Acridine-decorated cyclometallated gold(III) complexes: synthesis and anti-tumour investigations

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Abstract

(C^N) and (C^N^C) cyclometalated Au(III) represent a highly promising class of potential anticancer agents. We report here the synthesis of seven new cyclometalated Au(III) complexes with five of them bearing an acridine moiety attached *via* (N^O) or (N^N) chelates, acyclic amino carbenes (AAC) and *N*-heterocyclic carbenes (NHC). The antiproliferative properties of the different complexes were evaluated *in vitro* on a panel of cancer cells including leukaemia, lung and breast cancer cells. We observed a trend between the cytotoxicity and the intracellular gold uptake of some representative compounds of the series. Some of the acridine-decorated complexes were demonstrated to interact with ds-DNA using FRET-melting techniques.

Introduction

The worldwide approval of cisplatin for the treatment of several cancers in the late 1970s was the starting point of the modern use of metal complexes in medicine.[1] However, the poor selectivity of cisplatin for cancer cells over healthy fast growing cells induces severe side effects which limits the dose of chemotherapy given to patients,[2] and both intrinsic and acquired resistance reduces the effectiveness of platinum-based drugs.[3] For these reasons there has been an increasing interest in the development of alternatives to platinum-based compounds for therapeutic purposes, including iron, copper, ruthenium, titanium and goldcomplexes.[4-8] In particular, cyclometalated gold(III) complexes have been attracting significant interest.[9,10] Both bidentate, (C^N) or tridentate, (C^N^C), (C^N^N) or (N^C^N) cyclometalated

ligands have been used to stabilize the gold(III) centre, enabling the study of fundamental organometallic reactions, the characterization of catalytic intermediates, and the development of photoluminescent materials and of anticancer drug candidates.[11-13]

Henderson and coworkers introduced a series of neutral cyclometalated (C^N)Au(N^N) chelate complexes containing phenylenediamine and ethylenediamine moieties, with the nitrogen atoms bearing electron-withdrawing moieties. The derivatives were tested for their *in vitro* cytotoxicity against mouse leukaemia cells (P388) and some showed very promising results in comparison to cisplatin. Several different (C^N) cyclometalated ligands were used, including the 6-membered ring based on 2-benzyl pyridine, and more rigid 2-phenyl pyridine complexes, combined with (N^O) and (O^O) chelate ligands (Figure 1, structures **A** - **C**).[14,15] Other examples include (C^N)-(N^N) derivatives reported by Zhang *et al.*, such as the stable and water soluble cationic gold(III) complex, [Au(butyl-C^N)biguanidine]Cl, (Figure 1, structure **D**) which combined the lipophilic, cyclometalated (C^N) ligand and the hydrophilic, H-bonding NH_2 groups of the biguanidine ligand. The complex showed high cytotoxicity towards cancer cell lines, with IC_{50} values in the low micromolar range, as well as a high selectivity profile towards cancer cells over healthy CCD-19-Lu cells.[16]

Figure 1. Examples of previously reported cyclometallated gold(III) complexes tested for anticancer purposes.[14-18]

We have recently described the synthesis and anticancer activity of novel cyclometalated (C^N^{pz}^C) gold[III] complexes with acyclic amino carbene (AAC) ligands enabling the introduction of amines and

amino ester derivatives in the $[(C{\wedge}N^{pz}{\wedge}C)Au(carbene)]^+$ scaffold (Figure 1, structures **E** and **F**). The antiproliferative screening of these complexes identified them as promising potential chemotherapeutic agents.[17,18] Gold(III) *N*-heterocyclic carbene (NHC) complexes appear to be more stable towards reducing agents like GSH and are highly cytotoxic towards cancer cell lines; for example, complex **F** showed IC_{50} values in the micromolar and sub-micromolar range against a panel of cancer cell lines.[18] In spite of the structural and electronic similarity between gold(III) and platinum(II) compounds, the two metals appear to have very different intracellular targets and the binding of gold(III) complexes to DNA is often weak and reversible.[19] However, it is possible to enhance interactions of gold(III) complexes with double-stranded DNA by optimizing the choice of the ligand toward that application.[20]

Acridines are known to bind to DNA by intercalation; by the direct insertion of the three planar aromatic rings in between the base pairs of DNA which causes unwinding of the double helix.[21,22] For example, Janočková *et al.* synthesized a series of acridine analogues, (figure 2) and demonstrated their ability to inhibit cancer cell growth in acute promyelocytic leukaemia cells (HL60) with IC_{50} values in the low micromolar range. DNA binding studies towards calf thymus DNA showed a strong interaction through DNA intercalation resulting in the inhibition of certain enzymes such as topoisomerase 1 and 2.[21] Howell *et al.* also studied the DNA binding and in-vitro cytotoxicity of 9-aminoacridinecarboxamides (Figure 2), towards various types of tertiary DNA structures. The derivatives were shown to target G-quadruplex structures with some selectivity.[23] There are also examples of acridine motifs being used to enhance the cytotoxicity of metal-based chemotherapeutic agents with platinum, iron, iridium or rhodium metal centres. In all cases, the presence of the acridine increased the interactions of the complexes with DNA.[24,25]
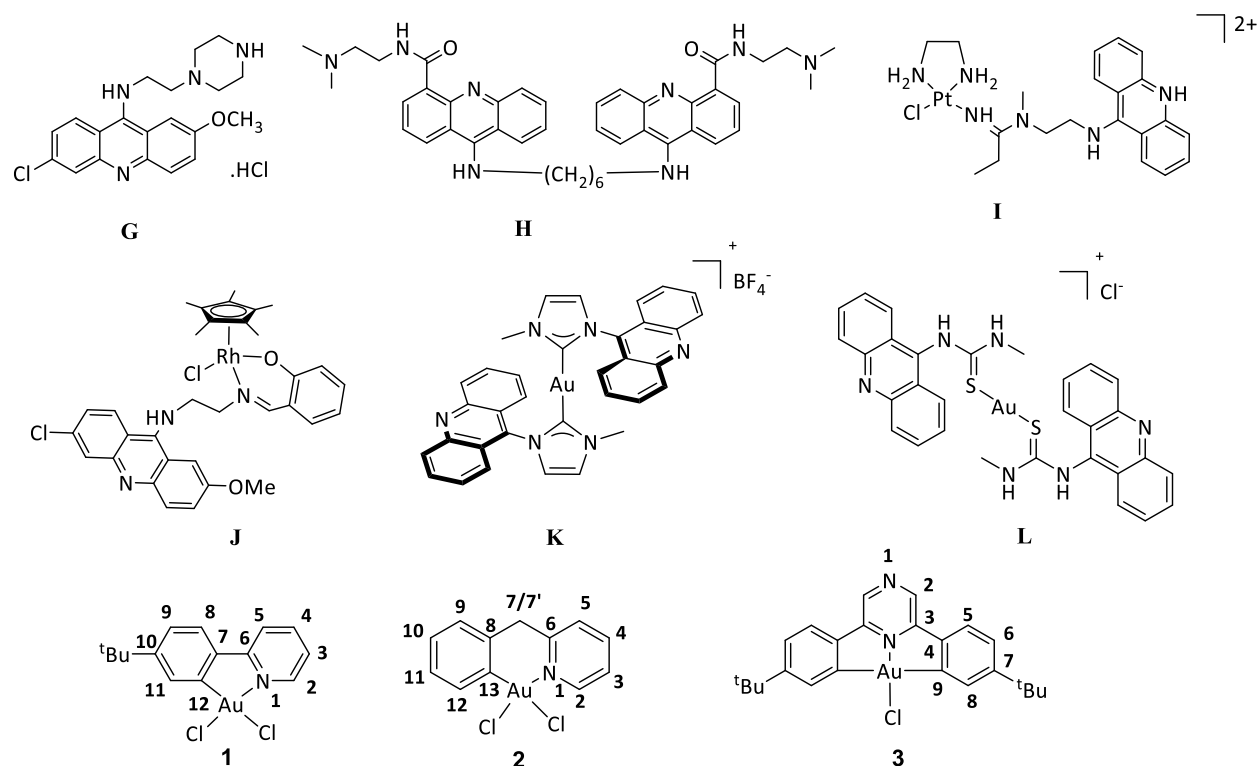
Figure 2. Examples of acridine derivatives and acridine-based metal complexes **G** – **L** tested for anticancer applications, and the structures of starting materials **1** – **3** showing the numbering scheme used for the NMR assignments.

Although examples of cytotoxic gold(I)-acridine hybrids have been reported with thiourea and NHC ligands (Figure 2, structures **K** and **L**),[26] to the best of our knowledge, no gold(III)-acridine conjugates have been described so far. In the present article, we report on the synthesis and anticancer activity of cyclometalated gold(III) complexes decorated with acridinyl substituents, based on the starting materials **1** – **3** (Figure 2). We focus on (i) neutral (C^N)-(N^O) and (C^N)-(N^N) derivatives, and (ii) (C^N^{pz}^C) pincer complexes where the acridine functionality is joined by either an AAC or an NHC ligand. The complexes were tested for their cytotoxicity towards three cancer cell lines, namely lung adenocarcinoma (A549), breast cancer (MCF-7) and leukaemia (HL60). The DNA binding abilities of the complexes towards dsDNA was also investigated using DNA FRET-melting assays.

Results and discussion

Synthesis and characterization

Following the work of Kilpin *et al.* who described the synthesis of cyclometalated (C^N)Au(III) complexes with (O^O), (N^O) and (N^N) ligands, we synthesized (N^O) and (N^N) chelate ligands bearing an acridine moiety. The reaction of 2-aminophenol with 2-(acridin-9-ylamino)-benzoic acid in the presence of *N,N'*-carbonyldiimidazole (CDI) did not proceed by coupling at the –COOH function as expected but instead gave the substituted phenol **L1**, with displacement of 2-aminobenzoic acid. The same reaction also occurred when o-phenylenediamine was used, affording **L2** (Scheme 1).
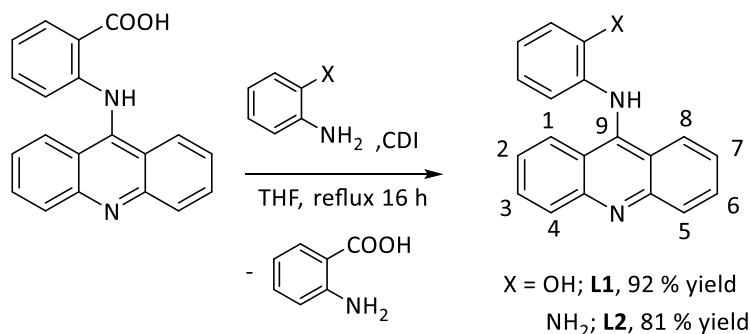
Scheme 1. Synthesis of the substituted acridine ligands **L1** and **L2**.

