Mo(II) complexes: A new family of cytotoxic agents?

Daniel Bandarra a, Miguel Lopes a, Telma Lopes a, Joana Almeida a, Marta S. Saraiva a, Maria Vasconcellos-Dias a, Carla D. Nunes a, Vitor Félix b, Paula Brandão c, Pedro D. Vaz a, Margarida Meireles a,⁎, Maria José Calhorda a

a Departamento de Química e Bioquímica, CQB, Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal
b Departamento de Química, CICECO and Secção Autónoma de Ciências da Saúde, Universidade de Aveiro, 3810-193 Aveiro, Portugal
c Departamento de Química, CICECO, Universidade de Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 12 May 2010
Received in revised form 5 July 2010
Accepted 6 July 2010
Available online 17 July 2010

Keywords:
Molybdenum
1,10-Phenanthroline
Antitumor activity
X-ray structure
Interaction with DNA

ABSTRACT

Several molybdenum complexes, [Mo(η3-C3H5)X(CO)2(N-N)] (N-N = 1,10-phenanthroline, phen: X = CF3SO3; T1, X = Br; B1, X = Cl; C1: N-N = 2,2′-bipyridyl, X = CF3SO3; T2, X = Br B2) and [W(η1-C3H5)Br(CO)3(phen)] (W1) have been synthesized and characterized. Their antitumor properties have been tested in vitro against human cancer cell lines cervical carcinoma (HeLa) and breast carcinoma (MCF-7) using a metabolic activity test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT), leading to IC50 values ranging from 3 to 45 μM, approximately. Most complexes exhibited significant antitumoral activity. Complexes B1 and T2 were chosen for subsequent studies aiming to understand their mechanism of action. Cellular uptake of molybdenum and octanol/water partition assays revealed that both B1 and T2 exhibit a selective uptake by cells and intermediate partition coefficients. The binding constants of B1 and T2 with ct DNA, as determined by absorption titration, are 2.08 (±0.98)×105 and 3.68 (±2.01)×105 M−1, respectively. These results suggest that they interact with DNA changing its conformation and possibly inducing cell death, and may therefore provide a valuable tool in cancer chemotherapy.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Organometallic complexes play a major role in many fields of chemistry, as precursors to new materials [1–3], in catalysis [4,5] and in applications to medicine [6,7]. Their versatility is associated with the possibility of finely tuning the stereoelectronic properties of metal centers by changing ligands, oxidation state and electronic configuration.

In the last decades, after the success of cis-platin, cis-[PtCl2(NH3)2], as antitumor agent [8], the interest in the use of transition metal complexes in medicine has grown rapidly. In particular, the search for new compounds that could overcome cell resistance and toxicity problems associated with platinum complexes led to the study of other metal containing antitumor drugs. In 1979, Köpf and Köpf-Maier reported the antitumor action of several metal-based complexes with Ti, V, Nb, Mo and Re [9], and later, several researchers reported interesting anticancer activities for neutral and charged metalloccene derivatives, amplifying the number of possible clinically useful drugs [10–14].

A number of molybdenum containing molecules have since then been described to display cancerostatic activity. These include Na2MoO4 [15], molybdenum alone [16], heteropolyacid Mo salts [17], polyoxomolybdates [18], and Mo complexes bound to small carborane ligands and chiral octahedral complexes [19,20]. Portuguese researchers in 2005 studied several molybdenum(II) compounds, concluding that they were very efficient cytotoxic agents against six cell lines, and filed a patent [21]. Since molybdenum is an essential trace metal for organisms, plays a crucial role as cofactor for important enzymes [22], and is transported and excreted as the anion [MoO4]2−, its low toxicity and its effects on metabolism should make possible the use of complexes of this metal as therapeutic agents. The mechanisms of action of most of the organometallic complexes of molybdenum are far from being understood. Since cell growth is slowed down by those compounds, it seems conceivable that the inhibitory activity might in some way be related to DNA damage. This can be due to a direct action on the DNA molecule (e.g. intercalation in the double helix) or by the oxidative action of oxygen free radicals generated by the chemical agents.

