Research Article

iMedPub Journals
http://www.imedpub.com/

Structural Chemistry & Crystallography Communication
ISSN 2470-9905

2015
Vol. 1 No. 1:7

DOI: 10.21767/2470-9905.100007

Structural Characterization and Cytotoxic Activity of Heteroleptic Copper (II) Complexes with L-Dipeptides and 5-NO_2-Phenanthroline. Crystal Structure of [Cu(Phe-Ala)(5-NO_2-Phen)]·$4H_2O$

Sebastián Iglesias[1], Natalia Alvarez[1], M Gabriela Kramer[2], María H Torre[1], Eduardo Kremer[1], Javier Ellena[3], Antonio J Costa-Filho[4] and Gianella Facchin[1]

1 Facultad de Química, General Flores 2124, Universidad de la República, Montevideo, Uruguay
2 Department of Biotechnology, Instituto de Higiene, Facultad de Medicina, Universidad de la República (UdelaR), Av. Alfredo Navarro 3051, Montevideo, Uruguay
3 Instituto de Física de São Carlos, Universidade de São Paulo, C.P. 369, 13560-970, São Carlos (SP), Brazil
4 Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 14040-901, Ribeirão Preto (SP), Brasil

Corresponding author: Gianella Facchin

gfacchin@fq.edu.uy

Facultad de Química, General Flores 2124, Universidad de la República, Montevideo, Uruguay.

Tel: +598 2 924 9739

Citation: Iglesias S, Alvarez N, Kramer MG, et al. Structural Characterization and Cytotoxic Activity of Heteroleptic Copper (II) Complexes with L-Dipeptides and 5-NO2-Phenanthroline. Crystal Structure of [Cu(Phe-Ala)(5-NO2-Phen)]·4H2O. Struct Chem Crystallogr Commun. 2015, 1:1.

Abstract

Objective: To prepare a new series of [Cu(L-dipeptide)(5-NO_2-phen)] complexes and study the influence of the nitro group on the structure, DNA interaction and cytotoxic activity, aimed at finding new copper compounds with antitumor activity.

Methodology and results: A series of mononuclear mixed ligand copper(II) complexes of the type [Cu(L-dipeptide)(5-NO_2-phen)]·nH_2O (where L-dipeptide: Ala-Phe, Phe-Ala, Phe-Val and Phe-Phe) were synthesized and characterized. The crystal structure of [Cu(Phe-Ala)(5-NO_2-phen)]·$4H_2O$ was solved by X-Ray diffraction. The complexes present square-based pyramidal coordination geometry. UV-Vis spectroscopy results suggest that the coordination observed in solid state is maintained in solution. The complexes bind to isolated DNA, as studied by Circular Dichroism. Biological experiments showed that all the complexes induce cell death in HeLa (human cervical adenocarcinoma) and MDA-MB-231 (human metastatic breast adenocarcinoma) cell lines. Their cytotoxic activity is higher than that of the Cisplatin.

Conclusions: Four new [Cu(L-dipeptide)(5-NO_2-phen)]·nH_2O were prepared, which present good cytotoxic activity. The introduction of the nitro group on the phen impaired DNA binding and cytotoxic activity.

Keywords: Copper complexes; Dipeptide; 5-NO_2-phen; DRX; DNA interaction; Cytotoxic activity

Received: Oct 02, 2015, **Accepted:** Oct 28, 2015, **Published:** Oct 31, 2015

Introduction

Coordination chemistry has provided medicine with drugs for clinical anticancer chemotherapy as well as a wider group of candidate compounds in different stages of research with promising activities. With the aim to obtain new antitumor metallo-drugs, complexes of several metals other than Pt are being studied [1]. In particular, under the hypothesis that endogenous metals may be less toxic than Pt, several copper complexes were studied looking for cytotoxic activity [2]. As a consequence, a variety of Cu-complexes were found to present high antitumor activity, as tested *in vitro* on several cancer cell lines, and a few of them on *in vivo* experiments [2,3]. One of the most relevant examples of active Cu-complexes are the coordination compounds Casiopeinas®, heteroleptic complexes containing an aromatic diimine and a coligand, two of which are already approved for clinical trials as antitumor drugs [4]. In this series of hetereolopetic compounds it was found that the

aromatic diiminic ligand was the most influent ligand in relation to the biological activity, with subtle differences in its structure influencing strongly its activity [5,6].

