Water-soluble CuII and MnII bis-phenanthroline octanedioate complexes with unprecedented nano and picomolar in vitro cytotoxicity as promising leads for chemotherapeutic drug development

Andrew Kellett
*Technological University Dublin*

Mark O'Connor
*Technological University*

Malachy McCann
*NUI Maynooth*

Orla Howe
*Technological University*

See next page for additional authors

Recommended Citation
doi:10.21427/D7KW5B
Water-soluble Cu$^{II}$ and Mn$^{II}$ bis-phenanthroline octanedioate complexes with unprecedented nano and picomolar in vitro cytotoxicity as promising leads for chemotherapeutic drug development

Andrew Kellett, a Mark O’Connor, a Malachy McCann, b Orla Howe, a Alan Casey, a Paurai McCarron, a,b Mary McNamara, a Sean Kennedy, a Donald D. May, c Philip Skell, a Denis O’Shea a and Michael Devereux. a

Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

Introduction

The development of self-cleaving chemical nucleases is regarded as the paradigm of redox-active metal-based chemotherapeutics. DNA targeted agents capable of inducing single stranded or double stranded scission have found clinical application within cancer chemotherapy. Other applications within this class include; probing of DNA-specific structures, mapping of protein and DNA interactions, gene regulation and signal transduction. 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 catalytic potential to support the one-electron oxidation/reduction reactions necessary to drive activation of C-H deoxyribose bonds.

In the presence of Cu$^{2+}$, the oxidative formation of π radical cations within marine-based products tambjamine E, prodigiosin and pyrimol 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 Cu$^{2+}$ compounds have also demonstrated significant in vitro chemotherapeutic potential against leukaemia and ovarian cancer cells, some of which had cisplatin resistance.

The discovery of the first synthetic chemical nuclease, [Cu(phen)$_2$]$^{2+}$ (phen = 1,10-phenanthroline, Figure 1), has sparked intense effort toward the development of new bis-phen agents capable of enhanced DNA cleaving compared to the parent complex. The DNA cleaving limitations of [Cu(phen)$_2$]$^{2+}$ include (i) a high dissociation constant of the second coordinated phenanthroline ligand and (ii) the need for exogenous reductant to generate the active species [Cu(phen)$_2$]$^{+}$. The dissociation problem (i) was solved by Meunier, Pitie et al through the advent of clip-phen, whereby two phenanthroline ligands are connected at the 2’ or 3’ position by a serinol bridge. Recently this laboratory has reported the first self-cleaving bis-phen system, [Cu(phen)$_2$(phthalate)] (phthalate = o-, m-, p-phthalate, Figure 1), capable of inducing single-stranded DNA scission in the absence of exogenous reductant or oxidant.
Figure 4. Relaxation of pUC18 by 1 and 2. \(^{(a)}\) Cleavage was carried out at 37°C then analyzed by agarose gel electrophoresis \(^{(a)}\) (a) 2hr incubation in the presence 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. \(^{(b)}\) 2hr incubation in the presence of added ascorbate (at twice complex concentration), lane 1: DNA alone; lanes 2-5: 1, 5, 10, 20 µM 1; lanes 6-9: 1, 5, 10, 20 µM 2. \(^{(c)}\) 2hr incubation of 20 µM 1 in the absence of added oxidant or reductant, lane 1: + 100 mM Na₂EDTA; lane 2: sat. At atmosphere. \(^{(d)}\) 2hr incubation of 20 µM 2 with added ascorbate (at twice complex concentration), lane 1: + 100 mM Na₂EDTA; lane 2: sat. At atmosphere.

Table 2. SOD concentrations of complexes 1 and 2 equivalent to effect 1U of Bovine Erythrocyte SOD activity (50% Inhibition) and Catalase mimicic potential of 1 and 2 examined as a function of 

\[ \text{H}_2\text{O}_2 \text{ disproportionation} \]

