Research Article

Synthesis, Characterization and Biological Properties of Metal Complexes with an N/S/O Functionalized Ligands

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ABSTRACT

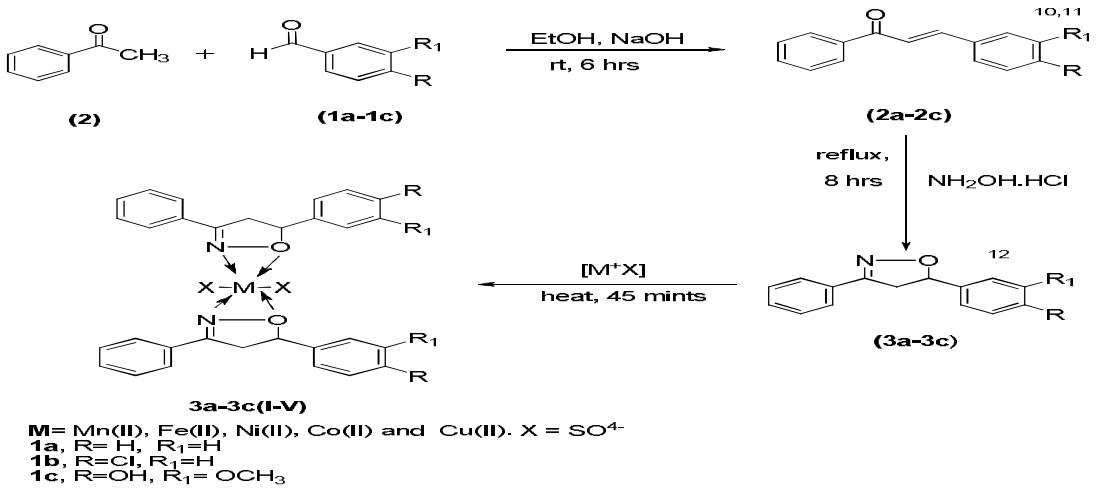
The synthesis of N/S/O functionalized Isoxazole ligands and their complexes of Mn(II), Fe(II), Ni(II), Co(II) and Cu(II) ions by base condensed reaction of various aldehydes and acetophenones are described. The complexes $[M(Ln)X_2]$ where n=1, 2, 3. Were characterized on the basis of ^1H NMR, magnetic properties data, elemental analysis, electronic absorption spectra to confirm the stability of metal complexes. The newly synthesized complexes were also evaluated for their antioxidant and antimicrobial activities. Among the synthesized complexes $[Co(L_3)Cl_2]$ and $[Cu(L_3)SO_4]$ exhibited dominant antioxidant activity with IC_{50} value more when compared to other complexes, against radicals, various microorganisms and further, the results were compared with the corresponding standards.

Keywords: Isoxazole ligands, metal complexes, antioxidant activity, antimicrobial activity.

INTRODUCTION

A wide range of biological activities of isoxazole derivatives include pharmacological properties such as hypoglycemic, analgesic, anti-inflammatory, anti-bacterial, anti-cancer, anti-HIV activity and also agrochemical properties like herbicidal and soil fungicidal activity and have applications as pesticides and insecticides[1]. Studies of a new kind of chemotherapeutic Schiff bases are now attracting the attention of biochemists[2]. Schiff bases derived from an amine and any aldehyde are a class of compounds which co-ordinate to metal ions via the azomethine nitrogen[3, 4]. Chelating ligands containing O and N donor atoms show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions[5-7]. It is well known that several Schiff base complexes have anti-inflammatory, anti-pyretic, analgesic, anti-diabetic, anti-bacterial[8], anti-cancer and anti-HIV activity[9] and the antimicrobial activity of 3-amino-5-methyl Isoxazole Schiff bases with methoxy salicylaldehyde, naphthaldehyde and their metal complexes[10,11].was reported earlier and it was found that these compounds show increased activity when administered as metal complexes rather than as organic compounds. Based on the above, in the continuation of our work on the metal complexes in the present investigation we synthesized substituted Isoxazole derivatives and screened their anti microbial activity with good potency.

EXPERIMENT



M= Mn(II), Fe(II), Ni(II), Co(II) and Cu(II). X = SO^{4-}
1a, R= H, R_1=H
1b, R=Cl, R_1=H
1c, R=OH, R_1= OCH_3

Synthesis of ligands

General procedure for Synthesis of chalcones (2a-2c)

The mixture of substituted benzaldehyde (1a-1c) (1 mmol) with acetophenone (2) (1 mmol)was taken in a round bottom flask and dissolved in15 ml of ethanol. To this reaction mixture sodium hydroxide (1.5 mmol) was added. The mixture was stirred at room temperature for 6 hrs. After completion of reaction (monitored by TLC) using hexane:ethylacetate (6:4) and further the mixture was isolate the product by filtration with a funnel and washed with cold water several times and dried solid products were obtained in reasonably good yields (2a-2c).

