Experimental Results (2022), 3, e22, 1–12
doi:10.1017/exp.2022.20

LIFE SCIENCE AND BIOMEDICINE

NOVEL-RESULT

Potential interactions between metal-based phenanthroline drugs and the unfolded protein response endoplasmic reticulum stress pathway

Tadhg O'Leary[1,2,*], Pauraic McCarron[2,3], Michael Devereux[1,2,4] and Steve Meaney[1,2]

[1]School of Biological and Health Sciences, College of Sciences and Health, Technological University Dublin – City Campus, Dublin, Ireland, [2]Centre for Biomimetic and Therapeutic Research, Environmental Sustainability and Health Institute, Technological University Dublin – Grangegorman Campus, Dublin, Ireland, [3]School of Science and Computing, Technological University Dublin – Tallaght Campus, Dublin, Ireland, and [4]College of Sciences and Health, Technological University Dublin – City Campus, Dublin, Ireland
***Corresponding author:** Email: tadhgmol@gmail.com

(Received 02 June 2022; Revised 06 September 2022; Accepted 09 September 2022)

Abstract

The unfolded protein response has recently been implicated as a mechanism by which 1,10-phenanthroline-containing coordination compounds trigger cell death. We explored the interaction of two such compounds —one containing copper and one containing manganese—with endoplasmic reticulum (ER) stress. Pre-treatment with anisomycin significantly enhanced the cytotoxic activity of both metal-based compounds in A2780, but only the copper-based compound in A549 cells. The effects of pretreatment with tunicamycin were dependent on the nature of the metal center in the compounds. In A2780 cells, the cytotoxic action of the copper compound was reduced by tunicamycin only at high concentration. In contrast, in A549 cells the efficacy of the manganese compound cells was reduced at all tested concentrations. Intriguingly, some impact of free 1,10-phenanthroline was also observed in A549 cells. These results are discussed in the context of the emerging evidence that the ER plays a role in the cytotoxic action of 1,10-phenanthroline-based compounds.

Key words: Metal-Phenanthroline drugs; ER Stress; Cytotoxicity; Anti-cancer

Introduction

The cytotoxic and microbicidal effects of 1,10-phenanthroline (1,10-phen) transition metal coordination complexes are well established (reviewed by McCann et al., 2012; Viganor et al., 2017). The mechanistic details of these effects are not fully determined, but multiple routes of action are likely. A consistent feature of these complexes is their capacity to directly generate or promote the formation of reactive oxygen species capable of inducing DNA damage (Anbu et al., 2013; Kellett et al., 2011a; 2011b; Roy et al., 2008). The accumulation of DNA damage and activation of one or more cell death pathways, is a consistent finding in studies of 1,10-phen-containing compounds (Deegan *et al.*, 2007; Kellett et al., 2011b; Slator et al., 2017). In recent years, however, evidence for the involvement of other cellular processes has begun to emerge.

The ability of 1,10-phen to inhibit the proteasome has been reported, specifically via an effect on the regulatory lid subunit protein RPN11, a zinc-dependent metalloprotease (Mansour et al., 2015; Song et al., 2016; 2017). The strong chelative action of 1,10-phen of transition metals likely underlies this effect.

1,10-Phen compounds containing copper have been shown to inhibit the proteasome and induce apoptosis in PC-3 and MDA-MB-231 cancer cells lines, but not in nontumorigenic origin MCF-10A cells (Crowley, 2019; Zhang et al., 2013; 2017) as well as in SKOV-3 and A549 cells (Crowley, 2019). Slator et al. (2017) demonstrated that a 1,10-phen-based compound containing manganese increased mitochondrial superoxide content and induced autophagy in SKOV-3 cells.

There is also some evidence for a role of endoplasmic reticulum (ER)-stress in the action of 1,10-phen-containing compounds. Wu et al. (2014) reported that a dinuclear 1,10-phen-copper complex could induce the expression of several ER-stress proteins in HepG2 cells, including Grp78, XBP-1, and CHOP. Copper(II)–phenanthroline complexes containing imidazolidine-2-thione derivatives were shown to induce apoptosis *via* the unfolded protein response (UPR) (Moráň et al. (2019). The toxicity was significantly inhibited in the presence of tauroursodeoxycholic acid (TUDCA), which prevents the dissociation of Grp78 from PERK, and thus attenuates the UPR (Yoon et al., 2016). Very recently, a novel copper(II)-phenanthroline complex containing salubrinal (a protein translation inhibitor) was reported to induce cell death *via* ER-stress in some, but not all, cell types studied (Masuri et al., 2020).

