Journal of Medicinal Chemistry

Article

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Synthesis, Characterization, and in Vitro Studies of Bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I/III) Complexes

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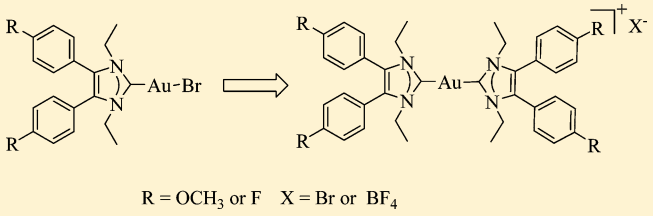
Supporting Information

ABSTRACT: Cationic bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I) complexes with 4-OCH_3 or 4-F substituents in the aromatic rings and Br^- (**3a,b**) or BF_4^- (**7a,b**) counterions were synthesized, characterized, and investigated for tumor growth inhibitory properties in vitro. Analogous to auranofin, the N-heterocyclic carbenes (NHCs) were also combined with a phosphine ligand (triphenylphosphine, **4a,b**) and 2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-glucopyranosyl-1-thiolate (**5a,b**). The growth inhibitory effect against MCF-7, MDA-MB 231, and HT-29 cells, which is more than 10-fold higher than that of cisplatin or 5-FU, was independent of the oxidation state (Au(III), **6a,b**) and the anionic counterion. Bis[1,3-diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene]gold(I) bromide **3b** as the most cytotoxic compound reduced the growth of MCF-7 cells with IC_{50} = 0.10 μM (cisplatin, 1.6 μM; 5-FU, 4.7 μM). The thioredoxin reductase (TrxR), the estrogen receptor (ER), and the cyclooxygenase (COX) enzymes, which have to be considered as possible targets based on the drug design, can be excluded from being involved in the mode of action.



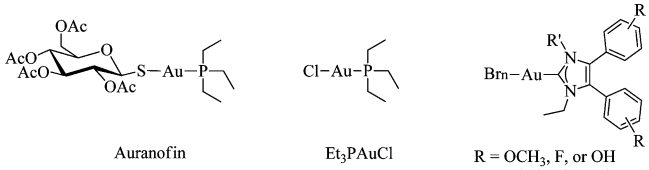
INTRODUCTION

Platinum complexes such as cisplatin and carboplatin are metal-based drugs, which are widely used in cancer chemotherapy. However, their use is extremely hampered by severe side effects and development of resistance during the therapy.[1−8] In order to overcome these disadvantages, current strategies in the development of novel metallodrugs focused more and more on the use of transition metal complexes containing improved organic ligands.[1−8]

The attention for N-heterocyclic carbenes (NHCs) typically used as ligands in complexes designed for catalytic applications[9−11] strongly increased during the past years since their suitability as carrier ligands for cytotoxic metal complexes was demonstrated. The simplicity of synthesis, functionalization, and isolation of NHCs and the success in complexation with a large variety of hard/soft metal ions make NHCs excellent ligands, thus allowing an easy fine-tuning of both the physicochemical properties and the reactivity in biological medium of the final NHC metal complexes.[1,2,10−30]

Auranofin, which has been used for the treatment of severe rheumatoid arthritis, and its chloro analogue Et_3PAuCl (Scheme 1) are very promising lead structures for the design of metal complexes with a mode of action different from that of cisplatin.[10−26,30−35] Both complexes cause antitumor activity mainly through inhibition of the enzyme thioredoxin reductase (TrxR).[36−41] Replacement of the triethylphosphine by an NHC ligand with pharmacological activity (e.g., hormonal activity, cyclooxygenase inhibitory properties) could lead to new antitumor agents with multitarget interaction including the TrxR.[10−26,33]

Scheme 1. Structures of Auranofin, Et_3PAuCl, and Au-NHC Complexes Derived from 4,5-Diarylimidazoles

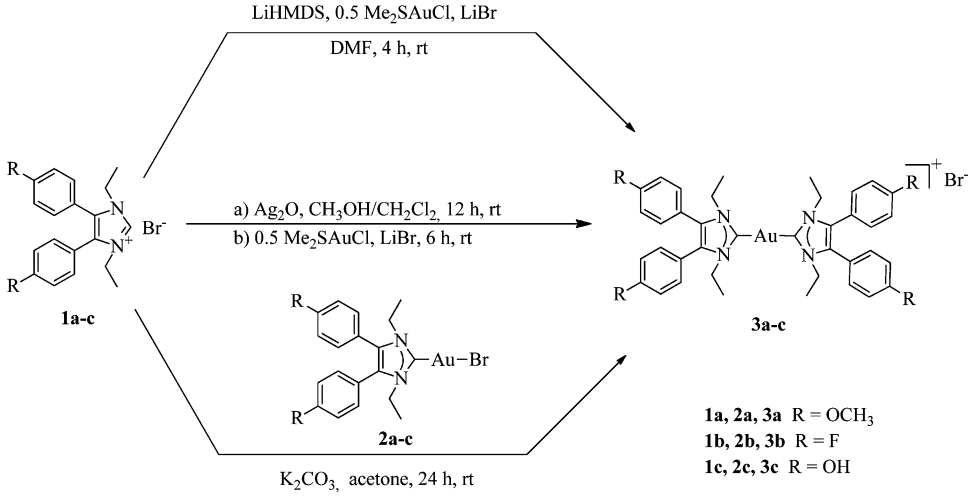


In continuation of our structure−activity relationship (SAR) study with NHC-silver halide complexes derived from 4,5-diarylimidazoles which possess good growth inhibitory effects against mammary and colon carcinoma cells,[42] we developed a series of analogously substituted neutral NHC-gold halide complexes (Scheme 1). All complexes inhibited the TrxR and showed tumor cell growth inhibitory effects comparable to those of cisplatin, auranofin, and Et_3PAuCl.[43]

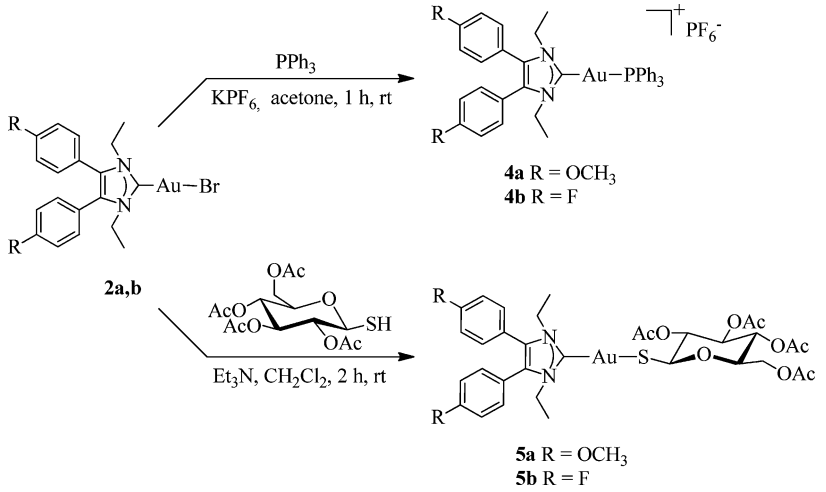
Encouraged by these promising results, we investigated the influence of the halide exchange at the Au-NHC complexes on their pharmacological properties. We selected the 4-F and 4-

Received: November 2, 2011
Published: March 19, 2012

Scheme 2. Synthesis Routes of 3a−c



Scheme 3. Synthesis Routes of 4a,b and 5a,b



OCH_3 (4-OH) substituted bromo[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I) complexes and exchanged the bromide by a second NHC ligand (Au(I)-bisNHC) to realize the concept of bivalent drugs or in relation to auranofin by 2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-glucopyranosyl-1-thiolate as well as a phosphine ligand (triphenylphosphine).

RESULTS AND DISCUSSION

Synthesis and Structural Characterization. The synthetic routes to the cationic Au(I)-bisNHC complexes **3a−c** are outlined in Scheme 2. Imidazolium salts **1a−c** and NHC-Au(I)-Br **2a−c** were synthesized according to our previously published methods.[42,43]

Three synthetic routes were investigated for the synthesis of **3a−c**. In method A[17,20,44] a DMF solution of Me_2SAuCl is treated with a solution containing 2 equiv of **1a−c** previously deprotonated with lithium bis(trimethylsilyl)amide (LiHMDS). This reaction gave **3a−c** in high yields (55−80%). The second procedure (method B) involved the Ag(I)-NHC transfer method, whereby **1a−c** were treated with silver oxide in CH_2Cl_2 and the resulting Ag(I)-NHC complex was allowed to react with a half equimolar amount of Me_2SAuCl.[45] This procedure gave **3a−c** in moderate yields (30−60%). Additional lithium bromide is necessary in both cases to ensure that sufficient amounts of bromide ions are available for the formation of Au(I)-bisNHC bromide. In the third synthetic route (method C)[46] **2a−c** were dissolved with an equimolar amount of **1a−c** in the presence of K_2CO_3. This procedure was also very effective and gave **3a−c** in high yields (80−90%).

