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Crystal structure of [Cu(*N*-quinolin-8-yl-*p*-toluenesulfonamidate)$_2$]: study of its interaction with DNA and hydrogen peroxide

Benigno Macías[a,*], María V. Villa[a], Emilio Fiz[a], Isabel García[a], Alfonso Castiñeiras[b], Marta Gonzalez-Alvarez[c], Joaquín Borrás[c]

[a]*Departamento de Química Inorgánica, Facultad de Farmacia, Universidad de Salamanca, Campus Unamuno, 37007 Salamanca, Spain*
[b]*Departamento de Química Inorgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain*
[c]*Departamento de Química Inorgánica, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain*

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Abstract

A new copper complex with *N*-quinolin-8-yl-*p*-toulenesulfonamide has been prepared and characterised. The compound crystallises in the triclinic system, space group *P1*, with $a=13.457(3)$, $b=15.067(5)$, $c=18.589(3)$ Å; $\alpha=112.05(2)$, $\beta=93.92(2)$, $\gamma=108.30(2)$ ° and $Z=4$. The geometry of the Cu(II) ion is distorted square planar. The *N*-quinolin-8-yl-*p*-toulenesulfonamidate anion behaves as a bidentate ligand through the $N_{sulfonamidate}$ and $N_{quinoline}$ atoms. The complex does not cleave DNA in the presence of hydrogen peroxide.

Keywords: Copper(II) complexes; Sulfonamide; Crystal structure; DNA interaction

1. Introduction

Copper, an essential element, has received considerable attention with regard to its presence in normal blood plasma and serum components. It has been established that copper-dependent enzymes are required for hemoglobin synthesis, growth, keratinisation, pigmentation, bone formation, reproduction and fertility. Reports abound in the literature on the active role of copper complexes in the control of inflammation.

The study of the interactions of nucleic acids with transition metal complexes has developed remarkably during the last decades, since it allows us to obtain information on the distribution of certain specific centres along the polymeric chain of nucleic acids, as well as to know how certain proteins that regulate the expression of the genetic information are able to select to very precisely particular centres of the chain [1,2]. These interactions can even give rise to splitting of the nucleic acid chains, which is carried out generally either by a red-ox reaction or by a hydrolysis reaction, so that the metallic complexes behave as nucleases [3]. One of the more widely studied complexes that has lead to positive results is $[Cu(phen)_2]^+$, which is able to break the DNA chain in the presence of H_2O_2 by a mechanism similar to that observed in the Fenton reaction [4,5]. The nuclease activity of other copper complexes with ligands containing aromatic rings has also been studied [6–10]. We report here the synthesis, characterisation, and properties of a new complex of Cu(II) with *N*-quinolin-8-yl-*p*-toluenesulfonamide. The cleavage reaction of DNA in the presence of the title compound and hydrogen peroxide shows a different behaviour when the reaction takes places in the presence of the Cu(II) chloride and hydrogen peroxide.

2. Experimental

2.1. Materials and methods

8-Aminoquinoline and toluene-4-sulfonyl chloride were provided by Fluka and 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 ICP spectrometer (Perkin-Elmer model 2380 Plasma 2).

FT-IR spectra were recorded using KBr mulls and a

*Corresponding author. Tel.: +34-923-294-524; fax: +34-923-294-515.

E-mail address: bmacias@gugu.usal.es (B. Macías).

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Perkin-Elmer FT-IR 1730 instrument. Electronic spectra were recorded on a Shimadzu UV-240 double beam spectrophotometer with a diffuse reflectance accessory and a Hewlett-Packard 8452A diode spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained in methanol on a Bruker DX400 instrument.

Magnetic susceptibility measurements at room temperature were taken with a fully automatized Aztec DSM8 pendulum-type susceptometer. Mercury tetrakis(thiocyanato)cobaltate(II) was used as a susceptibility standard. Corrections for diamagnetism were estimated from Pascal's constants. Electron paramagnetic resonance spectra were recorded at X-band frequencies with a Bruker ER 200D.

Molecular masses were measured by Servicio de Masas (Universidad Autónoma de Madrid, Spain) by the FAB method with samples held on a nitrobenzyl alcohol (NBA) matrix and L-SIMS ionisation mode, in a VG Autospec apparatus; the source was maintained at 30°C and 35 keV, and Cs^{+} ion were used.

