European Journal of Chemistry 5 (2) (2014) 201-208

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Synthesis of some transition metal complexes of novel 1-methylpyrazole-3-aldehyde-4-(2-pyridyl) thiosemicarbazone: Spectroscopic and in vitro biological activity studies

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ARTICLE INFORMATION

DOI: 10.5155/eurjchem.5.2.201-208.952

Received: 22 October 2013
Received in revised form: 22 November 2013
Accepted: 26 November 2013
Online: 30 June 2014

KEYWORDS

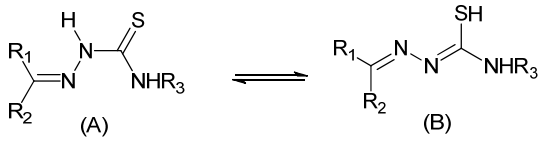
Spectra
Synthesis
Antifungal activity
Thiosemicarbazone
Molecular modeling
Antibacterial activity

ABSTRACT

Four new mixed ligand metal(II) complexes with 1-methylpyrazole-3-aldehyde-4-(2-pyridyl)thiosemicarbazone (MPAPT) and 1,10-phenanthroline are reported. These complexes namely Cu(MPAPT)(1,10-phen)(Cl)] (**1**), [Ni(MPAPT)(1,10-phen)(Cl)] (**2**), [Mn(MPAPT)(1,10-phen)(Cl)].H_2O (**3**) and [Co(MPAPT)(1,10-phen)(Cl)].H_2O (**4**), were characterized by elemental analysis, spectral (IR, ^1H NMR and UV-Vis) and magnetic moment measurements. The magnetic and spectral data indicates octahedral structure for all complexes. Metal complexes have been modeled using parameterized PM3 semi-empirical method. The free ligand and its M(II) chelates have been screened for their antimicrobial activities. The antimicrobial screening demonstrated that, the Cu(II) complex have the maximum and broad activities among the investigated complexes.

1. Introduction

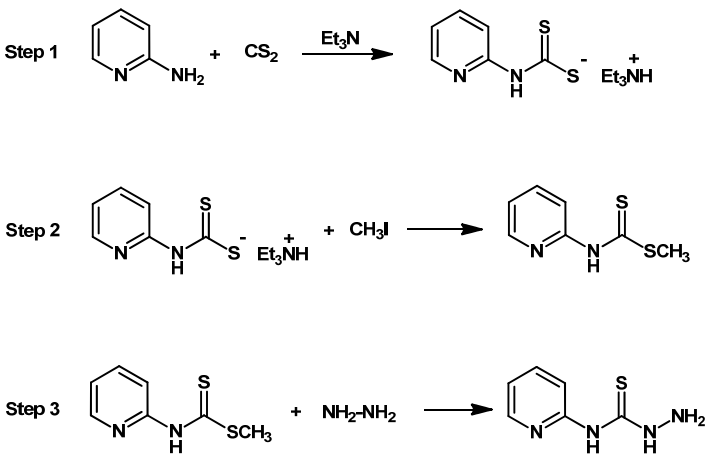
Thiosemicarbazones have been extensively studied because they have a wide range of actual or potential medical applications [1-6] which include notably antiparasital [5], antibacterial [6] antitumor activities [7], antiviral [8], fungicidal [9] and antineoplastic [10]. In general, thiosemicarbazones are obtained by condensation of the corresponding thiosemicarbazide with aldehydes or ketones. Thiosemicarbazones (TSCNs) exist in the tautomeric thione (A) and thiol (B) forms (Scheme 1).



Scheme 1

The chemistry of transition metal complexes of heterocyclic thiosemicarbazone ligands has been receiving considerable attention primarily because of their bioinorganic relevance [11,12]. There have been attempts [13] to determine structural correlation's between transition metal ion complexes of heterocyclic thiosemicarbazones and their wide spectrum of biological applications. In several cases, the pharmacological action of the thiosemicarbazons is enhanced due to their ability to chelate transition metal ions [14,15]. It is well authenticated that a NNS tridentate system is present in most of the thiosemicarbazones having carcinostatic potency and possessing substantial in *vitro* activity against various human tumour lines [16,17]. In the past, it has been shown that the metal complexes are more effective than their parent ligands in anticancer activity [18,19]. The inhibitory action of these compounds is attributed to their chelating properties to different metal ions that can be found in biological systems, which microorganisms need in their metabolism [20]. Additionally, one of the many reasons is that the binding affinity of metals to proteins or enzymes will change the interaction process of them with DNA, thereby affecting the DNA replication and cell proliferation [19]. Earlier studies on the biological properties of thiosemicarbazones and their metal complexes have concluded that the biologically active thiosemicarbazone molecules are planar and contain a pyridine ring or derivatives giving rise to NNS tridentate system [21,22].

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.5.2.201-208.952



Scheme 2

1,10-Phenanthroline (Scheme 2) is the parent of an important class of chelating agents. The choice of phenanthroline is mainly due to two factors. This heteroaromatic moiety can provide a further binding site for metal cations. It is rigid, and provides two aromatic nitrogens whose unshared electron pairs can act co-operatively in binding cations [23]. The π-electron deficiency makes phenanthroline an excellent π-acceptor. Moreover, 1,10-phenanthroline, the ligand moiety of the ternary complexes presented in this work is of considerable interest also according to the biological or pharmacological properties (antifungal, antimycoplasma and antiviral) of some of its metal complexes [24]. With this in mind, it seems therefore of considerable interest to synthesize and characterize M(II) complexes with the novel 1-methylpyrazole-3-aldehyde-4-(2-pyridyl)thiosemicarbazone (MPAPT) and 1,10-phenanthroline (1,10-phen) with different bivalent transition metal ions Co(II), Cu(II), Mn(II) and Ni(II). Additionally, our objective is also to study the antibacterial, antifungal and antitumor activities of the synthesized compounds.

2. Experimental

2.1. Materials and instrumentation

All starting materials were purchased from Fluka, Riedel and Merck and used as received. The microchemical analysis of the separated solid compounds was carried out at the Department of Chemistry, Faculty of Science, King Abdul-Aziz University, Jeddah, 21589, KSA. The analyses were performed twice to check the accuracy of the analyses data. Infrared spectra were recorded on 8001-PC FT-IR Shimadzu spectrophotometer using KBr pellets. The solid reflectance spectra were measured on a Shimadzu 3101 PC spectrophotometer. The room temperature magnetic susceptibility measurements for the complexes were determined by the Gouy balance using $Hg[Co(SCN)_4]$ as a calibrant. Electron paramagnetic resonance (EPR) has matured into a powerful, versatile, non-destructive, and non-intrusive analytical method. EPR signals were recorded at room temperature by using a Bruker EMX spectrometer (X-band) product of Bruker, Germany. The operating conditions are, microwave power = 0.201 mW, modulation amplitude = 4.00 Gauss, modulation frequency = 100 kHz, sweep width = 200 Gauss, microwave frequency = 9.775 GHz, time constant = 81.92 ms and sweep time = 20.97 s. The detection limits of EPR technique depends on the type of sample, sample size, detector sensitivity, frequency of the incident microwave radiation.

