Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 394–402
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and characterization of cobalt(II), nickel(II), copper(II) and zinc(II) complexes with Schiff base derived from 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine ☆

Kiran Singh [a,*], Manjeet Singh Barwa [a], Parikshit Tyagi [b]

[a] *Department of Chemistry, Kurukshetra University, Kurukshetra-136119, Haryana, India*
[b] *Department of Microbiology, Kurukshetra University, Kurukshetra-136119, Haryana, India*

Received 17 May 2006; received in revised form 25 September 2006; accepted 19 October 2006
Available online 16 January 2007

Abstract

A few (1:1) and (1:2) metal complexes of cobalt(II), nickel(II), copper(II) and zinc(II) have been isolated with ligand derived from the condensation of 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine with 2-acetylpyridine (L^1) and characterized by elemental analysis, conductivity measurements, infrared, electronic, ^{1}H NMR spectral data, magnetic and thermogravimetric analyses. Due to insolubility in water and most of the common organic solvents and infusibility at higher temperatures, all the complexes are thought to be polymeric in nature. A square-planar geometry was suggested for copper(II) and octahedral proposed for cobalt(II), nickel(II) and zinc(II). Some of the chemically synthesized compounds have been screened *in vitro* against the three Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*) and two Gram-negative (*Salmonella typhi* and *Escherichia coli*) organisms. It is observed that the coordination of metal ion has pronounced effect on the microbial activities of the ligand. The metal complexes have higher antimicrobial effect than the free ligands.

Keywords: 2-Acetylpyridine; *as*-Triazine; Transition metal complexes; Antibacterial activity; ^{1}H NMR

1. Introduction

Metal complexes of N and S chelating ligands have attracted considerable attention [1] because of their interesting physicochemical properties, pronounced biological activities and as models of the metalloenzyme active sites. It is well known that N and S atoms play a key role in the coordination of metals at the active sites of numerous metallobiomolecules. Triazine derivatives [2] are known to possess antitubercular, antibacterial, fungicidal, hypotensive and hypothermic activities. Vancomycin has been a drug of last report for the treatment of MDR. But the recent emergence of vancomycin-resistant enterococci (VRE) and vancomycin-intermediate resistant *Staphylococcus aureus* (VISA) is raising serious public health concern [3]. A number of Schiff bases and their transition metal complexes have been investigated by various techniques for different purposes (Figs. 1 and 2). Schiff-base metal complexes [4–7] have been widely studied because they have industrial, antifungal, antibacterial, anticancer and herbicidal applications. They serve as models for biologically important species and find applications in biomimetic catalytic reactions. Chelating ligands containing N and S as donor atoms [8,9] show broad biological activity [10–13] and are of special interest because of the variety of

Abbreviations: B. subtilis, *Bacillus subtilis*; CFU, colony forming unit; MHA, Muller Hinton agar; MTCC, Microbial Type Culture Collection and Gene Bank; MIC, minimum inhibitory concentration; *S. typhi*, *Salmonella typhi*; SCDA, soyabean casein digest agar; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*.

☆ The paper was presented at 93rd session of the Indian Science Congress, Hyderabad, no. 15, 2006.

* Corresponding author.
E-mail address: manjeetbarwa@rediffmail.com (M.S. Barwa).

0223-5234/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.10.016

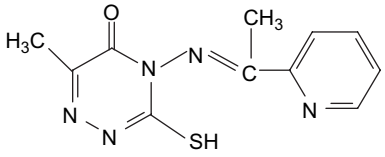


Fig. 1. Structure of Schiff base.

ways in which they are bonded to metal ions [14]. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities. Our ongoing research has established that non-biologically active compounds become biologically active and less biologically active compounds become more active upon coordination/chelation with metal ions.

Numerous compounds containing 1,2,4-triazines moiety [15,16] are well known in natural materials and show interesting biological and antiviral properties. Many derivatives of 1,2,4-triazine compounds form colored complexes with different metal ions and can be used as analytical reagent for their determinations. In the preceding communication complex formation of cobalt(II), nickel(II), copper(II) and zinc(II) ions with 4-(2-acetylpyridenylideneamino)-3-mercapto-6-methyl-5-oxo-1,2,4-triazine(L^1) (Fig. 1), which contain nitrogen and sulfur donor atoms [17], was investigated using different physicochemical techniques. Some compounds have been screened against various pathogenic bacterial strains of *S. aureus* MTCC 3160, *Staphylococcus epidermidis* MTCC 2639, *Bacillus subtilis* MTCC 121, *Salmonella typhi* MTCC 733 and *Escherichia coli* MTCC 51 by minimum inhibitory concentration method [18].

2. Chemistry

2.1. Methods

2.1.1. Synthesis of ligand

4-Amino-3-mercapto-6-methyl-5-oxo-1,2,4-*as*-triazine (AMMOT) was prepared by reported procedure [19]. Schiff base namely, 4-(2-acetylpyridenylideneamino)-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (L)(ApMMOT) was prepared by refluxing a mixture of equimolar quantities of the *as*-triazine (AMMOT) and the corresponding ketone, namely, 2-acetylpyridine, by using absolute ethanol as a solvent. After 4 h of refluxing, the light brown reaction mixture was kept at room temperature overnight and the product was filtered and recrystallized from the same solvent.

