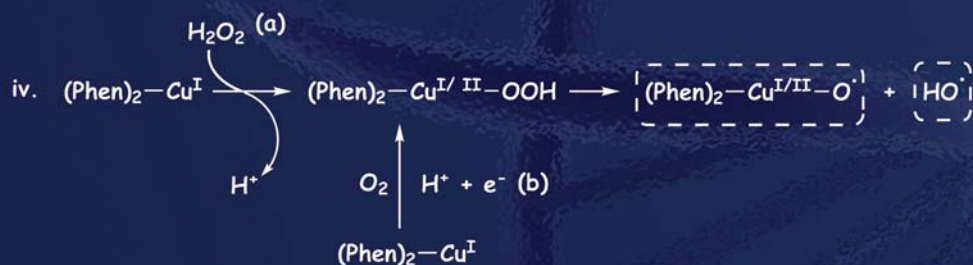
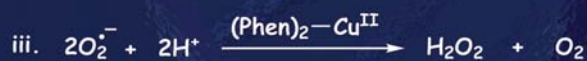
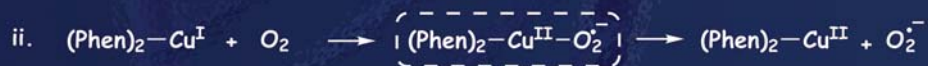
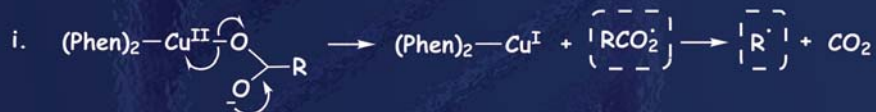
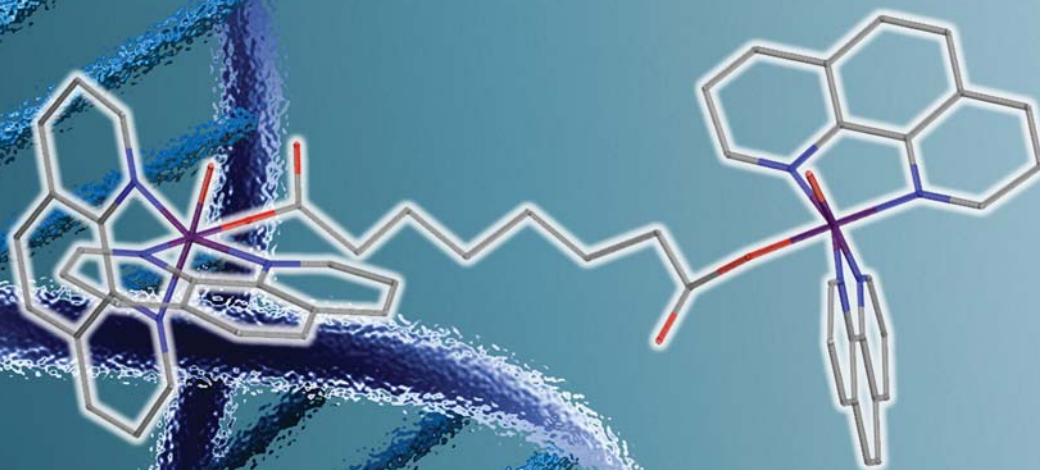


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CONCISE ARTICLE

Water-soluble bis(1,10-phenanthroline) octanedioate Cu²⁺ and Mn²⁺ complexes with unprecedented nano and picomolar *in vitro* cytotoxicity: promising leads for chemotherapeutic drug development†Andrew Kellett,^{‡*a} Mark O'Connor,^a Malachy McCann,^b Orla Howe,^a Alan Casey,^a Pauraic McCarron,^{ab} Kevin Kavanagh,^c Mary McNamara,^a Sean Kennedy,^a Donald D. May,^d Philip S. Skell,^e Denis O'Shea^a and Michael Devereux^{*a}

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Dinuclear Cu²⁺ and Mn²⁺ bis-phenanthroline octanedioate compounds exhibit rapid, unprecedented nano and picomolar *in vitro* cytotoxicity against human-derived colorectal cancer lines (HT29, SW480 and SW620) and are less cytotoxic toward non-cancerous normal human keratinocyte cells (HaCaT). Both complexes displayed greater *in vivo* drug tolerance compared to cisplatin when examined using the insect *Galleria mellonella*. The compounds are potent generators of intracellular reactive oxygen species within HT29 cells, display avid DNA binding and induce O₂-dependent cleavage of supercoiled pUC18 DNA. The Cu²⁺ complex was found to display self-cleaving nuclease activity and a mechanism of deoxyribose C–H bond activation is proposed, based on interactions with the superoxide anion and hydrogen peroxide along with DNA cleavage observations under anaerobic conditions and with an excess of the metal chelator EDTA.