We reported in an earlier work the antitumor activity of Mo(II) complexes associated with ferrocene, but the results were disappointing [23], even though the ferricinium ion by itself and other derivatives display some activity [24]. On the other hand, complexes [Mo(η3-C3H5)X(CO)2(N-N)], with X = Br, CF3SO3 and N-N = 2-(2′-pyridyl)benzimidazole or 2-(2′-pyridyl)imidazole exhibited a high activity against several cell lines [25]. In this study, we describe the noticeable antitumor activity of a family of 2,2′-bipyridyl and 1,10-
phenanthroline molybdenum complexes with halide and triflate counter-anions, and perform a series of biological studies in order to provide new insights on the mechanisms of action of these compounds.

2. Experimental

2.1. Materials

Commercially available reagents and all solvents were purchased from standard chemical suppliers. Molybdenum hexacarbonyl and 2,2′-bipyridyl were purchased from Fluka, allyl bromide from Sigma-Aldrich, 1,10-phenanthroline 1-hydrate from Panreac, and octanol from Riedel-de Haën, Germany. The RPMI 1640 cell culture medium, fetal bovine serum (FBS) were purchased from LONZA Co. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was purchased from Sigma Chemical Co, USA. Solvents were dried using common procedures. Dichloromethane was distilled over CaH₂, and n-hexane over Na/benzophenone, under nitrogen. The syntheses of the complexes were carried out under nitrogen atmosphere using Schlenk tube techniques. The complexes [MoBr(η⁴-C₅H₆)(CO)₂(CH₂CN)₂], [MoBr(η⁴-C₅H₆)(CO)₂(N-N)] (N-N=2,2′-bipyridyl, bpy), [MoCl(η⁴-C₅H₆)(CO)₂(phen)] [26] and [W(η⁴-C₅H₆)Br(CO)₂(phen)] [27] were synthesized according to literature procedures. Calf thymus DNA (ct DNA) was purchased from Sigma Chemical Co Ltd. and a stock solution was prepared by dilution in a buffer solution (50 mM NaCl/5 mM Tris–HCl, pH 7.1) followed by stirring at 4 °C for two days. This solution was stored at 4 °C. The stock solution of ct DNA gave a ratio of UV absorbance at 260 and 280 nm (A₂₆₀/A₂₈₀) of 1.87, indicating that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:10 dilution using ε = 6600 M⁻¹ cm⁻¹ [23]. MTT was dissolved (5 mg/mL) in phosphate buffer saline pH 7.2.

2.2. Methods

Infrared spectra were measured on a Nicolet 6700 spectrometer. Samples were run as KBr pellets. The intensity of reported IR signals is defined as s = strong, m = medium, and w = weak. NMR spectra were recorded on a Bruker Avance-400 spectrometer in CDCl₃ or deuterated DMSO. The splitting of proton resonances in the reported 1H NMR spectra is defined as s = singlet, d = doublet, t = triplet, and m = multiplet. UV–Vis spectra were recorded on a Shimadzu UV-2450 equipped with a Peltier cell for temperature control. High-resolution mass spectrometry (HR-MS) measurements were accomplished using electrospray ionization technique (ESI). All experiments were performed on an ApexQe FTICR Mass Spectrometer from Bruker Daltonics equipped with a combined Apollo II electrospray/MALDI ion source and a 7 T actively shielded superconducting magnet. Samples were introduced at a flow rate of 120 μL/h into the ESI source using an infusion pump. The applied spray potential was 4.5 kV and the capillary temperature was set at 200 °C. All remaining parameters were optimized to ensure the highest abundance possible for the ions of interest. All MS data were acquired in the positive ion mode, the full scan spectra being recorded in the m/z 50–1200 range. MS² with collision induced dissociation (CID) experiments were performed with argon, and the collision energy was gradually increased until the precursor and the product ions could both be observed in the MS² spectrum, though the precursor peak intensity was reduced compared to its original relative intensity. Prior to all MS experiments a calibration step was performed with a 2.8 × 10⁻⁶ M solution of polyethylene glycol 200 (PEG200) in HPLC grade methanol acidified with 0.1% (v/v) of formic acid. All errors of measured m/z ions were found to be below 5 ppm compared to expected values.