2.1.2. Synthesis of metal(II) complexes

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

3. Pharmacology

3.1. In vitro antibacterial assay

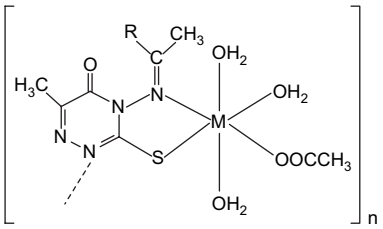
The newly synthesized ligand and their corresponding metal(II) chelates were screened *in vitro* for their antibacterial activity against above mentioned bacteria using minimum inhibitory concentration (MIC) method [18]. MIC is the lowest concentration of the antimicrobial agents that prevents the development of visible growth of microorganism after overnight incubation. MIC of chemically synthesized compounds against test bacteria namely *S. aureus* (MTCC 3160), *S. epidermidis* (MTCC 2639), *B. subtilis* (MTCC 121), *Sa. typhi* (MTCC 733) and *E. coli* (MTCC 51) was determined by reported methods [20]. All the test culture were streaked on SCDA and incubated overnight at 37 °C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland standard [21] by preparing bacterial suspension of 3–5 well-isolated colonies of same morphological type selected from a SCDA plate culture. The cultures were further diluted to 10-fold to get inoculum size of 1.2×10^7 CFU/ml. A stock solution of 4 mg/ml of each compound was prepared in DMSO and was appropriately diluted to get final concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, and 0.03 μg/ml. Standard antibiotics (cefaclor and cefuroxime axetil) were also diluted in same manner. Each dilution of 320 μl was added to 20 ml molten and cooled MHA (separate flasks were taken for each dilution). After thorough mixing, the medium was poured into sterilized Petri plates. The test bacterial cultures were spotted in a predefined pattern by aseptically transferring 5 μl of each bacterial culture on the surface of solidified agar–agar plates and incubated at 35 °C for 24 h.

4. Results and discussions

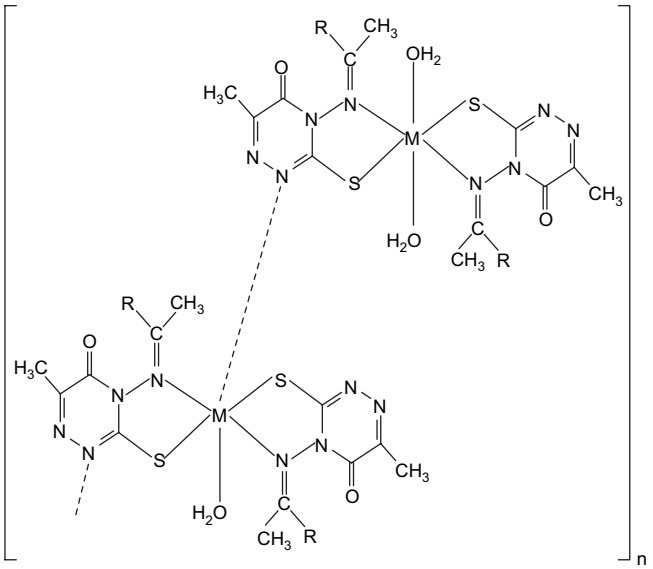
Infrared spectral data have been interpreted based on literature data reported. IR spectra of the ligand and its metal complexes are reported in Tables 1 and 3, shows a characteristic band due to ν(N–H) at 3220 cm^{-1} and a strong band at 1125 cm^{-1} is due to ν(C=S) [22] in the ligand, ApMMOT. However, as expected, in the metal complexes a new band was observed in the region 780–740 cm^{-1} for ν(C–S) with the disappearance of the band ν(C=S). Metal–sulfur bond formation further supported the confirmation of the coordination of the metal towards sulfur, by a band in the region 380–340 cm^{-1} in the far IR spectra [22].

The presence of coordinated water [22] molecules in the metal complexes is indicated by a broad trough band in the region 3600–3000 cm^{-1}. A strong band in the region 2100–1990 cm^{-1} has been assigned to $\nu(OOCCH_3)$ in the (1:1) (metal/ligand) complexes.

A strong band at 1601 cm^{-1} in the free ligand was assigned to the azomethine frequency $\nu(N{=}C{-}CH_3)$ which shifts [23–28] towards ±5–10 cm^{-1} in the spectra of metal complexes indicating that coordination through nitrogen atom is due to the reduction of double bond character of carbon–nitrogen bond of the azomethine group. Formation of metal–nitrogen

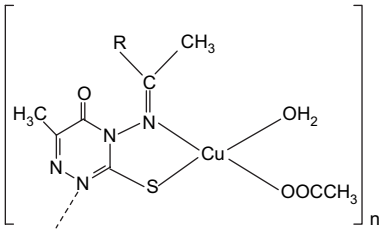


For (1:1)