2.2. Synthesis

2.2.1. Synthesis of 4-(2-pyridyl)-3-thiosemicarbazone

4-(2-Pyridyl)-3-thiosemicarbazone is prepared in three steps as follow (Scheme 2).

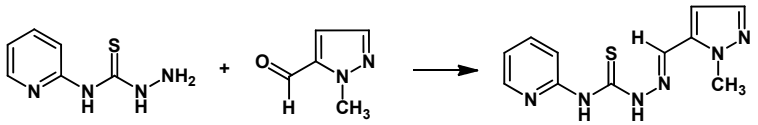
Step 1: Preparation of triethylammonium N-2-pyridyldithio carbamate: 2-Aminopyridine (0.3 mol, 27.5 g), carbon disulphide (0.3 mol, 18 mL) and triethylamine (0.3 mol, 45 mL) were warmed to give a clear solution. Two phases separated rapidly and the whole mixture was shaken at room temperature for 24 h, the whole had then solidified. The product was filtered and then washed with ether and air-dried. The lemon-yellow plates formed were triethylammonium *N*-2-pyridyldithiocarbamate, M.p.: 85 °C.

Step 2: Preparation of methyl-2-pyridyldithiocarbamate: Methanol (100 mL) was added to triethylammonium *N*-2-pyridyldithiocarbamate (49.0 g, 0.18 mol), followed by methyl iodide (13 mL, 0.2 mol). After 1 h, water was added into the solution and pale yellow needles of methyl-2-pyridyldithio carbamate were formed, M.p.: 101 °C.

Step 3: Preparation of 4-(2-pyridyl)-3-thiosemicarbazide: A mixture of 0.26 mol (50.0 g) of methyl-2-pyridyldithio carbamate and 26 mL (0.80 mol) of hydrazine hydrate in 20 mL of absolute ethanol was heated for 10 min. An abundant amount of crystalline precipitate of 4-(2-pyridyl)-3-thiosemi carbazide was formed, M.p.: 193 °C. Anal. calcd. for $C_6H_8N_4S$: C, 42.84; H, 4.79; N, 33.31. Found: C, 42.30; H, 4.62; N, 33.10%.

2.2.2. Synthesis of 1-methylpyrazole-3-aldehyde-4-(2-pyridyl)thiosemicarbazone (MPAPT)

The general route of synthesis (Scheme 3) is shown in the following: Equimolar amounts of 4-(2-pyridyl)-3-thiosemicarbazone (0.1682 g, 1 mmol) in 25 mL ethanol with an ethanolic solution (25 mL) of 1-methyl-1-*H*-pyrazole-5-carboxaldehyde (0.110 g, 1 mmol). The reaction mixture was then refluxed on a hot plate for 2-3 h. The obtained precipitates were separated out, filtered off, washed with diethyl ether and dried overnight under silica gel. The proposed chemical structure of the prepared thiosemicarbazone compound is in a good agreement with the stoichiometries concluded from their analytical data and confirmed by the IR spectral data. FT-IR (KBr, ν, cm^{-1}): 3188 (ν_{N1H}), 3168 (ν_{N2H}), 1648 ($\nu_{C=Nazomethine}$), 1549 ($\nu_{C=Npyridine}$), 1300, 825 ($\nu_{C=S}$), 1068 (νN-N), 622 ($\delta_{C=Npyridine}$). Anal. calcd. for $C_{11}H_{12}N_6S$: C, 50.75; H, 4.65; N, 32.28. Found: C, 50.90; H, 4.70; N, 32.38%.



Scheme 3

2.2.3. Synthesis of thiosemicarbazone complexes

All complexes were prepared by refluxing an ethanolic solution of the thiosemicarbazone (MPAPT) ligand (1 mmol) and 1,10-phenanthroline heterocyclic base (1 mmol) with an ethanolic solution of metal salt (1 mmol) in the molar ratio 1:1:1. The reaction mixtures were refluxed on a water bath for 4 h and allowed to cool to room temperature overnight. The precipitated complexes were then filtered off, washed with petroleum ether and dried overnight in a vacuum desiccator.

[Cu(MPAPT)(1,10-phen)(Cl)] complex (**1**): Yield: 77 %. FT-IR (KBr, ν, cm^{-1}): 3208 (ν_{N1H}), 1602 ($\nu_{C=Nazomethine}$), 1591 ($\nu_{N=C}$), 1525 ($\nu_{C=Npyridine}$), 1279, 825 ($\nu_{C=S}$), 1070 (νN-N), 615 ($\delta_{C=Npyridine}$), 452 (ν_{M-N}), 275 (ν_{M-Npy}), 343 (ν_{M-S}), 250 (ν_{M-Cl}). Anal. calcd. for $C_{23}H_{19}N_8SCuCl$: C, 51.30; H, 3.56; N, 20.81. Found: C, 51.21; H, 3.46; N, 20.70%.

[Ni(MPAPT)(1,10-phen)(Cl)] complex (**2**): Yield: 73 %. FT-IR (KBr, ν, cm^{-1}): 3215 (ν_{N1H}), 1608 ($\nu_{C=Nazomethine}$), 1581 ($\nu_{N=C}$), 1527 ($\nu_{C=Npyridine}$), 1270, 810 ($\nu_{C=S}$), 1070 (νN-N), 612 ($\delta_{C=Npyridine}$), 448 (ν_{M-N}), 340 (ν_{M-S}), 283 (ν_{M-Npy}), 248 (ν_{M-Cl}). Anal. calcd. for $C_{23}H_{19}N_8SNiCl$: C, 51.76; H, 3.59; N, 21.00. Found: C, 51.71; H, 3.46; N, 21.01%.

[Co(MPAPT)(1,10-phen)(Cl)].H_2O complex (**3**): Yield: 70 %. FT-IR (KBr, ν, cm^{-1}): 3220 (ν_{N1H}), 1601 ($\nu_{C=Nazomethine}$), 1580 ($\nu_{N=C}$), 1530 ($\nu_{C=Npyridine}$), 1268, 805 ($\nu_{C=S}$), 1082 (νN-N), 613 ($\delta_{C=Npyridine}$), 455 (ν_{M-N}), 340 (ν_{M-S}), 287 (ν_{M-Npy}), 255(ν_{M-Cl}). Anal. calcd. for $C_{23}H_{19}N_8SCoCl.H_2O$: C, 50.05; H, 3.84; N, 20.31. Found: C, 50.01; H, 3.76; N, 20.27%.

[Mn(MPAPT)(1,10-phen)(Cl)].H_2O complex (**4**): Yield: 77 %. FT-IR (KBr, ν, cm^{-1}): 3222 (ν_{N1H}), 1610 ($\nu_{C=Nazomethine}$), 1575 ($\nu_{N=C}$), 1529 ($\nu_{C=Npyridine}$), 1271, 811 ($\nu_{C=S}$), 1080 (νN-N), 611 ($\delta_{C=Npyridine}$), 448 (ν_{M-N}), 345 (ν_{M-S}), 290 (ν_{M-Npy}), 263 (ν_{M-Cl}). Anal. calcd. for $C_{23}H_{19}N_8SMnCl.H_2O$: C, 50.42; H, 3.86; N, 20.45. Found: C, 50.38; H, 3.43; N, 20.38%.

