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SUPPORTING INFORMATION

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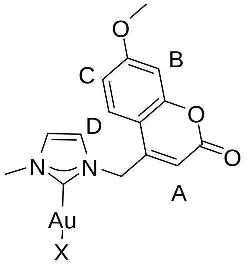
Title: New Gold(I) Organometallic Compounds with Biological Activity in Cancer Cells

Author(s): Benoît Bertrand, Andreia de Almeida, Evelien P. M. van der Burgt, Michel Picquet, Anna Citta, Alessandra Folda, Maria Pia Rigobello, Pierre Le Gendre, Ewen Bodio,* Angela Casini*

Experimental Section

General Remarks. All reactions were carried out under an atmosphere of purified argon using Schlenk techniques. Solvents were dried and distilled under argon before use. The precursor [AuCl(tht)] (Uson R. et al, Inorg Synth. 1989, 26, 85-91) has been synthesized according to literature procedure. All other reagents were commercially available and used as received. All the analyses were performed at the "Plateforme d'Analyses Chimiques et de Synthèse Moléculaire de l'Université de Bourgogne". The identity and purity (≥ 95%) of the complexes were unambiguously established using high-resolution mass spectrometry, elemental analysis, NMR and IR spectrometries. Exact mass of the synthesized complexes were obtained on a Thermo LTQ Orbitrap XL. Elemental analyses were performed on a Thermo Electron Flash EA 1112 Series analyzer. ^{1}H- (300.13, 500.13 or 600.23 MHz) and ^{13}C- (125.77 or 150.90 MHz) NMR spectra were recorded on Bruker 300 Avance III, 500 Avance III or 600 Avance II spectrometers. Chemical shifts are quoted in ppm (δ) relative to TMS (^{1}H and ^{13}C) using the residual protonated solvent (^{1}H) or the deuterated solvent (^{13}C) as internal standards. Infrared spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer (Golden Gate ATR) and far infrared spectra were recorded on a Bruker Vertex 70v FT-IR spectrophotometer (ATR Diamant).



Scheme S1: General coumarin ^{1}H and ^{13}C labelling used for the attribution of the NMR signals.

- **3-[(7-methoxy-2-oxo-2H-chromen-4-yl)methyl]-1-methylimidazolium bromide (1)**

A two-neck round-bottom flask was charged under argon with 1 g of 4-(bromomethyl)-7-methoxy-2*H*-chromen-2-one (3.76 mmol) which was suspended in THF (50 mL). 1-methylimidazole (0.31 mL, 3.76 mmol) was added dropwise and the mixture was refluxed overnight. After removing of the THF under vacuum, the obtained white powder was suspended into dry dichloromethane and a large amount of diethyl ether. The suspension was filtered and the residue was dried under vacuum to afford the pure product as a white powder (1.26 g, 96 % yield).

^{1}H NMR (DMSO-d6, 300.13 MHz, 300 K): 3.88 (s, 3 H, N-Me), 3.89 (s, 3 H, O-Me), 5.78 (s, 2 H, N-CH_2), 5.89 (s, 1 H, CH^A), 7.02 (dd, 1 H, $^3J_{H-H}$ = 9.0 Hz, $^4J_{H-H}$ = 2.7 Hz, CH^C), 7.08 (d, 1 H, $^4J_{H-H}$ = 2.7 Hz, CH^B), 7.76 (d, 1 H, $^3J_{H-H}$ = 9.0 Hz, CH^D), 7.80 (s, 1 H, CH_{Im}), 7.84 (s, 1 H, CH_{Im}), 9.22 (s, 1 H, N=CH-N^+).

^{13}C{^{1}H} (DMSO-d6, 75.78 MHz, 300 K): 36.6 (s, N-CH_3), 48.8 (s, N-CH_2), 56.6 (s, O-CH_3), 101.6 (s, CH^B), 110.7 (s, CH^A), 113.1 (s, CH^C), 123.4 (s, CH_{Im}), 124.7 (s, CH_{Im}), 126.1 (s, CH^D), 138.2 (s, N=CH-N^+), 149.8 (s, C_{quat}-CH^D), 155.5 (s, C_{quat}-OC(O)), 160.2 (s, *C*(O)), 163.4 (s, C_{quat}-OMe).

