Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information

A gold(I) biscarbene complex with improved activity as TrxR inhibitor and cytotoxic drug: comparative studies with different gold metallodrugs

Claudia Schmidt[1], Lucia Albrecht[2], Shantheya Balasupramaniam[1], Rainer Misgeld[3], Bianka Karge[4], Mark Brönstrup[4], Aram Prokop[3], Knut Baumann[1], Stephan Reichl[2] and Ingo Ott[1]

[1]Institute of Medicinal and Pharmaceutical Chemistry, Technische Universität Braunschweig, Beethovenstrasse 55, 38106 Braunschweig, Germany
[2]Institute of Pharmaceutical Technology, Technische Universität Braunschweig, Mendelsohnstrasse 1, 38106 Braunschweig, Germany
[3]Department of Pediatric Oncology, Children's Hospital Cologne, Amsterdamer Strasse 59, 50735 Cologne, Germany
[4]Department of Chemical Biology, Helmholtz Centre for Infection Research and German Centre for Infection Research (DZIF), Inhoffenstrasse 7, 38124 Braunschweig, Germany

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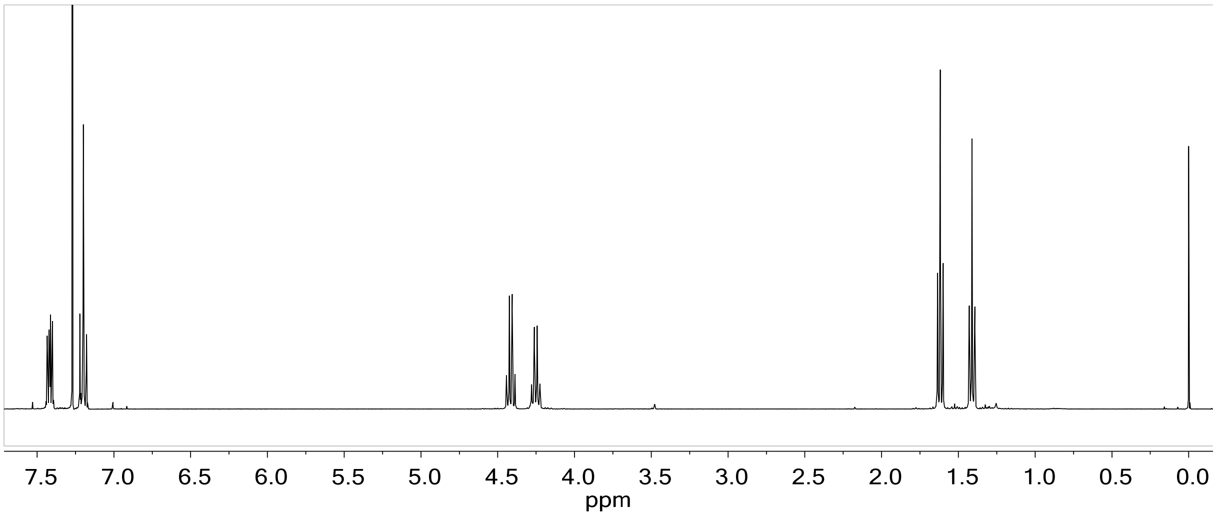

Figure SI1: ^{1}H-NMR spectrum of biscarbene **3** (400 MHz in $CDCl_3$)

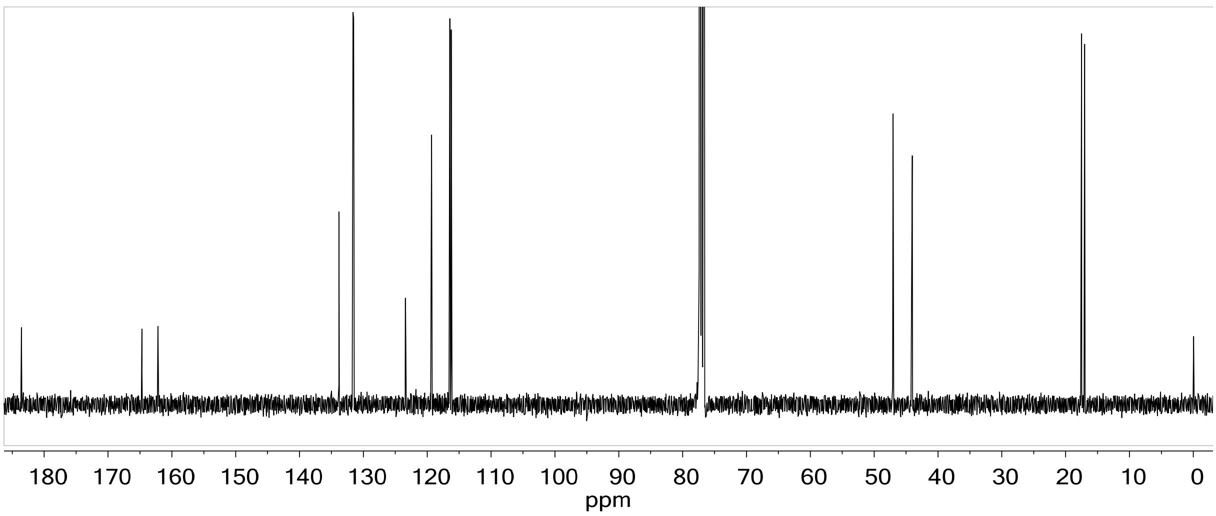

Figure SI2: ^{13}C-NMR spectrum of biscarbene **3** (400 MHz in $CDCl_3$)

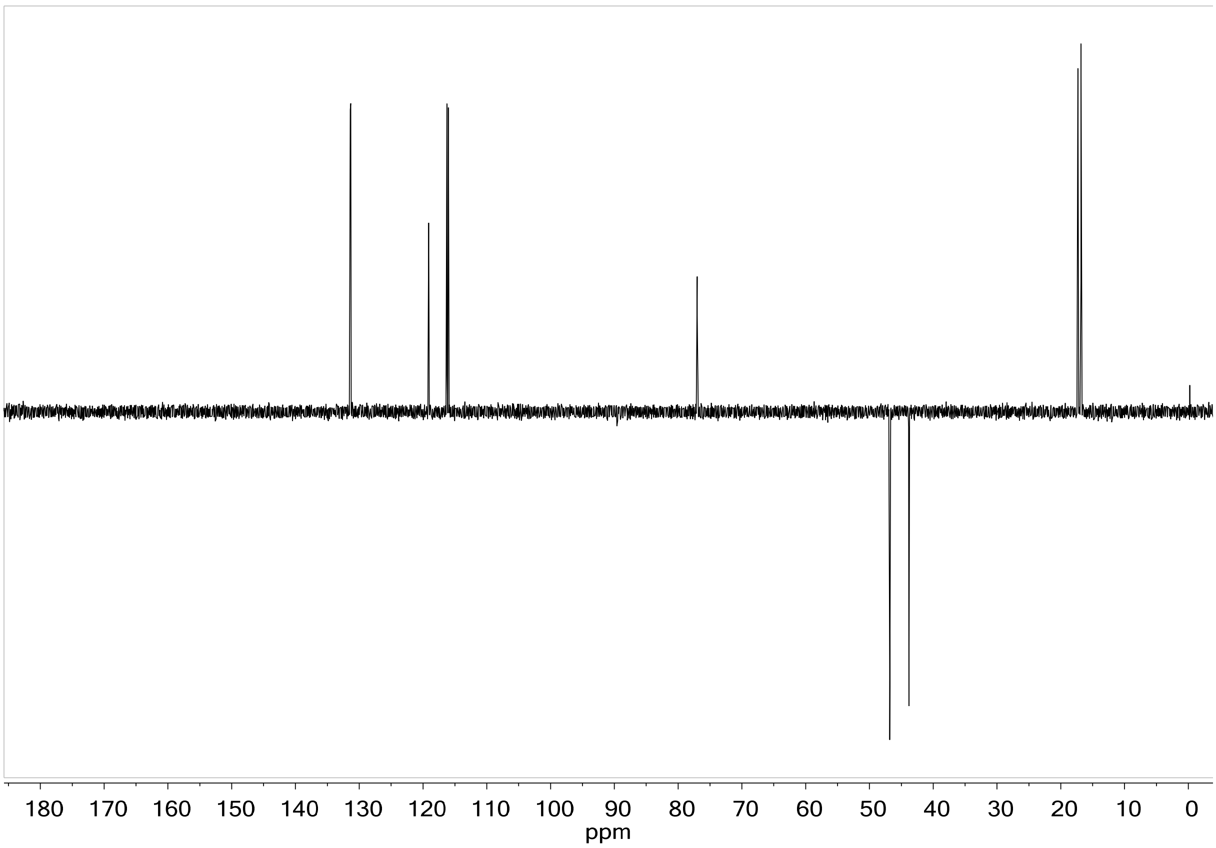

Figure SI3: ^{13}C-NMR-DEPT135 spectrum of biscarbene **3** (400 MHz in $CDCl_3$)