To expand studies of 1,10-phen and ER-stress, we explored the interaction between two 1,10-phen-containing compounds—{[Cu(3,6,9-tdda)(phen)2]·3H_2O·EtOH}n (Cu-Phen) and {[Mn (3,6,9-tdda)(phen)2]·3H_2O·EtOH}n (Mn-Phen) (where phen = 1,10-phenanthroline and 3,6,9-tddaH2 = 3,6,9-trioxaundecanedioic acid)—and either anisomycin and tunicamycin, both established inducers of ER-stress (Majumder et al., 2012; Mawji et al., 2007; Oslowski & Urano, 2011; Tang et al., 2018).

Methods

Materials

The synthesis of {[Cu(3,6,9-tdda)(phen)2].3H_2O.EtOH}n (Cu-Phen) and {[Mn(3,6,9-tdda) (phen) 2]·.3H_2O·EtOH}n (Mn-Phen) (where phen = 1,10-phenanthroline and 3,6,9-tddaH2 = 3,6,9-trioxaundecanedioic acid) is described in Gandra et al. (2017). 1,10-Phenanthroline was purchased from Sigma Aldrich (Merk), while anisomycin and tunicamycin were purchased from Cayman chemical. Anisomycin and tunicamycin were dissolved in DMSO, aliquoted, and stored at −20°C until required. 1,10-Phen was dissolved in methanol up to 24 hr before use.

Cell culture conditions

A2780 (ovarian cancer) and A549 (alveolar adenocarcinoma cancer cell lines were cultured in RPMI1640, supplemented with 10% fetal bovine serum and 1% L-glutamine, and incubated in a humidified atmosphere of 5% CO_2 in air at 37°C. Both cell lines were obtained from the internal cell bank at Technological University Dublin (TU Dublin City Campus) and commercially through the European Collection of Authenticated Cell Cultures (ECACC). Cells were plated at 1×10^4 cells/well for A2780 and 5×10^3 cells/well for A549 cells in black-walled 96-well plates. These cell densities are based on previous optimization trials in our laboratory.

Treatment with ER-stress-inducing agents

A2780 and A549 cells were pretreated with either 100 nM of anisomycin for 24 hr or 5 nM of tunicamycin for 8 hr, followed by treatment with Cu-phen, Mn-phen, or 1, 10-phen for 24 hr. Pretreatment concentrations and incubation times were optimized in previous experiments. To ensure sensitivity to changes in cytotoxic action, concentrations of 1,10-phen-based compounds were chosen to include non- and modestly toxic concentrations, based on previous work in our laboratory which determined the IC_{50} and cytotoxic range of the compounds (Table 1).

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press

Table 1. IC_{50} values for 1,10-phenanthroline-based compounds in A2780 and A549 cells

	IC_{50} μM (95% CI)		
Cell line	Cu-phen	Mn-phen	1,10-phen
A2780	2 (0.5–3.3)	45 (39–51)	495 (475–515)
A549	3.7 (3.5–3.9)	45 (37–54)	328 (275–382)

Note. Cells were treated for 24 hr with each compound and assayed using the Alamar blue cell proliferation assay. IC_{50} values were calculated using GraphPad prism 5.0.

Cell viability

Cell viability was evaluated via the resazurin reduction assay using empirically optimized conditions for each cell type. A 0.44 mM solution of resazurin salt (sigma) in PBS (pH 7.4), equivalent to the commercial Alamar Blue reagent (O'Brien et al., 2000), was prepared in house. Following drug exposure for the specified time, 10 μl of resazurin was added to each well at 10% of final well volume and incubated for 2 hr with A2780 cells and 4 hr with A549 cells. Fluorescence was detected using a *MULTISKANGO* spectrophotometer (ThermoFischer) at excitation of 530 nm and emission of 590 nm.

Statistical analysis

Data was first expressed as a percentage of nontreated control cells and then normalized to cells treated with anisomycin or tunicamycin only to account for affects either compound had on cell viability. Data were analyzed using Microsoft excel and graphs were generated using GraphPad Prism Version 5.00. Results were compared using the Students *t*-test with values $p < .05$ considered significant.

Results

Anisomycin treatment

All results shown below are corrected for the direct impact of anisomycin. Pretreatment of A2780 cells with anisomycin resulted in a modest yet significant increase in the cytotoxic action of Cu-phen, Mn-phen, and 1,10-phen (Figure 1). The apparent loss of the effect at 5 μM of Cu-phen is attributed to the pronounced cytotoxic action at this concentration masking any anisomycin-induced effects. This also prevented further studies at this concentration of Cu-phen. In A549 cells an almost identical effect was observed following 500 nM Cu-phen treatment (Figure 2). In contrast, there was no impact on the efficacy of Mn-phen and only a very minor impact on 1,10-phen. Anisomycin reduced the viability of A2780 cells by 42 ± 2.1% and A549 cells by 30 ± 5.1%.

