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Cytotoxicity of Mechanochemically Prepared Cu(II) Complexes

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4 ABSTRACT: Despite evidence that mechanochemistry has the potential to be a
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7 revolutionary manufacturing technique, the Pharma industry is still reluctant to introduce
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10 these new technologies into their manufacturing processes. Therefore, it is now time to
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13 pioneer the implementation of mechanochemistry in industry, by analysing the bioactive
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16 and physicochemical properties of mechanochemical products. Herein, we report the
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19 mechanochemical synthesis of a Cu(II) metallodrug, that has been previously
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22 investigated in solution. Interestingly, we found that ball milling leads to the formation of
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25 a novel Cu(II) complex of octahedral geometry, whereas conventional synthesis resulted
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28 in a square-pyramidal complex. In addition, we report for the first time, the enhanced
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31 cytotoxicity of this Cu(II) metallodrug, towards ovarian cancer cell lines, as a result of its
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34 mechanochemical preparation.
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43 KEYWORDS: Mechanochemistry • Metallodrugs • Active Pharmaceutical Ingredients
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46 (APIs) • Cytotoxicity • Green Chemistry
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55 INTRODUCTION

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Significant advances have been made in the field of solvent-free synthesis, with IUPAC naming mechanochemistry as one of the top ten innovations that will change the world.[1]

Many areas of inorganic, organic and material syntheses have been explored by ball milling in a mixer mill (Figure 1a). These include C-C bond formations,[2a,b] preparation of porous materials (*e.g.* Metal Organic Frameworks (MOFs))[3] and formation of

Mixer Ball Mill

Planetary Ball Mill

Twin-Screw Extruder

coordination compounds, as well as composite and alloy materials.[4] Mechanochemistry provides a route to many known chemical transformations, but it has also led to the formation of products typically unobtainable *via* conventional methods.[5]

Figure 1. a) Mixer ball mill (< 5 g scale), b) planetary ball mill (< 500 g scale) and c) a twin-screw extruder (kg hr⁻¹).

As a result of growing interest into the field, scale-up has been an important topic, with many research groups utilising planetary mills to investigate this as a batch process (Figure 1b).[6] In this context, we have demonstrated the continuous solvent-free synthesis of several organic and inorganic materials using Twin-Screw Extrusion (TSE),[7] a method already established in food production and in the formulation of Active Pharmaceutical Ingredients (APIs) (Figure 1c).[8] Employing a laboratory sized extruder gives us the opportunity to scale-up solvent-free synthesis to a kg hr^{-1} scale, and with larger extruders available, further scale-up to ton hr^{-1} quantities is easily achievable.

One area of particular interest in mechanochemistry is the synthesis of APIs, and there are several examples in the literature of preparing these compounds by ball milling,[9] such as in the case of glibenclamide[10], and more recently by TSE, the synthesis of the hydrazone-based antibiotic, Nitrofurantoin,[11]. However, the Pharma industry are still hesitant about implementing mechanochemical synthesis into their manufacturing processes. One of the main reasons for this is that mechanochemistry is still a relatively

novel topic, and most of the research over the last two decades has focused solely on the mechanochemical synthesis process, *i.e.* identifying reaction types that can be carried out without a solvent. There are, however, relatively few studies on how mechanochemically-prepared materials can be implemented in the real world, *i.e.* when they are used for their intended application. Until very recently, there were no reports yet on the biological activity of compounds obtained mechanochemically. However, Bantreil and Gaillard *et al.*[12] have recently published the synthesis of cytotoxic silver n-heterocyclic carbenes by ball milling, wherein the final complexes demonstrated IC₅₀ values as low as 21 nM, making them 256 times more potent than cisplatin.[12]

