European Journal of Medicinal Chemistry 45 (2010) 774–781

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Metal-based biologically active agents: Synthesis, characterization, antibacterial and antileukemia activity evaluation of Cu(II), V(IV) and Ni(II) complexes with antipyrine-derived compounds

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ARTICLE INFO

Article history:
Received 14 July 2009
Received in revised form
22 October 2009
Accepted 23 October 2009
Available online 4 November 2009

Keywords:
Schiff bases
Antimicrobial activity
Leukemia cells HL-60
Copper complexes
Antipyrine

ABSTRACT

The paper presents the synthesis of complex combinations of Cu(II), V(IV) and Ni(II) with Schiff bases obtained through the condensation of 4-amino-1,5-dimethyl-2-phenyl-1*H*-3-pyrazol-3(2*H*)-one (antipyrine) with 2-hydroxybenzaldehyde, 4-hydroxy-5-methoxyisophthalaldehyde and 4,5-dihydroxy isophalaldehyde respectively. The characterization of newly formed complexes was done by ^{1}H NMR, ^{13}C NMR, UV–VIS, IR, EPR spectroscopies and molar electric conductibility studies. The effect of these complexes on proliferation of human leukemia cells (HL-60) and their antibacterial activity against *Staphylococcus aureus* var. Oxford 6538, *Escherichia coli* ATCC 10536 and *Candida albicans* ATCC 10231strains were studied and compared with those of free ligands.

1. Introduction

Schiff base complexes are considered to be among the most important stereochemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety [1]. Schiff bases are potential anticancer drugs and, when administered as their metal complexes, the anticancer activity of these complexes is enhanced in comparison to the free ligand [2].

Schiff bases of 4-aminoantipyrine and its complexes present a great variety of biological activity ranging from antitumor, fungicide, bactericide, anti-inflammatory, and antiviral activities [3–5]. The number of transition metal complexes of Cu(II), Ni(II) and V(IV) with oxygen and nitrogen donor Schiff base derivatives of 4-aminoantipyrine is limited. A small number of papers describe the synthesis and characterization of these compounds based on aminoantipyrine Schiff bases [6–14].

In a previous paper [15] we presented the synthesis of Cu(II) complexes derived from the newly Schiff base ligands obtained by condensation of 4-amino-antipyrine with 2-hydroxybenzaldehyde **L^{1}** and terephthalic aldehyde **L^{4}** using $CuCl_2 \cdot 2H_2O$ and $CuSO_4 \cdot 5H_2O$ salts, respectively.

This paper is a continuation of our previous research and it presents the synthesis and characterization of new complexes of Cu(II) with Schiff base **L^{1}**, using other salts of Cu(II) as well as other complexes of V(IV) and Ni(II). Also, this paper presents the synthesis and the characterization of some complexes of Cu(II) and V(IV) with newly Schiff bases (Fig. 1) obtained by condensation of 4-amino-antipyrine with 4-hydroxy-5-methoxyisophthalaldehyde, **L^{2}** and 4,5-dihydroxy-isophtalaldehyde **L^{3}**, respectively. The biochemical effects involved in the anti-proliferative activity of ligands and synthesized complexes in human HL-60 promyelocytic leukemia cells were investigated. The complexes and ligands were also tested for their in vitro antibacterial activity against *Staphylococcus aureus* var. Oxford 6538, *Escherichia coli* ATCC 10536 and *Candida albicans* ATCC 10231 strains using the paper disc diffusion method [16] (for the qualitative determination) and the serial dilutions in liquid broth method [17] (for determination of MIC).

2. Results and discussion

2.1. Chemistry

The present Schiff bases **L^{2}**- **L^{3}** (Fig. 1) were prepared under the method described elsewhere [15], by refluxing in methanol

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doi:10.1016/j.ejmech.2009.10.034

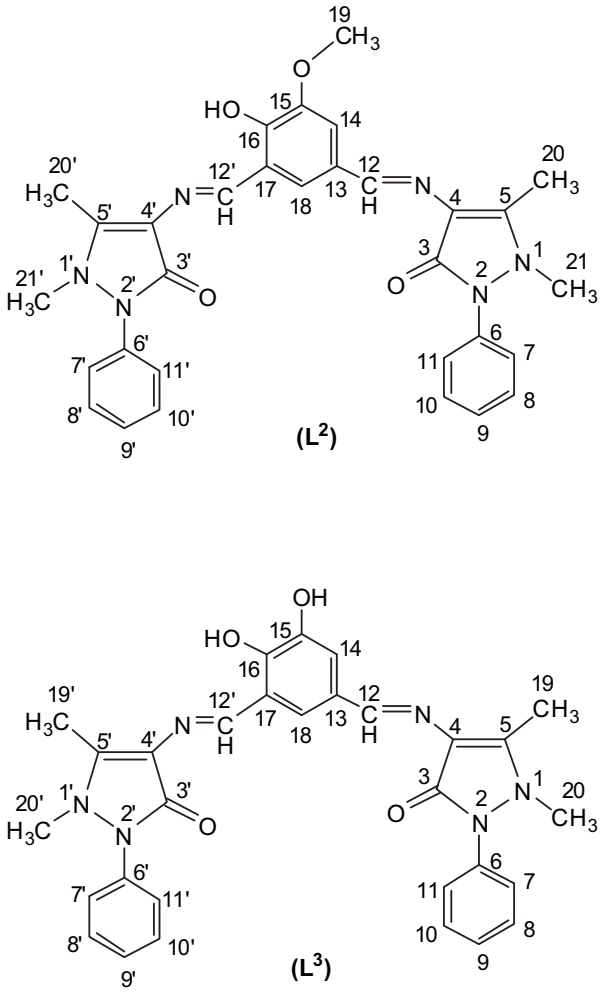


Fig. 1. The structures of ligands $\mathbf{L^2}$, $\mathbf{L^3}$.

(30–40 mL) an equimolar mixture of 4-amino-1,5-dimethyl-2-phenyl-1*H*-3-pyrazol-3(2*H*)-one with 4-hydroxy-5-methoxy isophthalaldehyde or 4,5-dihydroxyisophalaldehyde. The structures of these formed Schiff bases were established by IR, UV, ^{1}H NMR and ^{13}C NMR spectroscopies. These Schiff bases were further used for the complexation reaction with Cu^{+2}, VO^{+2} and Ni^{+2} metal ions, using the following metal salts: $CuSO_4{\cdot}5H_2O$ (for complexes **1**, **2**, **8**, **10**), $Cu(NO_3)_2$ $3H_2O$ (for **3**), $Cu(ClO_4)_2{\cdot}6H_2O$ (for **4**), $Cu(OAc)_2{\cdot}H_2O$ (for **5**), $NiCl_2{\cdot}6H_2O$ (for **7**) and $VOSO_4{\cdot}2H_2O$ (for **6, 9, 11**) (Merck). The obtained complexes are microcrystalline solids which are stable in air and decompose above 250 °C (Table 1) (except for complexes **1, 5, 6**). They are insoluble in organic solvents such as acetone and chloroform, but soluble in methanol, DMF and DMSO. The molar conductance of the soluble complexes in DMF showed values indicating that **(2)**, **(3)**, **(5)** and **(7)** (10–15 ohm^{-1} cm^2 mol^{-1}) are non-electrolytes and **(1)**, **(4)**, **(6)**, **(8–11)** (80–90 ohm^{-1} cm^2 mol^{-1}) are electrolytic in nature [18]. With the elemental analyses data of the Schiff bases (reported in experimental) and their complexes (Table 1) are in agrement with structures of the ligands as shown in Fig. 1 and with of the formulae of the complexes as shown in Fig. 2.

