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# TrxR Inhibition and Antiproliferative Activities of Structurally Diverse Gold *N*-Heterocyclic Carbene Complexes

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**Abstract**

Gold compounds with *N*-heterocyclic carbene (NHC) ligands have been largely described as potent thioredoxin reductase (TrxR) inhibitors and effective anticancer agents. However, despite these promising aspects structure-activity-relationship (SAR) studies still remain limited. In this study a structurally diverse library of gold(I) and gold(III) NHC complexes was investigated for inhibitory capacity against TrxR and for antiproliferative activity in HT-29 human colon adenocarcinoma cells with the aim of identifying valid SAR. Overall results indicated that the bioactivity, carried by the gold center, is intimately linked to the chemical properties of the residues at the NHC scaffold as well as other ligands coordinated to the gold atom. Although a direct correlation between $IC_{50}$ values for cytotoxicity and those for enzyme inhibition could not be established, the inhibition of TrxR represented an important parameter to achieve a good cytotoxic activity.

## Introduction

Gold and its complexes have a long tradition in medicine, and research on their potential in medicinal chemistry has experienced a renaissance over the last decades.[1-4] Complexes like aurothiomalate, aurothiosulfate or auranofin were recognized to be effective drugs for the treatment of rheumatoid arthritis.[5] The cellular pathophysiology of rheumatoid arthritis is related to the malfunction of biochemical pathways also relevant for cancer development (e.g. concerning the metabolism of cytokines such as IL-2 or proteins involved in the signal translation such as p38).[6-8] Consequently, gold(I) complexes have also been intensively investigated as new anticancer drugs and nowadays bioinorganic medicinal chemists have a large number of bioactive structures at their disposal.[2, 3, 9, 10] Among them, *N*-heterocyclic carbene (NHC) complexes of the Arduengo type represent a very promising class of molecules.[11] This was in part motivated by their high stability that is comparable (if not better) than those of phosphane ligands. Moreover, the combination of a NHC moiety with a gold center led to molecules with an interesting biological profile and gold(I) NHC complexes proved to have a different mode of action than the traditional platinum anticancer drugs, barely targeting DNA.[12-15] They affect the functionality of mitochondria by depolarizing the organelle membrane, stimulate reactive oxygen species (ROS) production, perturb important cellular regulation systems such as the antioxidant network and induce apoptosis via the intrinsic apoptotic pathway.[9, 14, 16-20]

Between the numerous hypotheses concerning the molecular mode of drug action, the inhibition of the enzyme thioredoxin reductase (TrxR) seems to play a central role in the pharmacology of gold complexes.[2, 3] TrxR is a ubiquitous flavoprotein in charge of regeneration of the functionality of small molecules (e.g. thioredoxin, glutathione), which are oxidized by different xenobiotics or enzymes belonging to the antioxidant network, and responsible for controlling the cellular redox homeostasis. The catalytic activity of TrxR is carried out by the so called Trx-motif present in the active site of the enzyme. This characteristic structure consists of a cysteine-AA-AA-cysteine peptidic sequence in the N-terminal motif and a selenocysteine-AA-AA-cysteine peptidic sequence at the C-terminal of the protein (where AA stands for any amino acid other than cysteine).[21, 22] Gold is a soft Lewis acid that can be expected to display a good affinity to soft donor atoms like selenium. It has been also reported that, because of their increased metabolism, cancer cells over-express TrxR.[23, 24] Consequently, the selective inhibition of this key enzyme represents a valuable parameter for the development of new gold-based anticancer drugs. Several effective anticancer agents were shown to inhibit TrxR efficiently. Gold complexes resulted to be the strongest and most selective inhibitors for this enzyme nowadays discovered, in many cases with $IC_{50}$ values in the nanomolar range.[23, 25, 26]

Despite their effective biological profile and an increasing pool of active derivatives, reports that aim at establishing structure-activity relationships for gold NHC complexes remain rather rare. We report here a screening of 20 structurally diverse gold NHC complexes (**1a** - **7**) with the aim of

identifying correlations between TrxR inhibition, cytotoxicity and structural features of the compounds. The selected examples consist of compounds with a gold(I) or gold(III) central atom with coordinated halides, thiolates as well as structurally diverse NHC ligands and thus they form a library of structurally diverse gold NHC complexes that is suitable to identify structure-activity-relationships (see figure 1).

