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Gold(I) *N*-Heterocyclic Carbene Complexes with Naphthalimide Ligands as Combined Thioredoxin Reductase Inhibitors and DNA Intercalators

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Organometallic conjugates consisting of a gold(I) *N*-heterocyclic carbene (NHC) moiety and a naphthalimide were prepared and investigated as cytotoxic agents that interact with both DNA and the disulfide reductase enzyme thioredoxin reductase (TrxR). The complexes were potent DNA intercalators related to their naphthalimide partial structure, inhibited TrxR as a consequence of the incorporation of the gold(I) moiety, and triggered efficient cytotoxic effects in MCF-7 breast and HT-29 colon adenocarcinoma cells. Strong effects on tumor cell metabolism were noted for the most cytotoxic complex, chlorido[1-(3′-(4″-ethylthio-1″,8″-naphthalimid-*N*″-yl))-propyl-3-methyl-imidazol-2-ylidene]gold(I) (**4 d**). In conclusion, the conjugation of naphthalimides with gold(I) NHC moieties provided a useful strategy for the design of bioorganometallic anticancer agents with multiple modes of action.

Introduction

1,8-Naphthalimides are a category of substances exhibiting high antitumor activities based on an effective intercalation into DNA and other effects such as apoptosis induction.[1] Some of these compounds (e.g., amonafide, Figure 1) were studied as anticancer agents in clinical trials but did not progress to extended therapeutic application. Optimization of naphthalimide anticancer drugs is an ongoing process that has led to a high number of very potent cytotoxic substances that hold great promise for anticancer drug development.[2]

One promising strategy is the combination of the naphthalimide moiety with metal fragments aiming at the design of cytotoxic agents that act via multiple modes of action. To the best of our knowledge, this concept was used for the first time by Navarro-Ranninger and colleagues who reported in 1999 on platinum(II)-naphthalimide conjugates, which combine the concept of intercalation with that of DNA platination (see Figure 1 for an example).[3] Interestingly, these complexes were able to circumvent cisplatin resistance.

In 2009, we reported on naphthalimides containing gold(I)-phosphane partial structures that were attached via a thiolate to the aromatic region of the naphthalimide moiety.[4] The complexes combined intercalation with gold(I) based thioredoxin reductase (TrxR) inhibition as a non-DNA-directed mechanism, and thus they opened options for the design of multitarget naphthalimides. Moreover, these conjugates induced apoptosis and inhibited angiogenesis, which was related to the presence of the naphthalimide structure. The uptake of gold into the nuclei of cancer cells could be confirmed by atomic absorption spectroscopy and that of the naphthalimide structure by fluorescence microscopy. In recent reports, the cytotoxicity and anticancer properties of naphthalimide metal conjugates with platinum,[5] ruthenium[6] and other metals[5a] have been addressed. One interesting example is a fluorescent platinum(II) terpyridine complex, which was efficiently taken up into tumor cells and induced apoptosis.[5b]

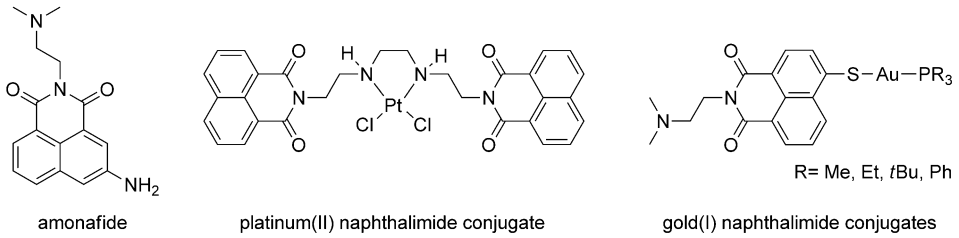

Figure 1. Amonafide and examples of metal naphthalimide conjugates.

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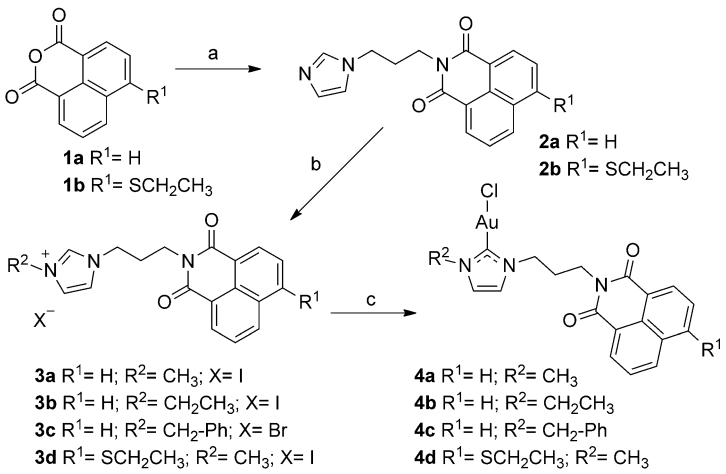

Scheme 1. *Reagents and conditions*: a) 1-(3′-aminopropyl)-imidazole, reflux in ethyl acetate for 8 h; b) alkyl halide, reflux in toluene for 12 h; c) Ag_2O in methanol/CH_2Cl_2, RT for 6 h, followed by addition of chlorido(dimethyl sulfide)gold(I) in CH_2Cl_2, RT for 4–24 h.