Introduction

The development of self-cleaving chemical nucleases is regarded as the paradigm of redox active metal-based chemotherapeutics. DNA targeting agents capable of inducing single stranded or double stranded scission have found clinical application within cancer chemotherapy.¹ Other applications within this class of compound include, the probing of DNA-specific structures, mapping of protein and DNA interactions, gene regulation and signal transduction.^{2,3} Thus, explorations toward the discovery and development of natural or synthetic chemical nucleases are major topics of interest. Redox-active, transition-metal-based chemical nucleases are particularly important due to their

potential to catalytically support the one-electron oxidation/reduction reactions necessary to drive activation of C–H deoxyribose bonds.

In the presence of Cu²⁺, the oxidative formation of π radical cations within marine-based products, tambjamine E,⁴ prodigiosin⁵ and pyrimol,⁶ have recently been shown to mediate self-cleaving DNA damage, *i.e.* scission which does not require the presence of added oxidant or reductant. These Cu²⁺ compounds have also demonstrated significant *in vitro* chemotherapeutic potential against leukaemia and ovarian cancer cells, some of which were resistant to cisplatin.^{5,6}

The discovery of the first synthetic chemical nuclease, [Cu(phen)₂]²⁺ (phen = 1,10-phenanthroline, Fig. 1), has sparked intensive effort toward the development of new bis(phen) agents with enhanced DNA cleaving ability.⁷ The DNA cleaving limitations of [Cu(phen)₂]²⁺ include, (i) a high dissociation constant of the second coordinated phen ligand⁸ and (ii) the need for exogenous reductant to generate the active Cu⁺ species, [Cu(phen)₂]⁺. The dissociation problem was solved by Meunier, Pitie *et al.*^{9–11} through the advent of clip-phen, whereby two phen

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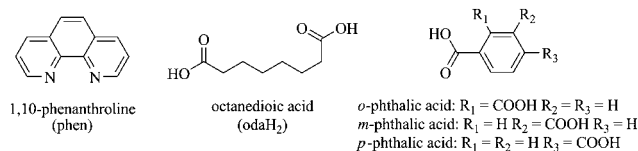


Fig. 1 Molecular structures of phen, odaH₂ and the phthalates.

ligands are connected at the 2' or 3' position by a serinol bridge. Recently, we have reported the first self-cleaving bis-phen system, $[\text{Cu}(\text{phen})_2(\text{phthalate})]$ (phthalate = *o*-, *m*-, *p*-phthalate, Fig. 1), capable of inducing single-stranded DNA scission in the absence of exogenous reductant or oxidant.¹²

While mononuclear $[\text{Cu}(\text{phen})_2(\text{phthalate})]$ complexes displayed excellent chemotherapeutic potential against colon (HT29), breast (MC-F7) and prostate (DU145) cancer lines, their water solubility is poor. Of the phthalate group of Cu^{2+} complexes the cationic, dinuclear species $[\text{Cu}_2(\text{phen})_4(\mu_2\text{-}p\text{-phthalate})]^{2+}$ proved to be the most active DNA-binding, self-cleaving chemotherapeutic agent. To that end, in the current study, we have investigated the application of water soluble Cu^{2+} and Mn^{2+} dinuclear, cationic, bis-phen octanedioate (oda) (Fig. 1) systems as nuclease mimetics and determined their ability to induce cancer cell death through the redox-generation of reactive oxygen species (ROS). Arguments for the possible formation of a π carboxyl radical within the Cu^{2+} system, which cleaves DNA by self-activation, are also presented.

$[\text{Cu}_2(\mu_2\text{-oda})(\text{phen})_4(\text{ClO}_4)_2]$ (**1**) (Fig. 2), $\{[\text{Mn}_2(\mu_2\text{-oda})(\text{phen})_4(\text{oda})_2]^{2-}[\text{Mn}_2(\mu_2\text{-oda})(\text{phen})_4(\text{H}_2\text{O})_2]^{2+}\}$ (**2**) (Fig. 3), were prepared according to the literature methods.^{13,14} The coordination environment about each of the Cu^{2+} ions in **1** is approximately square-pyramidal with the metals being linked *via* a bridging oda^{2-} ligand. In the double complex salt, **2**, the environment about each Mn^{2+} is octahedral with both metals in the cation and anion being bridged oda^{2-} in a similar fashion to **1**. Whereas each of the Mn^{2+} centres of the anionic subunit in **2** contain a unidentate oda^{2-} ligand coordinated in the apical position, the cationic unit contains bound water at these equivalent positions.

