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Oestradiol post-functionalized gold(I) bis(1,2,3-triazol-5-ylidene) complex exhibits high activity for ER α -positive breast cancer cells (MCF-7)

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Au(I) N-heterocyclic carbene (NHC) complexes have shown promising cytotoxicity against cancer cells, yet improving their selectivity remains a key challenge. In this study, a modular strategy to enhance tumor targeting by post-functionalizing an Au(I) bis-aNHC_{trz} complex (trz = 1,2,3-triazole-5-ylidene) with oestradiol *via* copper-catalyzed click chemistry is introduced. The resulting conjugate shows high cytotoxicity in the nanomolar range and markedly increased accumulation in ER α -positive breast cancer cells compared to its non-functionalized analogue. This work demonstrates the potential of hormone-based vectors to guide gold complexes selectively to hormone receptor-positive cancer cells.

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Introduction

Chemotherapy remains one of the most widely used cancer treatments. In England, 28% of patients who received a cancer diagnosis between 2013 and 2016 were treated with chemotherapy.¹ However, chemotherapy is usually accompanied by adverse effects ranging from mild discomfort to life-threatening complications. Studies indicate that nearly all cancer patients (97.4%) experience side effects during chemotherapy,² while 0.6% die due to complications directly caused by chemotherapy.³ Therefore, the development of novel anti-cancer agents that minimize side effects while effectively targeting cancer cells is of great importance.

The goal is to specifically identify and eliminate cancer cells with high precision, sparing healthy tissue and reducing the overall burden on patients. With the rise of cancer incidence worldwide and the subsequent increase in therapy,⁴ there is an urgent need for more selective and less toxic therapies. Particularly, breast cancer, the second most diagnosed cancer type, is estimated to rise from 2.3 million cases worldwide in 2020 to 3 million in 2040.⁵

Among emerging strategies, gold(I) complexes, especially Au(I) N-heterocyclic carbene (NHC) complexes, have attracted

significant interest due to their strong cytotoxic effects against various cancer cell lines, including MCF-7 breast cancer cells.^{6,7} In particular, Au(I) bis-NHC complexes have been repeatedly reported to reach IC₅₀ values (a given concentration that reduces the number of viable cells by 50%) in the nanomolar range.^{8,9} Due to the strong sigma-donating abilities of NHC, the Au(I)-NHC bond is stable under physiological conditions.¹⁰ Furthermore, a handful of Au(I) NHC complexes have shown selectivity towards cancerous cell lines over healthy cells,^{11,12} but this trait is not universal. To enhance selectivity, some research groups have conjugated targeting vectors to Au(I) NHC complexes.^{13–15}

Notably, the groups of Ott and Nolan have utilized steroids, such as oesthisterone, as ligands in Au(I) NHC complexes and investigated their cellular uptake in MCF-7 cells.¹³ Previous studies indicate that steroids, as small-molecule drug conjugates (SMDCs), can selectively deliver cytotoxic compounds to target cells and enhance cellular uptake of the compound.¹⁶ Since 70% of breast cancer cells are hormone receptor-positive (ER α +), exhibiting oestrogen or progesterone receptors, using oestrogens as vectors can potentially increase cellular uptake and selectivity in most breast cancer cells.¹⁷ Investigations of the complexes described by Ott and Nolan's groups determined a higher increase in cellular uptake for complexes containing oesthisterone compared to complexes without oesthisterone in MCF-7 cells (Fig. 1).¹³ Investigation of mestranol as a ligand for Au(I) NHC complexes resulted in a lower anti-proliferating activity of the mestranol-incorporating complex than the corresponding complex without mestranol.¹⁴

Utilizing steroids *via* coordination to a metal complex starkly influences the active metal centre, and thereby, the