2.3. Molecular modeling

An attempt to gain a better insight on the molecular structure of these synthesized thiosemicarbazone compounds and their M(II)-complexes, geometric optimization and conformation analysis has performed using semiempirical parameterized PM3 method as implemented in HyperChem 7.5 [25]. Convergence criteria were set to 0.01 kcal/mol.Å for PM3 calculations.

2.4. Biological activity

2.4.1. Antibacterial and antifungal activities

The antibacterial investigation of the free (MPAPT) ligand or its Mn(II), Co(II), Cu(II) and Ni(II) complexes was carried out using a modified Kirby-Bauer disc diffusion method [26]. The test was done against filamentous fungi as (*Aspergillus flavus* (RCMB 02568), *Pencicillium italicum* (RCMB 03924), and *Aspergillus flavus* (RCMB 02568)) at 30 °C for 24-48 hours; Gram (+) bacteria as (*Bacillus subtillis* RCMB 010067 and *Staphylococcus aureus* RCMB 010028); Gram (-) bacteria as (*Pseudomonas aeuroginosa* (RCMB 010043), and *Escherichia coli* (RCMB 010052)). The standard antibacterial agents used are *Gentamicin* for Gram-, *Ampicillin* for Gram+ and *Amphotericin B* (Antifungal agent) served as positive controls while DMSO, which exhibited no antimicrobial activity, was used as a negative control. The agar used is Mueller-Hinton agar that is rigorously tested for composition and pH. Plates were incubated with filamentous fungi at 30 °C for 24-48 hours and with bacteria at 35-37 °C for 24-48 hours [27] and then the diameters of inhibition zones were measured in millimeters using slipping calipers of the National Committee for Clinical Laboratory Standards (NCCLS) [28,29].

2.4.2. In vitro cytotoxicity

The synthesized M(II) complexes were screened for their cytotoxicity against colon carcinoma (HCT116) and larynx carcinoma (HEP2) cells by using the protocol of SRB assay [30]. Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of plate. Different concentrations of the test chemical compound were added to the cell monolayer. Triplicate wells were prepared for each individual dose and IC_{50} is the mean of three values. Monolayer cells were incubated for 48 h at 37 °C in air with 5 % CO_2. After 48 h, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with *tris*-EDTA buffer. Color intensity is measured in an ELISA reader. The average drug concentration (μg/cm^3) for 50 % inhibition of tumor cell-growth was determined by plotting the surviving fraction versus drug concentration for each tumor cell line.

3. Results and discussion

The isolated thiosemicarbazone compound is formed by the interaction of 4-(2-pyridyl)-3-thiosemicarbazone with 1-methyl-1*H*-pyrazole-5-carboxaldehyde in a molar ratio 1:1 under reflux conditions (Scheme 3). The formulation of the isolated compounds based on the elemental analysis, IR, and electronic spectra. The solid compounds are air stable. These compounds are colored, insoluble in H_2O and other common organic solvents like methanol and ethanol but soluble in dimethylformamide (DMF) and dimethylsulphoxide (DMSO). Attempts to obtain single crystal suitable for X-ray determination were unsuccessful, thus molecular modeling for MPAPT thiosemicarbazone compound and its M(II)-complexes were investigated.

3.1. Infrared spectra

The characteristic IR peaks of the free MPAPT thiosemicarbazone compound and its metal(II) complexes reveal each of the following: The stretching vibrations of azomethine functionality ν(C=N) was observed near 1648 cm^{-1}. The lower shift of ν(C=N) stretching vibration of the azomethine in the complexes indicating the participation of the azomethine nitrogen in coordination [31]. The ν(N-N) of the thiosemicarbazone ligand is found at 1068 cm^{-1}. The increase in frequency of this band in the spectra of complexes due to the increase in the double bond character of N-N [32] is an evidence for the enethiolization of the thiosemicarbazone ligand and the coordination via the azomethine nitrogen. The ν(N^1H) and hydrazine ν(N^2H) were observed at 3188 and 3168 cm^{-1}. The pyridine in-plane deformation mode at 622 cm^{-1} in the spectrum of the ligand shifts to 611-615 cm^{-1} in spectra of

the above complexes, suggesting coordination of the heteroaromatic nitrogen. In the spectra of all complexes, the bands assigned to the newly formed (N=C)* band due to the thiolating of the (C=S) groups are located in the range 1575-1591 cm^{-1}. The IR spectrum of the free 1,10-phen ligand shows a very stronger bands at ~1570 cm^{-1} due to stretching frequency of >C=N present in 1,10-phenanthroline moiety. This band was shifted to lower frequencies in the complexes ~25-28 cm^{-1}, which clearly indicate that the coordination of the two nitrogen atoms of the neutral 1,10-phen ligand to M(II) ion upon complexation [33]. In the ligand spectra, the strong band observed in the range 1549 cm^{-1} is assigned to ν(C=N) stretching vibration [34] of pyridine ring. In the spectra of complexes, this band was not observed at the same frequencies and the same intensities. It shifted after coordination to lower energies by *ca*. 19-24 cm^{-1}, indicating coordination via azomethine nitrogen [35]. The coordination positions of the MPAPT thiosemicarbazone in the M(II) complexes are confirmed by assigning the strong bands observed in the far IR spectra of the complexes. The bands observed at 448-455 and 340-345 cm^{-1} are assigned to ν(M-N) [36] and ν(M-S) as suggested by Lever [37], respectively. The broad band centered at 3460-3490 cm^{-1} in the spectra of both Co(II) and Mn(II) complexes may be due to hydrated water. In the literature, the bands appearing between 160 and 300 cm^{-1} are allotted to the vibration of the M-X bonds where M = metal and X = Cl or Br [38,39]. In our case the ν(M-Cl) frequencies appearing between 248-263 cm^{-1} are in good agreement with the reported values in the literature. Based on the above spectral evidences, it is confirmed that the thiosemicarbazone ligands lost the N^2 proton and coordinated to the M(II) ion as mononegative tridentate anion, coordinating via the azomethine and pyridine nitrogen atoms and the thiolate sulfur atom after deprotonation.

3.2. Electronic spectra

The probable assignments for the bands in the region 27345 and 32989 cm^{-1} are due to the n → π* and π → π* transitions of thiosemicarbazone compound respectively. Always n → π* transitions occurs at a lower energy than π → π* transitions [40]. In the spectra of M(II) complexes the bands observed in the region 26521-26920 cm^{-1} are assigned to S→M charge-transfer band [41]. The bands in the range 30870-31108 cm^{-1} observed in the spectra of all M(II) complexes are assigned as Cl → M charge-transfer transitions [42]. The shift of the π → π* bands to the longer wavelength region is the result of the C=S bond being weakened and conjugation system being enhanced after the formation of the complex [43]. The magnetic moment of Mn(II) complex (6.01 B.M.) is corresponding to five unpaired electrons, as expected for high spin $3d^5$ systems [44]. The electronic spectrum of this complex show two bands at 20850 and 24120 cm^{-1} attributable to $^6A_{1g} \rightarrow {}^6T_{1g}(G)$ and $^6A_{1g} \rightarrow {}^6T_{2g}(G)$, respectively, suggesting an octahedral structure [44].

