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Caffeine-Based Gold(I) N-Heterocyclic Carbenes as Possible Anticancer Agents: Synthesis and Biological Properties

Benoît Bertrand, Loïc Stefan, Marc Pirrotta, David Monchaud, Ewen Bodio, Philippe Richard, Pierre Le Gendre, Elena Warmerdam, Marina H. de Jager, Geny M.M. Groothuis, Michel Picquet, and Angela Casini

Abstract

A new series of gold(I) N-heterocyclic carbene (NHC) complexes based on xanthine ligands have been synthesized and characterized by mass spectrometry, NMR, and X-ray diffraction. The compounds have been tested for their antiproliferative properties in human cancer cells and nontumorigenic cells *in vitro*, as well as for their toxicity in healthy tissues *ex vivo*. The bis-carbene complex $[\text{Au}(\text{caffein-2-ylidene})_2][\text{BF}_4]$ (complex 4) appeared to be selective for human ovarian cancer cell lines and poorly toxic in healthy organs. To gain preliminary insights into their actual mechanism of action, two biologically relevant in cellulo targets were studied, namely, DNA (more precisely a higher-order DNA structure termed G-quadruplex DNA that plays key roles in oncogenetic regulation) and a pivotal enzyme of the DNA damage response (DDR) machinery (poly-(adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP-1), strongly involved in the cancer resistance mechanism). Our results indicate that complex 4 acts as an efficient and selective G-quadruplex ligand while being a modest PARP-1 inhibitor (i.e., poor DDR impairing agent) and thus provide preliminary insights into the molecular mechanism that underlies its antiproliferative behavior.

Introduction

Following the discovery of the cytotoxic properties of cisplatin by Rosenberg at the end of the 1960s, [\(1\)](#) the interest for metal-based anticancer treatments increased tremendously. However, in spite of their great success, administration of platinum compounds presents important drawbacks such as severe side-effects and development of drug resistance, which limit their domain of applicability. [\(2\)](#) Therefore, many different inorganic and organometallic compounds have been developed and evaluated for anticancer activity, including platinum, ruthenium, iron, and gold complexes. [\(3-7\)](#) The latter have been the object of intense studies by our group among others. [\(8, 9\)](#)

In spite of their promising antiproliferative effects, the risk in developing gold compounds for biological applications is their remarkable oxidizing character of the gold(III)/gold(I) centers and a tendency to reduce to gold(I)/gold(0) leading to extensive and unselective cell damage, as well as to a compound's possible inactivation in an aqueous environment. This is particularly true within the fairly reducing intracellular milieu. Therefore, different families of organometallic gold complexes were

synthesized in which the presence of a direct carbon–gold bond greatly stabilizes the gold oxidation state and guarantees more controlled chemical speciation in biological systems. In general, both organometallic gold(I) and gold(III) compounds have increased stability compared to the classical gold-based coordination complexes, allowing the design of gold compounds in which the redox properties and ligand exchange reactions can be modulated to achieve selective activation in diseased cells.

Within this frame, in the last years, gold(I) *N*-heterocyclic carbenes (NHCs) have transformed from niche compounds to some of the most popular scaffolds in medicinal inorganic chemistry. (10, 11) In fact, several studies have described the promising anticancer activities of gold(I) NHC complexes in vitro and in a few cases also in vivo. (12) With respect to the possible mechanisms of action, the antiproliferative effects of gold NHC compounds have been shown to be mediated by strong antimitochondrial effects via inhibition of the seleno-enzymes thioredoxin reductase (TrxR), involved in maintaining the redox homeostasis of cells. (13-15) Thus, for example, cytotoxic compounds based on a benzimidazol-2-ylidene core, such as the chloro-(1,3-dimethylbenzimidazol-2-ylidene)gold(I) complex (1, [AuCl(Me₂BIm)]) Figure 1), (16) as well as on an imidazol-2-ylidene scaffold, (17-19) have been reported to be efficient inhibitors of TrxR. However, it should be noted that a general direct correlation between TrxR inhibition and cytotoxicity of gold NHC complexes could not always be demonstrated, and this indicates that other mechanisms besides TrxR inhibition might contribute to the overall pharmacological profile.

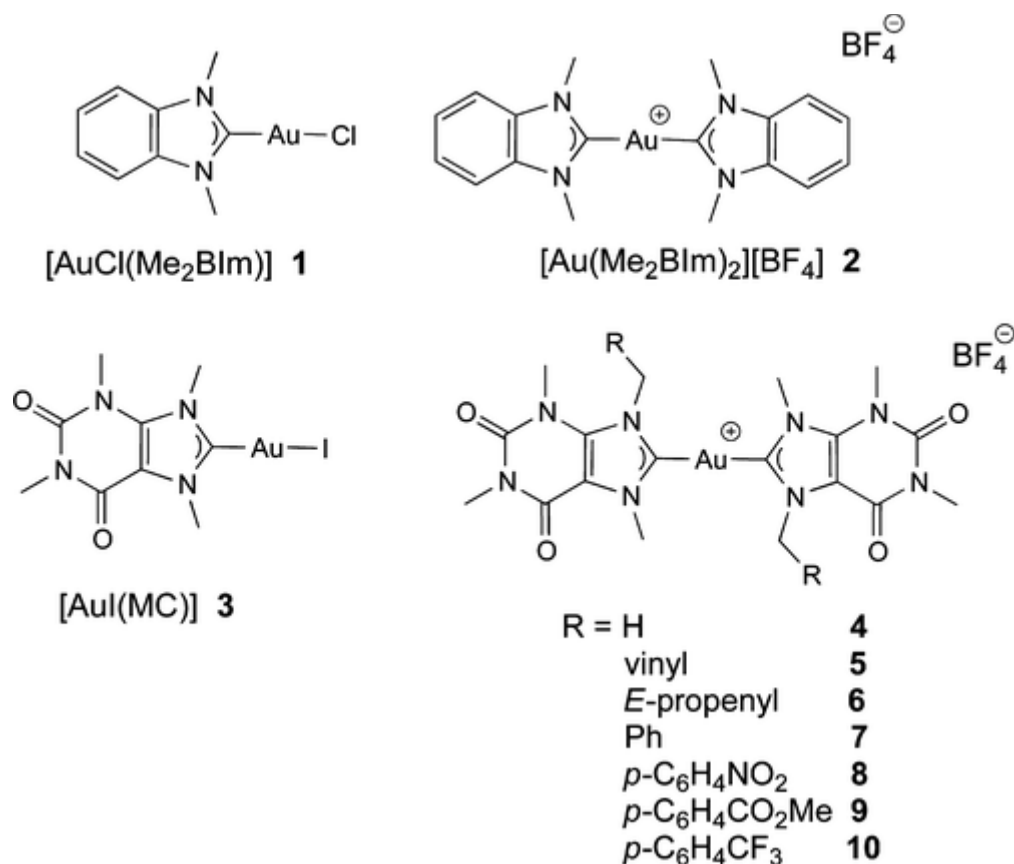


Figure 1. Structure of the gold(I) NHC complexes discussed in this study.