The reaction of the (C^N)gold(III) dichlorides **1** and **2** with 2-acetamidophenol in refluxing methanol in the presence of excess trimethylamine afforded high yields of the products **4** and **5**, respectively (Scheme 2), as previously reported.[15] Complex **6** was synthesized by reacting **1** with 1,2-diacetamidobenzene and silver oxide in refluxing dichloromethane. The reaction of **2** with 1,2-diacetamidobenzene/Ag$_2$O was also attempted but caused opening of the less rigid 6-membered ring.

Complex **6** was poorly soluble in the deuterated NMR solvents and therefore signals in both the ^{1}H and particularly the ^{13}C{^{1}H} NMR were poorly resolved.
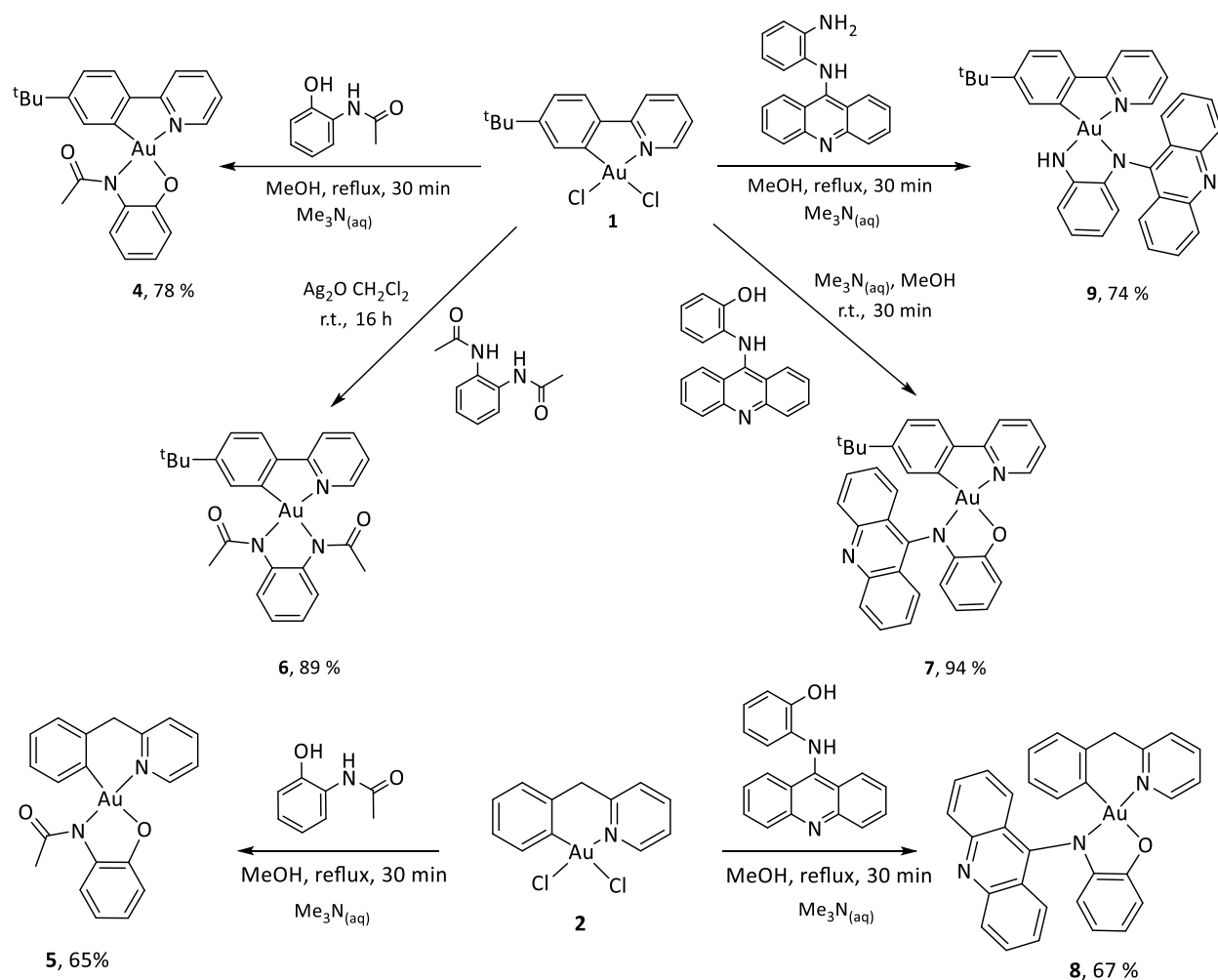
Scheme 2. Synthesis of the (C^N) cyclometalated gold(III) complexes **4-9**.

Complex **7** was obtained in high yield by reacting **1** with **L1** in refluxing methanol in the presence of an excess of trimethylamine, followed by precipitation with water. The reaction of **2** and **L1** under the same conditions was also successful and gave **8** in moderate yield. The corresponding (C^N)-(N^N) complex, complex **9** was obtained in good yield by the reaction of **1** with **L2** under the same conditions. Once again, the reaction was also attempted with the 2-benzyl pyridine gold dichloride starting material but caused ring opening.

At 25 °C solutions of **8** in CD_2Cl_2 gave broadened NMR spectra indicative of hindered rotation. These sharpen on cooling to -10°C and show well-resolved signals (see ESI, Figure S10) of the chemically inequivalent acridine protons H^{22-25}. The characteristic AB system of two doublets for the methylene bridge at 4.43 ppm and 3.91 ppm was observed, indicating the diastereotopic nature of these protons. For the three acridine-based complexes **7-9**, only one set of signals was observed for each proton,

demonstrating the formation of only one isomer in each case, as was also seen for the simpler (N^O) and (N^N) complexes **4-6**.

Slow diffusion of light petroleum into dichloromethane solutions of complexes **7** and **9** yielded crystals suitable for X-ray diffraction (Figure 3). In both compounds the square planar geometry of the gold(III) centres is slightly distorted due to the small bite angles of the cyclometalated ligand [respective C11-Au-N1 angles 81.1°(**7**) and 80.9° (**9**)]. In **7**, the acridine-substituted nitrogen atom is in *trans* position to the nitrogen of the cyclometalated ligand, placing it on the same side as the *tert*-butyl substituent, while the O atom, as the weakest *trans* influence ligand, is *trans* to the stronger C-donor. The adopted conformation is therefore a reflection of the electronic preferences. The acridine moiety is twisted out of the coordination plane, with an Au1-N2-C22-C23torsion angle of 110 °, presumably to avoid steric hindrance with the *tert*-butyl substituent. As suggested by the NMR spectra, only one isomer of the complex was obtained.

In complex **9**, on the other hand, the second ligand is an N^N chelate, with both donor atoms having similar electronic properties. Therefore, in this case a conformation is adopted which places the acridine substituent distal from the t-butyl group, to minimise steric interactions. Here, too, the acridine moiety is twisted out of the coordination plane (torsion angle Au1-N3-C22-C23 67.3°).
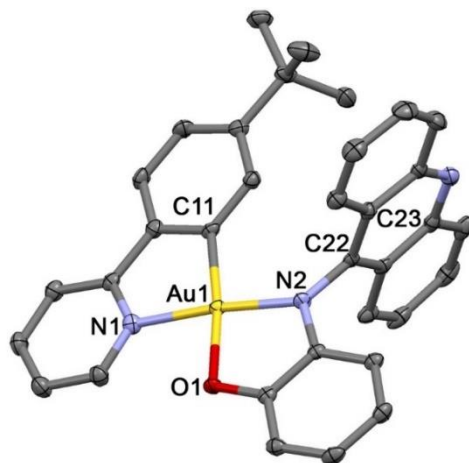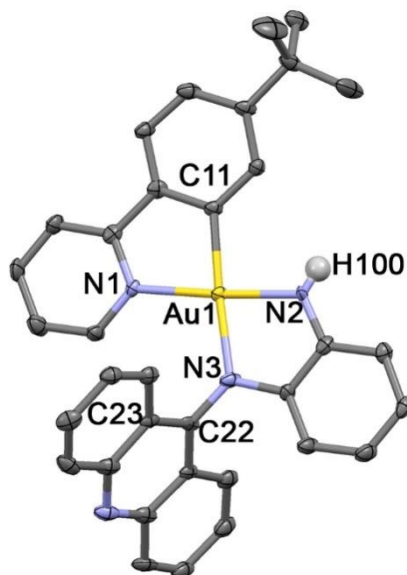
Figure 3. Top: crystal structure of complex **7**·CH$_2$Cl$_2$ (dichloromethane and hydrogen atoms omitted for clarity). Selected bond distances (Å) and angles (°). Au1-N1 2.028(3), Au1-C11 2.034(4), Au1-O1 2.049(3), Au1-N2 1.990(3), C11-Au1-N1 81.1(1), C11-Au1-N2 103.8(1), N2-Au1-O1 82.5(1), O1-Au1-N1 92.7(1), N1-Au1-N2 174.3(1), C11-Au1-O1 173.1(1), torsion Au1-N2-C22-C23 110.4(3). Bottom: Crystal structure of complex **9**·CH$_2$Cl$_2$ (dichloromethane and hydrogen atoms omitted for clarity). Selected bond distances (Å) and angles (°). Au1-N1 2.063(7), Au1-C11 2.015(6), Au1-N2 1.956(8), Au1-N3 2.081(6), N1-Au1-C11 80.9(3), C11-Au1-N2 95.2(3), N2-Au1-N3 80.3(3), N3-Au1-N1 103.9(3), Au1-N2-H100 119(6), N1-Au1-N2 175.4(3), C11-Au1-N3 173.0(3), torsion Au1-N3-C22-C23 67.3(9).

The pyrazine-based pincer complex (C^N^{pz}^C)AuCl (**3**) was synthesized as reported previously.[27] Conversion of **3** into the cationic xylylisocyanide complex [(C^N^{pz}^C)Au(CNxyl)]$^+$, followed by reaction with 9-aminoacridine afforded the functionalized acyclic amino carbene complexes **10** (Scheme 3). The yield was reduced due to the competitive hydrolysis of the highly electrophilic isocyanide adduct to the gold(III) formamide byproduct, as previously observed.[17]
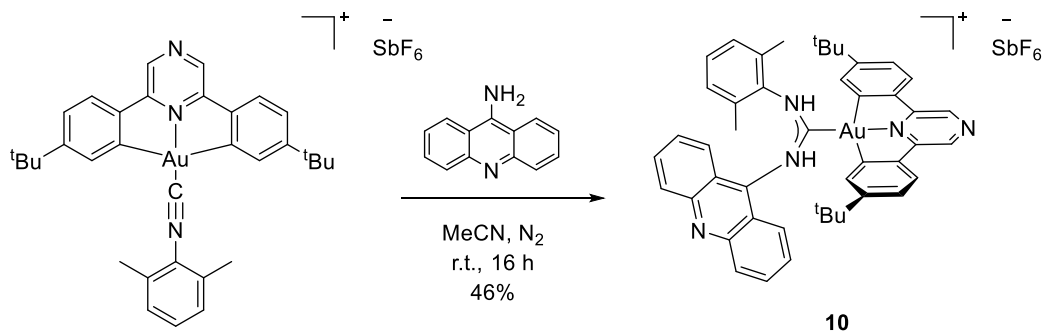
Scheme 3. Synthesis of the acridine functionalized [(C^N^{pz}^C)Au^{III}(AAC)]$^+$ **10**.