2.1.1. NMR spectra

The NMR spectra of free ligands were determined in DMSO-d_6. The ^{1}H NMR spectral data are reported along with the possible assignments. All the protons were found to be in the expected regions [19]. It was also observed that DMSO did not have any coordinating effect on the ligands or their metal complexes.

2.1.2. Electronic spectra and magnetic moments

The geometry of the metal complexes has been deduced from electronic spectra and magnetic moment data of the complexes (Table 1). The complex $[Cu_2(C_{18}H_{16}N_3O_2)_2]SO_4$ **(1)** shows a very low magnetic moment measured at room temperature, 0.62 BM per copper center. The subnormal magnetic moment indicates that the copper centers are strongly coupled anti-ferromagnetic spin-spin interactions through molecular association for square-planar geometry (Fig. 2) [20–25], or indicates the fact that a *super-exchange* interaction is probably occurs [26a]. The reflectance spectra exhibit broad absorption band at 14710 cm^{-1} specific for most complexes of Cu(II) [26b] and a weak band at 20408 cm^{-1}. The values of the magnetic moment (1.88–1.94 BM) of the solid complexes $[Cu(C_{18}H_{16}N_3O_2)_2]$ **(2)** and **(3)** as well as $[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]ClO_4$ **(4)** indicate the presence of an unpaired electron on Cu(II) ion.

The reflectance spectra exhibit three absorption bands of medium intensity for the complexes **(2)** and **(3)**: 14320 cm^{-1}, 15640 cm^{-1}, and 18270 cm^{-1}, which may be attributed to the transitions $d_{x^2-y^2} \rightarrow d_z^2$, $d_{x^2-y^2} \rightarrow d_{xz,yz}$ and $d_{x^2-y^2} \rightarrow d_{xy}$ characteristic to a distorted octahedral geometry. The reflectance spectra for complex **(4)** present a single absorption band at 14890 cm^{-1} corresponding to the d–d transition, indicating the low C_{2v} symmetry of the Cu^{2+} ion [26c]. The magnetic moment value (1.78 BM) of the complex $[Cu(C_{18}H_{16}N_3O_2)_2(H_2O)_2(ac)_2]$ **(5)** indicates that a *super-exchange* interaction is occurring, probably $p_\sigma \rightarrow e_\sigma$ or $p_\pi \rightarrow t_{2g}$ [26d,e]. The reflectance spectra of this complex showed two weak, low-energy bands at 13880 cm^{-1} and 19050 cm^{-1} attributed to the transitions $d_{x^2-y^2} \rightarrow d_{xz,yz}$ and $d_{x^2-y^2} \rightarrow d_{xy}$ specific to a distorted octahedral geometry and a strong high-energy band at 26320 cm^{-1}, assigned to ligand→metal charge transfer [27,28].

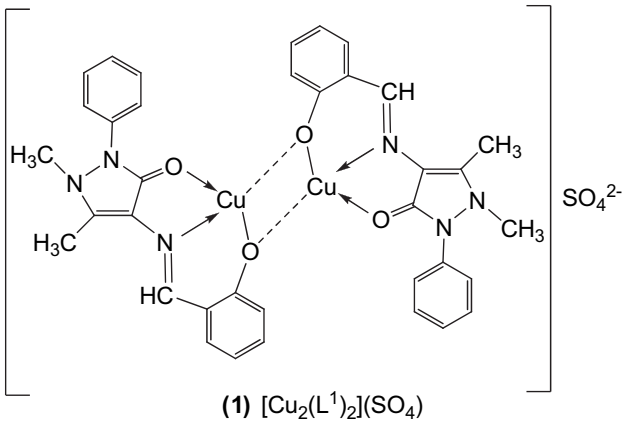
Also, at room temperature the magnetic moment values (2.02, 2.04 BM) of the solid complexes $[Cu(C_{31}H_{30}N_6O_4)]SO_4$ **(8)** and $[Cu(C_{30}H_{28}N_6O_4)]SO_4$ **(10)** indicate the presence of an unpaired electron on Cu(II) ion in an ideal square-planar environment [20]. The electronic spectra of complexes **(8)** and **(10)** showed two weak, low-energy bands at 14980, 20870 cm^{-1} and 15160, 21290 cm^{-1} respectively, which may be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions, for their square-planar geometry [29]. The magnetic moment values (1.51–1.73 B.M) for the VO^{+2} complexes $[VO(C_{18}H_{16}N_3O_2)(H_2O)]_2SO_4$ **(6)**, $[VO(C_{31}H_{30}N_6O_4)]SO_4$ **(9)** and $[VO(C_{30}H_{28}N_6O_4)]SO_4$ **(11)** suggest the presence of an unpaired electron and a strongly distorted geometry. All these complexes are paramagnetic and their values show the existence of monomeric species of oxovanadium (IV) [29].

The electronic spectra of complex **(6)** exhibit three bands. The first band at ≈14250 cm^{-1} can be attributed to a d-d transition ($d_{xy} \rightarrow d_{xz;yz.}$). The second band at ≈20250 cm^{-1} can be assigned to a $d_{xy} \rightarrow d^{22}_{x-y}$ transition. The third band at about 24520 cm^{-1} is attributed to a $d_{xy} \rightarrow d_{x^2-y^2}$ transition. The electronic spectra of complexes **(9)** and **(11)** exhibit two bands of low intensity at 13680, 18940 cm^{-1} and 13180, 19120 cm^{-1} respectively, due to the ligand-field transitions $d_{xy} \rightarrow d_{xz;yz.}$ and $d_{xy} \rightarrow d_{x^2-y^2}$, both characteristic for an oxovanadium(IV) chromophore. A third d-d transition was probably obscured by the tail of the ligand-to-metal or intra-ligand charge-transfer absorption [30]. The value of μ_{eff} (3.5 BM) obtained for complex $[Ni(C_{18}H_{16}N_3O_2)_2]$ **(7)** corresponds to two unpaired electrons per Ni(II) ion for their six-coordinated configuration [20]. The Ni(II) complex exhibited three spin-allowed bands at 10360, 16220 and 28940 cm^{-1} assignable to