In view of these promising results, we designed new organometallic gold(I) complexes with a NHC ligand based on a caffeine scaffold as possible cytotoxic agents. Caffeine bears an imidazole ring so that it is a natural precursor of NHC; moreover, this natural compound, widely consumed in beverages, and its analogues have recently drawn attention for their possible therapeutic applications as anticancer agents. [\(20\)](#)

We thus report herein on the synthesis and in-depth characterization of the in vitro/ex vivo properties of seven new caffeine-based Au(I) NHC complexes, complexes **3–10** (Figure [1](#)), bearing the methylated caffeine-2-ylidene (MC) ligand and its N7-substituted theophylline analogues. All compounds were tested in vitro against different human cancerous cell lines (i.e., A2780, A2780/R, SKOV3, and A549) along with noncancerous cells (i.e., HEK-293T). For comparison purposes, the gold(I) NHC derivative **1** and its bis-carbene analogue **2** ($[\text{Au}(\text{Me}_2\text{Blm})][\text{BF}_4]$ Figure [1](#)) ([16](#), [21](#)) were also synthesized and tested against the aforementioned cells. To gain preliminary mechanistic insights, we screened the interactions of bis-NHC complexes **2**, **4–10** with both quadruplex- and duplex-DNA, on the basis of our recent results that support the idea that complex **4** can be a selective G-quadruplex interacting compound. [\(22\)](#)

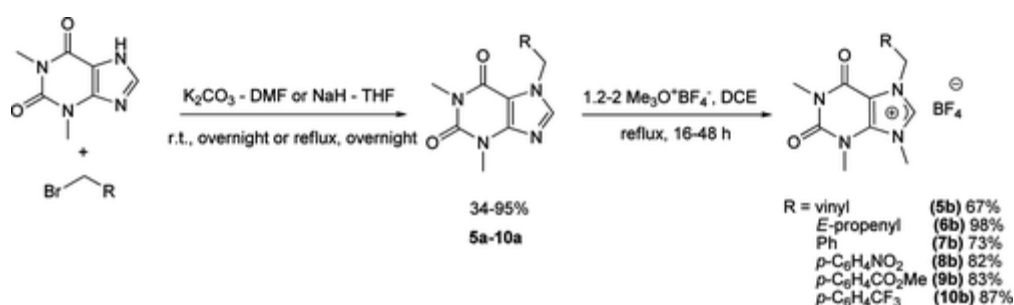
Thus, the properties of these complexes were further investigated: first, **2** and **4** were tested ex vivo in precision-cut tissue slices (PCTS) of liver, kidney, and colon to assess the compounds' toxicity in healthy organs; [\(23\)](#) PCTS are viable ex vivo explants of tissue with a reproducible and well-defined thickness, containing cells in their natural environment. Notably, this technique is a FDA-approved model for drug toxicity and metabolism studies. Second, compounds **2–4** were tested for the inhibition of the zinc-finger protein poly-(adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP-1), inspired by our recent report on the potent inhibition of this zinc-finger enzyme by Au(I) and Au(III) compounds. [\(24, 25\)](#) PARPs are key enzymes in the DNA damage response (DDR), binding to single-stranded breaks and base-excision sites to facilitate repair processes, and therefore, they are essential proteins involved in cancer resistance to chemotherapies, including cisplatin. [\(26\)](#) Studying both DNA interacting and DDR pivotal enzyme inhibitory properties enabled us to gain preliminary insights into the actual mechanism of action of these new gold(I) NHC complexes.

Results and Discussion

Synthesis and Structural Characterization

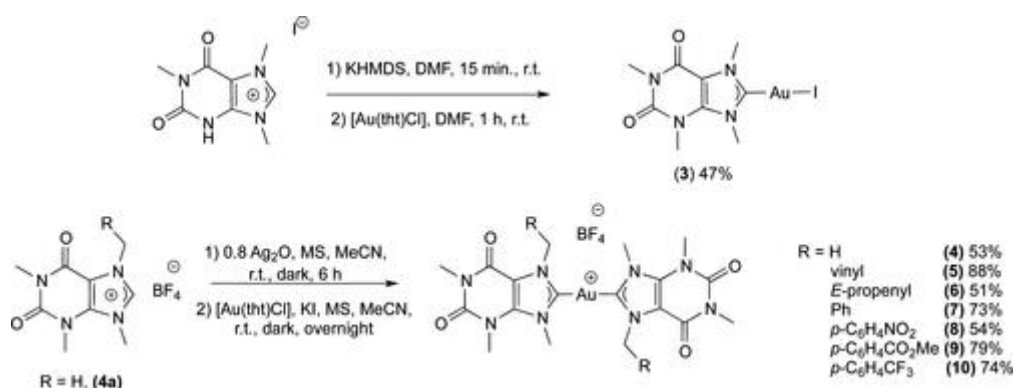
Initially, methylcaffeinium iodide and tetrafluoroborate were synthesized according to published methods by quaternization of caffeine using Meerwein's salt in refluxing 1,2-dichloroethane or a large excess of iodomethane in refluxing DMF. [\(27, 28\)](#) Due to a lack of nucleophilicity, quaternization of caffeine indeed requires harsh reaction conditions and is limited to methyl or benzyl substituents. [\(27-29\)](#) To enlarge the scope of these ligands, we considered an alternative route starting from theophylline. Thus, according to previously reported data by Petch et al., [\(30\)](#) theophylline was allowed to react with two equivalents of substituted allyl or benzyl bromide in the presence of

potassium carbonate in dry DMF at room temperature (Scheme 1). The resulting N7-substituted theophyllines **6a–10a** were obtained in moderate to good yields (51–95%) by precipitation upon addition of water. *N*-allyltheophylline (**5a**) did not precipitate in these conditions and was difficult to isolate; thus, it was obtained by deprotonation of theophylline using NaH in dry THF and addition of allyl bromide (34% yield). The next step consisted in the methylation of the N7-substituted theophyllines (**5a–10a**) with Meerwein's salt, affording the corresponding theophyllinium tetrafluoroborates (**5b–10b**) in refluxing 1,2-dichloroethane in good yields (Scheme 1).