Upon formation of **10**, a broad NH singlet appears in the ^{1}H spectrum at 10.34 ppm and the upfield H^2 shift to 9.13 ppm confirms that a reaction had occurred and suggests that the acyclic amino carbene has been formed. The signal for H^8 at 9.96 ppm is also in good agreement with H^8 signals observed for the AAC complexes synthesized previously.[17] The IR spectrum of **10** showed a carbene vibration at 1586 cm^{-1} and two NH stretches at 3388 cm^{-1} and 3270 cm^{-1}.

Complex **11** was synthesized in good yield by reacting an acridine functionalized imidazolium salt with silver oxide, followed by the addition of **3** and potassium hexafluorophosphate by adapting a known procedure (Scheme 4).[28] The slight downfield shift in the signal for H^2 and a larger downfield shift in H^8 from 7.88 to 7.21 ppm in the ^{1}H NMR spectrum and the appearance of a signal at δ_C 154.9 ppm for the Au-bound NHC suggested carbene formation.
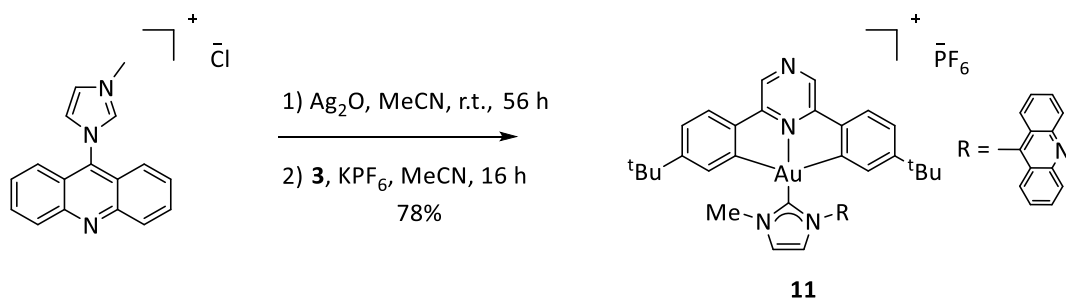
Scheme 4. Synthesis of the acridine-functionalized [(C^N^{pz}^C)Au^{III}(NHC)]$^+$ **11**.

Biological Activity

Although insoluble in aqueous cell culture medium, all the complexes and their ligand precursors were soluble enough in DMSO not to precipitate when diluted in 100 µM aqueous cell medium with 1% of DMSO. Complexes **1-11** and the acridine ligand precursors **L1** and **L2** were screened for their antiproliferative properties *in vitro* on a panel of human cancer cell lines, including lung adenocarcinoma

cells (A549), breast adenocarcinoma (MCF-7), and promyelocytic leukaemia (HL60). The IC_{50}, (the concentration at which cell viability is reduced to 50%), was then determined using a colorimetric MTS assay after 72 h of incubation, with the cisplatin included for comparison (see Experimental part for details). The results are summarized in Table 1.

Table 1: Cytotoxic effects of complexes **1-11** and ligands **L1** and **L2** in comparison to cisplatin towards different human cancer cell lines after 72 h of incubation.

Complex	$IC_{50} \pm SD$ (µM)[a]		
	A549	**MCF-7**	**HL60**
1	43.6 ± 4.1	10.8 ± 3.5	6.0 ± 0.5
2	>100	38.7 ± 4.7	12.8 ± 0.3
3[b]	> 50	8.6 ± 1.1	3.7 ± 0.3
4	12.7 ± 2.5	5.2 ± 1.0	2.9 ± 0.7
5	10.8 ± 2.7	3.6 ± 1.4	2.7 ± 0.4
6	22.7 ± 2.5	9.9 ± 2.1	2.5 ± 0.1
7	16.6 ± 2.8	2.7 ± 0.9	1.4 ± 0.4
8	19.9 ± 3.9	4.3 ± 0.4	1.5 ± 0.1
9	17.2 ± 4.4	6.7 ± 1.6	6.3 ± 0.1
10	15.0 ± 2.0	7.5 ± 0.6	20.7 ± 0.4
11	7.6 ± 0.6	1.5 ± 0.1	1.1 ± 0.1
L1	>100	>100	>100
L2	23.9 ± 3.5	14.4 ± 0.9	16.9 ± 0.9
Cisplatin	33.7 ± 3.7[b]	21.2 ± 3.9[b]	3.7 ± 0.3[b]

[a] Mean ± the standard error of at least three independent experiments. [b] Values from ref. 18

Complexes **4-11** showed greater or comparable activity to cisplatin under the conditions used in this assay and were overall more toxic than the gold dichloride precursors, complexes **1** and **2**. The free ligands, **L1** and **L2**, showed reduced toxicity particularly **L1** which was inactive against all of the cell lines tested, with an IC_{50} value of >100 µM against all three. **L2** also showed reduced toxicity in comparison to the gold complexes, which indicates that the gold[III] centre contributes significantly to the cytotoxicity.

Overall, the mono-cyclometalated (C^N)-(N^X) complexes, **4-9** showed greater activity than cisplatin against both the MCF-7 and the A549 human cancer cell lines and comparable activity against the HL60 cell line. Complexes **4-9** all showed similar activities against the MCF-7 cell lines, with

IC_{50}values in the 2-10 µM range. Complexes **5**, **7** and **8** showed particularly high toxicity with values between 2-4 µM. IC_{50} values for the A549 cell line were slightly higher, between 10-20 µM for all of the complexes. Here, the simpler structures, complexes **4** and **5** showed the greatest overall toxicity.

Changing the (C^N) cyclometalated ligand from the rigid 5-ring (phenyl pyridine)to the flexible 6-ring ligand (2-benzylpyridine) appeared to have no significant effect on the cytotoxicity of the complexes. For example, for the HL60 leukaemia cell line, complexes **4** and **5** have IC_{50}values of 2.9 and 2.7 µM, respectively. The same trend was found for complexes **7**(1.4 µM) and **8**(1.5 µM). This is different to results obtained by Kilpin *et al*., who found that complexes containing a six-membered cyclometalated ring displayed higher cytotoxicity than those with a five-membered cyclometalated ring.[14]

For all three cell lines, the (N^O) chelates appeared to be slightly more cytotoxic than the corresponding N^N chelates, which is in contrast to the values obtained for the free acridine ligands, **L1** and **L2**. For example, for the A549 lung cancer cell line the (N^O) chelate, complex **4,** has an IC_{50} of 12.7 µM, while the corresponding (N^N) chelate **6** has an IC_{50} of 22.7 µM. The same pattern can be seen for the acridine-functionalized derivatives: **7**, the (N^O) chelate, is more toxic than its (N^N) analogue **9** against the all three cancer cell lines. Here the effect is most notable against the leukaemia HL60 cell line, (IC_{50} of 1.4 and 6.3 µM for **7** and **9**, respectively).

As a comparison of complexes **4** *versus* **7** and **5** *versus* **8** shows, adding an acridine functionality appeared not to increase the overall cytotoxicity of the mono-cyclometalated (C^N)-(N^X) complexes. This also holds for the (N^N) chelates **6** compared to **9**, where the toxicity differences due to acridine are not significant.

The [(C^N^{pz}^C)Au(III)(AAC)]$^+$ complex **10** showed a higher cytotoxicity than cisplatin against both the MCF-7 and the A549 cancer cell lines, although it showed reduced activity against the HL60 cells (20.7 µM). These values are in good agreement with data on AAC complexes we reported earlier, particularly when considering the amino ester derivatives (e.g. GlyOEt – IC_{50} values of 6.4, 13.0 and 16.7 µM for MCF-7, A549 and HL60 respectively).[17]

The other (C^N^{pz}^C) pincer complex, **11** with acridine functionality bound to the NHC ligand, showed the highest overall toxicity, with values in the low µM regions for all three cell lines. It was more than ten times more toxic than cisplatin against MCF-7 cells and 3-4 times more toxic against the A549 and HL60 cell lines. Although our incubation time is longer (72 h instead of 24 h), it is interesting to note that **11** is active against A549 cells while the related Au^I complex [Au(MeImAcr)$_2$]$^+$ is not.[26b] The higher

cytotoxicity of the $[(C{\wedge}N^{pz}{\wedge}C)Au(NHC)]^+$ complexes with respect to the $[(C{\wedge}N^{pz}{\wedge}C)Au(AAC)]^+$ complexes is in line with our previous results.[17]

Cellular uptake into MCF-7 cells

The cellular uptake and the accumulation of drugs inside the cell are both major factors that influence the cytotoxicity of prospective anticancer agents.[29] Inductively coupled plasma-mass spectrometry, (ICP-MS) was used to quantify the amount of intracellular gold and thus measure the amount of compound taken up by the cell. In order to see whether the cytotoxicity of the complexes was influenced by their ability to be taken up by the cell, complexes **2**, **6**, **7** and **11** were chosen for uptake studies, since they show a range of antiproliferative activities against MCF-7 cells ranging from poor (IC_{50}(**2**) of 38.7 µM) to high (IC_{50}(**11**) of 1.5 µM) *via* intermediate activities (IC_{50}(**6**) and IC_{50}(**7**) of 9.9 µM2.7 µM respectively). Moreover, that selection of compounds includes a dichloro complex (**2**), a simple $(N{\wedge}X)$ chelate (**6**) and examples of acridine derivatives with $(C{\wedge}N)$ and $(C{\wedge}N^{pz}{\wedge}C)$ cyclometalated ligands (**7** and **11** respectively). MCF-7 cells were incubated for 6 h with 10 µM concentrations of each of the complexes in 1% DMSO. The results of three independent experiments are depicted in Figure 4.
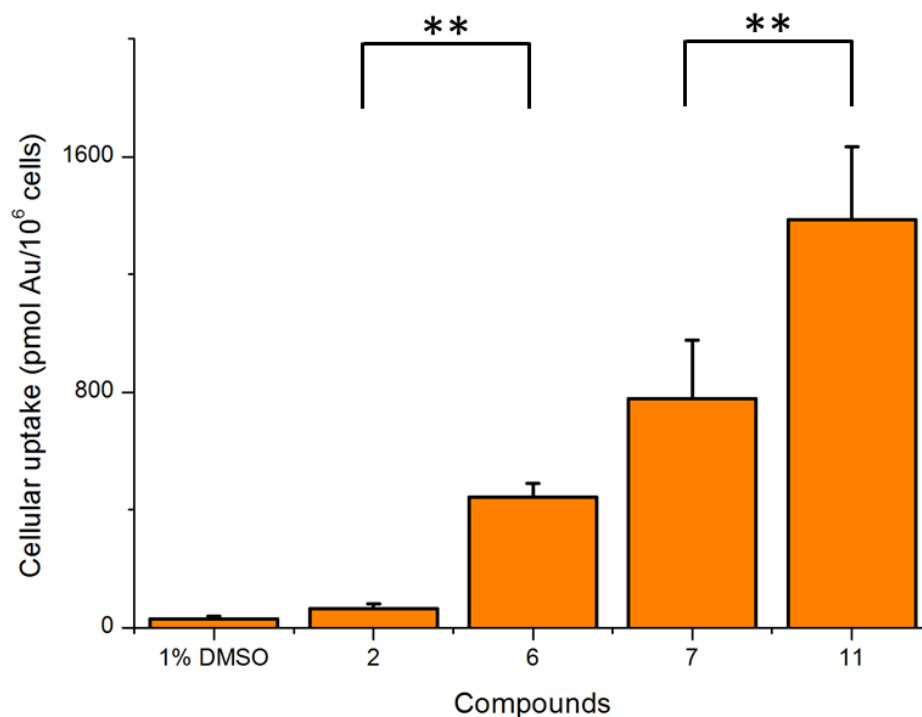
Figure 4. Cellular uptake of complexes **2**, **6**, **7** and **11** and a DMSO control in MCF-7 cells after 6 h of treatment at 10 µM in 1% DMSO. Data represent the average ± standard deviation of three experiments. The significance of the results was analysed by t-test. **p value < 0.01.

