



## SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF HETEROCYCLIC 1,3,4 THIADIAZOLE DERIVATIVES AND IT'S METAL COMPLEXES

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**Abstract:** A Novel heterocyclic 1,3,4 Thiadiazole derivatives and it's metal complexes of Fe(III),Co(II) have been Synthesized successfully in a alcoholic medium. The synthesized derivatives as a ligand were confirmed by IR,Uv-Vis, <sup>1</sup>HNMR, <sup>13</sup>CNMR and Mass spectral analysis, Molar Conductance and elemental analysis whereas, Metal complexes is analyzed by IR,Uv-Vis,Magnetic susceptibility, Molar Conductance, elemental analysis. The ligand and its metal complexes show moderate to excellent antifungal activity against *A. Niger* and *F. Oxysporum* and antibacterial activity against *S. aureus* and *B.subtilis* using Kirby-Bauer disc diffusion method.

**Keywords:** Thiadiazole, Heterocyclic ligand, Metal complexes, Antifungal activity.

### Introduction

Nowadays substituted 1,3,4-thiadiazole is having important application in pharmaceutical field due to these researchers having more attention towards these moieties which exhibit =N-C-S- moiety<sup>i</sup> The aromatic ring of 1,3,4 thiadiazole derivatives increases the biological activities also five membered ring system stability in vivo increases the less toxicity in higher vertebrates and human beings<sup>ii</sup>. The substitution of -CH=CH- by -S in pyridazine is bioisostere of 1,3,4-thiadiazole<sup>iii-iv</sup>.The biosteric replacement of ring with another ring increases the lipophilicity and pharmaceutical properties. Due to more oral absorption and good cell permeability. The interaction with the receptor increases because of substitution of homocyclic ring to heterocyclic ring for the synthesis of different compounds<sup>v</sup> 1,3,4-Thiadiazole derivatives are important building blocks in medicinal and biological chemistry efforts<sup>vi-vii</sup>.1,3,4-Thiadiazoles to triazoles and pyrazoles may lead to the assumption that thiadiazoles display similar coordination behaviors towards metal ions. However, the introduction of a sulfur atom to the five membered diazole ring influences the geometry and electronic properties of the molecule and thus changes metal ion coordination behaviors. Although 1,3,4-thiadiazoles have a set of three potential donor atoms (NNS), coordination via the sulfur atom has rarely been observed<sup>viii</sup>. The use of 1,3,4-thiadiazoles proves beneficial for the preparation of complexes with unsymmetrical chelating ligands<sup>ix</sup>1,3, 4 thiadiazole also

acts as ligands, increases biological activity by forming complex<sup>x</sup> During last few years more focus on different Thiadiazole derivative because of their important biological properties such as anticancer<sup>xi</sup>, anti-inflammatory<sup>xii</sup>, analgesic<sup>xiii</sup>, antituberculosis<sup>xiv</sup>, antihypertensive<sup>xv</sup>, Antimicrobial<sup>xvi</sup>, anticonvulsants<sup>xvii</sup>, Antioxidant<sup>xviii</sup>.