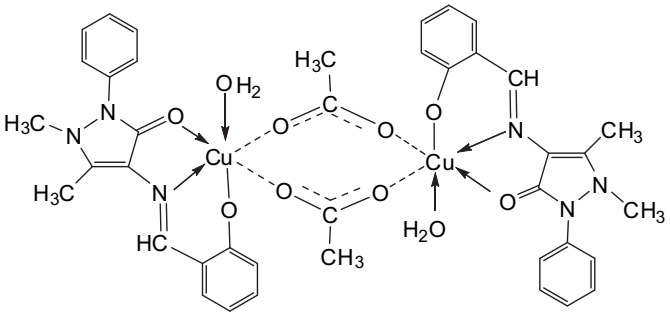
Table 1
Physical and analytical data of the metal complexes **(1-11)** and ligands $\mathbf{L^{1(*)}}$, $\mathbf{L^2}$ and $\mathbf{L^3}$

Comp No	Molecular formula	Molecular mass	Yield (%)	M.p. (^{0}C)	μ eff (BM)	Elemental analysis (%) calc (found)				IR (cm^{-1})	ν^{2-}_{SO4} (cm^{-1})		$\epsilon_{(\lambda,,\ nm)}$	λ_{max} (cm^{-1})
						C	H	N	M					
$\mathbf{L^1}$	$C_{18}H_{16}N_3O_2$													
(1)	$[Cu_2(L^1)_2]SO_4$	835.83	67	205-207	0.62	51.73	3.82	10.05	15.21	1645(C=O),1134(Ar-OH), 1585(C=N)	1148	613	ϵ_{490}	14710; 20408
	$C_{36}H_{32}Cu_2N_6O_8S$					(51.95)	(3.65)	(9.21)	(15.03)				15×10^3	
(2)	$[Cu(L^1)_2]$	676.22	78	>250	1.94	63.94	4.73	12.43	9.40	1628(C=O),1126(Ar-OH), 1585(C=N)			ϵ_{550}	14320;15640;
	$C_{36}H_{32}CuN_6O_4$					(64.05)	(4.58)	(12.31)	(9.28)				6.3×10^3	18270
(3)	$[Cu(L^1)_2]$	676.22	83	>250	1.92	63.94	4.73	12.43	9.40	1620(C=O),1130(Ar-OH), 1584(C=N)			ϵ_{550}	14320;15640;
	$C_{36}H_{32}CuN_6O_4$					(64.10)	(4.58)	(12.34)	(9.26)				6.8×10^3	18270
(4)	$[Cu(L^1)(H_2O)_2]ClO_4$	505.37	79	>250	1.88	42.78	3. 95	8. 31	12.57	1624(C=O),1120(Ar-OH), 1582(C=N)			ϵ_{670}	14890
	$C_{18}H_{20}CuClN_3O_8$					(42.90)	(3.72)	(8.14)	(12.38)				4.3×10^3	
(5)	$[Cu_2(L^1)_2(H_2O)_2(OAc)_2]$	893.88	81	238-240	1.78	53.75	4.69	9.40	14.22	1622(C=O),1120(Ar-OH), 1598(C=N)			ϵ_{525}	13880;19050
	$C_{40}H_{42}Cu_2N_6O_{10}$					(53.89)	(4.47)	(9.31)	(14.13)				10×10^3	
(6)	$[VO(L^1)(H_2O)]_2SO_4$	878.10	74	201-202	1.51	49.21	4.09	9.56	11.60	1664(C=O),1130(Ar-OH), 1584(C=N)	1145	610	ϵ_{410}	14250; 20250; 24520
	$C_{36}H_{36}V_2N_6O_{12}S$					(49.38)	(3.83)	(9.32)	(11.43)				5×10^3	
(7)	$[Ni(L^1)_2]$	671.37	85	>250	3.5	64.40	4.76	12.52	8.74	1642(C=O),1128(Ar-OH), 1581(C=N)			ϵ_{345}	10360; 16220; 28940
	$C_{36}H_{32}NiN_6O_4$					(64.67)	(4.52)	(12.31)	(8.59)				13×10^3	
$\mathbf{L^2}$	$C_{31}H_{30}N_6O_4$	550.61	87	153-154	-	67.62	5.44	15.26	-	1664(C=O),1134(Ar-OH),				
						(68.01)	(5.27)	(15.11)	-	1610(C=N)				
(8)	$[Cu(L^2)]SO_4$	710.22	65	>250	2.02	52.43	4.22	11.83	8. 95	1623(C=O),1130(Ar-OH),	1145	620	ϵ_{480}	14980; 20870
	$C_{31}H_{30}CuN_6O_8S$					(52.58)	(4.05)	(11.65)	(8.81)	1583(C=N)			16×10^3	
(9)	$[VO(L^2)]SO_4$	713.61		>250	1.73	52.18	4.20	11.78	7.14	1679(C=O),1138(Ar-OH),	1149	612	ϵ_{530}	13680; 18940
	$C_{31}H_{30}VN_6O_9S$					(52.33)	(3.98)	(11.59)	(6.93)	1590(C=N)			4.8×10^3	
$\mathbf{L^3}$	$C_{30}H_{28}\ N_6O_4$;	536.58	80	199-200	-	67.15	5. 21	15.66	-	1655(C=O),1138(Ar-OH),				
						(67.36)	(5.07)	(15.32)	-	1610(C=N)				
(10)	$[Cu(L^3)]SO_4$	696.19	59	>250	2,04	51.76	4.02	12.07	9.13	1629(C=O),1136(Ar-OH),	1142	604	ϵ_{470}	15160; 21290
	$C_{30}H_{28}CuN_6O_8S$					(51.94)	(3.84)	(11.88)	(8.94)	1579(C=N)			14×10^3	
(11)	$[VO(L^3)]SO_4$	699.59	53	>250	1.60	51.50	4.00	12.01	7. 28	1673(C=O),1131(Ar-OH),	1147	609	ϵ_{525}	13180; 19120
	$C_{30}H_{28}VN_6O_9S$					(51.77)	(3.87)	(11.88)	(7.09)	1578(C=N)			4.8×10^3	

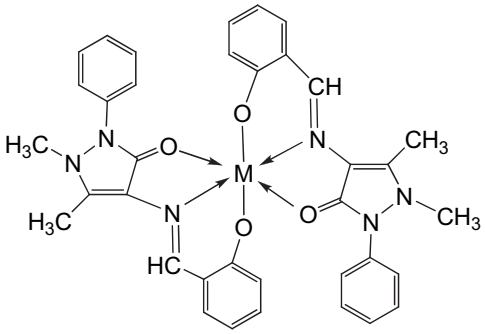
(*) $\mathbf{L^1}$ was prepared and described in [15]



