Synthesis, Characterization and Bio- Activity of Metal Complexes of Isatin Derivative

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ABSTRACT: A new series of transition metal complexes of Cu(II), Mn(II), Cd(II), Zn(II), Ni(II) and Co(II) derived from Isatin - anthranilic acid Schiff base and mixed ligand metal complexes have been synthesized and characterized in the light of spectral studies like IR, UV, and ^{1}H- NMR. The synthesized metal complexes have been screened for their invitro antibacterial activities by zone of inhibition method against *Proteus vulgaris, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Micrococcus and Salmonella typhi "H"*. The antibacterial screening showed that cadmium complex possesses higher activity towards *Staphylococcus aureus*

Keywords: Isatin, Anthranilic acid and antibacterial activity.

INTRODUCTION

Oxindole are endogenes compound with a long history and found in mammalian body fluids and tissues, ubiquitously distributed in the central nervous system, that have shown an extensive range of biological effects including antibacterial [1], antifungal [2], anticonvulsant [3], antiviral [4] and antiproliferative activity [5].Metal complexes with Schiff bases of Isatin derivatives exhibited remarkable biological activity and also used as pro-drug. Isatin and their derivatives have an important biological activities like anticonvulsant action [3], antiviral activity [4], insecticide, fungicide, anti-inflammatory [6], analgesic [7], anti-tubercular [8], antibacterial [1] and anti-depressant [9].

Schiff bases of Isatin were reported to possess antibacterial [1], antifungal [2], antiviral [4], antiproliferative activity [5], antiprotozoal [10], antiamoebic [11] and anthelmintic activities [12]. They also exhibit significant anticonvulsant activity apart from other pharmacological properties.

A considerable number of Isatin metal complexes are now found to possesess antitumor activity [13]. Isatin metal complexes exhibited invitro antibacterial effect against E.coil, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus anthracis, Staphylococcus faecalis, Proteus mirabilis, Entrobacter species.

Knowing the importance of Isatin, its derivatives and their metal complexes as revealed by literature, We planned to syntheses few newer Isatin Schiff base transition metal complexes derived from Isatin and anthranilic acid and mixed ligand complexes using Isatin and p-Anisidine.

EXPERIMENTAL

Materials and Analytical Methods

Isatin, Anthranilic acid, p-Ansidine, Metal salts and solvents were of analytical reagent (A.R.) grade. All the melting points were determined by open capillary tube and are uncorrected. The electronic absorption spectra of Isatin Schiff base and metal complexes in the range of 200nm-800nm in methanol were recorded on the shimadzu spectrophotometer. FT-IR spectra of Schiff base of Isatin and their metal complexes, mixed ligand complexes were recorded on shimadzu spectrophotometer using potassium bromide pellets in the range of 400-4000cm^{-1}. ^{1}H-NMR spectra of the Schiff base of Isatin in DMSO-d^6 was recorded on 300MHz Bruker instrument using tetramethylsiliane as internal standard.

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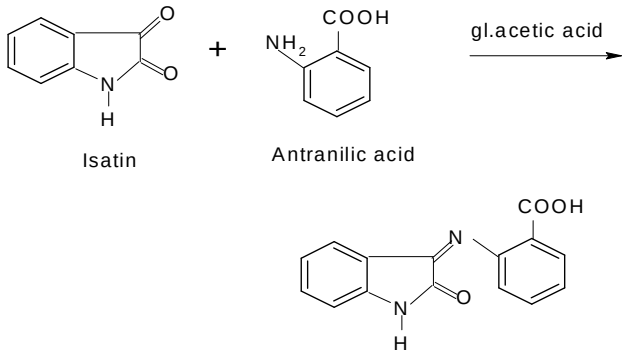
Synthesis of Schiff base of Isatin

The synthesis of Schiff base is schematically presented as Scheme (Figure 1). The reaction mixture of ethanolic solution (30ml) of Isatin (0.01M) and ethanolic solution (30ml) of Anthranilic acid (0.01M) was stirred for 2 hours with 3-4 drops of glacial acetic acid. The product obtained after the evaporation of solvent was filtered and recrystallized from ethanol.



