CHEMISTRY
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ChemPubSoc Europe

## Accepted Article

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**To be cited as:** *Chem. Eur. J.* 10.1002/chem.202001014

**Link to VoR:** http://dx.doi.org/10.1002/chem.202001014

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10.1002/chem.202001014


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# Potent anticancer efficacy of first-in-class Cu(II) and Au(III) metaled phosphorus dendrons with distinct cell death pathways

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**Abstract:** First-in-class Cu(II) and Au(III) metaled phosphorus dendrons were synthesized and showed significant antiproliferative activity against several aggressive breast cancer cell lines. The data suggest that the cytotoxicity increases with reducing the length of the alkyl chains, whereas the replacement of Cu(II) by Au(III) considerably increases the antiproliferative activity of metaled phosphorus dendrons. Very interestingly, we found that the cell death pathway is related to the nature of the metal complexed by the plain dendrons. Cu(II) metaled dendrons showed a potent caspase-independent cell death pathway; whereas Au(III) metaled dendrons displayed a caspase-dependent apoptotic pathway. The complexation of plain dendrons with Au(III) increased the cellular lethality *versus* dendrons with Cu(II) and promoted the translocation of Bax into the mitochondria and the release of Cytochrome C (Cyto C).

## Introduction

The discovery of cisplatin (*cis*-diaminodichloroplatinum(II)) in 1965 by Rosenberg, through a serendipitous approach, played a pivotal role in the discovery of many metallo-drugs used today in the treatment of cancer.[1] Cisplatin is the first approved anticancer drug based on an inorganic complex, and it is now used to treat a wide range of cancers.[2] This built the foundation of the modern era for the rapid development of anticancer metallo-drugs using several types of metals such as palladium, ruthenium, osmium, iron, gold, copper, and rhodium.[3] Note that other metals such as nickel, cadmium, chromium, and arsenic induced carcinogenesis effects.[4] The main limitation of metallo-drugs is their potential instability in the blood and their oxidation profile.[5]

Nanotechnology represents a huge multidisciplinary field encompassing chemistry, biology, physics, engineering, etc., and it has appeared to be a benefit to humanity in a wide variety of multidisciplinary domains including therapeutic domains (termed nanomedicine) such as in the fields of cancer, cardiovascular disease, and central nervous system disorders.[6] Thus, the rational design of nanoparticles encapsulating or complexing anticancer drugs (such as doxorubicin liposomes (Doxil®) and paclitaxel albumin (Abraxane®), which have been U.S. Food and Drug Administration (FDA) approved in 1995 and 2005, respectively) plays a pivotal role to treat cancer.[7]

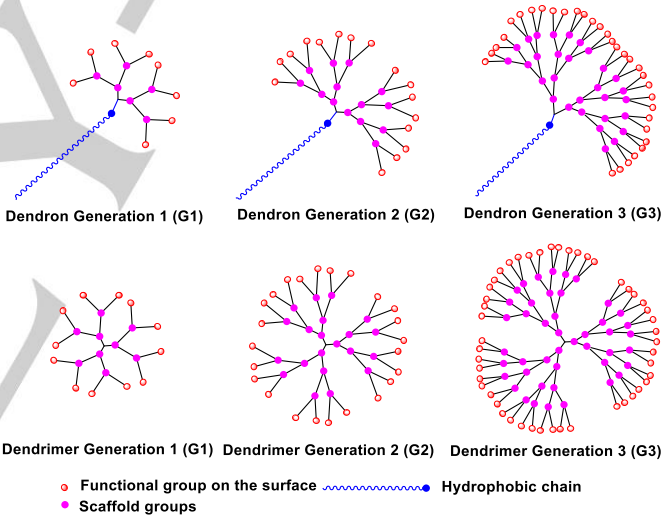


**Figure 1.** Schematic 2D chemical structures of dendrons and corresponding dendrimers.

Within the nanomedicine field, the remarkably unique and tunable properties of monodisperse dendrimers and dendrons have offered the opportunity to open new avenues in the domain of oncology. They are mainly used as nanocarriers of drugs, small interfering RNA (siRNA), and anti-sense therapy (gene delivery), as well as in diagnostics.[8] Dendrimers are hyperbranched globular macromolecules, they are repetitively branched molecules with a branched mode of growth and are prepared by iterative reactions. Dendrons are also monodisperse, owning wedge-shaped dendrimer sections with multiple terminal groups. They generally have a single distinct chemically addressable group at the focal point.[9]

The 2D schematic difference between dendrons and dendrimers (generations 1, 2 and 3) is illustrated in Figure 1. Due to their high degree of molecular uniformity, and perfect