2.3. Synthesis of molybdenum(II) complexes

2.3.1. [Mo(η⁴-C₅H₆)(CF₃SO₃)₂(1,10-phenanthroline)] (T₁)

Thallium triflate (TlCF₃SO₃) (0.353 g, 1 mmol) was added to a solution of [MoBr(η⁴-C₅H₆)(CO)₂(1,10-phenanthroline)] (0.453 g, 1 mmol) in acetonitrile (20 mL), and the mixture was refluxed for 5 h. A white solid of Tlblr was formed and filtered with celite. The solid was washed 3 times with acetonitrile. The filtrate was evaporated and the solid residue dissolved in dichloromethane. Addition of n-hexane resulted in the formation of red crystals after a few days. Yield: 76% (0.397 g)

IR (KBr disc) (cm⁻¹): 3435 (m); 2068 (m); 1947 (vs); 1847 (vs); 1729 (m); 1710 (m); 1604 (m); 1519 (m); 1479 (m); 1426 (m); 1319 (s); 1311 (s); 1327 (s); 1204 (s); 1180 (vs); 1014 (s); 956 (w); 930 (w); 851 (s); 780 (m); 725 (s); 580 (m); 522 (m); 493 (w).

1H NMR (400 MHz, DMSO-d₆): δ 1.39 (d, Hanti); 3.50 (d, Hsyn); 3.79 (d, Hanti); 3.76 (d, Hsyn); 4.06 (s); 11.63 (s); 1287 (s); 1237 (s); 1204 (s); 1180 (vs); 1014 (s); 956 (w); 930 (w); 851 (s); 780 (m); 725 (s); 580 (m); 522 (m); 493 (w).

HR-ESI-MS (m/z): calcd for [C₁₇H₁₃N₂O₂Mo]⁺: 375.0029; obsd, 375.00229; error, +0.3 ppm.

2.3.2. [Mo(η⁴-C₅H₆)(CF₃SO₃)(CO)₂(2,2′-bipyridyl)] (T₂)

Thallium triflate (TlCF₃SO₃) (0.353 g, 1 mmol) was added to a solution of [MoBr(η⁴-C₅H₆)(CO)₂(2,2′-bipyridyl)] (0.429 g, 1 mmol) in acetonitrile (20 mL), and the mixture was refluxed for 5 h. A white solid of TlBr was formed and filtered with celite. The solid was washed 3 times with acetonitrile. The filtrate was evaporated and the solid residue dissolved in dichloromethane. Addition of n-hexane resulted in the formation of red crystals after a few days. Yield: 72% (0.359 g)

IR (KBr disc) (cm⁻¹): 3436 (m); 3069 (m); 1947 (vs); 1863 (vs); 1602 (s); 1573 (m); 1495 (m); 1474 (s); 1441 (s); 1389 (w); 1312 (s); 1302 (s); 1287 (s); 1237 (s); 1219 (s); 1174 (s); 1158 (s); 1127 (w); 1109 (w); 1077 (m); 1034 (s); 930 (m); 795 (m); 764 (s); 734 (m); 657 (w); 650 (w); 630 (m); 577 (m); 570 (m); 516 (m); 504 (m); 439 (m); 418 (m).

1H NMR (400 MHz, DMSO-d₆): δ 1.63 (d, Hanti); 3.76 (d, Hsyn); 4.06 (m, Hsyn); 6.79 (t, H₂/H₆); 8.08 (t, H₂/H₆); 8.16 (d, H₄/H₆); 9.2 (s, H₁/H₈).

