SYNTHESIS, SPECTRAL CHARACTERISATION AND BIOLOGICAL STUDIES OF NiII, CuII, ZnII AND CdII COMPLEXES WITH 1,2,4-TRIAZOLE BASED BIDENTATE SCHIFF BASE

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ABSTRACT
Complexes of NiII, CuII, ZnII and CdII metal ions with a novel Schiff base ligand 4-(((3-ethyl-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxy-6-nitrophenol (HTNP) were synthesised, thoroughly characterized by spectral techniques i.e. IR, 1H-NMR, fluorescence, electronic spectra and also structurally examined by elemental analysis, thermal analysis and magnetic moment measurements. A significant enhancement in the fluorescence intensity of HTNP was observed after complexation. Comparative in vitro antimicrobial activities of HTNP and its metal complexes were studied and these novel compounds were found to show moderate to significant antibacterial activity. The results also indicated that antimicrobial activity of the Schiff base ligand (HTNP) gets considerably increased on complexation.

Keywords: Antibacterial Activity, Fluorescence, 1H-NMR, Metal Complexes, Schiff Base, 1,2,4-Triazole.

INTRODUCTION
The study and preparation of bioactive heterocyclic compounds having the magical moiety of 1,2,4-triazole have earned numerous attention because this moiety can exert non-covalent interactions which can enhance the solubility and the capability of binding to biomolecular targets, moreover triazole derivatives display antibacterial, fungicidal, antileishmanial, antihypertensive and antihyperthermic activities. Deliberation towards Schiff bases preparation is due to their facile synthesis, good solubility in common solvents and their wider applications in the pharmaceutical fields. It has been established that in Schiff bases, azomethine (-CH=N-) linkage is responsible for the biological activities. Considering the significant biological importance of triazole analogs and azomethine linkage, numerous triazole based Schiff bases have been reported to possess antimicrobial, anti-inflammatory, antidegenerative, anti-HIV activities. In addition to this, the chelation of Schiff base with transition metal ions improves their stability as well as enhances the pharmaceutical activities. Viewing the pronounced impact of triazole based Schiff base metal complexes and in the continuation of our lab work, 4-Hydroxy-3-methoxy-5-nitrobenzaldehyde was condensed with 4-amino-3-ethyl-5-mercapto-s-triazole in an endeavor to form higher biological active compounds that could resist bacterial and fungal species. This paper reports the synthesis, structural explication and biological studies of novel NiII, CuII, ZnII and CdII complexes with new Schiff base i.e. 4-(((3-ethyl-5-mercapto-4H-1,2,4-triazol-4-yl)imino)methyl)-2-methoxy-6-nitrophenol (HTNP).

EXPERIMENTAL
Material and Methods
All the reagents and solvents were obtained commercially. The melting point of the ligand and its metal complexes were determined in open capillaries in an electrical apparatus (using silicon oil 350 cSt) and may be uncorrected. Perkin-Elmer IR spectrophotometer was used to record the IR spectra of compounds. 1H-NMR (400 MHz) was noted in DMSO-d6 as a solvent on Bruker Avance-III. The electronic spectra were noted by using T 90 (1100-200 nm) UV/Vis spectrophotometer. The fluorescence properties were measured by using T 90 (200 nm) UV/Vis spectrophotometer.

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investigated on SHIMADZU RF-5301PC spectrophotometer. ESR of the Cu\textsuperscript{II} complexes was recorded using JES-FA200 ESR spectrometer with X and Q-band. Thermal studies of the complexes were conducted on a Perkin Elmer (Pyris Diamond).

**General Procedure**

HTNP was synthesized by condensation of 4-amino-3-ethyl-5-mercapto-s-triazole and 4-Hydroxy-3-methoxy-5-nitrobenzaldehyde in absolute alcohol under reflux for about four hours as per Scheme-1. Completion of the reaction and purity of HTNP was ensured by TLC method. The obtained yellow solid product was filtered off, rinsed with cold alcohol, desiccated and recrystallized with ethanol.

![Scheme-1: Synthesis of the ligand HTNP](image)

The metal complexes of HTNP were synthesized by the reaction of ligand (HTNP) with metal ions (Ni\textsuperscript{II}, Cu\textsuperscript{II}, Zn\textsuperscript{II}, and Cd\textsuperscript{II}) as acetate in 1:1 and 1:2 (M\textsuperscript{II}:HTNP) molar ratio taking ethanol as solvent under reflux condition.\textsuperscript{13} Metal contents were determined gravimetrically by reported methods.\textsuperscript{14}

**RESULTS AND DISCUSSION**

The ligand (HTNP) showed good solubility in almost all the organic solvents whereas the resulting metal complexes were not soluble in most of the organic solvents instead shown solubility in DMF and DMSO. The physical measurements and analytical results of the HTNP and its metal complexes exhibiting 1:1 and 1:2 (M\textsuperscript{II}:HTNP) stoichiometries are entered in Table-1.