Due to the lack of knowledge on the activity of mechanochemically prepared APIs, before mechanochemical manufacture is implemented into the Pharma industry, it is vital that we understand and identify any differences in the physicochemical and pharmacokinetic properties of these products. Furthermore, APIs prescribed in a solid form, *i.e.* a tablet, need to be dissolved in the body to be effective. If the dissolution rates differ from that expected, this will affect parameters such as API half-life (time required for an API

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3 concentration in the body to be reduced by one half).[13] This can also be extended to
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7 intravenous solutions and various drug delivery platforms, *e.g.* skin patches,[14] as
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10 differences in the pharmacokinetic factors can affect how quickly an API is absorbed by
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13 the patient's plasma and then eliminated.[15]
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21 The use of metals for pain relief and the effective management or treatment of diseases
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23 and infections is well reported.[16] Furthermore, coordination of a metal to an already
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27 known API has been seen to increase the dissolution rates of the latter, which may have
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31 an impact in overcoming drug resistance.[17] This has been particularly true with regards
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34 to the coordination of Cu(II) to various Non-Steroidal Anti-Inflammatory Drugs (NSAIDs).
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38 Enhanced potency is achieved *via* the higher dissolution rates of the copper complex
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41 compared to the free ligand, as well as possible Cu(II)-Cu(I) redox cycling processes
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Currently, the only Cu(II)-NSAID approved by the Medical and Healthcare products Regulatory Agency (MHRA) is a Cu(II) dimer of indomethacin,[19a] which is utilised for the treatment of inflammation and arthritis in domestic animals.[19b] However, a number of Cu(II)-NSAIDs have been shown to exhibit promising chemosuppressive and chemopreventive properties and therefore deserve further investigation. For example, *in vitro* studies carried out by Cini *et al.*[20,21] demonstrated high anti-proliferative activity of $[\text{Cu}(\text{HPir})_2(\text{DMF})_2]$ (where H_2Pir = piroxicam, Figure 2) against Small Cell Lung (SCL), Non-Small Cell Lung (NSCL), ovarian and melanoma tumours.[21] This advantageous behaviour is proposed to arise from Cu(II)-NSAIDs directly intercalating with ctDNA, disrupting the DNA backbone, and thus reducing cell viability, as confirmed by Circular Dichroism (CD) spectroscopy.[22] In addition, Alexandrova *et al.*[23] considered the antiproliferative effects of metal complexes with meloxicam (H_2Mel , a NSAID) acting as a ligand, and showed that $[\text{M}(\text{HMel})_2(\text{H}_2\text{O})_2]$ (where $\text{M} = \text{Cu}, \text{Zn}, \text{Co}, \text{Ni}$) exhibited improved cytotoxic effects compared to uncoordinated H_2Mel .

Figure 2. Structure of [Cu(HPir)₂(DMF)₂], as reported by Cini *et al.* [20]

Described herein is the solvent-free preparation of potential chemotherapeutic agents.

We wish to demonstrate that APIs prepared under solvent-free conditions exhibit the same cytotoxic behaviour as their conventionally prepared counterparts. We believe that this work is the next logical and important step in demonstrating that mechanochemical synthesis has excellent potential for API manufacture.

RESULTS AND DISCUSSION

Considering the relevance of Cu(II)-NSAIDs in cancer research, we focused our investigations on the ball mill synthesis of Cu(II) meloxicam complexes, $[\text{Cu}(\text{HMeI})_2(\text{S})_x]$ (where S = DMF or an alternative coordinating ligand, and $x = 1$ or 2). *In vitro* studies have shown that the mechanochemically prepared derivatives exhibit similar or enhanced cancer cell death within 48 hrs, compared to the conventionally prepared compound, of which the solvent-based synthesis and cytotoxicity has been reported previously.[21] This was desirable as it offered a basis upon which our cytotoxicity data could be compared to. Although these Cu(II) complexes are not yet available on the market as chemotherapeutic agents, this work shows for the first time that solvent-free manufacturing processes can be established without loss of biological activity.