DNA binding studies

In order to examine the interaction of compounds **1** and **2** with DNA, competitive ethidium bromide displacement and fluorescence quenching experiments with calf thymus DNA (CT-DNAp) were conducted (Table 1).[†] Complexes **1** and **2** were found to have high apparent DNA binding constants (K_{app}), and low fluorescence quenching values (Q), indicating that both systems have a high affinity for binding to DNA. High Q values (>20 μM) are generally found for “classical” DNA intercalants, whereas lower values between 2–15 μM are typically obtained for minor groove ligands or hybrid molecules.^{15,16}

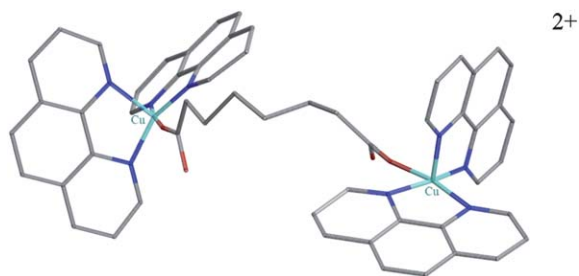


Fig. 2 Molecular structure of the $[\text{Cu}_2(\mu_2\text{-oda})(\text{phen})_4]^{2+}$ cation in complex **1**.

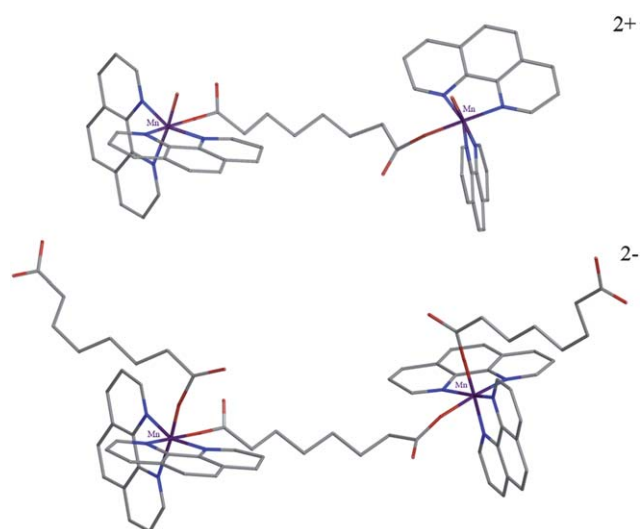


Fig. 3 Molecular structures of the dimeric cation and anion subunits in the Mn^{II} double salt complex $\{[\text{Mn}_2(\mu_2\text{-oda})(\text{phen})_4(\text{oda})_2]^{2-}[\text{Mn}_2(\mu_2\text{-oda})(\text{phen})_4(\text{H}_2\text{O})_2]^{2+}\}$ (**2**).

DNA cleavage reactions

The relaxation of supercoiled pUC18 DNA (SC, Form I) into open circular (OC, Form II) and linear (LC, Form III) conformations was used to quantify the relative cleavage efficiency of **1** and **2**.[†] To investigate the DNA self-cleaving ability of complexes **1** and **2**, SC DNA was exposed to both complexes over a concentration range of 1–50 μM for 20 h in the absence of added H_2O_2 or reductant (Fig. 4(a)). Only complex **1** exhibits concentration-dependant self-cleaving of SC DNA (Form I) to OC (Form II), with complete depletion of the parent SC band (I \rightarrow II) at 20 μM (lane 5 (a)). In the presence of added reductant (ascorbate) (Fig. 4(b)) both complexes exhibit enhanced DNA scission during a shorter time-frame (2 h) and at a lower concentration of added complex. While both **1** and **2** induced complete relaxation to OC Form II (lanes 4 and 8), only the Cu^{2+} complex, **1**, was found to induce efficient double stranded scission (I \rightarrow III) at a concentration of 20 μM , which was evident by the absence of a band in lane 5. In order to identify what role molecular O_2 and the metal ions ($\text{Cu}^{2+}/\text{Mn}^{2+}$) play within DNA cleavage, experiments were conducted under anaerobic conditions in an atmosphere saturated with argon and, separately, in the presence of 100 mM Na_2EDTA , under aerobic conditions

Table 1 Apparent DNA binding constants (K_{app}) and fluorescence quenching (Q) values for **1** and **2**.[‡] Assay conditions; C_{50}/K_{app} : final volume 2 mL, 1.2 μM EtBr, 1 μM CT-DNAp, 10 mM TES, 0.1 mM Na_2EDTA , pH 7.0; Q : final volume 2 mL, 2.0 μM EtBr, 20 μM CT-DNAp, 2 mM NaOAc buffer, 9.3 mM NaCl, 0.1 mM Na_2EDTA , pH 5.0

Complex	C_{50}^a (μM)	K_{app}^b	Q^c
1	46.89	2.55×10^5	22.64
2	28.46	4.20×10^5	43.13

^a C_{50} = concentration required to reduce fluorescence by 50% (competitive). ^b $K_{\text{app}} = K_e \times 1.26/C_{50}$ where $K_e = 9.5 \times 10^6 \text{ M (bp)}^{-1}$. ^c Q = equivalent concentration required to reduce fluorescence by 50% (quenching).