HR-ESI-MS (m/z): calcd for [C₁₇H₁₃N₂O₂Mo]⁺: 351.00285; obsd, 351.00315; error, +0.9 ppm.

2.4. Crystallography

X-ray single crystal data of [Mo(η⁴-C₅H₆)(CF₃SO₃)(CO)₂(1,10-phenanthroline)] (T₁) and [Mo(η⁴-C₅H₆)(CF₃SO₃)(CO)₂(2,2′-bipyridyl)] (T₂) were collected on a CCD Bruker APEX II at 150 (2 K) using graphite monochromatized Mo-Kα radiation (λ = 0.71073 Å).

The selected crystal was positioned at suitable distance from the CCD, 45 mm for T₁ and 35 mm for T₂, and the corresponding spots were measured with counting time of 60 s for T₁ and 10 s for T₂. Data reductions were carried out with SAINT-NT suite from Bruker AXS. Data of both complexes were corrected for absorption effects through the multi-scan method using the same software. The structures were solved with the SHELXS and refined by full-matrix least squares with the SHELXL-97 program using the SHELX97 software package [28]. All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were introduced in the structure refinement with individual isotropic temperature factors equal 1.2 times to those they are attached. The final R values obtained for complexes T₁ and T₂ together with pertinent crystallographic data are summarized in Table 1. Molecular diagrams were drawn with PLATON [29].

2.5. Physical measurements

2.5.1. Cell cultures

HeLa (cervical carcinoma) and MCF-7 (breast carcinoma) cell lines were maintained in RPMI 1640, while NIE-115 (neuroblastoma) cells
were grown in DMEM. Both media were supplemented with 10% FBS, 200 U/mL penicillin, 100 μg/mL streptomycin and 0.3 g/mL l-glutamine in a humidified atmosphere of 95% air/5% CO2 at 37 °C.

2.5.2. Cytotoxicity assay by MTT

The MTT assay was used to determine the cell viability as an indicator for the sensitivity of the cells to the complexes [Mo(η5-C5H5)(CF3SO3)(CO)2(phen)] (T1) and [Mo(η5-C5H5)(CF3SO3)(CO)2(bpy)] (T2).

2.5.2. Cytotoxicity assay by MTT

The MTT assay was used to determine the cell viability as an indicator for the sensitivity of the cells to the complexes [Mo(η5-C5H5)(CF3SO3)(CO)2(phen)] (T1) and [Mo(η5-C5H5)(CF3SO3)(CO)2(bpy)] (T2).

Table 1

<table>
<thead>
<tr>
<th>Complex</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>[Mo(η5-C5H5)(CF3SO3)(CO)2(phen)] [N-N]</td>
<td>[Mo(η5-C5H5)(CF3SO3)(CO)2(bpy)] [N-N]</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C₈H₆MoN₂O₅S</td>
<td>C₈H₆MoN₂O₅S</td>
</tr>
<tr>
<td>Space group</td>
<td>P2₁/n</td>
<td>P2₁/n</td>
</tr>
<tr>
<td>a[Å]</td>
<td>6.6085(2)</td>
<td>6.6085(2)</td>
</tr>
<tr>
<td>b[Å]</td>
<td>17.0354(6)</td>
<td>9.7660(5)</td>
</tr>
<tr>
<td>c[Å]</td>
<td>33.1497(10)</td>
<td>13.9669(7)</td>
</tr>
<tr>
<td>R(int)</td>
<td>0.882</td>
<td>0.882</td>
</tr>
<tr>
<td>μ[mm⁻¹]</td>
<td>1.863</td>
<td>1.871</td>
</tr>
<tr>
<td>R1,w</td>
<td>0.0616</td>
<td>0.0616</td>
</tr>
<tr>
<td>R2</td>
<td>0.0925</td>
<td>0.0925</td>
</tr>
<tr>
<td>Unique reflections,</td>
<td>8767 [0.0520]</td>
<td>4108 [0.0288]</td>
</tr>
<tr>
<td>Final R indices</td>
<td>0.0389, 0.0818</td>
<td>0.0246, 0.0647</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

