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Short Communication

Synthesis, characterization and biological studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with bidentate Schiff bases derived by heterocyclic ketone

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Abstract

A series of metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) have been synthesized with newly prepared biologically active ligands. These ligands were prepared by the condensation of 4-amino-5-mercapto-3-methyl-*s*-triazole (AMMT), 4-Amino-3-ethyl-5-mercapto-*s*-triazole (AEMT) with 2-acetylpyridine. The structure of the complexes have been proposed by elemental analyses, spectroscopic data i.e. IR, ^{1}H NMR, electronic and magnetic measurements. Thermal studies of the complexes are also reported. Antibacterial activities of 10 complexes have been studied in vitro. Heterocyclic bidentate Schiff bases were associated with substantially higher antibacterial activities than some commercial antibiotics.

Keywords: 1,2,4-Triazoles; Schiff bases; Antibacterial activity; Anticancer activity; ^{1}H NMR; Metal complexes

1. Introduction

During recent years coordination compounds of biologically active ligands [1–3] have received much attention. Chelation causes drastic change in the biological properties of the ligands and also the metal moiety. It has been reported that chelation is the cause and cure of many diseases including cancer. A number of Schiff base complexes [4–7] have been tested for antibacterial activities and they have been found antibacterial [8–11], antifungal [10–12], anticancer [13,14], and herbicidal [15] activities. However, for the period of antibiotics using in clinical practice, steady growth of clinically significant bacteria tolerance to these preparations has been observed. This is likely to be an unavoidable process. Selection of resistant to antibiotic mutants is especially rapid in population of opportunistic pathogenic microorganism [16], which, in turn, often act as donors of resistance genes for particularly dangerous infections agents. Recently, the number of diseases, caused by multidrug resistant gram-positive microorganism, has been continuously increasing. The ability of microorganism to become resistant to major therapies used against them has long been recognized and becomes increasing apparent [17]. Increasing antimicrobial resistance (AMR) presents major threats to public health because it reduces the effectiveness of antimicrobial treatment leading to increased morbidity, mortality and health care expenditure [18].

In view of the scanty informations available on the substitution complexes of transition metals and promoted from the biological activities of such complexes are reported in the present communication along with the structural elucidation by various physico-chemical methods.

Abbreviations: AMMT, 4-amino-5-mercapto-3-methyl-*s*-triazole; AEMT, 4-amino-3-ethyl-5-mercapto-*s*-triazole; *B. subtilis*, *Bacillius subtilis*; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; *E. coli*, *Escherichia coli*; *S. dysenterieae*, *Shigella dysenterieae*; *Pseudomonas* sp., *Pseudomonas* sp..

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2. Chemistry

2.1. Methods

2.1.1. Synthesis of the ligands

A series of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles [19–21] were synthesized. 4-Amino-5-mercapto-3-methyl-*s*-triazole (AMMT) and 4-amino-3-ethyl-5-mercapto-*s*-triazole (AEMT) were prepared by reported procedure [22]. Schiff bases namely, 4-acetylpyridenylideneamino-5-mercapto-3-methyl-*s*-triazole (ApMMT) and 4-acetylpyridenylideneamino-3-ethyl-5-mercapto-*s*-triazole (ApEMT), were prepared by refluxing a mixture of equimolar quantities of different *s*-triazoles (AMMT and AEMT) with 2-acetylpyridine. Ethanol was used as the solvent. The reaction mixture kept at room temperature and the product was filtered and recrystallized from ethanol.

ApMMT: Color Creamish, Yield 70%, m.p. 219–222 °C.
Anal. CHN $C_{10}H_{11}N_5S$.

ApEMT: Color Creamish, Yield 77%, m.p. 144–146 °C.
Anal. CHN $C_{11}H_{13}N_5S$.