The complexes **3a−c** were characterized by NMR and MS. Analogous to NHC-Au(I)-Br complexes,[43] the ^1H NMR spectra of bivalent Au-bisNHC analogues lack of the NCHN resonance found in the case of unbound NHC ligands and the ^{13}C NMR spectra include characteristic Au−C peaks. Compared to the ^1H NMR signals of **2a−c**, those of **3a−c** remain unaffected upon coordination of an additional NHC ligand. In the ^{13}C NMR spectra the resonances of the carbenoid C-atoms located at 181.4−182.7 ppm are significantly downfield shifted ($\Delta\delta \approx 10$ ppm) compared to **2a,b**.[43] This observation is in accordance with a lower Lewis acidity of the metal center due to the coordination of an additional strong *σ*-donor.[44,46] Furthermore, metal binding did not change the ^1H NMR and ^{13}C NMR signals of ethyl and phenyl groups.

The formation of complexes was further supported by positive mode ESI mass spectrometry, which documented a base peak corresponding to the $[M - Br]^+$ fragment for **3a−c**.

The cationic NHC-Au(I)-triphenylphosphines **4a,b** were prepared from **2a,b** by Br/PPh_3 exchange (Scheme 3) and were

dx.doi.org/10.1021/jm3000196 | J. Med. Chem. 2012, 55, 3713−3724

Scheme 4. Synthesis Routes of 6a,b, 7a,b, and 8a,b

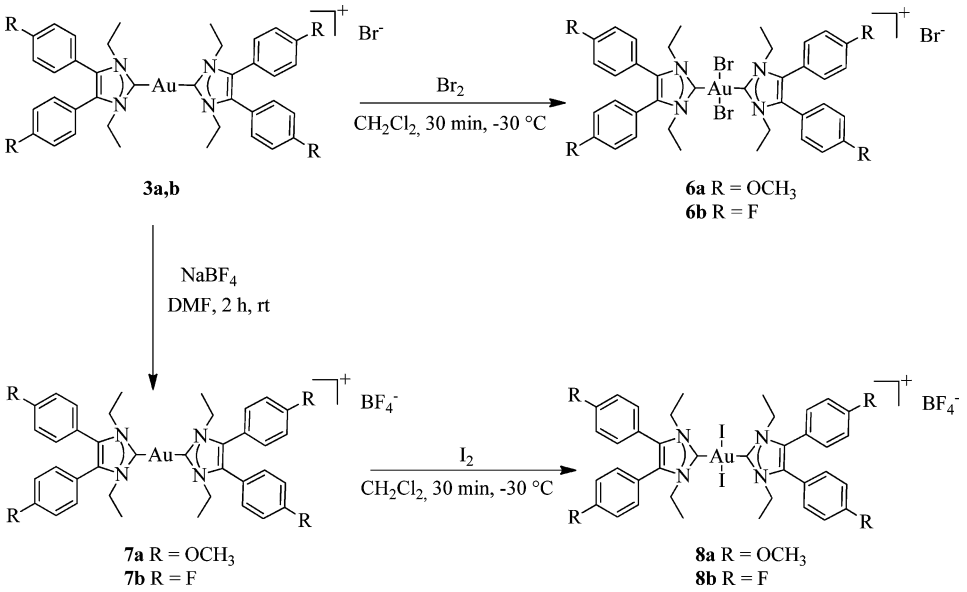


Table 1. Selected X-ray Data for 7a

wavelength	0.71069 Å
crystal system	monoclinic
space group	$P2_1/n$
unit cell dimensions	a = 11.063(5) Å
	b = 24.987(5) Å, β = 107.193(5)°
	c = 15.603(5) Å
volume	4120(2) Å^3
Z	4
density (calculated)	1.542 Mg/m^3
absorption coefficient	3.634 mm^{-1}
F(000)	1920
crystal size	0.37 × 0.06 × 0.05 mm^3
θ range for data collection	1.59−29.32°
index range	$-15 \le h \le 15$, $-34 \le k \le 33$, $-21 \le l \le 21$
reflections collected	44056
independent reflections	11155 [R_{int} = 0.1764]
absorption correction	integration
T_{min}	0.5687
T_{max}	0.8786
data/restraints/parameters	11155/0/503
goodness-of-fit on F^2	0.869
final R indices [$I > 2\sigma(I)$]	R1 = 0.0559, wR2 = 0.1070
R indices (all data)	R1 = 0.1749, wR2 = 0.1623
largest diff peak and hole	0.684 and −2.310 e·Å^{-3}

isolated as hexafluorophosphate salts.[15] The ^{1}H NMR spectra and the ESI mass spectra (base peak corresponding to the molecular peak of the complex cation) of **4a,b** showed the same tendencies as described above and correspond to the expected structure.

A modified procedure of Berners-Price et al.[15] was used for the synthesis of the neutral NHC-Au(I)-2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiolato complexes **5a,b**. The NHC-Au(I)-Br complexes **2a,b** were respectively dissolved in CH_2Cl_2 and reacted with 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiol under basic conditions (triethylamine, Scheme 3). The crude oily mixtures were purified by flash column chromatography, followed by a recrystallization from ethanol/water to give the analytically pure products as white solids.

Both complexes showed the same spectroscopic characteristics as the analogues mentioned above. The absence of the S−H signal in the ^{1}H NMR spectra pointed to S-coordination of the glucopyranosylthiolate unit,[15] which was further supported by positive mode ESI mass spectrometry (identification of [M + H]$^+$ and [M + Na]$^+$ fragments).

A convenient and gentle method to get the Au(III) complexes **6a,b** (Scheme 4) is the oxidation of the readily available Au(I)-bisNHC complexes **3a,b** with a slight excess of bromine.[44] Dibromo-Au(III)-bisNHC complexes **6a,b** were isolated as orange powders in high yields.[44] Exchange of the bromide counterion in **3a,b** by tetrafluoroborate yielding the complexes **7a,b** is easily performed with an excess of $NaBF_4$ in DMSO. The following treatment with elemental iodine gave

dx.doi.org/10.1021/jm3000196 | J. Med. Chem. 2012, 55, 3713−3724

the diiodo-Au(III)-bisNHC complexes **8a,b** as reddish-orange powders.[46] Both oxidative addition reactions are straightforward and do not require the exclusion of air or moisture.

The ^{1}H NMR spectra of Au(III)-bisNHC complexes **6a,b** and **8a,b** were similar to those of the corresponding Au(I)-bisNHC complexes **3a,b**. In the ^{13}C NMR spectra of **6a,b** and **8a,b**, the NCN resonances are upfield shifted by 30−40 ppm compared to **3a,b**. This ^{13}C shift is apparently based on the higher Lewis acidic metal center (higher oxidation state) and is within the reported range for Au(III) carbene complexes of imidazolium analogues.[24,43,44,46] The formation of complexes **6a,b** and **8a,b** was further supported by their ESI mass spectra, which documented base peaks corresponding to the $[M - Br]^{+}$and $[M - BF_4]^{+}$ fragments, respectively.

To gain insight into the coordination chemistry and structural parameters of these complexes, **7a** was crystallized by slow evaporation of a concentrated acetone solution and characterized by X-ray diffraction (Table 1).

Complex **7a** exists in a linear structure [C1−Au−C22, 176.8(4)°] as depicted in Figure 1. The distances of Au−C1 (2.044 Å) and Au−C22 (2.036 Å) are in good agreement with reported values.[44,46] Similar to **2b**, whose crystal structure was published previously,[43] neither aurophilic nor notable $\pi-\pi$ interactions were observed for **7a** in the solid state.

