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Mechanochemical Synthesis of Biologically Active Transition Metal Complexes of 1*H*-4,4,6-Trimethyl-3,4-dihydropyrimidine-2-thione

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A new series of metal complexes using 1*H*-4,4,6-trimethyl-3,4-dihydro-2-pyrimidinethione (HTMPT) ligand were synthesized mechanochemically and also evaluated for antibacterial and DNA photocleavage potential. Complexes of 1*H*-4,4,6-trimethyl-3,4-dihydro-2-pyrimidinethione (HTMPT) $[M(tmpt)_2(H_2O)_n]$ (M = Cu^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}; n = 2 and M = Zn^{2+}, Cd^{2+}, Pd^{2+}; n = 0) were synthesized in 1:2 metal ligand ratio. All the metal complexes were characterized by ^{1}H NMR, FTIR, UV-vis, ESI-mass spectrometry, molar conductivity, magnetic and thermal measurements. ^{1}H NMR and IR results showed that the coordination occurs *via* N and S forming four-membered cyclic ring. Mass spectrum showed the formation of 1:2 metals to ligand stoichiometry. The antibacterial activity of synthesized compounds was evaluated by agar well diffusion method. All the synthesized compounds were evaluated for their DNA nicking activity and found most of metal complexes exhibited good DNA photocleavage activities.

Keywords: Transition metal(II) complexes, 2-Pyrimidinethione, Biological activity, Thermal study.

INTRODUCTION

Resistance to antimicrobial agents is a serious problem these days, it is the need of the time to develop new classes of antibiotics and some biologically active compounds, which ultimately would target the pathogens life cycle and lipoid layer of the organisms. We are focusing on potential metal complexes, their design and synthesis of having such biological activities [1-3].

Pyrimidine and its derivative are such active components of antibiotics, antimicrobials, anticonvulsants, antispasmatics, antineoplastics (*e.g.* bleomycin) and antidiabetogenics. Number of these derivatives have been used in veterinary drugs, crop-disease control and seed dressing [4-7], antibacterial [8-10], antifungal [11,12], antileishmanial [13], anti-inflammatory [14,15], analgesic [16], antihypertensive [17], antipyretic [18], antiviral [19,20], antidiabetic [21], antiallergic [22], anti-convulsant [23], antioxidant [24,25], antihistaminic [26], herbicidal [27], anticancer activities [28,29] and many of pyrimidine derivatives are reported to possess potential central nervous system (CNS) depressant properties [30] and also act as calcium channel blockers [31].

Interesting and stimulating findings on transition metal complexes of pyrimidine-2-thione lead us to a discovery of an arsenal of bioclinical functions by this synthetic agent. In this paper, an attempt were made to synthesize various transition metal(II) complexes of pyrimidine -2-thione, which are being examined notably for their biological activities and of particular interest in the coordinating ability of these ligands. This work reports on the synthesis and physico-chemical characterization of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Pd(II) complexes with 1*H*-4,4,6-trimethylpyrimidine-2-thione. All the complexes have been characterized by elemental analysis, magnetic moment, electronic and IR spectroscopy and conductivity. Present findings stimulate us to explore the synthesis of new metal complexes of 1*H*-4,4,6-trimethyl-3,4-dihydropyrimidine-2-(1*H*)-thione (Htmpt) and investigate their biological properties.

EXPERIMENTAL

All reactions were checked by thin layer chromatography (TLC) using pet ether/ethyl acetate (7.5:2.5) as mobile phase. The spots were visualized using a UV lamp. The elemental analyses (C,H,N,S) was obtained from a LECO 9320 analyzer. The ^{1}H NMR spectra of ligand and complexes were recorded on Bruker 400 MHz instrument with DMSO-d_6 as the solvent, using tetramethylsilane (TMS) as the internal standard. IR spectra were measured on Nicolet-iS50 FTIR affinity in 4000-200 cm^{-1} range using Reflectance mode. The electronic spectra were recorded using Shimadzu UV 1800 instrument in DMSO as solvent. Mass spectra were recorded on Agilent mass spectrometer. All the melting points were determined using open capillary method and are uncorrected. The Perkin Elmer (Pyris Diamond) instrument was used to carry out thermal analysis of metal complex in atmospheric air (0-1000 ºC) at a heating rate of 10 ºC min^{-1} using alumina powder as reference. Molar conductivity measurements were conducted at room temperature on a YSI Model 32 conductivity bridge. Gel electrophoresis cleavage experiments were achieved with the help of Axygen electrophoresis supported by Genei power supply with a potential range 50-500 Volts.

Bacteria: Non-pathogenic strain of bacteria *Escherichia coli* was sourced from Department of Biotechnology, MMU Mullana the concerned strain was originally sourced from IMTECH, Chandigarh, according to which the strain was designated as MTCC 1195. Bacterial strain was routinely grown in Luria-Bertani (LB) broth media aerobically at 37 ºC with shaking at 100 rpm. Cell growth was monitored spectrophotometrically at 600 nm. Media was supplemented with antibiotic like chloramphenicol as a drug of control. DNA photo-cleavage studies were performed on plasmid DNA.

Synthesis of metal complexes: Ligand was synthesized by reported method [32].

General procedure: In first step, ligand (0.1 mol) was treated with solid sodium hydroxide (0.1 mol) in 1:1 ratio in a mortar and dried in desiccator for 2-3 days and then in the second step metal salts (0.05 mol) of Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pd(II) in appropriate amount were mixed in the ligand at room temperature and dried in sunlight for 4-5 h. Metal(II) complexes having dark colour thus obtained, then washed with methanol followed by petroleum ether wash to remove any unreacted metal traces.

1*H*-4,4,6-Trimethyl-3,4-dihydropyrimidine-2-thione (Htmpt) (3): Yield 74%, m.p.: 273 ºC; MS: *m/z* [M+1]$^+$; 157; IR (KBr, ν_{max}, cm^{-1}): 3204 (N_1H *str.*), 3117 (N_3H *str.*), 1700 & 1647 (N_1H & N_3H bend), 1567 (C=N+C=C *str.*) 1168 (C=S *str.*); ^{1}H NMR (DMSO): δ = 2.14 (s, 9H of $3CH_3$-), 4.57 (s, H of -C=C-H), 3.5 (s, 1H of N_1H), 5.83 (s, 1H of N_3H), Anal. calcd. (found) % for $C_7H_{12}N_2S$: C, 63.13 (61.15); H, 6.86 (5.88); N, 16.98 (15.12); S, 12.10 (10.90). UV/vis [λ (nm)]: 297.

