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Copper(II), Nickel(II) complexes of N-heteroaromatic hydrazone: Synthesis, Characterization and *in vitro* antimicrobial evaluation

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ABSTRACT

The present paper describes the synthesis, spectral characterization and antimicrobial activity of the Cu(II), Ni(II) complexes prepared with N^1*-(2-hydroxy benzilidine)-2-phenyl isonicotino hydrazide. The complexes were characterized by elemental analysis, Infrared, NMR and Electronic spectra. From the obtained data, the proposed molecular formula of the complexes are* $[MX(H_2L)H_2O]$ *where, M = Cu(II), Ni(II) and X = OAc or Cl. The ligand* (H_2L) *coordinates to the central metal through ketonic oxygen, azomethine nitrogen and phenolic oxygen atoms. The antibacterial and anti fungal activity of the Cu(II), Ni(II) complexes and their parent ligand have been studied in-vitro. The complexes exhibited lethal effect on gram positive, gram negative bacteria and fungi than their parent ligand. It has also been observed that concentration of compounds played a vital role in increasing the degree of inhibition; as the concentration increases, the activity also increased.*

Key words: Isonicotino hydrazide, Tridentate Schiff base, Metal complexes, Biological activity.

INTRODUCTION

Hydrazones are organic compounds characterized by the presence of –NH-N=CH- group in the molecule. Acylhydrazones have an additional donor site like C=O, which provides the versatility and flexibility of these compounds. Hydrazide derivatives represent an overwhelming and rapid developing field in modern medicinal chemistry. Considerable importance has been given to hydrazide derivatives due to their antimicrobial, antitubercular, antitumour, analgesic and antiinflammatory,trypanocidal, leishmanicidal, anti-HIV, anthrax lethal factor inhibitory, antidiabetic and antimalarial properties [1-10]. Furthermore, abilities of aroyl hydrazones to coordinate to metals either in keto or enol tautomeric form make them attractive as ligands. Aroyl hydrazones derived from the condensation of salicylaldehyde and its derivatives with alkyl and aroyl hydrazones play a very important role in coordination chemistry [11-15]. As the salicylaldehyde aroyl hydrazone schiffbase is concerned, it can act as a tridentate ligand both in the monoanionic and dianionic forms and hence can form very stable chelates with metal ions. Hydrazone compounds display a versatile behavior in metal coordination and their biological activity is often increased by bonding to transition metals. For example, the biological activity of d-metal complexes of N-heteroaromatic hydrazones of 2-pyridinecarboxaldehyde is often higher in comparison to the corresponding free ligand, with copper (II) complexes being the most active among all tested complexes [16].

Starting from these premises, this paper presents the synthesis and characterization of new aroyl hydrazone, N^1-(2-hydroxy benzilidine)-2-phenyl isonicotino- hydrazide (L) and studying its ligating behavior by coordinating to Cu(II) and Ni(II) (scheme 1). The study also includes antibacterial and antifungal activity of the free aroyl hydrazone and its metal complexes.

EXPERIMENTAL SECTION

2.1: Materials

All reagents and solvents (Sigma-Aldrich) used were of analytical reagent grade (A.R) and used without further purification. Thermo Nicolet FT-IR, Nicolet-200, USA spectro- photo meter was used to record the IR-spectra (4000-400 cm^{-1}) of the free aroyl hydrazone and its metal complexes as KBr pellets. Elemental analysis was carried out using Perkin-elmer 2400 elemental analyzer and ^{1}H-NMR spectra was recorded on Bruker AMX-300 MHz spectrometer operating at 300MHz. Electronic spectral studies of the compounds was carried out using Schimadzu UV 1800 spectrophotometer.

2.2: Synthesis of ligand (H_2L) (1)

An ethanolic solution of ethyl 2-phenylisonicotinate (0.001 mol.) was taken in a round bottom flask and to this hydrazine hydrate (0.001 mol.) and 2-3 drops of pyridine were added with constant stirring. The resulting mixture was heated under reflux for 2 hrs, colourless solid (2-phenyl isonicotino hydrazide) was formed and it was separated by filtration and dried in vacuum. To the ethanoic solution of 2-phenyl isonicotino hydrazide (0.001 mol.), salicylaldehyde (0.001 mol.) in ethanol was added with constant stirring. The resulting mixture was heated under reflux for 3 hrs. Yellowish product (N'-(2-hydroxybenzylidene)-2-phenylisonicotinohydrazide) was formed (scheme 1) which was separated out by filtration, washed with cold ethanol and dried in vacuum.

