Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b00480 • Publication Date (Web): 14 May 2015

Anticancer agents: does a phosphonium behave like a gold(I) phosphine complex? Let a "smart" probe answer!

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ABSTRACT:

Gold phosphine complexes, such as auranofin, have been recognized for decades as antirheumatic agents. Clinical trials are now underway to validate their use in anticancer or anti-HIV treatments. However, their mechanisms of action remain unclear. A challenging question is whether the gold phosphine complex is a prodrug that is administered in an inactive precursor form, or rather that the gold atom remains attached to the phosphine ligand during treatment. In this study, we present two novel gold complexes, which we compared to auranofin and to their phosphonium analogue. The chosen ligand is a phosphine-based smart Probe, whose strong fluorescence depends on the presence of the gold atom. The *in vitro* biological action of the gold complexes and the phosphonium derivative were investigated and a preliminary *in vivo* study in healthy zebrafish larvae allowed us to evaluate gold complex biodistribution and toxicity. The different analyses carried out showed that these gold complexes were stable and behaved differently from phosphonium and auranofin, both *in vitro* and i*n*

vivo. Two-photon microscopy experiments demonstrated that the cellular targets of these gold complexes are not the same as those of the phosphonium analogue. Moreover, despite similar IC_{50} values in some cancer cell lines, gold complexes displayed a low toxicity *in vivo*, in contrast to the phosphonium salt. They are therefore suitable for future *in vivo* investigations.

INTRODUCTION

Among metal-based therapeutic agents, gold(I) derivatives have attracted an increasing interest, mainly due to their antirheumatic and anti-cancer properties.[1] Two molecules, auranofin and aurothiomalate, are currently undergoing clinical trials for the treatment of certain cancers.[2] Several mechanistic studies support the idea that mitochondria, thioredoxin reductase and pathways of oxidative phosphorylation are among the primary intracellular targets.[3] Nevertheless, there is a lack of information concerning the understanding of the chemical behavior of gold complexes, in particular the gold phosphine compounds. It is still unclear which of: the whole complex; the phosphine ligand; the gold atom; or degradation products are responsible for the biological activity of these gold complexes. Phosphonium derivatives are well known for being cytotoxic and for targeting mitochondria.[4] If the P-Au bond is not strong enough, the phosphine may be toxic itself, alkylated *in situ* to generate the phosphonium salt, or oxidized to phosphine oxide. Moreover, when the structures of the gold complexes are examined, it can be seen that the gold(I) is isolobal to H^+. This could give a phosphonium character to the phosphine-Au fragment. It is possible that phosphonium-like behavior could explain the biological properties of these compounds.

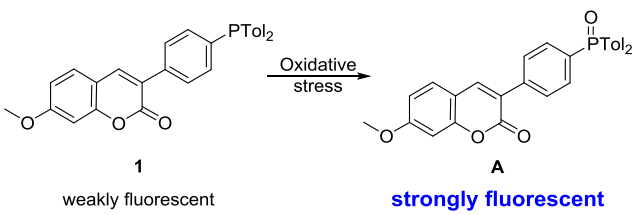
In the present study, we aimed to address this issue. More precisely, we set out to answer the following three questions:

1. When Au(I) is electronically isolobal to the proton, do phosphonium and the phosphine-Au(I) complex behave in the same way?

2. Is the P-Au bond stable both *in vitro* and *in vivo*, or does the phosphine-Au(I) complex function as a prodrug?

3. If the phosphine decoordinated from the metal center, does it induce the generation of a phosphonium salt *in situ*, or is the phosphine itself toxic?

To answer these questions, we used two different compounds, which are structurally very similar to each other: a gold complex and its phosphonium derivative. Furthermore, we studied an auranofin analogue of this complex. To assess their behavior, it is essential to be able to track these compounds, at least *in vitro*. One way to follow such compounds is through fluorescent analysis, where the fluorescence intensity changes dramatically, depending on the presence, or not, of the gold atom on the phosphorus atom. We were particularly interested by previous studies concerning coumarin-phosphines.[5] In these analyses, the fluorescence of coumarin was quenched by the phosphine group, but a strong fluorescence enhancement was observed upon phosphine oxidation (Scheme 1).[5a] This phenomenon enabled the authors to study biological behavior such as oxidative stress. In the present study, we wanted to take advantage of this property by masking the phosphorus lone pair of the probe either by alkylation or by complexing it to a metal atom. As a result, the phosphonium derivative and the gold complex would become strongly fluorescent, in contrast to the free ligand. This would allow us to determine the biological stability of the phosphine-Au bond by fluorescence imaging and to compare the behavior of the coumarin-phosphine-Au derivatives to that of the phosphonium analogue.



