Synthesis, Characterization and Biological Activity of Two New Copper (II) Complexes with N-sulfonamide Ligand

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Despite the fact that a large number of chemotherapeutic anticancer agents have been discovered, cancer still remains a great cause of deaths worldwide. The purpose of our researches is to discover a new antitumor drug. In this aim, two new Cu(II) complexes, [Cu(L)2(py)2(H2O)2](C1) and [Cu(L)2(phen)](C2) with a new ligand, N-(5-trifluoromethyl-[1,3,4]-thiadiazole-2-yl)-benzensulfonamide(HL) were synthesized. The complexes were characterized by elemental analysis, spectral and magnetic determinations. The nuclease activity studies of the complexes confirm their capacity to cleavage the DNA molecule. Both complexes have in vitro antioxidant activity (DPPH, FRAP methods), in vitro (using xanthine/xanthine oxidase system) and in vivo (using S.cerevisiae) SOD mimetic activity. The results of MTT assay on two carcinoma cell lines (HeLa and WM35) indicate that both complexes have antitumor activity, but (C2) has a superior activity compared with (C1) and with Cisplatin. On normal fibroblast (HDFa), (C1) showed toxicity comparable with Cisplatin, but (C2) showed a lower one. Bacterial assays were also performed (by the disk diffusion method) and both complexes have antibacterial activity against S. aureus, E. coli, P. aeroginosa and B. cereus. All the biological studies are in concordance and show that both complexes have biologic activity but (C2) is much more active.

Keywords: oxidative DNA cleavage, antioxidant capacity, SOD-mimetic activity, cytotoxicity, antibacterial properties

Metal ions are important for the course of the living organisms’ vital functions where they occur under the form of complex or chelated combinations, as well as for the drugs control and analyses [1-4]. Copper exhibits considerable biochemical action either as an essential trace metal or as a constituent of various exogenously administered compounds in humans [5,6].

Current interest in Cu complexes is stemming from their potential use as antimicrobial, antiviral, anti-inflammatory, antitumor agents, enzyme inhibitors, or chemical nuclease [7-11].

The study of N-substituted heterocyclic sulfonamides ligands showed their ability to coordinate biologically important metallic ions. The sulfonamides structures are varied and complex, and offer multiple possibilities to coordinate metallic ions depending on the type of donor atoms in the molecule (O, S, N), the stereochemistry of the compounds, the nature of the metallic center that can form complexes, the nature of the anion of the salt that offers the central ion, the synthesis parameters [12].

Copper complexes as artificial nuclease have been shown to possess in vitro anticancer activity and a lower toxicity than platinum drugs. Many of them are active against platinum drug resistant tumor cell lines. In contrast to platinum compounds which covalently bind DNA nucleobases, copper derivatives usually form non-covalent interactions with DNA (intercalation, electrostatic forces of attraction or major/minor groove binding). For the majority of copper complexes reported as DNA cleavage agents, their mode of action is mediated via the formation of ROS generated either by oxidation of Cu(II) to Cu(III) or by reduction from Cu(II) to Cu(I) in the presence of oxidizing (dihydrogen peroxide or molecular oxygen) or reducing agents (ascorbic acid) [13-15].

The synthesis of Cu(II) complexes with N-substituted heterocyclic sulfonamides has greatly increased in the past twenty years, due to the diversity of biological activity of the resulting compounds. In this context, for the past several years our group worked on the synthesis of such compounds in order to obtain new antitumor agents and we described the DNA cleavage, SOD-like and antitumor activity of several copper sulfonamide complexes [16-21].

In this paper we report the synthesis, the physico-chemical characterization of two new Cu(II) complexes with a N-substituted sulfonamide and we demonstrate their antioxidant, SOD-mimetic, antitumor and antibacterial activities.

Experimental part
Materials and physical measurements
2-amino-5-fluoromethyl-[1,3,4]-thiadiazole, sulfonyl chlorides, pyridine and phenantroline were provided by Aldrich and were used without further purification.

