

Synthesis, Characterization and Antimicrobial Studies of Mn(II), Ni(II) and Cu(II) Mixed Ligand Complexes Derived from Isatinphenylhydrazone and Nitrogen / Sulphurmonodentate Ligands

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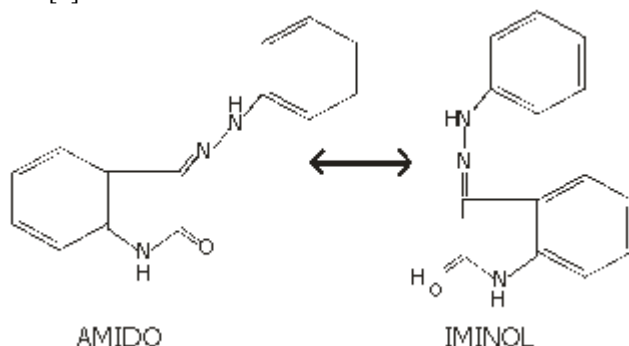
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Abstract: A Condensation reaction of isatin and phenylhydrazine yielded the primary ligand- isatinphenylhydrazone which combined with the metal(II) ions and the secondary ligands to give the metal(II) mixed ligand complexes. These were characterized using elemental analysis, molar conductivity, infrared and electronic absorption spectroscopy. Data obtained revealed the primary ligand to be bidentate coordinating to metal ion through NO hetero-atoms. The complexes formed are octahedrally configured existing in a 2:1:2 molar ratio of primary ligand :metal: secondary ligand. The antimicrobial studies carried out show enhanced growth inhibitory activities among the complexes with copper(II) mixed ligand complexes being the most effective.

Keywords: Isatinphenylhydrazone, bidentate, mixed ligand complexes, antimicrobial activities

1. Introduction

A new Schiff base, isatinphenylhydrazone with the formula $C_{14}H_{11}N_3O$ represented by IPH was obtained from condensation reaction of isatin and phenylhydrazine. This ligand constitute on important heterocyclic biologically functional compound. Its coordination to Mn(II), Ni(II) and Cu(II) through NO donor atoms along with the secondary ligand result in the formation of stable polymeric complexes. As a Schiff base, isatinphenylhydrazone formation simply involved the nucleophilic addition of the amine group of the hydrazine to the carbonyl carbon in isatin moiety [1]. This introduction of the C=O group into the hydrazine chromosphere tend to increase the selection delocalization and denticity of the hydrazine moiety thus makes the resulting compound stronger Lewis base. Also, the tautomerism in hydrazine gives another special and interesting feature to the compound. The compound exhibits amido-iminol tautomerism where in solid state the amido form predominate and in solution the iminol form exist [2].



[Tautomerism of substituted phenylhydrazone]

This property offers the possibility for the formation of different types of complexes. Thus this hydrazine derivative can coordinate to the metal either as a neutral amido form or

as deprotonated iminolate ion. Effective conjugation over the entire moiety is increased by the enolization of the ligand and thereby improves the electron delocalization [3]. The ligand versatility and varied coordination modes depends on these factors such as the tautomerism, reaction condition, stability of resulting complex, number and nature of the substituents on the hydrazine skeleton [4]. The complexation of this ligand, isatinphenylhydrazone to metals like Mn(II), Ni(II) and Cu(II) are implicated in the enhancement of their antimicrobial activities [5]. The therapeutic effect of these complexes lie in their ability to bond with deoxyribonucleic acid of the cells which is done through the synergistic activity of the transition metals. When this happens the DNA strands are cleaved and this result in seizure of normal metabolic activities of the cells leading to the antiproliferation of the tumor/cancerous cells and even death of cells in microbes. As a continuation of our research work on structure-activity relation studies on hydrazones and derivatives, this paper reports the synthesis, spectral characterization of the mixed ligand complexes and their antimicrobial activities on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus anthracis*, *Candida albicans* and *Aspergillus niger*.

