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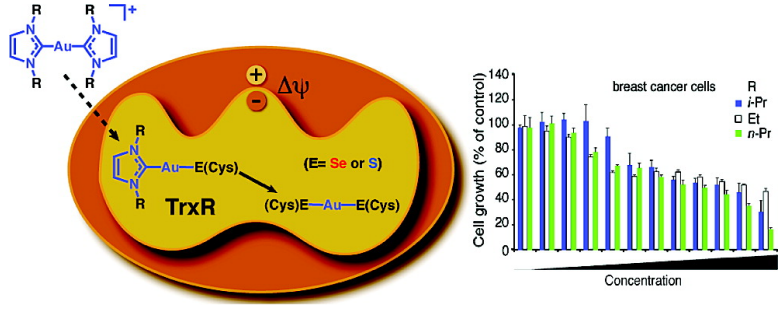

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Mitochondria-Targeted Chemotherapeutics: The Rational Design of Gold(I) *N*-Heterocyclic Carbene Complexes That Are Selectively Toxic to Cancer Cells and Target Protein Selenols in Preference to Thiols

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Herein we describe a new approach to mitochondria-targeted antitumor agents, in the design of Au(I) *N*-heterocyclic carbene (NHC) compounds that combine both selective mitochondria targeting and selective thioredoxin reductase inhibition properties within a single molecule.

Recent developments in understanding the central place of mitochondria as regulators of programmed cell death have stimulated great interest in targeting them in new approaches to cancer chemotherapy.[1] One approach involves the use of delocalized lipophilic cations (DLCs), which can pass readily through the lipid bilayer and then concentrate within mitochondria, driven by the large mitochondrial membrane potential ($\Delta\psi_m$) generated by the respiratory chain.[2] DLCs can selectively accumulate in mitochondria of cancer cells as a consequence of the elevated $\Delta\psi_m$ that is a feature of many tumor cells.[3] A variety of structurally diverse DLCs have shown antitumor activity,[2] and two predictive models suggest that selectivity for cancer cells over normal cells can be "tuned" by adjusting the lipophilicity (log *P*).[4]

The thioredoxin (Trx)/thioredoxin reductase (TrxR) system (in both cytoplasm and mitochondria) plays a major role in the regulation of the cellular redox state. An increase in Trx and TrxR activities has been correlated with evasion of apoptosis and acceleration of tumor growth,[5] while the inhibition of TrxR can lead to apoptosis of cancer cells.[6,7] Consequently, the selenoenzyme TrxR has emerged as an important new drug target.[8,9] While many compounds have been shown to be potent inhibitors of purified TrxR enzymes, only a few studies report their inhibition in cells.[10] Au(I) complexes such as Auranofin [Au(I)(PEt_3)(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-thioglucose-S)] are particularly potent inhibitors of mammalian TrxR,[7,8,11,12] their activity attributed to Au(I) binding to the C-terminal redox active −Cys-Sec− center.[12] They are usually also potent inhibitors of the closely related but Se-free enzyme glutathione reductase (GR), due to the high thiol reactivity that is characteristic of linear Au(I) complexes. The crystal structure of GR inhibited by a (phosphole)AuCl complex (a highly potent *in vitro* inhibitor of both TrxR and GR)[12] shows Au(I) bound to the active site Cys thiols with almost linear S−Au−S coordination.[12] Similarly, while Auranofin inhibits the growth of tumor cells *in vitro*,[13] its high reactivity toward protein thiols limits its antitumor activity *in vivo*.[14]

NHCs have similar donor properties to phosphines, and metal−NHC complexes are of great current interest, particularly for catalytic applications.[15] Their biomedical applications are as yet relatively unexplored.[16,17] An attractive feature of NHC chemistry is the relative ease with which a series of structurally similar complexes with varying lipophilicity can be prepared from simple imidazolium salt precursors. For a family of five linear, cationic Au(I) NHC complexes $[(R_2Im)_2Au]^+$ (R = Me, *i*-Pr, *n*-Bu, *t*-Bu, and Cy), the log *P* values vary within the range −1.09 to 1.73. Their ability to induce cyclosporin A-sensitive swelling in isolated rat liver mitochondria correlates with lipophilicity,[17] and we suggested that these compounds could potentially target mitochondrial cell death pathways.[17]