Elemental analyses (C, N, H, S) were performed with Perkin-Elmer elemental analyser. IR spectra were recorded using KBr disks on a Perkin-Elmer FT-IR 1730 instrument, in the 4000-400 cm\(^{-1}\) range. Electronic paramagnetic spin

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resonance spectrum was performed at room temperature with a Bruker ER 200D instrument. Solid UV-Vis spectra were recorded on a Shimadzu UV-2101 PC spectrophotometer. Mass spectra for the ligand were recorded using the FAB technique, on a VG Autospectrometer. Magnetic susceptibility measurements were performed at room temperature according to the Faraday method, using \( \text{Hg(Co(SCN))}_2 \) as standard.

**Synthesis**

**Synthesis of the ligand N-(5-trifluoromethyl-[1,3,4]-thiadiazole-2-yl)-benzensulphonamide (HL)**

The ligand was prepared by reacting 2-amino-5-trifluoromethyl-[1,3,4]-thiadiazole (1 mmol) with benzzenesulfonyl chloride (2 mmol) in a pyridine solution (6 mL). The mixture was refluxed at 60°C for 60 min; 10 mL of very cold water was then added in the mixture, continuously stirring. The white precipitate formed was filtered and further recrystallized using ethanol.

**Synthesis of the complex [Cu(L)_2(phen)] ([C])**

A pyridine solution (25 mL pyridine : H_2O = 2 : 3) containing 1 mmol ligand (HL) was added to a solution of CuSO_4 . 5H_2O (4 mmol CuSO_4 . 5H_2O in 20 mL pyridine : H_2O = 1 : 1), dropwise and continuously stirring. The resulting dark-blue mixture was stirred for 2 hours at room temperature. Slow evaporation of the solvent during one month allowed the formation of blue crystalline powder. The solid electronic spectra show a peak at 322 nm, due to \( \pi-\pi^* \) transition that appear within the heterocyclic ring. The mass spectra show a molecular peak with a value of 310; the peak is characteristic for the protonated form of the ligand (HL-H)^+.

**In vitro antioxidant activity**

**DPHP radical scavenging assay**

The radical scavenging activity of the (C)_1 and (C)_2 complexes towards the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured according to Kikuzaki et al. [22]. The sample solution (50 µL, 20 mg/mL in DMSO) was mixed thoroughly with a solution of DPPH in methanol (1 mL, 0.4 mM) and brought up with methanol to 5 mL. The mixture was shaken vigorously using a vortex and left to stand for 30 min at room temperature, in the dark. After that, the absorbance was measured at 517 nm using a Genesys-10 spectrophotometer. The DPPH solution without sample solution was used as control sample (A_{0}). The ability to scavenge the DPPH radical was calculated using the following formula:

\[
\% \text{Inhibition} = \left[\frac{A_{0} - A_{\text{Sample}}}{A_{0}}\right] \times 100
\]

**FRAP method (ferric reducing antioxidant power)**

The FRAP method is a simple spectrophotometric method that assesses the antioxidant power of the complexes, being based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reductant, at an acid pH. The stock solutions included: 300 mM acetate buffer; 270 mg FeCl_3· 6H_2O dissolved in 50 mL distillated water; 150 mg TPTZ and 150 µL HCl, dissolved in 50 mL distilled water. The working FRAP solution was freshly prepared by mixing 50 mM acetate buffer, 5 mL FeCl_3· 6H_2O solution and 5 mL TPTZ solution. Trolox was used as a standard solution, the calibration curve was made for concentrations between 0 and 300 µM, having a correlation coefficient \( R^2 = 0.9956 \) and the regression equation \( y = 0.0017x + 0.0848 \), where \( y \) represents absorbance detected at 595 nm. The results are expressed as µmol Trolox equivalents (TE)/100 mL extract [23,24].

