Anticancer Gold *N*-Heterocyclic Carbene Complexes: A Comparative in vitro and ex vivo Study

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A series of organometallic Au[I] *N*-heterocyclic carbene (NHC) complexes was synthesized and characterized for anticancer activity in four human cancer cell lines. The compounds' toxicity in healthy tissue was determined using precision-cut kidney slices (PCKS) as a tool to determine the potential selectivity of the gold complexes ex vivo. All evaluated compounds presented cytotoxic activity toward the cancer cells in the nano- or low micromolar range. The mixed Au[I] NHC complex, (*tert*-butylethynyl)-1,3-bis-(2,6-diisopropylphenyl)imidazol-2-ylidene gold(I), bearing an alkynyl moiety as ancillary ligand, showed high cytotoxicity in cancer cells in vitro, while being barely toxic in healthy rat kidney tissues. The obtained results open new perspectives toward the design of mixed NHC–alkynyl gold complexes for cancer therapy.

Metals and metal complexes have been used for medicinal applications since ancient times. For example, copper was the first metal described as a sterilizing agent between 2600 and 2200 BCE (Smith Papyrus). Furthermore, the Ebers Papyrus from 1500 BCE describes the use of copper to reduce inflammation and the use of iron to treat anemia, while at roughly the same time, zinc was being used to heal wounds.[1,2] In recent years, medicinal inorganic chemistry has become a rapidly growing field with a broad range of medical applications for inorganic and metal-based compounds, including mineral supplements (Fe, Zn, Cu, Se), diagnostics (Gd, Mn, Ba, I), antimicrobial (Ag), anticancer (Pt), antiulcer (Bi) and antiarthritic (Au) agents, among others.[3,4]

The interest in developing new metal-containing therapeutic compounds increased largely after the success of cisplatin in the treatment of solid malignancies.[5] Unfortunately, platinum(II)-containing compounds present several limitations such as toxicity in healthy tissues, restricted spectrum of activity and development of resistance.[5,6]

Subsequently, an extensive number of metal-containing compounds were described with interesting cytotoxic activities, displaying diverse mechanisms of action and pharmacological profiles.[7–10] Within this framework, gold-based complexes are particularly interesting due to their different possible oxidation states (e.g., Au[I] and Au[III]), stability and ligand exchange reactions, which confer them different mechanisms of activity relative to cisplatin.[4,11,12]

Early studies on the anticancer activity of the Au[I] complex auranofin ([Au[I](2,3,4,6-tetra-*O*-acetyl-1-(thio-kS)-b-d-glucopyranosato)(triethylphosphine)]), presently used in the clinic to treat severe rheumatoid arthritis, revealed activity levels similar to cisplatin in vitro,[13] which subsequently led to a large number of Au[I] complexes being evaluated for antiproliferative effects. Interestingly, most of the anticancer gold complexes reported so far, appear to exert their activity via the interaction with protein targets,[12–14] and only in a few cases DNA binding has emerged as a likely route to cancer cell death.[15,16]

Among the various families of gold compounds tested for their anticancer effects in the last decade, a variety of organometallic gold(I/III) *N*-heterocyclic carbene (NHC) complexes were designed, featuring anticancer activity in the micromolar or sub-micromolar range in vitro.[17–21] Specifically, gold(I) NHC derivatives exert their effects via different pathways, including: 1) mitochondrial damage (common for cationic gold(I) biscarbene complexes, which behave as delocalized lipophilic cations (DLC) being able to accumulate selectively inside the mitochondria of cancer cells due to their higher mitochondrial membrane potential);[21–25] 2) inhibition of the seleno-enzyme thioredoxin reductase;[26–28] 3) inhibition of protein tyrosine phosphatases (PTP);[29] and 4) stabilization of DNA G-quadruplexes.[30–32] Moreover, gold(I) NHC complexes are appealing from a synthetic point of view due to their high stability with respect to ligand exchange reactions, relatively easy synthetic procedures, possibility of functionalization leading to increased structural diversity, as well as tunable lipophilic–hydrophilic properties to enhance biological activity.[11,19,33,34]

Therefore, herein we report the synthesis of four Au[I] NHC complexes (1–4; Figure 1) including: mono- (1) and bis-NHC (2) compounds featuring a 1-butyl-3-methylimidazol-2-yilidene ligand. This ligand was chosen to obtain gold complexes possessing favorable lipophilicity, in line with previous studies.[33] Based on the same NHC scaffold, an auranofin-type complex 3 was obtained, where the NHC moiety replaces the phosphane ligand. Moreover, a mixed NHC-alkynyl complex 4 was synthe-

