

U.G.C. MINOR RESEARCH PROJECT

[FINAL REPORT]

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Title of project: **Synthesis, Characterization and antimicrobial activities of Novel hetero Cyclic Ligand**

Objectives of the project:

1. To synthesis 1,3,4-Oxadiazole derivatives.
2. To synthesis and characterization of 1,3,4-Oxadiazole p-amino salicylic acid clubbed molecules.
3. To monitor antimicrobial activity.

Work done so far:

We purchased all chemicals from local dealer and all chemicals were analytical grade. In initial stage we synthesized 5-(4-Pyridinyl)1,3,4-Oxadiazole-2-thione(POT). The method is as follows.

Isoniazide was dissolved in ethanol and equimolar quantities of carbon disulfide and potassium hydroxide were added to this solution. The contents were refluxed for 4 hours. Distilled water was then added followed by neutralization with dilute HCl. A solid mass was filtered and crystallized from methanol. The product was bright yellow crystals. M.P. 260⁰ C.

In second stage, we synthesized 3-(4-Carboxy-3-hydroxy phenyl-amino methyl)-5-(4-Pyridinyl) 1,3,4-Oxadiazole-2-thione(PCHAOT). The method is as follows:

A mixture of POT, Formaldehyde and 4-Amino salicylic acid (PAS) in ethanol was refluxed for 4 hours. Then ethanol was distilled off and pasty mass obtained. It was titrated with petroleum ether (40-60⁰ C). The solid designated as PCHAOT was isolated and dried in air. Yield was 70%. M.P. was 151⁰ C.

There after we examined its elemental analysis, IR and NMR features. The work mentioned above already completed.

Elemental Analysis

C₁₈H₁₈N₄O₄S : M.W.= 386 gm/mole

	C%	H%	N%
Calculated	55.95	4.65	14.55

Found	55.90	4.5	14.0
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Acid Value:

Theoretical: 165 mg KOH/1g. Sample

Found : 160 mg KOH/1g. Sample

IR Features:

1480-1520 cm^{-1}	1,3,4, Oxadiazole
3030,1500,1600 cm^{-1}	Aromatic
1680 cm^{-1}	CO of COOH
3200-3600 cm^{-1}	OH
3340 cm^{-1}	Ter.NH-
2850-2920 cm^{-1}	CH ₂

NMR :

(DMSO)

7.2 –7.6 ppm	Multiplate aromatic
2.56 ppm (1H)	Singlet CH ₂
12.2 ppm (1H)	Singlet (COOH)
3.9 ppm (1H)	Singlet (OH)
9.1ppm (1H)	Singlet (NH)
1.2ppm (6H)	Doublet (CH ₃)
2.97ppm (2H)	Multiplate (CH)

Synthesis of metal complexes of PCHAOT:

Formation of CHPOT-metal complexes:

The Cu²⁺, Co²⁺, Ni²⁺ and Zn²⁺ metal ion complexes of PCHAOT have been prepared in similar manner. The procedure is as follow:

To a solution of PCHAOT (0.1mole) in ethanol-acetone (1:1) mixture (150ml), 0.1N

KOH solution was added drop wise with stirring. The sticky precipitates were obtained at neutral P^H. These were dissolved by addition of water up to clear solution. It was diluted to 250ml. by water and was known as stock solution. 25ml of the stock solution (which contains 0.01 mole(PCHAOT) was added drop wise to the solution of metal salt (0.005 mole for divalent metal ions) in water at room temperature.

Sodium acetate or ammonia was added up to complete precipitation. The precipitates were digested on water bath at 80⁰ C for 2 hrs. The digested precipitates of complex were filtered washed with water and air-dried. It was amorphous powder. Yield was almost quantitative. The details are given in Table-1.

Measurement:-

The elemental analysis for C, H, and N were carried out on Thermofinigen Flash 1101 elemental analyzer. IR spectra of PCHAOT and its metal complexes were scanned on a Nicolet 760 FTIR spectrophotometer in KBr. The metal content of the metal complexes were performed by decomposing a weighed amount of each metal complex followed by EDTA titration as reported in literature [11]. Magnetic susceptibility measurement of the entire metal complex was carried out at room temperature by the Gouy method. Mercury tetrathiocyanatocobaltate(II). Hg [Co (NCS)₄] was used as a calibrant. The diffused reflectance spectra of solid metal complex were recorded on Beckman DK Spectrophotometer with solid reflectance attachments, MgO was employed as the reflectance compound.

