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Gold(I) *N*-heterocyclic carbene complexes with an "activable" ester moiety: Possible biological applications

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ABSTRACT

While *N*-heterocyclic carbenes (NHC) are ubiquitous ligands in catalysts for organic or industrial synthesis, their potential to form transition metal complexes for medicinal applications has still to be exploited. Within this frame, new Au(I)–NHC compounds have been synthesized and structurally characterized via different methods. The solid state structure of one of these compounds was also established by X-ray crystallography. Of note, three of them bear a pentafluorophenolic ester group as a possible "activable" moiety for further functionalization, which allowed tethering an alkyl amine ligand or another Au(I)–phosphine complex featuring a pendant amine function via microwave activation. The obtained compounds have been tested for their antiproliferative effects in human ovarian cancer A2780 cells, and in non-tumorigenic human embryonic kidney HEK-293T cells, showing promising anticancer properties and a certain selectivity towards cancerous cells.

Introduction

Currently, metal-based drugs are used in clinic in a regular basis as anticancer chemotherapic agents. In fact, the platinum-based drugs cisplatin, carboplatin and oxaliplatin, are present in more than 75% of anticancer chemotherapic cocktails [1,2]. However, despite their great clinical success, these drugs present major drawbacks such as the limited spectrum of action, development of resistance, and severe side effects that narrowed their range of applicability. To overcome such drawbacks, one of the most explored strategies consisted in the replacement of platinum by other transition metals. This approach already gave promising results in the case of ruthenium, iron, gold and titanium coordination and organometallic compounds among others [3–8]. In particular, gold(I) complexes have appeared in the last decades as very potent cytotoxic agents [9–12]; the most famous example being ((2,3,4,6-tetra-*O*-acetyl-1-(thio-κS)-β-D-glucopyranosato)(triethylphosphine)gold(I)) (auranofin) already in the clinic as anti-arthritis agent (Fig. 1) [13]. As a matter of fact, gold is the most noble of the elements and it certainly holds a central place in the world of finance, art and jewelry. Nowadays, the medicinal uses of gold compounds are the subject of intense studies. Conspicuous experimental evidence has been gathered so far to suggest that the pronounced antiproliferative effects caused by gold compounds most likely arise from innovative mechanisms of action in comparison to established anticancer drugs.

Following the successful application of gold phosphine complexes as antitumor agents, Berners-Price and coworkers have pioneered the application of a variety of cationic mononuclear gold(I) *N*-heterocyclic (NHC) biscarbene complexes as potential chemotherapeutic agents (an example is reported in Fig. 1) [14]. Since then a number of Au(I)–NHC carbene compounds have been synthesized and characterized for their biological properties, and the studies on this family of organometallic compounds have been reviewed on a regular basis in the past few years [15–17]. Indeed, Au(I)–NHC compounds present a variety of different derivatization possibilities associated to the possible presence of an ancillary ligand coordinated to gold, in addition to the Au–NHC bond (e.g. phosphines, thiols, as well as a second NHC ligand, as depicted in Fig. 1) [18,19]. Contrary to platinum derivatives, it has been shown

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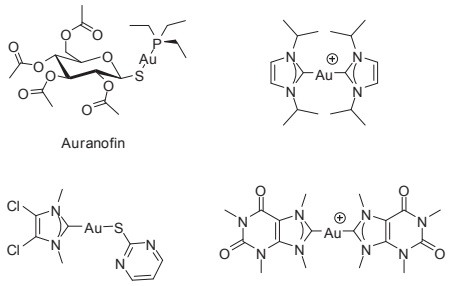


Fig. 1. Examples of currently studied Au(I) compounds with cytotoxic properties.

that several Au(I)–NHC complexes induce apoptosis via targeting mitochondria, but also through the interaction with different proteins/enzymes (e.g. thioredoxin reductase) [20,21].

Of note, physicochemical and biological properties of metal complexes have been improved through various strategies, among which the concept of *multinuclearity*. Indeed, a number of platinum [22], ruthenium and gold-based homo- or heteropolynuclear complexes, either bi- or polymetallic, have been developed by us and others and biologically tested [23–28].

