(Dipyrido[3,2-a:2′,3′-c]phenazine)(glycinato)copper(II) perchlorate: A novel DNA-intercalator with anti-proliferative activity against thyroid cancer cell lines

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1. Introduction

There is nowadays increasingly growing interest toward the search of new metal complexes showing anticancer properties, in particular regarding ruthenium [1,2] and platinum [3] derivatives. The main motivation of this research field is to find new active compounds toward selected cancer cells with reduced side effects and able to overcome the known "drug resistance" phenomenon occurring with classical anticancer drugs [4].

Anaplastic thyroid carcinoma (ATC) is a rare but aggressive thyroid cancer. Besides radical surgery and radiotherapy, the combination of chemotherapeutic drugs, such as doxorubicin and cisplatin, is at present considered the best treatment strategy, although without noticeable therapeutic success [5]. The chemotherapeutic effect of the classic anticancer drugs, doxorubicin and cisplatin, is related to their tight binding to DNA. In particular, cisplatin covalently binds to the N7 atom of guanine [6,7] while doxorubicin is a DNA-intercalator [8].

Recently, copper(II) complexes have been considered as potential anticancer compounds and, together with those of ruthenium(II), they are considered among the most promising alternatives to cisplatin as anticancer agents [9,10]. Interestingly, such anticancer activity has been usually related to their DNA-binding properties.

Among the DNA-binders, particular interest has been devoted to DNA-intercalators. In fact, it has been shown that the intercalation process is often related to interesting biological effects [11]. It has been reported that transition metal complexes with planar aromatic ligands are often more effective than the isolated ligands [12–14]. Several studies have been conducted with heteroleptic complexes of the dipyrido[3,2-a:2′,3′-c]phenazine (dppz) ligand, in particular with \([\text{M} \text{(phen)}_2 \text{dppz}]^{2+/3+}\) complexes ([M=Ru²⁺, Co³⁺, Cu²⁺, Fe²⁺, Ni²⁺, phen=1,10-phenanthroline]) [12,13,15–21] showing that the DNA intercalation of dppz occurs from the major groove side [12,22]. Such compounds, in particular \([\text{Ru(phen)}_2 \text{dppz}]^{2+}\), have been recently used in cellular imaging studies and for virus tracking [23]. Indeed, all known metal complexes of dppz, independently of the nature of the ancillary ligands, are strong DNA-intercalators [24–26]. Although several studies have been reported on ruthenium(II) complexes containing planar aromatic ligands [27–32], no DNA-intercalation has been observed for the related complexes with copper(II) [15,16] and platinum(II) [17,18]. Recently, copper(II) complexes have been considered as potential anticancer compounds and, together with those of ruthenium(II), they are considered among the most promising alternatives to cisplatin as anticancer agents [9,10].
intercalating agents, with values of DNA-binding constants within $10^4$–$10^7$ M$^{-1}$ [14,24–28]. Furthermore, it has been recently shown that a cationic derivative of the dppz ligand shows analogous water solubility and DNA-binding constant of the mentioned dppz metal complexes [29,30]. Remarkably, many of these dppz complexes present promising biological properties [31]. For example, the DNA binding of [CuII(dppz)$_2$](BF$_4$)$_2$, [CuII(dppz)(NO$_3$)$_2$]NO$_3$ and [CuII(dppz)$_2$(NO$_3$)$_2$]NO$_3$ has been related to biological activity against Leishmania mexicana [26,32]. Recently, the synthesis and DNA-binding of heteroleptic copper complexes of dppz and amino acids have been reported [33–37]. Such compounds, *inter alia*, have been proposed as models of bleomycins, naturally occurring anti-tumor glycopeptide antibiotics. Deprotonated amino acids are in fact efficient chelating ligands providing stable metal complexes [38].

We have recently studied the interaction of different copper(II) complexes with native calf thymus DNA [39–43]. In a few cases, the cytotoxic activity of these copper(II) complexes was correlated to their DNA-binding properties [40,43].

