



SYNTHESIS, CHARACTERIZATION AND CHELATING PROPERTIES OF NOVEL AZOPYRAZOLES

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ABSTRACT

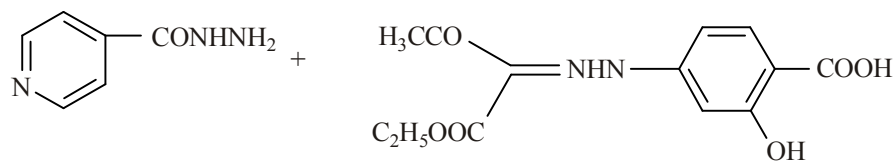
Reaction between isoniazid and 4-[(2E)-2-(1-ethoxy-1,3-dioxobutan-2-ylidene)hydrazinyl]-2-hydroxybenzoic acid in glacial acetic acid furnishes 2-hydroxy-4-{2-[3-methyl-5-oxo-1-(pyridin-4-ylcarbonyl)-1,5-dihydro-4H-pyrazol-4-ylidene]hydrazinyl} benzoic acid (IN-ASA). The transition metal complexes of Cu²⁺, Co²⁺, Ni²⁺, Mn²⁺ and Zn²⁺ of IN-ASA have been prepared and characterized by elemental analyses, spectral studies, magnetic moment determination, molar conductivity measurement and microbicidal activity.

Key words: Isoniazid, 4-[(2E)-2-(1-ethoxy-1, 3-dioxobutan-2-ylidene) hydrazinyl]-2-hydroxybenzoic, Metal chelates, Spectral studies, Magnetic moment, Antibacterial, Antifungal.

INTRODUCTION

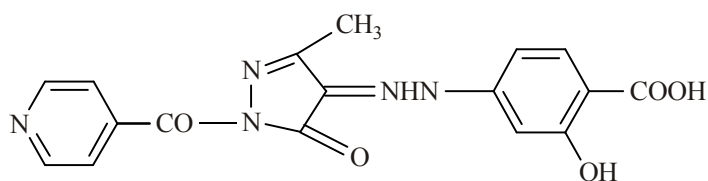
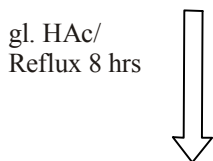
Hydrazones have been demonstrated to possess, among other, antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular and antitumoral activities. Isoniazids are associated with diverse pharmaceutical activities such as antitubercular¹⁻⁸, antibacterial, antiactinomycotic, antitubercular, antidepressant, antiseptic, antimalarial⁹, antimycobacteria¹⁰ effect, antibacterial¹, insecticidal², fungicidal³, antimicrobial⁴, antagonist⁵, anthelmintic⁶⁻⁸, anti-inflamatory⁹ etc. Thus, with a salicylic acid moiety, this may offer good chelating ligand with better microbicidal activity. Hence, the present communication comprises the complexation studies of isoniazide- 4-[(2E)-2-(1-ethoxy-1,3-dioxobutan-2-ylidene)hydrazinyl]-2-hydroxybenzoic acid. The research scheme is given in **Scheme 1**.

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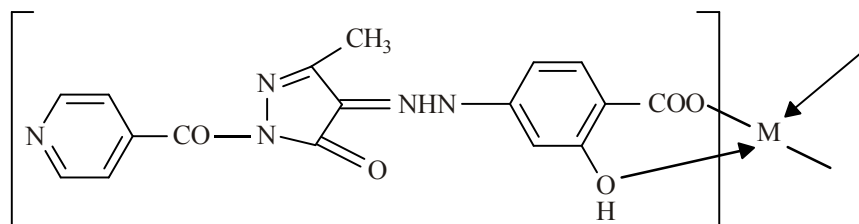
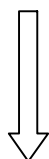
Isoniazid

4-[(2E)-2-(1-ethoxy-1,3-dioxobutan-2-ylidene)hydrazinyl]-2-hydroxybenzoic acid



2-Hydroxy-4-{2-[3-methyl-5-oxo-1-(pyridin-4-ylcarbonyl)-1,5-dihydro-4H-pyrazol-4-ylidene]hydrazinyl}

(IN - ASA)



IN - ASA Metal chelates

Where M : Cu^{2+} , Ni^{2+} , Zn^{2+} , Mn^{2+} and Co^{2+} **Scheme 1**

EXPERIMENTAL

Materials

Isoniazid¹¹ and 4-[(2E)-2-(1-ethoxy-1, 3-dioxobutan-2-ylidene) hydrazinyl]-2-hydroxy benzoic acid¹² were prepared by reported methods. All other chemicals used were of analytical grade.