Two heteroleptic Cu-5-NO_2-1,10-phenanthroline complexes were reported to present potent antitumor activity *in vitro*, bringing the attention to this phen derivative as a ligand for further development of heteroleptic complexes with antitumor activity [7]. The 5-NO_2-1,10-phenanthroline (5-NO_2-phen) ligand presents different hydrophobicity and hydrogen bonding properties than the 1,10-phenanthroline (phen). It acts as an electron-attractor substituent, modifying the electronic properties of the molecule. These properties may influence their complexes reactivity, possibly showing different ability to bind to DNA than Cu-phen complexes [8-11]. As a result, their cytotoxic activity may be different. This difference possibly extends to heteroleptic 5-NO_2-phen complexes.

Our research group has previously studied homoleptic Cu-L-dipeptide complexes [12-15] and heteroleptic Cu-L-dipeptide-phen complexes [16]. While both present cytotoxic activity (IC_{50} Cu-L-dipeptide 20-50 µM, IC_{50} Cu-L-dipeptide-phen 1-20 µM on HeLa cells) phen containing complexes present higher activity showing the relevance of the phen ligand to the activity [16].

To move on with these studies, heteroleptic Cu(II) complexes with L-dipeptide and 5-NO_2-1,10-phenanthroline were studied with the aim to evaluate the influence of the 5-NO_2-phen moiety in their structure, DNA binding and cytotoxic activity. As coligands, four L-dipeptides were used, selecting those which presented good cytotoxic activity in the Cu-L-dipeptide-phen series (L-Ala-Phe, L-Phe-Ala, L-Phe-Val and L-Phe-Phe) [16]. In this context, this work reports the synthesis, characterization and DNA binding of four new [Cu(L-dipeptide)(5-NO_2-1,10-phenanthroline)] compounds. In addition, their cytotoxic activity was evaluated in two cancer cell lines of human origin. To the best of our knowledge, no previous report on cytotoxic activity of copper complexes with dipeptides and 5-nitro-phen as ligands has been published to the date.

Materials and Methods

Reagents for synthesis and biochemical studies were used as commercially available: Copper salts (Fluka), L-dipeptides, 5-NO_2-1,10-phenanthroline (5-NO_2-phen, SIGMA) and Calf thymus DNA (CT-DNA, SIGMA). Distilled water was used for all the experiments.

Synthesis of the complexes and analytical characterization

Ternary Cu-dipeptide-5-NO_2-phen complexes (where L-dipetide: Ala-Phe, Phe-Ala, Phe-Val or Phe-Phe) were obtained as follows: 0.1 mmol of dipeptide were dissolved in 100 mL of warm water and 0.1 mmol of $CuSO_4 \cdot 5H_2O$ was added. The pH was adjusted to 7 with a 0.1 M NaOH solution. A solution of 0.1 mmol of 5-NO_2-phen in 10 mL of ethanol was added. A blue solution was obtained. The compounds were isolated by evaporation at 50-60° C, until blue crystals were formed. Yield 60-70%. To obtain single crystals, a small amount of the solution was allowed to slowly evaporate at room temperature. Blue crystals, suitable for X-ray diffraction, were only obtained for [Cu(Phe-Ala)(5-NO_2-phen)]·$4H_2O$.

Elemental analysis: [Cu(Ala-Phe)(5-NO_2-phen)]·$11H_2O$ (***C1***) Calc. for $C_{24}H_{43}CuN_5O_{16}$: C, 39.97, N, 9.71, H, 6.01 Found: C, 40.32, N, 9.64, H, 5.61; [Cu(Phe-Ala)(5-NO_2-phen)]·$4H_2O$ (***C2***) Calc. for $C_{24}H_{29}CuN_5O_9$: C, 48.44, N, 11.77, H, 4.91 Found: C, 48.09, N, 11.80, H, 4.90; [Cu(Phe-Val)(5-NO_2-phen)]·$4H_2O$ (***C3***) Calc. for $C_{26}H_{33}CuN_5O_9$: C, 50.12, N, 11.24, H, 5.34 Found: C, 50.08, N, 11.27, H, 5.32; [Cu(Phe-Phe)(5- NO_2-phen)]·$3{,}5H_2O$ (***C4***) Calc. for $C_{30}H_{32}CuN_5O_{8.5}$: C, 54.42, N, 10.58, H, 4.87 Found: C, 54.41, N, 10.64, H, 4.74.

Physical measurements

Chemical analyses for carbon, nitrogen, hydrogen and sulfur were performed with a Carlo Erba analyzer. Infrared spectra were recorded with a Bomen FT-IR spectrophotometer from 4000 to 400 cm^{-1} using KBr disks. UV–visible (UV–vis) spectra of the complexes in solution were carried out with a Milton Roy Spectronic 3000 spectrophotometer, using 1 cm path length quartz cells. Circular Dichroism spectra were recorded on a Jasco 720 instrument using 1 cm path length quartz cells.