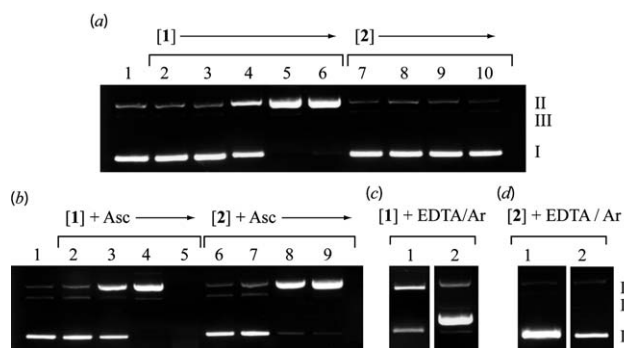


Fig. 4 Relaxation of pUC18 by **1** and **2**.† Cleavage was carried out at 37 °C then analyzed by agarose gel electrophoresis (a) 20 h incubation in the absence of added oxidant or reductant. Lane 1: DNA alone; lanes 2–6: 1, 5, 10, 20, 50 μM **1**; lanes 7–10: 5, 10, 20, 50 μM **2**. (b) 2 h incubation in the presence of added ascorbate (at twice the complex concentration). Lane 1: DNA alone; lanes 2–5: 1, 5, 10, 20 μM **1**; lanes 6–9: 1, 5, 10, 20 μM **2**. (c) 20 h incubation of 20 μM **1** in the absence of added oxidant or reductant. Lane 1: **1** + 100 mM Na₂EDTA; lane 2: sat. Ar atmosphere. (d) 2 h incubation of 20 μM **2** with added ascorbate (at twice the complex concentration). Lane 1: **2** + 100 mM Na₂EDTA; lane 2: sat. Ar atmosphere.

(Fig. 4 (c) and (d)). The Mn²⁺ system was examined at a concentration of 20 μM over a 2 h period in the presence of ascorbate, while the Cu²⁺ system was tested over a longer time period (20 h) and without added reductant. The Mn²⁺ complex, **2**, did not cleave DNA in the absence of O₂ and presence of EDTA, however, complex **1** was found to induce some DNA damage in the presence of excess metal chelator (EDTA) and slight relaxation in the absence of O₂.

Redox activity

Since the catalytic interaction of [Cu(phen)₂]²⁺ and its reduced form, [Cu(phen)₂]⁺, with the superoxide radical (O₂^{•-}) and hydrogen peroxide (H₂O₂) are imperative for cleaving the phosphodiester backbone in DNA,^{17–19} we have examined the interaction of complexes **1** and **2** with both these species. Superoxide was generated enzymatically by the xanthine/xanthine-oxidase system and quantified photometrically by the detector molecule, nitro-blue-tetrazolium (NBT). Both **1** and **2** show potent SOD (superoxide dismutase) mimetic activity, with the Mn²⁺ complex being an exceptional catalyst (1 U SOD = 24.6 nM) (Fig. 5 and Table 2). The catalase (CAT) mimetic activity of complexes **1** and **2** was determined by measuring the volume of

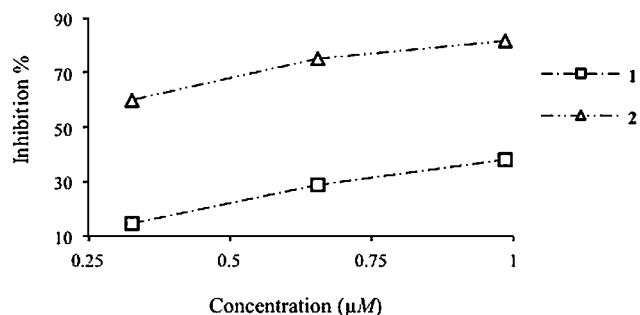
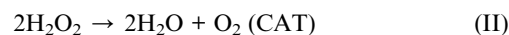
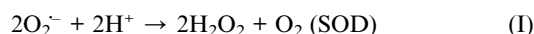


Fig. 5 SOD activity profiles for complexes **1** and **2**.