Ligand A

Figure 1: Scheme of the Synthesis of Schiff Base
Melting point - 105 °C Percentage of Yield = 80%

Synthesis of Metal (II) Acetate Complexes

A solution of ligand **A** (0.266, 0.001M) in aqueous ethanol (1:1,50ml) was added to a solution of metal (II) acetate (0.0005M) in aqueous ethanol (1:1, 50ml). The above solution was allowed to stand for overnight. The product was filtered off, washed with aqueous ethanol (1:1) and dried in air as described in scheme (Figure 2). The colour, yield and melting point of synthesized complexes were recorded and listed in table 1.

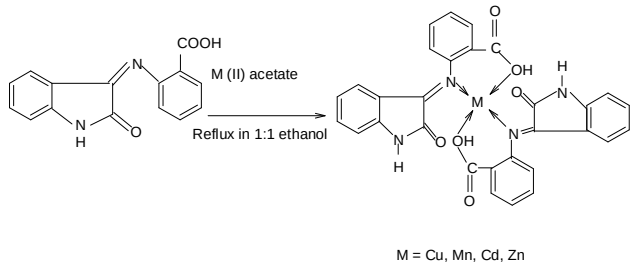


Figure 2: Scheme of Product Processes

Synthesis of Metal (II) Chloride Complexes

A solution of ligand A (0.266, 0.001M) in ethanol was added to an ethanolic solution of metal (II) chloride (0.001M).The mixture was stirred for 2 hour. The product was filtered off, washed with ethanol and dried in air. The colour, yield and melting point of synthesized complexes were recorded and listed in table 1.

Synthesis of Mixed Ligand Metal (II) Chloride Complexes and Acetate Complexes

A solution of primary ligand Isatin (0.001M) in aqueous ethanol (1:1, 50ml) and the secondary ligand p-Anisidine (0.001M) in aqueous ethanol (1:1, 50ml) were added to a solution of metal(II) chloride or acetate (0.002M) in aqueous ethanol (1:1, 100ml). The above mixture was allowed to stand for overnight. The product formed was filtered off, washed with aqueous ethanol (1:1) and dried in air. The colour, yield and melting point of synthesized complexes were recorded and listed in table 1.

Antibacterial Activity Determination

We have recorded the antibacterial activity of Isatin Schiff base metal complexes and mixed ligand complexes against *Proteus vulgaris, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Micrococcus and Salmonella typhi "H"* at Venture Institute, Pasumalai, Madurai District, Tamil Nadu. Ceftazidime was used as standard and Disc Diffusion Method was adopted.

Preparation of Test Sample

10 mg of each sample were taken and fractions were separately dissolved in 1 µl water to obtained corresponding stock solution of 10 µl.

Disc Diffusion Method

The pathogenic culture is spreaded in sterile MULLER HINTON AGAR PLATES (MHA) medium. Whatmann no. 1 filter paper is used to prepare disc with the help of punching machine. The discs are sterilized in autoclave and loaded with suitable concentration of test extracts. The test extracts loaded discs are placed in bacterial culture lawn on nutrient agar plates. Then the plates are incubated at 37 ºC for 2-3days.After incubation period the zone of inhibition is measured and recorded.

RESULT AND DISCUSSION

The ligand was synthesized by acid catalyzed condensation of Anthranilic acid with the Isatin in ethanol (Figure 3). The condensation proceeds

as usual, selectively on the carbonyl in position 3 in the Isatin ring. The Isatin Schiff base, its transition metal complexes and mixed ligand complexes of Isatin and p-Anisidine synthesized in our laboratory were characterized by of UV, IR and ^{1}H-NMR spectroscopy.

