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# Synergetic anticancer activity of gold porphyrin appended to phenyl tin malonate organometallic complexes

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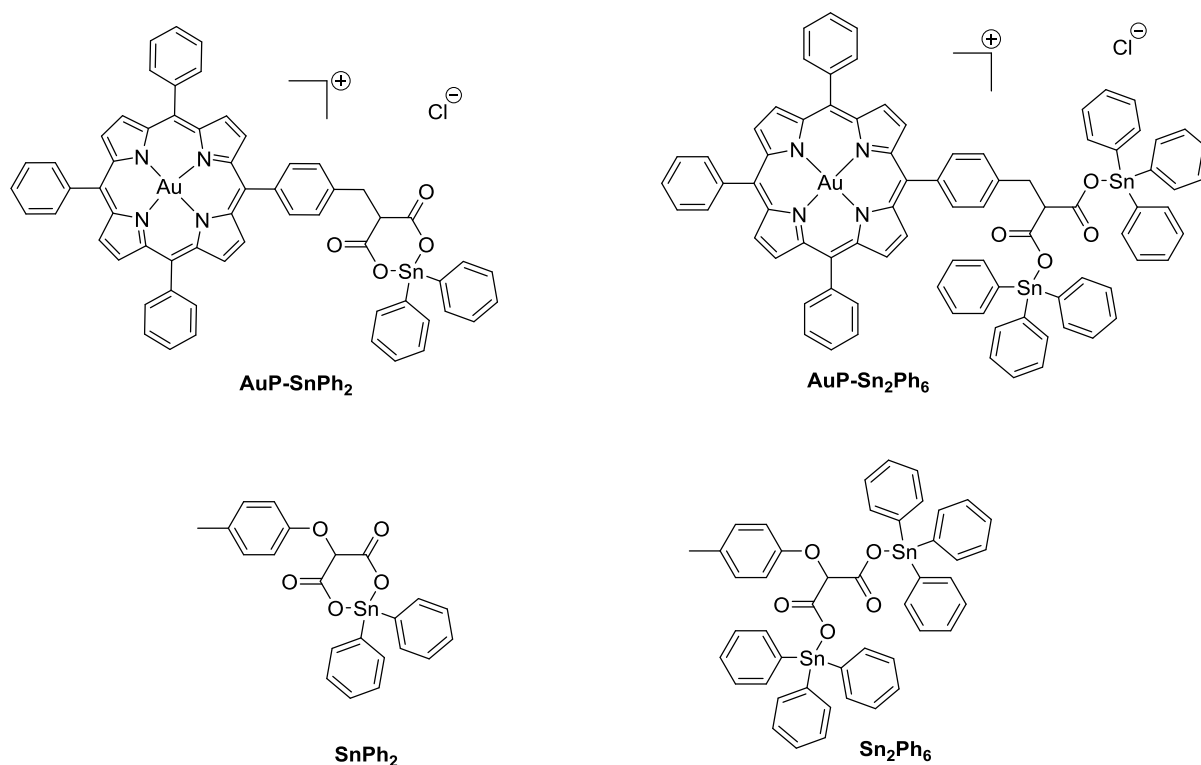
## Abstract

The discovery of novel anticancer chemotherapeutics is fundamental to treat cancer more efficiently. Towards this goal, two dyads consisting of a gold porphyrin appended to organotin(IV) entities were synthesized and their physicochemical and biological properties were characterized. One dyad contains a gold porphyrin connected to tin(IV) cation *via* a malonate and two phenyl ligands (**AuP-SnPh<sub>2</sub>**), while the second contains two tin(IV) cations each chelated to one carboxylic acid group of the malonate and three phenyl ligands (**AuP-Sn<sub>2</sub>Ph<sub>6</sub>**). The mode of chelation of Sn(IV) to the malonate was elucidated by IR spectroscopy and <sup>119</sup>Sn NMR. In the solid state, the complexes exist as coordination polymers in which the tin is penta-coordinated and bridged to two different malonate units. In solution the chemical shifts of <sup>119</sup>Sn signals indicate that the tin complexes are in the form of monomeric species associated with a tetra-coordinated tin cation. The therapeutic potential of these new compounds was assessed by determining their cytotoxic activities on human breast cancer cells (MCF-7) and on healthy human fibroblasts (FS 20-68). The study reveals that the dyads are more potent anticancer drugs than the mixture of their individual components (gold porphyrin and reference tin complexes). Therefore, the covalent link of organotin complexes to a gold porphyrin induces a synergistic cytotoxic effect. The dyad **AuP-SnPh<sub>2</sub>** presents high cytotoxicity (0.13 μM) against MCF-7 along with a good selectivity for cancer cells *versus* healthy cells. Finally, it was also shown that the dyad **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** exhibits a very high anticancer activity (LC<sub>50</sub> = 0.024 μM), but the presence of two tin units induces a strong cytotoxic on healthy cells too (LC<sub>50</sub> = 0.032 μM). This study underscores, thus, the potential

of the association of gold porphyrin and organotin complexes to develop anticancer metallo-drugs.

## Introduction

Cancer remains one of the most important causes of mortality in the world. The development of new and more selective anticancer drugs constitutes, therefore, a priority to strive against this disease. Organometallic compounds are attractive weapons against cancers and they have, so far, contributed towards the development of efficient modern medicinal anticancer treatments. For examples, the famous cisplatin and its derivatives, carboplatin and oxaliplatin are potent cytotoxic metallodrugs, which have been routinely used for decades in clinical treatment for various types of cancer (prostate, testicle, ovary, bladder and lung).<sup>1</sup> The development of resistance mechanism of certain cancers and the high toxicity for healthy cells leading to important secondary side effects prompted coordination chemists to investigate the anticancer activity of other families of complexes with fewer side effects. Towards this goal, many coordination and organometallic compounds with rhenium,<sup>2-3</sup> ruthenium,<sup>4-5</sup> iron,<sup>6</sup> titanium,<sup>7</sup> rhodium,<sup>8</sup> iridium,<sup>3, 8</sup> osmium,<sup>3</sup> gold<sup>9</sup> and tin<sup>10</sup> metals were prepared and their cytotoxic activities were evaluated. More specifically, gold porphyrins show valuable anticancer activities as they represent stable Au(III) complexes in physiological environment.<sup>9, 11-12</sup> Indeed, Che and co-workers reported in 2003, that gold meso tetrakis-phenyl porphyrin (**AuTPP**) was a powerful cytotoxic drug against several cancer cells lines.<sup>12-15</sup> Subsequent investigations on gold porphyrins support a mode of action involving the heat-shock protein HSP60.<sup>16-18</sup> On the other hand, tin organometallic compounds, particularly with carboxylate and mercapto ligands, present promising cytotoxic activity without resistance development found with classical chemotherapeutics.<sup>10, 19</sup> Like platinum metallodrugs, it is accepted that organotin compounds directly interact with DNA, causing cell death either by apoptotic or necrotic mechanisms.<sup>19</sup> Previous works have been devoted to free base porphyrin/tin complex conjugates, but free base porphyrin has little anticancer activity and is very hydrophobic like the tin complex.<sup>20-22</sup> In this study, a cationic gold porphyrin, which is less hydrophobic than the corresponding free base and whose cytotoxic activity toward cancer cells was clearly evidenced,<sup>12, 14-15</sup> was covalently appended to a tin malonate complex in order to investigate the possibility of amplifying the chemotherapeutic activity and the selectivity of these conjugates (Chart 1). One conjugate contains one tin organometallic moiety bearing two phenyl groups in its coordination sphere ( $\text{SnPh}_2$ ), while in the second conjugate, the tin is bound to three phenyls ( $\text{SnPh}_3$ ). Phenyl was selected as organometallic moiety around tin(IV), because it was shown in previous studies, that this type of motifs provides the highest anticancer activity.<sup>23-24</sup> On the other hand, malonic acid ligand was chosen to append tin(IV) cation because as a hard Lewis acid it has a strong affinity to carboxylate.<sup>25-26</sup> In addition, it is known that tin(IV) complexes present anticancer activity and particularly those with carboxylate ligands.<sup>10, 27</sup>