FT-IR (ATR, cm^{-1}): 3122, 3064, 2847, 1705, 1607, 1564, 1515, 1464, 1439, 1400, 1342, 1286, 1270, 1211, 1169, 1137.

ESI-MS (DCM/MeOH), *positive mode exact mass* for $C_{15}H_{15}N_2O_3^+$ (271.10772): measured *m*/*z* 271.10664 $[M-Br]^+$.

Anal. Calc. for $C_{15}H_{15}N_2O_3Br$: C, 51.30, H, 4.31, N, 7.98 %. Found: C, 51.27, H, 4.05, N, 8.03 %.

- **3-[(7-methoxy-2-oxo-2*H*-chromen-4-yl)methyl]-1-methylimidazol-2-ylidene gold(I) chloride (2)**

A round-bottom flask was charged with **1** (200 mg, 0.57 mmol) and Ag_2O (106 mg, 0.46 mmol) which were suspended in dichloromethane (150 mL). The mixture was reacted for 6 h at room temperature in the dark. [AuCl(tht)] (182 mg, 0.57 mmol) was then added and the reaction was stirred overnight at room temperature in the dark. After filtration through Celite, the filtrate was concentrated under reduced pressure. Upon addition of a large amount of diethyl ether, a pale yellow precipitate was formed and was collected by filtration. The residue was dried under vacuum to afford the pure product as a pale yellow powder (215 mg, 75 % yield).

^{1}H NMR ($CDCl_3$, 300.13 MHz, 300 K): 3.89 (s, 3 H, N-Me), 3.92 (s, 3 H, O-Me), 5.55 (s, 2 H, N-CH_2), 5.68 (s, 1 H, CH^A), 6.85 (d, 1 H, $^4J_{H-H}$ = 2.7 Hz, CH^B), 6.89 (dd, 1 H, $^3J_{H-H}$ = 9.0 Hz, $^4J_{H-H}$ = 2.7 Hz, CH^C), 7.00 (d, 1 H, $^3J_{H-H}$ = 1.8 Hz, CH_{Im}), 7.06 (s, 1 H, $^3J_{H-H}$ = 1.8 Hz, CH_{Im}), 7.52 (d, 1 H, $^3J_{H-H}$ = 9.0 Hz, CH^D).

^{13}C{^{1}H} ($CDCl_3$, 75.78 MHz, 300 K): 38.6 (s, N-CH_3), 51.1 (s, N-CH_2), 55.9 (s, O-CH_3), 101.5 (s, CH^B), 110.3 (s, CH^A), 110.8 (s, C_{quat}-CH_2), 112.9 (s, CH^C), 120.9 (s, CH_{Im}), 122.9 (s, CH_{Im}), 124.5 (s, CH^D), 148.8 (s, C_{quat}-CH^D), 155.6 (s, C_{quat}-OC(O)), 160.2 (s, *C*(O)), 163.4 (s, C_{quat}-OMe), 173.5 (s, $C_{carbene}$).

FT-IR (ATR, cm^{-1}): 3129, 2943, 1709, 1610, 1558, 1515, 1465, 1429, 1397, 1348, 1331, 1288, 1245, 1207, 330.

ESI-MS (DCM/MeOH), *positive mode exact mass* for $C_{15}H_{14}N_2O_3AuClNa^+$ (525.02507): measured *m*/*z* 525.02328 $[M+Na]^+$.

Anal. Calc. for $C_{15}H_{14}N_2O_3AuCl$: C, 35.84, H, 2.81, N, 5.57 %. Found: C, 35.74, H, 3.00, N, 5.50 %.

- **3-[(7-methoxy-2-oxo-2*H*-chromen-4-yl)methyl]-1-methylimidazol-2-ylidene gold(I) (thio-*β*-D-glucose tetraacetate) (3)**

A Schlenk tube was charged under argon with thio-*β*-D-glucose tetraacetate (36 mg, 0.10 mmol) which was dissolved in degassed acetone (3 mL). 1 M NaOH (0.1 mL, 0.10 mmol, 1 eq.) was added and the mixture was stirred for 30 min at room temperature. The mixture was then transferred onto a solution of **1** (50 mg, 0.10 mmol) in 5 mL of degassed acetone at 0°C. At the end of the addition, the ice bath was withdrawn and the mixture was stirred for 3 h at room temperature in the dark. The solvent was removed under vacuum. Dichloromethane was added and the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure. An off-white precipitate was formed after addition of a large amount of *n*-pentane. The precipitate was filtered and dried under vacuum to give the pure product as an off-white powder (58 mg, 71 % yield).