As a continuation of this research line, in the present paper we report on the synthesis and characterization, DNA-binding and anti-proliferative activity against two ATC human cell lines of a copper(II) complex of dppz and glycine. In fact, considering that both copper [44,45] and amino acids are present within the intracellular environment, in our opinion they could confer increased bio-compatibility to the resulting dppz containing metal complex. Furthermore, it has been shown that the presence of glycine in transition metal complexes confers on them interesting anti-cancer properties [46–50].

2. Experimental

2.1. Materials and methods

Solvents and reagents (reagent grade) were all commercial and used without further purification. Lyophilized calf thymus DNA (Fluka, BioChemika) was resuspended in 1.0 mM tris-hydroxymethylammonomethane (Tris–HCl) pH = 7.5 and dialyzed as described in the literature [51]. DNA concentration, expressed in monomer units ([DNAphosphate]), was determined by UV spectrophotometry using 7000 M$^{-1}$ cm$^{-1}$ as a molar absorption coefficient at 260 nm [52]. All experiments were carried in Tris–HCl aqueous buffer at pH = 7.5.

UV–visible (UV–vis) absorption spectra were recorded, at 25 °C, on a Varian UV–vis Cary 1E double beam spectrophotometer, equipped with a Peltier temperature controller, using 1 cm path-length cuvettes. The titration was carried out adding increasing amounts of DNA stock solution to a complex solution with constant concentration.

Circular dichroism spectra were recorded at 25 °C on a Jasco J-715 spectropolarimeter, using 1 cm path-length quartz cells. The titration was carried out adding increasing amounts of complexes stock solutions to a DNA solution with constant concentration.

Viscosity measurements were performed on a Ubbelodhe micro-viscometer, using 1 cm path-length quartz cells. The kinematic viscosity was calculated by the following equation:

$$\eta = \frac{\gamma}{\theta (\gamma / \theta)^{1/3}}$$

where $\eta$ is the effective viscosity of the DNA solution alone. The kinematic viscosity was calculated by the following equation:

$$\eta = -K (\gamma - 0), \quad K = 0.01 \text{ mm}^2/\text{s}$$

and $\gamma = 0.04$ s are two characteristic constants of the Ubbelodhe micro-viscometer, and $t$ (in s) is the efflux time.

All the spectroscopic and viscometry measurements were recorded after 24 h of the sample preparation.

2.2. Synthesis and characterization

The dppz ligand was synthesized and characterized as reported [28,54]. Cu(gly)dpdzClO$_4$ (1) (gly = glycinate) was synthesized by modifying a general synthetic method previously reported [33,37]. In detail, a mixture of glycine (75.1 mg, 1.0 mmol) and NaOH (40 mg, 1.0 mmol) in 10 ml of deionized water was added to an aqueous solution (25 ml) of Cu(ClO$_4$)$_2$·6H$_2$O (370.0 mg, 1.0 mmol) under constant stirring for 30 min. Subsequently, an ethanol solution (10 ml) of the heterocyclic base dppz (282.0 mg, 1.0 mmol) was added dropwise. The resulting white-blue mixture was stirred for 4 h at room temperature. The precipitate was filtered, washed with diethyl ether and cold aqueous methanol before drying under vacuum. The solid was recrystallized in a 50% v/v H$_2$O/MeOH solution, yielding blue crystals suitable for X-ray diffraction analysis. Yield = 85%.

The product was characterized by elemental analysis, FT-IR and UV–vis spectroscopy. CuC$_2$H$_4$N$_4$O$_6$Cl a) anal. calcd.: C, 46.25; H, 2.72; N, 13.48; found: C, 46.30; H, 2.47; N, 13.10. b) FT-IR (cm$^{-1}$): (Nujol): 3353w, 2900mb, 1631s, 1582s, 1645vs, 1376vs, 1095s, 1075s, 826w, 765m, 732s, 625m, 582w, 431w. c) Electronic absorption in aqueous Tris–HCl 1 mM buffer (nm (ε, M$^{-1}$ cm$^{-1}$)): 275 (5.77x10$^4$), 359 (1.15x10$^5$), 377 (1.19x10$^5$).