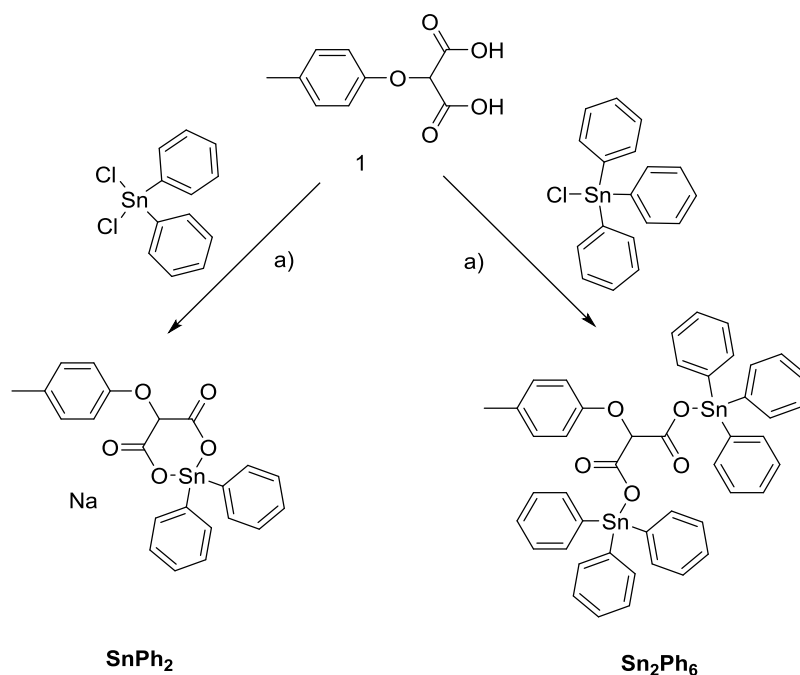


**Chart 1.** Structures of the compounds investigated in the study.

Overall, this study shows that the two gold porphyrin-tin dyads **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** exhibit superior cytotoxicity compared to the equimolar mixtures of their individual components **AuTPP-Malonic acid** (Porphyrin **2**) and **SnPh<sub>2</sub>** or **Sn<sub>2</sub>Ph<sub>6</sub>**. In addition and very interestingly, they display higher level of cytotoxicity toward MCF-7 carcinoma cell line than healthy cells, underscoring thus the potential of these associations to develop anticancer metallo-drugs.

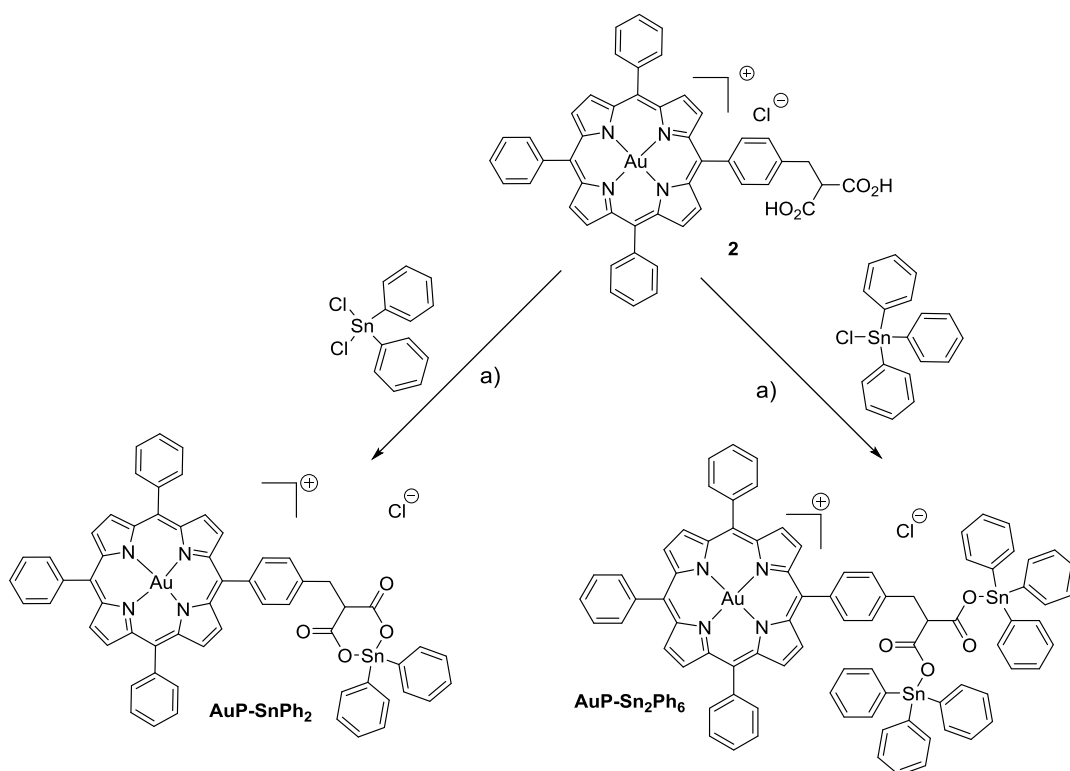
### Synthesis of the compounds

The synthesis of the reference tin compounds (**SnPh<sub>2</sub>** and **Sn<sub>2</sub>Ph<sub>6</sub>**) is illustrated in Scheme 1. The di-carboxymalonate p-cresol **1** ligand was prepared as previously described.<sup>28</sup> The dicarboxylato tin complexes **SnPh<sub>2</sub>** and **Sn<sub>2</sub>Ph<sub>6</sub>** were respectively obtained in 71% and 93 % yield by initial deprotonation of the carboxylic acid groups with triethylamine before being reacted with the tin phenyl precursor containing one or two chloro ligands.<sup>28</sup>



**Scheme 1.** Synthetic route for the preparation of the reference tin complexes **SnPh<sub>2</sub>** and **Sn<sub>2</sub>Ph<sub>6</sub>**. Reagents and conditions: a) THF, TEA, RT, 12h, 71% for **SnPh<sub>2</sub>** and 93 % for **Sn<sub>2</sub>Ph<sub>6</sub>**.