**In vitro SOD mimetic activity**

The in vitro SOD mimetic activities of the complexes were assayed using the Oberley and Spitz method with some minor modifications [25]. Xanthine (1.5 x 10^4 M) and xanthine oxidase in 50 mM potassium phosphate buffer, \( pH = 7.8 \) were used to generate a reproducible and constant flux of superoxide anions. These were detected by the reduction of nitro blue tetrazolium (NBT) (5.6 x 10^5 in base pairs), and 6µL of activating agent solution (H_2O_2 ascorbic acid) in a threefold molar excess relative to the concentration of the complex. The resulting solutions were incubated for 1 h at 37°C, after which a quench buffer solution (3µL) consisting of bromophenol blue (0.25%), xylene cyanole (0.25%) and glycerol (30%) was added. The solution was then subjected to electrophoresis on 0.8% agarose gel in 0.5 x TBE buffer (0.045 M Tris, 0.045 M boric acid, and 1 mM EDTA) containing 5µL/100 mL of a solution of ethidium bromide (10 mg/mL) at 100 V for 2 h. The bands were photographed on a capturing system (Gelprinter Plus TDI).

To test for the presence of reactive oxygen species (ROS) generated during strand scission and for possible complex-DNA interaction sites, various reactive oxygen intermediate scavengers and groove binders were added to the reaction mixtures. The scavengers used were 2,2,6,6-tetramethyl-4-piperidone (0.5 M), dimethylsulfoxide (DMSO) (14 M), \( \tau \)-butyl alcohol (10.5 M), sodium azide (NaN_3) (400 mM), superoxide dismutase (SOD) (15 units). In addition, a chelating agent of copper (I), neocuproine (36µL), along with the groove binder distamycin (80µM) were also assayed. Samples were treated as described above [16,19].
which has the ability to delete/insert the SOD 1 gene supplemented with 10% fetal bovine serum (Hyclone), 1
lines were maintained in DMEM (Sigma-Aldrich),
cell line (HDF α) (ATCC Manassas, Virginia, USA). The cell
line (WM35) and a human normal fibroblastic epithelial
cervical carcinoma line (HeLa), a human melanoma cell
were poured into Petri dishes and allowed to solidify at
70 µm) were added to the growth medium. Cell suspensions
of DMSO:EtOH (1:4) at increasing concentrations (30, 50,
PLUS Spectrofotometer.
reagents for these assays were obtained from Sigma and
plots of % inhibition versus complex concentration. All
experiments, 0.1 mL complex solution + 0.1 mL xanthine
oxidase were added to 0.8 mL of solution containing 0.09
mL of potassium phosphate buffer (pH = 7.8), 0.025 mL
of NBT and 0.085 mL of xanthine. To determine the SOD
activities of the complexes, the percentage inhibition of
NBT reduction was used. The formation of uric acid from
xanthine was followed at 310 nm. The % inhibition of
enzyme activity was subtracted from that of NBT. The
IC50 values (the concentration of complex required to yield
50% inhibition of NBT reduction) were determined from
plots of % inhibition versus complex concentration. All
reagents for these assays were obtained from Sigma and
the determinations were performed with a SPECCORD 200
PLUS Spectrofotometer.