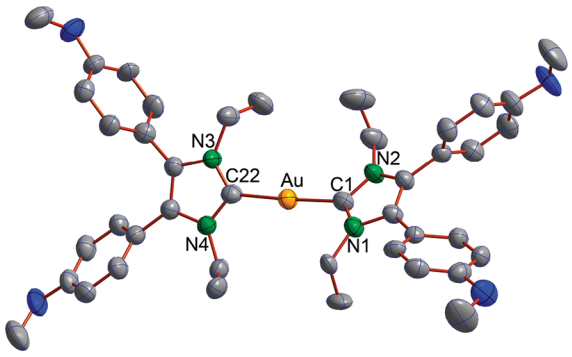


Figure 1. Ellipsoid representation of the structure of the complex cation of **7a**. Selected bond lengths [Å] and angles [deg] are as follows: Au−C22 2.036(9), Au−C1 2.044(9), N1−C1 1.32(1), N2−C1 1.33(1), N3−C22 1.36(1), N4−C22 1.33(1), C22−Au−C1 176.8(4), N1−C1−Au 126.4(6), N2−C1−Au 126.7(7), N4−C22−Au 125.4(6), N3−C22−Au 127.5(6), N4−C22−N3 106.5(8), N1−C1−N2 106.8(8).

Finally, all novel gold complexes are sufficiently stable in solid state and solution. If the complexes were dissolved in $CDCl_3$, methanol-d_4, methanol-d_4/D_2O (1:1), or DMSO-d_6, no changes of the spectra would be observed during storage at room temperature.

Antiproliferative Effects. A crystal violet assay was used to study the tumor cell growth inhibitory effects. All complexes as well as the established antitumor drugs 5-fluorouracil (5-FU) and cisplatin were screened against MCF-7 and MDA-MB 231 breast cancer as well as HT-29 colon carcinoma cells. The experiments were performed according to established procedures.[42,43,47]

Cytostatic effects (100% inhibition compared to the control) of the complexes were reached below 10 μM. Therefore, it is guaranteed that the water solubility (all complexes above 0.025 mg/mL (about 20 μM)) did not limit the determination of the IC_{50} (Table 2). The time-dependent antiproliferative effects of **3b** at the three cancer cell lines are presented in Figure 2.

Table 2. Growth Inhibitory Effects (IC_{50} [μM]) against Breast and Colon Cell Lines[a]

compd	MCF-7	MDA-MB 231	HT-29
2a[b]	1.4 ± 0.1	3.7 ± 0.7	2.9 ± 0.1
2b[b]	1.1 ± 0.3	3.9 ± 0.1	2.3 ± 0.1
2c[b]	4.5 ± 0.3	>20	17.0 ± 2.8
3a	0.17 ± 0.05	0.54 ± 0.11	0.43 ± 0.01
3b	0.10 ± 0.02	0.34 ± 0.01	0.23 ± 0.01
3c	0.30 ± 0.07	1.55 ± 0.04	0.47 ± 0.18
4a	0.22 ± 0.01	0.70 ± 0.22	0.42 ± 0.04
4b	0.21 ± 0.10	0.67 ± 0.11	0.37 ± 0.02
5a	1.26 ± 0.09	1.44 ± 0.20	3.19 ± 0.21
5b	0.81 ± 0.12	1.34 ± 0.12	3.22 ± 0.02
6a	0.15 ± 0.01	0.49 ± 0.01	0.32 ± 0.01
6b	0.13 ± 0.01	0.50 ± 0.10	0.33 ± 0.01
7a	0.13 ± 0.01	0.56 ± 0.07	0.36 ± 0.01
7b	0.15 ± 0.01	0.40 ± 0.01	0.62 ± 0.02
8a	0.15 ± 0.01	0.63 ± 0.01	0.26 ± 0.03
8b	0.13 ± 0.02	0.77 ± 0.09	0.30 ± 0.01
cisplatin	1.6 ± 0.5	7.8 ± 0.8	4.1 ± 0.3
5-FU	4.7 ± 0.4	9.6 ± 0.3	7.3 ± 1.0

[a]The IC_{50} was determined as the concentration causing 50% decrease in cell growth after 72 h of incubation and calculated as the mean of at least two or three independent experiments. [b]Data from ref 43.

Cisplatin characteristically reduced the cell growth of MCF-7 (IC_{50} = 1.6 μM), MDA-MB 231 (IC_{50} = 7.8 μM), and HT-29 (IC_{50} = 4.1 μM) cells. As expected, 5-FU was less active than cisplatin in these three cell lines (MCF-7, IC_{50} = 4.7 μM; MDA-MB 231, IC_{50} = 9.6 μM; HT-29, IC_{50} = 7.3 μM) because it is well-known that the used cells are very sensitive to platinum complexes. However, this finding does not reflect the real clinic situation. In contrast to 5-FU which is used for the treatment of colorectal cancer and pancreatic cancer and sometimes in the treatment of breast cancer, especially against aggressive inflammatory breast cancer, cisplatin is until yet not established in the chemotherapy of these tumor entities. Nevertheless, cisplatin is the standard reference compound for metal complexes and has been used in this study.

As already reported,[35,40,43] auranofin caused comparable effects as cisplatin against MCF-7 cells (IC_{50} = 1.1 μM) and was slightly more active against HT-29 cells (IC_{50} = 2.6 μM). Et_3PAuCl was less active at MCF-7 and HT-29 cell lines (IC_{50} = 3.2 and IC_{50} = 5.3 μM, respectively),[34,40,43] while the NHC-Au(I)-Br complexes **2a,b** reduced the cell growth (MCF-7, IC_{50} = 1.4 and IC_{50} = 1.1 μM, respectively; MDA-MB 231, IC_{50} = 3.7 and IC_{50} = 3.9 μM, respectively; HT-29, IC_{50} = 2.9 and IC_{50} = 2.3 μM, respectively) to a higher extent than cisplatin.[43] Complex **2c** showed a preference for MCF-7 cells with an IC_{50} of 4.5 μM compared to >17 μM determined for the other cell lines.[43]

Exchange of the Br^{-} ligand in **2a,b** by 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiolate (→**5a,b**) marginally increased the growth inhibitory effects at the MDA-MB 231 cell line (IC_{50}= 1.44 and IC_{50} = 1.34 μM, respectively) and in the case of **5b** at MCF-7 cells (IC_{50} = 0.81 μM), too. The effects at HT-29 cells remained unchanged.

Interestingly, the use of a triphenylphosphine ligand led to a 5-fold higher activity despite the formation of cationic species. Complexes **4a,b** were cytotoxic at nanomolar concentrations, more pronounced at MCF-7 (IC_{50} ≈ 0.21 μM) than at HT-29

dx.doi.org/10.1021/jm3000196 | J. Med. Chem. 2012, 55, 3713−3724

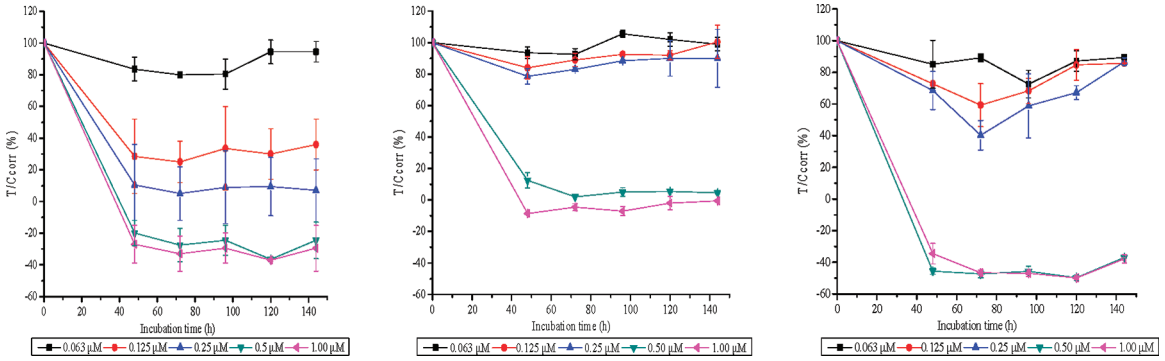


Figure 2. Time-dependent antiproliferative effects of complex **3b** on MCF-7 (left), MDA-MB 231 (middle), and HT-29 (right) cells.

Table 3. Cellular Uptake of Complexes after 6 and 24 h in MCF-7 and HT-29 Cells[a]

	μg of gold per mg of protein					
	6 h		24 h		μM, 6 h	
compd	MCF-7	HT-29	MCF-7	HT-29	MCF-7	HT-29
2b	0.067 ± 0.009	0.022 ± 0.002	0.064 ± 0.031	0.022 ± 0.002	38.3	21.9
3b	0.93 ± 0.06	0.70 ± 0.12	1.0 ± 0.1	1.0 ± 0.2	531.5	696.1
4b	0.47 ± 0.11	0.34 ± 0.03	0.42 ± 0.15	0.49 ± 0.03	269.4	344.3
5b	0.35 ± 0.06	0.032 ± 0.002	0.32 ± 0.03	0.038 ± 0.007	200.2	31.5
7b	1.0 ± 0.2	0.63 ± 0.10	0.39 ± 0.02	0.80 ± 0.17	584.6	632.1
Et_3PAuCl	0.28 ± 0.02	0.052 ± 0.002	0.15 ± 0.03	0.082 ± 0.018	160.8	52.4

[a]Results were calculated from the data of three independent experiments.

