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Oxidative DNA damage of mixed copper(II) complexes with sulfonamides and 1,10-phenanthroline
Crystal structure of [Cu(*N*-quinolin-8-yl-*p*-toluenesulfonamidate)$_2$(1,10-phenanthroline)]

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Abstract

Mixed coordination compounds of Cu(II) with sulfonamides and 1,10-phenanthroline as ligands have been prepared and characterised. Single crystal structural determination of the complex [Cu(*N*-quinolin-8-yl-*p*-toluenesulfonamidate)$_2$(phen)] shows Cu(II) ions are located in a highly distorted octahedral environment, probably as a consequence of the Jahn–Teller effect. The FT-IR and electronic paramagnetic resonance (EPR) spectra are also discussed. The mixed complexes prepared undergo an extensive DNA cleavage in the presence of ascorbate and hydrogen peroxide. Two of the complexes have higher nucleolytic efficiency than the bis(*o*-phenanthroline)copper(II) complex.

Keywords: Copper(II) complexes; Sulfonamides; 1,10-Phenanthroline; Nuclease activity

1. Introduction

The ability of many metal ions and coordination compounds to form highly reactive free radicals is well known. Because of this property, many research teams have used these compounds to split the chain of nucleic acids in predetermined sites of the chain [1–8].

One of the metals most widely used for this aim has been copper; many complexes of this metal with different types of ligands have been tested as potential chemical nucleases [3,9,10], and, among them, those of 1,10-phenanthroline have shown the most encouraging results, probably because of their ability to intercalate between the bases of DNA [3,5,11,12].

We have reported in previous papers on the synthesis and characterisation of Cu(II) complexes with different sulfonamides containing aromatic rings: *N*-quinolin-8-yl-*p*-toluenesulfonamide (Hqtsa), *N*-quinolin-8-yl-benzenesulfonamide (Hqbsa) and *N*-quinolin-8-yl-naphthalenesulfonamide (Hqnsa), in order to be tested as potential chemical nucleases [13,14]. The studies carried out so far with plasmid pUC18 in the presence of hydrogen peroxide did not report outstanding activities for these complexes, probably because of their stability against redox reactions, or, alternatively, because the spatial orientation of the sulfonamide ligands is not adequate enough to allow intercalation between the DNA bases.

For this reason, and bearing in mind the known ability of 1,10-phenanthroline to be intercalated between the DNA chains, here we report the preparation of new mixed complexes with this ligand and the above mentioned sulfonamides, and the nuclease activity has also been studied.

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2. Experimental

2.1. Materials and methods

8-Aminoquinoline and sulfonyl chlorides were provided by Fluka and 1,10-phenanthroline from Aldrich. All reagents used were of analytical grade.

Chemical analyses for carbon, hydrogen, and nitrogen were performed on a 2400 elemental analyser from Perkin-Elmer. Copper was determined on an inductively coupled plasma (ICP) spectrometer (Perkin-Elmer model 2380 Plasma 2). FT-IR spectra were recorded using KBr mulls and a Perkin-Elmer FT-IR 1730 instrument. Electron paramagnetic resonance spectra were recorded at X-band frequencies with a Bruker ER 200D.

2.2. Synthesis of the compounds

Preparation of the complexes with sulfonamide ligands, $[Cu(qbsa)_2]$, $[Cu(qtsa)_2]$, $[Cu(qnsa)_2]$ has been reported elsewhere [13,14]. To prepare the mixed complexes, the above complexes were dissolved in DMF (*N*,*N*-dimethylformamide) and, while being continuously stirred, 1,10-phenanthroline was added (1:10 molar ratio). For the (qbsa) and (qtsa) derivates, orange crystals are formed after 1 week, while for the (qnsa) derivate a brown precipitate is formed, which cannot be crystallised by a further dissolution. Only the complex formed with (qbsa) was suitable for molecular structure determination by single crystal X-ray diffraction.

Analysis: $[Cu(qbsa)_2(phen)]$: calculated for $CuC_{42}H_{30}N_6O_4S_2$: C 62.3; H 3.7; N 10.4; Cu 7.9. Found: C 61.9; H 3.9; N 10.6; Cu 8.1. IR ν_{max} (cm^{-1}): 1318 and 1128 ν_{asym} and ν_{sym}; 580 and 567 δ_{SO_2}; 948 ν_{S-N}; 691 ν_{C-S}.

$[Cu(qtsa)_2(phen)]$: calculated for $CuC_{44}H_{34}N_6O_4S_2$: C 63.0; H 4.1; N 10.0; Cu 7.6. Found: C 62.8; H 4.3; N 10.0; Cu 7.5. IR ν_{max} (cm^{-1}): 1314 and 1139 ν_{asym} and ν_{sym}; 573 and 551 δ_{SO_2}; 936 ν_{S-N}; 657 ν_{C-S}.