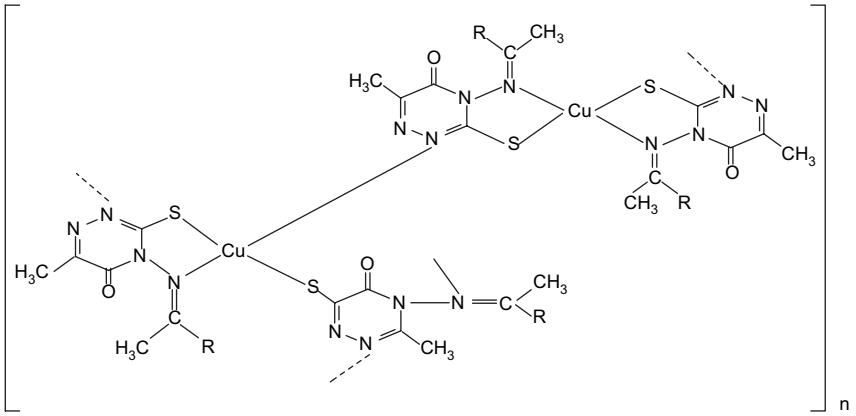


For (1:2)

M = Co(II), Ni(II) and Zn(II) for (1:1) and (1:2)



For (1:1)



For (1:2)

Where R = 

Fig. 2.

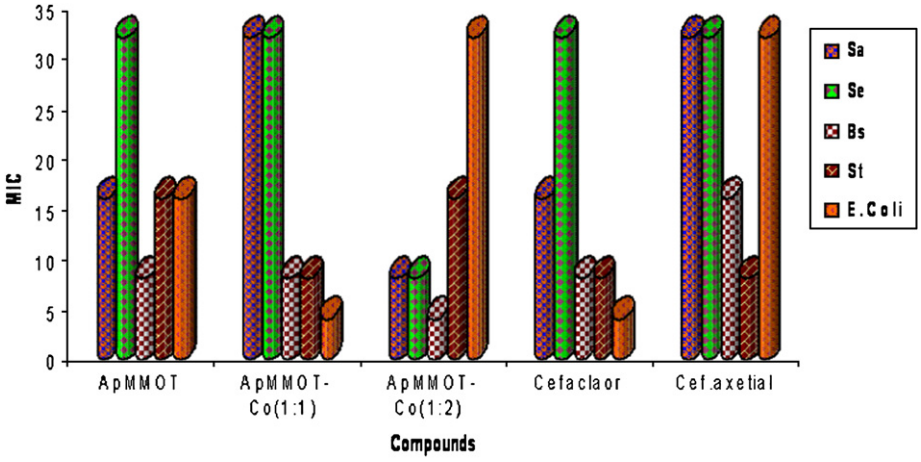


Fig. 3. Comparison of MIC of three compounds with commercial antibiotics.

bond is further supported by the appearance of a band in the region 540–480 cm^{-1} in far IR spectra. The free ligand exhibit a band at 1665 cm^{-1}, assigned to ν(C=O) [2] at the 5-position of the six-membered triazine ring. In the metal derivatives, no significant change was observed, indicating that the carbonyl oxygen may not participate in the coordination.

The above discussion indicate that the nature of ApMMOT is bidentate and shows the corrdination through N and S atoms to the metal.

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

The Co(II) complexes, ApMMOT–Co (1:1) and ApMMOT–Co (1:2) showed bands at 1007, 1007 nm and 604, 603 nm for ν_1 and ν_3, respectively. They exhibit $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(\nu_1)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)(\nu_3)$ transitions, respectively, and are suggestive of octahedral geometry [29,30] around the cobalt ions.

The Nickel complexes ApMMOT–Ni (1:1) and ApMMOT–Ni (1:2) showed bands at 1007, 1006; 600, 602 and 434, 427 nm for ν_1, ν_2 and ν_3, respectively. These bands are assigned to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(\nu_1)$; $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(\nu_2)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(\nu_3)$, respectively, consistent with their well-defined octahedral configuration [29,30].

The copper complexes namely ApMMOT–Cu (1:1) and ApMMOT–Cu (1:2) showed bands at 501 and 500 nm, respectively. This band assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition, a characteristic band of square-planar geometry [29,30].

Magnetic susceptibility measurements of the complexes ApMMOT–Co (1:1), ApMMOT–Co (1:2), ApMMOT–Ni (1:1), ApMMOT–Ni (1:2), ApMMOT–Cu (1:1) and ApMMOT–Cu (1:2) give magnetic moment values (Table 2) μ_{eff} of 4.7, 4.4, 3.2, 2.9, 1.8 and 1.9 BM, respectively, at room temperature. The magnetic measurements show the presence of three, two and one unpaired electrons in Co(II), Ni(II) and Cu(II) complexes, respectively, with their octahedral and square-planar environment [31].

In NMR spectra of complexes we observed a shift of electron density from the ligand to the metal. The signal of azomethine protons de-shielded [4,32] in the spectra of metal complexes was found to occur at 2.35 and 2.38 ppm, as compared to its Schiff base at 2.20 ppm after complexations to the metal ion inferring coordination through azomethine nitrogen atom of the ligands. Disappearance of –S*H* protons in the spectra of complexes supported the deprotonation of the thiol group.