^{1}H NMR ($CDCl_3$, 300.13 MHz, 300 K): 1.95 (s, 3 H, CH_3-COO), 2.00 (s, 3 H, CH_3-COO), 2.02 (s, 6 H, 2 CH_3-COO), 3.67-3.72 (m, 1 H, CH_{sugar}), 3.87 (s, 3 H, N-Me), 3.95 (s, 3 H, O-Me), 4.06 (dd, 1 H, $^2J_{H\text{-}H}$ = 12.3 Hz, $^3J_{H\text{-}H}$ = 2.4 Hz, $CH_{A\text{-}B,sugar}$), 4.20 (dd, 1 H, $^2J_{H\text{-}H}$ = 12.3 Hz, $^3J_{H\text{-}H}$ = 4.8 Hz, $CH_{A\text{-}B,sugar}$), 4.99-5.11 (m, 4 H, 4 CH_{sugar}), 5.57 (dd, 1 H, $^2J_{H\text{-}H}$ = 16.8 Hz, $^3J_{H\text{-}H}$ = 1.2 Hz, $CH_{A\text{-}B}$-N), 5.67 (d, 1 H, $^2J_{H\text{-}H}$ = 16.8 Hz, $CH_{A\text{-}B}$-N), 5.75 (s, 1 H, CH^A), 6.86 (d, 1 H, $^4J_{H\text{-}H}$ = 2.4 Hz, CH^B), 6.92 (dd, 1 H, $^3J_{H\text{-}H}$ = 8.7 Hz, $^4J_{H\text{-}H}$ = 2.4 Hz, CH^C), 6.96 (d, 1 H, $^3J_{H\text{-}H}$ = 1.8 Hz, CH_{Im}), 7.03 (d, 1 H, $^3J_{H\text{-}H}$ = 1.8 Hz, CH_{Im}), 7.67 (d, 1 H, $^3J_{H\text{-}H}$ = 8.7 Hz, CH^D).

^{13}C{^{1}H} ($CDCl_3$, 125.77 MHz, 300 K): 20.6 (s, *C*H_3-COO), 20.7 (s, *C*H_3-COO), 20.8 (s, *C*H_3-COO), 21.2 (s, *C*H_3-COO), 38.3 (s, *C*H_3-N), 50.9 (s, *C*H_2-N), 55.9 (s, *C*H_3-O), 63.0 (s, *C*$H_{2\text{-}sugar}$), 69.0 (s, *C*H_{sugar}), 74.3 (s, *C*H_{sugar}), 75.8 (s, *C*H_{sugar}), 77.6 (s, *C*H_{sugar}), 83.1 (s, *C*H_{sugar}), 101.4 (s, *C*H^B), 110.6 (s, C_{quat}-CH_2), 111.0 (s, *C*H^A), 112.9 (s, *C*H^C), 120.7 (s, *C*H_{Im}), 122.8 (s, *C*H_{Im}), 125.0 (s, *C*H^D), 149.3 (s, C_{quat}-CH^D), 155.6 (s, C_{quat}-OC(O)), 160.4 (s, *C*$(O)_{coum.}$), 163.3 (s, C_{quat}-OMe), 169.6 (s, *C*$(O)_{sugar}$), 169.9 (s, $C_{carbene}$), 170.2 (s, *C*$(O)_{sugar}$), 170.7 (s, *C*$(O)_{sugar}$).

FT-IR (ATR, cm^{-1}): 2946, 1735, 1613, 1561, 1463, 1430, 1368, 1288, 1219, 1147, 1031, 373.

ESI-MS ($CDCl_3$/MeOH), *positive mode exact mass* for $C_{29}H_{33}N_2O_{12}AuSNa^+$ (853.13119): measured *m*/*z* 853.12907 $[M+Na]^+$.