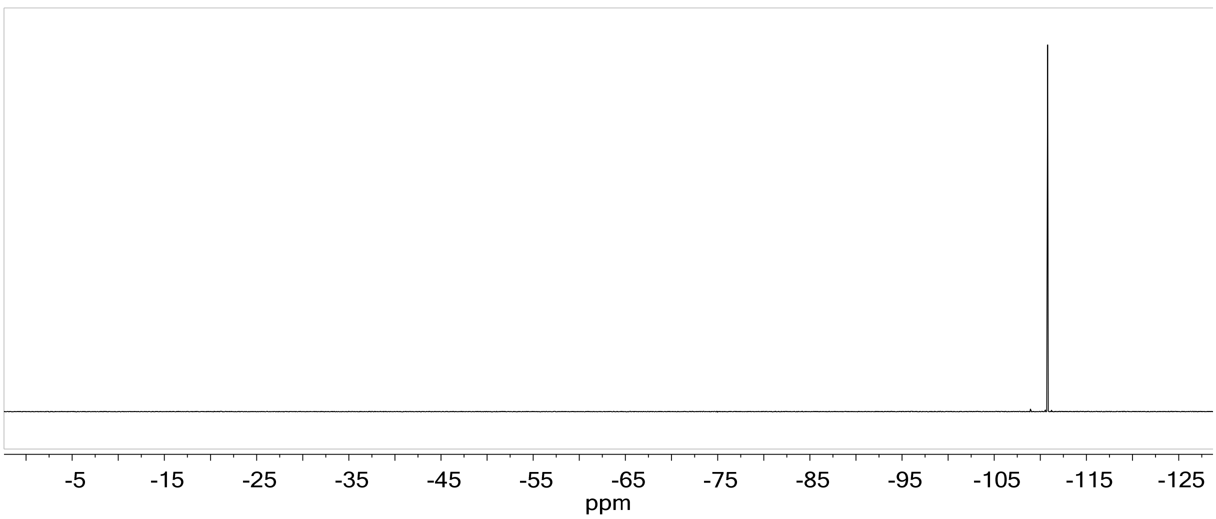

Figure SI4: ^{19}F-NMR spectrum of biscarbene **3** (400 MHz in $CDCl_3$)

2.) ESI-MS stability tests of monocarbene 2

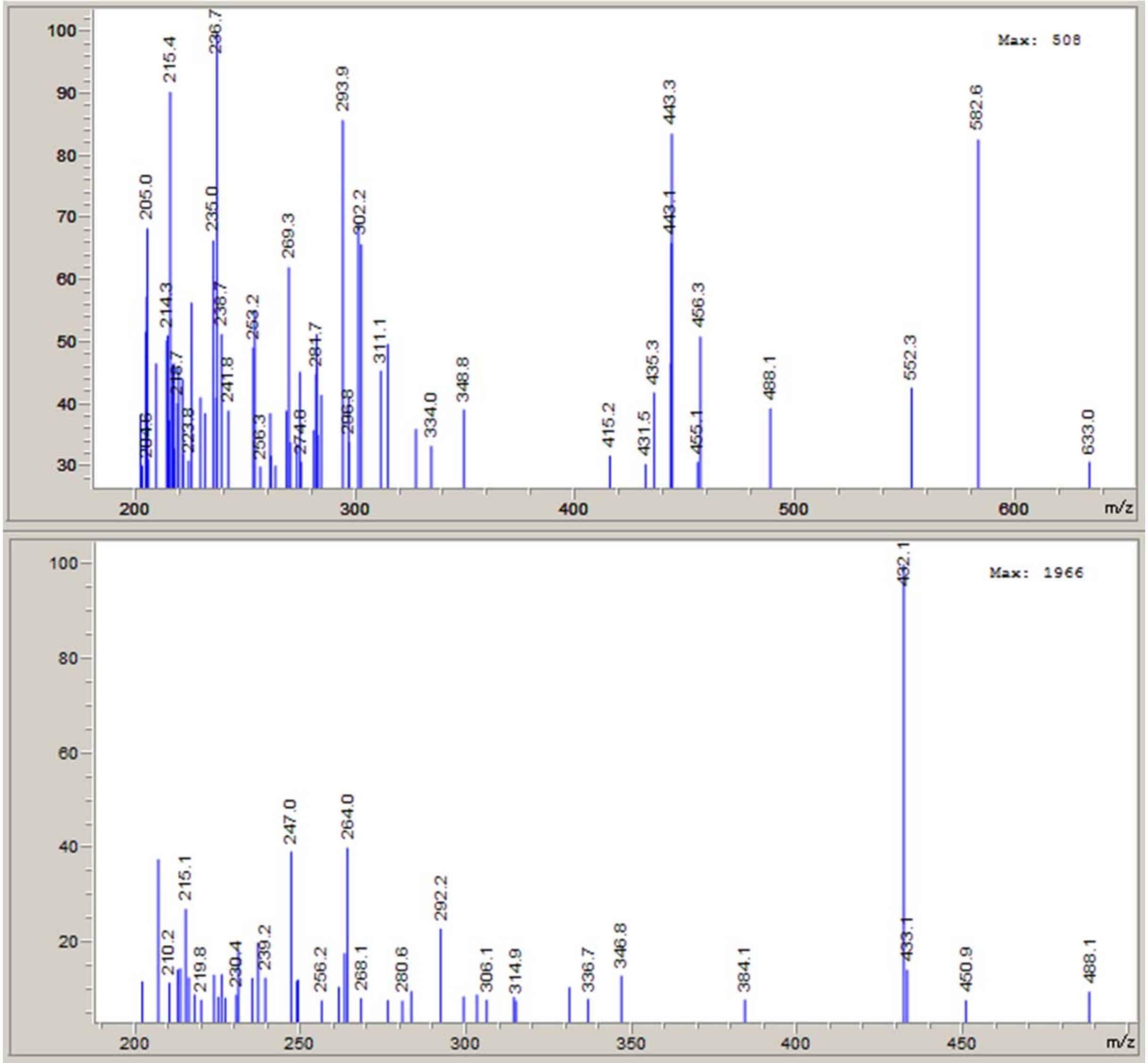

Figure SI5: Preliminary stability tests of monocarbene **2** (100 µM) in water (1% DMF) after 75 min. Positive scan mode: m/z 200-750; Retention time: 0.066 min (top), 0.672 min (bottom).

Spectra (top) at 0.066 min			Spectra (bottom) at 0.672 min		
m/z	Abundance [%]	Structure	m/z	Abundance [%]	Structure
219.20	45.9	NHC^+ (**1**)	219.80	7.9	NHC^+ (**1**)
431.50	30.3	NHC-Au-OH	432.10	100.0	NHC-Au-OH
488.10	39.4	NHC-Au-DMF	488.10	9.6	NHC-Au-DMF
633.00	30.7	$(NHC)_2Au^+$ (**3**)			

Table SI1: intensities of selected masses of preliminary stability tests of monocarbene **2** (100 µM) in water (1% DMF) after 75 min, positive scan mode: m/z 200-750.

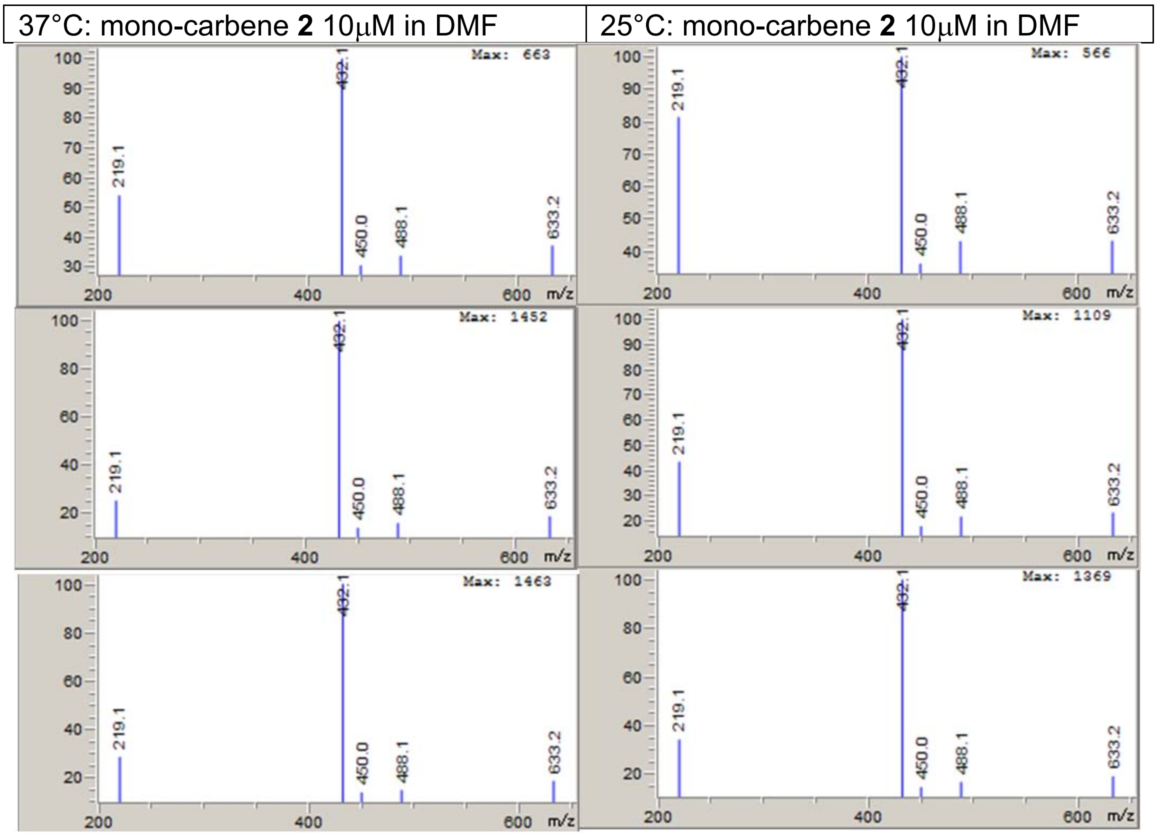

Figure SI6: Stability of monocarbene **2** (concentration: 10 μM) in water (1% DMF) time points: 1min, 15 min, 75 min, masses of interest (m/z: 219.15, 432.10, 450.05, 488.15 or 493.10, 633.20) in positive SIM mode.