There was a clear correlation between cellular uptake and the *in vitro* cytotoxicity of the complexes, with the most cytotoxic complexes showing the highest levels of cell uptake (see Figure 6 and ESI, Figure S1). This suggests that the primary limitation for the poorly toxic complexes is their very low uptake into the cell. Such a correlation between cellular uptake and antiproliferative activity has also been observed for other gold complexes, including [(C^N^{pz}^C)Au(NHC]$^+$ complexes.[18,30] The increase in cell uptake seems to follow the increasing lipophilicity of the complexes, which might suggest an internalization *via* passive diffusion, reminiscent of the process recently reported by Casini *et al.* for a phosphine analogue of **2**.[31]

DNA binding assays

Acridine derivatives are known to interact with duplex DNA as well as higher order DNA structures and this can influence the cytotoxic properties of the compounds.[22,23] DNA intercalators are of interest as probes in the study of ligand-DNA interactions and also as potential therapeutic agents.[32] DNA intercalation is a reversible mode of DNA-ligand binding which consists of the direct insertion of planar aromatic moieties in between the base pairs of DNA, which causes unwinding to the double helix. There are several examples of gold(III) complexes acting as DNA intercalators, including both aminoquinoline,[20a] and terpyridine[20b] derivatives, which have been shown to bind to intracellular DNA by intercalation of the planar gold(III) coordination plane into the DNA base pairs. To determine whether or not the acridine-functionalized complexes interact with DNA in the present cases, a Förster resonance energy transfer (FRET) DNA melting assay was used to measure the ability of **4**–**11, L1** and **L2** to stabilize double stranded DNA. FRET is used as a tool for detecting spatial relationships between two fluorophores and involves measuring the changes in fluorescence as FRET donor and acceptor moieties are brought closer together or moved further apart as a result of DNA stabilization or denaturation. As a result of the large differences in fluorescence between folded and unfolded DNA structures it is possible to measure the interaction of a particular drug molecule with the DNA.[33] Results are expressed as the changes in DNA melting temperature (ΔT_m) when double stranded DNA (0.2 µM) is dosed with 50 µM concentrations of each drug (Figure 5).
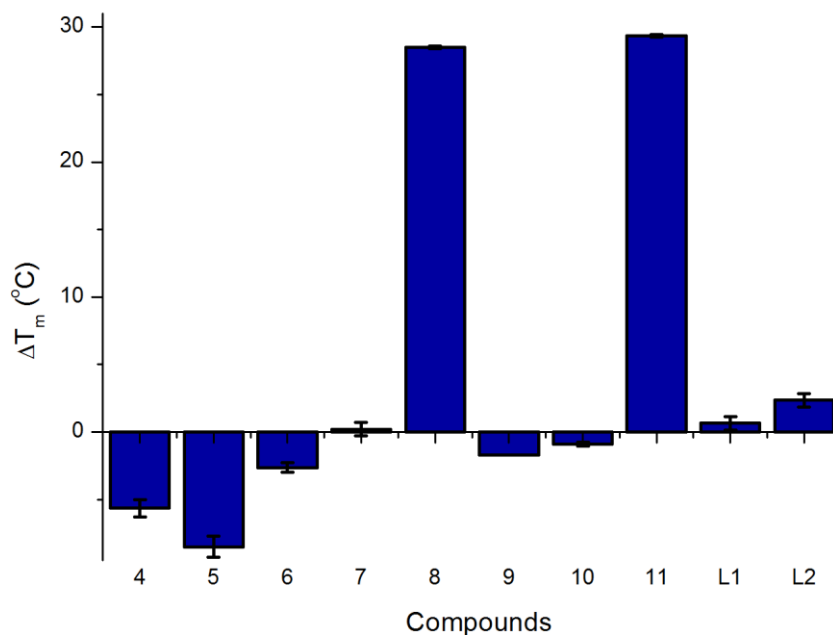
Figure 5. Stabilization of double-stranded DNA (0.2 µM) by complexes **4-11** and **L1/L2** at 50 µM concentrations measured by FRET DNA melting assay. Data represents the average and standard deviation of two separate experiments.

Out of the eight complexes tested, only two showed the ability to stabilize double-stranded DNA. The acridine-free complexes **4, 5** and **6** actually appeared to slightly destabilize ds DNA. These results confirm the poor interaction between gold(III) and ds-DNA. None of the (4-^{t}Bu-phenylpyridine)-based complexes **7** and **9** interacted with ds DNA, possibly due to the steric hindrance provided by the t-Bu substituted C^N ligand. The same phenomenon might be responsible for the lack of interaction of the AAC complex **10**. Complex **8**, the acridine functionalized 2-benzylpyridine derivative, showed high levels of DNA stabilization. This suggests that it is interacting with the ds DNA in some manner, potentially due to the hemi-lability of (C^N) ligand which could favour intercalation by decreasing steric hindrance. Interestingly, the [(C^N^{pz}^C)Au(NHC)]$^+$ complex **11** appeared to show significant interaction with ds-DNA, while the closely related complex **F** (Figure 1) lacking the acridine moiety interacted with ds-DNA rather poorly.[18] However, considering the poor DNA intercalation behaviour of the acridine ligands **L1** and **L2** and the lack of interaction of most of the acridine-decorated complexes, it seems likely that the

interactions observed for **8** and **11** are due to mechanisms other than the simple intercalation of the acridine moiety. Overall, considering that **7** and **8** present very similar cytotoxicities but drastically different interactions with DNA, these data suggest that acridine-DNA interactions have little bearing on the cytotoxicity mechanism of this series of compounds.

Reaction with Glutathione

Reduction of gold(III) complexes by glutathione (GSH) to gold(I) and gold(0) products is a typical feature of this class of complexes.[34] The stability of **11** and **7** towards reduction by GSH was therefore explored by ^{1}H NMR spectroscopy. Each complex was mixed at room temperature with reduced GSH in a 1:1 mixture of DMSO-d_6 and D_2O and monitored by over time. We had previously shown that a benzimidazole-based NHC complex of (C^N^{pz}^C)Au(III) (Figure 1, structure **F**) is stable towards reduction by GSH.[18] By contrast, the acridine substituted [(C^N^{pz}^C)Au(NHC)]$^+$ complex **11** appeared less stable, and over a 24 hour period the gradual formation of oxidized glutathione (GSSG) was observed, along with the disappearance of signals for reduced GSH (Figure 6). One can hypothesize that the higher steric hindrance around gold due to the acridine substituent on the NHC could facilitate the protolytic Au-C bond cleavage leading to S-coordination of GSH and subsequent reduction of the gold(III) ion, following a reaction pathway similar to the one recently found for the reaction of adamantylthiol with (C^N^C)Au pincer compounds.[35] The same pattern was seen for the acridine substituted (C^N)-(N^O) chelate complex, **7** (Figure S2).
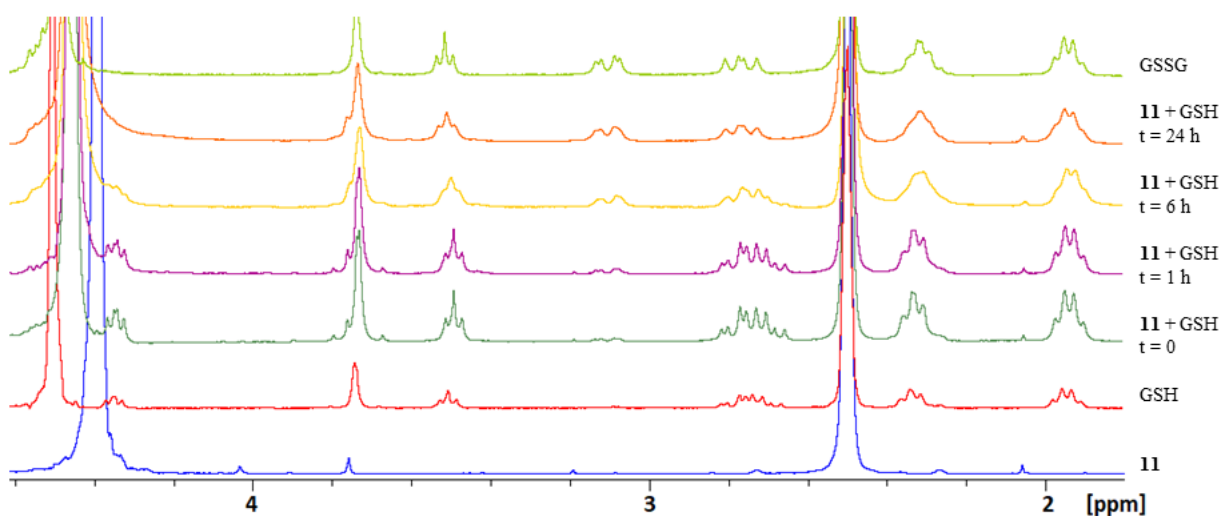

Figure 6. ^{1}H NMR spectra of a 1:1 mixture of **11** with GSH at room temperature, in comparison with the starting materials, **11**, GSH and GSSG (DMSO-d_6/D_2O 1:1).