2.2. Synthesis of the ligand

The ligand was prepared by reacting 8-aminoquinoline (0.721 g, 5 mmol) with toluene-4-sulfonyl chloride (0.953 g, 5 mmol) in a pyridine solution (10 ml). The mixture was refluxed at 110–120°C for 30 min, then the solution was cooled to 70°C and added to a beaker containing 10 ml of distilled water. A brown solid immediately appears which was filtered and washed with water until no pyridine was present in the filtrate (yield 88%).

2.2.1. Analysis

Calculated for $C_{16}H_{14}N_2O_2S$: C 64.4; H 4.7; N 9.4. Found: C 64.3; H 4.6; N 9.4%.

2.3. Synthesis of the complex

Fifty ml of a methanolic solution of copper(II) acetate (0.1996 g, 1 mmol) was slowly added with stirring to 100 ml of a methanolic solution containing 0.5965 g (2 mmol) of *N*-quinolin-8-yl-*p*-toluenesulfonamide. The solution turns dark brown and after 2–3 days brown crystals appear (yield 90%).

2.3.1. Analysis

Calculated for $CuC_{32}H_{26}N_4O_4S_2$: C 58.4; H 4.0; N 8.5; Cu 9.6. Found: C 58.1; H 4.0; N 8.2; Cu 9.8.

2.4. X-ray structure determination of $CuC_{32}H_{26}N_4O_4S_2$

A brown prismatic crystal of $[Cu(C_{16}H_{13}N_2O_2S)_2]$ 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 with $21.631<\theta<44.903$ using a Enraf Nonius CAD4 automatic diffractometer [11]. Data were collected at 293 K using Cu Kα radiation ($\lambda=1.54184$ Å) and the ω-scan technique, and corrected for Lorentz and polarization effects [12]. A semi-empirical absorption correction (ψ-scans) was made [13].

The structure was solved by direct methods [14] and subsequent difference Fourier maps, and refined on F2 by a full-matrix least-squares procedure using anisotropic displacement parameters [15]. All hydrogen atoms were located in their calculated positions (C–H, 0.93–0.97 Å) and were refined using a riding model. The contribution of the density of the disordered methanol solvate molecules was subtracted from the measured structure factors using SQUEEZE [16]. Subsequent refinement then converged with *R* factors and parameter errors significantly better than for all attempts to model the solvent disorder. Atomic scattering factors from International Tables for X-ray Crystallography [16]. Molecular graphics from PLATON99 [17]. A summary of the crystal data, experimental details, and refinement results is given in Table 1.

2.5. Cleavage of pUCI8 by the copper complex

The copper complex is insoluble in the different aqueous buffer solutions used in DNA cleavage processes. Conse-

Table 1
Crystal data and structure refinement for $[Cu(C_{16}H_{13}N_2O_2S)_2]$

Empirical formula	$CuC_{32}H_{26}N_4O_4S_2$
Formula weight	658.23
Temperature	293(2) K
Wavelength	1.54184 Å Cu Kα
Crystal system	Triclinic
Space group	*P1* (No. 2)
Crystal size	0.25×0.15×0.15 mm
Unit cell dimensions:	
$a=13.457$ (3)	$\alpha=112.05$ (2)°
$b=15.067$ (5) Å	$\beta=93.92$ (2)°
$c=18.589$ (3) Å	$\gamma=108.30$ (2)°
Volume	3241.3(14) $Å^3$
Z	4
Calculated density	1.349 Mg/m^3
θ range for data collection	2.62–75.88°
Reflections collected	13 913
Unique reflections	13 482
F(000)	1356
Absorption coefficient	2.494 mm^{-1}
Max. and min. transmission	0.983 and 0.924
Index ranges	$-16<h<16$, $-17<k<18$, $-23<l<0$
Final *R* indices [$I>2\sigma(I)$]:	
$R_1=0.0547$	$R_{w2}=0.1300$
R indices (all data):	
$R_1=0.1375$	$R_{w2}=0.1558$
Largest diff. peak and hole	0.256 and −0.419 e $Å^{-3}$

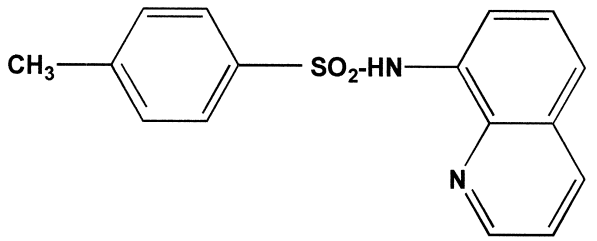


Fig. 1. *N*-Quinolin-8-yl-*p*-toluenesulfonamide.

quently we used a DMF solution following the method reported by Reddy and Reddy [18].