The Co(II) complexes generally give rise to three absorption bands in the visible region under the influence of the octahedral field by the excitation of the electron from the ground state $^4T_{1g}$ (F) to the excited states $^4T_{2g}$ (F), $^4A_{2g}$ (F) and $^4T_{1g}$ (P). In the Co(II) complex, only two bands are observed at 9935 cm^{-1} $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1) and 20,423 cm^{-1} $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3) as reported in many octahedral cobalt(II) complexes [45,46]. The ν_2 transition was not observed due to very weak intensity. The value of the magnetic moment 4.71 B.M. (normal range for octahedral Co(II) complexes is 4.3-5.2 B.M.) is additional evidence for an octahedral geometry around the Co(II) ion [47].

The copper(II) complex exhibit magnetic moment of 1.82 B.M. at room temperature. This value is quite close to the spin allowed values expected for a S = 1/2 system and may be indicative of a distorted octahedral geometry around copper(II) ion. This copper(II) complex displays a broad band at 14585 cm^{-1} due to $^2B_{1g} \rightarrow {}^2E_g$ assigned to *d-d* transitions of a distorted octahedral environment [48,49].

The electronic spectra of the nickel(II) complexes exhibited absorption bands at 10675 and 17540 cm^{-1}, attributable to $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g} \rightarrow {}^3T_{1g}(P)$, transitions, respectively, in an octahedral geometry [45,49]. Also, the value of the magnetic moment (3.21 B.M.) may be taken as additional evidence for their octahedral structure. On the basis of the above observations, it is tentatively suggested that all of the complexes show an octahedral geometry in which the MPAPT thiosemicarbazone ligand act as monoanion tridentate ligand, 1,10-phen acts as bidentate ligand and in addition to the monodentate chloride ion. These possibly accommodate themselves around the metal atom in such a way that a stable chelate ring is formed giving, in turn, stability to the metal complexes.

3.3. ESR spectrum of [Cu(MPAPT)(1,0-phen)Cl] complex

ESR spectroscopy is a direct measurement of electron spin when there are unpaired electrons within a chemical structure and thus provides a way to investigate the electronic spin state and oxidation state of the coordinated metal ion. Also, the ESR spectra of the complexes is important in studying the metal ion environment in the complexes, such as geometry, nature of ligation sites from the ligand to the metal, and the degree of covalence of the metal-ligand bonds. The room temperature powder ESR spectrum of [Cu(MPAPT)(1,10-phen)Cl] exhibits an axial signal with two g values ($g_{||}$ = 2.140, $g_{\perp}$ = 2.031). The $g_{||}$ and $g_{\perp}$ values are computed from the spectrum using DPPH free radical as "g" marker. The ordering of g values ($g_{||} > g_{\perp} > 2.003$) observed for copper(II) complex indicates that the unpaired electron is localized in (d_{x2-y2}) orbital [50] of the Cu(II) ion (spectroscopic state $^2B_{1g}$) and the spectral features are characteristics of axial symmetry [51]. $g_{||}$ is a moderately sensitive function for M-L bond nature. Kivelson and Neiman [40] have reported the $g_{||}$ value < 2.3 for covalent character of metal-ligand bond and $g_{||}$ value > 2.3 for ionic character. By applying this criterion, the $g_{||}$ value is less than 2.3 is an indication of significant covalent bonding in copper(II) complex. Based on elemental analysis, IR, electronic spectra and ESR data, Cu(II) complex has octahedral structure. The geometric parameter G, which is a measure of the exchange interaction between copper centers in the polycrystalline compound, is calculated using Hathway expression ($G = g_{||}-2.0023/g_{\perp}-2.0023$) [52]. According to Hathway, if the value of G > 4, the exchange interaction is negligible in solid complexes but G < 4 indicates considerable interaction in solid complexes. In the Cu(II) complex reported in this paper the G value (4.797) > 4, suggesting the absence of copper-copper exchange interactions in the solid state.

3.4. Conductivity measurements

Conductivity measurements in non-aqueous solutions have frequently been used in structural studies of metal chelates within the limits of their solubility. These measurements were provided a method for testing the degree of ionization of the complexes, the molar ions that a complex liberates in solution, the higher will be its molar conductivity and vice versa. The non-ionized complexes have negligible value of molar conductance. It is clear from the conductivity data (Table 1) that the complexes present seem to be nonelectrolytes (2.01-3.81 $\Omega^{-1}.cm^2.mol^{-1}$). Also the molar conductance values indicate that the anions were existed inside the coordination sphere which was also confirmed from the chemical analysis and also Cl^- ion is not precipitated by addition of $AgNO_3$ solution. Hence, the conductivity measurements of the metal(II)-chelates confirm the proposed general formulae of those chelates as suggested depending upon the results of elemental analyses, UV-Vis, ESR and IR spectra.

Table 1. Molar conductance, magnetic moment and electronic spectral data (cm^{-1}) of the complexes.

Compounds	Λ_M [a]	$\mu_{eff.}$ (B.M.)	*d-d*	Transitions
[Cu(MPAPT)(1,10-phen)Cl] (**1**)	2.01	1.82	14585	$^2B_{1g} \rightarrow {}^2E_g$
[Ni(MPAPT)(1,10-phen)Cl] (**2**)	2.54	3.21	10675	$^3A_{2g} \rightarrow {}^3T_{1g}(F)$
			17540	$^3A_{2g} \rightarrow {}^3T_{1g}(P)$
[Co(MPAPT)(1,10-phen)Cl].H_2O (**3**)	3.15	4.71	9935	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$
			20423	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
[Mn(MPAPT)(1,10-phen)Cl].H_2O (**4**)	3.81	6.01	20850	$^6A_{1g} \rightarrow {}^6T_{1g}(G)$
			24120	$^6A_{1g} \rightarrow {}^6T_{2g}(G)$

[a] Molar conductance measured for 10^{-3} M DMSO solution, Ω^{-1} cm^2 mol^{-1}.

Table 2. Antibacterial activity of thiosemicarbazone ligand and its metal complexes.

Compounds	Diameter of inhibition zone (in mm) [a]			
	G(-)		G(+)	
	Pseudomonas aeuroginosa **(RCMB 010043)**	***Escherichia coli*** **(RCMB 010052)**	***Bacillus subtillis*** **(RCMB 010067)**	***Staphylococcus aureus*** **(RCMB 010028)**
MPAPT	13.2±0.34	-	19.4±0.44	18.1±0.25
[Cu(MPAPT)(1,10-phen)Cl] (**1**)	17.7±0.19	14.1±0.37	24.7±0.52	27.2±0.45
[Ni(MPAPT)(1,10-phen)Cl] (**2**)	15.4±0.22	12.2±0.14	21.5±0.42	24.7±0.14
[Co(MPAPT)(1,10-phen)Cl].H_2O (**3**)	14.2±0.34	11.1±0.43	20.3±0.49	22.9±0.35
[Mn(MPAPT)(1,10-phen)Cl].H_2O (**4**)	12.7±0.25	10.1±0.25	18.8±0.57	21.8±0.39
Standard [b]	17.3±0.15	22.3±0.18	27.4±0.18	32.4±0.10

[a] Mean zone of inhibition in mm±standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (1 mg/mL) concentration of tested samples.
[b] The standard antibacterial agents are Gentamicin for G(-) and Ampicillin for G(+).

Table 3. Antifungal activity of thiosemicarbazone ligand and its metal complexes.