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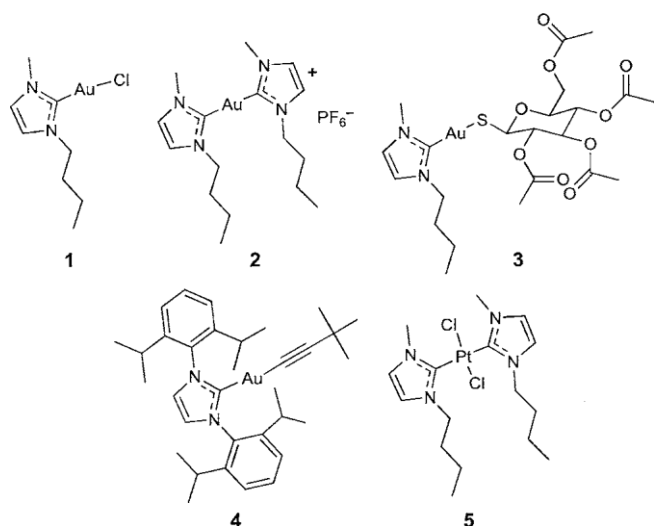
Figure 1. Structures of reported metal NHC complexes.

sized, with a 1,3-bis-(2,6-diisopropylphenyl)imidazol-2-ylidene ligand. The latter was chosen because it was impossible to stabilize the mixed NHC-alkynyl metal complexes using the 1-butyl-3-methylimidazol-2-ylidene ligand.

It should be noted that, recently, Au^I complexes of the type (alkynyl)Au^I(phosphane), containing an anionic alkynyl group as well as a neutral phosphane ligand, have been shown to possess interesting anticancer effects in vitro and in vivo.[35,36] However, to the best of our knowledge the anticancer properties of mixed NHC-alkynyl Au^I complexes have not been reported so far. Finally, for comparison purposes and to evaluate the effect of the metal ion on the biological activity, the Pt^{II} (5) analogue of 2 was also synthesized.

The biological activity of our series of Au^I NHC complexes was evaluated both in vitro and ex vivo. Specifically, the cytotoxicity of the compounds was tested in four cancer cell lines, namely a p53 wild-type and a p53 null variant of HCT 116 (colorectal carcinoma), MCF-7 (breast adenocarcinoma) and A375 (malignant melanoma). Additionally, the new compounds were tested for their toxicity on healthy rat kidney tissue ex vivo using precision-cut kidney slices (PCKS).[37] In PCKS, all cells remain in their natural environment maintaining the original cell–cell and cell–matrix contacts, which are absent in classical 2D cell cultures in vitro.[37,38] This technique is an FDA-approved model for drug toxicity and metabolism studies.[37,38] Recently, we have successfully used the precision-cut tissue slices technique to study the toxic effects of experimental anticancer organometallic compounds,[31,39,40] aminoferrocene-containing prodrugs,[41] ruthenium-based kinase inhibitors,[42] as well as supramolecular metallacages as possible drug delivery systems.[43]

The carbene (1-butyl-3-methylimidazol-2-ylidene)gold(I)-chlorido Au(BMIm)Cl (1) and the bis-carbene bis(1-butyl-3-methylimidazole-2-yilidene)gold(I) hexafluorophosphate [Au(B-MIm)$_2$]PF$_6$ (2) were synthetized according to previously reported procedures,[34] and adapted established protocols.[33,44] Thus, synthesis of compound 1 was achieved by a transmetalating route from the correspondent Ag^I carbene.

Compound 2 was synthetized by reaction of Au(SMe$_2$)Cl with 2 equiv of 1-methyl-3-butyl-2-ylidene, prepared in situ by deprotonation of the correspondent imidazolium salt with LiHMDS.

Complex 3 is a new analogue of auranofin bearing thio-b-glucose-tetraacetate as ancillary ligand while replacing the phosphine with the NHC carbene ligand. The compound was obtained in high yields by adapting a procedure previously published by Baker et al.[45] that allows the substitution of a chlorido ligand using K$_2$CO$_3$ in CH$_2$Cl$_2$. The compound was found to be soluble in chlorinated solvents (dichloromethane and chloroform), in acetone and in DMSO. This new gold(I) complex was characterized in solution by ^{1}H and ^{13}C NMR in CDCl$_3$ (Figures S1 and S2 in the Supporting Information). The signals are consistent with those reported for similar compounds, confirming the proposed structure.[33,44]

The most diagnostic feature in the ^{13}C NMR spectrum of compound 3 (Figure S2) is the carbene carbon signal at 183.7 ppm, which shows a downfield shift compared with the signal at 171.9 ppm of the corresponding precursor 1 with chlorido as ancillary ligand, most likely due to the better donating ability of the thiolate ligand.[44] The signal of the thiol carbon (C1') shows a downfield shift from 78.9 ppm to 83.0 ppm as a consequence of the coordination to the gold(I) center. In the ^{1}H NMR spectrum (Figure S1) the coordination of the thiolate is confirmed by the absence of the S H resonance. In FT-IR spectrum the most diagnostic feature is the band of the carbonyls at $\tilde{n} = 1744$ cm^{-1}. (Figure S3).