Synthesis of 2-hydroxy-4-{2-[3-methyl-5-oxo-1-(pyridin-4-ylcarbonyl)-1,5-dihydro-4H-pyrazol-4-ylidene]hydrazinyl}benzoic acid (IN-ASA)

Formation of IN-ASA

A mixture of isoniazide (IN) (0.02 mole) and 4-[(2E)-2-(1-ethoxy-1,3-dioxobutan-2-ylidene)hydrazinyl]-2-hydroxybenzoic acid (ASA) (0.02 mole) in glacial acetic acid (70 mL) was heated under reflux for 8 hours. Subsequently, glacial acetic acid was distilled off and then a lump mass was obtained. It was triturated with petroleum ether (40-60°C). The solid was designated as IN-ASA. It was isolated and dried in air. Yield was 62%. It's melting point was 173-75°C (uncorrected).

Elemental analysis : C₁₇H₁₃N₅O₅ (367.32):

	C %	H %	N %
Calculated:	55.59	3.57	19.07
Found :	55.52	3.50	19.00

Acid value Theoretical: 152.45 mg KOH/1 g. Sample.
 Found: 152.6 KOH/1 g Sample.

IR Features	1630 cm ⁻¹	C=N of pyrazolone
	3030, 1500, 1600 cm ⁻¹	Aromatic.
	1680 cm ⁻¹	CO of COOH
	3200-3600 cm ⁻¹	OH
	2950,1370cm ⁻¹	CH ₃
	1348cm ⁻¹	C-N stretch of pyridine

NMR	δ ppm		
(DMSO)	7.2 – 7.6 (7H)	Multiplet	Aromatic
	1.6 (3H)	Singlet	CH ₃
	10.1 (1H)	Singlet	(COOH)
	3.9 (1H)	Singlet	(OH)
	2.8 (1H)	Singlet	(NH)

Synthesis of metal chelates of IN-ASA

The Cu²⁺, Co²⁺, Ni²⁺, Mn²⁺ and Zn²⁺ metal ion chelates of IN-ASA have been prepared in a similar manner. The procedure is as follows -

To a solution of IN-ASA (36.73 g, 0.1 mole) in ethanol-acetone (1 : 1 v/v) mixture (150 mL), 0.1 N KOH solution was added dropwise with stirring. The pasty precipitates were obtained at neutral pH. These were dissolved by addition of water to make a clear solution and diluted to 250 mL and used as a stock solution. 25 mL of the stock solution containing 0.01 mole IN-ASA was added dropwise to the solution of metal salt (0.005 mole for divalent metal ions in water) at room temperature. Sodium acetate or ammonia was added to complete the precipitation. The precipitates were digested on a water bath at 80°C for 2 hours. The digested precipitates of chelates were filtered, washed with water and air dried. It was amorphous powder. Yield was almost quantitative. The detail are given in Table 1.

Table 1: Analytical data of the metal chelates of HL (IN-ASA)

Compd.	Empirical formula	Mol. Cal. g/mol	Yield (%)	Elemental analysis							
				C %		H%		N%		M%	
				Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
HL (IN-ASA)	C ₁₇ H ₁₃ N ₅ O ₅	367.32	62	55.59	55.52	3.57	3.50	19.07	19.00	-	-
(L) ₂ Cu ²⁺	C ₃₄ H ₂₂ N ₁₀ O ₁₀ Cu ²⁺ 2H ₂ O	830.14	60	49.14	48.98	3.13	3.08	16.86	16.77	7.64	7.60
(L) ₂ Ni ²⁺	C ₃₄ H ₂₂ N ₁₀ O ₁₀ Ni ²⁺ 2H ₂ O	824.69	55	49.47	49.38	3.15	3.11	16.97	16.88	7.12	7.07

Cont...

Compd.	Empirical formula	Mol. Cal. g/mol	Yield (%)	Elemental analysis							
				C %		H%		N%		M%	
				Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
(L) ₂ Co ²⁺	C ₃₄ H ₂₂ N ₁₀ O ₁₀ Co ²⁺ 2H ₂ O	824.93	58	49.45	49.37	3.15	3.12	16.97	16.89	7.14	7.09
(L) ₂ Mn ²⁺	C ₃₄ H ₂₂ N ₁₀ O ₁₀ Mn ²⁺ 2H ₂ O	820.93	59	49.69	49.60	3.16	3.14	17.05	16.98	6.69	6.64
(L) ₂ Zn ²⁺	C ₃₄ H ₂₂ N ₁₀ O ₁₀ Zn ²⁺ 2H ₂ O	831.40	54	49.07	48.98	3.12	3.09	16.84	16.76	7.86	7.81

Measurements

The elemental analysis for C, H and N were carried out on elemental analyzer TF-EA.1101 (Italy). IR spectra of IN-ASA and its metal complexes were scanned on a Nicolet 760 FTIR spectrophotometer in KBr. The NMR spectrum of IN-ASA was scanned on Bruker NMR spectrophotometer using DMSO solvent. The metal content of the metal chelates were determined by decomposing a weighed amount of each metal complex followed by EDTA titration as reported in literature¹².