2. Experimental

Synthesis of isatinphenylhydrazone

Isatin (0.736g, 0.005mmol) was dissolved in aqueous methanol, to this solution was added 3 drops of glacial acetic acid and phenylhydrazine (0.541g; 0.005mmol). The resulting mixture was boiled under reflux for two hours, during which the colour of the solution gradually changed from orange to yellow. The solution was allowed to cool at room temperature overnight. The precipitate formed was filtered and recrystallized from aqueous methanol. The precipitate was dried over fused calcium chloride in a desiccator. The percentage yield was 67%

Synthesis of Metal (II) mixed ligand complexes of isatinphenylhydrazones with ammonia orthiophene (where metal (II)--Mn(II), Ni(II) and Cu(II))

Solutions of the above named metal(II)hydrated chlorides (0.002mole) in aqueous methanol was added to to methanolic solution of isatinphenylhydrazone (0.949g , 0.004mol) while stirring for 20 minutes. 20ml of ammonia or 20ml of thiophene respectively was added to the above mixture and boiled under reflux for 1hour. The resulting solutions were left to stand overnight. The precipitates formed were filtered, washed with methanol and dried over fused calcium chloride in a dessicator. The percentage yield are shown in Table 1.

Antimicrobial Studies

The synthesized compounds were screened against bacteria and fungi following standard and well reported procedure, the Paper Disk Diffusion method [6].

[7][8][9]. Nutrient agar was used for bacteria and Sabourand Dextrose agar was used for the fungi

3. Result and Discussion

The complexes were synthesized by reacting the primary ligand (IPH), hydrated metal(II) salt with the respective monodentate secondary ligands in a 2:1:2 molar ratio using methanol as the reaction medium. The compounds formed were stable in air, variedly coloured ranging from yellow, brown to dirty green and soluble in coordinating solvents such as DMSO, DMF, acetone and methanol. The melting points of the synthesized compound are shown in Table 1 with other physicochemical properties. They are relatively high ranging from 101°C to 230°C. This seems to suggest that the chelates do not exist in simple monomeric forms but rather as polymeric compounds.

Table 1: Physicochemical Properties

Compound	Molecular Formula	% yield	Mp (°C)	Colour	Found (Calcd) %				Molar Conductance ($\Omega^{-1}\text{mol}^{-1}\text{cm}^{-2}$)
					C	H	N	M	
IPH	$\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}$ 237.27	67.5	215.7	Yellow	70.81 (70.39)	4.64 (4.66)	17.70 (17.58)		
$\text{Mn}(\text{IPH})_2(\text{NH}_3)_2\text{Cl}_2$	$\text{MnCl}_2\text{C}_{28}\text{H}_{28}\text{N}_8\text{O}_2$	54.3	228.2	violet	52.7 (52.5)	4.4 (4.3)	17.6 (17.4)	8.5 (8.4)	3.65
$[\text{Ni}(\text{IPH})_2(\text{NH}_3)_2]\text{Cl}_2$	$\text{NiCl}_2\text{C}_{28}\text{H}_{28}\text{N}_8\text{O}_2$	63.4	209.3	Brown	54.09 (54.10)	4.02 (3.88)	15.78 (15.66)	9.45 (9.87)	0.91
$\text{Cu}(\text{IPH})_2(\text{NH}_3)_2\text{Cl}_2$	$\text{CuCl}_2\text{C}_{28}\text{H}_{28}\text{N}_8\text{O}_2$	48.6	220.5	Green	52.3 (52.9)	4.4 (4.3)	17.4 (16.98)	9.8 (9.7)	4.25
$\text{Mn}(\text{II})[(\text{IPH})_2(\text{Th})]\text{Cl}_2$	$\text{MnCl}_2\text{C}_{32}\text{H}_{30}\text{N}_6\text{O}_2\text{S}_2$	32.8	219.3	Pale pink	53.4 (52.90)	4.2 (4.2)	11.7 (11.7)	7.5 (7.5)	2.78
$\text{Ni}(\text{II})[(\text{IPH})_2(\text{Th})]\text{Cl}_2$	$\text{NiCl}_2\text{C}_{32}\text{H}_{30}\text{N}_6\text{O}_2\text{S}_2$	47.3	214.1	Brown	53.2 (53.5)	4.2 (4.15)	11.7 (11.4)	8.1 (8.0)	1.90
$\text{Cu}(\text{II})[(\text{IPH})_2(\text{Th})]\text{Cl}_2$	$\text{CuCl}_2\text{C}_{28}\text{H}_{26}\text{N}_6\text{O}_2\text{S}$	54.8	210.6	Dirty green	52.1 (52.3)	4.0 (4.3)	13.0 (13.4)	9.9 (10.3)	4.20