Crystal structure determination of [Cu(Phe-Ala)(5-NO_2-phen)]·$4H_2O$, C2

Data for a suitable single crystal for complex ***C2*** was collected at 293 [2] K on an Enraf-Nonius FR590 Kappa-CCD diffractometer using graphite monochromated MoKα radiation (0,71073 Å). Bruker AXS Collect software was used for data collection and the HKL Denzo-Scalepack program suite for data reduction. The structure was solved by direct methods using SIR-92 [17] and refined with SHELXL-2014 [18]. Multi-scan absorption correction was applied [19]. SHELXL-2014 and ORTEP-3 programs were used within the WinGX [20]. Molecular structure graphics were prepared using MERCURY program [21]. All non-hydrogen atoms were refined using anisotropic displacement parameters. Carbon bonded hydrogen atoms were stereochemically position and refined isotropically using the *riding-model*, thermal parameters set at 1.2 times the U_{iso} of the C atom they are bonded to Amino and water hydrogen atoms were found in the difference Fourier map, positionally fixed and their thermal parameters set to 1.2 times the U_{iso} of the nitrogen atom for the amine and 1.5 times the U_{iso} of the oxygen atom for the water molecules. Summary of the crystallographic data, experimental details and refinement results are listed in **Table 1**.

Binding to DNA: CD studies

Absorption titration measurements were carried out on Calf Thymus-DNA in phosphate buffer solution (pH=7.4) as follows. To a 2.5 mL sample of 1.7×10^{-4} M CT-DNA 10 µL aliquots of the complexes 2.0×10^{-4} M in buffer were added. The CD spectra were registered after 15 m of incubation at room temperature.

Cytotoxicity studies

Cell lines were obtained from the American Type Culture Collection (ATCC): HeLa (CCL-2™, human cervical adenocarcinoma), MDA-MB-231 (HTB-22™, human metastatic breast adenocarcinoma). HeLa cells were grown in high glucose (4.5 g/L) Dulbecco's Modified Eagle Medium (DMEM) and MDA-MB-231 cells were grown in RPMI Medium both containing stable L-Glutamine

and supplemented with 10% Fetal Bovine Serum (FBS, GE Healthcare). Cells were removed enzymatically from flasks using 0.01% Trypsin-EDTA solution. Cultured cells were incubated at 37°C on 5% CO_2 atmosphere for 48 hours, containing solutions of the Cu complexes (final concentrations ranging from 1 to 50 µM). Cell viability in response to the complexes was determined by a colorimetric assay using Cell Counting Kit – 8 (Fulka) on 15.000 cells grown in 96-well plates. The kit utilizes a water-soluble tetrazolium salt that is reduced by dehydrogenases in cells to give a yellow colored product (formazan). The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. Absorbance of converted dye was measured at 450 nm using a micro plate reader. Cu-complexes solutions were prepared in 5% DMSO in water (due to poor solubility in water). The higher concentration of DMSO in culture medium was 0.5% (for the 100 µM test) and proved not to induce cytotoxicity. The IC_{50} was estimated from the dose-response curves, deviation between duplicates did not exceed 10%.

Results

Crystal structure

5-NO_2-phen copper complexes have not been widely studied yet. There are only six X-ray crystal structures of copper complexes containing 5-NO_2-phen reported to the date. None of them contains dipeptides as co-ligand [7,22-26]. The crystal structure of a new complex: [Cu(L-Phe-Ala)(5-NO_2-phen)]·4H_2O (**C2**) was obtained. The *ortep* type view of the compound with anisotropic displacement ellipsoids (50% probability) and numbering scheme is shown in **Figure 1**. The complex presents a coordination sphere where the copper ion is situated in a distorted squared pyramidal environment, equatorially coordinated to two N and one O atom from the dipeptide, and one N atom from the phen. The coordination sphere is completed by one N atom of the phen in the apical position. **Table 2** shows bond distances and angles around the copper atom. The 5-NO_2-phen ligand presents a usual bite distance (2.687 Å *vs* 2.64 mean distance for Cu(II)- 5-NO_2-phen complexes according to the Cambridge Structural Data Base) and angle (77.20° *vs* 81.08° mean angle) and it is in a nearly orthogonal position with the Cu-peptide moiety. The distortion of the pyramidal environment was evaluated by means of τ factor which varies from 0 for perfect square pyramidal to 1 for trigonal-bipyramid geometry [27]. τ=0.16, therefore the contribution of the trigonal-bipyramid geometry is 16%.