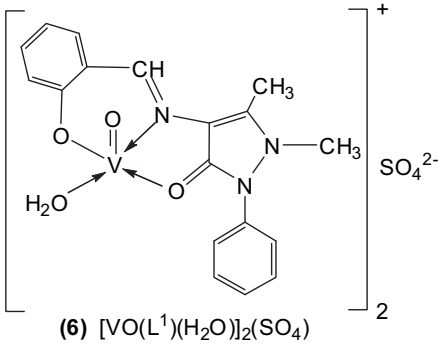
(1) $[Cu_2(L^1)_2](SO_4)$



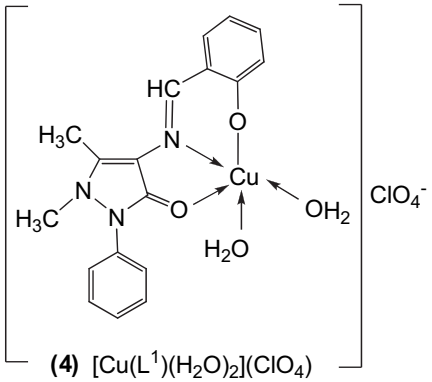
(5) $[Cu_2(L^1)_2(H_2O)_2(OAc)_2]$



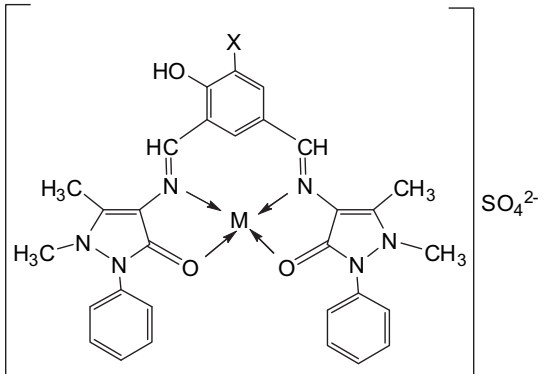
(2) $[Cu(L^1)_2]$ -from $CuSO_4x5H_2O$
(3) $[Cu(L^1)_2]$ - from $CuNO_3x3H_2O$
(7) $[Ni(L^1)_2]$



(6) $[VO(L^1)(H_2O)]_2(SO_4)$



(4) $[Cu(L^1)(H_2O)_2](ClO_4)$



X=OCH3, **(8)** $[Cu(L^2)](SO_4)$
(9) $[VO(L^2)](SO_4)$

X=OH, **(10)** $[Cu(L^3)](SO_4)$
(11) $[VO(L^3)](SO_4)$

Fig. 2. Proposed structures of the newly obtained metal complexes.

$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (υ_1), $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (υ_2) and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$ (υ_3) transitions, which are characteristic for their octahedral geometry [20,31]. For all the complexes was calculated molar absorption (Table 1).

2.1.3. IR spectra and coordination mode

The most relevant bands in the infrared spectra of the complexes are presented in Table 1. IR spectra of the ligands showed the absence of the bands at $\approx 1740\ cm^{-1}$ and $\approx 3400\ cm^{-1}$

due to carbonyl υ(C=O) and amino υ(NH_2) stretching vibrations and, instead, a new band assigned to azomethine υ(CH=N) linkage appeared at ≈1612 cm^{-1} [32]. This suggested that amino and carbonyl groups of the starting reagents (4-amino-antipyrine and substituted benzaldehyde, respectively) have been converted into their corresponding Schiff bases (Fig. 1).

Bands at 1614–1610 cm^{-1} due to C=N stretching frequencies in the free ligands shift towards lower values (1590–1578 cm^{-1}) in all complexes indicating that the azomethine nitrogen atom was coordinated. Bands at 1664–1653 cm^{-1} due to C=O stretching frequencies of antipyrine moiety, in the free ligands shift towards lower values in the spectra of the complexes of the Cu^{+2} and Ni^{+2} ions and towards higher values in all complexes of VO^{+2}. Comparing the spectra of Cu^{+2} ion complexes with the ones of VO^{+2}, be noticed that the υ(C=O) frequencies of the VO^{+2} complexes spectra are moving by 11–18 cm^{-1} in a positive way as compared to the same frequencies of the ligands. This displacement can be attributed to the electronic donation of the base to the vanadium (N → V), which increases the electron density on the metal d-orbitals, and consequently the $p_\pi \rightarrow d_\pi$ donation from the oxygen atom to vanadium is expected to be reduced [30]. This behavior indicates the fact that the carbonyl oxygen atom of the antipyrine residue was coordinated. The IR spectra of complex **(5)** exhibit two bands at 1580 and 1425 cm^{-1} which are characteristic for ν_{asym} (COO^-) and ν_{sym}(COO^-) of the symmetrical group COO^- present in bridging complexes [32].

Also, the specific band of aromatic-OH (1140 cm^{-1}) from the free ligand $\mathbf{L^1}$ [15] moves towards smaller wave numbers in the IR spectra of the complex combinations **(1–7)** (1120–1134 cm^{-1}). This effect always indicates the coordination of the ligand through the oxygen atom of the OH phenolic group.

The IR spectra of the VO^{+2} complexes exhibit a very strong band at 990–975 cm^{-1} attributed to the stretching vibration of the terminal V=O bond [33,34]. Based on these observations it may be appreciated that the $\mathbf{L^1}$ coordinate tridentate mononegatively around the metallic ion and the $\mathbf{L^2}$ and $\mathbf{L^3}$ coordinate neutral tetradentately.

2.1.4. EPR spectroscopy

EPR spectra of the polycrystalline powders were recorded at room temperature. The values of *g* factors were assessed following the method described by J. W. Searl [35] and are presented in Table 2. The *g* tensor values of Cu(II) complex can be used to derive the ground state. In tetragonal and square planar complexes the unpaired electron lies in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state with the $g_{//} > g_\perp > 2.003$ [36–40]. From the observed values for complexes **(1-3)** and **(8)**, it is clear that $g_\parallel > g_\perp$. These data are in agreement with those obtained from the electronic spectra and confirm the tetragonal geometry for complexes **(2)** and **(3)** and the square planar geometry for complexes **(1)** and **(8)**. The term of the fundamental state is thus defined by the orbital $d_{x^2-y^2}$. From the values of the *g* factors it may be determined the geometric parameter G, representing a measure of the exchange interaction between the Cu(II) centers in polycrystalline compounds following the formula $G = (g_\parallel - 2)/(g_\perp - 2)$ [28]. If $G < 4$, it is considered the existence of some exchange interactions between the Cu(II) centers and if $G > 4$, the exchange interactions are neglected. Thus, in case of complex **(1)**, the geometric parameter $G = 3.68$ confirms the existence of some exchange interactions between the Cu(II) centers. For the complexes **(2), (3)** and **(8),** the geometric parameter G is however higher than 4.

