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Synthesis, spectroscopic characterization and radiosensitizing properties of acetato-bridged copper(II) complexes with 5-nitroimidazole drugs

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ABSTRACT

Three novel acetato-bridged dinuclear copper(II) complexes with 5-nitroimidazoles (CuAcNtrim) and the known copper–acetato–metronidazole have been prepared by an environment-friendly route and spectroscopically characterized. The CuAcNtrim compounds of formula $[Cu_2(\mu\text{-}O_2CCH_3)_4Ntrim_2]$, where Ntrim = metronidazole (**1**), secnidazole (**2**), tinidazole (**3**) or nimorazole (**4**), exhibit dimeric copper–acetato paddle-wheel structures with Ntrim axial ligands coordinated to copper(II) ions through the N_3 atoms of the imidazole rings. EPR data indicate antiferromagnetic behavior for this novel series of copper complexes. The constant coupling has been found to decrease along with the increasing of basicity of the Ntrim axial ligand. The CuAcNtrim complexes and the correspondent Ntrim parent drugs have shown radiosensitizer properties for Hep2 (human larynx cancer) cell line *in vitro*. The best enhancement of radiosensitizer activity upon coordination of the Ntrim drug to copper(II) has been found for the nimorazole compound which has the strongest Cu–Ntrim bond and exhibits the highest lipophilicity within the series of CuAcNtrim complexes.

1. Introduction

Many classes of transition metal complexes are able to enhance cellular radiation damage both *in vitro* and *in vivo*. Three principal mechanisms have been suggested for radiosensitization by metal complexes: DNA-binding with subsequent consequences to repair processes, thiol depletion and electron-affinic mechanism implying reduction of the metal complex and subsequent fixation of damage on the intracellular target of radiation, the DNA [1]. Copper(II) ion is well known to modify the radiation response in both mammalian and bacterial cells [2,3]. The radiosensitizing mechanism in mammalian cells may involve reduction of copper(II) to copper(I) [2]. More recently, it has been found that: radiosensitization process may be related to radiation induced DNA damage [4]; biological damage sensitized by copper ions might involve nucleobases [5]; and copper complexes with different structural features can bind with double-helical DNA and promote double-strand DNA damage [6]. Steady-state and pulse radiolysis studies with nucleic acid bases and polynucleotides showed that copper(II) ion and its complexes can be effective in sensitizing radiation-induced nucleobase damage [7,8].

Metal complexes with imidazoles have been investigated as radiosensitizers for tumors cells [1,9]. Particularly, nitroimidazole drugs exhibit radiosensitizer activity that increases hypoxic cells sensibility to radiation to enhance treatment efficiency in cancer radiotherapy – in addition to a wide variety of other therapeutic properties [10,11]. Metronidazole [*Metrim*, 2-(2-methyl-5-nitro-imidazol-1-yl)ethanol] (Fig. 1) a member of 5-nitroimidazole (Ntrim) class of drugs, sensitizes efficiently hypoxic cells to γ-radiation induced damage. However, it shows toxicity in the clinical doses required for radiation therapy of cancer cells. The complexing ability of Metrim to copper(II) was studied for gamma radiolysis and radiosensitization of thymine [12,13], and structural and magnetic properties of a copper–acetato–Metrim compound have been reported [14,15]. However, no studies involving hypoxic cells was found for this compound although the influence of copper(II) ion and some synthetic aminocarboxylic derivatives on radiosensitivity of *Escherichia coli* has been described recently [16].

The present work describes the interaction of copper(II)–acetato (CuAc, $[Cu_2(\mu\text{-}O_2CCH_3)_4(H_2O)_2]$) with Metrim and three other 5-nitroimidazole drugs shown in Fig. 1: secnidazole [*Secnim*, 1-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol]; tinidazole [*Tinim*, 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole] and

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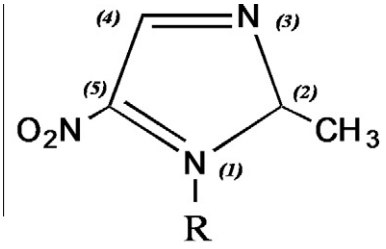

R = CH_2CH_2OH; ***Metrim***

R = $CH_2CH(OH)CH_3$; ***Secnim***

R = $CH_2CH_2SO_2CH_2CH_3$; ***Tinim***

R = $CH_2CH_2(NCH_2CH_2OCH_2CH_2)$; ***Nimim***

Fig. 1. General structure of 5-nitroimidazole (Ntrim).

nimorazole [*Nimin*, 4-[2-(5-nitroimidazol-1-yl)ethyl]morpholine]. The novel copper–acetato–Ntrim (CuAcNtrim) complexes have been prepared, spectroscopically and magnetically characterized and investigated for radiosensitizer properties.

2. Experimental

2.1. Materials

All reagents and solvents were reagent grade and used without further purification: metronidazole (Sigma), secnidazole (Rhodia), tinidazole (Pfizer), nimorazole (Abbot), copper acetato hydrate (Mallinckrodt) and solvents (Merck).

2.2. Preparation of CuAcNtrim complexes

Complexes **1**–**4** were prepared by the following general procedure: ethanolic hot solutions of CuAc (30 cm^3) and the correspondent Ntrim drug (20 cm^3) [CuAc/Ntrim molar ratios were according to 1:3.5 for compound **1** (0.40:0.60 g/g) and 1:3 for compounds **2** (0.25:0.35 g/g), **3** (0.25:0.46 g/g) and **4** (0.25:0.42 g/g)] were mixed and stirred at room temperature for approximately 2 h. The volume of the solvent was reduced to 10 cm^3 by slow evaporation at room temperature, except for **2** which had the volume reduced to 5 cm^3 by heating the solution at 60 °C. The solids were filtered off, washed with ethanol and dried *in vacuum*.