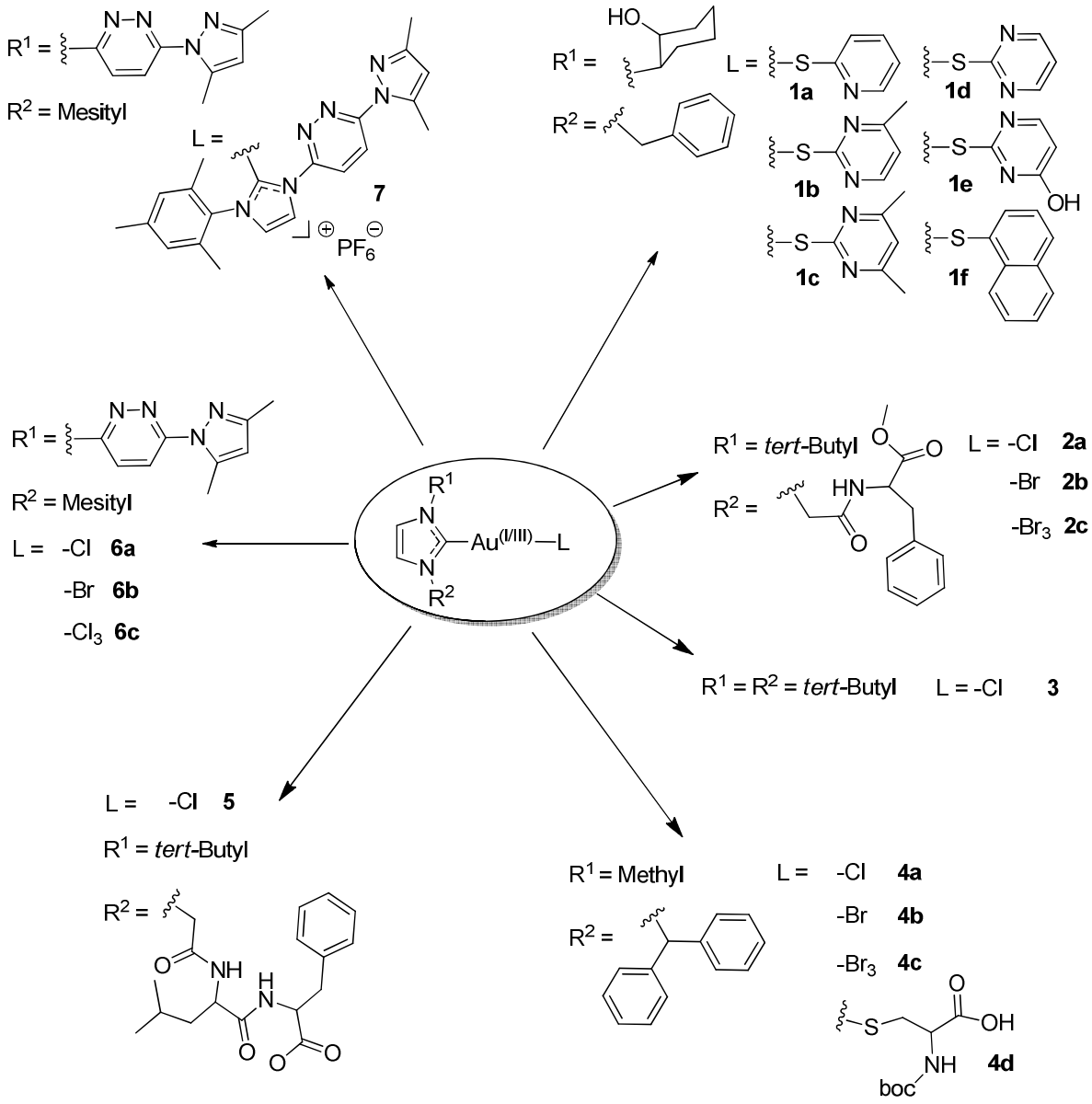


**Figure 1**: Gold NHC complexes investigated in this study

## Synthesis

The preparation and characterization of complexes **2** – **7** was reported recently.[27, 28]

The thiolato complexes **1a**-**1f** were prepared by the reaction of the (NHC)Au(I)Cl complexes with the *in situ* generated sodium salt of the appropriate thiol. The resulting (NHC)Au(I)thiolato complexes were obtained in good yields as colorless, beige, yellow or grey solids which are soluble in halogenated solvents, acetone and methanol. The new compounds were characterized by $^{1}$H and $^{13}$C NMR spectroscopy and elemental analysis. In one case, the solid-state structure was determined by X-ray diffraction.

The most diagnostic feature in the $^{13}$C-NMR spectra of the (NHC)Au(I)thiolato compounds is the chemical shift of the carbene carbon atom. In the halide complexes the signals are observed at around 170 ppm, while those of the thiolato compounds are observed at approximately 180 ppm.

The structure of the 1-naphthylthiolato derivative **1f** was solved by single crystal X-ray crystallography (see Figure 2 for an ORTEP plot and Table S1 for full geometrical parameters) and was found to have dimeric structure in the solid state. The angle about the metal centre is close to linearity (175.1°) and the Au-C bond length is in the expected range for carbene gold complexes. The compound forms dimers through an intermolecular aurophilic interaction [Au(1)···Au(1*) = 3.27 Å], a feature which is often observed in the solid-state structures of gold(I) complexes.

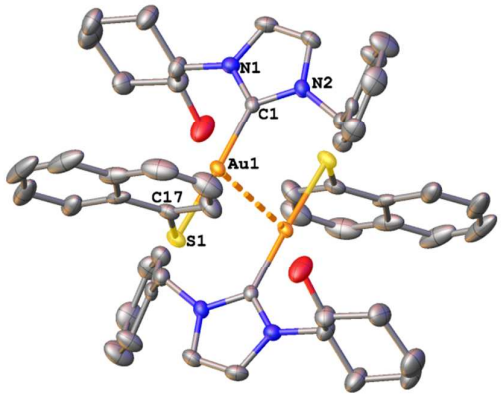


**Figure 2**: Molecular structure of **1f** as determined by single crystal X-ray crystallography. Ellipsoids show 30% probability levels. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (deg): C1-Au1 2.013(4), S1-Au1 2.302(1), Au1-Au1* 3.2688(2), C1-Au1-S1 175.1(1).