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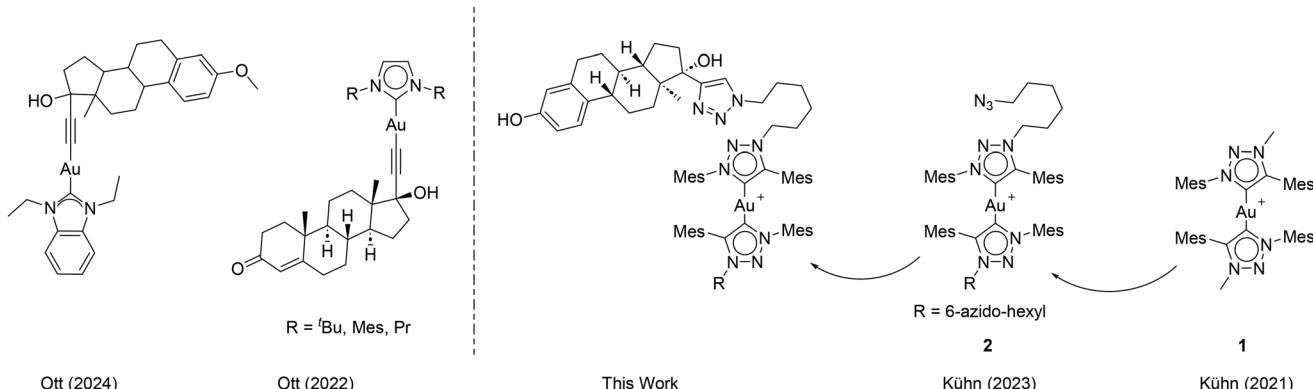


Fig. 1 Au(I) NHC complexes bearing oestrogen derivative inspiring this work.

affinity of the gold centre towards the biological target can be hindered, hence a decrease in cytotoxicity. Additionally, using alkyne ligands increases the lability of the compound, as the alkyne gold bond can easily be cleaved in the presence of thiols.¹⁸ The loss of the targeting vector decreases the selectivity and may lead to deactivation of the compound. To address these challenges, the classical approach of SMDCs offers a more robust strategy. Thereby, the targeting vector is connected to the ligand *via* a linker. A concept not new to metal-organic medicinal chemistry.^{19,20}

Previously, we have reported $[\text{Au}(\text{i})(\text{aNHC}_{\text{trzMe}})_2]\text{PF}_6$ ($\text{aNHC}_{\text{trzMe}} = 1,4\text{-dimesityl-3-methyl-1,2,3-triazole}$) **1**, which contains a NHC rarely explored in medicinal organometallic chemistry: mesoionic (“abnormal”) carbenes (Fig. 1). Complex **1** is highly cytotoxic against MCF-7 cells, showing high stability towards thiol-containing compounds, such as L-glutathione (GSH) and L-cysteine, as well as selectivity towards cancerous cells over healthy cells.⁸ Building upon this platform, we developed an Au(I) aNHC gold complex $[\text{Au}(\text{i})(\text{aNHC}_{\text{trzAzide}})_2]\text{I}$ ($\text{aNHC}_{\text{trzMe}} = 1,4\text{-dimesityl-3-(6-azidohexyl)-1,2,3-triazole}$) **2**, which features an azido-hexyl functional group in place of the triazole-bound methyl group, enabling facile post-functionalization *via* copper-catalysed click chemistry (Fig. 1).²¹ This work reports the post-functionalization of Au(I) bis-aNHC_{trz} complexes with oestradiol *via* copper-catalysed azide alkyne cycloaddition (CuAAC). The synthesized oestrogen-containing complexes are investigated for their cytotoxicity and selectivity towards oestrogen-receptor-positive and oestrogen-receptor-negative cancer and healthy cell lines.

Results and discussion

Synthesis

Based on previous work,²¹ the functionalization of complex **2** *via* copper-catalysed click chemistry has been explored. As the alkyne component, the synthetic oestrogen hormone ethinyl oestradiol was chosen. Ethinyl oestradiol, a compound used in common contraceptives, was selected due to its commercial availability at a low price.²² The synthetic oestrogen hormone

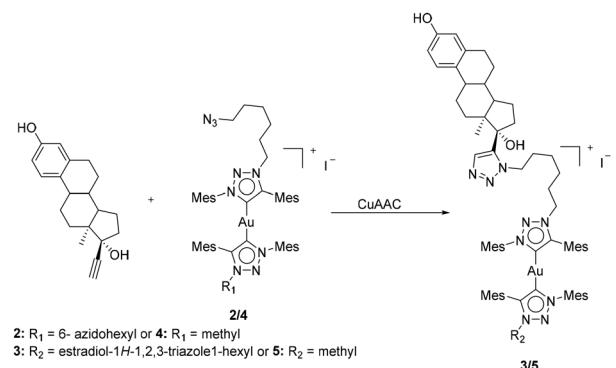


Fig. 2 Synthesis procedure of **3** and **5** *via* copper-catalysed click chemistry.

ethinyl oestradiol was “clicked” with the azido group of **2** under the same conditions reported before (Fig. 2).²¹ However, under the reported conditions, reaction of **2** with oestradiol does not give compound **3** in reasonable yield and purity.