<table>
<thead>
<tr>
<th>Complex</th>
<th>Concentration Equivalent to 1U Bovine SOD (µM)</th>
<th>Number of \text{H}_2\text{O}_2\text{molecules}</th>
<th>Number of \text{H}_2\text{O}_2\text{molecules}</th>
<th>Number of \text{H}_2\text{O}_2\text{molecules}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.300</td>
<td>6 x 10^4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.024</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Since the catalytic interaction of \([\text{Cu(phen)}_2]^{2+}\) and its reduced form, \([\text{Cu(phen)}_2]^-\), with the superoxide radical \(\text{O}_2^-\) and hydrogen peroxide \(\text{H}_2\text{O}_2\) are imperative for cleaving the phosphodiester backbone in DNA, 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 nitro-blue-tetrazolium (NBT). Both complexes 1 and 2 show potent SOD (superoxide dismutase) mimicic activity with the Mn\(^{2+}\) system being an exceptional catalyst (1U SOD = 24.6 nM) (Figure 5 & Table 2). The catalase mimetic activity of complexes 1 & 2 were examined as a function of oxygen evolution. After the addition of a 30% v/v solution of \(\text{H}_2\text{O}_2\) to 1 & 2 the O₂ gas evolved was quantified volumetrically (Table 2). Only the Mn\(^{2+}\) complex (2) was capable of decomposing \(\text{H}_2\text{O}_2\) and its activity can also be described as exceptional in this regard (6 x 10^{-1} \text{H}_2\text{O}_2\text{molecules} disproportionated in 5 min). Overall, both complexes 1 & 2 interacted with superoxide to produce hydrogen peroxide (I), but, only the Mn\(^{2+}\) complex (2) appears capable of disproportionate the resulting peroxide (II).

\[ \text{I.} \quad 2\text{O}_2^- + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O}_2 + \text{O}_2 \quad (\text{SOD}) \]
\[ \text{II.} \quad 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \quad (\text{CAT}) \]
Antitumour Activity

<table>
<thead>
<tr>
<th></th>
<th>HT29 24 hr</th>
<th>HT29 96 hr</th>
<th>SW480 24 hr</th>
<th>SW480 96 hr</th>
<th>SW640 24 hr</th>
<th>SW640 96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>phen</td>
<td>&lt;200</td>
<td>9,240</td>
<td>&lt;200</td>
<td>10,700</td>
<td>160,00</td>
<td>10,700</td>
</tr>
<tr>
<td>1</td>
<td>9,610</td>
<td>&gt;0.001</td>
<td>11.30</td>
<td>0.220</td>
<td>31.00</td>
<td>1.220</td>
</tr>
<tr>
<td>2</td>
<td>108,00</td>
<td>0.092</td>
<td>7.460</td>
<td>0.261</td>
<td>58.50</td>
<td>0.342</td>
</tr>
<tr>
<td>cisplatin</td>
<td>166,00</td>
<td>4.810</td>
<td>&lt;200</td>
<td>1.290</td>
<td>&lt;200</td>
<td>7.030</td>
</tr>
</tbody>
</table>

The cytotoxicity of 1 & 2 along with the free phen ligand and the clinical antitumour agent cisplatin, were measured at 24 and 96 hr intervals using a standard MTT assay against three progressive colorectal human-derived tumour cell lines (Table 3). Both complexes display remarkable cytotoxicity against all three lines. Low-micromolar LD\(_50\) activities for both complexes were found after 24 hrs of exposure and, significantly, these activities reached the nano- and picomolar level after 96 hrs. As the colorectal tumour lines progress (HT29→SW480→SW620) activity of complex 1, over 96 hrs, reduces from pico- to low micromolar, while the activity of 2 remained consistently in the low-mid nanomolar region. It is worth noting that while cisplatin displays significant low-micromolar cytotoxicity against all tumour lines after 96 hrs, the activity of 1 & 2, in this period, is superior to this agent by a factor between 1×10\(^{-3}\) – 1×10\(^{-2}\).

Cellular Reactive Oxygen Species (ROS) Study

In order to elucidate the relationship between cytotoxicity and ROS generation, complexes 1 & 2, along with phen and cisplatin, were exposed to colorectal cancer cells, HT29, pre-treated with the intracellular ROS indicator 2,7’-dichlorofluorescin diacetate (DCFH-DA). In the presence of endogenously generated ROS, DCFH-DA becomes oxidised to release the fluorophore 2,7’-dichlorofluorescin (DCF). Results are expressed relative to the fluorescent response of the positive control (+Ctrl), hydrogen peroxide, which is considered a potent generator of ROS. Corrections were made using a negative control (-Ctrl), which represents the natural level of cellular ROS generated. Results were recorded at 15, 30, 60, 120 and 180 min intervals and are shown in Figure 6. Complex 2 was found to be an exceptional generator of ROS with greater activity relative to H\(_2\)O\(_2\) (+Ctrl) across the concentration range 1000-250 nM and approximate equal activity to hydrogen peroxide at 125 nM. It is worth commenting that this species was almost seven times more active than the next most effective ROS generator, phen. The activity of the copper(II) complex (1) was somewhat lower than both free phen and complex 2, indeed 1 needed to be assessed across a higher concentration range (100,000-195 nM). The clinical agent cisplatin was the least active of all tested compounds. This is unsurprising considering it is non-catalytic, it has also been well established that cisplatin only becomes cytotoxic upon its hydrolysis to [Pt(NH\(_3\))\(_2\)OH\(_2\)]\(^-\), which generally occurs between 48-96 hrs.