Here we report on a first series of novel gold(I) complexes containing naphthalimide ligands (Scheme 1). The gold(I) center was introduced in form of a gold(I) *N*-heterocyclic carbene (NHC) moiety, an organometallic functional group that exhibits promising properties for anticancer drug design (e.g., TrxR inhibition, apoptosis induction) as reported by us and others.[7] The gold(I) NHC center is incorporated into the side chain of the naphthalimides at the position of an essential amino nitrogen.[1a] Protonation of the side chain nitrogen under physiological conditions leads to a positively charged species that is thought to interact with the negatively charged phosphate backbone of the DNA, and this interaction facilitates the intercalation of the flat aromatic ring system into the DNA. Of note, effective interaction with the DNA can also be achieved by incorporation of a quaternized cationic nitrogen center in the respective position in the side chain.[8]

This report provides a proof of concept that, upon introduction of imidazole-based gold(I) NHC fragments into the mentioned position of cytotoxic naphthalimides, active metallodrugs can be obtained. These carry essential properties of both the naphthalimide and the gold(I) NHC partial structure. The synthesis, characterization and biological evaluation of the new complexes is described.

Results and Discussion

Chemistry

Naphthalimides with imidazole-containing side chains, namely **2a** and its thioether derivative **2b**, were prepared by heating the respective naphthalic anhydrides **1a** and **1b** with 1-(3′-aminopropyl)-imidazole at reflux in ethyl acetate. The resulting naphthalimides were heated at reflux with an excess of an akylhalide in toluene in order to obtain derivatives containing imidazolium halides in the side chain (**3a**–**d**). The formation of the target gold(I) NHC complexes **4a**–**d** was achieved by reacting **3a**–**d** with Ag_2O, which led to the formation of silver intermediates, and by a subsequent transmetallation reaction using chlorido(dimethylsulfide)gold(I). Isolation and purification of **4a**–**d** was performed by filtration over Celite, recrystallisation in diethylether and drying over P_2O_5.

In the ^{1}H NMR spectra of the gold(I) NHC compounds **4a**–**d**, the absence of the imidazole C^2 proton signal at $\delta = 9.1$–9.3 ppm is an example confirming the formation of the organometallic carbon–gold bond. The high purity of **3a**–**d** and **4a**–**d** was determined by elemental analyses, which differed less than 0.5% from the theoretical values.

The interesting photophysical properties and often intense fluorescence emissions of naphthalimides and also of various transition metal complexes are well known, and many examples have been reported as efficient fluorescence sensors or imaging probes.[9] Recently, we had reported on naphthalimides with thioether or gold(I)-thiolate partial structures and the imaging of their cellular uptake and intracellular localization by fluorescence microscopy.[4,9f] In this context, we measured UV/Vis and fluorescence spectra exemplarily for the series **2a**–**4a** with the perspective of performing cellular localization studies by means of fluorescence microscopy. However, the obtained results indicated that the fluorescence emission of **2a**–**4a** was too weak to perform this kind of experiments (for details, see the Supporting Information).

Effects on tumor cell proliferation and inhibition of TrxR

The triggering of antiproliferative effects of the compounds was investigated in two tumor cell lines, namely MCF-7 human breast adenocarcinoma and HT-29 colon carcinoma cells (Table 1). The gold-free imidazolium cations **3a**–**d** caused moderate cytotoxic potency against HT-29 cells with IC_{50} values in the range of 27–57 μM, but afforded stronger activity in MCF-7 cells ($IC_{50} = 4$–18 μM). Transformation into gold(I) NHC complexes **4a**–**d** led to a strong increase in cytotoxic activity in both cell lines in the case of **4a**, **4b** and **4d**, whereas with

Table 1. Antiproliferative effects in HT-29 and MCF-7 cells and melting point shifts of the respective compounds in 10 mM phosphate buffer (pH 7.2) at a [complex]/[DNA] ratio of 0.1.

Compd	R^1	R^2	IC_{50} [μM][a] HT-29	IC_{50} [μM][a] MCF-7	ΔT_m [°C]	IC_{50} [μM][a] TrxR
3a	H	CH_3	56.8 ± 0.7	9.2 ± 0.2	3	> 1
3b	H	CH_2CH_3	31.3 ± 8.9	17.1 ± 1.7	4	n.d.
3c	H	CH_2-Ph	43.1 ± 10.2	5.2 ± 0.6	10	n.d.
3d	SCH_2CH_3	CH_3	27.5 ± 16.2	4.6 ± 3.6	n.d.	> 1
4a	H	CH_3	4.0 ± 0.7	4.6 ± 1.5	11	0.28 ± 0.12
4b	H	CH_2CH_3	4.9 ± 1.8	6.3 ± 0.6	6	n.d.
4c	H	CH_2-Ph	18.2 ± 5.9	5.8 ± 2.9	4	n.d.
4d	SCH_2CH_3	CH_3	1.9 ± 0.3	2.1 ± 0.5	6	0.40 ± 0.13

[a] Data represent the mean ± SEM of 2–3 experiments. n.d. = not determined.

complex **4c** this effect was only observed in HT-29 but not in MCF-7 cells.