Anal. Calc. for $C_{29}H_{33}N_2O_{12}AuS$: C, 41.93, H, 4.00, N, 3.37, S, 3.86 %. Found: C, 41.54, H, 4.20, N, 3.27, S, 3.09 %.

- **3-[(7-methoxy-2-oxo-2*H*-chromen-4-yl)methyl]-1-methylimidazol-2-ylidene gold(I) (thiolato-*β*-D-glucose) (4)**

A flame-dried Schlenk tube was charged under argon with sodium thiolate-β-D-glucose (26 mg, 0.12 mmol) which was suspended in methanol (3 mL). This mixture was transferred onto a solution of **2** (60 mg, 0.12 mmol) in 6 mL of dichloromethane at 0°C. After the end of the addition, the ice bath was withdrawn and the mixture was stirred for 3 h at room temperature. The solution was filtered under argon through Celite using a flame-dried sintered glass. Upon concentration under reduced pressure, and addition of a large amount of a mix diethylether/*n*-pentane, a white precipitate was formed. The precipitate was filtered and dried under vacuum to afford the product as an adduct with one equivalent of NaCl (58 mg, 68 % yield).

^{1}H NMR (DMSO-d6, 600.23 MHz, 300 K): 2.50 (dt, 1 H, $^3J_{H\text{-}H}$ = 9.0 Hz, $^3J_{H\text{-}H}$ = 3.6 Hz, CH_{sugar}), 2.93-3.04 (m, 3 H, 3 CH_{sugar}), 3.24-3.28 (m, 1 H, $CH_{2\text{-}sugar}$), 3.55 (ddd, 1 H, $^2J_{H\text{-}H}$ = 11.4 Hz, $^3J_{H\text{–}H}$ = 5.4 Hz, $^3J_{H\text{-}H}$ = 1.8 Hz, CH_{sugar}), 3.82 (s, 3 H, NCH_3), 3.87 (s, 3 H, OCH_3), 3.97 (broad s, 1 H, OH), 4.35 (pseudo t, 1 H, $^3J_{H\text{-}H}$ = 5.7 Hz, CH_2-OH), 4.47 (d, 1 H, $^3J_{H\text{-}H}$ = 9.0 Hz, CH_{sugar}), 4.71-4.73 (m, 2 H, 2 OH), 5.59 (s, 1 H, CH^A), 5.64 (s, 2 H, NCH_2), 7.01 (dd, 1 H, $J_{H\text{-}H}$ = 8.4 Hz, $^4J_{H\text{-}H}$ = 1.8 Hz, CH^C), 7.05 (s, 1 H, CH^B), 7.52 (s, 2 H, 2 CH_{Im}), 7.86 (d, 1 H, $^3J_{H\text{-}H}$ = 8.4 Hz, CH^D).

$^{13}C\{^1H\}$ NMR (DMSO-d6, 125.77 MHz, 300 K): 37.5 (broad s, N*C*H$_3$), 49.9 (broad s, N*C*H$_2$), 56.0 (s, O*C*H$_3$), 61.4 (s, *C*H$_{2\text{-}sugar}$), 70.5 (s, *C*H$_{sugar}$), 77.4 (s, *C*H$_{sugar}$), 80.1 (s, *C*H$_{sugar}$), 81.1 (s, *C*H$_{sugar}$), 85.1 (s, *C*H$_{sugar}$), 101.2 (s, *C*H^B), 109.3 (broad s, *C*$_{quat}$-CH$_2$), 110.5 (s, *C*H^A), 112.4 (s, *C*H^A), 121.9 (broad s, *C*H$_{Im}$), 123.3 (broad s, *C*H$_{Im}$), 125.8 (s, *C*H^D), 151.5 (s, *C*$_{quat}$-CH^D), 154.9 (s, *C*$_{quat}$-OC(O)), 159.8 (s, *C*(O)), 162.8 (s, *C*$_{quat}$-OMe), *ca.* 171 ($C_{carbene}$ not directly observed, see below).

FT-IR (ATR, cm^{-1}): 3365, 2909, 1710, 1610, 1559, 1515, 1463, 1399, 1349, 1289, 1146, 1020, 840, 558, 179, 171.