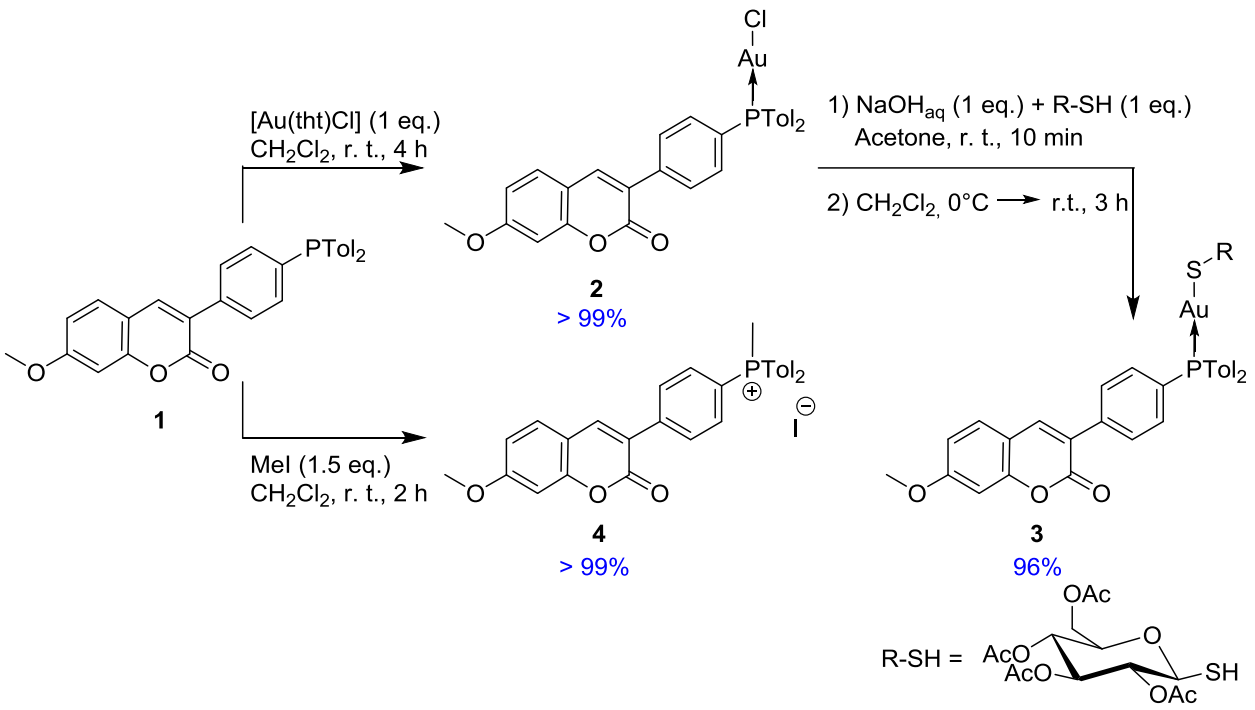
Scheme 1. Coumarin-phosphine ligand **1** and the corresponding oxide (A) described by Pratt and coll..[5a]

Thus, below we present the synthesis, characterization and photophysical properties of three new derivatives, as well as studies of the different compounds *in vitro* and *in* vivo in zebrafish larvae.

RESULTS AND DISCUSSION

Chemical syntheses

The coumarin-phosphine ligand **1** was synthesized in two steps according to the procedure described by Pratt and coll. (Scheme 2).[5a] This compound was then reacted with [Au(tht)Cl] (tht = tetrahydrothiophene) to afford quantitatively the gold complex **2**. In previous studies, we and others found that replacing the chlorido ligand of a phosphine-Au(I) complex by thioglucose tetraacetate often resulted in a significant improvement of its biological properties.[6] This reaction was carried out in high yield, by reacting the Au-Cl complex **2** with *in situ* generated thiolate, to obtain complex **3**. The phosphonium analogue **4** was quantitatively prepared by alkylating ligand **1** with iodomethane.



Scheme 2. Synthesis of compounds **2**, **3** and **4**.

The formation of these derivatives was monitored by ^{31}P NMR. A significant shift of the NMR signal was observed when ligand **1** (singlet at -7.6 ppm) was reacted to give the complexes (singlets at 31.4 ppm for complex **2** and at 35.6 ppm for complex 3) or phosphonium derivative **4** (singlet at 21.2 ppm). The broad shape of the complex **3** singlet is characteristic of P-Au-S compounds.[6a, 6c] The far infrared spectrum of complex **3** displayed a new band at 371 cm^{-1} confirming the formation of an S-Au bond.[7]

All the products were fully characterized and the structures of compounds **1**, **2** and **4** were confirmed by X-ray diffraction analysis of monocrystals (Figure 1; see supporting information for the Ortep representation of ligand **1**[8]). The comparison of complex **2** and phosphonium **4** Ortep views combined with a PM6[9] theoretical study indicates that compounds **2** and **4** are very similar in terms of 3-dimensional structure (see supplementary information for details). This hypothesis was confirmed by their similar UV-visible spectra, see below.