Table 2 Concentrations of complexes **1** and **2** equivalent to effect 1 U of bovine erythrocyte SOD activity (50% Inhibition) and the catalase mimetic potential of **1** and **2** examined as a function of H₂O₂ disproportionation

Complex	Concentration equivalent to 1 U bovine SOD (μM)	Number of H ₂ O ₂ molecules disproportionated by one molecule of complex in the first 5 min ¹⁴
1	1.300	0
2	0.024	6 × 10 ³

evolved O₂ from disproportionate H₂O₂ (30% v/v solution). Only the Mn²⁺ complex (**2**) was capable of decomposing H₂O₂ and its activity can also be described as exceptional in this regard (6 × 10³ H₂O₂ molecules disproportionated in 5 min). Overall, both **1** and **2** interacted with superoxide to produce hydrogen peroxide (equation I), the Mn²⁺ complex being the most active. Furthermore, it was only the Mn²⁺ complex (**2**) which appeared capable of disproportionating the peroxide molecules (equation II) resulting from the superoxide conversion.



Antitumour activity

The cytotoxicity of **1** and **2**, the free phen ligand and the clinical antitumour agent, cisplatin, were measured at 24 and 96 h intervals in triplicate using a standard MTT assay against three progressive colorectal human-derived tumour cell lines {HT29, SW480 (Dukes' B) and SW620 (Dukes' C)} along with a non-cancerous normal human keratinocyte line (HaCaT).† The average cytotoxicity data and the associated SD obtained were then used to calculate LD₅₀ values (±95% CI) for each cell line and time point (Table 3). Both complexes displayed remarkable cytotoxicity against all three cancerous lines. LD₅₀ activities in the low micromolar range were found for both complexes after 24 h exposure and, significantly, these activities reached the nano- and picomolar level after 96 h. As the

Table 3 LD₅₀ (at ±95% CI) values for complexes **1** and **2**, the free ligand phen and the antitumour agent cisplatin, against colorectal cancer lines HT29, SW480 and SW620 along with non-cancerous human keratinocyte line HaCaT. Experiments were conducted independently in triplicate over a period of 24 and 96 h†

	Antitumour Activity LD ₅₀ (μM)						
	HT29		SW480		SW620		HaCaT
	24 h	96 h	24 h	96 h	24 h	96 h	96 h
phen	>200	9.240	>200	10.70	160.00	10.700	5.500
1	9.610	<0.001	11.30	0.220	31.00	1.220	0.719
2	108.00	0.092	7.460	0.261	58.50	0.342	0.851
cisplatin	166.00	4.810	>200	1.290	>200	7.030	3.450

colorectal tumour lines progress (HT29 → SW480 → SW620) the LD₅₀ value for **1**, over a 96 h period, fell from pico- to low micromolar concentrations, while the activity of **2** remained consistently in the low- to mid-nanomolar region. It is notable that while cisplatin displays significant low-micromolar cytotoxicity against all tumour lines after 96 h, the activity of **1** and **2**, in the same time period is much superior (by a factor between approximately $1 \times 10^1 - 5 \times 10^3$). Over a 96 h time period, the LD₅₀ values of cisplatin and phen against the normal non-cancerous HaCaT cell line are approximately equivalent to the values obtained against cancerous lines HT29, SW480 and SW620. However, significant differences in the HaCaT cell line, were found for complexes **1** and **2**, particularly when compared to the HT29 cell line. Compound **2** was found to be 9.25 times less cytotoxic toward HaCaT cells when compared to HT29, while, compound **1** was 700 times less cytotoxic when compared to the HT29 cell line. One possible way to explain the enhancement of toxicity towards cancer cells in this study may relate to the activation of the oncogene, *p53*, a vital tumour-suppressor gene that functions by inducing apoptosis and preventing gene amplification and which is found mutated in many forms of human cancer including HT29, SW480 and SW620 carcinomas.²⁰ It has already been demonstrated that phen and its coordinated metal adducts enhance the *in vitro* activity of *p53* and can trigger apoptosis in *p53* negative cell lines,²¹ however, it is not clear at present if this oncogene plays any significant role in the toxicity profiles of these phen reagents.