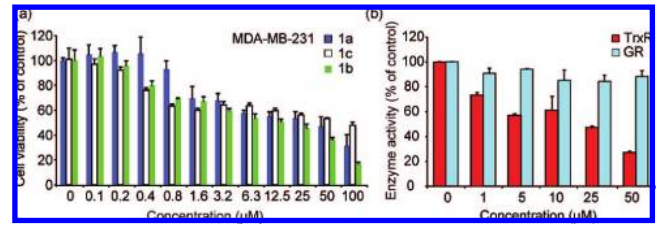

Figure 1. (a) MDA-MB-231 cells were treated with increasing concentrations of **1a**, **1b**, and **1c**, and cell growth was measured after 24 h using a cell titer assay. (b) Cells incubated with increasing concentrations of **1a** were lyzed, and GR and TrxR activities were measured and expressed as % of control. Data are means ± SD ($n = 3$).

Based on this previous work we chose the *i*-Pr derivative (**1a** (Cl^- salt) log $P = -0.29$) and prepared two new $[(R_2Im)_2Au]^+$ complexes with substituents R = *n*-Pr (**1b** (Br^- salt) log $P = -0.02$) and Et (**1c** (Br^- salt) log $P = -0.84$) to fine-tune the lipophilicity in the intermediate range. We compared the effects of increasing concentrations of **1a**, **1b**, and **1c** on cell growth of two highly tumorigenic breast cell lines, MDA-MB-231 and MDA-MB-468, and normal human mammary epithelial cells (HMEC) (Figure 1a and Figure S1a, Supporting Information). All three Au(I) NHC complexes are selectively toxic to both breast cancer cell lines but not to the normal cells, and the degree of selectivity correlates with their log *P* values. **1a** (R = *i*-Pr), with intermediate lipophilicity, shows the most optimal selectivity and cytotoxic potency compared with the other two compounds and was chosen as the lead candidate for further studies. The loss of total ATP after 24 h in the breast cancer cells, but not in the HMEC cells, further confirms the selective toxicity of **1a** (Figure S1b).

MDA-MB-231 cells were treated with **1a**, and the amount of Au present in mitochondria isolated from cells was measured by ICP-MS. The accumulation of **1a** into mitochondria is driven by the $\Delta\psi_m$, since in the presence of $\Delta\psi_m$ >85% of the Au was found within mitochondria, and on dissipation of the $\Delta\psi_m$ with the uncoupler FCCP, the distribution was reversed into the cytoplasm (Table S1). The $\Delta\psi_m$ was lowered after a 12 h treatment with 5 μM **1a** (Figure S2a), suggesting that uncoupling of the $\Delta\psi_m$ may

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Scheme 1

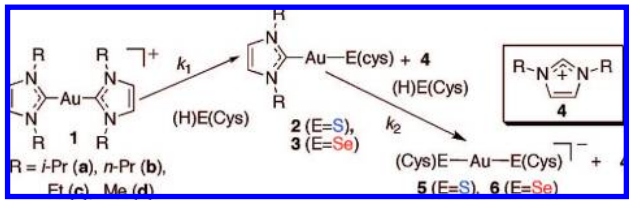

Table 1. Estimated Rate Constants for the Reactions of **1a** and **1d** with Cysteine (Cys) and Selenocysteine (Sec) at 37 °C, pH 7.2[a]

	Cys		Sec	
complex	k_1 (10^{-2} M^{-1} s^{-1})	k_2 (10^{-2} M^{-1} s^{-1})	k_1 (10^{-2} M^{-1} s^{-1})	k_2 (10^{-2} M^{-1} s^{-1})
1d (R = Me)	2.5 ± 0.3	0.48 ± 0.08	50 ± 3	42.4 ± 0.5
1a (R = *i*-Pr)	1.1 ± 0.2	0.24 ± 0.07	18 ± 1	18.6 ± 0.6

[a] **1a/1d** (1.7 mM), Cys/Sec (8.3 mM) in 0.1 M PBS, pH 7.2; values are derived from the mean of two independent experiments (see Table S2).

lead to cell death. Caspase-9 and caspase-3 were induced in cells treated with **1a** under the same conditions (Figure S2b) indicating that **1a** causes selective cancer cell death through a mitochondrial apoptotic pathway.