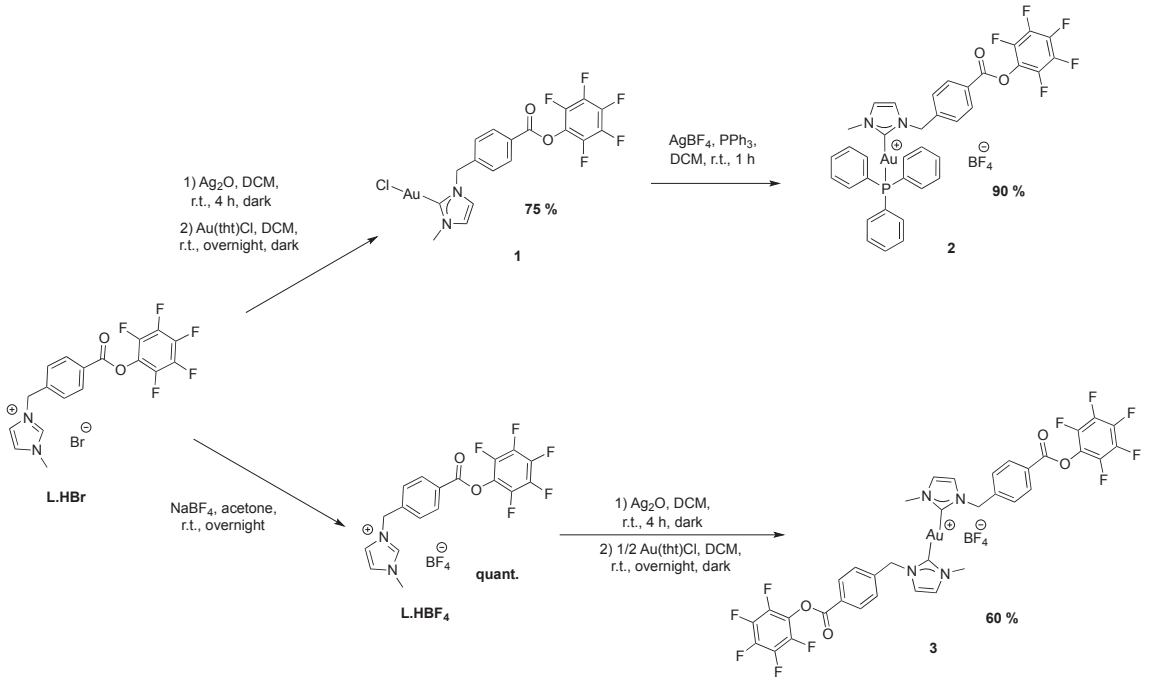
In this context, we describe here the synthesis and characterization of new Au(I)–NHC compounds among which three of them bear a pentafluorophenolic ester group as a possible "activable" moiety for further functionalization. In order to prove this concept, one of the Au(I)–NHC complexes was tethered *a posteriori* to an amine ligand or to another Au(I)–phosphine complex bearing a pendant amine function using microwave activation. In the latter case, a dinuclear Au(I) complex was obtained. The success of this strategy opens towards a quick route to a great diversity of structures allowing further exploration of synergies. Assessment of the antiproliferative properties of the new compounds in human ovarian cancer cells (A2780) and in a model of healthy cell (human embryonic kidney HEK-293T) is also reported demonstrating the suitability of these scaffolds for biological applications.

Results and discussion

Synthesis and structural characterization

In order to easily derivatize a NHC-ligand allowing further efficient coupling with an amine moiety, we selected the procedure previously described by Metzler-Nolte et al. in the case of ruthenium and rhodium functionalized complexes [29]. Thus, the "proligand" 1-methyl-3-(4-(perfluorophenoxycarbonyl)benzyl)imidazolium bromide (**L.HBr**, Scheme 1) was obtained in two steps starting from commercial 4-bromomethylbenzoic acid and pentafluorophenol, the corresponding ester being then reacted with one equivalent of 1-methylimidazole. The Au(I) complex **1** was obtained in 75% yield by a transmetallation reaction from the *in situ* formed Ag(I) complex according to the general method described by Lin et al., and subsequent transfer of the NHC ligand to the gold(I) precursor [AuCl(tht)] [30]. The formation of **1** was assessed by ^{1}H NMR spectroscopy where the disappearance of the singlet of the imidazolium proton at 9.37 ppm was noticed. The shift of the signal of the corresponding carbon in $^{13}C\{^{1}H\}$ NMR from 139.0 ppm in the imidazolium salt to 172.4 ppm confirmed the formation of the carbene complex [18]. An unsymmetrical cationic NHC/phosphine complex (**2**) was also synthesized in 90% yield by reacting **1** with triphenylphosphine in the presence of silver tertrafluoroborate as a chloride abstractor (Scheme 1). The coordination of the triphenylphosphine ligand was assessed by $^{31}P\{^{1}H\}$ NMR, the phosphorus atom giving a broad singlet at 40.7 ppm characteristic of cationic Au(I)–phosphine complexes [31]. Additionally, in $^{13}C\{^{1}H\}$ NMR the signal corresponding to the carbene was shifted from 172.4 ppm to 186.4 ppm, as expected for coordination to a cationic Au(I) center. Moreover, crystals of **2** suitable for X-ray diffraction have been obtained by slow evaporation of a dichloromethane/pentane (1/4) solution. The crystal structure of this complex was solved and shows the typical linear two-coordinated geometry of Au(I) cation (Fig. 2).

In order to obtain a cationic bis-NHC metal derivative, we used a classical method [32] starting from salts containing a non-halide



Scheme 1. Synthesis of the different Au(I)–NHC complexes bearing the pentafluorophenol ester moiety.

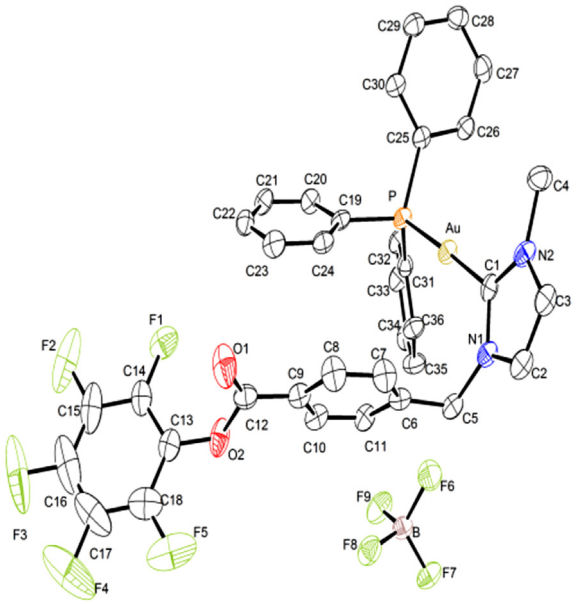


Fig. 2. Ortep view of compound **2**. Selected bond distances (Å) and angle (°): Au–P 2.2788(13); Au–C(1) 2.034(5); P–Au–C1 177.70(16).

anion, which is then reacted with silver oxide yielding the silver bis-NHC complex, acting as a halide abstractor and ligand transfer agent. **L.HBr** was thus reacted quantitatively with an excess of sodium tetrafluoroborate to lead to the tetrafluoroborate analogue **L.HBF$_4$**. The latter was reacted with silver(I) oxide and half an equivalent of [AuCl(tht)] to afford the cationic bis-NHC complex **3** in 60% yield (Scheme 1), whose formation was confirmed by ^{13}C{^{1}H} NMR spectroscopy, where the carbene gave a signal at 184.4 ppm characteristic of this type of cationic Au(I) complexes [33].