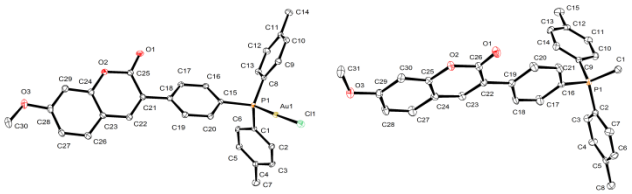


Figure 1. (left) Ortep view of complex **2**. (right) Ortep view of a phosphonium **4**.[8]

Photophysical studies

The photophysical properties of coumarin-phosphine ligand **1** and the different complexes were studied in dichloromethane, and are presented in Table 1.

Table 1. Photophysical data of the different compounds in CH_2Cl_2 at 298K.

N°	λ_{abs} (nm)	λ_{em} (nm)	ε ($M^{-1}.cm^{-1}$)	ϕ_f (%)[a]	B_r[b] ($M^{-1}.cm^{-1}$)	$B_r/B_{r(1)}$
1	348	430	25100	3	753	1
2	350	432	25700	83	21330	28
3	349	432	26200	83	21750	29
4	355	441	34300	91	31210	41

[a]: Reference: diphenylanthracene (Φ_f = 0.97, λ_{exc} = 355 nm, in cyclohexane).[10]

[b]: Brightness = $\varepsilon.\Phi_f$. (λ_{abs} = wavelength of maximum absorption; λ_{em} = wavelength of maximum emission; ε = molar absorption coefficient; ϕ_f =fluorescence quantum yield; B_r = brightness; $B_{r(1)}$ = brightness for compound **1**)

The coumarin-phosphine ligand **1** had an absorption maximum at 348 nm in CH_2Cl_2, with a molar absorption coefficient of 25100 $M^{-1}.cm^{-1}$.[5a] Alkylation of the phosphine induced a bathochromic shift of the absorption and emission spectra. Furthermore, this alkylation resulted in an increase of epsilon to 34300 $M^{-1}.cm^{-1}$. Complexing the phosphine onto Au(I) did not significantly influence the epsilon value.

The photophysical spectra of complex **3** are shown in Figure 2. A fluorescence curve corresponding to the coumarin moiety can be observed around 430 nm for compounds **1**, **2** and **3** (Figure 2 and see supplementary information) and 441 nm for compound **4** (see supplementary information).

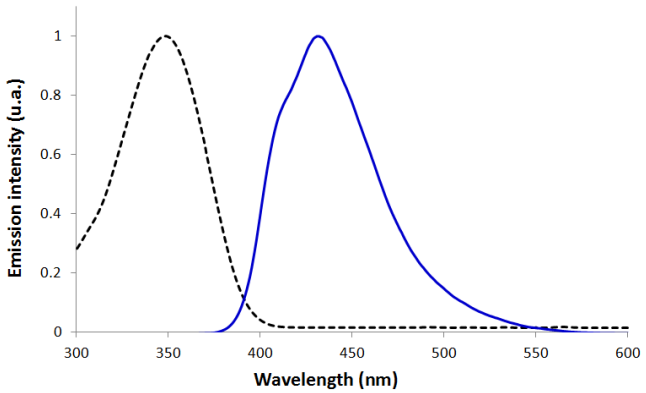


Figure 2. Absorption (black dashed line) and emission (blue solid line) spectra of complex **3.**

Spectra were determined in CH_2Cl_2, at 298K.

The quantum yield of the coumarin-phosphine ligand **1** is low (3%). This quenching can be explained by intramolecular Photoinduced Electron Transfer (PET) from the phosphine to the fluorophore. We verified that the PET mechanism was not intermolecular by recording the fluorescence spectrum of phosphonium **4** in the presence of 10 molar equivalents of tricyclohexylphosphine. Effectively, during this experiment no change in fluorescence emission was recorded, whereas if the PET were intermolecular, a significant quenching of phosphonium **4** fluorescence would have been observed. Alkylation of the phosphine strongly enhanced the coumarin fluorescence by preventing PET (Φ = 91%). Complexing of Au(I) to **1** induced the same phenomenon, with a quantum yield of fluorescence of 83% of the resulting complexes **2** and **3**. Another important factor, which characterizes a fluorophore for biological applications, is the brightness Br = $\varepsilon.\Phi_f$. This takes into account both the absorption and emission

properties of the fluorescent probe. Alkylation of the coumarin-phosphine ligand **1** induced an increase of brightness by a factor of 41, while that of the Au(I) complexes was increased by a factor of 29.