ESI-MS (MeOH), *positive mode exact mass* for $C_{21}H_{25}N_2O_8AuSNa^+$ (685.08893): measured *m*/*z* 685.08564 $[M+Na]^+$, ESI-MS (MeOH), *negative mode exact mass* for $C_{21}H_{25}N_2O_8AuSCl^-$ (697.06802): measured *m*/*z* 697.07103 $[M+Cl]^-$.

Anal. Calc. for $C_{21}H_{25}N_2O_8AuS.NaCl$: C, 34.99, H, 3.50, N, 3.89, S, 4.45 %. Found: C, 34.38, H, 3.64, N, 4.08, S, 3.21 %.

Remark: $^{13}C\{^1H\}$ NMR signals of **4** corresponding to the carbons of the imidazole ring appeared very broad and no signal of the carbenic carbon appeared. However, by ^1H-^{13}C HMBC correlation NMR spectroscopy, we could observe a correlation spot between both the *N*-methyl and the methylene bridge signals in the ^{1}H spectrum and a signal at around 181 ppm in the ^{13}C{^{1}H} spectrum.

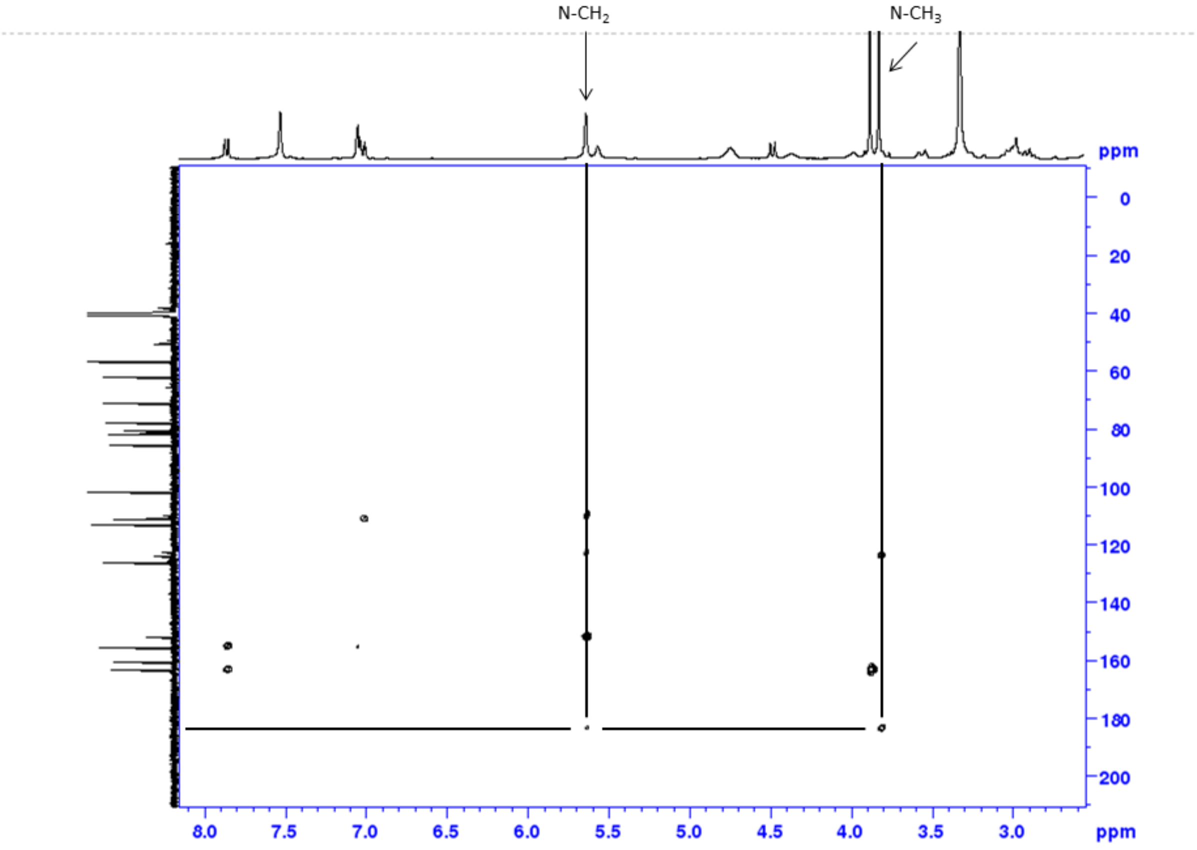


Figure S1 - ^{13}C-^{1}H HMBC NMR spectrum of **4** in DMSO

Fluorescence measurements

The steady-state fluorescence emission and excitation spectra were obtained by using a JASCO FP8560 spectrofluorometer instrument. All fluorescence spectra were corrected for instrument response. The fluorescence quantum yield (Φ_F) was calculated from equation 1.