Color: Yellow, Yield: 79.11%, Elemental analysis, found (calcd.) for $C_{19}H_{15}O_2N_3$: C, 71.89 (71.91); H, 4.76 (4.83); N, 13.24 (13.36); O, 10.08 (10.14) %. IR data (KBr, cm^{-1}); 3183 ν(O-H), 3041 ν(N-N), 1648 ν(C=O), 1604 ν(C=N), 1542 and 1473 ν(C=C), 1367 ν(C-N). ^{I}H-NMR (DMSO-d6); δ8.70 (1H, s, NCH), δ7.20-8.40 (5H, m, phenyl), δ7.70 (1H, s, pyridine), δ8.85 (1H, d, pyridine), δ7.20 (1H, d, pyridine), δ 11.04(1H, d, OH). Uv/Vis (DMF, nm); 304.50, 291.0 and 242.0. M/z: 317.12.

Scheme 1. Synthesis of N^1-(2-hydroxy benzilidine)-2-phenyl isonicotino hydrazide.

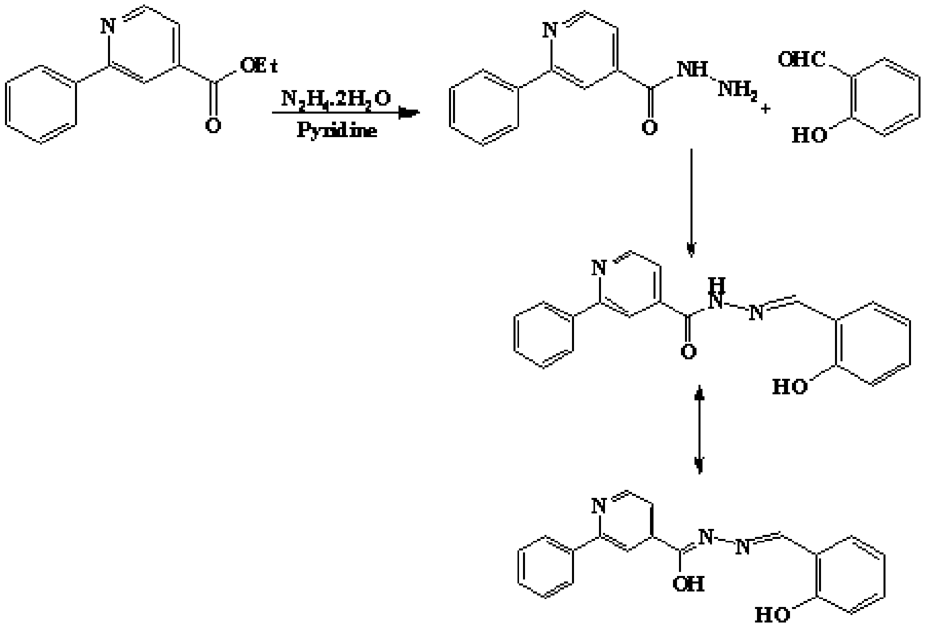


Table: 1 Analytical data and other details of the compounds

Compound	Colour	M.P (^{o}C)	Found (calculated)					Molar conductance ($\Omega^{-1}mol^{-1}cm^2$)
			C	H	O	N	M	
H_2L	Colourless	152	71.89 (71.91)	4.76 (4.83)	10.08 (10.14)	13.24 (13.36)	-	-
$[Cu(H_2L)(OAc)H_2O]$	Bluish green	238	55.32 (55.43)	3.98 (3.84)	17.55 (17.63)	9.22 (9.28)	13.94 (14.08)	18
$[Ni (H_2L)Cl_2 H_2O]$	Pale green	254	49.29 (48.89)	3.27 (3.34)	10.37 (10.24)	9.08 (9.16)	12.68 (12.44)	23

2.3: Synthesis of metal complexes

To the 20 mL of hot ethanolic solution of aroyl hydrazone (1 mmol.) 20 mL of appropriate metal solution (1 mmol.) in the same solvent was added with constant stirring. The reaction mixture was refluxed for 3 hrs resulting in formation of respective metal complexes as coloured precipitates (scheme 2). The coloured complexes obtained were filtered and washed with hexane and dried in vacuum.