$C_{14}H_{32}N_4S_2O_4Mn$ [Mn(tmpt)$_2$(H$_2$O)$_2$]: Yield 88%; m.p.: 258 ºC; MS: *m/z* 366 [M+1]$^+$; (KBr, ν_{max}, cm^{-1}): 3441 (O-H *str.*), 3196 (N_1H str), 1659 (N_1H bend.), 1568 (C=N+C=C), 1321, 1275, 1170 (N_1H, CN, NCS, CS coupling), 637 (C-S *str.*), 413 (M-N), 286 (M-S). Anal. calcd. (found) % for $C_{14}H_{32}N_4S_2O_4Mn$: C, 41.68 (40.20); H, 6.90 (5.10); N, 13.80 (11.70); S, 15.80 (13.10); O, 7.90 (7.00). UV/vis [λ (nm)]: 310.

$C_{14}H_{34}N_4S_2O_4Co$ [Co(tmpt)$_2$(H$_2$O)$_2$]: Yield 90%; m.p. 298 ºC; MS: *m/z* 370 [M+1]$^+$; (KBr, ν_{max}, cm^{-1}): 3411 (O-H *str.*), 3199 (N_1H *str.*), 1703 (N_1H bend.) 1567 (C=N+ C=C), 1322, 1276, 1171 (N_1H, CN, NCS, CS coupling), 673 (C-S *str.*), 302 (M-N), 254 (M-S); Anal. calcd. (found) % for $C_{14}H_{32}N_4S_2O_4Co$: C, 41.30 (45.00); H, 6.90 (4.95); N, 13.90 (10.20); S, 15.80 (15.83); O, 13.50 (11.40). UV/vis [λ (nm)]: 556, 290.

$C_{14}H_{32}N_4S_2O_4Ni$ [Ni(tmpt)$_2$(H$_2$O)$_2$]: Yield 91%; m.p. 280 °C; MS: *m/z* 370 [M+1]$^+$; (KBr, ν_{max}, cm^{-1}): 3437 (OH *str.*), 3197 (N_1H *str.*), 1689 (N_1H bend.), 1569 (C=N+C=C), 1322, 1277, 1178 (N_1H, CN, NCS, CS coupling), 679 (C-S *str.*), 386 (M-N), 263 (M-S); Anal. calcd. (found) % for $C_{14}H_{32}N_4S_2O_4Ni$: C, 41.30 (40.10); H, 6.90 (6.91); N, 13.70 (13.20); S, 15.70 (15.80); O, 7.80 (7.00). UV/vis [λ (nm)]: 660, 405.

$C_{14}H_{26}N_4S_2O_4Cu$ [Cu(tmpt)$_2$]·2H$_2$O: Yield 89%; m.p. 293 ºC; MS: *m/z* 375 [M+1]$^+$; (KBr, ν_{max}, cm^{-1}): 3411 (O-H *str.*), 3203 (N_1H *str.*), 1698 (N_1H bend.), 1568 (C=N+ C=C), 1323, 1276, 1171 (N_1H, CN, NCS, CS coupling), 643 (C-S *str.*), 314 (M-N), 223 (M-S); Anal. calcd. (found) % for $C_{14}H_{32}N_4S_2O_4Cu$: C, 40.80 (40.70); H, 6.90 (5.80); N, 13.60 (13.40); S, 15.50 (15.51); O, 7.70 (7.20). UV/vis [λ (nm)]: 535, 478.

$C_{14}H_{24}N_4S_2O_4Zn$ [Zn(tmpt)$_2$]·2H$_2$O: Yield 92%; m.p. 286 ºC; MS: *m/z* 376 [M+1]$^+$; (KBr, ν_{max}, cm^{-1}): 3441 (O-H *str.*), 3205 (N_1H *str.*), 1637 (N_1H bend.), 1568 (C=N + C=C), 1328, 1238, 1179 (N_1H, CN, NCS, CS coupling), 671 (C-S *str.*), 327 (M-N), 246 (M-S); Anal. calcd. (found) % for $C_{14}H_{32}N_4S_2O_4Zn$: C, 40.60 (40.10); H, 6.80 (6.90); N, 13.50 (13.10); S, 15.50 (14.85); O, 7.70 (7.10). UV/vis [λ (nm)]: 340.

$C_{14}H_{24}N_4S_2O_4Cd$ [Cd(tmpt)$_2$]·H$_2$O: Yield 90%; m.p. 422 ºC; MS: *m/z* 423 [M+1]$^+$; (KBr, ν_{max}, cm^{-1}): 3411 (O-H *str.*), 3202 (N_1H *str.*), 1655 (N_1H bend) 1567 (C=N+ C=C), 1326, 1275, 1171 (N_1H, CN, NCS, CS coupling), 640 (C-S *str.*), 323 (M-N), 253 (M-S); Anal. calcd. (found) % for $C_{14}H_{32}N_4S_2O_4Cd$: C, 37.90 (37.95); H, 5.90 (5.50); N, 12.60 (12.00); S, 14.50 (13.80); O, 3.60 (3.10). UV/vis [λ (nm)]: 390.

$C_{14}H_{24}N_4S_2Pd$ [Pd(tmpt)$_2$]: Yield 93%; m.p. 416 ºC; MS: *m/z* 417 [M+1]$^+$; (KBr, ν_{max}, cm^{-1}): 3197 (N_1H *str.*), 1686 (N_1H bend.), 1567 (C=N+C=C), 1327, 1280, 1170 (N_1H, CN, NCS, CS coupling), 680 (C-S *str.*), 410 (M-N), 280 (M-S); Anal. calcd. (found) % for $C_{14}H_{32}N_4S_2O_4Pd$: C, 40.20 (40.10); H, 5.70 (5.60); N, 13.50 (13.20); S, 15.30 (14.90). UV/vis [λ (nm)]: 410.

***In vitro* antibacterial activity:** To explore antibacterial activities of the synthesized metal complexes, Agar well diffusion method was used taking DMSO as solvent against various pathogenic strains of bacteria (*Bacillus subtilis* (5021), *S. aureus* (2063), *P. syringae* (5102), *P. aeruginosa* (5029). These strains were procured from Maharishi Markandeshwar Medical College, Maharishi Markandeshwar (Deemed to be University), Mullana, India. A nutrient agar (taken as medium, 25 mL) was poured into Petri plates and these plates were swabbed with 100 µL inocula of each test bacterium and kept for 15-20 min for adsorption. Wells were bored into the seeded agar plates using sterile cork borer (10 mm diameter). The stock solutions of the test compounds and standard were prepared in DMSO

at a concentration of 2000 µg/mL. From the stock solution, two-fold dilution of the compounds (2, 4, 8, 10 µg/mL) were inoculated (50 µL) to the corresponding wells in the seeded agar plates. All the plates were then incubated at 37 ºC for 24 h. The antibacterial activity of each synthesized compound was evaluated by measuring the zone of growth of inhibition with zone reader (Hi-Antibiotic zone scale) and the MIC was determined as the lowest concentration of the compound tested that was able to inhibit visible growth of bacteria. In each investigation DMSO was used as a negative control, whereas ampicillin, oxacillin or penicillin G was used as a reference drug.