Preparation of the gold(I) alkynyl compound 4 was performed according to already established procedures,[46,47] by reacting 3,3-dimethyl-1-butyne with the precursor gold(I) NHC carbene (Au(IPr)Cl) in presence of a strong base (tBuOK) in MeOH. The compound was characterized in solution by ^{1}H and ^{13}C NMR in CDCl$_3$ (Figure S4 and S5). The ^{13}C NMR spectrum is featured by the signal of the carbene carbon at low field (d= 191.9 ppm). Furthermore, the quaternary carbons of the alkynyl moiety are downfield shifted (d = 114.1 and 112.5 ppm) relative to the signals in the precursor (d= 92.7 and 66.5 ppm) owing to the coordination to gold(I). In the ^{1}H NMR he signal of the four isopropyl protons is a diagnostic feature, featuring a septet at 2.60 ppm as well as the corresponding doublets of the methyl groups at 1.34 and 1.18 ppm. Another characteristic feature of the spectrum is the singlet of the three methyl groups of the alkynyl moiety at 1.10 ppm. In the FT-IR spectrum the weak band of the triple bond at $\tilde{n} = 2116$ cm^{-1} can be recognized (Figure S6).

Compound 5 is a platinum(II) derivative bearing the same NHC ligand of compounds 1, 2, 3. It was synthesized by trans-metalating routes from the correspondent Ag^I carbene by adapting previously reported procedures.[48] Thus, 5 was obtained by holding Ag(BMIm)Cl at reflux with 0.5 equiv of K$_2$PtCl$_4$ in CH$_2$Cl$_2$ for 4 days.

The ^{1}H NMR spectrum of 5 in CDCl$_3$ (Figure S7) shows the superposition of two sets of signals (shifted of d 0.03 ppm) due to the presence of two isomers. [195]Pt NMR spectrum confirms the presence of two complexes in which the metal nucleus resonates at close chemical shifts (d= 3177.8,

3179.0 ppm; Figure S8). The nature of the isomers can be better elucidated through ^{13}C NMR spectrum (Figure S9). It suggests the presence of the two rotamers *trans–syn* and *trans–anti* as frequently observed for nickel(II) and palladium(II) complexes of two unsymmetrical NHC ligands.[49–51] As a matter of fact, only one carbenic carbon signal at chemical shift typical of *trans*-bis(NHCs)PtX$_2$ complexes is present (d= 167.3 ppm).[48] The formation of the *cis* isomer can therefore be excluded, as it would have given a carbenic carbon signal at significantly upfield shifted chemical shift as reported for similar compounds (d 132–150 ppm).[52,53]

The stability of gold compounds 3 and 4 was monitored by ^{1}H NMR spectroscopy of mixtures of water and [D$_6$]DMSO during 7 days at room temperature. The complexes were found to be stable in solution in these conditions (Figures S10–S11). Moreover, the two complexes were reacted with 1.5 equivalents of d,l-homocysteine, as an intracellular model nucleophile, in CD$_3$OD. ^{1}H NMR spectra revealed that no reaction with 3 occurred after 24 h. Indeed, neither the signals of the complex nor of homocysteine showed any variation (Figures S12–14).

In contrast, compound 4 was found to react with homocysteine with the formation of a new species that could be identified as the product of the substitution of the alkyne with the thiol. Indeed, a series of ^{1}H NMR spectra registered during 24 h shows the progressive conversion of 4 into a new complex (Figure S15), most likely involving coordination of the amino acid to the gold center with substitution of the alkynyl moiety. In fact, after 24 h, new signals in the region of aromatic protons are visible (from d= 7.55, 7.50, 7.36 ppm of the precursor to d= 7.62 ppm, d= 7.54, 7.37 ppm), the signal of one of the doublets of the methyl groups in the isopropyl moiety shifted from d= 1.23 to 1.26 ppm. Another relevant feature is the simultaneous progressive disappearance of the signal of the methyl groups of the alkyne at d= 1.03 with the formation of a new signal at d= 1.22 ppm, consistent with the presence of a dissociated 3,3-dimethyl-butyne moiety (Figure S16–S18). Furthermore, the integration of the signals of coordinated homocysteine with those of the NHC moiety led us to suppose that only the alkyne was substituted by the amino acid nucleophile under these conditions (Figure S19). This hypothesis was further confirmed by the ^{13}C NMR spectrum of the mixture after 24 h, where the carbenic carbon signal is still present (d= 173.7 ppm; Figure S20).

The antiproliferative properties of the Au[I] NHC complexes 1–4 and of cisplatin and auranofin as comparison, were assessed using the MTT assay in the human cancer cell lines HCT116 p53 wt, HCT116 p53 null, MCF-7 and A375. All the tested Au[I] complexes presented antiproliferative effects in the evaluated cell lines in the low micromolar range (Table 1). Instead, the Pt[II] complex 5 was scarcely active (EC$_{50}$ > 50 mm). The latter result is in accordance with other studies on Pt[II] bis-NHC complexes with chlorido ligands (see Ref. [20] and citations therein) featuring moderate cytotoxic effects in vitro. Notably, the compounds 1, 2 and 3 showed superior activity, in the nanomolar or low micromolar range, compared with cisplatin and auranofin in three of the four cell lines, A375 being the exception.