As mentioned above, TrxR is an important cancer target and many gold(I) complexes are strong inhibitors of this enzyme with IC_{50} values in the submicromolar range.[10] Comparing the cytotoxicity values of the gold(I) species **4a**–**d** indicates a preference for those complexes containing the sterically less demanding methyl group on one of the imidazole nitrogens ($R^2 = CH_3$). Accordingly, **4a** and **4d** were selected as examples to study the inhibition of TrxR. The gold-free analogues **3a** and **3d** were selected as the corresponding negative references. In fact, **4a** and **4d** effectively inhibited TrxR activity with IC_{50} values of 0.28 ± 0.12 and 0.40 ± 0.13 μM, respectively, in an assay that measures the activity of purified TrxR exposed to the compounds. In contrast, **3a** and **3d** did not cause inhibition of TrxR up to the highest investigated concentration of 1.0 μM. The TrxR inhibition values of **4a** and **4d** are comparable with those obtained with gold(I) complexes of the type $[Au^{I}Cl(PR_3)]$,[11] a benzimidazolylidene-containing NHC complex [AuCl(NHC)][7d] or the recently reported gold(I) naphthalimide derivative.[4b] This indicates that the gold(I) NHC fragment contributes the expected TrxR inhibiting properties of the here reported gold naphthalimide conjugates.

Glutathione reductase was used as a reference enzyme that is structurally and functionally related to TrxR and is commonly inhibited less efficiently by gold species and also does not represent a preferred anticancer drug target. This enzyme was inhibited by **4a** and **4d** only at much higher concentrations ($IC_{50} = 221.2 \pm 0.12$ μM for **4a** and $IC_{50} = 15.6 \pm 2.8$ μM for **4d**) confirming an appropriate selectivity of the complexes for inhibiting the tumor-relevant enzyme TrxR.

Interaction with DNA

The interaction with calf thymus CT-DNA was evaluated by melting point and circular dichroism (CD) experiments.

By measuring the thermal denaturation temperature changes (ΔT_m) of DNA upon exposure to substances, their efficacy of intercalation can be evaluated. For this purpose, DNA was incubated with the compounds in a 1:10 ratio, and the melting points were recorded. Values for **3a**–**c** and **4a**–**d** ranged between 3 °C and 11 °C, which are in accordance with differing intercalative potentials of the naphthalimides as well as their gold(I) NHC complexes (Table 1). On the basis of their ΔT_m values, the most effective intercalators were **3c** and **4a**. In the case of the imidazolium derivatives **3a**–**c**, increasing the size of the residues (R^2) at the imidazole nitrogen was accompanied by an increase of ΔT_m values, whereas this led to a decrease of the ΔT_m values in the case of gold(I) NHC derivatives **4a**–**c**.

Characteristic changes can be induced in the CD spectra of DNA in the presence of small molecules. The CD spectrum of DNA exhibits a negative peak at approximately 245 nm, which is caused by the helical B conformation, and a positive peak at approximately 275 nm that is caused by base stacking. Changes in the range of 220–350 nm thus provide important information on changes in the conformation of the nucleic acid structure.

The CD spectra obtained with the imidazolium cations **3a**–**c** did not show strong changes in the intensity of the peak at 245 nm; however, the shape of this signal was deformed (Figure 2). The positive signal at 275 nm was increased. Both effects were most marked for **3c**, and in general they indicate that the imidazolium derivatives have a substantial influence on the stacking of the bases with only minor modifications in the helical conformation. Comparable effects had been observed previously using the same assay with different naphthalimides.[9f] While **4a** induced similar changes compared to **3a**–**c**, derivatives **4b**–**d** caused stronger modifications in the intensity and shape of the band at 245 nm and strongly increased the intensity of the positive signal at 275 nm. Complexes **4b** and **4d** showed the strongest influence on the CD spectra.

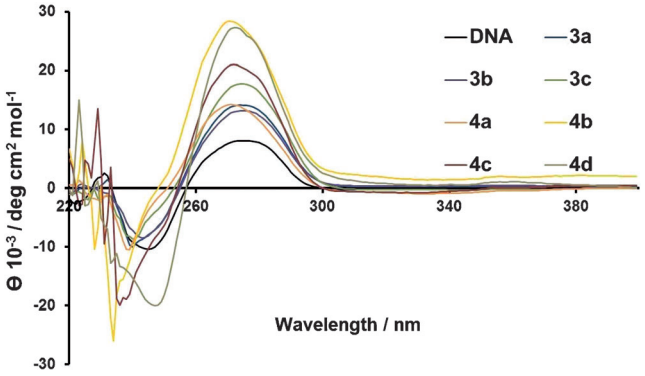

Figure 2. CD spectra of CT-DNA and mixtures of the respective compounds with CT-DNA {[compound]/[DNA] = 0.2 for [DNA] = *M*(bp)} in 10 mM phosphate buffer (pH 7.2) after an incubation period of 1 h.

In summary the observed changes indicate that the gold(I) NHC derivatives were efficient intercalators that also distorted the B conformation of DNA.

Effects on cellular metabolism

The influence of 5 and 10 μM of selected compounds **3a**, **4a** and **4d** on the cellular metabolism of MCF-7 cells was monitored online by using a metabolic sensor chip analysis system (BIONAS), which allows to monitor the cellular oxygen consumption (respiration rate, Figure 3a), changes on the cellular morphology (impedance, Figure 3b), as well as cell stress (extracellular acidification rate, Figure 3c).[12]

With the gold-free **3a** and complex **4a** a slight decrease of the cellular respiration was noted but no relevant changes of cell impedance or extracellular acidification. Complex **4d** induced a stronger and dose-dependent decrease of the respiration rate. At the higher exposure concentration of **4d** (10 μM) this was accompanied by immediate effects on the extracellular acidification and delayed (after 8 h of exposure) effects on the cell impedance. All effects of **4d** were irreversible (no recovery of the metabolic parameters after washing the cells with drug-free medium, see the final RM phase in Figure 3)