The difference in fluorescence efficiency between coumarin-phosphine ligand **1** and the gold complexes **2** and **3** could be exploited to monitor the stability of the complexes and their behavior *in vitro*. The release of Au(I) should induce a strong quenching of fluorescence. To trace this phenomenon, the fluorescence intensity of complex **2** was followed, in the presence of 10 molar equivalents of tricyclohexylphosphine as a competitor ligand (see supporting information for details of the corresponding NMR study). The release of Au(I) from complex **2** was almost immediate and this was accompanied by strong quenching of the coumarin fluorescence (Figure 3). This validates our concept of smart probe.

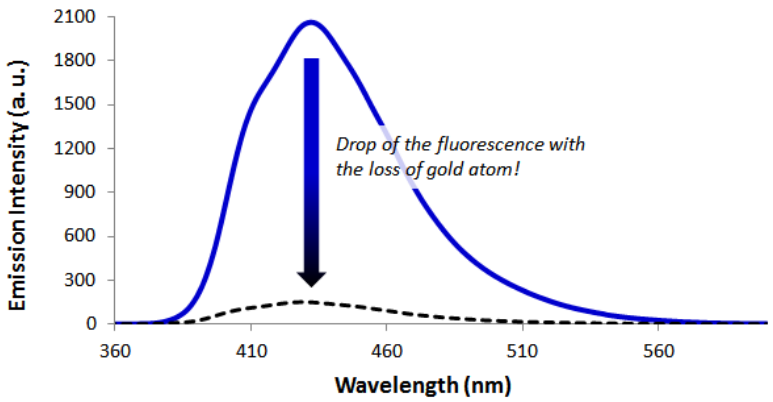


Figure 3. Emission intensity of complex **2** before (blue solid line) and after (black dashed line) the addition of 10 molar equivalents of tricyclohexylphosphine (a. u. = arbitrary unit).

Biological studies

The anti-proliferative activity of the four compounds was tested in human cancer cell lines from mammary (MDA-MB-231 and MCF-7) or colon (SW480 and HCT-116) origins. These cell lines in particular were chosen for two reasons. Firstly, mammary and colon cancers are very frequent cancers, with poor prognosis when diagnosed at a late stage. Secondly, these cells differ in their status of the p53 tumor suppressor gene. HCT-116 and MCF-7 cells express WT p53, whereas SW480 and MDA-MB-231 cells express a mutated form of the p53 gene. The sensitivity of cancer cells in general towards treatment is often dependent on p53[11], which is mutated in almost 50% of all human cancers. These mutations are associated with a lack of response to certain chemotherapeutic drugs.[13] Auranofin is an interesting compound in this context, as it has been reported to induce apoptosis *via* oxidative stress, but independently of p53.[14] Thus it may represent the first of a group of compounds able to kill tumor cells irrespective of their p53 status. Such compounds could be used for the elaboration of new strategies to overcome chemotherapy resistance. The gold phosphine complexes **2** and **3** displayed substantial toxic effects with similar IC_{50} values ranging between 33 and 50.1 μM in mammary and colon cancer cell lines. However, these compounds were less potent cytotoxic agents than the phosphonium analogue **4**, which gave an IC_{50} value of 10.1 to 33.4 μM (Table 2). The toxicity of these compounds was independent of the p53 status since no difference was observed between cells expressing WT p53 and cells expressing mutated p53. No IC_{50} value could be attributed to the coumarin-phosphine ligand **1**, confirming its non-toxicity.

Table 2. Determination of the IC_{50} values of compounds **1**, **2**, **3** and **4** in SW480, MDA-MB-231, MCF-7 and HCT-116 cells.

The IC_{50} values (μM) of compounds **1**, **2**, **3** and **4** were determined at 48h in SW480, MDA-MB-231, MCF-7 and HCT-116 cells by MTS assays. The indication N. D. (not determined) means that IC50 was > 100 μM.

N°	SW480	MDA	MCF7	HCT116
1	N.D.	N.D.	N.D.	N.D.
2	42.0 ± 11.7	33.1 ± 14.8	49.0 ± 1.3	37.8 ± 17.1
3	33.6 ± 14.1	46.5 ± 4.8	50.1 ± 0.1	49.9 ± 0.3
4	25.7 ± 0.3	10.1 ± 0.2	33.4 ± 14.2	28.4 ± 4.5

We used two-photon fluorescence microscope and a fluorimeter to investigate whether the differences in toxicity between gold complexes and phosphonium could be due to variability in cell accumulation or subcellular location of the different compounds. SW480 cells or MDA-MB-231 cells were incubated for a short time (15 min) with 25 or 50 μM of the coumarin-phosphine derivatives (compounds **1**-**4**) and fluorescence was measured at different times after treatment. Phosphonium **4** accumulated rapidly in the cells as was attested by the high fluorescence intensity (see supplementary information for details) and localized to the cytoplasm (Figure 4). In contrast, gold phosphine complexes **2** and **3** accumulated at the plasma membrane as small aggregates. The coumarin-phosphine ligand **1** gave a very weak fluorescent signal, localized in the cytoplasm, confirming that free coumarin-phosphine corresponds to the inactive form of the probe (Figure 4). Fluorescence measured with all the compounds is dose-dependent and stable when observed 48 h after application to the cells (data not shown). These results demonstrated that both the fluorophore and the P-Au bond are stable *in vitro* for at least 48 h. The weak fluorescence observed in the case of coumarin-phosphine ligand **1** indicated that this molecule did not

undergo protonation, alkylation or oxidation *in vitro*. The results concerning oxidation are in agreement with the study of Pratt's group [5a].