The DTG curve of Co(ApMMOT)OAc·$3H_2O$ showed that the three water molecules were lost at 96, 185 and 235 °C corresponding to the mass loss of 3.85% (calc. 4.16%), 8.07% (calc. 8.33%) and 12.24% (calc. 12.50%) on TG curve (Fig. 4). After 235 °C, the organic part started decomposing, giving metal–triazine at 397 °C with a mass loss of 53.44% on TG curve (calc. 53.70%), as indicated by the DTA curve [14,33] In the temperature range 397–424 °C, all the triazine part decomposed with the mass loss of 78.93% (calc. 78.66%) and finally formation of CoS took place at 424 °C.

Table 1
Spectral and analytical data of Schiff bases

Schiff base	M. P. (°C)	IR (cm^{-1})	^{1}H NMR (DMSO-d_6) (ppm)	Calc. (found) %			Yield (%)
				C	H	N	
L(ApMMOT) $C_{11}H_{11}N_5OS$ [261.4]	194–196	3220, 1665, 1601, 1125	13.7 (s, 1H, SH), 7.2–8.8 (m, 4H, Aromatic-*H*), 2.4 (s, 3H, triazine-*H*), 2.20 (s, 3H, CH_3–C=N)	50.57 (50.23)	4.21 (3.89)	26.82 (26.46)	70

Table 2
Physical and analytical data of the metal complexes

S. no.	Metal complex, mol. weight, mol. formula	M.P. (°C) (d)	B.M. (μ_{eff})	Calc. (found)%				Yield (%)
				C	H	N	M	
1.	Co(ApMMOT)OAc·3H_2O, [432.1], $C_{13}H_{19}N_5CoO_6S$	>260	4.7	36.11(35.83)	4.40(4.12)	16.21(15.91)	13.66(13.30)	77
2.	Ni(ApMMOT)OAc·3H_2O, [432.4], $C_{13}H_{19}N_5NiO_6S$	>280	3.2	36.11(36.37)	4.40(4.24)	16.20(15.87)	13.58(13.22)	87
3.	Cu(ApMMOT)OAc·H_2O, [400.8], $C_{13}H_{15}N_5ZnO_4S$	>260	1.8	38.90(38.65)	3.74(3.21)	17.46(17.13)	15.84(15.70)	74
4.	Zn(ApMMOT)OAc·3H_2O, [438.2], $C_{13}H_{19}N_5ZnO_6S$	>232	–	35.62(35.43)	4.34(4.13)	15.98(15.75)	14.93(14.72)	79
5.	Co(ApMMOT)$_2$·2H_2O, [614.7], $C_{22}H_{24}N_{10}NiO_4S_2$	>260	4.4	42.93(42.73)	3.90(3.59)	22.76(22.69)	9.59(10.02)	80
6.	Ni(ApMMOT)$_2$·2H_2O, [615.3], $C_{22}H_{24}N_{10}NiO_4S_2$	>276	2.9	42.93(42.69)	3.90(3.74)	22.76(22.54)	9.54(9.33)	84
7.	Cu(ApMMOT)$_2$, [583.9], $C_{22}H_{24}N_{10}CuO_2S_2$	>270	1.9	45.21(44.98)	3.42(3.06)	23.97(23.66)	10.88(10.59)	76
8.	Zn(ApMMOT)$_2$·2H_2O, [621.1], $C_{22}H_{24}N_{10}ZnO_4S_2$	>284	–	42.51(42.42)	3.86(3.71)	22.54(22.09)	10.53(10.17)	77

The sequence for thermal degradation of the complex Co(ApMMOT)OAc·3H_2O are given below:

$$\mathrm{Co(ApMMOT)OAc\cdot 3H_2O} \xrightarrow[-3\mathrm{H_2O}]{96-235^\circ\mathrm{C}} \mathrm{Co(ApMMOT)OAc} \xrightarrow[-\mathrm{OAc},\ -\text{org moiety}]{235-397^\circ\mathrm{C}} \mathrm{Co-}\textit{as}\mathrm{-triazine} \xrightarrow[-\textit{as}-\text{triazine}]{397-424^\circ\mathrm{C}} \mathrm{CoS}$$

The complex Co(ApMMOT)$_2$·2H_2O lost its two water molecules at 96 and 164 °C with the mass loss of 2.87% (calc. 3.08%) and 5.89%(calc. 6.17%) on the TG curve. At higher temperature, the organic part decomposed. The DTG curve (Fig. 5) indicated the formation of Co-*as*-triazine, which was further supported by the DTA curve in the temperature region 164–360 °C and a mass loss of 42.97% (calc. 43.22%) on TG curve. The first part of the triazine lost at 672 °C with a mass loss of 67.23% (calc. 67.40%) and the decomposition of the second part of the triazine, left behind CoS at 900 °C with a mass loss of 85.88% (calc. 86.10%) on the TG curve.