Cellular reactive oxygen species study

In order to elucidate the relationship between cytotoxicity and reactive oxygen species (ROS) generation, complexes **1** and **2**, along with metal-free phen and cisplatin, were exposed to HT29 colorectal cancer cells which had been pre-treated with the intracellular ROS indicator 2',7'-dichlorofluorescein diacetate (DCFH-DA).[†] In the presence of endogenously generated ROS, DCFH-DA is oxidised to release the fluorophore 2',7'-dichlorofluorescein (DFC). Results are shown as increases in cellular ROS levels after drug exposure and are expressed in comparison to the ROS level of unexposed controls (-Ctrl). A positive control of 0.5 μM hydrogen peroxide (+Ctrl) was utilised as it is considered a potent generator of ROS. Results were recorded at 15, 30, 60, 120 and 180 min intervals and are shown in Fig. 6. The Mn²⁺ complex **2** was found to be an exceptional ROS generator with greatest activity, relative to H₂O₂ (+Ctrl), across the concentration range 1000–250 nM and registering approximate equal activity to 0.5 μM hydrogen peroxide at 125 nM. It is worth commenting that **2** was almost seven times more active than the next most effective ROS generator, phen. The activity of the Cu²⁺ complex, (**1**), even when assessed across a much higher concentration range (100,000–195 nM) was considerably lower than that of metal-free phen and complex **2**. The clinical agent, cisplatin, was the least active of all. This is not surprising considering that it is non-catalytic. Furthermore, it is known that cisplatin only becomes cytotoxic upon hydrolysis to [Pt(NH₃)₂(OH)₂], which generally occurs 48–96 h post intravenous administration.²²

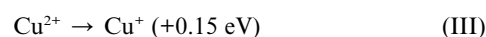
In vivo drug tolerance

Larvae of the insect *Galleria mellonella* (the greater wax moth) were employed to assess the *in vivo* cytotoxic tolerance of complexes **1** and **2**, metal-free phen and cisplatin. Larvae of *G. mellonella* have been widely used as a convenient and inexpensive *in vivo* screening model to assess the therapeutic potential of novel antimicrobial drugs.^{23,24} They have yielded results that are considered comparable to those obtained using mammalian models.²⁵ The innate defences of insects, including *G. mellonella*, like those of mammals consist of structural and passive barriers as well as humoral and cellular responses within the haemolymph (analogous to the blood of mammals).²⁶ Indeed cellular responses within the haemolymph are often activated by signal transduction systems comparable to mice.²⁷

Testing was carried out in triplicate using ten healthy *G. mellonella* larvae in the 6th developmental stage. Compounds were tested across the concentration range 5000–100 μg mL⁻¹ (333–13 mg kg⁻¹ average body weight) with sterile test solutions being administered *via* hypodermic injection. Larvae were incubated at 30 °C for 72 h with survival being monitored at 24 h intervals and significance being determined using the log rank (Mantel-Cox) method. Death was assessed by the lack of movement in response to stimulus together with discolouration. Results are presented (Table 4) as the mean % kill (± standard deviation) resulting from exposure to the tested compound. Larvae exposed to high concentrations of the compounds (5000 and 2000 μg mL⁻¹) showed poor tolerance. However, at the lower concentration ranges (1000–200 μg mL⁻¹) significant differences were observed. Larvae had the highest tolerance to complexes **1** and **2** (50 and 40% kill at 67 mg kg⁻¹, respectively, and 0% kill at 33 mg kg⁻¹). Cisplatin was the least well tolerated of the test compounds, with high toxicity (60% kill) being observed at 500 μg mL⁻¹ (33 mg kg⁻¹). This value for cisplatin correlates well with the known LD₅₀ value for this drug (32.7 mg kg⁻¹ body weight) in the mouse model (oral exposure) but appears somewhat higher than the reported mouse intravenous LD₅₀ value (11 mg kg⁻¹ body weight).²⁸ These differences could arise in mammals due to the dose-limiting toxicity of cisplatin toward renal tubular damage.²⁸

Proposed DNA self-cleaving mechanism

One possible mechanism by which the Cu²⁺ complex, **1**, self-activates the phosphodiester backbone in DNA is *via* the formation of a π carboxylate radical which concomitantly leads to the formation of the reduced Cu⁺ species, [Cu(phen)₂]⁺ (Scheme 1). Aliphatic carboxylate radicals are known to react by hydrogen abstraction in close competition with decarboxylation.^{29,30} Generation of a π carboxylate radical within complex **1** would depend on the strength of the HOMO d orbital overlap on Cu²⁺ with the oxygen carboxylate. From previous X-ray crystallographic studies it is known that the Cu–O bond length in **1** is 1.974 Å and that it is significantly shorter than the equivalent Mn–O bond in **2** (2.147 Å).^{13,14} However, some degree of caution must be exercised when invoking this radical theory based on metal-oxygen (carboxylate) bond lengths since the Cu–O bond length may alter when **1** binds to DNA.