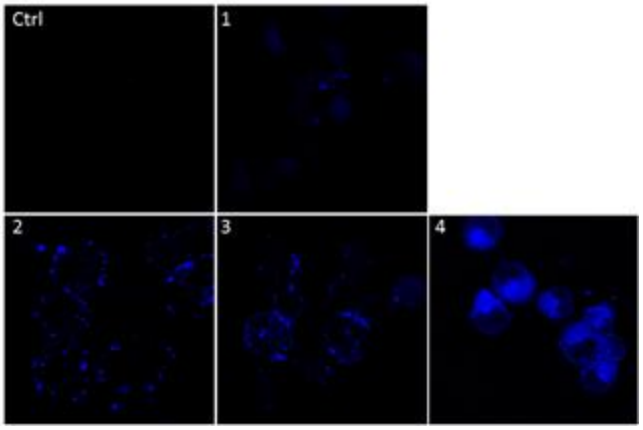


Figure 4. Two-photon fluorescence microscopy experiments in the MDA-MB-231 cell line. Cells were treated with 50 µM of metal compounds **1**, **2**, **3** and **4** for 15 min at 37 °C and excitation was at 750 nm.

The punctuated and plasma membrane localization of complexes **2** and **3** seems to occur in lipid rafts, which are cellular domains that concentrate plasma membrane proteins and lipids involved in the regulation of numerous cell functions, including signaling, trafficking, adhesion, migration and growth. These functions seem to be due to the location in the rafts of a large panel of receptors and signaling proteins involved in carcinogenesis and metastasis, such as EGFR, VEGFR, Ras (a small GTPase), Focal Adhesion Kinase, Src (a tyrosine-protein kinase Proto-oncogene)[15] . To verify this hypothesis, cells were pre-treated with methyl-β-cyclodextrin (mβCD) (10 mM, Figure 5) or Nystatin A (50 µg/mL, see supplementary information) to disrupt these micro-domains. Nystatin A is a cholesterol scavenger that disrupts the rafts and forces the cytoplasmic accumulation of their components (see supporting information for details). In the presence of either mβCD or Nystatin A, the gold phosphine complexes **2**

and **3** re-located to the cytoplasm, lending weight to the hypothesis of their accumulation in lipid rafts in cancer cells.

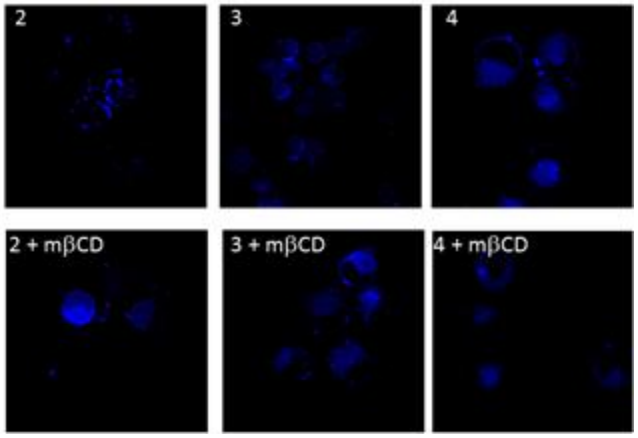


Figure 5. Two-photon fluorescence microscopy experiments of MDA-MB-231 cells treated with metal compounds **2**, **3,** or **4**.

Cells were treated with 50 µM of the metal compounds for 15 min at 37 °C after incubation with (lower panels), or without (upper panels), methyl-β-cyclodextrin (mβCD) (10 mM 1 h at 37°C). Excitation was at 750 nm.

It can be proposed that the location of the two phosphine gold complexes in lipid rafts would increase their interaction with these oncogenic proteins and affect their cell toxicity.