(IC_{50} ≈ 0.40 μM) and MDA-MB 231 cells (IC_{50} ≈ 0.68 μM), independent of the substituent at the NHC.

A further increase in activity was determined with cationic Au(I)-bis(NHC) complexes; **3a,b** were about 10-fold more active than **2a,b** (**3b** to higher extent than **3a**). **3b** showed the highest activity among the new complexes with outstanding IC_{50} = 0.10 μM at MCF-7 cells. Unexpectedly, the 4-OH substituted complex **3c** caused strong growth inhibition too, with selectivity for MCF-7 (IC_{50} = 0.30 μM) and HT-29 cells (IC_{50} = 0.47 μM). In contrast to **2c**, which was inactive at MDA-MB 231 cell, its IC_{50} = 1.55 μM even surpassed that of cisplatin.[43]

The influence of the counterion and the oxidation state on the in vitro antitumor effects is low. The IC_{50} values of bromides (**3a,b**) are nearly the same as those of tetrafluoroborate salts (**7a,b**). Oxidation of **3a,b** with Br_2 yielding **6a,b** only slightly reduced the growth inhibitory effects in the case of **3b** → **6b** (see Table 2).

Finally, the exchange of the bromide ligands in **6a,b** by iodide increased the IC_{50} values at MDA-MB 231 cells from about 0.50 to 0.63 μM (**8a**) and 0.77 μM (**8b**). The effects at MCF-7 and HT-29 cells remained unchanged.

In conclusion, the attempt to increase the antitumor potency of NHC-Au-Br complexes was successful if the bromide ligand was exchanged by PPh_3 or a further NHC ligand. The resulting complexes were more active than cisplatin and 5-FU with IC_{50} values in the low nanomolar range. As this initial biological screening of fluoro- and methoxy-substituted compounds showed comparable results and no substantial differences between Au(I) complexes and Au(III) complexes, we mainly focused on fluoro-substituted Au(I) complexes (**3b**, **4b**, **5b**, and **7b**) in the following experiments.

The time−activity curves of **3b** and **4b** at concentrations ranging from 0.063 to 1 μM are presented in Figure 2 and Figure S1. In contrast to cisplatin[42] and **2a,b**[43] both complexes caused cytocidal effects (T/C_{corr} < 0%) at 1 μM. At higher concentrations their curves are characterized by a fast onset of activity with maximal effects after an incubation time of 48 h. Cisplatin achieved its maximal effect at the end of the experiment. Furthermore, both figures show, in the cases of **3b** and **4b**, only a marginal recuperation of the tumor cells after a prolonged exposition time. Therefore, the development of drug resistance is very unlikely.[42,43,47]

Cellular Uptake Studies. It is well-known that uncharged molecules can cross the cell membrane more easily by passive diffusion than ionic drugs. Because the majority of the new complexes are permanent cations, it is of interest to study their cellular uptake. In this context an extremely valuable parameter is the cellular molar concentration, which can be calculated from the quotient of metal and cellular protein amounts, the mean cellular volume, and the mean cellular protein content.[34,35,40,41,43,47] Relation to the extracellular concentration allows the calculation of the accumulation grade.

To quantify the intracellular drug levels in HT-29 and MCF-7 cells, we used a recently described electrothermal atomic absorption spectroscopy (ETAAS) method, which was developed for the bioanalysis of gold complexes and based on the standard addition method.[34,35] Experiments were performed using an extracellular concentration of 10 μM and a short exposure period (6 and 24 h). Under these conditions no significant loss of cell biomass occurred due to toxic effects. Results were corrected for the respective protein content of the samples, and values are accordingly presented as microgram of gold per milligram of protein (see Table 3).

With the exception of complex **7b**, for which a 60% reduced intracellular gold content was measured, all complexes hold their Au levels in MCF-7 cells over an incubation time of 24 h. In HT-29 cells an even higher metal concentration was measured.

dx.doi.org/10.1021/jm3000196 | J. Med. Chem. 2012, 55, 3713−3724

Table 4. Uptake of Complexes into the Nuclei of HT-29 and MCF-7 Cells (ng of Gold per mg of Nuclear Protein)[a]

	2b	3b	4b	5b	7b	Et_3PAuCl
MCF-7	81.4 ± 36.5	160.1 ± 29.9	225.9 ± 127.9	237.2 ± 60.8	162.0 ± 34.7	176.8 ± 80.3
HT-29	9.5 ± 4.1	200.6 ± 59.0	216.1 ± 61.9	22.8 ± 5.7	216.3 ± 84.9	56.6 ± 14.3

[a]Results were calculated from the data of three independent experiments.

The accumulation depends on the complex structure. Each modification on **2b** increased the intracellular gold content: exchange of the Br^- by 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiolate from 0.067 (**2b**) to 0.35 μg/mg (**5b**) in MCF-7 cells (6 h) and from 0.022 to 0.032 μg/mg in HT-29 cells (6 h). The use of PPh_3 or NHC further increased the uptake. Independent of the counterion, the highest drug level was determined for the Au(I)-bisNHC complexes **3b** and **7b**. The measured amounts (**3b**/**7b**) of 0.93/1.0 μg/mg in MCF-7 and 0.70/0.63 μg/mg in HT-29 cells correspond with accumulation grades of 53.2/58.5 in MCF-7 and 69.6/63.2 in HT-29 cells which are higher than those of cisplatin (2.3/2.9)[43] and Et_3PAuCl (16.1/5.2).

The cellular uptake values for complexes are in accordance with their growth inhibitory potency. In vitro cytotoxicity assays showed that **3b**, **4b**, and **7b** were more active than **2b**, **5b**, and Et_3PAuCl. Furthermore, comparable cytotoxicities for **3b**, **4b**, and **7b** in both cell lines were obtained.

Nuclear Uptake Studies. Prompted by the favorable results of the cellular uptake results, we investigated whether the cytotoxic effects of gold complexes might be a consequence of a metal accumulation in the nucleus, which was already confirmed by Ott et al.[33,34,40,41] for the [*N*-(*N*′,*N*′-dimethylaminoethyl)-1,8-naphthalimide-4-sulfide](triethylphosphine)-gold(I) complex.

Therefore, the nuclei of MCF-7 and HT-29 cells treated with **2b**, **3b**, **4b**, **5b**, **7b**, or Et_3PAuCl were isolated by a short sucrose gradient and investigated for their gold content using ETAAS. The results were calculated as nanogram of gold per milligram of nuclear protein (see Table 4).

Complex **2b** caused a gold content in the nuclei of MCF-7 cells (81.4 ng/mg) that was half of that obtained with Et_3PAuCl (176.8 ng/mg). The metalation of the nuclei was more efficient with the Au(I)-bisNHC complexes **3b** and **7b** (~160 ng/mg) and the auranofin derivatives **4b** and **5b** (~230 ng/mg).

Another situation was found in HT-29 cells. In the nuclei of cells treated with **2b** only an amount of 9.5 ng/mg was determined, much lower than in MCF-7 cells. Exchange of the bromide by 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiolate (**5b**) increased the gold amount only to 22.8 ng/mg. Both values are below that obtained with Et_3PAuCl (56.6 ng/mg). The most effective metal uptake in the nuclei of 200−216 ng/mg was achieved with the cationic complexes, meaning an about 20-fold higher accumulation grade in the nuclei compared to the parent compound **2b**.

In conclusion, the accumulation studies point to an active transport of the charged complexes through the cell membrane or by a passive transport as stable ionic pairs. In the nuclei the cationic complexes can bind electrostatically at the negative charged phosphate backbone of the DNA and can participate on the damage of the cells. The exact DNA binding mode is still unclear but will be investigated in detail in a forthcoming study. Nevertheless at this stage of the study it could be demonstrated that the NHC ligands are suitable for directing the complexes in high amounts to the nuclei.

Pharmacological Profile. On the basis of the history of the drug design (see Introduction), the complexes are derivatives of auranofin, a competent thioredoxin reductase inhibitor,[33−41,43,48−52] and the NHC are derivatives of synthetic hormones (estrogen receptor binder) and cyclooxygenase inhibitors.[42,43,53−55] These targets must be considered as targets of the novel complexes, too.