Electronic Spectral Studies

Electronic absorption spectra of synthesized Isatin Schiff base and its metal complexes, mixed ligand metal complexes, the wave length of absorption maxima (λ_{max}) were found to lie in the range from 230 – 464nm as listed in table 1. All the complexes were coloured.

^{1}H NMR Spectral Studies

^{1}H NMR spectrum of Isatin Schiff base have shown the presence of aromatic indole nucleus signals at 6.6 ppm (doublet), 6.5 ppm (triplet), 6.4 ppm (doublet) and carboxylic acid signal at 10.1 ppm (singlet) and aromatic nucleus signals at 5.8 ppm (doublet), 6.02 ppm (triplet), 6.18 ppm (triplet) and 5.69 ppm (doublet). Hence the Schiff base may have the structure.

Table 1
Melting Point, Yield and UV Spectral Data of Metal Complexes of Isatin

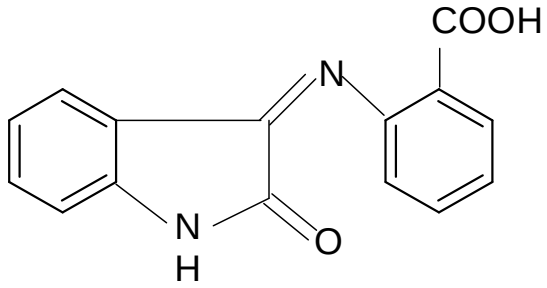
S. No.	*Compound*	*Colour*	*Melting Point*	*Percentage of Yield*	*ë $_{max}$ (nm)*
1.	A_2Cu	Green	275°C-278 °C	85%	416-464
2.	ACu	Dark green	230 °C	88%	332
3.	A_2Mn	Pink	240 °C	90%	435
4.	AMn	Orange yellow	246 °C	86%	239
5.	A_2Cd	Yellow	248 °C-250 °C	82%	414-422
6.	A_2Zn	Pale yellow	254 °C	96%	283
7.	AZn	Yellow	220 °C-220 °C	94%	303
8.	ANi	Yellow	275 °C	85%	408
9.	ACo	Brown	262 °C	85%	407
10.	INCu	Black	240 °C	93%	409
11.	INMn	Yellowish pink	265 °C	90%	416
12.	INCd	Yellowish brown	235 °C	88%	414
13.	INZn	Yellow	221 °C	94%	288
14.	INCo	Brown	244 °C	80%	449
15.	INNi	Dark brown	265 °C	86%	417

A – Isatin anthranilic acid Schiff base

I – Isatin N- p-Anisidine

IR Spectral Studies

Isatin have shown ν_{-NH}= 3190 cm^{-1}, ν_{-CO} = 1740 & 1620cm^{-1}and ν_{-NH} = 1400-1100 cm^{-1} (bending vibration) Stretching frequencies and Isatin-Anthranilic acid Schiff base (Ligand A) have shown IR spectral frequencies at 3473.80cm^{-1} (ν_{-OH}), 3373.5cm^{-1} (ν_{-NH}), 1728.22 &1616.35cm^{-1} (ν_{-CO}) and 1604cm^{-1} ($\nu_{-C=N}$).



Isatin-3-N-2-carboxy phenyl imine

Figure 3: Structure of Ligand

As we have synthesized complexes of different transition metal salts of two different anionic parts, we would like to compare the structural variation of the complexes. IR spectral datas of simple and mixed ligand complexes were listed in table 2 & 3.

In all the complexes of Isatin Schiff base, we observed that N-H stretching frequencies lies around 3194-3116cm^{-1}and C=O stretching frequencies in the range 1728-1628 cm^{-1}. This showed that these two groups of Isatin doesn't involved in bonding.