Table-1: The Physical Measurements and Analytical Results of the HTNP and its Metal Complexes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Colour</th>
<th>mp (°C)</th>
<th>Elemental Analysis, % Found (Calcd.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C %</td>
</tr>
<tr>
<td>Schiff Base (HTNP)</td>
<td>Yellow</td>
<td>(230-232)</td>
<td>44.20 (44.58)</td>
</tr>
<tr>
<td>Ni(TNP)(OAc)•3H\textsubscript{2}O</td>
<td>Brick red</td>
<td>(278-280)[a]</td>
<td>30.96 (34.02)</td>
</tr>
<tr>
<td>Ni(TNP)•2H\textsubscript{2}O</td>
<td>Brick red</td>
<td>(269-271)[a]</td>
<td>37.21 (38.98)</td>
</tr>
<tr>
<td>Cu(TNP)(OAc)•H\textsubscript{2}O</td>
<td>Reddish brown</td>
<td>(261-263)[a]</td>
<td>36.12 (36.32)</td>
</tr>
<tr>
<td>Cu(TNP)\textsubscript{2}</td>
<td>Dark brown</td>
<td>(265-267)[a]</td>
<td>39.69 (40.70)</td>
</tr>
<tr>
<td>Zn(TNP)(OAc)•3H\textsubscript{2}O</td>
<td>Pastel yellow</td>
<td>(300-302)[a]</td>
<td>32.98 (33.57)</td>
</tr>
<tr>
<td>Zn(TNP)•2H\textsubscript{2}O</td>
<td>Buff</td>
<td>(309-311)[a]</td>
<td>37.25 (38.64)</td>
</tr>
</tbody>
</table>
STUDIES OF Ni\textsuperscript{II}, Cu\textsuperscript{II}, Zn\textsuperscript{II} AND Cd\textsuperscript{II} COMPLEXES

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<table>
<thead>
<tr>
<th></th>
<th></th>
<th>(3.75)</th>
<th>(18.78)</th>
<th>(8.76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd(TNP)(OAc)•3H\textsubscript{2}O</td>
<td>Pale yellow</td>
<td>(295-297)\textsuperscript{[a]}</td>
<td>29.78 (30.69)</td>
<td>3.21 (3.83)</td>
</tr>
<tr>
<td>Cd(TNP)\textsubscript{2}•2H\textsubscript{2}O</td>
<td>Buff</td>
<td>(281-283)\textsuperscript{[a]}</td>
<td>34.98 (36.34)</td>
<td>2.77 (3.53)</td>
</tr>
</tbody>
</table>

\textsuperscript{[a]} Decomposition temperature

Spectroscopic Characterization

Infrared Spectra

The mode of chelation of HTNP with metal ions can be assigned by comparing IR spectral data of the ligand with its metal complexes. A significant band at 1580 cm\textsuperscript{-1} was detected owing to the presence of azomethine group in the free ligand which get shifted towards the lower frequency region by 20-35 cm\textsuperscript{-1} indicating the bonding of azomethine nitrogen with metal ions.\textsuperscript{15} Another absorption band at 2810 cm\textsuperscript{-1} was also noticed, showing the presence of –SH group in HTNP ligand which get disappeared in metal complexes confirming the formation of M-S bond via deprotonation.\textsuperscript{16} Bands in the region 314-325 cm\textsuperscript{-1}, 466-482 cm\textsuperscript{-1} and 740-780 cm\textsuperscript{-1} in the far-IR spectra of metal complexes were assigned to ν(M-S), ν(M-N) and ν(C-S) accordingly which further justified the formation of M-N and M-S bond.\textsuperscript{17} IR spectrum of HTNP showed the sharp band at 3434 cm\textsuperscript{-1} by cause of ν(OH) stretching vibrations and presence of broadband around 3300-3590 cm\textsuperscript{-1} in spectra of metal complexes indicates the existence of coordinated H\textsubscript{2}O molecules which was further ensured by TGA studies. A strong band due to ν(OCOCH\textsubscript{3}) was also observed at 1744 cm\textsuperscript{-1} in the case of 1:1 (M\textsuperscript{II}:HTNP) metal complexes.

\textsuperscript{1}H-NMR Spectra

The structure of HTNP and the mode by which this ligand gets bonded to the metal ions was confirmed by \textsuperscript{1}H-NMR spectra as in Fig.-1. The Schiff base showed a characteristic singlet peak due to azomethine proton (-CH= N-) at 9.82 ppm which get shifted on complexation indicating the coordination of N atom of the group (-CH= N-) with metal ions.\textsuperscript{18} A singlet due to –SH proton was observed at 13.77 ppm and this peak was not observed in complexes illustrating deprotonation and bonding through a sulphur atom.\textsuperscript{19} A singlet due to coordinated acetate group was also observed around 1.80 ppm in case of 1:1 (M\textsuperscript{II}:HTNP) metal complexes of Zn\textsuperscript{II} and Cd\textsuperscript{II}.

