the free heterocycles is shown in Table 1. Diacetylbenzimidazole derivative (L^1) showed maximum activity which favors the reported explanation[4] showing the accessibility of the two carbonyls in the molecule. This activity trend is followed by the activity shown by oxadiazole derivative (L^4) which again favors the report by Saegusa *et al.*[13] where the oxa-

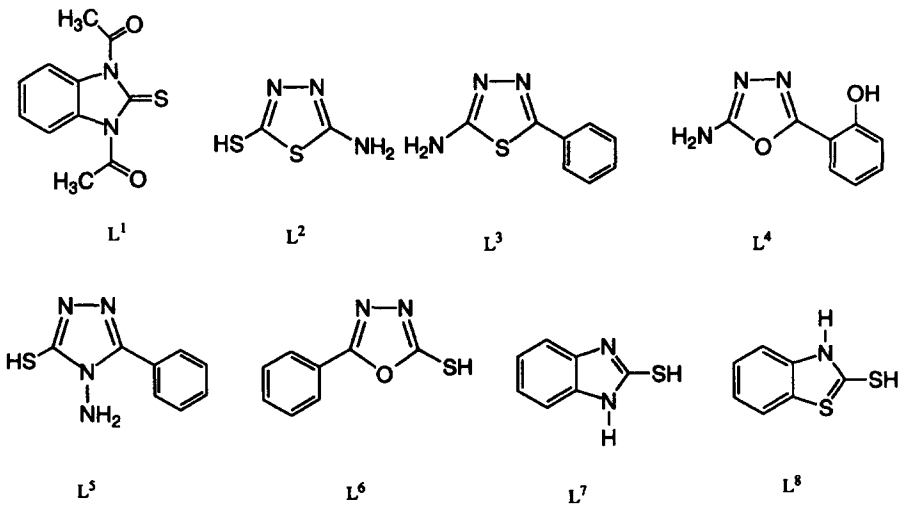


Figure 1.

diazoles are reported to interact with the amino acids resulting in new product which may be responsible for the activity. The oxadiazole derivative (L^4) when mixed as a co-ligand with diacetylbenzimidazole derivative (L^1) and complexed with $FeSO_4 \cdot 7H_2O$ showed significant activity as compared to the free oxadiazole (L^4). Furthermore, it was again observed that sulfate bridged complexes were found to be better active as compared to their chloro-bridged analogs. But the presence of oxadiazole (L^4) as a co-ligand is found to be important as the sulfate bridged systems containing other co-ligands were found to be inactive.

Antibacterial results shown in Table 2 indicate that free heterocycles (L^5–L^8) are insensitive against all bacteria except some activity of triazole (L^5) and oxadiazole (L^6) against *Vibrio cholerae non O-1, Klebsiella* and *Salmonella.* Free $FeCl_3$ also did not show significant activity excèpt in the case of *Salmonella, Vibrio cholerae non O-1* and *Vibrio cholerae.* However, $[Fe_2S^*_2(L)_2]$ and $[Fe_2Cl_2(L)_2]$ type complexes (LH = different deprotonated heterocycles) have been found to show better activity as compared to their free heterocycles which may be explained on the basis of chelation theory[5] allowing better intracellular penetrability of the complexes. Among these complexes the order of the activity is $[Fe_2Cl_2(L^6)_2] > [Fe_2S^*_2(L^8)_2] > [Fe_2S^*_2(L^6)_2] \approx [Fe_2Cl_2(L^5)_2] \approx [Fe_2Cl_2(L^8)_2] > [Fe_2Cl_2(L^7)_2]$ (S*: bridged sulfur). Thus the chloro-bridged complexes of oxadiazole derivatives have been found to be the best candidate for antibacterial activity against most of the bacteria including *Viblio cholerae.* However, this is a preliminary report and any generalization of the correlation of the activity with the structure of the compounds calls for further investigation.