2.1.2. Synthesis of metal complexes

Aqueous ethanolic solution of metal acetates of Co(II), Ni (II), Cu(II) and Zn (II) were added to the hot ethanolic solution of the ligand in (1:1) and (1:2) molar ratios, which resulted in the precipitation of metal derivatives in all the cases. The product formed were filtered, washed with warm water, ethanol and finally with acetone and dried on water bath.

ApMMT-Co(1:1): Color Brown, Yield 79%, m.p. > 220 °C(d).
Anal. CHN $Co(C_{10}H_{10}N_5S)OAc{\cdot}3H_2O$.
ApMMT-Co(1:2) : Color Light brown, Yield 77%, m.p. > 220 °C(d)
Anal. CHN $Co(C_{10}H_{10}N_5S)_2{\cdot}2H_2O$.
ApMMT-Ni(1:1) : Color Green, Yield 68%, m.p. > 280 °C(d).
Anal. CHN Ni $(C_{10}H_{10}N_5S)$ $OAc{\cdot}3H_2O$.
ApMMT-Ni(1:2): Color Light green, Yield 78%, m.p. > 260 °C(d).
Anal. CHN $Ni(C_{10}H_{10}N_5S)_2{\cdot}2H_2O$.
ApMMT-Cu(1:1): Color Creamish, Yield 78%, m.p. > 280 °C(d).
Anal. CHN $Cu(C_{10}H_{10}N_5S)OAc{\cdot}H_2O$.
ApMMT-Cu(1:2): Color Creamish, Yield 63%, m.p. > 280 °C(d).
Anal. CHN $Cu(C_{10}H_{10}N_5S)_2$.
ApMMT-Zn(1:1): Color Creamish, Yield 81%, m.p. > 260 °C(d).
Anal. CHN $Zn(C_{10}H_{10}N_5S)OAc{\cdot}3H_2O$.
ApMMT-Zn(1:2):Color Creamish, yield 85%, m.p. > 260 °C (d)
Anal. CHN $Zn(C_{10}H_{10}N_5S)_2{\cdot}2H_2O$.
ApEMT-Co(1:1) : Color Dark brown, yield 71%, m.p. > 280 °C (d).
Anal. CHN $Co(C_{11}H_{12}N_5S)OAc{\cdot}3H_2O$.
ApEMT-Co (1:2): Color Dark brown, Yield 79%, m.p. > 280 °C (d)
Anal. CHN $Co(C_{11}H_{12}N_5S)_2{\cdot}2H_2O$.
ApEMT- Ni (1:1): Color Light brown, yield 76%, m.p. > 260 °C (d).
Anal. CHN $Ni(C_{11}H_{12}N_5S)OAc{\cdot}3H_2O$.
ApEMT-Ni(1:2):Color Light brown, yield 85%, m.p. > 220 °C (d).
Anal. CHN $Ni(C_{11}H_{12}N_5S)_2{\cdot}2H_2O$.
ApEMT- Cu (1:1) :Color Creamish, yield 87%, m.p. > 280 °C (d).
Anal. CHN $Cu(C_{11}H_{12}N_5S)OAc{\cdot}H_2O$.
ApEMT- Cu (1:2): Color Creamish, yield 61%, m.p. > 260 °C (d).
Anal. CHN $Cu(C_{11}H_{12}N_5S)_2$.
ApEMT- Zn (1:1):Color Light brown, yield 82%, m.p. > 250 °C (d).
Anal. CHN $Zn(C_{11}H_{12}N_5S)OAc{\cdot}3H_2O$.
ApEMT- Zn (1:2):Color Light brown, yield 84%, m.p. > 220 °C (d).
Anal. CHN $Zn(C_{11}H_{12}N_5S)_2{\cdot}2H_2O$.