In order to determine the structural similarity between this 5-NO_2-phen complex with its previously reported analogue containing phen [16] the crystal structures of [Cu(Phe-Ala)(5-NO_2-phen) and [Cu(Phe-Ala)(phen)] were compared by means of the solid form module in the Mercury program. The restrictions applied to the search of similarity by the module were 20% distance and 10 degree angle tolerance. The comparison led up to a 97% similarity of the complexes' powder diffraction patterns. When comparing one molecule of each there is a root mean square (*rms*) of 0.19 Å. Comparison of cell parameters are shown in **Table 3** and the diagrams resulting from the comparison are shown in **Figure 2**. The compounds are isostructural. Moreover,

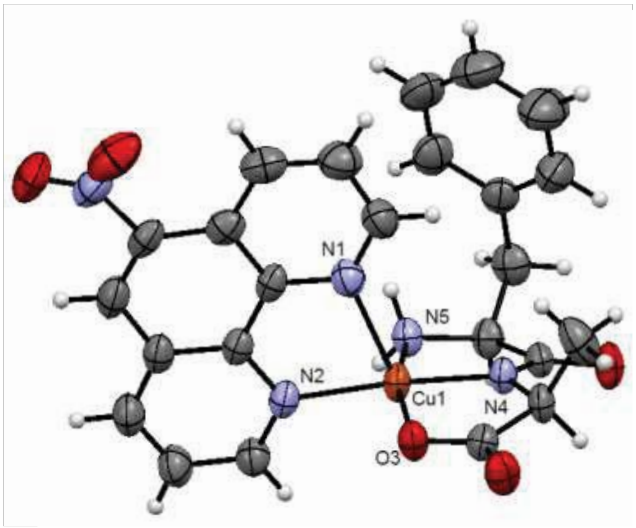

Figure 1 ORTEP representation of the asymmetric unit of [Cu(Phe-Ala)(5-NO_2-phen)]·4H_2O (C2). Water molecules were omitted for simplicity.

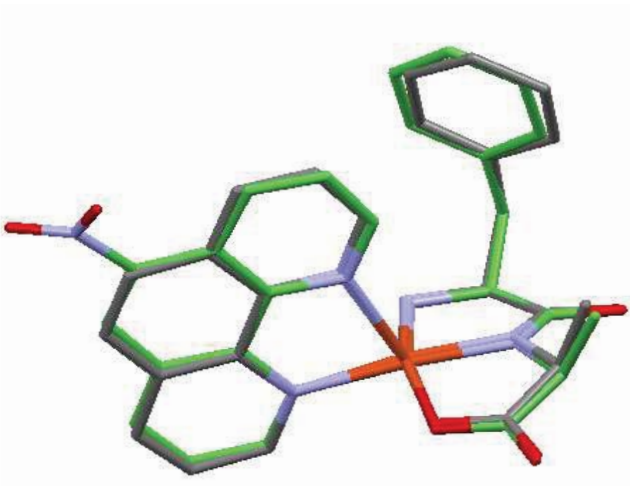

Figure 2 Diagram showing the results of the solid form module comparison tool for [Cu(Phe-Ala)(5-NO2-phen)] and [Cu(Phe-Ala)(phen)].

the spatial disposition of atoms around the coordination center and throughout the crystal lattice was practically identical.

Infrared spectra of the complexes

The infrared spectra of the complexes present several common features. They are also similar to those of the Cu-dipeptide-phen complexes [16]. For instance, a broad, very strong peak around 1600 cm^{-1} attributed to ν(C=O)+ν(C-N)+$ν_{as}$(COO), indicative of the coordinated dipeptide moiety [12,13,28]. Absorption peaks corresponding to ring stretching frequencies of the 5-NO_2-phen are slightly modified in relation to the free ligand and appear around 1515 cm^{-1} and 1420 cm^{-1}, in agreement with the coordination of the 5-NO_2-phen [29]. Other distinctive bands, like the ones due to C-H rocking in the 5-NO_2-phen, appear from 1200 cm^{-1} to 720 cm^{-1}, as shown in **Table 4**. Taking into account the similarity found in the infrared spectra of the complexes it can be

Table 1 Crystal data and structure refinement for C2.