Electronic Spectra

The electronic spectra of Ni\textsuperscript{II} and Cu\textsuperscript{II} complexes were analyzed in DMF solvent of 10\textsuperscript{-5} M concentration at room temperature. Octahedral geometry of Ni\textsuperscript{II} complexes were confirmed by the electronic spectra as three bands were observed in the region 10,182-10,189 cm\textsuperscript{-1}, 17,921-18,315 cm\textsuperscript{-1} and 22,522-22,779 cm\textsuperscript{-1} due to $^3T_{2g}(F) ← {^2}A_{2g}(F)$ ($v_1$), $^3T_{1g}(F) ← {^2}A_{2g}(F)$ ($v_2$), $^3T_{1g}(P) ← {^2}A_{2g}(F)$ ($v_3$) transitions accordingly.\textsuperscript{19} Covalent character of ligand to metal bond and octahedral environment around Ni\textsuperscript{II} ion was further ensured by calculating crystal-field parameters viz. Dq, B, β, β% as given in Table-2. Ni(TNP)(OAc)•3H\textsubscript{2}O and Ni(TNP)\textsubscript{2}•2H\textsubscript{2}O were found to have magnetic moment values of 2.96-3.10 BM ensuring two unpaired electrons and octahedral geometry for both the complexes.\textsuperscript{17} As the magnetic
moment values of Cu\textsuperscript{II} complexes were 1.82-1.86 BM which suggested one unpaired electron and square planar geometry around Cu\textsuperscript{II} ion which was further supported by the presence of an absorption band at 18,518-19,047 cm\textsuperscript{-1} that corresponds to \(^2\text{A}_{1g} \leftarrow \text{B}_{1g}(\nu_1)\) transition, indicating square-planar geometry.\textsuperscript{20}

Fluorescence Studies
The ligand (HTNP), its 1:1 and 1:2 (M\textsuperscript{II}:HTNP) complexes were investigated for fluorescence properties at room temperature in 10\textsuperscript{-3} M DMF solution with the excitation wavelength set at 280 nm. It was observed that all metal complexes exhibit enhanced fluorescence intensity than the ligand in order of HTNP < Cu\textsuperscript{II} < Ni\textsuperscript{II} < Cd\textsuperscript{II} < Zn\textsuperscript{II}. The intensified fluorescence properties of the metal complexes in comparison with the free ligand, are the result of chelation of the ligand to metals, which attributes energy loss reductions by thermal vibrational decay as ligand rigidity get increased on chelation.\textsuperscript{21} This indicates better chelation behavior of the Schiff base ligand. The results also emphasize on photochemical applications of these complexes.\textsuperscript{22} The merged spectra of the ligand (HTNP) and its 1:1 (M\textsuperscript{II}:HTNP) complexes with Ni\textsuperscript{II}, Cu\textsuperscript{II}, Zn\textsuperscript{II} and Cd\textsuperscript{II} are shown in Fig.-2.

Electron Spin Resonance (ESR) Spectra
The ESR spectra (X-band) of powdered Cu\textsuperscript{II} complexes at room temperature, showed axial behaviour having g values as g\textsubscript{||} = 2.300, g\textsubscript{\perp} = 2.092, G = 3.33 for Cu(TNP)(OAc)\textsuperscript{•}H\textsubscript{2}O and g\textsubscript{||} = 2.224, g\textsubscript{\perp} = 2.064, G = 3.58 for Cu(TNP)\textsubscript{2} as per Fig.-3. The g\textsubscript{||} values of these complexes (≤ 2.3) corroborate the covalent nature of the metal-ligand bond.\textsuperscript{23} The presence of unpaired e\textsuperscript{-} in d\textsubscript{x2−y2} orbital is revealed by the observed trend of g values i.e. g\textsubscript{e} (2.002) < g\textsubscript{\perp} < g\textsubscript{||}, which is also consistent with the square planar geometry of Cu\textsuperscript{II} complexes.\textsuperscript{24}

