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Cobalt and Zinc Compounds Bearing 1,10-Phenanthroline-5,6-dione or 1,3,5-Triaza-7-phosphaadamantane Derivatives – Synthesis, Characterization, Cytotoxicity, and Cell Selectivity Studies

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The compounds [mPTA][$CoCl_4$] (**1**, mPTA = *N*-methyl-1,3,5-triaza-7-phosphaadamantane cation), [$CoCl(H_2O)(DION)_2$]-[BF_4] (**2**, DION = 1,10-phenanthroline-5,6-dione), [$Zn(DION)_2$]-Cl_2 (**3**) and [ZnCl(κO-PTA=O)(DION)][BF_4] (**4**) were synthesized by reaction of $CoCl_2$ with [mPTA]I or DION and $ZnCl_2$ with DION or 1,3,5-triaza-7-phosphaadamantane-7-oxide (PTA=O) and DION, respectively. All complexes are water soluble and have been characterized by IR, far-IR, ^1H, ^{13}C and $^{31}P\{^1H\}$ NMR spectroscopy, ESI-MS, elemental analyses and single-crystal X-ray diffraction structural analysis (for **1**). They were screened against the human tumour cell lines HCT116, HepG2 and MCF7. Complexes **2** and **3** exhibit the highest in vitro cytotoxicity and show lower cytotoxic activities in normal human fibroblast cell line than in HCT116 tumour cell line, which demonstrates their slight specificity for this type of tumour cell.

Introduction

The coordination chemistry of 1,3,5-triaza-7-phosphaadamantane (PTA, Figure 1a) has experienced a rapid expansion owing to the water solubility of its transition-metal complexes,[1] which enables their application, for example, as aqueous phase catalysts,[1,2] antitumour agents[1,3,4,5] or photoluminescent materials.[1,6,7]

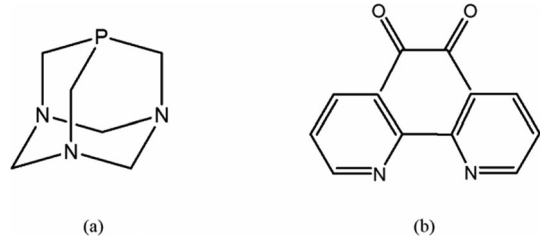

Figure 1. Structures of (a) PTA and (b) DION.

PTA and its derivatives usually display the *P*-coordination mode[1–11] but an increasing number of *N*-,[12–15] *P*,*N*-[16,17] or *P*,*N*,*N*-coordinated[18] PTA complexes have been reported. As expected, the oxide PTA=O does not exhibit the *P*-coordination mode but the *N*-coordination mode instead.[12a]

The *P*-coordinated Co^{III} PTA compound [$Co(NCS)_3$-$(PTA)_3$]·EtOH and the *N*-coordinated Co^{II} PTA=O complex *trans*-[$Co(NCS)_2$(κ*N*-PTA=O)$_2$(H_2O)$_2$] were the first Co compounds with such phosphane ligands.[12a] Other examples of Co and Zn compounds bearing PTA and its derivatives have further been reported.[13,16b,19]

1,10-Phenanthroline-5,6-dione (DION, Figure 1b) is also a versatile ligand for the assembly of metal–organic materials;[20,21] however, its diketone functionality can easily be

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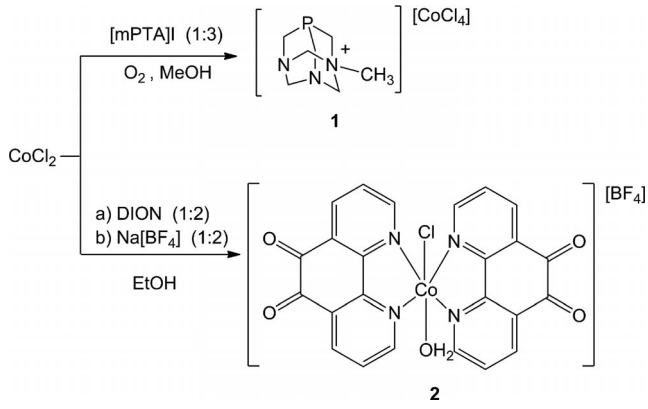
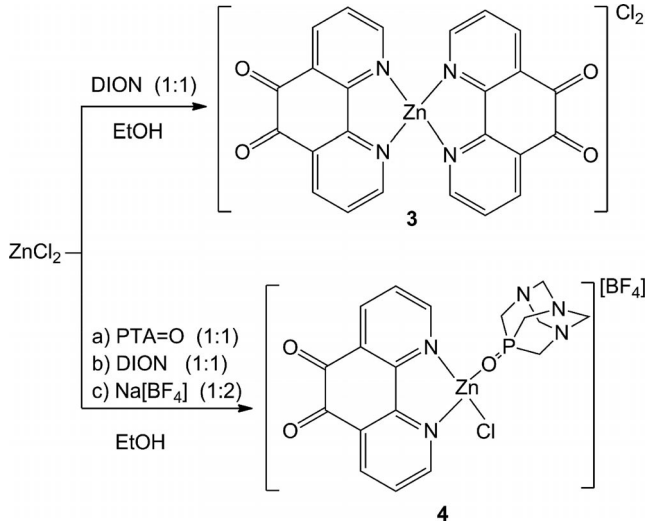
transformed to other chelating groups such as diamines or adioximes.[22] Therefore, DION is relatively prone to give undesirable products either in the course of the main reaction or in the usually slow procedures required to obtain good crystals for X-ray diffraction analysis.