Conclusion

A new series of mono cyclometalated (C^N)(N^X)Au(III) complexes as well as two bis-cyclometalated (C^N^{pz}^C) pincer complexes were synthesized, with and without acridine functionality. These complexes were tested for their cytotoxicity towards three cancer cell lines. These studies identified complex **11**, the NHC derivative, as the most promising candidate, with IC$_{50}$ values in the low µM range for all three cell lines. However, the introduction of an acridine functionality did not appear to increase the cytotoxicity of the complexes. Cell uptake studies demonstrated a correlation between cellular uptake and the *in vitro* cytotoxicity of the complexes and indicated that cellular uptake is the primary limitation for non-toxic gold(III) complexes. DNA binding studies showed that two of the derivatives, complex **8** and the NHC derivative **11**, demonstrate high levels of DNA stabilization suggestive of DNA intercalation. The lack of a correlation between the cytotoxicity data and the interaction with ds-DNA may suggest that the addition of the acridine moiety onto these scaffolds did not result in improved DNA targeting. Taken together, those data suggest that DNA is probably not the main target of that class of compounds and that more experiments are required to fully elucidate its involvement in the mechanism of action of these complexes. Moreover, both the pincer NHC complex [(C^N^{pz}^C)Au(NHC)]$^+$**11** and the mono-cyclometalated (C^N)Au(N^O) derivative **7** were prone to reduction by glutathione at a time scale commensurate with incubation, which suggests that these compounds are reduced to gold(I) species in the cellular environment. Further work is required to investigate the localization and accumulation of those complexes within the cells and other possible modes of action of these complexes.

Materials and methods

General remarks. When required, manipulations were performed using standard Schlenk techniques under dry nitrogen or in a MBraun glove box. Nitrogen was purified by passing through columns of supported P$_2$O$_5$ with moisture indicator, and activated 4 Å molecular sieves. Anhydrous solvents were freshly distilled from appropriate drying agents. ^{1}H and ^{13}C[^{1}H] spectra were recorded using a Bruker AvanceDPX-300 spectrometer. ^{1}H NMR spectra (300.13 MHz) were referenced to the residual protons of the deuterated solvent used. ^{13}C[^{1}H] NMR spectra (75.47 MHz) were referenced internally to the D-coupled ^{13}C resonances of the NMR solvent. Elemental analyses were carried out at London Metropolitan University. Complexes **1**, **2**, **3** and **5** were synthesized following reported procedures.[15,27,36,37]

Synthesis of complex 4

A mixture of 2-(4-*t*-butyl)phenyl pyridine gold dichloride, (**1**), (0.200 g, 0.418 mmol), 2-acetamidophenol (0.100 g, 0.661 mmol) and aqueous trimethylamine (2 mL) was refluxed in methanol (30 mL) for 30 min. The reaction was then cooled and water (40 mL) was added causing an immediate orange precipitation. The flask was then placed in the freezer for two days. The orange solid was filtered and purified by dissolving in minimal dichloromethane (2 mL) and precipitating the product with an excess of light petroleum (bp. 40-60 °C) (20 mL). This was filtered and dried under vacuum (0.181 g, 0.325 mmol, 78%). Anal. Calcd. for $C_{23}H_{23}AuN_4O_2.2H_2O$ (592.45): C, 46.63; H, 4.59; N, 4.73. Found: C, 46.23; H 4.21; N 4.76. ^1H NMR (CD_2Cl_2, 300 MHz, 298 K): 8.97 (d, $^3J_{H-H}$ = 5.1 Hz, 1H, H^2), 8.07 (t, $^3J_{H-H}$ = 8.1 Hz, 1H, H^4), 7.83 (d, $^3J_{H-H}$ = 8.1 Hz, 1H, H^5), 7.75 (d, $^4J_{H-H}$ = 1.5 Hz, 1H, H^{11}), 7.51-7.40 (m, 2H, H^{3+8}), 7.37 (dd, $^3J_{H-H}$ = 8.3 Hz, $^4J_{H-H}$ = 1.5 Hz, 1H, H^9), 7.22 (dd, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.3 Hz, 1H, $H^{14/17}$), 6.84 (m, 1H, $H^{15/16}$), 6.66 (dd, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.3 Hz, 1H, $H^{14/17}$), 6.49 (m, 1H, $H^{15/16}$), 2.32 (s, 3H, H^{20}), 1.35 (s, 9H, ^tBu). $^{13}C[H]$ NMR (CD_2Cl_2, 75 MHz): 173.7 (s, C^{19}), 164.2 (s, C^{12}), 161.3 (s, $C^{6/7}$), 155.5 (s, $C^{6/7}$), 147.0 (s, C^2), 144.7 (s, C^{10}), 144.3 (s, $C^{13/18}$), 142.7 (s, C^4), 139.4 (s, $C^{13/18}$), 133.0 (s, C^{11}), 125.4 (s, C^3), 124.8 (s, C^8), 124.2 (s, C^9), 123.8 (s, $C^{14/17}$), 123.4 (s, $C^{14/17}$), 120.2 (s, C^5), 115.6 (s, $C^{15/16}$), 115.3 (s, $C^{15/16}$), 35.3 (s, $C(CH_3)_3$), 30.8 (s, C($CH_3)_3$), 25.2 (s, C^{20}).

*Synthesis of complex **6***

A mixture of 2-(4-*t*-butyl)phenyl pyridine gold dichloride, (**2**) (0.200 g, 0.418 mmol), 1,2-diacetamidobenzene (0.081 g, 0.421 mmol) and silver^I oxide (200 mg, 0.863 mmol) was added to dichloromethane (30 mL) and refluxed for 24 h. The mixture was then cooled, filtered through celite and the filtrate was concentrated under vacuum. A large excess of diethyl ether (20 mL) was added to precipitate the product as a pale-yellow, fluffy, solid. This was filtered and dried under vacuum (0.224 g, 0.375 mmol, 89%). Anal. Calcd. for $C_{25}H_{26}AuN_3O_2$ (597.47): C, 50.26; H, 4.39; N, 7.03. Found: C, 50.12; H 4.38; N 6.97. ^1H NMR (CD_2Cl_2, 300 MHz, 298 K): 9.30 (s, 1H, H^2), 8.02 (t, $^3J_{H-H}$ = 7.8 Hz, 1H, H^4), 7.79 (d, $^3J_{H-H}$ = 7.8 Hz, 1H, H^5), 7.55 (d, $^4J_{H-H}$ = 1.4 Hz, 1H, H^{11}), 7.48 (d, $^3J_{H-H}$ = 8.0 Hz, 1H, H^8), 7.41-7.28 (m, 3H, $H^{9+3+14/17}$), 7.09 (m, 1H, $H^{14/17}$), 6.99 (dt, $^3J_{H-H}$ = 7.3 Hz, $^4J_{H-H}$ = 1.4 Hz, 1H, $H^{15/16}$), 6.91 (dt, $^3J_{H-H}$ = 7.3 Hz, $^4J_{H-H}$ = 1.4 Hz, 1H, $H^{15/16}$), 2.27 (s, 3H, $H^{20/22}$), 2.15 (s, 3H, $H^{20/22}$), 1.34 (s, 9H, ^tBu). $^{13}C[H]$ NMR (CD_2Cl_2, 75 MHz): 174.1 (s, $C^{19/21}$), 173.1 (s, $C^{19/21}$), 164.6 (s, C^{12}), 154.8 (s, C^2), 153.3 (s, $C^{6/7}$), 145.5 (s, C^{10}), 142.2 (s, C^4), 140.5 (s, $C^{13/18}$), 130.8 (s, C^{11}), 125.5 (s, $C^{3/9}$), 124.6 (s, $C^{15/16}$), 124.2 (s, C^8), 124.0 (s, $C^{14/17}$), 123.0 (s, $C^{3/9}$), 122.7 (s, $C^{15/16}$), 119.7 (s, C^5), 35.3 (s, $C(CH_3)_3$), 30.8 (s, C($CH_3)_3$).

*Synthesis of complex **7***

A mixture of 2-(4-*t*-butyl)phenyl pyridine gold dichloride, (**1**), (0.235 g, 0.491 mmol), ligand **L1** (0.300 g, 1.048 mmol) and aqueous trimethylamine (2 mL) was refluxed in methanol (30 mL) for 30 min. The

reaction was then cooled and water (40 mL) was added causing an immediate dark brown precipitation. The flask was placed in the freezer for two days. The brown solid was filtered off and purified by dissolving in dichloromethane (2 mL), followed by precipitation an excess of light petroleum (bp. 40-60 °C) (20 mL). This product was filtered off and dried under vacuum (0.273 g, 0.394 mmol, 80%). Anal. Calcd. for $C_{34}H_{28}AuN_3O$ (691.19): C, 59.05; H, 4.08; N, 6.08. Found: C, 58.84; H 4.13; N 6.02. ^1H NMR (CD$_2$Cl$_2$, 300 MHz, 298 K): 9.61 (dd, $^3J_{H-H}$ = 5.9 Hz, $^4J_{H-H}$ = 1.0 Hz, 1H, H^2), 8.49 (dd, $^3J_{H-H}$ = 8.7 Hz, $^4J_{H-H}$ = 0.7 Hz, 2H, H$^{21/24}$), 8.30 (d, $^3J_{H-H}$ = 8.7 Hz, 2H, H$^{21/24}$), 8.08 (dt, $^3J_{H-H}$ = 8.1 Hz, $^4J_{H-H}$ = 1.0 Hz, 1H, H^4), 7.82 (d, $^3J_{H-H}$ = 8.1 Hz, 1H, H^5), 7.75 (m, 2H, H$^{22/23}$), 7.54 (m, 1H, H^3), 7.48-7.36 (m, 3H, H$^{8+22/23}$), 7.01 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 1.7 Hz, 1H, H^9), 6.85 (dd, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.2 Hz, 1H, H$^{14/17}$), 6.48 (dt, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.2 Hz, 1H, H$^{15/16}$), 6.12 (dt, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.2 Hz, 1H, H$^{15/16}$), 5.39 (dd, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.2 Hz, 1H, H$^{14/17}$), 5.23 (d, $^4J_{H-H}$ = 1.7 Hz, 1H, H^{11}), 0.35 (s, 9H, ^{t}Bu). $^{13}C[H]$ NMR (CD$_2$Cl$_2$, 75 MHz): 163.3 (s, C^2), 156.1 (s, C^{12}), 155.2 (s, $C^{6/7}$), 153.3 (s, $C^{6/7}$), 151.0 (s, C^{19}), 150.9 (s, C^{20}), 147.5 (s, C^{10}), 142.5 (s, $C^{13/18}$), 141.9 (s, C^4), 140.2 (s, $C^{13/18}$), 130.3 (s, C^{21}), 130.2 (s, C^{22}), 127.4 (s, C^9), 126.2 (s, C^{23}), 125.4 (s, C^8), 124.5 (s, C^{25}), 124.3 (s, C^{24}), 124.0 (s, C^{11}), 123.5 (s, C^3), 120.2 (s, C^5), 118.4 (s, $C^{15/16}$), 117.0 (s, $C^{15/16}$), 115.1 (s, $C^{14/17}$), 113.7 (s, $C^{14/17}$), 34.4 (s, $C(CH_3)_3$), 29.9 (s, C(CH_3)$_3$).