Compound	EC$_{50}$ [mm][a]			
	HCT116 p53wt	HCT116 p53null	MCF-7	A375
1	0.5 0.3	0.6 0.2	0.8 0.4	2 1
2	0.6 0.4	0.24 0.09	1.1 0.3	1.0 0.6
3	0.8 0.2	1 0.4	2 0.8	1.2 0.2
4	9 3	8.8 0.9	6 2	10 1
5	>50	>50	>50	ND
auranofin	5.1 0.7	7.10 0.01	7 2	1.3 0.8
cisplatin	11 3	20.6 0.9	12 2	3.7 0.9

Table 1. EC$_{50}$ values of Au[I] NHC complexes in various human cancer cell lines in comparison with auranofin and cisplatin after 72 h incubation.

[a] Values were calculated using a nonlinear fitting of log[concentration] versus response and are presented as the mean SD of at least three independent experiments; ND: not determined.

Overall, no significant differences could be observed between the EC$_{50}$ values for the mono-carbene (1), bis-carbene (2), and auranofin-analogue (3) in the different cancer cell lines. Interestingly, the alkynyl derivative (4) was effective, albeit ~ 4–10-fold less potent than the other gold NHC complexes. However, in general 4 was markedly more active or as active as cisplatin, except in the A375 cell line. Additionally, the lack of differences observed between the HCT116 p53 wild-type and p53 null cells treated with the gold complexes indicates that their toxicity mechanism is independent of p53 activity, in contrast to cisplatin. The latter is known to induce rapid p53-dependent apoptosis and, as a secondary effect, p53-independent cell-cycle arrest.[54,55]

Due to their potent cytotoxic effects in cancer cells, complexes 1–4 were tested for their possible toxicity in an ex vivo model in healthy rat kidney tissue using the PCKS technology.[37,38] Kidney was selected because cisplatin is well known to induce severe nephrotoxicity in patients.[56] Hence, kidney slices were incubated with various concentrations of each gold complex, and after 24 h the viability of the tissues was determined measuring the ATP content (Table 2 and Figure 2). Auranofin and cisplatin were also tested for comparison.

The gold complexes, including auranofin, displayed a concentration dependent toxicity profile, with complexes 1 and 2 as the most toxic, with TC$_{50}$ below 1 mm. Interestingly, compounds 1–3 were more toxic than cisplatin (~ 6–12-fold). Remarkably, complex 4 showed no toxicity up to 50 mm. For com-

Compound	TC$_{50}$ [mm][a]	TC$_{50}$(PCKS)/EC$_{50}$(cells)
1	0.8 0.3	0.4–1.6
2	0.8 0.7	1.3–3.3
3	2.1 0.4	1.1–2.6
4	>50	> 5.0–8.3
auranofin	2.9 1.4	0.4–2.2
cisplatin	12 6	0.6–3.2

Table 2. Toxicity of Au[I] NHC complexes in PCKS in comparison with auranofin and cisplatin.

[a] Values were calculated using a nonlinear fitting of log[concentration] versus response and are presented as the mean SD of at least three independent experiments.
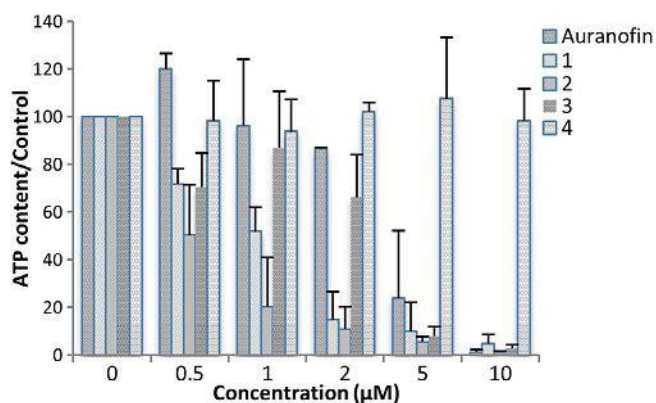
Figure 2. Viability of rat (PCKS) relative to the controls (untreated slices) after treatment with complexes 1–4 and auranofin for 24 h. The error bars show the standard deviation of at least three independent experiments. We performed an ANOVA analysis with each individual experiment as random effect and a Tukey HSD post-hoc test for pairwise comparisons.
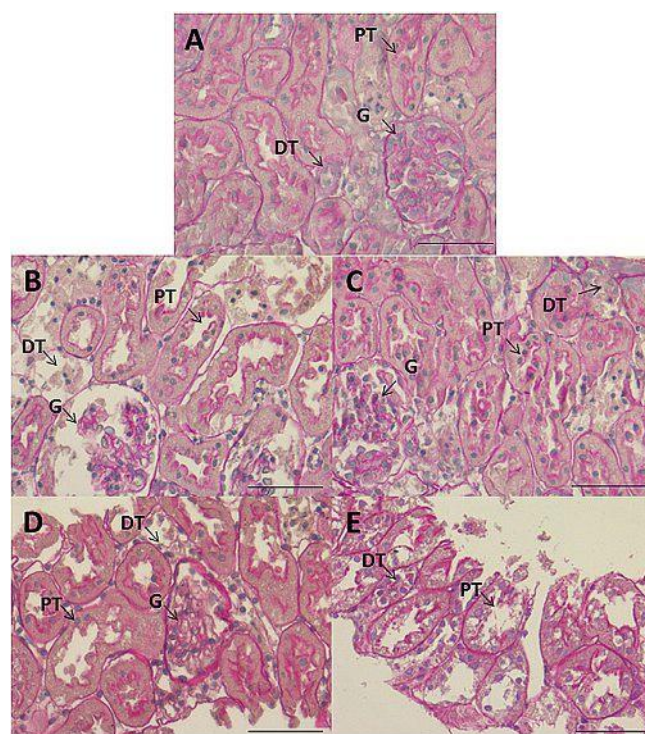