The sequence for thermal degradation of the complex Co(ApMMOT)$_2$·2H_2O are given below:

$$\mathrm{Co(ApMMOT)_2\cdot 2H_2O} \xrightarrow[-2\mathrm{H_2O}]{96-164^\circ\mathrm{C}} \mathrm{Co(ApMMOT)_2} \xrightarrow[-\text{org moiety}]{164-360^\circ\mathrm{C}} \mathrm{Co-}(\textit{as}-\text{triazine})_2 \xrightarrow[(-\textit{as}-\text{triazine})_2]{360-900^\circ\mathrm{C}} \mathrm{CoS}$$

Table 3
Spectral data of metal complexes

S. no.	IR (cm^{-1})	λ_{max} (nm)	^{1}H NMR (DMSO-d_6) (ppm)
1.	3600–3000, 2073, 1683, 1600, 771, 505, 344	1007, 604	–
2.	3600–3200, 1994, 1679, 1601, 776, 483, 362	1007, 600, 434	–
3.	3600–3200, 2100, 1667, 1601, 770, 519, 372	501	–
4.	3550–3100, 2084, 1661, 1589, 780, 527, 351	–	7.3–8.7 (m, 4H, Aromatic-*H*), 2.4 (s, 3H, triazine-*H*), 2.35 (s, 3H, $-CH_3-C{=}N$)
5.	3600–3000, 1679, 1607, 770, 491, 359	1007, 603	–
6.	3600–3100, 1668, 1604, 767, 498, 375	1006, 602, 427	–
7.	1685, 1597, 775, 514, 364	500	–
8.	3500–3100, 1666, 1599, 780, 531, 333	–	7.4–8.5 (m, 4H, Aromatic-*H*), 2.4 (s, 3H, triazine-*H*), 2.38 (s, 3H, $-CH_3-C{=}N$)

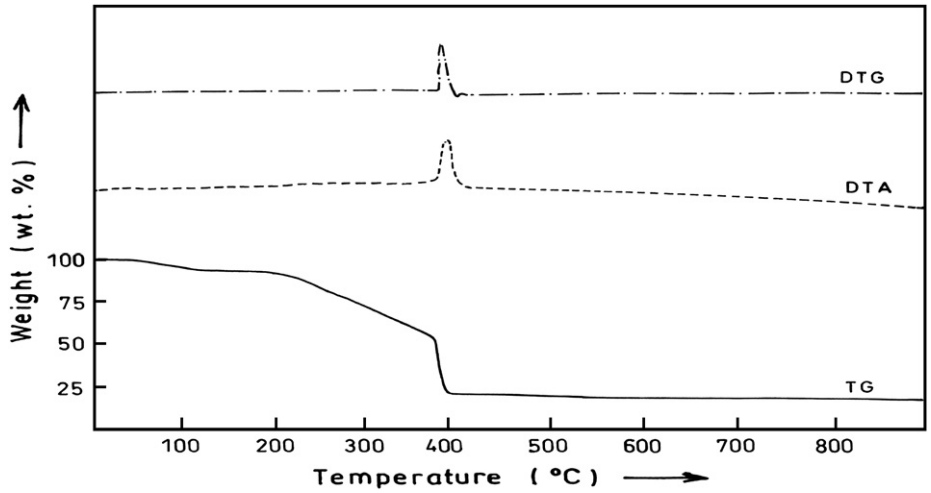


Fig. 4. Thermoanalytical curves of Co(ApMMOT)OAc·3H_2O.

The antibacterial activity of the three synthesized compounds *viz*. ApMMOT and its Co (1:1) and Co (1:2) complexes have been tested *in vitro* against *S. aureus*, *S. epidermidis*, *B. subtilis*, *Sa. typhi* and *E. coli* by reported method. ApMMOT–Co (1:2) was found most active against *S. aureus*, *S. epidermidis* and *B. subtilis* at MIC 8, 8 and 4 μg/ml, respectively. The complex of ApMMOT–Co (1:1) was also found to be inhibitory at concentrations 8 and 4 μg/ml against *Sa. typhi* and *E. coli*. The antibacterial results evidently show that the activity of the ligand became more pronounced and significant when coordinated to the metal ions [34]. This enhancement in activity may be due to an efficient diffusion of the metal complexes in to the bacterial cell and/or interaction with the bacterial cell [35]. But at remaining concentrations ApMMOT–Co (1:2) against *S. aureus*, *S. epidermidis* and *B. subtilis* and ApMMOT–Co (1:2) against *Sa. typhi* and *E. coli* are found less potent/equal as compared to ligand ApMMOT. The activities of these two cobalt complexes were also compared (Fig. 3) with two commercial antibiotics (cefaclor and cef. axetil). In some cases the results were found to be more potent than the standard antibiotics (Table 4).

5. Conclusions

Activities of the cobalt complexes were found more potent than the ligand itself and two commercial antibiotics namely, cefaclor and cef. axetil at some concentrations which have been mentioned in Table 4.

With the help of various physicochemical techniques, geometries of the newly synthesized compounds have been proposed.

Due to insolubility in water and common organic solvents, and infusibility at higher temperatures all the complexes are thought to be polymeric in nature [2,4,5].