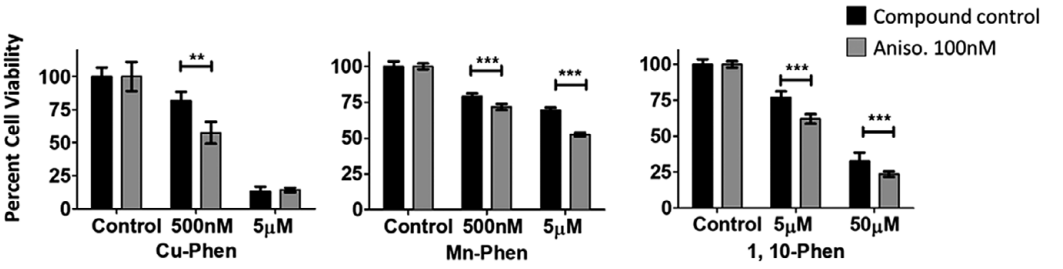

Figure 1. Effects of Anisomycin (100 nM) on the efficacy of 1,10-phenanthroline-based compounds in A2780 cells. Cells were pretreated with anisomycin as described in methods for 24 hr, followed by treatment with Cu-phen, Mn-phen, and 1,10-phen for a further 24 hr. Results are expressed as mean percent viability compared to cells without cotreatment, ±1 standard deviation of a representative sample of two independent experiments ($^{**}p < .01$, and $^{***}p < .001$).

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press

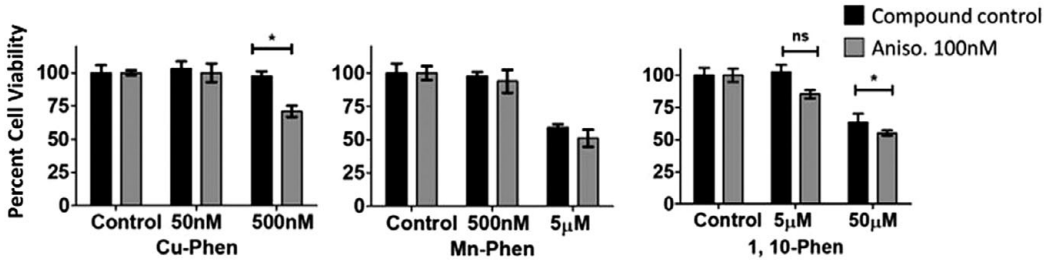

Figure 2. Effects of Anisomycin (100 nM) on the efficacy of 1,10-phenanthroline-based compounds in A549 cells. Cells were pretreated with anisomycin as described in methods for 24 hr, followed by treatment with Cu-phen, Mn-phen, and 1,10-phen for a further 24 hr. Results are expressed as mean percent viability compared to cells without cotreatment, ±1 standard deviation of a representative sample of two independent experiments (*$p < .05$, ns, not significant).

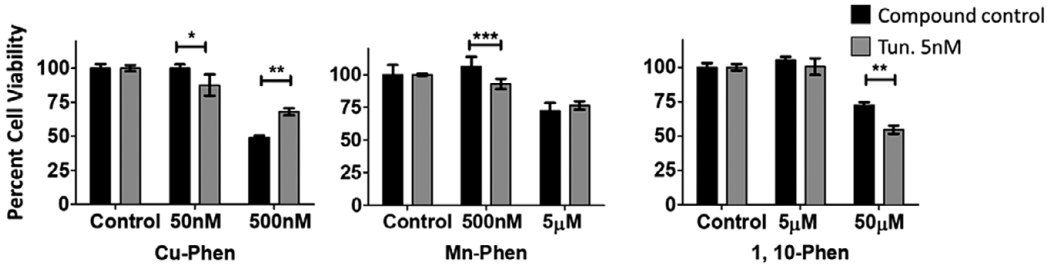

Figure 3. Effects of Tunicamycin (5 nM) on the efficacy of 1,10-phenanthroline-based compounds in A2780 cells. Cells were pretreated with Tunicamycin as described in methods for 8 hr, followed by treatment with Cu-phen, Mn-phen, and 1,10-phen for a further 24 hr. Results are expressed as mean percent viability compared to cells without cotreatment, ±1 standard deviation of a representative sample of two independent experiments (*$p < .05$, **$p < .01$, and ***$p < .001$).

Tunicamycin treatment

All results shown below are corrected for the direct impact of tunicamycin. Pretreatment with tunicamycin resulted in very variable effects, dependent on both cell type, compound type, and concentration. In A2780 cells, treatment with low concentrations of Cu-phen and Mn-phen slightly increased the cytotoxic action of both compounds. Similar potentiation of cytotoxic action was observed at high concentrations of 1,10-phen. In contrast, treatment of cells with a higher concentration of Cu-phen inhibited the action of Cu-Phen and eliminated the effect of Mn-phen (Figure 3). Tunicamycin reduced A2780 cell viability by 35 ± 2.2% and A549 cell viability by 40 ± 2.1%.