Synthesis of complex 8

A mixture of 2-benzylpyridine gold dichloride, (**2**), (0.200 g, 0.459 mmol), ligand **L1** (0.178 g, 0.622 mmol) and aqueous trimethylamine (2 mL) was refluxed in methanol (30 mL) for 30 min. The reaction was then cooled and water (40 mL) was added causing an immediate dark brown precipitation. The flask was then placed in the freezer for two days. The brown solid was then filtered and purified by dissolving in minimal dichloromethane and acetone (3 mL). the product was precipitated with an excess of light petroleum (bp. 40-60 °C) (20 mL), filtered off and dried under vacuum (0.201 g, 0.309 mmol, 67%). Anal.Calcd.for $C_{31}H_{22}AuN_3O.5H_2O$ (739.58): C, 50.35; H, 4.36; N, 5.68. Found: C, 50.63; H 3.36; N 7.45. ^1H NMR (CD$_2$Cl$_2$, 300 MHz, 263 K): 9.23 (d, $^3J_{H-H}$ = 5.6 Hz, 1H, H^2), 8.51 (d, $^3J_{H-H}$ = 8.6 Hz, 1H, H$^{22/25}$), 8.46 (d, $^3J_{H-H}$ = 8.6 Hz, 1H, H$^{22/25}$), 8.21 (d, $^3J_{H-H}$ = 8.6 Hz, 1H, H$^{22/25}$), 8.03 (dt, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.4 Hz, 1H, H^4), 7.95 (d, $^3J_{H-H}$ = 8.6 Hz, 1H, H$^{22/25}$), 7.80 (m, 1H, H$^{23/24}$), 7.67 (d, $^3J_{H-H}$ = 7.7 Hz, 1H, H^5), 7.62-7.46 (m, 3H, H$^{3+23/24}$), 7.25 (t, $^3J_{H-H}$ = 8.6 Hz, 1H, H$^{23/24}$), 6.78-6.71 (m, 2H, H$^{15/18+9/12}$), 6.55 (t, $^3J_{H-H}$ = 7.4 Hz, 1H, H$^{16/17}$), 6.47 (t, $^3J_{H-H}$ = 7.4 Hz, 1H, H$^{10/11}$), 6.16 (t, $^3J_{H-H}$ = 7.4 Hz, 1H, H$^{16/17}$), 5.99-5.84 (m, 2H, H$^{9/12+10/11}$), 5.69 (dd, $^3J_{H-H}$ = 7.4 Hz, $^4J_{H-H}$ = 1.0 Hz, 1H, H$^{15/18}$), 4.43 (d, $^2J_{H-H}$ = 15.3 Hz, 1H, H$^{7/7'}$), 3.91 (d, $^2J_{H-H}$ = 15.3 Hz, 1H, H$^{7/7'}$). $^{13}C[H]$ NMR (CD$_2$Cl$_2$, 75 MHz): 157.2 (s, C^{13}), 157.1 (s, $C^{6/8}$), 151.7 (s, $C^{6/8}$), 151.5 (s, $C^{14/19}$), 150.7 (s, C^2), 141.7 (s, C^4), 138.5 (s, $C^{14/19}$), 132.3 (s, $C^{20/21/26}$), 130.2 (s, $C^{22/25}$), 130.1 (s, $C^{9/12}$), 129.8 (s, $C^{22/25}$), 129.5 (s, $C^{23/24}$), 126.4 (s, $C^{9/12}$), 126.4 (s, $C^{10/11}$), 126.4 (s, $C^{10/11}$), 126.1

(s, C^5), 125.6 (s, $C^{23/24}$), 124.7 (s, C^3), 118.3 (s, $C^{16/17}$), 116.7 (s, $C^{16/17}$), 115.4 (s, $C^{15/18}$), 113.4 (s, $C^{15/18}$), 47.8 (s, $C^{7/7'}$).

*Synthesis of complex **9***

A mixture of 2-(4-*t*-butyl)phenyl pyridine gold dichloride, (**1**), (0.200 g, 0.418 mmol), ligand **L2** (0.177 g, 0.620 mmol) and aqueous trimethylamine (2 mL) was refluxed in methanol (30 mL) for 30 min. The reaction was then cooled and water (40 mL) was added causing an immediate light brown precipitation. The flask was then placed in the freezer for two days. The brown solid was then filtered and purified by dissolving in minimal dichloromethane (2 mL). The product was precipitated with an excess of light petroleum (bp. 40-60 °C) (20 mL), filtered off and dried under vacuum (0.214 g, 0.310 mmol, 74%). Anal. Calcd. for $C_{34}H_{29}AuN_4 \cdot 3H_2O$ (744.65): C, 54.84; H, 4.74; N, 7.52. Found: C, 54.25; H; 4.20 N; 7.41. ^{1}H NMR (CD$_2$Cl$_2$, 300 MHz, 298 K): 8.40 (dd, $^3J_{H-H}$ = 8.7 Hz, $^4J_{H-H}$ = 0.7 Hz, 2H, H$^{21/24}$), 8.24 (d, $^3J_{H-H}$ = 8.7 Hz, 2H, H$^{21/24}$), 7.78-7.68 (m, 4H, H$^{22/23+2+4/5}$), 7.64-7.55 (m, 2H, H$^{3+4/5}$), 7.41 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 1.7 Hz, 1H, H^9), 7.35 (m, 2H, H$^{22/23}$), 6.97 (dd, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.1 Hz, 1H, H$^{14/17}$), 6.44 (t, $^3J_{H-H}$ = 7.7 Hz, 1H, H$^{15/16}$), 6.36-6.18 (m, 3H, H$^{8+11+15/16}$), 5.62 (dd, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.1 Hz, 1H, H$^{14/17}$), 1.46 (s, 9H, ^{t}Bu). ^{13}C[H] NMR (CD$_2$Cl$_2$, 75 MHz): 164.6 (s, C^2), 155.3 (s, C^{12}), 154.5 (s, $C^{6/7}$), 150.5 (s, $C^{6/7}$), 149.6 (s, C^{19}), 148.0 (s, C^{20}), 145.6 (s, C^{10}), 145.0 (s, $C^{13/18}$), 140.4 (s, C^4), 139.7 (s, $C^{13/18}$), 130.2 (s, $C^{21/24}$), 130.0 (s, $C^{21/24}$), 126.5 (s, C^{25}), 126.0 (s, C^9), 125.5 (s, $C^{22/23}$), 124.9 (s, C^8), 124.8 (s, $C^{22/23}$), 124.6 (s, C^{11}), 122.3 (s, C^3), 120.1 (s, C^5), 118.2 (s, $C^{15/16}$), 116.3 (s, $C^{15/16}$), 113.3 (s, $C^{14/17}$), 112.6 (s, $C^{14/17}$), 35.6 (s, C(CH$_3$)$_3$), 31.0 (s, C(CH$_3$)$_3$).

*Synthesis of complex **10***

A mixture of (C^N^{pz}^C)AuCl, (**3**), (0.060 g, 0.104 mmol), 2,6-dimethylphenyl isocyanide (0.016 g, 0.125 mmol), AgSbF$_6$ (0.043 g, 0.125 mmol) and a few pellets of 4A molecular sieves were combined in a flame-dried Schlenk flask under a nitrogen atmosphere with dry dichloromethane (15 mL). The mixture was left to stir at room temperature for 4 h. A white precipitate of AgCl was removed by filtration through celite, and the filtrate collected under an N$_2$ atmosphere. The solvent was evaporated to a minimum and the product precipitated with an excess of light petroleum (bp. 40-60 °C). The supernatant was removed and the residue dried under vacuum to yield a yellow solid. 9-amino acridine (0.028 g, 0.146 mmol) was added to a flame-dried Schlenk flask. The solution was sonicated for 30 min and transferred to a separate flame-dried Schlenk flask containing the [(C^N^{pz}^C)Au(2,6-dimethylphenyl isocyanide)]SbF$_6$. The reaction was then stirred for 16 h at room temperature and the precipitate removed from solution via filtration through a celite plug. Next, the solvent was removed under vacuum and the solid residue re-dissolved in dichloromethane (2 mL). The product was precipitated using a 2:1 mixture of light petroleum

(bp. 40-60 °C) / diethyl ether (5 mL). After removal of the solvent, the residue was dried under vacuum to yield a dark yellow solid. Proton NMR showed a mixture of the desired product and the formamide hydrolysis product. The solid was washed twice with dry dichloromethane (40 mL) followed by light petroleum (10 mL) and dried under vacuum (0.053 g, 0.048 mmol, 46%). Anal. Calcd. for $C_{46}H_{45}AuF_6N_5Sb$ (1100.62): C, 50.20; H, 4.12; N, 6.36. Found: C, 49.97; H 4.03; N 6.35. ^1H NMR ((CD$_3$)$_2$SO, 300 MHz, 298 K): 10.34 (s, 1H, NH), 9.13 (s, 2H, H^2), 8.26 (d, $^3J_{H-H}$ = 8.2 Hz, 2H, H$^{20/23}$), 7.96 (d, $^4J_{H-H}$ = 1.5 Hz, 2H, H^8), 7.85 (d, $^3J_{H-H}$ = 8.4 Hz, 2H, H^5), 7.74-7.57 (m, 4H, H$^{20/23+21/22}$), 7.30 (dd, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 1.5 Hz,2H, H^6), 7.25-7.14 (m, 3H, H^{14+15}), 6.96 (t, 2H, $^3J_{H-H}$ 7.5 Hz, 2H, H$^{21/22}$), 2.56 (s, 6H, H^{16}), 1.20 (s, 18H, ^{t}Bu). ^{13}C[H] NMR (CD$_3$CN, 75 MHz): 167.6 (s, C^9), 156.0 (s, C^4), 155.6 (s, C^3), 144.7 (s, C^7), 139.9 (s, C^2), 139.6 (s, C^{13}), 135.1 (s, C^8), 134.0 (s, $C^{20/23}$), 128.6 (s, C^{15}), 127.7 (s, C^{14}), 127.6 (s, $C^{20/23}$), 127.6 (s, C^{12}), 127.0 (s, C^5), 124.5 (s, C^6), 123.6 (s, $C^{21/22}$), 123.5 (s, $C^{21/22}$), 117.8 (s, C^{18}), 117.8 (s, C^{19}), 117.8 (s, C^{24}), 35.7 (s, C(CH$_3$)$_3$), 31.4 (s, C(CH_3)$_3$), 19.3 (s, C^{16}). v_{max} (neat)/cm^{-1}: 3388 (NH), 3270 (NH) 2957 (^{t}Bu), 2867 (Ar), 1586 (carbene).