Antifungal activity of the free heterocycles (L^5–L^8), free $FeCl_3$ and their complexes of the type $[Fe_2S^*_2(L)_2]$ and $[Fe_2Cl_2(L)_2]$ are shown in Table 3. At higher concentration, viz. 500 and 1000 ppm mL^{-1}, almost all

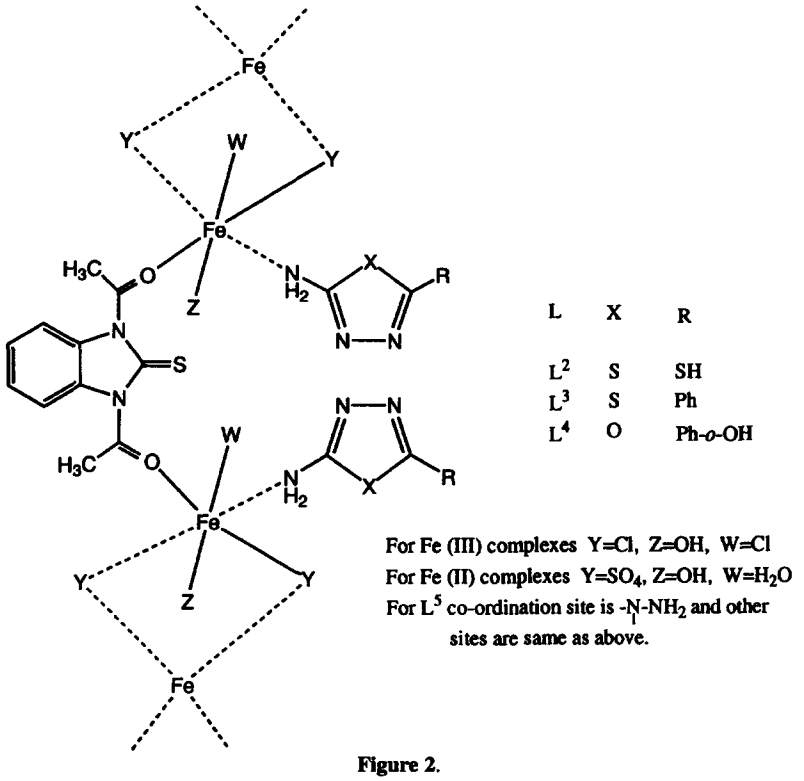


Figure 2.

Table 1. Antitumor activity of ligands and complexes against P388 cells

compound	IC_{50} values ($\mu g\ mL^{-1}$)	compounds	IC_{50} values ($\mu g\ mL^{-1}$)
L^1	5.0	$[FeL^1_{1/2}L^4Cl_2 \cdot OH]$	32.0
L^2	> 100	$[FeL^1_{1/2}L^5Cl_2 \cdot OH]$	> 100
L^3	> 100	$[Fe^*L^1_{1/2}L^2SO_4 \cdot OH \cdot H_2O]$	88.0
L^4	11.0	$[Fe^*L^1_{1/2}L^3SO_4 \cdot OH \cdot H_2O]$	> 100
L^5	75.0	$[Fe^*L^1_{1/2}L^4SO_4 \cdot OH \cdot H_2O]$	6.5
$[FeL^1_{1/2}L^2Cl_2 \cdot OH]$	> 100	$[Fe^*L^1_{1/2}L^5SO_4 \cdot OH \cdot H_2O]$	> 100
$[FeL^1_{1/2}L^3Cl_2 \cdot OH]$	> 100		

*Oxidized Fe perhaps due to aerial oxidation.[8]

Table 2. Antibacterial activity (MIC) of ligands and complexes at 250 μg mL^{-1}

organism \ compound	1	2	3	4	5	6	7	8	9	10	11	12	13
Escherichia coli	-	-	-	-	-	-	-	+	+	-	+	+	-
Klebsiella	-	+	-	-	-	-	-	-	+	-	+	-	-
Salmonella	-	+	-	-	+	-	-	-	+	+	+	+	+
Vibrio cholerae non O-1	+	-	-	-	+	+	+	-	-	+	+	+	+
Shigella dysenteriae	-	-	-	-	-	-	+	-	+	+	+	-	+
Aeromonas hydrophila	-	-	-	-	-	-	+	-	+	+	+	-	+
Pseudomonas	-	-	-	-	-	-	+	-	-	+	-	-	-
Plesiomonas shigelloides	-	-	-	-	-	-	+	-	-	+	+	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-
Vibrio cholerae	-	-	-	-	+	-	-	-	+	-	+	+	+

