

## Synthesis, characterization, antibacterial, antifungal and anthelmintic activities of a new 5 - nitroisatin Schiff base and its metal complexes

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The copper (II), cobalt (II), nickel (II) and zinc (II) complexes of 5 - nitroisatin Schiff base (L) (L= Schiff base derived from 5-nitroisatin and 2-methyl-4-nitroaniline) were synthesized and characterized. The authenticity of the ligand and its metal complexes has been established by micro analysis, IR, NMR, LC/MS, UV-VIS and electrical conductance measurements. The ligand acts as a bidentate agent in which the carbonyl oxygen and the azomethine nitrogen of 5-nitroisatin are involved in co-ordination. Square planar geometry was proposed for the Cu (II) and Ni (II) complexes and tetrahedral geometry was proposed for the Co (II) and Zn (II) complexes. The ligand and its metal complexes have been screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, etc. and for antifungal activity against *Aspergillus niger*, *Aspergillus flavous*, etc. The Schiff base and its complexes were also screened for anthelmintic activity on earthworms. Both samples displayed significant activities.

**Key words:** Schiff base, Isatin, Antibacterial activity, Antifungal activity

### INTRODUCTION

Isatin (2,3-dioxindole or indole-2,3-dione) is an endogenous compound identified in humans, and its effect has been studied in a variety of systems. The biological properties of isatin include a range of actions in the brain and offer protection against certain types of infections. Isatin has a range of actions such as CNS-MAO inhibition, anticonvulsant and anxiogenic activities. 5-Nitroisatin with 2-methyl-4-nitro aniline derivatives was found to exhibit interesting applications in physiological studies. Many isatin-derived compounds possess a wide spectrum of medicinal properties and thus have been studied for activity against tuberculosis, leprosy, fungal, viral and bacterial infections, etc. [1– 4].

Z.H. CHOCHAN *et al.* have prepared Schiff bases of isatin with 2-aminothiozoles and its metal complexes. It was observed that such compounds would carry medicinal properties mainly as anticonvulsants. In the present study, we mainly deal with the synthesis and characterization of a novel Schiff base formed by the condensation of 5-nitroisatin with 2-methyl-4-nitroaniline and its complexes with cobalt (II), nickel (II), copper (II)

and zinc (II). Thus another class of isatin incorporating metal-ligand as antibacterials, antifungals and anthelmintics has been introduced. The ligand and its metal complexes have been characterized by IR, NMR, UV-visible, LC/MS, molar conductance, magnetic moment and elemental analysis data.

The resulting Schiff base and its metal complexes were screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *S. typhi* and *Pseudomonas aeruginosa* and for antifungal activity against *Aspergillus niger*, *Aspergillus flavous* and *Candida Albicans* by the agar-well diffusion method. The anthelmintic activity on earthworms (*Pheretima posthumous*) of the ligand and its metal complexes was also tested by a reported method [5]. The ligand and its complexes showed varying activity against the strains and their activity was enhanced on coordination/chelation.

### EXPERIMENTAL

#### Material and Methods

All chemicals were purchased from Sigma – Aldrich and used as such. The CHN analysis was carried out by using CHNS analyser of Elementar Vario III. IR spectra were recorded on a Perkin

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Elmer IR spectrometer. NMR spectra were recorded on a Varion FT – NMR apparatus. Mass spectra were recorded using a LC/MS instrument. UV-visible spectra were recorded on the UV-visible spectrophotometer of Shimadzu Hitachi. The melting points were determined in open capillaries and are uncorrected; conductance measurements were measured on a Systronics conductivity meter in DMF. \

#### Preparation of the Schiff base (L)

Schiff base ligand was prepared according to the following procedure. Ethanolic solutions of 5-nitroisatin (Fig. 1) (0.01 mol, 1.92 g) in 50 ml and 2-methyl-4-nitroaniline (Fig. 2) (0.01 mol, 1.52 g)

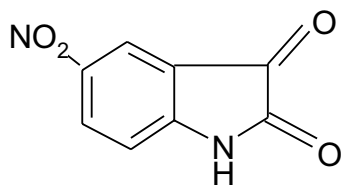


Fig. 1.

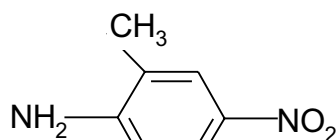


Fig. 2.