Compounds	Diameter of inhibition zone (in mm) [a]		
	Aspergillus flavus **(RCMB 02568)**	***Pencicillium italicum*** **(RCMB 03924)**	**Geotricum candidum (RCMB 05097)**
MPAPT	14.6±0.38	15.4±0.24	17.7±0.24
[Cu(MPAPT)(1,10-phen)Cl] (**1**)	19.6±0.50	19.2±0.38	24.5±0.15
[Ni(MPAPT)(1,10-phen)Cl] (**2**)	17.8±0.28	16.5±0.54	22.4±0.32
[Co(MPAPT)(1,10-phen)Cl].H_2O (**3**)	16.6±0.45	15.7±0.24	20.6±0.75
[Mn(MPAPT)(1,10-phen)Cl].H_2O (**4**)	15.6±0.37	14.8±0.37	17.8±0.73
Amphotericin (B)	23.7±0.10	21.9±0.12	28.7±0.22

[a] Mean zone of inhibition in mm±standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (1 mg/mL) concentration of tested samples.

3.5. Biological studies

3.5.1. Antimicrobial activity

To assess the biological potential of the synthesized compounds, the thiosemicarbazone ligand and its metal complexes were tested against different species of bacteria and fungi. The parent (MPAPT) ligand and its complexes are water insoluble; therefore, the antimicrobial test was carried out in DMSO. The results of the antimicrobial test of the parent ligand and its metal complexes against Gram positive and Gram negative are given in Table 2 while the results of the antifungal activity against was given in Table 3. In testing the antimicrobial activity of these compounds, we used more than one test organism to increase the chance of detecting antibiotic principles in tested materials. The organisms used in the present investigations included two Gram positive (*Staphylococcus aureus* and *Bacillus subtillis*) and two Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*). The diffusion agar technique was used to evaluate the antibacterial activity of the synthesized mixed ligand complexes [53-56]. The medium used for growing the culture was nutrient agar. Metal ions are adsorbed on the cell walls of the microorganisms, disturbing the respiration processes of the cells and thus blocking the protein synthesis that is required for further growth of the organisms. Hence, metal ions are essential for the growth-inhibitory effects [57]. The synthesized compounds were found to be more toxic compared with the parent free ligand against the same micro-organism and under the identical experimental conditions. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials, so lipophilicity is an important factor controlling the antifungal activity. Upon chelating, the polarity of the metal ion will be reduced due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. In addition, chelation allows for the delocalization of π-electrons over the entire chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity facilitates the penetration of the complexes into lipid membranes, further restricting proliferation of the microorganisms. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the microbial cells or on differences in the ribosomes of the cells [58]. All of the metal complexes possess higher antifungal activity than the free ligand [59,60]. Although the exact biochemical mechanism is not completely understood, the mode of action of antimicrobials may involve various targets in the microorganisms. These targets include the following.

(i) The higher activity of the metal complexes may be due to the different properties of the metal ions upon chelation. The polarity of the metal ions will be reduced due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. Thus, chelation enhances the penetration of the complexes into lipid membranes and the blockage of metal binding sites in the enzymes of the microorganisms [61].

(ii) Tweedy's chelation theory predicts that chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with donor groups and possible electron delocalization over the entire ring. This consequently increases the lipophilic character of the chelates, favoring their permeation through the lipid layers of the bacterial membrane [62].

(iii) Interference with the synthesis of cellular walls, causing damage that can lead to altered cell permeability

Table 4. Effect of synthesized metal complexes on tumor cell growth in vitro (IC_{50} values in µg/mL).

Compounds	IC_{50}	
	HCT116	HEP2
[Cu(MPAPT)(1,10-phen)Cl] (**1**)	0.63	0.41
[Ni(MPAPT)(1,10-phen)Cl] (**2**)	0.91	0.66
[Co(MPAPT)(1,10-phen)Cl].H_2O (**3**)	1.11	0.78
[Mn(MPAPT)(1,10-phen)Cl].H_2O (**4**)	1.35	0.81
Doxorubicin (standard)	0.69	0.40

IC_{50} =cytotoxic dose at 50 %, i.e. the drug concentration to inhibit the growth of the cancer cells by 50 %.

characteristics or disorganized lipoprotein arrangements, ultimately resulting in cell death.

(iv) Deactivation of various cellular enzymes that play a vital role in the metabolic pathways of these microorganisms.

(v) Denaturation of one or more cellular proteins, causing the normal cellular processes to be impaired.

(vi) Formation of a hydrogen bond through the azomethine group with the active centers of various cellular constituents, resulting in interference with normal cellular processes [63-65].

The tested complexes were more active against Gram(+) than Gram(-) bacteria, it may be concluded that the antibacterial activity of the compounds is related to cell wall structure of the bacteria. It is possible because the cell wall is essential to the survival of bacteria and some antibiotics are able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids, but in contrast, Gram negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins. These differences in cell wall structure can produce differences in antibacterial susceptibility and some antibiotics can kill only Gram-positive bacteria and is ineffective against Gram-negative pathogens [66,67]. It is worth noting that the comparison of antimicrobial activity of the compounds against the selected types of bacteria (Figure 1 and 2) indicates that Cu(II) > Ni(II) > Co(II) > Mn(II).

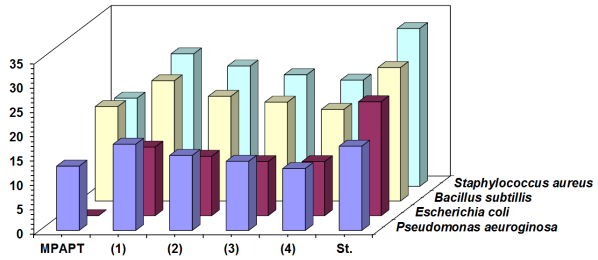


Figure 1. Antibacterial activity of M(II)-complexes towards different types of bacterial strains.

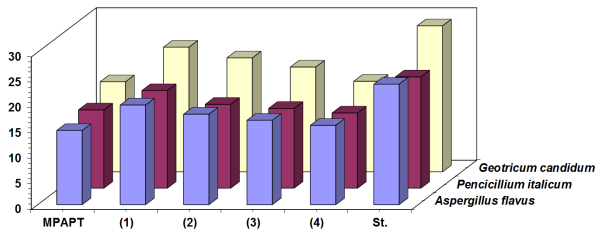


Figure 2. Antifungal activity of M(II)-complexes towards different types of fungal strains.

3.5.2. Cytotoxic activities

Cytotoxic study of the compounds against Colon Carcinoma (HCT116) and Larynx Carcinoma (HEP2) cells indicate that, [Cu(MPAPT)(1,10-phen)Cl] complex show significant activity against (HCT116) and (HEP2) cells with IC_{50} values of 0.41 µg/ml (Figure 3) and 0.63 µg/mL respectively. Thus, [Cu(MPAPT)(1,10-phen)Cl] complex is chemotherapeutically significant (Table 4). IC_{50} is the concentration which can reduce the growth of cancer cells by 50 %. Results show that the growth inhibition of tumor cells is due to apoptosis (programmed cell death) in all M(II)-complexes. Induction of apoptosis was recently thought to be one of the mechanisms of the antitumor effect of *cis*-platin [68].