Procedure for synthesis of ligands (L_1), (L_2) and (L_3)

A mixture of compounds (2a-2c) (1 mmol) is taken in a 100 ml of round bottom flask was dissolved in 15 ml of ethanol. To this reaction mixture hydroxylamine hydrochloride is added (2 mmol) shake well and then sodium hydroxide (1.5 mmol) was added. The reaction mixture was stirred and reflux for 8 hrs. The progress of reaction is followed by TLC. After completion of the reaction, the mixture pours into 50ml of ice water. The separated product was filtered off and washed with cold water for several times and dried afforded ligands (L_1), (L_2) and (L_3).

Preparation of metal complexes

Ethanolic solution of ligands (L_1), (L_2) and (L_3) (2 mmol) was slowly added to ethanolic solution of $MX_2.nH_2O$ (1 mmol, where X =Cl, Y= SO^{4-}, n = 0, 1, 2, 3…) and the mixture was kept on a water bath at 70 ^oC with continuous stirring for one hour. The solid complex formed was filtered and washed with ethanol then with ether and dried over sodium sulphate ($NaSO_4$). The probable geometry of metal complexes as shown in (Figure 1).

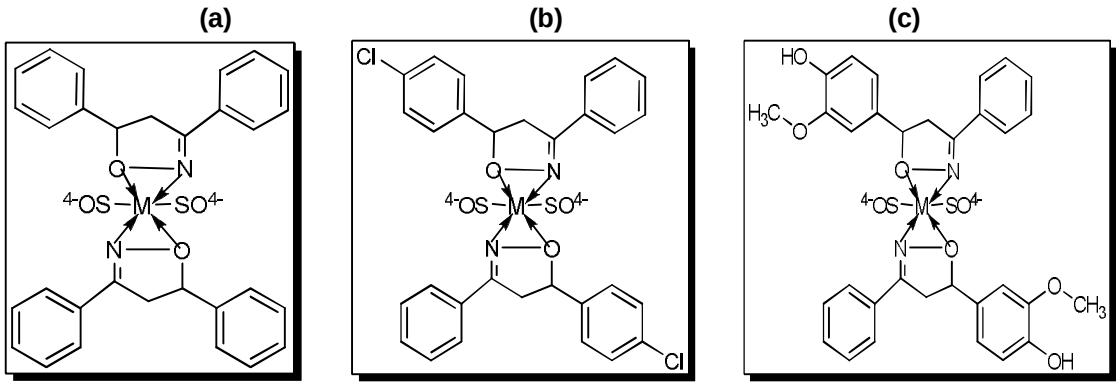


Fig. 1: Probable geometry of metal complexes

RESULTS AND DISCUSSION

Antioxidant evaluation

Procedure

The evaluation of antioxidant activity of newly synthesized compounds was done by DPPH radical scavenging activity assay[12].Internal standard BHA and the synthesized compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 µM, 25 µM, 50 µm, 100 µM, 200 µM and 500 µM) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction[13-16]. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

Radical scavenging activity (%) = [(A_o-A_1)/A_o X 100]

Where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound.

Evaluation of antioxidant activity for the newly synthesized complexes was done by using *in vitro* assay such as 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity. The antioxidant properties were expressed as 50% inhibitory concentration (IC_{50}) values (Table 1). The DPPH radical scavenging evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity (RSA) of specific compounds. The reaction of synthesized metal complexes with stable DPPH free radical indicates their free radical scavenging ability. In the present study, different electron withdrawing and electron donating groups attached to phenyl ring as substituent which is linked to sulfonyl groups were studied for antioxidant efficacy. All the synthesized metal complexes showed certain degree of antioxidant activity. Among the synthesized complexes $[Co(L_3)Cl_2]$ and $[Cu(L_3)SO_4]$ exhibited dominant antioxidant activity (Table 1) with IC_{50} value more when compared to other complexes (Figure 2), this may be presence of methoxy group on and also presence of hydroxyl group on phenyl ring. Whereas, the remaining Ni(II), Fe(II) complexes showed the least antioxidant activity. The above SAR correlation study reveals that, the nature of the functional group present phenyl ring influences the antioxidant activity.

Table 1: Concentration required for 50% scavenging (IC_{50}) of DPPH radicals by the complexes and the standard antioxidant Ascorbic acid. Each value represents mean ± SD (n=3)

IC_{50} table

S.No.	Compounds	IC_{50} (µM) [a]
1.	$[Ni(L_1)SO_4]$	>500
2.	$[Ni(L_2)SO_4]$	456±0.13
3.	$[Ni(L_3)SO_4]$	>400
4.	$[Cu(L_1)SO_4]$	296±0.53
5.	$[Cu(L_2)SO_4]$	122±0.21
6.	$[Cu(L_3)SO_4]$	56±0.56
7.	$[Co(L_1)Cl_2]$	198±0.14
8.	$[Co(L_2)Cl_2]$	78±0.21
9.	$[Co(L_3)Cl_2]$	59±0.22
10.	$[Fe(L_1)SO_4]$	325±0.51
11.	$[Fe(L_2)SO_4]$	256±0.23
12.	$[Fe(L_3)SO_4]$	95±0.55
Std.	Ascorbic acid	63±0.05

The values are expressed as µM concentration. Lower IC_{50} values indicate higher radical scavenging activity (Figure 2).