$$\frac{\Phi_F}{\Phi_{FR}} = \frac{n^2}{n_R^2} \times \frac{\int_0^\infty I_F(\lambda_E, \lambda_F) d\lambda_F}{\int_0^\infty I_{FR}(\lambda_E, \lambda_F) d\lambda_F} \times \frac{1-10^{-A_R(\lambda_E)}}{1-10^{-A(\lambda_E)}}$$

Equation 1

Φ_F and Φ_{FR} are fluorescence quantum yields of the compound and the reference respectively. $A(\lambda_E)$ and $A_R(\lambda_E)$ are the absorbance at the excitation wavelength, and n is the refractive index of the medium. I_F and I_{FR} are fluorescent intensities of the compound and the reference respectively. The reference system used was 9,10-diphenylanthracene (ϕ = 0.955 in cyclohexane, λ_{ex} = 366 nm).[3] The data are shown in Table 1. Absorption and emission spectra of compound **1** are displayed in figure S2 (the same behaviour was observed for complexes **2**, **3** and **4**).

Table 1: Spectroscopic data of the studied compounds

Compound	*Solvent*	*λ_{abs}/nm*	*ε/$M^{-1}.cm^{-1}$*	*λ_{em}/nm*	*Φ_F(%)^{a,b}*	*Brightness (ε. Φ_F) / $M^{-1}.cm^{-1}$*
1	CH_2Cl_2	327	11144	405	2	223
2	CH_2Cl_2	324	12369	393	2	247
3	CH_2Cl_2	323	10048	391	1	100
4	H_2O	326	6955	404	1	70

[a] λ_{exc} = 361 nm ; [b] Using 9,10-diphenylanthracene as reference, Φ_F = 0.955 in cyclohexane, λ_{exc} = 366 nm. All Φ_F are corrected from refractive index.

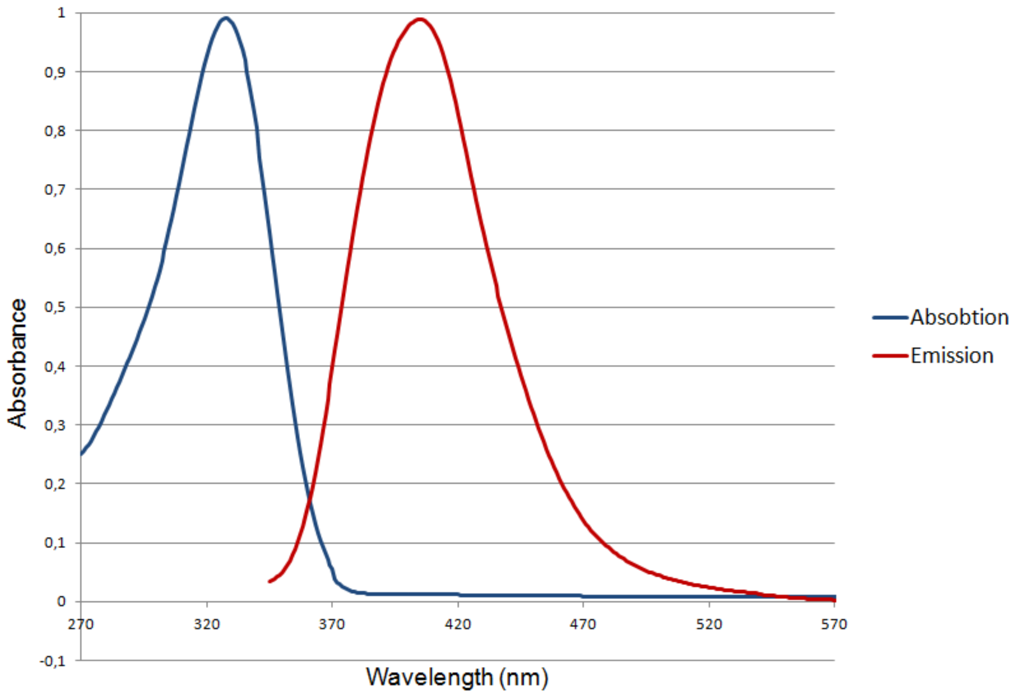


Figure S2 – Typical photophysical profile of compounds **1-4** (here absorption and emission spectra of **1** are displayed).