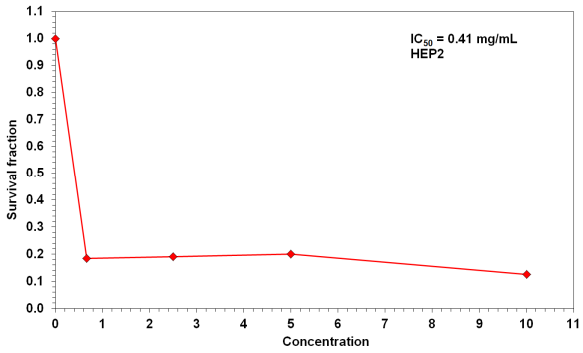


Figure 3. Effect of Cu(II) complex on surviving fraction of HEP2 tumor cell.

3.6. Molecular modeling

The atomic numbering scheme as given in Figures 4-6 and the theoretical geometry structures for the ligand and some of its metal complexes are calculated. The molecular parameters: total energy, binding energy, isolated atomic energy, electronic energy, heat of formation, dipole moment, HOMO and LUMO were calculated and represented in Table 5. A comparison between the bond length of the ligand and its complexes is illustrated. All the active groups taking part in coordination have bonds longer than that already exist in the ligand (like C=N and C=S). The lower HOMO energy values show that molecule donating electron ability is the weaker. On contrary, the higher HOMO energy implies that the molecule is a good electron donor. LUMO energy presents the ability of a molecule receiving electron [69].

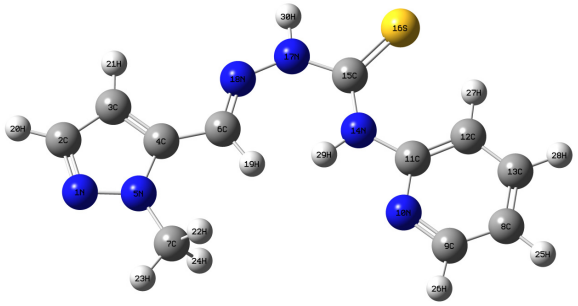


Figure 4. The optimized structural geometry of MPAPT along with the atom numbering scheme.

Table 5. Some energetic properties of MPAPT thiosemicarbazone and its complexes calculated by PM3 method.

Ligand	Total energy (kcal/mol)	Binding energy (kcal/mol)	Electronic energy (kcal/mol)	Dipole moment (Debye)	HOMO (eV)	LUMO (eV)
MPAPT	-60965.8	-3093.26	-4001356.6	4.55	-8.94	-1.51
[Cu(MPAPT)(1,10-phen)Cl] (**1**)	-138355.50	-5845.52	-1299306.8	7.91	-3.95	-1.45
[Ni(MPAPT)(1,10-phen)Cl] (**2**)	-135067.27	-5967.66	-1268318.8	19.47	-6.36	-2.29
[Co(MPAPT)(1,10-phen)Cl].H_2O (**3**)	-129390.70	-6156.55	-1231416.2	8.50	-3.62	-1.55
[Mn(MPAPT)(1,10-phen)Cl].H_2O (**4**)	-120098.41	-5907.57	-1181854.9	10.19	-4.30	-1.31

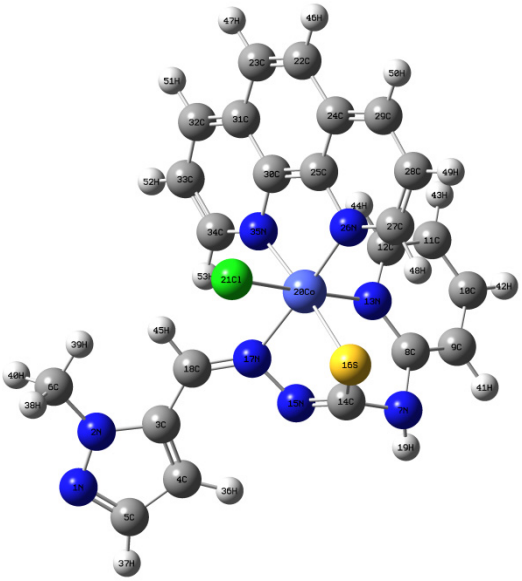


Figure 5. The optimized structural geometry of [Co(MPAPT)(1,10-phen)Cl] (**3**) along with the atom numbering scheme.

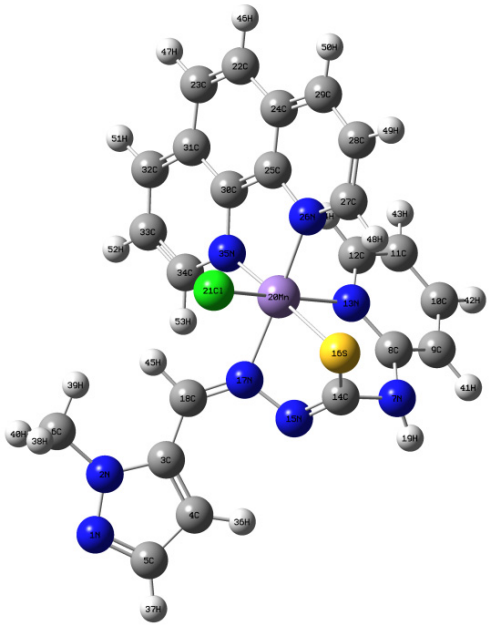


Figure 6. The optimized structural geometry of [Mn(MPAPT)(1,10-phen)Cl] (**4**) along with the atom numbering scheme.

The bond lengths and bond angles of [Co(MPAPT)(1,10-phen)Cl] complex as a representative example of M(II) compounds are given in supplementary data. A drawing of MPAPT-thiosemicarbazone ligand is shown in Figure 4 while Co(II) and Ni(II) complexes with the atomic numbering scheme are shown in Figures 5 and 6. The coordination results in the changes of bond lengths and angles of the thiosemicarbazone moiety, as expected, thus when the bond lengths in the coordinated thiosemicarbazone ligand are compared with those in the free thiosemicarbazone ligand, it is seen that coordination elongates the thiosemicarbazone moiety's C–S bond from 1.664 Å to 1.709-1.787 Å and contracts adjacent N–C(S) bond from 1.420 Å to 1.325-1.347 Å in which is consistent with the C–S acquiring a partial single bond and N–C(S) a partial double bond character. These changes in bond lengths are attributable to stabilization of the iminothiolate form of the thiosemicarbazone ligand upon complexation *via* loss of the hydrazinic proton [70]. This means that, C–S distances which are in the range of single bond character being some of the largest found for M(II) complexes (typical bond lengths being C(sp^2)–S 1.706 Å in $(MeS)_2C{=}C(SMe)_2$ and C=S 1.630 Å in naphthylphenylthioketone) [71,72]. This also confirms the IR and spectral data which assumed that the C=S on coordination gains C–S character. Similar structural features are known for other metal complexes of such ligands that have the same coordination sites [70,73]. The other bond lengths and angles also suffer some changes, but not significantly. In general, the M–S bond length is longer than that of M–Cl for the all M(II) complexes and the M–N bond length is shorter than M–Cl bond length showing that the bond length obeyed this order M–S > M–Cl > M–N. In all complexes, $C{=}N_{py}$ bond distance of the pyridine ring is elongated. Owing to the formation of the M-N bond which makes the C=N bond weaker as a result of coordination. The bond angles of the thiosemicarbazone moiety of MPAPT are altered somewhat upon coordination and may be reduced or increased on complex formation as a consequence of bonding. The bond angles around the M(II) center (≃90 °) prove that the geometric is octahedral as proposed by the different tools of analysis mentioned previously. Finally, from the interpretation of elemental analysis, spectral data (infrared, electronic and ESR) as well as magnetic susceptibility measurements at room temperature, conductivity measurements and QM calculations, it is possible to draw up the tentative octahedral structures of the metal complexes.