1: L^5, **2**: L^6, **3**: L^7, **4**: L^8, **5**: $FeCl_3$, **6**: $[Fe_2S^*_2(L^5)_2]$, **7**: $[Fe_2S^*_2(L^6)_2]$, **8**: $[Fe_2S^*_2(L^7)_2]$, **9**: $[Fe_2S^*_2(L^8)_2]$, **10**: $[Fe_2Cl_2(L^5)_2]$, **11**: $[Fe_2Cl_2(L^6)_2]$, **12**: $[Fe_2Cl_2(L^7)_2]$, **13**: $[Fe_2Cl_2(L^8)_2]$.
(-) Insensitive, (+) sensitive, *bridged sulfur.

Table 3. Antifungal activity of ligands and their Fe(III) complexes

compound	*Helminthosporium oryzae*			*Alternaria alternata*			*Curvularia lunata*		
	concentration (μg mL^{-1}) / inhibition (%)								
	250	500	1000	250	500	1000	250	500	1000
L^5	75	100	100	80	100	100	95	100	100
L^6	45	66	85	55	75	90	60	75	95
L^7	57	75	100	60	85	100	70	95	100
L^8	75	100	100	80	100	100	95	100	100
$FeCl_3$	75	80	100	80	94	100	90	100	1 00
$[Fe_2S^*_2(L^5)_2]$	38	52	70	40	60	80	50	85	90
$[Fe_2S^*_2(L^6)_2]$	40	65	75	45	75	85	55	75	92
$[Fe_2S^*_2(L^7)_2]$	58	80	90	60	75	95	65	95	100
$[Fe_2S^*_2(L^8)_2]$	70	85	98	77	95	100	90	100	100
$[Fe_2Cl_2(L^5)_2]$	55	85	100	65	95	100	75	100	100
$[Fe_2Cl_2(L^6)_2]$	77	85	100	85	97	100	90	100	100
$[Fe_2Cl_2(L^7)_2]$	62	77	95	67	85	100	75	100	100
$[Fe_2Cl_2(L^8)_2]$	52	67	93	60	75	100	90	100	100

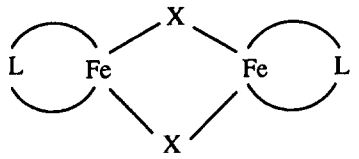
*Bridged sulfur.

compounds showed significant activity. However, activity is very much related with the type of fungi used, e.g. *Curvularia lunata* has shown highest activity followed by *Alternaria alternata* and then *Helminthosporium oryzae.*

At lower concentration, the chloro-bridged complexes derived from oxadiazole derivatives (L^6) showed highest activity, greater than the free oxadiazole and comparable to free $FeCl_3$ and free benzothiazole against all fungi but highest against *Curvularia lunata.* Furthermore, the correlation of activity with the structure of the complexes could not be generalized again since the activity of the free benzothiazole and free $FeCl_3$ even at lowest concentration (250 ppm) is found to be significant. The higher activity shown by the benzothiazole could definitely be correlated with the chelation[5] of the benzothiazole with the biologically important trace elements present in the fungi. The interaction of $FeCl_3$ with the functional group present in some of the enzymes may bring abnormality in the enzymatic function which could be the probable reason for the significant activity shown by free $FeCl_3$.

Experimental

The ligand 1,3-diacetyl-2*H*-benzimidazole-2-thione (L^1) and various heterocycles (L^2–L^5) acting as co-ligands in their Fe(II)/Fe(III) complexes have been synthesized and characterized as mentioned in our earlier communication.[8] The heterocycles (L^7 and L^8) were commercially available and used as such. The sulfido-bridged dinuclear Fe(III) complexes bearing heterocycles (L^5–L^8) as terminal ligands have also been reported by us recently.[10,11] In order to correlate the activity of these disulfido-bridged complexes their chloro analogs $[Fe_2Cl_2(L)_2]$ type complexes were also prepared by the addition of $FeCl_3$ (3 mmol, 486.6 mg in 20 mL of dry MeOH) to deprotonated L (9 mmol each in 50 mL of dry MeOH containing NaOMe 9 mmol; 207 mg Na in 15 mL of dry MeOH) under a dry nitrogen atmosphere with stirring for 10–15 h. The respective solutions thus obtained were filtered and the filtrates concentrated under reduced pressure to give the solid products which were further washed with methanol and dried *in vacuo*. Their elemental analyses were found to be consistent with the general composition as



where X= Cl / S, Fe= Fe (II) / Fe (III)

Figure 3.