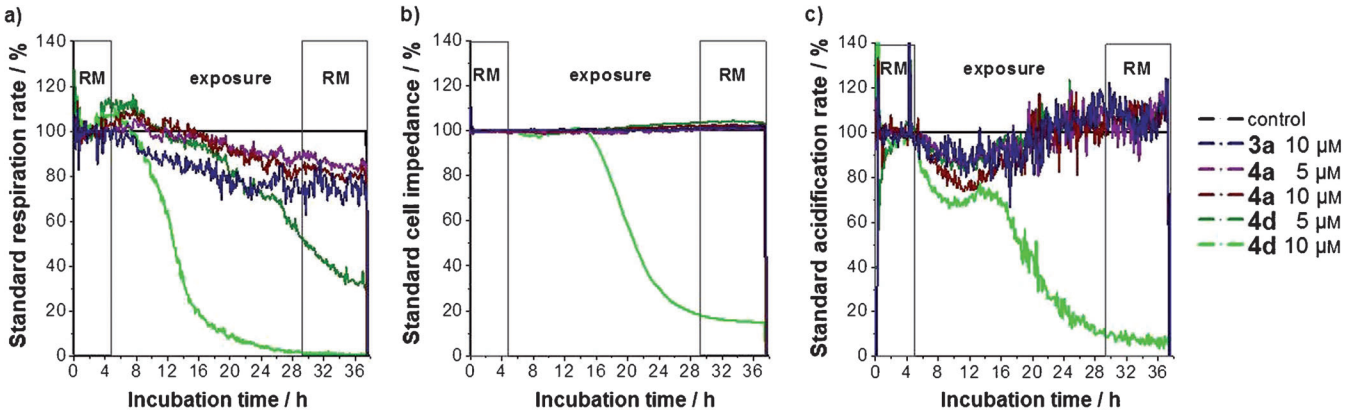

Figure 3. Real-time response of MCF-7 cell metabolism in response to **3a**, **4a** and **4d**. Treatment (exposure) started after 5 h of equilibration and was continued for 24 h. a) Standard respiration rate, b) standard cell impedance, and c) acidification rate; RM = running medium (without compound).

and are in good agreement with the high cytotoxicity of this compound as well as the strong effects on DNA observed in the CD studies.

Conclusions

Naphthalimide conjugates with a gold(I) NHC partial structure in the side chain were prepared and studied biologically in comparison to the respective imidazolium precursors. Compounds **2a**–**4a** exhibited only low fluorescence emission in solution, and hence fluorescence microscopy studies in cells were not performed. However, it could be speculated that compounds **3d** or **4d** might show stronger emissive properties based on their thioether substituents on the naphthalimide, which might open options for cellular bioimaging in future studies.[4,9f]

The introduction of the gold(I) NHC fragment into the side chain led to higher cytotoxic activity, with **4c** in MCF-7 cells as the only exception. Strong effects on cell metabolism (respiration, cell impedance, extracellular acidification) were noted for **4d**, which was the most cytotoxic complex. Effective inhibition of the enzyme TrxR was confirmed for selected examples as a likely contributing factor. Moreover, the gold(I) NHC partial structure led to an enhanced interaction with DNA. Hence, it can be concluded that the conjugation of naphthalimides with gold(I) NHC moieties provides an useful strategy for the design of bioorganometallic anticancer agents with multiple (nonrelated) modes of action. In this context, the combination of TrxR inhibition with DNA intercalation appears particularly interesting because these are mechanisms that are both relevant in anticancer research but not directly linked with each other (redox- and DNA-metabolism, respectively).

From a broader viewpoint, the here reported gold compounds represent conjugates between an active drug (naphthalimide) and a metal–NHC fragment. The chemical biology and medicinal chemistry of this type of organometallic moiety has been intensively studied over the last years but so far it has not been used frequently for modifying the properties of existing bioactive agents or in the context of drug targeting. Metal–NHC conjugates with peptides[13] or caffeine[14] are interesting, but rare, where this strategy has been applied very successfully.

Together with these works, our results may suggest that the metal NHC moiety could be used as an organometallic functional group or as a pharmacophore in medicinal chemistry and drug design. Further application and evaluation of this general strategy appears promising and is the subject of ongoing research.

Experimental Section

General

All reagents and solvents were used as received from Sigma Aldrich or Fluka. Rat liver thioredoxin reductase (TrxR), baker's yeast glutathione reductase (GR) and trypsin from bovine pancreas were obtained from Sigma Aldrich. ^{1}H NMR spectra were recorded on a Bruker DRX-400 AS NMR System, ^{13}C NMR spectra on a Bruker AV II-600 AS NMR System, and MS spectra on a FinniganMAT4515. The purity of the target compounds (>95%) was confirmed by elemental analysis (Flash EA112, Thermo Quest Italia). For all compounds undergoing biological evaluation, the experimental values differed less than 0.5% from the calculated ones. The synthesis of **1b**[9f,15] and **2a**[6] is described elsewhere.

