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 mononucleophile complexes and their gold(III) analogues (NHC)Au(III)Cl₂ 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 mononucelophosphate (NHC)Au(I)Cl complexes triggered much stronger inhibition of bacterial TrxR than cationic bis-carbene 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 bis-carbene 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 mononucleophile 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)Cl₂. The structures of the carbene ligands contain ethyl groups on the nitrogen side chains, in continuation of our previous work with this type of...
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). 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 1H- and 13C-NMR, mass spectrometry, 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 1H-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 13C-NMR signal of C2 upon complex formation, an effect previously reported by Sivaram et al. For the gold(III) complexes, mass spectrometry confirmed the presence of multiple chlorido ligands ([2M–Cl]n and [2M–Cl]2) signals for 1c, [M–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 CAuCl2 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–C1 bond is observed, making it longer than the cis Au–Cl bonds. A search of the Cambridge Database,Version 5.41, gave 38 hits (47 molecules) for the fragment (carbene)–AuCl2, 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’ 3.945(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”. We have drawn attention to the presence of such interactions between [AuX3]+ anions (X = Cl, Br). 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. A further short, linear contact C7–H7···Cl1 \[H···Cl 2.279(12) Å, with an angle of 170°, operator x, 1+y, z\] may reasonably be...
regarded as a hydrogen bond. The net effect is to form ribbons of dimers parallel to the b axis (Figure 3). Molecules of 4c 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 2. Packing diagram of compound 1c, viewed perpendicular to the ac plane. Thick dashed lines indicate short Cl–Cl contacts. Hydrogen atoms are omitted for clarity.

Figure 3. Packing diagram of compound 3b 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 2b 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 2c, with an MIC value of 16.8 μM, was approximately four times as active as the gold(I) analogue 2b (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 1b–4b and 1c–4c and the reference compound K[Au(CN)2] inhibited the enzyme efficiently, with IC50 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 2b. 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.

| Antibiotic   | MRSA  | E. faecium | E. coli | P. aeruginosa | A. baumannii | K. pneumoniae |
|--------------|-------|------------|---------|---------------|--------------|---------------|
| 1b           | 2.8 ± 1.0 | 0.3 ± 0.1 | 6.9 ± 7.3 | 2.3 ± 1.5       | 2.9 ± 3.4    |
| 1c           | <0.2 | 0.6 ± 0.2 | >30 | 85.1 ± 20.1   | 3.8 ± 2.4    | 18.7 ± 9.2  |
| 2a           | 1.6 ± 0.7 | 2.3 ± 0.0 | 18.7 ± 0.0 | 74.9 ± 0.0   | 23.1 ± 0.0   | 18.7 ± 0.0   |
| 2b           | 0.8 ± 0.4 | 1.2 ± 0.0 | 39.3 ± 0.0 | 118.0 ± 43.1  | 84.2 ± 2.4   | 65.6 ± 32.1 |
| 2c           | 1.1 ± 0.0 | 4.2 ± 0.0 | 39.1 ± 25.6 | >30       | 3.5 ± 1.2   | 16.8 ± 0.0   |
| 3b           | 2.2 ± 0.0 | 11.5 ± 5.0 | >30 | >60   | 17.2 ± 0.0  | 91.8 ± 39.7  |
| 3c           | 1.6 ± 0.5 | 3.7 ± 0.0 | 34.9 ± 8.6 | 59.8 ± 0.0   | 7.5 ± 0.0   | 24.9 ± 8.6   |
| 4b           | 2.4 ± 0.6 | 5.6 ± 2.4 | 50.1 ± 16.7 | >30       | 16.7 ± 0.0   | 66.8 ± 0.0   |
| 4c           | 1.8 ± 0.0 | 3.6 ± 0.0 | 53.4 ± 8.4 | 56.2 ± 0.0   | 7.3 ± 0.0   | 29.1 ± 0.0   |
Auranofin and K[Au(CN)2(NHC) complexes were generally stronger in MDA-MB-231 and lung carcinoma cells, MDA-MB-231 breast adenocarcinoma cells effects. The antiproliferative potential of gold NHC complexes is well afforded comparable values, confirming the appropriate reproducibility of the assay procedure. 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 3. Inhibition of bacterial TrxR as IC50 values ± standard deviation (n = 3).