The poor conversion might be attributed to the formation of reactive oxygen species-mediated decomposition. Therefore, the reaction was performed under exclusion of air with a mixture of copper turnings and CuSO_4 in a DCM/water suspension. This modified synthesis pathway yields only low conversion over several days.

The reaction was then further modified: CuSO_4 /sodium ascorbate and the substrates **2** and ethinyl oestradiol in different solvent mixtures (*t*-BuOH/water or *t*-BuOH/water/acetone) did not yield any conversion over several days.

Lastly, the reaction with Cu/CuSO_4 in a DCM/water mixture with a mixture of argon/air resulted in an insignificant conversion after 6 days.

After column chromatography (gradient DCM : methanol), no clean elemental analysis was obtained for compound **3** and very low yields (<10%) were achieved. The difficulty in synthesizing compound **3** can be attributed to its high lipophilicity, assigned to the lipophilic nature of oestradiol ($\log P$ value of 2.69)²³ and to the two hexyl chains, paired with the size and slight charge of compound **3**. This hinders the CuAAC, which



generally contains hydrophilic copper catalysts. The cationic character, combined with its high lipophilicity and organometallic nature, restricts the available purification methods. Both, the poor yields and purity prompted us to a redesign of the complex, namely through the synthesis of a complex with only one functional backbone. Another key motivation for the modification was the antiproliferation activity of complex **2** compared to complex **1** in MCF-7 cells. The previously reported IC_{50} values after 48 h incubation are 84 nM and 261 nM for complexes **1** and **2**, respectively (Table 1). These results suggest that exchanging the methyl group with hexyl groups decreases the cytotoxicity in MCF-7 cells. Therefore, designing a complex with only one hexyl group instead of two hexyl groups is expected to benefit the antiproliferative activity. Complex **4** [$Au(i)aNHC_{trzMe}NHC_{trzAzide}I$] was synthesized through ligand exchange reaction of [$Au(NHC_{trzMe}Cl)$] with 1,4-dimesityl-3-(6-azidohexyl)-1,2,3-triazole in the presence of base (K_2CO_3). The reaction was monitored *via* ESI-MS. Reaction control showed reorganization of the complexes, resulting in small traces of complexes **1** and **2** present in the reaction mixture.

Au NHC bonds are considered one of the strongest metal–NHC interactions, with several reports showing their stability in the presence of sulphur-containing molecules, despite the strong sulphur affinity to gold.²⁴ Complex **1** was also tested against sulphur-containing molecules and did not react with them in a period of 72 h.⁸ However, reorganization of heteroleptic $Au(i)$ bis-NHC complexes over time is known. Huynh and co-workers reported the ligand scrambling of heteroleptic $Au(i)$ bis-NHC complexes to their corresponding homoleptic counterparts over time.²⁵

Complex **4** can be obtained in pure form after purification by column chromatography. All three complexes (**1**, **2**, and **4**) can be separated using a 12 : 1 DCM : methanol mixture. Complex **4** was used as a substrate for the click reaction with ethynyl oestradiol to form complex [$Au(i)aNHC_{trzMe}HHC_{trzoestradiol}I$] (**5**). The reported reaction conditions²¹ were improved for this synthesis by adding a mixture of $CuSO_4$ /copper turning as a catalyst.

The reaction of complex **5** was monitored *via* electrospray ionization mass spectrometry (ESI-MS), and after 7 days, the reaction was complete. The copper impurities were removed by column chromatography. The pure (>95%), colourless complex **5** was characterized by elemental analysis, ESI-MS, and 1H - ^{13}C -NMR spectroscopy. Trace amounts of complexes **1** and **3** can be observed in the ESI-MS spectrum after 7 days. If ligand scrambling occurs, complexes **1** and **3** can be removed from **5** through column chromatography together with traces of copper.