2.3. X-ray data collection and structure refinement

Sea-green single crystals suitable for X-ray structural analysis were selected from the crystals obtained from an H$_2$O/MeOH solution. Data were collected at room temperature with a Bruker APEX II CCD area-detector diffractometer and graphite-monochromated Mo-K$_\alpha$ radiation ($\lambda = 0.71073$ Å). Data collection, cell refinement, data reduction and absorption correction by multi-scan method were performed by Bruker software [55]. The structure was solved by direct methods using SHELXS97 [55]. The non-hydrogen atoms were refined anisotropically by the full-matrix least-squares method on $F^2$ using SHELXL97 [56]. All the H atoms were introduced in calculated positions and constrained to ride on their parent atoms. The crystal and experimental data are listed in Table 1. Selected bond angles and distances are given in Table 2. Atomic coordinates, the full list of bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates and hydrogen bonds are reported as supplementary material in Tables S1–S5, while the pictures are shown in Figs. 1 and S1–S3.

CCDC ID: 883024 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre (CCDC) via www.ccdc.cam.ac.uk/data_request/cif.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Crystal data and structure refinement for 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
<td>C20H15ClCuNS06</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>519.35</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>296(2)K</td>
</tr>
<tr>
<td><strong>Wavelength</strong></td>
<td>0.70173 Å</td>
</tr>
</tbody>
</table>
| **Crystal system, space group** | Monoclinic, P2_1/
| **Unit cell dimensions** | a = 17.3944(5), $\alpha = 90^\circ$; 
| | b = 8.2657(2), $\beta = 97.228^\circ$; 
| | c = 13.8451(4), $\gamma = 90^\circ$. |
| **Volume** | 1974.79(9) Å$^3$ |
| **Z, Calculated density** | 4.1629 Mg/m$^3$ |
| **Absorption coefficient** | 1.287 mm$^{-1}$ |
| **F(000)** | 984 |
| **Crystal size** | 0.23 x 0.16 x 0.14 mm |
| **Theta range for data collection** | 2.73 to 28.09 deg. |
| **Limiting indices** | -22 ≤ h ≤ 22, -10 ≤ k ≤ 10, -18 ≤ l ≤ 18 |
| **Reflections collected / unique** | 65632 / 4798 [R(int) = 0.00658] |
| **Completeness to theta = 28.09** | 99.7 % |
| **Refinement method** | Full-matrix least-squares on $F^2$ |
| **Data / restraints / parameters** | 4798 / 0 / 335 |
| **Goodness-of-fit on $F^2$** | 1.075 |
| **Final R indices | R1 = 0.0336, wR2 = 0.0814 |
| **R indices (all data)** | R1 = 0.0474, wR2 = 0.0911 |
| **Largest diff. peak and hole** | 0.559 and -0.3695 e. Å$^{-3}$ |
2.4. Biological essays

2.4.1. Cell lines and culture conditions

The two human ATC cell lines 8505c and SW1736 were maintained and grown in RPMI 1640 medium (RPMI = Roswell Park Memorial Institute) and DMEM-high glucose (DMEM = Dulbecco’s Modified Eagle Medium), respectively, containing 10% heat-inactivated fetal bovine serum, 1% l-glutamine, 1% streptomycin and penicillin, under standard culture conditions, at 37 °C and 5% CO2. All cell culture products were purchased by PAA Laboratories, UK.

2.4.2. Drugs

Stock solutions of 1 and of cisplatin were diluted with sterile serum-free culture medium to the desired concentration immediately before each experiment.

2.4.3. Cytotoxicity assay

8505c and SW1736 cell lines were plated in 96-well plates with 100 μl medium/well, at the seeding density of 3 × 10^3 cells/well and incubated overnight. After 24 h cells were treated with 1 and with cisplatin at the following concentrations: 0, 1, 5, 10 and 20 μM and cultured up to 72 h. Due to its known cytotoxicity, 20 μM cisplatin was not used. The cell viability was evaluated performing a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), on both cell lines. At the time point 10 μM/well of MTT solution was added and, after 4 h, removed and replaced by 100 μl of DMSO, to stop the reaction. The plates were incubated for 5 min at room temperature and the values of optical density detected by UV absorption spectrum.