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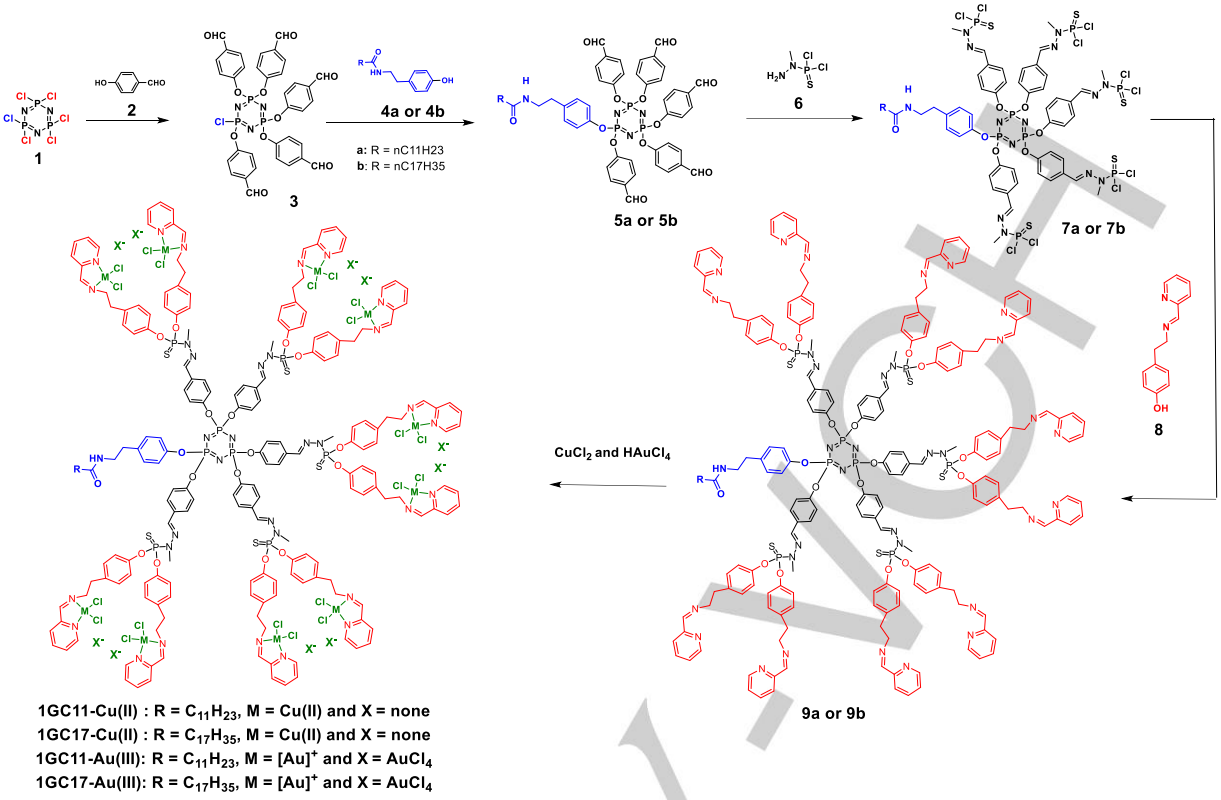


**Figure 2.** Synthetic pathways of **9a, 9b**, and metaled phosphorus dendrons **1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III),** and **1GC17-Au(III).**

control of their shape, size, and surface groups, dendrimers and dendrons offer a variety of drug-design options and important suitable alternatives for the delivery of active biomolecules in oncology applications.[9-10] Interestingly, several examples of dendrimer- conjugated metallo-drugs have been pointed out such as poly (alkylidenimine) dendrimers functionalized with Ru metal,[11] and multivalent Cu(II)-conjugated phosphorus dendrimers developed by several of us (S. M., A-M. C.; and J-P. M.).[12] Thus, in 2013, novel Cu(II)-phosphorus dendrimers - generations 1 (12 Cu(II)), 2 (24 Cu(II)), and 3 (48 Cu(II)) - were prepared in good overall yield and displayed moderate to good antiproliferative properties against KB (epidermal carcinoma) and leukaemia HL60 cell lines (promyelocyte). The most potent phosphorus dendrimer came from generation 3 (termed 1G3-Cu(II), Figure S1), with 48 Cu(II) on the surface. 1G3-Cu(II) also showed sustainable antiproliferative activities against a panel of tumor cells such as HCT116 (human colon cancer), MCF7 (hormone-responsive breast cancer), OVCAR8 (ovarian carcinoma), and U87-MG (human glioblastoma-astrocytoma, epithelial-like) cancer cell lines. Outstandingly, 1G3-Cu(II) demonstrated a good safety ratio based on its $IC_{50}$ non-cancer cells/$IC_{50}$ cancer cells ratio. MRC5 (proliferative human lung fibroblasts) and the quiescent EPC (endothelial progenitor cells, Cyprinus carpio) have been selected as non-cancer cell lines. The $IC_{50}$s against cancer cell lines ranged between ~200 and ~800 nM, and against non-tumor cell lines between 800 and 1400 nM. Recently, Del Olmo, N. S *et al*. introduced the same bidentate chelator with Cu(II) in the carbosilane dendrimer series affording similar *in vitro* antiproliferative activities.[13]

Thereafter, in line with our previous work (*vide supra*), S. Mignani and J-P. Majoral *et al.* conjugated the most potent G3 phosphorus dendrimer with Au(III) in place of Cu(II) giving **1G3-Au(III)**.[12, 14] The complexation of the dendrimer with Au(III) strongly increased the antiproliferative activity against both KB and leukemia HL-60 cancer cell lines *versus* the corresponding **1G3-Cu(II)**, showing $IC_{50}$s in the low nanomolar range while maintaining a good safety ratio.

Taking inspiration from the potent antiproliferative activity of the phosphorus dendrimers **1G3-Cu(II)** and **1G3-Au(III)**, in this manuscript we describe the synthesis and strong antitumor properties of four first-in-class multivalent Cu(II) and Au(III) metaled phosphorus dendrons (generation 1, 10 terminal groups) bearing two different types of linear alkyl chains (termed **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III)** and **1GC17-Au(III)**). Outstandingly, Cu(II)-phosphorus dendrons and Au(III)-phosphorus dendrons displayed a distinct cell death pathway.