Synthesis of complex 11

[(MeImAcr)H]Cl (0.070 g, 0.232 mmol) and silver oxide (0.043 g, 0.186 mmol) and acetonitrile (15 mL) were combined in a Schlenk and stirred for 56 h. (C^N^{pz}^C)AuCl, (**3**), (0.133 g, 0.232 mmol) and KPF$_6$ (0.128 g, 0.695 mmol) were then added to the flask and the reaction was stirred for a further 16 h before being filtered through a celite plug. The filtrate was concentrated under vacuum and the product was precipitated using a 2:1 mixture of light petroleum (bp. 40-60 °C) / diethyl ether (5 mL), and after removing the solvent, dried under vacuum to yield a dark yellow solid (0.170 g, 0.180 mmol, 78%). Anal. Calcd. for $C_{41}H_{39}AuF_6N_5P$.5MeCN (1148.99): C, 53.31; H, 4.74; N, 12.19. Found: C, 52.92; H, 5.10; N, 11.80. ^1H NMR (CD$_3$CN, 300 MHz, 298 K): 8.68 (s, 2H, H^2), 8.12-8.02 (m, 3H, H^{11+19}), 7.96 (d, 1H, $^3J_{H-H}$ = 1.9 Hz, H^{12}), 7.88-7.77 (m, 4H, H^{16+18}), 7.55 (d, 2H, $^3J_{H-H}$ = 8.3 Hz, H^5), 7.45 (t, 2H, $^3J_{H-H}$ = 7.8 Hz, H^{17}), 7.33 (dd, 2H, $^3J_{H-H}$ = 8.3 Hz, $^4J_{H-H}$ = 1.7 Hz, H^6), 7.21 (d, 2H, $^4J_{H-H}$ = 1.7 Hz, H^8), 4.15 (s, 3H, H^{13}), 1.30 (s, 18H, ^{t}Bu). ^{13}C[H] NMR (CD$_3$CN, 75 MHz): 166.1 (s, C^9), 156.8 (s, C^4), 156.3 (s, C^3), 154.9 (s, C^{10}), 146.6 (s, C^{20}), 144.6 (s, C^7), 140.5 (s, C^{14}), 139.3 (s, C^2), 133.3 (s, C^{18}), 133.0 (s, C^8), 128.9 (s, C^{17}), 127.0 (s, C^{12}), 126.9 (s, C^{19}), 126.7 (s, C^5), 126.3 (s, C^{11}), 125.2 (s, C^6), 123.5 (s, C^{16}), 122.2 (s, C^{15}), 39.1 (s, C^{13}), 35.1 (s, C(CH$_3$)$_3$), 30.3 (s, C(CH_3)$_3$).

Synthesis of L1

2-(Acridin-9-ylamino)-benzoic acid (0.600 g, 2.100 mmol) and *N,N'*-carbonyldiimidazol(CDI) (0.341 g, 2.103 mmol) were placed in a flame dried flask, fitted with a condenser and stirred in dry tetrahydrofuran (20 mL) under a nitrogen atmosphere, for 15 min. 2-amino phenol (0.301 g, 2.758 mmol) was then added

and the reaction was stirred for a further 15 min before being heated to reflux overnight. After cooling to room temperature, the orange solid was filtered and washed twice with light petroleum (bp. 40-60 °C) before being dried under vacuum (0.551 g, 1.925 mmol, 92%). ^{1}H NMR ((CD$_2$)SO, 300 MHz, 298 K): 10.18 (s, 1H, H^8), 8.26 (d, $^3J_{H-H}$ = 8.8 Hz, 1H, H$^{3/6}$), 8.18 (d, $^3J_{H-H}$ = 7.9 Hz, 1H, H$^{3/6}$), 8.08-7.88 (m, 5H, H$^{4/5+11+14}$) 8.28-7.50 (m, 4H, H^{12+13}), 7.03 (t, $^3J_{H-H}$ = 7.9 Hz, 1H, H$^{4/5}$). ^{13}C[H] NMR ((CD$_2$)SO, 75 MHz): 156.8 (s, $C^{2/7/9}$), 152.9 (s, $C^{2/7/9}$), 140.5 (s, $C^{2/7/9}$), 136.1 (s, $C^{11/14}$), 131.9 (s, $C^{4/5}$), 130.5 (s, $C^{10/15}$), 128.1 (s, $C^{3/6}$), 128.0 (s, $C^{12/13}$), 125.9 (s, $C^{3/6}$), 124.6 (s, $C^{12/13}$), 121.0 (s, $C^{10/15}$), 119.9 (s, $C^{11/14}$), 117.8 (s, $C^{4/5}$), 114.1 (s, C^q).

*Synthesis of **L2***

4-(Acridin-9-ylamino)-benzoic acid (1.500 g, 4.772 mmol) and *N,N'*-carbonyldiimidazol (CDI) (0.774 g, 4.773 mmol) were placed in a flame dried flask, fitted with a condenser, and stirred in dry tetrahydrofuran (50 mL) under a nitrogen atmosphere for 15 min. *O*-phenylenediamine (0.675 g, 6.242 mmol) was then added and the reaction was stirred for a further 15 min before being heated to reflux overnight. After cooling to room temperature, the orange solid was filtered and washed twice with light petroleum (bp. 40-60 °C) before being dried under vacuum (1.102 g, 3.862 mmol, 81%). ^{1}H NMR ((CD$_2$)SO, 300 MHz, 298 K): 8.24 (d, $^3J_{H-H}$ = 8.1 Hz, 2H, H$^{11/14}$), 8.02 (d, $^3J_{H-H}$ = 8.1 Hz, 2H, H$^{11/14}$), 7.90 (t, $^3J_{H-H}$ = 8.1 Hz, 2H, H$^{12/13}$), 7.33 (t, $^3J_{H-H}$ = 8.1 Hz, 2H, H$^{12/13}$), 7.18 (t, $^3J_{H-H}$ = 7.7 Hz, 1H, H$^{4/5}$), 7.05 (d, $^3J_{H-H}$ = 7.7 Hz, 1H, H$^{3/6}$), 6.89 (d, $^3J_{H-H}$ = 7.6 Hz, 1H, H$^{3/6}$), 6.60 (t, $^3J_{H-H}$ = 7.6 Hz, 1H, H$^{4/5}$). ^{13}C[H] NMR ((CD$_2$)SO, 75 MHz): 156.5 (s, $C^{2/7/9}$),145.1 (s, $C^{2/7/9}$), 140.3 (s, $C^{2/7/9}$), 135.3 (s, $C^{12/13}$), 129.9 (s, $C^{4/5}$), 127.2 (s, $C^{3/6}$), 126.2 (s, $C^{11/14}$), 125.0 (s, $C^{10/15}$), 123.7 (s, $C^{12/13}$), 119.2 (s, $C^{11/14}$), 116.9 (s, $C^{4/5}$), 116.3 (s, $C^{3/6}$),113.6 (s, $C^{10/15}$).

X-ray crystallography

Description of the crystals: The crystals were mounted on a MiTeGen MicroMesh and fixed in a cold nitrogen stream. Diffraction intensities were recorded at 298, 173 or 140 K on a Rigaku HG Saturn724+ (2×2 bin mode) diffractometer or an Oxford Diffraction Xcalibur-3 instrument, both equipped with Mo-Kα radiation. Data collection, refinement and reduction were performed using the CrystalClear-SM Expert 3.1 b27 or CrysAlisPro software and the absorption correction was applied at this stage.[38]All structures were solved using SHELXS/T and refined by full-matrix least-squares methods on F^2 with SHELXL.[39] Non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were included in idealised positions. No missed symmetry was reported by PLATON.[40] Computer programs used in this analysis were run through WinGX.[41] Scattering factors for neutral atoms were taken from reference.[42]

Antiproliferation assay

The human A549 and HL60 cancer cell lines (from ECACC) were cultured in RPMI 1640 medium with 10% fetal calf serum, 2 mM L-glutamine, 100 U mL−1 penicillin and 100 µg mL−1 streptomycin (Invitrogen). The cells were maintained under a humidified atmosphere at 37 °C and 5% CO_2. The human MCF-7 cancer cell line (from ECACC) was cultured in DMEM medium with 10% fetal calf serum, 2 mM L-glutamine, 100 U mL−1 penicillin and 100 µg mL−1 streptomycin (Invitrogen). The cells were maintained under a humidified atmosphere at 37 °C and 5% CO_2. Inhibition of cancer cell proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) and following the manufacturer's instructions. Briefly, the cells (3×10^4 per 100 µL for HL60, 8×10^3 per 100 µL for A549 and MCF-7) were seeded in 96-well plates and left untreated or treated with 1 µL of DMSO (vehicle control) or 1 µL of complexes diluted in DMSO at different concentrations, in triplicate for 72 h at 37 °C with 5% CO_2. Following this, MTS assay reagent was added for 4 h and absorbance measured at 490 nm using a Polarstar Optima microplate reader (BMG Labtech). IC_{50} values were calculated using GraphPad Prism Version 5.0 software.

Uptake study

MCF-7 cells were grown in 75 cm^2 flasks up to 70% of confluence in 10 mL of culture medium. Compounds **2**, **5**, **6** and **11** were added to the flasks (100 µL of 1 mM solution in DMSO) and incubated for 6 h at 37 °C with 5% CO_2. Negative controls were used by incubating cells with DMSO alone under the same conditions. After removal of the medium and washing of the cells with PBS pH 7.4, the cells were detached using a trypsin solution. After quenching of trypsin with fresh medium, centrifugation and removal of the supernatant, the cell pellet was resuspended into 1 mL of PBS pH 7.4 and split into twice 500 µL for metal and protein quantification. The number of cells (expressed per million cells) of each sample was determined by measuring the protein content of the treated samples using a BCA assay (ThermoFischer Scientific) corrected by the amount of protein/10^6 cells determined for each cell type by measuring the protein content of an untreated sample and dividing by the corresponding number of cells measured with a hematocytometer following a reported procedure. Microwave digestion was used to solvate the samples to liquid form. Nitric acid and hydrogen peroxide were used in a Milestone Ethos 1 microwave system using SK-10 10 place carousel. The digest was ramped to 200 °C in 15 minutes holding at 200 °C for 15 min. The sample was weighed into a microwave vessel before digestion, and decanted and rinsed into a pre- weighed PFA bottle after digestion. ICP-MS samples were spiked with rhodium internal

standard and run on a Thermo X series 1 ICP-MS. The isotopes selected were ^{63}Cu, ^{65}Cu, ^{107}Ag, ^{109}Ag and ^{197}Au. Certified standards and independent reference were used for accuracy. Acid blanks were run through the system and subtracted from sample measurements before corrections for dilution.

FRET assay.