<table>
<thead>
<tr>
<th>Bond distances Å and angles (°) for 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(1) – O(2)                          1.932 (2)</td>
</tr>
<tr>
<td>Cu(1) – N(1)                          2.008 (2)</td>
</tr>
<tr>
<td>Cu(1) – N(5)                          2.011 (2)</td>
</tr>
<tr>
<td>Cu(1) – N(2)                          2.015 (2)</td>
</tr>
<tr>
<td>Cu(1) – O(1)#1                       2.251 (2)</td>
</tr>
<tr>
<td>Cu(1) – O(3)                          2.741 (2)</td>
</tr>
<tr>
<td>N(3) – C(5)                           1.322 (3)</td>
</tr>
<tr>
<td>N(3) – C(9)                           1.351 (4)</td>
</tr>
<tr>
<td>O(1) – C(20)                          1.239 (3)</td>
</tr>
<tr>
<td>O(1) – Cu(1)#2                       2.251 (2)</td>
</tr>
<tr>
<td>O(2) – C(20)                          1.275 (3)</td>
</tr>
<tr>
<td>C(21)–C(20)                           1.504 (4)</td>
</tr>
<tr>
<td>Cu(1) – Cu(1)#1                     5.8940 (2)</td>
</tr>
<tr>
<td>O(2) – Cu(1) – N(1)                   167.55 (7)</td>
</tr>
<tr>
<td>O(2) – Cu(1) – N(5)                   84.73 (8)</td>
</tr>
<tr>
<td>N(1) – Cu(1) – N(5)                   99.30 (8)</td>
</tr>
<tr>
<td>O(2) – Cu(1) – N(2)                   91.97 (7)</td>
</tr>
<tr>
<td>N(1) – Cu(1) – N(2)                   81.37 (8)</td>
</tr>
<tr>
<td>N(5) – Cu(1) – N(2)                   166.45 (8)</td>
</tr>
<tr>
<td>O(2) – Cu(1) – O(1)#1                 98.02 (7)</td>
</tr>
<tr>
<td>N(1) – Cu(1) – O(1)#1                 93.82 (7)</td>
</tr>
<tr>
<td>N(5) – Cu(1) – O(1)#1                 89.19 (8)</td>
</tr>
<tr>
<td>N(2) – Cu(1) – O(1)#1                 104.30 (7)</td>
</tr>
<tr>
<td>C(5) – N(3) – C(9)                    116.8 (2)</td>
</tr>
<tr>
<td>C(20) – O(1) – Cu(1)#2                132.70 (17)</td>
</tr>
<tr>
<td>C(20) – O(2) – Cu(1)                  116.22 (16)</td>
</tr>
<tr>
<td>N(5) – C(21) – C(20)                  111.6 (2)</td>
</tr>
</tbody>
</table>

Fig. 1. Perspective drawing of 1 with the atomic numbering scheme. Thermal ellipsoids are drawn at the 50% probability level. A: Fragment of 1D polymeric chain; B: coordination around the Cu atom.
at 550 nm with a Microplate Reader, Model 550 (Bio-Rad). The concentrations that kill or inhibit cell growth by 50% (IC50) were estimated from the resulting concentration–response curves, using the Gossa-fit software. Each experiment was repeated at least three times. The percentage of cell viability was calculated as follows: [(A[drug] − A[blank])/(A[control] − A[blank])] × 100, where A is the absorbance.

Subsequently, SW1736 and 8505c cells were seeded at 3 × 10⁴ cells/well and incubated overnight. The next day SW1736 and 8505c cell lines were treated with 1 μM at the concentration of 2.5 μM and 6 μM, respectively, for 48 h at a cell density of 1 × 10⁵ cells in a 75 cm² flask, then scraped and lysed at 37 °C for 2 h in a buffer containing 10 mM Tris–HCl (pH = 8.0), 0.1 M EDTA (pH 8.0), 0.5% SDS, and 20 μg/ml RNase, DNase-free. Cell lysates were treated with proteinase K at 100 μg/ml at 57 °C, overnight. Cellular genomic DNA was precipitated with NaCl 5 M and isopropanol for 30 min at −20 °C. The precipitate was washed in 70% ethanol and resuspended in 10 μg/ml Tris/EDTA. DNA concentration was evaluated by the Flurochrome (Invitrogen), then DNA (10 μg) was resolved by electrophoresis in 1.5% agarose gel and stained in 5 μg/ml gelRed.