Scheme 1. Synthesis of the Theophyllinium Tetrafluoroborates **5b–10b**

The neutral compound [Au(MC)] (**3**) was synthesized adapting a procedure described by Berners-Price et al., consisting of the deprotonation of the iodide salt of the methylated caffeinium to afford the free carbene, which was then reacted with [Au(tht)Cl] (Scheme 2). (31) The different cationic complexes **4–10** were synthesized via the commonly used silver carbene route by reacting caffeinium and theophyllinium tetrafluoroborates (**5b–10b**) with silver oxide and then with [Au(tht)Cl] (Scheme 2). (21) All new Au(I) complexes have been characterized by ¹H and ¹³C NMR and by ¹⁹F NMR in the case of complex **10**. The ¹H NMR spectra of complexes **3–10** are rather similar to those of the corresponding imidazolium salts, the signal of the imidazolium proton (8.80 < δ < 9.49 ppm) was however obviously absent. In the ¹³C NMR spectra, a significant shift is observed for the signal corresponding to the carbenic carbon moving from δ = 139 ppm for the imidazolium salts to δ = 187 ppm for the Au(I) carbene. It is worth noting that for all cationic complexes **4–10** the ¹H NMR spectra display a perfect symmetry of the molecule, both carbene ligands giving rise to the same set of signals even by decreasing the NMR acquisition temperature down to 213 K. This isochrony can be explained by the fast rotation of the ligand around the Au–C_{carbene} axis with respect to the NMR time scale. This observation is in good agreement with the work of Berners-Price in the case of bis(1-ethyl-3-methylimidazol-2-ylidene)Au(I) complex. (14)



Scheme 2. Syntheses of the Caffeine- and Theophylline-Based NHC Au(I) Complexes (3–10)

All cationic complexes have been subjected to high-resolution mass spectrometry studies to measure their exact mass. Additionally, suitable crystals for X-ray structure determination were grown from complexes **3**, **7**, and **10**, and their solid state structures have been solved (see experimental section); however, the structure of complex **4** has been previously reported. (22) The structures reveal the typical linear two-coordinated geometry of the Au(I) cation with the angles C–Au–I (for **3**) and C–Au–C (for **4**, **7**, and **10**) being 177.66(12)°, 176.02(16)°, 178.23(28), and 175.00(39)°, respectively (Figure 2). The main difference in the solid state structures of the cationic gold(I) heterobis(carbene) complexes **4**, **7**, and **10** lies in the relative position of the two purine ligands. Whereas both purine rings in complex **4** are coplanar with a dihedral angle of 2.27(16)°, they are tilted from 73.37(12)° and 83.42(20)° in complexes **7** and **10**, respectively. We could not rationalize this difference as resulting from strong intra or intermolecular interactions and we therefore suspect a low energy difference between planar and orthogonal conformers. It is worth mentioning that a planar cationic silver complex isostructural to **4** has been described, (32) whereas cationic gold complexes bearing two benzyl substituted imidazole-based NHC ligands oriented in orthogonal planes have been also reported in the literature. (33)

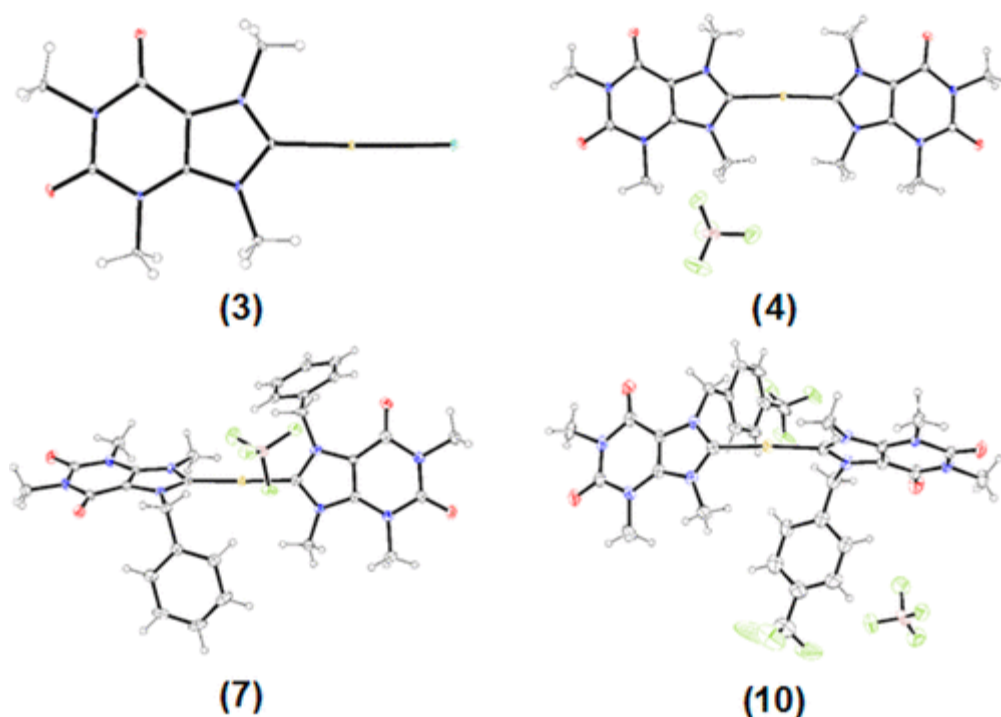
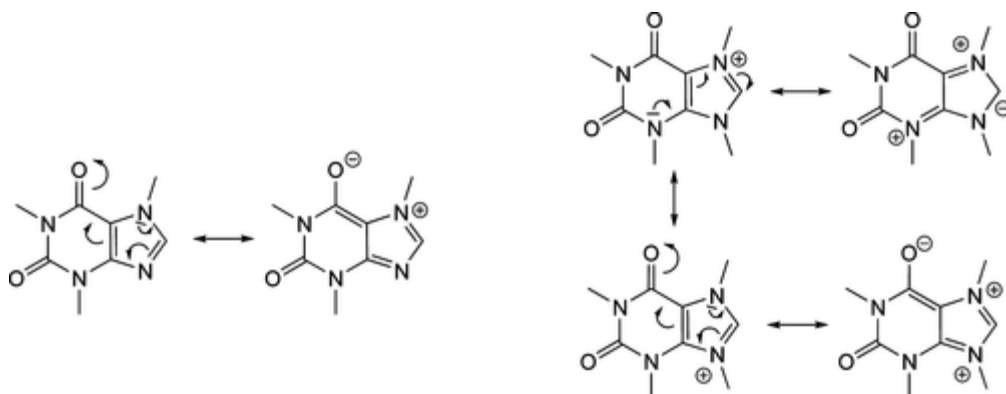


Figure 2. ORTEP views of compounds **3**, **4**, **7**, and **10**. Solvent molecules are omitted for clarity.