Gel electrophoresis for DNA photocleavage: The DNA photocleavage activity by metal complexes was studied on agarose gel electrophoresis. Supercoiled plasmid DNA (5 µL) was added to metal complex (40 µg) and incubated at 37 ºC for 1 h. Agarose (0.8%) gel was melted and prepared in 1X TAE (*Tris*-acetate-EDTA; *Tris* 10 mm, EDTA 0.01mm, pH 8.0) buffer. To prepare the mould, edges of clean dry plexiglas platform was sealed by adhesive tapes and then the gel mixture was poured into this platform fixed with a comb to form slots. The gel was allowed to set in the mould. After 2-3 h, comb and tapes were removed and the mould was immersed in the electrophoresis tank containing 1X TAE buffer. DNA samples and loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol) were mixed together and loaded into the slots prepared in the submerged gel. Electrophoresis was carried out for 2 h at 90 V. The gel was stained with ethidium bromide, (1.0 µg/mL) viewed under UV trans-illuminator (Bio-Rad UV Trans-illuminator 2000) and photographed.

RESULTS AND DISCUSSION

All the complexes are powdery solids, coloured and stable at the normal atmosphere. The elemental analysis supports the formation of 2:1 metal complexes. All complexes were soluble in DMSO. However, in mechano-chemical approach, reactions were completed early and gave desired products with good yields and purity. Therefore, the present method has many advantages over conventional methods like no solvent requirement, simplicity, easy operation, eco friendly and less expensive with excellent yields of desired products.

Conductance and elemental analysis: Table-1 showed the data for observed conductance and elemental analysis for the synthesized complexes. To determine the electrolytic nature of the synthesized metal complexes their conductance were recorded in 10^{-4} M DMSO at room temperature. The observed low conductance values for all the metal complexes indicated their non-electrolytic nature [33-35].

IR spectra: Distinctive IR bands of the ligand were assigned at 3204, 3117, 1700, l657, 1567, 1434, 1168 cm^{-1} which are assigned to ν(N_1H), ν(N_3H), (N_1H and N_3H bend.), ν(C=N + C=C), ν(N-C=S), ν(C=S) vibrations, respectively [36,37]. IR confirmed the thione form of ligand in the solid state supported by the presence of NH *str.* vibrations.

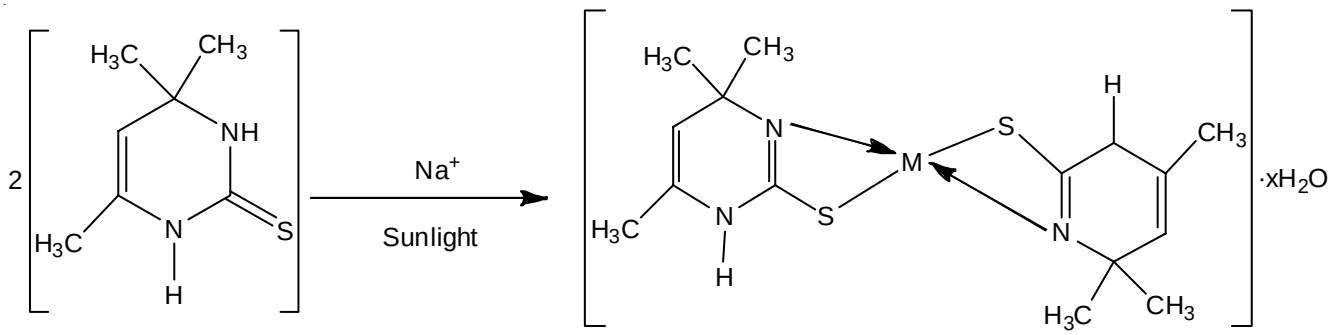
Upon complexation, the changes in ν(C=S) vibration near 1168 cm^{-1} in the free ligand towards upward frequency in all metal complexes from 1179 cm^{-1} to 1170 cm^{-1} as well C-S band appearance near 680-637 cm^{-1}, this confirmed the involvement of thione sulfur in coordination with the metal ion [36-38] and due to sulfur-metal (S-M) bond formation the expected single bond character of carbon-sulphur (C-S) bond increases, consequently the contribution of the –N=C-S^- increases and a noticable change is visible in frequency of the bands assigned to C=C, C=N, NCS, CS [39-41].

This the disappearance of N_3H *str.* and bending at 3117 and 1700-1600 cm^{-1} gives best indication of contribution of nitrogen in the ligand. Thus, M-ligand bond formed [42-45]. Also, low frequency region of the spectra revealed the presence of a new medium-intensity band between 413-223 cm^{-1} due to ν(M–N) and ν(M–S) vibrations [41,42,45,46]. A broad medium intensity band in the range of 3371-3445 cm^{-1} due to free ν(OH) stretching frequencies are observed may be due to the presence of water molecule [47] (also confirmed by TGA studies). The data revealed that Htmpt behaved as anionic bidentate ligand, coordinating the metal ions through the thione S and the deprotonated cyclic N(3) atoms (**Scheme-I**) [7,15-17,25] forming four membered ring. The IR spectral data with the proposed structures of metal complexes is shown in Table-2.

^{1}H NMR spectra: ^{1}H NMR data of ligand Htmpt and its metal(II) complexes [$M(tmpt)_2$] [M = Cu^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Pd^{2+}] are shown in Table-3. The ^{1}H NMR spectrum of Htmpt shows signals due to 3(CH_3), C=C-H, N_3-H and N_1-H at δ 1.34, 1.42, 1.48, 4.9 8.3 and 9.3 respectively. In the ^{1}H NMR spectra of [$M(tmpt)_2$], the singlet due to N_3H disappeared while other reported protons are shifted upfield, confirming that coordination occurs through deprotonated cyclic N atom at (3rd position) this fact appeared to be in good agreement with the IR data [48]. The NMR spectra of Cu(II), Ni(II), Co(II), Mn(II) complexes show broad peaks due to the para-