Synthesis

3′-(4″-Ethylthio-1″,8″-naphthalimid-*N*″-yl)-1-propyl-imidazol (2b): Compound **1b** (258.2 mg, 1 equiv) and 1-(3-aminopropyl)-imidazole (1.5 equiv) were held at reflux in EtOAc (40 mL) for 6 h. The solution was cooled to RT, and the product was isolated by precipitation. The obtained white solid was filtered off and dried over P_2O_5 to give **2b** as a white powder (yield not determined): ^{1}H NMR ([D$_6$]DMSO): δ = 8.53–8.46 (m, 2H, ArH), 8.34 (d, ^3J = 7.9 Hz, 1H, ArH), 7.85 (dd, ^3J = 8.4, 7.4 Hz, 1H, ArH), 7.72 (d, ^3J = 8.0 Hz, 1H, ArH), 7.66 (dd, ^4J = 1.1 Hz, 1H, NC*H*N), 7.22 (dd, ^4J = 1.1 Hz, 1H, CH imidazole), 6.88 (dd, ^4J = 1.1 Hz, 1H, CH imidazole), 4.05 (dt, ^2J = 12.0 Hz, ^3J = 7.2 Hz, 4H, NC*H*$_2$-), 3.28 (q, ^3J = 7.3 Hz, 2H, SC*H*$_2$CH$_3$), 2.09 (p, ^3J = 7.1 Hz, 2H), 1.39 ppm (t, ^3J = 7.3 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 163.30 (CO), 163.25 (CO), 144.43 (ArC), 137.20 (NCHN), 130.94 (ArC), 130.40 (ArC), 129.46 (ArC), 128.53 (ArC), 128.29 (ArC), 127.59 (ArC), 127.07 (ArC), 122.75 (CH imidazole), 122.66 (CH imidazole), 119.19 (ArC), 118.37 (ArC), 44.06

(NCH_2-), 37.25 (NCH_2-), 29.32 (SCH_2-), 25.17 ($NCH_2CH_2CH_2N$), 13.37 (SCH_2CH_3); Anal. calcd for $C_{20}H_{19}N_3O_2S$: C 65.73, H 5.24, N 11.50, found: C 65.51, H 5.11, N 11.46.

General procedure for the synthesis of 3a–d: Compound **2a** or **2b** (0.5 mmol, 1 equiv) and an excess of the respective alkyl halide (3 equiv) were heated to reflux in toluene (30 mL) for 24 h. After cooling to RT, the solvent was removed in vacuo, and the remaining solid was resuspended in tetrahydrofurane (THF). The precipitate was filtered off and dried in vacuo and over P_2O_5, resulting in a white solid, which corresponded to the desired pure product.

1-(3′-(1″,8″-Naphthalimid-*N*″-yl))-propyl-3-methylimidazolium iodide (3a): White powder (201.3 mg, 90%): ^{1}H NMR ([D$_6$]DMSO): δ = 9.12 (dd, ^4J = 1.8 Hz, 1H, NC*H*N), 8.56–8.44 (m, 4H, ArH), 7.90 (dd, ^3J = 8.3, 7.3 Hz, 2H, ArH), 7.81 (dd, ^4J = 1.8 Hz, 1H, imidazole CH), 7.71 (dd, ^4J = 1.8 Hz, 1H, imidazole CH), 4.29 (t, ^3J = 6.6 Hz, 2H, NC*H*$_2$CH$_2$-), 4.12 (t, ^3J = 6.5 Hz, 2H, NCH$_2$C*H*$_2$CH$_2$-), 3.86 (s, 3H), 2.24 ppm (dt, ^2J = 13.6 Hz, ^3J = 6.6 Hz, 1H, NCH$_2$C*H*$_2$CH$_2$N); ^{13}C NMR ([D$_6$]DMSO): δ = 163.66 (CO), 136.65 (N*C*HN), 134.35 (ArC), 131.26 (ArC), 130.69 (ArC), 127.42 (ArC), 127.20 (ArC), 123.50 (ArC), 122.22 (CH imidazole), 122.08 (CH imidazole), 46.91 (N*C*H$_2$-), 36.68 (N*C*H$_2$-), 35.74 (N*C*H$_3$), 28.40 ppm (NCH$_2$*C*H$_2$CH$_2$N); Anal. calcd for $C_{19}H_{18}IN_3O_2$: C 51.02, H 4.06, N 9.39, found: C 50.63, H 4.02, N 9.17.

1-(3′-(1″,8″-Naphthalimid-*N*″-yl))-propyl-3-ethylimidazolium iodide (3b): White powder (126.9 mg, 55%): ^{1}H NMR ([D$_6$]DMSO): δ = 9.20 (dd, ^4J = 1.8 Hz, 1H, NC*H*N), 8.53–8.45 (m, 4H, ArH), 7.89 (dd, ^3J = 8.2, 7.3 Hz, 2H, ArH), 7.84 (dd, ^4J = 1.8 Hz, 1H, CH imidazole), 7.82 (dd, ^4J = 1.8 Hz, 1H, CH imidazole), 4.29 (t, ^3J = 6.6 Hz, 2H, NC*H*$_2$-), 4.20 (q, ^3J = 7.3 Hz, 2H, NC*H*$_2$CH$_3$), 4.12 (t, ^3J = 6.5 Hz, 2H, NC*H*$_2$-), 2.34–2.20 (m, 2H, NCH$_2$C*H*$_2$CH$_2$N), 1.41 ppm (d, ^3J = 7.3 Hz, 2H, NCH$_2$C*H*$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 163.63 (CO), 135.81 (N*C*HN), 134.34 (ArC), 131.24 (ArC), 130.68 (ArC), 127.39 (ArC), 127.18 (ArC), 122.32 (CH imidazole), 122.02 (CH imidazole), 46.95 (N*C*H$_2$CH$_3$), 44.18 (N*C*H$_2$-), 36.65 (N*C*H$_2$-), 28.26 (NCH$_2$*C*H$_2$CH$_2$N), 14.95 (NCH$_2$*C*H$_3$); Anal. calcd for $C_{20}H_{20}IN_3O_2$: C 52.07, H 4.37, N 9.11, found: C 52.06, H 4.21, N 8.79.