2.5.3. Cellular uptake studies

HeLa cells were seeded in 100 mm dishes at 4 × 10⁵ cells/mL and cultures were exposed to compounds in final concentrations of 0, 10, 50 and 100 μM for 48 h. Following treatment, cell cultures were trypsinized as described [31], and sedimented. The Mo content of cells was determined by inductively coupled plasma mass spectrometry (ICP-MS) by standard protocol at the University of Vigo, Spain.

2.5.4. Octanol/water partition coefficient

Water-saturated octanol and octanol-saturated water were prepared by shaking equal volumes of octanol and water for 5 h and allowing the mixture to separate into the respective phases for 24 h. Twenty micromolar solutions of complexes T2 and B1 were prepared in water-saturated octanol and their absorbance was analyzed by UV spectrophotometry. Three and six milliliters of drug solution were then added to 40 mL of octanol-saturated water. These solutions were shaken vigorously for 2 h. The aqueous phase was separated ensuring that there was no contamination from the octanol phase, and each of these solutions was analyzed by UV spectrophotometry to obtain the absorbance of the compounds.

2.5.5. Interaction of complexes with DNA

Calf thymus DNA (ctDNA) solutions of various concentrations (0–100 μM) were added to 20 μM buffered solutions (5 mM Tris, 50 mM NaCl, pH 7.2) of the metal complexes. The same amount of ctDNA solution was added to the reference cell in order to correct for the contribution of the increasing DNA concentration. Absorption spectra were recorded after equilibration at 37.0 °C for 10 min. The intrinsic binding constant, K, was determined from a plot of D/Δε Δap vs. D according to Eq. (1) [32]

\[
D / \Delta \varepsilon_{ap} = D / \Delta \varepsilon + 1 / (\Delta \varepsilon \times K)
\]

where D is the concentration of DNA in base pairs, Δε Δap = |εA - εt|; εA = Aabs/complex, and Δε = [εA - εt], with εA and εt corresponding to the extinction coefficient of the DNA-bound complex and unbound complex, respectively.

3. Results and discussion

3.1. Synthesis and spectroscopic studies

Complexes [Mo(η5-C5H5)X(CO)2(N-N)] [N-N=1,10-phenanthroline: X=CF₃SO₃, T1, X=Cl C1; N-N = 2,2’-bipyridyl, X=CF₃SO₃ T2, X=Br B2] and [W(η5-C5H5)X(CO)2(phen)] [W1] were synthesized in order to study their biological activity in vitro. All the complexes with X = Br were obtained directly from reaction between [MBr(η5-C5H5)(CO)2(phen)] T1, with Mo, [26]W, [27]) and the diimine ligand, which led to the substitution of the two nitride ligands (similarly for X = Cl). Reaction of [Mo(η5-C5H5)Br(CO)2(N-N)] with Tl(CF3SO3) in CH3CN resulted in loss of the halide and coordination of the triflate ion, as reported for other complexes [25]. The triflate complexes T1 and T2 were structurally characterized by single crystal X-ray diffraction (see 3.2).

The FTIR spectra of T1 showed the two strong typical bands assigned to the stretching modes of the octahedral coordinated ligand at 1936 and 1847 cm⁻¹, also observed at 1947 and 1864 for T2. Several bands characteristic of the coordinated triflate ion and the diimine ligand were also observed for both complexes.