m/z	Structure	Abundance at 37 °C			Abundance at 25 °C		
		1 min	15 min	75 min	1 min	15 min	75 min
219.15	NHC^+ (**1**)	342	369	400	461	485	475
432.10	NHC-Au-OH	633	1452	1520	566	1109	1369
450.05	NHC-Au-Cl	195	203	205	206	202	205
488.15	NHC-Au-DMF	215	232	220	254	244	235
633.20	$(NHC)_2Au^+$ (**3**)	237	274	231	246	262	266

Table SI2: Intensities of MS stability studies of monocarbene **2** in 1 % DMF

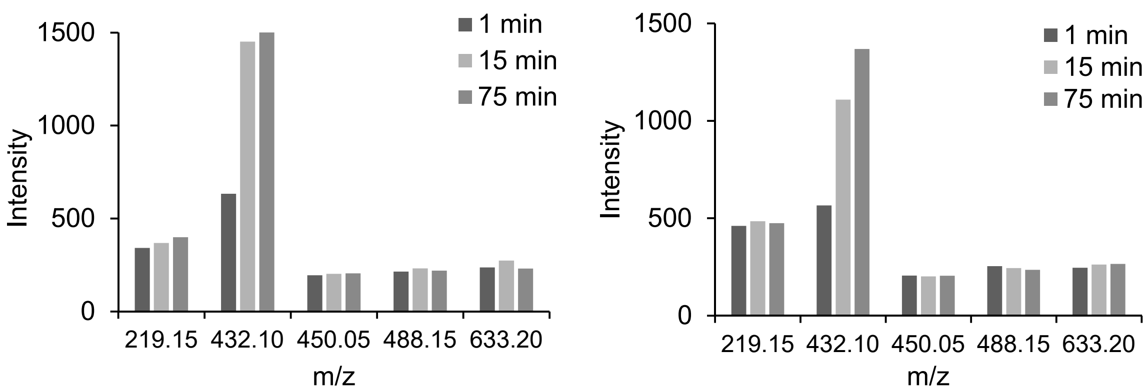

Figure SI7: Intensities of MS stability studies of monocarbene **2** in 1 % DMF (left at 37 °C, right at 25 °C)

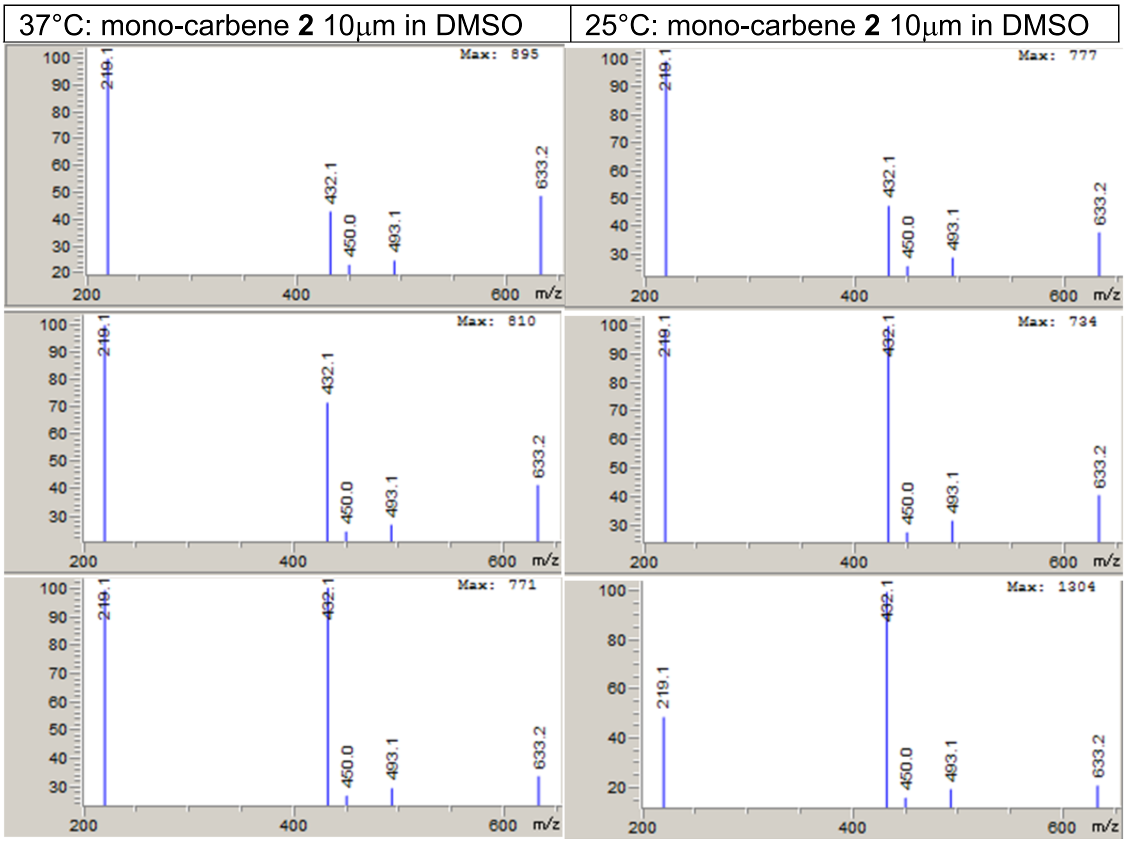

Figure SI8: Stability of mono-NHC complex **2** in water (1% DMSO) time points: 1min, 15 min, 75 min.

m/z	Structure	Abundance at 37 °C			Abundance at 25 °C		
		1 min	15 min	75 min	1 min	15 min	75 min
219.15	NHC^+ (**1**)	777	725	638	895	810	773
432.10	NHC-Au-OH	370	734	1304	385	580	734
450.05	NHC-Au-Cl	201	202	209	207	198	209
493.10	NHC-Au-DMSO	225	232	254	221	218	226
633.20	$(NHC)_2Au^+$ (**3**)	296	298	273	437	336	257

Table SI3: Intensities of MS stability studies of complex **2** in 1 % DMSO

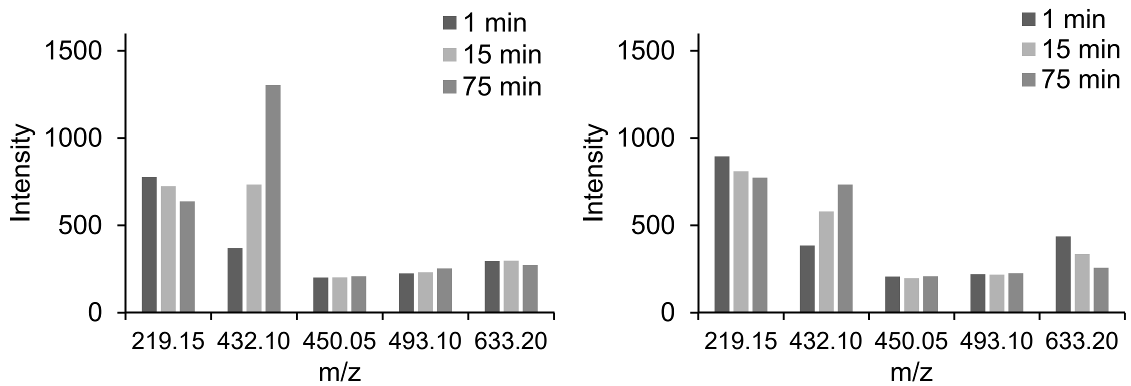

Figure SI9: Intensities of MS stability studies of complex **2** in 1 % DMSO (left at 37 °C, right at 25 °C)

3.) Photometric Measurements (UV-Vis)

UV/VIS measurements were performed on a Specord 40 Photometer (Analytik Jena) and the software Winspect was used for calculations (range: 200 – 600 nm in 0.5 nm steps and a scan speed of 5 nm/s).

All test compounds were solved in DMF and diluted with DMEM cell culture medium (without phenolred or any other supplements) to a final compound concentration of 50 µM (0.1% v/v DMF) and incubated at 37 °C throughout the whole experiment. 1.0 mL of these solutions were transferred to a quartz cuvette and measured time-dependently (0, 1, 2, 4 and 8 hours). A reference spectrum of a blank, which contains only DMF (0.1% v/v) in DMEM was measured before and automatically subtracted by the software.