**Inhibition of enzymatic activities**

The inhibition of the target enzyme TrxR was measured using an established assay, which is based on the NADPH dependent reduction of the disulfide bond of the substrate 5,5´-dithiobis(-2-nitrobenzoic acid) (DTNB or Elmanns reagent) by the isolated enzyme (see table 1).[29] Glutathione reductase (GR) inhibition was studied for some of the complexes for reference purposes.

| Compound | TrxR (μM) | GR (μM) | Selectivity GR/TrxR (x-fold) | HT-29 $IC_{50}$ (μM) |
|---|---|---|---|---|
| **1a** | 0.42 ± 0.12 | n.d | --- | 7.0 ± 3.5 |
| **1b** | 0.32 ± 0.07 | n.d | --- | 4.9 ± 0.1 |
| **1c** | 3.3 ± 0.9 | n.d | --- | 7.6 ± 0.3 |
| **1d** | 0.43 ± 0.03 | n.d | --- | 5.2 ± 0.6 |
| **1e** | 0.49 ± 0.15 | n.d | --- | 12.1 ± 2.4 |
| **1f** | 1.1 ± 0.3 | n.d. | --- | 4.2 ± 0.2 |
| **2a** | 2.95 ± 0.7 | 16.7 | 5.6 | 63.8 ± 6.7 * |
| **2b** | 5.35 ± 1.42 | 24.5 | 4.6 | n.d. |
| **2c** | 6.89 ± 1.00 | 17.8 | 2.6 | 125.8 ± 49.7 * |
| **3** | 5.32 ± 1.24 | 17.9 | 3.4 | n.d. |
| **4a** | 5.08 ± 1.00 | > 50 | > 9.8 | 2.8 ± 1.7 * |
| **4b** | 4.14 | 25.1 | 6.1 | n.d. |
| **4c** | > 50 | > 50 | --- | 12.7 ± 1.2 * |
| **4d** | 7.23 ± 1.67 | > 50 | > 6.9 | 10.5 ± 1.9 * |
| **5** | 0.36 ± 0.2 | 8.7 | 24.2 | 45.3 ± 0.12 |
| **6a** | 0.69±0.19 | 23.2±2.7 | 33.6 | n.d. |
| **6b** | 1.2±0.4 | 7.8±2.1 | 6.5 | 7.1 ± 2.4 |
| **6c** | 6.4±1.4 | 56.2±1.4 | 8.8 | 0.26 ± 0.06 |
| **7** | 0.47±0.14 | 4.4±1.1 | 9.4 | 5.1 ± 1.1 |

**Table 1**: Inhibition of TrxR and GR and antiproliferative effects in the HT-29 cancer cell line triggered by the gold NHC complexes; n.d.: not determined; * data taken from reference [27]

Many complexes showed an effective inhibition of the target enzyme TrxR with $IC_{50}$ values in the low micromolar or submicromolar range, which is comparable or even lower as observed for "organic" inhibitors (e.g. PX12).[30] Some interesting conclusions can be drawn from the obtained data.

The most effective inhibitors of TrxR with $IC_{50}$ values well below 1 μM were **1a, 1b**, **1d**, **1e**, which contain a thiophenolate derived ligand, **5** with a peptidic side chain, as well as the pyrazol/pyridazinyl containing compounds **6a** and **7**. The majority of the complexes (**1c**, **1f**, **2a** - **4b**,

**4d**, **6b, 6c**) displayed more moderate activity ($IC_{50}$ values in the range of 1 -10 μM), complex **4c** was not active (no $IC_{50}$ values below 50 μM). In the series **1a** - **1f**, the comparably lower activity of **1c** and **1f** could be the consequence of steric hindrance caused by the two methyl groups of **1c** or the additional annellated benzene ring of **1f**. An increased steric demand at the thiolate supposedly counteracts ligand exchange processes with the selenocysteine side chain in the TrxR binding site. Similar to the thiophenolate moiety of **1a** – **1f**, the halide or thiolate ligands of **2a** - **6c** represent leaving groups that can undergo ligand exchange processes. Not surprisingly these compounds were active inhibitors with **4c** as the only exception. The nature of the halide (Br vs Cl) is of minor relevance with a slight preference for chlorine. Comparison of the pairs **2a**/**2b**, **4a**/**4b** and **6a**/**6b** indicates that for the chlorido complexes **2a** and **6a** (but not for **4a**) the activity against TrxR is slightly elevated. These minor differences may be due to only small kinetic differences between the two halido ligands. The influence of the oxidation state of the gold central atom can be evaluated by comparing **2b** vs **2c**, **4b** vs **4c** and **6a** vs **6c**. In all cases the gold(I) derivatives were more effective TrxR inhibitors than the gold(III) complexes with strongly increased potency in the case of **4b** and **6a**. Compounds **2a**, **2b**, **3**, **4a**, **4b**, **5** and **6a**, **6b** contain a halido ligand coordinated to a gold(I) center but among them they differ in the substituents at the nitrogen atoms of the NHC ligand, which enables some comparisons to be made. The most active enzyme inhibitors of this group are **5** and **6a**, which carry on one NHC nitrogen atom a rather bulky and lipophilic *tert*-butyl or mesityl group and on the other nitrogen atom a more polar peptide moiety or a pyrazol/pyridazinyl containing side chain. Interestingly, **5** is approximately one order of magnitude higher active than **2a**. These two compounds differ only in an additional leucine in the $R^2$ side chain of **5**. It can thus be speculated that this particular position could be important for a proper interaction with the active site of TrxR. Finally, **7** represents a cationic lipophilic complex, for which anti-mitochondrial effects might play a role due to the facilitated mitochondrial targeting of such compounds.[31] These data are in excellent agreement with recent published studies on other cationic NHC-Au-NHC complexes as well as NHC-Au-L species.[14, 17, 32]

GR is an enzyme with close functional and structural relationship to TrxR. The most striking difference between the two enzymes is that GR does not contain a selenocysteine in its binding sites and carrys a cysteine at the respective position. Based on its higher acidity the selenocysteine exhibits a stronger affinity to the soft gold(I) center and this enables the convenient design of gold based TrxR inhibitors with a strong selectivity for TrxR over GR. A number of 14 of the 20 investigated complexes was subjected to studies on GR inhibition, which clearly confirmed the expected preferential interaction with TrxR (see table 1).