Table 2: Magnetic moment and reflectance spectral data of metal chelates of IN –ASA ligand

Metal chelate	Magnetic moment μ_{eff} (B.M.)	Molar conductivity $\Omega\text{m}^{-1}\text{cm}^2\text{mol}^{-1}$	Absorption band (cm^{-1})	Transitions
(L) ₂ -Cu ²⁺	1.97	37.3	22690 15864	C.T ${}^2\text{T} \rightarrow {}^2\text{T}_{2\text{g}}$
(L) ₂ -Ni	3.88	36.0	14694 22987	${}^3\text{A}_{2\text{g}} \rightarrow {}^3\text{T}_{1\text{g}}(\text{P})$ ${}^3\text{A}_{1\text{g}} \rightarrow {}^3\text{T}_{1\text{g}}(\text{F})$
(L) ₂ -Co	4.56	35.6	15378 22716	${}^4\text{T}_{1\text{g}}(\text{F}) \rightarrow {}^4\text{T}_{2\text{g}}(\text{F})$ ${}^4\text{T}_{1\text{g}}(\text{F}) \rightarrow {}^3\text{A}_{2\text{g}}$
(L) ₂ -Mn	5.16	37.7	15377 17650 22966	${}^6\text{A}_{1\text{g}} \rightarrow {}^4\text{T}_{1\text{g}}({}^4\text{Eg})$ ${}^6\text{A}_{1\text{g}} \rightarrow {}^4\text{T}_{2\text{g}}({}^4\text{G})$ ${}^6\text{A}_{1\text{g}} \rightarrow {}^4\text{T}_{1\text{g}}({}^4\text{G})$

Zn²⁺ - Complex is diamagnetic in nature.

Magnetic susceptibility measurements of all the metal complexes were carried out at room temperature by the Gouy method. Mercury tetrathiocyanatocobalate (II), Hg [Co (NCS)₄], was used as a calibrant. The diffused reflectance spectra of solid metal complexes were recorded on a Backman DK spectrophotometer with a solid reflectance attachment; MgO was employed as the reflectance compound. The electrical conductivity of all the complexes was measured in acetonitrile at 10⁻³ M concentration. All these analyses are given in Table 2.

Antifungal activity

The fungicidal activities of all the compounds were studied at 1000 ppm concentration *in vitro* against plant pathogenic organisms and the results are listed in Table 3. The antifungal activities of all the samples were measured by cup plate method¹³. Each of the plant pathogenic strains were grown on potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200 g, dextrose 20 g, agar 20 g and water 1 litre. 5 days old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120° C for 15 min at 15 atm pressure. These media were poured into sterile petri plate and the organisms were inoculated after cooling the petri plate. The percentage inhibition for fungi was calculated after 5 days using the formula -

$$\text{Percentage of inhibition} = \frac{100(x - y)}{x} \quad \dots(1)$$

where, x = Area of colony in control plate and y = Area of colony in test plate.

The fungicidal activities of all the compounds are reported in Table 3.

Table 3: Antifungal activity of ligand IN-ASA and its metal chelates

Sample	Zone of inhibition of fungus at 1000 ppm (%)						
	PE	BT	N	T	RN	AN	TL
IN-ASA	63	63	65	65	68	60	63
(L) ₂ -Cu ²⁺	75	82	77	83	78	73	72
(L) ₂ -Ni ²⁺	72	80	75	80	76	76	76

Cont...

Sample	Zone of inhibition of fungus at 1000 ppm (%)						
	PE	BT	N	T	RN	AN	TL
(L) ₂ -Co ²⁺	75	75	73	76	75	75	73
(L) ₂ -Mn ²⁺	66	74	70	72	73	71	72
(L) ₂ -Zn ²⁺	71	79	73	78	73	70	73

PE = *Penicillium expansum*

BT = *Botrydepladia thiobromine*

N = *Nigrospra sp.*

T = *Trichothesium sp.*

RN = *Rhizopus nigricans*

AN = *Aspergillus niger.*

TL = *Trichoderma lignorum*

RESULTS AND DISCUSSION

The parent ligand IN-ASA was an amorphous brown powder, soluble in various solvents like dioxane, DMSO and DMF. The results of elemental analysis of the ligand are reported earlier. They are consistent with the predicted structure as shown in **Scheme 1**.