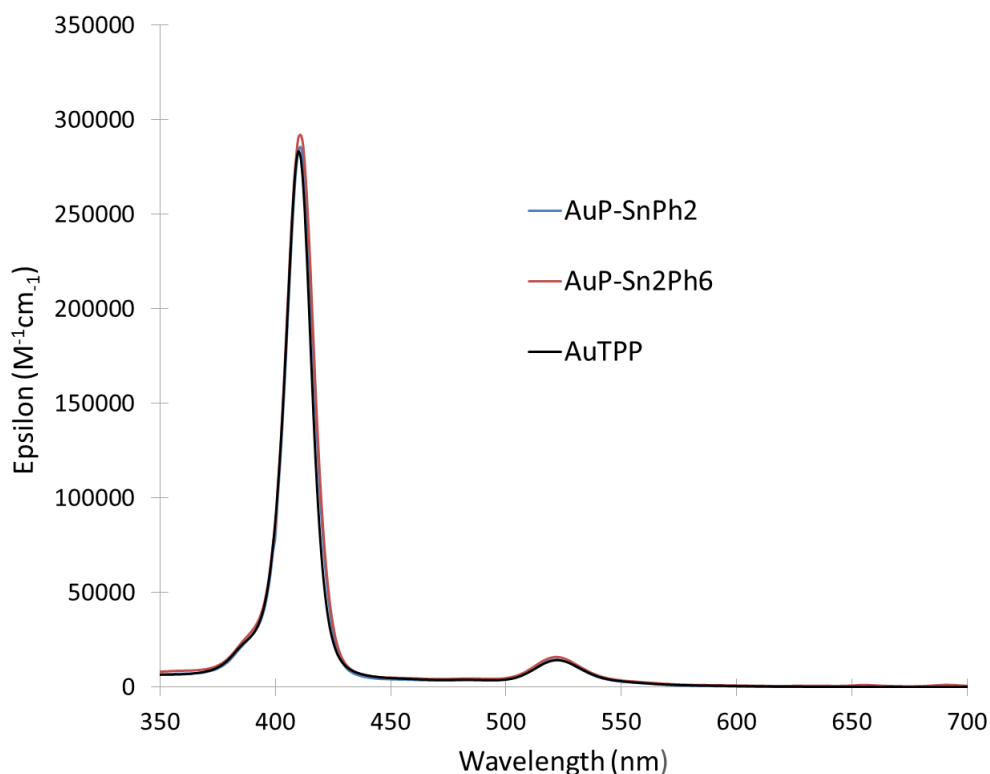
The preparation of the dyads made of gold porphyrin/tin complex required the porphyrin **2** as key intermediate, which was synthesized as previously described (Scheme 2).<sup>29</sup> The introduction of the tin complex was accomplished with the same strategy as that described for the reference complexes. The conjugates were obtained in 98% and 80% yields for **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** respectively. The complexes were satisfyingly characterized by proton NMR, high resolution mass spectrometry and elementary analyses. The dyads **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** were soluble in DMSO, THF, DMF, chloroform and acetone.



**Scheme 2.** Synthetic route for the preparation of the dyads composed of gold porphyrin linked to tin complex. Reagents and conditions: a) THF, TEA, RT, 12h, 98% for **AuP-SnPh<sub>2</sub>** and 80 % for **AuP-Sn<sub>2</sub>Ph<sub>6</sub>**.

### UV-Vis Electronic Absorption Spectra

The absorption spectra of the gold porphyrin/tin complex dyads along with that of the reference gold tetrakis phenyl porphyrin (**AuTTP**) recorded in dichloromethane are shown in Figure 1, and the spectroscopic data are gathered in Table 1. The spectra of the dyads are essentially dominated by the transitions of the gold(III) porphyrin unit, since the Sn complex does not exhibit any absorption band in the visible region (Figure S5) and there is no communication between the porphyrin and the tin complex. This is consistent with previously published conjugates composed of a dye appended to a complex with non- $\pi$ -conjugated spacer.<sup>29-31</sup> More specifically, the dyads exhibit the characteristic absorption bands of gold tetrakisaryl porphyrins, with a blue shift of Soret (at 412 nm) and the Q-bands (around 530 nm) owing to the back bonding between gold(III) and the porphyrin ligand, which stabilizes the HOMO level and increases the HOMO–LUMO gap with respect to that of the parent free base or zinc porphyrins.<sup>32</sup> Unsurprisingly, the presence of the appended tin complex does not alter the spectrum of the gold porphyrin owing to the deconjugation induced by the methylene spacer between the porphyrin and the Sn complex.



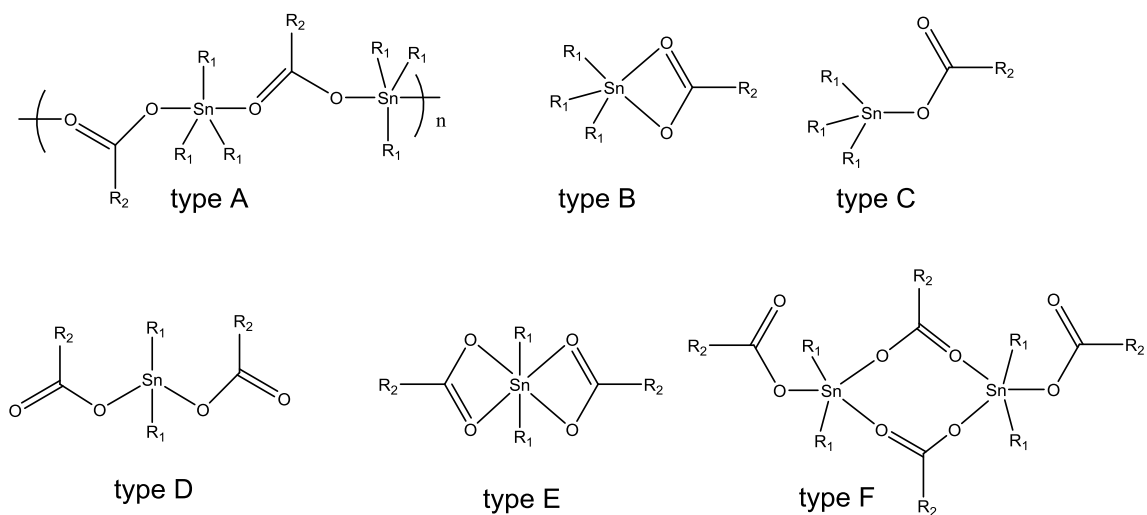
**Figure 1.** Absorption spectra of the porphyrin derivatives recorded in dichloromethane.