## Materials and methods:

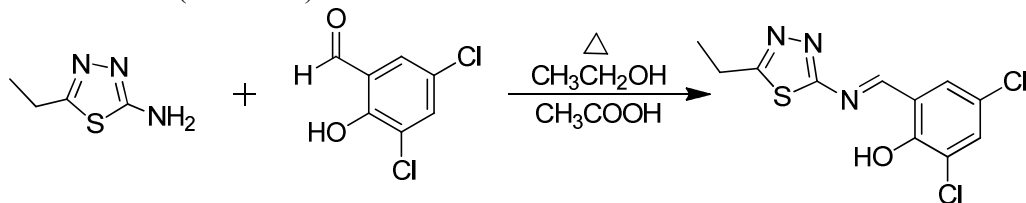
### Experimental

All the chemical of analytical grade. All salts are metal nitrates i.e.  $\text{CO}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (Sigma-Aldrich) were purchased from Sigma-Aldrich and used without further purification. 3,5-dichloro-2-hydroxybenzaldehyde and 5-ethyl-1,3,4-thiadiazol-2-amine from Sigma-Aldrich and Alfa Aesar used without further purification. Distilled Ethanol used for synthesis of metal complexes and ligand diethyl ether (Sigma-Aldrich). IR Spectra recorded on Perkin Elmer Spectrometer in range 4000-400  $\text{cm}^{-1}$  KBr pellets.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Spectra were recorded on BRUKER AVANCE III HD NMR 500 MHz spectrophotometer. Room Temperature magnetic moments by Guoy's method in B.M. Electronic Spectra using DMSO on Varian Carry 5000 Spectrometer. Molar Conductance measurements in dry DMSO having  $1 \times 10^{-3}$  concentration on Systronics conductivity bridge at room temperature. Elemental analysis (C, H, N) were carried out by using perkin Elmer 2400 elemental analyzer. Mass Spectra were recorded on Bruker IMPACT HD. Thermo gravimetric analysis is a technique in which mass of the compound measured with respect to time as the temperature changes in an atmosphere of nitrogen, helium or in vacuum. TGA can be used to evaluate the thermal stability of metal complexes. The temperature range usually selected  $25^\circ\text{C}$  to  $1000^\circ\text{C}$  for metal complex.

### Biological Activity:

Schiff Base and their metal complexes evaluated in vitro their antibacterial activity against two Gram-Positive bacteria, viz, *B. Subtilis*; *S. aureus*, Two fungal strains *A. niger* and *F. oxysporum* by Kirby-Bauer disc diffusion method. The fungal and bacterial strains sub-cultured on PDA and Nutrient Agar. The stock solution ( $1 \text{ mg mL}^{-1}$ ) was pre-prepared in DMSO solution. The stock solution again diluted by using sterilized water to dilution in 500 ppm. The bacteria were subculture in agar medium and disc were kept incubated for  $37^\circ\text{C}$  at 24 hrs. The standard antibacterial drug Miconazole and Ciprofloxacin was also screen under same condition for comparison. Activity was measure and calculated by zone of inhibition (mm) surrounding discs. The experimental value compare with standard drug value Miconazole for the Antifungal activity and Ciprofloxacin for the antibacterial activity.

**Synthesis of Schiff base Ligand:** The mixture of 1:1 3,5-dichloro-2-hydroxybenzaldehyde (1.91g, 0.01mol) with 5-ethyl-1,3,4-thiadiazole-2-thiol (1.29g, 0.01 mol) dissolved in ethanol. Then add Few drops of glacial acetic acid was added. The resultant mixture stirred for 3-4 hrs the colored precipitate of Ligands was obtained. Then wash with Ethanol recrystallized with Ethanol and Ether then dried in air. The purity of compound was checked by TLC using Silica Gel method (Scheme.1)

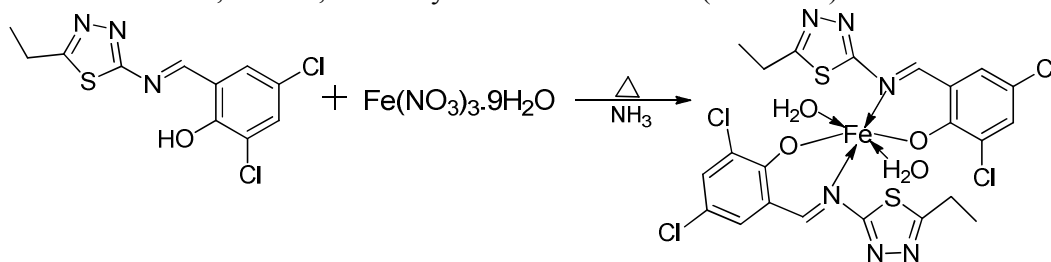


5-ethyl-1,3,4-thiadiazol-2-amine 3,5-dichloro-2-hydroxybenzaldehyde 2,4-dichloro-6-((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methylphenol

### Scheme.1 Synthesis of ligands

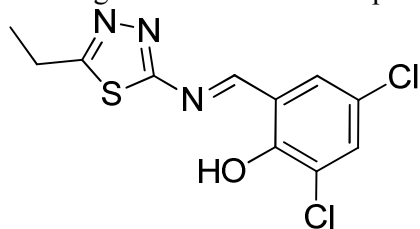
**Synthesis of Metal Complexes:** The metal complexes were prepared by mixing of  $\text{CO}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  with (30 ml) ethanolic solution of Ligand in (metal: ligand)

1:2 ratio. The resulting mixture refluxed on water bath for 5-6hr. A colored product obtain washed with ethanol, filtered, and recrystallised with ethanol (Scheme.2).