$[Fe_2Cl_2(L)_2]$. The spectral (IR, UV/vis) magnetic and conductance's data were found to agree with the earlier reported structure[10] as shown in Figure 3.

Assay of cytotoxic activity against P388 cells

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well plate.[14] The cytotoxic activity of the complexes along with free ligands was examined against P388 lymphocyctic leukemia cells. The assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Mouse P388 leukemia cells (2×10^4 cells mL^{-1}) were inoculated in each well with 100 μL mL^{-1} of RPMI-1640 medium (Nissui Pharm. Co., Ltd) supplemented with 5% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd) and kanamycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 μL) were added to the cultures at day 1 after the transplantation. At day 3, 20 μL of MTT solution (5 mg mL^{-1}) per well was added to each cultured medium. After a further 4 h of incubation, 100 μL of 10% SDS –0.01 N HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. The optical density measurements were made using a microplate reader (Tohso MPR-A4i) with a two wavelength system (550 and 700 nm). In all these experiments, three replicate wells were used to determine each point. The results (IC_{50} values) shown in Table 1 are expressed by the concentration of each compound which achieved 50% reduction of growth in sample-treated cells with respect to the control.

Assay of antibacterial activity

The minimum inhibition concentration (MIC) method[15] was followed to test the antibacterial activity of the complexes as well as of the free ligands. The general procedure adopted was as follows. Freshly prepared concentrations (1000, 500, 250 and 125 ppm mL^{-1}) in DMSO (2 mL) containing different compounds were added to sterile Mueller–Hinton agar (MHA) cooled to 45–50 °C and mixed well then poured into Petri dishes, 20 mL being poured in each of 100-mm plates to give a depth of about 4 mm. These plates were dried prior to experiment and each plate was divided into equal sectors. The overnight broth cultures of the test strain and control strain of *Escherichia coli* NCTC 10418 were diluted 1:1000 in phosphate buffer solution (PBS, pH 7.4) to a concentration of about 10^4–10^5 cells mL^{-1}, and aliquots of 0.01 mL of the diluted cultures were spotted on the different sectors on the agar plates containing several dilution of the compounds and the inocula were allowed to dry and then the plates were incubated at 37 °C overnight. Additionally, all the strains were also inoculated on a control plate without compounds. The result (MIC) shown in Table 2 are expressed by the lowest concentration of the compound mL^{-1} of medium that completely inhibited the visible growth of the corresponding test strain.

Assay of antifungal activity

The poisoned food method of Grover and Moore[16] was used to evaluate the antifungal activity of free ligands and the metal complexes. The general procedure adopted was as follows. Potato dextrose agar medium (Potato dextrose agar, 20 g in 1000 mL of distilled H_2O) was prepared and sterilized for 30 min under 15 lb $inch^{-2}$ pressure. The medium was then cooled up to 40 °C and supplemented with 10 mg of streptomycin to prevent bacterial contamination. The fungitoxic activity of compound was determined at 1000, 500 and 250 ppm mL^{-1} concentrations using DMSO as solvent. Ten milliliters of the medium was poured into pre-sterilized Petri dishes (7.5 cm diameter). Fungal discs were taken from seven-day-old culture of the test fungi maintained separately and were incubated to the center of the poured plates and then kept in an incubation chamber at 28 ± 1 °C for 6 days. Requisite control sets were kept in parallel to the treated sets, and the experiment was repeated in triplicate for each concentration of the compound under investigation. The colony diameter of mycelial growth of test fungi at the treatment and control sets were measured in mutually perpendicular directions after 7 days, and the percentage of the mycelial inhibition was calculated by the mean value of the colony diameters. The result of antifungal activity against *Helminthosporium oryzae, Alternaria alternata* and *Curvularia lunata* are shown in Table 3.

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