Table 2: Selected IR Spectral Band

Compound	$\nu\text{N-H}$	$\nu\text{N-N}$	$\nu\text{C=O}$	$\nu\text{C=N}$	$\nu\text{M-O}$	$\nu\text{M-N}$	$\nu\text{M-S}$
IPH	3597w	1068s	1681s	1610s			
$\text{Mn}(\text{IPH})_2(\text{NH}_3)_2\text{Cl}_2$	3342br	1022	1640sh	1575m	387w	465m	443m
$[\text{Ni}(\text{IPH})_2(\text{NH}_3)_2]\text{Cl}_2$	3386v,w	1104w	1625m	1621w	395v,w	501v,w	413m
$\text{Cu}(\text{IPH})_2(\text{NH}_3)_2\text{Cl}_2$	3254br	1017	1621sh	1540m	377m	498m	407br
$\text{Mn}(\text{II})[(\text{IPH})_2(\text{Th})]\text{Cl}_2$	3312br	1013	1615m	1552w	342m	430m	401m
$\text{Ni}(\text{II})[(\text{IPH})_2(\text{Th})]\text{Cl}_2$	3332br	1032	1611m	1532w	332m	427s	402br
$\text{Cu}(\text{II})[(\text{IPH})_2(\text{Th})]\text{Cl}_2$	3421m	1053	1590w	1529w	340w	488s	418m

*br=broad, w= weak, m= medium, sh = sharp, s = small

Infrared Spectra of Ligand and Metal(II) complexes

The selected Infrared spectra bands of the ligand and metal complexes are shown in Table 2. The stretching vibration bands for νNH appear within $3312\text{--}3597\text{cm}^{-1}$. The bands at 1681cm^{-1} and 1601cm^{-1} in the spectrum of the ligand are attributed to $\nu\text{C=O}$ and $\nu\text{C=N}$ carbonyl and azomethine groups respectively which on complexation were found to have shifted. The bands observed at 1610cm^{-1} due to azomethine group of the IPH were found to shift to lower vibration frequencies 1575cm^{-1} and 1540cm^{-1} in the spectra of Mn(II) and Cu(II) complexes. While the stretching vibration band of C=N bond, appearing in the IR spectra of the Ni(II) complexes had shifted to higher

frequency (1621cm^{-1}). These shifts in C=N bands of complexes are affirmation of the involvement of the azomethine nitrogen in chelation [11]. Similarly the change in intensity of the C=O band in Cu(II) complexes and the shifts to higher frequencies in Mn(II) and Ni(II) complexes all indicate the coordination of the carbonyl oxygen to the metal ions. Bands in range of $330\text{--}490\text{cm}^{-1}$ are all due to the stretching vibrations of $\nu\text{M-O}$, $\nu\text{M-N}$ and $\nu\text{M-S}$ of the complexes