Empirical formula	$C_{24}H_{29}N_5O_9Cu$
Formula weight	595.06
Temperature	293(2) K
Wave length	0.71073 Å
Crystal system	Monoclinic
Space group	C2
Unit cell dimensions	
a=22.9400(8) Å	α=90°
b=11.7710(5) Å	β=112.860(2)°
c=10.6910(5) Å	γ=90°
Volume	2660.1(2) Å^3
Z	4
Density (calculated)	1.486 Mg/m^3
Absorption coefficient	0.882 mm^{-1}
F(000)	1236
Crystal size	0.292 × 0.206 × 0.124 mm^3
Theta range for data collection	3.122 to 26.716°
Index ranges	-26<=h<=28 -14<=k<=14 -10<=l<=13
Reflections collected	10731
Independent reflections	5407 [R(int)=0.0274]
Completeness to theta=25.242°	99.7%
Absorption correction	Multi-scan
Maximum and minimum transmission	0.896 and 0.804
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5407 / 1 / 353
Goodness-of-fit on F2	1.037
Final R indices [I>2sigma(I)]	R_1=0.0367, wR^2=0.0777
R indices (all data)	R_1=0.0502, wR^2=0.0833
Absolute structure parameter	-0.021(7)
Largest different peak and hole	0.347 and -0.343 e.Å^{-3}

assumed that the four studied complexes present a coordination similar to that observed for the [Cu(Phe-Ala)(5-NO_2-phen)]·4H_2O complex **(C2)**.

Characterization in aqueous solution: UV-visible

Table 5 presents the wavelength of the maxima of the visible spectra of the complexes. They present a broad peak around 625 nm with a shoulder at about 800 nm, characteristic of copper in penta coordinated environments [30]. The λ_{max} values are similar to those of their [Cu(dipeptide)(phen)] analogs [16]. In an attempt to evaluate if the coordination observed in solid state is maintained in aqueous solution, UV-visible spectra of the complexes were analyzed using the work of Prenesti et al. who have previously developed visible spectra/structure correlations for Cu(II) complexes [31-33].

The expected λ_{max} for the observed coordination is 610-630 nm composed by: 580 nm (octahedral arrangement with an N_3O equatorial coordination scheme) [31] plus 30-40 nm due to the presence of one N axially coordinated [33]. This value is in accordance with the observed values for this series of [Cu(dipeptide)(5-NO_2-phen)] complexes. This analysis suggests that the same coordination observed in solid state is maintained for the major species in aqueous solution at the studied concentration (around 5 × 10^{-3} M). Similar results were previously reported for [Cu(Gly-Gly)(5-NO_2-phen)] [34].

Binding to DNA: CD studies

The CD spectrum of CT-DNA was monitored alone, in the presence of 5-NO_2-phen ligand and the complexes. Spectra for the ligand 5-NO_2-phen is presented in **Figure 3** (spectra for the other compounds can be found as supplementary material). Upon addition of the ligand 5-NO_2-phen to CT-DNA, an intensity increase in the positive band of the spectrum is observed. Similar results are observed for all the complexes. These observation shows that 5-NO_2-phen (and its complexes) binds to isolated DNA [35]. It also suggests an intercalative mode of binding of the 5-NO_2-phen and its complexes to the DNA, as observed for other Cu-5-NO_2-phen heteroleptic complexes [7]. The stacking of the complex molecules, possible through the 5-NO_2-phen moiety, between the base pairs of DNA led to an enhancement in the positive band. These results are also similar to that obtained in the same conditions for the phen ligand. The extent of hyperchromism is qualitatively minor in the case of 5-NO_2-phen and its complexes (around 20% for 5-NO_2-phen and its complexes *vs* 50% for phen in the same experimental conditions) suggesting that the 5-nitro group impairs DNA binding. A similar behavior was also observed for a series of Ru mixed ligand complexes [36].

Cytotoxic activity

Table 6 presents the IC_{50} values for the studied complexes. All of them present higher cytotoxic activity (lower IC_{50}) on HeLa and MDA-MB-231 cells than Cisplatin. [Cu(dipeptide)(5-NO_2-phen)] complexes show similar activity to [Cu(5-NO_2-phen)$_2$$Cl_2$]. Compared with their phen analogs, the 5-NO_2-phen complexes present lower activity (for instance, [Cu(ala-phe)(phen)]) presents an IC_{50} 2 μM on HeLa cells [16]. This tendency was previously observed in the Casiopeinas series, where complexes containing