In vivo SOD mimetic activity
The protective effect of the complexes (C1) and (C2)
against free radicals produced by oxidative agents has been
determined.
The SOD-like activities of the complexes where evaluated using a strain of S.cerevisiae∆sod1 (ATCC69687),
which has the ability to delete/insert the SOD, gene
encoding the synthesis of Cu,Zn SOD. The characteristics of S.cerevisiae∆sod1 (ATCC96687) are: MAT aura 3-52 trp1-
289 his3-α1 leu 2-3112 sod1: URA3). The Cu,Zn SOD is the main SOD in the cell and it is localized in the
cytoplasm. Yeast cells were grown in YPD reach medium
(1% yeast extract, 2% peptone and 2% glycerol). The used
culture medium does not contain glucosse, it contains
glycerol instead, because in its presence the levures can
breath. This is determinant, as free radicals are going to be
generated during the breath processus taking place in the
mitochondria. Solid media contained 1.5%agar. Cell density
from cultures grown overnight was determined by cell
counting in a Neubauer hematemat. 106 cells were
resuspended in 15 mL of melted solid YPD media kept at
45°C. Solutions of the (C1) and (C2) complexes in a mixture
of DMSO:EtOH (1:4) at increasing concentrations (30, 50,
70 µm) were added to the growth medium. Cell suspensions
were poured into Petri dishes and allowed to solidify at
room temperature. Paper disks measuring 6 mm in diameter (Antibiotica test Blättenbach) containing 5µL of a 5
mM menadione solution in ethanol or 5 µL of a 17.5% H2O2
solution were solubilized with dimethyl sulfoxide (DMSO), while the positive control
was represented by Cisplatin (Ebewe Pharma Ges.m.b.H. Nfg. KG, Austria) in the same concentration as the complexes. Each experiment was carried out in triplicate.
The cellular viability was determined using the MTT assay
at 24, 48 and respectively 72 hours. The formazan particles
were solubilized with dimethyl sulfoxide (DMSO) (Sigma).
The absorbance was read at 550 nm using a spectro-
photometer (Bio-Rad, Hercules, CA, USA). The IC50 values
representing the concentration of complex required to inhibit 50% of cell proliferation were calculated from the dose-response curves using nonlinear regresion [18,20,27,28].

Antibacterial activity
Preliminary screening of (C1) and (C2) complexes against
two Gram-positive bacteria species: S. aureus ATCC 6538P,
B. cereus ATCC 14579 and against two Gram-negative bacteria species: E. coli ATCC 10536 and P. aeruginosa
ATCC 27853 was done using the disk diffusion method
[29-32]. As positive control Norfloxacin (Sigma-Aldrich)
was used [33]. The reference microbial strains were
obtained from American Type Culture Collection (ATCC,
Manassas, VA, USA).
The values of the minimum inhibitory concentration
were determined using the serial micro-broth dilution
method with Müller-Hinton broth, described by Carson et
al. [34]. All tests were duplicated and the MIC values were
expressed as mean ± standard deviation.

Statistics
All the experiments were conducted in triplicates and
data are displayed as Mean ± SD, representing the average
of independent experiments performed in triplicate. The IC50 values representing the complexes concentration
required to inhibit 50% of cell proliferation were calculated from the dose response curve using non-linear regression. Statistical values were generated using GraphPad Prism
version 5.0 for Windows, GraphPad Software, San Diego
California USA.

Results and discussions
The chemical structures of the complexes (C1), (C2) and
of the ligand (HL) are given in Figure 1.

The complexes structures
In complex (C1), Cu(NO3)2 chromophore type, the coordination geometry of the Cu(II) ion is slightly distorted
octahedral. The two deprotonated ligands by N ligand
atom, two pyridine’s molecules as monodentate ligand by N
and two water’s molecules by O, coordinate the metallic
ion. The structure for the complex (C1) shows that the Cu(II)
ion is four-coordinated and the chromophore type is CuN4.
The Cu(II) ion is bound symmetrically by two deprotonated
ligands and one phenantroline in a square-planar
environment (C1). The sulfonamide ligand (HL) coordinates the Cu(II) ion upon the deprotonation of the -NH-SO2-
molecule. Each ligand coordinates the metal ion through the
one Nphenantroline atom. The phenantroline’s molecules also
participate at the Cu(II) coordination as bidentate ligand by two nitrogen atoms N.