Table 1 IC_{50} values \pm SD (nM) of **1**, **2**, **4** and **5** and reference drugs cisplatin and auranofin in human cancer cell lines and human dermal fibroblasts after 48 h incubation determined by the MTT assay. SI = IC_{50} normal cells (HDF)/ IC_{50} MCF-7 cancer cells. n.d. = not determined

Compounds	A2780	MCF-7	MDA-MB-231	HDF	SI
Cisplatin ⁸	3600 ± 1300	$21\,000 \pm 6300$	$13\,800 \pm 4500$	4506 ± 408	0.2
Auranofin ⁸	430 ± 230	280 ± 140	1900 ± 700	298 ± 143	1.1
1 ⁸	360 ± 90	84 ± 16	63 ± 2	n.d.	n.d.
2 ²¹	26.6 ± 1.3	261 ± 75	n.d.	n.d.	n.d.
4	34.6 ± 14	43.1 ± 9.0	53.7 ± 11	193 ± 47	4.5
5	236 ± 47	80.9 ± 8.2	321 ± 86	262 ± 51	3.2



Table 2 Cellular uptake studies of **4** and **5** with/without oestradiol (E₂) or tamoxifen (TAM) in MCF-7 cell line by ICP-MS after 3 h of incubation time. Results are mean (\pm SD) of two independent experiments done with two replicates

Compounds	Cellular uptake (ng Au per 10 ⁶ cells)
4 , 10 μ M	9.5 \pm 2.5
5 , 10 μ M	65 \pm 2.2
5 , 10 μ M + E ₂ , 10 μ M	39 \pm 2.0
5 , 10 μ M + TAM, 10 μ M	55 \pm 2.5
5 , 20 μ M	115 \pm 11

by ICP-MS, the studies were conducted after 3 h incubation time. At this time point, the IC₅₀ value found for oestradiol is >100 μ M, which is much higher than the values obtained for tamoxifen (43.6 \pm 16.4 μ M), for complex **4** (10 μ M) and for complex **5** (20 μ M) (Table S1).

Complex **5** (10 μ M, 65 \pm 2.2 ng Au per 10⁶ cells) showed a nearly 7-fold increase in gold uptake compared to complex **4** (9.5 \pm 2.5 ng Au per 10⁶ cells) (Table 2). This striking difference can be assigned to the presence of oestrogen. The uptake of **5** at the IC₅₀ value (20 μ M) increased almost twice (115 \pm 11 ng Au per 10⁶ cells), as expected.

Competition studies with oestradiol and tamoxifen are also included. These drugs have an opposing effect in oestrogen receptor-positive breast cancer cells, where tamoxifen blocks oestradiol from binding to oestrogen receptors, while oestradiol promotes the growth of oestrogen receptor-positive cells.

The studies with complex **5** in the presence of free oestradiol or tamoxifen indicate that such drugs compete with the metal complex for oestrogen receptors in the MCF-7 breast cancer cells, reducing cell uptake values. Notably, this effect was more pronounced for oestradiol (39 \pm 2.0 ng Au per 10⁶ cells) than for tamoxifen (55 \pm 2.5 ng Au per 10⁶ cells).

The lower cytotoxicity of **5** compared to **4**, despite the higher uptake, can also be caused by oestrogen. Oestrogen transports into different parts of the cell, such as the nuclei,³¹ while complex **1**, which **5** is derived from, seems to be active through the mitochondrial pathway.⁸ However, **5** has the same activity in MCF-7 cells as the unmodified complex **1**. Despite the linker and oestrogen modification, **5** keeps its activity and gains selectivity towards ER α -positive cancer cells. A shortening of the alkyne chain from hexyl to propyl can, based on the findings, potentially increase activity in MCF-7 cells. Only one backbone-modification side of **1** was used for the addition of oestradiol, leaving one side potentially open for the addition of markers (radioactive, fluorescent), which could track **5** inside the cell or *in vivo*. Investigations into labelling complex **2** are currently underway.