| Compound          | IC50 [μM] |
|-------------------|-----------|
| K[Au(CN)2]       | 0.310 ± 0.112 |
| auranofin        | 0.210 ± 0.030 |
| Et,ImAuCl        | 1 b        | 0.243 ± 0.032 |
| Et,ImAuCl        | 1 c        | 0.550 ± 0.211 |
| Et,BrAuCl        | 2 b        | 0.183 ± 0.018 |
| Et,BrAuCl        | 2 c        | 0.270 ± 0.156 |
| Et,BiCO2,MeAuCl  | 3 b        | 0.267 ± 0.077 |
| Et,BiCO2,MeAuCl  | 3 c        | 0.252 ± 0.060 |
| Et,BiCO2,EtAuCl  | 4 b        | 0.450 ± 0.121 |
| Et,BiCO2,EtAuCl  | 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(III) NHC complexes were generally stronger in MDA-MB-231 and RC-124 cells (IC50 values in the range 4–11 μM for 1 b/1 c–4 b/4 c) than 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. Notably, the antibacterial effects against Gram-positive strains (see Table 2) were in general stronger than the growth-inhibiting effects against human cell lines.

Conclusion

A series of (NHC)Au(II)Cl monocarbene complexes and their gold (III) analogues (NHC)Au(III)Cl 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, antiptrypanosomal, antileishmanial or antimalarial effects have also been confirmed for this type of organometallics.[2,21]

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[26] 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.[3] Interestingly, inhibition of bacterial TrxR has been recently confirmed also for a antimicrobial silver NHC complex (K= 10.8 nM).[23]

The IC50 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 13C NMR were recorded on a DRX-400 AS, AVIIIHD 500 or AVII 600. A Finnigan LCQDeca or a Finnigan MAT 9S 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®Xt. Compounds 1a, 1b, 2a, 2b, 3a, 3b, and PhICl[14] were prepared as described in the literature.

Synthesis

Ethyl-1H-benzimidazole-5-carboxylate BICOEet

This compound was prepared according to a modified version of a published procedure.[24] Benzimidazole-5-carboxylic acid (650 mg, 4.716 mmol); yield: 317 mg (68.2 mmol, 49 %), pale brown powder. \(^{1}H\) NMR (500.1 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 8.21 (dd, \(J = 1.4, 0.7\) Hz, 1H, C(4)H), 7.53 (dd, \(J = 8.6, 0.7\) Hz, 1H, C(7)H), 4.85 (m, 4H, NCH\(_2\)CH\(_3\)), 4.46 (q, \(J = 7.1\) Hz, 2H, OCH\(_2\)CH\(_3\)), 1.57 (m, 6H, NCH\(_2\)CH\(_3\)), 1.45 (t, \(J = 7.1\) Hz, 3H, OCH\(_3\)CH\(_3\)).

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

The appropriate benzimidazolide (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-(dimethylsulfoxide)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-(ethoxy)carboxylbenzimidazol-2-ylidenegold(I)] 3b

The compound was prepared from 1,3-diethyl-5-(ethoxycarbonyl)benzimidazolide iodide 3a (500 mg, 1.388 mmol); yield: 317 mg (68.2 mmol, 49 %), pale brown powder. \(^{1}H\) NMR (600.1 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 8.21 (dd, \(J = 1.4, 0.7\) Hz, 1H, C(4)H), 8.16 (dd, \(J = 8.6, 0.7\) Hz, 1H, C(7)H), 4.85 (m, 4H, NCH\(_2\)CH\(_3\)), 4.00 (s, 3H, OCH\(_3\)CH\(_3\)), 1.57 (m, 6H, NCH\(_2\)CH\(_3\)), 1.45 (t, \(J = 7.1\) Hz, 3H, OCH\(_3\)CH\(_3\)).

Chlorido[1,3-diethyl-5-(ethoxy)carboxylbenzimidazol-2-ylidenegold(I)] 4b

The compound was prepared from 1,3-diethyl-5-(ethoxycarbonyl)benzimidazolide iodide 4a (300 mg, 0.794 mmol); yield: 176.5 mg (369 mmol, 46 %), grey-white powder. \(^{1}H\) NMR (500.3 MHz, CDCl\(_3\)): \(\delta\) (ppm) = 8.20 (dd, \(J = 1.4, 0.6\) Hz, 1H, C(4)H), 8.16 (dd, \(J = 8.6, 0.7\) Hz, 1H, C(7)H), 4.58 (m, 4H, NCH\(_2\)CH\(_3\)), 4.46 (q, \(J = 7.1\) Hz, 2H, OCH\(_2\)CH\(_3\)), 1.57 (m, 6H, NCH\(_2\)CH\(_3\)), 1.45 (t, \(J = 7.1\) Hz, 3H, OCH\(_3\)CH\(_3\)).