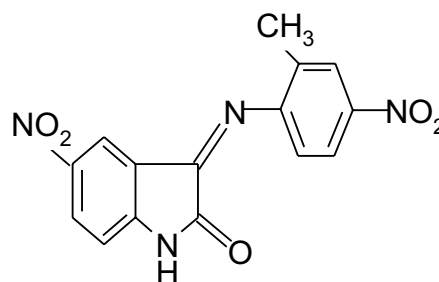


Fig. 3.

#### Preparation of complexes

The metal complexes were prepared by adding a solution of the metal salt (0.01 mol) in 50 ml absolute ethanol to 100 ml of an ethanolic solution of the ligand (0.01 mol) (1:1 ratio) and heating under reflux conditions at about 80–90°C for 2–4 h. Then the reaction mixture was evaporated to a small volume. On cooling, the metal complexes crystallized and were filtered, washed thoroughly with ethanol and dried under vacuum over fused calcium chloride.

#### Biological Activity

##### Antibacterial activity, antifungal activity and anthelmintic activity [in vitro] measurements

The Schiff base and its complexes were screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *S. typhi*, and *Pseudomonas aeruginosa*. The antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Candida Albicans* was checked by the agar-well diffusion method.

The following strains were used: bacteria - *Staphylococcus aureus* ATCC 25922, *Escherichia coli* ATCC 25923, *B. subtilis* ATCC 6633, *S. typhi* 19430, *Pseudomonas aeruginosa* ATCC 27853 and fungi - *Aspergillus niger* ATCC6275, *Aspergillus flavus* ATCC4797 and *Candida albicans*10231.

in 50 ml were mixed and refluxed for about 1 h. The reaction mixture was evaporated to a small volume and left to cool. The resulting Schiff base ligand (Fig. 3) precipitated on cooling and was filtered off, washed with ethanol and recrystallized from ethanol. The purity of the Schiff base ligand was monitored by TLC using 1:1 ethyl acetate and petroleum ether as an eluent. The product, separated by column chromatography, had a molecular weight of 326, corresponding to the molecular formula of the Schiff base  $C_{15}H_{10}N_4O_5$ [L]. The yield of Schiff base was 90%.

The bacteria and fungi were obtained from the Government Science College, Bangalore.

The anthelmintic activity of the ligand and its metal complexes was tested on earthworms (*Pheretima posthumous*) using a reported method.

The bacterial /fungal inoculums of two to six hours containing approximately  $10^5$  cfu/ml were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. The recommended amounts of 100  $\mu$ l of the test sample were introduced into the corresponding wells. The other wells supplemented with DMF and standard drugs served as negative and positive controls respectively; the plates were incubated immediately at 37°C for 24 h. Activity was determined by measuring the diameter of zones showing the complete inhibition in mm. Gentamycin (positive control) was used as a standard drug for antibacterial activity and nystatin (positive control) was used as a standard for antifungal activity. The positive controls were supplied by the Government Science College, Bangalore.

The Schiff base and its complexes were screened for anthelmintic activity on earthworms (*Pheretima posthumous*) using the standard piperazine citrate.

## RESULTS AND DISCUSSION

### Chemistry

This section deals with analytical data, conductivity measurements, IR, NMR, UV-visible and mass spectral data. All metal complexes are colored solids stable towards air and have high melting points (above 200°C). The complexes have the general formula  $[MLCl_2]$  where M= Co(II) or Zn(II) and  $[MLCl.H_2O]Cl$  where M= Cu(II) or Ni(II).

The complexes are insoluble in water and common organic solvents but are soluble in DMF and DMSO. Hence the molecular weights could not be determined. Analytical data of the complexes suggest that the metal to ligand ratio in all complexes is 1:1.

The analytical and physical data of the ligand and its complexes are described as follows.

