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Bioinorganic Chemistry of Co (II) and Mn (II) Complexes

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ABSTRACT

Objectives: Schiff base ligand 2-thio phenyl glyoxal anthranilic acid is used as the starting material for the synthesis of many biologically active compounds. The main objectives of the Study was to synthesize, characterization and biological evaluation of a octahedral complexes of Co (II) and Mn(II) containing 2- thiophenyl glyoxal anthranilic acid as ligand. **Methods:** Ethanolic solution of ligand 2-thiophenyl glyoxal anthranilic acid added into the ethanolic solution of metal acetate the resulting solution was refluxed for 2 hr the product thus obtained was filtered, washed with ethanol followed by ether and characterized by various spectral studies and biological significance. **Results:** The resulted complex was investigated by the help of elemental analysis, Molar Conductance, Magnetic moment, IR, Electronic and NMR Spectral Studies. Spectral data indicates that the geometry of the complexes are Octahedral. The result of biological and cytotoxic studies indicate that complexes are much more biologically active as compared to ligand fragments. **Conclusion:** The complexes are biologically active and were found to be effective in all the human cancer cell lines.

Key words: Antimicrobial, Cytotoxicity, Enzyme inhibition, Spectra, Synthesis.

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INTRODUCTION

Schiff bases containing penicillin and heterocyclic structural units with N, N donor atoms are considered the most prominent research area in the field of coordination chemistry.[1-6] The various donor atoms in them offer special ability for binding metals. Schiff base change the physiological, morphological and pharmacological activities of the compounds. Schiff base complexes have been used as drugs and have valuable antibacterial antifungal, anti-inflammatory and antitumor activities. A large number of Schiff bases and their complexes have been studied for their interesting and important properties e.g. their ability to reversibly bind oxygen, catalytic activity in hydrogenation of olefins and transfer of an amino group photochromic properties. The high affinity for the chelation of the Schiff bases towards the transition metal ions is utilized in preparing their solid complexes. In the present paper the Co (II) and Mn (II) complexes were synthesized with Schiff base ligand 2-Thio phenyl glyoxal anthranilic acid (TGAA). The coordination behavior of the ligand towards transition metal irons was fully investigated by various spectral techniques. Biochemical Behavior of the complexes were evaluated by antimicrobial, Enzyme inhibition activity and cytotoxic studies.

MATERIALS AND METHODS

Materials

All the Chemicals used were of analytical reagent grade and the solvents were dried and distilled before use according to a standard procedure. The organic solvents DMSO, DMF, Ethanol and Ether were of analytical reagent grade and used as such without further purification.

Methods

Synthesis of complexes

An ethanolic solution of ligand 2-Thiophenyl glyoxal anthranilic acid (TGAA) prepared by dissolving 1.04g ligand in 60 mL ethanol was added to 30-40 mL ethanolic solution of Metal acetate 0.50g Co $(OAC)_2.4H_2O$/0.40g $Mn(OAC)_2$ $4H_2O$ in round bottom flask. The resulting solution was refluxed over a water bath for about 2 hr. and cooled. The separated coloured products were filtered through suction, washed with ethanol followed by ether and dried in Vacuum over anhydrous $CaCl_2$.

	Yield
$Co[C_{13}H_8O_3NS]_2$	65%
$Mn[C_{13}H_8O_3NS]_2$	70%

Instrumentations

C,H,N were analyzed on carlo-Erba microanalyzer Model 1106 Metal contents estimated by standard procedures 7 IR spectra were recorded in the range 4000 cm^{-1} -100 cm^{-1} with a Bruker IFS66V in KBr and Polyethylene medium for all complexes. The Molar conductance of the complexes in DMF (10-3M) solution was measured at 27+3°C with an Elico Model Conductivity meter. Electronic spectra of the complexes were recorded on varian cary- 5E UV-Visible spectrophotometer NMR spectra were recorded or Bruker 300 Hz spectrophotometer using DMSO d6 as solvent chemical Shifts are reported in ppm relative to tetramethyl silane using the solvent signal as internal reference. The Magnetic moment measurement were mode on a Gouy Balance at room temperature using Hg[Co(SCN)4] as the Calibrant.

Biological activities

Antimicrobial activity

The Schiff base ligand and its complexes were investigated for antibacterial and antifungal activities against *Staphylococcus aureus* and *Streptococcus pyogenes* as gram positive bacteria and *Escherichia coli* and *Klebsiella pneumoniae* as gram negative and

the Fungi *Fusarium oxysporum* and as *Aspergillus fumigatus* by using disc agar diffusion method.