In A549 cells, there were marked differences between the effects of pretreatment on the action of Cu-phen and Mn-phen. There was only a very modest impact of tunicamycin on Cu-phen at the highest concentration tested, in contrast to the situation in A2870 cells (Figure 4). Surprisingly, we observed a robust protective effect of both Mn-phen and 1,10-phen in A549 cells at all concentrations tested. Indeed, in these cases, tunicamycin pretreatment appeared to prevent all cytotoxic action at all tested concentrations.

Discussion

The potential for metal-phen-based compounds to interact with the UPR pathway as a novel mechanism of action has recently been explored, particularly regarding copper-containing phenanthroline derivatives (Masuri et al., 2020; Moráň et al., 2019; Wu et al., 2014). This work investigated whether the cytotoxic potential of novel copper and manganese-containing phenanthroline compounds could be increased using the UPR-inducing drugs Anisomycin and Tunicamycin. Overall, these data indicate that Cu-Phen, Mn-Phen, and 1,10-phen interact with both UPR-inducing drugs to a modest degree and in a cell type specific manner. While major changes in cytotoxicity were not seen, the modest yet significant

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press

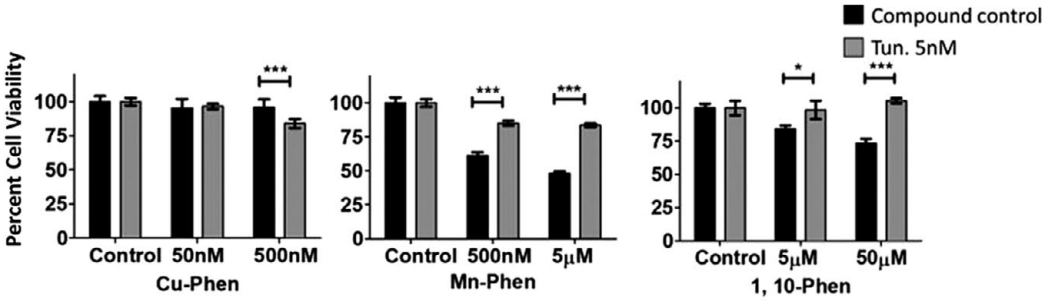

Figure 4. Effects of Tunicamycin (5 nM) on the efficacy of 1,10-phenanthroline-based compounds in A549 cells. Cells were pretreated with Tunicamycin as described in methods for 8 hr, followed by treatment with Cu-phen, Mn-phen, and 1,10-phen for a further 24 hr. Results are expressed as mean percent viability compared to cells without cotreatment, ±1 standard deviation of a representative sample of two independent experiments. (*$p < .05$, ***$p < .001$).

enhancement of metal-phen compound efficacy suggests that the UPR may play a role in the mechanism of action of the metal-based compounds, although it may not be the primary mediator of cell death.

Both Mn-Phen and 1,10-phen reacted with Anisomycin and Tunicamycin treatment in cell type specific manners. In A549 cells the efficacy of 1,10-phen is significantly enhanced by Anisomycin with a similar trend observed in A2780 cells indicating that 1,10-phen likely interacts with the UPR pathway albeit modestly. Mn-Phen interaction with the UPR-inducing drugs is highly contextual in relation to the cell type implemented. Mn-Phen did not interact with Anisomycin in A549 cells yet experienced modest increases in cytotoxicity with a high degree of significance in A2780 cells following Anisomycin treatment. Tunicamycin appeared to inhibit both Mn-phen and 1,10-phen activity in A549 cells while again modest yet highly significant increases in cytotoxicity were observed in A2780 cells. Collectively these data indicate that in a similar fashion to 1,10-phen Mn-phen does interact with the UPR pathway to a modest degree, however, the somewhat contradictory results observed between A549 and A2780 cells regarding Tunicamycin pretreatment are somewhat perplexing.

At the current state of knowledge, it is difficult to speculate about the mechanisms for this increase in viability. It may be a consequence that the mechanism of action of Mn-Phen is dependent on one or more glycoproteins, the concentration of which is reduced following Tunicamycin treatment in A549 cells but not A2780 cells. Glycans and glycoproteins are involved in the complex regulation of cell fate either promoting or inhibiting cell death in a context dependent manner (reviewed by Lichtenstein & Rabinovich, 2013; Seyrek et al., 2019). As such alterations to glycosylation status of proteins involved in regulating cell death pathways by Tunicamycin could have unexpected effects regarding cytotoxic insult by metal-phen compounds.

Furthermore, Shenkman et al. (2007) demonstrated that inhibition of both proteasomal and non-proteasomal protein degradation pathways by 1,10-phen was alleviated by the presence of cobalt and manganese, likely owing to metal dependant degradation enzymes. As inhibition of protein degradation would further signal ER-stress mediated cell death, the presence of manganese supplied both by Mn-phen and 1,10-phens chelative properties could reduce the accumulation of misfolded proteins thereby decreasing ER-stress and associated cell death signals. It may therefore be the case that disruption of glycoprotein cell death signaling by tunicamycin and alleviation of protein degradation pathways coincide to significantly reduce the cytotoxic potential of Mn-Phen and 1,10-phen in a cell type specific context. While the exact reasons for these effects are outside of the scope of the current work, further exploring this avenue of investigation in more detail would likely prove fruitful.