Conclusions

The successful post-functionalization of an Au(i) bis-aNHC_{trz} complex with oestradiol is reported. This oestradiol-containing compound (**5**) exhibits potential anticancer activity, with IC₅₀

values in the nanomolar range and pronounced cytotoxicity against the ER α + MCF-7 cell line. Additionally, complexes **4** and **5** both show high selectivity towards MCF-7 over HDF cells (SI > 3). This result confirms that complex **4** is also an interesting candidate for further investigations. Complex **5** shows a 7-fold higher cellular gold accumulation in MCF-7 cells than the oestradiol-free analogue **4**. This result, together with the decrease in cellular uptake in the presence of oestradiol and tamoxifen, indicates that the ER α receptor in MCF-7 cells appears to play a role in the uptake of compound **5**. Notably, compound **5** retains the cytotoxicity potency of the unmodified complex **1**, demonstrating that the targeted functionalization can be achieved without compromising efficacy or stability. These findings highlight compound **5** as a promising candidate for hormone receptor-targeted chemotherapy. Additionally, this work highlights the importance of attaching targeting vectors through covalent bonding to the metal centre, rather than coordination, thereby paving the way for future innovations in targeted therapy with Au(i) NHCs.

Experimental

Materials and methods

Reagents were purchased from Sigma-Aldrich, TLC, and abcr and used without further purification. All reactions were performed under aerobic conditions unless otherwise stated. Dry solvents were obtained by a Mbraun solvent purification system.

The NMR spectra were obtained on a Bruker AVANCE DPX 400 and AV500C. The chemical shifts are given in parts per million and referenced to the residual signal of deuterated solvent (dichloromethan-*d*₂: 5.20 ppm, dimethylsulfide-*d*₆: 2.50 ppm, acetonitrile-*d*₃: 1.96 ppm). Analytical reversed-phase HPLC-HESI-MS was performed on an UltiMate 3000 UHPLC focused chromatographic system (Dionex) connected to an LCG Fleet mass spectrometer (Thermo Scientific) equipped with a C18 column (Hypersil GOLD aQ, 150 \times 2.1 mm, 3 μ m). Linear gradients of 85–100% acetonitrile with 0.1% [v/v] TFA and 0.1% [v/v] TFA in water were applied over 25–30 min. Elemental analyses (C/H/N/S) were performed by the microanalytical laboratory of the Technical University of Munich using a HEKATech Euro EA-CHNS combustion analyzer.

General procedures for synthesis

3-(6-Azidohexyl)-1,4-dimesityl-1*H*-1,2,3-triazolium iodide,²¹ [Au(NHC_{trzMe})Cl],³² and, [Au(tht)Cl]³³ were synthesized according to literature procedures. The qualitative analysis was carried out through elemental analysis, HESI-MS, and NMR spectroscopy.

[Au(i)NHC_{trzMe}NHC_{trzAzide}]I (4). 150 mg of [Au(NHC_{trzMe})Cl] (271.8 μ mol, 1.00 equiv.) is dissolved in 16 mL of acetone, additionally, 151.8 mg of 3-(6-azidohexyl)-1,4-dimesityl-1*H*-1,2,3-triazolium iodide (271.8 μ mol, 1 equiv.) and 93.91 mg of K₂CO₃ (679.5 μ mol, 2.50 equiv.) are added to the solution. The reaction mixture is stirred for 24 h at 60 °C and afterward fil-