The solvent-based synthesis of $[\text{Cu}(\text{HMeI})_2(\text{DMF})]$ was carried out as reported by Cini *et al.*, [21] in which $(\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O})$ was refluxed with commercially sourced H_2MeI in EtOH for 2 hrs, followed by recrystallisation from DMF, resulting in the formation of the hydrated

complex $[\text{Cu}(\text{HMeI})_2(\text{DMF})] \cdot 0.25\text{H}_2\text{O}$ (Figure 3).[21] The green product obtained was characterised by Infrared (IR) spectroscopy and Powder X-ray Diffraction (PXRD), and the results were fully consistent with those reported for $[\text{Cu}(\text{HMeI})_2(\text{DMF})] \cdot 0.25\text{H}_2\text{O}$, including a good match between the PXRD pattern of the product and the simulated pattern from crystallographic data (see ESI, Figure S1(b)).[21] Single Crystal X-ray Diffraction (SXRD) studies have shown that one DMF molecule coordinates apically to the Cu(II) centre, adopting a square-pyramidal geometry instead of the more common octahedral shape.[21] This has been attributed to i) steric hindrance and ii) the formation of secondary interactions between the sulfoxide and the thiazolyl methyl groups, so that a solvent molecule cannot coordinate to the adjacent apical coordination site.[24]

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 \text{secondary} \\
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 \end{array}
 \begin{array}{c}
 \text{2} \\
 \text{methyl groups,}
 \end{array}
 \begin{array}{c}
 \text{interactions between the sulfoxide and the} \\
 \text{methyl groups,}
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 \begin{array}{c}
 \text{so that a solvent molecule cannot} \\
 \text{coordinate to the adjacent apical} \\
 \text{coordination site.}[24]
 \end{array}
 + \text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$$

i) EtOH/ Δ /2h
 ii) DMF

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 - H₂O

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Figure 3. Conventional synthesis of $[\text{Cu}(\text{HMeI})_2(\text{DMF})]\cdot 0.25\text{H}_2\text{O}$ carried out in EtOH, followed by recrystallisation from DMF, as reported by Cini *et al.*[21]

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3 Ball milling between $(\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O})$ and 2 mol. eq. of H_2Mel was initially attempted under
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7 neat conditions but was unsuccessful, most likely due to the absence of coordinating
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10 DMF. Therefore, the reaction was repeated with 1 mol. eq. of DMF, but this resulted in a
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13 black, viscous material that could not be characterised by PXRD. Since the solvent-based
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17 synthesis employs both EtOH and DMF, a *ca.* 1:1.1 mol eq. EtOH:DMF mixture was used
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21 in the ball mill reaction, with PXRD indicating that a partial reaction had taken place.
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29 Subsequent to this, the parameters required to drive the reaction to completion were
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32 investigated, with ball mill frequency (10 – 25 Hz), milling time (15 - 60 mins) and the
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36 amount of added solvent varied. PXRD was used to monitor the outcome of each reaction.
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40 The variable frequency experiments (1 hr, 1:1.1 mol. eq. EtOH:DMF) showed that at
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43 frequencies over 15 Hz there was loss of crystallinity in the final product. Additionally, in
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47 all cases the PXRD patterns contained sharp diffraction peaks at 2θ positions of *ca.* 38.4°
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50 and *ca.* 44.7° , attributed to residual quantities of $(\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O})$ (see ESI, Figures S3 –
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S6). However, by increasing the amount of EtOH:DMF to 2:2.2 mol. eq., a complete conversion to product was obtainable at a frequency of 15 Hz within 15 mins (Figure 4a).