(a) Ligand ( $C_{15}H_{10}N_4O_5$ ): color yellow, yield % 90.0, M. P. °C 180, elemental analysis %

calc./found C: 55.21/55.01, H: 3.07/3.04, N: 17.18/17.05

IR( $cm^{-1}$ ): 3342 (NH isatin), 1715(C=O), 1642(C=N)

$^1H$  NMR( $\delta$ ): isatin: 8.82(s), 7.86(d), 7.83(d); aniline: 7.86(d), 7.83(d), 7.08(d); methyl: 6.47(s)

N-H 11.65 (br)

UV( $cm^{-1}$ ): 27777, 36363

Mass spectra (m/z): 325.5

(b) Complex  $[CuLCl.H_2O]Cl$ : color light brown, yield % 87.4, M.P. °C:230(d), elemental

analysis % calc./found: C 37.66/37.52, H: 2.51/2.47, N: 11.72 /11.68,

molar conductance  $\lambda_{MS} cm^2 mol^{-1}$ :88

IR( $cm^{-1}$ ): 3345(NH isatin), 1700(C=O), 1621(C=N), 535(M-O), 479(M-N), 326(M-Cl)

UV( $cm^{-1}$ ): 26315,15748 ESR ( $\mu_{eff}$  BM): 1.85

(c) Complex  $[Co LCl_2]$ : color light brown, yield % 89.2, M.P. ° C242(d), elemental analysis %

calc./found: C 39.48/39.33, H: 2.19/2.16, N: 12.28/12.21, molar conductance  $\lambda_{MS} cm^2 mol^{-1}$ :29

IR( $cm^{-1}$ ): 3343 (NH isatin), 1701(C=O), 1622(C=N), 537(M-O), 475(M-N), 327(M-Cl)

UV( $cm^{-1}$ ): 27027,15503 ESR ( $\mu_{eff}$  BM): 4.51

(d) Complex  $[NiLCl.H_2O]Cl$ : color light brown, yield % 89.8, M.P. °C 236(d), elemental analysis %

calc./found: C: 38.16/38.04, H:2.54/2.50, N: 11.87 /11.81, molar conductance  $\lambda_{MS} cm^2 mol^{-1}$ : 71

IR( $cm^{-1}$ ): 3349(NH isatin), 1704(C=O), 1621(C=N), 536(M-O), 488(M-N), 334(M-Cl)

UV( $cm^{-1}$ ): 28571, 14814 ESR ( $\mu_{eff}$  BM): 3.68

(e) Complex  $[Zn LCl_2]$ : color: light brown, yield % 86.3, M.P. °C: 224(d), elemental analysis

% calc./found: C: 38.93/38.84, H: 2.16/2.13, N: 12.11/12.05, molar conductance  $\lambda_{MS} cm^2 mol^{-1}$ : 22

IR ( $cm^{-1}$ ): 3328(NH isatin), 1705(C=O), 1623(C=N), 542(M-O), 484(M-N), 332(M-Cl)

$^1H$  NMR( $\delta$ ): isatin: 8.90(s), 7.91(d), 7.89(d) aniline: 7.90(d), 7.95(d), 7.21(d) methyl: 6.47(s)

N-H12.65 (br)

UV( $cm^{-1}$ ): 28169,21978, ESR: diamagnetic

### IR Spectra

The infrared absorption frequencies along with their tentative assignments for the major absorption bands pertaining to the ligand and its complexes are presented in Table 3. In the present investigation the tentative assignments of the infrared bands for most of the major peaks are based on the assignments of previous workers.

The IR spectrum of the free ligand is characterized mainly by the strong bands at 3342, 1715 and 1642  $cm^{-1}$  which were attributed to the stretching frequencies of NH (aromatic), C=O and C=N (azomethine) groups, respectively and reflected in the spectral data of the metal complexes.

The IR spectra of the complexes showed a lower shift of wave numbers of both azomethine ( $\nu$  C=N) and heteroatomic moieties by about 15  $cm^{-1}$  respectively. The band located at 1700  $cm^{-1}$  in the ligand attributed to ( $\nu$  C=O) moiety of isatin also moved to the lower frequency side by about 15  $cm^{-1}$ . The  $\nu$  M-O,  $\nu$  M-N and  $\nu$  M-Cl bands in the IR region were also clearly seen. These data on comparison with the spectra of the chelates suggest the azomethine-N and isatin-O of the ligand [7-9].

### NMR Spectra

The proton NMR spectral data of the ligand and of the Zn complex are presented in Table 4. The number of protons calculated from the integration of  $^1H$  NMR spectra is in accordance with that expected from CHN analyses (Table 2) [10-11].

### Mass Spectra

The molecular weight 326 for  $C_{15}H_{10}N_4O_5$  and a molecular ion peak is found at 325.5 in the mass spectrum supporting the molecular formula of the Schiff base.