tered through Celite. The solvent is removed *in vacuo*. The resulting crude product is purified through column chromatography (1 : 12 methanol : DCM, R_f = 0.3, silica gel 0.06–0.2 mm, 60 Å, Thermo Scientific, d = 2.5 cm, h = 30 cm), yielding 118.1 mg of 4 (109.8 μmol , 40.0%) as a white powder. **1H-NMR** (400 MHz, acetonitrile- d_3 , 298 K) δ (ppm): 7.00 (s, 4H, CH_{mes}), 6.97 (s, 4H, CH_{mes}), 4.11 (t, 2H, $\text{CH}_2\text{-N}_{\text{trz}}$), 3.81 (s, 3H, Me-N_{trz}), 3.18 (t, 2H, $\text{CH}_2\text{-N}_3$), 2.43 (s, 6H, *o*-Me_{mes}), 2.41 (s, 6H, *o*-Me_{mes}), 1.82 (s, 6H, *p*-Me_{mes}), 1.82 (s, 6H, *p*-Me_{mes}), 1.75 (s, 12H, *o*-Me_{mes}), 1.75–1.70 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-N}_{\text{trz}}$), 1.45–1.41 (m, 2H, $\text{CH}_2\text{-CH}_2\text{-N}_3$), 1.27–1.16 (m, 4H, CH_2). **Anal. calc. for** [Au(i)NHC_{trzMe}NHC_{trzAzide}]I: found: C 52.68 H 5.56 N 11.46 calc.: C 52.57 H 5.54 N 11.74; **ESI-MS, positive (m/z):** [Au(NHC_{trzMe}NHC_{trzAzide})]⁺ calc. 946.46 found 946.84.

[Au(i)NHC_{trzMe}NHC_{trzestradiol}]I (5). Under air, 42.8 mg of complex 4 (39.85 μmol , 1.00 equiv.) and 11.81 mg ethyl oestradiol (38.95 μmol , 1.00 equiv.) are dissolved in 7.0 mL DCM. 2.5 mg of copper turnings (39.85 μmol , 1.00 equiv.) are added together with 9.95 mg CuSO₄ (39.85 μmol , 1.00 equiv.) and 3.0 mL of water. The reaction mixture is stirred at 30 °C, and the progress of the reaction is monitored *via* HESI-MS. After 7 d the two phases were extracted. The aqueous phase is extracted two times with DCM (2 × 6 mL). The organic phases are joined, washed with brine (2 × 10 mL), and dried over Na₂SO₄. The solvent is removed *in vacuo*. The crude product can be purified through column chromatography (1 : 12 methanol : DCM, silica gel 0.06–0.2 mm, 60 Å, Thermo Scientific, d = 2.5 cm, h = 30 cm), resulting in 30.1 mg of white powder 5 (21.96 μmol , 55.0%). **1H-NMR** (400 MHz, acetonitrile- d_3 , 298 K) δ (ppm): 7.77 (s, 1H, H_{trz}), 7.02–6.97 (m, 8H, H_{mes}, ar), 6.97–6.92 (d, 1H, CH_{estr}, ar), 6.61 (m, 1H, CH_{estr}, ar), 6.59 (s, 1H, H_{estr}, ar), 4.22 (t, 2H, CH₂-N_{trz}), 4.16 (t, 2H, CH₂-N_{trz}), 3.88 (s, 3H, Me-N_{trz}), 3.52 (s, 1H, OH_{estr}), 2.74 (m, 2H, CH_{estr}, al), 2.45–2.42 (s, 12H, *o*-Me_{mes}), 2.35–2.31 (m, 2H, CH_{2estr}), 2.09–2.03 (m, 3H, CH_{2estr}), 1.84 (s, 6H, *p*-Me_{mes}), 1.82 (s, 6H, *p*-Me_{mes}), 1.77 (s, 6H, *o*-Me_{mes}), 1.75 (s, 3H, *o*-Me_{mes}), 1.74 (s, 3H, 1.69 *o*-Me_{mes}), 169–1.18 (m, 10H, CH_{2hexyl}/CH_{2estr}), 1.13 (m, 4H, CH_{2hexyl}), 1.0 (s, 3H, Me_{estr}), 0.61 (dt, 1H, H_{estr}). **13C-NMR** (126 MHz, acetonitrile- d_3 , 298 K) δ (ppm): 176.21 (carbene), 175.99 (carbene), 156.22 (C_{estr,ar}-OH), 154.82 (C_{trz}), 147.27 (C_{trz-mes}), 146.78 (C_{trz-mes}), 141.61 (C_{mes}), 141.57 (C_{mes}), 141.46 (C_{mes}), 139.04 (C_{mes}), 138.84 (C_{mes}), 138.62 (C_{mes}), 136.28 (C_{mes}), 135.00 (C_{mes}), 134.89 (C_{mes}), 131.66 (C_{mes}), 129.95 (CH_{mes}), 129.92 (CH_{mes}), 129.61 (CH_{mes}), 129.46 (CH_{mes}), 126.92 (CH_{estr,ar}), 123.09 (C_{mes}), 122.88 (CH_{trz}), 116.11 (CH_{estr,ar}), 113.72 (CH_{estr,ar}), 82.80 (C_{estr}), 51.20 (CH₂-N_{trz}), 50.30 (CH₂-N_{trz}), 49.12 (CH_{estr}), 47.99 (C_{estr}-Me), 44.51 (CH_{estr}), 40.50 (CH_{estr}), 38.29 (CH_{2estr}), 37.69 (Me_{trz}), 33.83 (CH_{2estr}), 30.39 (CH_{2estr}), 30.30 (CH_{2estr}), 29.12 (CH_{2estr}), 28.33 (CH_{2estr}), 27.19 (CH_{2estr}), 26.10 (CH_{2estr}), 26.06 (CH_{2estr}), 24.23 (CH_{2estr}), 21.40 (*o*-Me_{mes}), 21.36 (*o*-Me_{mes}), 20.70 (*p*-Me_{mes}), 20.09 (*p*-Me_{mes}), 17.28 (*o*-Me_{mes}), 17.19 (*o*-Me_{mes}), 14.82 (Me_{estr}). **Anal. calc. for** [Au(i)NHC_{trzMe}NHC_{trzestradiol}]I: found: C 58.93 H 6.53 N 9.19 calc.: C 58.73 H 6.11 N 9.20; **ESI-MS, positive (m/z):** [Au(NHC_{trzMe}NHC_{trzestradiol})]⁺ calc. 1242.63 found 1242.81 [Au(NHC_{trzMe}NHC_{trzestradiol}) + H]²⁺ calc. 621.82 found 622.53.