**Antiproliferative effects and correlation to TrxR inhibition**

The triggering of antiproliferative effects by the complexes was investigated in HT-29 colon adenocarcinoma cells, which represent tissues obtained from a highly relevant human tumor. $IC_{50}$ values were calculated and are presented in table 1. The majority of the investigated complexes displayed promising cytotoxicity in the low micromolar range (2.8 – 12.7 μM). The most active complex was **6c**, complexes **2a**, **2c** and **5** in contrast displayed only low cytotoxicity ($IC_{50}$ values in the range of 40 – 130 μM). It has to be noted that in particular **5** was a strong inhibitor of TrxR activity but not cytotoxic. In order to establish possible correlations between TrxR inhibition and cytotoxicity, the $IC_{50}$ values obtained in the antiproliferative experiments were compared with those for TrxR inhibition (see figure 3).

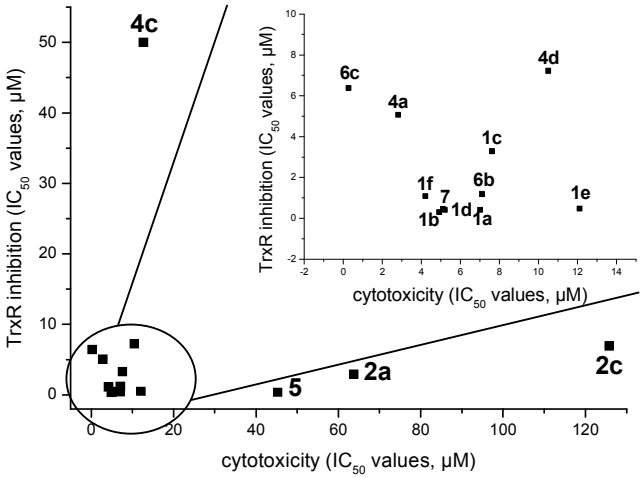


**Figure 3**: $IC_{50}$ values for TrxR inhibition plotted against $IC_{50}$ values for cytotoxicity; the insert represents an enlargement

The majority of the compounds showed a combination of low $IC_{50}$ values for both TrxR inhibition and cytotoxicity, which confirms that inhibition of TrxR can be an important contributing factor to trigger antiproliferative effects. However, an unambiguous correlation between the cytotoxicity of the complexes and the inhibition of TrxR could not be established and this may be related to the contribution of additional parameters affecting drug activity such as biodistribution and metabolisation, especially for compounds **2a**, **2c** and **5**. Conversely, compound **4c** shows excellent antiproliferative activity at low TrxR affinity, which might point to an activation mechanism *in vitro*.

**Conclusions**

An extended and structurally diverse set of gold(I) and gold(III) compounds with *N*-heterocyclic carbene ligands was investigated for the inhibition of the target enzyme TrxR and for the triggering of antiproliferative properties in the HT-29 colon carcinoma cell line. The majority of the complexes

presented a strong TrxR inhibition and led to antiproliferative effects in the range of established cytotoxic drugs (e.g. cisplatin, auranofin). Although a direct correlation cannot be claimed, the data strongly indicated that TrxR inhibition contributes largely to the mode of action of gold NHC complexes.

Analysis of the TrxR inhibition data helped to establish the following trends in structure-activity-relationships: i) Gold(I) NHC complexes were stronger TrxR inhibitors than gold(III) NHC complexes;ii) thiophenolate ligands lead to strong TrxR inhibition unless they are sterically hindered; iii) the insertion of a leucine-containing amino acid into the side chains at the NHC nitrogens resulted in strong TrxR inhibition.

Whereas the enhanced activity of thiophenolates is certainly related to kinetic reasons, the positive effect of the leucine containing residue may be related to specific binding characteristics in the enzymes active site. This clearly warrants further investigations and suggests that an optimization of the peptide side chain could lead to derivatives with increased TrxR inhibition.

The lower activity of gold(III) NHC complexes compared to gold(I) analogues, which has recently also been observed by Gust et al., might be the consequence of reduction of gold(III) upon reaction with the enzyme.[20] In this context it should be noted that the formation of gold(I) species from gold(III) upon reaction with proteins has been reported by Gabbiani, Messori et al.[33, 34] Moreover, reduction of gold(III) NHC to gold(I) NHC complexes in cells triggered by the reaction with thiols has been clearly confirmed by Chi-Ming Che and co-workers,[35] and this could in fact be a relevant mechanism for the good cytotoxic activity of compound **4c** in this study.

In summary our report clearly suggests that inhibition of TrxR activity is a major factor contributing to the biological profile of gold NHC derivatives and that subtle changes in the coordinated ligands at the gold central atom can lead to major changes in the interaction with the target enzyme.

**Acknowledgement**

Financial support by Deutsche Forschungsgemeinschaft (DFG, project FOR630) is gratefully acknowledged.

