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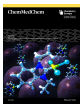

Gold(I) and Gold(III) N-Heterocyclic Carbene Complexes as Antibacterial Agents and Inhibitors of Bacterial Thioredoxin Reductase

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A series of (NHC)Au(I)Cl monocarbene complexes and their gold (III) analogues (NHC)Au(III)Cl_3 were prepared and investigated as antibacterial agents and inhibitors of bacterial TrxR. The complexes showed stronger antibacterial effects against the Gram-positive MRSA and *E. faecium* strains than against several Gram-negative bacteria. All complexes were efficient inhibitors of bacterial thioredoxin reductase, indicating that inhibition of this enzyme might be involved in their mechanism of action. The efficacy of gold(I) and gold(III) analogues was comparable in most of the assays. The cytotoxicity of the gold NHC compounds against cancer and human cells was overall weaker than the activity against the Gram-positive bacteria, suggesting that their optimization as antibacterials warrants further investigation.

Introduction

With increasing numbers of drug-resistant bacteria, the need for new and improved antibiotics has been growing rapidly. Besides the identification of new molecular targets, novel chemical structures are of the utmost interest for medicinal chemistry and drug development. The majority of registered antibiotics, however, belong to only a few chemical classes, such as penicillins or tetracyclines. In this context, metal-based compounds offer a pool of relatively unexplored chemical scaffolds for antibacterial drug design projects.[1]

Over the last 10–15 years, gold complexes have attracted major attention in inorganic medicinal chemistry, leading to very promising experimental drugs, in particular for anticancer indications.[2,3] Ever since an early report by Robert Koch on antimicrobial activity displayed by gold cyanide salts against *M. tuberculosis*,[4] there has been evidence for the possible application of gold compounds as a new type of antibacterials, and this has been substantiated by recent studies.[5]

We have recently reported on gold(I) complexes with *N*-heterocyclic carbene (NHC) ligands with antibacterial and anticancer activity.[6–8] NHC ligands offer the advantage of a wide variability and adaptability of the properties of the complexes. This makes a modular synthetic approach possible, allowing a broad spectrum of modifications to be evaluated and analysed.[9]

With regard to the application of gold compounds as antibacterial agents, their generally high activity as inhibitors of thioredoxin reductase (TrxR) offers an unconventional molecular mechanism of drug action. Notably, many Gram-positive pathogenic bacteria, including *S. aureus*, lack sufficient levels of glutathione, thus making their metabolism highly dependent on the activity of TrxR, which reduces oxidized thioredoxin (Trx).[10] In consequence, a functional Trx/TrxR system is critical for growth and survival of bacteria with low glutathione levels.

In our recent report we were able to confirm the effective inhibition of bacterial TrxR by gold complexes of the type (NHC)Au(I)Cl in combination with high activity against several Gram-positive pathogenic bacteria.[6] Initial structure-activity-relationships indicated that monocarbene (NHC)Au(I)Cl complexes triggered much stronger inhibition of bacterial TrxR than cationic biscarbene complexes of the type Au(I)$(NHC)_2^+$, which were also less active as antibacterial agents, but on the other hand triggered substantially higher antiproliferative activity against cancer cell lines.[7,8] The antibacterial activity of gold NHC biscarbene complexes was also decreased or lost when the organometallics were incorporated in macrocyclic structures.[11]

Here we report on the extension of our efforts in developing antibacterial monocarbene gold NHC complexes by comparing a series of (NHC)Au(I)Cl complexes with the respective gold(III) derivatives of the type (NHC)Au(III)Cl_3. The structures of the carbene ligands contain ethyl groups on the nitrogen side chains, in continuation of our previous work with this type of

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complexes.[6–8] The possible release of the carbene ligands would result in benzimidazolium cations that are probably non-toxic. The synthesis and characterization of the complexes are presented, together with a biological investigation as antibacterial TrxR inhibitors. Experiments with cancer cell lines were added to assess the selectivity of the compounds.

Results and Discussion

Chemistry

The target compounds were synthesised using established procedures with minor modifications (Scheme 1).[6,12–15] In the first step, the (benz)imidazolium salts **1a**–**4a** were formed via alkylation of the respective (benz)imidazoles **1**–**4** with iodoethane in the presence of potassium carbonate. The gold(I) NHC complexes **1b**–**4b** were synthesised in a two-step reaction via a silver intermediate, which was reacted with chlorido(dimethylsulfide)gold(I). The gold(III) compounds **1c**–**4c** were obtained by oxidation of **1b**–**4b** using dichlorophenyliodane.

The products were characterized by ^{1}H- and ^{13}C-NMR, mass spectroscopy, and elemental analysis. These data were in good agreement with the proposed structures. The transformation of the (benz)imidazolium salts **1a**–**4a** to the gold(I) complexes **1b**–**4b** was confirmed by the absence of the C2-hydrogen signal in the spectra of the products; the metal-free precursors **1a**–**4a** show a signal for this hydrogen in the range 10.1-11.3 ppm. Whereas the ^{1}H-NMR spectra of the gold(I) complexes **1b**–**4b** are very similar to those of the respective gold(III) analogues **1c**–**4c**, there is a notable downfield shift (ranging from 25.6 ppm for **4b**/**4c** to 31.7 ppm for **1b**/**1c**) in the ^{13}C-NMR signal of C2 upon complex formation, an effect previously reported by *Sivaram et al.*[16] For the gold(III) complexes, mass spectrometry confirmed the presence of multiple chlorido ligands ($[2\,M\text{-}5Cl]^+$ and $[2M\text{–}Cl]^+$ signals for **1c**, $[M\text{–}Cl+MeOH]^+$ for **2c** and $[M+Na]^+$ signals for **3c** and **4c**). Elemental analyses confirmed the high purity of all target compounds with deviations below 0.41% from the calculated values. Additionally, crystal structures for four complexes were obtained, confirming the structures of **1c**, **2c**, **3b** and **3c** (Figure 1; ellipsoids are shown at the 50% level).