A typical reaction was carried out by mixing 3 μl of the Cu(II) complex 100 μM in DMF, 2 μl of 0.25 μg/μl pUCI8, and 3 μl of H_2O_2 10 mM. The resulting solution contains 15 μM of the complex, 0.025 μg/μl pUCI8, and 1.5 mM of H_2O_2. After allowing the sample to incubate at 25°C for 3 h, 3 μl of a quench buffer solution containing 0.25% bromophenol blue, 0.25% xylene cyanole, and 30% glycerol was added. Then the solution was subjected to electrophoresis on a 0.7% 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.

3. Results and discussion

The reaction of 8-aminoquinoline with toluene-4-sulfonyl chloride gives rise to the sulfonamide N-derivative shown in Fig. 1.

When a methanol solution of this ligand is added to a Cu(II) acetate solution, single crystals of the title compound, Cu[*N*-quinolin-8-yl-*p*-toluenesulfonamidate]$_2$, suitable for X-ray structure determination, appear.

3.1. Description of the crystal structure

The crystal structure contains two independent molecules shown in Fig. 2. The main difference between them is the orientation of the SO_2 groups. Selected bond distances and angles are given in Table 2.

Copper is four-coordinated with two nitrogen atoms from each of two quinolinesulfonamidate ligands. The Cu–O distances are larger than 2.9 Å, so there is no interaction between copper and oxygen atoms. The geometry around copper can be best described as distorted square planar. The $Cu–N_{sulfonamidate}$ bond lengths are 1.930 and 1.945 Å, slightly less than the $Cu–N_{quinoline}$ distances of 1.990 and 2.001 Å. Both are in the range observed for copper complexes with similar ligands [6,19]. As expected from steric considerations, the quinoline rings are *trans* with respect to the copper ion. Quinolinesulfonamidate acts as a bidentate ligand through the $N_{sulfonamidate}$ and the $N_{quinoline}$ atoms, forming a five-membered ring with the copper ion.

3.2. Mass spectrometry

Fig. 3 shows the mass spectrum of the complex. The signal at $m/z=659.6$ corresponds to the molecular ion of the complex. Weaker peaks appear at $m/z=360.1$, corresponding to the species resulting after the loss of one ligand from the original molecular ion, and at 503.1 and 347.1, which arise from the loss of one or two 4-sulfonyltoluene fragments, respectively.

3.3. FT-IR spectroscopy

The positions of the most significant bands are shown in Table 3. Note the two bands due to the anti-symmetric and symmetric vibrations of the SO_2 group [20,21]. These bands are clearly distinguished in the spectrum of the