4. Conclusions

The condensation reaction of 4-(2-pyridyl)-3-thiosemicarbazone with 1-methyl-1*H*-pyrazole-5-carbox-aldehyde in the molar ratio (1:1) afforded the corresponding 1-methylpyrazole-3-aldehyde-4-(2-pyridyl)thiosemicarbazone compound (MPAPT). The bonding of ligands to the metal ion is confirmed by analytical, spectral and magnetic measurements. The IR spectra showed that, thiosemicarbazone compound present in the thione form in the solid state. In the absence of X-ray single crystal data of the current synthesized complexes and based on the physicochemical studies and geometrical optimization, a tentative structure could be proposed as shown in Figures 4-6. M(II)-complexes are formulated as [M(MPAPT)(1,10-phen)Cl].nH_2O where MPAPT is the deprotonated thiosemicarbazone ligand. In these complexes the thiosemicarbazone ligand is coordinated to the metal(II) ion as a tridentate anion, coordinating via the azomethine nitrogen, pyridine nitrogen atoms and the thiolate sulfur atom after deprotonation. 1,10-Phen is coordinated as neutral bidentate ligand via the two pyridine nitrogen atoms.

From elemental analysis, IR, spectral, thermal analysis, magnetic and conductance measurements, all M(II)-complexes are nonelectrolytes with octahedral structure. In this work, it was found that the M(II) compounds show significantly different levels of biological activity. The antibacterial, antifungal, screening data revealed that newly generated compounds are potential antimicrobial agents. Cu(II) complex showed a higher antitumor activity versus larynx carcinoma.

Supplementary material

Supporting information for this article is available on the WWW under http://www.eurjchem.com or from author. The bond lengths and bond angles of [Co(MPAPT)(1,10-phen)Cl] complex as a representative example of M(II) compounds are given in Table S1.