Molecular dimensions (Table 1) may be regarded as normal, with only small deviations from ideal bond angles at the gold atoms. The carbene ligands and the $CAuCl_3$ units are all effectively planar (max. r.m.s. deviation 0.018 Å) and mutually perpendicular (but less so for **1c**). A slight but consistent *trans* influence of the carbene ligand on the Au1–Cl1 bond is observed, making it longer than the *cis* Au–Cl bonds. A search of the Cambridge Database,[17] Version 5.41, gave 38 hits (47 molecules) for the fragment C(carbene)–$AuCl_3$, whereby we deleted one serious outlier; the *trans* Au–Cl bond length then averaged 2.313(9) and the *cis* bond length 2.279(12) Å, with an average Au–C bond length of 2.002(12) Å.

The crystal packing of **1c** involves short intermolecular contacts between chlorine atoms [Cl1···Cl1′ 3.6447(8) Å, operator *-x*, 1-*y*, 1-*z*, and Cl2···Cl2′ 3.4945(9) Å, operator *-x*, 1-*y*, *-z*]. These combine to form zigzag chains of molecules parallel to the *c* axis (Figure 2). Such short halogen-halogen contacts are a special case of "halogen bonds".[18] We have drawn attention to the presence of such interactions between $[AuX_4]^-$ anions (X = Cl, Br).[19]

Molecules of **3b** are linked to form inversion-symmetric pairs by aurophilic interactions [Au1···Au1′ 3.3537(3) Å, operator 1-*x*, 1-*y*, *-z*). Such contacts are frequently observed for gold(I) compounds.[20] A further short, linear contact C7–H7···Cl1 [H···Cl 2.68 Å, angle 170°, operator *x*, 1+*y*, *z*] may reasonably be

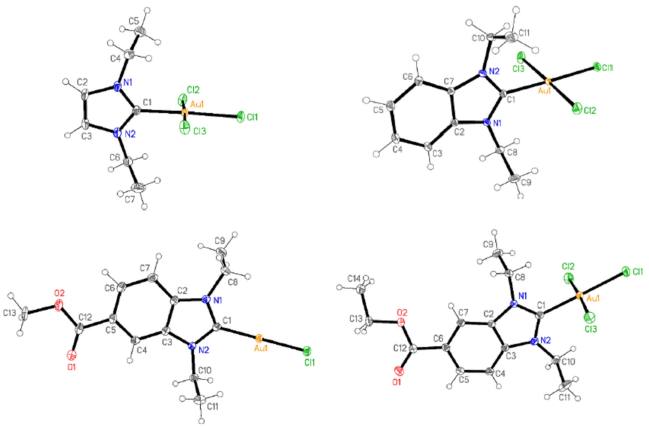
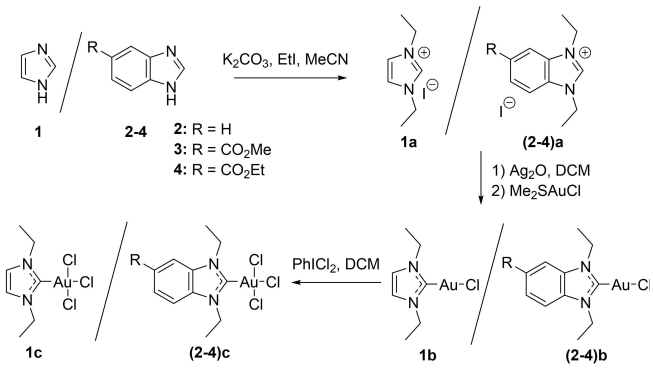


Figure 1. Molecular structures of **1c** (top left), **2c** (top right), **3b** (bottom left) and **4c** (bottom right).



Scheme 1. Synthesis of the target compounds.

Table 1. Selected molecular dimensions (Å or °).

Compound	1c	2c	3b	4c
Au1-Cl1	2.3199(4)	2.3151(3)	2.2852(8)	2.3054(5)
Au1-Cl2	2.2791(4)	2.2767(3)	–	2.2820(6)
Au1-Cl3	2.2814(4)	2.2757(3)	–	2.2868(5)
Au1-C1	1.9974(16)	2.0087(11)	1.978(3)	2.004(2)
C1-Au1-Cl1	178.28(5)	178.20(3)	179.41(9)	179.01(6)
C1-Au1-Cl2	89.34(5)	90.52(3)	–	88.18(6)
C1-Au1-Cl3	88.06(5)	87.29(3)	–	88.43(6)
Interpl. angle carbene/$AuCl_3$	77.2(1)	86.79(2)	–	89.75(3)

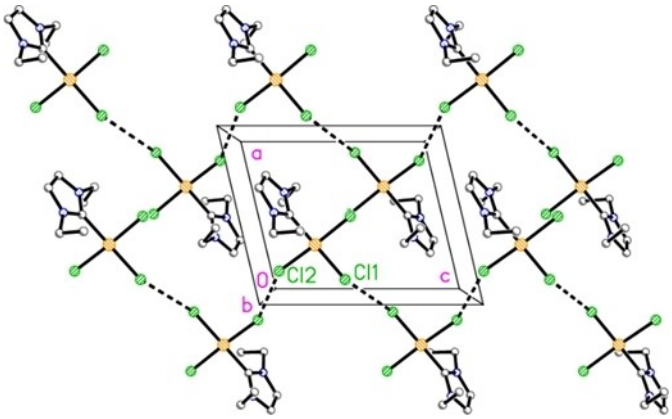


Figure 2. Packing diagram of compound **1 c**, viewed perpendicular to the *ac* plane. Thick dashed lines indicate short Cl···Cl contacts. Hydrogen atoms are omitted for clarity.

regarded as a hydrogen bond. The net effect is to form ribbons of dimers parallel to the *b* axis (Figure 3). Molecules of **4 c** are linked by the hydrogen bond C5–H5···O1 [H···O 2.35 Å, angle 148°, operator 2-*x*, 1-*y*, 1-*z*] to form inversion-symmetric pairs.