There has been great interest in the antitumour potential of metal complexes since the discovery of the antitumour activity of cisplatin.[23] The lack of selectivity of some anticancer agents towards cancer cells and the development of acquired resistance to the therapeutic drugs are major obstacles to cancer treatment.[24,25] The evaluation of the antitumour potential of newly developed metal complexes is of utmost importance for the establishment of structure–antitumour activity relationships and ultimately for drug development, in an effort to circumvent the main disadvantages of the currently used chemotherapeutic agents. DION is cytotoxic against human kidney and hepatocellular adenocarcinomas and has an estimated IC_{50} value lower than that of cisplatin.[26] This ligand is of great biological interest owing to its redox activity[27] and ability to covalently bind proteins and interact with DNA.[28] Platinum complexes with DION have been reported in the literature[29] and, in some cases, their anticancer and antimicrobial activities have been investigated. Similarly, complexes bearing PTA have shown antitumour activity against mammary adenocarcinoma[30] and human ovarian carcinoma.[31] Owing to possible ligand dissociation in cellular conditions, metal ions that can promote an antitumour response offer the advantage that they are active species on their own. For example, there are studies supporting the ability of zinc and cobalt ions to promote apoptosis.[32]

We now report the synthesis of the new and water-soluble Co and Zn compounds [mPTA][$CoCl_4$] (**1**), [$CoCl(H_2O)(DION)_2$][BF_4] (**2**), [$Zn(DION)_2$]Cl_2 (**3**) and [ZnCl(κ*O*-PTA=O)(DION)][BF_4] (**4**). Considering the known antineoplasic characteristics of both metal ions, the PTA and DION ligands and their biological interest, we performed the screening of the cytotoxicity and the cytoselectivity of all the complexes in this work, as well as of PTA=O and DION.



Scheme 1.



Scheme 2.

Results and Discussion

Synthesis and Spectroscopic Characterization

Treatment of a methanolic or ethanolic solution of $CoCl_2$ with [mPTA]I or DION in the presence of Na[BF_4] at room temperature afforded the Co^{III} compound [mPTA][$CoCl_4$] (**1**) or the Co^{II} complex [$CoCl(H_2O)(DION)_2$][BF_4] (**2**), respectively (Scheme 1).

The reaction of an ethanolic solution of $ZnCl_2$ with DION in the absence or presence of PTA=O gives [$Zn(DION)_2$]Cl_2 (**3**) or [ZnCl(κ*O*-PTA=O)(DION)][BF_4] (**4**), respectively (Scheme 2).

Compounds **1**–**4** were isolated as microcrystalline solids and characterized by IR, far-IR and NMR (**1**, **3** and **4**) spectroscopy, ESI^+-MS (in MeOH and H_2O), elemental analyses and single-crystal X-ray diffraction (for **1**). They are air stable in the solid state and in solution, soluble in H_2O, MeCN, dimethyl sulfoxide (DMSO) and $Me_2C(O)NH_2$, sparingly soluble in MeOH and EtOH, and insoluble in Me_2CO, *n*PrOH, CH_2Cl_2, $CHCl_3$, Et_2O, CCl_4 and C_6H_6. Crystals of **1** suitable for X-ray analysis were obtained upon cooling the reaction solution to 4 °C in air.

The IR spectrum of **1** exhibits the typical set of vibrations of $[mPTA]^+$, whereas that of **2** shows those of coordinated H_2O (3410 and 1574 cm^{-1}) and of the $[BF_4]^-$ counterion (centred at ca. 1071 cm^{-1}). The latter bands are also displayed by **4**. The DION complexes **2**–**4** show characteristic absorptions at 1699, 1574 (**2**), 1702, 1572 (**3**) and 1697, 1575 (**4**) cm^{-1} assigned to ν(CO), which indicate the chelation of the ligand through the N atoms. The IR spectrum of **4** shows the presence of the P=O group with a band at 1132 cm^{-1}, shifted to lower wavenumbers compared to that of the free PTA=O (1165 cm^{-1}) in accord with the *O*-coordination of the phosphane ligand.[12a]

The ^1H and $^{31}P\{^1H\}$ NMR spectra of **1** in DMSO reveal the expected resonances of free $[mPTA]^+$, although they are broadened owing to the paramagnetism of the anionic

metal component. For instance, the $^{31}P\{^1H\}$ NMR spectrum of **1** exhibits a broad resonance centred at δ –87.3 ppm, a value similar to that of the free mPTA cation.[13] The $^{31}P\{^1H\}$ NMR spectrum of **4** shows a singlet at δ –2.4 ppm, which corresponds to the coordinated PTA=O ligand.[12a]

In the ESI$^+$-MS spectrum of **2** in H_2O/MeOH, the $[CoCl(DION)_2]^+$ ion is observed. In the spectra of the Zn compounds **3** and **4**, the fragments $[Zn(DION)Cl]^+$ and $[Zn(DION)(PTA=O)]^+$, respectively, are detected.