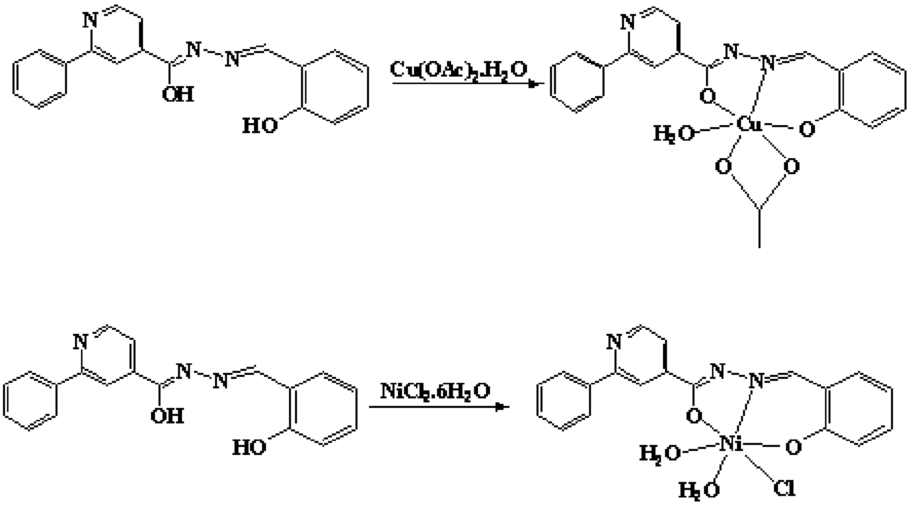
1. $[Cu (H_2L)(OAc)H_2O]$ (2)

Color: dark green, Yield: 76 %, m.p. 238 ^{o}C. Anal.calc. for $C_{21}H_{18}CuO_5N_3$ found (calcd.); C, 55.32 (55.43); H, 3.98 (3.84); N, 9.22 (9.28); O, 17.55 (17.63); Cu, 13.94 (14.08) %. FT-IR (KBr, cm^{-1}); 1605 ν(C=N), 1433 ν(C=C), 1198 ν (C-N), 1147 ν(N-N), 693-957 ν(C-H), 761 ν(Cu-O), 424 ν(Cu-N). Uv/Vis (DMF, nm); 401.00, 312.50, 247.00, 203.50. M/z: 455.05

2. [Ni $(H_2L)Cl_2$ H_2O] (3)

Color: pale green, Yield: 72%, m.p. 254 ^oC. Anal.calc.for $C_{19}H_{15}Cl_2NiO_3N_3$: found (calcd.); C, 49.29 (51.22); H, 3.27 (3.85); N, 9.08 (9.43); O, 14.32 (14.37); Cl, 7.96 (7.89); Ni, 13.21(13.17) %. FT-IR (KBr, cm^{-1}); 1612 ν(C=N), 1435 ν(C=C), 1200 ν(C-N), 1150 ν(N-N), 693-957 ν(C-H), 520 ν(Ni-N), 590 ν(Ni-O). Uv/Vis (DMF, nm); 790.0, 419.0, 376.50, 289.50, 261.0. M/z: 462.98

Scheme 2. Synthesis of Cu and Ni complexes of N^1-(2-hydroxy benzilidine)-2-phenyl Isonicotino hydrazide.



2.4: Antimicrobial activity

Anti bacterial assay

The metal complexes and their parent ligand were tested in vitro against gram positive (Staphylococcus aureus) and gram negative bacteria (E-coli) with different concentrations by disc diffusion technique [17, 18]. 25 mL of sterilized nutrient agar media (NA) was poured in a petri-plate and after solidification 0.1 mL of the test bacteria was spread over the medium using spreader. 63.5, 125,250 µg mL^{-1} concentrations of free ligand and its metal complexes were prepared by dissolving the compounds in DMF. The discs of Whatmann No.1 filter paper with a diameter 5 mm each were soaked in test compound solutions of aforesaid concentrations and placed at four equidistant places at a distance of 2 cm from the centre in the inoculated petriplates. Filter paper disc treated with DMF served as control and streptomycin was used as a standard drug. The petriplates were kept in refrigerator for 24h for pre-diffusion. Finally petriplates were incubated at 30 ^0C for 24h. All the studies were made in duplicate for each of the compound. Average of two independent readings for each compound was recorded.