Cell viability assay

The human lung cancer cell line A549, human breast cancer MCF-7 cells and human ovarian cancer A2780 cells, (obtained from the European Centre of Cell Cultures ECACC, Salisbury, UK) were cultured in DMEM (A549, MCF-7) and RPMI (A2780) both containing GlutaMax-I supplemented with 10% FBS and 1% penicillin/streptomycin (all from Invitrogen), at 37°C in a humidified atmosphere of 95% of air and 5% CO_2 (Heraeus, Germany). Non-tumoral human embryonic kidney cells HEK-293T were cultivated in DMEM medium with GlutaMax-I, 10% FBS and 1% penicillin/streptomycin, incubated in the same conditions as other cell lines. For evaluation of growth inhibition, cells were seeded in 96-well plates (Costar, Integra Biosciences, Cambridge, MA) at a concentration of 10000 cells/well and grown for 24 h in complete medium. Solutions of the compounds were prepared by diluting a freshly prepared stock solution (10^{-2} M in DMSO) of the corresponding compound in aqueous media (RPMI or DMEM depending on the cell lines). The percentage of DMSO in the culture medium never exceeded 0.2%: at this concentration DMSO has no effect on the cell viability. Cisplatin (Sigma-Aldrich) stock solutions were prepared in MilliQ water. Afterwards, the intermediate dilutions of the compounds were added to the wells (200 μL) to obtain a final concentration ranging from 0 to 200 μM, and the cells were incubated for 72 h. Following 72 h drug exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the cells at a final concentration of 0.50 mg.ml^{-1} incubated for 3-4 h, then the culture medium was removed and the violet formazan dissolved in DMSO. The optical density of each well (96-well plates) was quantified in quadruplicate at 540 nm using a multi-well plate reader and the percentage of surviving cells was calculated from the ratio of absorbance between treated and untreated cells. The IC_{50} value was calculated as the concentration reducing the proliferation of the cells by 50% and is presented as a mean (± SE) of at least three independent experiments.

Estimation of enzyme activities inhibition in vitro

Highly purified cytosolic thioredoxin reductase (TrxR1) was prepared from rat liver, according to Luthman and Holmgren, (M. Luthman and A. Holmgren, Biochemistry, 1982, 21, 6628-6633) Mitochondrial thioredoxin reductase (TrxR2) was purified from isolated rat liver mitochondria following the procedure of Rigobello et al. (M. P. Rigobello and A. Bindoli, Methods Enzymol., 2010, 474, 109-122.) Thioredoxin reductases activity was determined by measuring the ability of the enzyme to directly reduce DTNB in the presence of NADPH. (M. Luthman and A. Holmgren, *Biochemistry*, 1982, **21**, 6628-6633) Aliquots of highly purified TrxR1 (30 nM) and TrxR2 (30 nM) in 0.2 M Na, K-phosphate buffer (pH 7.4), 5 mM EDTA, 0.25 mM NADPH were pre-incubated for 5 min with the Au-NHC coumarin derivatives. Afterwards, the reaction was started with 1 mM DTNB, and monitored spectrophotometrically at 412 nm for about 10 min.

Yeast glutathione reductase was obtained from Sigma (St. Louis Mo, USA) and used without further purification. Glutathione reductase activity was measured in 0.2 M Tris HCl buffer (pH 8.1), 1 mM EDTA, and 0.25 mM NADPH after 5 min pre-incubation with the gold complexes. The assay was initiated by the addition of 1 mM GSSG and followed spectrophotometrically at 340 nm.