TABLE-1
COLOURS, MELTING POINTS AND ELEMENTAL ANALYSIS OF Htmpt AND ITS METAL COMPLEXES

Ligand/Complexes	Colour	m.p. (°C)	Yield (%)	Elemental analysis (%): Found (calcd.)					Molar conductance (ohm^{-1} cm^2 mol^{-1})
				C	H	N	S	O	
Htmpt	White	273	74%	53.6 (53.1)	7.6 (6.8)	17.7 (17.9)	20.5 (20.1)	–	1.0
$[Mn(tmpt)_2(H_2O)_2]$	Brown	258	88%	41.8 (39.2)	6.5 (5.1)	13.8 (10.7)	15.9 (12.1)	7.9 (6.0)	1.1
$[Co(tmpt)_2(H_2O)_2]$	Pink violet	298	90%	41.5 (45.0)	6.4 (4.95)	13.8 (10.2)	15.8 (8.3)	7.9 (14.4)	2.0
$[Ni(tmpt)_2(H_2O)_2]$	Green	280	91%	41.6 (47.1)	6.4 (5.3)	13.9 (11.2)	15.8 (8.8)	7.9 (10.0)	1.6
$[Cu(tmpt)_2]\cdot 2H_2O$	Light green	293	89%	40.9 (46.9)	5.8 (4.8)	13.6 (11.4)	15.6 (8.5)	7.8 (11.1)	1.9
$[Zn(tmpt)_2]$	White	286	92%	44.8 (50.1)	5.8 (4.9)	14.9 (13.15)	17.0 (8.85)	–	1.5
$[Cd(tmpt)_2(H_2O)_2]\cdot 3H_2O$	Cream	422	90%	36.7 (47.9)	5.6 (4.5)	12.2 (11.0)	13.9 (8.8)	6.9 (4.9)	1.6
$[Pd(tmpt)_2]$	White	416	93%	40.3 (47.1)	5.3 (4.6)	13.5 (11.2)	15.4 (7.9)	–	1.4



Scheme-I

TABLE-2
KEY IR SPECTRAL BANDS (cm^{-1}) OF Htmpt AND ITS METAL(II) COMPLEXES

Ligand/Complexes	ν(OH)	ν(N_1H)	ν(N_3H)	δ(N_1H) δ(N_3H)	ν(C=N) + ν(C=C)	ν(NCS)	ν(C=N) ν(N-C=S) ν(C=S)	ν(C-S)	ν(MN)	ν(MS)
Htmpt	–	3204	3117	1700 1667	1567	1434	1386 1275 1168	–	–	–
[Mn(tmpt)$_2$(H_2O)$_2$]	3441	3196	–	1659 –	1568	1514	1321 1275 1179	637	413	286
[Co(tmpt)$_2$(H_2O)$_2$]	3411	3199	–	1703 –	1567	1420	1322 1276 1171	673	302	254
[Ni(tmpt)$_2$(H_2O)$_2$]	3437	3197	–	1689 –	1569	1412	1322 1277 1178	679	386	263
[Cu(tmpt)$_2$]·2H_2O	3411	3203	–	1698 –	1568	–	1323 1276 1171	643	314	223
[Zn(tmpt)$_2$]	3375	3205	–	1637 –	1568	–	1328 1238 1179	671	327	246
[Cd(tmpt)$_2$(H_2O)$_2$]·3H_2O	3411	3202	–	1655 –	1567	–	1326 1275 1171	640	323	253
[Pd(tmpt)$_2$]	3429	3166	–	1686 –	1567	–	1327 1280 1170	680	410	280

TABLE-3
^{1}H NMR DATA OF Htmpt AND ITS METAL COMPLEXES

Ligand/Complexes	^{1}H NMR (DMSO-d_6) (ppm)
Htmpt	1.34,1.42,1.48 (s,3 CH_3), 4.9(s,C=C-H), 8.9(s,N_{pym}-H), 8.6(s,N_1-N-H) 7.76-7.91(m,Ar C-H)
[Zn(tmpt)$_2$]	1.28, 1.38, 1.43 (3 CH_3), 5.02(C=C-H), 8.97(s,N_1-N-H) 7.36-7.99(m, Ar C-H)
[Cd(tmpt)$_2$(H_2O)$_2$]·3H_2O]	1.30, 1.39, 1.42 (3 CH_3), 4.83(C=C-H), 8.93 (s,N_1-N-H) 7.78-7.91(m, Ar C-H)
[Pd(tmpt)$_2$]	1.27, 1.37, 1.43 (3 CH_3), 5.02(C=C-H), 8.96(s,N_1-N-H) 7.38-7.99(m, Ar C-H)

magnetic behaviour of Cu(II), Ni(II), Co(II), Mn(II) metal ions [49]. This observation agrees with the reported results, which further tells the formation of metal(II) complexes.

Mass spectra: ESI-mass spectra has supported the molecular ion formation of representative Htmpt, [Mn(tmpt)$_2$(H_2O)$_2$], [Co(tmpt)$_2$(H_2O)$_2$], [Cu(tmpt)$_2$(H_2O)$_2$], [Ni(tmpt)$_2$(H_2O)$_2$], [Zn(tmpt)$_2$] and [Pd(tmpt)$_2$] and [Cd(tmpt)$_2$(H_2O)$_2$] complexes. The mass fragmentation of Htmpt and selected metal(II) complexes are presented in Table-4. An intense molecular ion peak at *m/z* 157 (M^++1) in mass spectrum of Htmpt corresponds to its molecular formula (calcd. 156). The spectra of the complexes show fragmentation patterns corresponding to the successive degradation and stepwise ligand loss of the complex. The mass spectrum of Htmpt exhibits an intense peak at *m/e* 157 (calcd. 156), in agreement with the assigned formula [Htmpt+1]$^+$. The mass spectrum of [Mn(tmpt)$_2$(H_2O)$_2$] shows peak at *m/z*

TABLE-4
MASS FRAGMENTATION DATA OF Htmpt AND SOME SELECTED METAL COMPLEXES (M^++1) MODE

Ligand/Complexes	(M^++1) mode
Htmpt	127.1,157
$[Mn(tmpt)_2(H_2O)_2]$	157,197, 203,225,366,402
$[Ni(tmp)_2(H_2O)_2]$	127, 157, 179, 370,406
$[Co(tmp)_2(H_2O)_2]$	157, 370
$[Cu(tmp)_2]\cdot 2H_2O$	157, 375, 411
$[Zn(tmpt)_2]$	375.1, 376.1
$[Cd(tmpt)_2(H_2O)_2]\cdot 3H_2O$	157, 423.41
$[Pd(tmpt)_2]$	157, 417.42