### 3. Results and discussion

#### 3.1. Structural characterization

The synthesis of complex 1 straightforwardly proceeds at room temperature, by mixing water solutions of Cu(ClO₄)₂·6H₂O and of sodium glycinate, with an ethanol solution of dppz, being the three reactants in equimolar ratio, as sketched in the Scheme 1. The crystal structure of 1 consists of a zig-zag 1D polymeric chain elongating parallel to the crystallographic b axis (see Fig. 1A). The copper is bonded to the bidentate N,N-donor dppz and monoanionic N,O-donor glycinate ligand through the amine nitrogen and carboxylate oxygen atoms; the second carboxylic oxygen atom is axially bonded to an equivalent symmetric copper atom. The X-ray structural features show how the perchlorate anion is inter-planar distance of 3.671 Å of each monodimensional polymer chain (Fig. S1). The whole molecular packing is also determined by several hydrogen bonds and the usual van der Waals interactions.

#### 3.2. Biological activity

ATC is an aggressive and usually fatal tumor with median survival of 4–12 month after diagnosis [5]. Rapid proliferation and heterogeneity of ATC lesions, probably due to the nature of stem cells, make therapy difficult [59,60]. The current standard treatment includes surgery, chemotherapy and radiotherapy, although it has been shown not to be curative [61]. The anticancer compounds rose is quantitatively and qualitatively different and the therapeutic applications are limited by toxicity and by drug resistance [62,63]. Neither doxorubicin, nor other drugs such as cisplatin, bleomycin, fluorouracil or cyclophosphamide, showed any real efficacy in controlling the disease [64–66].

We have tested the potential anti-proliferative in vitro activity of the copper complex 1 on two ATC cell lines: 8505c (Braf WT/V600E) and SW1736 (Braf WT/V600E) and we have compared the results to cisplatin treatment, the most common DNA crosslinker interfering with cell division by mitosis, used both in vitro and in vivo chemo-toxicity experiments [67,68]. Both cell lines showed a dose-dependent cytotoxic profile when exposed at the treatment of 1, as well as of cisplatin (see Fig. 2).

After 48 h of drug exposure the IC₅₀ resulted cell lines related. In particular, 1 showed a good antiproliferative activity in SW1736 cell lines, with an IC₅₀ value of 2.86 ± 0.54 μM, to be compared with that of cisplatin, IC₅₀ = 2.50 ± 0.40 μM. Remarkably, 1 exhibited an excellent cytotoxic activity against 8505c, with an IC₅₀ value of 1.05 ± 0.48 μM, considerably lower than the IC₅₀ value shown by cisplatin against the same cell line, 6.04 ± 0.78 μM. In fact, the exposure to 1 for 72 h showed a stronger lethal effect with respect to cisplatin treatment (Fig. 3A–B), detectable already at 24 h of treatment. In details, when SW1736 were treated with 1 at 2.5 μM after 24 h, the viability decrease was about 20% lower than that due to cisplatin treatment (see Fig. 3A); at 48 and at 72 h the viability was 49% and 33%, respectively (Fig. 3A). On the other hand, when 8505c was treated with 1 at 1 μM, the viability rate was reduced to 53%, 50% and 24%, at 24, 48 and 72 h, respectively (Fig. 3B). In both ATC cell lines a time-dependent effect of the IC₅₀ was detected, with an optimum at 72 h (see Fig. 3A–B). The synthesis of complex 1 straightforwardly proceeds at room temperature, by mixing water solutions of Cu(ClO₄)₂·6H₂O and of sodium glycinate, with an ethanol solution of dppz, being the three reactants in equimolar ratio, as sketched in the Scheme 1. The crystal structure of 1 consists of a zig-zag 1D polymeric chain elongating parallel to the crystallographic b axis (see Fig. 1A). The copper is bonded to the bidentate N,N-donor dppz and monoanionic N,O-donor glycinate ligand through the amine nitrogen and carboxylate oxygen atoms; the second carboxylic oxygen atom is axially bonded to an equivalent symmetric copper atom. The X-ray structural features show how the perchlorate anion is inter-planar distance of 3.671 Å of each monodimensional polymer chain (Fig. S1). The whole molecular packing is also determined by several hydrogen bonds and the usual van der Waals interactions.