3. Pharmacology

3.1. In vitro antibacterial assay

Antibacterial assay of 10 synthesized compounds viz. ApMMT, ApMMT-Ni (1:1), ApMMT-Ni (1:2), ApMMT-Zn (1:1), ApMMT-Zn (1:2), ApEMT, ApEMT-Co (1:1), ApEMT-Co (1:2), ApEMT-Cu (1:1), and ApEMT-Cu (1:2) were done in vitro by the reported method [23]. The stock solution (1 mg ml^{-1}) of the test chemical was prepared by dissolving 10 mg of the test chemical in 10 ml of respective solvent for each compound. The compounds ApMMT, ApEMT, ApMMT-Ni (1:1), ApMMT-Ni (1:2), ApEMT-Cu (1:1) and ApEMT-Cu (1:2) were dissolved in *N*,*N*-dimethyl formamide (DMF), ApMMT-Zn (1:1) and ApMMT-Zn (1:2) in dimethyl sulfoxide (DMSO) and remaining two compounds i.e. ApEMT-Co (1:1) and ApEMT-Co (1:2) were soluble in acetic acid. The stock solution was suitably diluted with sterilized distilled water to get dilution of 500, 100, and 50 μg ml^{-1}. Control for each dilution was prepared by diluting 10 ml of respective solvent instead of stock solution with sterilized distilled water.

Four bacteria, namely *Bacillus subtilis* (gram +ve, non-motile), *Escherichia coli*, *Shigella dysenterieae* and *Pseudomonas* sp. (gram –ve, non-motile) were used for the antibacterial assay. Spread-plate method was used for all the four bacteria. For this 24-h-old broth cultures were diluted up to 10^{-3}. Fifty microliters of the test chemical of required dilution, and 50 μl of 24-h-old culture broth of 10^{-3} dilutions were mixed to make a total volume of 0.1 ml. The mixture was then spread on the surface of nutrient agar plates with the help of a sterilized spreader. For control, 50 μl of respective dilution of solvent used for a particular microorganism was added in to the broth medium. All the plates were incubated at 35 °C for 24 h and the colonies observed in the test and control plates were counted.

4. Result and discussions

Electronic spectrum of the complexes Co(II), Ni(II) and Cu (II) were taken. The test solution was prepared by dissolving the compounds in the respective solvent.

The complexes ApMMT-Co (1:1), ApMMT-Co (1:2) were dissolved in DMF and ApEMT-Co (1:1) and ApEMT-Co (1:2) in acetic acid. Cobalt complexes generally exhibits two distinct bands in the region 8000–12,000 and 18,000–20,000 cm^{-1}, respectively. These bands may be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1) and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$($\nu_3$) transitions, suggesting octahedral geometry [24]. The cobalt complexes viz. ApMMT-Co (1:1), ApMMT-Co (1:2), ApEMT-Co (1:1) and ApEMT-Co (1:2) showed bands at 11,001, 10,965, 10,299, 10,299 cm^{-1} and 18,416, 18,537, 20,619, 18,692 cm^{-1} for ν_1 and ν_3, respectively.

The Nickel complexes ApMMT-Ni (1:1) and ApMMT-Ni (1:2) were dissolved in DMF and ApEMT-Ni (1:1) and ApEMT-Ni (1:2) in methanol. Nickel complexes generally exhibits three bands, which are characteristic of octahedral geometry [24]. These bands in the region 7000–13,000, 13,000–19,000 and 20,000–27,000 cm^{-1} may be assigned due to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$; $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\nu_3)$ transitions, respectively. The nickel complexes viz. ApMMT-Ni (1:1), ApMMT-Ni (1:2), ApEMT-Ni (1:1) and ApEMT-Ni (1:2) showed bands at 10,965, 10,989, 10,953, 10,953; 16,584, 16,527, 16,542, 16,590 and 18,727, 18,519, 26,954, 27027 cm^{-1} for ν_1, ν_2 and ν_3, respectively.