2.2.1. $[Cu_2(Ac)_4(Metrim)_2]$ (**1**)

Yield 60%. *Anal.* Calc. for $C_{20}H_{30}N_6O_{14}Cu_2$: C, 34.0; H, 4.3; N, 11.9. Found: C, 34.3; H, 4.1; N, 12.0%. Decomposition: $T > 150$ °C. FTIR, ν (cm^{-1}): 3432m br, 3153 w, 3150–2800 w, 1623 vs, 1549 m, 1475m, 1432 s, 1369m, 1268m, 1188m, 1144 w, 1084 w, 1043 w, 988 w, 830 w, 745 w, 685m, 627 w.

2.2.2. $[Cu_2(Ac)_4(Secnim)_2]$ (**2**)

Yield 57%. *Anal.* Calc. for $C_{22}H_{34}N_6O_{14}Cu_2$: C, 36.0; H, 4.7; N, 11.5. Found: C, 36.3; H, 4.4; N, 11.5%. Decomposition: $T > 175$ °C. FTIR, ν (cm^{-1}): 3342 m br, 3137 w, 3130–2800 w, 1619 vs 1541 m, 1512 sh, 1492 m, 1474 s, 1425 s, 1372 m, 1300 w, 1269 m, 1193 m, 1148 w, 1133 w, 990 w, 830 w, 739 w, 682 m, 626 w.

2.2.3. $[Cu_2(Ac)_4(Tinim)_2]$ (**3**)

Yield 70%. *Anal.* Calc. for $C_{24}H_{38}N_6S_2O_{16}Cu_2$: C, 33.6; H, 4.5; N, 9.8. Found: C, 33.4; H, 4.0; N, 9.7%. Decomposition: $T > 175$ °C. FTIR, ν (cm^{-1}): 3432m br, 3158 w, 3140–2900 w, 1629 vs, 1556 s, 1519 w, 1492 m, 1478 m, 1458 sh, 1427 vs, 1373 m, 1365 m, 1351 m, 1310 s, 1267 s, 1209 m, 1173 w, 1134 s, 1062–866 w, 830 mw, 792 mw, 742 mw, 682 m, 627 w.

2.2.4. $[Cu_2(Ac)_4(Nimim)_2]$ (**4**)

Yield 60%. *Anal.* Calc. for $C_{26}H_{40}N_8O_{14}Cu_2$: C, 38.3; H, 4.9; N, 13.7. Found: C, 38.3; H, 4.6; N, 14.0%. Decomposition: $T > 150$ °C. FTIR, ν (cm^{-1}): 3430 w br, 3137 w, 3130–2800 w, 1629 vs, 1537 s, 1521 s, 1472 s, 1434 s, 1370 s, 1300 w, 1263 w, 1236 w, 1201 w, 1170 w, 1141 w, 1125 m, 1112 s, 1016 w, 933 w, 860 w, 828 w, 741 w, 684 m, 648 m, 627 w.

2.3. Physical measurements

Elemental analyses were performed by the Analytical Center of the Chemistry Institute in São Paulo University using a Perkin–Elmer 2400 CHN analyzer. Atomic emission analyses for copper were conducted on Spectroflame Co. equipment (λ 327.396 nm). FTIR spectra were recorded on a BOMEM MB-100 (as KBr pellets; 4000–400 cm^{-1}) and an ABB BOMEM MB-102 (as Nujol mulls/CsI windows; 500–190 cm^{-1}) spectrophotometers. UV–Vis spectra in solution were recorded on a Hitachi U-3000 or a Shimadzu UV-1650PC spectrophotometer and spectra of solids were registered on a Guided-wave 260 equipped with optical fiber or a Shimadzu UV-2401PC (samples diluted with $BaSO_4$) equipped with an integration sphere X-band spectrophotometer. EPR spectra were obtained at room temperature on a Bruker EMX instrument using polyethylene tubes and DPPH (α,α′-diphenyl-β-picrylhydrazyl) as frequency calibrant ($g = 2.0036$). Room temperature magnetic susceptibilities were measured by Faraday technique with a Cahn electrobalance 7500 in a magnetic field of 1T with the calibrant $Hg[Co(SCN)_4]$ (16.44×10^{-6} c.g.s. units/Gauss). Variable-temperature (30–300 K) magnetic susceptibility measurements were carried out on powdered samples with a SQUID magnetometer under applied magnetic field of 500 Oe. The susceptibility data were corrected for diamagnetic contributions of the constituent atoms.

2.4. Biological assays

2.4.1. Preparation of CuAc, CuAcNtrim and Ntrim solutions

Compound CuAc and derivatives **1**–**4** were individually dissolved in water to give ~3 mmol dm^{-3} concentration stock-solutions. Then, 3.0 cm^3 of these stock-solutions were diluted with 2.0 cm^3 MEM (Minimum Eagle's Medium supplemented by 10% fetal calf serum) giving the correspondent 100%-work-solutions. Sterilization was performed by filtering the 100%-work-solutions through a 0.22 μm Millipore filter membrane and final concentrations determined by atomic emission were (in mmol dm^{-3}): 0.16 (CuAc); 0.95 (**1**); 1.54 (**2**); 1.50 (**3**) and 1.60 (**4**). Subsequently, these sterile 100%-work-solutions were serially diluted with MEM to 50%, 25%, 12.5% and 6.25% giving final solutions within a range from ~1600 to 160 μmol dm^{-3} drug concentrations. The solutions of the Ntrim organic drugs were prepared by similar procedures giving 100%-work-solution concentrations (mmol dm^{-3}) of 43.7 (Metrim), 40.7 (Secnim), 15.4 (Tinim) and 37.2 (Nimim).

2.4.2. Cytotoxicity assays

Cytotoxicity assays were carried out *in vitro* by using cell culture of mouse connective tissue NCTC clone 929 obtained from American Type Culture Collection (ATCC) by neutral red uptake methodology according to Ciapetti et al. [17].