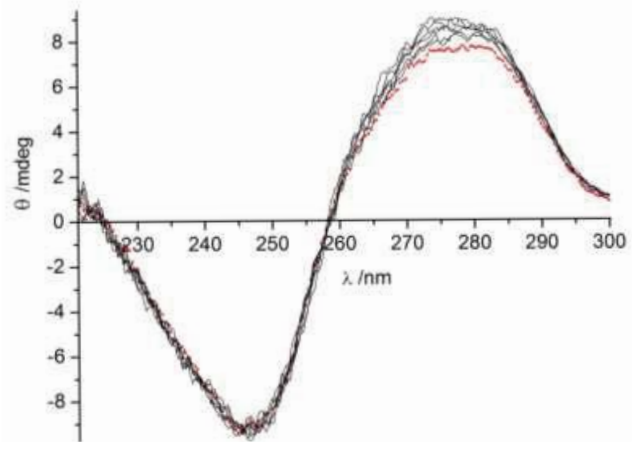

Figure 3 CD spectra of DNA alone (red dotted line) and in the presence of 5-NO_2-phen (black solid lines).

Table 2 Selected bond lengths and angles.

Selected Bond Length (Å)		Cu1 N5	2.011(3)
Cu1 N4	1.891(4)	Cu1 N2	2.025(4)
Cu1 O3	1.993(3)	Cu1 N1	2.271(4)
Selected angles (°)			
N4 Cu1 O3	83.39(13)	N5 Cu1 N2	94.72(13)
N4 Cu1 N5	83.40(13)	N4 Cu1 N1	109.10(14)
O3 Cu1 N5	164.08(13)	O3 Cu1 N1	96.02(13)
N4 Cu1 N2	173.55(18)	N5 Cu1 N1	96.68(14)
O3 Cu1 N2	97.43(14)	N2 Cu1 N1	77.21(15)

Table 3 Cell parameters comparison.

Compound	Space group	a	b	c	α	β	γ
[Cu(phe-ala)(phen)]·4H_2O	C2	22.5305(4) Å	11.5646(2) Å	10.7493(2) Å	90°	116.108(1)°	90°
[Cu(phe-ala)(5-NO_2-phen)]·4H_2O	C2	22.9400(8) Å	11.7710(5) Å	10.6910(5) Å	90°	112.860(2)°	90°

Table 4 Wavenumber (cm^{-1}) of representative absorption bands in the complexes and in free 5-NO_2-phen, as well as their tentative assignment.

Assignment	ν(C=O)+ν(C-N)+ $\nu(COO)_{as}$	ν(C=C)	ν(C=N)	ρ(C-H)	ρ(C-H)	ρ (C-H)	ρ(C-H)
5-NO_2-phen	---	1518	1422	1148	1081	832	734
[Cu(ala-phe)(5-NO_2-phen)]	1589	1514	1420	1150	1079	836	734
[Cu(phe-ala)(5-NO_2-phen)]	1594	1514	1419	1152	1079	836	734
[Cu(phe-val)(5-NO_2-phen)]	1580	1514	1417	1155	1079	836	734
[Cu(phe-phe)(5-NO_2-phen)]	1591, 1576	1514	1417	1150	1079	836	734

Table 5 Wavelength of the maxima of the visible spectra of the complexes (λ_{max}) and molar absorbance (ε_M).

Compound	λ_{max}	ε_M ($M^{-1}cm^{-1}$)
C1	621	132
C2	631	140
C3	623	141
C4	627	145

Table 6 Cytotoxic activity (expressed by IC_{50}) of the studied complexes against HeLa and MDA-MB-23 cell lines.

Compound	IC_{50} (μM) Hela	IC_{50} (μM) MDA MB 231
[Cu(Ala-Phe)(5-NO_2-phen)]	13	4.0
[Cu(Phe-Ala)(5-NO_2-phen)]	>20	8.4
[Cu(Phe-Val)(5-NO_2-phen)]	14	4.8
[Cu(Phe-Phe)(5-NO_2-phen)]	>20	9.3
[Cu(5-NO_2-phen)$_2$]Cl_2	16	5.8
Cisplatin	50	30

5-NO_2-phen had larger IC_{50} than their counterparts with phen [6], showing the relevance of the phen moiety on the activity of the complexes. This reduction of the cytotoxic activity is possibly related to the fact that the nitro group impairs DNA binding. The electron-attractor effect of the nitro substituent on the phen can also influence the reactivity of the Cu center in the complexes and therefore their activity. The activity of the complexes makes them good candidates to perform *in vivo* antitumor tests comparing they tissue distribution, which may be different from that of the related phen complexes.