1-(3′-(1″,8″-Naphthalimid-*N*″-yl))-propyl-3-benzylimidazolium bromide (3c): White powder (188.2 mg, 79%): ^{1}H NMR ([D$_6$]DMSO): δ = 9.33 (dd, ^4J = 1.8 Hz, 1H, NC*H*N), 8.52–8.47 (m, 4H, ArH), 7.90 (dd, ^3J = 8.2, 7.3 Hz, 2H, ArH), 7.87 (dd, ^4J = 1.8 Hz, 1H, CH imidazole), 7.83 (dd, ^4J = 1.8 Hz, 1H, CH imidazole), 7.47–7.36 (m, 5H, NCH$_2$(C$_6$*H*$_5$)), 5.45 (s, 2H, NC*H*$_2$Ph), 4.37–4.25 (m, 2H, NC*H*$_2$-), 4.11 (t, ^3J = 6.5 Hz, 2H, NC*H*$_2$-), 2.31–2.17 ppm (m, 2H, NCH$_2$C*H*$_2$CH$_2$N); ^{13}C NMR ([D$_6$]DMSO): δ = 163.67 (CO), 136.34 (N*C*HN), 134.75 (ArC), 134.37 (ArC), 131.29 (ArC), 130.70 (ArC), 128.95 (ArC), 128.67 (ArC), 128.21 (ArC), 127.45 (ArC), 127.21 (ArC), 122.79 (CH imidazole), 122.47 (CH imidazole), 122.11 (ArC), 51.90 (NC*H*$_2$Ph), 47.06 (N*C*H$_2$-), 36.62 (NH*C*H$_2$-), 28.28 ppm (NCH$_2$*C*H$_2$CH$_2$N); Anal. calcd for $C_{25}H_{22}BrN_3O_2$: C 63.03, H 4.65, N 8.82, found: C 62.88, H 4.56, N 8.66.

1-(3′-(4″-Ethylthio-1″,8″-naphthalimid-*N*″-yl))-propyl-3-methylimidazolium iodide (3d): White powder (yield not determined): ^{1}H NMR ([D$_6$]DMSO): δ = 9.12 (dd, ^4J = 1.8 Hz, 1H, NC*H*N), 8.56–8.49 (m, 2H, ArH), 8.37 (d, ^3J = 8.0 Hz, 1H, ArH), 7.89 (dd, ^3J = 8.5, 7.4 Hz, 1H, Ar H), 7.80 (dd, ^4J = 1.8 Hz, 1H, CH imidazole), 7.76 (d, ^3J = 8.0 Hz, 1H, ArH), 7.71 (dd, ^4J = 1.8 Hz, 1H, CH imidazole), 4.28 (dd, ^3J = 6.5 Hz, 2H, NC*H*$_2$-), 4.09 (t, ^3J = 6.5 Hz, 2H, NC*H*$_2$-), 3.85 (s, 3H, NC*H*$_3$), 3.30 (q, ^3J = 7.3 Hz, 2H, SC*H*$_2$CH$_3$), 2.28–2.16 (m, 2H, NCH$_2$C*H*$_2$CH$_2$N), 1.40 ppm (t, ^3J = 7.3 Hz, 3H, SCH$_2$C*H*$_3$); ^{13}C NMR ([D$_6$]DMSO): δ = 163.43 (CO), 163.39 (CO), 144.62 (ArC), 136.65 (N*C*HN), 131.00 (ArC), 130.46 (ArC), 129.60 (ArC), 128.56 (ArC), 127.62 (ArC), 127.15 (ArC), 123.50 (ArC), 122.71 (CH imidazole), 122.22 (CH imidazole), 118.30 (ArC), 46.91 (N*C*H$_2$CH$_2$-), 36.63 (NCH$_2$*C*H$_2$-), 35.74 (N*C*H$_3$), 28.39 (S*C*H$_2$CH$_3$), 25.19 (NCH$_2$*C*H$_2$CH$_2$N), 13.37 ppm (SCH$_2$*C*H$_3$); Anal. calcd for $C_{21}H_{22}IN_3O_2S$: C 49.71, H 4.37, N 8.28, found: C 49.95, H 4.40, N 8.15.

General procedure for synthesis of the gold NHC complexes 4a–d: The respective imidazolium halide **3a–d** (0.25 mmol, 1 equiv) and Ag_2O (1 equiv) were added to a dried Schlenk tube. The mixture was back flashed with N_2 (3×), and then dry CH_2Cl_2 (10 mL) was added. The flask was closed, and the mixture was stirred for 4–24 h at RT in the dark. A solution of chlorido(dimethyl sulfide)-gold(I) in CH_2Cl_2 (73.6 mg, 0.25 mmol, in 10 mL) was added, and the reaction mixture was stirred for another 4–24 h at RT in the dark. The obtained suspension was filtered over Celite (281 nm), and the solvent was removed at 900 mbar and 35 °C to prevent decomposition. The obtained solid was recrystallized from Et_2O and dried over P_2O_5.