The initial FRET melting screen was performed using a fluorescence resonance energy transfer (FRET) DNA melting based assay. The sequence used was DS$_{FRET}$ FAM-d(TAT-AGC-TAT-A-HEG(18)-TAT-AGC-TAT-A)-TAMRA-3′). The labelled oligonucleotide (donor fluorophore FAM is 6-carboxyfluorescein; acceptor fluorophore TAMRA is 6-carboxytetramethyl-rhodamine) were prepared as a 220 nM solution in 10 mM sodium cacodylate buffer at the indicated pH with 100 mM sodium chloride and then thermally annealed. Strip-tubes (QIAgen) were prepared by aliquoting 20 μL of the annealed DNA, followed by 0.5 μL of the compound solutions. Control samples for each run were prepared with the same quantity of DMSO with the DNA in buffer. Fluorescence melting curves were determined in a QIAgen Rotor-Gene Q-series PCR machine, using a total reaction volume of 20.5 μL. Measurements were made with excitation at 470 nm and detection at 510 nm. Final analysis of the data was carried out using QIAgen Rotor-Gene Q-series software and Origin or Excel.

Electronic Supporting Information

Correlation between IC$_{50}$ and cell uptake, NMR spectra.CCDC numbers 1850050 (**7**·CH$_2$Cl$_2$) and 1850051 (**9**·CH$_2$Cl$_2$) contain the crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

References

1   B. Rosenberg, L.Vancamp and T. Krigas, *Nature*, 1965, **205**, 698-699.

2   R. Oun, Y. E. Moussa andN. J. Wheate, *Dalton Trans.,*2018, **47**, 6645-6653.

3   L. Galluzzi, L. Senovilla, I. Vitale, J. Michels, O. Kepp, M. Castedo and G. Kroemer, *Oncogene*, 2012, **15**, 1896-1883.

4   G. Jaouen, A. Vessières and S. Top, *Chem. Soc. Rev.,*2015, **44**, 8802–8817.

5   C. Santini,M. Pellei, V. Gandin, M. Porchia,F. Tisato and C. Marzano, *Chem. Rev.,*2014, **114**,815−862.

6   G. Süss-Fink, *Dalton Trans.,* 2010,**39**, 1673−1688.

7   M. Cini, T.D. Bradshaw and S. Woodward, *Chem. Soc. Rev.,*2017, **46**, 1040–1051.

8   (a) I Ott, *Coord. Chem. Rev.,*2009, **253**, 1670−1681. (b) B. Bertrand and A. Casini, *Dalton Trans.,*2014, **43**, 4209−4219;(c) T. T. Zou, C. T. Lum, C. N. Lok, J. J. Zhang and C. M. Che, *Chem. Soc. Rev.,*2015, **44**, 8786−8801.

9   S. Jürgens, F. E. Kühn and A. Casini, *Curr. Med. Chem.,*2018, **25**, 437-461.

10   B. Bertrand, M. R. M. Williams and M. Bochmann, *Chem. Eur. J.,* 2018, DOI: 10.1002/chem.201800981.

11   M. Joost, A. Amgoune and D. Bourissou, *Angew. Chem. Int. Ed.,*2015, **54**, 15022-15045.

12   (a) C. Bronner and O. S. Wenger, *Dalton Trans.,*2011, **40**, 12409–12420; (b) W.-P.To, D.-L.Zhou, G. S. M. Tong, G. Cheng, C. Yang and C.-M.Che, *Angew. Chem. Int. Ed.,*2017, **56**, 14036 – 14041;(c) L. Currie, J. Fernandez-Cestau, L. Rocchigiani, B. Bertrand, S. J. Lancaster, D. L. Hughes, H. Duckworth, S. T. E. Jones, D. Credgington, T. J. Penfold and M. Bochmann, *Chem. Eur. J.,*2017, **23**, 105-113.

13   (a) R. W.-Y.Sun, C.-N.Lok, T. T.-H. Fong, C. K.-L. Li, Z. F. Yang, T. Zou, A. F.-M. Siu and C.-M.Che, *Chem. Sci.,* 2013, **4**, 1979–1988; (b) N. Cutillas, G. S. Yellol, C. de Haro, C. Vicente, V. Rodriguez and J. Ruiz, *Coord. Chem. Rev.,* 2013, **257**, 2784–2797; (c) S. K. Fung, T. Zou, B. Cao, P.-Y.Lee, Y. M. E. Fung, D. Hu, C.-N.Lok and C.-M.Che, *Angew. Chem. Int. Ed.* 2017, **56**, 3892 – 3896.

14   K. J. Kilpin, W. Henderson and B. K. Nicholson, *Polyhedron*, 2007, **26**, 434-447.

15   C. H. A. Goss, W. Henderson, A. L. Wilkins and C. Evans, *J. Organomet.Chem.,*2003, **679**, 194-201.

16   J. J. Zhang, R. W. Y. Sun and C. M. Che, *Chem. Commun.,*2012, **48**, 3388-3390.

17   M. Williams, A. I. Green, J. Fernandez-Cestau, D. L. Hughes, M. A. O'Connell, M. Searcey, B. Bertrand and M. Bochmann, *Dalton Trans.,*2017, **46**, 13397-13408.

18   B. Bertrand, J. Fernandez-Cestau, J. Angulo, M. M. D. Cominetti, Z. A. E. Waller, M. Searcey, M. A. O'Connell and M. Bochmann, *Inorg. Chem.,*2017, **56**, 5728-5740.

19   L. Messori, P. Orioli, C. Tempi and G. Marcon, *Biochem. Biophys. Res. Commun.,*2001, **281**, 352-360.

20   (a) T. Yang, C. Tu, J. Y. Zhang, L. P. Lin, X. M. Zhang, Q. Liu, J. Ding, Q. Xu and Z. J. Guo, *Dalton Trans.,* 2003, 3419-3424; (b) P. F. Shi, Q. Jiang, Y. M. Zhao, Y. M. Zhang, J. Lin, L. P. Lin, J. Ding and Z. J. Guo, *J. Biol. Inorg. Chem.,*2006, **11**, 745-752.

21   J. Janockova, J. Plsikova, J. Kasparkova, V. Brabec, R. Jendzelovsky, J. Mikes, J. Koval, S. Hamulakova, P. Fedorocko, K. Kuca and M. Kozurkova, *Eur. J. Pharm. Sci.,*2015, **76**, 192-202.

22   L. A. Howell, R. Gulam, A. Mueller, M. A. O'Connell and M. Searcey, *Bioorg. Med. Chem. Lett.,*2010, **20**, 6956-6959.

23   L. A. Howell, R. A. Bowater, M. A. O'Connell, A. P. Reszka, S. Neidle and M. Searcey, *ChemMedChem*, 2012, **7**, 792-804.

24   C. L. Smyre, G. Saluta, T. E. Kute, G. L. Kucera and U. Bierbach, *ACS Med. Chem. Lett.,*2011, **2**, 870-874.

25   A. C. Matsheku, M. Y. H. Chen, S. Jordaan, S. Prince, G. S. Smith and B. C. E. Makhubela, *Appl. Organomet.Chem,*2017, **31**, 3852-3865.

26   (a)L. C. Eiter, N. W. Hall, C. S. Day, G. Saluta, G. L. Kucera and U. Bierbach, *J. Med. Chem.,*2009, **52**, 6519-6522; (b) R. Visbal, V. Fernández-Moreira, I. Marzo, A. Laguna and M. C. Gimeno, *Dalton Trans.,* 2016, **45**, 15026-15033;(c) S. A. Perez, C. de Haro, C. Vicente, A. Donaire, A. Zamora, J. Zajac, H. Kostrhunova, V. Brabec, D. Bautista and J. Ruiz, *ACS Chem. Biol.,*2017, **12**, 1524-1537.

27   J. Fernandez-Cestau, B. Bertrand, M. Blaya, G. A. Jones, T. J. Penfold and M. Bochmann, *Chem. Commun.,*2015, **51**, 16629–16632.

28   M. C. Gimeno, A. Laguna and R. Visbal, *Organometallics,*2012, **31**, 7146-7157.

29   S. Spreckelmeyer, C. Orvig and A. Casini, *Molecules, 2014*, **19**, 15584-15610.

30   (a) 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 and A. Casini, *Inorg. Chem.,*2011, **50**, 9472-9480; (b) P.-E.Doulain, R. Decréau, C. Racoeur, V. Goncalves, L. Dubrez, A. Bettaieb, P. Le Gendre, F. Denat, C. Paul, C. Goze and E. Bodio, *Dalton Trans.,*2015, **44**, 4874-4883;(c) C. Schmidt, B. Karge, R. Misgeld, A. Prokop, M. Brönstrup and I. Ott, *Med. Chem. Commun.*2017, **8**, 1681-1689.

31   S. Spreckelmeyer, N. Estrada-Ortiz, G. G. H. Prins, M. van der Zee, B. Gammelgaard, S. Stürup, I. A. M. de Graaf, G. M. M. Groothuis and A. Casini, *Metallomics,*2017, **9**, 1786-1795.

32   M. Searcey, P. N. Martin, N. M. Howarth, B. Madden and L. P. G. Wakelin, *Bioorg. Med. Chem. Lett.,*1996, **6**, 1831-1836.

33   A. De Cian, L. Guittat, M. Kaiser, B. Sacca, S. Amrane, A. Bourdoncle, P. Alberti, M. P. Teulade-Fichou, L. Lacroix and J. L. Mergny, *Methods,* 2007,**42**, 183-195.

34   (a) A. Casini, M. A. Cinellu, G. Minghetti, C. Gabbiani, M. Coronnello, E. Mini and L. Messori, *J. Med. Chem.*,2006, **49**, 5524-5531;(b) T. Zou, C. T. Lum, S. S.-Y. Chui and C.-M.Che, *Angew. Chem. Int. Ed.,* 2013,**52**, 2930-2933.

35   L. Currie, L. Rocchigiani, D. L. Hughes and M. Bochmann, *Dalton Trans.,*2018, **47**, 6333-6343.

36   E. C. Constable and T. A. Leese, *J. Organomet. Chem.,* 1989, **363**, 419-424.

37   M. A. Cinellu, A. Zucca, S. Stoccoro, G. Minghetti, M. Manassero and M. Sansoni, *J. Chem. Soc. Dalton Trans.,* 1995, 2865-2872.

38   Programs CrysAlisPro, Oxford Diffraction Ltd., Abingdon, UK, 2010.

39   G. M. Sheldrick, *ActaCryst.*2008, **A64**, 112-122.

40   (a) A. L. Spek, PLATON – A Multipurpose Crystallographic Tool, Utrecht University, Utrecht, The Netherlands, 2006; (b) A. L. Spek, *ActaCryst.*1990, **A46**, C34.

41   L. J. Farrugia, *J. Appl. Crystallogr.,* 1999,**32**, 837-838.

42   'International Tables for X-ray Crystallography', Kluwer Academic Publishers, Dordrecht.Vol. C. 1992, pp. 500, 219 and 193.

43   S. J. Coles and P. A. Gale, *Chem. Sci*. 2012, **3**, 683-689.