### ESR Spectra

The X-band ESR spectrum of the powdered sample of the copper complex was recorded at room temperature in solid state on X-band at a frequency of 9.1 GHz under magnetic field of 3000 G. The spectral profile is typical of axial type

**Table 1.** Thermo gravimetric analysis of the complexes

Compound	Stage	Temperature °C	Weight Loss (%) Calculated/Found	Species Lost	Residue
[CuLCl.H <sub>2</sub> O]Cl	1	100	7.43/7.38	Uncoordinated Cl	CuO
	2	210	11.19/11.15	Coordinated Cl+H <sub>2</sub> O	
	3	320	68.20/68.15	Ligand	
[Co LCl <sub>2</sub> ]	1	100	15.60/15.49	Coordinated Cl	Co <sub>2</sub> O <sub>3</sub>
	2	300	71.50/71.34	Ligand	
[Ni LCl.H <sub>2</sub> O]Cl	1	100	7.53/7.39	Uncoordinated Cl	NiO
	2	210	11.34/11.18	Coordinated Cl+H <sub>2</sub> O	
	3	330	69.11/69.05	Ligand	
[Zn LCl <sub>2</sub> ]	1	100	15.36/15.24	Coordinated Cl	ZnO
	2	310	70.50/70.32	Ligand	

( $g_{\parallel} > g_{\perp}$ ) implying  $d_{x^2-y^2}$  ground state. The  $g_{\parallel}$  and  $g_{\perp}$  are found to be 2.26 and 2.10, respectively.

The  $g_{av}$  is related to  $g_{\parallel}$  and  $g_{\perp}$  by the equation  $g_{av} = (g_{\parallel} + 2g_{\perp}) / (g_{\parallel} + 2)$  and is calculated to be 2.6. Since the value of  $g_{\parallel}$  is less than 2.6, a considerable covalent character of the complex in the metal-ligand bond has suggested [12].

#### Thermogravimetric studies

The TGA studies of the complexes were carried out in nitrogen atmosphere at a rate of 10° per minute up to 700°C. The data are presented in Table 1. In the thermal decomposition studies, a general pattern of uncoordinated chloride, coordinated chloride, water and ligand finally gave the respective oxides at a higher temperature.

#### UV-visible and magnetic susceptibility measurements

The electronic spectrum of the copper complex exhibits bands at 26315  $\text{cm}^{-1}$  and 15748  $\text{cm}^{-1}$  which can be assigned to  $2B_{1g} \rightarrow 2A_{1g}$  and  $2B_{1g} \rightarrow 2E_{1g}$  transitions. These transitions, as well as the measured value of the magnetic moment ( $\mu_{\text{eff}} = 1.85 \mu_B$ ) suggest a square-planar stereochemistry of the compound. The visible electronic absorption spectrum of the cobalt (II) complex is dominated by the highest energy  $4A_{2g} \rightarrow 4T_{1g}$  (P) transition, which is a typical one for tetrahedral Co (II) complexes. The magnetic moment value (4.51  $\mu_B$ ) and the light brown color of the cobalt (II) complex also suggest tetrahedral stereochemistry. The electronic spectrum of the nickel complex shows two bands at 28571  $\text{cm}^{-1}$  and 14814  $\text{cm}^{-1}$  which are attributed to  $1A_{1g} \rightarrow 1A_{2g}$  and  $1A_{1g} \rightarrow 1B_{2g}$  transitions. These transitions, as well as the measured value of the magnetic moment (3.68  $\mu_B$ ) suggest a square-planar stereochemistry of the compound. Since the zinc ion has a  $d^{10}$  configuration, the absorption at 28169  $\text{cm}^{-1}$  could be assigned to a charge transfer transition. However, taking into account the

spectrum and the configuration of the zinc (II) ion, a tetrahedral geometry could be assumed for its complex [13–14].

## BIOLOGICAL ACTIVITIES

### A) Antibacterial activity

In view of their potent biological activity, the ligand and its metal complexes were tested against five bacteria, viz., *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* which are representatives of gram positive bacterial groups and *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* and *Salmonella Typhi* which are representative of gram negative bacterial groups. These bacterial strains are chosen as they are potential pathogens of human beings. The biological screening was conducted by the disc method according to [5, 15]. The enhancement in the activity can be explained on the basis of the chelation theory and the results obtained are presented in Table 2. The ligand and its metal complexes exhibit more activity at higher concentration.