(calcd. 546.9) $[Co(tmpt)_2(H_2O)_2]$ shows peak at *m/z* 551.4 (calcd. 550.9) corresponding to molecular ion $[Co(tmpt)_2]^+$. Two more peaks at *m/z* 587.9 and 306 were observed due to $[Co(tmpt)_2+2\ H_2O)]^+$ (calcd. 586.9) and $[Co(tmpt)]^+$ (calcd. 304.9), respectively. The mass spectrum of $[Cu(tmpt)_2(H_2O)_2]$ shows peak at *m/z* 556.5 (calcd. 555.5) corresponding to molecular ion $[Cu(tmpt)_2]^+$. Another two peaks at *m/z* 592.5 and 309.5 are also observed due to $[Cu(tmpt)_2\cdot 2H_2O]^+$ (calcd. 591.5) and $[Cu(tmpt)]^+$ (calcd. 309.5), respectively. $[Zn(tmpt)_2(H_2O)_2]$ complex exhibits three peaks at *m/z* 594.4, 558.4 and 312.4 due to $[Zn(tmpt)_2\cdot 2H_2O)]^+$ (calcd. 593.4) $[Zn(tmpt)_2]^+$ (calcd. 558.4) and $[Zn(tmpt)]^+$ (calcd. 311.4) fragments, respectively. The spectrum of $[Cd(tmpt)_2]$ shows peaks at *m/z* 642.4 and 359.4 corresponding to $[Cd(tmpt)_2]^+$ (calcd. 641.4) and $[Cd(tmpt)]^+$ (calcd. 358.4) fragments, The mass spectrum of $[Pd(tmpt)_2]$ shows peaks at *m/z* 599.4 and 353.4 corresponding to $[Pd(tmpt)_2]^+$ (calcd. 598.4) and $[Pd(tmpt)]^+$ (calcd. 352.4) fragments, respectively [42,43,50,51].

Magnetic measurements and electronic spectra: The electronic and magnetic data of ligand and its metal(II) complexes are given in Table-5. The electronic spectra provided enough information regarding the arrangements of the ligands around the metal ions. The ligand showed the following two signals centered at 284 nm and 313 nm, respectively. These absorption bands suggested the π–π* and n–π* transitions due to azomethine moiety [52].

These bands showed blue shift in case of synthesized metal complexes as compared to ligand suggesting the involvement of nitrogen group in coordination. Co(II) showed bands at 556 and 615, Cu(II) at 651, 406, 333 and Ni(II) at 553, 412, 311 nm due to *d–d* transitions [53,54], respectively. The observed bands suggested the octahedral geometry around the mentioned metal ions, also confirmed by magnetic moment observed at

TABLE-5
ELECTRONIC AND MAGNETIC MOMENT DATA OF LIGAND AND METAL COMPLEXES

Ligand/Complexes	Magnetic moments (B.M.)	Transitions λ_{max} (nm)
Htmpt	–	297
$[Mn(tmpt)_2(H_2O)_2]$	5.83 (Ocahedral)	310, 307, 289
$[Ni(tmp)_2(H_2O)_2]$	3.34 (Ocahedral)	660, 405, 311
$[Co(tmp)_2(H_2O)_2]$	4.95 (Ocahedral)	556, 290
$[Cu(tmp)_2]\cdot 2H_2O$	1.82 (Ocahedral)	535, 478, 333
$[Zn(tmpt)_2]$	Diamagnetic	340, 294
$[Cd(tmpt)_2(H_2O)_2]\cdot 3H_2O$	Diamagnetic	390, 295
$[Pd(tmpt)_2]$	Diamagnetic	410, 342

4.95, 1.82, 3.34 B.M. for Co(II), Cu(II), Ni(II), respectively [53-55]. In case of Mn(II) very weak band was observed at 560 nm due to spin forbidden nature of these transitions and magnetic moment 5.83 B.M. indicative of octahedral geometry [52]. The complexes with Zn(II), Cd(II), Pd (II) show bands at 340, 397, 351 nm *i.e.* no band in visible region as expected for d^{10} system and should be diamagnetic and square planer [56-58].

Thermal studies: TG analysis was carried out for $[Cu(tmpt)_2]\cdot 2H_2O$ and $[Cd(tmpt)_2]\cdot 2H_2O$ from 0 to 80 ºC under nitrogen atmosphere (Fig. 1). The decomposition temperature, pyrolyzed products, % mass loss of the complexes and the ash (%) are given in Table-6. The thermograms for both of these complexes show four-five decomposition steps. In $[Cu(tmpt)_2]\cdot 2H_2O$ complex, 0-80 ºC results in a mass loss of 9% (calcd. 8.78%) corresponding to loss of two lattice water molecules [44,51,59]. The 2nd and 3rd step (80-210 and 210-310 ºC) corresponds to removal of $–CH_3$-C-N_1-H and $–C_4H_7$ [59,60]. Moiety with mass loss of 9.5% (calcd. 10.2%) and 13.5% (calcd. 13.4%). The fourth step 400-600 ºC corresponds to a mass loss C_2H_4N of the complex leaving CuO residue with carbide and sulfide.

Four decomposition steps were observed, in case of $[Cd(tmpt)_2]\cdot 2H_2O$ complex. The first step 0-90 ºC results in a mass loss of 7.9% (calcd. 7.5%) corresponding to elimination of two lattice water molecules. Removal of $–CH_3$-C-N_1-H and $–C_5H_7$ moiety with mass loss of 12.2% (calcd. 12.5%) and 14.6% (calcd. 14.3%) observed in 2nd and 3rd step (90-300 and 300-430 ºC) and in the 4th step 430-760 ºC corresponds to a mass loss C_4H_6N of the complex leaving CdO residue contaminated with CdC_2 and CdS [59,60]. The decomposition of the complexes ended with metal oxide formation.

TABLE-6
THERMOGRAVIMETRIC DATA OF $[Cu(tmpt)_2H_2O]\cdot 2H_2O$, $[Cd(tmpt)_2(H_2O)_2]\cdot 2H_2O$ COMPLEXES

Compound	Decomposition stages and assignment	Temp. (°C)	Weight loss found (calcd.)
$[Cu(tmpt)_2]\cdot 2H_2O$ $[C_{14}H_{26}N_4CuO_3S_2]$	1. Lattice water (hydrated water)	0-80	9.0 (8.78)
	2. C_2H_4N	80-210	10.2 (9.5)
	3. C_4H_8 elimination	210-310	13.5 (13.8)
	4. C_4H_7	310-400	13.4 (13.5)
	5. C_2H_4N elimination	400-700	10.2 (10.1)
$[Cd(tmpt)_2(H_2O)_2]\cdot 3H_2O$ $[C_{14}H_{30}N_4CoO_4S_2]$	1. Lattice water (hydrated water)	0-90	7.9 (7.9)
	2. C_2H_4N	90-300	12.2 (12.5)
	3. $C_6H_{11}N_2$ elimination	300-430	14.6 (14.3)
	4. C_6H_6S	430-760	11.99 (12.0)