To assess whether thiol- and selenol-containing proteins could be possible targets for these Au(I) NHC complexes we followed the reactions of **1a** and **1d** (1.7 mM) with Cys or Sec (8.3 mM) at 37 °C in 0.1 M phosphate buffered saline (pH 7.2), by observing time dependent changes in the ^{1}H NMR resonances of the imidazoyl H4/H5 protons (Figure S3−S4).

The intermediates and products observed are consistent with a two-step reaction (Scheme 1) involving the successive substitution of the two NHC ligands to form either $[Au(Cys)_2]^-$ (**5**) or $[Au(Sec)_2]^-$ (**6**). Estimated rate constants k_1 and k_2, based on a single set of concentrations, are shown in Table 1 and were derived from fitting the speciation plots to the kinetic model depicted in Scheme 1. All rate constants are 2- to 3-fold higher for the reactions of **1d** (R = Me) compared to **1a** (R = *i*-Pr), consistent with an associative mechanism in which the bulkier *i*-Pr substituents more effectively shield the Au atom from attack by the entering ligand or produce a more sterically hindered 3-coordinate transition state. While the crystal structures of **1a** and **1d** show the gold atom is relatively open and naked with mutually planar carbene ligands, in solution there is evidence for free rotation about the Au−C bond.[17] The substituent on the imidazolium salt precursors can thus be tailored to finely modulate the reactivity of $[(R_2Im)_2Au]^+$ complexes in ligand substitution reactions with protein (Cys/Sec) residues.

The estimated rate constants are 20- to 80-fold higher for the reactions of **1a** and **1d** with Sec, compared to Cys, reflected by the dramatically different completion times (Sec 0.5 to 2 h; Cys 35−55 h, Figures S3−S4). This trend reflects the difference in pK_a values (Cys 8.5;[18] Sec 5.2[19]) so that at pH 7.2 the selenol is fully ionized and the thiol is not. An important difference for the two-step reactions of both complexes is the relative magnitude of the two rate constants, similar for reactions with Sec, whereas for the Cys reactions k_1 is 5-fold higher than k_2. The higher lability of the NHC ligand in the Au(I) selenolate intermediate (**3**) compared to the thiolate intermediate (**2**) has important implications for the ability of these complexes to irreversibly inhibit TrxR, as an intermediate Sec−Au−NHC species would readily undergo irreversible ligand substitution, e.g. with a neighboring Cys residue, to form a Se−Au−S species.

To investigate whether **1a** can inhibit TrxR activity in cells, we treated MDA-MB-231 cells with increasing concentrations of **1a** for 6 h. TrxR activity was inhibited by ∼50% with 5 μM **1a**, whereas no inhibition of intracellular GR activity occurred under these conditions (Figure 1b). TrxR plays an important role in the regulation of cell growth, and its inhibition may contribute to the onset of apoptosis in breast cancer cells.

In summary, by fine-tuning the ligand exchange reactions at the Au(I) center, we have designed lipophilic, cationic Au(I) complexes that selectively induce apoptosis in cancer cells but not normal cells and allow selective targeting of mitochondrial selenoproteins, such as TrxR. Our work paves the way to new approaches for the development of mitochondria-targeted chemotherapeutics.

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Supporting Information Available: Full experimental procedures, Figures S1−S4, Tables S1−S2, and SCIENTIST model used to determine the rate constants k_1 and k_2. This material is available free of charge via the Internet at http://pubs.acs.org.

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