The cells were maintained in MEM (Minimum Eagle's Medium with 10% fetal calf serum, 0.1 mmol dm^{-3} non-essential amino acids and 1.0 mmol dm^{-3} sodium piruvate). Cells were detached with 0.2% trypsin and 0.02% EDTA, and the cellular suspension was adjusted to 5×10^3 to 5×10^4 cells cm^{-3}. A 0.2 cm^3 volume

of this suspension was seeded in each well of 96 microplate-wells and incubated (INC: incubation under humidified air atmosphere, 5% CO_2, at 37 °C) for 24 h. After that, culture medium was replaced by 0.2 cm^3 of each CuNtrim or Ntrim solution, in triplicate. Extract of PVC used as negative control and 0.02 % phenol solution used as positive control received the same dilution treatment. Each well of control-cells received 0.2 cm^3 MEM. The microplate was maintained in INC for 24 h. After this time, the culture medium and solutions were replaced by neutral red dye-containing MEM (50 μg cm^{-3}). After incubation (INC) for 3 h, the microplates were washed twice with phosphate buffer (PBS) and 1% $CaCl_2$ in 0.5% formaldehyde solutions. Each well received 0.2 cm^3 of 1% acetic acid in 50 % ethanol and the optical densities (OD) were measured at 540 nm with an ELISA reader spectrophotometer Sunrise from Tecan. The determination of the cytotoxicity index (IC_{50}), *i.e.*, the concentration of the solution which injures or kills 50% of cell population in the assay, was based on the graphic of cell viability percentages (in relation to cell control 100 % viability) in function of the concentrations of CuNtrim or Ntrim solutions.

2.4.3. Gamma-radiation lethal dose (γ-LD_{50}) assays

Experiments were carried out *in vitro* under anaerobic conditions for Hep2 cell line (human larynx cancer cell of American Type Culture Collection (ATCC-CCL23) by adapted neutral red uptake methodology [17]. The cells were maintained in MEM and were detached with 0.2% trypsin and 0.02 % EDTA. A suspension of about 1.0×10^5 cells cm^{-3} was seeded in each well of 96 microplate-wells and incubated in INC for 24 h. After that, culture medium was changed by fresh MEM and the microplate was introduced into partially opened plastic boxes and enclosed into plastic bags under microaerophilic conditions created by using CampyGen sachets (Oxoid). Then, the samples were exposed to γ-rays from a panoramic ^{60}Co source with 0.89 Gy min^{-1} dose rate for periods of time correspondent to doses of 0; 50 and 100 Gy. Culture medium were changed again and the samples were incubated (INC) for 24 h. The culture medium was replaced by a solution of neutral red dye-containing MEM (50 μg cm^{-3}) and the procedure was followed according to that described in Section 2.4.2. The cell viability was calculated in relation to control cell – non-irradiated microplate (zero Gy = 100%) and the γ-radiation lethal dose (γ-LD_{50}) was determined.

2.4.4. Radiosensitizer assays

The experimental procedure was similar to that described for γ-LD_{50} assays (Section 2.4.3). However, before being submitted to microaerophilic conditions, the microplate culture medium was replaced by a 100 μmol dm^{-3} CuAcNtrim or MEM containing Ntrim. The samples were exposed to ^{60}Co γ-rays for periods of time correspondent to doses of 0; 3; 6 and 10 Gy which were lower than those used for determination of γ-LD_{50}.

3. Results and discussion

3.1. Synthesis

The preparation of CuAcMetrim previously reported in the literature [14,15] was carried out by heating under reflux for 6 h an ethanolic mixture of CuAc and Metrim. In the present work, this compound has been re-prepared by an alternative environment-friendly route which was also found to be appropriate to synthesize the other three novel copper(II)–acetato–Ntrim complexes. The products from reactions of CuAc with Ntrim drugs in ethanol, at room temperature (~25 °C), are formed by the replacement of the two axial water molecules in the precursor by two Ntrim drugs and exhibit general formula $[Cu_2(\mu\text{-}O_2CCH_3)_4(Ntrim)_2]$.

3.2. Electronic and Infrared spectra

The absorption bands observed in the electronic absorption spectra of the copper(II) complexes **1**–**4** are listed in Table 1. Methanol solutions of these complexes show an intense UV band at λ_{max} ~300 nm ($\varepsilon \approx 30.000$–$45.000$ mol^{-1} dm^3 cm^{-1}) with shoulder at ~235 nm. These bands are located at similar maximum wavelengths for the correspondent non-coordinated 5-nitroimidazoles drugs and might be ascribed to Ntrim intraligand (IL) transitions. The broad absorption band at Vis–NIR region (Table 1) with λ_{max} centered around 700 nm ($\varepsilon \approx 140$–180 mol^{-1} dm^3 cm^{-1}) is assigned to the characteristic copper(II) *d*–*d* transition (band I: $d_{xy,yz} \rightarrow d_{x^2-y^2}$) [18–20]. The other two expected bands (band II, charge transfer, ca. 300 nm, and band III, ca. 256 nm) for copper(II) complexes are probably overlapped with Ntrim ligand bands. The UV–Vis–NIR reflectance spectra show that the broad band assigned to copper(II) *d*–*d* transition is centered at λ_{max} in the region of 700–750 nm (Table 1) for the CuAcNtrim compounds in solid state. The energy of this electronic transition might depend on the nature of the Ntrim axial ligand according to the order Nmim > Tinim > Secnim ~ Metrim. However, a reliable correlation cannot be made because of the broadness of these bands (for example, the copper(II) *d*–*d* transition for compound **1** in microcrystalline form has been previously reported at a lower λ_{max} (710 nm) [14,15]). Additionally, the spectra of all CuAcNtrim complexes have a shoulder located at ~370 nm that might be ascribed to band II and give evidence for a carboxylato-bridged structure for the dinuclear copper(II) complexes with axial 5-nitroimidazoles [4, 21].