$[Cu(qnsa)_2(phen)]$: calculated for $CuC_{50}H_{34}N_6O_4S_2$: C 66.0; H 3.8; N 9.2; Cu 7.0. Found: C 65.8; H 3.5; N 9.2; Cu 7.1. IR ν_{max} (cm^{-1}): 1317 and 1123 ν_{asym} and ν_{sym}; 567 and 552 δ_{SO_2}; 959 ν_{S-N}; 662 ν_{C-S}.

2.3. X-ray crystallography of $[Cu(qtsa)_2(phen)]$

2.3.1. X-ray data collection and reduction

An orange prismatic crystal of $[Cu(L)_2(phen)]$ was mounted on a glass fiber and used for data collection. Cell constants and an orientation matrix for data collection were obtained by least-squares refinement of the diffraction data from 25 reflections in the range of $17.243<\theta<28.682$ deg. on an Enraf Nonius CAD4 automatic diffractometer [15]. Data were collected at 213 K using Cu Kα radiation (λ=1.54184 Å) and the ω-scan technique, and corrected for Lorentz and polarization effects [16]. A semi-empirical absorption correction (ψ-scans) was made [17].

2.3.2. Structure solution and refinement

The structure was solved by direct methods [18] and subsequent difference Fourier maps, and refined on F^2 by a full-matrix least-squares procedure using anisotropic displacement parameters [19]. The H atoms were included in geometrically idealized positions employing appropriate riding models with isotropic displacement parameters constrained to 1.2U~(eq) of their carrier atoms. Atomic scattering factors were from the 'International Tables for X-ray Crystallography' [20]. Molecular graphics were from PLATON [21]. A summary of the crystal data, experimental details and refinement results is listed in Table 1.

2.4. Cleavage of pUC18 by copper complexes

A typical reaction was carried out by mixing 7 μl of cacodylate buffer, 6 μl of the Cu(II) complex $[Cu(qbsa)_2(phen)]$, 10 μM in cacodylate buffer, 1 μl of 0.25 μg/μl pUCI8, 3 μl of ascorbate and 3 μl of H_2O_2 2.5-fold molar excess relative to the concentration of the complex in cacodylate buffer (pH 6.0). The resulting solution contains 3 μM of the complex, 0.012 μg/μl pUCI8 (3.5 μM in nucleotides) and 7.5 μM of H_2O_2/ascorbate. After allowing the sample to incubate at 25 °C for different times, 3 μl of a quench buffer solution consisting of 0.25% bromophenol blue, 0.25% xylene cyanole and 30% glycerol was added. Then the solution was subjected to electrophoresis on a 0.8% agarose gel in 1× TBE buffer (0.045 M tris, 0.045 M boric acid and 1 mM edta) at 80 V for about 2 h. The gel was stained with 10 μg/ml ethidium bromide and photographed on a capturing system gel printer plus TDI. A similar process was carried out with different concentration for the complex $[Cu(qtsa)_2(phen)]$.

A typical reaction for complex $[Cu(qnsa)_2(phen)]$ was undertaken by mixing 7 μM of cacodylate buffer, 6 μl of complex 50 μM in DMF (due to its insolubility in cacodylate buffer), 1 μl of 0.25 μg/μl pUC18, 3 μl of ascorbate and 3 μl of H_2O_2 2.5-fold molar excess relative to the concentration of the complex in cacodylate buffer (pH 6). The resulting solution contains 15 μM of complex, 0.012 μg/μl pUC18 and 37.5 μM of H_2O_2/ascorbate. After allowing the sample to incubate at 25 °C for different times, 3 μl of a quench buffer solution consisting of 0.25% bromophenol blue, 0.25% xylene cyanole and 30% glycerol was added. Then the solution was subjected to electrophoresis on a 0.8% agarose gel in 1× TBE buffer (0.045 M tris, 0.045 M boric acid and 1 mM edta) at 80 V for about 2 h. The gel was stained with 10 μg/ml ethidium bromide and photographed on a capturing system gel printer plus TDI.

Table 1
Crystal data and structure refinement for $[Cu(qtsa)_2(phen)]$

Empirical formula	$C_{44}H_{34}CuN_6O_4S_2$	
Formula weight	838.43	
Temperature	213(2) K	
Wavelength	1.54184 Å	
Crystal system	Triclinic	
Space group	*P*-1 (No. 2)	
Unit cell dimensions		
	$a = 13.4239(16)$ Å	$\alpha = 63.052(11)°$
	$b = 13.515(3)$ Å	$\beta = 82.655(11)°$
	$c = 14.045(2)$ Å	$\gamma = 68.923(11)°$
Volume	2117.6(6) $Å^3$	
Z	2	
Calculated density	1.315 Mg/m^3	
Absorption coefficient	2.044 mm^{-1}	
F(000)	866	
Crystal size	0.30×0.25×0.20 mm	
θ range for data collection	5.10 to 64.96°	
Limiting indices	$-1 < h < 15$	
	$-15 < k < 15$	
	$-16 < l < 16$	
Reflections collected/unique	8212/7194 [R(int) = 0.0424]	
Completeness to $\theta = 64.96$	99.8%	
Max. and min. transmission	0.969 and 0.812	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	7194/0/514	
Goodness-of-fit on F^2	1.079	
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0546$, $\omega R_2 = 0.1687$	
R indices (all data)	$R_1 = 0.0829$, $\omega R_2 = 0.1796$	
Largest diff. peak and hole	0.573 and −0.575 $e.A^{-3}$	