The antibiotic chloramphenicol and tetracycline were used as standard reference in the case of Gram-negative and Gram-positive bacterial species, whereas Clotrimazole was used as standard reference for fungal species incubation time for 36h at 27°C in the case of bacteria and for 48h at 24°C in the case of fungus, inhibition of the organism which evidenced by clear zone surround each disk was measured and used to calculate men of inhibition zone.

Enzymatic activity

Enzymatic activity is carried out by cup-plate method. All the plates were incubated at 30°C for 48 hr in BOD incubator. The activity zone of the produced enzyme after incubation was developed by flooding the plates with an appropriate developing agent. The total diameter of zone of inhibition in mm was measured and the activity was calculated.

Cytotoxic studies

Further the synthesized metal complexes have been evaluated for their cytotoxic activity against a panel of different human cancer cell lies by using MTT (3-(4)5- dimethyl thiazole-2-yl)2,5- di phenyl tetrazolium bromide) assay.

RESULTS

Selection of ligand

Metal complexes with a variety of organic chelating ligand are also of current interest due to their biological activities. These include antimicrobial, enzymatic activity and cytotoxic studies.

The required amount of ligand 2-thiophenyl glyoxal anthranilic acid in ethanol was added to the ethanolic solution of Cobalt acetate/ Manganese acetate refluxed for 2 hr, The coloured product thus obtained were filtered washed with ethanol followed by ether and dried. Finally characterized by elemental analysis, molar conductance and spectral studies. The spectral datas indicates that the geometry of complexes are Octahedral and Biological studies showed that metal complexes are much more active as compared to ligand fragments.

Characterization of free ligand and its metal complexes

Elemental analysis, Molar Conductance and Melting point/ Decomposition temperature

In Table 1, The elemental analysis results (C.H,N,M), Melting point/ Decomposition temperature, The Molar Conductance of ligand and metal complexes.

IR Spectral Studies

Infrared spectra of free ligand shows a sharp band in the region of 1615-1590 cm^{-1} which may be attributed due to the stretching vibration of azomethine group (>C=N). This band is shifted towards lower frequency region in the spectra of metal complexes. Suggesting thereby the participation of imine nitrogen in complexation A strong band in the region 1730-1680 cm^{-1} has been observed in the IR spectra of ligand which in due to the presence of stretching vibration of carbonyl group (>C=O) coordination through this carbonyl oxygen to the central metal ion is confirmed by a negative Schiff in this frequency in the spectra of corresponding metal complexes.[8-10]

Electronic spectral Studies

In octahedrally surrounded Co(II) ions three bands in the region of 8000, 16000-13500 and 20000-17600 cm^{-1} are expected which may be assigned to 4 $T_{1g} \rightarrow {}^4T_{2g}$ (F)υ_1, ${}^4T_{1g} \rightarrow 4A_{2g}$ (F) υ_2 and ${}^4T_{1g} \rightarrow {}^4T_{1g}$(P) υ_3 Transition respectively. The ${}^4A_{2g}$ Transition is very weak and often appears as a shoulder The Mn (II) complex shows three bands at 17000,2500, 29800 cm^{-1} These spectral bands are assigned as the ${}^6A_{1g} \rightarrow {}^4T_{1g} \rightarrow {}^6A_{1g} \rightarrow {}^4T_{2g}$ and charge transfer (CT) respectively.[11-16]

NMR Spectral Studies

^{1}H NMR spectra of free Schiff base the signals were appeared in the range of 8.10 - 8.24 ppm due to (HC=N) proton however in the spectra of Co(II) and Mn(II) complexes the signals were observed in the upfield region of 8.25 – 8.34 ppm supporting the coordination of iminonitrogen atom to Co(II)/ Mn (II). There is no appreciable change in the Peak position corresponding to NH and aromatic proton. The ^{13}C NMR signals for the metal complexes of Co (II) and Mn (II) ions are assigned by the comparison with the spectra of corresponding free Schiff base ligand. A down field shift of CH=N group in the range of 172.6-174.5 ppm and for carboxyl carbon COO^- ion in the range of 192.6 – 195.4 ppm.[17] The NMR spectra of complexes indicates that the ligand coordinates through both the nitrogen atom of CH=N and the oxygen of COO^- ion

Biological activity

Enzyme inhibition activity

A comparative study of enzyme activity of the ligand and their metal complexes indicate that the enzyme activity of the ligand has considerably increased in the form of metal complexes. The ligand and metal complex play an competitive role with the substrate required for their production and thus the free availability of the substrate in reduced inhibiting the growth.[18-20] The results are given in Table 2 and Table 3.