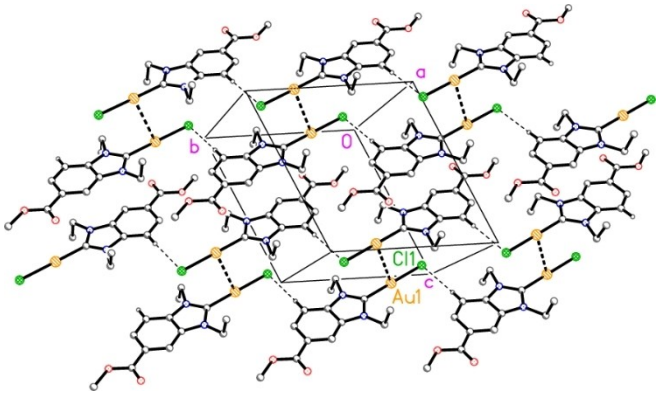


Figure 3. Packing diagram of compound **3 b** viewed perpendicular to the *ac* plane. Thin and thick dashed lines indicate H···Cl or Au···Au contacts respectively. All hydrogen atoms except H7 are omitted for clarity.

Antibacterial effects

The averaged minimal inhibitory concentration (MIC) was determined in two Gram-positive (*E. faecium*, methicillin resistant *S. aureus*, MRSA) and in four Gram-negative (*A. baumannii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*) pathogenic bacteria strains by a curve-fitting procedure (Table 2). Auranofin and various antibiotics served as references.

The gold NHC complexes displayed the highest activity against the Gram-positive strains MRSA (MIC values in the range of 0.8–2.8 μM) and *E. faecium* (MIC values in the range 1.2–11.5 μM) and were less active against the Gram-negative strains *E. Coli* (MIC values > 18 μM), *P. aeruginosa* (MIC values > 30 μM), *A. baumannii* (MIC values 3.5–17.2 μM) and *K. pneumoniae* (MIC values > 18 μM). We have already observed such a preference for Gram-positive strains,[6] although in the current study a substantially stronger activity against the Gram-negative *A. baumannii* was registered.

The results in the two most sensitive strains, MRSA and *E. faecium*, show no clear trend concerning the oxidation state of gold. Compound **2 b** was the most active gold NHC complex against Gram-positive pathogens; however, it remained less active than the gold-containing reference drug auranofin. Interestingly, in the two Gram-negative strains *A. baumannii* and *K. pneumoniae* a consistently higher inhibition of bacterial cell growth was noted for the gold(III) NHC complexes, compared to the respective gold(I) analogues. For example, against *K. pneumoniae* the gold(III) NHC complex **2 c**, with an MIC value of 16.8 μM, was approximately four times as active as the gold(I) analogue **2 b** (MIC value of 65.6 μM).

Inhibition of bacterial TrxR

The inhibition of isolated bacterial *E. coli* TrxR was determined in an enzymatic assay (Table 3). The gold complexes **1 b**–**4 b** and **1 c**–**4 c** and the reference compound $K[Au(CN)_2]$ inhibited the enzyme efficiently, with IC_{50} values in a narrow range of 0.2 to 0.6 μM, suggesting TrxR inhibition as a possible mechanism of antibacterial activity. The most active compounds were auranofin and **2 b**. Comparing gold(I) with gold(III) complexes, no clear trend could be observed.

Table 2. Mean MIC values [μM] ± standard error (n = 3-13); MRSA = methicillin-resistant *S. aureus*; amikacin (*P. aeruginosa*), linezolid (*S. aureus*) and ciprofloxacin (*E. faecium, E. coli, A. baumannii, K. pneumoniae*) were used as antibiotic references.

	MRSA	*E. faecium*	*E. coli*	*P. aeruginosa*	*A. baumannii*	*K. pneumoniae*
antibiotic	2.8 ± 1.0	n.d.	0.3 ± 0.1	6.9 ± 7.3	2.3 ± 1.5	2.9 ± 3.4
auranofin	< 0.2	0.6 ± 0.2	> 30	> 30	7.8 ± 7.1	> 30
1 b	2.8 ± 0.0	1.4 ± 0.0	22.4 ± 0.0	85.1 ± 20.1	3.8 ± 2.4	18.7 ± 9.2
1 c	1.6 ± 0.7	2.3 ± 0.0	18.7 ± 0.0	74.9 ± 0.0	2.3 ± 0.0	18.7 ± 0.0
2 b	0.8 ± 0.4	1.2 ± 0.0	39.3 ± 0.0	118.0 ± 43.1	8.4 ± 2.4	65.6 ± 32.1
2 c	1.1 ± 0.0	4.2 ± 0.0	39.1 ± 25.6	> 30	3.5 ± 1.2	16.8 ± 0.0
3 b	2.2 ± 0.0	11.5 ± 5.0	> 30	> 60	17.2 ± 0.0	91.8 ± 39.7
3 c	1.6 ± 0.5	3.7 ± 0.0	34.9 ± 8.6	59.8 ± 0.0	7.5 ± 0.0	24.9 ± 8.6
4 b	$2.4^{\pm 1}$.6	5.6 ± 2.4	50.1 ± 16.7	> 30	16.7 ± 0.0	66.8 ± 0.0
4 c	1.8 ± 0.0	3.6 ± 0.0	53.4 ± 8.4	58.2 ± 0.0	7.3 ± 0.0	29.1 ± 0.0

Table 3. Inhibition of bacterial TrxR as IC_{50} values ± standard deviation ($n = 3$).

Compound		IC_{50} [μM]
$TrK(Au(CN)_2)$		0.310 ± 0.112
auranofin		0.210 ± 0.030
$Et_2ImAuCl$	**1 b**	0.243 ± 0.032
$Et_2ImAuCl_3$	**1 c**	0.550 ± 0.211
$Et_2BIAuCl$	**2 b**	0.183 ± 0.018
$Et_2BIAuCl_3$	**2 c**	0.270 ± 0.156
$Et_2BICO_2MeAuCl$	**3 b**	0.267 ± 0.077
$Et_2BICO_2MeAuCl_3$	**3 c**	0.252 ± 0.060
$Et_2BICO_2EtAuCl$	**4 b**	0.450 ± 0.121
$Et_2BICO_2EtAuCl_3$	**4 c**	0.332 ± 0.179

Antiproliferative effects

The antiproliferative potential of gold NHC complexes is well known and was studied here in reference to the antibacterial effects.