The *g* tensor values for the complex **(4)** indicate a distorted trigonal bipyramide geometry (C_{2v}) [41]. The term of the fundamental state may be defined with the help of the geometric parameter R deduced from the relation $R = (g_2 - g_3)/(g_1 - g_2)$ [36]. If $R > 1$, the term of the ground state is defined by the orbital d_z^2, but if $R < 1$ it is defined by the orbital $d_{x^2-y^2}$. For the complex **(4)** ($R = 2.46$), it is clear that the ground state term is d_z^2. From the value of the R parameter for complex **(5)**, the term of the ground state is defined by $d_{x^2-y^2}$ orbital. This result is in agreement with the data obtained by electronic spectra. From the *g* tensor values for the complex **(10)**, there was determined the value of the parameter R indicating a term of the ground state defined by $d_{x^2-y^2}$ orbital.

2.2. Biological activity

2.2.1. Cell viability determination

The *in vitro* cytotoxicity of the ligands $\mathbf{L^2}$, $\mathbf{L^3}$ and some of their Cu(II), Ni(II) and VO(IV) complexes **(1)**, **(6)**, **(7)**, **(8)**, **(9)**, **(10)** and **(11)** on human promyelocytic leukemia cells (HL-60) was determined by a MTS-based assay. Included in this section are values for 4-(2-hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one $\mathbf{L^1}$, 4,4'-(1,4-phenylene bis(iminomethyl)-bis(1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one), $\mathbf{L^4}$ and copper(II) complex $[Cu_2(L^4)_2(SO_4)_2]$ **(12)**, previously obtained [15]. The cells were incubated 3 days in the presence of each ligand or complex at two concentrations (1 and 10 μM) and the results were expressed as the percentage of cell growth (Table 3). The cell proliferation of the control (untreated cells) was fixed to 100%. Any tested ligands and complexes inhibited the cell proliferation at a concentration of 1 μM, but complexes **(8)**, **(10)** and **(12)** shown a significant effect at 10 μM. The copper(II) complex **(12)** was clearly the most potent compound reducing the HL-60 cell proliferation to 40% whereas complexes **(8)** and **(10)** reduced the cell proliferation to 82% and

Table 2
The {g} parameter values of the Cu(II) complexes.

Nr	Complex	$g_\parallel$	$g_\perp$	g_1	g_2	g_3
(1)	$[Cu_2(C_{18}H_{16}N_3O_2)_2]SO_4$	2.247	2.067			
(2)	$[Cu(C_{18}H_{16}N_3O_2)_2]$	2.245	2.052			
(3)	$[Cu(C_{18}H_{16}N_3O_2)_2]$	2.243	2.060			
(4)	$[Cu(C_{18}H_{16}N_3O_2)(H_2O)_2]ClO_4$			2.263	2.209	2.076
(5)	$[Cu_2(C_{18}H_{16}N_3O_2)_2(H_2O)_2(OAc)_2]$			2.207	2.107	2.058
(8)	$[Cu(C_{31}H_{30}N_6O_4)]SO_4$	2.268	2.064			
(10)	$[Cu(C_{30}H_{28}N_6O_4)]SO_4$			2.461	2.178	2.066

Table 3
Effect of a series of ligands $\mathbf{L^1}$–$\mathbf{L^4}$, and complexes **(1)**, **(6–12)** at two concentrations of 1 and 10 μM on the HL-60 cell proliferation.[a]

Compound	Survival cell fraction (%) at 1 μM	Cell growth inhibition (%) at 1 μM	Survival cell fraction (%) at 10 μM	Cell growth inhibition (%) at 10 μM
CTL	100 ± 2	–	100 ± 2	–
$\mathbf{L^1}$	110 ± 3	–	113 ± 2	–
(1)	119 ± 2	–	94 ± 2	6
(6)	112 ± 1	–	110 ± 2	–
(7)	116 ± 2	–	98 ± 1	2
$\mathbf{L^2}$	119 ± 4	–	94 ± 3	6
(8)	90 ± 1	10[b]	81 ± 4	19[b]
(9)	110 ± 1	–	110 ± 2	–
$\mathbf{L^3}$	99 ± 2	–	101 ± 4	–
(10)	103 ± 4	–	69 ± 3	31[b]
(11)	114 ± 2[b]	–	145 ± 5 [b]	–
$\mathbf{L^4}$	115 ± 3	–	111 ± 1	–
(12)	104 ± 4	–	41 ± 2	59[b]
Doxo[c]	20 ± 2	80[b]	5 ± 1	95[b]

[a] The results ± SD are reported as the survival fraction of cells (%). Cell growth inhibition in % was also reported for active compounds.
[b] Significantly different from the control ($P > 95\%$).
[c] The potent cytotoxic agent doxorubicin (Doxo) was used as a positive control.

70%, respectively. Since no effect was observed with the ligand ($\mathbf{L^2}$, $\mathbf{L^3}$ and $\mathbf{L^4}$) only, the inhibition of malignant cell proliferation observed with **(8)**, **(10)** and **(12)** is the result of a copper-ligand complexation. On the other hand, the vanadium complexes **(9)** and **(11)**, which are analogues to the copper complexes **(8)** and **(10)**, did not reduce the cell proliferation. In fact, the vanadium complex **(11)** stimulated the cell proliferation of HL-60 cells.

2.2.2. Antibacterial activity

A comparative study of MIC values of the Schiff bases $\mathbf{L^2}$-$\mathbf{L^3}$ and its complexes indicate that, generally, the metal complexes have a better activity than the free ligands. This is probably due to the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of chelating theory [35]. On chelating, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and blocks the metal binding sites on enzymes of micro-organisms.

The synthesized Schiff base compounds have comparable and similar inhibitory effects (low to moderate MIC values 256 and 512 μg/mL) on the growth of tested strains (Table 4). The antibacterial results evidently show that the activity of the Schiff base compounds became more pronounced when coordinated to the metal ions. Hence, all the complexes show greater bactericidal and fungicidal activities against *E. coli* (MIC = 128 μg/mL) and *C .albicans* (MIC = 128 μg/mL) as compared to their corresponding Schiff bases (exception for **(7)**, which exert a visible decrease of antimicrobial action).

The structure of the tested compounds seems to be the principal factor influencing the antimicrobial activity. The presence of anionic groups outside the coordination site, exerts a number of changes on antimicrobial activity of the tested complexes. So, in case of **(1)**, **(4)**, **(8–11)**, the presence of SO_4^{2-} moiety induced a visible increase of their action against all bacterial species taken in the study. Compound **(3)**, was found to have low activity against *S. Aureus*, *E. coli* and *C. albicans* (MIC = 512 μg/mL) probably due to the presence of more bulkier ClO_4^- anion. The most active tested compounds are **(9)** and **(11),** (MIC = 64–128 μg/mL), probably due to the simultaneous presence of VO^{2+} and SO_4^{2-}.