Examination of IR spectrum (not shown) of IN-ASA reveals that broad band of phenolic hydroxyl stretching was observed at 3200-3600 cm⁻¹ as well as additional absorption bands at 3030, 1500 and 1600 cm⁻¹ were characteristics of the salicylic acid. The strong bands at 1680 cm⁻¹ are assigned to C=O. The NMR data also confirm the structure of IN-ASA.

The metal chelate of IN-ASA with ions Cu²⁺, Co²⁺, Ni²⁺, Mn²⁺ and Zn²⁺ vary in colours. On the basis of the proposed structure in **Scheme 1**, the molecular formula of the IN-ASA ligand is C₁₇H₁₃N₅O₅. This upon complexation coordinates with one central metal atom at four coordination sites and with two water molecules. Therefore, the general molecular formula of the resulting metal chelate is [C₁₇H₁₁O₅N₅]₂M.2H₂O for divalent metal ions. This has been supported by results of elemental analysis reported in Table 1. The data are in agreement with the calculated values.

Inspection of the IR spectra (not shown) of metal chelates reveals that all the spectra are identical in all respects. The comparison of IR spectrum of the parent ligand IN-ASA with that of its each metal chelates has revealed certain characteristics differences.

One of the significant differences to be expected between the IR spectrum of the parent ligand and its metal chelates is the presence of more broadened bands in the region of 3200-3600 cm^{-1} for the metal chelates, as the oxygen of the O-H group of the ligands forms a coordination bond with the metal ions^{14,15}. Another noticeable difference is the bands due to the COO^- anion at 1600 cm^{-1} in the IR spectrum of the each metal chelates. The band at 1400 cm^{-1} in the IR Spectrum of HL assigned to in-plane OH. Absorption¹⁴⁻¹⁶ is shifted towards higher frequency in the spectra, which is confirmed by a weak bands at 1105 cm^{-1} corresponding to C-O-M stretching¹⁴⁻¹⁶. Thus, all of these characteristics features of the IR studies suggested the structure of the metal chelates.

Examination of data of the metal contents in each compound revealed a 1 : 2 metal : ligand (M : L) stoichiometry in all the chelates of divalent metal ions.

Magnetic moments (μ_{eff}) of the metal chelates are given in Table 2. Examination of these data reveals that all chelates other than that of Zn^{2+} , are paramagnetic in nature while that of Zn^{2+} is diamagnetic.

The diffused electronic spectrum of the $[\text{Cu}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ metal complex shows broad bands at 15864 and 22690 cm^{-1} due to the ${}^2\text{T} \rightarrow {}^2\text{T}_{2g}$ transition and charge transfer, respectively suggesting a distorted octahedral structure¹⁷⁻¹⁹ for the $[\text{Cu}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ complex, which is further confirmed by the higher value of μ_{eff} of the $[\text{Cu}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ complex. The $[\text{Ni}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ and $[\text{Cu}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ complexes gave two absorption bands respectively at 14694; 22987 and 15864; 22690 cm^{-1} corresponding to ${}^4\text{T}_{1g} \rightarrow {}^2\text{T}_{1g}$ and ${}^4\text{T}_{1g}(\text{p})$ transitions. Thus, the diffused absorption bands in the reflectance spectra and the value of the magnetic moments μ_{eff} indicate an octahedral configuration for the $[\text{Ni}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ and $[\text{Cu}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ complexes. The spectra of $[\text{Mn}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ shows weak bands at 15377, 17650 and 22966 cm^{-1} , which are assigned to the transitions ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}({}^4\text{G})$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}({}^4\text{G})$ and ${}^6\text{A}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}$, respectively, suggesting an octahedral structure for the $[\text{Mn}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ chelate. As the spectrum of the $[\text{Zn}(\text{IN-ASA})_2(\text{H}_2\text{O})_2]$ chelate is not well resolved, it is not interpreted but its μ_{eff} value shows that it is diamagnetic in behaviour as expected.

Conductivities of all the complexes were measured in acetonitrile solvent and all the complexes were found to be electrolytic²⁰ in nature of 1 : 2 type and molar conductivity values are in the range of 36.0-39.3 $\text{Ohm}^{-1} \text{cm}^{-1}$.

The antifungal activities of all the compounds were measured for various plant pathogens. Inspection of the results (Table 3) indicates that all compounds are toxic against

fungi. Out of all the compounds, copper chelate was more toxic than other. These compounds almost inhibit the growth fungi by about 70%. Hence, these metal chelates can be employed as garden fungicides. Further work in this direction is in progress.

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