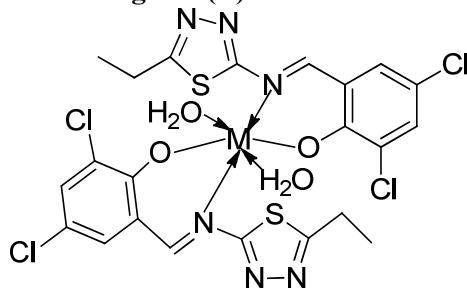


**Scheme 2.**Synthesis of metal complexes

**Results and Discussion:** The ligand (Fig.1) and its transition metal complexes (Fig.2) of 2,4-dichloro-6-(((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol are stable at room temperature in solid state. The ligand is soluble in organic solvent DMSO, DMF and a metal complex is easily soluble in DMSO. The synthesized complexes having 1:2 metal to ligand stoichiometric ratio. The structure data of Ligands and its metal complexes as shown in Table 1. Spectral data shows formation of ligand and its metal complexes.



**Figure1:** Structure of Schiff base Ligands(L).



**Figure 2:** Proposed Structures of metal complexes M: Fe (III),Co (II).

Entry	Products (3)	Time (h)	Yield (%)	M.P (°C)
L	 L	4-5	69	150

MC <sub>1</sub>		1-2	70	>300
MC <sub>2</sub>		1-2	73	>300

Table 1. Structure of Ligands and Metal Complexes

**Characterization data of Ligands 2,4-dichloro-6-((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol (Table.1,L)** :Light Yellow;M.F. C<sub>11</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>OS;Yield : 69% ; M.P.150°C;Molar Cond.(DMSO 1×10<sup>-3</sup>conc., ohm<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>):9.02; UV(DMSO, cm<sup>-1</sup>): 284 (π→π\* tran. of benzene ring), 366 (n→π\* azomethine moieties and phenolic -OH.); IR(KBr Cm<sup>-1</sup>) : ν =3321 (O-H str. in aromatic ring),ν= 1652 (C=N azomethine), ν=1488 (-C=N-N=C str.in Thiadiazole ring), ν=1259 (C-O Phenolic), ν=1028 (N-N Thiadiazole ring), ν=754 (C-S-C str.in thiadiazole ring) ; <sup>1</sup>H NMR (400 MHZ.DMSO d<sub>6</sub>) δ ppm: δ = 11.14(s,1H,Ar-OH), δ =9.21 (s,1H,CH=N), δ = 7.09-7.92( m,2H,Ar-CH), δ =1.19 (t,3H -CH<sub>3</sub>), δ =3.11 (q,2H -CH<sub>2</sub>-).<sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 400 MHZ,) δ ppm : δ =150.2-121.1 (C1-C6 Aromatic), δ =161.1 (C7,-C=N- Azomethine), δ =155.2 (C8 Thiadiazole ring), δ = 168.6 (C9 Thiadiazole ring), δ = 24.3 (C10 -CH<sub>2</sub>-), δ = 14.2 (C11 -CH<sub>3</sub>);MS (70 eV) m/z :302.18 [M+H,100%],Anal.Calcd.For C<sub>11</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>OS :C, 43.72; H, 3.00;N, 13.91; O, 5.29; S, 10.61.Found : C, 43.02; H, 2.85; N, 13.75; O, 5.25; S, 10.55.