The present investigations of antimicrobial screening data revealed that all of the newly synthesized compounds exhibited poor activity compared to that of the control drugs. Because the MIC values are not spectacular, no statistical calculations were made.

Table 4
Antibacterial activities of ligands $\mathbf{L^1}$–$\mathbf{L^3}$ and complexes **(1–11)** as MIC values (μg/mL).

Compound	Gram-negative bacteria[a]		Fung[b]
	Sa	Ec	Ca
$\mathbf{L^1}$	512	256	512
(1)	256	128	128
(2)	512	1024	512
(3)	512	512	512
(4)	256	128	128
(5)	512	128	128
(6)	256	128	128
(7)	512	1024	1024
$\mathbf{L^2}$	512	256	128
(8)	128	128	128
(9)	256	128	128
$\mathbf{L^3}$	512	–	128
(10)	256	128	128
(11)	128	64	64
$CuSO_4\ 5H_2O$	1024	512	512
$Cu(NO_3)_2\ 3H_2O$	1024	1024	–
$Cu(ClO_4)_2\ 6H_2O$	–	1024	1024
$Cu(OAc)_2\ H_2O$	512	512	–
$NiCl_2\ 6H_2O$	–	–	1024
$VOSO_4\ 2H_2O$	512	1024	512
Tetracycline	0.19	1.5	–
Fluconazol	–	–	2

[a] **Sa** (*Staphylococcus aureus* var. Oxford 6538); **Ec** (*Escherichia coli* ATCC 10536).
[b] **Ca** (*Candida albicans* ATCC 10231).

3. Conclusions

The analytical and physico-chemical analyses confirmed the composition and the structure of the newly obtained complex combinations. The IR, electronic transition and {g} tensor value data lead to the conclusion that the Cu^{2+} ion takes different geometries depending on the nature of used metallic salts, solvent (complexes **(1)** and **(2)**) and ligand type. In all complexes the ligand $\mathbf{L^1}$ acts as mononegative tridentate, around the metallic ion, and the ligands $\mathbf{L^2}$ and $\mathbf{L^3}$ coordinate neutral tetradentate.

Results of antitumor activity screening indicated that the ligand only has no cytotoxic effects at the two tested concentrations. Interestingly, the complex **(12)** greatly reduced the malignant HL-60 cell growth. The sensitivity spectrum of the microbial strains towards the ligands and the corresponding complexes was determined by qualitative and quantitative methods. The quantitative antimicrobial activity test results proved that both the ligand and the complex combinations have specific anti-microbial activity, depending on the microbial species tested. The antibacterial assay against other Gram-negative and Gram-positive strains is in progress, because none of the presented compounds is effective against the tested micro-organisms in comparison with used drugs.

4. Experimental protocols

4.1. Chemistry

The required chemicals were of analytical reagent grade and were purchased from Merck and Chimopar Bucharest. All manipulations were performed using materials as received. Electronic spectra were recorded using the Jasco V-550 spectrophotometer, in diffuse reflectance, using MgO dilution matrices. IR spectra were recorded with a BioRad FTS 135 spectrophotometer in the 4000–400 cm^{-1} region using KBr pellets. EPR spectra were recorded on an ART-6 a spectrometer, equipped with a field modulation unit at 100 kHz. Measurements were effected in the X band, on microcrystalline powder at room temperature. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$, with the Bruker DRX 400 spectrometer. The molar conductivity was determined with CONSORT-C533 conductometer. The chemical elemental analysis for the determination of C and N was done with the Carlo-Erba LA-118 microdosimeter whereas the AAS-1N Carl-Zeiss-Jena spectrometer was used for the determination of Cu(II) and Ni(II). Vanadium was determined following the method described by Fries J. and Getrost H. [42].

4.1.1. General procedures for the synthesis of the Schiff bases $\mathbf{L^1}$, $\mathbf{L^2}$ and $\mathbf{L^3}$

$\mathbf{L^1}$: *4-(2-hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one* has been prepared by the method described elsewhere [15].

$\mathbf{L^2}$: *4,4′-(4-hydroxy-5-methoxy-1,3-phenylenebis(iminomethyl))bis(1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one).* A solution of 4-hydroxy-5-methoxy-isophthalaldehyde (0.018 g, 1 mmol) in

methanol (10 mL) was added to a solution of 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (0.0203 g, 1 mmol) in methanol (20 mL). The mixture was refluxed for 1 h, then stirred for 3 h at room temperature and left at the same temperature for one day. The resulting precipitate of intense yellow color was filtered, washed with methanol and dried.

Yield: 87%; M.p. 153-154 °C. Anal. Calc. (%) for $C_{31}H_{30}N_6O_4$ (550.61 g/mol): C-67.62; N-15.26; Found: C-68.01; N-15.11; IR (KBr, cm^{-1}): The IR spectrum of the obtained ligand confirms the occurrence of the absorption band of 1610 cm^{-1}, specific for the azomethinic group [26,27]; 1664 (s, C═O), 1144 (Ar-OH); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.16 (s; 3H; H-20); 2.42 (s; 3H; H-20′); 3.15 (s; 3H; H-21); 3.22 (s; 3H; H-21′); 3.89 (s; 3H; H-19); 7.35-7.57 (m; 10H; H-7-11; H-7′-11′); 7.80 (s; 1H; H-14); 7.86 (s; 1H; H-18); 9.50 (s; 1H; H-12); 9.73 (s; 1H; H-12′).

^{13}C-NMR (DMSO-d_6; δ, ppm):163.7 (C-3; C-3'); 160.5 (C-12; C-12′); 154.2 (C-16); 153.6 (C-15); 150.3 (C-5; C-5′); 133.9 (C-6; C-6′); 129.2 (C-8; C-10; C-8′; C-10′); 127.4 (C-13); 123.9 (C-7; C-11; C-7′; C-11′); 122.8 (C-9; C-9′); 122.1 (C-18); 119.6 (C-17); 113.5 (C-14); 111.1 (C-4; C-4′); 55.8 (C-19); 34.5 (C-21′); 33.9 (C-21); 14.2 (C-20′); 13.4 (C-20). The same method was applied for the preparation of **L^3**.