Figure 3. Morphology of rat kidney slices. A) 24 h control incubation, B) complex 1, C) complex 4, D) auranofin, E) cisplatin. The Au[I] complexes were incubated at 5 mm for 24 h, whereas cisplatin was present at 25 mm for 24 h. PT: proximal tubule, DT: distal tubule, G: glomerulus. Scale bars indicate 50 mm.

pounds 1–3 the safety margin for toxicity was small to absent with a ratio of TC_{50} PCKS/EC_{50} cells between 0.4 and 3.3. However, for compound 4 the TC_{50} PCKS/EC_{50} cells ratio cells was higher than 5.0 to 8.3 indicating selective toxicity toward cancer cells over healthy kidney tissue.

The differential effects of complexes 1 and 4 (5 mm concentration) on kidney slices were further assessed by histomorphology using periodic acid–Schiff staining (PAS) to evaluate slice integrity and particularly to visualize the basement membranes and epithelial brush border in the proximal tubule cells, as reported in the experimental section. After 24 h incubation, the untreated kidney slices show minor morphological changes, indicated by pyknosis and swelling of some of the tubular cells (Figure 3 A). Marked effects were observed upon treatment with complex 1, which induced dilatation of Bowman's space in the glomerulus and necrosis of the distal tubule cells, as well as discontinuation of the brush border in the proximal tubule cells at some sites (Figure 3 B). In contrast, exposure of slices to complex 4 (Figure 3 C) did not induce significant morphological changes relative to controls. Auranofin treatment led to damage of the distal tubule cells and loss of nuclei from the proximal tubule cells. Moreover, cisplatin treatment (25 mm corresponding to 2-fold the TC_{50}, Figure 3 E) showed damage to the proximal tubular cells with loss of nuclei and discontinuation of the brush border; additionally, damage of the distal tubule is evident, as previously reported.[57]

The broad spectrum and synthetic possibilities in organometallic chemistry allowed us to develop and study different NHC ligands "fine-tuning" the physicochemical properties of the resulting Au[I] complexes, and, possibly, achieving selectivity toward cancer tissue. Herein, we reported the synthesis of complexes 3 and 4, as a continuation of our previous work where we described the synthesis and very preliminary biological evaluation of complexes 1 and 2.[34] In this study, complexes 1–4 showed interesting cytotoxic activity against HCT116 p53 wild-type and p53 null, MCF-7 and A357, in the low micromolar range. Instead, the Pt[II] complex 5 was poorly

cytotoxic, indicating the essential role of Au[I] ions in the biological activity. However, complexes 1–3 and auranofin displayed severe toxicity in healthy kidney tissue (PCKS), even higher than cisplatin, indicating that these compounds, when administered in vivo, may also induce severe nephrotoxicity. Conversely, the mixed NHC and alkynyl complex 4 appears to be at least 5-fold less toxic in healthy tissue, while maintaining antiproliferative effects at the low micromolar concentration. The decreased bioactivity of this compound may be partly due to its lower stability in the presence of nucleophiles, such as sulfur donor ligands. However, the selectivity of 4 for cancer cells with respect to healthy tissues is still promising in comparison with the other tested Au[I] NHC complexes. This initial result prompts us to develop new mixed organometallics with enhanced selectivity against cancer cells. Moreover, ongoing studies in our labs are aimed at further investigating the mechanisms of action of toxicity in cancer cells and kidney slices. So far, pilot studies using mass spectrometry (MS) analysis of complexes 1–4 showed no reactivity toward model protein targets (cytochrome c and lysozyme), while 1 and 2 could bind to the copper chaperon protein Atox-1 upon complete ligand loss.[34] Overall, we believe that it is important that toxicology studies, as those presented here using our ex vivo model, should be conducted as early as possible on new experimental metallodrugs to select the optimal chemical scaffolds and to orient the drug design at its early stages.

Experimental Section

General: Unless stated otherwise the reactions were performed under inert atmosphere of nitrogen in anhydrous conditions. Solvents and reagents were used without prior treatments. NMR spectra were recorded on a Varian Gemini 200 BB instrument (^{1}H, 200 MHz; ^{13}C, 50.3 MHz; ^{195}Pt, 42.8 MHz) at room temperature; frequencies are referenced to the residual resonances of the deuterated solvent. UV/Vis spectra were recorded on an Agilent Cary 60 spectrophotometer. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Spectrum One PerkinElmer instrument equipped with a UATR unit.