All the copper complexes namely ApMMT-Cu (1:1), ApMMT-Cu (1:2), ApEMT-Cu (1:1) and ApEMT-Cu (1:2) were dissolved in DMF. Copper complexes showed one broad band at ~18,500 cm^{-1}, which is assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition, a characteristic of square–planer geometry [24]. The visible spectrum of ApMMT-Cu (1:1), ApMMT-Cu (1:2), ApEMT-Cu (1:1) and ApEMT-Cu (1:2) showed bands 18,553, 18,450, 18,416 and 18,519 cm^{-1}, respectively.

Important infrared (IR) bands for the ligands appear at (cm^{-1}): 3250, 2700, 1630–1615, 1100.

Important IR bands for complexes appear at (cm^{-1}): 3500–3200, 1750–1720 (1:1), ± 10–20 (1630–1615), 800–750, 750, 720–700, 540–480, 380–340.

The IR spectrum of the ligands shows characteristic bands due to ν(N–H) and ν(S–H) at 3250 and 2700 cm^{-1}, respectively [25]. Another band at 1100 cm^{-1} is assigned to ν(C=S) [25]. The deprotonation of thiol group is indicated by the absence of a band in metal complexes at 2700 cm^{-1}, which appears due to ν(S–H) in the spectra of ligands indicating there by complexation through sulfur. A new band appears at ~ 750 cm^{-1} which is assigned to ν(C–S) and it further confirms coordination through sulfur atom. Metal–sulfur bond formation is further confirmed by a band in the region 380–340 cm^{-1} in the far IR-spectra.

The presence of coordinated water in the complexes [25] is indicated by a broad trough band in the region 3500–3200 cm^{-1} and two weaker bands in the region 800–750 and 720–700 cm^{-1} due to ν(–OH) rocking and wagging mode of vibrations, respectively [26]. A strong band in the region 1750–1720 cm^{-1} has been assigned to ν($OOCCH_3$) in (1:1) (metal/ligand) complexes.

A strong band in the region 1630–1615 cm^{-1} in the free ligands assigned to ν(N=C–CH_3) exhibits ± 10–20 cm^{-1} shifting [27–29] in the spectra of complexes indicating coordination through azomethine nitrogen of Schiff bases and this can be explained by the donation of electrons from nitrogen to the empty d-orbitals of the metal atoms. Formation of metal–nitrogen bond is further supported by the presence of a band in the region 540-480 cm^{-1}.

Thus the IR spectral data results provide strong evidences for the complexation of the potentially multidentate ligands.

Proton NMR peaks of the ligands in DMSO-d_6 appear at:

ApMMT	13.0 (s, 1H, S**H**), 7.6–8.8 (m, 4H, Aromatic-**H**), 1.3 (s, 3H, Alkyl-**H**), 1.9 (s, 3H, $-C(=N)-CH_3$)
ApEMT	13.5 (s, 1H, S**H**), 7.4–8.8 (m, 4H, Aromatic-**H**), 1.14 (t, 3H, $-CH_2C\mathbf{H}_3$), 1.25 (q, 2H, $-CH_2CH_3$), 2.1 (s, 3H, $-C(=N)-CH_3$)

Proton NMR peaks of Zn complexes in DMSO-d_6 appear at:

ApMMT-Zn (1:1):	7.4–8.5(m,4H, Aromatic-**H**), 1.87 (s,3H,Alkyl-**H**), 2.25 (s, 3H, $-C(=N)-CH_3$)
ApMMT-Zn (1:2):	7.4–8.5(m,4H,Aromatic-**H**), 1.90(s, 3H, Alkyl-**H**),2.21 (s, 3H, $-C(=N)-CH_3$)
ApEMT-Zn (1:1):	7.4–8.5 (m,4H, Aromatic-**H**), 1.10(t, 3H,-$CH_2C\mathbf{H}_3$), 1.20 (q,2H,-$C\mathbf{H}_2CH_3$), 2.5 (s, 3H, $-C(=N)-CH_3$)
ApEMT-Zn (1:2):	7.4–8.5(m,4H,Aromatic-**H**), 1.10(t, 3H, $-CH_2C\mathbf{H}_3$), 1.20 (q,2H,-$C\mathbf{H}_2CH_3$), 2.55 (s, 3H, $-C(=N)-CH_3$)