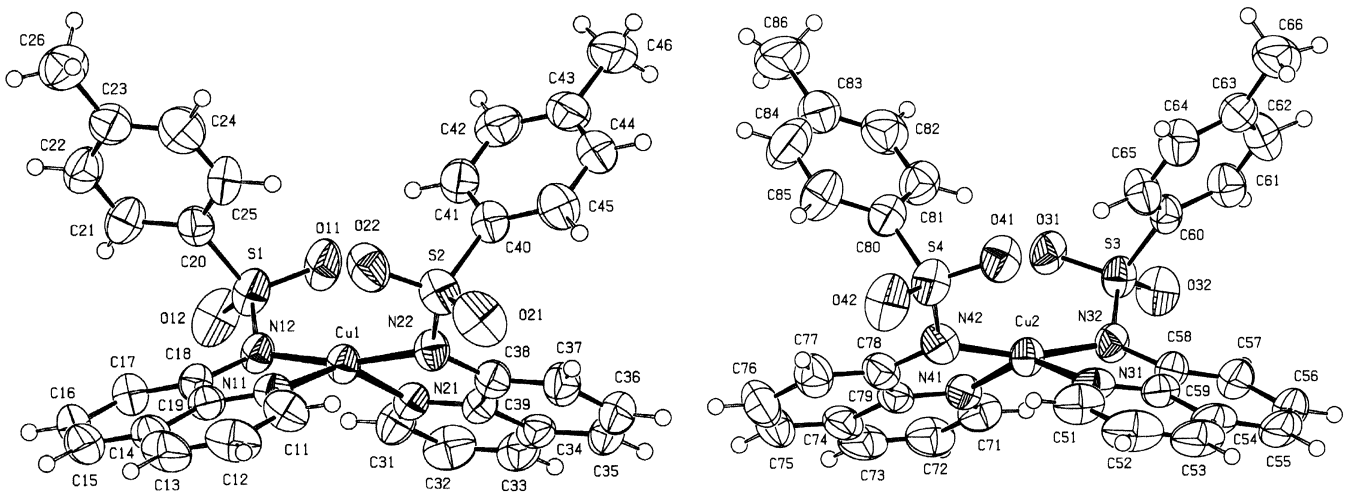


Fig. 2. ORTEP diagram for the Cu-complexes with the atom-labelling scheme.

Table 2
Selected bond lengths (Å) and angles (°)

Cu(1)–N(12)	1.930
Cu(1)–N(22)	1.945
Cu(1)–N(11)	1.990
Cu(1)–N(21)	2.001
Cu(1)–O(11)	3.018
Cu(1)–O(21)	4.229
Cu(2)–N(42)	1.929
Cu(2)–N(32)	1.936
Cu(2)–N(31)	1.995
Cu(2)–N(41)	2.001
Cu(2)–O(31)	2.972
Cu(2)–O(41)	3.026
N(12)–Cu(1)–N(22)	166.06
N(12)–Cu(1)–N(11)	82.93
N(22)–Cu(1)–N(11)	102.96
N(12)–Cu(1)–N(21)	100.30
N(22)–Cu(1)–N(21)	82.38
N(11)–Cu(1)–N(21)	144.35
N(12)–Cu(1)–O(11)	53.28
N(22)–Cu(1)–O(11)	115.10
N(11)–Cu(1)–O(11)	129.01
N(21)–Cu(1)–O(11)	76.07
N(12)–Cu(1)–O(21)	154.49
N(22)–Cu(1)–O(21)	20.94
N(11)–Cu(1)–O(21)	84.11
N(21)–Cu(1)–O(21)	103.03
O(11)–Cu(1)–O(21)	123.53
N(42)–Cu(2)–N(32)	164.17
N(42)–Cu(2)–N(31)	103.83
N(32)–Cu(2)–N(31)	82.38
N(42)–Cu(2)–N(41)	82.84
N(32)–Cu(2)–N(41)	100.42
N(31)–Cu(2)–N(41)	145.39
N(42)–Cu(2)–O(31)	112.25
N(32)–Cu(2)–O(31)	54.30
N(31)–Cu(2)–O(31)	127.64
N(41)–Cu(2)–O(31)	77.49
N(42)–Cu(2)–O(41)	53.17
N(32)–Cu(2)–O(41)	116.71
N(31)–Cu(2)–O(41)	73.89
N(41)–Cu(2)–O(41)	131.16
O(31)–Cu(2)–O(41)	98.64

complex, probably due to the different spatial disposition of this group, as shown in Fig. 2. Another important feature in the spectrum of the complex is that the band at 3260 cm^{-1} present in the spectrum of the ligand disappears, indicating deprotonation of the amino group bonded to the metal ion. Also, the S–N stretching frequency is shifted about 30 cm^{-1} towards higher wavenumbers in the complex, probably due to the deprotonation in the ligand of the N atom next to S.

3.4. NMR spectroscopy

Table 4 lists the signals of the ^{1}H and the ^{13}C spectra for the ligand [22].

3.5. Magnetic susceptibility, electronic spectra and EPR spectrum

The magnetic moment at room temperature is 1.75 BM, close to the spin only magnetic moment [23].