Ligand/ metal complexes of Co(II) and Mn(II) on diffusion inside cells affect their Mitochondrial action causing a death to them. There could be a ligand or metal exchange reaction on diffusion of these species insides the cells, Thus rapturing the cell structure Ligand may be more toxic to the enzymatic cell in combination with Metal ions vice-versa. It is evident from Table 3 that the bacteria *Staphylococcus aureus* produced cellular, Protease and amylase in liquid culture medium containing three different specific at the optimum pH values of their production the order of production of these enzymes under identical experimental conditions has been observed as Amylase > Protease> cellulose.

Antimicrobial Activity

The free ligand and metal complexes were evaluated against bacterial and fungal species. The results are given in Table 4.

Metal chelates bear polar and non-polar properties together this makes them suitable for permeation to the cells and tissues. In addition, chelation may enhance or suppress the bio-chemical potential of bioactive organic species. Changing hydrophobicity and lipophilicity probably leads to bringing down. The solubility and permeability barriers of cell Further lipophilicity which control the rate of entry of molecules into the cell in modified by coordination so the metal complexes can become more active than free ligand. Chelation considerably reduces the polarity of the Metal ion because of particular sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring as a result the lipophilicity of Metal complexes enhances the Penetration of the complexes into lipid membranes and blocking of the Metal binding sides in the enzymes of micro-organisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the Proteins that restricts further growth of the organism.[21-23]

Table 1: Physical and analytical data of synthesized compounds.

S.No.	Compound	Molecular Formula	Colour	%Analysis C	Found/Calc H	N	M	MP (0C) / Decom Temp.	Molar Conductace (Ω) ohm^{-1} cm^2 mol^{-1}
1	TGAA	$C_{13}H_9O_3N_S$	Orange Red	60.40 (60.23)	3.63 (3.47)	5.56 (5.41)	-	187-190	-
2	Co[TGAA]	$Co[C_{13}H_8O_3NS]_2$	Green	54.17 (54.27)	2.63 (2.78)	4.64 (4.87)	10.39 (10.25)	302	9.02
3	Mn[TGAA]	$Mn[C_{13}H_8O_3NS]_2$	Red	60.40 (60.46)	3.18 (3.10)	5.00 (5.42)	10.59 (10.63)	295	6.29

Table 2: Substrate used for the Production of enzymes, their optimum pH, developing agent and color of the developed zone

S.No.	Enzyme Produced	Substrate Used	pH of the Medium	Developing Agent	Colour of Deloped Zone
1.	Cellulose	Carboxy methyl Cellulose	5.3	3% Lead Actual Soluion	Pearl White
2.	Protease	Gelatin	6.8	--	Opal Black Ground
3.	Amylase	Starch	5.3	0.1 N Solution of Iodine	Violet Black Ground

Table 3: Enzyme inhibition Activity (in mm) of synthesized compound at 30°C after 48 hrs

S.No.	Compound	Enzyme		
		Cellulose	Protease	Amylase
1.	TGAA (L)	3.0	4.0	7.0
2.	Co $[C_{13}H_9O_3NS]_2$	1.0	2.0	3.0
3.	Mn $[C_{13}H_9O_3NS]_2$	1.0	2.0	4.0

Table 5: Half Maximum inhibitory concentration of synthesized Metal complexes IC_{50} (a) values expressed in µM

Compound	MCF–7[(b)]	OVCAR-3[(c)]	HT- 29 [(d)]
$Co[C_{13}H_8O_3NS]_2$	1.62	2.50	4.58
$Mn[C_{13}H_8O_3NS]_2$	8.24	9.46	10.20
Doxorubicin	1.73	1.12	1.01

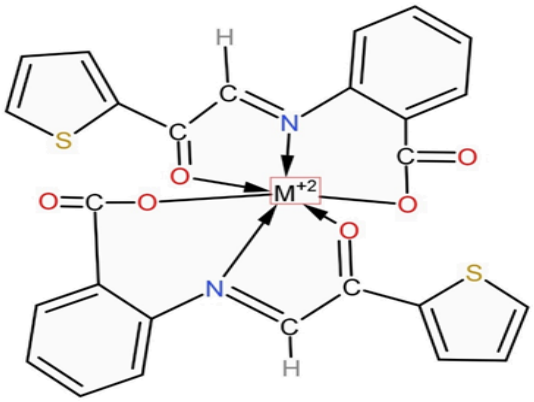

Figure 1: Chemical Structure of Metal Complex. M = Mn (II)/Co (II).