3. Results and discussion

3.1. Description of the crystal structure of the $[Cu(qtsa)_2(phen)]$ complex

An ORTEP drawing of the complex $[Cu(qtsa)_2(phen)]$ including the atomic numbering scheme is shown in Fig. 1. Selected bond distances and angles are presented in Table 2.

All three ligands act as bidentate, forming five-member rings with the metal cation. The local environment of Cu(II) cations corresponds to a distorted octahedron, and

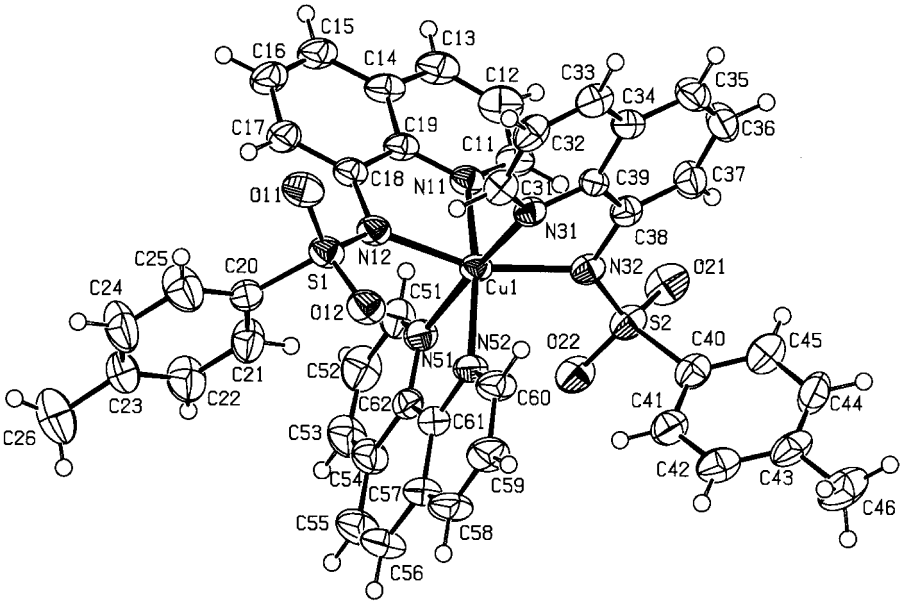

Fig. 1. Molecular structure of $[Cu(qtsa)_2(phen)]$ showing the atom numbering scheme.

Table 2
Selected bond lengths (Å) and angles (°) for [Cu(qtsa)$_2$(phen)]

Cu(1)–N(52)	1.997(3)
Cu(1)–N(11)	2.001(3)
Cu(1)–N(32)	2.071(4)
Cu(1)–N(12)	2.102(4)
Cu(1)–N(51)	2.342(3)
Cu(1)–N(31)	2.468(3)
N(52)–Cu(1)–N(11)	163.94(13)
N(52)–Cu(1)–N(32)	94.31(14)
N(11)–Cu(1)–N(32)	92.15(14)
N(52)–Cu(1)–N(12)	98.40(14)
N(11)–Cu(1)–N(12)	80.36(14)
N(32)–Cu(1)–N(12)	158.17(13)
N(52)–Cu(1)–N(51)	76.71(13)
N(11)–Cu(1)–N(51)	87.26(13)
N(32)–Cu(1)–N(51)	110.73(13)
N(12)–Cu(1)–N(51)	89.52(13)
N(52)–Cu(1)–N(31)	95.32(12)
N(11)–Cu(1)–N(31)	100.66(12)
N(32)–Cu(1)–N(31)	71.79(12)
N(12)–Cu(1)–N(31)	89.35(12)
N(51)–Cu(1)–N(31)	171.69(11)

can be considered as the result of adding the new ligand (phen) to the original structure of the [Cu(qtsa)$_2$] complex. Such an addition gives rise to important modifications in the bond lengths and bond angles in the new complex with respect to the starting one, in order to leave room enough to accommodate the new ligand in the coordination sphere of the Cu(II) cations. Two of the Cu–N distances, Cu–N(31) and Cu–N(51) are markedly larger than the other four; this difference can be related to a severe Jahn–Teller distortion, despite that the chelated rings usually tend to restrict such a distortion. This phenomenon has been previously reported for several Cu(II) complexes, such as that formed by this cation with 2,2′-bipyridyl and, hexafluoroacetylacetonate, [Cu(hfa)$_2$(bipy)], where two of the Cu–N bond lengths are 0.33 Å larger than the other four [22,23].