As previously mentioned, caffeine and N-substituted theophylline react more sluggishly with MeI than their methylimidazole or methylbenzimidazole analogues. Therefore, we expected a weaker σ -bond donor ability of the corresponding xanthine-derived NHC ligands. Such expectation is at variance with the similitude in the Au–C_{carb} bond lengths, confirmed in our caffeine- and theophylline-derivatives in regard to imidazole-

based NHC complexes: 2.004(4) Å for **3** (mean = 2.007 Å for imidazole-based AuI complexes, 2 structures in the CCDC) and a mean of 2.023 Å for **4**, **7**, and **10** (mean = 2.021 Å for 84 imidazole-based structures in the CCDC). This apparent discrepancy can be rationalized if we simply consider the mesomeric forms of caffeine and of the methylated caffeine, the latter being isolobal to the gold complex (see Scheme 3). The carbonyl group of the dioxypyrimidine ring next to the imidazole moiety withdraws electrons through delocalization and thus decreases electron density on the imidazole ring. Conversely, the opposite NMe group of the dioxypyrimidine ring shows a +M effect and releases electrons toward the imidazolium ring. Consequently, two significantly different bond lengths are observed in complex **3**, **4**, **7**, and **10** for C_{carb}—N: 1.338(3) Å and 1.369(3) Å as means over seven pairs of bonds, (34) the shorter bond being located on the side of the conjugated carbonyl group. Thus, the dioxypyrimidine fragment inhibits the reactivity of the caffeine toward simple alkylation but may act as push–pull substituent giving rise to stable carbene gold complex as attested by the Au–C_{carbene} bond lengths in complex **3**, **4**, **7**, and **10**.



Scheme 3. Mesomeric Forms of Caffeine (Left) and Methyl Caffeinium (Right)

In Vitro Cell Viability Assays

The antiproliferative properties of the Au(I) NHC complexes **1–10** (with cisplatin used as comparison) were assessed by monitoring their ability to inhibit cell growth using the MTT assay in the human ovarian cancer A2780 cell line, its cisplatin resistant variant (A2780/R), in human ovarian cancer SKOV3 cells, as well as in the human lung cancer A549 cell line. In addition, in order to evaluate the compounds' selectivity for cancerous compared to healthy cells, the gold complexes were also tested in human embryonic kidney HEK-293T cells. Several conclusions can be drawn in light of the results displayed in Table 1: First, all compounds elicit moderate antiproliferative properties against the tested cancerous cell lines (IC₅₀ values lying in the micromolar range), with the notable exception of complex **2** (IC₅₀ values lying in the nanomolar range), although the MC ligand is completely nontoxic in all the selected cell lines (IC₅₀ > 200 μM). Second, the new compounds display certain selective antiproliferative properties, being not cytotoxic for HEK-293T (neither for A459) but fairly active against the human ovarian cancer cells A2780 and A2780/R. This first series of results enthrones complex **4** as the most promising compound, yet being less active than cisplatin against A2780 cells (IC₅₀ = 16.2 vs 5.2 μM, respectively) but almost 2-fold

more potent against A2780/R cells ($IC_{50} = 15.6$ vs $35 \mu\text{M}$, respectively), though poorly effective against SKOV3 and A549 cancer cells ($IC_{50} > 60 \mu\text{M}$) and with very low activity in noncancerous cells ($IC_{50} > 100 \mu\text{M}$). Notably, the neutral compound **3**, although moderately cytotoxic in all the tested cell lines, is also as poorly selective as compound **2**.

Table 1. IC_{50} Values of Au(I) NHC Complexes against Various Cancer Cell Lines and Non-Cancerous Cells HEK-293T Compared to Cisplatin after 72 h Incubation at 37°C ^{a,b}

complex	IC_{50} (μM)				
	A2780	A2780/R	SKOV3	A549	HEK-293T
1	10.9 ± 1.0			17.8 ± 4.8	10.9 ± 2.4
2	0.54 ± 0.12		0.75 ± 0.29	5.9 ± 2.2	0.20 ± 0.09
3	37 ± 15	49 ± 15	37.3 ± 9.8	25.4 ± 2.2	22.9 ± 6.9
4	16.2 ± 2.1	15.6 ± 2.7	62.7 ± 7.8	>100	>100
5	26.0 ± 2.2	17.2 ± 1.7	60 ± 14	52.8 ± 5.2	42.0 ± 4.0
6	28.4 ± 4.0	25.8 ± 1.7	25.6 ± 4.5	46.7 ± 5.6	38.7 ± 8.3
7	12.4 ± 0.2	17.1 ± 0.4	21.8 ± 2.3	47.7 ± 0.6	32.5 ± 4.4
8	23.4 ± 4.0	20.7 ± 2.8	53.8 ± 4.6	90.0 ± 4.8	82 ± 13
9	21.9 ± 2.4	22.1 ± 3.2	37.6 ± 7.2	56.0 ± 7.9	84 ± 11
10	13.1 ± 2.4	17.8 ± 1.7	30.3 ± 3.4	26.1 ± 2.1	37.9 ± 2.1
cisplatin	5.2 ± 1.9	35 ± 7	13.2 ± 3.5	8.0 ± 0.5	11.0 ± 2.9

^a Mean \pm SE of at least three determinations or mean of three independent experiments performed with quadruplicate cultures at each tested concentration.

^b Solutions of the gold complexes were prepared by diluting a freshly prepared stock solution (10^{-2} M in DMSO) of the corresponding compounds in cell culture medium. The stability of the complexes in DMSO was checked: after 20 h at room temperature no degradation and no ligand replacement by DMSO was observed. Cisplatin stock solutions were prepared in Milli-Q water.

DNA-Binding Properties

In order to gain initial mechanistic insights, we subsequently investigated the properties of all bis-NHC complexes, namely **2**, **4–10**, as G-quadruplex DNA stabilizers, inspired by our recent results that highlighted the exquisite quadruplex-selectivity of complex **4** (also named AuTMX₂) against a panel of alternative DNA secondary structures. (22) G-quadruplexes are peculiar nucleic acid architectures adopted by guanine-rich DNA and RNA sequences, whose stability originates in the stacking of contiguous G-quartets (a planar and cyclic K⁺-promoted association of four guanines in a Hoogsteen hydrogen-bonding arrangement). (35) Quadruplexes are currently intensively studied, because they are suspected to play important roles in key cellular

events: quadruplex-forming DNA sequences are indeed found both in eukaryotic telomeres (36) and in promoter regions of identified oncogenes. (37) Their stabilization by selective small molecules (also called G-quadruplex ligands) (38) is thus currently investigated as a mean to control key cellular events (telomere homeostasis and chromosomal stability, as well as regulation of oncogene expression). Moreover, increasing evidence now points toward a major role of quadruplex ligands as DNA damaging agents; (39) this observation is particularly important in light of DDR defects of most cancer cells, in which PARP (and PARP-inhibitors) are playing a pivotal role (vide infra). (40) In other words, among the new strategies currently implemented in cancer chemotherapy, the development of drugs targeting quadruplexes is extremely versatile and promising. (41) In this context, complex 4 was recently reported as an exquisitely selective quadruplex-DNA interacting compounds, on the basis of the possible bioinspired recognition between the accessible G-quartet of the quadruplex and the Au(I) NHC complex that mimics the native guanine/K⁺ assembly. (22)