The cell growth inhibitory effects were determined in A549 lung carcinoma cells, MDA-MB-231 breast adenocarcinoma cells and RC-124 kidney epithelial cells (Table 4). $K[Au(CN)_2]$ and auranofin were used as references.

Whereas the metal free ligands **1 a**–**4 a** were inactive or only marginally active, all the gold complexes exhibited good to moderate cytotoxicity. However, the gold reference compounds auranofin and $K[Au(CN)_2]$ remained the most cytotoxic complexes in this study. The antiproliferative effects of the gold(I/III) NHC complexes were generally stronger in MDA-MB-231 and RC-124 cells (IC_{50} values in the range 4–11 μM for **1 b/1 c**–**4 b/4 c**) than in in A549 cells, where the complexes reached activities of 10–24 μM. The efficient toxicity against RC-124 cells showed that there was no relevant selectivity for tumor tissue. Complex **1 b** was the only example of a gold(I) complex that triggered higher cytotoxicity than the respective gold(III) analogue (**1 c**). In the case of the pairs **2 b/2 c**, **3 b/3 c** and **4 b/4 c** the differences were not significant. The antiproliferative activity of **1 b** and **2 b** had been studied in a previous report and had afforded comparable values, confirming the appropriate reproducibility of the assay procedure.[6] Notably, the antibacterial effects against Gram-positive strains (see Table 2) were in general stronger than the growth-inhibiting effects against human cell lines.

Table 4. Antiproliferative activity as IC_{50} values (μM) ± standard deviation ($n = 2$-4).

Compound	A549	MDA-MB-231	RC-124
$K(Au(CN)_2)$	1.0 ± 0.2	0.3 ± 0.1	0.1 ± 0.0
auranofin	4.2 ± 0.6	1.2 ± 0.3	0.4 ± 0.3
1 a	> 100	> 100	> 100
1 b	13.5 ± 1.3	6.2 ± 0.8	6.1 ± 2.6
1 c	23.6 ± 0.6	10.6 ± 0.0	7.0 ± 2.4
2 a	> 50	> 100	57.0 ± 8.9
2 b	10.0 ± 0.4	8.8 ± 0.6	4.7 ± 1.1
2 c	11.6 ± 0.8	6.7 ± 0.5	6.3 ± 2.0
3 a	51.4 ± 4.8	> 100	> 100
3 b	24.0 ± 0.8	9.5 ± 1.0	7.2 ± 3.7
3 c	19.3 ± 0.6	9.1 ± 0.5	6.4 ± 1.9
4 a	50.8 ± 2.5	> 100	> 100
4 b	11.6 ± 0.5	5.2 ± 0.4	4.0 ± 2.6
4 c	13.2 ± 0.5	4.7 ± 0.2	5.5 ± 0.9

Conclusion

A series of (NHC)Au(I)Cl monocarbene complexes and their gold (III) analogues $(NHC)Au(III)Cl_3$ were prepared, analytically characterized, and investigated as organometallic antibacterial drugs. Whereas the anticancer activity of gold NHC complexes has been intensively studied over the last years, the possible application of gold NHC complexes as anti-infectives has been explored to a much smaller extent. However, antitrypanosomal, antileishmanial or antimalarial effects have also been confirmed for this type of organometallics.[3,21]

The complexes triggered higher antibacterial effects against the Gram-positive MRSA and *E. faecium* than against the Gram-negative strains (*A. baumannii*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*). Such preference for Gram-positive bacteria by monocarbene gold(I) complexes had already been observed in our previous study[6] and a screening of the literature indicates that some other complexes of this type had shown similar effects. [22]

All target complexes were efficient inhibitors of bacterial TrxR, suggesting that inhibition of this enzyme might be involved in the mechanism of the antibacterial activity and confirming our previous results.[6] Interestingly, inhibition of bacterial TrxR has been recently confirmed also for a antimicrobial silver NHC complex ($K_i = 10.8$ nM).[23]

The IC_{50} values for the antiproliferative activity against cancer and human cells were generally higher than the MIC values against the Gram-positive bacteria and were dependent on the individual cell line, indicating that monocarbene gold NHC complexes have a selectivity index in principle, which however needs to be increased by structurally optimized analogues. Notably, some of the complexes reported here have been studied in different cell lines in an assay that uses almost confluent cell layers, and afforded lower cytotoxicity under these experimental conditions (unpublished results).

In summary, this report confirms for the first time the antibacterial effects of gold(III) NHC species and their efficient inhibition of bacterial TrxR. With the exception of the activity against some Gram-negative bacteria, the efficacy of the studied gold(I) and gold(III) analogues was comparable. Further investigation of monocarbene gold NHC complexes for the development of novel antibiotics is worthwhile.

Experimental Section

General

The reagents were purchased from Sigma-Aldrich, Alfa Aesar or TCI and used without additional purification steps. All reactions were performed without precautions to exclude air or moisture. ^1H and ^{13}C NMR were recorded on a DRX-400 AS, AVIIIHD 500 or AVII 600. A Finnigan LCQDeca or a Finnigan MAT 95 was used to record the ESI mass spectra. The elemental analyses were performed using a

Flash EA 1112 (Thermo Quest CE Instruments). Absorption measurements for TrxR inhibition and antiproliferative activity were performed on a Perkin-Elmer 2030 Multilabel Reader VICTOR™ X4. Compounds **1a**, **1b**,[6] **2a**, **2b**,[12] **2c**[13] and $PhICl_2$[14] were prepared as described in the literature.