In NMR spectra of complexes we observed a shift of electron density from the ligands to metal. The signal of azomethine protons deshielded in the spectra of metal complexes were found to occur in the range (2.21–2.55) ppm, as compared to its Schiff bases (1.9–2.1) ppm after complxations to the metal ion inferring coordination through azomethine nitrogen atom [30] of the ligands. Disappearance of –SH protons in the spectra of complexes supported the deprotonation of the thiol group.

Magnetic susceptibility measurements of the complexes (ApMMT-Ni (1:1), ApMMT-Ni (1:2) and ApMMT-Co (1:1), ApMMT-Co (1:2) give a magnetic moment values μ_{eff} of 2.92, 2.96 and 3.92, 4.62 BM, respectively, at room temperature. The magnetic moment value of ApMMT-Co (1:1) is 3.92 BM (close to spin-only value) which is may be due to quenching of orbital contribution and ligand effect [31]. This value is lower than the spin-only value of 4.3–5.2 BM for three unpaired electrons.

Thermal behavior of the complexes are almost, the same. Hence only two complexes namely Ni(ApMMT)OAc·$3H_2O$ and Co(ApEMT)OAc·$3H_2O$ are discussed in detail.

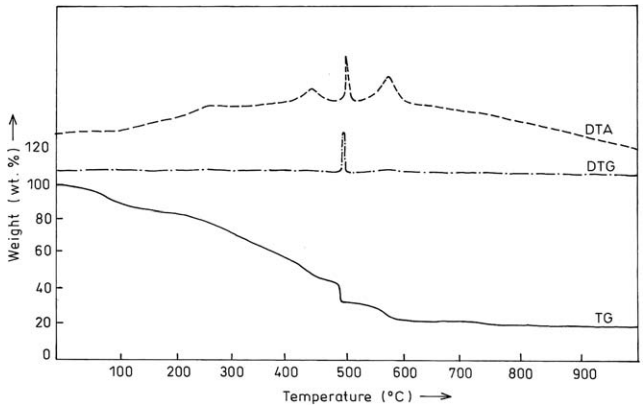

Fig. 1. Thermoanalytical curves of Ni (ApMMT) OAc. $3H_2O$.

The DTG Curve of Ni(ApMMT)OAc·$3H_2O$ (Fig. 1) showed the three water molecules were lost at 60, 80 and 150 °C corresponding to the mass loss of 3.98% (calc. 4.46%), 8.71% (calc. 8.92%) and 13.05% (calc. 13.37%) on TG curve [32–34]. After 150 °C, the organic part starts decomposing, giving metal-triazole at 460° with a mass loss of 53.58% on TG curve (calc. 53.99%), as indicated by the DTA curve (Fig. 1). In the temperature range 460–698 °C, all the triazole part decomposed, with the mass loss of 77.08% (calc. 77.53%) and finally formation of NiS took place at 698 °C.

The sequence for thermal degradation of the complex Ni (ApMMT)OAc·$3H_2O$ are given below:

$$\mathrm{Ni\ (ApMMT)OAc.3H_2O} \xrightarrow[-3H_2O]{60-150\,^\circ} \mathrm{Ni}$$

$$\mathrm{(ApMMT)\ OAc} \xrightarrow[-OAc,-Org\ moiety]{150-460\,^\circ} \mathrm{Ni}-s-\mathrm{triazole} \xrightarrow[-s-triazole]{460-698\,^\circ} \mathrm{NiS}$$

The complex Co(ApEMT)OAc·$3H_2O$ loses its all the three water molecules at 60, 102 and 187 °C with the mass loss of 4.13% (Calc. 4.30%), 8.19% (calc. 8.61%) and 12.68% (calc. 12.92%) on TG curve (Fig. 2) [32–34]. At higher temperature, the organic part decomposed. The DTG curve indicated the formation of Ni-*s*-triazole, which was further supported by DTA curve (Fig. 2) at 540 °C and a mass loss on TG curve 51.74% (calc. 52.15%). The formation of end product took place at 760 °C with the decomposition of *s*-triazole part remaining behind CoS with a mass loss of 77.93% (calc. 78.23%).