L^3: *4,4′-(4,5-dihydroxy-1,3-phenylenebis(iminomethyl)-bis(1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one)*

Yield: 80%; M.p. 199–200 °C. Anal. Calc. (%) for $C_{30}H_{28}N_6O_4$ (536.58 g/mol): C-67.15; N-15.66; Found: C-67.36; N-15.32; IR (cm^{-1}, KBr): 1610 (m, C═N), 1655 (s, C═O), 1138 (Ar-OH); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.16 (s; 3H; H-19); 2.42 (s; 3H; H-19′); 3.13 (s; 3H; H-20); 3.21 (s; 3H; H-20′); 7.29-7.57 (m; 10H; H-7-11; H-7′-11′); 7.60 (s; 1H; H-14); 7.88 (s; 1H; H-18); 9.45 (s; 1H; H-12); 9.70 (s; 1H; H-12′).

^{13}C-NMR (DMSO-d_6; δ, ppm): 162.5 (C-3; C-3′); 160.1 (C-12; C-12′); 154.6 (C-16); 150.8 (C-5; C-5′); 147.5 (C-15); 133.5 (C-6; C-6′); 128.9 (C-8; C-10; C-8′; C-10′); 127.9 (C-13); 123.7 (C-7; C-11; C-7′; C-11′); 122.9 (C-9; C-9′); 122.6 (C-18); 120.4 (C-17); 117.9 (C-14); 110.8 (C-4; C-4′); 34.2 (C-20′); 33.0 (C-20); 13.8 (C-19′); 13.1 (C-19).

4.1.2. General procedure for the preparation of the metal complexes (**1–11**)

Synthesis of the copper(II) complexes with 4-(2-hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one. An ethanol solution (15 mL) of Schiff base **L^1** (0.307 g, 1 mmol) was added to $CuSO_4 \cdot 5H_2O$ (0.250 g, 1 mmol) dissolved in distilled water (10 mL). This solution was refluxed for 2 h and left at room temperature for 3 days. A brown-red precipitate was formed for **1**. The product was separated by filtration, purified by washing with cold methanol and then with ether. If a methanol solution of the Schiff base is used, the obtained product **2** is green.

All other complexes were synthesized by the same method. The elemental analysis confirms the molecular formula. The physical and analytical data are presented in Table 1.

4.2. Cytotoxicity assay

4.2.1. Ppreparation of test solutions

Stock solutions of the investigated compounds (**L^1**–**L^4**, **(1)**, **(6–12)**) were prepared in dimethylsulfoxide (DMSO) at a concentration of 10 mM and diluted with nutrient medium to various working concentrations. DMSO was used instead of ethanol due to solubility problems.

4.2.2. Cell culture

Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, USA) containing *L*-glutamine (2 nM), antibiotics (100 IU penicillin/mL, 100 μg streptomycin/mL) and supplemented with 10% (v/v) fetal bovine serum (FBS), in a 5% CO_2 humidified atmosphere at 37 °C. Cells were currently maintained in continuous exponential growth with twice a week dilution of the cells in culture medium.

4.2.3. Cell proliferation assay

The cell proliferation assay was performed using 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium inner salt (MTS) (Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable cells. In brief, triplicate cultures of 1×10^4 cells in a total of 100 μL medium in 96-well microtitre plates (Becton Dickinson and Company, Lincoln Park, NJ, USA) were incubated at 37 °C, 5% CO_2. Compounds were dissolved in DMSO to prepare the stock solution of 1×10^{-2} M. These compounds were diluted at the appropriate concentration (1 or 10 μM) with culture media, added to each well and incubated for 3 days. Following each treatment, 20 μL MTS was added to each well and incubated for 4 h. MTS is converted to water-soluble blue formazan by a dehydrogenase enzyme present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). The results were reported as the percentage of cell proliferation inhibition compared to the control (basal cell proliferation = 100%).

4.3. Antibacterial activity

Qualitative determination of antimicrobial activity was done using the disk diffusion method. Suspensions in sterile peptone water from 24-h cultures of microorganisms were adjusted to 0.5 McFarland. Muller–Hinton Petri dishes of 90 mm were inoculated using these suspensions. Paper disks (6 mm in diameter) containing 10 μL of the substance to be tested (at a concentration of 2048 μg/mL in DMSO) were placed in a circular pattern in each inoculated plate. Incubation of the plates was done at 37 °C for 18–24 h. DMSO impregnated discs were used as negative controls. Toxicity tests of the solvent, DMSO, showed that the concentrations used in antibacterial activity assays did not interfere with the growth of the microorganisms. Reading of the results was done by measuring the diameters of the inhibition zones generated by the test substance. Tetracycline and fluconazol were used as reference substances.

Determination of MIC was done using the serial dilutions in liquid broth method. The materials used were 96-well plates, suspensions of microorganism (0.5 McFarland), Muller-Hinton broth (Merck) and stock solutions of each substance to be tested (2048 μg/mL in DMSO). The following concentrations of the substances to be tested were obtained in the 96-well plates: 1024, 512, 256, 128, 64, 32, 16, 8, 4 and 2 μg/mL. After incubation at 37 °C for 18–24 h, the MIC for each tested substance was determined by microscopic observation of microbial growth. It corresponds to the well with the lowest concentration of the tested substance where microbial growth was clearly inhibited.

Acknowledgement

The authors thank the Organic of Chemistry Department at ICECHIM Bucharest, Romania for microanalysis, Horia Hulubei National Institute of Physics and Nuclear Engineering Bucharest for help with EPR spectroscopy and Medicines Control Laboratory Bucharest for antimicrobial activity assays. Thank also to Jenny Roy who performed the HL-60 cell proliferation assay.