The electronic spectra of the four CuAcNtrim complexes in solution have been monitored in a time range from 0 h to 27 h. No significant absorbance changes have been observed neither for UV (~235 (sh), 300 nm) nor for VIS (~700 nm) bands. These results indicate that the copper complexes exhibit good stability in solution since dissociation of Ntrim ligands has not been detected to be appreciable in the range of time investigated here.

Selected absorption bands in FTIR spectra of compounds **1**–**4** are shown in Table 1 together with main bands of the non-coordinated Ntrim drugs for comparison. The ν_a(OCO) and ν_s(OCO) stretchings of acetato ligands in CuAcNtrim spectra appear at wavenumber regions of 1619–1629 cm^{-1} and 1425–1434 cm^{-1}, respectively, giving $\Delta\nu$(COO) values of ~190–200 cm^{-1} in good agreement with symmetrical bridging coordination mode of the acetato ligands [22]. The δ(OCO) and ρ(OCO) angular deformations are located around 685 and 627 cm^{-1} respectively. The main Ntrim bands are tentatively ascribed here by comparison with the IR spectra of some other imidazoles and their metal complexes [15,22–24]. The major changes in CuAcNtrim spectra in relation to the correspondent Ntrim ligand spectra occur in the region of the imidazole ring vibrations. The typical ν(C=N) imidazole ring stretching bands in CuAcNtrim spectra are shifted to higher wavenumbers (1537–1556 cm^{-1}) in relation to those of the correspondent non-coordinated Ntrim drugs (1525–1536 cm^{-1}) and indicate that the coordination of Ntrim to copper(II) is through the N_3 atom of the imidazole ring. The comparison of wavenumbers for the CuAcNtrim series shows a slight increasing of the ν(C=N) vibrational frequency along with the following order for Ntrim axial ligands: Nimim < Secnim < Metrim < Tinim. This order is in good agreement with the basicity [25] of the Ntrim drug axial ligand – the most basic drug Nimim coordinates to Cu(II) more tightly and hence the CuAcNimim exhibits the weakest C=N_3 ring bond. On the other hand, the $\nu_a(NO_2)$ (~1475 cm^{-1}) and $\nu_s(NO_2)$ (~1370 cm^{-1}) stretching vibrations remain in approximately the same position as observed for the non-coordinated Ntrim drugs. The splitting of these two bands, $\Delta\nu(NO_2)$ ~105 cm^{-1}, is similar for all CuAcNtrim complexes and suggests that the –NO_2 group is not involved in the coordination of Ntrim drugs to the copper(II) [15]. The frequency of

Table 1
Selected UV–Vis–NIR and FTIR** spectral data for compounds **1**–**4**.

	Compounds **1**–**4** *(Ntrim drug)*			
	1 (Metrim)	**2** (Secnim)	**3** (Tinim)	**4** (Nimim)
λ_{max} (nm);	235 ^{sh}	235 ^{sh}	236 ^{sh}	230 ^{sh}
[$\varepsilon \times 10^3$, mol^{-1} dm^3 cm^{-1}]	309 [38]	309 [45]	310 [36]	296 [34]
(methanol solutions)	702 [0.14]	700 [0.18]	700 [0.18]	700 [0.18]
λ_{max} (nm);	307	310	315	302
(solid state – diffuse reflectance)	370 ^{sh}	370 ^{sh}	370 ^{sh}	374 ^{sh}
	~750 br	~750 br	~720 br	~700 br
Selected FTIR bands				
ν_a (OCO)	1623 vs	1619 vs	1629 vs	1629 vs
ν_s (OCO)	1432 s	1425 s	1427 vs	1434 s
ν (C=N)	1549 m (1536 s)	1541 m (1528 s)	1556 s (1523 s)	1537 s (1525 s)
ν_a (NO_2)	1475 m (1476 s)	1474 s (1489 s)	1478 m (1478 s)	1478 s (1471 s)
ν_s (NO_2)	1369 m (1370 s)	1372 m (1379 s)	1373 m (1375 s)	1372 s (1370 s)
ν ($NCNO_2$)	830 w (826 m)	830 w (825 m)	830 mw (829 m)	828 w (824 m)
ν_a (SO)	–	–	1310 s (1302 vs)	–
ν_s (SO)	–	–	1134 s (1123 vs)	–
ν (COC)	–	–		1112 s (1112 s)
δ (OCO)	685 m	682 m	682 m	684 m
ρ (OCO)	627 w	626 w	627 w	627 w
ν (Cu–O)	352 w	350 w	354 w	360 w
ν (Cu–N)	265 w	260 w	265 w	278 w

** Wavenumbers in cm^{-1}: br = broad; vs = very strong; s = strong; m = medium; mw = medium-weak; w = weak; sh = shoulder.

the $\nu(NCNO_2)$ vibration mode of Ntrim drugs is also not significantly changed upon their coordination to the metal ions. Typical bands of the substituent groups: ν_a(SO) of Tinim and ν(COC) of Nimim, appear for the correspondent CuAcNtrim complexes at approximately the same wavenumbers that those observed for non-coordinated Ntrim drugs. The ν_s(SO) of Tinim however is slightly shifted to higher wavenumber for the complex indicating that this group might be involved in intermolecular interactions. The involvement of the $-SO_2$ group in other interactions might be the reason for the highest shifting observed for ν(C=N) upon coordination of Tinim to copper(II) ions. The unique band found for CuAcNtrim complexes at ~350 cm^{-1} might be assigned to copper-(*O*-acetato) stretching. The new bands at ~260–280 cm^{-1} which are not present for CuAc or Ntrim might be due to copper-(N_3) stretchings [26,27]. The highest value for ν(Cu–N) is found for complex CuAcNimim (278 cm^{-1}) giving additional evidence that Cu–Nimin bond is stronger than the Cu–Ntrim bonds of its analogs.