**Table 1.** Maximum absorption wavelengths and molar extinction coefficients of the porphyrins recorded in dichloromethane at room temperature.

Compound	$\lambda_{\text{abs}}/\text{nm}$ ( $\log \epsilon/\text{M}^{-1} \text{cm}^{-1}$ )
<b>AuP-SnPh<sub>2</sub></b>	412 (5.44); 532 (3.98)
<b>AuP-Sn<sub>2</sub>Ph<sub>6</sub></b>	412 (5.45); 529 (4.11)
<b>AuTPP</b>	409 (5.44); 527 (4.11)

### Infra-Red and <sup>119</sup>Sn NMR spectroscopic studies

Carboxylate ligand can chelate tin(IV) cation according to several potential binding modes (Figure 2).<sup>10, 33-34</sup> In solid state, tri-organo tin(IV) complexes are usually polymeric compounds with cis-trigonal bipyramidal geometry having five-coordinate tin sites under the bridging mode (type A).



**Figure 2.** Illustration of the main binding modes of tri-organo tin(IV) and di-organo tin(IV) complexes with carboxylate ligand.

Particularly in solution, the complexes can exist as monomers either under a five-coordinate chelating bridged mode (type B and E) or as four-coordinate tin with carboxylate acting as a monodentate ligand (types C and D). ATR-IR and  $^{119}\text{Sn}$  NMR spectra can be used as tools to diagnose the mode of coordination of tin complexes.<sup>26, 33, 35-36</sup> More specifically, concerning IR spectroscopy, the binding mode can be deduced from the wavenumber difference ( $\Delta\nu = \nu_{\text{as}} - \nu_{\text{s}}$ ) between the carboxylate antisymmetric ( $\nu_{\text{as}}$ ) and symmetric ( $\nu_{\text{s}}$ ) stretches.<sup>34, 37-38</sup> If this difference is larger than  $250\text{ cm}^{-1}$ , tin(IV) is involved in a tetrahedral geometry (type C and D binding modes), while values between  $250$  and  $150\text{ cm}^{-1}$  indicate a bridging structure (types A and F). When  $\Delta\nu$  is lower than  $150\text{ cm}^{-1}$ , then carboxylate is bidentate and binds to Sn(IV) under the bridged chelating mode (types B and E). The strong asymmetric stretching band of the carbonyl group in the acid form of compounds **1** and **2**, situated around  $1700\text{ cm}^{-1}$  has completely disappeared and is shifted to lower energy after the reaction with chloro phenyl tin derivatives, besides the strong stretching band of Sn-O linkage is clearly visible at  $450\text{ cm}^{-1}$  for both **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** (Figures S1-S4 in ESI materials). These two features are supplementary indications that complexation of tin by the carboxylate has occurred. The wavenumber values of the stretching bands of the carboxylate before and after complexation of tin(IV) and the  $^{119}\text{Sn}$  chemical shifts are gathered in Table 2 and the spectra are shown in Figures S1-S4 in ESI materials. The  $^{119}\text{Sn}$  chemical shifts of the



conjugates **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** could not be determined owing to their insufficient solubility to record reliable NMR spectra.

Table 2. Wavenumber values of the stretching bands of the compounds recorded in the solid state by IR-ATR spectroscopy and <sup>119</sup>Sn chemical shift (δ) recorded in THF solution.

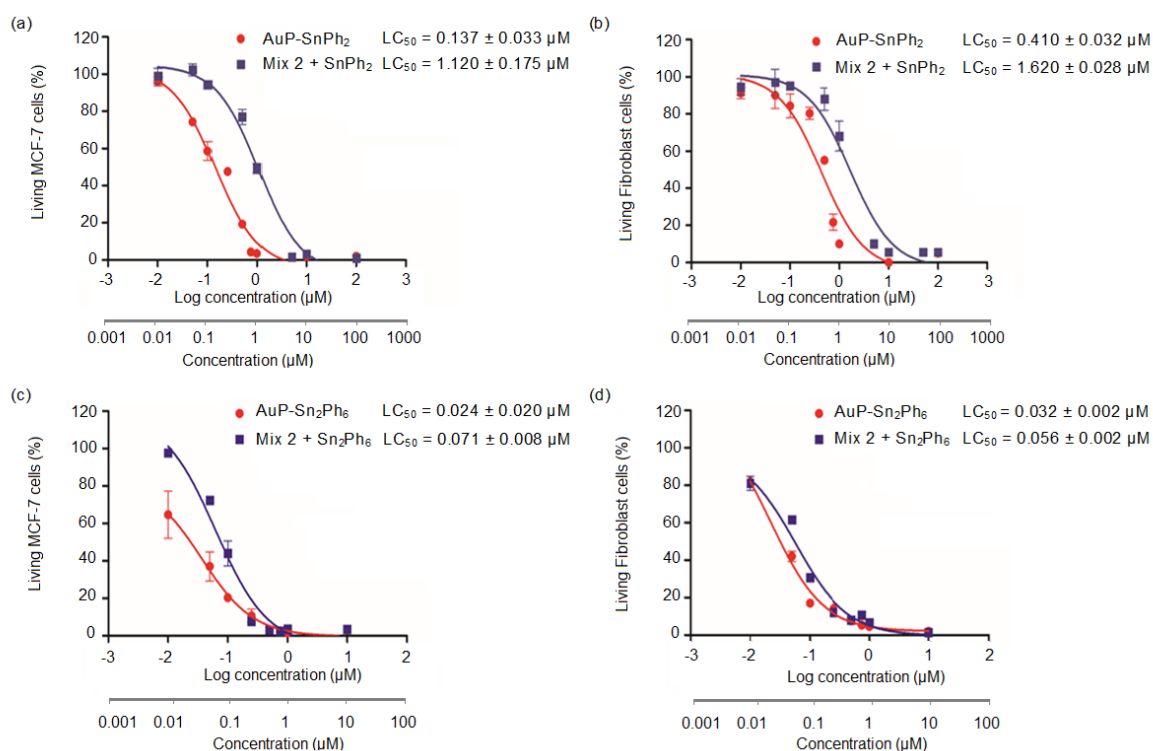
Compound	v(Sn-O) cm <sup>-1</sup>	v <sub>as</sub> (OCO) cm <sup>-1</sup>	v <sub>s</sub> (OCO) cm <sup>-1</sup>	Δv	δ( <sup>119</sup> Sn) ppm
<b>1</b>	-	1706	1399	307	-
<b>SnPh<sub>2</sub></b>	446	1592	1429	163	-172
<b>Sn<sub>2</sub>Ph<sub>6</sub></b>	453	1624	1428	196	-226
<b>2</b>	-	1726	1358	368	-
<b>AuP-SnPh<sub>2</sub></b>	450	1598	1429	169	n.r.
<b>AuP-Sn<sub>2</sub>Ph<sub>6</sub></b>	450	1643	1428	215	n.r.