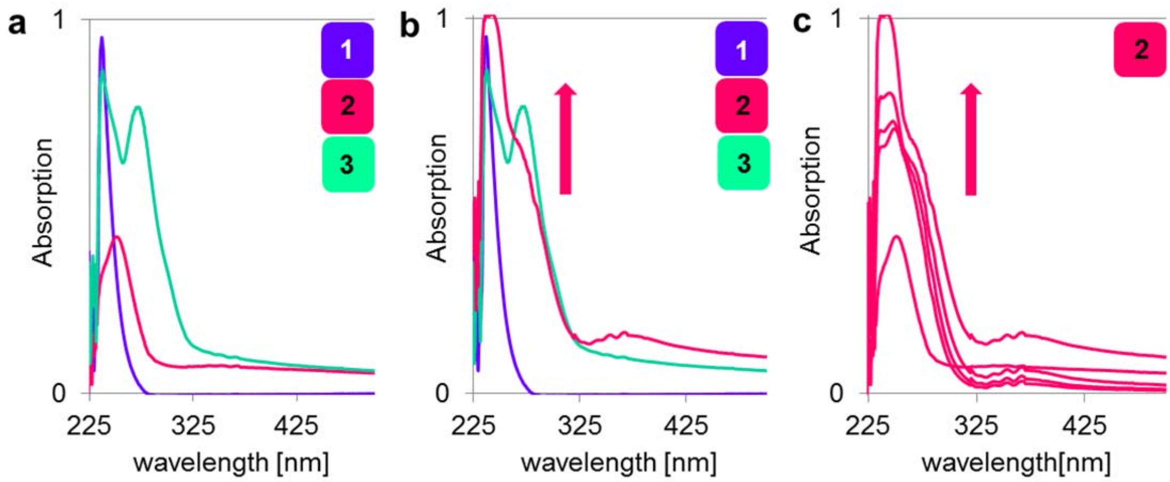

Figure SI10: Photometric measurements in DMEM cell culture medium without phenolred)
a) **1** (λ_{max} 237 nm), **2** (λ_{max} 252 nm) and **3** (λ_{max} 237 nm/272 nm) after 0 hours **b**) **1** (λ_{max} 237 nm), **2** (λ_{max} 248 nm/268 nm) and **3** (λ_{max} 252 nm/272 nm) after 8 hours and **c**) **2** over 8 hours (λ_{max} 252 nm → λ_{max} 248 nm/268 nm)

The spectra at the beginning of the experiment (0 h, Fig. SI10a) showed that it is to some extent possible to compare compounds **1**, **2** and **3** by UV-Vis spectroscopy. Importantly, **3** showed an additional maximum at 272 nm, which was not present in **1** and **2**. After 8 hours of exposure the spectra of **1** and **3** did not show significant changes, whereas the spectrum of **2** had experienced major alterations, which could be explained with formation of **1** and **3** (increase of absorbance and appearance of a shoulder at approx. 272 nm, Fig. SI10b). The changes in the spectrum of **2** appeared in a time dependent manner (Fig. SI10c).

4.) HPLC-MS (ESI) stability tests for precursor 4-phenylimidazolium iodide 1

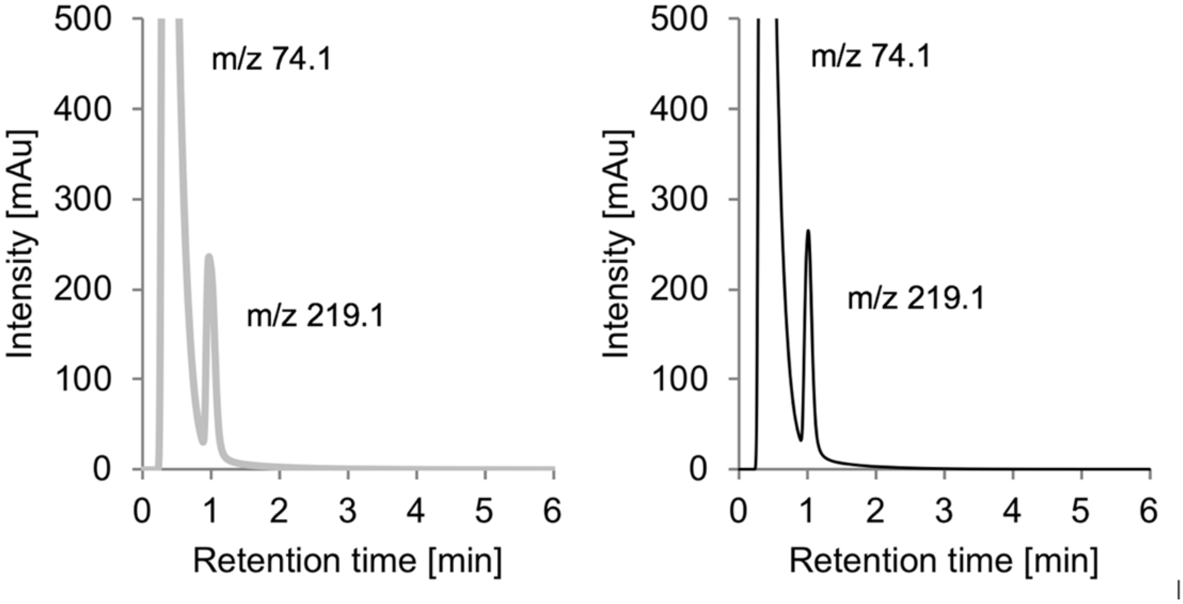

Figure SI11: Stability of NHC ligand **1** in DMF; grey line: chromatogram after 0 hour; black line: chromatogram after 96 hours; Peaks: m/z (0.22 min) = 74.1 (DMF), m/z (1.01 min) = 219.1 (cation of ligand **1**)

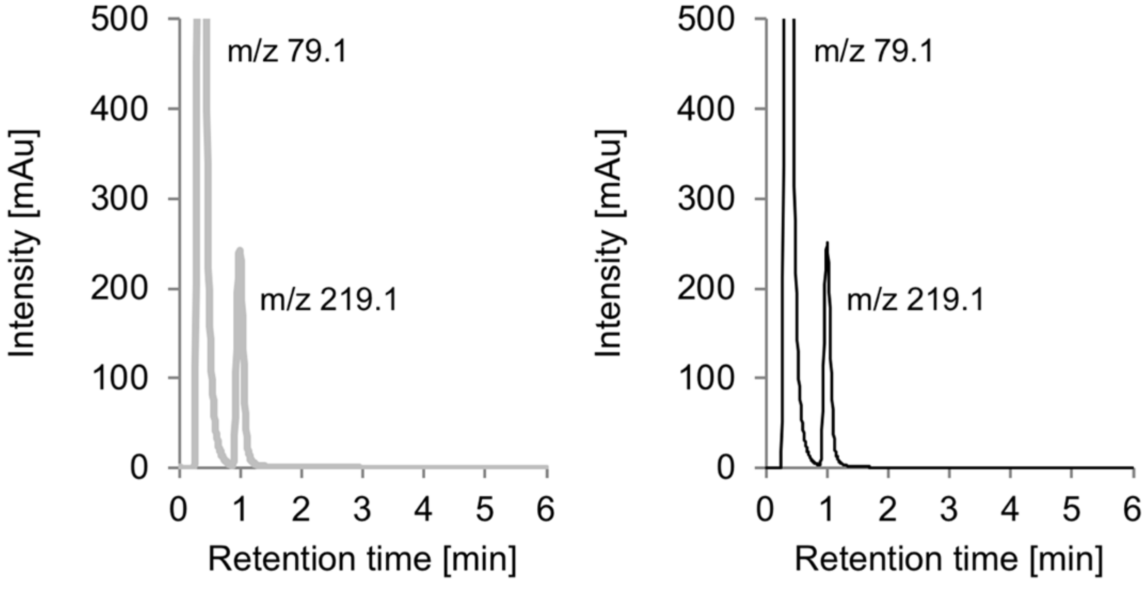

Figure SI12: Stability of NHC ligand **1** in DMSO; grey line: chromatogram after 0 hour; black line: chromatogram after 96 hours; Peaks: m/z (0.23 min) = 79.1 (DMSO), m/z (1.00 min) = 219.1 (cation of ligand **1**)

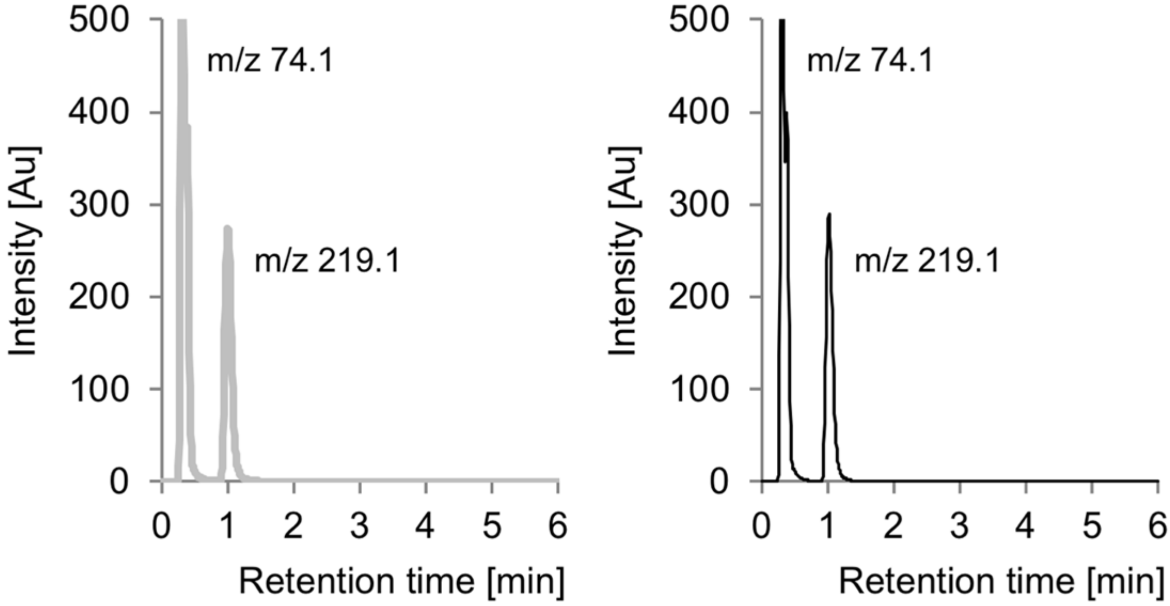

Figure SI13: Stability of NHC ligand **1** in water; grey line: chromatogram after 0 hour; black line: chromatogram after 96 hours; Peaks: m/z (0.19 min) = 74.1 (DMF), m/z (1.01 min) = 219.1 (cation of **1**)