Cu-Phen is seen to interact with Anisomycin and Tunicamycin in both cells lines and supports the hypothesis that this compound interacts with the UPR stress pathway, possibly due to a combination of previously reported proteasome inhibition by copper-phen complexes (Zhang et al., 2017). While a higher degree of activity between Cu-Phen and ER stressinducing agents was expected, these findings are supported by previous observations reported within the literature (Masuri et al., 2020; Moráň et al., 2019; Wu et al., 2014), and further, add to the mounting evidence of interactions between ER-stress mediated

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press

cell death and Cu-Phen compounds. Using an alternative inhibitory approach, for example, using ER stress alleviators such as TUDCA over ER stress inducers (Anisomycin, Tunicamycin) may demonstrate a higher degree of interaction between compound and pathway.

Interestingly higher concentrations of Cu-Phen appeared to experience an inhibition of cytotoxicity similar to that of Mn-Phen following treatment with Tunicamycin. However, this inhibition is observed in A2780 cells regarding Cu-Phen yet Mn-Phen is inhibited only in A549 cells and is enhanced in A2780 cells following Tunicamycin treatment. Many of the reasons for these phenomena have already been speculated and this specific instance of compound inhibition may be a combination of both cell type specific effects regarding not just Tunicamycin as previously thought but also cytotoxic mechanisms of the compounds in both cell lines.

To fully determine the extent the UPR plays in compound cytotoxicity the implementation of a multipronged approach would likely be required. Expression analysis of UPR-associated proteins (IRE1α, XBP-1, ATF4, and CHOP) (Walter & Ron, 2011) and implementing various inhibitors involved in UPR signaling would likely add supporting evidence to the observations, particularly regarding Cu-Phen. Inhibitors of the autophagic process would also shed light on whether the Mn-Phen compound described shares similarities with the analogous manganese-phen compound already shown to induce autophagic cell death as described by Slator et al. (2017). Sequentially combining both UPR inhibitors with inhibitors of autophagy could also shed light on the degree of any potential crosstalk between the two pathways in relation to signaling cell death following treatment with Mn-phen. A change in cytotoxicity in response to one or both inhibitors would likely determine this.

Taken together these results implicate the potential of the UPR pathway in mediating the effects of novel metal-based phen compounds.

Conclusions

Emerging evidence suggests that 1,10-phen-based drugs may interact with ER stress pathways. Pretreatment with the stress-inducing agents anisomycin or tunicamycin followed by treatment with novel copper and manganese compounds revealed evidence of potential interaction, although cell-specific effects were apparent. However, these data may also highlight a potentially conserved mechanism of action for 1,10-phen-based compounds—interaction with the ER and triggering of ER stress and the UPR.

Abbreviations

1,10-phen	1,10-phenanthroline
Aniso	anisomycin
Cu-phen	copper phenanthroline compound
DMSO	dimethyl sulfoxide
ER	endoplasmic reticulum
Mn-phen	manganese phenanthroline compound
Tun	tunicamycin
UPR	unfolded protein response

Acknowledgments. The authors would like to thank the School of Biological and Health Sciences, TU Dublin and the Environmental and Sustainability Institute for facilitating this project.

Data availability statement. Readers can contact the authors if they want access to such materials.

Funding statement. This work was funded by the Fiosraigh PhD Scholarship program as part of Technological University Dublin.

Conflict of interest. The authors declare no conflicts of interest.

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press

Authorship contributions. T.O.L: Devised and carried out experiments and writing; S.M.: Supervised experiments and writing; M.D.: Supervision and intellectual input and revision of manuscript; P.M.: Synthesis and characterization of compounds.

References

Anbu, S., Killivalavan, A., Alegria, E. C. B. A., Mathan, G., & Kandaswamy, M. (2013). Effect of 1,10-phenanthroline on DNA binding, DNA cleavage, cytotoxic and lactate dehydrogenase inhibition properties of Robson type macrocyclic dicopper(II) complex. *Journal of Coordination Chemistry*, **66**, 3989–4003. https://doi.org/10.1080/00958972.2013.858136

Crowley, A. (2019). *The generation of novel metal-folate-phenanthroline complexes and phenanthroline-folate conjugates with potential as chemotherapeutic agents.* https://doi.org/10.21427/232k-5777

Deegan, C., McCann, M., Devereux, M., Coyle, B., & Egan, D. A. (2007). In vitro cancer chemotherapeutic activity of 1,10-phenanthroline (phen), [Ag2(phen)3(mal)]·2H2O, [Cu(phen)2(mal)]·2H2O and [Mn(phen)2(mal)]·2H2O (malH2=malonic acid) using human cancer cells. *Cancer Letters*, **247**(2), 224-233. https://doi.org/10.1016/j.canlet.2006.04.006