Incorporation of the 1,10-phenanthroline ligand also modifies the bond angles of the two already existing sulfonamide ligands, especially the N(31)–Cu(1)–N(32) angle, which becomes slightly closed from ca. 83° in the [Cu(qtsa)$_2$] complex [13] to ca. 72° in the mixed complex. The same origin would explain that among all nitrogen atoms in *trans* positions, the N(12)–Cu(1)–N(32) angle decreases to 158.17°, although in this case the repulsions between the sulfonyl groups might be also important.

3.2. FT-IR spectroscopy

Among the IR bands originated by the ligands, those due to the SO_2 group are the most informative. Bands due to the antisymmetric and symmetric vibration modes of the S=O bonds are recorded close to 1315 and 1130 cm^{-1}, ca. 20 cm^{-1} lower than those corresponding to the free ligands [13,14]; such a decrease can be related to the electron transfer from the deprotonated, negatively charged N atom to the sulfonyl oxygen atoms, which results in partial single-bond character for the S–O bonds [24]. Such electron transfer increases the bond order of the S–N moiety, thus accounting for the 25 cm^{-1} shift towards higher wavenumbers of the band due to the stretching mode of this bond. The lack of bands close to 3200 cm^{-1}, which were originally present in the spectra of the free ligands confirm deprotonation of the N–H bonds.

3.3. EPR spectra

Polycrystalline EPR spectra of the three complexes at room temperature are axial. In complex [Cu(qbsa)$_2$(phen)] the parallel region is resolved with hyperfine splitting from copper, as is shown in Fig. 2. The EPR parameters calculated by simulation [25] are $g_{\parallel}=2.275$, $g_{\perp}=2.075$ and $A_{\parallel}=144\times10^{-4}$ cm^{-1} for complex [Cu(qbsa)$_2$(phen)], $g_{\parallel}=$

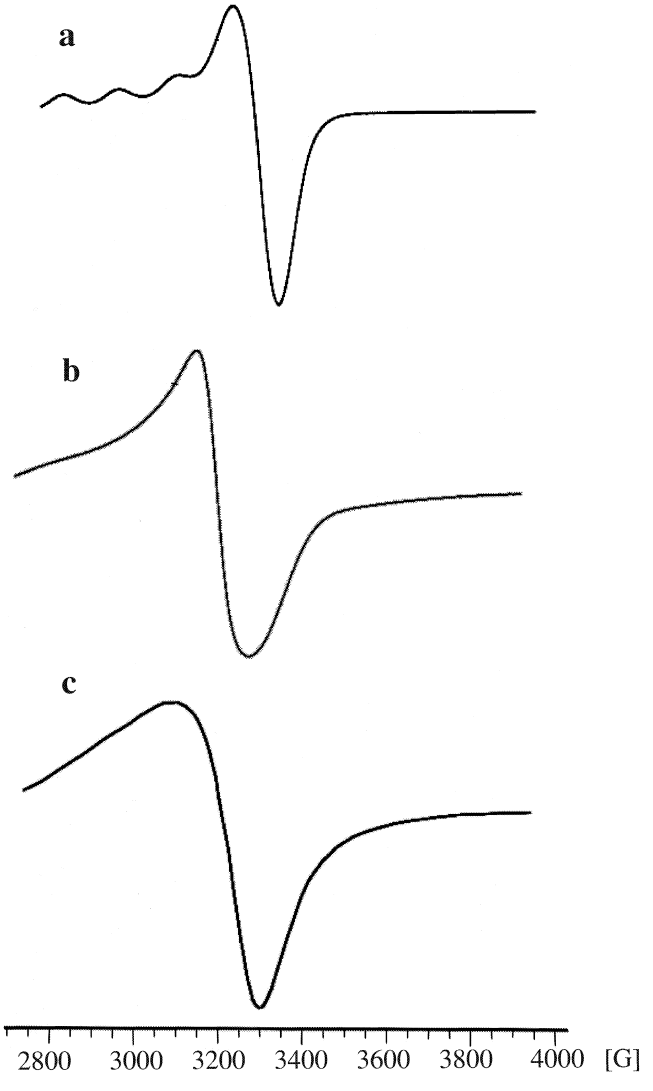

Fig. 2. Polycrystalline EPR spectrum of (a) [Cu(qbsa)$_2$(phen)], (b) [Cu(qtsa)$_2$(phen)] and (c) [Cu(qnsa)$_2$(phen)].