Thermal Analysis
Thermogravimetric analysis of Ni(TNP)(OAc)\textsuperscript{•}3H\textsubscript{2}O, Cu(TNP)(OAc)\textsuperscript{•}H\textsubscript{2}O, Zn(TNP)(OAc)\textsuperscript{•}3H\textsubscript{2}O and Cd(TNP)(OAc)\textsuperscript{•}3H\textsubscript{2}O were carried out up to 1000 °C at a heating rate of 10 °C/min. The number of stages, ranges of decomposition temperature, percentage of weight losses and decomposition products are discussed as per Fig.-4. Decomposition of the Ni(TNP)(OAc)\textsuperscript{•}3H\textsubscript{2}O complex occurred in three stages. The initial mass loss accounted for the removal of three coordinated H\textsubscript{2}O molecules in 60-210 °C range of

Table-2: Electronic Spectral Data and Crystal-field Parameters for Ni\textsuperscript{II} Complexes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Transitions (cm\textsuperscript{-1})</th>
<th>Dq (cm\textsuperscript{-1})</th>
<th>B (cm\textsuperscript{-1})</th>
<th>(\nu_2/\nu_1)</th>
<th>(\beta)</th>
<th>(\beta) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni(TNP)(OAc)\textsuperscript{•}3H\textsubscript{2}O</td>
<td>10,189, 17,921, 22,779</td>
<td>1018.9</td>
<td>675.5</td>
<td>1.75</td>
<td>0.648</td>
<td>35.2</td>
</tr>
<tr>
<td>Ni(TNP)\textsuperscript{•}2H\textsubscript{2}O</td>
<td>10,182, 18,315, 22,522</td>
<td>1018.2</td>
<td>686.0</td>
<td>1.79</td>
<td>0.658</td>
<td>34.1</td>
</tr>
</tbody>
</table>

Fig.-2: Fluorescence Spectra for HTNP and its Complexes of 1:1 (M\textsuperscript{II}:HTNP) Stoichiometry.
temperature and found mass loss was 10.90% (Calcd. 10.93%). The second stage corresponded to the elimination of organic moiety through observed mass loss 48.71% (Calcd. 48.61%) between 210 and 290 °C. The last degradation step with an observed mass loss of 29.27% (Calcd. 28.55%) between the temperature range of 290-810 °C, confirmed the removal of triazole moiety followed by nickel oxide as a leftover product. Similarly, Zn(TNP)(OAc)•3H₂O and Cd(TNP)(OAc)•3H₂O complexes also exhibited breakdown in three steps for the elimination of three coordinated H₂O molecules, organic and triazole moieties. Cu(TNP)(OAc)•H₂O complex displayed three decomposition steps in TGA thermogram. The starting decomposition step was accompanied by the loss of a coordinated H₂O molecule from 60-230 °C with mass loss of 4.33% (Calcd. 3.89%). The second decomposition showed loss of organic moiety in 230-700 °C range with mass loss of 50.49% (Calcd. 51.89%). The final decomposition corresponded to the elimination of triazole moiety with mass loss of 29.49% (Calcd. 30.48%) at about 700-930 °C.

![X-band ESR Spectra of the CuII Complexes at Room Temperature.](image1)

![TGA Curves for Metal Complexes of 1:1 (MII:HTNP) Stoichiometry.](image2)

### Biological Evaluation

The antimicrobial studies were explored by agar well diffusion method in vitro for the novel ligand (HTNP) and its metal complexes versus two G– bacterial strains i.e. *P. aeruginosa* and *E. coli*, two G+ bacterial strains i.e. *S. aureus* and *B. subtilis* and a fungal strain i.e. *C. albicans*. Ciprofloxacin and Amphotericin B were assayed as reference drugs against the bacterial and fungal strains respectively. The outcomes concluded that the chelation process has improved the antimicrobial activity of free HTNP. This higher bio-activity of the complexes in comparison to free HTNP can be explain by the overtone’s concept of cell permeability and Tweedy-chelation theory. Out of all the tested compounds, ZnII and CdII complexes displayed the best antibacterial potency. The rest of the synthesized compounds have shown low to moderate antibacterial activity. Some of the tested compounds were also found to be moderately
active against *C. albicans* (MIC 50 μg/mL). A thorough comparison of MIC values for HTNP and its metal complexes have been shown in Fig.-5.

**CONCLUSION**

The structure and bidentate nature (through azomethine-N and thiol-S) of HTNP were confirmed by IR and 1H-NMR studies. Spectral data concluded about coordination geometry i.e all metal complexes had octahedral geometry except Cu\textsuperscript{II} complexes which acquired square planar geometry. Thermal analysis ensured about several H\textsubscript{2}O molecules were coordinated to the central metal ion. ESR spectra were also in good coincidence with the proposed structure of Cu\textsuperscript{II} complexes. Enhancement in fluorescence intensity after complexation suggested that these complexes can have a pronounced impact in the field of chemosensors. The chelation process had improved the biological activity of HTNP and among the metal complexes, Zn\textsuperscript{II} and Cd\textsuperscript{II} complexes were found to be the most bioactive.

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**REFERENCES**


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