Glutathione peroxidase activity was performed by the following procedure: (X Liu, K E Pietsch, S J Sturla, *Chem. Res. Toxicol.*, *24* (2011) 726–736) aliquots of GPx from bovine erythrocytes (0.02 U) were incubated with gold compounds in a total volume of 0.5 mL of 50 mM Hepes buffer (pH 7.0) containing 3 mM EDTA and 0.3 mM NADPH at 25°C. After 5min, 4 mM GSH and 25 nM glutathione reductase were added. After 2 min of incubation the reaction was started by the addition of 200 µM tert-butyl hydroperoxide and monitored spectrophotometrically at 340 nm as decrease of NADPH.

BIAM assay

TrxR (1 µM) pre-reduced in presence of NADPH was incubated with different concentrations of complexes for 30 min at room temperature, in 20mM Tris-HCl buffer (pH 7.4) containing 200 µM NADPH, and 1 mM EDTA. After incubation, 8 µL of the reaction mixture was removed and added to 50 µM biotinylated iodoacetamide (BIAM) in 0.1 M Hepes- Tris pH 6.0.(J. Fang and A. Holmgren *J. Am. Chem. Soc.*, 2006, *128* (6), pp 1879–1885) Samples were incubated at room temperature for additional 30 min to alkylate the remaining -SH groups in the enzyme. Then, BIAM-modified enzyme was mixed with loading buffer and the mixture was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) on a 7.5 % gel, and the separated proteins were transferred to a nitrocellulose membrane. Proteins labelled with BIAM were detected with horseradish peroxidase-conjugated streptavidin and enhanced chemiluminescence detection.

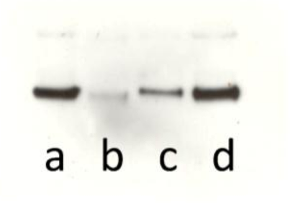
Determination of TrxR and GR activities in cell lysates

A2780 cells (1x10^6) were incubated for 48 h with 10 µM of compounds **1**-**4**, with refresh at 24 h. After incubation, cells were harvested and washed twice with ice-cold PBS. Each sample was lysed with a modified RIPA buffer. (E. Schuh et al., J. Med. Chem., 2012, 55, 5518-5528) After 40 min of incubation at 0 °C, lysates were centrifuged at 14000 x g for 5 min. The obtained supernatants were tested for enzyme activities. Aliquots (50 µg) of lysates were subjected to thioredoxin reductase determination in a final volume of 250 µl of 0.2 M Na, K-phosphate buffer (pH 7.4), 5 mM EDTA, and 2 mM DTNB. After 2 min the reaction was started with 0.3 mM NADPH. In cell lysates glutathione reductase activity was also estimated using 50 µg protein/ml as reported above.

Fluorescence microscopy

Cells (A2780) were seeded (5 x 10^5 for each sample) and grown on 8 well microscope plates, coated with Poly-L-Lysine hydrobromide (Sigma-Aldrich, P6516) with a complete medium. After 24 h, cells were incubated with various concentrations of the complexes in RPMI, without FCS for 2 h at 37 °C and 4°C. At the end of incubation, cells were rapidly washed with cold PBS and then fixed with 2% paraformaldehyde for 30 min at 4°C. For the visualization of PI, cells were permeabilized with 0.2% Triton X-100 for 20 min at 4°C and treated with 1μg/μl of PI for 10 min at room temperature. Cells were washed once with PBS and then analyzed by confocal microscopy. As preparation for visualization, the plate wells were removed and glycerol was used to cover the slide with a glass cover slip. The fluorescence was analysed using a Leica DM4000 B Automated Upright Microscope, equipped with the appropriate filters. PI was excited at 547 nm (emission wavelength 572 nm) and the compounds at 358 nm (emission wavelength 461 nm, DAPI filter). The acquired images were obtained using individual filters and a combined image, overlaying the fluorescence acquired with the two filters, was obtained using the Leica microscope software.

Figure S4 - Alkylation of TrxR1 with BIAM following treatment with gold(I) complexes. Thioredoxin reductase was incubated as indicated in the Experimental section at pH 6.0.



a= control
b= compound 3
c= compound 2
d= compound 1

Figure S4 - Thioredoxin reductase (TrxR) and Glutathione reductase (GR) activities in A2780 cell lysates. Cells were subjected to 48 h treatment in presence of 10 µM of **3**. TrxR and GR activities were performed in 50µg proteins.