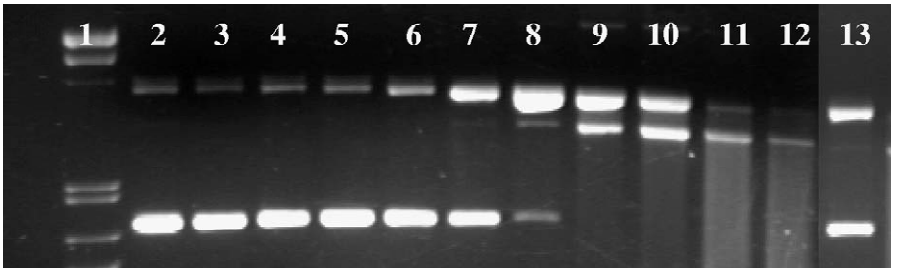

Fig. 3. Agarose gel electrophoresis of pUC18 plasmid DNA treated with 3 μM complex [Cu(qbsa)$_2$(phen)] or $CuSO_4 \cdot 5H_2O$ in the presence of the ascorbate/H_2O_2 tandem 2.5× for different incubation times at 37 °C. 1, Marker; 2, control DNA T_{inc} = 100 min; 3, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 20 min; 4, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 40 min; 5, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 60 min; 6, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 80 min; 7, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 100 min; 8, complex 3 μM T_{inc} = 20 min; 9, complex 3 μM T_{inc} = 40 min; 10, complex 3 μM T_{inc} = 60 min; 11, complex 3 μM T_{inc} = 80 min; 12, complex 3 μM T_{inc} = 100 min; 13, $CuSO_4 \cdot 5H_2O$ + phenanthroline 3 μM T_{inc} = 80 min.

2.26 and $g_\perp = 2.09$ for complex [Cu(qtsa)$_2$(phen)] and $g_\parallel = 2.27$ and $g_\perp = 2.10$ for complex [Cu(qnsa)$_2$(phen)]. As $g_\parallel > g_\perp$ in the complexes, the unpaired electron must be in the $d_{x^2-y^2}$ (or d_{xy}) orbital. The EPR parameters of the three complexes are very similar suggesting that the coordination polyhedron around copper(II) in these complexes are also equivalent.

4. Nuclease activity of the complexes

The ability of the title complexes to perform DNA cleavage has been studied by gel electrophoresis using supercoiled pUC18 DNA in cacodylate buffer (pH 6.0). The three complexes on reaction with DNA in the presence of a H_2O_2/ascorbate mixture as reducing agent present high nuclease activity (Figs. 3–5). In all the figures the nucleolytic activity of the complexes is compared with that of the copper(II) salt at the same concentrations of copper(II), reducing agents and electrophoretic times. From Fig. 3 we can observe that at 20 min of reaction time, the DNA supercoiled (form I) practically disappears while specially nicked (form II) and linear (form III) appreciate, however the same concentration of copper(II)