3.2.1. EPR spectra and magnetic susceptibility behavior

Effective magnetic moments, EPR and magnetic parameters for compounds **1**–**4** are shown in Table 2. The effective magnetic moments (μ_{eff}) per copper(II) atom at room temperature are in the range of 1.40–1.50 BM. This range includes the value of CuAc (μ_{eff} = 1.45 BM) and other dinuclear copper(II) compounds [15,28] and suggests the existence of dimeric structures with interaction between the two copper atoms for CuAcNtrim complexes.

The EPR spectra at X-band frequency for powdered compounds **1**–**4** show three signals located at 150–1500, 4540–4640 and 5930–6040 G (Fig. 2) which are respectively assigned to H_{z1}, $H_\perp$ and H_{z2} [29]. Small resonance signals at ~3000–4000 G might be ascribed to the presence of monomer impurities which are often observed for carboxylato-bridged copper(II) compounds. The EPR spectra of the dinuclear copper(II) compounds arise from the combined effects of the anisotropic and antisymmetric exchange and from the classical dipole–dipole interaction. It can be fitted satisfactorily using the following spin Hamiltonian (Eq. (1)) for isolated copper(II) dimers (S = 1) [29,30]:

$$H = DS_z^2 + E(S_x^2 - S_y^2) + \beta(g_z H_z S_z + g_x H_x S_x + g_y H_y S_y) \quad (1)$$

Table 2
Magnetic moments (μ_{eff}) per copper(II) atom, EPR parameters and magnetic parameters for compounds **1**–**4**.

	1	**2**	**3**	**4**
μ_{eff} (BM)	1.53 1.55 [15]	1.55	1.48	1.40
$g_\perp$	2.03	2.01	2.05	2.04
$g_{//}$	2.16	2.16	2.18	2.19
g_{iso} *	2.07	2.07	2.09	2.09
D_{total} (cm^{-1})	0.27	0.29	0.30	0.30
$2J/k$ (cm^{-1})	−390	−374	−423	−319
p	0.012	0.01	0.06	0.01
Nα		630×10^{-6}	-340×10^{-6}	40×10^{-6}

* $g_{iso} = 1/3\ (g_{//} + 2\,g_\perp)$.

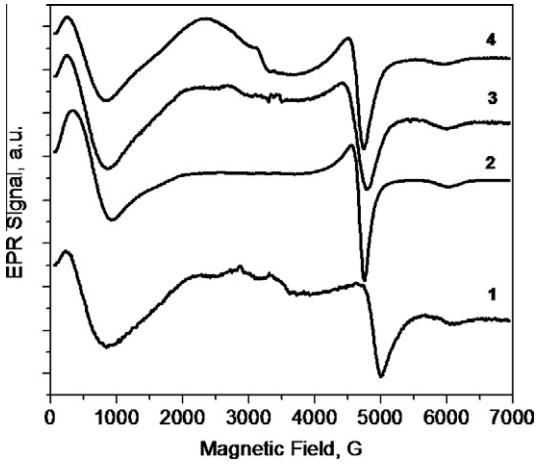

Fig. 2. EPR spectra of compounds **1**–**4**.

where D and E are the zero-field splitting parameters, β is the Bohr magneton, and x, y and z are the principal axes of a coordinating system that is fixed with respect to the Cu–Cu bond. EPR spectroscopic parameters from room temperature measurements are shown in Table 2. The spectral features together with the g

and the D values compare well with those found for a large number of reported dimeric copper(II)–carboxylato complexes with axial symmetry [31–34]. The EPR data are characteristic of tetragonal structures ($g_{//} > g_{\perp}$) where the unpaired electron is in a $d_{x^2-y^2}$ orbital [35].

The magnetic susceptibility values were plotted as a function of the temperature and the representative plot for compound **2** is shown in Fig. 3. A broad maximum at the temperature of approximately 230 K and a clear trend of decreasing values at lower temperatures are typical of discrete antiferromagnetically ordered systems [30]. The susceptibility value increases below 20 K, probably due to the presence of small paramagnetic impurity.

The observed magnetic behavior can be described by the Bleaney–Bowers equation combined with an additional term which accounts for the paramagnetic contribution [36] (Eq. (2)):

$$\chi = 2N\beta^2 g^2/kT[3 + \exp(-2J/kT)]^{-1}(1-\rho) + [N\beta^2 g^2/2kT]p + N\alpha, \quad (2)$$

where all the terms have their usual definitions and ρ is the percentage of paramagnetic impurity. The spectroscopic splitting factor g_{iso} (obtained from the EPR spectrum) was used as a constant in the fitting processes. This approach was used successfully for compounds **1**–**4** and the best fit parameters are shown in Table 2.

The value of $|2J/k|$ increases along with the following order based on the Ntrim axial ligand of the dimeric CuAcNtrim carboxylatos: Nimim < Secnim < Metrim < Tinim. Therefore, in this series of compounds there is a general trend for coupling constant to decrease as the Ntrim axial ligand becomes better electron-donor. The strength of the antiferromagnetic coupling decreases along with the increasing of the basicity of the axial Ntrim drug-ligand. A plot of the $|2J/k|$ values for the CuAcNtrim complexes as a function of the pKa of the Ntrim drugs (pKa values used here were taken from the literature [25]) shows a $|2J/k|$ *vs.* pKa correlation that is approximately linear (Fig. 4). The influence of several factors on the constant couplings for carboxylato-bridged dinuclear copper(II) compounds has been examined by computational models and shows that the replacement of axial H_2O molecules by NH_3 molecules in $[Cu_2(\mu\text{-}O_2CCH_3)_4(H_2O)_2]$, for example, reduces the strength of constant coupling by 5% [37]. The present work shows that the replacement of the less basic Tinim by the most basic Nimim reduces the strength of the antiferromagnetic coupling by 25%. Therefore, by changing the Ntrim ligand according to the basicity it is possible to modulate the constant coupling for this series of CuAcNtrim compounds for which structures are shown in Fig. 5.