Interaction of the Enzyme TrxR. As discussed above (Introduction), the TrxR inhibition is a well accepted principle to reduce tumor cell growth.[17,26,33−41,43] Auranofin and related gold complexes demonstrated high affinity for TrxR, leading to inactivation due to the formation of a covalent bond to the Se center of the enzyme.[17,26,33−41,43,48−52]

The inhibitory potentials of the novel gold complexes **2a−c** as well as Et_3PAuCl and auranofin (as positive controls) were measured by the dithiobis(nitrobenzoic acid) (DTNB) reduction assay using isolated rat liver TrxR and were recently published.[26,40,43]

The structures of the complexes clearly determined the TrxR inhibitory effects (Table 5). While the bivalent Au(I)-bisNHC complexes **3a−c**, **6a,b**, **7a,b**, and **8a,b** were nearly inactive against TrxR (IC_{50} > 2 μM), the Ph_3P and glucopyranosylthiolate derivatives **4a,b** and **5a,b** caused a 50% inhibition of the enzyme at about half of the concentration used for **2a,b**. Compared to Et_3PAuCl and auranofin (IC_{50} of 25.8 and 18.6 nM, respectively), however, all complexes were less active and a correlation between the inhibition of TrxR and cytotoxicity does not exist.

Table 5. Inhibition of Thioredoxin Reductase (TrxR)[a]

compd	IC_{50} [nM]	compd	IC_{50} [nM]
2a[b]	1505.0 ± 27.1	**5b**	527.7 ± 33.5
2b[b]	815.4 ± 74.3	**6a**	4028.7 ± 374.1
2c[b]	4371.3 ± 322.1	**6b**	5163.0 ± 104.1
3a	>10000	**7a**	3430.6 ± 249.2
3b	>10000	**7b**	6786.2 ± 616.5
3c	>10000	**8a**	2521.5 ± 84.3
4a	700.1 ± 40.2	**8b**	2388.3 ± 96.1
4b	424.3 ± 50.4	auranofin	18.6 ± 7.2
5a	784.1 ± 155.2	Et_3PAuCl	25.8 ± 13.0

[a]The IC_{50} was determined as the concentration causing 50% decrease of enzyme activity and calculated as the mean of three independent experiments. [b]Data from ref 43.

Reaction with Glutathione. Thiols such as the tripeptide glutathione play an important role for inactivation of cisplatin and many other metal based drugs. Auranofin is biologically processed in the same way and metabolized in ligand exchange processes. This "thiol-based" metabolism of gold(I) complexes hampered so far the development of novel drug candidates out of this class.[37,56] Therefore, it was of interest to study the interaction of our complexes with glutathione under physiological conditions.

In the used assay, an excess of the respective gold complex (166.7 μM) was incubated with reduced glutathione at 37 °C.

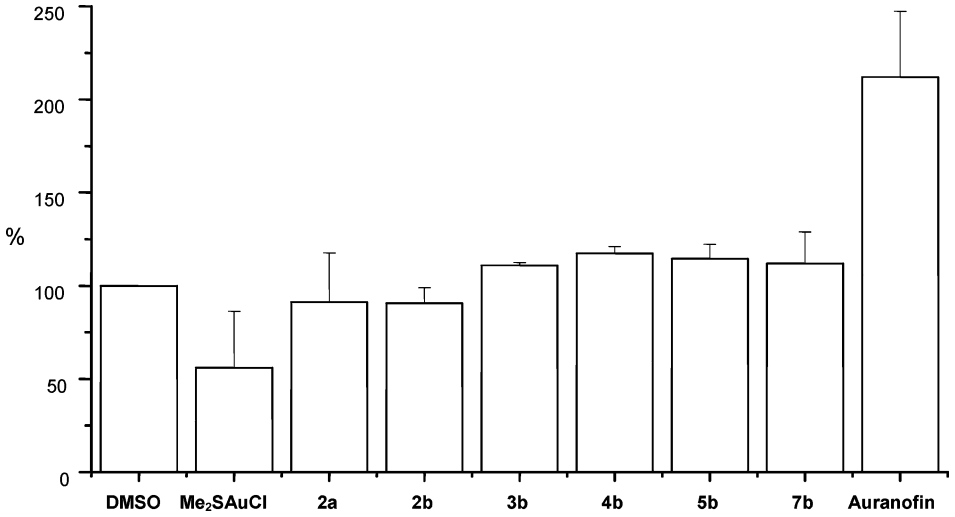


Figure 3. Interaction of Au(I) complexes with glutathione and DTNB: percentage building of TNB after 60 min of incubation.

After 60 min, oxidized GSH was quantified by reduction of DTNB to 2-nitro-5-thionitrobenzoic acid (TNB). Under the chosen conditions auranofin strongly increased the TNB building by 212% (Figure 3) compared to a DMSO control (100%).[26,43,52] In contrast, similar to **2a** (91%) and **2b** (90%), the selected Au(I)-NHC complexes **3b** (111%), **4b** (117.5%), **5b** (114.5%), and **7b** (112.0%) did not significantly induce the oxidation of GSH. Accordingly, the choice of NHC ligands probably provides a useful strategy for the design of gold(I) drugs with enhanced biological stability.

ER Interaction and the Inhibition of COX. As the NHC ligands described in this paper are derivatives of 2,4,5-triarylimidazoles designed as ER ligands,[42,43,53−55] we investigated if the ER is involved in the mode of action in hormone-dependent MCF-7 cells. For this purpose, ERα-positive MCF-7 breast cancer cells stably transfected with the plasmid ERE_{wtc}luc (MCF-7-2a cells) and U2-OS cells transiently transfected with the plasmid pSG5-ERα (U2-OS/α) or pSG5-ERβ FL (U2-OS/β) and the reporter plasmid p(ERE)2-luc+ were used to evaluate ER subtype selectivity on molecular level.[53−55,57] Unfortunately, all complexes did not induce agonistic or antagonistic properties (data not shown). Therefore, an ER-mediated cytotoxicity can be excluded.

A further possible target of triarylimidazole derivatives are the cyclooxygenase enzymes (COX-1/2) as demonstrated in an earlier study.[42,43,55] Both subtypes are the targets not only for the design of anti-inflammatory drugs but also in cancer chemotherapy.[55] Recently, it was verified that auranofin inhibited the formation of eicosanoids related to the activity of COX-1 and 12-lipoxygenase (LOX) in human platelets.[33] In addition, our previous results showed that **2b** possesses very promising COX inhibitory properties with COX-1 selectivity at 10 μM (inhibition of COX-1, 100%; inhibition of COX-2, 0%).[43]

In order to estimate if this is limited to monovalent NHC-Au complexes, we examined the relative COX inhibition at 10 μM exemplarily for **3b** by ELISA. Aspirin and **2b** were used for comparison. At the concentration used, aspirin showed only marginal activity at COX-1 (29% inhibition) and was inactive at COX-2. Unlike complex **2b** the Au(I)-bisNHC complex **3b** was inactive at COX-1 and COX-2, so it was decided to stop further experiments at that moment.

CONCLUSION

In this SAR study we demonstrate that gold complexes bearing two 1,3-diethyl-4,5-diarylimidazol-2-ylidene ligands represent effective cytostatics with growth inhibitory effects against MCF-7, MDA-MB 231, and HT-29 cells up to 10-fold higher than cisplatin or 5-FU. Oxidation from Au(I) to Au(III) or variation of the counterion of the cationic Au(I)-bisNHC complexes did not change their cell growth-inhibitory capacities. They were effective accumulated in the tumor cells and located in high amounts in the nuclei.

The mode of action is still unclear. Direct DNA damage, modification of the cell cycle, mitochondrial damage involving thioredoxin reductase inhibition, proteasome inhibition, modulation of specific kinases, and other cellular processes that eventually trigger apoptosis must be taken into account. First investigations indicated that thioredoxin reductase, the estrogen receptor, and the cyclooxygenase enzymes, which must also be considered as targets based on our drug design, can be excluded. Further studies on the mode of action are in progress and will be presented in a forthcoming paper.

EXPERIMENTAL SECTION

General. The following instrumentation was used: for ^{1}H and ^{13}C NMR spectra, Bruker ADX 400 spectrometer operated at 400 or 100 MHz (internal standard, tetramethylsilane); for ESI-TOF spectra, Agilent 6210 ESI-TOF, Agilent Technologies, Santa Clara, CA, U.S.; for elemental C, H, N analysis, PerkinElmer 240 B and C analyzer. All complexes reported in the manuscript have a purity of >95%. Chemicals were obtained from Sigma-Aldrich (Germany) and used without further purification. Reactions were all monitored by TLC, performed on silica gel plates 60 F254 (Merck, Darmstadt/Germany). Visualization on TLC was achieved by UV light. Column chromatography was performed with Merck silica gel 60H, grain size of <0.063 mm, 230 mesh ASTM (Darmstadt/Germany).