Chlorido[1-(3′-(1″,8″-naphthalimid-*N*″-yl))-propyl-3-methyl-imidazol-2-ylidene]gold(I) (4a): White powder (45.5 mg, 33%): ^{1}H NMR ($CDCl_3$) δ = 8.61 (dd, ^3J = 7.3 Hz, ^4J = 1.1 Hz, 2H, ArH), 8.24 (dd, ^3J = 8.4 Hz, ^4J = 1.1 Hz, 2H, ArH), 7.78 (dd, ^3J = 8.2 Hz, ^3J = 7.3 Hz, 2H, ArH), 7.24 (d, ^4J = 1.9 Hz, 1H, CH imidazolylidene), 6.96 (d, ^4J = 1.9 Hz, 1H, CH imidazolylidene), 4.32 (t, ^3J = 6.8 Hz, 2H, NC*H*$_2$CH$_2$-), 4.24 (t, ^3J = 7.0 Hz, 2H, NC*H*$_2$CH$_2$-), 3.84 (s, 3H, NC*H*$_3$), 2.34 ppm (p, ^3J = 6.9 Hz, 2H, NCH$_2$C*H*$_2$CH$_2$N). ^{13}C NMR ($CDCl_3$) δ = 171.53 (NHC), 164.26 (CO), 134.28 (ArC), 131.64 (ArC), 131.50 (ArC), 128.20 (ArC), 127.03 (ArC), 122.37 (ArC), 121.70 (CH imidazolylidene), 120.73 (CH imidazolylidene), 49.20 (N*C*H$_2$-), 38.34 (N*C*H$_3$), 37.39 (N*C*H$_2$-), 29.47 ppm (NCH$_2$*C*H$_2$CH$_2$N). Anal. calcd for $C_{19}H_{17}N_3ClO_2Au$: C 41.36, H 3.11, N 7.62, found: C 40.99, H 2.91, N 7.19.

Chlorido[1-(3′-(1″,8″-naphthalimid-*N*″-yl))-propyl-3-ethyl-imidazol-2-ylidene]gold(I) (4b): White powder (14.2 mg, 10%): ^{1}H NMR ($CDCl_3$) δ = 8.61 (dd, ^3J = 7.1, 1.1 Hz, 2H, ArH), 8.24 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 2H, ArH), 7.78 (dd, ^3J = 8.2 Hz, ^3J = 7.3 Hz, 2H, ArH), 7.24 (d, ^3J = 2.0 Hz, 1H, CH imidazolylidene), 6.99 (d, ^3J = 1.9 Hz, 1H, CH imidazolylidene), 4.32 (t, ^3J = 6.8 Hz, 2H, NC*H*$_2$CH$_2$-), 4.25 (t, ^3J = 7.0 Hz, 2H, NC*H*$_2$CH$_2$-), 4.23 (q, ^3J = 7.3 Hz, 2H, NC*H*$_2$CH$_3$), 2.35 (dt, ^3J = 6.9 Hz, 2H, NCH$_2$C*H*$_2$CH$_2$N), 1.47 ppm (t, ^3J = 7.3 Hz, 3H, NCH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ = 170.52 (NHC), 164.27 (CO), 134.27 (ArC), 131.65 (ArC), 131.50 (ArC), 128.21 (ArC), 127.03 (ArC), 122.38 (ArC), 120.74 (CH imidazolylidene), 119.82 (CH imidazolylidene), 49.29 (N*C*H$_2$-), 46.60 (N*C*H$_2$-), 37.41 (N*C*H$_2$-), 29.44 (NCH$_2$*C*H$_2$CH$_2$N), 16.46 ppm (NCH$_2$*C*H$_3$). Anal. calcd for $C_{20}H_{19}N_3ClO_2Au$: C 42.46, H 3.38, N 7.43, found: C 42.51, H 2.93, N 7.07.

Chlorido[1-(3′-(1″,8″-naphthalimid-*N*″-yl))-propyl-3-benzyl-imidazol-2-ylidene]gold(I) (4c): White powder (17.3 mg, 11%): ^{1}H NMR ($CDCl_3$): δ = 8.61 (dd, ^3J = 7.3 Hz, ^4J = 1.2 Hz, 2H, ArH), 8.25 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 2H, ArH), 7.78 (dd, ^3J = 8.2, 7.3 Hz, 2H, ArH), 7.47–7.29 (m, 5H, NCH$_2$(C$_6$*H*$_5$)), 7.23 (d, ^4J = 2.0 Hz, 2H, CH imidazolylidene), 6.89 (d, ^4J = 2.0 Hz, 1H, CH imidazolylidene), 5.39 (s, 2H, NC*H*$_2$Ar,), 4.35 (t, ^3J = 6.8 Hz, 2H, NC*H*$_2$CH$_2$CH$_2$N), 4.27 (t, ^3J = 7.0 Hz, 2H, NC*H*$_2$CH$_2$CH$_2$N), 2.37 ppm (dt, ^3J = 7.0 Hz, 2H, NCH$_2$C*H*$_2$CH$_2$N); Anal. calcd for $C_{25}H_{21}N_3ClO_2Au$ (% calcd/found): C 47.82, H 3.37, N 6.69, found: C 47.93, H 3.41, N 6.41.