*Calculated in parentheses

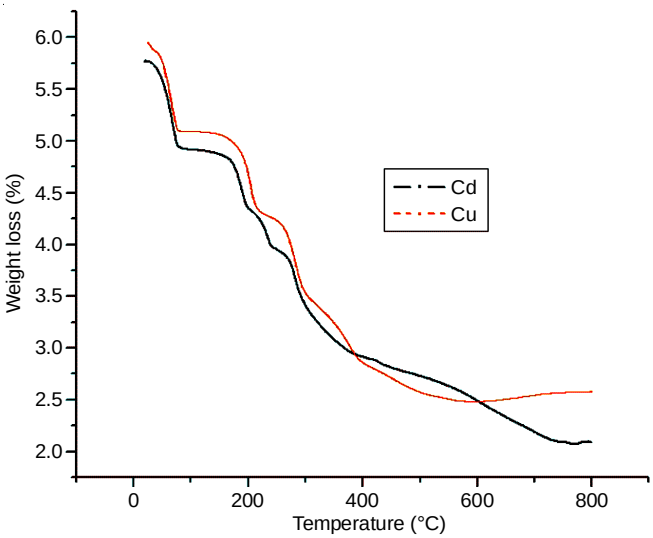


Fig. 1. TGA curves for Cd and Cu Complex with HTMPT

Biological assay

Antibacterial activity: All the synthesized seven metal (II) complexes were tested for their *in vitro* antibacterial activity against two Gram-positive (*B. subtilis* and *S. aureus*) and two Gram-negative bacteria (*P. syringae* and *P. aeruginosa*) (Table-7). Penicillin G and oxacillin were used as standard drugs for comparison of antibacterial activity with synthesized compounds.

It is observed that the antibacterial action of ligand is significantly enhanced on chelation with transition metal ions. The chelation reduces the polarity of the metal ion because of the partial sharing of its positive charge with the donor groups [61-63] and possible π-electron deloca-lization within the whole chelate ring during coordination. As a result of this, increase in the lipophilic character of metal chelates takes place, so as to make it more permeable through the lipid layer of microorganisms thus destroying them more violently. Once the compound enters into the microbial cell, it inhibits the growth of microorganism by binding at the active site of enzymes, which is involved in various essential bio-chemical processes including protein synthesis and cell respiration.

The mode of action of metal(II) complexes involves the formation of hydrogen bonds with azomethine group by the active sites leading to interference with the cell wall synthesis. The hydrogen bond formation damages the cytoplasmic membrane and the cell permeability may also be altered leading to cell death. The order of bioactivity of complexes found was Ni(II) > Cd(II) > Co(II) > Zn(II) > Cu(II) > Pd(II) > Mn(II) > ligand. This higher activity of complexes can be explained on the basis of chelation, the polarity of Ni(II), Cd(II), Co(II) & Zn(II) ions is found to be reduced to a greater extent, due to overlap of the ligand orbital and partial sharing of the positive charge of the metal ions with donor groups. Therefore, Ni(II), Cd(II), Co(II) & Zn(II) ions are easily adsorbed on the surface of the cell wall of microorganisms. The adsorbed Ni(II), Cd(II), Co(II) & Zn(II) ions disturbs the respiratory process of the cell and blocks the synthesis of protein. This, in turn, restricts further growth of the organisms [64].

Plasmid DNA photocleavage studies: Antibacterial activity was followed by DNA photocleavage activity, which was determined using puc18 plasmid DNA. It was surprising to note that transition metal complex containing nickel and cobalt exhibited promising [65] cleavage activity. Complex having nickel cleaves the plasmid to reveal form I (super coiled) and form II (open circular) structures at 25 µg concentration and the form III (nicked) structure was observed when 40 µg of the complex was used. Cobalt complex has exhibited similar activities at 40 and 60 µg to reveal form II and form III structures, respectively (Fig. 2). However, there was markable increase in cleaving ability in case of metal complexes as compared to free ligand due to non-covalent interactions of metal complexes with DNA, which results in the binding of complex followed by cleavage of DNA. The difference in the cleavage potential

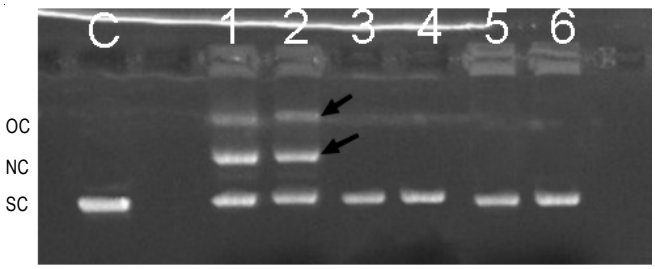


Fig. 2. DNA Photocleavage activity of Htmpt and M[tmpt]$_2$ [Lane C has the control plasmid and lane 1, 2 has the complexes containing nickel and cobalt respectively. The upper arrow displays the form II structure and the lower arrow points toward the form III structure of DNA resulted from the nicking activity of the complexes]

TABLE-7
ANTIBACTERIAL ACTIVITY RESULTS OF HTMPT AND ITS Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Pd(II) COMPLEXES

Compounds	Diameter of growth of zone inhibition (mm)			
	Bacillus subtilis (NCIM-5021)	*S. aureus* (NCIM-2063)	*P. aeruginosa* (NCIM-5029)	*P. syringae* (NCIM-5102)
Htmpt	10	10	10	10
$[Mn(tmpt)_2(H_2O)_2]$	11	10	10	13
$[Co(tmpt)_2(H_2O)_2]$	17	21	18	16
$[Ni(tmpt)_2(H_2O)_2]$	32	23	33	30
$[Cu(tmpt)_2]\cdot 2H_2O$	14	21	19	12
$[Zn(tmpt)_2]$	23	25	20	19
$[Cd(tmpt)_2]\cdot 3H_2O$	30	23	24	20
$[Pd(tmpt)_2]$	12	12	11	11
Penicillin G	35	31	37	38
Oxacillin	36	36	37	38

can be due to the different degree of binding capabilities of different metal complexes. In addition to these results, it was also observed that H_2O_2 had no additional effect on the nicking activity of the complexes. When rest of the complexes and the ligand were subjected to plasmid cleavage activity, it was noted that they failed to provide nicks in the plasmid but degrades the DNA at 400 µg.