General Procedure for the Synthesis of 3a−c. Method A. LiHMDS (104 mg, 0.62 mmol) was added to a solution of the respective imidazolium salt (**1a−c**, 0.55 mmol) in DMF (3 mL). The resulting solution was stirred for 15 min, and a solution of Me_2SAuCl (81 mg, 0.275 mmol) and LiBr (477 mg, 5.5 mmol) in DMF (1 mL) was added. The suspension was stirred for an additional 4 h. Then water (10 mL) was added, and a gray precipitate formed. The precipitate was collected and washed with water (3 × 10 mL). Column chromatography on silica gel (CH_2Cl_2/CH_3OH) followed by recrystallization from CH_2Cl_2/*n*-hexane yielded pure gold complexes.

dx.doi.org/10.1021/jm3000196 | J. Med. Chem. 2012, 55, 3713−3724

Method B. To a stirred solution of the respective imidazolium salt (**1a−c**, 0.16 mmol) in CH_2Cl_2/methanol (1:1.2 mL) was added silver(I) oxide (21 mg, 0.09 mmol) under N_2, and the reaction mixture was allowed to stir for 12 h in the dark. Me_2SAuCl (23.5 mg, 0.08 mmol) and LiBr (139 mg 1.6 mmol) were added, and the suspension was stirred for an additional 6 h under N_2. The resultant gray precipitate was filtered over a bed of Celite. The colorless filtrate was isolated, and the volatiles were removed under reduced pressure to yield a colorless oil. Column chromatography on silica gel (CH_2Cl_2/CH_3OH) followed by recrystallization from CH_2Cl_2/*n*-hexane yielded pure gold complexes.

Method C. K_2CO_3 (1.8 g, 13 mmol) was added to a mixture of the respective imidazolium salt (**1a−c**, 10 mmol) and the NHC-Au(I)-Br complex (**2a−c**, 10 mmol) in acetone (30 mL). The mixture was stirred for 24 h. The solvent was removed under reduced pressure. CH_2Cl_2 (10 mL) was added and filtered through Celite. The solvent was removed under reduced pressure and the residue was recrystallized from CH_2Cl_2/*n*-hexane, yielding pure gold complexes.

Bis[1,3-diethyl-4,5-bis(4-methoxyphenyl)imidazol-2-ylidene]gold(I) Bromide 3a. Method A: yield 77.3%. Method B: yield 55.6%. Method C: yield 92.1%. A white solid. ^{1}H NMR ($CDCl_3$): δ 1.41 (t, 12H, CH_2CH_3, J = 7.2 Hz), 3.81 (s, 12H, OCH_3), 4.24 (q, 8H, CH_2CH_3, J = 7.2 Hz), 6.90 (d, 8H, ArH, J = 8.4 Hz), 7.16 (d, 8H, ArH, J = 8.4 Hz). Anal. Calcd for $C_{42}H_{48}AuBrN_4O_4 \cdot 0.1H_2O$: C, 53.01; H, 5.11; N, 5.89. Found: C, 52.57; H, 5.25; N, 5.90.

Bis[1,3-diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene]gold(I) Bromide 3b. Method A: yield 73.2%. Method B: yield 60.3%. Method C: yield 88.2%. A white solid. ^{1}H NMR ($CDCl_3$): δ 1.42 (t, 12H, CH_2CH_3, J = 7.2 Hz), 4.29 (q, 8H, CH_2CH_3, J = 7.2 Hz), 7.09 (t, 8H, ArH, J = 8.4 Hz), 7.24−7.27 (m, 8H, ArH). Anal. Calcd for $C_{38}H_{36}AuBrF_4N_4$: C, 50.62; H, 4.02; N, 6.21. Found: C, 50.83; H, 4.11; N, 6.52.

Bis[1,3-diethyl-4,5-bis(4-hydroxyphenyl)imidazol-2-ylidene]gold(I) Bromide 3c. Method A: yield 55.1%. Method B: yield 32.1%. Method C: yield 78.1%. A white solid. ^{1}H NMR (DMSO-d_6): δ 1.32 (t, 12H, CH_2CH_3, J = 7.2 Hz), 4.12 (q, 8H, CH_2CH_3, J = 7.2 Hz), 6.78 (d, 8H, ArH, J = 8.4 Hz), 7.16 (d, 8H, ArH, J = 8.4 Hz), 9.82 (s, 4H, ArOH, exchangeable by D_2O). Anal. Calcd for $C_{38}H_{40}AuBrN_4O_4$: C, 51.07; H, 4.51; N, 6.27. Found: C, 51.11; H, 4.63; N, 6.29.

General Procedure for the Synthesis of 4a and 4b. A solution of potassium hexafluorophosphate (100 mg, 0.543 mmol), the respective NHC-Au(I)-Br complex (**2a** or **2b**) (0.291 mmol), and triphenylphosphine (100 mg, 0.380 mmol) in acetone (8 mL) was stirred for 1 h. The mixture was filtered, and the solid was extracted with additional acetone (2 × 3 mL). The filtrates were evaporated under reduced pressure. The residue was washed with petroleum ether (2 × 5 mL) and water (2 × 5 mL), dried in vacuo, and recrystallized from CH_2Cl_2/*n*-hexane to give the product as a white solid.

[1,3-Diethyl-4,5-bis(methoxyphenyl)imidazol-2-ylidene](triphenylphosphine)gold(I) Hexafluorophosphate 4a. Yield: 75.2% of a white solid. ^{1}H NMR (DMSO-d_6): δ 1.36 (t, 6H, CH_2CH_3, J = 7.2 Hz), 3.79 (s, 6H, OCH_3), 4.21 (q, 4H, CH_2CH_3, J = 7.2 Hz), 6.88 (d, 4H, ArH, J = 8.4 Hz), 7.17 (d, 4H, ArH, J = 8.4 Hz), 7.54−7.59 (m, 15H, ArH). Anal. Calcd for $C_{42}H_{46}AuF_6N_2O_2P_2 \cdot H_2O$: C, 50.36; H, 4.83; N, 2.80. Found: C, 49.96; H, 4.45; N, 2.52.

[1,3-Diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene](triphenylphosphine)gold(I) Hexafluorophosphate 4b. Yield: 69.3% of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.39 (t, 6H, CH_2CH_3, J = 7.2 Hz), 4.23 (q, 4H, CH_2CH_3, J = 7.2 Hz), 7.08 (t, 4H, ArH, J = 8.4 Hz), 7.23−7.26 (m, 4H, ArH), 7.50−7.66 (m, 15H, ArH). Anal. Calcd for $C_{40}H_{40}AuF_8N_2P_2 \cdot 0.5CH_2Cl_2$: C, 48.54; H, 4.12; N, 2.80. Found: C, 48.72; H, 3.66; N, 3.12.

General Procedure for the Synthesis of 5a and 5b. The respective NHC-Au(I)-Br complex (**2a** or **2b**) (0.460 mmol) and 2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiol (175 mg, 0.480 mmol) were dissolved in CH_2Cl_2 (8 mL), and triethylamine (70 μL, 0.500 mmol) was added. The reaction was monitored by TLC (CH_2Cl_2) and was complete after 2 h. The solvent was removed under reduced pressure and after chromatography on silica (CH_2Cl_2/petroleum ether) and recrystallization from EtOH/H_2O a fluffy white solid was left.

[1,3-Diethyl-4,5-bis(4-methoxyphenyl)imidazol-2-ylidene](2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiolato)gold(I) 5a. Yield: 42.1% of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.34 (t, 6H, CH_2CH_3, J = 7.2 Hz), 1.97 (3H, s, OAc), 2.00 (3H, s, OAc), 2.03 (3H, s, OAc), 2.11 (3H, s, OAc), 3.80 (s, 6H, OCH_3), 4.12−4.25 (m, 7H, CH_2CH_3, β-D-glucopyranosyl H), 5.09−5.15 (m, 4H, β-D-glucopyranosyl H), 6.86 (d, 4H, ArH, J = 8.4 Hz), 7.10 (d, 4H, ArH, J = 8.4 Hz). Anal. Calcd for $C_{35}H_{44}AuN_2O_{11}S$: C, 46.82; H, 4.94; N, 3.12. Found: C, 46.87; H, 4.89; N, 3.11.