X-ray Crystal Structure

The structure of **1** was determined by single-crystal X-ray diffraction analysis. The perspective drawing of the cobalt complex anion and the $[mPTA]^+$ counterion is shown in Figure 2, selected bond lengths and angles are given in the caption, and the crystallographic data are summarized in Table 1. The coordination environment around the Co^{III} atom is, according to the Houser criteria,[33] an almost perfect tetrahedron (τ_4 = 0.95) filled by four chloride anions, and the Co–Cl bond lengths range from 2.3618(13) to 2.2693(19) Å. Most of the bonding parameters in the $[mPTA]^+$ cation are analogous to those reported for other similar salts, such as $[mPTA]_2[ZnI_2Cl_2]$[13] or $[mPTA]_2[Co(NCS)_4]$,[12a] and the C–N bonds of the alkylated N11 atom (av. 1.578 Å) are longer than those of the other N atoms (av. 1.462 Å). Moreover, the cations are positioned relatively close to the Co complex anions (minimum Co···P distance of 4.908 Å), which enables nonclassic hydrogen-bond interactions of the type C–H···Cl in the 3.681(7)–3.801(5) Å range.

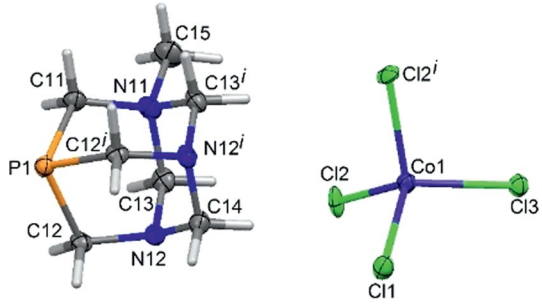


Figure 2. Molecular structure of **1** with atomic numbering scheme. Selected bond lengths [Å] and angles [°]: C11–P1 1.874(7), C12–P1 1.848(5), C11–N11 1.503(10), C13–N11 1.543(7), C15–N11 1.481(10), C12–N12 1.480(6), C13–N12 1.438(7), C14–N12 1.467(6), Cl1–Co1 2.2693(19), Cl2–Co1 2.3618(13), Cl3–Co1 2.2700(18); N11–C11–P1 113.7(5), N12–C12–P1 114.7(3), Cl3–Co1–Cl1 111.69(7), Cl3–Co1–Cl2 107.37(4). Symmetry operation for equivalent atoms: (*i*) 1/2 – *x*, *y*, *z*.

Table 1. Crystallographic data for **1**.

	1
Empirical formula	$C_7H_{15}Cl_4CoN_3P$
Formula weight	372.92
Crystal system	orthorhombic
Space group	*Pmnb* (No. 62)
a [Å]	9.1817(2)
b [Å]	14.0870(4)
c [Å]	17.3613(3)
V [Å^3]	2245.56(9)
Z	4
T [K]	150
Density (calcd.) [Mg/m^3]	1.103
μ [mm^{-1}]	1.297
F(000)	752.0
Unique reflections	2175
Observed reflections	1883
R_{int}	0.0392
R_1,[a] wR_2[b] [$I > 2\sigma(I)$]	0.0633, 0.1991
R_1,wR_2 (all data)	0.0696, 0.2037
Goodness-of-fit on F^2	1.075

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = [\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]]^{1/2}$.

Viability Assays

The cytotoxic potential of the compounds was investigated by the quantification of cell viability after the exposure of cells to each of the complexes for an incubation period of 48 h. All complexes induced a decrease in cellular viability in a dose-dependent manner (Figures 3 and S1), and our data clearly demonstrate that complexes bearing DION as a ligand (**2** and **3**) are much more active against the tested neoplastic cell lines than the mPTA compound **1** (Figures 3 and S1, Table 2).

Indeed, as observed in Table 2, complex **4**, which has one PTA=O and one DION ligands, has a higher IC_{50} value for all of the tested tumour cell lines (0.373 ± 0.067, 2.45 ± 0.49 and 1.68 ± 0.22 μM for HCT116, HepG2 and MCF-7 cell lines, respectively) than **2** (0.206 ± 0.023, 0.582 ± 0.034 and 0.69 ± 0.25 μM for HCT116, HepG2 and MCF-7 cell lines, respectively) and **3** (0.217 ± 0.022, 0.978 ± 0.036 and 0.73 ± 0.18 μM for HCT116, HepG2 and MCF-7 cell lines, respectively), both of which contain two DION molecules. To confirm that the observed cytotoxic potential originated from the metal compounds and not the ligand itself, we have also performed MTS assays for free PTA=O, [mPTA]I and DION [Figure 4, MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium].