The electronic spectra of the complex in the solid state and in aqueous solution show a band at 560 nm (ε=80 M^{-1} cm^{-1}) attributed to a d–d transition, and a band at 460 nm (ε=1700 M^{-1} cm^{-1}) assigned to a metal-to-ligand charge transfer (MLCT) transition. Furthermore, two bands appear at 370 nm (ε=20 500 M^{-1} cm^{-1}) and at 270 nm (ε=40 000 M^{-1} cm^{-1}) due to ligand-to-ligand transitions. As the coordination polyhedron around the Cu(II) ion is square planar, we propose that the d–d band at 560 nm corresponds to a $B_{1g}\rightarrow A_{1g}$ transition, while the $B_{1g}\rightarrow B_{2g}$ and $B_{1g}\rightarrow E_{g}$ transitions must be overlapped by the intense MLCT band [24].

The polycrystalline powder EPR spectrum is shown in Fig. 4. It is an axial spectrum with EPR parameters $g_{\parallel}$=2.28, $g_{\perp}$=2.08 and $A_{\parallel}$=180 G. These values suggest a square planar geometry [25], in agreement with the crystal structure.

4. Cleavage of pUCI8 by copper(II) complex and copper(II) salt

An important difference can be concluded from the electrophoresis results for the title complex and the Cu(II) chloride (Fig. 5). The Cu(II) chloride at 200 mM gives the nicked form (II) of DNA, at 300 mM it gives the linear form (III), and at 400 and 500 mM the gel shows smearing, while the Cu(II) complex in the concentration range from 100 mM to 500 mM does not lead to any type of cleavage of DNA.

From the mechanism proposed by Sigman et al. [5] for the Cu(1,10-phenanthroline)$_2$ complex, the Cu(II) complex is first reduced to form the Cu(I)-1,10-phenanthroline complex, which then reacts with DNA to make a (1,10-phenanthroline)-Cu(I)–DNA complex. This complex then reacts with hydrogen peroxide to form a 'copper-oxene' radical that is the species responsible for the cleavage of the DNA.

Douglas [26] proposes another mechanism for the reaction of the Cu(1,10-phenanthroline)$_2$ complex in the presence of the reductant and dioxygen, that implies the formation of hydroxyl radical or other O_2-derived species that are responsible of the cleavage of DNA.

We suggest that the Cu(II) chloride in the presence of hydrogen peroxide gives rise to these radical species that cleave the DNA, as we can observe in lanes 3–7 of Fig. 5. The complex [Cu(*N*-quinolin-8-yl-*p*-toluenesulfonamidate)$_2$] behaves differently because there is no DNA cleavage at any concentration. The title compound does