[1,3-Diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene](2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiolato)gold(I) 5b. Yield: 53.1% of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 6H, CH_2CH_3, J = 7.2 Hz), 1.97 (3H, s, OAc), 2.01 (3H, s, OAc), 2.03 (3H, s, OAc), 2.11 (3H, s, OAc), 4.08−4.29 (m, 7H, CH_2CH_3, β-D-glucopyranosyl H), 5.08−5.15 (m, 4H, β-D-glucopyranosyl H), 7.06 (t, 4H, ArH, J = 8.4 Hz), 7.16−7.19 (m, 4H, ArH). Anal. Calcd for $C_{33}H_{38}AuF_2N_2O_9S$: C, 45.37; H, 4.38; N, 3.21. Found: C, 45.57; H, 4.54; N, 3.27.

General Procedure for the Synthesis of 6a and 6b. The respective Au-bisNHC bromide complex (**3a** or **3b**) (0.14 mmol) was dissolved in CH_2Cl_2 (20 mL), and bromine (23.9 mg, 0.15 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise at −30 °C. The resultant solution was stirred for 30 min at −30 °C and for another 2 h at ambient temperature. The solvent as well as the excess of bromine was removed under reduced pressure. The residue was recrystallized from CH_2Cl_2/*n*-hexane to yield an orange powder.

Dibromo-bis[1,3-diethyl-4,5-bis(4-methoxyphenyl)imidazol-2-ylidene]gold(III) Bromide 6a. Yield: 89.5% of an orange powder. ^{1}H NMR (DMSO-d_6): δ 1.37 (t, 12H, CH_2CH_3, J = 7.2 Hz), 3.77 (s, 12H, OCH_3), 4.22 (q, 8H, CH_2CH_3, J = 7.2 Hz), 7.01 (d, 8H, ArH, J = 8.4 Hz), 7.39 (d, 8H, ArH, J = 8.4 Hz). Anal. Calcd for $C_{42}H_{48}AuBr_3N_4O_4 \cdot 1.2CH_2Cl_2$: C, 42.83; H, 4.19; N, 4.62. Found: C, 43.13; H, 4.67; N, 5.01.

Dibromo-bis[1,3-diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene]gold(III) Bromide 6b. Yield: 90.3% of an orange powder. ^{1}H NMR (DMSO-d_6): δ 1.37 (t, 12H, CH_2CH_3, J = 7.2 Hz), 4.25 (q, 8H, CH_2CH_3, J = 7.2 Hz), 7.32 (t, 8H, ArH, J = 8.8 Hz), 7.55−7.59 (m, 8H, ArH). Anal. Calcd for $C_{38}H_{36}AuBr_3F_4N_4 \cdot 2.2CH_2Cl_2$: C, 38.68; H, 3.26; N, 4.49. Found: C, 38.48; H, 3.45; N, 4.88.

General Procedure for the Synthesis of 7a and 7b. To a solution of the respective Au(I)-bisNHC bromide complex (**3a** or **3b**) (1 mmol) in DMF (8 mL) was added $NaBF_4$ (378 mg, 10 mmol). The solution was stirred for 2 h. Then water (10 mL) was added, and a gray precipitate formed. The precipitate was collected, washed with water (3 × 10 mL), and recrystallized from CH_2Cl_2/*n*-hexane, yielding the pure gold complexes.

Bis[1,3-diethyl-4,5-bis(methoxyphenyl)imidazol-2-ylidene]gold(I) Tetrafluoroborate 7a. Yield: 93% of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.41 (t, 12H, CH_2CH_3, J = 7.2 Hz), 3.81 (s, 12H, OCH_3), 4.24 (q, 8H, CH_2CH_3, J = 7.2 Hz), 6.90 (d, 8H, ArH, J = 8.4 Hz), 7.16 (d, 8H, ArH, J = 8.4 Hz). Anal. Calcd for $C_{42}H_{48}AuBF_4N_4O_4$: C, 52.73; H, 5.06; N, 5.86. Found: C, 52.40; H, 5.27; N, 6.02.

Bis[1,3-diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene]gold(I) Tetrafluoroborate 7b. Yield: 95% of a white solid. ^{1}H NMR ($CDCl_3$): δ 1.40 (t, 12H, CH_2CH_3, J = 7.2 Hz), 4.26 (q, 8H, CH_2CH_3, J = 7.2 Hz), 7.09 (t, 8H, ArH, J = 8.4 Hz), 7.24−7.27 (m, 8H, ArH). Anal. Calcd for $C_{38}H_{36}AuBF_8N_4$: C, 50.24; H, 3.99; N, 6.17. Found: C, 50.29; H, 3.80; N, 6.11.

General Procedure for the Synthesis of 8a and 8b. The respective Au(I)-bisNHC tetrafluoroborate complex (**7a** or **7b**) (0.130 mmol) was dissolved in CH_2Cl_2 (20 mL), and elemental iodine (35.2 mg, 0.140 mmol) dissolved in CH_2Cl_2 (10 mL) was added dropwise at −30 °C. The resulting solution was stirred for 30 min at −30 °C and for another 2 h at ambient temperature. Evaporation of the solvent under reduced pressure yielded a reddish orange solid, which was washed several times with ether. The solid was then dissolved in CH_2Cl_2 and filtered through Celite. The solvent was removed under vacuum and recrystallized from CH_2Cl_2/*n*-hexane to get a red powder.

dx.doi.org/10.1021/jm3000196 | J. Med. Chem. 2012, 55, 3713−3724

Diiodo-bis[1,3-diethyl-4,5-bis(4-methoxyphenyl)imidazol-2-ylidene]gold(III) Tetrafluoroborate 8a. Yield: 82.1% of a red solid. ^{1}H NMR ($CDCl_3$): δ 1.41 (t, 12H, CH_2CH_3, J = 6.4 Hz), 3.83 (s, 12H, OCH_3), 4.26 (q, 8H, CH_2CH_3, J = 6.4 Hz), 6.93 (d, 8H, ArH, J = 8.4 Hz), 7.22 (d, 8H, ArH, J = 8.4 Hz). Anal. Calcd for $C_{42}H_{48}AuBF_4I_2N_4O_4$: C, 41.68; H, 4.00; N, 4.63. Found: C, 41.87; H, 4.11; N, 4.41.

Diiodo-bis[1,3-diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene]gold(III) Tetrafluoroborate 8b. Yield: 75.4% of a red solid. ^{1}H NMR ($CDCl_3$): δ 1.44 (t, 12H, CH_2CH_3, J = 7.2 Hz), 4.31 (q, 8H, CH_2CH_3, J = 7.2 Hz), 7.16 (t, 8H, ArH, J = 8.4 Hz), 7.34−7.37 (m, 8H, ArH). Anal. Calcd for $C_{38}H_{36}AuBF_8I_2N_4$: C, 39.27; H, 3.12; N, 4.82. Found: C, 39.32; H, 3.05; N, 4.65.

X-ray Crystallography. The intensities for the X-ray determinations were collected on a STOE IPDS 2T instrument with Mo Kα radiation (λ = 0.710 73 Å) at 200 K. Standard procedures were applied for data reduction and absorption correction. Structure solution and refinement were performed with SHELXS97 and SHELXL97.[58] Hydrogen atom positions were calculated for idealized positions and treated with the "riding model" option of SHELXL. More details on data collections and structure calculations are summarized in Table 1 and Supporting Information.

Water Solubility. An amount of 10 μL of a 100 mM gold complex stock solution (DMSO) was added to 990 μL of water at 37 °C. The sample was shaken at 37 °C overnight and was centrifuged at 20 000 rpm and 37 °C for 30 min. The concentration of the complex was calculated by the gold content in the solution measured by ETAAS.

Cytotoxicity. The human MCF-7, MDA-MB 231 breast cancer cell, and HT-29 colon cancer cell lines were obtained from the American Type Culture Collection. All cell lines were maintained as a monolayer culture in L-glutamine containing Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose (PAA Laboratories, Austria), supplemented with 5% fetal bovine serum (FBS; Biochrom, Germany) in a humidified atmosphere (5% CO_2) at 37 °C.

The experiments were performed according to established procedures with some modifications.[42,43,47] In 96-well plates, an amount of 100 μL of a cell suspension in culture medium at 7500 cells/mL (MCF-7 and MDA-MB 231) or 3000 cells/mL (HT-29) was plated into each well and was incubated for 3 days under cell culture conditions. After the addition of various concentrations of the test compounds, cells were incubated for up to the appropriate incubation time. Then the medium was removed, the cells were fixed with glutardialdehyde solution 1%, and stored under phosphate buffered saline (PBS) at 4 °C. Cell biomass was determined by crystal violet staining, followed by extraction of the bound dye with ethanol and a photometric measurement at 590 nm. Mean values were calculated, and the effects of the compounds were expressed as percent treated/$control_{corr}$ values according to the following equation:

$$T/C_{corr}[\%] = \frac{T - C_0}{C - C_0} \times 100$$

where C_0 is control cells at the time of compound addition, C is control cells at the time of test end, and T is probes/samples at the time of test end.