Cytotoxicity assays
For the cell survival the cell lines were plated (1x105
cells/ well) in 96-well plates for 24h in normal propagation
media. The culture medium was then replaced with
complete medium containing two type of complexes (C1)
and (C2) in 5 different concentrations (50 µM, 25 µM, 125
µM, 2 µM and 0.2 µM). The compounds were initially
dissolved in DMSO, followed by serial dilutions in media
until the final concentration of DMSO was less than 1%.
The negative controls were represented by cell lines
cultivated in normal expansion medium added with the
same amount of diluted DMSO, while the positive control
was represented by Cisplatin (Ebewe Pharma Ges.m.b.H. Nfg. KG, Austria) in the same concentration as the complexes. Each experiment was carried out in triplicate.
The cellular viability was determined using the MTT assay
at 24, 48 and respectively 72 hours. The formazan particles
were solubilized with dimethyl sulfoxide (DMSO) (Sigma).
The absorbance was read at 550 nm using a spectro-
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one Nphenantroline atom. The phenantroline’s molecules also
participate at the Cu(II) coordination as bidentate ligand by two nitrogen atoms N.
Spectroscopic properties
Comparing the IR spectra of the complexes and of the ligand, the most remarkable modifications appear in the vibrational bands of the thiadiazolic ring, $\nu$(S-O) and $\nu$(S-N) (in the sulfonamidic group). The band corresponding to the stretching vibrations of the thiadiazolic ring is shifted towards lower frequencies for both complexes: from 1550 cm$^{-1}$ for (HL) to 1493 cm$^{-1}$ for (C1) and from 1490 cm$^{-1}$ for (C2). The modifications prove the involvement of the thiadiazolic nitrogen in the coordination of the cooper ion; an electronic transfer between the thiadiazolic and sulfonamidic nitrogen atoms may occur upon deprotonation of the sulfonamidic nitrogen -N(-)SO$_2$. The bands attributed to (S=Oas) and (S=Os) in the structure of both complexes, are shifted towards lower frequencies (15-20 cm$^{-1}$); these modifications can be related to the deprotonation of the sulfonamidic nitrogen and subsequent conjugation between the atoms of the group (N, S, O). The phenomenon is confirmed by the shifting of the S-N vibrational band toward higher frequencies for both complexes as compared to the ligand (932 cm$^{-1}$ and 938 cm$^{-1}$ for the complexes and 915 cm$^{-1}$ for the ligand). The IR spectra, with all the modifications mentioned above, display a similar pattern to all Cu(II) complexes described in literature where N-substituted sulfonamides act as ligands. The deprotonation of the sulfonamidic nitrogen, and the coordination of the cooper atom through the nitrogen atoms in the heterocyclic ring are characteristic for this type of complexes [35]. The characteristic bands for the pyridine molecule that coordinates the metal ion cannot be identified due to the superposition of these bands with those of the ligand. The spectrum of the (C1) complex presents bands at 1125, 1087 and 730 cm$^{-1}$, which are attributed to the coordinated phenanthroline molecule [36]. In general, the IR spectra are similar to those for the other copper N-sulfonamide compounds [37]. The changes of the IR characteristic bands are mainly due to the sulfonamide deprotonation.

The solid electronic spectra exhibit a maximum at 590 nm, for (C1) and at 595 nm, for (C2), due to d-d transitions; the peaks are characteristic for octahedral distorted and respectively square-planer geometries. The peaks that appear at 392 nm for (C1) and at 405 nm for (C2) are due to the charge transfer between the ligand and the metal ion [38,39].

EPR and magnetic properties
The complexes display effective magnetic moments of 1.93 MB (C1) and 1.83 MB (C2). This behavior is characteristic for monomers, with no metal-metal interactions (1.72 - 2.20 MB) and indicates the presence of a single unpaired electron in a 3d orbital for the metal ion [39].