To investigate the molecular mechanisms leading to gold phosphine-mediated cell death, we tested the effects of these compounds on superoxide production, as phosphonium salts and auranofin are known to induce the production of mitochondrial superoxide anions.[4] SW480 and MDA-MB-231 cells were incubated for 1 h with these compounds (50 µM) before treatment with dihydroethidium (a fluorescent superoxide indicator) and flow cytometry analysis. The phosphonium analogue **4** and auranofin induced a high production of ROS in mammary or colorectal cancer cells, which was time dependent, whereas no superoxide anion production was observed with the coumarin-phosphine ligand **1** or the gold complexes

2 and **3** (Figure 6 and Figure S24). These results showed that the signaling pathways induced by the gold phosphine complexes **2** and **3** must be different from those induced by the phosphonium analogue **4**. For example, ROS is produced in the presence of auranofin, but not in the presence of complex **3**. One hypothesis for this difference is the location of complexes in the lipid rafts. Thus, cells were pre-treated with nystatin A (50 µg/mL). Then, ROS production was no longer detected, confirming that the mechanism of action of gold complexes **2** and **3** differs from that of phosphonium analogue **4** and auranofin.

The previous results also highlight the strong influence of the phosphine substituents on mitochondria. Indeed, we recently observed this same phenomenon with another phosphine fluorophore.[6a]

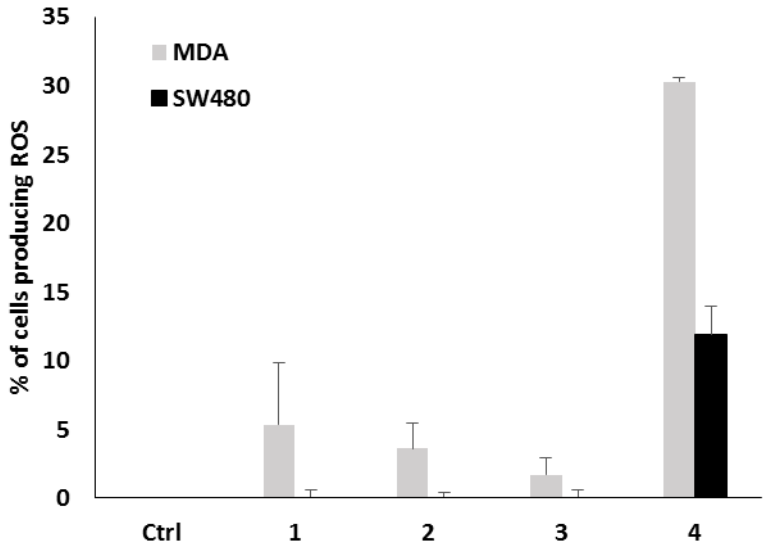


Figure 6. ROS detection in MDA-MB-231 and SW480 cells measured by flow cytometry using the dihydroethidium dye after incubation with compounds **1**, **2**, **3** and **4**.

Cells were treated with gold compounds at 50µM for 1 h. Results are expressed as the % of cells that produce ROS, after subtraction of control fluorescence.

The biological behavior of these different compounds was studied *in vivo* using the zebrafish larvae model. We observed dose-dependent toxicity in 4dpf larvae treated with compounds **2**, **3** and **4** but not with the coumarin-phosphine ligand **1** (Table 3). In this assay, (Table 3), compounds **3** and **4** at 100 µM both killed all larvae, although compound **4** was more toxic than complex **3** as evidenced by a lower threshold for 100% larvae death. In contrast, complex **2** was only mildly toxic at 100 µM. Ligand **1** did not show significant *in vivo* toxicity, in agreement with *in vitro* results. *In vivo* toxicity thus depends on the nature of the ligand linked to the gold atom. These results suggest that despite its relatively modest IC_{50} value, compound **2** is worth investigating further *in vivo*.

Table 3. Determination of mortality of 4dpf zebrafish larvae after 24 h incubation with the different compounds. Footnote: Mortality was evaluated by % larvae death, total larvae death is shaded in dark grey and high mortality in pale grey, for DMSO control larvae 1.8 ± 1.6% mortality was observed

Compound	10µM	25µM	50µM	75µM	100µM
1	3.7±3.2	7.9±3.4	7.9±3.4	10.8 ± 5.0	4.2±3.6
2	3.7±3.2	3.7±3.2	4.2±3.6	12.0±6.3	22.7±14.6
3	15.3±8.4	7.4±6.4	14.9±3.2	76.8±11.4	100.0±0.0
4	0.0±0.0	14.5±2.4	81.8±11.7	100.0±0.0	100.0±0.0