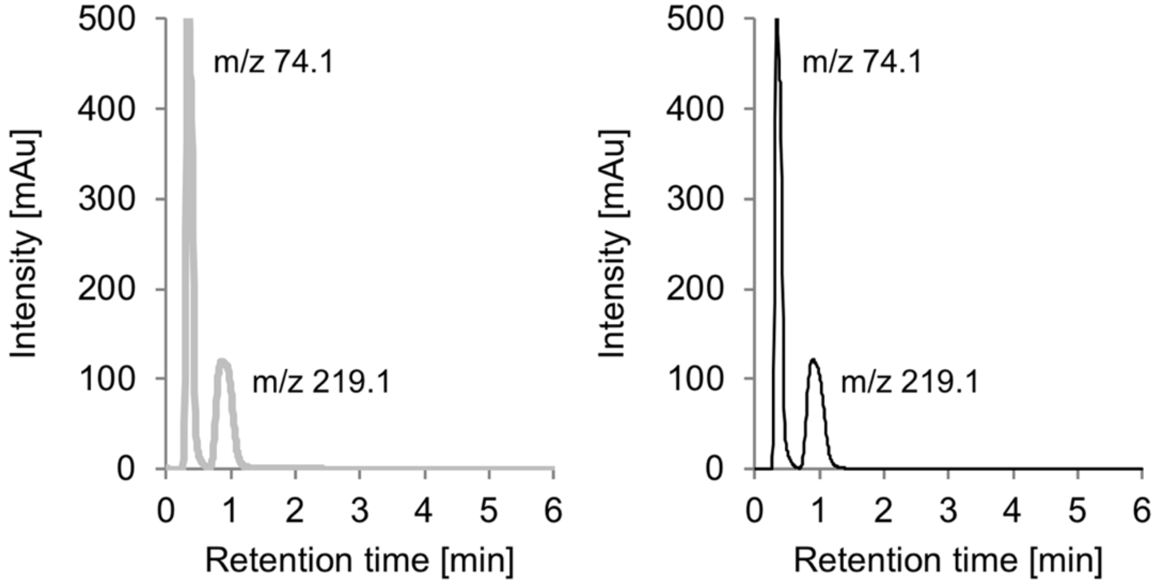

Figure SI14: Stability of NHC ligand **1** in PBS buffer; grey line: chromatogram after 0 hour; black line: chromatogram after 96 hours; Peaks: m/z (0.20 min) = 74.1 (DMF), m/z (0.53 min) = 219.1 (cation of ligand **1**)

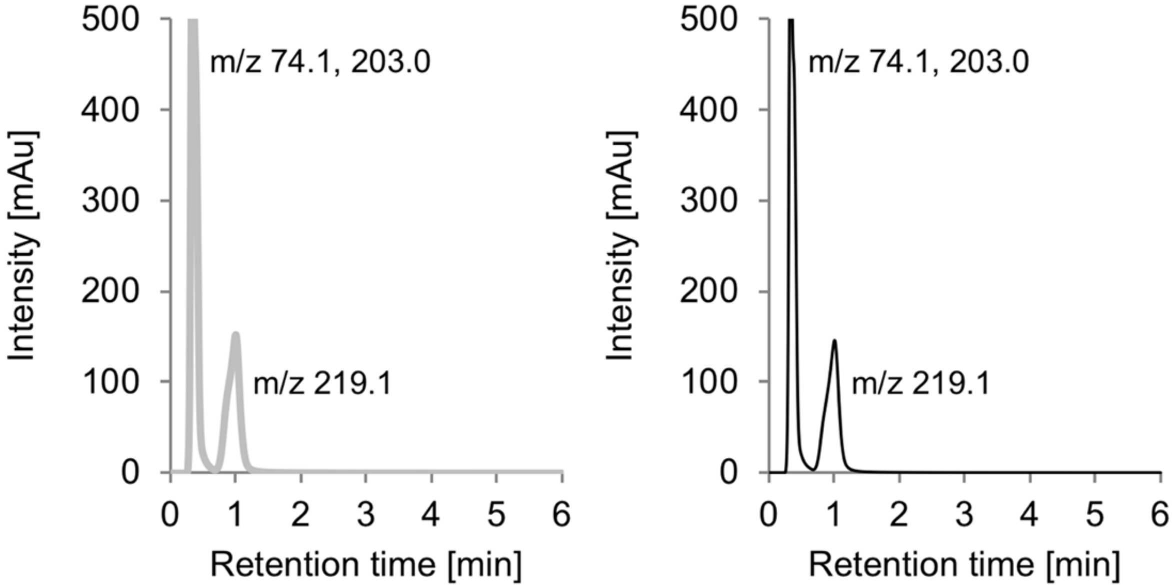

Figure SI15: Stability of NHC ligand **1** in DMEM cell culture medium (without supplements); grey line: chromatogram after 0 hour; black line: chromatogram after 96 hours; Peaks: m/z (0.20 min) = 74.1 (DMF) and 203.0 (DMEM matrix), m/z (1.01 min) = 219.1 (cation of ligand **1**)

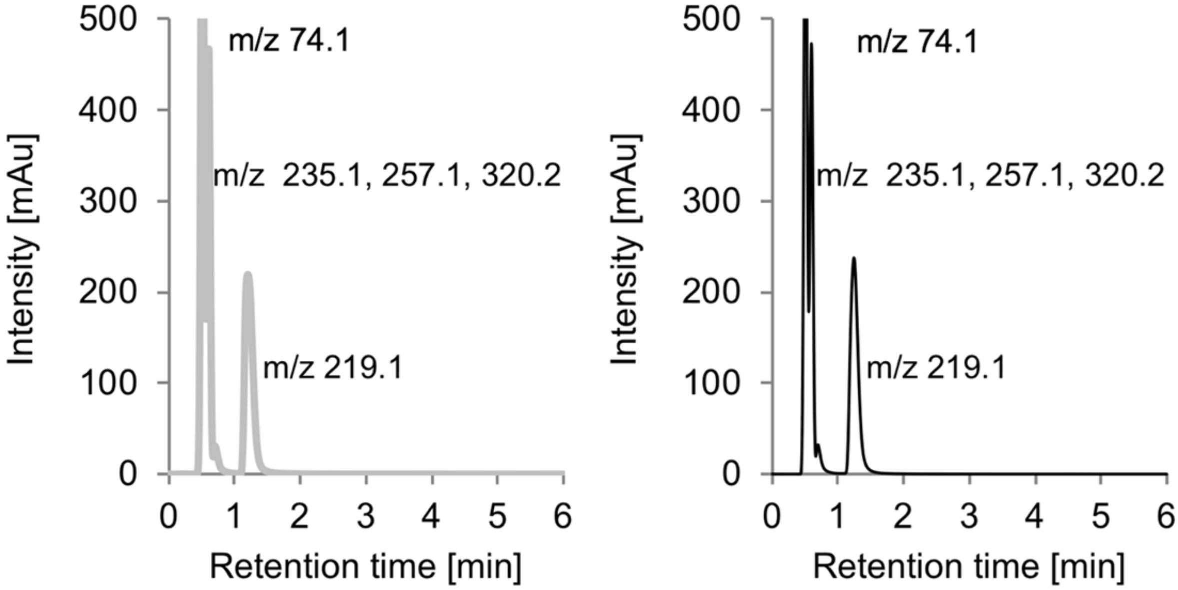

Figure SI16: Stability of NHC ligand **1** in 1-Octanol; grey line: chromatogram after 0 hours; black line: chromatogram after 96 hours; Peaks: m/z (0.31 min) = 74.1 (DMF), m/z (0.36 min) = various masses (octanol impurities), m/z (1.15 min) = 219.1 (cation of ligand **1**)

5.) HPLC-MS (ESI) stability tests for biscarbene 3

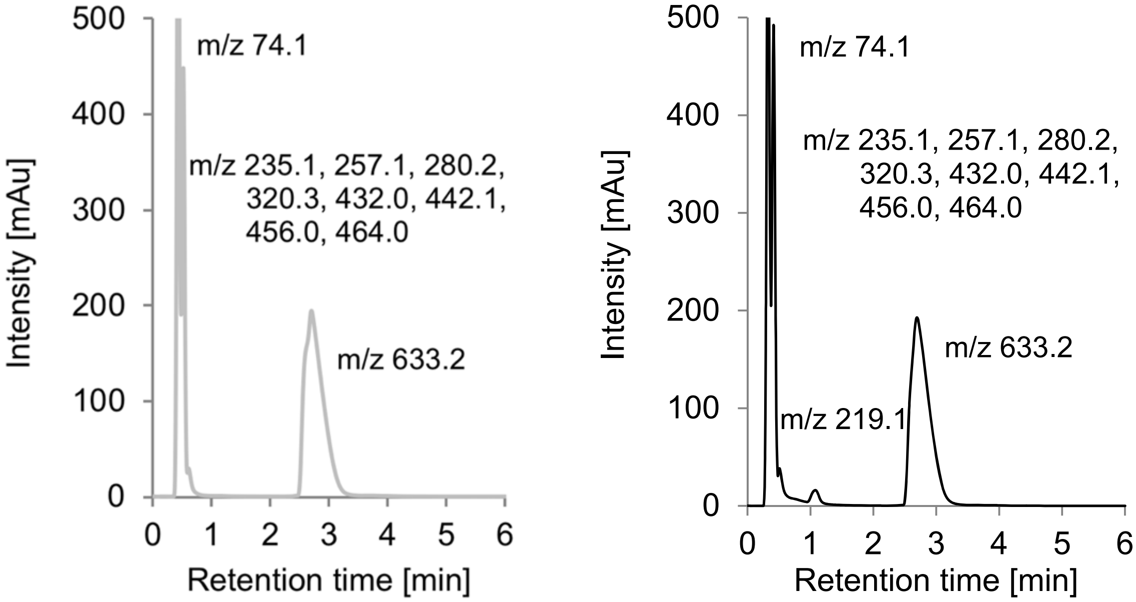

Figure SI17: Stability of bis-NHC complex **3** in n-Octanol at 0 hours; black line: 96 hours; Peaks: m/z (0.20 min) = DMF, m/z (0.25 min) = various masses (octanol impurities); m/z (1.04 min) = 219.1 (cation of ligand **1**), m/z (2.41 min) = 633.2 (cation of biscarbene **3**)