Conclusions

Four new complexes were synthesized and characterized. They present square-based pyramidal coordination geometry with a dipeptide and a 5-NO_2-phen coordinated to the Cu(II) center, perpendicular to each other. UV-vis spectra suggest no change in coordination upon dissolution. The complexes bind to isolated DNA, possibly by intercalation. The extent of binding is lower than that observed for Cu-dipeptide-phen complexes. The complexes present cytotoxic activity against the studied cell lines, higher than Cisplatin. The introduction of the nitro group in the phen reduces the cytotoxic activity of the complexes.

Acknowledgements

The authors thank CSIC and PEDECIBA Química and Biología (Uruguay); CNPq and FAPESP (Brazil) and CAPES/UdelaR (Brazil-Ururguay bilateral research grant) for financial support.

Supplementary data

CCDC 1055554 contains the supplementary crystallographic data for C2. This data can be obtained free of charge via https://summary.ccdc.cam.ac.uk/structure-summary-form, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, Fax: (+44) 1223-336-033. CD spectra of DNA alone and in the presence of the studied compounds **(Figure S1 – S5)**.

References

1 Rijt SH, Sadler PJ (2009) Current applications and future potential for bioinorganic chemistry in the development of anticancer drugs. Drug Discov Today 14: 1089–1097.

2 Santini C, Pellei M, Gandin V, Porchia M, Tisato F, et al. (2014) Advances in Copper Complexes as Anticancer Agents. Chem Rev 114: 815–862.

3 Marzano C, Pellei M, Tisato F, Santini C (2009) Copper complexes as anticancer agents. Anti-Cancer Agents Med Chem 9: 185-211.

4 Serment-Guerrero J, Cano-Sanchez P, Reyes-Perez E, Velazquez-Garcia F, Bravo-Gomez ME, et al. (2011) Genotoxicity of the copper antineoplastic coordination complexes casiopeinas®. Toxicol In Vitro 25: 1376-1384.

5 Bravo-Gómez ME, García-Ramos JC, Gracia-Mora I, Ruiz-Azuara L (2009) Antiproliferative activity and QSAR study of copper (II) mixed chelate [Cu (N–N)(acetylacetonato)] and [Cu (N–N)(glycinato)] complexes. J Inorg Biochem 103: 299-309.

6 Bravo-Gómez ME, Manzanilla D S, Garibay J F, Muciño–Hernández M A, Ángel Mendoza, et al. (2012) Secondary Ligand Effects on the Cytotoxicity of Several Casiopeína's Group II Compounds. J Mex Chem Soc 56: 85-92.

7 Li X-W, Li X, Li Y-T, Wu Z-Y, Yan C-W (2012) Synthesis and structures of binuclear copper (II) complexes bridged by N-(5-chloro-2-hydroxyphenyl)-N′-[3-(dimethylamino) propyl] oxalamide ligand: DNA-binding properties and cytotoxic activities. J Organomet Chem 700: 48-57.

8 Veal JM, Rill RL (1988) Sequence specificity of DNA cleavage by bis (1, 10-phenanthroline) copper (I). Biochemistry Mosc 27: 1822-1827.

9 Alvarez N, Facchin G, Torre M (2011) Antioxidant and pro-oxidant properties and antiproliferative activity of homoleptic and heteroleptic copper complexes, potential antitumoral species. Met Ions Biol Med: 117-122.

10 Thomas AM, Nethaji M, Mahadevan S, Chakravarty AR (2003) Synthesis, crystal structure, and nuclease activity of planar mono-heterocyclic base copper (II) complexes. J Inorg Biochem 94: 171-178.

11 Ni Y, Lin D, Kokot S (2006) Synchronous fluorescence, UV–visible spectrophotometric, and voltametric studies of the competitive interaction of bis (1, 10-phenanthroline) copper (II) complex and neutral red with DNA. Anal Biochem 352: 231-242.

12 Facchin G, Torre MH, Eduardo Kremer, Piro OE, Castellano EE, et al (2000) Structural and spectroscopic characterization of two new Cu (II)-dipeptide complexes. Z Naturforsch B 55: 1157-1162.

13 Facchin G, Torre MH, Kremer E, Piro OE, Castellano EE, et al. (2002) Synthesis and characterization of three new Cu (II)-dipeptide complexes. J Inorg Biochem 89: 174-180.

14 Facchin G, Gianella F, Eduardo K, Enrique JB, Eduardo EC, et al. (2006) Structural characterization of a series of new Cu-dipeptide complexes in solid state and in solution. Polyhedron 25: 2597-2604.

15 Facchin G, Eduardo K, Baranb JE, Castellanoc EE, Pirod OE, et al. (2003) Cu (II) complexation with His–Gly and His–Ala. X-ray structure of [Cu (his–gly)2(H2O)2]• 6H2O. Inorg Chim Acta 355: 408-413.