Biodistribution analyses were also performed *in vivo* in 6dpf zebrafish larvae (see Figure 7 for details and supporting information for additional images). A Mito Tracker Green FM dye (MT, a fluorophore that targets mitochondria), was used for co-localization purposes because phosphoniums are known to target mitochondria. Thus, a blue signal corresponds to the biological molecule indicated (**1** to **4,** emission

recorded at 445 nm) and a green one to MT (emission recorded at 525 nm). Mitochondria rich cells (MRCs) present in neuromasts and the nasal epithelium were labelled using MT. Cranial and trunk neuromasts appear as punctuate dots. Representative signals are indicated by yellow arrows and epithelial nasal cells by red ones (MT larvae).[16] All the compounds tested were detected in the digestive system, with the highest accumulation being observed for coumarin-phosphine ligand **1,** then complex **3**, whereas only moderate signals were observed in this area for compounds **2** and **4**. For coumarin-phosphine ligand **1,** a diffuse staining was observed throughout the larval body. This is not due to native phosphine**,** but to that which has undergone oxidation to phosphine oxide (**A,** Schema 1). Indeed, coumarin-phosphine ligand **1** oxidizes when it is left for a prolonged time in an aerobic solution, here aquarium water. This indicated that the *in vivo* toxicity of compound **A** is not significant.[5a] Concerning compound **4**, a clear specific phosphonium staining was observed. This corresponded to MRCs of the nasal epithelium and hair cells of neuromasts, as evidenced by the MT signal. While phosphine oxide **A** staining also overlaps with the MT dye, neither compounds **2** nor **3** interfered with MT labeling, suggesting that gold complexes **2** and **3** do not target mitochondria. No more MT staining was observed in mitochondria after treatment with compound **4**, suggesting a mitochondria-dependent toxic effect of **4.** In control larvae, a green background signal of autofluorescence was observed in the yolk sac (black arrows), whereas no blue background signal was observed.

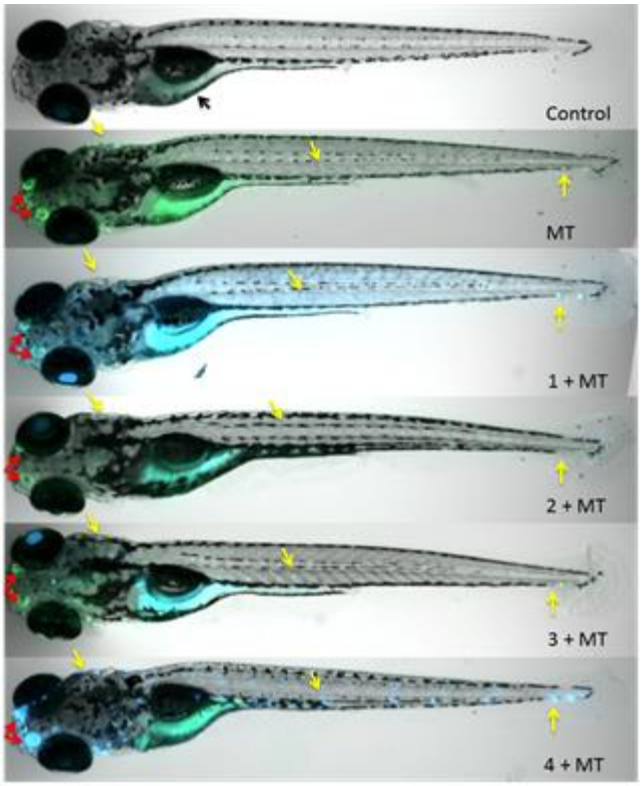


Figure 7. Biodistribution of compounds **1**, **2**, **3** and **4** in 6dpf zebrafish larvae.

Zebrafish larvae were subjected to fluorescence imaging after 1 h incubation with 10 µM of one of the four biological compounds under study, as indicated (**1**-**4**), followed by 1 h labeling with 25 nM MT. The experimental compounds give a blue fluorescence, whereas MT gives a green one. Yolk sac: black arrow; nasal epithelium: red arrows; representative cranial and trunk neuromasts: yellow arrows.

CONCLUSION

In this study, we report the synthesis of three new phosphine derivatives that display strong fluorescence properties. Two of these were gold complexes, whereas the third was a phosphonium analogue. Their characterization and especially the combination of X-ray diffraction analyses and theoretical calculations highlighted their structural similarities. We studied the stabilities of the two gold complexes both *in vitro* in cancer cells and *in vivo* in the zebrafish larvae model. The P-Au bond is stable in these systems for at

least 48 h and the difference in terms of *in vivo* toxicity between complexes **2** and **3** suggests the importance of the nature of the ligand attached to the gold atom. The coumarin-phosphine ligand **1** did not display toxicity, either *in vitro* or *in vivo*. The absence of fluorescence *in vitro* indicated that no alkylation or oxidation of the phosphorus had occurred at least over 48 h after treatment, while diffuse *in vivo* fluorescence suggested the oxidation of compound **1**. So even if the phosphine is decoordinated from the metal center, it should not immediately be toxic.