Fungicidal assay

The preliminary fungitoxicity screening of the compounds with different concentrations was performed in vitro against the test fungi, R. bataticola and A. alternata by food poison technique [19, 20]. Stock solutions of compounds were prepared by dissolving the compounds in DMF. Chlorothalonil was used as standard and DMF served as control. Appropriate quantities of the compounds in DMF were added to potato dextrose agar medium in order to get a concentrations of 63.5, 125, 250 µg mL^{-1} of compound in the medium. The medium was poured in to a set of two petriplates under aseptic conditions in a laminar flow hood and allowed the medium to be solidified. A mycelial disc of 0.5 cm in diameter was cut from the periphery of the 7 days old culture and it was aseptically inoculated upside down in the centre of the petriplates containing the medium. These treated petriplates were incubated at 26 ± 1 ^{0}C until fungal growth in the control petriplates was almost complete. The mycelial growth of the fungi (mm) in each petriplates was measured diametrically and growth inhibition was calculated and the results of the fungicidal screening are listed in table-5.

RESULTS AND DISCUSSION

The scheme of synthesis of hydrazone was shown in scheme 1. Colours, melting points, molar conductivity and elemental analysis of the hydrazone and its complexes are summarized in table-1. The molar conductivity measurements in DMSO, clearly showed that the compounds are non-electrolytes. (E)-N'-(2-hydroxybenzylidene)-2-phenylisonicotinohydrazide was prepared from 2-phenyl isonicotino hydrazide and salicylaldehyde and its metal complexes were prepared as shown scheme 1. All metal complexes are insoluble in common organic solvents and soluble in DMF and DMSO.

3.1: Infrared spectra

The characteristic IR bands of the complexes differed from the free ligand H_2L, and provided significant indications regarding the coordination and bonding sites of the ligand. Relevant characteristic bands of all the compounds are listed in table 2. The strong band at 1604 cm^{-1} due to C=N group of the free ligand is shifted to higher values (1606 cm^{-1}), indicating coordination via azomethine nitrogen in complexes [21]. The azomethine nitrogen coordination is further evidenced by presence of new band at 424 and 520 cm^{-1} assignable to ν(M-N) for the complexes. The spectrum of the free ligand exhibited a broad band at 3183 cm^{-1} which is assigned to the phenolic –OH group. The disappearance of this band in the spectra of complex is an evidence for the ligand coordination around the metal ion in its deprotanated form [22]. This causes ν(C-O) shift to lower frequencies and a new bands in the range 761 and 590 cm^{-1} assigned to ν(M-O) is found in the complex spectra, which confirms the coordination of ligand via phenolic oxygen [23]. The strong band at 1648 cm^{-1} due to ν(C=O) group of the free ligand is shifted to lower values in the complexes, indicating coordination via ketonic oxygen and it is further evidenced by presence of new band at 465 cm^{-1} [24].

Table: 2 Characteristic IR absorption bands (cm^{-1}) of ligand and metal complexes

Compound	ν(C=N)	ν(O-H)	ν(C=O)	ν(M-N)	ν(M-O)	ν(N-N)	ν(C=C)	ν(C-N)
H_2L	1604	3183	1648	-	-	1147	1542, 1473	1367
$[Cu(H_2L)(OAc)H_2O]$	1606	-	1516	424	761	1119	1433	1198
$[Ni (H_2L)Cl_2 H_2O]$	1612	-	1535	520	590	1125	1445	1205

3.2: Uv/Vis spectra

Electronic spectra of the ligand and complexes in DMF solution are presented in table-3. Ligand exhibited intraligand transitions at 291 nm due to π–π* transition and at 304.50 nm due to n–π* transition of azomethine moiety. This band is shifted to higher energy in the complexes. The bands of the Cu(II) complex exhibited bands at 203.5, 247.0, 312.5 and 401.0 nm corresponding to π–π* of aromatic ring, L→Cu charge transfer, azomethine moiety, and d-d transitions respectively. The bands of the Ni(II) complex exhibited bands at 261.0, 289.5, 376.5, and 419 nm corresponding to L→Ni charge transfer, π–π* intraligand, n–π* and d-d transitions.

Table: 3 Electronic spectral data the ligand and metal complexes

Compound	λ max (nm)
H_2L	304.50, 291.00, 242.50
$[Cu (H_2L)(OAc)H_2O]$	401.00, 312.50, 247.00, 203.50
$[Ni (H_2L)Cl_2 H_2O]$	790.00, 419.00, 376.50, 289.50, 261.00

3.3: ^{1}H-NMR-Spectra

The ^{1}H-NMR spectra of ligand showed signals at δ8.70, δ7.20-8.30, δ7.70, δ8.85, δ7.20 and δ 11.26 due to NCH, phenyl, pyridine, and OH protons respectively. There is absence of signal at 11.04 in the complex indicating the involvement of –OH group in the complexation. Ligand exhibited three bands at δ7.20, 7.70 and 8.85 due to the three pyridine protons, which are shifted slightly downfield (δ 6.59-7.64) incase of complexes implying the shift of electron cloud towards the positive metal center [25].