Gandra, R. M., Mc Carron, P., Fernandes, M. F., Ramos, L. S., Mello, T. P., Aor, A. C., Branquinha, M. H., McCann, M., Devereux, M., & Santos, A. L. S. (2017). Antifungal Potential of Copper(II), Manganese(II) and Silver(I) 1,10-Phenanthroline Chelates Against Multidrug-Resistant Fungal Species Forming the Candida haemulonii Complex: Impact on the Planktonic and Biofilm Lifestyles. *Frontiers in Microbiology*, 8. https://doi.org/10.3389/fmicb.2017.01257

Kellett, A., O'Connor, M., McCann, M., Howe, O., Casey, A., McCarron, P., Kavanagh, K., McNamara, M., Kennedy, S., May, D. D., Skell, P. S., O'Sheaa, D., & Devereux, M. (2011a). Water-soluble bis(1,10-phenanthroline) octanedioate Cu^{2+} and Mn^{2+} complexes with unprecedented nano and picomolar in vitro cytotoxicity: Promising leads for chemotherapeutic drug development. *MedChemComm*, **2**, 579–584. https://doi.org/10.1039/c0md00266f

Kellett, A., O'Connor, M., McCann, M., McNamara, M., Lynch, P., Rosair, G., McKee, V., Creaven, B., Walsh, M., McClean, S., Foltyn, A., O'Shea, D., Howe, O., & Devereux, M. (2011b). Bis-phenanthroline copper(II) phthalate complexes are potent in vitro antitumour agents with "self-activating" metallo-nuclease and DNA binding properties. *Dalton Transactions*, **40**, 1024–1027. https://doi.org/10.1039/c0dt01607a

Lichtenstein, R. G., & Rabinovich, G. A. (2013). Glycobiology of cell death: when glycans and lectins govern cell fate. *Cell Death & Differentiation*, **20**(8), 976-986. https://doi.org/10.1038/cdd.2013.50

Majumder, M., Huang, C., Snider, M. D., Komar, A. A., Tanaka, J., Kaufman, R. J., Krokowski, D., & Hatzoglou, M. (2012). A novel feedback loop regulates the response to endoplasmic reticulum stress via the cooperation of cytoplasmic splicing and mRNA translation. *Molecular and Cellular Biology*, **32**, 992–1003. https://doi.org/10.1128/mcb.06665-11

Mansour, W., Nakasone, M. A., von Delbrück, M., Yu, Z., Krutauz, D., Reis, N., Kleifeld, O., Sommer, T., Fushman, D., & Glickman, M. H. (2015). Disassembly of Lys11 and mixed linkage polyubiquitin conjugates provides insights into function of proteasomal deubiquitinases Rpn11 and Ubp6. *Journal of Biological Chemistry*, **290**, 4688–4704. https://doi.org/10.1074/jbc.M114.568295

Masuri, S., Cadoni, E., Cabiddu, M. G., Isaia, F., Demuru, M. G., Moráň, L., Buček, D., Vaňhara, P., Havel, J., & Pivetta, T. (2020). The first copper(II) complex with 1,10-phenanthroline and salubrinal with interesting biochemical properties. *Metallomics*, **12**, 891–901. https://doi.org/10.1039/d0mt00006j

Mawji, I. A., Simpson, C. D., Gronda, M., Williams, M. A., Hurren, R., Henderson, C. J., Datti, A., Wrana, J. L., & Schimmer, A. D. (2007). A chemical screen identifies anisomycin as an anoikis sensitizer that functions by decreasing FLIP protein synthesis. *Cancer Research*, **67**, 8307–8315. https://doi.org/10.1158/0008-5472.CAN-07-1687

McCann, M., Kellett, A., Kavanagh, K., Devereux, M., & Santos, A. L. S. (2012). Deciphering the antimicrobial activity of phenanthroline chelators. *Current Medicinal Chemistry*, **19**, 2703–2714. https://doi.org/10.2174/092986712800609733

Moráň, L., Pivetta, T., Masuri, S., Vašíčková, K., Walter, F., Prehn, J., Elkalaf, M., Trnka, J., Havel, J., & Vaňhara, P. (2019). Mixed copper(II)–phenanthroline complexes induce cell death of ovarian cancer cells by evoking the unfolded protein response. *Metallomics*, **11**, 1481–1489. https://doi.org/10.1039/C9MT00055K

Oslowski, C. M., & Urano, F. (2011). Measuring ER stress and the unfolded protein response using mammalian tissue culture system. *Methods in Enzymology*, **490**, 71–92. https://doi.org/10.1016/B978-0-12-385114-7.00004-0

O'Brien, J., Wilson, I., Orton, T., & Pognan, F. (2000). Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *European Journal of Biochemistry*, **267**(17), 5421–5426. https://doi.org/10.1046/j.1432-1327.2000.01606.x