Afterward, with the aim of obtaining a bimetallic complex, we imagined the grafting of a Au(I)–phosphine complex, bearing a pendant amine function, directly to the Au(I)–NHC complex through the activated pentafluorophenol ester. However, in order to setup the reaction conditions, 2-phenylethylamine was used as a test compound. Therefore, **1** was reacted with one equivalent of the amine in acetonitrile under microwave irradiation and the best reaction conditions were found to be heating at 80 °C for 30 min (Scheme 2); higher temperatures lead to partial decomposition of the gold complex even when using a shorter reaction time. It is also worth noting that no additional base was used to prevent any degradation of the carbene. The coupling product (**4**) was simply purified by precipitation and obtained in very good yield (87%).

Applying these optimized reaction conditions, we then reacted complex **1** with the 2-aminoethyldiphenylphophine gold(I) chloride complex and obtained the desired bimetallic complex (**5**) in very good yield (86%) (Scheme 2).

Table 1
Cell Viability IC$_{50}$ Values of compounds **1**–**5** in human ovarian carcinoma cell line A2780 or in human embryonic kidney cells (HEK-293T) after 72 h incubation.

Compound	IC$_{50}$ (μM)	
	A2780	HEK-293T
1	53.0 ± 2.4	122.0 ± 6.2
2	5.2 ± 0.7	11.2 ± 0.5
3	19.7 ± 2.0	41.5 ± 1.4
4	–	–
5	2.2 ± 0.4	6.2 ± 0.7
Auranofin	1.2 ± 0.5	1.7 ± 0.3

In both reactions leading to **4** and **5**, ^{19}F{^{1}H} NMR on the resulting complex shows the disappearance of the signals of pentafluorophenol. Moreover, the ^{1}H spectrum displays a downshift of all signals of the NHC moiety when compared to the spectrum of the starting complex **1**, an upfield shift of the signal of the N–CH$_2$ in the ethylenic linker from 2.9 ppm to 3.5 ppm and the appearance of a broad singlet between 6.5 and 7.0 ppm corresponding of the NH. Additionally, in ^{13}C{^{1}H} spectroscopy, the signal of the C(O) is shifted from 161 ppm in the activated ester to 166 ppm in the amide confirming the reaction of the perfluorinated ester moiety. Finally, the comparison of the IR spectra shows a shift of the vibration band of the carbonyl group from 1715 cm^{-1} to 1640 cm^{-1}. Both complexes were also characterized by high-resolution mass spectrometry and elemental analysis, all these data being in agreement with the formation of the amide linkage.

In an attempt to enlarge the scope of polymetallic complexes, both cationic complexes **2** and **3** were reacted with one or two equivalents of 2-aminoethyldiphenylphosphine gold(I) chloride respectively. However, even though the coupling through the activated ester occurred, a redistribution of the ligands was observed, yielding to a mixture of products in both cases.

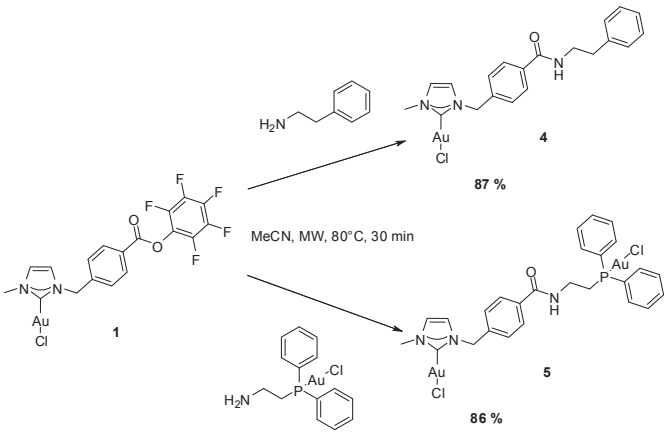
Antiproliferative activities

The new compounds were screened for their antiproliferative properties in human ovarian cancer cell lines sensitive (A2780) using the classical MTT assay (see Experimental section for details). In addition, in order to evaluate the compounds' selectivity for cancerous compared to healthy cells, the complexes were also tested in human embryonic kidney HEK-293T cells.

A dose-dependent inhibition of cell growth was observed in all cell lines with IC$_{50}$ values ranging from ca. 2 to 122 μM after 72 h incubation as depicted in Table 1, the most effective compound being the dinuclear Au(I) derivative **5**. Differently from the reference gold(I) complex auranofin, which is very cytotoxic in all the tested cell lines, the new compounds display certain selective antiproliferative activities, being at least 2-fold less cytotoxic in the non-tumorgenic HEK-293T cells.