Within this frame, the interactions of complexes 2, 4–10 with quadruplex-DNA were assessed via FRET-melting assays, implemented in a competitive manner: briefly, experiments were performed with the most classically used quadruplex-forming oligonucleotide F21T which mimics the human telomere sequence (FAM-d[⁵G₃(T₂AG₃)₃]-TAMRA). (42) The FRET-melting principle is schematically presented in Figure 3A: it relies on the temperature-promoted unfolding of an oligonucleotide doubly labeled with a FRET pair (herein fluoresceine phosphoramidate (FAM or F) and tetramethyl-6-carboxyrhodamine (TAMRA or T)); the stability imparted by a ligand (expressed as $\Delta T_{1/2}$ values, in °C), readily monitored through the modification of the FRET phenomenon (*fluorescence resonance energy transfer*, Figure 3A), enables an easy quantification of its apparent affinity for quadruplex-DNA. Herein, FRET-melting experiments were carried out with F21T in absence or presence of an excess of the unlabeled duplex-DNA competitor ds17 (d[⁵C₂AGT₂CGTAGTA₂C₃]³/d[⁵G₃T₂ACTACGA₂CTG₂]³) to assess the quadruplex-versus-duplex selectivity. Results (summarized in Figure 3B,C) are interesting because they parallel to some extent the antiproliferative results: indeed, only four complexes exceeded the affinity threshold (i.e., $\Delta T_{1/2} = 10$ °C, Figure 3B), namely, 2, 4, 8, and 9 (with $\Delta T_{1/2} = 13.4, 14.0, 10.7,$ and 13.4 °C, respectively), and only complex 4 from that series exceeded the selectivity threshold (i.e., normalized $\Delta T_{1/2} = 50\%$, Figure 3C) with normalized $\Delta T_{1/2} = 89$ and 69% in presence of 15 and 50 equivalents of ds17, respectively. These results, along with that of antiproliferative studies, enabled us to select complexes 2 and 4 as the most interesting prototypes for further studies, the former being highly active but unselective (elevated antiproliferative effects on cancer cells and noncancerous cells, and DNA affinities whatever its structure) and the latter being both active and exquisitely selective (toxic for cancerous cell lines only, high affinity and selectivity for quadruplex-DNA only, reminiscently of what has been observed during our previous investigations). (22)

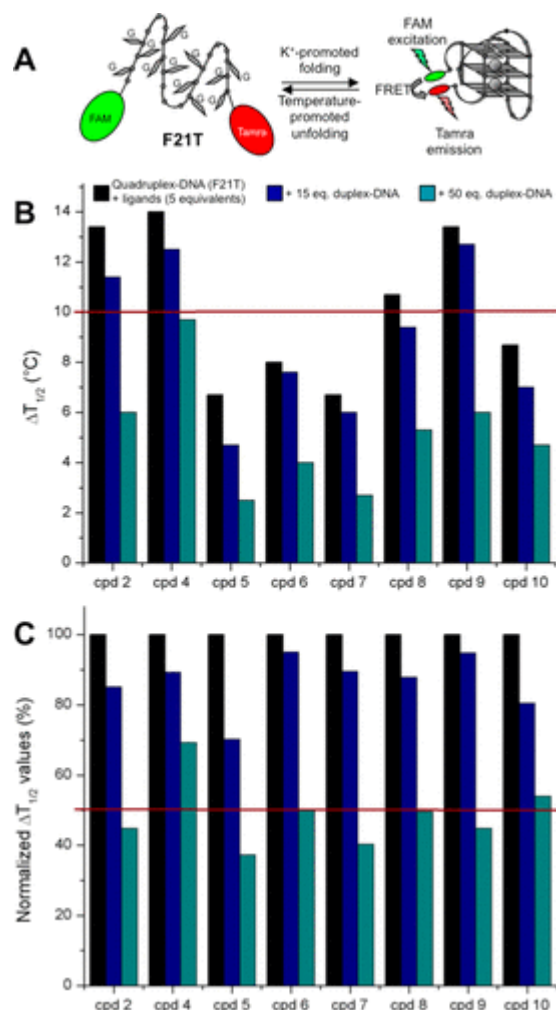


Figure 3. FRET-melting principle (A) and DNA binding properties of complexes **2**, **4–10**: quadruplex affinity (B) and selectivity (C) evaluated via competitive FRET-melting assay carried out F21T (0.2 μ M), Au(I) complexes (1.0 μ M) without (black bars) or with 15 (blue bars) or 50 equiv (green bars) of ds17.

We can postulate that the two unsubstituted (i.e., flat) phenyl rings found at the periphery of complex **2** make it an efficient intercalator able to slither in between two base pairs (the major duplex-DNA binding site). Conversely, the two 1,3-dimethyluracil moieties found at the periphery of complex **4** create a steric hindrance that prevents intercalation between base pairs to some extent, thereby making it a weaker duplex-DNA interacting compound. Given that the access of the quadruplex binding site is less sterically demanding (being based on the ligand stacking onto the external quartet), both complexes display roughly comparable quadruplex stabilization ability (Figure 3B). Therefore, the difference of quadruplex selectivity between complexes **2** and **4** probably originates solely in the lower affinity for duplex structures of the latter.

Ex Vivo Toxicity Studies

Afterward, complexes **2** and **4** were tested for their possible toxic effects in healthy organs *ex vivo* in rat liver, colon, and kidney tissues using the PCTS technology. (23) Thus, tissue slices have been incubated with different concentrations of each gold complex, and after a certain incubation time (24 h for liver and kidney, 5 h for colon slices, respectively), the viability of the tissues has been determined measuring the ATP content. The obtained results are presented in Figure 4.