## Experimental

### Chemistry

Unless otherwise stated, all manipulations were carried out without taking precautions to exclude air and moisture. All chemicals and solvents (HPLC grade or better) were sourced commercially and used as received. The 1-benzyl-3-(2-hydroxycyclohexyl)imidazol-2-ylidene gold chloride was prepared as described in the literature.[36]

$^{1}$H and $^{13}$C NMR spectra were recorded on Bruker Avance 400 or Bruker Avance III 600 MHz spectrometers and are referenced to external TMS. IR spectra were run as KBr disks on a Bruker Tensor 27 instrument. Elemental analyses were performed by staff of the microanalytical laboratory of the University of Wuppertal.

*X- ray crystallography* Diffraction data were collected at 150 K using an Oxford Diffraction Gemini E Ultra diffractometer, equipped with an EOS CCD area detector and a four-circle kappa goniometer. For the data collection the Mo source emitting graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) was used. Data integration, scaling and empirical absorption correction was carried out using the CrysAlis Pro program package (Oxford Diffraction Ltd, 2009). The structures were solved using Direct Methods or Patterson Methods and refined by Full-Matrix-Least-Squares against $F^2$. The non-hydrogen atoms were refined anisotropically and hydrogen atoms were placed at idealized positions and refined using the riding model. All calculations were carried out using the program Olex2.[37] Full crystallographic and refinement parameters as well as tables with bond lengths and angles are included in the supporting information. The crystal structure data are deposited in the Cambridge Crystallographic Data Centre (CCDC) and the CCDC number for compound 1f is 927677. These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/data_request/cif.





**Synthesis**

Complexes **2** - **7** were prepared as recently described.[27, 28]

General procedure for the synthesis of [(NHC)Au(SR)] complexes**1a** - **1f**: The thiol (1.5 equiv.) and NaOMe (2 equiv.) was dissolved in MeOH (15 ml) and stirred for 10 min. The (NHC)Au(I)Cl complex (1 equiv.) was added, the mixture was stirred at room temperature for 6 h and 5 ml 2 M KOH in MeOH was added. After stirring for short time the solvent was concentrated in vacuum and water was added until appearance of a precipitate. The solid was isolated by filtration and washed with water and subsequently dried in air.

**(3-Benzyl-1-(2'-hydroxycyclohexyl)imidazol-2-ylidene)gold(I)(pyridin-2''-thiolato) (1a)**

The beige solid was precipitated with $Et_2O$, 67.6 % (0.07 mmol, 39.0 mg).

$^{1}$H-NMR ($CD_2Cl_2$, 600 MHz, [ppm]): *δ* 8.18 (dd, 1H, *J* =1.1 Hz, *J* =4.9 Hz, $CH_{thiolate}$ (H6)), 7.42 - 7.39 (m, 5H, Ph), 7.38 - 7.36 (m, 1H, $CH_{thiolate}$ (H3)), 7.32 (dt, 1H, *J* =1.9 Hz, *J* =7.7 Hz, $CH_{thiolate}$ (H4)), 7.11 (d, 1H, *J* =1.9 Hz, NCHCHN), 7.00 (d, 1H, *J* =1.9 Hz, NCHCHN), 6.87 (ddd, 1H, *J* =0.9 Hz, *J* =5.1 Hz, *J* =6.9 Hz, $CH_{thiolate}$ (H5)), 5.50 (d, 1H, *J* =14.9 Hz, $CH_2$-Ph), 5.36 (d, 1H, *J* =14.9 Hz, $CH_2$-Ph), 4.76 - 4.72 (m, 1H, OCH or NCH ($C_6H_{10}$)), 3.63 (dt, 1H, *J* =4.3 Hz, *J* =10.6 Hz, OCH or NCH ($C_6H_{10}$)), 2.31 - 2.29 (m, 1H, $CH_2$ ($C_6H_{10}$)), 2.17 - 2.14 (m, 1H, $CH_2$ ($C_6H_{10}$)), 1.89 - 1.83 (m, 3H, $CH_2$ ($C_6H_{10}$)), 1.58 - 1.52 (m, 1H, $CH_2$ ($C_6H_{10}$)), 1.48 - 1.39 (m, 2H, $CH_2$ ($C_6H_{10}$)); $^{13}$C-NMR ($CD_2Cl_2$, 151 MHz, [ppm]): *δ*182.8 (C-Au), 168.3 (C-S), 147.8 ($CH_{thiolate}$(C6)), 135.9 ($C_{ipso}$-Ph), 135.3

($CH_{thiolate}$(C4)), 128.9 ($CH_{Ph}$), 128.3 ($CH_{Ph}$), 127.9 ($CH_{Ph}$), 126.6 ($CH_{thiolate}$(C3)), 120.4 (NCHCHN), 117.8 (NCHCHN), 117.6 ($CH_{thiolate}$(C5)), 73.6 (OCH or NCH ($C_6H_{10}$)), 67.2 (OCH or NCH ($C_6H_{10}$)), 54.9 ($CH_2$-Ph), 35.6 ($CH_2$ ($C_6H_{10}$)), 31.5 ($CH_2$ ($C_6H_{10}$)), 25.0 ($CH_2$ ($C_6H_{10}$)), 24.7 ($CH_2$ ($C_6H_{10}$)). Anal. Calc. for $C_{21}H_{24}N_3OSAu$ [%]: cal.: C, 44.76; H, 4.29; N, 7.46; S, 5.69; found: C, 44.71; H, 5.03; N, 7.39; S, 5.20. Yellow luminescence is observed after excitation with UV-light (366 nm, 254 nm).