DION has a much higher cytotoxicity than free m[PTA]-I and PTA=O for both HCT116 and HepG2 tumour cell lines, corresponding to a much lower IC_{50} value (Figure 4 and Table 2). Nevertheless, the IC_{50} values for **2** and **3** for HCT116, HepG2 and MCF-7 tumour cell lines are two to three times lower than the corresponding values for DION (Figures 3 and 4, Table 2). DION was previously shown by Deegan and collaborators[26] to be more cytotoxic than cisplatin, which is also in agreement with our results (Table 2 and Figure S2), and to affect tumour and nonmalignant cells equally. These authors also showed its ability to inhibit DNA synthesis based on an intercalation-independent mechanism.[26] The cytotoxic activity of ruthenium complexes bearing PTA in breast adenocarcinoma cell lines[30]

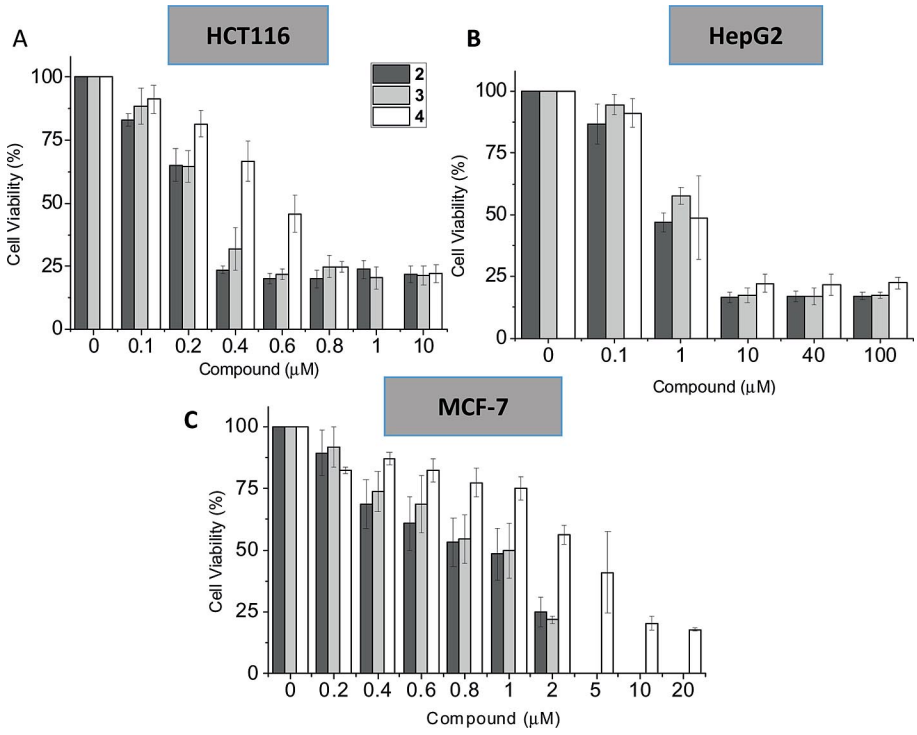


Figure 3. Effect of **2** (dark grey bars), **3** (light grey bars) and **4** (white bars) on the cellular viability of human colorectal (HCT116, A), hepatocellular (HepG2, B) and breast (MCF-7, C) carcinoma cell lines. Cells were incubated for 48 h in the presence of water (0 μM; vehicle control) or each of the complexes. The results are expressed as mean ± standard error of the mean (SEM) percentage compared to controls from at least three independent experiments. All the results are statistically significant with $p < 0.01$ (as compared to control for each compound and cell line).

Table 2. IC_{50} values for **1**, **2**, **3**, **4**, DION, PTA=O and [mPTA]I on human colorectal (HCT116), hepatocellular (HepG2) and breast (MCF-7) carcinoma cell lines. The results are expressed as mean ± SEM from at least three independent experiments.

Tumour cell line	Compound	IC_{50} (SEM) [μM][a]
HCT116	**1**	540 (14)
	2	0.206 (0.023)
	3	0.217 (0.022)
	4	0.373 (0.067)
	DION	0.68 (0.14)
	PTA=O	n.d.
	[mPTA]I	>1000 (20)
HepG2	**1**	413 (28)
	2	0.582 (0.034)
	3	0.978 (0.036)
	4	2.45 (0.49)
	DION	1.99 (0.44)
	PTA=O	n.d.
	[mPTA]I	>1500 (25)
MCF-7	**2**	0.69 (0.25)
	3	0.73 (0.18)
	4	1.68 (0.22)
	DION	1.28 (0.13)
	PTA=O	n.d
	[mPTA]I	n.d

[a] n.d.: not detected for the studied concentrations.

and their ability to interact with DNA was also previously reported.[34]

Compounds **2** and **3**, with two DION ligands, are the most active ones, in particular, against HCT116 tumour cell line (Figure 3 and Table 2). Indeed, in our tested conditions, their IC_{50} values in HCT116 tumour cell line [0.206 ± 0.023 (**2**) and 0.217 ± 0.022μM (**3**)] are ca. 70 and two times lower than the IC_{50} values for the commonly used antitumour drugs cisplatin (15.2 ± 0.55 μM, Figure S2) and doxorubicin (0.49 ± 0.08 μM, Figure S2), respectively. Their water solubility may also allow direct parenteral administration. With the exception of the HCT116 tumour cell line, for which both the cobalt complex **2** and the zinc complex **3** show similar IC_{50} values (Table 2), **2** displays a more cytotoxic potential than **3** (Figure 3 and Table 2).