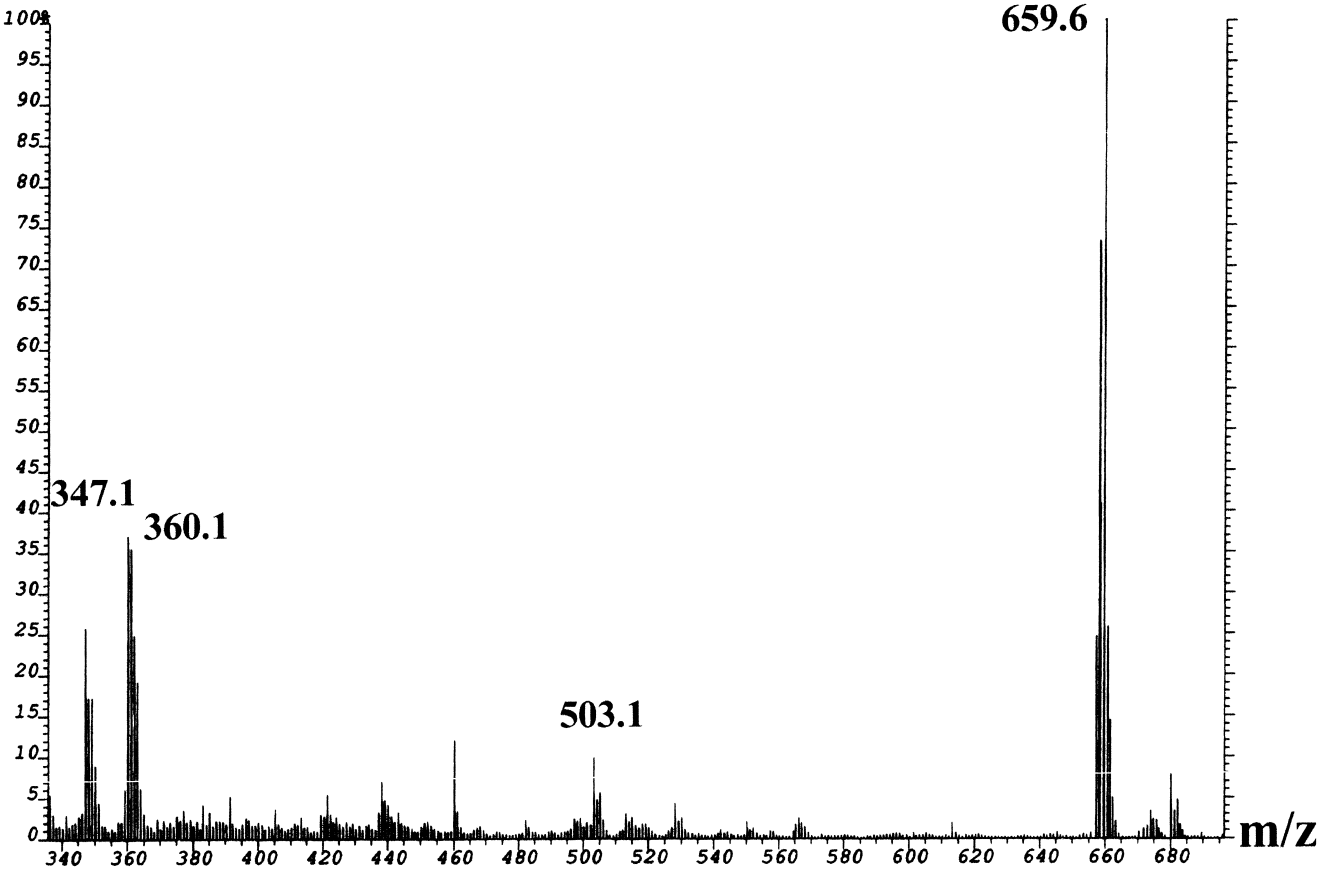


Fig. 3. FAB$^+$/MS spectra for the complex $CuC_{32}H_{26}N_4O_4S_2$.

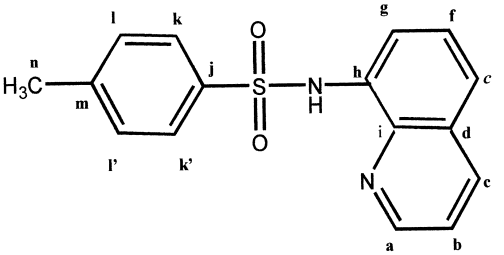
not have nuclease properties under the conditions of the experiment.

The lack of nuclease activity in the complex might be due to (a) the stability of the Cu(II) complex, which does not react with H_2O_2, so the adduct between a Cu(I) complex and DNA is not obtained; or (b) the possibility that once the Cu(II) complex reacts with H_2O_2, the Cu(I)–

Table 3
Selected IR bands (cm^{-1})

Compound	SO_2	N–H	S–N	C–S
Ligand	1307 ν_{as}			
	1160 ν_s	3260	922	666
	570			
	545			
Complex	1131 ν_{as}			
	1283 ν_{as}			
	1144 ν_s	–	955	665
	1117 ν_s			
	577			
	551			

Table 4
Chemical shifts (ppm) from ^1H and ^{13}C NMR spectra of *N*-quinolin-8-yl-*p*-toluenesulfonamide



	^1H	^{13}C
a	8.78	150.24
b	7.73	123.14
c	7.80	123.91
d	–	127.59
e	8.20	117.49
f	7.20	123.55
g	7.53	106.34
h	–	137.50
i	–	129.76
j	–	137.81
k,k′	7.47	130.40
l,l′	7.17	128.31
m	–	145.27
n	2.26	21.25