![Scheme 1. Synthesis of the copper(II) complex 1.](image-url)
dependent cell death, we have performed the DNA ladder assay. As shown in Fig. 3D, DNA fragmentation, i.e. degradation into multiples of 180-base pair long fragments, that is a typical hallmark of apoptosis [69], was detected in both SW1736 and 8505c IC50 treated with 1, as well as in cisplatin incubated cells.

On the whole, these data confirm the excellent in vitro anti-proliferative activity of the title copper complex, that is superior than that shown by cisplatin in the case of 8505c ATC cell lines. It is worth mentioning that metal complexes of gallium(III), tested on proliferative activity of the title copper complex, that is superior than that shown by cisplatin in the case of 8505c ATC cell lines. It is worth mentioning that metal complexes of gallium(III), tested on the same 8505c cell lines, have recently shown IC50 values of more than 10 times higher [63,70].

3.3. DNA-binding experiments

All the spectroscopic and especially viscosity measurements performed on buffered aqueous solutions of 1 and DNA, confirm that the title cationic copper complex is a strong DNA-intercalator.

In detail, the UV-vis absorption spectrum of 1 in aqueous solution, black line in Fig. 4, shows an intense band at 275 nm and a weaker multiplet in the range 340–400 nm, which is the typical infra-ligand (IL) band of coordinated dppz [28]. Such IL band was used to monitor the interaction with DNA, because the latter shows no absorption in such wavelength range. In fact, the absorption spectrum is considerably modified by the addition of increasing amounts of DNA. In particular, the maximum at 275 nm undergoes a bathochromic shift of about 6 nm up to plateau at [DNAphosphate]/[1] molar ratios of about 5.

Moreover, the band at 377 nm shows also a hypochromic effect, i.e. a decrease of the intensity, of about 50%, with an isobestic point at 385 nm. The latter is indicative of the presence of only two copper(II) complex species in solution, i.e. free and bound to DNA. The UV-vis titration data shown in Fig. 4 were analyzed by the method proposed by Carter et al. [71] to determine the intrinsic binding constant (Kb) and the stoichiometry (s) of the 1-DNA system. In particular, s represents the binding size in base pairs, i.e. the number of DNA base pairs involved in the bond with the metal complex. In detail, the absorbance values at 375 nm were plotted in the inset of Fig. 4 as (εf − εb)/((ε0 − εf)) vs the molar concentration of DNA in monomeric units ([DNAphosphate]) and analyzed by Eqs. (1a) and (1b) [28]. In Eqs. (1a) and (1b), Cf is the total concentration of the metal complex, εf, equal to 1.19×10^4 M⁻¹ cm⁻¹, was determined by a calibration curve of the isolated metal complex in aqueous solution, following the Beer-Lambert law. εb, equal to 6.12×10^3 M⁻¹ cm⁻¹, was determined from the plateau of the plot, where further addition of DNA did not cause any changes in the absorption spectrum. Finally, ε0 was determined as the ratio between the measured absorbance and the analytical molar concentration of 1. The Kb and s values obtained were (2.1±0.1)×10^6 M⁻¹ and 0.42±0.01, respectively.

\[
\frac{\varepsilon_f - \varepsilon_b}{\varepsilon_0 - \varepsilon_f} = \frac{b^2 \frac{2K_bC_f[DNA_{phosphate}]}{s}}{2K_bC_f} \frac{1}{2}
\]  

(1a)

\[
b = 1 + K_bC_f + \frac{K_b[DNA_{phosphate}]}{2s}
\]

(1b)

Noteworthy, these values of Kb and of s are very similar to those obtained by the same absorption titration method for the interaction of [Ru(phen)2dppz]2+ with DNA [27]. This result indicates a similarity between the title copper complex 1 and [Ru(phen)2dppz]2+ both in the strength and in the stoichiometry, hence in the interaction mechanism, of the metal complex–DNA binding.