References

[1] (a) V. Alexander, Chem. Rev. 95 (1995) 273–342;
(b) A.D. Granvoskii, A.L. Nivorozhkin, V.I. Minkin, Coord. Chem. Rev. 126 (1993) 1–26.
[2] (a) E.M. Hodnett, W.J. Dunn, J. Med. Chem. 13 (1970) 768–770 J. Med. Chem. 15(1972) 339–345;
(b) D. Kessel, A.F.A. Sayyab, E.M.H. Jaffar, A.H.H.A. Lanil, Iraqian J. Sci. 22 (1981) 312–316;
(c) J. Chakraborty, R.N. Patel, J. Indian Chem. Soc. 73 (1996) 191–193.
[3] (a) A.M. Farghaly, A. Hozza, Pharmazie 35 (1980) 596–601;
(b) T. Hitoshi, N. Tamao, A. Hideyuki, F. Manabu, Takayuki Polyhedron 16 (1997) 3787–3794.
[4] M.E. Hossain, M.N. Alam, J. Begum, A.M. Akbar, M. Nazimuddin, F.E. Smith, R.C. Hynes, Inorg. Chim. Acta 249 (1996) 207–213.
[5] D. Chiaramonte, J.M. Steiner, J.D. Broussard, K. Baer, S. Gumminger, E.M. Moeller, D.A. Williams, R. Shumway, Can. J. Vet. Res. 67 (2003) 183–188.
[6] (a) D.X. West, H. Gebremedhin, R.J. Butcher, J.P. Jasinski, A.E. Liberta, Polyhedron 12 (1993) 2489–2497;
(b) A.F. Kolodziej, Prog. Inorg. Chem. 41 (1994) 493–598.
[7] N. Rama, J. Dhaveethu Raja, A. Sakthivel, J. Chem. Sci. 119 (2007) 303–310.
[8] P.M. Selvakumar, E. Suresh, P.S. Subramanian, Polyhedron 26 (2007) 749–756.
[9] R.K. Agarwal, L. Singh, D. Sharma, L. Singh, H. Agarwal, Bioinorg. Chem. Appl. (2006). doi:10.1155/BCA/2006/29234 Article ID 29234.
[10] N. Raman, A. Kulandaisamy, C. Thangaraja, Trans. Met. Chem. 29 (2004) 129–135.
[11] K.Z. Ismail, Trans. Met. Chem. 25 (2000) 522–528.
[12] A. El-Dissouky, A.K. Shehata, G. El-Mahadey, Polyhedron 16 (1997) 1247–1253.
[13] G. Shankar, R.R. Premkumar, S.K. Ramalingam, Polyhedron 5 (1986) 991–994.
[14] T. Radhakrishnana, P.T. Joseph, C.P. Prabhakaran, J. Inorg. Nucl. Chem. 38 (1976) 2212–2217.
[15] T. Rosu, S. Pasculescu, V. Lazar, C. Chifiriuc, Cernat Molecules 11 (2006) 904–914.
[16] A. Barry, Procedures and theoretical considerations for testing antimicrobial agents in agar media. in: Lorian (Ed.), Antibiotics in Laboratory Medicine, fifth ed. Williams and Wilkins, Baltimore, 1991.
[17] National Committee for Clinical Laboratory Standard, NCCLS: Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; Approved guideline, Document M45-A, 26(19), Willanova, PA, USA, 1999.
[18] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81–122.
[19] (a) W.W. Simmons, The Saddler Handbook of Proton NMR Spectra. Sadtler Research Laboratorics, Inc, Philadelphia, 1978;
(b) D.J. Pasto, Organic Structure Determination. Prentice Hall International, London, 1969.
[20] (a) G. Wilkinson, D.R. Gillard, A.J. McCleverty, Comprehensive Coordination Chemistry, vol. 1, Pergamon Press, New York, 1987, p. 274;
(b) R.L. Carlin, Transition Metal Chemistry, second ed. Marcel Decker, New York, 1965.
[21] (a) P. Zanello, S. Tamburini, P.A. Vigato, G.A. Mazzocchin, Coord. Chem. Rev. 77 (1987) 165–273;
(b) P. Guerriero, S. Tamburini, P.A. Vigato, Coord.Chem. Rev. 139 (1995) 17–23.
[22] J.W. Yu, R.J. Tao, X.Y. Zhou, D.M. Jui, D. Liao, Polyhedron 13 (1994) 951–955.
[23] F. Lam, R.J. Wang, T.C.W. Mak, K.S. Chan, J. Chem. Soc., Chem. Commun. (1994) 2439–2440.
[24] A. Benzekri, P. Dubourdeaux, J.M. Latour, P. Rey, J. Laugier, J. Chem. Soc. Dalton Trans. (1991) 3359–3365.
[25] H. van Crawford, W. Hatfield, Inorg. Chem. 16 (1977) 1336–1341.
[26] (a) P.W. Anderson, Phys. Rev. 79 (1950) 350–356;
(b) J.K. Nag, D. Das, S. Pal, C. Sinha, Proc. Indian Acad. Sci. (Chem. Sci.) 113 (2001) 11–20;
(c) S. Tyagi, B.J. Hathaway, J. Chem. Soc., Dalton Trans. (1983) 199–203;
(d) E. Koenig, G. Koenig, Landolt–Bornstein, vol. 10, Springer-Verlag, Berlin, 1982, 1979, Group 11;
(e) E. Koenig, G. Koenig, Landolt-Bornstein, vol. 11, Springer-Verlag, Berlin, 1983, 1980, Group 11, vol. 12a, 12b (1984).
[27] A.P.B. Lever, Inorganic Electronic Spectroscopy, second ed. Elsevier Science, New York, 1984, pp. 557–559, 509–521.
[28] A.P.B. Lever, J. Lewis J. Chem. Soc. (1963) 2552–2556.
[29] G. Wilkinson, D.R. Gillard, A.J. McCleverty, Comprehensive Coordination Chemistry, vol. 1, Pergamon Press, New York, 1987, p. 271.
[30] A.L. Doadrio, J. Sotelo, A. Fernandez-Ruano, Quím. Nova 25 (2002) 525–528.
[31] C.J. Balhausen, An Introduction to Ligand Field. McGraw Hill, New-York, 1962.
[32] K. Nakamoto, Infrared Spectra of Inorganic and Coordination Compounds. Wiley and Sons, New York, 1986.
[33] A.L. Doadrio Villarejo, G. Pérez, C.V. Ragel, Synth. React. Inorg. Met. Org. Chem. 23 (1993) 949–962.
[34] J. Martínez, R. Lozano, J. Román, A. Doadrio, J.L. Peña, Polyhedron 5 (1986) 1341–1349.
[35] J.W. Searl, R.C. Smith, S.J. Wyard, Proc. Phys. Soc. 78 (1961) 1174–1176.
[36] B.J. Hathaway, D.E. Billing, Coord. Chem. Rev. 5 (1970) 143–207.
[37] (a) V. Suresh Babu, A. Ramesh, P. Raghuram, R.R. Naidu, Polyhedron 16 (1997) 607–612;
(b) S. Djebbar-Sid, O. Benali-Baitich, J.P. Deloume, Polyhedron 16 (1997) 2175–2182.
[38] R.S. Drago, M.J. Desmond, B.R. Corden, K.A. Miller, J. Am. Chem. Soc. 105 (1983) 2287–2296.
[39] R.K. Ray, G.R. Kauffman, Inorg. Chem. Acta 173 (1990) 207–214.
[40] (a) K. Jayasubramanian, S.A. Samath, S. Thambidurai, R. Murugesan, S.K. Ramalingam, Trans. Met. Chem. 287 (1995) 76–82;
(b) V.S.X. Anthonisamy, R. Murugesan, Chem. Phys. Lett. 287 (1998) 353–359;
(c) V.S.X. Anthonisamy, R. Anantharam, R. Murugesan, Spectrochim. Acta Part A 55 (1998) 135–142.
[41] J.U.R. Wasson, D.M. Klassen, H.W. Richardson, W.E. Hatfield, Inorg. Chim. Acta 16 (1977) 1906–1914.
[42] J. Fries, H. Getrost, Organic Reagents for Trace Analysis. Merck Darmstadt, 1977, pp. 394–405.