Chlorido[1-(3′-(4″-ethylthio-1″,8″-naphthalimid-*N*″-yl))-propyl-3-methyl-imidazol-2-ylidene]gold(I) (4d): White powder (yield not determined): ^{1}H NMR ($CDCl_3$): δ = 8.62 (dd, ^3J = 7.3 Hz, ^4J = 1.1 Hz, 1H ArH), 8.59 (dd, ^3J = 8.4 Hz, ^4J = 1.2 Hz, 1H, ArH), 8.48 (d, ^3J = 7.9 Hz, 1H, ArH), 7.76 (dd, ^3J = 8.5, 7.3 Hz, 1H, ArH), 7.56 (d, ^3J = 7.9 Hz, 1H, ArH), 7.24 (d, ^4J = 1.9 Hz, 1H, CH imidazolylidene), 6.95 (d, ^4J = 1.9 Hz, 1H, CH imidazolylidene), 4.31 (t, ^3J = 6.8 Hz, 2H, NC*H*$_2$CH$_2$-), 4.22 (t, ^3J = 7.0 Hz, 2H, NC*H*$_2$CH$_2$-), 3.84 (s, 3H, NC*H*$_3$),

3.21 (q, $^3J=7.4$ Hz, 2H, SCH_2CH_3), 2.33 (p, $^3J=6.9$ Hz, 2H, $NCH_2CH_2CH_2N$), 1.49 ppm (t, $^3J=7.4$ Hz, 3H, SCH_2CH_3); ^{13}C NMR ($CDCl_3$): $\delta=171.51$ (NHC), 164.15 (CO), 164.12 (CO), 145.93 (ArC), 131.78 (ArC), 131.08 (ArC), 130.46 (ArC), 129.60 (ArC), 128.41 (ArC), 126.64 (ArC), 122.80 (ArC), 122.64 (ArC), 121.67 (CH imidazolylidene), 120.76 (CH imidazolylidene), 118.66 (ArC), 49.20 (NCH_2CH_2-), 38.35 (NCH_2CH_2-), 37.35 (NCH_3), 29.48 (SCH_2CH_3), 26.38 ($NCH_2CH_2CH_2N$), 13.52 ppm (SCH_2CH_3); Anal. calcd for $C_{21}H_{21}N_3ClSO_2Au$: C 41.22, H 3.46, N 6.87, found: C 41.05, H 3.34, N 6.47.

Biology

Antiproliferative effects and enzymatic inhibition assays: Experiments on cell proliferation were performed according to recently described procedures.[7d] To determine the inhibition of the enzymes TrxR, GR established microplate reader based assays were performed.[7d]

Interaction with DNA: An Analytik Jena SPECORD 200 equipped with a Peltier temperature controller was employed for UV/Vis measurements and CD spectra were recorded on a Jasco J-715 instrument. DNA melting curves were recorded on the SPECORD 200 at 1 °C steps for 1:5 complex/DNA mixtures in a 10 mM phosphate buffer (2.96 mM KH_2PO_4, 7.04 mM Na_2HPO_4) at pH 7.2. Thermal denaturation temperatures (T_m) were calculated by determining the midpoints of the melting curves from the first order derivatives. Concentrations of calf thymus CT-DNA as *M*(base pairs) were determined spectrophotometrically using the molar extinction coefficient $\varepsilon_{260}=-13200\ M^{-1}cm^{-1}$.

Effects on cell metabolism: Online measurement of cell metabolism and morphological changes was performed using a Bionas2500 sensor chip system (Bionas, Rostock, Germany). The metabolic sensor chips (SC 1000) include ion-sensitive field-effect transistors to record pH changes, an oxygen electrode to monitor oxygen consumption, and interdigitated electrode structures to measure impedance under the cell layer. Approximately 1.5×10^5 cells were seeded directly onto each sensor chip in 450 μL of *N*,*N*-dimethylethanolamine (DMAE; PAA Laboratories, E15-883) with 1% penicillin/streptomycin and 10% (*v*/*v*) fetal calf serum (FCS; PAA) and incubated at 37 °C, 5% CO_2, and 95% humidity for 24 h. The cell number used resulted in approximately 80–90% confluence of the cells on the chip surface after 24 h. This was the starting condition for online monitoring. Sensor chips with cells were then transferred to the Bionas2500 analyzer in which medium is continuously exchanged in 8 min cycles (4 min exchange of medium and 4 min without flow) during which the parameters were measured. The running medium used during analysis was Dulbecco's modified Eagle medium (DMEM) without carbonate buffer and only weakly buffered with 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) (1 mM) and reduced FCS (0.1%). For drug activity testing, the following steps were included: (1) 5 h equilibration with running medium (RM), (2) drug incubation with substances freshly dissolved in medium at indicated concentrations and indicated incubation time, (3) a regeneration step in which cells are again fed with running medium without substances, (4) at the end of each experiment the cell membrane was damaged by addition of 0.2% Triton X-100 to obtain a basic signal without living cells on the sensor surface as a negative control.

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Keywords: DNA · gold · naphthalimides · N-heterocyclic carbenes · thioredoxin reductase

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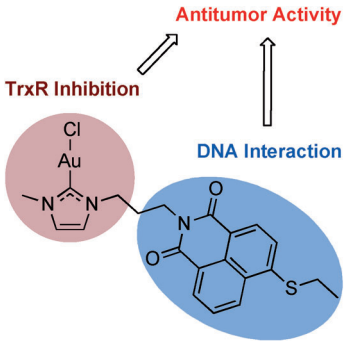
Gold(I) *N*-Heterocyclic Carbene Complexes with Naphthalimide Ligands as Combined Thioredoxin Reductase Inhibitors and DNA Intercalators



Multiple modes of action: The conjugation of naphthalimides with gold(I) NHC moieties led to higher cytotoxic activity, strong effects on cell metabolism, and effective inhibition of TrxR for selected compounds. Moreover, the gold(I) NHC partial structure led to an enhanced interaction with DNA.