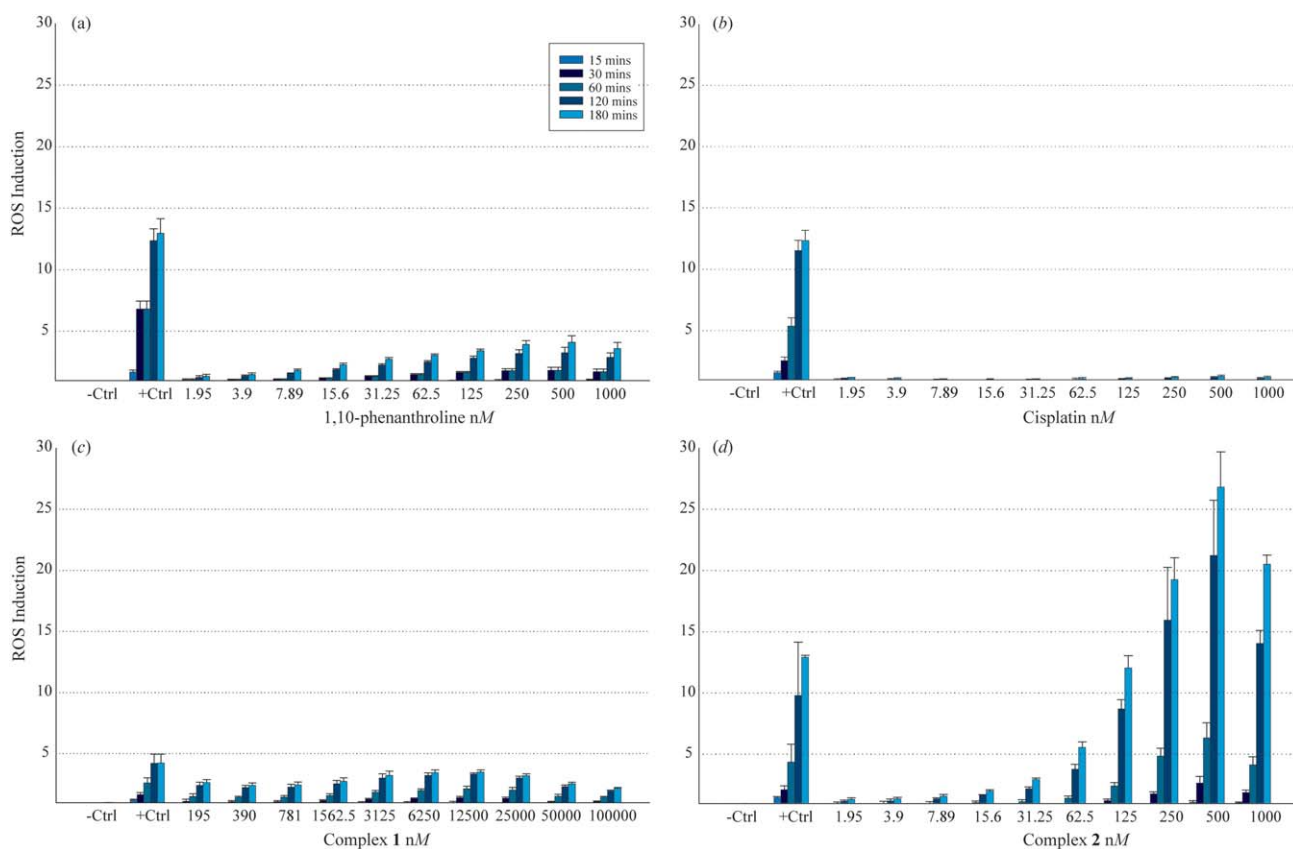
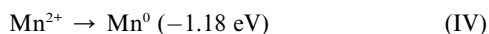


Fig. 6 Generation of endogenous reactive oxygen species (ROS) within the cancer cell line HT29 after exposure to; (a) the free ligand 1,10-phenanthroline, (b) the clinical antitumour agent cisplatin, (c) complex **1** and (d) complex **2**.



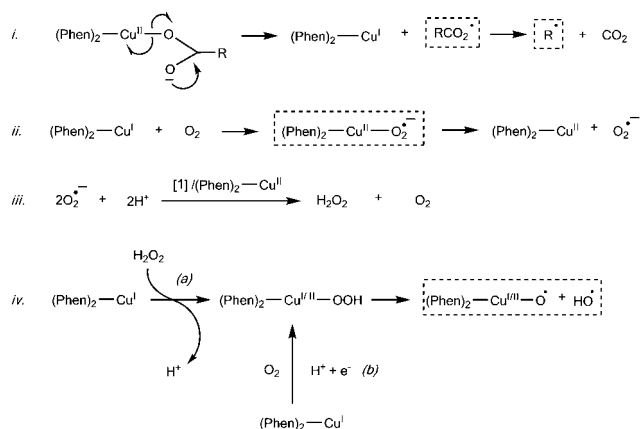
Considering the standard reduction potentials for Cu^{2+} (III) and Mn^{2+} (IV) could explain why the Cu^{2+} complex, **1**, self-activates SC DNA while the Mn^{2+} complex, **2**, does not. Carboxylate anions could not be considered potent reducing agents and so metal ions that may show this effect are ions, like Cu^{2+} , that have very low oxidation potentials between the M^n and M^{n-1} state.