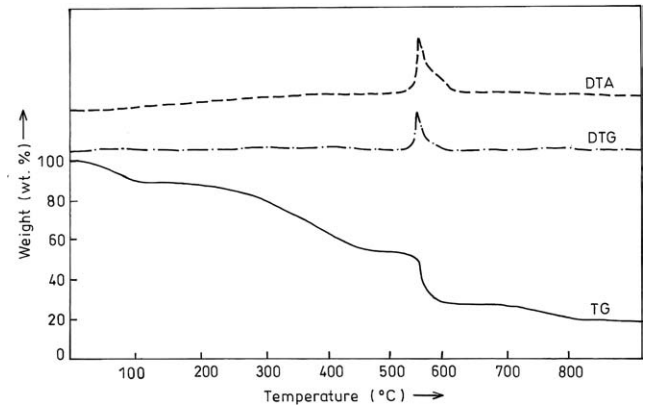

Fig. 2. Thermoanalytical curves of Co (ApEMT) OAc. $3H_2O$.

The sequence of thermal degradation of the complex follows Co(ApEMT)OAc·$3H_2O$ are given below:

$$\mathrm{Co(ApEMT)OAc.3H_2O} \xrightarrow[-3H_2O]{50-190\,^\circ} \mathrm{Co}$$

$$\mathrm{(ApEMT)OAc} \xrightarrow[-OAc,-Org\ moiety]{200-540\,^\circ} \mathrm{Co}-s-\mathrm{triazole} \xrightarrow[-s-triazole]{540-760\,^\circ} \mathrm{COS}$$

The antibacterial activity of 10 synthesized compounds viz. ApMMT ApMMT-Ni (1:1), ApMMT-Ni (1:2), ApMMT-Zn (1:1), ApMMT-Zn (1:2), ApEMT, ApEMT-Co (1:1), ApEMT-Co (1:2), ApEMT-Cu (1:1), and ApEMT-Cu (1:2) were tested in vitro against *B. subtilis*, *E. coli*, *S. dysenterieae* and *Pseudomonas* sp. by reported method [23]. Out of 10 compounds tested, ApEMT-Co (1:1) and ApEMT-Co (1:2) were most active against all the four test bacteria at all the four concentrations (500, 100, 50 and 10 μg ml^{-1}) showing maximum inhibition (Table 1). The compounds ApMMT, ApEMT, ApMMT-Ni (1:1) and ApMMT-Ni (1:2) were also found to be inhibitory at all the four concentrations against *E. coli* and *B. subtilis* but they were completely inactive against *S. dysenterieae* and *Pseudomonas* sp. ApMMT-Zn (1:2) was also found to be inhibitory against *Pseudomonas* sp. and *E. coli* at all the four concentrations while the activity of the compound was low against *B. subtilis* and *S. dysenterieae* at all the concentrations. ApEMT-Cu (1:1) and ApEMT-Cu (1:2) were found completely inactive against all the bacteria. The activities of the compounds were also compared with the three commercial antibiotics (ciprofloxacin, chloramphenicol and streptomycin) and were found to be more potent than commercial antibiotics (Table 1 and Fig. 3).

5. Conclusions

With the help of various physico-chemical techniques, geometries of the newly synthesized compounds have been proposed. Cobalt compounds were found most active and copper compounds were completely inactive against all the test bacteria at all the concentration. The activities of the cobalt compounds were found more potent than some commercial antibiotics.