Metal (II) complexes of ligand A using metal(II)acetate have shown IR spectral values range from 3394- 3271.27cm^{-1} (ν_{-OH}), 1581-1458 cm^{-1} ($\nu_{-C=N}$). The decrease in the –OH frequency and –C=N frequencies indicated that they may involved in bonding with metal atoms. Therefore we observed that irrespective of the anionic counter part of metal salts both the complexes involved the bonding of –OH group, nitrogen atom of –C = N with metal atom. Further the bonding was proved by M-O stretching frequency range within 455-424.34 cm^{-1} and M-N stretching frequency range from 524- 478.35cm^{-1}. Hence the complex may have the structure.

Mixed Ligand Metal Complexes of Isatin and p-Anisidine

Mixed ligand copper complex have shown the following IR spectral values.

ν_{-NH} = 3450(b, w) - 3055 (b, w) cm^{-1}

ν_{-C-O-C} = 1249.16-1242 cm^{-1}

ν_{M-N-} = 547.78 - 501.49cm^{-1}

The decrease in the $-NH_2$ frequency of p-Anisidine and disappearance of Isatin -NH frequencies indicated that they may involved in bonding with metal atom. The bonding was proved by M-N stretching. Hence the complex may have the structure.

Antibacterial Activity

The antibacterial activity of the synthesized metal complexes against *Proteus vulgaris, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Micrococci and Salmonella typhi "H"* micro organisms by zone of inhibition studies, revealed that cadmium (II) complex was found to have higher activity against *Staphylococcus aureus* compared to other micro-organisms. The maximum activity was observed at 10ìl concentration by zone of inhibition studies. Isatin Schiff base of cadmium, zinc and manganese complexes were found to possess activity against *Klebsiella pneumoniae* and Isatin Schiff base of copper, zinc and nickel complexes were found to have activity against *Escherichia coli.*

Table 2
IR Spectral Data of Isatin – Anthranilic Acid Schiff base Metal Complexes

S. No.	Complexes	$\nu_{-OH}(cm^{-1})$	$\nu_{-NH}(cm^{-1})$	$\nu_{-CO}(cm^{-1})$	$\nu_{C=N}(cm^{-1})$	$\nu_{M-O}(cm^{-1})$	$\nu_{M-N}(cm^{-1})$
1.	A	3473.80	3373.5	1728.22 1616.35	1604	-	-
2.	A2Cu	3271.27	3116	1728(w) 1610(b)	1573	424.34	478.35
3.	ACu	3271.27	3116	1706(w) 1604(s)	1550	424.34	478.35
4.	A2Mn	3302.12	3140	1678 1589.34	1543.05	424	516.21
5.	AMn	3348.42	3140	1681.63 1620.21	1581.63	425	524.64
6.	A2Cd	3286.70	3132.40	1705.07 1620.21	1535.34	423	516.92
7.	A2Zn	3302.13	3124.68	1597.06 1543.05	1458.18	416.62	516.92
8.	AZn	3302.13	3132.40	1728.22 1612.49	1543.05	416.62	455.20
9.	ANi	3302.13 (w)	3194.12	1728.22 1620.21	1543.05	455.2	478.34
10.	ACo	3394(b)	3100(b)	1728.22 1620.21	1543.05	455.2	478.35

Table 3
IR Spectral Data of Isatin – p- Ansidine Metal Complexes

S. No.	Complexes	$\nu_{NH}(cm^{-1})$	$\nu_{C=O}(cm^{-1})$	$\nu_{C-O-C}(cm^{-1})$	$\nu_{M-N}(cm^{-1})$
1.	INCu	3450(b,w) 3348(b,w)	1728.2 1612	1249.16	547.78 501.49
2.	INMn	3448(w) 3224(b)	1735.9 161249	1242.16	578.64 532.35
3.	INCd	3448.72(w) 3224.98(b)	1735.93 1612.49	1249.16	578.64 532.35
4.	INZn	3400(w) 3240(b)	1735.93 1612.49	1242.16	578.64 532.35
5.	INCo	3400(b) 3062.96(w)	1697.36 1612.49	1249.87	540.07 501.49
6.	INNi	3400(b,w) 3055.24(w)	1697.36 1612.49	1249.01	540.07 501.49