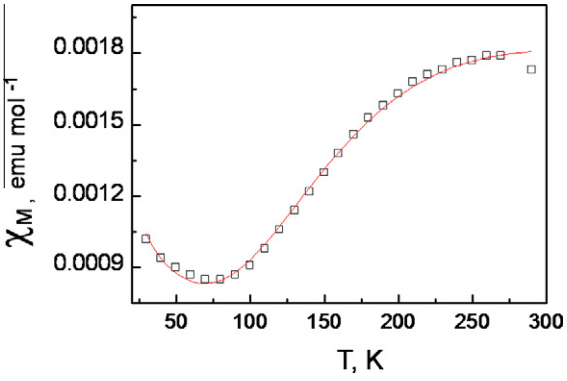

Fig. 3. Temperature dependence of the magnetic susceptibility, χ_M, for dimeric compound **2**.

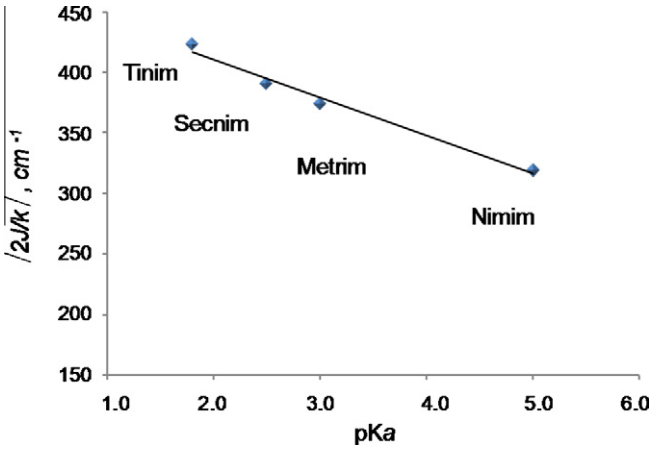

Fig. 4. Plot of the constant couplings for CuAcNtrim as a function of p*K*a values [25] of the Ntrim ligands (the value of p*K*a for Secnim has been considered as similar to that found for the closest analog $C_7H_{13}N_3O_4$ (or DA 3838).

3.3. Biological assays

The results obtained from the *in vitro* biological assays are summarized in Table 3.

3.3.1. Cytotoxicity assays

The IC_{50} values determined from cytotoxicity assays (NCTC clone 929 cell line) indicate that CuAcNtrim compounds are more cytotoxic to cells than the correspondent non-coordinated Ntrim drugs (>10 mmol dm^{-3}). CuAcMetrim (**1**) is approximately twice less cytotoxic than the other three analogs (**2**, **3** and **4**) which show similar behavior. The IC_{50} data were used further to establish the non-toxic levels (100 μmol dm^{-3}) for the radiosensitizer assays of the CuAcNtrim complexes.

3.3.2. Gamma-radiation lethal dose assays

The radiation lethal dose, *i.e.*, the radiation dose that kills 50% cellular population, was determined based on plots (not shown) of cell viability logarithms as function of radiation doses (0; 50 and 100 Gy). The value of γ-LD_{50} found for Hep 2 cells (34.1 Gy) provided the radiation dose level to be used for radiosensitizer assays.

3.3.3. Radiosensitizer assays

The cell suspensions were exposed to CuAcNtrim compounds at concentrations (0.100 mmol dm^{-3}) which were below their toxic levels (IC_{50}). Radiosensitizer properties of CuAcNtrim complexes and Ntrim drugs were evaluated based on experimental data for radiation doses of 0, 3, 6 and 10 Gy. Plots of survival fraction (S) logarithms as function of radiation doses (Fig. 6) were adjusted to a quadratic linear model (α,β-model) [38]:

$$\ln S = -\alpha D - \beta D^2 \quad (3)$$

where S is the survival fraction, *i.e.*, the ratio between the number of survival cells for a determined radiation dose and the number of survival cells for a non-irradiated sample; D is the radiation dose; α and β are constants that correspond, respectively, to one event and two independent events produced by radiation.

Survival curves on Fig. 6 show that the value of ln S decreases along with the increasing of radiation doses for all CuAcNtrim compounds. Values of D_s, *i.e.*, the radiation dose required for survival fraction S, correspondent to $S = 0.5$ (50% cell survival) and $S = 0.8$ (80% cell survival) are summarized in Table 3.