Conclusion

In search of better antimicrobial active agents, a bidentate ligand and metal(II) complexes of the first transition series were synthesized by the mechanochemical method. The advantages of this method are, shorter reaction times, higher yields and milder conditions. This could be of significant use in industrial applications in the pharmaceutical and/or fine chemical industries. The metal(II) complexes were also evaluated for their DNA photocleavage ability as well as the DNA gyrase inhibition tendency with an expectation to find efficient DNA nicking and antibacterial agents. It has been observed that Cu(II) and Ni(II) complexes were found to exhibit good DNA fragmentation capacity as compared to ligand and other metal(II) complexes. Some major modifications in the structure of ligand may lead to escalation of better DNA nicking agents in near future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Z.H. Chohan and C.T. Supuran, *J. Enzyme Inhib. Med. Chem.*, **20**, 463 (2005); https://doi.org/10.1080/10485250500219765
2. K. Singh, Y. Kumar and R.K. Pundir, *Synth. React. Inorg. Met.-Org. Nano-Met. Chem.*, **40**, 836 (2010); https://doi.org/10.1080/15533174.2010.522646
3. A. Altundas, N. Sari, N. Colak and H. Ögütcü, *Med. Chem. Res.*, **19**, 576 (2010); https://doi.org/10.1007/s00044-009-9214-8
4. R.S. Nimthong, S.C. Chamchong, C.J. Pakawatchai, J. Mokhagul and Y. Wattanakanjana, *Acta Crystallogr. Sect. E Struct. Rep. Online*, **69**, 1266 (2013); https://doi.org/10.1107/S1600536813019223
5. M.A. Gonzalez, S.J. Carrington, N.L. Fry, J.L. Martinez and P.K. Mascharak, *Inorg. Chem.*, **51**, 11930 (2012); https://doi.org/10.1021/ic3018216
6. Y. Wu, R. Sa, Q. Li, Y. Wei and K. Wu, *Chem. Phys. Lett.*, **467**, 387 (2009); https://doi.org/10.1016/j.cplett.2008.11.073
7. C.R. Maldonado, M. Quirós and J.M. Salas, *Polyhedron*, **27**, 2779 (2008); https://doi.org/10.1016/j.poly.2008.06.001
8. P. Sharma, N. Rane and V.K. Gurram, *Bioorg. Med. Chem. Lett.*, **14**, 4185 (2004); https://doi.org/10.1016/j.bmcl.2004.06.014
9. O. Prakash, V. Bhardwaj, R. Kumar, P. Tyagi and K.R. Aneja, *Eur. J. Med. Chem.*, **39**, 1073 (2004); https://doi.org/10.1016/j.ejmech.2004.06.011
10. M. Botta, M. Artico, S. Massa, A. Gambacorta, M.E. Marongiu, A. Pani and P. La Colla, *Eur. J. Med. Chem.*, **27**, 251 (1992); https://doi.org/10.1016/0223-5234(92)90009-P
11. W. Wu, W. Lan, C. Wu and Q. Fei, *Front. Chem.*, **9**, 695628 (2021); https://doi.org/10.3389/fchem.2021.695628
12. S. Maddila, S. Gorle, N. Seshadri, P. Lavanya and S.B. Jonnalagadda, *Arab. J. Chem.*, **9**, 681 (2016); https://doi.org/10.1016/j.arabjc.2013.04.003
13. V.J. Ram, N. Haque and P.Y. Guru, *Eur. J. Med. Chem.*, **27**, 851 (1992); https://doi.org/10.1016/0223-5234(92)90121-G
14. M. Amir, S.A. Javed and H. Kumar, *Indian J. Pharm. Sci.*, **69**, 337 (2007); https://doi.org/10.4103/0250-474X.34540
15. S.M. Sondhi, S. Jain, A.D. Dwivedi, R. Shukla and R. Raghubir, *Indian J. Chem.*, **47B**, 136 (2008).
16. P. Sethi, P. Dogra, G.K. Gupta, S.I. Mostafa and S. Kaur, *Res. J. Chem. Environ.*, **22**, 73 (2018).
17. K. Rana, B. Kaur and B. Kumar, *Indian J. Chem.*, **43B**, 1553 (2004).
18. P.A.S. Smith and R.O. Kan, *J. Org. Chem.*, **29**, 2261 (1964); https://doi.org/10.1021/jo01031a037
19. J. Balzarini and C. McGuigan, *J. Antimicrob. Chemother.*, **50**, 5 (2002); https://doi.org/10.1093/jac/dkf037
20. H. Kimura, T. Katoh, T. Kajimoto, M. Node, M. Hisaki, Y. Sugimoto, T. Majima, Y. Uehara and T. Yamori, *Anticancer Res.*, **26**, 91 (2006).
21. H. Lee, B. Kim, J. Ahn, S. Kang, J. Lee, J. Shin, S. Ahn, S. Lee and S. Yoon, *Eur. J. Med. Chem.*, **40**, 862 (2005); https://doi.org/10.1016/j.ejmech.2005.03.019
22. P.F. Juby, T.W. Hudyma, M. Brown, J.M. Essery and R.A. Partyka, *J. Med. Chem.*, **22**, 263 (1979); https://doi.org/10.1021/jm00189a009
23. A.K. Gupta and H.P. Sanjay, *Indian J. Pharmacol.*, **26**, 227 (1994).
24. A.A. Abu-Hashem, M.M. Youssef and H.A.R. Hussein, *J. Chin. Chem. Soc.*, **58**, 41 (2011); https://doi.org/10.1002/jccs.201190056
25. A.A. Abu-Hashem, M.F. El-Shehry and F.A. Badria, *Acta Pharm.*, **60**, 311 (2010).
26. S.A. Rahaman, Y. Rajendra Pasad, P. Kumar and B. Kumar, *Saudi Pharm. J.*, **17**, 255 (2009); https://doi.org/10.1016/j.jsps.2009.08.001
27. Y. Nezu, M. Miyazaki, K. Sugiyama, I. Kajiwara, *Pestic. Sci.*, **47**, 103 (1996); https://doi.org/10.1002/(SICI)1096-9063(199606)47:2<103::AID-PS396>3.0.CO;2-Z
28. A. Mahapatra, T. Prasad and T. Sharma, *Futur. J. Pharm. Sci.*, **7**, 123 (2021); https://doi.org/10.1186/s43094-021-00274-8
29. F. Xie, H. Zhao, L. Zhao, L. Lou and Y. Hu, *Bioorg. Med. Chem. Lett.*, **19**, 275 (2009); https://doi.org/10.1016/j.bmcl.2008.09.067
30. A.L.S. Rodrigues, J.M. Rosa, V.M. Gadotti, E.C. Goulart, M.M. Santos, A.V. Silva, B. Sehnem, L.S. Rosa, R.M. Gonçalves, R. Corrêa and A.R.S. Santos, *Pharmacol. Biochem. Behav.*, **82**, 156 (2005); https://doi.org/10.1016/j.pbb.2005.08.003
31. B. Kumar, B. Kaur, J. Kaur, A. Parmar, R.D. Anand and H. Kumar, *Indian J. Chem.*, **41B**, 1526 (2002).
32. R.A. Mathes, F.D. Stewart and F. Swedish Jr., *J. Am. Chem. Soc.*, **70**, 1452 (1948); https://doi.org/10.1021/ja01184a046
33. A.A. Osowole, I. Ott and O.M. Ogunlana, *Int. J. Inorg. Chem.*, **2012**, 206417 (2012); https://doi.org/10.1155/2012/206417
34. M.I. Husain, M.A. Shukla and S.K. Agarwal, *J. Indian Chem. Soc.*, **56**, 306 (1979).
35. M. Patel, M. Chhasatia and B. Bhatt, *Med. Chem. Res.*, **20**, 220 (2011); https://doi.org/10.1007/s00044-010-9310-9
36. K. Nakamoto, Infrared Spectra and Raman Spectra of Inorganic and Coordination Compounds, John Wiley & Sons: New York, (1997).
37. M. Sonmez, A. Levent and M. Sekerci, *Synth. React. Inorg. Met.-Org. Chem.*, **33**, 1747 (2003); https://doi.org/10.1081/SIM-120026545
38. A.K. Mishra, J. Jacob and K. Müllen, *Dyes Pigments*, **75**, 1 (2007); https://doi.org/10.1016/j.dyepig.2006.05.025