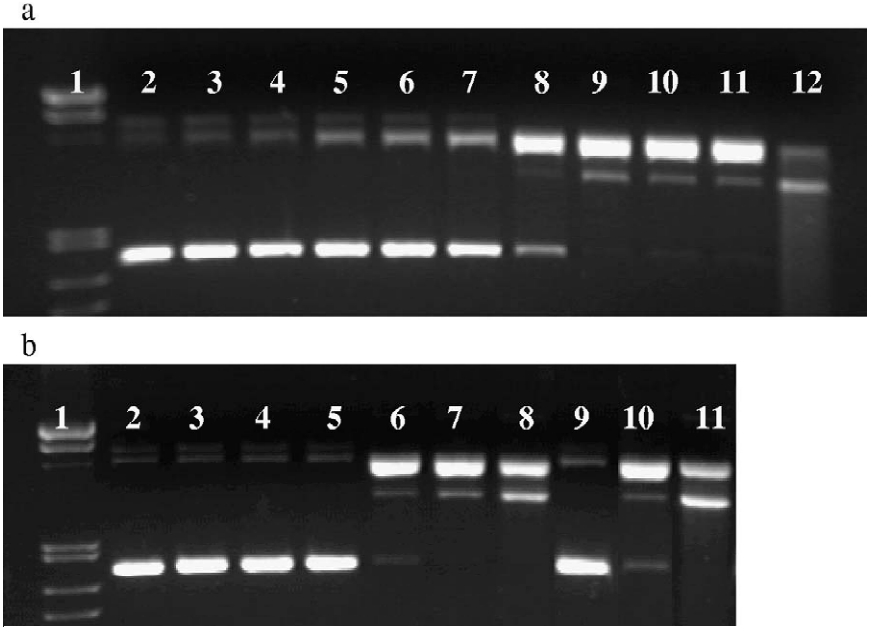

Fig. 4. (a) Agarose gel electrophoresis of pUC18 plasmid DNA treated with 3 μM complex [Cu(qtsa)$_2$(phen)] or $CuSO_4 \cdot 5H_2O$ in the presence of the ascorbate/H_2O_2 tandem 2.5× for different incubation times at 37 °C. 1, Marker; 2, control DNA T_{inc} = 100 min; 3, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 20 min; 4, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 40 min; 5, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 60 min; 6, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 80 min; 7, $CuSO_4 \cdot 5H_2O$ 3 μM T_{inc} = 100 min; 8, complex 3 μM T_{inc} = 20 min; 9, complex 3 μM T_{inc} = 40 min; 10, complex 3 μM T_{inc} = 60 min; 11, complex 3 μM T_{inc} = 80 min; 12, complex 3 μM T_{inc} = 100 min. (b) Agarose gel electrophoresis of pUC18 plasmid DNA treated with different concentrations of complex [Cu(qtsa)$_2$(phen)] or $CuSO_4 \cdot 5H_2O$ in the presence of the ascorbate/H_2O_2 tandem 1× for 10 or 20 min of incubation time at 37 °C. T_{inc} = 10 min; 1, marker; 2, control DNA; 3, $CuSO_4 \cdot 5H_2O$ 6 μM; 4, $CuSO_4 \cdot 5H_2O$ 12 μM; 5, $CuSO_4 \cdot 5H_2O$ 15 μM; 6, complex 6 μM; 7, complex 12 μM; 8, complex 15 μM; T_{inc} = 20 min; 9, $CuSO_4 \cdot 5H_2O$ 15 μM; 10, $CuSO_4 \cdot 5H_2O$ + phenanthroline 15 μM; 11, complex 15 μM;.

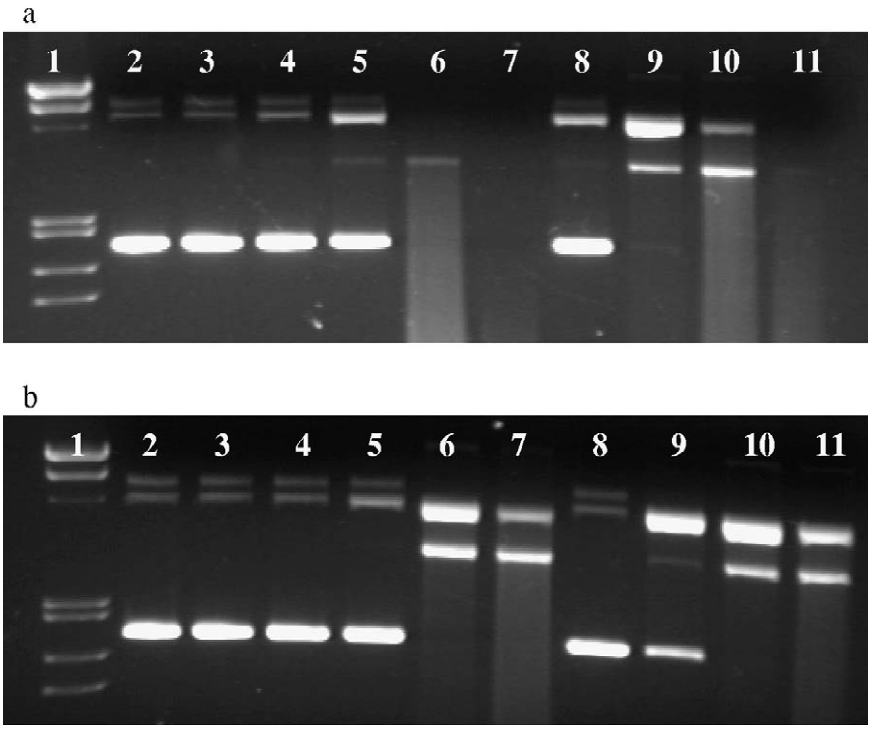

Fig. 5. Agarose gel electrophoresis of pUC18 plasmid DNA treated with different concentrations of complex $[Cu(qnsa)_2(phen)]$ or $CuCl_2 \cdot 2H_2O$ in the presence of the ascorbate/H_2O_2 tandem 10× (a) or 2.5× (b) for 60 min of incubation time at room temperature. 1, Marker; 2, control DNA; 3, $CuCl_2 \cdot 2H_2O$ 15 μM; 4, $CuCl_2 \cdot 2H_2O$ 30 μM; 5, $CuCl_2 \cdot 2H_2O$ 45 μM; 6, $CuCl_2 \cdot 2H_2O$ 60 μM; 7, $CuCl_2 \cdot 2H_2O$ + phenanthroline 60 μM; 8, complex 15 μM; 9, complex 30 μM; 10, complex 45 μM; 11, complex 60 μM.

sulphate only presents DNA supercoiled (see lanes 8 and 3). The comparison between lanes 5 and 10 clearly shows the higher nuclease activity of the complex $[Cu(qbsa)_2(phen)]$ with respect to copper(II). The same conclusions can be deduced from lanes 5 and 10 of Fig. 4a for the complex $[Cu(qtsa)_2(phen)]$ or lanes 5 and 10 of Fig. 5a for the complex $[Cu(qnsa)_2(phen)]$. These results permit us to deduce that the complexes have more potent nuclease activity efficiency than the free copper(II) and this property is due to the nature of the complexes and not the presence of copper(II).