To gain more insights into the possible in vitro selectivity of **2** and **3** for tumour cells, MTS assays were also performed in normal human fibroblasts (Figure 5). The cytotoxicity of **2**, **3** and **4** and of the free DION ligand was lower in normal fibroblasts than in HCT116 tumour cells (Figure 5). Indeed, there are still 84 and 77.5% of viable fibroblasts for **2** and **3** (at a concentration of 0.5 μM), respectively, and 83.4 and 94% of viable fibroblasts for **4** and DION (at 1 μM), respectively (Figure 5). The IC_{50} values of **2** and **3** in the normal fibroblasts are 0.62 and 0.60 μM, respectively. Interestingly, at the concentrations of **2** and **3** corresponding to their IC_{50} values for the HCT116 tumour cell line, there are 94.1 and 89.4% of viable fibroblasts, respectively (Table 2 and Figure 5).

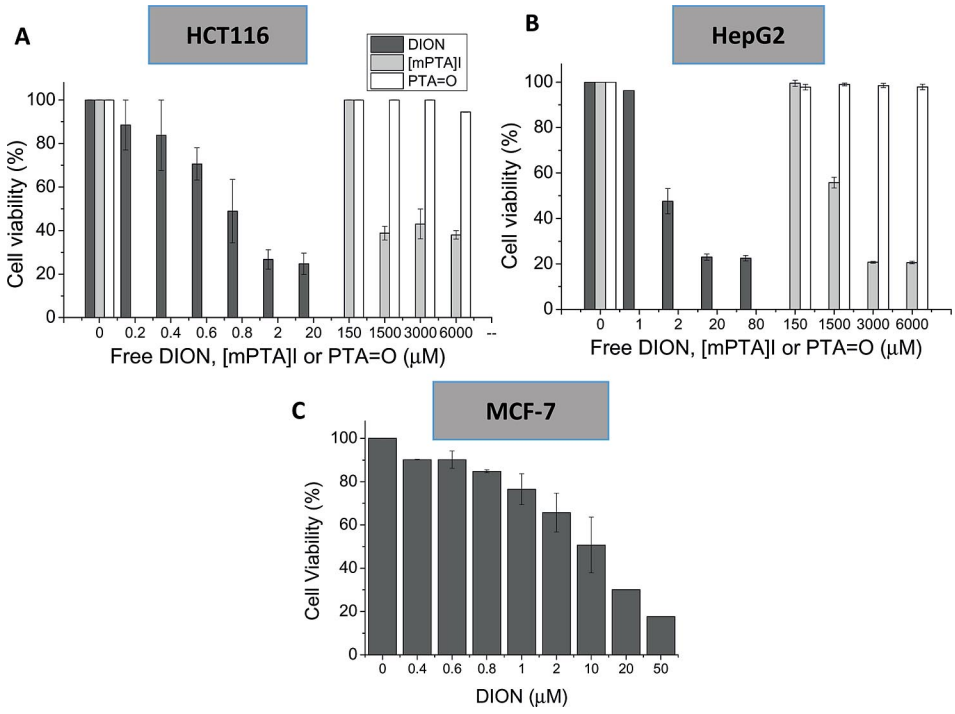


Figure 4. Effect of free DION (dark grey bars), [mPTA]I (light grey bars) and PTA=O on the cellular viability of three human tumour cell lines: colorectal carcinoma (HCT116, A), hepatocellular carcinoma (HepG2, B) and breast carcinoma (MCF-7, C). Cells were exposed to water (vehicle control, 0 μM) or each one of the free ligands for 48 h. The results are expressed as mean ± SEM percentage compared to controls from at least three independent experiments. The results are statistically significant with $p < 0.01$ (as compared to control for each compound and cell line).

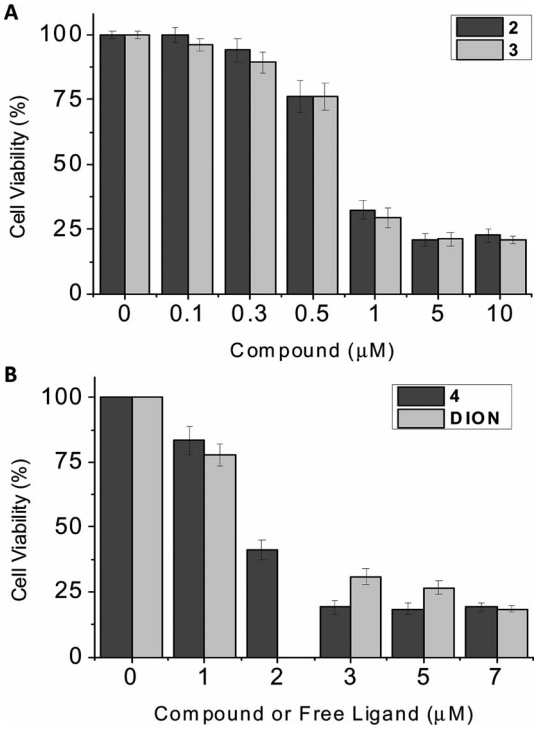


Figure 5. Effect of **2**, **3** (A), **4** and the free DION ligand (B) on the cellular viability of normal fibroblast cell lines. Cells were exposed to water (vehicle control, 0 μM) or to each of the compounds for 48 h. The results are expressed as mean ± SEM percentage compared to controls from at least three independent experiments. The results are statistically significant with $p < 0.01$ (as compared to control for each compound and cell line).