The polycrystalline X-band EPR spectrum of the complex (C1) is axial and for the complex (C2) is rhombic. The parallel region is resolved with hyperfine splitting from cooper. The EPR parameters calculated by simulation are $g_\parallel = 2.26$, $g_\perp = 2.05$ and $A_\parallel = 154 \times 10^{-4}$ cm$^{-1}$ for complex (C1) and $g_\parallel = 2.030$, $g_\perp = 2.070$, $g_\perp = 2.140$ and $A_\parallel = 190 \times 10^{-4}$ cm$^{-1}$ for complex (C2) [40]. The calculated parameters suggest that the unpaired electron is in a d$_{x^2-y^2}$ or a d$_{xy}$ orbital [41]. According to the Bertini classification, the value of $A_\parallel$ can be correlated with the geometry of the complex [42].

The structures of the complexes (C1) and (C2) resulting from the elemental analysis and the spectral data are shown in Figure 1.

DNA cleavage
The ability of the complexes to cleave DNA was studied using supercoiled pUC18 DNA in DMF: cacodylate buffer (0.1 M, pH 6.0) a molar proportion (1:39)in the presence of H$_2$O$_2$/ascorbic acid, 3.0-fold excess relative to the complex concentration. Control experiments with CuSO$_4$ were also carried out under the same experimental conditions.

![Fig. 1. Structures of the complexes (C1), (C2) and of the ligand (HL)](image)

The results are presented in Figure 2(a), 2(b).

![Fig. 2(a). Electroferogram in agarose gel of the pUC18 plasmid treated with the complex (C1)](image)

Complex (C1) at 18 µM (Figure 2(a), lanes 10) produced a partial conversion of closed circular (NC) conformation DNA into its supercoiled form (SC). At 24 µM, degradation of the supercoiled form to open circular DNA (Figure 2(a), lanes 11) was observed. Under the same conditions, CuSO$_4$ exhibited less nucleolytic activity than either of the complexes (lanes 4-7).

![Fig. 2(b). Electroferogram in agarose gel of the pUC18 plasmid treated with the complex (C2)](image)
copper(II) complex, which is a well-known and very efficient chemical nuclease, we found that at a dose of 24 µM, complex \((C_1)\) was less active than \([\text{Cu}(\text{phen})]^{2+}\).

It appears that complex \((C_1)\) can destroy DNA in stages, forming the circular form at a concentration of 18 µM and then the linear form at a concentration of 24 µM. At the last concentration, the circular and linear forms coexist (lane 11), while at 18 µM the circular and the helicoidal form coexist (lane 10). Initially, the cleavage is realized at a single point of the DNA chain, leading to the circular form. A second cleavage then occurs at another point of the chain, leading to the linear form.

For complex \((C_2)\) (Figure 2(b)), the DNA cleavage starts at 6 µM, giving an appreciable amount of plasmid Form II (lane 10). Then, at 9 µM the nicked circular and linear forms appear (lane 11). At higher concentrations (lanes 12 and 13), smearing is observed. CuSO\(_4\), at the highest concentration, 15 µM, does not induce any DNA cleavage. Overall, the activity of complex \((C_2)\) is higher than that of the bis(o-phenanthroline) copper complex (compare line 10). As a consequence, \(\text{Cu}^2+\) can be defined as a strong nuclease agent in the presence of \(\text{H}_2\text{O}_2\) or ascorbic acid. When the concentrations of complexes \((C_1)\) and \((C_2)\) are increased, whilst keeping those of reducing agents and DNA pUC18 plasmid constant, the amount of SC (Form I) decreases whereas that of NC (Form II) increases. If we compare complex \((C_1)\), containing sulfonamide ligands, with complex \((C_2)\), which also incorporates 1,10-phen ligand, we can say that the introduction of the phenantroline ligand increases the nuclease activity of the resulting complex. Phenantroline also enhances the activity of the complex for complete destruction of the DNA molecule (smearing)

In the presence of an oxidizing agent like hydrogen peroxide, \(\text{Cu}^2+\) complexes with phenantroline ligand are known to initiate an oxidative attack on C-1 and C-4 of the 2-deoxyribose moiety, leading to the destruction of DNA [43]. The planar aromatic rings of the sulfonamide ligand may allow the complex molecules be intercalated between the DNA base pairs. This would be followed by the destruction of the nuclease activity, caused by the production of ROS in its close vicinity [44,45].