Table 4
Antibacterial Activity of Isatin Schiff Base Transition Metal Complexes

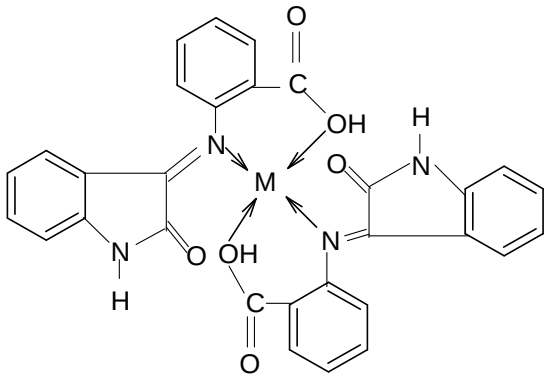
Organisms	*Compound Zone of inhibition (mm)*								
	A_2Cu	A_2Mn	A_2Cd	A_2 Zn	A Zn	AMn	ANi	ACo	ACu
Staphylococcus aureus sp	A	NA	A	A	NA	A	NA	A	NA
Bacillus subtilis	NA	NA	NA	NA	NA	NA	NA	NA	NA
Proteus vulgaris	NA	NA	NA	NA	NA	NA	NA	NA	NA
E.coli	A	NA	NA	NA	A	NA	A	NA	NA
Klebsiella pneumoniae	NA	A	A	A	A	NA	A	NA	NA
Pseudomonas aeruginosa	NA	A	A	NA	NA	NA	NA	NA	NA
Salmonellatyphi "H"	NA	NA	NA	NA	NA	NA	NA	NA	NA
Micrococcus sp	A	A	A	A	NA	NA	NA	NA	NA

CONCLUSION AND SUMMARY

Isatin Schiff base and mixed ligand complexes dervied from Isatin p-Ansidine transition metal complexes were synthesized in our laboratory. The yields and melting point of the complexes were recorded. All the complexes were found to be coloured.

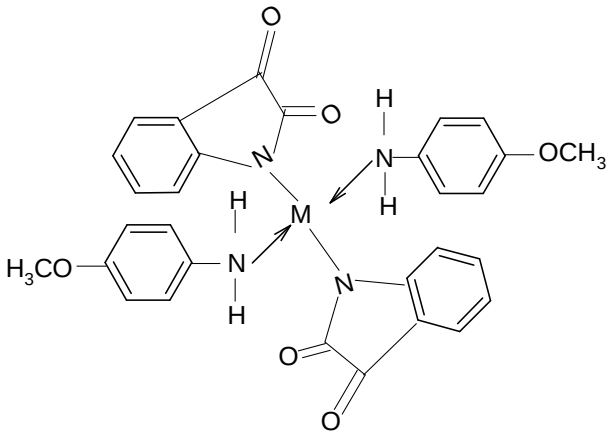
UV spectra of synthesized metal complexes have shown absorption maxima (λ_{max}) from 230 – 464nm. IR and ^{1}H-NMR spectral values of Isatin-Anthranilic acid Schiff base proved the structure (Figure 3).

Comparing the IR spectra of Isatin Schiff base and their transition metal complexes, we observed that nitrogen atom of -C = N and –OH of carboxylic acid groups involved in bond formation with metal atoms like Cu, Mn, Cd, Zn, Co and Ni and we observed four co-ordinated geometry for the metal complexes as given in (Figure 4). In the case of mixed ligand complexes of Isatin, nitrogen atom of Isatin was involved in bonding with different metal atoms (Figure 5).



M = Cu, Mn, Cd, Zn, Co,Ni

Figure 4: Structure of Chelate 1



M = Cu, Mn, Cd, Zn, Co, Ni

Figure 5: Structure of Chelate 2

The antibacterial activities of the synthesized metal complexes by zone of inhibition studies, revealed that cadmium (II) complex was found to have higher activity against *Staphylococcus aureus* compared to other micro-organisms.

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