## Results and Discussion

As shown in Figure 2, we prepared four different dendrons bearing two distant linear alkyl chains ($C_{11}H_{23}$ and $C_{17}H_{35}$), and bearing ten *N-(*pyridin-2-ylmethylene) ethanamine groups to complex Cu(II) and Au(III) to afford **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III)** and **1GC17-Au(III)**. These dendrons were prepared with good overall yields. The regioselective

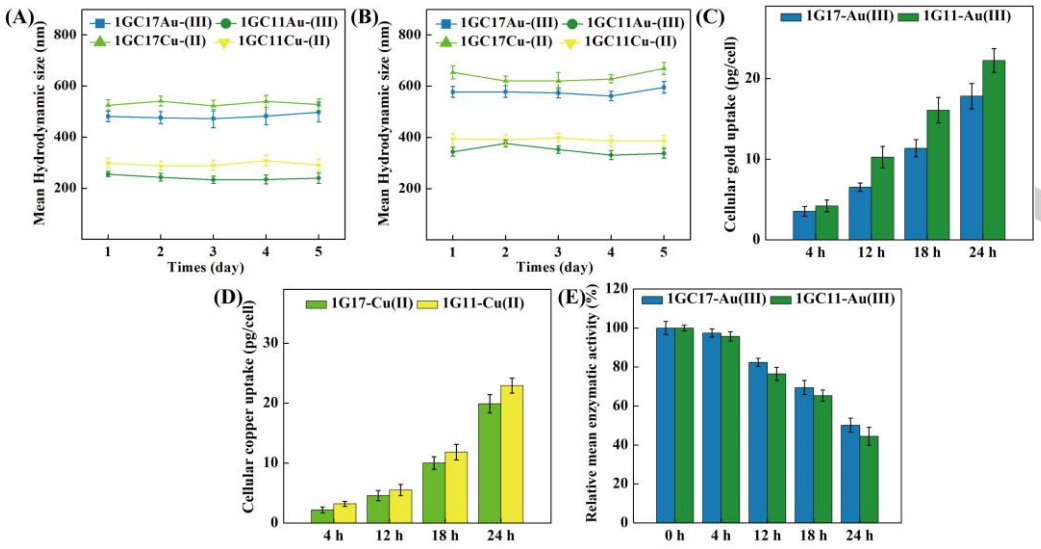


**Figure 3.** Hydrodynamic size of the metaled dendrons in normal saline (A) and medium (B) at different time periods; Cell uptake in 4T1 cells after incubation with Au(III) metaled dendrons (C) or Cu(II) metaled dendrons (D) at the dendrons concentration of 10 μM for different time periods; Thioredoxin reductase activity of 4T1 cells incubation with **1GC17-Au(III)** and **1-GC11-Au(III)** at the dendrons concentration of 10 μM (E).

condensation of 4-hydroxybenzaldehyde (**2**) with the hexachlorocyclotriphosphazene (**1**) (THF, room temperature) afforded the **$AB_5$** monomer (**3**) in 76% yield. Then, **3** was treated with the amino-phenol-derivatives **4a** and **4b** (cesium carbonate, THF, room temperature) to give the dendrons **5a** and **5b** in 85% yield. Thereafter, the resulting dendrons were treated with (1- methylhydrazinyl)phosphonothioic dichloride (**6**) (dichloromethane, room temperature) to afford **7a** and **7b** (90% yield), which were treated with 4-(2-((pyridin-2-ylmethylene)amino)ethyl)phenol (**8**) (cesium carbonate, THF, room temperature), to give the dendrons **9a** and **9b** in 85% yield. Finally, the complexation of **9a** and **9b** with $CuCl_2$ and $HAuCl_4$ (dimethylformamide, room temperature) gave the corresponding metaled-dendrons **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III)** and **1GC17-Au(III)** in 78–81% yield. **1GC11-Cu(II)** and **1GC17-Cu(II)** contain 10 $CuCl_2$ on their surface, whereas **1GC11-Au(III)** and **1GC17-Au(III)** incorporate 10 $[AuCl_2]^+/[AuCl_4]^-$ on their surface. Monitoring the progress of the reaction was performed using $^{31}P$ NMR, $^{1}H$ NMR, and $^{13}C$ NMR spectral analysis and inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis, which corroborate the structure of the metaled phosphorus dendrons (Supporting Information).

The hydrodynamic sizes of the four metaled dendrons were analyzed *via* dynamic light scattering (DLS). The results are shown in Table S1. Interestingly, the hydrodynamic sizes of the metaled dendrons with shorter alkyl chain length (C11) (~255 nm) are smaller than those with the longer alkyl chain length ($C_{17}$) for the same metal (~ 298 nm). Meanwhile, the presence of different metal on the surface plays a role in hydrodynamic size of the metaled dendrons.

To explore the stability of the metaled dendrons, the hydrodynamic sizes of the metaled dendrons dispersed in both normal saline and complete cell culture medium at 37 ºC were monitored at different time periods. As shown in Figure 3A-B, Au(III) and Cu(II) metaled dendrons exhibited approximately similar hydrodynamic sizes at different time periods in both media, indicating their good colloidal stability.

The primary objective of this study was to investigate the antiproliferative activity of the four metallo-dendrons prepared. Two different breast cancer cell lines were chosen for antiproliferative activity: 1) 4T1, mouse breast adenocarcinoma cells which are highly tumorigenic and invasive unlike most tumor models used; and 2) MCF-7, human breast adenocarcinoma cells which are widely studied as epithelial cancer cells, as well the normal fibroblast NIH-3T3 cells and the human fetal lung fibroblast cells MRC5 for safety purposes.

**Table 1.** Anti-proliferative activities of dendrons **9a**, **9b**, **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III))** and **1GC17-Au(III).**

| | $IC_{50}$ ($\mu$M)[a] | | | |
|---|---|---|---|---|
| | **4T1*** | **MCF-7*** | **NIH-3T3*** | **MRC5*** |
| **9a** | >100 | >100 | >100 | NT |
| **9b** | >100 | >100 | >100 | NT |
| **1GC11-Cu(II)** | 0.585±0.11 | 1.489±0.27 | 1.491 ± 0.22 | 2.46 ±1.20 |
| **1GC17-Cu(II)** | 1.025±0.09 | 2.755±0.25 | 4.075 ± 0.65 | 2.66 ± 0.64 |
| **1GC11-Au(III))** | 0.164±0.04 | 0.286±0.07 | 0.468 ± 0.12 | 2.71 ± 0.71 |
| **1GC17-Au(III)** | 0.339±0.06 | 0.802±0.15 | 0.132 ± 0.06 | 3.18 ± 0.27 |

[a] Cells were incubated dendrons at eight concentrations (0.01 to 50 μM) for 72 h and the data were represented as "mean ± SD" (n = 3).