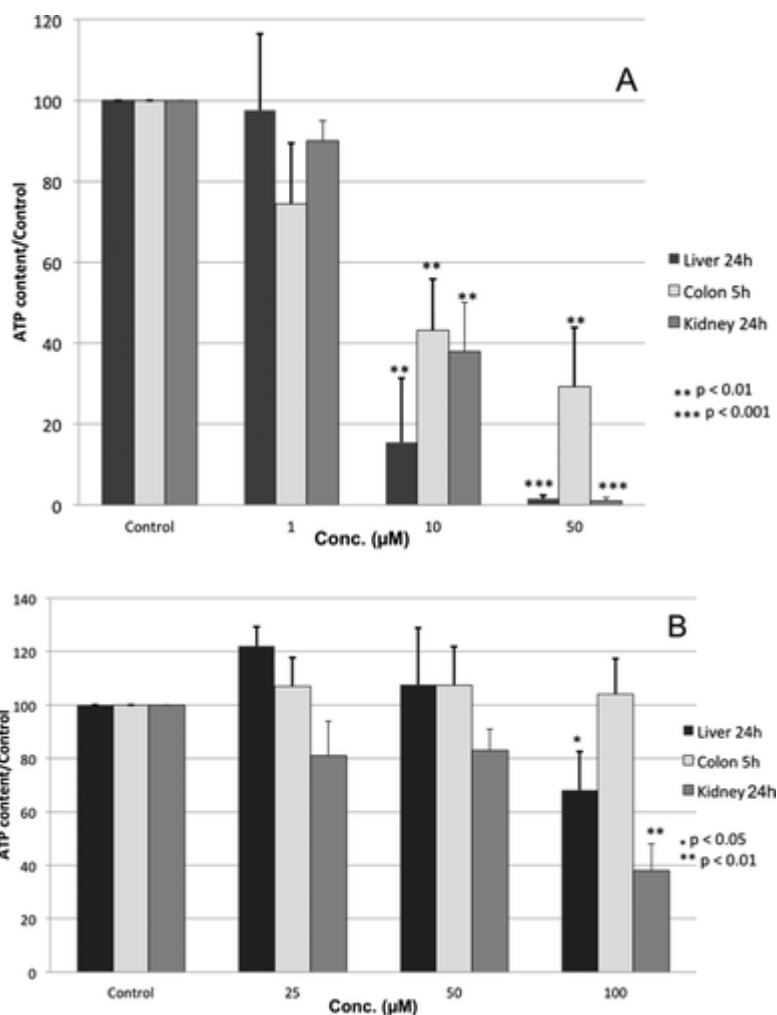


Figure 4. Viability of rat liver, colon, and kidney PCTS after treatment with **2** (A) and **4** (B). The obtained values were statistically analyzed using a *t*-test comparing treated samples with controls.

As it can be observed, although **2** is highly cytotoxic already at 10 µM concentration in liver, kidney, and colon slices (Figure 4A), complex **4** shows some toxicity mainly at 100 µM and 24 h incubation in liver, as well as in kidney slices (Figure 4B). The effects of **4** on the precision-cut kidney slices (PCKS) were also assessed by histomorphology. The data are shown in Figure 5 and confirm the findings made using the ATP data. The kidney slices were only slightly affected by the compound up to 50 µM during 24 h incubation in comparison to the controls; however, PCKS incubated with 100 µM **4** show necrosis indicated by the loss of nuclei, particularly in the cortex area. The highest concentration also causes loss of normal histologic architecture, as

well as swelling and vacuolation of the tubular epithelium. Notably, even at the highest concentration, the glomerulus is not affected, as previously reported in the case of cisplatin in a similar ex vivo model. (43) Overall, although the ex vivo data cannot be directly compared to the in vitro antiproliferative activities, the results obtained in PCTS are in line with those observed for the same compounds in HEK-293T cells (i.e., compound **2** being markedly more toxic than **4** in both cases).

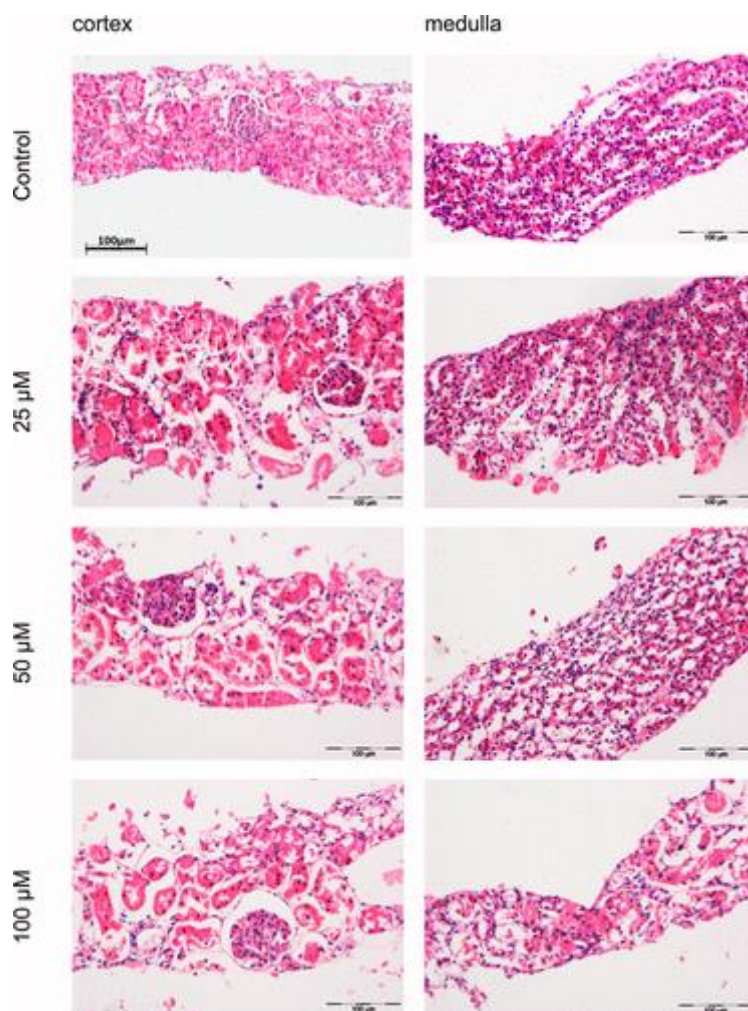


Figure 5. Histomorphology of PCKS (3 mg) treated with different concentrations of compound **4** for 24 h. Slices were stained with hematoxylin-eosin. The original magnification is 20x.

PARP-1 Activity Assay

Finally, inspired by our recent results that indicate that some cytotoxic gold compounds are efficient inhibitors of the zinc-finger protein PARP-1, we tested complexes **2–4** on the purified human enzyme. PARP-inhibitors are currently highly investigated for their selective cytotoxicity properties: they are indeed poorly toxic to normal cells but are highly active against homologous recombination (HR)-defective cells, notably BRCA-defective breast and ovarian cancers. PARP inhibitors can thus be considered as DDR inhibitors, (44) which can be used in combination with classical DNA damaging agents

for optimizing the therapeutic outcome. In other words, PARP-inhibiting properties of complex **4** were investigated to decipher if it can be used as a double-edged sword, acting as both a DNA damaging drug (quadruplex interaction) and DDR impairing agent (PARP inhibition). PARP-1 inhibition was indeed observed with all compounds **2** ($IC_{50} = 1.20 \pm 0.40 \mu\text{M}$), **3** ($IC_{50} = 0.45 \pm 0.10 \mu\text{M}$), and **4** ($IC_{50} = 0.96 \pm 0.13 \mu\text{M}$), albeit in a lower range than other cytotoxic gold(III) and gold(I) complexes (showing IC_{50} values in the low nanomolar range). [\(25\)](#) These results are not surprising per se, because the PARP-1 inhibition by Au(I) complexes is likely to occur via a direct gold binding to the zinc finger domain of the protein after exchange of the MC and iodido ligands. Because such ligands are less prone to undergo ligand exchange reactions than, for example, chlorido and N-donor ligands, they impede the metal center (gold) to covalently bind to the zinc finger domain. Altogether, these results are interesting as they indicate that the highly selective antiproliferative properties of complex **4** probably originate in a mechanism relying rather on the noncovalent interaction with peculiar DNA structures than on the covalent inhibition of DDR-related enzymes. Nonetheless, they also clearly highlight that massive efforts have now to be invested to decipher precisely the actual mechanism of action of gold(I) NHC complexes.