Due to insolubility in water and common organic solvents, and in fusibility at higher temperatures all the complexes are thought to be polymeric in nature [35,36].

The tentative structures for complexes (1:1) and (1:2) (Fig. 4) are based on elemental analyses, IR, ^{1}H NMR, electronic, magnetic measurements and thermal studies.

6. Experimental protocols

6.1. Chemistry

All the chemicals and solvents were of Anala 'R' grade. The metal contents were estimated using standard methods [37].

Table 1
In vitro antibacterial spectrum of chemically synthesized compounds

Compound	Conc. (μg ml^{-1})	Percentage inhibition			
		B. subtilis	*E. coli*	*S. dysenterieae*	*Pseudomonas* sp.
ApMMT	500	85.66	100	56	Nil
	100	80	100	36.66	Nil
	50	76.44	96.46	Nil	Nil
ApMMT-Ni (1:1)	500	100	87.66	Nil	Nil
	100	88.28	56.66	Nil	Nil
	50	49.45	Nil	Nil	Nil
ApMMT-Ni (1:2)	500	100	66.66	Nil	Nil
	100	78	33.45	Nil	Nil
	50	39.23	Nil	Nil	Nil
ApMMT-Zn (1:1)	500	Nil	Nil	Nil	Nil
	100	Nil	Nil	Nil	Nil
	50	Nil	Nil	Nil	Nil
ApMMT-Zn (1:2)	500	Nil	20	Nil	100
	100	Nil	16.67	Nil	100
	50	Nil	10	Nil	100
ApEMT	500	100	100	Nil	Nil
	100	100	100	Nil	Nil
	50	86.44	100	Nil	Nil
	10	69.99	81.04	Nil	Nil
ApEMT-Co (1:1)	500	100	100	100	100
	100	100	100	100	100
	50	89.66	91.22	88.00	100
	10	57.66	61.21	78.00	79.43
ApEMT-Co (1:2)	500	100	100	98.33	100
	100	86.67	100	91.32	97.31
	50	83.34	93.21	86.66	90.44
	10	57	68.87	59.00	69.36
ApEMT-Cu (1:1)	500	Nil	Nil	Nil	Nil
	100	Nil	Nil	Nil	Nil
	50	Nil	Nil	Nil	Nil
ApEMT-Cu (1:2)	500	Nil	Nil	Nil	Nil
	100	Nil	Nil	Nil	Nil
	50	Nil	Nil	Nil	Nil
Ciprofloxacin	500	86.96	89.29	93.85	100
	10	51.67	48.58	45.72	57.32
Chloramphenicol	500	94.08	88.47	92.43	85.22
	10	46.67	68.74	53.13	55.00
Streptomycin	500	91.9	94.62	84.35	89.7
	10	56.34	54.00	49.24	63.12