Roy, S., Hagen, K. D., Uma Maheswari, P., Lutz, M., Spek, A. L., Reedijk, J., & van Wezel, G. P. (2008). Phenanthroline derivatives with improved selectivity as DNA-targeting anticancer or antimicrobial drugs. *ChemMedChem*, **3**, 1427–1434. https://doi.org/10.1002/cmdc.200800097

Shenkman, M., Tolchinsky, S., & Lederkremer, G. Z. (2007). ER stress induces alternative nonproteasomal degradation of ER proteins but not of cytosolic ones. *Cell Stress and Chaperones*, **12**, 373–383. https://doi.org/10.1379/CSC-281.1

Slator, C., Molphy, Z., McKee, V., & Kellett, A. (2017). Triggering autophagic cell death with a di-manganese(II) developmental therapeutic. *Redox Biology*, **12**, 150–161. https://doi.org/10.1016/j.redox.2017.01.024

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press

Song, Y., Li, S., Ray, A., Das, D. S., Qi, J., Samur, M. K., Tai, Y.-T., Munshi, N., Carrasco, R. D., Chauhan, D., & Anderson, K. C. (2017). Blockade of deubiquitylating enzyme Rpn11 triggers apoptosis in multiple myeloma cells and overcomes bortezomib resistance. *Oncogene*, **36**, 5631–5638. https://doi.org/10.1038/onc.2017.172

Song, Y., Ray, A., Das, D. S., Samur, M. K., Carrasco, R. D., Munshi, N. C., Chauhan, D., & Anderson, K. C. (2016). Deubiquitylating enzyme Rpn11/POH1/PSMD14 as therapeutic target in multiple myeloma. *Blood*, **128**, 4469. https://doi.org/10.1182/blood.v128.22.4469.4469

Tang, M.-B., Li, Y.-S., Li, S.-H., Cheng, Y., Zhang, S., Luo, H.-Y., Mao, C.-Y., Hu, Z.-W., Schisler, J. C., Shi, C.-H., & Xu, Y.-M. (2018). Anisomycin prevents OGD-induced necroptosis by regulating the E3 ligase CHIP. *Scientific Reports*, **8**, 6379. https://doi.org/10.1038/s41598-018-24414-y

Viganor, L., Howe, O., McCarron, P., McCann, M., & Devereux, M. (2017). The antibacterial activity of metal complexes containing 1,10-phenanthroline: potential as alternative therapeutics in the era of antibiotic resistance. *Current Topics in Medicinal Chemistry*, **17**, 1280–1302. https://doi.org/10.2174/1568026616666161003143333

Walter, P., & Ron, D. (2011). The unfolded protein response: from stress pathway to homeostatic regulation. *Science*, **334**, 1081–1086. https://doi.org/10.1126/science.1209038

Wu, J., Chen, W., Yin, Y., Zheng, Z., & Zou, G. (2014). Probing the cell death signaling pathway of HepG2 cell line induced by copper-1,10-phenanthroline complex. *Biometals*, **27**, 445–458. https://doi.org/10.1007/s10534-014-9710-2

Yoon, Y. M., Lee, J. H., Yun, S. P., Han, Y.-S., Yun, C. W., Lee, H. J., Noh, H., Lee, S.-J., Han, H. J., & Lee, S. H. (2016). Tauroursodeoxycholic acid reduces ER stress by regulating of Akt-dependent cellular prion protein. *Scientific Reports*, **6**, 39838. https://doi.org/10.1038/srep39838

Zhang, Z., Bi, C., Schmitt, S. M., Fan, Y., Dong, L., Zuo, J., & Ping Dou, Q. (2013). 1,10-phenanthroline promotes copper complexes into tumor cells and induces apoptosis by inhibiting the proteasome activity. *Journal of Biological Inorganic Chemistry*, **17**, 1257–1267. https://doi.org/10.1007/s00775-012-0940-x.1

Zhang, Z., Wang, H., Yan, M., Wang, H., & Zhang, C. (2017). Novel copper complexes as potential proteasome inhibitors for cancer treatment. *Molecular Medicine Reports*, **15**, 3–11. https://doi.org/10.3892/mmr.2016.6022

Cite this article: O'Leary T, McCarron P, Devereux M, Meaney S (2022). Potential interactions between metal-based phenanthroline drugs and the unfolded protein response endoplasmic reticulum stress pathway. *Experimental Results,* 3, e22, 1–12. https://doi.org/10.1017/exp.2022.20

Peer Reviews

Reviewing editor: Dr. Eray Metin Guler

Minor revisions requested.

doi:10.1017/exp.2022.20.pr1

Review 1: Potential interactions between metal-based phenanthroline drugs and the Unfolded Protein Response ER stress pathway

Reviewer: Dr. Mustafa Gokce

Bezmialem Vakif University, Istanbul, Turkey, 34093

Date of review: 13 June 2022

Conflict of interest statement. "Reviewer declares none"

Comment

Comments to the Author: -Page 5, Line 2: The title is not clear, and the abbreviations should not bu used in title.