Conclusions

This study was initiated in the frame of ongoing studies in our laboratories aiming at developing new organometallic gold compounds as anticancer agents. In fact, as demonstrated by numerous studies, regulating the reactivity and redox chemistry of gold compounds via the optimization of an appropriate organometallic scaffold may constitute a strategy to achieve selectivity for cancer tissues, a feature that is often lacking with other types of coordination with gold complexes. Overall, the possibility of “fine-tuning” the stability of organometallic gold complexes while maintaining their biological activity and decreasing their side-effects is extremely attractive in the field of metallodrug development. Moreover, the combination of very stable organometallic gold moieties with bioligand functionalization, such as caffeine-type ligands, holds also great promise for further investigation. [\(45\)](#)

In this context, we reported here on a series of new caffeine-based gold(I) NHC complexes that have been synthesized and tested for their antiproliferative activities in different cancerous and nontumorigenic cell lines in vitro in comparison to 1,3-dimethylbenzimidazol-2-ylidene derivatives. The bis-carbene caffeine-based complex $[\text{Au}(\text{MC})_2][\text{BF}_4]$ **4** has shown interesting anticancer properties in vitro against the human ovarian cancer cell line A2780 and its cisplatin resistant variant A2780/R, though it appeared to be poorly toxic in non cancerous HEK-293T cells in vitro, as well as in healthy tissues ex vivo. This latter result is of particular importance for the future development of new metallodrugs with reduced side-effects.

Interestingly, complex **4** has also been proved to be an efficient and selective quadruplex-interacting agent. To date, a number of G-quadruplex stabilizing small molecules have been synthesized, but they often lack selectivity when incubated with

duplex DNA. (46) Among them, metal complexes occupy an increasingly important role, (47) but only very few of the studies reported up to now have been devoted to the study of Au complexes. (22, 48, 49) Most importantly, the gold compounds investigated so far are mainly gold(III) coordination complexes. Thus, the results presented herein further bolster the interest of gold compounds in the quest for valuable G-quadruplex ligands, notably in light of the enticing quadruplex selectivity of the bis-carbene complex **4**. Notably, the cationic derivative **2** efficiently stabilizes quadruplex-DNA albeit with a very poor selectivity, which may account for its high but indiscriminate antiproliferative properties and enhanced toxicity *ex vivo*.

This series of results is also interesting in that it provides some invaluable—yet preliminary—insights into the mechanism that may underlie the observed antiproliferative behavior of this class of compounds: the covalent linking of PARP-1 enzyme seems rather unlikely, but the noncovalent interaction of the intact gold complexes (that is, without any loss of carbenic ligand) with higher-order nucleic acids structures appears efficient. Even more interesting is the parallel that can be drawn between the quadruplex and cytotoxicity selectivity, although further studies are necessary to confirm direct DNA binding in cells.

In light of very recent results, (50, 51) we are currently assessing the ability of these gold complexes to recognize other quadruplexes, notably RNA quadruplexes, (52) aiming at combining these investigations with cellular uptake and biodistribution studies in order to better fathom the molecular basis of their intracellular action. We hope to report on these results soon.

Furthermore, the insertion of substituents in the N7 position did not lead to a substantial improvement of the biological effects in terms of cytotoxicity and G-quadruplexes selectivity; however, further chemical design is ongoing in our laboratories aimed at expanding this family of compounds using theobromine instead of theophylline as NHC building block, which allows for inserting functional groups in the back of the carbene ligand (N1 position) while keeping the two methyl substituents in N7 and N9 positions. (53)

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Notes

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References

- (1) Rosenberg, B.; Vancamp, L.; Krigas, T. *Nature* 1965, 205, 698.
- (2) Rabik, C. A.; Dolan, M. E. *Cancer Treat. Rev.* 2007, 33, 9.

- (3) Nobili, S.; Mini, E.; Landini, I.; Gabbiani, C.; Casini, A.; Messori, L. *Med. Res. Rev.* 2010, 30, 550.
- (4) Nagy, E. M.; Ronconi, L.; Nardon, C.; Fregona, D. *Mini-Rev. Med. Chem.* 2012, 12, 1216.
- (5) Gianferrara, T.; Bratsos, I.; Alessio, E. *Dalton Trans.* 2009, 37, 7588.
- (6) Vessieres, A.; Top, S.; Beck, W.; Hillard, E.; Jaouen, G. *Dalton Trans.* 2006, 529.
- (7) Reedijk, J. *Eur. J. Inorg. Chem.* 2009, 1303.
- (8) Casini, A. *J. Inorg. Biochem.* 2012, 109, 97.
- (9) Casini, A.; Messori, L. *Curr. Top. Med. Chem.* 2011, 11, 2647.
- (10) Oehninger, L.; Rubbiani, R.; Ott, I. *Dalton Trans.* 2013, 42, 3269.
- (11) Liu, W. K.; Gust, R. *Chem. Soc. Rev.* 2013, 42, 755.
- (12) Zou, T. T.; Lum, C. T.; Chui, S. S. Y.; Che, C. M. *Angew. Chem., Int. Ed.* 2013, 52, 2930.
- (13) Hickey, J. L.; Ruhayel, R. A.; Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Filipovska, A. *J. Am. Chem. Soc.* 2008, 130, 12570.
- (14) Baker, M. V.; Barnard, P. J.; Berners-Price, S. J.; Brayshaw, S. K.; Hickey, J. L.; Skelton, B. W.; White, A. H. *Dalton Trans.* 2006, 3708.
- (15) Bindoli, A.; Rigobello, M. P.; Scutari, G.; Gabbiani, C.; Casini, A.; Messori, L. *Coord. Chem. Rev.* 2009, 253, 1692.
- (16) Rubbiani, R.; Kitanovic, I.; Alborzina, H.; Can, S.; Kitanovic, A.; Onambebe, L. A.; Stefanopoulou, M.; Geldmacher, Y.; Sheldrick, W. S.; Wolber, G.; Prokop, A.; Wolf, S.; Ott, I. *J. Med. Chem.* 2010, 53, 8608.
- (17) Schuh, E.; Pfluger, C.; Citta, A.; Folda, A.; Rigobello, M. P.; Bindoli, A.; Casini, A.; Mohr, F. *J. Med. Chem.* 2012, 55, 5518.
- (18) Citta, A.; Schuh, E.; Mohr, F.; Folda, A.; Massimino, M. L.; Bindoli, A.; Casini, A.; Rigobello, M. P. *Metallomics* 2013, 1006.
- (19) Rubbiani, R.; Can, S.; Kitanovic, I.; Alborzina, H.; Stefanopoulou, M.; Kokoschka, M.; Monchgesang, S.; Sheldrick, W. S.; Wolf, S.; Ott, I. *J. Med. Chem.* 2011, 54, 8646.
- (20) Daly, J. W. *Cell. Mol. Life Sci.* 2007, 64, 2153.
- (21) Wang, H. M. J.; Lin, I. J. B. *Organometallics* 1998, 17, 972.
- (22) Stefan, L.; Bertrand, B.; Richard, P.; Le Gendre, P.; Denat, F.; Picquet, M.; Monchaud, D. *ChemBioChem* 2012, 13, 1905.
- (23) de Graaf, I. A. M.; Olinga, P.; de Jager, M. H.; Merema, M. T.; de Kanter, R.; van de Kerkhof, E. G.; Groothuis, G. M. M. *Nat. Protoc.* 2010, 5, 1540.