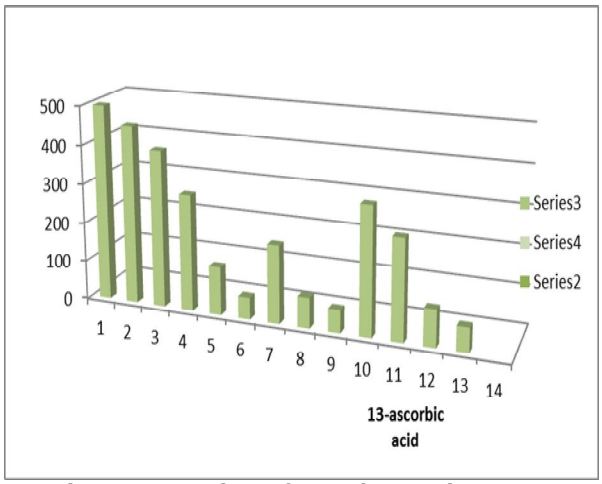


Fig. 2: IC_{50} value of metal complexes

Antimicrobial activity

Procedure

The bacterial sub-cultures *Pseudomonas aeurogenosa* and *Escherichia coli* were obtained form Biotechnology department, UOM. The anti-bacterial action of the ligand L_1, L_2 and L_3 their complexes of Co(II), Ni(II) Cu(II) and Fe(II) was checked by paper disc method[17]. The compounds were dissolved in acetone (1.0 mg ml^{-1}) and the discs of Whattmann filter paper No.41 having the diameter 4mmwere prepared and soaked in it. These soaked discs were placed on nutrient agar plates inoculated with bacteria. These plates were incubated for 36 h at 30 ◦C. The inhibition zone was observed after 36 h. Acetone was used as a control and gentamycin as a standard drug. The complexes shows effective microbial activity as shown in the (Table 2).

Table 2: Antimicrobial activity table of metal complexes

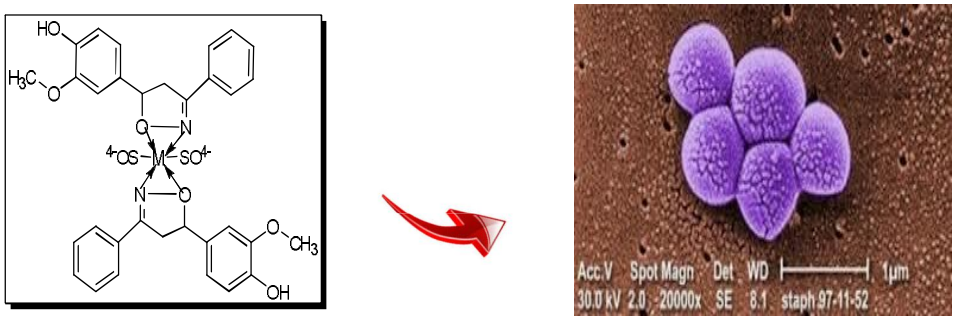
Complex/ligands	*E. coli*	*P. aeurogenosa*	*R. oryzae*	*A. niger*
DDHI	+	+	+	+
Ni(II)	++	++	+++	+++
Cu(II)	+++	++	+++	+
Co(II)	++	+	++	++
Fe(II)	+	++	+	++
CPDI	++	+	++	++
Ni(II)	++	+	++	+++
Cu(II)	+++	++	+++	++
Co(II)	++	+	++	+
Fe(II)	+	++	+	+
MPDP	+	+	-	+
Ni(II)	++	++	+++	++
Cu(II)	++	+	++	+
Co(II)	+++	++	++	+++
Fe(II)	+	+	++	++

***Note*:** Maximum zone of inhibition was represented as +++ (20–35 mm). Medium zone of inhibition as ++ (10–20 mm). Minimum zone of inhibition as + (5–10 mm).

CONCLUSION

Series of novel Isoxazole based metal complexes were synthesized and their antioxidant activity have been evaluated. In antioxidant DPPH assay, all the synthesized complexes showed certain degree of antioxidant activity. Initially scaffolds L_1, L_2 and L_3 exhibited less activity, Further, coordinated with metal significantly increased the antioxidant activity. The presence of hydroxyl and methoxy substituent's on the phenyl ring, in complexes $[Cu(L_3)SO_4]$ and $[Co(L_3)Cl_2]$ exhibited more antioxidant activity than the standard. Whereas, the other metal complexes showed less antioxidant efficacy. The Structural-Activity Relationship studies revealed that, the nature of functional (electron donating groups) on phenyl ring are may be play important role in antioxidant. On the basis of their activity, these complexes were identified as viable leads for further studies.

Graphical abstract



Highlights

- Synthesis of metal complexes of N/S/O functionalized ligands has been described by simple and efficient protocol.
- Metal complexes were characterized by physico-chemical and spectral techniques.
- Synthesized metal complexes were screened for antioxidant and antimicrobial activity.
- Some of metal complexes shows dominant antioxidant activity.
- Some of metal complexes showed excellent antimicrobial activity.

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