Conclusion

We have reported here the synthesis of five neutral or cationic Au(I)–NHC complexes in good yields. Among them, three present the pentafluorophenolic ester functionality as a useful "activable" ester for further functionalization, and chlorido, NHC or



Scheme 2. Synthesis of the different Au(I)–NHC complexes through reaction of the activated ester moiety.

triphenylphosphine as a second ligand. A stoichiometric base-free method for grafting an alkyl amine on gold(I) carbenes in high yields using microwave irradiation is also reported, which allowed the synthesis of two new NHC complexes: a monometallic Au(I)–NHC complex and a homobimetallic Au(I) complex with a NHC–phosphine bridging ligand. Each derivative has been fully characterized by classical methods (^{1}H, ^{13}C{^{1}H}, ^{19}F{^{1}H} and ^{31}P{^{1}H} NMR spectroscopy, IR spectroscopy, high resolution mass spectrometry and elemental analysis). The structure of compound **2** has also been solved by X-ray diffraction.

Although more biological studies should be undertaken to further investigate the mechanisms of action of this new series of compounds, preliminary *in vitro* antiproliferative assays have revealed the promising cytotoxic properties of the compounds in cancer cells with respect to non-tumorigenic ones. Overall, we are confident that these results will allow new possibilities of fine-tuning of the chemico-physical properties of organometallic Au(I)–NHC scaffolds for biological applications.

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 precursors [AuCl(tht)] [34] and [AuCl($PPh_2(CH_2)_2NH_2$)] have been synthesized according to literature procedures [28,30]. 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 and NMR. Exact mass of the synthesized complexes were obtained on a Thermo LTQ Orbitrap XL. ^{1}H-(300.13, 500.13 or 600.23 MHz), ^{13}C-(125.77 or 150.90 MHz) and ^{19}F-(282.38 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) and $CFCl_3$ (^{19}F), using the residual protonated solvent (^{1}H) or the deuterated solvent (^{13}C) as internal standards. Alternatively, 85% H_3PO_4 (^{31}P) and $CFCl_3$ as an external standard (^{19}F). Infrared spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer (Golden Gate ATR). X-ray diffraction data for **3** were collected on a Nonius Kappa CCD at 115 K. Microwave reactions were carried on in an Anton Paar Monowave 300 apparatus.

Synthesis

*1-Methyl-3-{4-[(perfluorophenoxy)carbonyl]benzyl}imidazolium tetrafluoroborate (**L.HBF$_4$**)*

A round-bottom flask was filled with 279 mg of **2** (0.60 mmol) and 200 mg of $NaBF_4$ (1.81 mmol) in 20 mL of acetone. The reaction was maintained at room temperature overnight. After removing of acetone under vacuum, the obtained white solid was partially dissolved in 30 mL of dichloromethane, and filtrated through paper to give a colorless solution. Dichloromethane was then evaporated under vacuum to lead to the pure product (98% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 3.95 (s, 3H, Me) 5.70 (s, 2H, CH_2), 7.74 (d, 2H, $^{3}J_{H-H}$ = 8.5 Hz, 2 CH_{Ph}), 7.84 (d, 1H, $^{3}J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 7.90 (d, 1H, $^{3}J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 8.28 (d, 2H, $^{3}J_{H-H}$ = 8.5 Hz, 2 CH_{Ph}), 9.37 (s, 1H, $NCHN^{+}$). ^{19}F{^{1}H} NMR ($CDCl_3$, 282.38 MHz): −150.4 (m, BF_4), −153.6 (dt, 2F, $^{3}J_{F-F}$ = 25.4 Hz, $^{4}J_{F-F}$ = 2.8 Hz, F_{ortho}), −157.5 (t, 1F, $^{3}J_{F-F}$ = 22.6 Hz, F_{para}), −162.4 (m, 2F, F_{meta}). FT-IR (ATR, cm^{-1}): 3166, 1759, 1613, 1574, 1518, 1473, 1451, 1422, 1248, 1170, 1049. Anal. Calc. for $C_{18}H_{11}F_9N_2O_2B$: C, 45.99, H, 2.57, N, 5.96%. Found: C, 45.51, H, 2.39, N, 6.19.

*Chlorido(1-methyl-3-{4-[(perfluorophenoxy)carbonyl]benzyl} imidazol-2-ylidene)gold(I) (**1**)*

A round-bottom flask was filled with 146 mg of **L.HBr** (0.31 mmol), 60 mg of Ag_2O (0.26 mmol), molecular sieves 4 Å (MS 4 Å) (200 mg) in 16 mL of dichloromethane. The mixture was reacted for 4 h at room temperature in the dark. Afterward, 101 mg of [AuCl(tht)] (0.31 mmol) dissolved into 5 mL of dichloromethane was added dropwise to the previous mixture and reacted overnight at room temperature in the dark. After a filtration over celite, the volatiles were removed under vacuum to give the product as a white solid. (75% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): 3.88 (s, 3H, Me), 5.49 (s, 2H, CH_2), 6.93 (d, 1H, $^{3}J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 7.00 (d, 1H, $^{3}J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 7.47 (d, 2H, $^{3}J_{H-H}$ = 8.5 Hz, 2 CH_{Ph}), 8.18 (d, 2H, $^{3}J_{H-H}$ = 8.5 Hz, 2 CH_{Ph}). ^{13}C{^{1}H} NMR ($CDCl_3$, 75.48 MHz): 38.4 (*Me*), 54.5 (CH_2), 120.5 ($CH_{im.}$), 122.7 ($CH_{im.}$), 127.4 (C_1), 128.3 (CH_{Ph}), 131.5 (CH_{Ph}), 136.3 ($C_{perfluoro}$), 139.7 ($C_{perfluoro}$), 141.4 ($C_{perfluoro}$), 141.9 (C_4), 143.0 ($C_{perfluoro}$), 161,9 (*C*=O), 172.4 ($C_{carbene}$). ^{19}F{^{1}H} NMR (DMSO-d_6, 282.38 MHz): −152.5 (dt, 2F, $^{3}J_{F-F}$ = 25.4 Hz, $^{4}J_{F-F}$ = 2.8 Hz, F_{ortho}), −157.7 (t, 1F, $^{3}J_{F-F}$ = 22.6 Hz, F_{para}), −162.2 (m, 2F, F_{meta}). FT-IR (ATR, cm^{-1}): 3094.3, 1753.4, 1610.7, 1516.4, 1466.9, 1414.0. ESI–MS (DMSO/MeOH), *positive mode exact mass* for $C_{18}H_{11}AuClF_5N_2O_2$ + Na (636.99869): measured *m/z* 636.99561 $[M + Na]^{+}$. Anal. Calc. for $C_{18}H_{11}AuClF_5N_2O_2$: C, 35.17, H, 1.80, N, 4.56%. Found: C, 34.58, H, 1.73, N, 4.47%.