Conclusions

We have synthesized and characterized new Co and Zn compounds with 1,10-phenanthroline-5,6-dione or 1,3,5-triaza-7-phosphaadamantane derivatives. Their solubility and stability in water encouraged us to test their biological activity.

Our results for the high cytotoxicity of DION in culture medium are in agreement with previous work.[26] Nevertheless, the water-soluble cobalt and zinc complexes bearing DION (**2** and **3**, respectively) exhibit the highest in vitro cytotoxicity, which is higher than those observed in our conditions and also described by others for cisplatin and doxorubicin (Figure S2).[35,36]

These in vitro antiproliferative activities are much higher than that exhibited by cisplatin, and **2** and **3** show lower cytotoxic activities in normal human fibroblast cell line (IC_{50} = 0.62 and 0.60 μM, respectively) than in HCT116 tumour cell line (IC_{50} = 0.205 and 0.206 μM, respectively), which demonstrates their slight specificity for this type of tumour cell and is a positive feature for further development.

Although HCT116, HepG2 and MCF-7 are epithelial-type tumour cells, these results, particularly those for **2**, are very promising and viability assays will also be performed with normal breast epithelial cell line (MCF10A) to confirm the in vitro specificity of **2** and **3** to colorectal carcinoma cell lines.

Experimental Section

General Materials and Procedures: Syntheses of all complexes were performed in air at room temperature. $CoCl_2$ (Aldrich), $CoCl_2·6H_2O$ (Aldrich), $ZnCl_2$ (Aldrich) and $Na[BF_4]$ (Aldrich) were used as received, and DION,[20] PTA=O[37] and [mPTA]I[38] were synthesized in accordance with literature methods. IR spectra (4000–400 cm^{-1}) were recorded with a BIO-RAD FTS 3000 MX spectrophotometer with samples as KBr pellets; wavenumbers are in cm^{-1}; abbreviations: vs, very strong; s, strong; m, medium. Far-IR (400–200 cm^{-1}) spectra were recorded with a Vertex 70 spectrophotometer with samples as CsI pellets. ^1H, ^{13}C and ^{31}P NMR spectra were recorded with Bruker Avance II+ 300 and 400 MHz (Ultra Shield TM Magnet) spectrometers. Chemical shift (δ) values are in ppm relative to $SiMe_4$ (^1H and ^{13}C) or 85% H_3PO_4 (^{31}P). Coupling constants are in Hz; abbreviations: s, singlet; d, doublet; t, triplet; m, complex multiplet; br, broad. ESI^+/ESI^- mass spectra were obtained with a VARIAN 500-MS LC ion trap mass spectrometer [solvent: H_2O, MeOH; flow: 20 μL/min; needle spray voltage: ± 5 kV, capillarity voltage: ± 100 V; nebulizer gas (N_2): 35 psi; drying gas (N_2): 10 psi; drying gas temperature (N_2): 350 °C]. The C, H, N elemental analyses were performed by the Microanalytical Service of the Instituto Superior Técnico.

[mPTA][CoCl$_4$] (1): To a methanolic solution (15 mL) of anhydrous $CoCl_2$ (200 mg, 1.55 mmol) was added a methanolic solution (10 mL) of [mPTA]I (1.39 g, 4.65 mmol). The reaction mixture was stirred at room temperature for 24 h. The microcrystalline pink product was collected by filtration and dried in vacuo. Crystals suitable for single-crystal X-ray diffraction were obtained by slow evaporation of a MeOH/DMSO solution, yield 21.0% (120.7 mg). Complex is soluble in H_2O ($S_{25\,°C} \approx 2.0\ mg\,mL^{-1}$) and DMSO. $C_7H_{15}Cl_4CoN_3P·H_2O$ (390.95): calcd. C 21.51, H 4.38, N 10.75; found C 21.71, H 4.30, N 11.10. MS (ESI+): m/z = 172 $[mPTA]^+$. IR (KBr): $\tilde{\nu}$ = 2964, 2950, 1453 (m), 1406 (s), 1313 (m) 1291 (m), 1119 (m), 1093 (s br), 1022 (s), 983 (m), 918 (m), 897 (m), 814 (m), 556 (br, mPTA bands) cm^{-1}. Far-IR (CsI): $\tilde{\nu}$ = 328, 292, 264, 248 cm^{-1}. ^1H NMR ([D$_6$]DMSO): δ = 2.61 (s, 3 H, N^+CH_3), 3.76 and 3.95 [$^2J_{H^A,H^B}$ = 14, $^2J_{H^A,P}$ = 7.6, $^2J_{H^B,P}$ = 3.6 Hz, 4 H, PCH^AH^BN], 4.34 and 4.54 [$^2J_{H^A,H^B}$ = 12.8 Hz, 2 H, NCH^AH^BN], 4.41 (s, 2 H, PCH_2N^+), 4.86 and 4.95 [$^2J_{H^A,H^B}$ = 11.9 Hz, 4 H, $NCH^AH^BN^+$] ppm. $^{13}C\{^1H\}$ NMR ([D$_6$]DMSO): δ = 46.2 (s, PCH_2N), 48.6 (s, N^+CH_3), 55.3 (s, PCH_2N^+), 68.5 (s, NCH_2N), 79.4 (s, NCH_2N^+) ppm. $^{31}P\{^1H\}$ NMR ([D$_6$]DMSO): δ = –87.3 (s) ppm.