The DNA strand scission chemistry of the complexes $[Cu(qbsa)_2(phen)]$ and $[Cu(qtsa)_2(phen)]$ has been studied with the reaction time. In both cases an increase of reaction time produced an increase of the DNA cleavage (see lanes 8–12 of Figs. 3 and 4a). Besides, it can be deduced that cleavage takes place with formation of the nicked form and then the double strand takes place. If we compare Figs. 3 and 4a we can deduce that the complex $[Cu(qbsa)_2(phen)]$ has more nuclease efficiency than the complex $[Cu(qtsa)_2(phen)]$ (for instance, see lanes 10 of both figures).

The influence of the concentration of the nucleolytic agent can be observed in lanes 6, 7 and 8 of Fig. 4b and in lanes 8, 9, 10 and 11 of Fig. 5a. An increase of complex concentration gives rise to an increase in the DNA cleavage.

As the title complexes are mixed complexes with phenanthroline, we have compared the nucleolytic activity of the title complexes with the bis(*o*-phenanthroline)copper complex. Lane 11 of Fig. 3 of the complex $[Cu(qbsa)_2(phen)]$ and lane 11 of Fig. 4b of the complex $[Cu(qtsa)_2(phen)]$ compared with lane 13 of Fig. 3 and lane 10 of Fig. 4b, clearly indicates that these complexes are more potent than the bis(*o*-phenanthroline)copper complex, especially the complex $[Cu(qbsa)_2(phen)]$. However, complex $[Cu(qnsa)_2(phen)]$ has similar nuclease activity to the bis(*o*-phenanthroline)copper complex (see lanes 7 and 11 of Fig. 5).

These results show that the complexes $[Cu(qbsa)_2(phen)]$ and $[Cu(qtsa)_2(phen)]$ present considerable more nuclease activity than the copper–phenanthroline complex.

4.1. Investigation of the active species

Copper, in the presence of oxygen donor species, is thought to be able to form different oxidative intermediates, depending on the specific complex and conditions. A nondiffusable copper-oxene intermediate has been invoked in some cleavage reactions [26] while in others, Fenton-type chemistry, which involves release of diffusible hydroxyl radicals, has been suggested [27]. In order to study the active species, standard scavengers of reactive oxygen intermediates were included in the electrophoretic studies (Fig. 6a–c).