*(1-Methyl-3-{4-[(perfluorophenoxy)carbonyl]benzyl}imidazol-2-ylidene)(triphenylphosphine)gold(I) tetrafluoroborate (**2**)*

A Schlenk tube was filled with 40 mg of **1** (0.065 mmol) and 18.7 mg of PPh_3 (0.072 mmol) which were dissolved into 4 mL of distilled dichloromethane. $AgBF_4$ in solution in methanol was added dropwise at room temperature. The reaction was maintained for one hour during which a white precipitate appeared and became gray after some minutes. After a filtration over fritte with celite, the volatiles were removed under vacuum to afford a colorless oil which gave rise to a white precipitate after being washed with Et_2O. The white precipitate was washed with Et_2O to give the pure product. (90% yield). Crystals suitable for X-ray diffraction have been obtained by slow evaporation of a solution in dichloromethane/pentane (1/4). ^{1}H NMR ($CDCl_3$, 500 MHz): 3.99 (s, 3H, Me), 5.54 (s, 2H, CH_2), 7.31 (d, 1H, $^{3}J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 7.37 (m, 8H, 6 $H_{ortho/P}$ + 2 CH_{Ph}), 7.48 (d, 1H, $^{3}J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 7.50 (pseudo-t, 6H, $^{3}J_{H-H}$ = 7.3 Hz, 6 $H_{meta/P}$), 7.57 (t, 3H, $^{3}J_{H-H}$ = 7.5 Hz, 3 $H_{para/P}$), 7.98 (d, 2H, $^{3}J_{H-H}$ = 8.5 Hz, 2 CH_{Ph}). ^{13}C{^{1}H} NMR ($CDCl_3$, 125.76 MHz): 38.7 (*Me*), 54.3 (CH_2), 123.6 ($CH_{im.}$), 123.8 ($CH_{im.}$), 126.7 (C_1), 128.0 ($C_{ipsoPPh3}$, $^{1}J_{P-C}$ = 56.6 Hz), 128.2 ($CH_{paraPPh3}$), 129.7 ($C_{métaPPh3}$, $^{3}J_{P-C}$ = 11.3 Hz), 131.3 (CH_{Ph}), 132.4 (CH_{Ph}), 133.9 ($C_{orthoPPh3}$, $^{2}J_{P-C}$ = 13.8 Hz), 137.1 ($C_{perfluo}$), 139.1 ($C_{perfluo}$), 140.5 ($C_{perfluo}$), 142.5 ($C_{perfluo}$), 143.8 (C_4), 162,2 (*C*=O), 186.4 ($C_{carbene}$). ^{19}F{^{1}H} NMR ($CDCl_3$, 470.59 MHz): −152.6 (dt, 2F, $^{3}J_{F-F}$ = 25.4 Hz, $^{4}J_{F-F}$ = 2.8 Hz, F_{ortho}), −152.9 (m, BF_4), −157.6 (t, 1F, $^{3}J_{F-F}$ = 22.6 Hz, F_{para}), −162.0 (m, 2F, F_{meta}). ^{31}P{^{1}H} NMR ($CDCl_3$, 202.46 MHz): 40.7 (s). FT-IR (ATR, cm^{-1}): 1758.2, 1612.8, 1520.0, 1475.3, 1437.5. ESI–MS (DMSO/MeOH), *positive mode exact mass* for $[C_{36}H_{26}AuF_5N_2O_2P]^{+}$ (841.13121): measured *m/z* 841.12857 $[M - BF_4]^{+}$. Anal. Calc. for $C_{36}H_{26}AuF_5N_2O_2PBF_4$: C, 46.58, H, 2.82, N, 3.02%. Found: C, 46.57, H, 2.92, N, 3.04%.

*Bis(1-methyl-3-{4-[(perfluorophenoxy)carbonyl]benzyl}imidazol-2-ylidene)gold(I) tetrafluoroborate (**3**)*

A round-bottom flask was filled with 84.4 mg of **L.HBF$_4$** (0.18 mmol), 33 mg of Ag_2O (0.14 mmol), and molecular sieves 4 Å (MS 4 Å) (100 mg) in 10 mL of dichloromethane. The mixture was