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

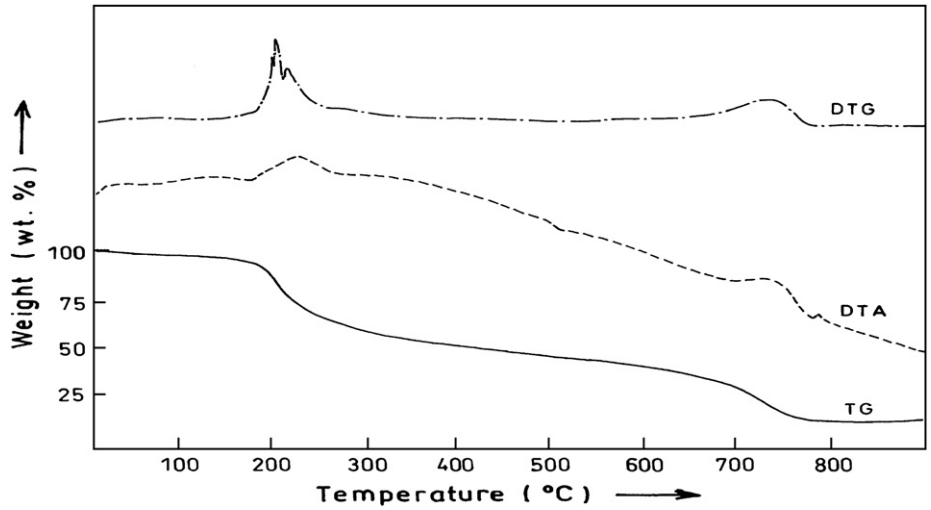


Fig. 5. Thermoanalytical curves of Co(ApMMOT)$_2$·2H_2O.

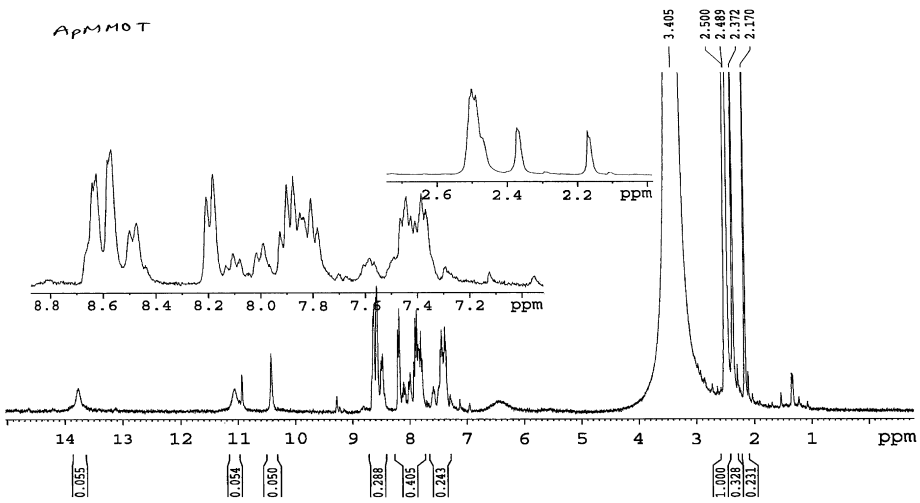


Fig. 6. PMR spectra of the Schiff base, ApMMOT.

6. Experimental protocols

6.1. Chemistry

Reagents used: hydrazine hydrate, carbon disulphide, pyruvic acid, 2-acetylpyridine, cobalt acetate, nickel acetate, copper acetate, zinc acetate, ethanol, dimethyl sulfoxide, acetone, diethyl ether, DMSO-d_6. All the chemicals and solvents used were of Anala 'R' grade.

The metal contents were estimated using standard methods, gravimetric methods, cobalt was estimated as cobalt pyridine thiocyanate, nickel as nickel dimethylglyoximate, copper as cuprous thiocyanate and zinc as zinc ammonium phosphate [36].

Two solid media namely Muller–Hinton agar (MHA; beef infusion 300 g/l, casein acid hydrolysate 17.5 g/l, starch 1.5 g/l, agar–agar 17 g/l and distilled water 1000 ml, adjusted to pH 7.4) and soyabean casein digest agar (SCDA; casein enzymatic hydrolystate 17.0 g/l, papain digest of soyabean 3.0 g/l, NaCl 5.0 g/l, dipotassium phosphate 2.5 g/l, dextrose 2.5 g/l, distilled water 1000 ml, adjusted to pH 7.3) were used for the biological assays.

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

Analyses indicated by CHN were within ±0.4% of the theoretical values on elemental analyzer, Perkin–Elmer 2400, at Punjab University, Chandigarh.

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) (Figs. 6–8).