n.r.= not recorded owing to insufficient solubility of the dyads for suitable <sup>119</sup>Sn spectra

In the diphenyl tin(IV) compounds (**SnPh<sub>2</sub>** and **AuP-SnPh<sub>2</sub>**), the Δv values are between 250 and 150 cm<sup>-1</sup> indicating that in the solid state, these complexes are probably associated to form coordination polymer with carboxylate acting as bidentate ligand (type F) but in a bridged mode owing to the intramolecular nature of the two carboxylate groups of malonate. For the tri-phenyl tin(IV) compounds (**Sn<sub>2</sub>Ph<sub>6</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>**), the Δv values are between 250 and 150 cm<sup>-1</sup> meaning that they also exist as coordination polymers (type A) in which the tin is penta-coordinated and bridged to two different malonate units. The chemical shifts δ(<sup>119</sup>Sn) of organo-tin compounds cover a broad range from 200 ppm to -550 ppm. It has been shown that diphenyl tin(IV) are tetra-coordinated when δ(<sup>119</sup>Sn) is situated in the range of +30 to -220 ppm, while penta-coordinated ones are situated between -250 ppm and -340 ppm.<sup>39</sup> Consequently, compounds **SnPh<sub>2</sub>** and **AuP-SnPh<sub>2</sub>** are most likely tetra-coordinated in solution as the carbonyl dissociates and is probably replaced by a solvent molecule. On the other hand, δ(<sup>119</sup>Sn) of penta-coordinated trialkyl organotin of complexes lie in the range -100 to -330 ppm, while tetra-coordinated are between +200 to -60 ppm.<sup>26, 40</sup> Since the chemical shifts of <sup>119</sup>Sn signal in reference complex **Sn<sub>2</sub>Ph<sub>6</sub>** is -226 ppm, it is concluded that this complex and most probably the parent compound **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** are penta-coordinated in solution.

## Biological tests

In order to determine the therapeutic potential of these new compounds, the cytotoxic activity on human breast cancer cells (MCF-7) and healthy fibroblasts (FS 20-68) were studied. Figure 3 shown their semi logarithmic representations (Figure 3). The red curves correspond to the evolution of the percent of living cells in function of dyads concentrations (**AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>**). The toxicity of the dyads was compared with their equimolar mixture of gold porphyrin **2** and the reference tin complex **SnPh<sub>2</sub>** or **Sn<sub>2</sub>Ph<sub>6</sub>** (blue curves). Towards this goal, cells were incubated 72 h in darkness, with increasing concentrations of each compound (from 0.01 to 100  $\mu\text{M}$  as final concentration). As shown in all cases, dyads were more toxic than mixtures whether it is **AuP-SnPh<sub>2</sub>** or **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** and whether it is on



cancer or healthy cells (Fig 3a-d).

**Figure 3.** Cytotoxic study of **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** in comparison with **2** + **SnPh<sub>2</sub>** and **2** + **Sn<sub>2</sub>Ph<sub>6</sub>**, respectively. (a) Human breast cancer cells (MCF-7) and (b) healthy fibroblasts cells (FS 20-68) were incubated 72 h with increasing concentrations of **AuP-SnPh<sub>2</sub>** or **2** + **SnPh<sub>2</sub>** and maintained in darkness. (c) Human breast cancer cells (MCF-7) and (d) healthy fibroblasts cells (FS 20-68) were incubated 72 h with increasing concentrations of **AuP-**

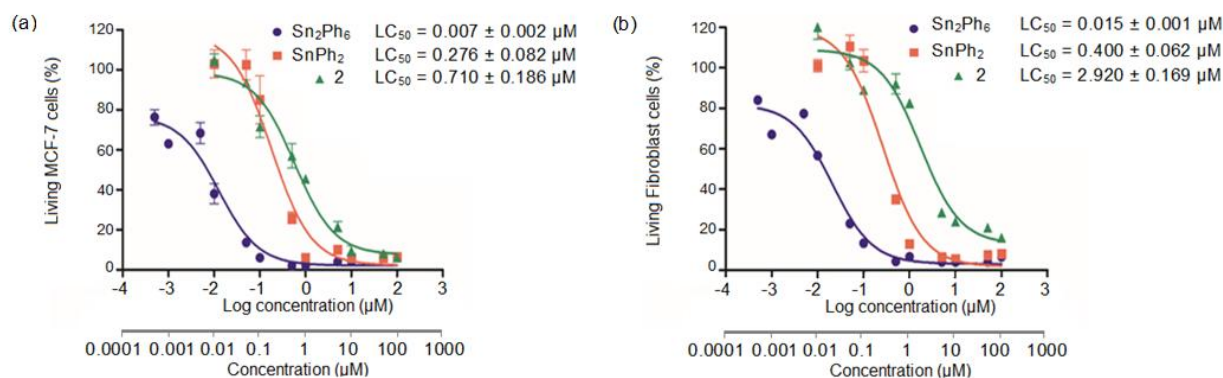
**Sn<sub>2</sub>Ph<sub>6</sub>** or **2** + **Sn<sub>2</sub>Ph<sub>6</sub>** and maintained in darkness. Values are means ± standard deviations of 3 experiments.

These classical sigmoidal dose-response curves of cytotoxicity obtained when plotted as a logarithmic function of the concentration (μM) permitted to determine drug concentrations leading to 50% cell mortality (LC<sub>50</sub>) (Fig 3a-d). **AuP-SnPh<sub>2</sub>** dyad exhibited a 3 folds lower LC<sub>50</sub> on cancer cells (0.137 ± 0.033 μM) than on healthy cells (0.410 ± 0.032 μM), demonstrating a significant selectivity for cancer cells. The LC<sub>50</sub> values showed also that on cancer cells, the toxicity of **AuP-SnPh<sub>2</sub>** dyad was 9 fold higher (0.137 ± 0.033 μM) than equimolar mixture of its components (AuTPP-Malonic acid **2** and **SnPh<sub>2</sub>**) (1.120 ± 0.175 μM). This underscored the potential of such association to develop metallodrugs in which the anticancer efficiency would be exacerbated.