### B) Antifungal Activity

The antifungal activity of the ligand and its metal complexes was tested against the fungi *Aspergillus niger*, *Aspergillus flavous* and *Candida albicans* and the results obtained are presented in Table 3. The ligand and its metal complexes exhibit more activity at higher concentration.

### C) Anthelmintic activity [in vitro]

Anthelmintic activity of the ligand and its metal complexes was tested on earthworms (*Pheretima posthuma*) by a reported method [5, 15]. Normal saline has no effect till 10 hours. The standard, piperazine citrate, took 18 minutes till the death of the worms and the results obtained are presented in Table 4. Amongst the complexes under investigation, the complexes of Cu(II), Co(II),

**Table 2.** Antibacterial activity of the Ligand and its complexes [Concentration(( $\mu\text{g/ml}$ )]

Compound	50	100	150	200	250	300	350	400	450	500
$\text{C}_{15}\text{H}_{10}\text{N}_4\text{O}_5(\text{L})$ a,b,c,d,e↓	-	-	-	+	++	++	++	++	++	+++
	-	-	+	+	+	+	++	++	++	+++
	+	+	+	+	+	+	++	++	++	+++
	-	-	+	+	+	+	++	++	++	+++
[CuLCIH <sub>2</sub> O]Cl	+	+	++	++	++	++	++	++	++	+++
	+	+	++	++	++	++	++	++	++	+++
	+	+	+	++	++	++	++	++	++	++
	+	+	+	+	+	++	++	++	++	+++
[Co LC <sub>2</sub> ]	+	+	++	++	++	++	++	++	+++	+++
	+	+	+	++	++	++	++	++	++	+++
	+	+	+	++	++	++	++	++	+++	+++
	+	+	+	+	+	++	++	++	++	+++
[Ni LC <sub>2</sub> H <sub>2</sub> O]Cl	+	++	++	++	++	++	++	++	+++	+++
	+	+	+	+	+	++	++	++	+++	+++
	-	-	-	+	+	+	++	++	++	++
	+	+	+	+	+	+	++	++	++	+++
[Zn LC <sub>2</sub> ]	+	+	+	+	++	++	++	+++	+++	+++
	+	+	+	++	++	++	++	++	++	+++
	-	-	-	-	+	+	++	++	++	++
	+	+	+	+	+	++	++	++	++	+++
Negative Control(DMF)					Nil					
Standard(mm)Positive control					a-25,b-26,c-22,d-29,e-27					

(a) Escherichia coli (b); S. aureus; (c) Bacillus subtilis; (d) S. typhi; (e) Pseudomonas aeruginosa ( $\mu\text{g/ml}$ ). DMF(control): Nil, No effect up to 24 hours. Key for interpretation: a) - : Inactive Less than 10 mm; b) + Weakly active between 10 – 14 mm. c) ++ : Moderately active between 15 – 17 mm and d) +++: Highly active above 18 mm

**Table 3.** Anti fungal activity measurements of the ligand and its complexes[Concentration(( $\mu\text{g/ml}$ )]

Compound	50	100	150	200	250	300	350	400	450	500
$\text{C}_{15}\text{H}_{10}\text{N}_4\text{O}_5(\text{L})$ a,b,c↓	+	+	+	+	++	++	++	++	++	++
	+	+	+	+	++	++	++	++	++	++
	+	+	+	+	++	++	++	++	++	++
[CuLCIH <sub>2</sub> O]Cl	-	+	+	+	+	++	+++	+++	+++	+++
	-	+	+	+	+	++	+++	+++	+++	+++
	-	+	+	+	+	++	+++	+++	+++	+++
[Co LC <sub>2</sub> ]	-	+	+	+	+	++	++	+++	+++	+++
	-	+	+	+	+	++	++	+++	+++	+++
	-	+	+	+	+	++	++	+++	+++	+++
[Ni LC <sub>2</sub> H <sub>2</sub> O]Cl	-	-	+	+	+	++	+++	+++	+++	+++
	-	-	+	+	+	++	+++	+++	+++	+++
	-	-	+	+	+	++	+++	+++	+++	+++
[Zn LC <sub>2</sub> ]	-	+	+	+	+	++	+++	+++	+++	+++
	-	+	+	+	+	++	+++	+++	+++	+++
	-	+	+	+	+	++	+++	+++	+++	+++
Negative Control(DMF)					Nil					
Standard(mm)Positive control					a-23,b-21,c-25					

Ni(II) and Zn(II) showed higher activity than the standard.