Table 4: Antimicrobial activities of Ligand and Metal complexes inhibition zone in mm concentration 100 µg/mL

S.No.	Test Compound	Gram-positive Bacteria		Gram-negative Bacteria		Fungi	
		S. aureus	*S. pyogenies*	*E. coli*	*K. pneumoniae*	*F. oxysporum*	*A. fumigatus*
1.	Tetracycline	27	25	--	--	--	--
2.	Chloramphenicol	--	--	30	28	--	--
3.	Clotrimazole	--	--	--	--	22	23
4.	Ligand (TGAA)	10	12	12	12	08	10
5.	$Co[C_{13}H_8O_3NS]_2$	22	18	20	17	13	13
6.	$Mn[C_{13}H_8O_3NS]_2$	11	14	16	17	14	10

Cytotoxic Studies

Preliminary screening of the synthesized metal complexes was carried out for their cytotoxic activity against a panel of selected human cancer cell lines such as MFC -7 (Breast), OVCAR-3 (ovarian) and HT-29 (Colon) by using MTT assay.[24-27] The result of this cytotoxic testing expressed as IC_{50} values is summarized in Table 5 Doxorubicin was used as a positive control. Co(II) and Mn(II) complexes were found to be effective in all the cell lines examined.

DISCUSSION

The elemental analysis and metal estimation of the complex led to the formula $M[C_{13}H_8O_3NS]_2$ where M= Mn (II)/Co (II).The experimental values are in good agreement with the theoretical values (given in parentheses). The low molar conductance values indicate that the complexes are non-electrolytic nature. IR spectra of ligand (TGAA) show a band of medium intensity in the region of 3500-3470 cm^{-1} due to the –OH stretching vibration of free- CO_2H group coordination of ligand as a consequence of deprotonation of – CO_2H group is evident by the disappearance of the above band in the IR spectra of respective metal complexes. Furthermore the asymmetrical and symmetrical vibrations of COO^- group appear at 1560-1530 cm^{-1} and 1340-1320 cm^{-1} $\Delta\upsilon$(as-s) value 220-210 cm^1 indicate the coordination through unidentate carboxylate group. Some New bands appeared in the IR spectra of metal complexes in the regin of 550-530 cm^{-1} 450-430 cm^{-1} and 335-325 cm^{-1} are probably due to formation of M-O, M-N and M-S respectively. On the basis of electronic spectral studies The Magnetic moment values of the Co(II) and Mn(II) complexes are 4.60 and 5.05 B.M. suggesting octahedral geometry. The free ligand NMR spectra has a characteristic NMR signal for carboxyl group proton in the 10.17-10.80 ppm range, the disappearance of this signal in the ^1H NMR spectra of metal complexes indicating the involvement of Carboxylate ion oxygen in chelation through deprotonation.

Suggested structure of the Complex

The proposed structure of complex based on the above mentioned physico-chemical, Spectral studies like elemental, molar conductance, magnetic moment, IR, Electronic, NMR. The tentative structure of the complex is shown in Figure 1. A comparative study of enzyme activity of the ligand and their respective metal complexes indicate that the enzyme activity of the ligand has considerably increased in the form of metal complexes. The ligand/ metal complexes play a competitive role with the substrate required for their production and thus the free availability of the substrate in reduced inhibiting the growth. The result of antimicrobial activity of the ligand and its metal complexes of Co(II) and Mn(II) indicate that complexes show more activity and the ligand has less activity against microorganism. The higher cytotoxic activity of Cobalt complex than Manganese complex may be attributed to the function of the Cobalt complex as a competitive inhibitor of Hemeoxygenase which is produced in large amounts in solid tumors in humans and animal tumor thus cobalt complex has the capacity to reduce the energy status in tumors as well as to enhance tumor hypoxia which also influences its antitumor activity probably through Cobalt biomolecule interaction.[28-30]

CONCLUSION

Schiff base and its Co(II) and Mn(II) complexes were prepared and characterized using the micro-analytical, conductance spectral analysis which reveal that ligand coordinating through the azomethine nitrogen and carbonyl oxygen atoms. The geometry of the complexes is assigned as octahedral. The complexes are biologically active and showed enhanced antimicrobial, Enzyme inhibition activity compared to the free ligand. Synthesized metal complexes were also found to be effective in all the human cancer cell lines.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

DMSO: Dimethylsulfoxide; **DMF:** Dimethylformamide; **IR:** Infrared Spectra **UV:** Ultraviolet Spectra; **NMR:** Nuclear Magnetic Resonance Spectra; **BOD:** Biological Oxygen Demand; **PPM:** Parts Per Million

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