**Bis(2,4-dichloro-6-((E)-((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenoxy)iron dihydrate (Table.1,MC<sub>1</sub>)**:Light Green; M.F. C<sub>22</sub>H<sub>20</sub>Cl<sub>4</sub>FeN<sub>6</sub>O<sub>4</sub>S<sub>2</sub>;Yield :70% ; M.P.>300°C; Molar Cond.(DMSO 1×10<sup>-3</sup>conc., ohm<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>):11.9;Magnetic moment (μ<sub>eff</sub> B.M.) :5.69;UV(DMSO, cm<sup>-1</sup>) :388,468,592 (π→π\*, n → π\*, d-d transition); IR(KBr Cm<sup>-1</sup>) : γ=3438 (O-H str. Water molecule), ν = 1630(C=N azomethine), ν =1480 (-C=N-N=C (str.in Thiadiazole ring), ν =1298 (C-O Phenolic), ν =1022 (N-N Thiadiazole ring), ν =757 (C-S-C str.in thiadiazole ring), ν =542 ( M-O bond), ν =463 ( M-N bond); Anal.Calcd.For C<sub>22</sub>H<sub>20</sub>Cl<sub>4</sub>FeN<sub>6</sub>O<sub>4</sub>S<sub>2</sub> : C, 38.06; H, 2.90; N, 12.11; Fe, 8.04; Found : C, 37.45; H, 2.02; N, 12.02; Fe, 7.85%.

**Bis(2,4-dichloro-6-((E)-((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenoxy)cobalt dihydrate(Table.1,MC<sub>2</sub>)**:Light Red; M.F. C<sub>22</sub>H<sub>20</sub>Cl<sub>4</sub>CoN<sub>6</sub>O<sub>4</sub>S<sub>2</sub>;Yield :73% ; M.P.>300°C;

Molar Cond.(DMSO  $1 \times 10^{-3}$  conc.,  $\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$ ):9.2;Magnetic moment ( $\mu_{\text{eff}}$  B.M.) :5.02;UV(DMSO,  $\text{cm}^{-1}$ ) :321,493,709 ( $\pi \rightarrow \pi^*$ ,  $n \rightarrow \pi^*$ , d-d transition); IR(KBr  $\text{Cm}^{-1}$ ) :  $\nu=3446$  (O-H str. Water molecule),  $\nu = 1615$ (C=N azomethine),  $\nu = 1469$  (-C=N-N=C (str.in Thiadiazole ring),  $\nu = 1297$  (C-O Phenolic),  $\nu = 1026$  (N-N Thiadiazole ring),  $\nu = 756$  (C-S-C str.in thiadiazole ring),  $\nu = 542$  ( M-O bond),  $\nu = 457$ ( M-N bond); Anal.Calcd.For  $\text{C}_{22}\text{H}_{20}\text{Cl}_4\text{CoN}_6\text{O}_4\text{S}_2$ : C, 37.89; H, 2.89; N, 12.05; Co, 8.45; Found : C, 37.10; H, 2.13; N, 11.95; Co, 8.00; %.

### Results and Discussion:

The IR data of the spectra of 2,4-dichloro-6-(((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol Schiff base heterocyclic ligands shows peak in the IR spectra of the complexes are compared with those of the free ligand in order to determine the coordination sites that may be involved in a chelation. There are some guide peaks in the spectra of the ligand, which is different in metal complex helps to prove formation of metal complexes. The peak of ligands at  $3321 \text{ cm}^{-1}$  is due to -OH stretching frequency while in metal complexes is at range  $3438-3446 \text{ cm}^{-1}$  due to water molecule coordinated to metal in metal complexes. The azomethine peak of ligands is at  $1652 \text{ cm}^{-1}$  while complexes  $\text{MC}_1$  and  $\text{MC}_2$  in range  $1615-1630 \text{ cm}^{-1}$  frequency of absorption in complexes decreases because of the indicating the bonding of nitrogen of the azomethine group to the metal ion and this can be explained by the donation of electrons from the nitrogen to the empty d-orbital of the metal ion. The phenolic (C-O) stretching vibration of ligands and metal complexes is having major change due to the participation of oxygen in the C-O-M bond. The new bands observed in metal complexes in range  $542 \text{ cm}^{-1}$  and  $457-436 \text{ cm}^{-1}$  is due to the metal oxygen (M-O) and Metal Nitrogen (M-N) bond respectively while another peak of ligand and metal complexes no measurable change is observed<sup>xxix</sup>.