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

The compound was prepared from ethyl-1H-benzimidazole-5-carboxylate (897 mg, 4.716 mmol); yield: 1307 mg (3.493 mmol, 74 %), pale brown powder. \(^{1}H\) NMR (500.3 MHz, CDCl\(_3\)): \(\delta\) (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\(_2\)CH\(_3\)), 4.48 (q, \(J = 7.1\) Hz, 2H, OCH\(_2\)CH\(_3\)), 1.82 (dt, \(J = 9.2, 7.4\) Hz, 2H, NCH\(_2\)CH\(_3\)).

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

The appropriate benzimidazolide (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-(dimethylsulfoxide)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.

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

The compound was prepared from methyl-1H-benzimidazole-5-carboxylate (580 mg, 3.292 mmol); yield: 688 mg (1910 mmol, 58 %), pale brown powder. \(^{1}H\) NMR (400.4 MHz, CDCl\(_3\)): \(\delta\) (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\(_2\)CH\(_3\)), 4.02 (s, 3H, OCH\(_3\)CH\(_3\)), 1.82 (td, \(J = 7.4, 5.7\) Hz, 2H, OCH\(_2\)CH\(_3\)).
General procedure for synthesis of [(NHC)Au(III)]Cl complexes 1c, 3c, 4c

(NHC)Au(III)Cl complexes were prepared according to a modified reported procedure.[18] The respective (NHC)Au(III)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) 1c

The compound was prepared from chlorido(1,3-diethyl-imidazol-2-ylidene)gold(III)(l) 1b (100.0 mg, 0.280 mmol) yield: 69.2 mg (0.162 mmol, 58%) pale yellow powder; 1H-NMR (500.3 MHz, DMSO- d6) 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, NCH₂CH₃), 15.7 (NCH₂CH₃); MS (ESI): m/z = 607.1 (M+H)+; CHN (calc./found) C (29.5/29.5), H (3.01/3.24), N (5.23/5.02).

For compound 1c, 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-Kα radiation. Absorption corrections were implemented on the basis of multi-scans. The structures were refined anisotropically on F² using the programs SHEXL-2017 or –2018.[20] 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 tryctoycic 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 pneumoanae (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 inhibition 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 2–8×10⁶ 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 (Empire 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.[19] It is partly based on the procedure developed by Lu et al.[21] 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 (R² ≥ 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

Crystal Structure Determinations

For compound 3b, 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-Kα 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-Kα radiation.
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 5A 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 under 5% CO_2.

**Antiproliferative assay in tumorigenic and non-tumorgenic cells**

The antiproliferative effects were determined according to a previously published procedure.[vi] 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.

**Acknowledgements**

Financial support by the DFG (Deutsche Forschungsgemeinschaft, bilateral grant 382040922 for TU-B5 and HZI) and the Lower Saxony Ministry for Science and Culture for the doctoral program “Drug Discovery and Cheminformatics for New Anti-Infectives (iCA)” are gratefully acknowledged. Open Access funding enabled and organized by Projekt DEAL.

**Conflict of Interest**

The authors declare no conflict of interest.

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

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[vi] P. Bięgański, Ł. Szczupak, M. Arruebo, K. Kowalski, RSC Chem. Biol. 2021, 2, 368–386.
M. B. Harbut et al.,
R. Rubbiani et al.,
M. Gil-Moles, U. Basu, R. Misgeld, B. Karge, M. Brönstrup, I. Ott,
C. Schmidt, B. Karge, R. Misgeld, A. Prokop, K. Baumann, S. Reichl, I. Ott,
C. Schmidt, B. Karge, R. Misgeld, A. Prokop, K. Baumann, S. Reichl, I. Ott,
C. Schmidt, B. Karge, R. Misgeld, A. Prokop, K. Baumann, S. Reichl, I. Ott,
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C. Schmidt, B. Karge, R. Misgeld, A. Prokop, K. Baumann, S. Reichl, I. Ott,