3.4: Molar conductivity measurements

The complexes were dissolved in DMF and the molar conductivities of solutions at 25 ± 2 ^{0}C were measured and presented in table-1. From the results it is concluded that the complexes are found to have molar conductance values of 18-23 $\Omega^{-1}mol^{-1}cm^2$ indicating that these complexes are nonelectrolytes.

3.5: Structural interpretation

Based on the aforesaid IR, NMR and UV-Visible spectral data and elemental analysis the proposed structures shown in scheme 1 and scheme 2 of the H_2L and its Cu(II), Ni(II) complexes are confirmed. It is concluded that H_2L behaves as a mono negative tridentate ligand that coordinated to the metal ions via azomethine nitrogen, phenolic oxygen and ketonic oxygen.

3.6: Antimicrobial Activity

Anti bacterial assay

Anti bacterial activity of the ligand and its Cu(II), Ni(II) complexes against test bacteria S.aureus(Gram +ve) and E-Coli (Gram -ve) (table-4) showed that both Cu(II) and Ni(II) metal complexes of N^1-(2-hydroxy benzilidine)-2-phenyl isonicotino hydrazide are more active than the free ligand [26]. Copper complex of the ligand showed higher activity than the Nickel complex. Since copper salt has antimicrobial action a synergistic effect of ligand and metal acting together may be the reason for higher antibacterial activity of the copper complex. It has also been observed that concentration played a vital role in increasing the degree of inhibition; as the concentration increased, the activity also increased.

Table: 4 Anti bacterial activities of ligand and metal complexes

Compound	Bacteria tested	Diameter (mm) of compounds at concentrations (µg mL^{-1})		
		63.5	125	250
H_2L	*S.aureus*	-	11	16
	E-coli	-	-	10
$[Cu(H_2L)(OAc)H_2O]$	*S.aureus*	11	22	30
	E-coli	-	14	16
$[Ni (H_2L)Cl_2 H_2O]$	*S.aureus*	-	12	18
	E-coli	-	7	12
Streptomycin (standard)	*S.aureus*	14	25	30
	E-coli	16	26	40

Anti Fungicidal assay

Data in table-5 shows that the % inhibition values of the complexes of Cu(II) and Ni(II) on R. bataticola and A. alternatata are higher than that of the corresponding free ligand N^1-(2-hydroxy benzilidine)-2-phenyl isonicotino hydrazide. This indicates that, the complexation process decreased the fungicidal potency on these two tested fungi. The high fungi toxic effect of the complexes than the free ligand can be understood in tern of chelation theory which stated that, upon complexation the polarity of metal ion gets reduced which increases the lipophilicity of the metal complexes facilitating them to cross the cell membrane easily [27].

Table: 5 Fungicidal screening data of the ligand and their corresponding metal complexes

Compound	Fungus tested	Fungal inhibition (%) at concentrations (µg mL^{-1})		
		63.5	125	250
H_2L	*R. bataticola*	20.0	45.5	62.7
	A. alternata	22.6	48.2	56.4
$[Cu(H_2L)(OAc)H_2O]$	*R. bataticola*	32.5	57.4	76.6
	A. alternate	36.4	51.8	63.7
$[Ni (H_2L)Cl_2 H_2O]$	*R. bataticola*	26.2	50.1	65.4
	A. alternate	23.7	52.4	58.6
Chlorothalonil (standard)	*R. bataticola*	49.0	76.6	90.0
	A. alternate	46.0	80.0	98.0

CONCLUSION

A new aroyl hydrazone namely N^1-(2-hydroxy benzilidine)-2-phenyl isonicotino hydrazide and its Cu(II) and Ni(II) complexes were prepared and characterized using elemental analysis, ^1H NMR, FT-IR, Electronic spectra. Spectral data confirmed that ligand coordinated to the metal ion through azomethine nitrogen, phenolic oxygen and ketonic oxygen atoms. Molar conductivity values of the complexes indicated that all the complexes are nonelectrolites. The antibacterial and antifungal studies of the complexes showed significant activity. Copper complex of the ligand showed higher activity than the Nickel complex and free ligand. It has also been observed that concentration of compounds plays a vital role in increasing the degree of inhibition; as the concentration increased, the activity increased.

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