The elemental (CHNS) analysis of the ball milled product was close to that expected for $[\text{Cu}(\text{HMeI})_2(\text{DMF})] \cdot 0.25\text{H}_2\text{O}$ (see ESI), and its IR spectrum matched that of the conventionally synthesised complex, although the IR bands of the ball mill product were generally broader; for example, the $\nu(\text{CO})$ stretching band of coordinated DMF (*ca.* 1680 cm^{-1}) appeared as a shoulder on the broad band present at *ca.* 1570 cm^{-1} (see ESI, Figure S11). However, comparison of the PXRD pattern for the ball mill product with both the simulated[21] and solution PXRD patterns, did show some differences (Figure 4b). In particular, an additional diffraction peak at a 2θ value of *ca.* 26° was present in the pattern

of the ball

mill product.

a)

b)

Figure 4. PXRD pattern of the product obtained by ball milling $(\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O})$ and H_2Mel (1:2), at 15 Hz for 15 mins, in the presence EtOH:DMF (2:2.2), compared with (a) the

PXRD patterns of starting materials, and (b) the simulated pattern for $[\text{Cu}(\text{HMeI})_2(\text{DMF})] \cdot 0.25\text{H}_2\text{O}$ (CCDC: 654174).[21] (BM = Ball Mill)

The geometry of the ball mill product was then investigated as the source of differing PXRD patterns. As 2.2 mol. eq. of DMF was added to the reactions, it is feasible that DMF coordinates to both axial positions. Unfortunately, crystals suitable for SXRD were not obtained, which could have confirmed the geometry of the ball mill product.

IR spectroscopy did not reveal any information regarding the compound's geometry, as the spectrum reported for the solution-synthesised compound and that of the ball mill product were very similar, as noted above. The found CHNS composition indicates the presence of only one DMF molecule per Cu(II) atom, but the product had to be dried overnight in order to remove excess solvent prior to submitting the sample for elemental analysis. In these conditions, it is possible that some of the DMF was also removed.

PXRD was therefore our main route to hypothesising the final geometry of the ball mill product. Chen *et al.*[25] report formation of two different Cu(II) coordination complexes with the API, topiroxostat (ptpc). The authors prepared both an octahedral, $(\text{Cu}(\text{ptpc})_2(\text{NO}_3)_2(\text{DEF})_2)$ (DEF = diethylformamide), and a square-pyramidal, $([\text{Cu}(\text{ptpc})_2(\text{NO}_3)(\text{DMF})_2] \cdot \text{NO}_3)$, complex; obtaining the different geometries by changing the alkyl group in the dialkylformamide solvent.[25] SXRD was used to determine the geometry however, as this was unavailable to us, we considered the differences in the diffraction patterns reported by Chen *et al.*,[25] namely in the 2θ range of $5 - 10^\circ$ and at *ca.* 28° , and compared them to those we were observing. With regards to the former, diffraction occurs at different angles dependent on geometry, *i.e.* the octahedral complex diffracts at a 2θ of *ca.* 8.0° and *ca.* 7.2° for the square-pyramidal geometry. This is also observed herein, with the simulated pattern showing a diffraction peak at a 2θ value of *ca.* 5.6° [21] compared to the ball mill product diffraction peak at a 2θ value of *ca.* 6.1° . In addition, the PXRD pattern for Chen's octahedral complex has an isolated diffraction peak at a 2θ value of *ca.* 26.5° . [25] However, in the pattern for the square-pyramidal complex, an isolated diffraction peak at this 2θ value is not observed and instead a cluster of peaks

are seen, with a sharp reflection at a 2θ value of *ca.* 29° .^[25] Herein, we see that the simulated pattern does not contain any significant diffraction between a 2θ value of $25 - 30^\circ$,^[21] however the ball mill product does show an intense peak at a 2θ value of *ca.* 26° . Furthermore, Chen *et al.*^[25] do not report any significant differences between the IR spectra of the two compounds, in-line with our experimental observations.