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

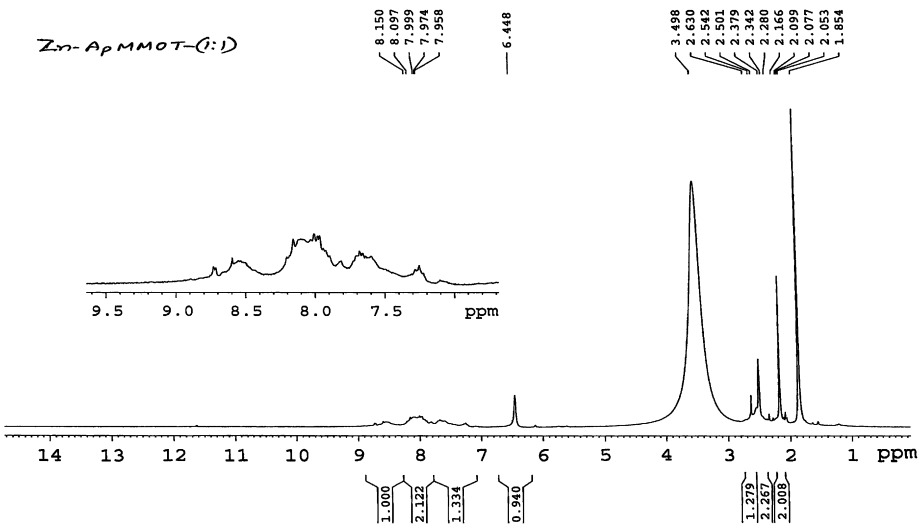


Fig. 7. PMR spectra of the metal complex, Zn(ApMMOT)OAc·$3H_2O$.

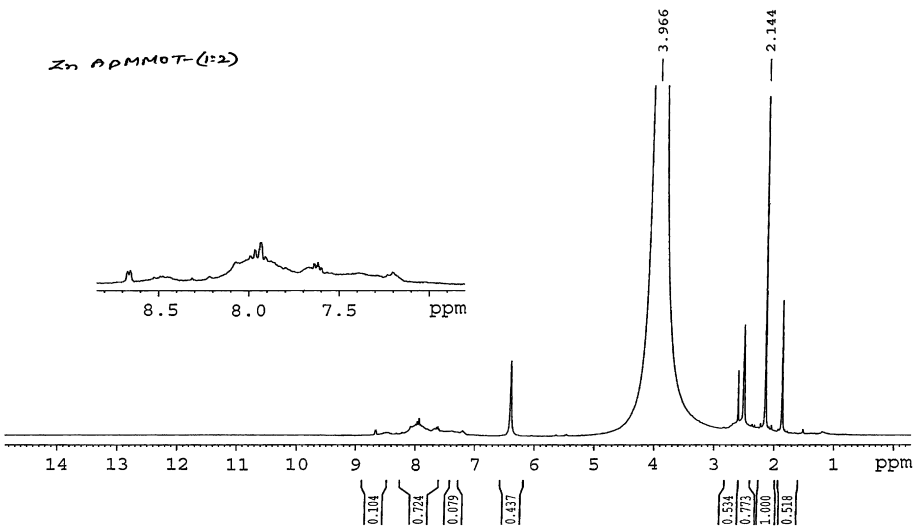


Fig. 8. PMR spectra of the metal complex, $Zn(ApMMOT)_2 \cdot 2H_2O$.

Thermal analyses of metal complexes were carried out in atmospheric air using a Perkin–Elmer (Pyris Diamond) instrument reference to alumina powder at IIC, IIT, Roorkee.

6.2. Biological evaluation

In vitro antibacterial activities were done by using minimum inhibitory concentration method. The medium (MHA and SCDA) adjusted to pH 7.0 was used for this purpose. All the test culture were streaked on SCDA and incubated overnight at 37 °C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland standard by preparing bacterial suspension of 3–5 well-isolated colonies of same morphological type selected from a SCDA plate culture. The cultures were further diluted to 10-fold to get inoculum size of 1.2×10^7 CFU/ml. A stock solution of 4 mg/ml of each compound was prepared in DMSO and was appropriately diluted to get final concentrations. Each dilution of 320 μl was added to 20 ml molten and cooled MHA. After thorough mixing, the medium was poured into sterilized Petri plates. The test bacterial cultures were spotted in a predefined pattern by aseptically transferring 5 μl of each bacterial culture on the surface of solidified agar–agar plates and incubated at 35 °C for 24 h and results were recorded.

Table 4
Minimum inhibitory concentration (MIC) of three compounds against test microorganism by using agar dilution assay technique

Compound	Sa	Se	Bs	St	*E. coli*
L^1	16	32	8	16	16
1	32	32	8	8	4
5	8	8	4	16	32
Cefaclor	16	32	8	8	4
Cef. axetil	32	32	16	8	32

Sa – *S. aureus* (MTCC 3160), Se – *S. epidermidis* (MTCC 2639), Bs – *B. subtilis* (MTCC 121), St – *S. typhi* (MTCC 733) and *E. coli* – *Escherichia coli* (MTCC 51).

Acknowledgement

This investigation received financial support from the University Grant Commission, New Delhi, No. F.12-47/2003(SR) UGC provided Project Fellowship to one of the authors (M.S.B.).