Synthetic procedures: The carbenes (1-butyl-3-methylimidazol-2-yilidene)silver(I)chlorido Ag(BMIm)Cl, (1-butyl-3-methylimidazol-2-yilidene)gold(I)chlorido Au(BMIm)Cl (1) and the bis-carbene bis(1-butyl-3-methylimidazole-2-yilidene)gold(I) hexafluorophosphate [Au(BMIm)$_2$]PF$_6$ (2) were synthetized according to procedures previously reported by us,[34] and their purity was confirmed by elemental analysis, resulting to be > 98 %.

Synthesis of thio-b-d-glucose-tetraacetate-(1-butyl-3-methyl)imidazol-2-ylidene-gold(I) (3): Precursor 1 was reacted with thio-b-d-glucose-tetraacetate to prepare compound 3. Compound 1 (0.1 mmol) was dissolved in 15 mL of CH$_2$Cl$_2$, 1 mmol of K$_2$CO$_3$ and
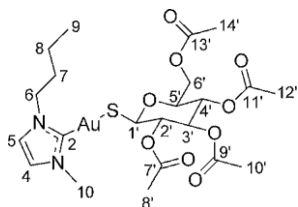
0.1 mmol of thio-b-glucose-tetracetate (tgt) were added to the solution. The suspension was stirred at room temperature for 10 min in the dark. The mixture was filtered and the solution was concentrated by evaporation to ∼ 2 mL. The product was precipitated with hexane and washed to obtain a light-brown solid (yield > 99 %). ^{1}H NMR (CDCl$_3$, 293 K): d = 6.90 (m, 2 H, H$_4$ and H$_5$), 5.12–5.03 (m, 3 H, tgt), 4.25–4.12 (m, 2 H, H$_6$, 3H tgt), 3.83 (s, 3 H, H$_{10}$), 3.76–3.68 (m, 1H, H$_{5'}$), 2.08 (s, 3H, OAc), 2.02 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 1.96 (s, 3 H, OAc), 1.83 (apparent quintet J = 7.4 Hz, 2 H, H$_{7a-b}$), 1.68 (s, H$_2$O), 1.37 (apparent sextet J = 7.4 Hz, 2 H, H$_{8a-b}$), 1.26 (hexane), 0.96 (t J = 7.4 Hz, 3 H, H$_9$), 0.86 ppm (hexane); ^{13}C NMR (CDCl$_3$, 293 K): d = 183.2 (C2), 170.8 (C=O), 170.3 (C=O), 169.9 (C=O), 169.7 (C=O), 121.4 (C4 or C5), 120.3 (C4 or C5), 83.3 (C1'), 77.9 (C2'), 75.8 (C5'), 74.5 (C3'), 69.2 (C4'), 63.2 (C6'), 50.9 (C6), 36.1 (C10), 33.4 (C7), 31.4 (hexane), 22.8 (hexane) 21.4 (OAc), 21.0 (OAc), 20.9 (2OAc), 19.8 (C8), 14.1 (hexane), 13.9 ppm (C9); IR (solid state): ñ = 2962 (w-m), 2916 (w), 2870 (vw), 1744 (m, CO), 1567 (vw), 1469 (w), 1412(w), 1258 (s), 1220 (m), 1086 (s), 1016 (vs), 911 (w-m), 865 (w-m), 795 (vs), 734 (w-m), 680 cm^{-1} (w-m); Anal. calcd (%) for C$_{22}$H$_{33}$AuN$_2$O$_9$S: C 37.70, H 4.75, N 4.00, found: C 37.64, H 4.65, N 3.98.

Synthesis of (tert-butylethynyl)-1,3-bis-(2,6-diisopropylphenyl)imidazol-2-ylidene gold(I) (4): The compound was prepared according to previously reported procedures.[46, 47] The colorless solid was obtained in 80 % yield. ^{1}H NMR (CDCl$_3$, 293 K): d = 7.48 (t, J = 7.4 Hz, 2 H, CH-aromatic), 7.28 (d, J = 7.4 Hz, 4 H, CH-aromatic), 7.06 (s, 2 H, CH-imidazole), 2.61 (septet, J = 6.9 Hz, 4 H, CH(CH$_3$)$_2$ isopropyl), 1.35 (d, J = 6.8 Hz, 12 H, CH(CH$_3$)$_2$ isopropyl), 1.18 (d, J = 6.8 Hz,