Although with the available data we cannot fully confirm the amount of DMF coordinated to Cu(II), comparisons with the literature and the information from the Crystal Structure Database (CSD), as discussed above, allows us to conclude that an octahedral complex of formula $[\text{Cu}(\text{HMeI})_2(\text{DMF})_x]$ (where $x = 2$), is the most likely product formed by ball milling, instead of the expected square-pyramidal complex.^[21] Additionally, we strengthen our argument for obtaining an octahedral complex later, when we discuss the preparation of $[\text{Cu}(\text{HMeI})_2(\text{THF})_2]$.^[26]

On account of the possible application of $[\text{Cu}(\text{HMeI})_2(\text{DMF})_x]$ as a chemotherapeutic NSAID, it is important to reduce the general toxicity (*i.e.* toxicity targeted towards all cells, not just cancer cells) of the complex. This could be achieved by replacing DMF with more innocuous solvents.[27] Therefore, the Liquid Assisted Grinding (LAG)[28] of $(\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O})$ and H_2MeI was carried out at 15 Hz for 2 hrs (to ensure a complete reaction), in a 2:2.2 mol. eq. mixture of $\text{EtOH}:\text{S}$, where S is acetonitrile (MeCN), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO) or H_2O . PXRD showed that regardless of the solvent used, the 2θ peak positions were largely similar in all cases (Figure 5), although some small variations were observed, associated with differences in the crystal

packing because of the various solvents employed.

Figure 5. PXRD patterns of Cu(II) meloxicam complexes $[\text{Cu}(\text{HMeI})_2(\text{S})_x]$ obtained upon varying the coordinating solvent, S, and milling at 15 Hz for 2 hrs.

Whilst most of these compounds have not previously been reported, $[\text{Cu}(\text{HMeI})_2(\text{THF})_2]$ has been shown to have an octahedral geometry[26] and the synthesis of $[\text{Cu}(\text{HMeI})_2(\text{H}_2\text{O})_2]$ has also been reported.[23] Crucially, the simulated PXRD pattern of $[\text{Cu}(\text{HMeI})_2(\text{THF})_2]$ and that obtained from the ball mill experiment matched well (see ESI, Figure S17). Furthermore, the PXRD patterns obtained for all the complexes, whereby

the solvent (S) was varied, were very similar, further supporting that synthesis by ball milling results in the formation of an octahedral complex, rather than square-pyramidal. This is a very interesting result, highlighting the advantage that mechanochemical synthesis can result in different compounds or phases, compared to those obtained in solution.

Importantly, the coordinated-solvent study shows that DMF, which is toxic to all cells, is not essential for the successful mechanochemical synthesis of a Cu(II) meloxicam complex.

The potency of mechanochemically prepared $[\text{Cu}(\text{HMeI})_2(\text{S})_x]$, where S = DMF, DMSO or H_2O , was assessed by Resazurin cell viability assay. Coordination of DMSO may enhance the anti-proliferative effects of $[\text{Cu}(\text{HMeI})_2(\text{S})_x]$, on account of the high affinity which DMSO has for cancer-causing, free-radical species. In addition, the ability of DMSO to permeabilise membranes, including the blood-brain barrier, may improve the targeted activity of the Cu(II)-NSAID complex towards various forms of brain cancer.[29] As H_2O

has been demonstrated to be less toxic to both healthy and cancer cells (compared with the other coordinating solvents investigated),[27] $[\text{Cu}(\text{HMeI})_2(\text{H}_2\text{O})_x]$ was selected for testing. In addition, $([\text{Cu}(\text{HMeI})_2(\text{DMF})].0.25\text{H}_2\text{O})$ prepared conventionally, was tested and employed as a benchmark for the ball mill products.