**(3-Benzyl-1-(2'-hydroxycyclohexyl)imidazol-2-ylidene)gold(I)(4''-methylpyrimidine-2''-thiolato) (1b)**

The solution of 1.6 equiv. 4-Methylpyrimidin-2-thiole and 3.1 equiv. NaOMe were stirred for 30 min and after addition of the solution was (NHC)Au(I)Cl stirred for 24 h. Yellow solid, 91.5 % (0.189 mmol, 109.3 mg)

$^1$H-NMR ($CDCl_3$, 400MHz, [ppm]): *δ* 8.14 (d, 1H, *J* =5.2 Hz, $CH_{thiolate}$), 7.39 - 7.32 (m, 5H, Ph), 7.02 (d, 1H, *J* =2.0 Hz, NCHCHN), 6.90 (d, 1H, *J* =1.9 Hz, NCHCHN), 6.69 (d, 1H, *J* =5.2 Hz, $CH_{thiolate}$), 5.50 (d, 1H, *J* =14.7 Hz, $CH_2$Ph), 5.33 (d, 1H, *J* =14.7 Hz, $CH_2$Ph), 4.74 (ddd, 1H, *J* =3.8 Hz, *J* =10.1 Hz, *J* =12.2 Hz, OCH or NCH ($C_6H_{10}$)), 3.67 (dt, 1H, *J* =4.2 Hz, *J* =10.3 Hz, OCH or NCH ($C_6H_{10}$)), 2.39 (s, 3H, $Me_{thiolate}$), 2.29 - 2.24 (m, 1H, $CH_2$ ($C_6H_{10}$)), 2.17 - 2.11 (m, 1H, $CH_2$, ($C_6H_{10}$)), 1.88 - 1.80 (m, 3H, $CH_2$, ($C_6H_{10}$)), 1.52 - 1.38 (m, 3H, $CH_2$, ($C_6H_{10}$)); $^{13}$C-NMR ($CDCl_3$, 101 MHz, [ppm]): *δ*182.3 (C-Au), 180.3 (C-S), 166.9 ($C_{ipso}$-Thiolat), 155.3 ($CH_{thiolate}$), 135.4 ($C_{ipso}$-Ph), 129.0 (*m*-$CH_{Ph}$), 128.5 (*p*-$CH_{Ph}$), 128.2 (*o*-$CH_{Ph}$), 120.2 (NCHCHN), 117.9 (NCHCHN), 114.8 ($CH_{thiolate}$), 73.6 (OCH or NCH ($C_6H_{10}$)), 67.1 (OCH or NCH ($C_6H_{10}$)), 55.1 ($CH_2$ ($C_6H_{10}$)), 35.5 ($CH_2$ ($C_6H_{10}$)), 31.7 ($CH_2$ ($C_6H_{10}$)), 25.0 ($CH_2$ ($C_6H_{10}$)), 24.6 ($CH_2$ ($C_6H_{10}$)), 24.1 ($Me_{thiolate}$). Anal. Calc. for $C_{21}H_{25}N_4OSAu \cdot 0.5$ MeOH [%]: calc.: C, 43.28; H, 4.75; N, 9.18; S, 5.25; found: C, 43.22; H, 5.07; N, 9.31; S, 4.97. White luminescence is observed after excitation with UV-light (366 nm, 254 nm).

**(3-Benzyl-1-(2'-hydroxycyclohexyl)imidazol-2-ylidene)(4'',6''-dimethylpyrimidine-2''-thiolato)gold(I) (1c)**

1.3 equiv. sodium-4,6-dimethylpyrimidin-2-thiolate was used and the NHC)Au(I)Cl complex was dissolved 10 ml $CH_2Cl_2$, the soltution was stirred for 24 h. Grey solid 70.8 % (0.15 mmol, 89.9 mg). $^1$H-NMR ($CDCl_3$, 600 MHz, [ppm]): *δ*7.39 - 7.33 (m, 5H, Ph), 7.02 (d, 1H, *J* =1.9 Hz, NCHCHN), 6.89 (d, 1H, *J* =1.9 Hz, NCHCHN), 6.55 (s, 1H, $CH_{thiolate}$), 5.55 (d, 1H, *J* =14.7 Hz, $CH_2$-Ph), 5.41 (d, 1H, *J* =14.7 Hz, $CH_2$-Ph), 4.59 - 4.54 (m, 1H, OCH or NCH ($C_6H_{10}$)), 3.91 (dt, 1H, *J* =4.4 Hz, *J* =10.4 Hz, OCH or NCH ($C_6H_{10}$)), 2.27 (s, 6H, $Me_{thiolate}$), 2.21 - 2.18 (m, 1H, $CH_2$ ($C_6H_{10}$)), 2.15 - 2.12 (m, 1H, $CH_2$ ($C_6H_{10}$)), 1.85 - 1.79 ( m, 3H, $CH_2$ ($C_6H_{10}$)), 1.49 - 1.33 (m, 3H, $CH_2$ ($C_6H_{10}$)); $^{13}$C-NMR ($CDCl_3$, 151 MHz, [ppm]): *δ*182.3 (C-Au), 179.6 (C-S), 166.0 ($C_{ipso}$-$Me_{thiolate}$), 135.5 ($C_{ipso}$-Ph), 129.0 (*m*-$CH_{Ph}$), 128.6 (*p*-$CH_{Ph}$), 128.3 (*o*-$CH_{Ph}$), 73.4 (NCH or OCH ($C_6H_{10}$)), 67.3 (NCH or OCH ($C_6H_{10}$)), 55.3 ($CH_2$-Ph), 35.2 ($CH_2$ ($C_6H_{10}$)), 32.4 ($CH_2$ ($C_6H_{10}$)), 24.9 ($CH_2$ ($C_6H_{10}$)), 24.5 ($CH_2$

($C_6H_{10}$)), 24.0 ($Me_{thiolate}$). Anal. Calc. for $C_{22}H_{27}N_4OSAu \cdot 1$ MeOH [%]: calc.: C, 44.23; H, 4.97; N, 8.97; S, 5.13; found: C, 43.98; H, 5.41; N, 9.54; S, 5.69. White luminescence is observed after excitation with UV-light (366 nm, 254 nm).