12 H, CH(CH$_3$)$_2$ isopropyl), 1.10 ppm (s, 9 H, C(CH$_3$)$_3$ tert-butylethynyl); ^{13}C NMR (CDCl$_3$, 293 K): d = 191.9 (NCN imidazole), 145.7 (CH aromatic), 134.7 (C aromatic), 130.4 (CH aromatic), 124.2 (CH aromatic), 123.2 (CH imidazole), 114.05 (Au-CC), 112.51 (Au-CC), 32.6 (C(CH$_3$)$_3$ tert-butylethynyl), 28.9 (CH(CH$_3$)$_2$ isopropyl), 28.3 (C(CH$_3$)$_3$ tert-butylethynyl), 24.5 (CH(CH$_3$)$_2$ isopropyl), 24.3 ppm (CH(CH$_3$)$_2$ isopropyl); IR (solid state): ñ = 3150 (w), 2966 (m/s), 2923 (m, sh), 2864 (w/m), 2116 (vw, CC), 1956 (vw), 1890 (vw), 1825 (vw), 1593 (w), 1720 (vw), 1677 (vw), 1650 (vw), 1558 (w), 1458 (s), 1410 (m), 1387 (w/m), 1364 (m), 1354 (w/m), 1343 (w/m), 1329 (w/m), 1275 (w), 1249 (m), 1208 (w/m), 1208 (w/m), 1181 (w/m), 1104 (w/m), 1082 (w/m), 1062 (w/m), 1032 (w), 981 (w), 944 (m), 805 (s), 764 (s), 731 (s), 697 (s), 56 cm^{-1} (w); ESI-MS (DMSO-MeOH), positive mode exact mass for [C$_{33}$H$_{46}$N$_2$Au]$^+$ (667.3321): measured m/z 667.3322 [M]$^+$; Anal. calcd (%) for C$_{33}$H$_{45}$AuN$_2$: C 59.45, H 6.80, N 4.20, found: C 59.39, H 6.58, N 4.18.

Synthesis of trans-dichloridobis(1-butyl-3-methylimidazole-2-yilidene)platinum(II) (5): K$_2$PtCl$_4$ (161 mg, 0.388 mmol) were suspended in 15 mL of CH$_2$Cl$_2$, and afterward 218 mg of Ag(BMIm)Cl (0.775 mmol) were added. The mixture was held at reflux for four days and then filtered on Celite. The solvent was removed under reduced pressure and the product was obtained in 86 % yield. ^{1}H NMR (CDCl$_3$, 293 K), syn and anti d = 6.80 (m, 2H, imidazole), 4.54–4.46 (m, 8H, NCH$_2$CH$_2$CH$_2$CH$_3$), 4.11, 4.08 (s+s, 12H, NCH$_3$), 2.14–1.96 (m, 8 H, NCH$_2$CH$_2$CH$_2$CH$_3$), 1.63 (H$_2$O), 146–1.43 (m, 8 H, NCH$_2$CH$_2$CH$_2$CH$_3$), 1.00 (t J^2 = 7.3 Hz, 6 H, NCH$_2$CH$_2$CH$_2$CH$_3$), 0.99 ppm (t, J^2 = 7.3 Hz, 6 H, NCH$_2$CH$_2$CH$_2$CH$_3$); ^{13}C NMR (CDCl$_3$, 293 K), syn and anti d = 167.3 (NCN), 121.3 (s + d, J_{Pt-c} = 23.3 Hz, CH imidazole), 120.1 (s + d, J_{Pt-c} = 24.5 Hz, CH imidazole), 50.0 (s + d, J_{Pt-c} = 18.9 Hz, NCH$_2$CH$_2$CH$_2$CH$_3$), 37.1 (NCH$_3$), 37.0 (NCH$_3$), 33.2 (NCH$_2$CH$_2$CH$_2$CH$_3$), 20.2 (NCH$_2$CH$_2$CH$_2$CH$_3$), 14.0 (NCH$_2$CH$_2$CH$_2$CH$_3$), 13.9 ppm (NCH$_2$CH$_2$CH$_2$CH$_3$); ^{195}Pt NMR (CDCl$_3$, 293 K), syn and anti d = 3177.8, 3179.0 ppm.

NMR stability studies in aqueous media and reactivity with homocysteine: Compound 3 (4 V 10^3 mmol) was dissolved in 0.4 mL of [D$_6$]DMSO/H$_2$O 60:40, while 4 (6 V 10^3 mmol) was dissolved in 0.4 mL of [D$_6$]DMSO/H$_2$O 80:20. ^{1}H NMR spectra were registered at various time intervals (0 h, 24 h, 1 week). Afterward, 5.0 V 10^3 mmol of 3 or 4.2 V 10^3 mmol of 4 were reacted with 1.5 equivalents of d,l-homocysteine in 0.4 mL of CD$_3$OD. Reactions were monitored by ^{1}H NMR (0 h, 6 h, 24 h) and ^{13}C NMR.

Cell lines: The human colorectal carcinoma HCT 116 p53 null and HCT 116 wild-type p53 variants (kindly provided by Dr. Gçtz Hartleben, University of Groningen), the human breast adenocarcinoma MCF-7 (Leibniz-Institut DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), and human malignant melanoma A375 (kindly provided by Prof. Sylvestre Bonnet, Leiden University) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing Glutamax supplemented with 10 % FBS and 1% penicillin/streptomycin (all from Invitrogen), at 378C in an incubator (Thermo Fisher Scientific, USA) under a humidified atmosphere of 95 % of air and 5% CO$_2$.