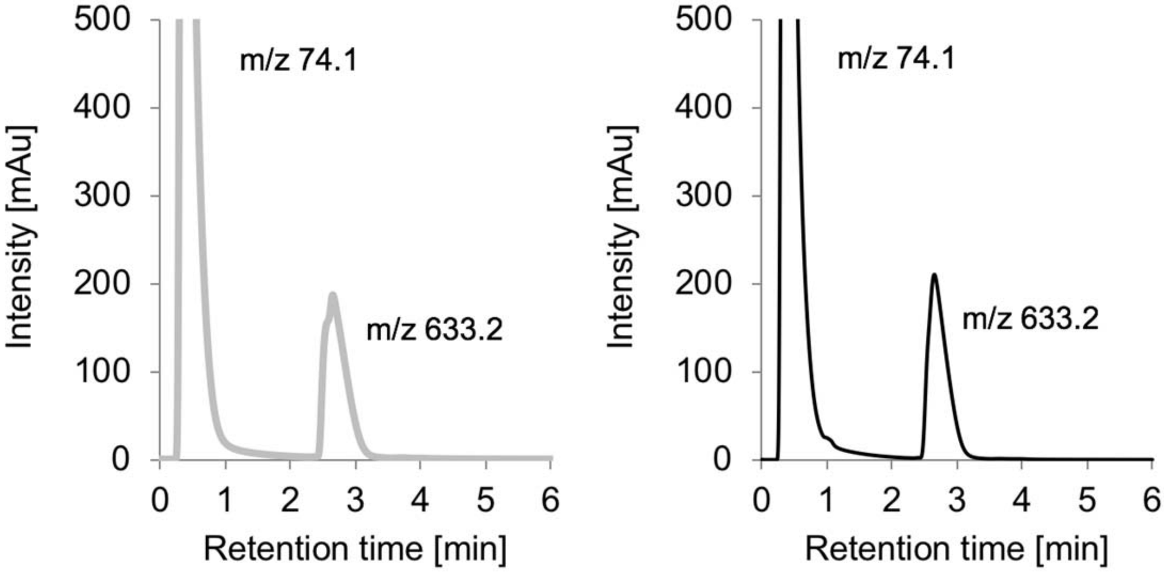

Figure SI18: Stability of bis-NHC complex **3** in DMF; grey line: chromatogram at 0 hours; black line: 96 hours; Peaks: m/z (0.24 min) = 74.1 (DMF) m/z (2.39 min) = 633.2 (cation of biscarbene **3**)

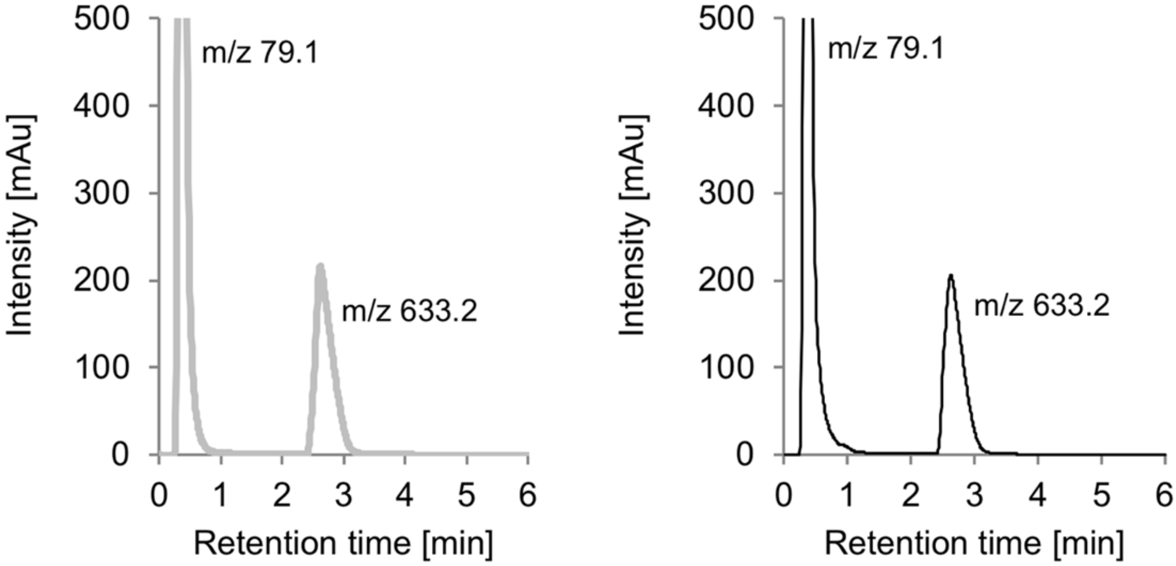

Figure SI19: Stability of bis-NHC complex **3** in DMSO; grey line: chromatogram at 0 hours; black line: 96 hours; Peaks: m/z (0.23 min) = 79.1 (DMSO) m/z (2.37 min) = 633.2 (cation of biscarbene **3**)

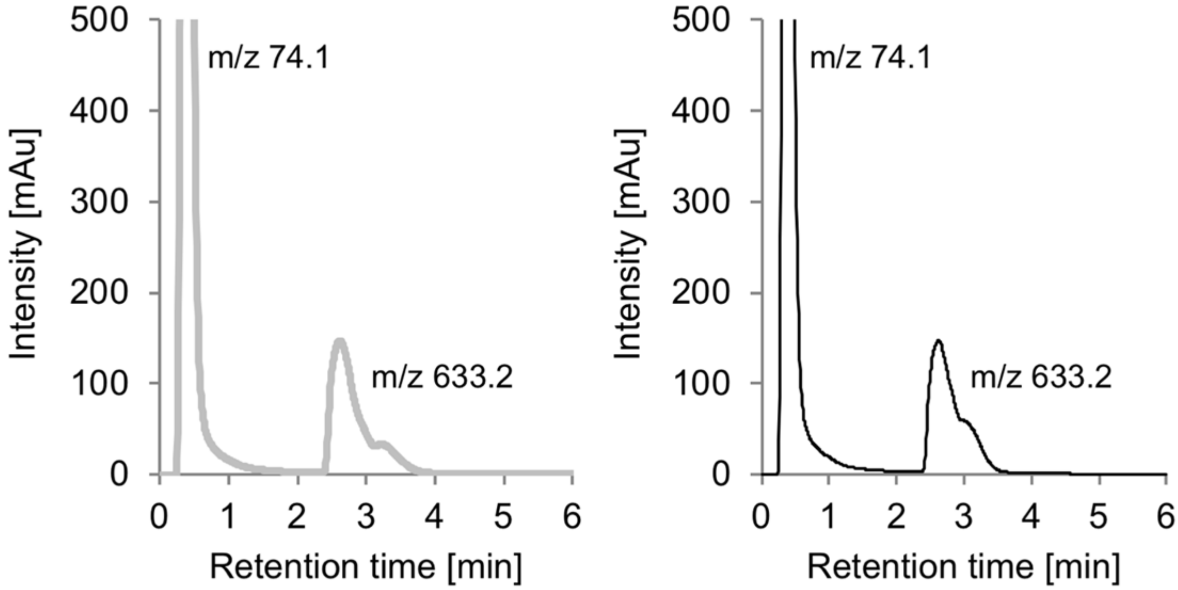

Figure SI20: Stability of bis-NHC complex **3** in water; grey line: chromatogram at 0 hours; black line: 96 hours; Peaks: m/z (0.23 min) = 74.1 (DMF) m/z (2.37 min) = 633.2 (cation of biscarbene **3**)