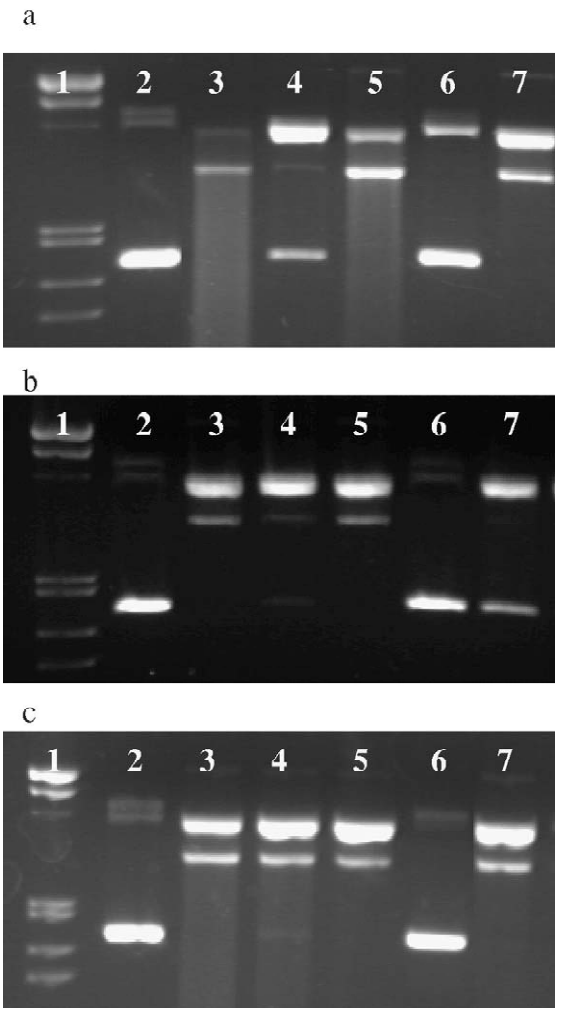

Fig. 6. Agarose gel electrophoresis of pUC18 plasmid DNA treated with the complexes with and without different oxidation inhibitors. (a) Complex [Cu(qbsa)$_2$(phen)] 3 μM, T=37 °C, H_2O_2/ascorbate 2.5×, T_{inc}=80 min, (b) complex [Cu(qtsa)$_2$(phen)] 15 μM, T=37 °C, H_2O_2/ascorbate 1×, T_{inc}=10 min, (c) complex [Cu(qnsa)$_2$(phen)] 60 μM, T=room temperature, H_2O_2/ascorbate 2.5×, T_{inc}=60 min. 1, Marker; 2, control DNA; 3, without inhibitor; 4, SOD (15 units); 5, NaN_3 (20 mM); 6, EDTA (20 mM); 7, DMSO (1 M).

One of the most interesting electrophoretic results of the three complexes takes place when the DNA cleavage experiment is done in the presence of the SOD enzyme in order to observe if the ion superoxide is one of the reactive species. Lanes 4 of Fig. 6a and b show a reduction of the DNA cleavage of the complex [Cu(qbsa)$_2$(phen)] and [Cu(qtsa)$_2$(phen)], suggesting the O_2^- is one of the reactive species that breaks the DNA. However lane 4 of Fig. 6c shows that SOD (superoxide dismutase) has a slight influence in the DNA cleavage of the complex [Cu(qnsa)$_2$(phen)].

Azide (lanes 5 of Fig. 6a–c) inhibits, to a little extent, DNA cleavage by the compound [Cu(qbsa)$_2$(phen)], indicating that probably $^1O_2$ is involved in the reaction. However since the azide ion can act as a ligand to copper(II), inhibition of the nuclease reaction may occur not by radical quenching, but by blocking of the coordination site on copper that is involved in radical formation.

The hydroxyl radical scavenger, dmso (lanes 7 of Fig. 6a–c), diminishes significantly the nuclease activity of the compound which is indicative of the involvement of the hydroxyl radical in the cleavage process.

When the electrophoretic processes take place in the presence of EDTA (ethylenediaminetetraacetic acid) in large excess (20 mM) the nuclease activity of the complexes [Cu(qtsa)$_2$(phen)] and [Cu(qnsa)$_2$(phen)] is totally reduced (see lanes 6 of Fig. 6b and c, respectively). However, in these conditions the complex [Cu(qbsa)$_2$(phen)] presents slight activity because we can appreciate nicked and supercoiled DNA (see lane 6 of Fig. 6a). These results are according to the formation of copper–EDTA complex which does not act as a nuclease agent because this complex does not react with hydrogen peroxide and as a consequence there is no formation of copper(I) [28].

In conclusion, the active oxygen species involved in the reaction are $^{\bullet}OH$ and O_2^-. The presence of these reactive oxygen species (ROS) exclude the formation of a copper-oxene such as proposed by Sigman in bis(*o*-phenanthroline)copper(II) [11]. According to the presence of hydroxyl and superoxide anion, we propose Fenton-type reactions

$$Cu^{II}(L) + e^- \rightarrow Cu^{I}(L)$$

$$Cu^{I}(L) + H_2O_2 \rightarrow Cu^{II}(L) + OH^- + OH^{\bullet}$$

or through the Haber–Weiss reaction [29]

$$Cu^{I}(L) + O_2 \leftrightarrows Cu^{II}(L) + O_2^-$$

$$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + OH^{\bullet}$$

The mechanism of the DNA strand scission of bis-(phenanthroline)copper(II) indicated that once the copper(II) complex is reduced by some reducing agent such as mercaptopropionic acid or ascorbate, and binds DNA, then one of the two phenanthrolines is dissociated and then hydrogen peroxide forms a copper-oxene reactive species that attacks the C1–H of the d-ribose.

We propose the following mechanism in the strand scission of DNA by the title complexes.

1. Reduction of copper(II) complex to copper(I) complex and dissociation of one of the sulfonamidate ligands.
2. Binding of the phenanthroline and sulfonamidate ligands to nucleic bases of DNA by stacking and/or hydrogen bonding interactions.
3. Fenton or Haber–Weiss reactions raising hydroxyl radical that breaks supercoiled strands in the nicked one and then in the linear one.

The higher DNA cleavage activity of the complex $[Cu(qbsa)_2(phen)]$ with respect to the complex $[Cu(qtsa)_2(phen)]$ can be due to the methyl group in the sulfonamidate ligand that can present a steric effect when the complex binds DNA prior to ROS participating in the scission reaction.

We also plan to continue to screen other copper(II) complexes in the hope of discovering other potentially practical inorganic complex nucleases.

5. Supplementary material

Atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates and isotropic thermal parameters, hydrogen bonds and observed and calculated structure factors are available from the authors upon request.

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