[CoCl(H$_2$O)(DION)$_2$][BF$_4$] (2): To an ethanolic solution (10 mL) of $CoCl_2·6H_2O$ (52 mg, 0.219 mmol) was added an ethanolic solution (10 mL) of 1,10-phenanthroline-5,6-dione (99.6 mg, 0.44 mmol). A saturated solution of $Na[BF_4]$ (47.9 mg, 0.44 mmol) in ethanol was added, and the reaction mixture was stirred at room temperature for 24 h. The brown product was collected by filtration, washed with Et_2O (3× 5 mL) and dried in vacuo, yield 44.6% (60.5 mg). Complex is soluble in H_2O ($S_{25\,°C} \approx 2.2\ mg\,mL^{-1}$), CH_2Cl_2 and DMSO. $C_{24}H_{15}BClCoF_4N_4O_5·1/4(C_{12}H_6N_2O_2)$ (671.51): calcd. C 48.25, H 2.33, N 9.38; found C 48.13, H 2.37, N 9.18. MS (ESI+): m/z = 514 $[CoCl(DION)_2]^+$, 239 $[Co(DION)_2]^+$. IR (KBr): $\tilde{\nu}$ = 3410 [br, ν(H_2O)], 1574 [br, ν(H_2O)], 1690 [ν(C=O)], 1514 [ν(C=N)], 1426 (s), 1306 (m) 1257 (m), 1210 (m), 1071 [s, ν(B–F), BF_4], 1021 (s br), 935 (s), 841 (m), 735 (m), 708 (m, DION bands) cm^{-1}. Far-IR (CsI): $\tilde{\nu}$ = 225 [ν(Co–Cl)]cm^{-1}.

[Zn(DION)$_2$]Cl$_2$ (3): To an ethanolic solution (10 mL) of $ZnCl_2$ (52.2 mg, 0.39 mmol) was added a yellow ethanolic solution (20 mL) of 1,10-phenanthroline-5,6-dione (89 mg, 0.39 mmol). The yellow reaction mixture was stirred at room temperature for 24 h. The yellow product was collected by filtration and dried in vacuo, yield 42.0% (90.7 mg). Complex is soluble in H_2O ($S_{25\,°C} \approx 2.0\ mg\,mL^{-1}$) and DMSO. $C_{24}H_{12}Cl_2N_4O_4Zn·1/2H_2O$ (562.96): calcd. C 51.16, H 2.33, N 9.95; found C 51.17, H 2.29, N 9.66. MS (ESI+): m/z = 372 $[Zn(DION)Cl]^+$, 242 $[Zn(DION)_2]^+$. IR (KBr): $\tilde{\nu}$ = 1702 [m, ν(C=O)], 1572 [m, ν(C–N)], 1474 (s), 1423 (m) 1313 (m), 1210 (m), 1097 (s br), 1072 (s), 1021 (m), 933 (m), 842 (m), 737 (m), 691 (s), 633 (s), 547 (s, DION bands) cm^{-1}. ^1H NMR (D_2O): δ = 8.88 (br., s, 2 H), 8.46 (br., s, 2 H), 7.66 (br., s, 4 H) ppm.

[ZnCl(κ*O*-PTA=O)(DION)][BF$_4$] (4): To an ethanolic solution (10 mL) of $ZnCl_2$ (29.0 mg, 0.219 mmol) were added PTA=O (37.9 mg, 0.219 mmol) and, after 20 min, an ethanolic solution (10 mL) of 1,10-phenanthroline-5,6-dione (49.8 mg, 0.22 mmol). A saturated solution of $Na[BF_4]$ (47.9 mg, 0.44 mmol) in ethanol was added, and the reaction mixture was stirred at room temperature for 24 h. The brown product was collected by filtration, washed with Et_2O (3× 5 mL) and dried in vacuo, yield 59.3% (73.9 mg). Complex is soluble in H_2O ($S_{25\,°C} \approx 2.2\ mg\,mL^{-1}$), CH_2Cl_2 and DMSO. $C_{18}H_{18}BClF_4N_5O_3PZn$ (569.02): calcd. C 37.96, H 3.19, N 12.30; found C 38.55, H 3.78, N 12.87. MS (ESI): m/z = 309 $[ZnCl(DION)]^+$, 271 $[ZnCl(PTA=O)]^+$, 447 $[Zn(DION)(PTA=O)]^+$. IR (KBr): $\tilde{\nu}$ = 1697 [m, ν(C=O)], 1576 [m, ν(C–N)], 1471 (s), 1283 [m, ν(C–H)], 1132 [s, ν(P=O)], 1020 [s, ν(B–F), BF_4], 972 (m), 904 (m), 806 (s br), 738 (s), 578 (m, PTA=O and DION bands) cm^{-1}. Far-IR (CsI): $\tilde{\nu}$ = 290 [ν(Zn–Cl)] cm^{-1}. ^1H NMR (MeOD): δ = 8.79 (br., s, 1 H), 8.65 (br., s, 2 H), 8.53 (br., s, 1 H), 7.92 (br., s, 2 H), 4.39 and 4.25 [$^2J_{H^A,H^B}$ = 14 Hz, 6 H, NCH^AH^BN], 3.98 [d, $^2J_{P,H}$ = 10.4 Hz, 6 H, PCH_2N] ppm. $^{31}P\{^1H\}$ NMR (D_2O): δ = –2.4 (s) ppm.