**(3-Benzyl-1-(2'-hydroxycyclohexyl)imidazol-2-ylidene)gold(I)(pyrimidine-2''-thiolato) (1d)**

1 ml 2 M aqueous KOH-solution was used instead of NaOMe and (NHC)Au(I)Cl was dissolved in 10 ml $CH_2Cl_2$. Colorless solid, 87.6 % (0.09 mmol, 52.6 mg).

$^1$H-NMR ($CDCl_3$, 600 MHz, [ppm]): $\delta$8.34 (d, 2H, $J$ =4.9 Hz, $m$-$CH_{thiolate}$), 7.40 - 7.35 (m, 5H, Ph), 7.03 (d, 1H, $J$ =1,9 Hz, NCHCHN), 6.91 (d, 1H, $J$ =1.9 Hz, NCHCHN), 6.83 (t, 1H, $J$ =4.9 Hz, $p$-$CH_{thiolate}$), 5.50 (d, 1H, $J$ =14.8 Hz, $CH_2$-Ph), 5.33 (d, 1H, $J$ =14.9 Hz, $CH_2$-Ph), 4.80 - 4.75 (m, 1H, NCH or OCH ($C_6H_{10}$)), 3.66 - 3.64 (m, 1H, NCH or OCH ($C_6H_{10}$)), 2.32 - 2.29 (m, 1H, $CH_2$ ($C_6H_{10}$)), 2.17 - 2.15 (m, 1H, $CH_2$ ($C_6H_{10}$)), 1.90 - 1.82 (m, 3H, $CH_2$ ($C_6H_{10}$)), 1.52 - 1.39 (m, 3H, $CH_2$ ($C_6H_{10}$)); $^{13}$C-NMR ($CDCl_3$, 101 MHz, [ppm]): $\delta$183.6 (C-Au), 180.6 (C-S), 156.4 ($m$-$CH_{thiolate}$), 135.4 ($C_{ipso}$-Ph), 129.0 ($m$-$CH_{Ph}$), 128.6 ($p$-$CH_{Ph}$), 128.2 ($o$-$CH_{Ph}$), 120.3 (NCHCHN), 117.8 (NCHCHN), 115.0 ($p$-$CH_{thiolate}$), 73.8 (NCH or OCH ($C_6H_{10}$)), 67.8 (NCH or OCH ($C_6H_{10}$)), 55.1 ($CH_2$-Ph), 35.7 ($CH_2$ ($C_6H_{10}$)), 31.5 ($CH_2$ ($C_6H_{10}$)), 25.0 ($CH_2$ ($C_6H_{10}$)), 24.7 ($CH_2$ ($C_6H_{10}$)). Anal. Calc. for $C_{20}H_{23}N_4OSAu \cdot 0.25$ NaCl [%]: calc.: C, 41.48; H, 3.97; N, 9.68; S, 5.53; found: C, 41.53; H, 4.55; N, 10.08; S, 5.94.

**(3-Benzyl-1-(2'-hydroxycyclohexyl)imidazol-2-ylidene)gold(I)(4''-hydroxypyrimidine-2''-thiolato) (1e)**

3.5 equiv. NaOMe were used, the crude product was dissolved in $CH_2Cl_2$, filtrated over celite and the solvent was concentrated under reduced pressure and the product precipitated with hexane. Grey solid 83.6 % (0.09 mmol, 53.4 mg). $^1$H-NMR ($CDCl_3$, 400 MHz, [ppm]): $\delta$10.32 (s, br, 1H, NH), 7.71 (d, 1H, $J$ =6.5 Hz, $CH_{thiolate}$), 7.42 - 7.33 (m, 5H, Ph), 7.07 (d, 1H, $J$ =1.6 Hz, NCHCHN), 6.95 (d, 1H, $J$ =1.6 Hz, NCHCHN), 6.07 (d, 1H, $J$ =6.6 Hz, $CH_{thiolate}$), 5.46 (d, 1H, $J$ =14.8 Hz, $CH_2$-Ph), 5.29 (d, 1H, $J$ =14.8 Hz, $CH_2$-Ph), 4.72 - 4.66 (m, 1H, OCH or NCH ($C_6H_{10}$)), 3.71 - 3.64 (m, 1H, OCH or NCH ($C_6H_{10}$)), 2.30 (dt, 1H, $J$ =5.8 Hz, $J$ =11.4 Hz, $CH_2$ ($C_6H_{10}$)), 2.16 - 2.10 (m, 1H, $CH_2$ ($C_6H_{10}$)), 1.93 - 1.73 (m, 3H, $CH_2$ ($C_6H_{10}$)), 1.55 - 1.38 (m, 3H $CH_2$ ($C_6H_{10}$)); $^{13}$C-NMR ($CDCl_3$, 151 MHz, [ppm]): $\delta$183.1 (C-Au), 168.2 (C-O), 163.4 (C-S), 155.1 ($CH_{thiolate}$), 135.1 ($C_{ipso}$-Ph), 129.1 ($CH_{Ph}$), 128.7 ($CH_{Ph}$), 128.1 ($CH_{Ph}$), 120.8 (NCHCHN), 118.0 (NCHCHN), 109.1 ($CH_{thiolate}$), 73.5 (NCH or OCH ($C_6H_{10}$)), 67.5 (NCH or OCH ($C_6H_{10}$)), 55.2 ($CH_2$-Ph), 35.1 ($CH_2$ ($C_6H_{10}$)), 31.5 ($CH_2$ ($C_6H_{10}$)), 24.9 ($CH_2$ ($C_6H_{10}$)), 24.4 ($CH_2$ ($C_6H_{10}$)) Anal. Calc. for $C_{20}H_{23}N_4OSAu \cdot 1.5$ MeOH [%]: calc.: C, 39.54; H, 4.28; N, 9.23; S, 4.28; found: C, 40.60; H, 4.32; N, 8.97; S, 4.23; comment: the carbon value has a deviation > 1.0% from the calculated value. However, experimental values for H, N and S were very close to the theoretical ones and biological results were comparable to those of related structures. It can thus be concluded that possible small impurities will not have influenced