The  $^1\text{H}$ - NMR peak of ligand (L) shows azomethine peak at  $\delta 9.21$  ppm, phenolic -OH at  $\delta 11.14$  ppm while aromatic protons resonate at range  $\delta 7.09-7.92$  ppm while methylene group resonate at  $\delta 3.11$  ppm and methyl group at  $\delta 1.19$  ppm confirms the formation of ligand<sup>xx</sup>.  $^{13}\text{C}$ -NMR peak of ligands aromatic region resonate at  $\delta 150.2-121.1$  ppm, azomethine carbon resonate at  $\delta 161.1$  ppm confirms the formation of ligands, While thiadiazole ring carbon resonate at  $\delta 155.2$  ppm and  $\delta 168.6$  ppm<sup>xxi</sup>. Mass Spectra of ligands shows peak at  $m/z 302.18$  [M+H, 100%], which is M+H peak at 100% intensity this peak support to the structure of the ligand. As per molar conductance data all ligands and metal complexes is non electrolytic in nature<sup>xxii</sup>.

The band appearing at 284 nm is due to transition of benzene ring of the ligand. Another band due to free ligands 366 nm due to transition for phenolic OH and azomethine moieties. These band shifts longer due to formation of Schiff base metal complexes<sup>xxiii</sup>. The magnetic moment value for Fe (III) complexes ( $\text{MC}_1$ ) is 5.69 B.M and UV spectra shows peak at 388 nm, 468 nm and 592 nm due to d-d transition confirms the octahedral geometry of complexes<sup>xxiv</sup>. The magnetic moment value for CO(II) complexes ( $\text{MC}_2$ ) is 5.02 B.M is close to octahedral complex spectra shows band at 321 nm, 493 nm and 709 nm shows that octahedral geometry of CO(II) complexes<sup>xxv</sup>.

### Antimicrobial activity

The antimicrobial activity in vitro on selected two gram positive bacteria *S. aureus* and *B. Subtlis* two fungi *A. niger* and *F. Oxysporum* was carried out. All of the tested synthesized heterocyclic ligand and metal complexes showed good biological activity against microorganism. The bactericidal and fungicidal investigation data of the compounds are given in Tables 2. The investigation shows that metal complexes shows more good bactericidal and fungicidal activity than Heterocyclic Ligand hence activity of metal

complexes increases due to chelation in metal complexes increase in delocalization of  $\pi$  electron on chelating ring and enhance the penetration of complexes in lipid membrane of microorganism and blocks the binding site enzymes of harmful microorganism there are some other factors also like lipophilicity, hydrophilicity solubility, Conductivity and bonding between M-L that increases the activity of complexes<sup>xxvi</sup>.

Compounds	Antibacterial Activity				Antifungal Activity			
	<i>S.aureus</i>		<i>B.subtilis</i>		<i>A.niger</i>		<i>F.oxysporum</i>	
	Diameter of inhibition Zone in mm	Diameter of inhibition Zone in mm	Diameter of inhibition Zone in mm	Diameter of inhibition Zone in mm	Diameter of inhibition Zone in mm	Diameter of inhibition Zone in mm	Diameter of inhibition Zone in mm	Diameter of inhibition Zone in mm
	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm	250 ppm	500 ppm
<b>Ligands(H L)</b>	19	17	20	22	21	00	00	22
<b>Fe Complex</b>	21	23	22	24	20	21	17	20
<b>Co Complex</b>	20	22	20	00	18	22	20	23
<b>Ciprofloxacin (Standard)</b>	32	34	31	33	----	----	----	----
<b>Miconazole (Standard)</b>	----	----	----	----	29	31	25	27

**Table 2. Antimicrobial activity of ligand and its Metal Complexes**

### Conclusion

The Present Novel Heterocyclic Synthesis, Characterization, Antibacterial and Antifungal study 2,4-dichloro-6-(((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenol (L) and Bis(2,4-dichloro-6-((E)-((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenoxy)iron dihydrate(MC<sub>1</sub>);Bis(2,4-dichloro-6-((E)-((5-ethyl-1,3,4-thiadiazol-2-yl)imino)methyl)phenoxy)cobalt dihydrate (MC<sub>2</sub>) by Conventional method. The antimicrobial activity data showed that Most of the metal complexes are more biologically potent as compared to that parent ligand against all pathogenic Microorganisms. These type of study helps to decrease emerging problems in drug resistance microorganism in health sciences.

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