When gold porphyrin **2** was functionalized with 2 tin residues to obtain **AuP-Sn<sub>2</sub>Ph<sub>6</sub>**, we observed that this dyad was highly cytotoxic (LC<sub>50</sub> of 0.024 ± 0.020 μM on cancer cells) which is about 4 or 5 folds more potent than **AuP-SnPh<sub>2</sub>**. In addition, this dyad exhibited greater cytotoxicity than the equimolar mixture of its individual components (AuTPP-Malonic acid **2** and **Sn<sub>2</sub>Ph<sub>6</sub>**). However, the selectivity towards cancer cells is lower than that of **AuP-SnPh<sub>2</sub>**, since the **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** LC<sub>50</sub> on cancer cells (0.024 ± 0.020 μM) is quite similar to that on healthy cells (0.032 ± 0.002 μM). This strong toxicity, close between cancer and healthy cells is a real drawback for an eventual biomedical application in which the secondary effects of such a chemotherapy could be dramatic.

All together, these data demonstrated that **AuP-SnPh<sub>2</sub>** dyad exhibited a higher toxicity against human breast cancer cells than healthy fibroblasts suggesting a stronger and specific effect on cancer cells. The cytotoxic potential of **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** dyad is 4 or 5 folds higher on breast cancer cells than that of **AuP-SnPh<sub>2</sub>**, highlighting the advantage of the complexation with two tin cations instead of one, but this strong effect is counteracted with a loss of specificity toward cancer cells demonstrated by an elevated cell death level of healthy fibroblasts. Interestingly, the dyad **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** is more than 35 times active than our previously reported best conjugates containing a gold porphyrin connected to a cis-platinum derivative.<sup>29</sup>

In order to better understand this biological activity that we supposed to be due to the intrinsic toxicity of **Sn<sub>2</sub>Ph<sub>6</sub>**, we performed cytotoxicity experiments of all separated compounds on cancer and healthy cells (Figure 4a, b). As we can see, tin complexes (red and blue curves) were more cytotoxic than gold porphyrin **2** (green curves). In addition, in each cell line **Sn<sub>2</sub>Ph<sub>6</sub>** exhibited the strongest cell death (blue curves).

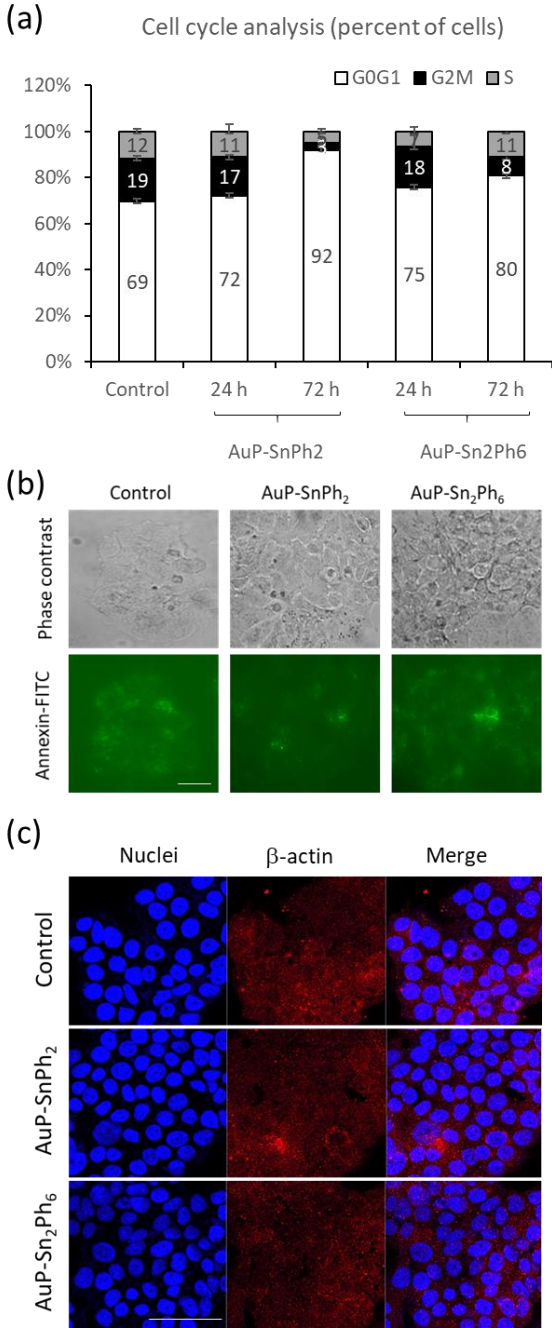


**Figure 4.** Cytotoxic study of **SnPh<sub>2</sub>**, **Sn<sub>2</sub>Ph<sub>6</sub>** and **2**. (a) Human breast cancer cells (MCF-7) and (b) healthy fibroblasts cells (FS 20-68) were incubated 72 h with increasing concentrations of **SnPh<sub>2</sub>**, **Sn<sub>2</sub>Ph<sub>6</sub>** and **2**, and maintained in darkness. Values are means ± standard deviations of 3 experiments.

The analysis of LC<sub>50</sub> values confirmed that **Sn<sub>2</sub>Ph<sub>6</sub>** was highly toxic for MCF-7 (0.007 ± 0.002 μM) and only two-fold less toxic on healthy fibroblasts (0.015 ± 0.001 μM). **SnPh<sub>2</sub>** exhibited a lower cytotoxic effect than **Sn<sub>2</sub>Ph<sub>6</sub>**, and the difference of this effect between cancer and healthy cells remains short (0.276 ± 0.082 μM and 0.400 ± 0.062 μM for MCF-7 and FS 20-68, respectively). This suggested an absence of discrimination between cancer and healthy cells by tin complexes avoiding a biomedical application free from significant side effects. In contrast, the gold porphyrin **2** was clearly less efficient to kill cancer cells (0.710 ± 0.186 μM), but demonstrated a discrimination with healthy cells (2.920 ± 0.169 μM) in which cytotoxic effect was more than 4 times lower. The grafting of high efficient tin complexes with gold porphyrin **2**, which is more apt to preferentially select cancer cells, is certainly a good strategy to obtain the best balance between efficiency and targeting on cancer cells.

In order to understand by which mechanism these dyads act on cancer cells, we performed a series of studies at the LC<sub>50</sub> concentrations of both dyads, such as (i) flow cytometer to

determine the modifications in cell cycle phases during treatment, (ii) detection of apoptosis using annexin V and (iii) cytoskeleton integrity via  $\beta$ -actin staining.