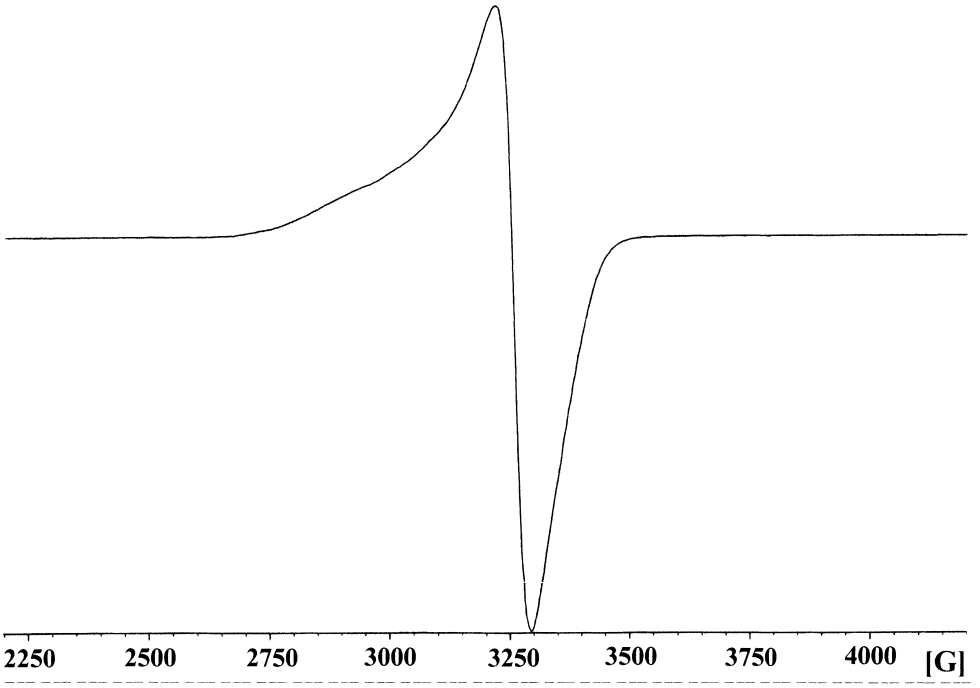


Fig. 4. Experimental polycrystalline X-band EPR spectrum at 298 K of the copper complex.

ligand complex is so stable that it does not react again with H_2O_2 to give radical species that cleave DNA, as occurs with the Cu(I)–neocuproine complex [27].

We can conclude that the title compound is not toxic to DNA at concentrations as high as 500 μM.

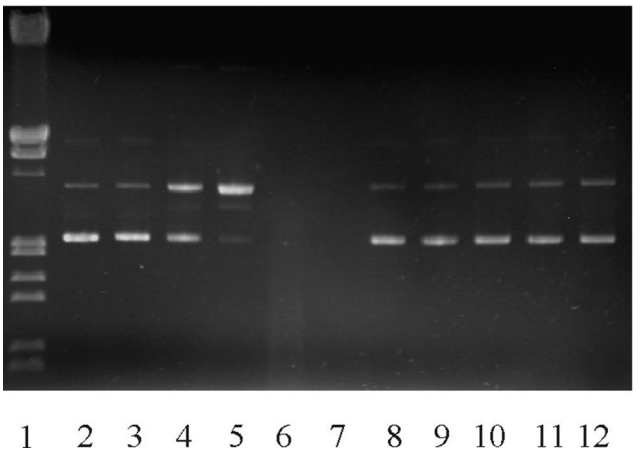


Fig. 5. Cleavage of pUCI8 by Cu(II) [*N*-quinolin-8-yl-*p*-toluenesulfonamidate]$_2$ in the presence of H_2O_2. Lanes (1) Lambda DNA/*Eco*RI+*Hin*dIII marker; (2) untreated pUCI8; (3) with added 100 μM Cu(II) and 2 mM H_2O_2; (4) with added 200 μM Cu(II) and 20 mM H_2O_2; (5) with added 300 μM Cu(II) and 30 mM H_2O_2; (6) 400 μM Cu(II) and 40 mM H_2O_2; (7) 500 μM Cu(II) and 50 mM H_2O_2; (8) 100 μM of the Cu((II) complex and 10 mM H_2O_2; (9) 200 μM Cu(II) complex and 20 mM H_2O_2; (10) 300 μM Cu(II) complex and 30 mM H_2O_2; (11) 400 μM of the Cu(II) complex and 40 mM H_2O_2; (12) 500 μM of the Cu(II) complex and 50 mM H_2O_2.

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.

6. Abbreviations

FAB	fast atomic bombardment
L-SIMS	liquid secondary ion mass spectrometry
TBE	name of the buffer whose composition is described in Section 2.5
TDI	is the trade mark of the capturing system gel printer

Acknowledgements

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