The ¹H NMR spectrum of T1 in deuterated DMSO displayed peaks of the 1,10-phenanthroline at 8.06 (H₂), 8.22 (H₄, H₅), 8.34 (H₆) 8.80 (H₈), 8.88 (H₈), 9.37 (H₇), and 9.50 ppm (H₆), reflecting the asymmetric coordination of the ligand, with one nitrogen trans to

![Fig. 1. Structures of complexes T1-2, B1-2, with numbering scheme.](Image 365x66 to 508x231)
the allyl and the second trans to one CO group (axial isomer). The allylic protons are observed at 1.39 (H_{ally}), 3.50 and 3.79 (H_{ally}), and 4.22 (H_{ally}) ppm in agreement with the presence of the axial isomer. The data for complex T2 are analogous (see 2.3).

High-resolution mass spectra (HR-MS) were acquired using the electrospray ionization (ESI) technique to confirm the proposed structure of complexes T1 and T2. Both the molecular mass and the isotopic pattern were analyzed. Although the ESI method is a very mild ionization technique, confirmation of the masses as formulated in Fig. 1, was not possible for both complexes T1 and T2. The detected m/z peaks corresponded in both cases to the desired complexes with the loss of the CF$_3$SO$_3$ ligands.

Thus, complex T1 has been successfully detected at m/z = 375.00299, making possible its formulation as [T1 − CF$_3$SO$_3$]$^+$, with an error of 0.9 ppm relative to the expected ion at m/z = 375.00289, which is in extremely good agreement. Collision induced dissociation (CID) experiments revealed the loss of both CO ligands. This afforded an ion at m/z = 319.01304 formulated as [T1 − 2CO − CF$_3$SO$_3$]$^+$, showing an error of 0.1 ppm relative to the expected ion at m/z = 319.01302. Another species was also detected at m/z = 365.01876 and was positively identified as [T1 − CH$_3$OSO$_2$CF$_3$ + CH$_2$O]$^+$, which probably arose from the exchange of the allyl ligand in the initial complex with methoxide, and shows an error of 4.8 ppm compared to the expected ion at m/z = 364.98212. This cation also loses both CO ligands originating an ion at m/z = 309.96370 corresponding to an oxidized species formulated as [Mo($\eta^1$-C$_3$H$_5$)O$_2$]$^+$ (error of 2.9 ppm compared to the expected ion at m/z = 309.96366). This last species may be originated from gas-phase reactions with the solvent (methanol).

Complex T2 was also detected as [T2 − CF$_3$SO$_3$]$^+$ at m/z = 351.00315. Compared to the expected mass at m/z = 351.00285, this represents an error of 0.9 ppm, in excellent agreement. CID experiments revealed the loss of both CO ligands, from [T2 − CF$_3$SO$_3$]$^+$ ion originating a species at m/z = 295.01363, as already described above for T1. This corresponds to a cation formulated as [T2 − 2CO − CF$_3$SO$_3$]$^+$ with an error of 2.2 ppm relative to the expected ion at m/z = 295.01297.

### 3.2. Crystal structures

The crystal structures of complexes [Mo($\eta^1$-C$_3$H$_5$)][CF$_3$SO$_3$][(CO)$_2$](N-N)] complexes, with N-N = phen (T1) or bpy (T2), are analogous (see 2.3).

#### 3.3. Cytotoxic studies

The molybdenum complexes were assayed for cytotoxic activity against HeLa (cervical carcinoma), MCF-7 (breast carcinoma) and identical Mo–N bond lengths and N–Mo–N angles, which are consistent with the identical coordination constraints imposed by phen and bpy ligands. In addition, these parameters, as well as the remaining ones listed in Table 2, agree well with those found for other related fac-Mo($\eta^1$-C$_3$H$_5$)(CO)$_2$ polypyridyl complexes, although some of them exist as the equatorial isomer, as observed for [Mo($\eta^1$-C$_3$H$_5$)Br(CO)$_2$(N-N)], with N-N = 2,2′-bipyridyl (B2) or 1,10-phenanthroline (B1) [33–36].
NIE-115 (neuroblastoma). These cells provide a readily available, easy to handle model cell line, against which all the complexes could be tested and compared. The cells were exposed to each of the compounds for a total of 48 h, to compare the results of the cell uptake experiments with the cytotoxicity. Using the colorimetric mitochondrial function-based MTT viability assay, the IC50 values (final concentration ≤ 0.5% DMSO) were calculated from dose–response curves obtained by nonlinear regression analysis. IC50 values are concentrations of drug required to inhibit tumor cell proliferation by 50%, compared to the control viability.