The IC_{50} was determined as the concentration causing 50% inhibition of cell proliferation and based on a Boltzmann fit (OriginPro 8). Results were calculated as the mean of at least two or three independent experiments.

TrxR Inhibition. To determine the inhibition of TrxR, an established microplate reader based assay was performed with minor modifications.[26,40,43] For this purpose, commercially available rat liver TrxR (from Sigma-Aldrich) was used and diluted with distilled water to achieve 2.0 U/mL. The compounds were freshly dissolved as stock solutions in DMSO. To each, 25 μL aliquots of the enzyme solution, each 25 μL of potassium phosphate buffer, pH 7.0, containing the compounds in graded concentrations or vehicle (DMSO) without compounds (control probe), were added, and the resulting solutions (final concentration of DMSO, 0.5% v/v) were incubated with moderate shaking for 75 min at 37 °C in a 96-well plate. To each well, an amount of 225 μL of the reaction mixture (1000 μL of reaction mixture consisted of 500 μL of potassium phosphate buffer, pH 7.0, 80 μL of 100 mM EDTA solution, pH 7.5, 20 μL of BSA solution 0.05%, 100 μL of 20 mM NADPH solution, and 300 μL of distilled water) was added, and the reaction was started by addition of 25 μL of a 20 mM ethanolic DTNB solution. After proper mixing, the formation of TNB was monitored with a microplate reader (Perkin-Elmer Victor X4) at 405 nm at 10 s intervals for 6 min. The increase in TNB concentration over time followed a linear trend ($r^2 \geq 0.99$), and the enzymatic activities were calculated as the slopes (increase in absorbance per second) thereof. For each tested compound, the noninterference with the assay components was confirmed by a negative control experiment using an enzyme-free solution. The IC_{50} values were calculated as the concentration of compound decreasing the enzymatic activity of the untreated control by 50% and are given as the mean of three independent experiments.

Reaction with Glutathione. The gold complexes were prepared as stock solutions in DMSO and diluted with potassium phosphate buffer, pH 7.0, to achieve a final concentration of 500 μM (DMSO, 0.2% v/v). To 25 μL of a 250 μM aqueous solutions of reduced glutathione, each 25 μL of the respective potassium phosphate buffer solution (containing the compounds or only the DMSO vehicle as control) and 25 μL of a 100 mM aqueous EDTA solution pH 7.5 were added, and the resulting solutions were incubated with moderate shaking in a 96-well plate at 37 °C for 60 min. To each well, an amount of 200 μL of the reaction mixture (1000 μL of reaction mixture consisted of 620 μL of potassium phosphate buffer, pH 7.0, 80 μL of 100 mM EDTA solution, pH 7.5, and 300 μL of distilled water) was added, and the reaction was started with the addition of 25 μL of a 20 mM ethanolic solution of DTNB. After proper mixing, the formation of TNB was monitored in a microplate reader (Perkin-Elmer Victor X4) at 405 nm. For each tested compound, the noninterference with the assay components was confirmed by a negative control experiment using a glutathione free solution. Results are presented as the mean of at least two independent experiments.[26,43]

Sample Preparation for Cellular Uptake Studies. The cellular uptake was measured according to a previously described procedure.[34,35] In brief, cells were grown until at least 70% confluency in 175 cm^2 cell culture flasks. Stock solutions of the gold complexes in DMF were freshly prepared and diluted with cell culture medium to the desired concentration (final DMF concentration of 0.1% v/v, final gold complex concentration of 10.0 μM). The medium in the cell culture flasks was replaced with 10 mL of fresh cell culture medium solutions containing the compounds, and the flasks were incubated at 37 °C/5% CO_2 for 6 h. Cell pellets were prepared by trypsinization of the cells and centrifugation (room temperature, 2000 rpm, 5 min). Cells were resuspended in twice distilled water, lysed by using a sonotrode, and appropriately diluted using twice distilled water. An aliquot was removed for the purpose of protein quantification by the Bradford method. The determination of the gold content of the samples was performed by ETAAS. Results were calculated from the data of three independent experiments and are given as microgram of gold per milligram of cellular protein.

Sample Preparation for Nuclear Uptake Studies. The nuclei of the tumor cells were isolated according to previously described procedures[34,40,41] with some minor modifications. Cells were grown in 175 cm^2 cell culture flasks until at least 70% confluency. The medium was removed and replaced with 10 mL of medium containing 10.0 μM drug. After 24 h of incubation at 37 °C in humidified atmosphere, the drug containing medium was removed, cells were trypsinized, resuspended in 10 mL of cell culture medium, isolated by centrifugation (1500 rpm, 5 min), and 0.5−1.0 mL of 0.9% NaCl solution was added. After centrifugation (1500 rpm, 5 min) pellets were resuspended in 300 μL of RSB-1 (0.01 M Tris-HCl, 0.01 M NaCl, 1.5 mM $MgCl_2$, pH 7.4) and left for 10 min in an ice bath. Swollen cells were centrifuged (2000 rpm, 5 min), resuspended in 300 μL of RSB-2 (RSB-1 containing each 0.3% v/v Nonidet-P40 and sodium desoxycholate), and homogenized by 10−15 up/down pushes in a 1 mL syringe with needle. Aliquots of 50 μL of the homogenisate

dx.doi.org/10.1021/jm3000196 | J. Med. Chem. 2012, 55, 3713−3724

were removed for determination of the total gold content and mixed with 500 μL of water. The homogenisate was centrifuged at 2500 rpm for 5 min, and the resulting crude nuclei were taken up in 150 μL of 0.25 M sucrose containing 3 mM $CaCl_2$. The suspension was underlayed with 150 μL of 0.88 M sucrose and centrifuged 10 min at 2500 rpm. The nuclei pellets were stored at −20 °C or immediately dissolved in 500 μL of water and disrupted by use of a sonotrode. The gold content of the samples was measured by ETAAS and the protein content by the Bradford method. Results are expressed as the mean of three independent experiments as nanogram of gold per milligram of nuclear protein.

Electrothermic Atomic Absorption Spectroscopy (ETAAS). ETAAS measurements were performed according to a previously published standard addition procedure with some minor modifications.[34,35] In brief, to 100 μL aliquots of the diluted lysates increasing amounts of aqueous gold standard solutions were added. All probes were adjusted to a final volume of 200 μL using twice distilled water. Each 20 μL of Triton X-100 (1%) and ascorbic acid (1%) were added, and the probes were measured as described below. The gold content of the lysates was accessed by the linear extrapolation method. A Vario 6 electrothermal atomic absorption spectrometer (Analytik Jena AG) was used for the gold measurements. Gold was detected at a wavelength of 242.8 nm with a bandpass of 0.8 nm. A deuterium lamp was used for background correction. Probes were injected at a volume of 25 μL into regular graphite wall tubes. Drying, atomization, and tube cleaning steps were performed as outlined in more detail in the literature.[34,35] The temperature for pyrolysis was set to 1200 °C. The mean AUC (area under curve) absorptions of duplicate injections were used throughout the study. The limit of gold detection using biological samples as described above was 1.7 μg/L.

Estrogen Receptor Interaction. Estrogen receptor interaction studies were performed according to previously described procedures without modifications.[53−55,57]

Inhibition of COX Enzymes. The inhibition of isolated ovine COX-1 and human recombinant COX-2 was determined with 10 μM of the respective compounds by ELISA ("COX inhibitor screening assay", Cayman Chemicals). Experiments were performed according to the manufacturer's instructions. Absorption was measured at 415 nm (Victor 2, Perkin-Elmer). Results were calculated as the mean of duplicate determinations.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Cystallographic information of **7a**, time-dependent antiproliferative effects of complex **4b**, and additional spectroscopic data of key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the China Scholarship Council (CSC) and the Deutsche Forschungsgemeinschaft (DFG) within FOR 630 "Biological Function of Organometallic Compounds". We are also grateful for technical support by Heike Scheffler, Gerhard Rubner, Andrey Molchanov, and Maxi Wenzel (Institute of Pharmacy, Freie Universität Berlin, Germany).

■ ABBREVIATIONS USED

TrxR, thioredoxin reductase; ER, estrogen receptor; COX, cyclooxygenase; NHC, N-heterocyclic carbene; SAR, structure−activity relationship; LiHMDS, lithium bis(trimethylsilyl)-amide; 5-FU, 5-fluorouracil; ETAAS, electrothermal atomic absorption spectroscopy; DTNB, dithiobis(nitrobenzoic acid); TNB, 2-nitro-5-thionitrobenzoic acid; LOX, lipoxygenase

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