the results to a significant extent. IR (KBr, [cm−1]) *v*= 1665 (*v*(C=O)). White luminescence is observed after excitation with UV-light (366 nm, 254 nm).

**(3-Benzyl-1-(2'-hydroxycyclohexyl)imidazol-2-ylidene)gold(I)(naphthalin-1''-thiolato) (1f)**

1 equiv. 1-Thionaphthole and 1 equiv. NaOH were stirred for 10 min. (NHC)Au(I)Cl was dissolved in 10 ml $CH_2Cl_2$ and the solution was stirred for 3 h. The solvent was removed under reduced pressure, the crude solid dissolved in $CH_2Cl_2$ and after filtration over celite the solvent was removed to give the colorless solid. 76.6 % (0.11 mmol, 69.0 mg). X-ray quality crystals for were grown from a solution of $CHCl_3$ and hexane.$^1$H-NMR ($CDCl_3$, 400 MHz, [ppm]): $\delta$8.94 (d, 1H, $CH_{Naph}$), 8.04 (d, 1H, $CH_{Naph}$), 7.76 (d, 1H, $CH_{Naph}$), 7.54 - 7.41 (m, 3H, $CH_{Naph}$), 7.37 - 7.28 (m, 5H, $CH_{Ph}$), 7.21 (t, 1H, *J* =7.6 Hz, $CH_{Naph}$), 6.98 (d, 1H, *J* =1.8 Hz, NCHCHN), 6.89 (d, 1H, *J* =1.7 Hz, NCHCHN), 5.42 (d, 1H, *J* =14.5 Hz, $CH_2$-Ph), 5.35 (d, 1H, *J* =14.7 Hz, $CH_2$-Ph), 4.38 - 4.30 (m, 1H, NCH or OCH ($C_6H_{10}$)), 3.97 - 3.90 (m, 1H, NCH or OCH ($C_6H_{10}$)), 2.14 - 2.09 (m, 2H, $CH_2$ ($C_6H_{10}$)), 1.92 - 1.83 (m, 1H, $CH_2$ ($C_6H_{10}$)), 1.80 - 1.77 (m, 2H, $CH_2$ ($C_6H_{10}$)), 1.36 - 1.26 (m, 3H, $CH_2$ ($C_6H_{10}$)); $^{13}$C-NMR ($CDCl_3$, 101 MHz, [ppm]): $\delta$181.0 (C-Au), 140.4 (C-S), 135.3 ($C_{ipso}$-Ph), 131.0 ($CH_{Naph}$), 129.0 (*o*-$CH_{Ph}$), 128.5 ($CH_{Naph}$), 128.0 (*p*-$CH_{Ph}$), 128.0 (*m*-$CH_{Ph}$), 127.8 ($CH_{Naph}$), 125.3 ($CH_{Naph}$), 125.0 ($CH_{Naph}$), 125.0 ($CH_{Naph}$), 124.0 ($CH_{Naph}$), 120.3 (C5), 118.9 (C4), 73.0 (NCH or OCH ($C_6H_{10}$)), 67.4 (NCH or OCH ($C_6H_{10}$)), 55.3 ($CH_2$-Ph), 34.8 ($CH_2$ ($C_6H_{10}$)), 32.9 ($CH_2$ ($C_6H_{10}$)), 24.8 ($CH_2$ ($C_6H_{10}$)), 24.1 ($CH_2$ ($C_6H_{10}$)). Anal. Calc. for $C_{26}H_{27}N_2AuOS$ [%]: calc.: C, 50.98; H, 4.44; N, 4.57; S, 5.23; found: C, 50.78; H, 4.85; N, 4.96; S, 5.23. White luminescence is observed after excitation with UV-light (366 nm, 254 nm).

**Biological Experiments**

Enzymatic inhibition and tumor cell proliferation experiments were performed as described in more detail in recent reports.[17, 29] For the studies of the enzymatic inhibition a DTNB reduction assay was applied. Cell proliferation was evaluated using the crystal violet assay. Data were generally obtained in 2-3 independent experiments and mean values with errors are presented. In some cases only results from one experiment could be obtained.

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