As demonstrated by the IC50 values listed in Table 3, all the molybdenum complexes, and to a smaller extent the tungsten complex, showed to be very effective as cytotoxic agents against the in vitro growth of various cancer cell lines. They exhibited activities with IC50 values ranging from 3 to 39 μM, approximately, in HeLa cells, 9 to 45 μM, approximately, in MCF-7 cells (except for W1, which showed less activity) and 4 to 60 μM approximately, in NIE-115 cells, with T2 and B1 being among the most potent. These results presented some interesting structure–activity relationships. All the complexes with 1,10-phenanthroline have comparable activity and achieved almost total inhibition (~10% cell viability) at the maximum concentration tested (1000 μM) for nearly all cell lines. In fact, their values are comparable to cis-platin, which has IC50 values below 10 μM with a range of cell lines [37,38]. It is interesting also to notice that the compounds with 2,2′-bipyridyl (T2 and B2) also have comparable activities and showed a noteworthy cytotoxicity, although not as impressive as the previous ones. For further comparison between these two complexes, two complexes (T2 and B1) have been chosen for complementary studies.

The effect of varying the total exposure time of cells to compounds T2 and B1 was also investigated. The dose–response curves for the treatment of HeLa cells with the compounds for 1, 2, 24, and 48 h are shown in Fig. 4 and the results of their cytotoxicity are summarized in Table 4. These results showed a decrease in the IC50 value of both complexes as the exposure time increases, although the difference between 24 h and 48 h does not seem relevant. This may be indicative that the maximum cytotoxic effect is established at 24 h of drug exposure time.

3.4. Cellular uptake studies

The intracellular Mo content was determined after treatment of HeLa cells with compounds T2 and B1, in order to figure out the effect of altering the ligands on Mo cell uptake. The correlation of the Mo cell uptake with the cytotoxicity also provides information regarding the intracellular cytotoxic potential of complexes. Each compound was tested at three different concentrations (10, 50 and 100 μM). These concentrations were chosen based on the cytotoxicity data reported previously. The intracellular Mo concentration for the treated cells is shown in Fig. 5.

The intracellular molybdenum levels of the cells treated with B1 revealed a significant increase, contrasting to the cells treated with T2. The cellular uptake is also dose dependent for compound B1. However, the relative concentration of intracellular molybdenum is very low as compared to the administered molybdenum in complexes; in the case of B1 there is less than 0.005 μmol of Mo/million cells, which corresponds to less than 0.5% of the administered molybdenum. In opposition, raising concentrations of T2 do not seem to be directly related to the intracellular molybdenum content. These results may explain the antitumoral potency of each compound when compared with each other, as B1 showed a stronger cytotoxicity effect than T2 (Table 3).

3.5. Octanol/water partition coefficient

Preliminary studies on the partition coefficients, P, were carried out to estimate how easily the compounds T2 and B1 are able to pass through a biological membrane. The P measurements are based on the difference in solubility that a given compound exhibits in an aqueous vs. a hydrophobic medium [39]. Complex B1 presented a hydrophobic behavior (log P = 0.760 ± 0.039), which is consistent with the high levels of intracellular molybdenum detected through cellular uptake studies. In what concerns compound T2, it was not possible to determine a conclusive value of log P. However, preliminary studies indicated that it possesses a less hydrophobic nature than B1, which would be in agreement with its cellular uptake results.