**Figure 5.** Mechanistic study of **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** cytotoxic effect. (a) Flow cytometer study after 24 and 72 h incubation with **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** at their respective LC<sub>50</sub> concentrations. Values are means  $\pm$  standard deviations of 2 experiments. (b) Annexin V-FITC staining for apoptosis markers detection after 72 h incubation time with

dyads ( $LC_{50}$ ). (c)  $\beta$ -actin staining by immunofluorescence, for cytoskeleton integrity analysis after 24 h incubation time with dyads ( $LC_{50}$ ).

It is well established that some chemotherapeutic agents are involved in interruption of the cell cycle.<sup>41-42</sup> Here we demonstrated that **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** induced a  $G_0G_1$  phase increase from 69% to 72% and 75% respectively, after only 24 h incubation time suggesting an arrest of cells in the quiescent state (Figure 5A). Importantly, 72 h incubation time of cells with **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** induced a stronger increase in  $G_0G_1$  phase from 69% to 92% and 80%, respectively. These data prove the arrest of cells before the restriction point avoiding the cell cycle entry.

We also performed in parallel, the analysis of the apoptotic marker annexin V level in MCF-7 cells, after **AuP-SnPh<sub>2</sub>** and **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** treatment (Figure 5B). We noted no difference between expression of Annexin V between control and dyads treatment.

Finally, the effect of dyads was studied on the cytoskeleton of cells, by  $\beta$ -actin staining before and after dyads treatment (Figure 5C). Immunofluorescence experiment performed here did not show any alterations in the structural integrity of the cells.

Altogether these data highlight the blocking of cancer cells in the cycle phase before the entry in cell cycle, inducing the quiescence of the cells that is logically connected with an absence of apoptosis markers as shown by the same level of annexin V-FITC labeling between control and treated cells, and also with an absence in the cytoskeleton of cells.

## Conclusion

For the first time, we report the synthesis and characterization of two new dyads consisting of gold porphyrin appended to phenyl tin malonate organometallic complexes. Their biological properties towards cancer and healthy cells were investigated and compared to the mixture of their corresponding individual components. The main finding of this work are: i) the dyads are more potent anticancer drugs than the mixture of their individual components. Therefore, the coordination of tin complex to the gold porphyrin induces a synergistic cytotoxic effect; ii) the dyad **AuP-SnPh<sub>2</sub>** is an interesting anticancer metallodrug

as it presents a very strong cytotoxicity and a good selectivity for cancer cells *versus* healthy cells; iii) **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** exhibits a very good anticancer activity with a higher LC<sub>50</sub> than that of **AuP-SnPh<sub>2</sub>**, but the presence of two tin units also induces a strong cytotoxic effect on healthy cells; iv) ultimately, tin complexes bring toxicity and the porphyrin brings more selectivity of cancer cells.

Overall, this study highlights the potential of the association of gold porphyrin with organotin compounds on anticancer therapy by acting on cell cycle and more particularly in blocking G<sub>0</sub>G<sub>1</sub> phase inducing quiescence of cancer cells. Additional studies are in due course in our laboratory to establish the mechanism of action of these new metallodrugs and to improve the cancer cells selectivity of the most efficient dyads.

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## Experimental part

### Synthesis of the compounds

#### *Generalities*

<sup>1</sup>H and <sup>119</sup>Sn{<sup>1</sup>H}<sup>24</sup> spectra were recorded on an *AVANCE 300 UltraShield BRUKER* and *AVANCE 400 BRUKER*. Chemical shifts for <sup>1</sup>H spectra are referenced relative to residual proton in the deuterated solvent (DMSO-d<sub>6</sub> δ = 2.50 ppm for <sup>1</sup>H). NMR spectra were recorded at room temperature, chemical shifts are written in ppm and coupling constants in Hz. Electrospray (ESI)-time of flight (TOF) mass spectrometry (MS) measurements were performed on a Xevo G2-XS QTOF spectrometer (Waters, USA) for ESI<sup>+</sup> and ESI<sup>-</sup>.

Chemicals were purchased from Sigma-Aldrich or Alfa Aesar and used as received. Thin-layer chromatography (TLC) was performed on silica sheets precoated with Merck 5735 Kieselgel 60F<sub>254</sub>. Column chromatography was carried out with Merck 5735 Kieselgel 60F (0.040-0.063 mm mesh). Compounds di-carboxymalonic acid p-cresol **1** ligand and porphyrin **2** were prepared according to literature procedures.<sup>29</sup>

**SnPh<sub>2</sub>**. A solution of **1** 50 mg (0.23 mmol) in THF (1 ml) was added dropwise to a solution of SnPh<sub>2</sub>Cl<sub>2</sub> 81 mg (0.23 mmol) in THF (1 ml) at room temperature. The reaction was stirred for 20 min, subsequently NEt<sub>3</sub> 66 μl (0.47 mmol) was added dropwise. The reaction was then stirred overnight. The solvent was evaporated and the residue was washed with water in order to remove triethylammonium salts. Yield: 71% (85 mg). <sup>119</sup>Sn{<sup>1</sup>H} NMR (THF-d<sub>8</sub>, 400 MHz) δ (ppm): -172. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz) δ (ppm): 7.75-7.73 (d, 4H, *J* = 7.7 Hz), 7.55-7.39 (m, 6H), 6.93-6.90 (d, 2H, *J* = 6.9 Hz), 6.23-6.20 (d, 2H, *J* = 6.2 Hz), 4.40 (s, 1H), 2.16 (s, 3H). HRMS (ES+) [M+Na]<sup>+</sup> *m/z* calcd for C<sub>22</sub>H<sub>18</sub>O<sub>5</sub>SnNa: 501.0064; found 501.0069 (Δ = 5 ppm). Elem. Anal. Exp. C, 53.95; H, 6.41; N, 2.4. calc. C, 53.88; H, 6.33; N, 2.5.

**Sn<sub>2</sub>Ph<sub>6</sub>**. A solution of **1** 30 mg (0.14 mmol) in THF (1 ml) was added dropwise to a solution of SnPh<sub>3</sub>Cl 110 mg (0.28 mmol) in THF (1 ml) at room temperature. The reaction was stirred for 20 min, subsequently NEt<sub>3</sub> 39 μl (0.28 mmol) was added dropwise. The reaction was then stirred overnight. The solvent was evaporated and the residue was washed with water in order to remove triethylammonium salts. Yield: 93% (120 mg). <sup>119</sup>Sn{<sup>1</sup>H} NMR (THF-d<sub>8</sub>, 400 MHz) δ (ppm): -226. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz) δ (ppm): 7.82-7.80 (d, 4H, *J* = 7.8 Hz), 7.68-7.65 (d, 8H, *J* = 7.6 Hz), 7.47-7.22 (m, 18H), 6.85-6.82 (d, 2H, *J* = 6.8 Hz), 6.44-6.41 (d, 2H, *J* = 6.4 Hz), 4.67 (s, 1H), 2.16 (s, 3H). Elem. Anal. Exp. C, 58.39; H, 5.8; N, 1.77. calc. C, 58.32; H, 5.7; N, 1.85.