reacted for 4 h at room temperature in the dark. Then 31 mg of [AuCl(tht)] (0.09 mmol) dissolved into 3 mL of dichloromethane were added dropwise to the previous mixture and reacted overnight at room temperature in the dark. After a filtration over fritte with celite, the volatiles were removed under vacuum to give the product as a white solid. (60% yield). ^{1}H NMR (DMSO-d_6, 300 MHz): 3.83 (s, 3H, Me), 5.53 (s, 2H, CH_2), 7.44 (d, 2H, $^3J_{H-H}$ = 8.5 Hz, 2 CH_{Ph}), 7.57 (d, 1H, $^3J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 7.65 (d, 1H, $^3J_{H-H}$ = 1.8 Hz, $CH_{im.}$), 8.06 (d, 2H, $^3J_{H-H}$ = 8.5 Hz, 2 CH_{Ph}). ^{13}C{^{1}H} NMR (DMSO-d_6, 125.77 MHz): 38.6 (*Me*), 53.9 (CH_2), 123.7 ($CH_{im.}$), 124.9 ($CH_{im.}$), 126.4 (C_1), 129.1 (CH_{Ph}), 131.8 (CH_{Ph}), 145.6 (C_4), 162.6 (C=O), 184.4 ($C_{carbene}$). ^{19}F{^{1}H} NMR (DMSO-d_6, 282.38 MHz): −148.34 (d, BF_4), −153.6 (d, 4F, $^3J_{F-F}$ = 25.4 Hz, F_{ortho}), −157.7 (t, 1F, $^3J_{F-F}$ = 22.6 Hz, F_{para}), −162.6 (m, 2F, F_{meta}). FT-IR (ATR, cm^{-1}): 1759.7, 1615.9, 1521.4, 1476.1, 1439.5. ESI–MS (DMSO/MeOH), *positive mode exact mass* for $[C_{36}H_{22}AuF_{10}N_4O_4]^+$ (961.11414): measured *m/z* 961.11676 $[M - BF_4]^+$. Anal. Calc. for $C_{36}H_{22}AuF_{14}N_4O_0B$: C, 41.24, H, 2.12, N, 5.34%. Found: C, 41.12, H, 2.11, N, 5.45%.

General procedure for the microwave-based coupling reactions

A microwave 10 mL-tube was charged with **1** and **H_2N–CH_2–R** (1 eq.) dissolved in distillated acetonitrile. The mixture was reacted in microwave oven (quick heating from r.t. to 80 °C, 850 W, stirring at 600 rpm) at 80 °C (temperature checked by IR probe) for 30 min (50 W, stirring at 600 rpm). After evaporation of the acetonitrile, the product was redissolved in dichloromethane and filtrated through celite. After partial removal of dichloromethane and addition of a large amount of pentane, the obtained precipitate was filtrated and dry under vacuum to give the pure product.

*Chlorido{1-methyl-3-[4-(2-phenylethylcarbamoyl)benzyl]imidazol-2-ylidene}gold(I) (**4**)*

1 (50 mg, 0.081 mmol) was dissolved in the tube into acetonitrile (4 mL) and 2-phenylethylamine (0.081 mmol, 10 μL) was added dropwise.

Product as a light yellow powder (38.9 mg, 87% yield). ^{1}H NMR ($CDCl_3$, 298 K, 300.13 MHz): 2.93 (t, 2H, $^3J_{H-H}$ = 6.9 Hz, CH_2–Ph), 3.71 (pseudo-q, 2H, $^3J_{H-H}$ = 6.9 Hz, CH_2–NH), 3.86 (s, 3H, N–Me), 5.37 (s, 2H, N–CH_2), 6.19 (broad s, 1H, NH), 6.88 (d, 1H, $^3J_{H-H}$ = 1.8 Hz, CH_{Im}), 6.94 (d, 1H, $^3J_{H-H}$ = 1.8 Hz, CH_{Im}), 7.23 (m, 3H, 3 CH_{Ph}), 7.32 (m, 4H, 2 $CH_{p\text{-}C6H4}$ + 2 CH_{Ph}), 7.66 (d, 2H, $^3J_{H-H}$ = 8.1 Hz, 2 $CH_{p\text{-}C6H4}$). {^{1}H}^{13}C NMR ($CDCl_3$, 300 K, 125.77 MHz): 35.6 (s, CH_2–Ph), 38.4 (s, N–CH_3), 41.2 (s, CH_2–NH), 54.6 (s, CH_2–N), 120.5 (s, CH_{Im}), 122.5 (s, CH_{Im}), 126.7 (s, CH_{arom}), 127.7 (s, CH_{arom}), 128.1 (s, CH_{arom}), 128.8 (s, CH_{arom}), 135.2 (s, C_{quat}–C(O)), 138.3 (s, C_{quat}–CH_2), 138.8 (s, $C_{quat\text{-}Ph}$), 166.8 (s, C=O), 172.0 (s, $C_{carbene}$). FT-IR (ATR, cm^{-1}): 3349, 3109, 2934, 1640, 1535, 1500, 1464, 1408, 1307, 1236, 1191. ESI–MS ($CDCl_3$/MeOH), *positive mode exact mass* for $[C_{20}H_{22}AuClN_3O]^+$ (552.11114): measured *m/z* 552.10972 $[M + H]^+$, *positive mode exact mass* for $[C_{20}H_{21}AuClN_3ONa]^+$ (574.09309): measured *m/z* 574.09147 $[M + Na]^+$. Anal. Calc. for $C_{20}H_{22}AuN_3O$: C, 43.45, H, 3.84, N, 7.60%. Found: C, 43.58, H, 2.72, N, 7.71%.