X-ray Structure Determination: A crystal of **1** was immersed in Cryo Oil, mounted in a Nylon loop and measured at a temperature of 150 K. Intensity data were collected with a Bruker AXS-KAPPA APEX II diffractometer with graphite monochromatic Mo-K_α (λ = 0.71073) radiation. Data were collected by using omega scans of 0.5° per frame, and a full sphere of data was obtained. The cell parameters were retrieved by using Bruker SMART software and refined by using Bruker SAINT[39a] on all the observed reflections. Absorption correction was applied by using SADABS.[39a] The structure was solved by direct methods by using the SHELXS-97 package[39b] and refined with SHELXL-97.[39b] Calculations were performed by using the WinGX system, version 1.80.03.[39c] All hydrogen atoms were inserted in calculated positions. There were disordered solvent molecules present in the structure. As no clear major site occupations were found for those molecules, it was not possible to model them. PLATON/SQUEEZE[39d] was used to correct the data, and a potential volume of 903 $Å^3$ was found with 232 electrons per unit cell worth of scattering. The electron count suggests the presence of one molecule of DMSO and one of methanol per unit cell. Least square refinements with anisotropic thermal motion parameters for all the non-hydrogen atoms and isotropic motion parameters for most of the remaining atoms were employed. CCDC-918591 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Cell Culture: HCT116 human colorectal carcinoma cells were grown in Dulbecco's modified Eagle's medium (DMEM, Invitrogen Corp., Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Invitrogen Corp.) and maintained at 37 °C in a humidified atmosphere of 5% CO_2. HepG2 human hepatocellular carcinoma cells were grown in

similar conditions, supplemented with 1% MEM nonessential amino acid (Invitrogen Corp.). MCF-7 cells were derived from pleural effusion of breast adenocarcinoma from a female patient and were grown in the same conditions and medium as the HepG2 cells. This cell line was kindly provided by A. S. Rodrigues [Human Molecular Genetics Research Centre (CIGMH), Department of Genetics, Faculty of Medical Sciences, Universidade Nova de Lisboa, Portugal]. Normal human fibroblasts were grown in the same conditions as the HepG2 and MCF-7 cell lines and were kindly provided by I. Carreira (Laboratory of Cytogenetics and Genomics, Faculty of Medicine, University of Coimbra, Portugal).

Compound Exposure for Dose-Response Curves: Cells were plated at 5000 cells/well in 96-well plates. Media was removed 24 h after platting and replaced with fresh media containing: 100–2000 μM of **2** and **1**, 0.1–100 μM of **4** and **3**, 0.2–80 μM of DION, 1000–6000 μM of PTA or sterile water (vehicle control; 0 μM). To assure a maximum volume of compound solution in a culture medium of 5% (v/v), stock solutions of each compound were prepared in sterile double distilled water whenever necessary.

Cisplatin [*cis*-diamineplatinum(II) dichloride, ≥99.9% trace metals basis] and doxorubicin hydrochloride (98.0–102.0%) were purchased from Sigma.

Viability Assays: After 48 h of cell incubation in the presence or absence of each compound, cell viability was evaluated with CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) by using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS). In brief, this is a homogeneous, colorimetric method to determine the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. The CellTiter 96 AQueous Assay is composed of solutions of MTS and an electron coupling reagent (phenazinemethosulfate, PMS). MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan product at 490 nm can be measured directly from 96-well assay plates without additional processing. The conversion of MTS into the aqueous soluble formazan product is accomplished by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product was measured with a Bio-Rad microplate reader Model 680 (Bio-Rad, Hercules, CA, USA) at 490 nm, as absorbance is directly proportional to the number of viable cells in culture.

Supporting Information (see footnote on the first page of this article): Effect of **1** on the cellular viability of human colorectal (HCT116) and hepatocellular HepG2 carcinoma cell lines (Figure S1), effect of cisplatin on the cellular viability of human colorectal (HCT116), adenocarcinoma (MCF7) and hepatocellular (HepG2) carcinoma cell lines, and effect doxorubicin on the cellular viability of human colorectal (HCT116) carcinoma cell line (Figure S2).

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