**AuP-SnPh<sub>2</sub>**. A solution of porphyrin **2** 50 mg ( $4.6 \times 10^{-2}$  mmol) in THF (2 ml) was added dropwise to a solution of SnPh<sub>2</sub>Cl<sub>2</sub> 19.26 mg ( $5.6 \times 10^{-2}$  mmol) in THF (1 ml) at room temperature. The reaction was stirred for 20 min, subsequently NEt<sub>3</sub> 13 μl ( $9.3 \times 10^{-2}$  mmol)



was added dropwise. The reaction was then stirred overnight. The solvent was evaporated. The complex was dissolved in THF and precipitated in a saturated solution of NaCl. The precipitated complex **AuP-SnPh<sub>2</sub>** was filtered, washed with water and dried. Yield: 98% (57 mg). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>, nm)  $\lambda_{\max}$  (log  $\epsilon$ /M<sup>-1</sup> cm<sup>-1</sup>): 412 (5.44); 532 (3.98). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz): 9.34-9.28 (m, 8H), 8.29-8.27 (dd, 6H, *J* = 1.1, 4.4 Hz), 8.03-7.90 (m, 15H), 7.42-7.27 (m, 8H), 3.48-3.46 (t, 1H, *J* = 3.4 Hz), 3.27-3.25 (d, 2H, *J* = 3.2 Hz). MS (ES<sup>+</sup>) M<sup>+</sup> *m/z* calcd for C<sub>60</sub>H<sub>40</sub>N<sub>4</sub>O<sub>4</sub>SnAu : 1193.1733; found 1193.1678 ( $\Delta$  = 4.6 ppm). Elem. Anal. Exp. C, 55.42; H, 3.47; N, 4.12. calc. C, 55.85; H, 3.94; N, 4.34.

**AuP-Sn<sub>2</sub>Ph<sub>6</sub>**. A solution of porphyrin **2** 64 mg ( $5.9 \times 10^{-2}$  mmol) in THF (2 ml) was added dropwise to a solution of SnPh<sub>3</sub>Cl 49 mg ( $12 \times 10^{-2}$  mmol) in THF (1 ml) at room temperature. The reaction was stirred for 20 min, subsequently NEt<sub>3</sub> 17  $\mu$ l ( $11 \times 10^{-2}$  mmol) was added dropwise. The reaction was then stirred overnight. The solvent was evaporated. The complex was dissolved in THF and precipitated in a saturated solution of NaCl. The precipitated complex **AuP-Sn<sub>2</sub>Ph<sub>6</sub>** was filtered, washed with water and dried. Yield: 80% (90 mg). UV-Vis (CH<sub>2</sub>Cl<sub>2</sub>, nm)  $\lambda_{\max}$  (log  $\epsilon$ /M<sup>-1</sup> cm<sup>-1</sup>): 412 (5.45); 529 (4.11). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz): 9.30-9.25 (m, 8H), 8.28-8.26 (dd, 6H, *J* = 1.2, 7.1 Hz), 8.12-7.68 (m, 25H), 7.42-7.37 (m, 18H). HRMS (ES<sup>+</sup>) [M]<sup>+</sup> *m/z* calcd for C<sub>84</sub>H<sub>60</sub>AuN<sub>4</sub>O<sub>4</sub>Sn<sub>2</sub>: 1623.2272, found 1623.2318 c ( $\Delta$  = 4.6 ppm). Elem. Anal. Exp. C, 58; H, 4.08; N, 2.88. calc. C, 57.69; H, 4.26; N, 3.2.

## Biological experiments

### Cell culture

Human breast adenocarcinoma cells (MCF-7) (purchased from ATCC) were cultured in Dulbecco Eagle's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). Adult Human Dermal Fibroblast cells (FS 20-68) were maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% FBS and 1% P/S. Both cell lines were allowed to grow in humidified atmosphere at 37 °C under 5 % CO<sub>2</sub>. For cytotoxic studies, the compounds (powder) are first diluted in DMSO at the concentration of 10 mM. Then, they are sonicated during 30 seconds and

diluted at the required concentrations from 0.01 to 100  $\mu\text{M}$  in culture medium of each cell line.

### **Cytotoxicity study**

MCF-7 and FS 20-68 cells were seeded into 96-well plates at a density of  $10^3$  cells/cm<sup>2</sup>. One day after, cells were incubated with or without different concentrations of compounds (from 0.01 to 100  $\mu\text{M}$ ) for 3 days. To quantify the percentage of living cells in each condition, cells were incubated 4 h with 0.5 mg mL<sup>-1</sup> MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide) in order to determine mitochondrial enzyme activity. Then, MTT precipitates were dissolved in ethanol/DMSO (1:1) solution and absorbance was measured at 540 nm.

### **Cell-cycle analysis**

Flow cytometric analysis was performed on MCF-7 cells seeded in 6-well plate and allowed to grow for 24 h. Cells were then treated with dyads at their respective LC<sub>50</sub> for 24 or 72 h. After treatment cells were harvested and fixed with 70% ethanol overday. The fixed cells were then incubated with 10 mg/mL RNase A and 1 mg/mL propidium iodide, in the dark, for 24 h at 4°C. Finally, DNA content of the cells was analyzed using BD FACSCalibur flow cytometer.

### **Immunofluorescent staining for $\beta$ -actin detection.**

MCF-7 cells allowed to grow for 24 h on cover slips and were treated with or without dyads at their respective LC<sub>50</sub> for 24 h. Then cells were fixed using paraformaldehyde (PFA) 3.7% for 20 min and permeabilized using 0.2% Triton X-100 for 4 min at room temperature. Actin was stained using a primary human anti-actin antibody (made from mouse) and an Alexa Fluor 568-conjugated anti-mouse antibody. Both of them were incubated 45 min at room temperature. Nuclei were counter-stained using Hoechst 33342. Representative images were obtained under Carl Zeiss confocal microscope, LSM 780.

### **Annexin V detection**

MCF-7 cells allowed to grow for 24 h on cover slips and were treated with or without dyads at their respective LC<sub>50</sub> for 72 h. Cells were incubated with 5  $\mu\text{L}$  of annexin V-FITC (from

abcam) for 5 min, in darkness and room temperature. Then, cells were rinsed twice and fixed using PFA 2% for 20 min. Representative images were obtained under fluorescent microscope Leica DM IRB.

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