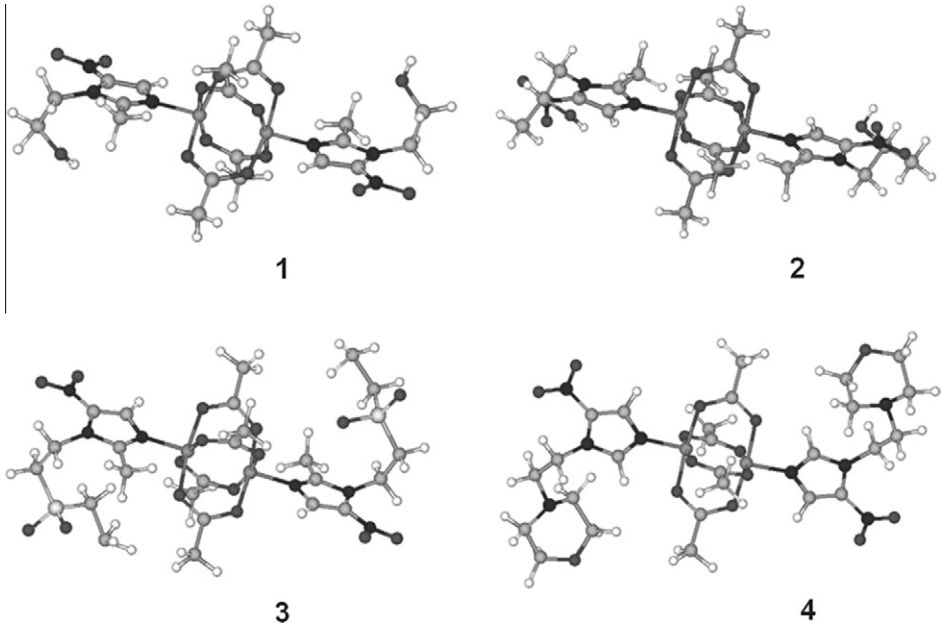

Fig. 5. Molecular structures of compounds **1**–**4**.

Table 3
Results obtained from *in vitro* biological assays.

Data from biological assays	Compounds **1**–**4** (non-coordinated drug)			
	1 (Metrim)	**2** (Secnim)	**3** (Tinim)	**4** (Nimim)
IC_{50} (mmol dm^{-3})	0.270 ± 0.001	0.150 ± 0.005	0.140 ± 0.019	0.150 ± 0.009
$D_{0.5}$ (Gy)	7.1 [8.2]	6.8 [4.0]	6.7 [5.3]	6.3 [8.6]
$ER_{0.5}$*	1.2 [1.0]	1.3 [2.1]	1.3 [1.6]	1.4 [1.00]
$D_{0.8}$ (Gy)	2.8 [3.0]	2.4 [1.4]	2.3 [1.8]	3.1 [4.0]
$ER_{0.8}$*	2.2 [2.0]	2.6 [4.4]	2.7 [3.4]	2.0 [1.5]
α ($\times 10^{-3}$ Gy^{-1})	64.7 [65.7]	86.8 [154.3]	76.5 [122.2]	35.8 [32.6]
$\beta^{1/2}$ ($\times 10^{-3}$ Gy^{-1})	67.1 [48.0]	50.0 [60.8]	64.8 [43.6]	108.6 [74.2]

*ER = D_S^0/D_S; D_S^0 = radiation dose without radiosensitizer (8.6 Gy for $S = 0.5$; 6.2 Gy for $S = 0.8$); D_S = radiation dose in the presence of radiosensitizer.

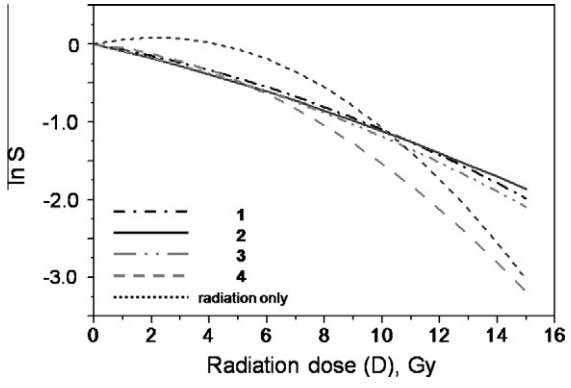

Fig. 6. Survival curves for Hep2 cells irradiated in hypoxic ambient in the presence of compounds **1**–**4** (100 μmol dm^{-3}) obtained by polynomial adjust to Eq. (4) (D = radiation at 0.89 Gy min^{-1} dose rate).

The radiation dose values that give 50% cell survival (D_{50}) for Hep2 cells in hypoxic condition in the presence of the radiosensitizers are in the range from 6.0 to 7.1 Gy. These values are approximately 5-fold lower than the γ-LD_{50} (34.1 Gy). Therefore, all CuAcNtrim complexes and Ntrim drugs show radiosensitizer activity and have the ability to enhance the gamma-irradiation effect for the same cell culture mortality. The $D_{0.5}$ values show a slight decreasing from compound **1** (7.1 Gy) to **4** (6.3 Gy). $ER_{0.5}$ values higher than 1.0 give evidence for radiosensitizer activity. The $ER_{0.5}$ enhancement ratios are approximately similar for all the CuAcNtrim complexes. However, these values indicate that compounds **1** and **4** show enhancement (20% and 40%, respectively) of radiosensitizer activity in relation to their Ntrim parent drugs. The best enhancement of radiosensitizer property upon coordination of Ntrim drugs is found for CuAcNimim (**4**). Values of $D_{0.8}$ (80% cell survival) are also shown in Table 3. These are approximately 17-fold lower than the γ-LD_{50} (34.1 Gy) and show a trend that is similar to that observed for $D_{0.5}$ within the series of CuAcNtrim and Ntrim drugs. However, $ER_{0.8}$ values are higher than $ER_{0.5}$ ratios and show larger differences among the CuAcNtrim complexes with the lowest value found for compound **4**. On the other hand, again compounds **1** and **4** exhibit enhancement (10 % and 25 %, respectively) of radiosensitizer activity in relation to their Ntrim parent drugs and the best enhancement of radiosensitization upon coordination of Ntrim is found for CuAcNimim (**4**).

The curve for the non-drug acetato-bridged copper(II) could not be adjusted to linear quadratic model and the values of D could not be calculated for this compound. However, the results (not shown) obtained for the non-drug indicate that the radiosensitizer effect of the CuAcNtrim complexes is not dependent only on the Ntrim drug ligands. Although the CuAcNtrim complexes have been found to be stable in solution up to 27 h, it is not possible to affirm that the molecular structure of CuAcNtrim is preserved in the biological

medium required for the radiosensitizer assays. However, since each molecule of CuAcNtrim carries two molecules of Ntrim drug (*i.e.*, 100 μmol dm^{-3} of copper complex corresponds to 200 μmol dm^{-3} of Ntrim), the parameters for radiosensitizer activity of CuAcNtrim complexes would be expected to be about 2-fold higher than those of the correspondent non-coordinated Ntrim drugs if the effect was attributed only to released axial Ntrim drugs. Therefore, a synergistic effect due to the presence of the metal and the Ntrim drug ligands might play important role on the radiosensitizer activity of the CuAcNtrim complexes.