Synthesis

Ethyl-1H-benzimiazole-5-carboxylate BICOOEt

This compound was prepared according to a modified version of a published procedure.[24] Benzimidazole-5-carboxylic acid (650 mg, 4.009 mmol) was suspended in absolute ethanol (10 mL), conc.H_2SO_4 (0.25 mL, cat.) was added and the solution was stirred under reflux for 26 h. The reaction mixture was poured onto ice/water, made alkaline with NaOH solution (5 N) and extracted with ethyl acetate (EtOAc, 3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo to yield the product. When necessary, the product was redissolved in EtOAc and washed with saturated Na_2CO_3 solution. The aqueous phase was once more extracted with EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was again removed in vacuo to isolate the purified product; yield: 453 mg (2.382 mmol, 59%), brown or pale brown powder; ^{1}H-NMR (500.3 MHz, $CDCl_3$): δ (ppm) = 8.44 (dd, *J* = 1.58, 0.68 Hz, 1 H, C(4)*H*), 8.25 (s, 1 H, C(2)*H*), 8.03 (dd, *J* = 8.51, 1.58 Hz, 1 H, C(6)*H*), 7.69 (dd, *J* = 8.51, 0.68 Hz, 1 H, C(7)*H*), 4.41 (q, *J* = 7.10 Hz, 2 H, OCH_2CH_3), 1.41 (t, *J* = 7.10 Hz, 3 H, OCH_2CH_3); ^{13}C-NMR (100.7 MHz, $CDCl_3$): δ (ppm) = 167.2 (1 C, *C*OO), 142.8 (1 C, NCHN), 140.7 (1 C, *C*(Ar)), 137.5 (1 C, *C*(Ar)), 125.4 (1 C, *C*(Ar)), 124.4 (1 C, *C*(Ar)H), 118.1 (1 C, *C*(Ar)H), 115.0 (1 C, *C*(Ar)H), 61.1 (1 C, OCH_2CH_3), 14.3 (1 C, OCH_2CH_3); MS (ESI): *m/z*: 162.9 $[M+H–Et]^+$, 190.9 $[M+H]^+$; CHN (calc./found) C (63.15/62.97), H (5.30/5.37), N (14.73/14.36).

Procedure for synthesis of (benz-)imidazolium iodides 3a and 4a

The appropriate benzimidazole (1 eq.) and K_2CO_3 (1 eq.) were suspended in acetonitrile (15 mL), ethyl iodide (5 eq.) was added, and the reaction mixture was stirred under reflux for 24 h. The solvent was removed in vacuo, the residue was suspended in dichloromethane (20 mL) and the solution filtered. The solvent was removed again and the residue was washed three times with tetrahydrofuran/*n*-hexane (2/5).

1,3-Diethyl-5-methoxycarbonyl-benzimidazolium iodide 3a

The compound was prepared from methyl-1H-benzimiazole-5-carboxylate (580 mg, 3.292 mmol); yield: 688 mg (1.910 mmol, 58%), pale brown powder; ^{1}H-NMR (400.4 MHz, $CDCl_3$) δ (ppm) = 11.27 (s, 1H, C(2)*H*), 8.44 (dd, *J* = 1.4, 0.7 Hz, 1H, C(4)*H*), 8.35 (dd, *J* = 8.8, 1.4 Hz, 1H, C(6)*H*), 7.85 (dd, *J* = 8.8, 0.7 Hz, 1H, C(7)*H*), 4.76 (qd, *J* = 7.4, 2.3 Hz, 4H, CH_2CH_3), 4.02 (s, 3H, OCH_3), 1.82 (td, *J* = 7.4, 5.7 Hz, 6H, CH_2CH_3); ^{13}C-NMR (100.7 MHz, $CDCl_3$) δ (ppm) = 165.1 (1 C, *C*OO), 143.7 (1 C, NCHN, *C*(2)), 133.9 (1 C, *C*(Ar)), 131.1 (1 C, *C*(Ar)), 129.4 (1 C, *C*(Ar)), 128.2 (1 C, *C*(Ar)H), 115.0 (1 C, *C*(Ar)H), 113.2 (1 C, *C*(Ar)H), 53.0 (1 C, OCH_3), 43.5 (1 C, CH_2CH_3), 43.4 (1 C, CH_2CH_3), 14.9 (1 C, CH_2CH_3), 14.8 (1 C, CH_2CH_3); MS (ESI): *m/z*: 233.1 $[M–I]^+$, 593.1 $[2M–I]^+$; CHN (calc./found) C (43.35/43.35), H (4.76/4.75), N (7.78/7.71).

1,3-Diethyl-5-ethoxycarbonyl-benzimidazolium iodide 4a

The compound was prepared from ethyl-1H-benzimiazole-5-carboxylate (897 mg, 4.716 mmol); yield: 1307 mg (3.493 mmol, 74%), pale brown powder; ^{1}H NMR (500.3 MHz, $CDCl_3$) δ (ppm) = 11.27 (s, 1H, C(2)*H*), 8.42 (dd, *J* = 1.4, 0.7 Hz, 1H, C(4)*H*), 8.35 (dd, *J* = 8.7, 1.4 Hz, 1H, C(6)*H*), 7.84 (dd, *J* = 8.7, 0.7 Hz, 1H, C(7)*H*), 4.76 (qd, *J* = 7.3, 4.2 Hz, 4H, NCH_2CH_3), 4.48 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 1.82 (dt, *J* = 9.2, 7.4 Hz, 6H, NCH_2CH_3), 1.46 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C-NMR (125.8 MHz, $CDCl_3$) δ (ppm) = 164.7 (1 C, *C*OO), 143.6 (1 C, NCHN, *C*(2)H), 133.8 (1 C, *C*(Ar)), 131.1 (1 C, *C*(Ar)), 129.8 (1 C, *C*(Ar)), 128.2 (1 C, *C*(Ar)H), 114.9 (1 C, *C*(Ar)H), 113.1 (1 C, *C*(Ar)H), 62.1 (1 C, OCH_2CH_3), 43.5 (2 C, NCH_2CH_3), 14.8(2 C, NCH_2CH_3), 14.3 (1 C, OCH_2CH_3); MS (ESI): *m/z*: 247.1 $[M–I]^+$; CHN (calc./found) C (44.93/44.95), H (5.12/5.09), N (7.49/7.31).

General procedure for synthesis of (NHC)Au(I)Cl complexes 3b and 4b

The appropriate benzimidazolium iodide (1 eq.) and silver oxide (0.6 eq.) were dissolved in dichloromethane (20 mL) and stirred at room temperature overnight with protection from light. Chlorido-(dimethylsulfide)gold(I) (1.1 eq.) was added and the mixture was further stirred for 60 h. The reaction mixture was filtered through celite, the solvent was removed in vacuo, the residue was washed with THF/*n*-hexane (2/5) and several times with diethyl ether. The product was dried in vacuo.