-Page 6, Line 30: 'ER' Write what the abbreviation indicates.

-Page 8, Line 99: ''Pretreatment concentrations and incubation times were optimised in previous experiments.'' Where is the source ?

-Page 8, Line 90: Where A2780 and A549 cells were obtained from should be indicated.

-Page 8, Line 94: ''These cell densities are based on previous optimisation trials in our laboratory'' Which trials ? You should add them.

-Page 8, Line 103: ''..based on previous work in our laboratory which determined the IC50 and cytotoxic range of the compounds.'' Which work ?

-Literature information on how anisomcyin and tunicamycin are ER stress-inducing agents is not given.

-The figures and their explanations appear elsewhere in the file. I assume that the figures at the beginning of the file go in order. But if not, this situation should be fixed.

-Two different cancer cells were studied, and the effects of the UPR pathway on these cancer cells were examined over concentrations. It has been stated that different effects can be seen in both cell lines even at the same concentrations. Why weren't healthy cell lines added to these cell lines (like Human Ovarian Surface Epithelial Cells or Primary Lung Fibroblast Cells) and a relative comparison was not made? In this way, a more complex inference could be made from the concentration-viability response in healthy cells.

-Overall, this was a study in which you stated that you were investigating the interactions between the -ER-induced stress pathway and target drug molecules. But as you can see, your method consists of creating an ER stress with a drug and examining the viability of target molecules and derivatives against concentration. In this case, before making an interpretation with the ER-induced stress pathway, for example, if a certain protein expression levels on that pathway were examined, it would be a more accurate pathway interpretation.

Score Card

Presentation

Is the article written in clear and proper English? (30%)	5/5
Is the data presented in the most useful manner? (40%)	5/5
Does the paper cite relevant and related articles appropriately? (30%)	4/5

Context

Does the title suitably represent the article? (25%)	3/5
Does the abstract correctly embody the content of the article? (25%)	4/5
Does the introduction give appropriate context? (25%)	4/5
Is the objective of the experiment clearly defined? (25%)	2/5

Analysis

Does the discussion adequately interpret the results presented? (40%)	4/5
Is the conclusion consistent with the results and discussion? (40%)	3/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	2/5

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press

doi:10.1017/exp.2022.20.pr2

Review 2: Potential interactions between metal-based phenanthroline drugs and the Unfolded Protein Response ER stress pathway

Reviewer: Dr. Kubra Bozali

Avans University of Applied Science, Breda, Breda, Netherlands, 4800 RA

Date of review: 16 June 2022

Conflict of interest statement. Reviewer declares none

Comment

Comments to the Author: In this manuscript, the authors described "Potential interactions between metal-based phenanthroline drugs and the Unfolded Protein Response ER stress pathway". The study will be very useful for the literature. The report is an interesting study, but it needed some suggestions for publication.

Here are the concerns for the authors;

Generally;

1. In the titlepage, "Tadhg O'Leary a, b, i, 1, Pauraic McCarron b, c,3 Michael Devereux a, b, d,3 Steve Meaney a, b,2,3", the numbers are not stated by the affilitations.

2. The references et. al in the text should not give italic.

3. In materials section, the DMSO abbreviation should be explained.

4. In methods section, "A2780 (ovarian cancer) and A549 (alveolar adenocarcinoma cancer cell lines were cultured in RPMI1640, supplemented with 10% foetal bovine serum and 1% L-glutamine, and incubated….". The names of the cell lines should be given with the product codes, and the medium of A549 should be checked and corrected.

5. The lot number and brand name of chemicals and kits should be given in the materials and methods section.

6. The words such "CO2" and "foetal bovine serum" should be corrected.

7. The p en t should be written in italic in statistical analysis.

8. The p values should be given in the results section.

9. All the text should be in justify form.

10. The figure legends should be more descriptive and detailed.

11. In the figures, the concentrations of Mn-Phen are nM and μM. This should be the same.

12. The discussion section should be enriched in terms of content and the number of references should be increased.

Score Card

Presentation

3.3 /5

Is the article written in clear and proper English? (30%)	4/5
Is the data presented in the most useful manner? (40%)	3/5
Does the paper cite relevant and related articles appropriately? (30%)	3/5

Context

Does the title suitably represent the article? (25%)	4/5
Does the abstract correctly embody the content of the article? (25%)	3/5
Does the introduction give appropriate context? (25%)	3/5
Is the objective of the experiment clearly defined? (25%)	3/5

Analysis

Does the discussion adequately interpret the results presented? (40%)	4/5
Is the conclusion consistent with the results and discussion? (40%)	3/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	2/5

https://doi.org/10.1017/exp.2022.20 Published online by Cambridge University Press