39. M.D. Gutierrez, R. Lopez, M.A. Romero and J.M. Salas, *Can. J. Chem.*, **66**, 249 (1988); https://doi.org/10.1139/v88-042
40. E. López-Torres and M.A. Mendiola, *Polyhedron*, **24**, 1435 (2005); https://doi.org/10.1016/j.poly.2005.03.093
41. G. Golub, H. Cohen, P. Paoletti, A. Bencini and D. Meyerstein, *J. Chem. Soc*., *Dalton Trans.*, **10**, 2055 (1996); https://doi.org/10.1039/DT9960002055
42. S. El-Sayed, B. Jean-Claude, I. Butler and S. Mostafa, *J. Mol. Struct.*, **1028**, 208 (2012); https://doi.org/10.1016/j.molstruc.2012.05.073
43. S.I. Mostafa and N. Hadjiliadis, *Transition Met. Chem.*, **33**, 529 (2008); https://doi.org/10.1007/s11243-008-9076-9
44. S.I. Mostafa, C. Papatriantafyllopoulou, S. Perlepes and N. Hadjiliadis, *Bioinorg. Chem. Appl.*, **2008**, 647873 (2008); https://doi.org/10.1155/2008/647873
45. K.M. Ibrahim, S.I. Mostafa, N. Nawar and Z.A. Younis, *Indian J. Chem.*, **43B**, 2294 (2004).
46. K. Nakamoto, Infrared Spectra of Inorganic and Coordination Compounds, John Wiley: New York (1963).
47. S.P. Perlepes, V. Lazaridou, B. Sankhla and J.M. Tsangaris, *Bull. Soc. Chim. Fr.*, **127**, 597 (1990).
48. S.I. Mostafa, M.A. Kabil, E.M. Saad and A.A. El-Asmy, *J. Coord. Chem.*, **59**, 279 (2006); https://doi.org/10.1080/00958970500266149
49. B.A. Kim and H. So, *Bull. Korean Chem. Soc.*, **20**, 1145 (1999); https://doi.org/10.5012/bkcs.1999.20.10.1145
50. P. Sethi, R. Khare and R. Choudhary, *Asian J. Chem.*, **32**, 2594 (2020); https://doi.org/10.14233/ajchem.2020.22813
51. S.I. Mostafa, *Transition Met. Chem.*, **32**, 769 (2007); https://doi.org/10.1007/s11243-007-0247-x
52. A.B.P. Lever, Inorganic Electronic Spectroscopy, Amsterdam: Elsevier (1984).
53. B.N. Figgis, J. Lewis and F.A. Cotton, Progress in Inorganic Chemistry, Interscience Publisher: NewYork (1964).
54. J. Lewis and R.G. Wilkins, Modern Coordination Chemistry, Interscience Publisher: New York (1964).
55. S.I. Mostafa, *Transition Met. Chem.*, **23**, 397 (1998); https://doi.org/10.1023/A:1006948815853
56. A. Golcu, M. Tumer, H. Demirelli and R.A. Wheatley, *Inorg. Chim. Acta*, **358**, 1785 (2005); https://doi.org/10.1016/j.ica.2004.11.026
57. A.A. Osowole and E.J. Akpan, *J. Eur. Appl. Sci.*, **4**, 14 (2012).
58. A.A. Osowole, R. Kempe, R. Schobert and K. Effenberger, *Synth. React. Inorg. Met. Org. Chem. Nano-Met. Chem.*, **41**, 825 (2011); https://doi.org/10.1080/15533174.2011.591310
59. A.A. Shabana, I.S. Butler, D.F.R. Gilson, B.J. Jean-Claude, Z.S. Mouhri, M.M. Mostafa and S.I. Mostafa, *Inorg. Chim. Acta*, **423**, 242 (2014); https://doi.org/10.1016/j.ica.2014.09.018
60. F.A. El-Morsy, B.J. Jean-Claude, I.S. Butler, S.A. El-Sayed and S.I. Mostafa, *Inorg. Chim. Acta*, **423**, 144 (2014); https://doi.org/10.1016/j.ica.2014.07.031
61. J.M. Andrews, *Antimicrob. Chemother.*, **48**, 5 (2001); https://doi.org/10.1093/jac/48.suppl_1.5
62. Z.H. Chohan, A. Scozzafava and C.T. Supuran, *J. Enzyme Inhib. Med. Chem.*, **17**, 261 (2002); https://doi.org/10.1080/1475636021000006261
63. Z.H. El-Wahab, M.M. Mashaly, A.A. Salman, B.A. El-Shetary and A.A. Faheim, *Spectrochim. Acta A*, **60**, 2861 (2004); https://doi.org/10.1016/j.saa.2004.01.021
64. M. Belicchi Ferrari, S. Capacchi, G. Pelosi, G. Reffo, P. Tarasconi, R. Albertini, S. Pinelli and P. Lunghi, *Inorg. Chim. Acta*, **286**, 134 (1999); https://doi.org/10.1016/S0020-1693(98)00383-1
65. Y. Liu, H. Chao, L. Tan, Y. Yuan, W. Wei and L. Ji, *J. Inorg. Biochem.*, **99**, 530 (2005); https://doi.org/10.1016/j.jinorgbio.2004.10.030