The proposed mechanism proceeds by the following steps: (i) after the intercalation of **1** to DNA (discussed in relation to Table 1), homolytic cleavage at the Cu–O bond generates the π carboxyl radical (---) and the reduced Cu^+ d^{10} complex $[\text{Cu}$

$(\text{phen})_2]^+$. The resultant carboxyl π radical ($\pi\text{-RCO}_2$) undergoes rapid conversion to a σ radical ($\sigma\text{-RCO}_2$) which decarboxylates to generate CO_2 and R. (ii) $[\text{Cu}(\text{phen})_2]^+$ reacts, as previously reported,³¹ with O_2 to generate the superoxide radical through an intermediate $(\text{phen})_2\text{-Cu}^{2+}\text{-O}_2^-$ which then decomposes to $[\text{Cu}(\text{phen})_2]^{2+}$ and O_2^- . (iii) Either complex **1** or $[\text{Cu}(\text{phen})_2]^{2+}$ subsequently react with O_2^- to generate H_2O_2 (as discussed in relation to Fig. 5 and Table 2). (iv) Since **1** does not disproportionate hydrogen peroxide a subsequent reaction of $[\text{Cu}(\text{phen})_2]^+$ with H_2O_2 in (a) or O_2 in (b) can generate known metal-oxo and hydroxyl radical bond activators ($(\text{phen})_2\text{-Cu}^{+2+}\text{-O}_2^-$ and HO).³¹ Since some nuclease activity was detected for **1** in the presence of the powerful metal chelator EDTA (Fig. 4(c)) it appears feasible that the carboxyl radical, and/or its breakdown product (R), is

Table 4 % Kill of *G. mellonella* larvae after exposures to 5000–100 $\mu\text{g mL}^{-1}$ of complexes **1** and **2**, the ligand phen and the clinical antitumour drug cisplatin over the period 72 h†

% Kill of <i>Galleria mellonella</i> larvae (72 h)					
Concentration $\mu\text{g mL}^{-1}$ (mg kg^{-1} bo dy weight) \pm S.D.					
	5000 (333)	2000 (133)	1000 (67)	500 (33)	200 (13)
phen	100 (\pm 0)	90 (\pm 1)	80 (\pm 0)	0 (\pm 0)	0 (\pm 0)
cisplatin	100 (\pm 0)	100 (\pm 0)	100 (\pm 0)	60 (\pm 1)	0 (\pm 0)
1	93.30 (\pm 0.58)	86.70 (\pm 0.58)	50 (\pm 1)	0 (\pm 0)	0 (\pm 0)
2	93.30 (\pm 1.15)	93.30 (\pm 0.58)	40 (\pm 1)	0 (\pm 0)	0 (\pm 0)



Scheme 1 Proposed mechanism of generating C–H bond activators (---).

capable of abstracting H from the phosphodiester backbone of DNA. In the absence of oxygen, the self-cleaving nuclease potential of **1** is diminished (Fig. 4 (c)), and since, in the proposed mechanism, steps (ii)–(iv) are O₂-dependent, it stands to reason that the nuclease potential of this copper complex would be less in the absence of these aerobically-generated oxo- and hydroxo-bond activators. Efforts are currently underway in our laboratory to establish direct experimental evidence for this proposed mechanism.

Conclusions

In summary, dinuclear, water-soluble [Cu₂(μ₂-oda)(phen)₄](ClO₄)₂ (**1**) and {[Mn₂(μ₂-oda)(phen)₄(oda)₂]²⁻[Mn₂(μ₂-oda)(phen)₄(H₂O)₂]²⁺} (**2**), in comparison to other mononuclear metal-phen adducts, represent a significant advancement in the area of DNA-targeted chemotherapeutics. These Cu²⁺ and Mn²⁺ bis(phen) octanedioate complexes have powerful and unprecedented cytotoxicity, encouraging cytoselectivity and *in vivo* drug tolerance. Both complexes are avid binders of DNA, and the Cu²⁺ complex has the capacity to self-cleave DNA, possibly through the generation of a π carboxyl radical. The Mn²⁺ complex is an exceptional redox catalyst that generates remarkable levels of intracellular ROS within HT29 colorectal cancer cells compared to hydrogen peroxide.

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