Chlorido(1,3-diethyl-5-methoxycarbonyl-benzimidazol-2-ylidene)gold(I) 3b

The compound was prepared from 1,3-diethyl-5-methoxycarbonyl-benzimidazolium iodide **3a** (500 mg, 1.388 mmol); yield: 317 mg (0.682 mmol, 49%), light yellow powder; ^{1}H-NMR (600.1 MHz, $CDCl_3$) δ (ppm) = 8.21 (dd, *J* = 1.4, 0.7 Hz, 1H, C(4)*H*), 8.16 (dd, *J* = 8.6, 1.4 Hz, 1H, C(6)*H*), 7.53 (dd, *J* = 8.6, 0.7 Hz, 1H, C(7)*H*), 4.58 (m 4H, NCH_2CH_3), 4.00 (s, 3H, OCH_3), 1.57 (m, 6H, NCH_2CH_3); ^{13}C-NMR (150.9 MHz, $CDCl_3$) δ (ppm) = 180.3 (1 C, NCAuN, *C*(2)), 166.1 (1 C, *C*OO), 135.6 (1 C, *C*(Ar)), 132.6 (1 C, *C*(Ar)), 126.7 (1 C, *C*(Ar)), 126.0 (1 C, *C*(Ar)H), 113.3 (1 C, *C*(Ar)H), 111.1 (1 C, *C*(Ar)H), 52.7 (1 C, OCH_3), 44.3 (2 C, NCH_2CH_3), 15.5 (2 C, NCH_2CH_3); MS (EI): *m/z*: 429.0 $[M–Cl]^+$, 464.0 $[M]^+$; CHN (calc./found) C (33.60/33.51), H (3.47/3.36), N (6.03/5.85).

Chlorido(1,3-diethyl-5-ethoxycarbonyl-benzimidazol-2-ylidene) gold(I) 4b

The compound was prepared from 1,3-diethyl-5-ethoxycarbonyl-benzimidazolium iodide **4a** (300 mg, 0.794 mmol); yield: 176.5 mg (0.369 mmol, 46%), grey-white powder; ^{1}H-NMR (500.3 MHz, $CDCl_3$) δ (ppm) = 8.20 (dd, *J* = 1.4, 0.6 Hz, 1H, C(4)*H*), 8.16 (dd, *J* = 8.6, 1.4 Hz, 1H, C(6)*H*), 7.52 (dd, *J* = 8.6, 0.6 Hz, 1H, C(7)*H*), 4.58 (m, 4H, NCH_2CH_3), 4.46 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 1.57 (m, 6H, NCH_2CH_3), 1.45 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ (ppm) = 180.2 (1 C, NCAuN, *C*(2)), 165.7 (1 C, *C*OO), 135.5 (1 C, *C*(Ar)), 132.6 (1 C, *C*(Ar)), 127.1 (1 C, *C*(Ar)), 125.9 (1 C, *C*(Ar)H), 113.2 (1 C, *C*(Ar)H), 111.0 (1 C, *C*(Ar)H), 61.7 (1 C, OCH_2CH_3), 44.2 (2 C, NCH_2CH_3), 15.5 (2 C, NCH_2CH_3), 14.4 (1 C, OCH_2CH_3); MS (EI): *m/z*: 415.0 $[M–Et-Cl]^+$, 431.0 $[M–Cl]^+$, 478.0 $[M]^+$; CHN (calc./found) C (35.13/35.36), H (3.79/3.75), N (5.85/5.72).

General procedure for synthesis of (NHC)Au(III)Cl_3 complexes 1 c, 3 c, 4 c

(NHC)Au(III)Cl_3 complexes were prepared according to a modified reported procedure.[16] The respective (NHC)Au(I)Cl complex (1 eq.) was dissolved in dichloromethane (4 mL) and stirred at room temperature with protection from light. Dichlorophenyliodane (1.4 eq.) was added and the mixture was stirred for 24 h. The solvent was removed and the residue was washed with *n*-hexane (3x), diethyl ether (3x) and cold chloroform (2x). The obtained solid was dried in vacuo at 40 °C.

Trichlorido(1,3-diethyl-imidazol-2-ylidene)gold(III) 1 c

The compound was prepared from chlorido(1,3-diethyl-imidazol-2-ylidene)gold(I) **1 b** (100.0 mg, 0.280 mmol) yield: 69.2 mg (0.162 mmol, 58 %) pale yellow powder; ^{1}H-NMR (500.3 MHz, DMSO-d_6) δ (ppm) = 8.20 (s, 2H, CH), 4.29 (m, ^{3}J = 7.3 Hz, 4H, NCH_2CH$_3$), 1.45 (m, ^{3}J = 7.3 Hz, 6H, NCH$_2$CH_3); ^{13}C-NMR (125.8 MHz, DMSO-d_6) δ = 135.2 (NCAuN, *C*2), 124.2 (*C*H), 45.3 (NCH$_2$CH$_3$), 15.4 (NCH$_2$CH$_3$); MS (ESI): *m/z*: 677.1 [2 M-5Cl]$^+$, 819.0 [2M–Cl]$^+$, 876.9 [2 M + Na]$^+$; CHN (calc./found) C (19.67/19.48), H (2.83/2.90), N (6.55/6.37).