In vitro cell viability assays: Cells in an exponential growth rate were seeded (8000 cells per well) in 96-well plates (Costar 3595) and grown for 24 h in complete medium. Solutions of the gold compounds were prepared by diluting a stock solution (10^2 m in DMSO, ethanol in the case of auranofin) in culture medium (DMSO or ethanol in the culture medium never exceeded 1 %). Subsequently, different dilutions of the compounds were added to the wells to obtain a final concentration from 0.5 to 100 mm. Following 72 h of exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoli-

um bromide (MTT) was added to the cells at a final concentration of 0.50 mgmL $^{-1}$ in phosphate-buffered saline solution (PBS, pH 7.4) and incubated for 2.5 h. The incubation medium was removed and the violet formazan crystals in the cells were dissolved in DMSO, and the optical density of each well was quantified at 550 nm, using a multi-well plate reader (ThermoMax microplate reader, Molecular Devices, USA). The percentage of surviving cells was calculated from the ratio of absorbance between treated and untreated cells. Each treatment was performed in quadruplicate. The EC_{50} value was calculated as the concentration causing 50 % decrease in cell number and is presented as a mean SD of at least three independent experiments.

Preparation of rat precision-cut kidney slices (PCKS) and toxicity studies ex vivo: Male Wistar rats (Charles River, France) of 250–300 g were housed under a 12 h dark/light cycle at constant humidity and temperature. Animals were permitted ad libitum access to tap water and standard lab chow. All experiments were approved by the committee for care and use of laboratory animals of the University of Groningen and were performed according to strict governmental and international guidelines. Kidneys were harvested (from rats anesthetized with isoflurane) and immediately placed in University of Wisconsin solution (UW, ViaSpan, 4 8C) until further use. After removing fat, kidneys were cut in half lengthwise using a scalpel, and cortex cores of 5 mm diameter were made from each half perpendicular to the cut surface using disposable Biopsy Punches (KAI medical, Japan). PCKS were made as described by de Graaf et al.[37, 38] The cores were sliced with a Krumdieck tissue slicer (Alabama R&D, Munford, AL, USA) in ice-cold Krebs–Henseleit buffer, pH 7.4 saturated with carbogen (95 % O_2 and 5% CO_2). Kidney slices weighing ~ 3 mg (150 mm thickness), were incubated individually in 12-well plates (Greiner bio-one GmbH, Frickenhausen, Austria), at 378C in Williams' medium E (WME, Gibco by Life Technologies, UK) with Glutamax-1, supplemented with 25 mm d-glucose (Gibco) and ciprofloxacin·HCl (10 mg mL $^{-1}$, Sigma–Aldrich, Steinheim, Germany) in an incubator (Panasonic Biomedical) in an atmosphere of 80 % O_2 and 5% CO_2 with shaking (90 rpm). Stock solutions of compounds 1–4, auranofin, and cisplatin were prepared as for the studies on cell lines. The final concentration of DMSO and ethanol during the PCKS incubation was always < 0.5 % to exclude solvent toxicity. For each concentration, three slices were incubated individually for 1 h in WME and subsequently, different dilutions of the compounds were added to the wells, to obtain a final concentration from 0.5 to 50 mm. After this, PCKS were incubated for 24 h. After the incubation, slices were collected for ATP and protein determination, by snap freezing them in 1 mL of ethanol (70 % v/v) containing 2 mm EDTA with pH 10.9. After thawing the slices were homogenized using a mini bead beater and centrifuged. The supernatant was used for the ATP essay and the pellet was dissolved in 5 n NaOH for the protein essay. The viability of PCKS was determined by measuring the ATP using the ATP Bioluminescence Assay kit CLS II (Roche, Mannheim, Germany) as described previously.[37] The ATP content was corrected by the protein amount of each slice and expressed as pmol(mg protein) $^{-1}$. The protein content of the PCKS was determined by the Bio-Rad DC Protein Assay (Bio-Rad, Munich, Germany) using bovine serum albumin (BSA, Sigma–Aldrich, Steinheim, Germany) for the calibration curve. The TC_{50} value was calculated as the concentration that decreases the viability of the slices by 50 %, in terms of ATP content corrected by the protein amount of each slice and relative to the slices without any treatment, and is presented as the mean SD of at least three independent experiments.

Histomorphology: Kidney slices were fixed in 4% formalin for 24 h and stored in 70 % ethanol at 4 8C until processing for morphology studies. After dehydration, the slices were embedded in paraffin and 4 mm sections were made, which were mounted on glass slides and PAS staining was used for histopathological evaluation. Briefly, the glass slides were deparaffinized, washed with distilled water, followed by treatment with a 1 % aqueous solution of periodic acid for 20 min and Schiff reagent for 20 min, the slides were rinsed with tap water, finally, a counterstain with Mayer's hematoxylin (5 min) was used to visualize the nuclei.

Statistics: A minimum of three independent experiments were performed with the cells, with four replicates for each condition. PCKS were prepared from three rats and in each experiment slices were exposed in triplicate. The EC_{50} and TC_{50} values were calculated as the concentration reducing the viability of the cells or slices by 50 %, relative to the untreated samples using a nonlinear fitting of log[compound concentration] versus response and is presented as the mean SD of at least three independent experiments. Statistical testing was performed with one-way ANOVA with each individual experiment as random effect. We performed a Tukey HSD post-hoc test for pairwise comparisons. In all graphs and tables the mean values and standard deviation (SD) are shown.

Conflict of interest

The authors declare no conflict of interest.

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