Firstly, we compared the anti-proliferative properties of two phosphorus dendrons bearing Cu(II) as the metal moiety and with two different linear alkyl chains, C11 (**1GC11-Cu(II)**) and C17 (**1GC17-Cu(II)**) against 4T1, MCF-7, NIH-3T3, and MRC5 cells. As shown in Table 1, in the absence of metal ions, the dendrons **9a** and **9b** did not display any inhibitory effect on cell proliferation ($IC_{50}$s >100μM) against the two cell lines (MCF-7 and NIH-3T3), whereas the introduction of Cu(II) increased the

antiproliferative activity against 4T1 and MCF-7 cells with $IC_{50}$s between 0.6 and 2.8 µM. The dendron **1GC11-Cu(II)** showed safety ratios of ~2.5 ($IC_{50}$ NIH-3T3/$IC_{50}$ 4T1), ~4 ($IC_{50}$ MRC5/$IC_{50}$ 4T1), 1 ($IC_{50}$ NIH-3T3/$IC_{50}$ MCF-7), and ~1.7 ($IC_{50}$ MRC5/$IC_{50}$ MCF-7), whereas, the dendron **1GC17-Cu(II)** showed safety ratios of ~4 ($IC_{50}$ NIH-3T3/$IC_{50}$ 4T1), ~3 ($IC_{50}$ MRC5/$IC_{50}$ 4T1), ~1.5 ($IC_{50}$ NIH-3T3/$IC_{50}$ MCF-7), and 1 ($IC_{50}$ MRC5/$IC_{50}$ MCF-7). Clearly, these data demonstrated that the complexation of phosphorus dendrons with Cu(II) boosts the antiproliferative activity *versus* nonmetallic dendrons, and the safety ratio was related to the metal considered, to the nature of the alkyl chain and to the nature of normal and tumoral cells used.

Thereafter, our attention was directed to the replacement of Cu(II) by Au(III). Previously, we showed that the replacement of Cu(II) in the phosphorus dendrimer generation 3 (**1G3-Cu(II)**) by Au(III) (**1G3-Au(III)**) strongly boosted the antiproliferative activities against the KB cancer cell lines and HL60 (promyelocytic) cell lines (*vide supra*) while keeping good a safety ratio. In this direction, we prepared the metallo-dendrons **1GC11-Au(III)** and **1GC17-Au(III)** (Figure 2). The antiproliferative activities of **1GC11-Au(III)** and **1GC17-Au(III)** are presented in Table 1.

As observed in the phosphorus dendrimer series (*vide supra*), the replacement of Cu(II) by Au(III) strongly improved the antiproliferative activity. **1GC11-Au(III)** and **1GC17-Au(III)** showed $IC_{50}$s of ~0.16–0.8 µM against 4T1 and MCF-7 cells. The improvement was between 3- and 5-times in favor of Au(III) *versus* Cu(II). Interestingly, the safety ratios for **1GC11-Au(III)** were ~3 and ~1.7, for 4T1 and MCF-7 *versus* NIH-3T3 cells, and ~17 and ~9, for 4T1 and MCF-7 *versus* MCR5 cells, respectively. The safety ratio for **1GC17-Au(III)** were ~0.3 and ~0.1, for 4T1 and MCF-7 *versus* NIH-3T3 cells, and ~11 and ~4, for 4T1 and MCF-7 *versus* MCR5 cells, respectively. As previously mentioned, the safety ratio for the considered metal is related to the nature of the alkyl chain and to the nature of normal cell lines used.

Then, we evaluated the cellular uptake of the metaled dendrons in 4T1 cells after the cells were treated with the compounds at different time periods up to 24 h (Figure 3C-D). Clearly, all compound increased the Au or Cu uptake with the extension of incubation time. Interestingly, the metaled dendrons with shorter alkyl chain length ($C_{11}$) exhibit more uptake than those with the longer alkyl chain length ($C_{17}$) for the same metal. Hence, for the same metal, the metaled dendrons with $C_{11}$ chain display a better therapeutic efficacy than those with $C_{17}$ chain (see Table 1).

Taken together, these data fully confirm the strong anticancer profile of phosphorus dendrons bearing linear alkyl chains ($C_{11}H_{23}$ and $C_{17}H_{35}$) and complexed with Au(III). As in the copper series, the nature of the alkyl chain plays a major role in the safety ratio profile.

In order to extend the panel of cancer cell line profile, we tested the antiproliferative activities of **1GC11-Cu(II)**, **1GC17- Cu(II)**, **1GC11-Au(III),** and **1GC17-Au(III)** against leukemia HL-60, human colon cancer HCT116, and the chronic myeloid leukemia cell line K562. As shown in Table 2, moderate antiproliferative activities were observed regardless of the metal complexed and the length of the alkyl chains, and $IC_{50}$s were between ~1.3 and ~8 µM.