- (24) Serratrice, M.; Edafe, F.; Mendes, F.; Scopelliti, R.; Zakeeruddin, S. M.; Gratzel, M.; Santos, I.; Cinellu, M. A.; Casini, A. *Dalton Trans.* 2012, 41, 3287.
- (25) Mendes, F.; Groessl, M.; Nazarov, A. A.; Tsybin, Y. O.; Sava, G.; Santos, I.; Dyson, P. J.; Casini, A. *J. Med. Chem.* 2011, 54, 2196.
- (26) Schreiber, V.; Dantzer, F.; Ame, J. C.; de Murcia, G. *Nat. Rev. Mol. Cell Biol.* 2006, 7, 517.
- (27) Schutz, J.; Herrmann, W. A. *J. Organomet. Chem.* 2004, 689, 2995.
- (28) Kascatan-Nebioglu, A.; Panzner, M. J.; Garrison, J. C.; Tessier, C. A.; Youngs, W. *J. Organometallics* 2004, 23, 1928.
- (29) Landaeta, V. R.; Rodriguez-Lugo, R. E.; Rodriguez-Arias, E. N.; Coll-Gomez, D. S.; Gonzalez, T. *Transition Met. Chem.* 2010, 35, 165.
- (30) Petch, D.; Anderson, R. J.; Cunningham, A.; George, S. E.; Hibbs, D. E.; Liu, R.; Mackay, S. P.; Paul, A.; Small, D. A. P.; Groundwater, P. W. *Bioorg. Med. Chem.* 2012, 20, 5901.
- (31) Baker, M. V.; Barnard, P. J.; Berners-Price, S. J.; Brayshaw, S. K.; Hickey, J. L.; Skelton, B. W.; White, A. H. *J. Organomet. Chem.* 2005, 690, 5625.
- (32) Kascatan-Nebioglu, A.; Melaiye, A.; Hindi, K.; Durmus, S.; Panzner, M. J.; Hogue, L. A.; Mallett, R. J.; Hovis, C. E.; Coughenour, M.; Crosby, S. D.; Milsted, A.; Ely, D. L.; Tessier, C. A.; Cannon, C. L.; Youngs, W. *J. Med. Chem.* 2006, 49, 6811.
- (33) Hirtenlehner, C.; Krims, C.; Holbling, J.; List, M.; Zabel, M.; Fleck, M.; Berger, R. J.; Schoefberger, W.; Monkowius, U. *Dalton Trans.* 2011, 40, 9899.
- (34) The reported standard deviations of the means are based on the pooled standard deviation calculated over the sample of the 14 Ccarb←N bonds ($s_{\text{mean}} = s_{\text{pooled}}/\sqrt{7}$).
- (35) Collie, G. W.; Parkinson, G. *N. Chem. Soc. Rev.* 2011, 40, 5867.
- (36) Xu, Y. *Chem. Soc. Rev.* 2011, 40, 2719.
- (37) Balasubramanian, S.; Hurley, L. H.; Neidle, S. *Nat. Rev. Drug Discovery* 2011, 10, 261.
- (38) Monchaud, D.; Teulade-Fichou, M. P. *Org. Biomol. Chem.* 2008, 6, 627.
- (39) Rodriguez, R.; Miller, K. M.; Forment, J. V.; Bradshaw, C. R.; Nikan, M.; Britton, S.; Oelschlaegel, T.; Xhemalce, B.; Balasubramanian, S.; Jackson, J. *Nat. Chem. Biol.* 2012, 8, 301.
- (40) Martin, S. A.; Lord, C. J.; Ashworth, A. *Curr. Opin. Genet. Dev.* 2008, 18, 80.
- (41) Balasubramanian, S.; Neidle, S. *Curr. Opin. Chem. Biol.* 2009, 13, 345.
- (42) De Cian, A.; Guittat, L.; Kaiser, M.; Sacca, B.; Amrane, S.; Bourdoncle, A.; Alberti, P.; Teulade-Fichou, M. P.; Lacroix, L.; Mergny, J. L. *Methods* 2007, 42, 183.

- (43) Vickers, A. E.; Rose, K.; Fisher, R.; Saulnier, M.; Sahota, P.; Bentley, P. *Toxicol. Pathol.* 2004, 32, 577.
- (44) Jackson, S. P.; Bartek, J. *Nature* 2009, 461, 1071.
- (45) Bertrand, B.; Casini, A. *Dalton Trans.* 2014, DOI: 10.1039/C3DT52524D.
- (46) Le, T. V. T.; Han, S.; Chae, J.; Park, H. J. *Curr. Pharm. Des.* 2012, 18, 1948.
- (47) Georgiades, S. N.; Abd Karim, N. H.; Suntharalingam, K.; Vilar, R. *Angew. Chem., Int. Ed.* 2010, 49, 4020.
- (48) Suntharalingam, K.; Gupta, D.; Miguel, P. J. S.; Lippert, B.; Vilar, R. *Chemistry* ← *Eur. J.* 2010, 16, 3613.
- (49) Sun, R. W.-Y.; Li, C. K.-L.; Ma, D.-L.; Yan, J. J.; Lok, C.-N.; Leung, C.-H.; Zhu, N.; Che, C.-M. *Chemistry* ← *Eur. J.* 2010, 16, 3097.
- (50) Di Antonio, M.; Biffi, G.; Mariani, A.; Raiber, E. A.; Rodriguez, R.; Balasubramanian, S. *Angew. Chem., Int. Ed.* 2012, 51, 11073.
- (51) Haudecoeur, R.; Stefan, L.; Monchaud, D. *Chemistry* ← *Eur. J.* 2013, 19, 12739.
- (52) Bugaut, A.; Balasubramanian, S. *Nucleic Acids Res.* 2012, 40, 4727.
- (53) Panzner, M. J.; Hindi, K. M.; Wright, B. D.; Taylor, J. B.; Han, D. S.; Youngs, W. J.; Cannon, C. L. *Dalton Trans.* 2009, 7308. *Inorganic Chemistry Article* dx.doi.org/10.1021/ic403011h | *Inorg. Chem.* 2014, 53, 2296–2303