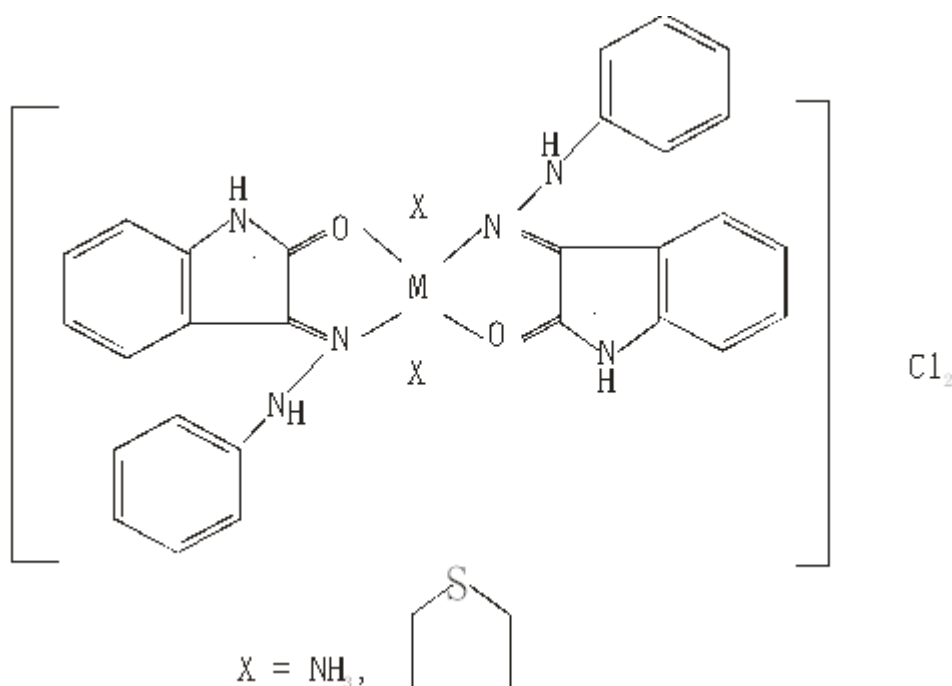
4. Electronic Spectra

The electronic spectra of metal(II) complexes displayed in Table 3 were the observed selected absorption bands. Four of these bands were within the ultraviolet region ca 42,553, 31,250, 28,986 and 25,974 cm^{-1} . They were assigned to $n - \sigma^*$, $\pi - \pi^*$, $n - \pi^*$ and $n - \pi^*$ transitions; attributed to intraligand transition, lone pair of electrons on oxygen atom and the double bond within the molecular moiety as shown figure 1 above and figure 2 below. In the spectra of the complexes many of the bands were bathochromically shifted while others remain unchanged or hypsochromically shifted. The shifts in the bands were indication of the ligand, IPH coordination to the metals. The absorption spectrum of $[\text{Mn}(\text{IPH})_2\text{X}_2]$ ($\text{X} = \text{ammonia}$ or thiophen) complexes show bands at 23,833 and 18,396 cm^{-1} assignable to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$ and ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g$ transitions. In the electronic spectrum of $[\text{Ni}(\text{IPH})_2\text{X}_2]$, the transitions are (13987cm^{-1}) ${}^3\text{A}_{1g} \rightarrow {}^3\text{T}_{2g}$, ($16,155\text{cm}^{-1}$) ${}^3\text{A}_{1g} \rightarrow {}^3\text{T}_{1g}$ (F) and ($22,522\text{cm}^{-1}$) ${}^3\text{A}_{1g} \rightarrow {}^3\text{T}_{1g}$ (P) respectively from ground states to excited states and are all spin allowed giving an octahedral geometry. The transitions in $\text{Cu}(\text{IPH})_2\text{X}_2$ complexes are broad low intensity bands at 15,385 cm^{-1} and 22,202 cm^{-1} assignable to d-d asymmetric transition which as reported by Jouad et al, 2005 result in the lengthening of the bond along z-axis. The ${}^2\text{E}_g$

and ${}^2\text{T}_{2g}$ states of $\text{Cu}(\text{II})$ (d^9 ion) in octahedral ligand field distortionally split into 3 transitions viz ${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$, ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$ and ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$. These 3 transitions since they are close in energy lie within the single broad band. The higher energy bands due to charge transfer are found at 27000 cm^{-1} and 21500 cm^{-1} assignable to S/N-Cu LMCT resulting in a distorted octahedral geometries [8][4].

Table 3: Electronic Absorption Spectral Data

Compound	Band (cm^{-1})	Assignment	Geometry
IPH	31,200 25,974	$\pi - \pi^*$ $n - \pi^*$	
$\text{Mn}(\text{IPH})_2(\text{NH}_3)_2\text{Cl}_2$ $\text{Mn}(\text{IPH})_2(\text{Th})_2\text{Cl}_2$	23,833 18,396	${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$ ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g$	Octahedral
$[\text{Ni}(\text{IPH})_2(\text{NH}_3)_2]\text{Cl}_2$ $[\text{Ni}(\text{IPH})_2(\text{Th})_2]\text{Cl}_2$	16,155 22,522	${}^3\text{A}_{1g} \rightarrow {}^3\text{T}_{2g}$, ${}^3\text{A}_{1g} \rightarrow {}^3\text{T}_{1g}$ (F) ${}^3\text{A}_{1g} \rightarrow {}^3\text{T}_{1g}$ (P)	Octahedral
$\text{Cu}(\text{II})[\text{IPH}_2(\text{NH}_3)_2]\text{Cl}_2$ $\text{Cu}(\text{II})[\text{IPH}_2(\text{Th})_2]\text{Cl}_2$	15,385 22,202	${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$ ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$ ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$	Octahedral