The ER values (Table 3) of the non-coordinated Ntrim drugs show that the order (Secnim > Tinim > Metrim ⩾ Nimim) of the Ntrim drug sensitization is not directly correlated to the order of the reduction potentials of the nitro group [39] which is: Nimim > Secnim > Tinim > Metrim. Moreover, the most lipophilic Nimin is also not the best Ntrim radiosensitizer drug in this *in vitro* experimental condition for human larynx cancer cell. It has been suggested in the literature that the biological activity of nitroimidazoles is dependent on the nitro group in the 5-position of the imidazole ring and also that the steric protection of the $-NO_2$ group by substituents in N1 and C2 is necessary. Apart from the essential nitro group that gives the molecules a high (less negative) 1-electron reduction potential, it has been proposed, for example, that the degree of antimicrobial activity depend on the substituents in C2 and N1, *i.e.*, it either enhances or ablates the resonance conjugation of the molecule by its special arrangement in the molecule as well as the hydrophilic/lipophilic properties of the molecule. Different substitutions at N1 and C2 also modify kinetics of these drugs. In addition, nitroimidazoles are able to bind plasma proteins [11]. Mechanism of action of nitroimidazole drugs to kill anaerobic protozoa involves several steps that include the reduction of the nitro group and binding of the reduced drug to DNA resulting in DNA damage (loss of helical structure, strand breakage, impaired template function) [11]. Therefore, the understanding of the behavior of the radiosensitizer property of drugs depends on several factors.

In the present work, since the α,β-mathematical model [38] was used to describe the survival curves of the CuNtrim compounds, an attempt has been made to compare the values of α and $\beta^{1/2}$ parameters in order to find if there was a relation between these values and the behavior of the drugs investigated here. It is important to mention that the α,β-model is not being suggested to explain the radiosensitizer property of the drugs. It has been used to compare the different results obtained for the enhancement of the radiosensitizer activity of CuAcNtrim complexes in relation to their Ntrim parent drugs. The model is based on the assumption that the cellular death is caused by the DNA damage and allows to estimate the type of events involved in the radiosensitizer process. A single event ($\alpha > \beta^{1/2}$) indicates that DNA double-strand breaks in one step while a double event ($\alpha < \beta^{1/2}$) shows that double-strand breaks in two independent steps [40]. Based on this model, we found that the radiosensitizer process involves a single event ($\alpha > \beta^{1/2}$) for Metrim, Secnim, Tinim, CuAcSecnim (**2**) and CuAcTinim (**3**). In contrast, a double (two independent) event ($\alpha < \beta^{1/2}$) is involved in the radiosensitizer process for Nimim, CuAcMetrim (**1**) and CuAcNimim (**4**). Therefore, it is possible to suggest that the Nimim non-coordinated drug and the correspondent CuAcNimin complex exhibit unique behavior in comparison with the other analogs with respect to the radiosensitizer process involving damage to DNA. The existence of double event might account for the enhancement of radiosensitizer property upon coordination of Nimim drug to copper(II) ions in CuAcNimim (**4**). Moreover, the slight enhancement of radiosensitization upon coordination of Metrim to copper(II) in CuAcMetrim (**1**) might be explained in a similar way since in this case a change from single event for the organic drug to double event for the metal complex appears to occur, although the value of α very close to that of $\beta^{1/2}$ for compound **1**.

Additionally, it should be mentioned that along the series of CuAcNtrim dimeric complexes, the CuAcNimim (**4**) is the derivative which carries the most lipophilic Ntrim drug. In contrast to the free Nimim pro-ligand drug, N_3 is coordinated to copper(II) in the complex. Consequently, the factor lipophilicity of the CuAcNimim complex may favor its incorporation into cells that might contribute in significant way for its radiosensitizer activity. A recent report has shown that the structure of metal complexes and the presence of electron affinic site(s) play important role in the cellular incorporation and accounts for the activity as radiosensitizer. Copper(II) ion and a copper(II)–NTA (nitrilo triacetic acid) complex, which are significantly uptaken, were found to radiosensitize *E. coli* cells, in contrast to complexes of ethylene diamine tetraacetic acid (EDTA) and imino diacetic acid (IDA) which were not incorporated into the cells [16].

4. Concluding remarks

In this work we have reported that 5-nitroimidazole drugs axially coordinate acetato-bridged copper(II) dimer through the N_3 atom of the imidazole ring by reacting CuAc with the correspondent Ntrim drugs at room temperature. The dimeric compounds show antiferromagnetic behavior and the constant coupling decreases along with the increasing of the basicity of the Ntrim axial drug ligand. All CuAcNtrim complexes and their correspondent Ntrim parent drugs show radiosensitizer activity for Hep2 cell line. The best enhancement of radiosensitizer activity upon coordination of Ntrim drug is found for CuAcNimim. It is an interesting result since this compound has the strongest Cu-Ntrim axial bond and exhibits the highest lipophilicity within the series of CuAcNtrim complexes investigated here. Consequently, for this series of metal compounds, the radiosensitizer property might depend on the molecular structure of the copper(II)–acetato–Ntrim complex. Furthermore, the enhancement of radiosensitizer activity upon axial coordination of Ntrim drug to acetato-bridged copper(II) moiety might be related to the type of the event in the radiosensitizer process involving DNA damage. Finally, the CuAcNimin is a novel metallodrug that deserves future detailed studies in respect to radiosensitizer activity.

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