The next study is the comparison of the antiproliferative activities of the generation 1 metaled phosphorus dendrimers bearing 12 metal groups and the dendrons of generation 1 bearing 10 metal groups (Figure 4). Table 2 presents the antiproliferative activities of the dendrimers **1G1-Cu(II)**, **1G1-Au(III)** and, the dendrons **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III)**, and **1GC17-Au(III)** against HL-60 and HCT116. The

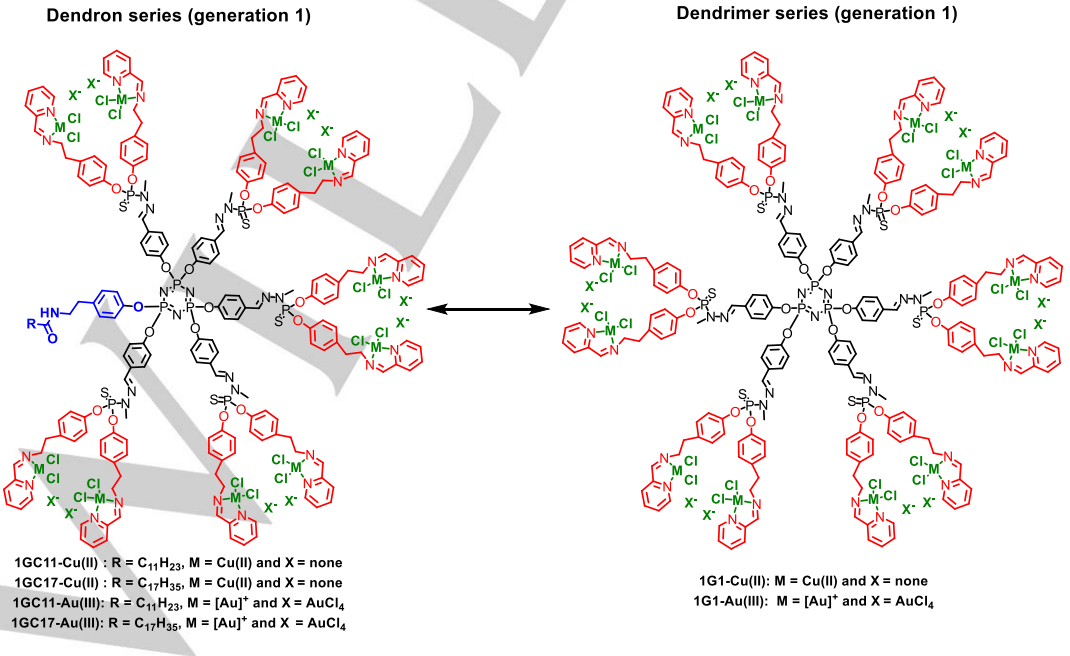


**Figure 4.** Schematic representation of **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III)**, **1GC17-Au(III)**, **1G1-Cu(II)** and **1G1-Au(III)**.

dendrimers **1G1-Cu(II)** and **1G1-Au(III)** are ~2 times more potent ($IC_{50}$s: 0.65 and 1 μM) than the corresponding dendrons **1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III)),** and **1GC17-Au(III)** against HL-60, and the same potencies have been observed against HCT116. This suggests that the scaffold of dendrimers and dendrons may play a role in regulating the different anticancer activities of the metal complexes.

**Table 2.** Anti-proliferative activities of **1GC11-Cu(II), 1GC17-Cu(II)**, **1GC11-Au(III)** and **1GC17-Au(III).**

| | | $IC_{50}$ (μM)[a] | | |
|---|---|---|---|---|
| | | HL-60* | HCT116* | K562* |
| Dendrons | 1GC11-Cu(II) | 1.93±1.37 | 3.37±0.87 | 2.36±0.20 |
| | 1GC17-Cu(II) | 2.65±0.65 | 3.03±0.254 | 7.8 ± 1.28 |
| | 1GC11-Au(III) | 1.33±0.25 | 2.09±0.23 | 1.46 ± 0.04 |
| | 1GC17-Au(III) | 1.34±0.30 | 4.44±0.87 | 2.31 ± 0.20 |
| Dendrimers | 1G1-Cu(II) | 1.00±0.20 | 3.26±0.71 | |
| | 1G1-Au(III) | 0.65±0.04 | 2.90±0.87 | |

[a] Cells were incubated dendrons and dendrimes at eight concentrations (0.01 to 50 μM) for 72 h and the data were represented as "mean ± SD" (n = 3).

In order to evaluate the cell death pathway of **1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III)** and **1GC17-Au(III)**, several studies were performed including the thioredoxin reductase activity, fluorescence imaging studies, flow cytometric analysis and Western blot assays.

Recent studies have revealed that several cytotoxic Au(III) complexes are potent thioredoxin reductase (TrxR) inhibitors, which is beneficial for the improved generation of reactive oxygen species.[1] To clarify if the intracellular TrxR can be inhibited by the Au(III) dendrons, the TrxR activity in 4T1 cells was evaluated. As shown in Figure 3E, after incubation of the Au(III) dendrons (10 μM) with cells for different time periods, the activity of TrxR decreased gradually with the incubation time. Obviously, gold metaled dendrons are effective inhibitors of TrxR. In addition, the Au(III) dendrons with C11 chain inhibited the TrxR activity more significantly than those with C17 chain, probably due to the fact that $C_{11}$ dendrons could be more significantly taken up by cells than the $C_{17}$ dendrons.

For the mechanisms of interaction of gold compounds with this TrxR enzyme, related researches were assumed that the "soft", catalytically relevant, selenate group should constitute the common anchoring site for gold-based inhibitors; this assumption was grounded on the concept that "soft" gold ions, are able to form strong bonds with "soft" Lewis donors.[15] Therefore, a reasonable mechanisms of the Au(III) metaled phosphorus dendrons could be that these compounds act as gold(III) carriers. The "soft" gold(III) could be released possiibly through a intracellular reductive environment when these metaled compounds engulfed by cells. Then, The "soft" gold(III) will reduce the activity of TrxR.