Antimicrobial Studies

The subcultured bacteria- *Staphylococcus aureus*, *Escherichia coli*, *Bacillus anthracis* and fungi- *Candida albicans*, *Asperigillus niger* were spread in plates containing Nutrient agar for bacteria and Sabouraud Dextrose agar for the fungi. The synthesized compounds were dissolved in DMF to a concentration of 1000 $\mu\text{g}/\text{ml}$. The Paper disks (0.7mm diameter size) were soaked in these test samples, drained and using a sterilized forceps, placed in the agar plates containing the bacteria and the fungi. This was repeated with the reference standards, ampicillin for bacteria and fluconazole for fungi. The temperature of the incubation was set at 37°C. These inoculated plates were then incubated for 36 hours for bacteria and 72 hours for

fungi. This was to allow enough time for the normal microbes growth. The zones of inhibition were then measured across the disks in mm. The results are as shown in Table 4. The data obtained clearly show a remarkable inhibitory action of the synthesized compounds on the growth and proliferation of the microbes. This is in conformity with many reported works on Schiff base complexes such as [4] [10] [11][17]. $\text{Cu}(\text{II})$ mixed ligand complexes were most sensitive, giving inhibitory zones ranging from 20.1mm to 25.0mm for both bacteria and fungi competing favorably with the standards. The inhibitory effectiveness of $\text{Mn}(\text{II})$ chelates were also significant ranging from 17.8mm for *S. aureus* to 19.3—19.8mm for *B. anthracis*, 19.7—19.9mm for *A. niger* (fungus) and 20.0—20.6mm for *E. coli* bacteria / *C. albicans* (fungus). A zone of

10.0mm diameter was considered to be ineffective inhibition zone but all the complexes inhibited well beyond this value showing that they all have inhibitory capacities at varying degrees. The modes of coordination of the primary ligand, isatinphenylhydrazone, in these complexes play a significant role in the observed efficiency. The resulting ring structures within the complexes as the primary ligand coordinates the metal ions confer greater stability but labilize the M—N or

M—S bond of the secondary ligand (ammonia and thiophen) making the penetration of cell membrane easier through displacement reaction of the monodentate ligands with DNA of the cells. Thus interfering with the normal metabolic reactions of the cell and thereby causing inhibition in the growth or even death of the cell.

Table 4: Zones of inhibition (1000µg/ml) measured in mm

COMPOUND	S. aureus,	E. coli	B. anthracis	C. albican	A. nigers
IPH	16.0	17.7	15.6	18.4	16.0
Mn(IPH) ₂ (NH ₃) ₂ Cl ₂	17.0	20.0	19.3	20.0	19.7
Mn(IPH) ₂ (Th) ₂ Cl ₂	17.8	20.6	19.8	20.3	19.9
[Ni (IPH) ₂ (NH ₃) ₂]Cl ₂	17.0	17.5	16.5	18.0	17.5
[Ni (IPH) ₂ (Th) ₂]Cl ₂	18.6	18.8	16.4	18.2	17.8
Cu(II)[IPH ₂ (NH ₃) ₂]Cl ₂	23.0	25.0	20.1	21.0	23.4
Cu(II)[IPH ₂ (Th) ₂]Cl ₂	22.8	19.9	20.3	23.2	24.2
Ampicillin / Fluconazole	25.0	23.3	22.8	25.2	26.2

5. Conclusion

Six novel mixed ligand complexes were synthesized based on 2: 1 :2 molar ratio of primary ligand to metal ion to secondary ligand. The chelates were found to be stable in air, variedly coloured and melt at relatively high temperature. Their low molar conductivity values indicate the non-electrolytic nature. Data obtained from infrared and electronic absorption spectral affirmed the bidentate coordination modes through the azomethine N atom and carbonyl oxygen, with the fifth and sixth metal sites occupied by N or S of ammonia and thiophen respectively-monodentate secondary ligands, thus giving octahedral configuration to the molecules. These mixed ligand complexes were screened against *Staphylococcus aureus*, *Echiarichia coli*, *Bacillus anthracis* (bacteria) *Candidasalbican* and *Asperigellusniger*(fungi) for sensitivity toward growth and proliferation. They were all found to be effective antimicrobial agents with Cu(II) chelates being most sensitive.

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