References

[1] Z.H. Chohan, M.A. Farooq, A. Scozzafava, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 17 (1) (2002) 1–7.
[2] G. Singh, Ph.A. Singh, A.K. Sen, K. Singh, S.N. Dubey, R.N. Handa, J. Choi, Synth. React. Inorg. Met. Org. Chem. 32 (1) (2002) 171–187.
[3] Y. Sugie, H. Hirai, T. Inagaki, M. Ishiguro, Y.J. Kim, Y. Kojima, T. Sakakibara, S. Sakemi, A. Sugiura, Y. Suzuki, L. Brennan, J. Duignan, L.H. Huang, J. Sutcliffe, N.J. Kojima, J. Antibiot. 54 (2001) 911–916.
[4] K. Singh, M.S. Barwa, P. Tyagi, Eur. J. Med. Chem. 41 (1) (2006) 147–153.
[5] K. Singh, D.P. Singh, M.S. Barwa, P. Tyagi, Y. Mirza, J. Enzyme Inhib, Med. Chem. 21 (5) (2006) 557–562.
[6] P.G. Cozzi, Chem. Soc. Rev. 33 (2004) 410–421.
[7] A.A. Jarrahpour, M. Zarei, Molbank M377, 2004.
[8] S. Chandra, J. Sangeetika, J. Indian Chem. Soc. 81 (2004) 203–206.
[9] P.G. More, R.B. Bhalvankar, J. Indian Chem. Soc. 81 (2004) 13–17.
[10] M. Yildiz, B. Dulger, S.Y. Koyuncu, B.M. Yapici, J. Indian Chem. Soc. 81 (2004) 7–12.
[11] M.S. Islam, M.A. Farooque, M.A.K. Bodruddoza, M.A. Mosaddik, M.S. Alam, J. Biol. Sci. (OnLine) 2 (12) (2002) 797–799.
[12] M.B. Ferrari, S. Capacchi, G. Pelosi, G. Reffo, P. Tarasconi, R. Albertini, S. Pinelli, P. Lunghi, Inorg. Chim. Acta 286 (2) (1999) 134–141.
[13] Z.H. Chohan, H. Pervez, K.M. Khan, A. Rauf, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 19 (1) (2004) 51–56.
[14] E. Canpolat, M. Kaya, J. Coord. Chem. 57 (14) (2004) 1217–1223.
[15] Z.H. Chohan, H. Pervez, A. Rauf, K.M. Khan, G.M. Maharvi, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 19 (2) (2004) 161–168.
[16] M. Mashaly, H.A. Bayoumi, A. Taha, Chem. Papers Chem. Zvesti. 53 (5) (1999) 299–308.
[17] V.I. Cesljevic, V.M. Leovac, P.N. Radivojsa, B. Gordic, G. Argay, J. Serb. Chem. Soc. 63 (12) (1998) 967–977.
[18] D. Greenwood, R. Slack, J. Peutherer, Medical Microbiology: A Guide to Microbial Infections: Pathogensis, Immunity, Laboratory Diagnosis and Control, 15th ed. (1997).

[19] A. Dornow, H. Menzel, P. Marx, Chem. Ber. 97 (1954) 2173.
[20] NCCLS, Method for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically Approved Standard, fifth ed. National Committee for Clinical Laboratory Standards, Villanova, PA, 2000.
[21] J. McFarland, J. Am. Med. Assoc. 14 (1907) 1176–1178.
[22] A. Kumar, G. Singh, Ph.A. Singh, K. Singh, R.N. Handa, S.N. Dubey, P.J. Squattrito, Proc. Natl. Acad. Sci. (India) 72A (II) (2002) 87–94.
[23] S. Gaur, B. Sharma, J. Indian Chem. Soc. 80 (2003) 841–842.
[24] K.B. Gudasi, P.B. Maravalli, T.R. Goudar, J. Serb. Chem. Soc. 70 (4) (2005) 643–650.
[25] B.D. Sharma, J.C. Bailar, J. Am. Chem. Soc. 77 (1955) 5476–5480.
[26] L.J. Bellamy, The Infrared Spectra of Complex Molecules, third ed. Methuen, London, 1996.
[27] R.M. Silverstein, G. Bassler, Morril, Spectrometric Identification of Organic Compounds, fifth ed. John Wiley and Sons, New York, 1991.
[28] K. Nakamoto, Infrared Spectra of Inorganic and Coordination Compounds Part B, fifth ed. Wiley Interscience, New York, 1997.
[29] A.B.P. Lever, Inorganic Electronic Spectroscopy, Elsevier, Amsterdam, 1984; M.H. Klingele, S. Brooker, Coord. Chem. Rev. 241 (1–2) (2003) 119–132.
[30] S.M.E. Khalil, Chem. Paper Chem. Zvesti. 54 (1) (2000) 12–18.
[31] C.J. Ballhausen, An Introduction to Ligand Field Theory, third ed. McGraw Hill, New York, 1962.
[32] B.S. Jhaumeer-Laulloo, M.G. Bhowon, Indian J. Chem. 42A (2003) 2536–2540.
[33] S.A. Sallam, Transit. Met. Chem. 30 (2005) 341–351.
[34] K. Singh, D.P. Singh, M.S. Barwa, P. Tyagi, Y. Mirza, J. Enzyme Inhib. Med. Chem., in press.
[35] Z.H. Chohan, H. Pervez, A. Rauf, A. Scozzafava, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 17 (2) (2002) 117–122.
[36] A.I. Vogel, A Text Book of Quantitative Chemical Analysis, fifth ed. Addison Wesley Longman, London, 1999.