Biological studies

Cytotoxic activity. The cytotoxic activity of the complexes was evaluated in the human ovarian A2780 and human dermal fibroblasts HDF (Sigma-Aldrich), breast MCF-7 and MDA-MB-231 (American Type Culture Collection) cancer cells. Cells were grown in RPMI (A2780) or DMEM + GlutaMAX (MCF-7 and MDA-MB-231) media supplemented with 10% FBS and maintained at 37 °C in an incubator with 5% CO₂. The cellular viability was measured by using the colorimetric MTT assay. For the assay, cells (~2 × 10⁴ cells per 200 μL medium) were seeded in 96-well plates and left to adhere for 24 h. Complexes were first diluted in DMSO and then in medium to prepare serial dilutions in the range 0.001–50 μM . The reference drugs cisplatin and auranofin were first diluted in water (cisplatin) or DMSO (auranofin) and then in medium to prepare serial dilutions in the range 0.1–100 μM . Each compound's dilution (200 μL) were added to the cells and incubated for 48 h at 37 °C. After, the medium was discarded and 200 μL of MTT solution in PBS (0.5 mg ml⁻¹) were applied to each well, following a similar procedure as previously described.³⁴ Results are shown as the mean ± SD of at least two independent experiments done with six replicates per condition.

Uptake studies. The cellular gold content in the MCF-7 cells was analyzed by a Quadrupole ICP-MS Thermo X-Series. Cells (~10⁶ cells per 5 mL medium) were exposed to the complexes 4 and 5 for 3 h at their IC₅₀ values (found at 3 h incubation), respectively 10 and 20 μM and also exposed to the complexes simultaneously incubated with the reference drugs oestradiol and tamoxifen at 10 μM for 3 h. After incubation at 37 °C, 5% CO₂, cells were washed with ice-cold PBS and centrifuged to obtain a cellular pellet. The gold content in the pellets was measured as previously described.³⁵

Author contributions

Melanie E. Hoffmann: conceptualisation, formal analysis, investigation, writing – original draft, funding acquisition, project administration, visualization. Fritz E. Kühn: conceptualisation, supervision, resources, data curation, writing – review and editing, funding acquisition, validation. Fernanda Marques: analysis, investigation, writing – review and editing, visualization. João D. G. Correia: writing – review and editing, resources, conceptualisation, data curation, supervision, validation.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article are included in the text or in the supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d5dt01763g>.

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