Functional studies showed that even if the molecules tested are closely related structurally, their cytotoxicity, sub-cellular location and biodistribution in the zebrafish larval model are clearly distinct. The cytoplasmic location of the phosphonium derivative **4** and its concentration in mitochondria-rich cells (MRCs) of the nasal epithelium and neuromasts (cluster of hair cells), combined with its ability to induce ROS production, could explain its toxicity *in vitro* and *in vivo*. In contrast, gold complexes **2** and **3** are unable to produce ROS and they accumulate in lipid rafts. This suggests a different mechanism of action from that of phosphonium derivatives and auranofin. Gold complexes **2** and **3** may induce cell toxicity by interaction with protein(s) contained in lipid rafts. Furthermore, the cytotoxicity of compounds **2**-**4** seems to be p53-independent, which should allow new avenues to be explored to overcome chemotherapy resistance.

Interestingly, compound **2** is toxic in tumor cells *in vitro*, especially in colon cancer cells, whereas it shows low toxicity in healthy zebrafish larvae. Thus, it may be an interesting drug to control the development of colon cancer cells as it is distributed throughout the digestive system, with limited side effects in normal cells. However, more studies are needed to precisely characterize the targets and the signaling pathways that these compounds could induce, before they can be considered as a new strategy for cancer therapy.

Finally, this study highlighted the value of designing traceable therapeutic agents to help identify targets and clarify the mechanisms of action of new drugs.

ASSOCIATED CONTENT

Supporting Information Available:

Procedures to synthesize compounds **2**, **3** and **4**; structural characterization data and spectra of compounds **1**, **2**, **3** and **4**; photophysical characterization data for these compounds and their spectra; X-ray structure data of compounds **1**, **2** and **4**; theoretical calculation of phosphonium analogue **4**; *in vitro* tests on compounds **1**, **2**, **3** and **4** (IC_{50} determination, two-photon fluorescence microscopy images, ROS measurements and uptake observations in living cells by fluorescence); *in vivo* tests on compounds **1**, **2**, **3** and **4** (toxicity assay and fluorescence imaging in zebrafish larvae). This material is available free of charge *via* the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENTS

We are grateful to the “Centre National de la Recherche Scientifique” (ICMUB, UMR CNRS 6302) for financial support. Support was also provided by the “Université de Bourgogne” and the “Conseil Régional de Bourgogne” through the 3MIM integrated project (“Marquage de Molécules par les Métaux pour l’Imagerie Médicale”) and PARI SSTIC n°6. Dr. Fanny Picquet, Marie José Penouilh and Dr. Michel Picquet are acknowledged for technical support. EB thanks Dr. Malacea for the crystallization of compound **4** and VL thanks Dr Charles Thomas for the mitotracker dye. PR thanks Dr Fleurat-Lessard for helpful discussions

concerning the molecular modeling study. We are grateful to Pr. Johanna Chluba for her oversight of the zebrafish facility and to Tatiana Lopez for fish handling and care; and to Pascale Winckler (DImaCell, UMR PAM A 02.102, Université de Bourgogne/Agrosup) and Benoît Gasquet (CellImap, IFR Santé Stic, Université de Bourgogne) for their help in two-photon and confocal microscopy studies. EB thanks COST Action TD1004 for financial support.

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(8) Crystal data:

For **1** $C_{30}H_{25}O_3P$, M = 464.47, triclinic, P-1 (No. 2, a = 6.29 Å, b = 9.533 Å, c = 20.743 Å, α = 99.499°, β = 95.427°, γ = 102.793°,V = 1185.1(12) Å^3, T = 115 K, Z = 2, μ (Mo K_α) = 0.146, 40464 reflections measured, 6822 unique (R_{int} = 0.0295) which were used in all calculations. The final wR_2 was 0.1054 (all data) and R_1 was 0.0399 (I > 2(I)). CCDC = 1014821.

For **2 .** $C_{30}H_{25}AuClO_3P$, M = 696.89, monoclinic, $P2_1/c$ (No. 14, a = 9.4608 Å, b = 13.6407 Å, c = 19.9564 Å, α = 92.341°, β = γ = 90°,V = 2573.26(9) Å^3, T = 115(2) K, Z = 4, μ (Mo K_α) = 5.914, 71125 reflections measured, 11461 unique (R_{int} = 0.0683) which were used in all calculations. The final wR_2 was 0.0636 (all data) and R_1 was 0.0313 (I > 2(I)). CCDC = 1014822 .

For **4 .** $C_{65}H_{62}Cl_6I_2O_6P_2$, M = 1467.58, orthorhombic, $Pca2_1$ (No. 29, a = 18.6546 Å, b = 18.6191 Å, c = 18.5054 Å, α = β = γ = 90°,V = 6427.5(4) $Å^3$, T = 100 K, Z = 4, μ (Mo $K_α$) = 1.327, 34584 reflections measured, 13908 unique (R_{int} = 0.0245) which were used in all calculations. The final wR_2 was 0.0975 (all data) and R_1 was 0.0414 (I > 2(I)). CCDC = 1014823.

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