*μ-(1-Methyl-3-{4-[(2-diphenylphosphinoethyl)-κP-carbamoyl] benzyl}imidazol-2-ylidene)-κC-bis(chlorido)gold(I) (**5**)*

1 (67 mg, 0.11 mmol) and (2-aminoethyldiphenylphosphine-κ*P*) chloridogold(I) (0.11 mmol, 50 mg) were dissolved in the tube into acetonitrile (5 mL). The product was recovered as a pale yellow powder (85 mg, 86% yield). ^{1}H NMR ($CDCl_3$, 298 K, 300.13 MHz): 2.90 (m, 2H, CH_2–P), 3.74 (m, 2H, CH_2–NH), 3.85 (s, 3H, N–Me), 5.36 (s, 2H, CH_2–N), 6.90 (broad s, 2H, NH + CH_{Im}), 6.96 (s, 1H, CH_{Im}), 7.28 (d, 2H, $^3J_{H-H}$ = 8.1 Hz, 2 $CH_{p\text{-}C6H4}$), 7.42–7.52 (m, 6H, 6 CH_{Ph}), 7.66–7.69 (m, 6H, 2 $CH_{p\text{-}C6H4}$ + 4 CH_{Ph}). {^{1}H}^{13}C NMR ($CDCl_3$, 300 K, 125.77 MHz): 28.2 (d, $^1J_{P-C}$ = 37.7 Hz, CH_2–P), 36.6 (d, $^2J_{P-C}$ = 5.0 Hz, CH_2–NH), 38.4 (s, N–CH_3), 54.5 (s, N–CH_2), 120.7 (s, CH_{Im}), 122.5 (s, CH_{Im}), 127.8 (s, $CH_{p\text{-}C6H4}$), 127.9 (s, $CH_{p\text{-}C6H4}$), 128.7 (d, $^1J_{P-C}$ = 61.6 Hz, C_{quat}–P), 129.4 (d, $^2J_{P-C}$ = 11.3 Hz, $CH_{ortho\text{-}Ph}$), 132.3 (d, $^4J_{P-C}$ = 1.3 Hz, $CH_{para\text{-}Ph}$), 133.2 (d, $^3J_{P-C}$ = 13.8 Hz, $CH_{ortho\text{-}Ph}$), 133.9 (s, C_{quat}–CH_2–N), 138.7 (s, C_{quat}–C(O)), 167.0 (s, C(O)), 171.8 (s, $C_{carbene}$). {^{1}H}^{31}P NMR ($CDCl_3$, 300 K, 202.45 MHz): 24.2 (broad s, –CH_2–*P*Ph_2–AuCl). FT-IR (ATR, cm^{-1}): 3345, 3127, 3054, 2992, 2925, 1648, 1532, 1500, 1466, 1435, 1406, 1308, 1283, 1233, 1187, 1104. ESI–MS (H_2O/MeOH), *positive mode exact mass* for $[C_{26}H_{26}Au_2Cl_2N_3OPNa]^+$ (914.04138): measured *m/z* 914.03946 $[M + Na]^+$. Anal. Calc. for $C_{26}H_{26}Au_2Cl_2N_3OP$: C, 35.00, H, 2.94, N, 4.71%. Found: C, 34.90, H, 2.42, N, 4.93%.

X-ray crystallography

Crystals of **2** were obtained by slow evaporation of a dichloromethane/pentane (1/4) solution.

Intensity data were collected on a Bruker APEX II at 115 K. The structure was solved by direct methods (SIR92) [35] and refined with full-matrix least-squares methods based on F^2 (SHELXL-97) [36] with the aid of the WINGX program [37]. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were included in their calculated positions and refined with a riding model. Crystallographic data are reported in Table 2. CCDC reference is 981279.

Antiproliferative assay

The human ovarian cancer cell line A2780 was obtained from the European Centre of Cell Cultures ECACC (Salisbury, UK) and were cultured respectively in RPMI medium containing GlutaMaxI 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 kindly provided by Dr. Maria Pia Rigobello (CNRS, Padova, Italy) and were cultivated in DMEM medium, added with GlutaMaxI (containing 10% FBS and 1% penicillin/streptomycin (all from Invitrogen)) and incubated at 37 °C and 5% CO_2. For evaluation of growth inhibition, cells were seeded in 96-well plates (Costar, Integra Biosciences, Cambridge, MA) at a concentration of 15,000 cells/well and grown for 24 h in complete

Table 2
Crystal data and structure refinement for **2**.

Empirical formula	C_{36} $H_{26}AuBF_9N_2O_2P$
Formula weight	928.33
Temperature	115(2) K
Wavelength	0.71073 Å
Crystal system, space group	Triclinic, P −1
Unit cell dimensions	a = 9.9996(5) Å α = 102.500(3)°
	b = 11.5364(6) Å β = 95.586(2)°
	c = 17.5029(10) Å γ = 100.972(2)
Volume	1914.91(18) Å^3
Z, calculated density	2, 1.610 Mg/m^3
Absorption coefficient	3.960 mm^{-1}
F(000)	904
Crystal size	0.2 × 0.2 × 0.2 mm
θ range for data collection	1.952–27.461°
Limiting indices	$-11 \le h \le 11$, $-14 \le k \le 14$, $-20 \le l \le 22$
Reflections collected/unique	11,851/7768 [*R*(int) = 0.0297]
Completeness to theta = 25.242	98.1%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	7768/0/470
Goodness-of-fit on *F*2	1.139
Final *R* indices [$I > 2\sigma(I)$]	*R*1 = 0.0399, *wR*2 = 0.0826
R indices (all data)	*R*1 = 0.0486, *wR*2 = 0.0882
Largest diff. peak and hole	1.082 and −0.739 e Å^{-3}

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 for the A2780 and HEK-293T, respectively). Afterward, the intermediate dilutions of the compounds were added to the wells (100 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 0.25 mg ml^{-1} incubated for 2 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 three times in tetraplicates at 540 nm using a multi-well plate reader, and the percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC_{50} value was calculated as the concentration reducing the proliferation of the cells by 50% and it is presented as a mean (±SE) of at least three independent experiments.