IGROV-1 and SKOV-3 ovarian cancer cell lines were initially incubated with 10 – 100 μM of $([\text{Cu}(\text{HMeI})_2(\text{DMF})].0.25\text{H}_2\text{O})$ (prepared *via* conventional synthesis), where the final DMSO concentration (< 2%) did not contribute to the overall toxicity. The cell viability was then measured after 48 and 72 hrs, with greater potency observed for SKOV-3 cells (see ESI, Figure S18), in line with that reported by Cini *et al.*[21] for $[\text{Cu}(\text{HPir})_2(\text{DMF})_2]$. Furthermore, it was determined that dramatic cell death occurred within the first 48 hrs at 100 μM . Subsequently, the toxicity of Cu(II) complexes obtained by ball milling, as well as the uncoordinated ligand, were tested in SKOV-3 cancer cells. 48 hrs post-incubation, the Cu(II) complex toxicity was concentration-dependent (from 10 - 100 μM). As the concentration of the uncoordinated ligand increased (from 50 to 100 μM), there was a

very small decrease in cell viability, *ca.* 5 - 10%, however, this drop in cell viability was too small for it to be considered as cytotoxic (Figure 6).

Figure 6. Cytotoxicity of various $[\text{Cu}(\text{HMel})_2(\text{S})_x]$ complexes. SKOV-3 cells were seeded in 96-well plates (1×10^4 cells/well), and next day incubated with different drug concentrations (10, 50 and 100 μM). Cell viability was assessed using Resazurin assay. The results are expressed as the mean $\pm$ SD of $n = 6$ samples of two independent experiments, calculated as a percentage of the control.

Upon closer analysis of the cytotoxic behaviour of the Cu(II) complexes, no significant decrease in cell viability can be seen at 10 μM . Furthermore, there does not appear to be a substantial difference between the cytotoxicity of the parent ligand and the Cu(II)

complexes. However, at 50 μM we see a decrease in cell viability for the Cu(II) complexes of *ca.* 10 - 20%, whilst at 100 μM , the cytotoxicity of all Cu(II) complexes significantly improved, with > 50% cell death being observed. Interestingly however, there was one exception, $[\text{Cu}(\text{HMeI})_2(\text{DMF})] \cdot 0.25\text{H}_2\text{O}$ prepared *via* conventional synthesis, showed only *ca.* 20% cell death at 100 μM , but importantly, this is still regarded as cytotoxic. It must also be noted that the treatments at a concentration of 100 μM (and the ball mill products at 50 μM) had a significance difference compared to the control and *versus* the conventional method of synthesis (see ESI Figure S19 for complete ANOVA analysis).

Unexpectedly, the ball mill product, $[\text{Cu}(\text{HMeI})_2(\text{DMF})_x]$, exhibited enhanced cytotoxic behaviour (*ca.* 30 - 40%), compared with that conventionally synthesised, on SKOV-3 cells, 48 hrs after treatment at 100 μM .

It must also be noted that the studies carried out here and those of Cini[21] and Alexandrov,[23] all agree that the Cu(II) complexes exhibited enhanced cytotoxicity,

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3 compared to the uncoordinated ligand. This is most probably due to the enhanced efficacy
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7 that tumour cells have for metal complexes or the positive redox potential offered by
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19 Although direct comparisons with the literature are difficult due to the different
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22 methodologies used, some conclusions can be drawn regarding the cell viability of
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26 products produced by ball milling, compared with those synthesised conventionally. For
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29 example, in comparison with the study by Cini *et al.*[21] on the effect of $[\text{Cu}(\text{HPir})_2(\text{DMF})_2]$
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32 on SKOV-3 cells, we can conclude that the H_2Pir derivative is five times more potent than
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36 its H_2Mel analogue. Within 48 hrs, $[\text{Cu}(\text{HPir})_2(\text{DMF})_2]$ exhibited a GI50 value of 20.0 μM ,
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39 *i.e.*, 50% cancer cell death is achieved within this time. In order for $([\text{Cu}(\text{HMel})_2(\text{DMF})_x])$ to
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42 reach a cell viability of *ca.* 50% within 48 hrs, 100 μM concentrations were required.
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46 However, $([\text{Cu}(\text{HMel})_2(\text{DMF})_x])$ has a greater potency towards SKOV-3 cancer cells than
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50 $[\text{Cu}(\text{HMel})_2(\text{H}_2\text{O})_2]$ towards HeLA cells (cervical cancer cell lines), as reported by
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54 Alexandrov *et al.*[23] Expressing our concentrations in the same units as these authors,
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we observe > 50% SKOV-3 cell death within 48 hrs, using a concentration of *ca.* 90 $\mu\text{g/mL}$. In order to obtain a similar cell viability result, HeLa cell lines would have to be treated with 500 $\mu\text{g/mL}$ solutions of $[\text{Cu}(\text{HMeI})_2(\text{H}_2\text{O})_2]$ over 96 hrs.