Trichlorido(1,3-diethyl-5-methoxycarbonyl-benzimidazol--2-ylidene)gold(III) 3 c

The compound was prepared from chlorido(1,3-diethyl-5-methoxycarbonyl-benzimidazol-2-ylidene)gold(I) **3 b** (118.7 mg, 0.250 mmol); yield: 130 mg (0.243 mmol, 97 %), yellow powder; ^{1}H-NMR (600.1 MHz, CDCl$_3$) δ (ppm) = 8.35 (d, *J* = 1.5 Hz, 1H, C(4)*H*), 8.26 (dd, *J* = 8.7, 1.4 Hz, 1H, C(6)*H*), 7.71–7.67 (m, 1H, C(7)*H*), 4.71 (m, *J* = 7.4 Hz, 4H, NCH_2CH$_3$), 4.03 (s, 3H, OCH_3), 1.70 (m, *J* = 7.4 Hz, 6H, NCH$_2$CH_3); ^{13}C-NMR (150.9 MHz, CDCl$_3$) δ (ppm) = 165.3 (1 C, *C*OO), 154.6 (1 C, NCAuN, *C*(2)), 136.0 (1 C, *C*(Ar)), 133.2 (1 C, *C*(Ar)), 128.1 (1 C, *C*(Ar)), 127.1 (1 C, *C*(Ar)H), 114.1 (1 C, *C*(Ar)H), 112.0 (1 C, *C*(Ar) H), 53.0 (1 C, OCH_3), 44.3 (2 C, NCH_2CH$_3$), 14.7 (2 C, NCH$_2$CH_3) ; MS (ESI): *m/z*: 557.0 [M + Na]$^+$, 1035.0 [2M–Cl]$^+$, 1093.0 [2 M + Na]$^+$; CHN (calc./found) C (29.15/29.56), H (3.01/3.24), N (5.23/5.02).

Trichlorido(1,3-diethyl-5-ethoxycarbonyl-benzimidazol-2--ylidene)gold(III) 4 c

The compound was prepared from chlorido(1,3-diethyl-5-ethoxycarbonyl-benzimidazol-2-ylidene)gold(I) **4 b** (125 mg, 0.261 mmol); yield: 106 mg (0.193 mmol, 74 %), pale yellow to pale orange powder; ^{1}H-NMR (500.3 MHz, CDCl$_3$) δ (ppm) = 8.33 (dd, *J* = 1.4, 0.7 Hz, 1H, C(4)*H*), 8.26 (dd, *J* = 8.7, 1.4 Hz, 1H, C(6)*H*), 7.68 (dd, *J* = 8.7, 0.7 Hz, 1H, C(7)*H*), 4.71 (m, *J* = 7.3 Hz, 4H, NCH_2CH$_3$), 4.49 (q, *J* = 7.2 Hz, 2H, OCH_2CH$_3$), 1.70 (m, *J* = 7.3 Hz, 6H, NCH$_2$CH_3), 1.46 (t, *J* = 7.2 Hz, 3H, OCH$_2$CH_3); ^{13}C-NMR (100.7 MHz, CDCl$_3$) δ (ppm) = 164.9 (1 C, *C*OO), 154.6 (1 C, NCAuN, *C*(2)), 135.9 (1 C, C(Ar)), 133.2 (1 C, C(Ar)), 128.6 (1 C, C(Ar)), 127.1 (1 C, C(Ar)H), 114.0 (1 C, C(Ar)H), 111.9 (1 C, C(Ar)H), 60.1 (1 C, OCH$_2$CH$_3$), 44.3 (2 C, NCH$_2$CH$_3$), 14.8 (1 C, NCH$_2$CH$_3$), 14.6 (1 C, NCH$_2$CH$_3$), 14.3 (1 C, OCH$_2$CH$_3$); MS (ESI): *m/z*: 571.0 [M + Na]$^+$, 1063.1 [2M–Cl]$^+$, 1121.0 [2 M + Na]$^+$; CHN (calc./found): C (30.59/30.25), H (3.30/3.39), N (5.10/4.88).

Crystal Structure Determinations

For compound **3 b**, the crystal was mounted in inert oil on a glass fibre and transferred to an Oxford Diffraction Xcalibur E diffractometer; intensity data were recorded using monochromated Mo-*K*α radiation. Other crystals were mounted in inert oil on nylon loops and transferred to a Rigaku/Oxford XtaLAB Synergy diffractometer; intensity data were recorded using mirror-focussed Mo-*K*α radiation. Absorption corrections were implemented on the basis of multi-scans. The structures were refined anisotropically on F^2 using the programs SHELXL-2017 or −2018.[25] Hydrogen atoms were included using rigid methyl groups or a riding model starting from calculated positions.

Crystallographic data are summarized in Table 5. Additionally, complete data have been deposited with the Cambridge Crystallographic Data Centre under the numbers CCDC 2085400–3. Copies of the data can be obtained free of charge from –www.ccdc.cam.ac.uk/data_request/cif.

Antibacterial screening

The following strains were used and maintained at 37 °C in MHB (21 g/L Müller Hinton, pH 7.4) or TSY (30 g/L trypticase soy broth, 3 g/L yeast extract, pH 7.0–7.2) medium. *Acinetobacter baumannii* (DSM 30007, ATCC 19606) in MHB, *Escherichia coli* (DSM1116, ATCC 9637) in TSY, *Klebsiella pneumoniae* (DSM 11678, ATCC33495) in MHB, *Pseudomonas aeruginosa* PA7 (DSM 24068) in MHB, *Enterococcus faecium* (DSM 20477, ATCC 19434) in TSY, *Staphylococcus aureus* MRSA (DSM 11822, ICB 25701) in TSY). Minimum inhibitory concentration (MIC) values were determined following a standardized protocol in broth dilution assays. The compounds and auranofin were serially diluted starting from 64 μg/ml using a pipetting robot (epMotion, Eppendorf, Germany). Starting inocula of 2–8 × 10^5 colony-forming units per ml in MHB or TSY media at 37 °C were used, and serial dilutions were carried out in 384-well microtiter plates in duplicate. After incubation of the plates for 20 h at 37 °C, the absorbance at 600 nm was measured to determine the MIC value (Enspire Multimode Microplate Reader, Perkin Elmer Inc.). The MIC values for the tested compounds were determined in three independent experiments by a curve-fitting procedure using the GraphPad Prism software (GraphPad Software, Inc.). Amikacin (*P. aeruginosa*), Linezolid (*S. aureus*), and Ciprofloxacin (all other strains) served as positive controls.