4T1 cells were selected due to the potent anti-proliferative activities of **1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III)** and **1GC17-Au(III)** (Table 1). With the purpose of evaluating the apoptotic features of 4T1 cells by the action of phosphorus dendrons with and without metals (**9a, 9b, 1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III)** and **1GC17-Au(III)**), morphological change were analyzed using the fluorescence microscopic imaging technique after 24 h at two concentrations of phosphorus dendrons, 10 μM (Figure 5A) and 20 μM (Figure 5B). As shown in Figure 5, significant morphological changes including granular apoptotic bodies and the appearance of membrane blebbing were observed (Figure S11 and S12, experimental section). As shown in Figure 5A, the four metaled phosphorus dendrons enhanced the frequency of apoptotic nuclei in 4T1 cells (marked with an arrow). Clearly, these morphological observations suggested extensive DNA cleavage to produce high molecular weight fragments (HMW) associated with weak chromatin condensation.[16]

The rate of the generation and the abundance of HMW fragments significantly depended on the following: 1) apoptotic agent used, 2) the duration of the stimulus, and 3) the cell type considered.[16] In this assay, 4T1 cells were treated with the four different metaled phosphorus dendrons at different

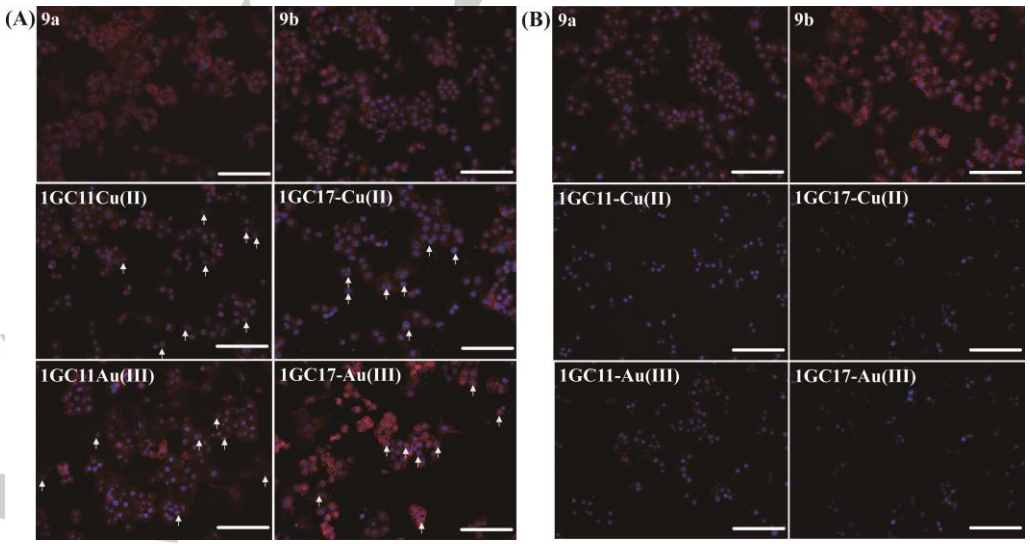


**Figure 5.** Fluorescence microscopic images of 4T1 cells treated with **9a, 9b, 1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III))** and **1GC17-Au(III)** at 10 μM for 24 h (A) and at 20 μM for 24 h (B). Scale bar in each panel represents 100 μm.

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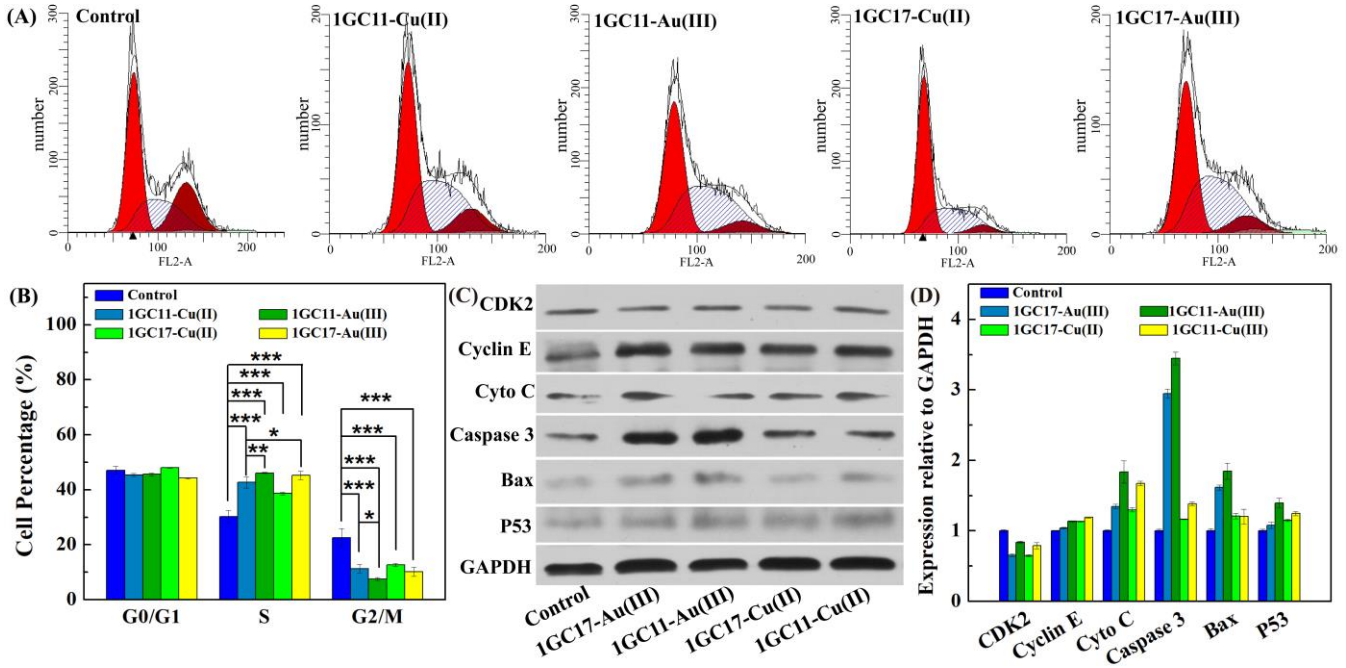