It is possible that the enhanced cytotoxicity of $[\text{Cu}(\text{HMeI})_2(\text{DMF})_x]$ prepared *via* ball milling, is a direct result of having two DMF molecules per one Cu(II) coordination site, as DMF is known to be cytotoxic in itself.[29] However, the difference in cytotoxicity between the ball mill and conventionally prepared complexes (*ca.* 30 - 40%) appears to be too significant to be the result of additional DMF alone.[29] Whilst further investigation is needed to determine the exact cause for this enhanced cytotoxicity, several factors may be taken into consideration. For example, it is known that relatively small variations in the coordination environment of Cu(I)/Cu(II) complexes can have a significant effect on their anticancer activity; *e.g.* the nature of the anion (X^-) has been reported to affect the cytotoxicity of $[\text{Cu}\{\text{NH}(\text{R})-(\text{CH}_2)_n-\text{NH}_2\}\text{LX}]^+$ ($\text{X} = \text{Cl}^-, \text{Br}^-$),[30] although the reasons for this are unclear. In other cases, similar effects have been related to alterations in the

Cu(II)/Cu(I) redox potential, which in turn affects the DNA binding and cleavage properties of the complex.[31] More significantly, it has been reported that octahedral Cu(II) complexes show enhanced anticancer activity compared to their square-pyramidal analogues.[32] Palaniandavar *et al.*[32a] found a DNA binding constant up to thirty times higher for their octahedral complex compared to the square-pyramidal derivative; with increased DNA binding translating to enhanced cytotoxic behaviour.[32b]

CONCLUSION

In conclusion, we have demonstrated that $[\text{Cu}(\text{HMeI})_2(\text{DMF})_x]$ can be successfully prepared by ball milling, under reduced solvent conditions (typically 2 mol. eq. of solvent required). Key to this work, we have shown, for the first time, that $[\text{Cu}(\text{HMeI})_2(\text{DMF})_x]$ prepared mechanochemically, exhibits similar toxicity towards SKOV-3 cells at 50 μM whilst exhibiting enhanced cytotoxicity at 100 μM . In addition, we have demonstrated that DMF can be replaced with innocuous solvents, which may result in the reduced death of healthy cells, whilst maintaining the chemotherapeutic properties of the complex. It was

also confirmed in all cases that the Cu(II) complexes resulted in greater SKOV-3 cell death than the NSAID, H₂Mel, alone.

The higher cytotoxicity found for the copper compounds described herein is an unexpected result that can be related to one or more aspects, as discussed above.

Although it is not possible to establish the exact causes with the currently available data, our work highlights the impact that the synthetic method has on the biological response of metal compounds. If more environmentally friendly mechanochemical synthesis is to be implemented by the Pharma industry, it is important to demonstrate that the activity of mechanochemical products remains at least the same as the solvent-based synthesised counterparts, and that the factors that may affect their activity are studied.

Supporting Information. The following file is available free of charge.

Electronic Supplementary Information (PDF): *McCalmont et al._ESI*

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Graphical Abstract:

SYNOPSIS: The enhanced cytotoxicity afforded by ball mill synthesis under minimal solvent conditions, is shown for a Cu(II) metallodrug of non-typical geometry.

574x313mm (120 x 120 DPI)