16 Iglesias S, Alvarez N, Torre MH, Kremer E, Ellena J, et al. (2014) Synthesis, structural characterization and cytotoxic activity of ternary copper (II)–dipeptide–phenanthroline complexes. A step towards the development of new copper compounds for the treatment of cáncer. J Inorg Biochem 139:117-123.

17 Altomare A, Cascarano G, Giacovazzo C Guagliardi A (1993) Completion and refinement of crystal structures with SIR92. J Appl Crystallogr 26: 343-350.

18 Sheldrick GM (2008) A short history of SHELX (Translated from English). Acta Crystallogr Sect A 64:112-122.

19 Blessing R (1995) An empirical correction for absorption anisotropy. Acta Crystallogr Sect A 51: 33-38.

20 Farrugia L (2012) WinGX and ORTEP for Windows: An update. Journal of Applied Crystallography 45: 849-854.

21 Macrae CF, Edgington PR, Patrick M, Pidcock E, Shields GP, et al. (2006) Mercury: visualization and analysis of crystal structures. Journal of Applied Crystallography 39: 453-457.

22 Xiao HP, Zhu LG (2003) Catena-Poly [[[di-μ-chloro-bis [(5-nitro-1, 10-phenanthroline) copper (II)]]-μ-terephthalato] bis (N, N′-dimethylformamide)]. Acta Crystallogr Sect Sect E Struct Rep Online 59: 964-966.

23 Dey S, Mukherjee T, Sarkar S, Evans HS, Chattopadhyay P (2011) 5-Nitro-1, 10-phenanthroline bis (N, N-dimethylformamide-K' O)-bis (perchlorato) copper (II): synthesis, structural characterization, and DNA-binding study. Transition Met Chem 36: 631-636.

24 Bush PM, Whitehead JP, Pink CC, Gramm EC, Eglin J, et al. (2001) Electronic and structural variation among copper (II) complexes with substituted phenanthrolines. Inorg Chem 40: 1871-1877.

25 Chen XF, Cheng P, Liu X, Zhao B, Liao DZ, et al. (2001) Two-dimensional coordination polymers of copper (II) with oxalate: lattice water control of structure. Inorg Chem 40: 2652-2659.

26 Wei YQ, Wu KC, Zhuang BT, Zhou ZF (2006) Synthesis, structures and luminescence of two copper(I)–dppm complexes, dppm=bis(diphenylphosphino)methane. J Coord Chem 59: 713-719.

27 Gispert JR (2000) Química de coordinación (Ediciones de la Universitat de Barcelona, Barcelona, España).

28 Nakamoto K (2009) Infrared and Raman spectra of inorganic and coordination compounds, applications in coordination, organometallic and bioinorganic chemistry (Wiley-interscience, New Jersey). 6th edn.

29 Yuan C, Peng Z, Pan Q, Li D, Shen Y (2006) Spectroscopic and theoretical studies on copper (II) complex of maleonitriledithiolate and 5-nitro-1,10-phenanthroline. J Mol Struct 789: 52-58.

30 Cotton FA, Wilkinson G, Murillo CA, Bochmann M, Grimes R (1999) Advanced inorganic chemistry. Wiley & Sons. New York.

31 Prenesti E, Daniele P, Prencipe M, Ostacoli G (1999) Spectrum–structure correlation for visible absorption spectra of copper (II) complexes in aqueous solution. Polyhedron 18: 3233-3241.

32 Prenesti E, Daniele PG, Toso S (2002) Visible spectrophotometric determination of metal ions: the influence of structure on molar absorptivity value of copper (II) complexes in aqueous solution. Anal Chim Acta 459: 323-336.

33 Prenesti E, Daniele PG, Berto S, Toso S (2006) Spectrum–structure correlation for visible absorption spectra of copper (II) complexes showing axial co-ordination in aqueous solution. Polyhedron 25: 2815-2823.

34 Zoroddu MA, Gladiali S, Marchettini N, Dallocchio R (1995) Synthesis and spectroscopic characterization of ternary complexes of copper (II) glycylglycine and substituted phenanthrolines. Transition Met Chem 20: 351-355.

35 Gore MG (2000) Spectrophotometry and Spectrofluorimetry. A Practical Approach. Oxford University Press, New york.

36 Pyle A, Pyle AM, Rehmann JP, Meshoyrer R, Kumar CV, et al. (1989) Mixed-ligand complexes of ruthenium (II): factors governing binding to DNA. J Am Chem Soc 111: 3051-3058