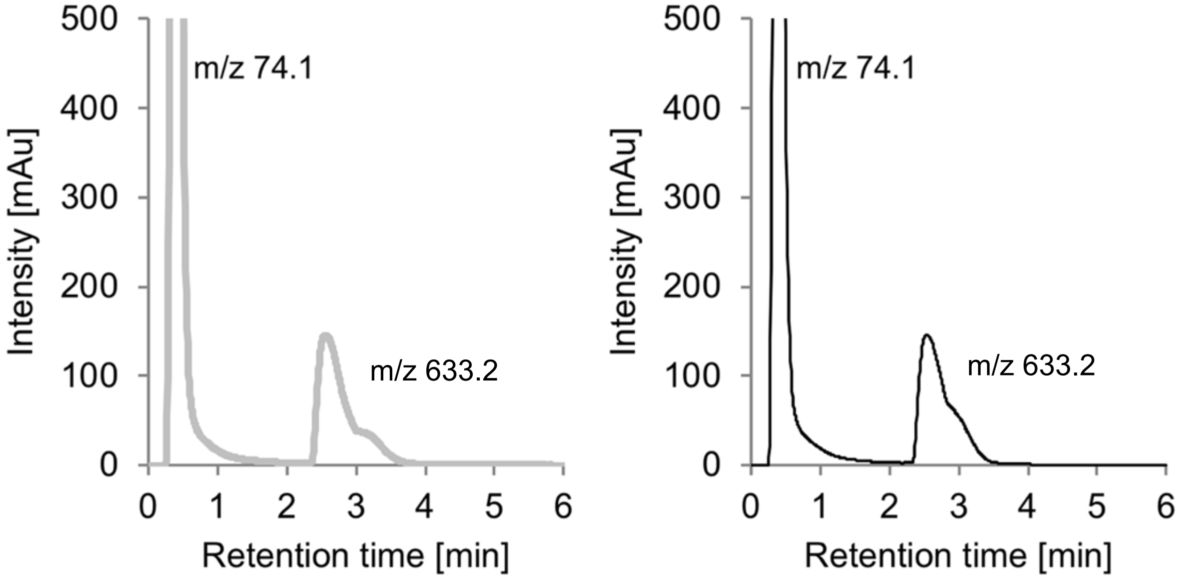

Figure SI21: Stability of bis-NHC complex **3** in PBS buffer; grey line: chromatogram at 0 hours; black line: 96 hours; Peaks: m/z (0.22 min) = 74.1 (DMF) m/z (2.32 min) = 633.2 (cation of biscarbene **3**)

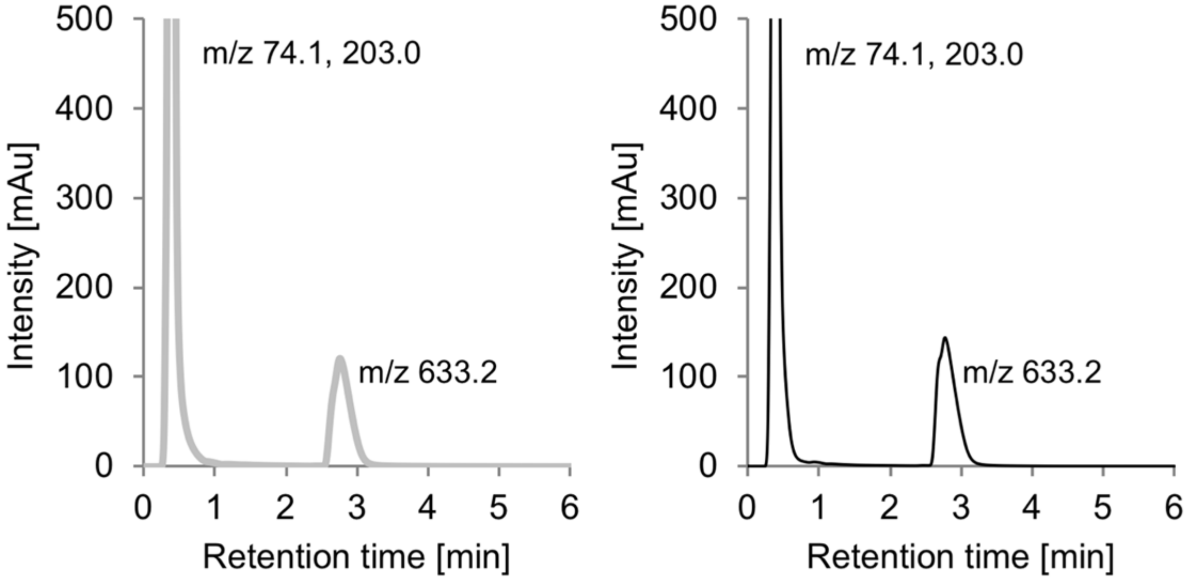

Figure SI22: Stability of bis-NHC complex **3** in DMEM cell culture medium (without any supplements); grey line: chromatogram at 0 hours; black line: after 96 hours; Peaks: m/z (0.23 min) = 74.1 (DMF) and 203.0 (DMEM matrix), m/z (2.46 min) = 633.2 (cation of biscarbene **3**)

6.) Protein binding

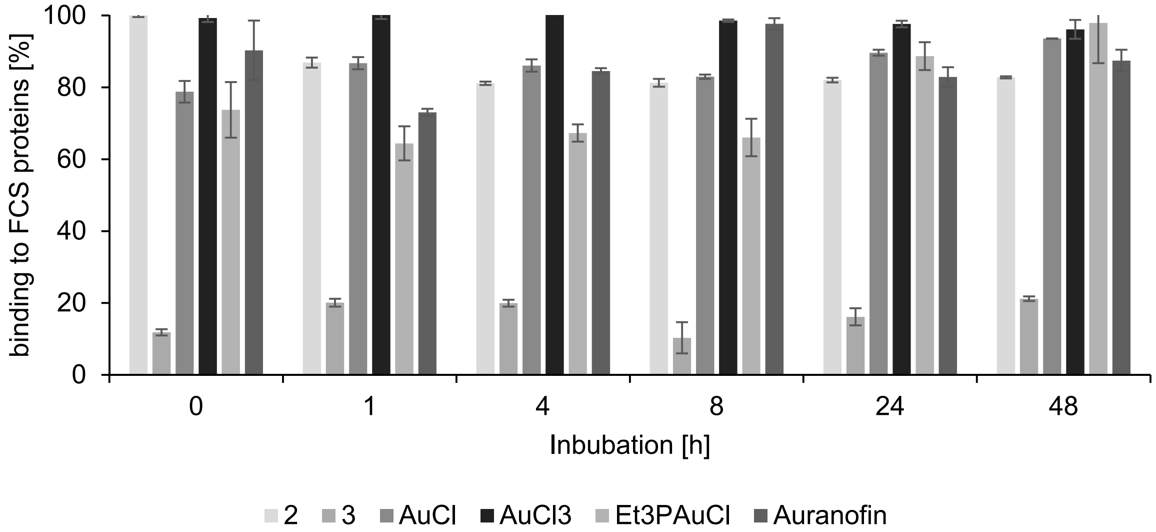

Figure SI23: Protein binding of **2** [12 µM][1], **3** [3 µM], Auranofin [3 µM], Et_3PAuCl [3 µM] and gold chlorides [12 µM] to fetal calf serum proteins

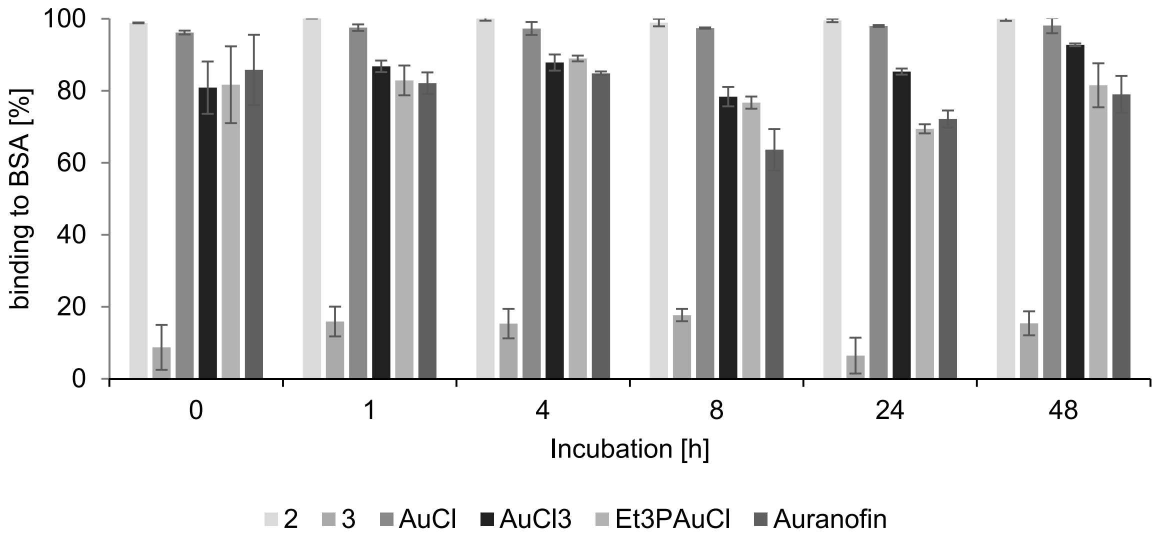

Figure SI24: Protein binding of **2** [12 µM][1], **3** [3 µM], Auranofin [3 µM], Et_3PAuCl [3 µM] and gold chlorides [12 µM] to bovine serum albumin

7.) Permeability studies and cellular uptake in Caco-2 cells

Permeability studies with Caco-2 cells were performed in order to investigate if the gold complexes are able to passage through intestinal cells, which is an important aspect for the absorption and distribution by oral administration. In this assay the drug is placed on the "apical" side of an insert, which is separated from the "basolateral" side by a permeable filter membrane on which the CaCo-2 cells were grown as a cell layer. After 6 hours the gold binding to BSA [%] binding to FCS proteins [%] content at both sides of the permeable filter membrane can be measured by HRCS-AAS (see experimental setup in Fig. SI25). However, in these experiments no gold could be detected neither in the apical aliquots nor in the basolateral aliquots with **2**, **3**, Et_3PAuCl or Auranofin. On the other hand, concentration- and time-dependent cellular uptake studies with the complexes in Caco-2 cells confirmed the accumulation of gold after exposure to the compounds (see Figs SI26 and SI27).