3.6. Absorption titration

Electronic absorption spectroscopy is universally employed to study the binding mode of DNA to complexes [40]. The absorption spectra of T2 in the absence and presence of ct DNA are shown in Fig. 6. The addition of ct DNA (0–50 μM bp−1) to T2 led to spectral changes with hyperchromism of the 299 nm band. For the absorbance data at 299 nm, a plot of D/Δεopt vs. D is shown in the inset of Fig. 6. This plot indicates that there is a linear relationship, with a binding constant value (K) of 2.08 (±0.03) × 105 M−1. The binding of B1 to ct DNA also led to similar spectral changes, with hyperchromism of the 272 nm absorption band. The K value for B1 was calculated to be 3.68 (±0.03) × 105 M−1.

The results indicate that the complex T2 binds more strongly than B1. However, both K values are lower than the observed for ethidium
bromide ($K_{EthBr} = 1.4 \times 10^6 \text{ M}^{-1}$), a typical classical intercalator [41]. Nevertheless, it is possible that intercalation with DNA might be one of the binding patterns, since the tested molybdenum complexes contain aromatic ligands (1,10-phenanthroline on $B1$ and 2,2’-bipyridyl on $T2$) with extended $\pi$ systems, which could intercalate in DNA [42].

3.7. Conclusions

Five molybdenum(II) and one tungsten(II) complexes were synthesized and tested for their cytotoxicity against HeLa, MCF-7, and N1E-115 cell lines in vitro. These complexes revealed to be very effective, exhibiting activities with IC_{50} values ranging from 3 to 45 $\mu$M. Two different molybdenum complexes, $T2$ and $B1$, showed that maximum cytotoxic effect is established after 24 h of drug exposure time. The intracellular molybdenum uptake occurs differently for these two complexes, with uptake being dependent of increasing drug concentration for $B1$, contrarily to $T2$. Accordingly, complex $B1$ displayed a hydrophobic behavior with a log $P$ of 0.760 ± 0.039, suggesting that $B1$ might be able to pass more easily through a biological membrane. The binding interactions of $B1$ and $T2$ with ct DNA could be assigned to intercalation, in agreement with their spectral changes (modest hyperchromicity). The binding constants are $2.08 (\pm 0.98) \times 10^5$ and $3.68 (\pm 2.01) \times 10^5 \text{ M}^{-1}$, at 37 °C, for $T2$ and $B1$, respectively, these values being lower than the one determined for the classical intercalator, ethidium bromide. Future work will address the intracellular localization of molybdenum, further studies with DNA molecules and other physical determination in order to obtain a clear understanding of the mechanism of action of these molybdenum compounds as they might prove to be of application in target-based cancer therapy.

Abbreviations

IC_{50} inhibitory concentration (dose causing 50% inhibition of cell growth)

bpy 2,2'-bipyridyl

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50} (\mu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 1 h</td>
</tr>
<tr>
<td>$T2$</td>
<td>54</td>
</tr>
<tr>
<td>$B1$</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4 Cytotoxities of compounds studied as measured by the MTT assay in HeLa cells.

Fig. 4. Treatment time dependence of the cytotoxicity of $T2$ and $B1$, respectively. The arrow indicates the variation of cell viability with the increasing incubation time.

Fig. 5. Comparison of the intracellular molybdenum concentration in HeLa cells after 48 h of exposure to compounds $T2$ and $B1$.

Acknowledgements

The authors are grateful to F. Antunes (FCUL) for providing the cell lines and to F. Martins (FCUL) for inestimable help in octanol partition studies. The authors thank the Portuguese National Mass Spectrometry Network (REDE/1501/REM/2005). MSS (SFRH/BD/48640/2008) thanks FCT for a research grant.

Appendix A. Supplementary data

References


Fig. 6. UV–Vis absorption spectra of T2 (20 μM) in Tris buffer in the presence of increasing amounts of ct DNA [DNA] = 0, 10, 20, 30, 40, and 50 μM. The arrow indicates the absorbance changes upon increasing DNA concentration. The inset is a plot of Δεap vs. D for the titration of DNA to complex. Absorbance was monitored at 299 nm.