Anti-fungal activity:-

The fungicidal activity of all compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms listed in Table-3 were used. The anti-fungal activity of all the samples was measured by cup plate method [12]. Each of the plant pathogenic strains on a potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200gms, dextrose 20gms, agar 20gms and water suspended (1000ppm)

in a PDA medium and autoclaved at 120 °C for 15 min. at 15 atm pressure. These medium were poured into sterile Petri plate and the organisms were inoculated after cooling the petri plates. The percentage inhibition for fungi was calculated after 5 days using the formula given below.

$$\text{Percentage of Inhibition} = \frac{100(X-Y)}{X}$$

Where X: Area of colony in control plate

Y: Area of colony in test plate

The fungicidal activity all compounds is shown in Table –3.

Result and discussion:

The parent ligand PCHAOT was an amorphous yellow powder, soluble in various solvents like dioxane, DMSO and DMF. The results of elemental analysis of the ligand reported in Table–I are consistent with the predicted structure as shown in Scheme-1. The ligand was synthesized as shown in the scheme-1.

Examination of IR spectrum (not shown) of PCHAOT reveals that a broad band of phenolic hydroxyl stretching is observed at 3200-3600 cm⁻¹ as well as additional absorption bands at 3030, 1500 and 1600 are characteristics of the salicylic acid[13,14].

The strong band at 1680 for C=O and band at 3400 for ter.N. The NMR data (shown in experimental part) also confirm the structure of PCHAOT.

The metal complexes of PCHAOT with the metal ions Cu²⁺, Co²⁺, Ni²⁺, and Zn²⁺ vary in colors.

On the basis of the proposed structure as shown in scheme-1.

The molecular formula of the PCHAOT ligand is C₁₈H₁₈N₄O₄S. Which upon complexation coordinates with one central PCHAOT atom at four coordination sites and with two water molecules. Therefore the general molecular formula of the resulting metal complex [C₁₈H₁₈N₄O₄S] M.2H₂O for divalent metal ions. This has been confirmed by results of elemental analysis of all the seven metal complexes and their parent ligand. The data of

elemental analysis reported in Table-1 are in arrangement with the calculated values of C, H, and N based on the above mentioned molecular formula of parent ligand as well as metal complexes..

Inspection of the IR Spectra (not shown) of metal complexes reveals that all the spectra are identical in all respects. The comparison of IR spectrum of the parent ligand PCHAOT with that of its each metal complex has revealed certain characteristics differences.

One of the significant difference to be expected between the IR spectrum of the parent ligand and its metal complexes is the presence of more broadened bands in the region of 3200-3600 cm^{-1} for the metal complexes as the oxygen of the O-H group of the ligand forms a coordination bond with the metal ions. [13-15]. This is explained by the fact that water molecules might have strongly absorbed to the metal complexes sample during their formation. Another noticeable is that the band due to the COO anion at 1600 cm^{-1} in the IR spectrum of each metal complex. The band at 1400 cm^{-1} in the IR spectrum of HL assigned to in plane of OH determination [13-15] is shifted towards higher frequency at the spectra of the metal complexes due to the formation of metal oxygen bonds. This has been further confirmed by a weak band at 1105 cm^{-1} corresponding to C-O-M stretching [12]. Thus all of these characteristics features of the IR studies suggested the structure of the metal complexes as shown in scheme.

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

Magnetic moment (μ_{eff}) each of the metal complex is given in Table-2.

Examination of these data reveals that all complexes other than that of Zn^{2+} are para-magnetic while those of Zn^{2+} diamagnetic.

The diffuse electronic spectrum of the $[\text{Cu HL}(\text{H}_2\text{O})_2]$ metal complexes shows broad bands at 15873 and 24691 cm^{-1} due to the ${}^2\text{T} \rightarrow {}^2\text{T}_{2g}$ transition and charge transfer, respectively suggesting a distorted octahedral structure [16-18] for the $[\text{CuHL}(\text{H}_2\text{O})_2]$ complex.