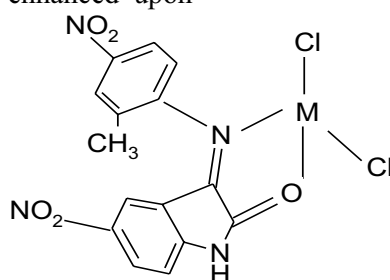
### CONCLUSIONS

The Schiff base acts as a bidentate ligand with azomethine nitrogen and negatively charged oxygen atom as donors. The probable geometry of the structure for the complexes of Co and Zn would be tetrahedral and for those of Cu and Ni - square planar (Figs. 4 and 5, respectively). The antibacterial and antifungal activity of the ligand is greatly enhanced upon complexation with metal ions. Almost all complexes are highly active at higher concentration (500 µg/ml) except a few that are moderately active. The anthelmintic activity of the ligand is greatly enhanced upon

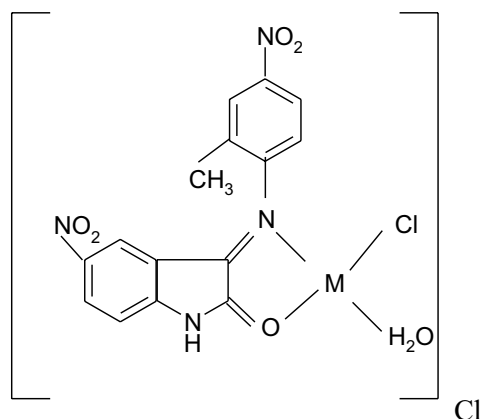
complexation with metal ions, particularly Co, Ni and Zn.

**Table 4.** Anthelmintic activity of the investigated compounds..

Compound	Time taken for paralysis and death of worms	
	Paralysis (min)	Death (min)
Blank Normal Saline	No effect for 10 hours	
Standard (Piperazine citrate)	10	15
C <sub>15</sub> H <sub>10</sub> N <sub>4</sub> O <sub>5</sub> (L)	9	14
[CuLCl.H <sub>2</sub> O]Cl	8	12
[Co LCl <sub>2</sub> ]	11	17
[Ni LCl.H <sub>2</sub> O]Cl	13	20
[Zn LCl <sub>2</sub> ]	21	27



**Fig 4.** Tetrahedral geometry M= Co and Zn.



**Fig. 5.** Square planar geometry. M= Cu and Ni.

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## СИНТЕЗА, ОХАРАКТЕРИЗИРАНЕ, АНТИБАКЕРИАЛНИ, ПРОТИВОГЪБИЧНИ И АНТИХЕЛМИНТНИ СВОЙСТВА НА 5-НИТРОИЗАТИН-ШИФОВА БАЗА И НЕЙНИТЕ КОМПЛЕКСИ

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(Резюме)

Синтезирани и охарактеризирани са комплексите на медта (II), кобалта (II), никела (II) и цинка (II) на 5 – нитроизатонива Шифова база (L) (L= Шифова база получена от 5-нитроизатин и 2-метил-4-нитроанилин). Автентичността на лигандите и тяхниѐ метални комплекси е установена чрез микроанализ, ИЧ-, ЯМР-, ГХ/МС-, UV-VIS-спектроскопии и измервания на електропроводността. Лигандите действат като би-дентатни агенти в които карбонилния кислород и азо-метиновия азот в 5-нитроизатина участват в координирането. За структурата на комплексите на Cu (II) и (II) е установена планарна квадратна геометрия, а за комплексите на Co (II) и Zn (II) – тетраедрична. Лигандите и тяхните комплекси са изпитани за антибактериална активност спрямо *Staphylococcus aureus*, *Escherichia coli* и др., за противогъбични свойства - спрямо *Aspergillus niger*, *Aspergillus flavous* и др. Шифовата база и нейните комплекси са изпитани и за антихелминтни свойства спрямо земни червеи. И двете групи съединения проявяват значителна активност.