The detection limit of gold in the HRCS-AAS measurements were 2.0 µM in MEM cell culture medium / distilled water [2:1] as well as the used KRB buffer solution. For comparison, the detection limit of gold in pure water was 0.031 µM and 0.125 µM in MCF-7 cell lysates as used for cellular uptake studies (protein concentration 1.0 mg/mL).

(For determining the detections limits, stock solutions (16 µM) of the respective compounds in either KRB or MEM cell culture medium/distilled water [2:1] were diluted and measured via HRCS-AAS until no gold signals could be detected. The last concentration where a signal could reliably be identified was defined as the detection limit.)

Based on the higher detection limits in the KRB buffer solution, a final conclusion on gold permeability could not be drawn. Further method development and a modified experimental setup is necessary to lower the thresholds of quantification for AAS in the respective medium and buffer-based matrices to investigate the permeability and accumulation behavior.

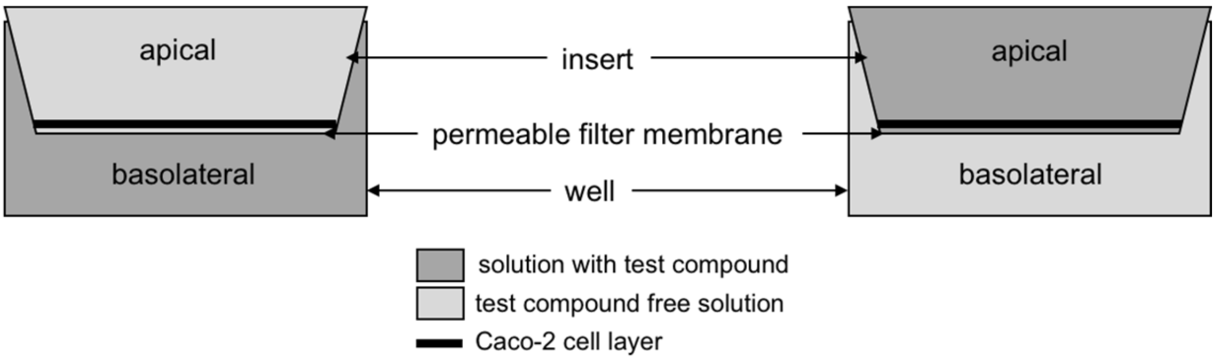

Figure SI25: Experimental setup of permeability tests in Caco-2 cells

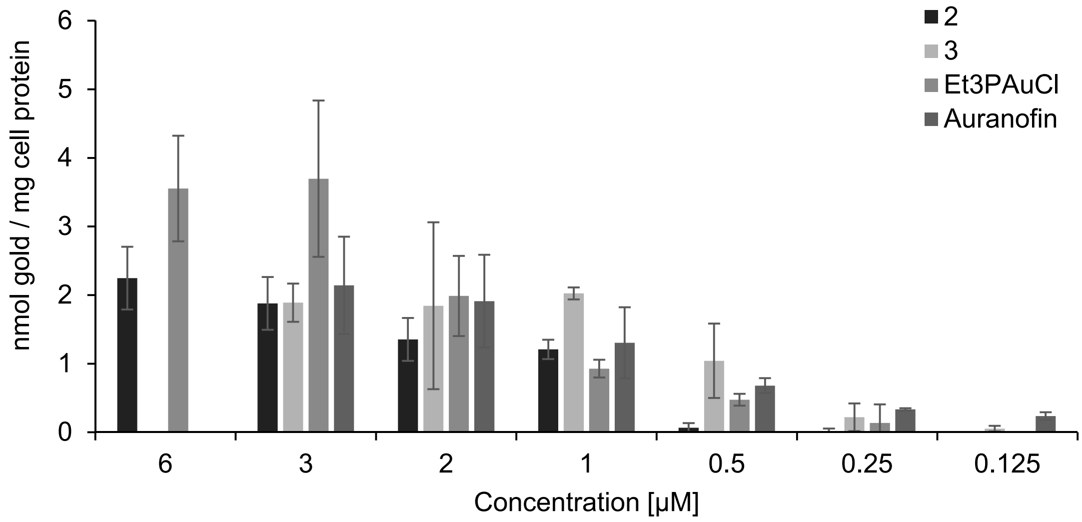

Figure SI26: Concentration dependent cellular uptake in Caco-2 cells of **2**/Et_3PAuCl (6 – 0.25 µM) and **3**/Auranofin (3 – 0.125 µM) after 6 hours.

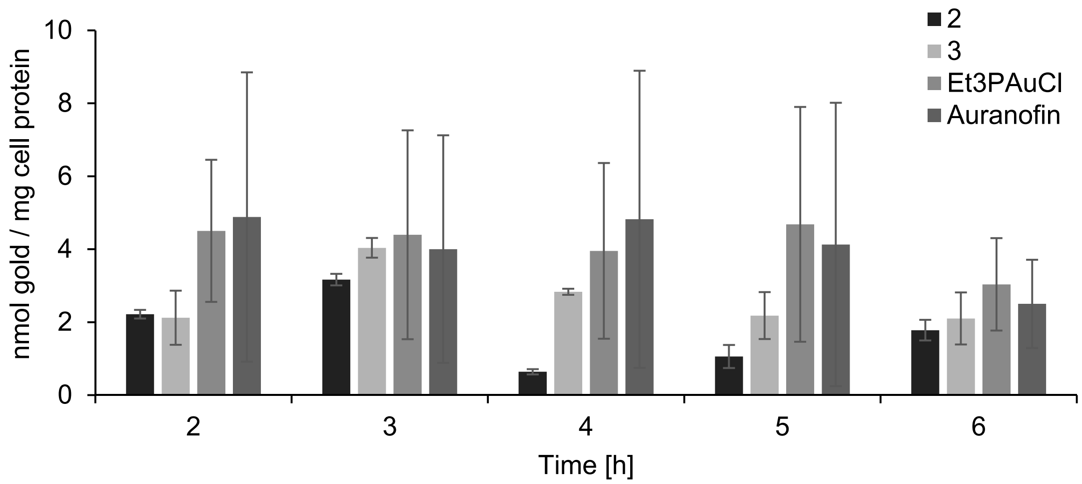

Figure SI27: Time dependent cellular uptake in Caco-2 cells of **2**/Et_3PAuCl (6 µM) and **3**/Auranofin (3 µM)

8.) Permeability tests in Caco-2 cells (Experimental)

For permeability studies an established in vitro barrier model derived from human epithelial colorectal adenocarcinoma Caco-2 cells was used, which simulates the absorption of compounds in the gastrointestinal tract from the intestinal lumen into the blood stream. For these studies in a 6-well plate 400,000 cells/well were seated on a filter membrane of an insert and incubated (37 °C / 5 % CO_2) in modified MEM cell culture medium until the transepithelial electrical resistance (TEER) parameter was between 250 and 300 Ω*cm^2 indicating that an appropriate cell layer barrier was formed (Fig. SI26). The cell culture medium was removed and the cells washed with a mixture of MEM cell culture medium/distilled water (2:1). The compounds were solved in DMF and diluted with the same mixture to final concentrations of either 3.0 µM (Auranofin / **3**) or 6.0 µM (Et_3PAuCl / **2**) in non-toxic concentrations (comment: the toxicity of the compounds against Caco-2 cells had been determined by MTT assays, data not shown). A volume of 2.0 mL of the respective dilutions were added on the apical site (on the insert membrane) and 3.0 mL of the medium/water mixtures without compounds were pipetted directly into the wells (basolateral site). The plates were incubated for 6 hours (37 °C / 5 % CO_2) under smooth shaking and aliquots (120 µL) were taken from the apical as well as basolateral side of the cell layer after the desired time. The gold content of the aliquots was determined by HR-CS AAS.

9.) Cellular uptake studies in Caco-2 cells (Experimental)

For each time point or test concentration a 75 cm^2 flask was prepared with 10 mL of Caco-2 cells (from DSMZ) in MEM (120,000 cells/mL) and cultivated for 72 hours (37 °C / 5 % CO_2). Test compounds were dissolved in DMF and diluted with distilled water. These solutions were diluted 1+9 with KRB (final DMF concentration 0.5% v/v). Cell culture medium was removed, the cells were washed with 5 mL KRB and the solution exchanged by 10 mL of the before mentioned mixture, containing the compounds in the desired concentrations. After incubation the medium was removed, the cells were washed with 5 mL PBS buffer and 10 mL fresh PBS buffer was added. The cells were scratched from the flask bottom; the suspension transferred into new tubes, centrifuged (5 min, 1096 g) and the supernatant was decanted. The obtained cell pellets were stored at –20 °C for further use. For metal and protein quantification the pellets were resuspended in demineralized water (0.5 mL) and lysed 30 min by ultra-sonication. The protein content of lysates was determined by the Bradford method and the metal content was determined by AAS as described in the main text.

10.) Reference

[1] Schmidt, C.; Karge, B.; Misgeld, R.; Prokop, A.; Franke, R.; Brönstrup, M.; Ott, I. Gold(I) NHC Complexes: Antiproliferative Activity, Cellular Uptake, Inhibition of Mammalian and Bacterial Thioredoxin Reductases, and Gram-Positive Directed Antibacterial Effects. *Chem. Eur. J.,* **2017**, *23*, 1869–1880.