**Figure 6. (**A) Cell cycle analysis of the 4T1 cells after incubation with **1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III))** and **1GC17-Au(III)** at the dendrons concentration of 10 μM for 24h; (B) The percentages of the cellular distribution in different cell cycle phases of the 4T1 cells; (C) Western Blot assay of the expression of protein related to S phase and apoptosis in 4T1 cells after incubation with different dendrons ([dendron] = 10 μM) for 24 h. The GAPDH protein was used as a reference; (D) Relative protein expression levels in 4T1 cells after incubation with the different dendrons ([dendron] = 10 μM) for 24 h.

concentrations for 24 h. At high concentrations (20 μM), metaled phosphorus dendrons stimulated the autophagosome process and the formation of HMW fragments (Figure 5B). These data suggest that the apoptosis is a consequence of direct damage produced on nuclear DNA and autophagosome, highlighting the strong proapoptotic potential of these novel metaled dendrons. In addition, when 4T1 cells were treated with **9a** and **9b**, no morphological changes of cells were observed when compared to control cells treated with PBS (Figure S11 and S12).

It is commonly accepted that the cytotoxicity of antiproliferative agents is primarily associated with cell cycle arrest in G1, S or G2/M phases which trigger the final apoptosis pathway and cell death. Since metaled phosphorus dendrons displayed the ability to promote autophagosome and apoptotic effects against 4T1 cells, we addressed the question about the perturbation effect of the metaled phosphorus dendrons on cell-cycle progression (Figure 6). To evaluate this effect, the impact of the metaled dendrons **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III))** and **1GC17-Au(III)** at 10 μM was investigated in 4T1 cells treated for 24 h. The flow cytometric analysis of the cell cycle distribution is presented in Figure 6A, whereas the percentages of the cellular distribution in different cell cycle phases (G0/G1, S, and G2/M) are given in Figure 6B.In the control group, cells contained 2n chromosomes (phase G0/G1, ~45 %) and few cells were in an active DNA synthesis stage (phase S, ~30 %) or already engaged in the mitosis process (phase G2/M, ~ 22 %).However, for Cu-metaled phosphorus dendrons (**1GC11-Cu(II)** and **1GC17-Cu(II)**), cells are in the mitosis phase (S) (~45%) without a substantial increase of the G0/G1 phase after a 24 h exposure. Particularly, phosphorus dendrons complexed with Au(III) (**1GC11-Au(III)** and **1GC17-Au(III)**) were more potent promoters of cell cycle arrest than Cu-complexed dendrons in 4T1 cells (phase G0/G1, ~ 45%; phase G2/M, ~ 10%; and phase S, ~ 45%).

In order to elucidate the molecular mechanism of the cell cycle arrest and apoptosis induced by the metaled phosphorus dendrons using 4T1 cells, the cell cycle- and apoptosis-related proteins were inspected using Western blotting (Figure 6C). The cyclin E protein is regularly synthesized during the cell cycle, reaching a peak in late G1 and early S phase.[17] CyclinE-CDK2 is essential for the progression through the G1-phase of the cell cycle and initiation of DNA replication (G1- and S-phase transitions).[18] As shown in Figure 6D, for the treatment with **1GC11-Cu(II), 1GC17-Cu(II), 1GC11-Au(III)** and **1GC17-Au(III)** during 24 h, up-regulation of Cyclin E and weak up-regulation of CDK2 were observed. However, for **1GC17-Cu(II)**, weak up-regulation of Cyclin E and CDK2 were observed, indicating that the cellular percentage of phase G0/G1 is similar for **1GC17-Cu(II)** and the control group (~45 %). Additionally, the expression of tumor suppressor P53 were also detected. P53 regulates the cell cycle restriction point which is related to the DNA damage checkpoint. In response to DNA damage, P53 is activated and turns on transcription of one of its downstream genes, then the downstream genes activate cyclin-Cdk complexes to stop DNA replication.[19] After treatment of the cells with metaled phosphorus dendrons, the cells displayed a high level of P53 *versus* the control group. Previous experiments showed that up-regulation of P53 induces G2/M cell cycle arrest.[20] Consequently, the metalated dendrons

influenced the expressions of cell cycle regulatory proteins which altered the cell cycle distribution. In addition, the expression of cytochrome C (Cyto C), Caspases-3, and Bax were also detected. Caspases-3 are known to play a pivotal role in both initiation and execution of apoptosis.[21] It has been reported that caspase-dependent apoptosis requires the release of proteins sequestered in the mitochondria of cancer cells but the high inner membrane potential prevents the opening of mitochondrial pores.[21] One of the key controls to open the mitochondrial membrane pores is the regulation of the level of Bax.[22] During the 24 h treatment with **1GC11-Au(III))** and **1GC17-Au(III)**, the cells displayed up-regulation of Cyto C, Caspase-3, and Bax, while 4T1 cells treated with the Cu(II)-dendrons did not display significative changes in the regulation of Cyto C, Caspase-3 and Bax when compared to the control cells treated with PBS. In a nutshell, the 4T1 cells treated with Au(III)-dendrons exhibited higher levels of caspase-3 and Bax than the group of Cu(II)-dendrons.