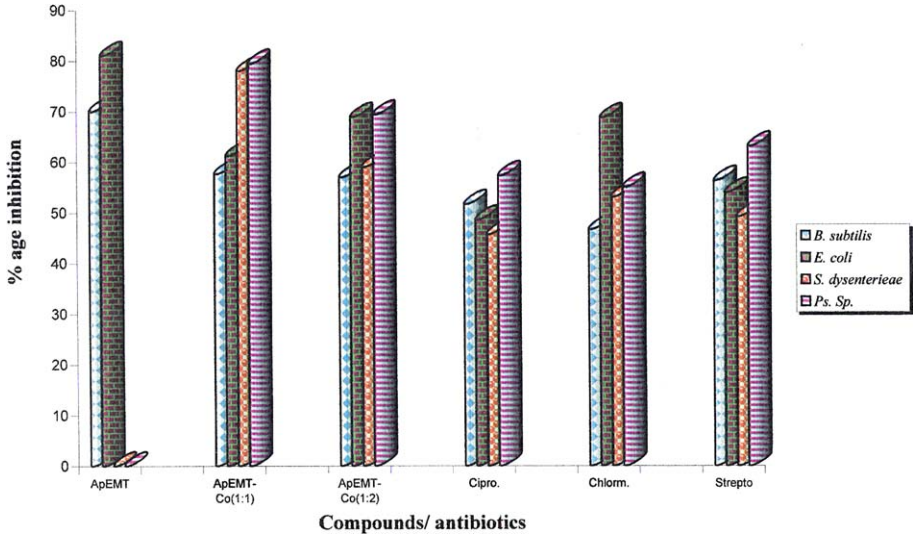

Fig. 3. In vitro antibacterial spectrum of chemically synthesized compounds at conc. 10 μg/ml.

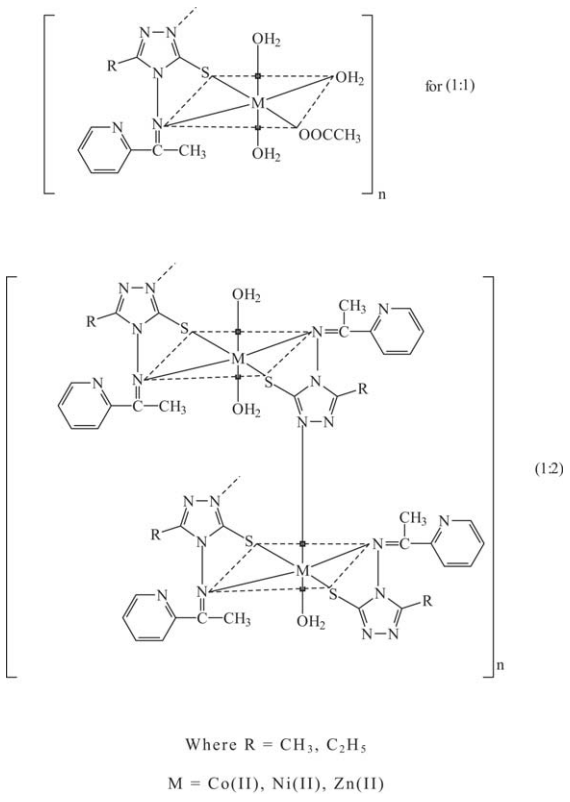

Fig. 4.

The medium used for screening of compounds for antibacterial activities was nutrient agar medium (NAM) whose composition is as follows: peptone - 5.0 g; beef extract - 3.0 g; NaCl - 5.0 g; Agar–Agar - 18.0 g; distilled water - 1000 ml; pH - 7.3.

Melting points were determined in open capillaries in electrical melting point apparatus.

Analyses indicated by CHN were within ± 0.4% of the theoretical values.

Electronic spectra of metal complexes were recorded in the region 1100–200 nm on a Hitachi U-2000 spectrophotometer.

IR Spectra were recorded in Beckman IR-20 spectrophotometer in KBr/Nujol mull in the range 4000–250 cm^{-1}.

Proton NMR spectra were recorded in DMSO-d_6 on a Brucker ACF 300 spectrometer at 300 MHz reference to Me_4Si (0.0 ppm).

Magnetic moments were measured at USIC, IIT, Roorkee, on vibrating sample magnetometer (model 155).

Thermal analyses of metal complexes were carried out in atmospheric air using a Perkin Elmer (Pyris Diamond) Instrument reference to Alumina Powder at USIC, IIT, Roorkee.

6.2. Biological evaluation

In vitro antibacterial activities were done by using spread-plate method. NAM adjusted to pH 7.0 was used for this purpose. Twenty milliliters sterilized NAM was poured to 90 mm sterilized and labelled petri plates. After solidification, 0.1 ml aliquot of each culture having dilution 10^{-3} was spread on the surface of agar plates with the help of sterilized spreader, all inoculated plates were incubated at 35 °C for 24 h in an inverted positions. Results were recorded by counting the bacterial colony of test and control plates.

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