Which is further confirmed by the higher value of μ_{eff} of the $[\text{CuHL}(\text{H}_2\text{O})_2]$ complex. The

$[\text{NiHL}(\text{H}_2\text{O})_2]$ and $[\text{Cu HL}(\text{H}_2\text{O})_2]$ complexes gave two absorption bands respectively

at 14990,23568 and 15888,23859 cm^{-1} corresponding to ${}^4\text{T}_{1g} \rightarrow {}^2\text{T}_{1g}$ and ${}^4\text{T}_{1g} (\text{p})$ transitions. Thus absorption bands of the diffuse, reflectance spectra and the value of the magnetic moments

(μ_{eff}) indicate and octahedral configuration for the $[\text{NiHL}(\text{H}_2\text{O})_2]$ and $[\text{Cu HL}(\text{H}_2\text{O})_2]$ complexes.

As the spectrum of the $[\text{Zn HL}(\text{H}_2\text{O})_2]$ polymer is not well resolved, it is not interpreted but its μ_{eff} value shows that it is diamagnetic as expected.

The antifungal activity of all the samples measured for various plant pathogens.

Inspection of the result shown in Tabel-3 indicates that all compounds are good toxic for fungi. Out of all the compounds copper complex is more toxic than others. These compounds almost inhibit the fungi about 70%. Hence produced metal complexes can be employed as garden fungicides. Further work in direction is in progress.

Scheme:

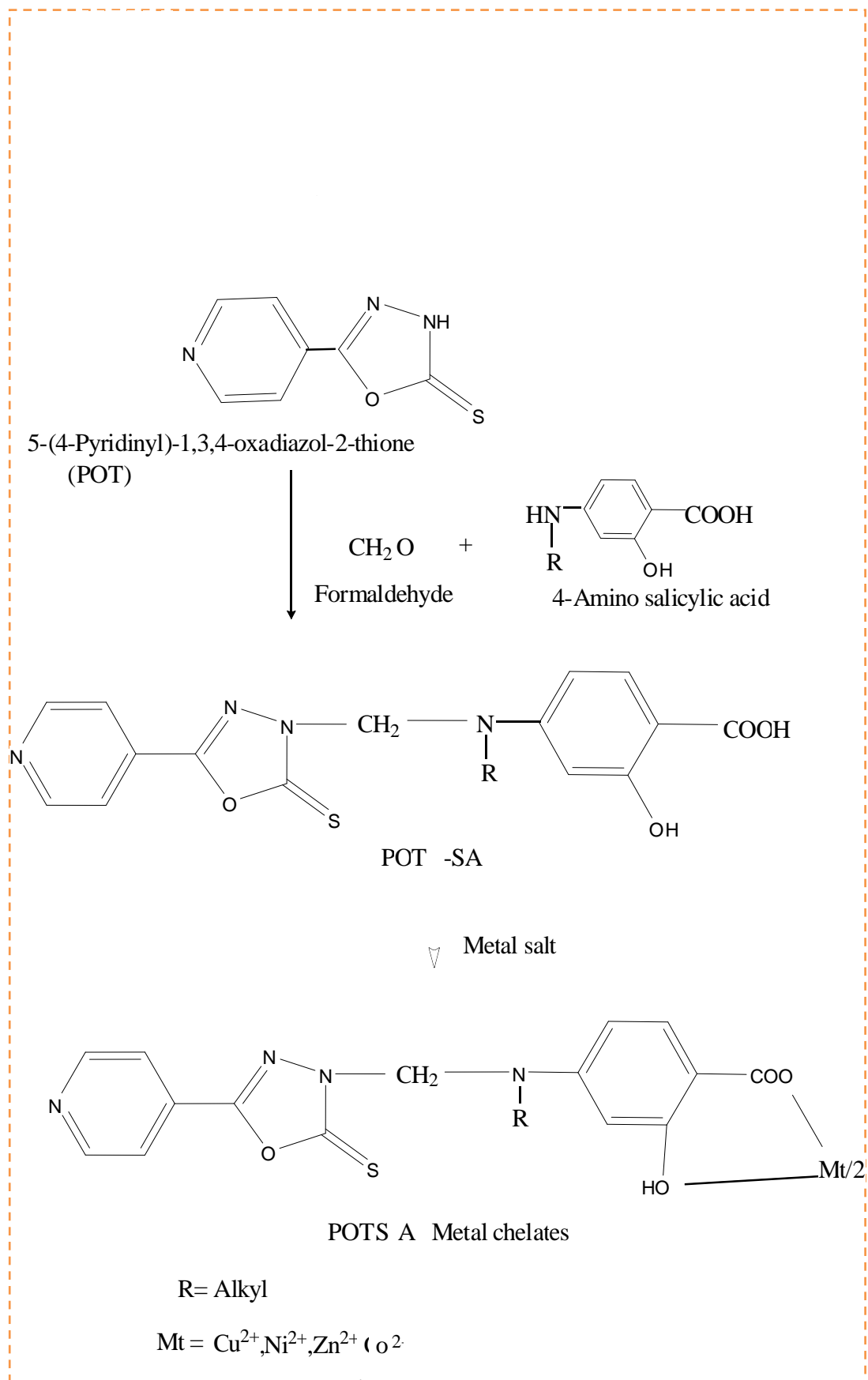


Table 1. Analytical and spectral data of PCHAOT ligand and its metal chelates.

Metal complexes	Molecular formula	Mol.Wt. gm/mol	Yield %	Elemental analysis.								%Metal analysis	
				C%		H%		N%		S%		Cald.	Found
				Cald.	Found	Cald.	Found	Cald.	Found	Cald.	Found		
(HL) ₂ Cu ²⁺	C ₃₆ H ₃₄ N ₈ O ₈ S ₂ Cu ²⁺ ·2H ₂ O	869.54	57	49.67	49.5	4.36	4.26	12.88	12.8	3.68	3.62	7.30	7.23
(HL) ₂ Ni ²⁺	C ₃₆ H ₃₄ N ₈ O ₈ S ₂ Ni ²⁺ ·2H ₂ O	864.69	63	49.96	49.7	4.39	4.31	12.94	12.8	3.71	3.63	6.77	6.72
(HL) ₂ Co ²⁺	C ₃₆ H ₃₄ N ₈ O ₈ S ₂ Co ²⁺ ·2H ₂ O	864.93	69	49.94	49.7	4.39	4.32	12.95	12.8	3.71	3.65	6.81	6.72
(HL) ₂ Zn ²⁺	C ₃₆ H ₃₄ N ₈ O ₈ S ₂ Zn ²⁺ 2H ₂ O	871.39	71	49.57	49.5	4.36	4.26	12.86	12.7	3.68	3.61	7.50	7.41

Table 2 : Reflectance Spectral Data of ML, Chelates (cm⁻¹).

Metal chelates	Absorption band(Cm ⁻¹)	Transitions	Magnetic moment(BM)
Cu- (PCHAOT) ₂ ·2H ₂ O	23,859	C.T	1.94
	15,888	² B _{1g} → ² A _{1g}	
Ni- (PCHAOT) ₂ ·2H ₂ O	23,568	³ A _{2g} → ³ T _{1g} (p)	2.92
	14,989	³ A _{1g} → ³ T _{1g} (f)	
Co- (PCHAOT) ₂ ·2H ₂ O	24,156	⁶ A _{1g} (f) → ⁴ T _{2g} (f)	3.94
	18,523	⁶ A _{1g} (f) → ⁴ T _{2g}	
	7,999	⁶ A _{1g} (f) → ⁴ T _{2g} (p)	
Zn-(PCHAOT) ₂ ·2H ₂ O	-	--	

Zn-(PCHAOT)₂.2H₂O is diamagnetic in nature.

Table-3 Antifungal activity of Ligand PCHAOT and its Metal complexes

Sample	Zone of inhibition of fungus at 1000 ppm(%)					
	Botrydepladia thiobromine	Nigrospora Sp.	Rhizopus Nigricans	Aeperginus niger	Amdida Albicans	Amdida Kruseigos candida glabrata405
PCHAOT	56	56	71	73	66	60
PCHAOTCo ²⁺	73	77	74	65	65	75
PCHAOTNi ²⁺	72	75	70	74	70	66
PCHAOTCu ²⁺	60	79	80	80	79	75
PCHAOTZn ²⁺	64	75	67	75	74	80

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