Taken together, these studies demonstrated that the dendrons complexed with Au(III) increased the cellular lethality (4T1 cells) and the cell death pathway is related to caspase-dependent process, promoting the translocation of Bax to the mitochondria and then the release of Cyto C, inducing the activation of the apoptosis process.

To sum up, Au(III)-complexed dendrons (**1GC11-Au(III)** and **1GC17-Au(III)**) clearly demonstrated their ability to promote cell death in a caspase-dependent pathway by facilitating the translocation of Bax to the mitochondria and the release of Cyto C, whereas Cu(II)-complexed dendrons (**1GC11-Cu(II)** and **1GC17-Cu(II)**) are weak activators of caspase-3. Also, copper-based dendrons showed weak antiproliferative activities in contrast with Au(III)-complexed ones. Table 3 summarizes the mechanism of actions of the phosphorus dendrons **1GC11-Cu(II)**, **1GC17-Cu(II)**, **1GC11-Au(III)** and **1GC17-Au(III)** *versus* phosphorus dendrimers **1G3** and **1G3-Cu**.

**Table 3.** General view of cell death pathway of phosphorus dendrons prepared in this study in comparison with the corresponding dendrimers.

| Nanoparticles | Translocation of Bax | Caspase-3 activation |
|---|---|---|
| **Phosphorus dendrons** | Strong effect (4T1): 1GC11-Au(III)) and 1GC17-Au(III) | Weak effect (4T1): 1GC11-Cu(II), 1GC17-Cu(II) |
| **Phosphorus dendrimers[12, 14]** | Strong effect (KB and HL60): 1G3-Cu(II) | Strong effect (KB and HL60): 1G3 |

## Conclusion

In summary, first-in-class Cu (II) and Au (III) metaled phosphorus dendrons (generation 1) bearing 10 $Cu(II)Cl_2$ or $[Au(III)Cl_2]^+$ on their surface, and with $C_{11}H_{23}$ and $C_{17}H_{35}$ linear alkyl chains were synthesized, and showed significant antiproliferative activity against cancer cell lines such as 4T1 and MCF-7 (breast cancer). The complexation of phosphorus dendrons with Cu(II) or Au(III) boosts its antiproliferative activity compared to nonmetallic dendrons. The safety ratio depends on the metal, the nature of the alkyl chain, and the nature of the normal cells used. The antiproliferative activities against 4T1 and MCF-7 cells showed $IC_{50}$s between 0.6 and 2.8 µM. Similar to the phosphorus dendrimers, the replacement of Cu(II) by Au(III) strongly improved the anti-proliferative activities. **1GC11-Au(III)** and **1GC17-Au(III)** dendrons showed $IC_{50}$s of ~0.16–0.8 µM against 4T1 and MCF-7 cells. In addition, short alkyl chain length of the dendrons renders Au(III) complexes with more significant antiproliferative activity to kill cancer cells. Cell death pathway analysis reveals that the metaled dendrons could alter the cell cycle- and apoptosis-related protein status of cells, resulting in cell cycle S-phase arrest and apoptosis. In particular, Au(III)-complexes induced the caspase-dependent cellular lethality by promoting the translocation of Bax to the mitochondria and the release of Cyto C, whereas the Cu(II)-complexes are weak activators of caspase-3, in line with their moderate antiproliferative activity in cancer cells.

Taken together, these studies showed that these first-in-class metaled phosphorus dendrons represent a novel class of anticancer nano-drugs, and their development will open new avenue to tackle cancer. We thus ought to see the emergence of novel dendron-based particles to be applied against difficult diseases such as cancers and distant metastases.

## Acknowledgements

This research is financially supported by the National Natural Science Foundation of China (21911530230, 21773026, and 81761148028) and Sino-French Cai Yuanpei Programme. S. Mignani and X. Shi acknowledges the support of FCT – Fundacao para a Ciencia e a Tecnologia with Portuguese Government funds through the CQM Strategic Project PEst-OE/QUI/UI0674/2013, and ARDITI-Agencia Regional parao Desenvolvimento da Investigacao Tecnologia through the project M1420-01-0145-FEDER-000005 – Centro de Quımica da Madeira – CQM+ (Madeira 14-20 Program). J-P. Majoral, A-M. Caminade and R. Laurent thank the CNRS (France) for financial support.

## Conflict of interest

There are no conflicts to declare.

**Keywords:** anti-proliferative activities • metaled phosphorus dendrons • fluorescence imaging • flow cytometric analysis

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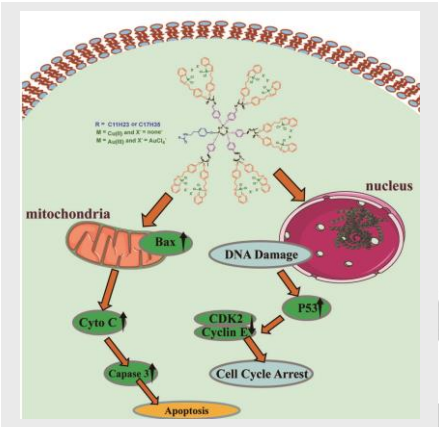
10.1002/chem.202001014




## Entry for the Table of Contents

# FULL PAPER

**First-in-class Cu(II) and Au(III) metaled phosphorus dendrons** were developed and displayed potent anticancer activity stemming from different cell death pathways. Cu(II) metaled dendrons showed a potent caspase-independent cell death pathway; whereas Au(III) metaled dendrons displayed a caspase-dependent apoptotic pathway. These metaled phosphorus dendrons represent a novel class of anticancer nano-drugs, and their development will open new avenue to tackle cancer.

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**Potent antitumoral efficacy of first-in-class Cu(II) and Au(III) metaled phosphorus dendrons with distinct cell death pathways**