Inhibition of bacterial TrxR (E.coli)

The TrxR (*E.coli*) inhibition assay was performed according to a previously published procedure.[6] It is partly based on the procedure developed by *Lu et al.*[26] and detects the formation of 5-TNB (5-thionitrobenzoic acid). Solutions of *E. coli* TrxR (35.4 U/mL) and of its substrate thioredoxin (Trx) *E.coli* (156 μg/mL) (both purchased from Sigma-Aldrich and diluted with distilled water) and fresh stock solutions of the test compounds (in DMF) were prepared. TE buffer (Tris-HCl 50 mM, EDTA 1 mM, pH 7.5) containing graded concentrations of the respective compounds (20 μL) or buffer without compounds (20 μL, as control) were mixed with TrxR solution (10 μL), Trx solution (10 μL), and a solution of NADPH (200 μM) in TE buffer (100 μL) in a well on a 96-well plate. As blank solution, 200 μM NADPH in TE buffer (100 μL) mixed with a DMF/buffer mixture (40 μL) was used (final concentrations of DMF: 0.5 % v/v). The solutions on the 96-well plate were incubated for 75 min at 25 °C with moderate shaking. A volume of 100 μL of a reaction mixture (TE buffer containing 200 μM NADPH and 5 mM DTNB) was added to each well to initiate the reaction. After thorough mixing, the formation of 5-TNB was monitored by a microplate reader at 405 nm in 35 s intervals (10 measurements). The values were corrected by subtraction of the values for the blank solution. The increase in concentration of 5-TNB followed a linear trend ($r^2 \geq$ 0.990) and the enzymatic activities were calculated as the gradients (increase in absorbance per second) thereof. Absence of interference with the assay components was confirmed by a negative control experiment for each test compound. The highest test compound concentration was used and the enzyme solution was

Table 5. Crystallographic data and structure refinement details.

	1c	2c	3b	4c
CCDC number	2085400	2085401	2085402	2085403
Formula	$C_7H_{12}AuCl_3N_2$	$C_{11}H_{14}AuCl_3N_2$	$C_{13}H_{16}AuClN_2O_2$	$C_{14}H_{18}AuCl_3N_2O_2$
M_r	427.50	477.56	464.69	549.62
Cryst. size (mm)	0.2×0.15×0.10	0.16×0.14×0.10	0.20×0.10×0.04	0.10×0.03×0.03
Crystal system	monoclinic	orthorhombic	triclinic	monoclinic
Space group	$P2_1/n$	*Pbca*	*P*(-1)	$P2_1/c$
Temperature (°C)	−173	−173	−173	−173
a (Å)	8.31009(18)	13.38840(17)	9.0249(4)	7.7709(2)
b (Å)	14.8691(3)	14.21271(16)	9.3241(7)	14.8464(4)
c (Å)	10.1260(2)	14.49339(17)	10.1589(6)	15.3816(4)
α (°)	90	90	114.783(6)	90
β (°)	103.407(2)	90	93.470(5)	101.737(3)
γ (°)	90	90	111.503(6)	90
V (Å^3)	1217.11	2757.88	698.52	1737.48
Z	4	8	2	4
D_x (Mg m^{-3})	2.333	2.300	2.209	2.101
λ (Å)	0.71073	0.71073	0.71073	0.71073
μ (mm^{-1})	12.7	11.2	10.7	8.9
Transmissions	0.542–1.000	0.511–1.000	0.524–1.000	0.534–1.000
F(000)	792	1792	440	1048
$2\theta_{max}$	76.7	104.3	59	68
Refl. measured	120169	613091	31317	82892
Refl. indep.	6607	15837	3586	6595
R_{int}	0.61	0.074	0.051	0.041
Parameters	120	156	175	202
wR(F^2, all refl.)	0.042	0.039	0.041	0.048
R(*F*, >4σ(*F*))	0.019	0.026	0.021	0.023
S	1.03	1.15	1.05	1.09
Max. Δ*p* (e Å^{-3})	1.6, −2.1	1.8, −2.6	1.3, −0.95	3.2, −0.83

replaced by TE buffer for this purpose. The IC_{50} values were calculated as the concentration of the compound decreasing the enzymatic activity of the positive control by 50% and are presented as the means and standard deviations of three repeated experiments.

Cell culture

A549 lung carcinoma cells and MDA-MB-231 breast cancer cells were maintained in Dulbecco's modified Eagle's medium (DMEM; 4.5 g/L D-glucose, L-glutamine, pyruvate), supplemented with fetal bovine serum superior, standardized (Biochrom GmbH, Berlin, 10% v/v) and gentamycin (50 mg/L) with a weekly passage. RC-124 healthy human kidney cells were maintained in McCoy's 5 A medium (modified with L-glutamine) supplemented with fetal bovine serum superior, standardized (Biochrom GmbH, Berlin) and gentamycin (50 mg/L) (10% v/v), and were also passaged weekly. For experiments with RC-124 cells, microtiter plates were pretreated as follows: sterilized gelatine solution (1.5%; 30 μL) was added to each well of a flat-bottomed 96-well plate and incubated for 1 h at 37 °C while covered with the lid. The excess solution was removed and the wells were washed with PBS (pH 7.4). The new cell culture medium was immediately added.

Antiproliferative assay in tumorigenic and non-tumorgenic cells

The antiproliferative effects were determined according to a previously published procedure.[6] A volume of 100 μL of A549 cells (710000 cells/mL), MDA-MB-231 cells (830000 cells/mL) and RC-124 cells (610000 cells/mL) was transferred into the wells of a 96-well plate (plates for RC-124 were pretreated as mentioned above) and incubated at 37 °C under 5% CO_2 for 72 h (MDA-MB-231, RC-124) or 48 h (A549). Stock solutions of the compounds were freshly prepared in dimethylformamide (DMF) and diluted with the respective cell medium to obtain various concentrations (final concentration of DMF: 0.1% v/v). After 72 h (A549) or 96 h (MDA-MB-231, RC-124) of exposure, the biomass of the cells was determined via crystal violet staining and the IC_{50} value was determined as the concentration that caused 50% inhibition of cell proliferation relative to an untreated control. The results were calculated as the mean values of three independent experiments, unless stated otherwise.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: antibacterial · gold · N-heterocyclic carbene · thioredoxin reductase

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