The involvement of ROS in the nuclease mechanism was determined by monitoring the quenching of DNA cleavage in the presence of certain ROS scavengers: DMSO, t-butyl alcohol, sodium azide, 2,2,6,6-tetramethyl-4-piperidone, distamycin, superoxide dismutase and neocuproine. We chose a concentration of 24 µM for \((C_1)\) and 12 µM for \((C_2)\). In view of the difference in nuclease activity for the two complexes. The assays were performed with \(\text{H}_2\text{O}_2\)/ascorbic acid (in 3.0-fold molar excess relative to the complex concentration) as an activating agent. The resulting electroferograms are presented in Figure 3.

In the presence of both DMSOand of t-butyl alcohol (lanes 4 and 5), we observed a decrease in degradation of the DNA. This can be explained by the presence of HO-radicals in the degradation of DNA, since these inhibitors act by quenching these free radicals.

The measurement of reducing power defines an important aspect of the antioxidant activity of the complexes. FRAP (mg TE/mL) for \((C_1)\) complex was found, 80±0.14 and for \((C_2)\) complex, 112±0.14, results that Fig. 3.Electroferogram in agarose gel of the pUC18 plasmid treated with the \((C_1)\) and \((C_2)\) complexes and various inhibiting agents.
confirm the superior antioxidant capacity of (C2) complex, fact demonstrated also by DPPH radical scavenging assay.

**In vitro SOD mimetic activity**

The SOD-like activities of (C1) and (C2) complexes were tested by an indirect method using the xanthine/xanthine oxidase system as the source of superoxide radicals. Complex (C1) was more potent than (C2); the IC50 values were found 0.115 μM (C1) and 0.223 μM (C2). These values are higher than that for native Cu2Zn2SOD (0.006 μM) (that means that the complexes are less active than native SOD) but similar to those of complexes with related sulfonamide ligands [18,37,52].

**In vivo SOD mimetic activity**

The in vivo SOD mimetic activity of (C1) and (C2) complexes was quantified by a method based on the protection against free radicals provided by the extracts to the yeast S. cerevisiae [23,26,52]. The SOD-mimetic activity of the complexes on cell growth with a Asod1 mutant treated with menadione or H2O2 had been evaluated. The oxidative stress was produced by two oxidative agents: menadione which toxicity is due to the superoxide radical production and H2O2 which toxicity is registered due to HO· radicals. It was been considered that the complex has a SOD-mimetic activity if a decrease of the diameter of the inhibition zone is registered versus the control zone. The efficacy was evaluated by comparison of the diameter of the inhibition area for the complex and control (Figure 4).

![Complex C1 and Complex C2](image)

**Table 1**

<table>
<thead>
<tr>
<th>Complex (μM)</th>
<th>Diameter of the inhibition area (cm)</th>
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<tbody>
<tr>
<td>Control (Menadione 3 mM; H2O2 17%)</td>
<td>8</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>50</td>
<td>5.6</td>
</tr>
<tr>
<td>70</td>
<td>5.6</td>
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</table>

The protection of the complexes against free radicals generated by H2O2 is lower than in the case of free radicals generated by menadione. (C1) complex produce a reduction of the inhibition diameter about 18.75-23.75% while (C2) complex between 30-33.75%. Nor in this case the protective action produced by the complexes does not depend on complexes concentration.