The CD spectrum of DNA is consistently modified by the addition of 1 (see Fig. 5). In detail, by increasing the amount of metal complex in solution, both negative and positive bands are monotonously shifted toward more positive values of the intensity and toward smaller wavelengths. Moreover, a slight induced negative CD band appears in the range 280–330 nm. Glycine was chosen also because it is not chiral and as a consequence it does not affect the circular dichroism of DNA. So, being achiral, these results show that the DNA conformation is perturbed only by the interaction with the copper complex. The observed modifications of the DNA CD spectrum reveal the occurrence of structural
changes of the DNA double helix following its interaction with 1 and are in agreement with the hypothesis of DNA-intercalation.

The viscosity measurements are the most indicative results of the occurrence of DNA-intercalation. It is fact known that molecules that are able to intercalate DNA typically induce unwinding of the double helix and elongation of the DNA rods, giving rise to an increase of solution viscosity [72]. As shown in Fig. 6, the relative viscosity of DNA solutions, in the presence of 1 increases already at very small values of [1]/[DNA-phosphate] molar ratios. Interestingly, the observed trend of the relative viscosity of DNA vs. the [1]/[DNA-phosphate] molar ratio is analogous to that obtained in the presence of known DNA intercalators, such as ethidium bromide [73].

It is well known that the accepted mechanism of the cytotoxic activity of cisplatin and doxorubicin, as anticancer drugs, relies on the capacity of

![Fig. 3. Evaluation of the cytotoxicity, by MTT essays, of cisplatin and of 1 on SW1736 and 8505c cells, at 24 h, 48 h and 72 h. A) Cytotoxic effect of 2.5 μM cisplatin and of 2.8 μM 1 in SW1736 cells. B) Cytotoxic effect of 6.0 μM cisplatin and of 1.0 μM 1 in 8505c cells. C) Cell phenotypes modification 1-treatment dependent. SW1736 cells and SW1736 cells plus 1 (upper); 8505c cells and 8505c cells plus 1 (lower). Both cell lines show a rounded shape after exposure to 1 for 48 h. D) DNA fragmentation in SW1736 and 8505c cells after 72 h. Lane-1: SW1736 CN; lane-2: SW1736 plus cisplatin; lane-3: SW1736 plus 1; lane-4: 8505c CN; lane-5: 8505c plus cisplatin; lane-6: 8505c plus 1; CN: cells without treatment; L: DNA laddering marker (100 bp).]

![Fig. 4. UV–vis titration of 1 with increasing amount of DNA in Tris–HCl 1.0 mM, pH=7.5. [1]=40 μM (black solid line); [DNA-phosphate]=2, 4, 8, 12, 16, 20, 28, 40, 80, 200 μM; inset: plot of (ε_b − ε_f)/(ε_a − ε_f), at 375 nm, vs. [DNA-phosphate] (see text).]

![Fig. 5. Circular dichroism spectra of DNA solutions in Tris–HCl 1.0 mM, pH=7.5, in the presence of increasing amounts of 1. [DNA-phosphate]= 50 μM (black solid line); [1]=0, 5, 12.5, 25, 35.7 μM.]
cisplatin to covalently bind the N7 atom of two guanine nucleobases [6,7] and of doxorubicin to intercalate into stacked DNA base pairs [8]. The DNA-binding experiments unequivocally show that 1 tightly binds DNA, presumably through the intercalation of the dppz moiety, although the occurrence of a covalent binding involving the metal atom cannot be excluded. In fact, the apical coordination sites of the copper atom in the [Cu(dppz)(gly)] unit can be replaced by coordinating N and O donor atoms of the DNA bases. As a consequence, it is possible to hypothesize that the mechanism of the cytotoxic activity of the title copper(II) compound could be induced by both DNA-intercalation and metal coordination, as recently pointed out by Liu and Sadler [11].

4. Conclusions

The heteroleptic dppz and glycinato complex of copper(II) 1 has been synthesized and characterized. Cytotoxicity assays provided evidence for a remarkable anti-proliferative activity of 1 against two human anaplastic thyroid cancer cell lines, SW1736 and 8505c, with an IC50 value of 2.53 ± 0.54 μM and 1.05 ± 0.48 μM, respectively. The latter, in particular, is lower than the analogous IC50 Value shown by cisplatin toward the same cell line, 6.03 ± 0.78 μM.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jinorgbio.2012.08.011.

References