References

[1]. Berber, I.; Cokmus, C.; Atalan, E. *Mikrobiologia* **2003**, *72*, 54-55.
[2]. Harbottle, H.; Thakur, S.; Zhao, White, D. G. *Anim. Biotechol.* **2006**, *17*, 111-124.
[3]. Lakshmi, B.; Avaji, P. G.; Shivananda, K. N.; Nagella, P.; Manohar, S. H.; Mahendra, K. N. *Polyhedron* **2011**, *30*, 1507-1515.
[4]. Chan, J.; Huang, Y.; Liu, G.; Afrasiabi, Z.; Sinn, E.; Padhye, S.; Ma, Y. *Toxicol. Appl. Pharm.* **2004**, *197*, 40-48.
[5]. Rosen, J.; Smith, H.; Wiggall, K. J.; Zhang, L.; Luengo, J. I. *J. Med. Chem.* **2002**, *45*, 3573-3575.
[6]. Du, X.; Guo, C.; Hansel, E.; Doyle, P. S.; Caffrey, C. R.; Holler, T. P.; McKerrow, J. H.; Cohen, F. E. *J. Med. Chem.* **2002**, *45*, 2695-2707.
[7]. Kovala-Demertzi, D.; Demertzis, M. A.; Filiou, E.; Pantazaki, A. A.; Yadav, P. N.; Miller, J. R.; Zheng, Y.; Kyriakidis, D. A. *Biometals* **2003**, *16*, 411-419.
[8]. Scovill, J. P.; Klayman, D. L.; Franchino, D. G. *J. Med. Chem.* **1982**, *25*, 1261-1264.
[9]. Klayman, L.; Scovill, J. P.; Bartosevich, J. F.; Bruce, J. *J. Med. Chem.* **1983**, *26*, 35-39.
[10]. Demertzi, D. K.; Demertzis, M. A.; Miller, J. R.; Papadopoulou, C.; Dodorou, C.; Filousis, G. *J. Inorg. Biochem.* **2001**, *86*, 555-563.
[11]. Gupta, P.; Basuli, F.; Peng, S. M.; Lee, G. H.; Bhattacharya, S. *Inorg. Chem.* **2003**, *42*, 2069-2074.
[12]. Singh, N. K.; Srivastava, A.; *Trans. Met. Chem.* **2000**, *25*, 133-140.
[13]. Li, J.; Zheng, L. M.; King, I.; Doyle, T. W.; Chen, S. H. *Curr. Med. Chem.* **2001**, *8*, 121-133.
[14]. Chandra, S.; Kumar, U.; Verma, H. S. *J. Saudi Chem. Soc.* **2003**, *7(3)*, 337-346.
[15]. Chandra, S.; Kumar, U.; Verma, H. S. *Oriental J. Chem.* **2003**, *19 (2)*, 355-359.
[16]. Saha, N. C.; Butcher, R. J.; Chaudhuri, S.; Saha, N. *Polyhedron* **2003**, *22*, 383-390.
[17]. Chandra, S.; Singh, G.; Tyagi, V. P.; Raizada, S. *Synth. React. Inorg. Met. Org. Chem.* **2001**, *31(10)*, 1759-1770.
[18]. Malon, M.; Travnicek, Z.; Marysko, M.; Zboril, R.; Maslan, M.; Marek, J.; Dolezal, K.; Rolcik, J.; Krystof, V.; Strnad, M. *Inorg. Chim. Acta* **2001**, *323*, 119-129.
[19]. Saha, D. K.; Padhye, S. Sinn, E.; Newton, C. Indian *J. Chem. A* **2002**, *41A*, 279-283.
[20]. Booth, B. A.; Moore, E. C.; Sartorelli, A. C. *Cancer Res.* **1971**, *31*, 228-234.
[21]. Tojal, J. G.; Orad, A. G.; Serra, J. L.; Pizarro, J. L.; Lezama, L.; Arriortua, M. I.; Rojo, T. *J. Inorg. Biochem.* **1999**, *75*, 45-54.
[22]. El-Sherif, A. A.; Aljahdali, M. S. *Inorg. Chim. Acta* **2013**, *407*, 58-68.
[23]. Sammes, P. G. Yahioglu, G. *Chem. Soc. Rev.* **1994**, *23*, 327-334.
[24]. Farrell, N. Transition Metal Complexes as Drugs and Chemotherapeutic Agents, Kluwer Academic, Dordrecht, 1989.
[25]. Hyper Chem. Version 7.5 Hypercube, Inc., 2003.
[26]. Bauer, A. W.; Kirby, W. M.; Sherris, C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, *45*, 493-496.
[27]. Liebowitz, L. D.; Ashbee, H. R.; Evans, E. G. V.; Chong, Y.; Mallatova, N.; Zaidi, M.; Gibbs, D. *Microbiol. Infet. Dis.* **2001**, *24*, 27-33.
[28]. Pfaller, M. A.; Burmeister, L.; Bartlett, M. A.; Rinaldi, M. G. *J. Clin. Microbiol.* **1988**, *26*, 1437-1441.
[29]. National Committee for Clinical Laboratory Standards, Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi: Proposed standard M38-A, NCCLS, Wayne, PA, USA, 2002.
[30]. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl Cancer Inst.* **1990**, *82*, 1107-1112.
[31]. El-Sherif, A. A.; Shehata, M. R.; Shoukry, M. M.; Barakat, M. H. J. *Spectrochim. Acta A* **2012**, *96*, 889-897.
[32]. Aljahdali, M. S. *Eur. J. Chem.* **2013**, *4(4)*, 434-443.
[33]. Maurya, R. C.; Mishra, D. D.; Jaiswal, S. K.; Dubey, *J. Synth. React. Inorg. Met. Org. Chem.* **1995**, *25*, 521–535.
[34]. El-Sherif, A. A.; Eldebss, T. M. *Spectrochim. Acta A* **2011**, *79*, 1803-1814
[35]. Kurup, M. R. P.; Joseph, M. *Synth. React. Inorg. Met. Org. Chem*. **2003**, *33*, 275-281.
[36]. Philip, V.; Suni, V.; Kurup, M. R. P.; Nethaji, M. *Polyhedron* **2004**, *23*, 1225-1233.
[37]. Lever, A. B. P. Inorganic Electronic Spectroscopy, 2nd Edition, Elsevier, Amsterdam, 1984.
[38]. El-Sherif, A. A.; Shoukry, M. M.; Abd-Elgawad, M. M. A. *Spectrochim. Acta A* **2012**, *98*, 307-321
[39]. Nakamoto, K. Infrared and Raman Spectra of Inorganic and Coordination Compounds, Wiley-Interscience, New York, 1986.
[40]. Philip, V.; Suni, V.; Kurup, M. R. P. *Polyhedron* **2006**, *25*, 1931-1938.
[41]. Sankaraperumal, A.; Karthikeyan, J.; Shetty, A. N.; Lakshmisundaram, R. *Polyhedron* **2013**, *50*, 264-269.
[42]. Suzuki, M.; Kanatomi, H.; Koyama, H.; Murase, I. *Bull. Chem. Soc. Japan* **1980**, *53*, 1961-1970.
[43]. Li, Q.; Tang, H.; Li, Y.; Wang, M.; Wang, L.; Xia, C. *J. Inorg. Biochem.* **2000**, *78*, 167-178.
[44]. Mohan, M.; Kumar, M. *Trans. Met. Chem*. **1985**, *10*, 255–258.
[45]. Singh, V. P.; Katiyar, A. *J. Pesticide Biochem. Physlog.* **2008**, *92*, 8-14
[46]. Aljahdali, M.; El-Sherif, A. A.; Hilal, R. H. *Eur. J. Chem*. **2013**, *4(4)*, 370-378.
[47]. El-Sherif, A. A. *J. Solution Chem.* **2010**, *39*, 1562–1581.
[48]. Lever, A. B. P.; Lewis, J.; Nyholm, R. S. *J. Chem. Soc*. **1963**, 2552- 2556.
[49]. Ballhausen, C. J. An Introduction to Ligand Field; 3rd Edn.; McGraw Hill: New York, 1962.
[50]. Hathaway, B. J.; Tomlinson, A. A. G. *Coord. Chem. Rev.* **1970**, *5*, 143-207.
[51]. Balasubramanian, B.; Krishnan, C. N. *Polyhedron* **1986**, *5*, 669-675.
[52]. Dudley, R. J.; Hathaway, B. J. *J. Chem. Soc. A* **1970**, 1725-1728.
[53]. Ferrari, M. B.; Capacchi, S.; Pelosi, G.; Reffo, G.; Tarasconi, P.; Al-bertini, R.; Pinelli, S.; Helicin, P. L. *Inorg. Chim. Acta* **1999**, *286*, 134-141.
[54]. Jayabalakrishnan, C.; Natarjan, K. *Synth. React. Inorg. Met. Org. Chem.* **2001**, *31*, 983-988.
[55]. Jeeworth, T.; Wah, H. L. K.; Bhowon, M. G.; Ghoorhoo, D.; Babooram, K. *Synth. React. Inorg. Met. Org. Chem.* **2002**, *30*, 1023-1038.
[56]. Dharmaraj, N.; Viswanathamurthi, P.; Natarajan, K. *Trans. Met. Chem*. **2001**, *26*, 105-112.
[57]. Pal, I.; Basuli, F.; Bhattacharya, S. *Chem. Sci.* **2002**, *114(4)*, 255-268.
[58]. Anjaneyula, Y.; Rao, R. P. *Synth. React. Inorg. Met. Org. Chem.* **1986**, *16*, 257-272.
[59]. Chohan, Z. H.; Arif, M.; Akhtar, M. A.; Supuran, C. T. *Bioinorg. Chem. Appl*. Vol. 2006, 1-11, DOI: 10.1155/BCA/2006/83131.
[60]. Chohan, Z. H.; Scozzafava, A.; Supuran, C. T. J. *Enzym. Inhib. Med. Chem.* **2003**, *18(3)*, 259-263.
[61]. Prasad, K. S.; Kumar, L. S.; Shekar, S. C.; Prasad, M.; Revanasiddappa, H. D. *J. Chem. Sci.* **2011**, *12*, 1-10.
[62]. Thangadurai, T. D.; Natarajan, K.; *Trans. Metal Chem*. **2001**, *26(4-5)*, 500-504.
[63]. Dharmaraj, N.; Viswanathamurthi, P.; Natarajan, K. *Trans. Metal Chem*. **2001**, *26(1-2)*, 105-109.
[64]. Joseyphus, R.; Nair, M. *Mycobiology* **2008**, *36*, 93-98.
[65]. Malhota, L.; Kumar, S.; Dhindsa, K. S. *Indian J. Chem. A* **1993**, *32*, 457-459.
[66]. Koch, A. L. *Clin. Microbiol. Rev*. **2003**, *16*, 673-678.
[67]. Collins, R. C.; Davis, R. E. *Acta Crystallogr. B* **1978**, *34*, 283-285.
[68]. Barry, M. A.; Behnke, C. A.; Eastman. A. *Biochem. Pharmacol.* **1990**, *40*, 2353-2362.
[69]. Sagdinc, S.; Koksoy, B.; Kandemirli, F.; Bayari, S. H. *J. Mol. Struct.* **2009**, *917*, 63-70.
[70]. Collins, C.; Davis, R. E. *Acta Crystallogr. B* **1978**, *34*, 283-285.
[71]. Dutta, S.; Basuli, F.; Peng, S. M.; Lee, G. H.; Bhattacharya, S. *New J. Chem.* **2002**, *26*, 1607-1613.
[72]. Arjunan, P.; Ramamurthy, V.; Ventakesan, K. *Acta Crystallogr. C* **1984**, *40*, 556-558.
[73]. Kraker, A.; Krezoski, S.; Schneider, J.; Minkel, D.; Petering, D. H. *J. Biol. Chem.* **1985**, *260*, 13710-13718.