Both complexes are able to protect efficiently against superoxide anions, but (C2) complex register a higher SOD-mimetic activity compared with (C1) complex. They could be considered as promising effective agents against toxicity of superoxide anion, improving significantly the growth of Asod1 strain. They supply the Cu2Zn2SOD deficiency of the mutant. These levels of protection are superior to those obtained for other complexes of Cu (II) with sulfonamide ligand cited in the literature [22,53,54]. For this reason both complexes are potential therapeutic agents in the prevention and treatment of diseases mediated by free radicals.

**Cytotoxicities of the complexes**

(C1) and (C2) complexes were examined for their antiproliferative properties. The in vitro cytotoxicity of (C1) and (C2) copper complexes were tested on two human carcinoma cells, a cervical line (HeLa) and a melanoma cell line (WM35); as well as on human normal fibroblastic epithelial cell line (HDFa), using Cisplatin as positive control. The response was quantified using 3-(4,5-dimethyl-2-thiazoloyl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. The cells were exposed to various concentrations of each complex (ranging between 0.2 and 50μM) at 24, 48 and 72 hours.

The results revealed a promising profile, the complexes expressing different levels of cytotoxicity. On both tumor cell lines: HeLa and WM35, complex (C1) was more potent as compared to (C2) and more potent than Cisplatin, the IC50 values being lower than those of Cisplatin. (C1) complex evoked also cytotoxicity, but a weaker one. No significant differences were found between IC50 values obtained for the two complexes at 24, 48 and 72 hours (Table 2).

The presence of phenanthroline in (C1) complex clearly increases the activity of the compound toward DNA cleavage. Due to the greater hydrophobic character of phenanthroline, the binding of (C1) complex to DNA is expected to be stabilized through greater hydrophobic interactions, which may contribute to its higher toxicity [18].

On human normal fibroblast (HDFa line), (C1) complex showed a toxicity comparable with Cisplatin, but (C2) complex showed a lower toxicity than Cisplatin (Table 2).

In this study we demonstrated that (C2) complex has a superior antitumor activity on both HeLa and WM35 human cell lines comparative with Cisplatin. In addition complex (C2) is less toxic on human HDFa cells compared to Cisplatin.
Another (C2) and (C1) complexes were found active against the four microorganisms, the MIC values are given in Table 3.

The results reveal that both complexes have similar antibacterial activity against *S. aureus*, *E. coli* and *P. aeroginosa*, but less than Norfloxacin. Against *B. cereus* both complexes have shown a better antibacterial activity and superior to Norfloxacin activity.

The antibacterial activity may be due to the following mechanisms: inhibition of ribonucleoside diphosphate reductase enzyme which helps in DNA synthesis; by oxidative rupture, creation of lesions in DNA strand or by binding to the nitrogen base of DNA or RNA results in inhibit base replication [55].

Conclusions

Two new Copper (II) complexes with N-substituted sulfonylamide were synthesized. The molecular structures for the complexes were attributed using the data obtained from elemental analysis, spectral (IR, UV-Vis, EPR) and magnetic determinations. The complexes have a superior nuclease activity as compared to the non-coordinated Cu(II) ion. The use of scavengers of ROS indicates that the hydroxyl and the superoxide anions are the main radicals that break the DNA strands. Both complexes have antioxidant, SOD mimetic and antibacterial activities. The complexes have nuclease activity as compared to the non-coordinated Cu(II) ion. Both complexes have shown a better antibacterial activity and superior to Norfloxacin activity.

The antibacterial activity may be due to the following mechanisms: inhibition of ribonucleoside diphosphate reductase enzyme which helps in DNA synthesis; by oxidative rupture, creation of lesions in DNA strand or by binding to the nitrogen base of DNA or RNA results in inhibit base replication [55].

Acknowledgement

This research was funded by the Ministry of Research and Innovation through Program 1 - Development of the National Research and Development System, Subprogram 1.2 - Institutional Performance - Projects for Financing the Excellence in CDI, Contract no. 37PFE/06.11.2018 as part of the project Increasing the institutional performance through consolidation and development of research directions within the USAMVCN.

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Manuscript received: 14.05.2019