Figure 6. Generation of exogenous 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.

In vivo Drug Tolerance

Larvae of the insect Galleria mellonella were employed to assess the in vivo cytotoxic tolerance of complexes 1 and 2, the ligand phen and the clinical antitumour agent cisplatin. Larvae of G. mellonella (the greater wax moth) have been widely used as a convenient and inexpensive in vivo screening model to assess the therapeutic potential of novel antimicrobial drugs. They have yielded results that are considered comparable to those obtained using mammalian models. 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). Indeed cellular responses within the haemolymph are often activated by signal transduction systems comparable to mice.

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>5000 (333)</th>
<th>2000 (333)</th>
<th>1000 (67)</th>
<th>500 (33)</th>
<th>200 (13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>phen</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>93.3</td>
<td>86.7</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>93.3</td>
<td>93.3</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>cisplatin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>60</td>
<td>0</td>
</tr>
</tbody>
</table>

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 (333-13 mg/kg average body weight) with sterile test solutions being administered via hypodermic injection. Larvae were incubated at 30°C for 72 hr with survival being monitored at 24 hr 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 tested compound. Galleria exposed to high drug concentrations of 5000 and 2000 µg/mL showed poor tolerance to all tested species. However, at lower concentration ranges 1000-200 µg/mL significant differences were observed. Tolerance of Galleria larvae exposed to complexes 1 and 2 was highest of all tested species (50 and 40% kill at 67 mg/kg respectively, and 0% kill at 33 mg/kg). The clinical antitumour agent cisplatin was the poorest tolerated tested species with high toxicity (60% kill) being observed at 500 µg/mL (33 mg/kg).

Encouragingly, the well-established rat LD\(_{50}\) of cisplatin (oral exposure) is reported as 25.8 mg/kg body weight, thus, it appears some agreement between these two models does exist.

Proposed DNA Self-Cleaving Mechanism

To conclude we would like to propose a mechanism by which of complex 1 self-activates the phosphodiester backbone in DNA via formation of a \(\pi\) carboxylate radical, concomitantly leading to the reduced formation of [Cu(phen)]\(^{+}\) (Figure 7). Aliphatic carboxylate radicals are known to react by hydrogen abstraction in close competition with decarboxylation. Generation of a \(\pi\) carboxylate radical within complex 1 would depend on the strength of the HOMO d orbital overlap on Cu\(^{+}\) with the oxygen carboxylate. X-ray crystallography has revealed that the bond length between Cu-O in 1 is 1.974 Å and is significantly shorter than the equivalent Mn-O bond in 2 which is 2.147 Å.
However, since the Cu-O bond length may be altered when 1 binds with DNA, it is impossible to know its exact length in vivo.

In summary, dinuclear Cu(II) bis-phen complexes of the dicarboxylate, octanedioate, represent a significant advancement compared to existing metal-phenanthroline adducts. Their application as new water-soluble DNA-targeted chemotherapeutics is highly significant given their powerful and unprecedented cytotoxicity, encouraging in vivo drug tolerance and unique modes of action. While both complexes are avid binders of DNA, additionally, the copper(II) system has the capacity to self-cleave DNA, possibly through the generation of a \( \pi \) carboxyl radical, while the manganese(II) system is an exceptional redox catalyst that generates unprecedented levels of intracellular ROS within colon cancer cells.

The authors wish to acknowledge financial support from the Dublin Institute of Technology Capacity Building Scheme for Strategic Research programme (CaBS). This work has been carried out (in part) within the structures of the Focas Research Institute, DIT, funded under The Irish National Development Plan with assistance from the European Regional Development Fund.

Notes and references

\( ^{a} \) Dublin Institute of Technology, Focas Research Institute, Camden Row, Dublin 8, Ireland. E-mail: andrew.kellett@dit.ie, Tel: +353 1 4027800; E-mail: michael.devereux@dit.ie, Tel: +353 1 4024680

\( ^{b} \) Chemistry Department, National University of Ireland, Maynooth, Co. Kildare, Ireland

\( ^{c} \) Electronic Supplementary Information (ESI) available: Experimental procedures, X-ray crystallographic data and biological evaluation studies See DOI: 10.1039/b000000x/