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References

[1] F. Muggia, N. Farrell, Crit. Rev. Oncol. Hematol. 53 (2005) 1–2.
[2] K.R. Barnes, S.J. Lippard, Met. Ions Biol. Syst. 42 (2004) 143–177.
[3] C.G. Hartinger, P.J. Dyson, Chem. Soc. Rev. 38 (2009) 391–401.
[4] A. Vessieres, S. Top, W. Beck, E. Hillard, G. Jaouen, Dalton Trans. (2006) 529–541.
[5] S. Komeda, A. Casini, A. Curr, Top. Med. Chem. 12 (2012) 219–235.
[6] A.F.A. Peacock, P.J. Sadler, Chem. Asian J. 3 (2008) 1890–1899.
[7] J. Claffey, M. Hogan, H. Müller-Bunz, C. Pampillón, M. Tacke, ChemMedChem 3 (2008) 729–731.
[8] G. Gasser, I. Ott, N. Metzler-Nolte, J. Med. Chem. 54 (2011) 3–25.
[9] E.R. Tiekink, Inflammopharmacology 16 (2008) 138–142.
[10] S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, L. Messori, Med. Res. Rev. 30 (2010) 550–580.
[11] I. Ott, Coord. Chem. Rev. 253 (2009) 1670–1681.
[12] B. Bertrand, A. Casini, Dalton Trans. (2014), http://dx.doi.org/10.1039/C3DT52524D.
[13] I.S. Kim, J.Y. Jin, I.H. Lee, S.J. Park, Br. J. Pharmacol. 142 (2004) 749–755.
[14] P.J. Barnard, M.V. Baker, S.J. Berners-Price, D.A. Day, J. Inorg. Biochem. 98 (2004) 1642–1647.
[15] W.K. Liu, R. Gust, Chem. Soc. Rev. 42 (2013) 755–773.
[16] A. Gautier, F. Cisnetti, Metallomics 4 (2012) 23–32.
[17] L. Oehninger, R. Rubbiani, I. Ott, Dalton Trans. 42 (2013) 3269–3284.
[18] W. Liu, K. Bensdorf, M. Proetto, U. Abram, A. Hagenbach, R. Gust, J. Med. Chem. 54 (2011) 8605–8615.
[19] R. Rubbiani, E. Schuh, A. Meyer, J. Lemke, J. Wimberg, N. Metzler-Nolte, F. Meyer, F. Mohr, I. Ott, MedChemComm 4 (2013) 942–948.
[20] A. Bindoli, M.P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori, Coord. Chem. Rev. 253 (2009) 1692–1707.
[21] A. de Almeida, B.L. Oliveira, J.D.G. Correia, G. Soveral, A. Casini, Coord. Chem. Rev. 257 (2013) 2689–2704.
[22] N. Farrell, Met. Ions Biol. Syst. 42 (2004) 251–296.
[23] C. Gabbiani, A. Casini, G. Kelter, F. Cocco, M.A. Cinellu, H.H. Fiebig, L. Messori, Metallomics 3 (2011) 1318–1323.
[24] M. Wenzel, B. Bertrand, M.-J. Heymin-Ondel, V. Comte, J.A. Harvey, P. Richard, M. Groessl, O. Zava, H. Amrouche, P.D. Harvey, P. Le Gendre, P.J. Dyson, M. Picquet, A. Casini, Inorg. Chem. 50 (2011) 9472–9480.
[25] F. Pelletier, V. Comte, A. Massard, M. Wenzel, S. Toulot, P. Richard, M. Picquet, P. Le Gendre, O. Zava, F. Edafe, A. Casini, P.J. Dyson, J. Med. Chem. 53 (2010) 6923–6933.
[26] N. Lease, V. Vasilevski, M. Carreira, A. de Almeida, M. Sanaú, P. Hirva, A. Casini, M. Contel, J. Med. Chem. 56 (2013) 5806–5818.
[27] E. Vergara, E. Cerrada, A. Casini, O. Zava, M. Laguna, P.J. Dyson, Organometallics 29 (2010) 2596–2603.
[28] E. García-Moreno, S. Gascón, M.J. Rodriguez-Yoldi, E. Cerrada, M. Laguna, Organometallics 32 (2013) 3710–3720.
[29] J. Lemke, N. Metzler-Nolte, Eur. J. Inorg. Chem. 21 (2008) 3359–3366.
[30] H.M.J. Wang, I.J.B. Lin, Organometallics 17 (1998) 972–975.
[31] R. Rubbiani, S. Can, I. Kitanovic, H. Alborzinia, M. Stefanopoulou, M. Kokoschka, S. Monchgesang, W.S. Sheldrick, S. Wolfl, I. Ott, J. Med. Chem. 54 (2011) 8646–8657.
[32] I.J.B. Lin, C.S. Vasam, Can. J. Chem. 83 (2005) 812–825.
[33] W. Liu, K. Bensdorf, M. Proetto, A. Hagenbach, U. Abram, R. Gust, J. Med. Chem. 55 (2012) 3713–3724.
[34] R. Uson, A. Laguna, M. Laguna, D.A. Briggs, H.H. Murray, J.J.P. Fackler, Inorg. Synth. 26 (1989) 85–91.
[35] A. Altomare, M.C. Burla, M. Camalli, G.L. Cascarano, C. Giacovazzo, A. Guagliardi, A.G.G. Moliterni, G. Polidori, R. Spagna, J. Appl. Crystallogr. 32 (1999) 115–119.
[36] G.M. Sheldrick, Acta Crystallogr. Sect. A 64 (2008) 112–122.
[37] L.J. Farrugia, J. Appl. Crystallogr. 32 (1999) 837–838.