Antitumor Agents

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Metal/N-Heterocyclic Carbene Complexes: Opportunities for the Development of Anticancer Metallodrugs

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In cancer therapy, there is an acute need to supersede the widely used platinum drugs because of the severe side effects and resistance phenomena which limit their clinical usefulness. Moreover, platinum compounds are not active against all types of cancer cells. Their target, DNA, is ubiquitously present is all type of cells, including healthy ones, which result in a systemic toxicity. To circumvent these drawbacks, exploration of novel metal complexes that target mitochondria with pharmacokinetic and pharmacodynamic properties has been considered.[1a] Among these, metal/N-heterocyclic carbene (NHC) complexes (especially gold) have recently shown very promising results. The benefits of using Au-NHCs (and other transition metals) as metallodrugs reside in the ability of the ligand to stabilize the metal (especially Au^I from disproportionation), in the strong metal–carbon bond (compared to metal phosphines such as auranofin), in the large diversity of structures easily accessible by (quite) simple synthetic pathways and hence, in the wide range of biological targets they can potentially affect.[1b–c] Therefore, the design of metal-NHCs targeting mitochondria (Figure 1) is an emerging area and might provide new alternatives for therapeutic treatments of cancer.[2]

The central place of mitochondria as regulators of apoptosis (the programmed cell death) is now well recognized and thus, it is not surprising that a lot of effort has been focused on targeting them to develop new approaches to cancer chemotherapy. Indeed, numerous proapoptotic signals converge on this organelle to induce mitochondrial outer membrane permeabilization (MOMP), which is lethal because of the caspase cascade activation and/or the release of caspase-independent death effectors such as apoptosis inducing factor (AIF). Skulachev cations,[3a] also called delocalized lipophilic cations (DLCs), penetrate into the mitochondria because of their strong negative membrane potential ($\Delta\psi m$). At first triphenylalkylphosphonium cations were used as "electric locomotives" to drive the entry of an attached pharmacophore (antioxidant) into mitochondria.[3b] The concept of DLCs has been extended to the anticancer field by utilizing the cationic phosphine/gold(I) complexes [Au(dppe)$_2$]Cl or [Au(d2pypp)$_2$]Cl, and more recently homoleptic Au^I-NHC complexes. It was shown that Au^I-NHCs increase reactive oxygenated species (ROS) production inside the mitochondria, thus ultimately leading to cell death. In these cases, the carbene ligand is the lipophilic auxiliary, which can be fine-tuned, and the metal is considered the reactive center interacting with proteins, namely those containing cysteine and selenocysteine. Berners-Price and coworkers demonstrated that a series of Au^I-NHCs (**1**; Scheme 1) induce mitochondrial swelling as a function of their lipophilicity and inhibit the selenoenzyme thioredoxin reductase (TrxR), an enzyme overexpressed in numerous human cancers.[4a] Importantly, TrxR plays a major role in the regulation of the cellular redox state and its inhibition causes

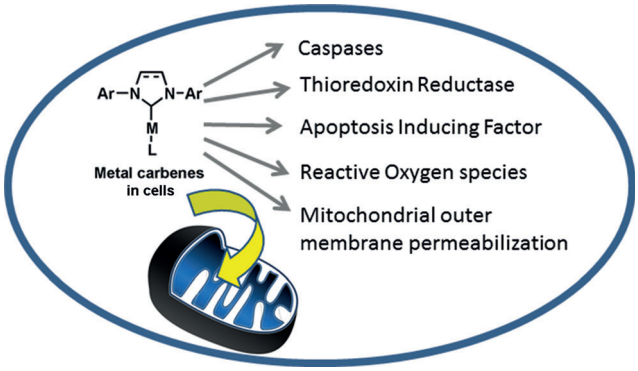
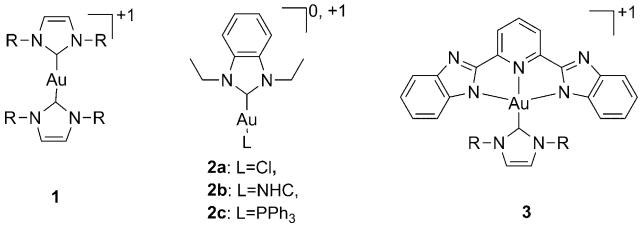


Figure 1. Targets of metal-NHCs: mitochondria and proteins.



Scheme 1. Gold-NHCs targeting mitochondria and inhibiting TrxR.

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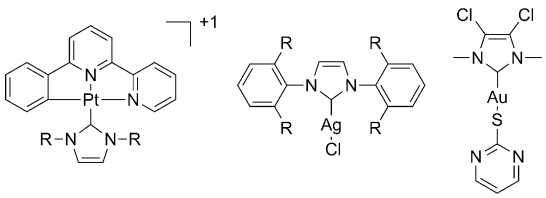
the over-production of ROS. Both actions occur by MOMP and end up with the release of cytochrome *c* inducing the caspase cascade, and ultimately DNA fragmentation. Selectivity for TrxR in living cells is demonstrated by its predominant inhibition over glutathione reductase (GR), a closely related enzyme which does not contain selenocysteine. Regarding the compounds **2**, the effective uptake of gold into mitochondria was evaluated on MCF-7 cells and shows an increase in the level of gold with the order **2a** < **2b** ≪ **2c**, which reflects their charge and lipophilicity.[4b,c]

The proof of the localization in mitochondria was recently obtained by Che and co-workers by taking advantage of the proluminescence of the DLC gold(III) complex **3**.[5] The strong emission of the 2,6-bis(benzimidazol-2-yl)pyridine (H_2BPB) ligand is quenched by the presence of the Au^{III} center but is switched on by the release of the free H_2BPB upon the reduction into Au^{I} by glutathione (GSH). Therefore, blue fluorescence localized within the mitochondria was detected after only few minutes of exposure to HeLa cells. This process is reminiscent of what is reported for the Pt^{IV} prodrugs for which a two-electron reduction by GSH occurs to release the active drugs. Once reduction occurs, inhibition of TrxR in HeLa cells occurs with further activation of apoptotic caspases 3, 7, 9, and PARP (which indicate a direct or indirect DNA strand break). The occurrence of antimitochondrial effects is not only linked to cationic $Au^{I/III}$-NHCs. An impressive 300-fold increased cytotoxicity (versus Cisplatin) on HeLa cells is observed for the photoluminescent cationic Pt^{II}-NHC **4**, which colocalizes with mitotracker (Scheme 2).[6] This strong activity is ascribed to the survivin



Scheme 2. Metal-NHCs reacting on Survivin, TrxR, and AIF.

protein inhibition, an inhibitor of apoptosis, highly expressed in most cancers and associated with chemotherapy resistance. Recent results indicate that the action of metal-NHCs does not depend solely on the presence of a net positive charge. Indeed, it has been shown that heteroleptic Ag^{I}-NHCs possesses strong cytotoxic potential[1b,c] and it has been demonstrated that proapoptotic Ag^{I}-NHCs (**5**), localizing at the mitochondrion, provoke their membrane potential depolarization and a high ROS production.[7a] Interestingly, silver induces the death of cancer cells independent of the classical caspase cascade by translocation of AIF and caspase 12. Here, the exact cellular targets (proteins) remain to be determined, but a report indicates that Ag^{I}-NHCs display a more pronounced inhibition of TrxR than gold.[7b] Neutral the Au^{I}-NHC **2a** also provokes cell death by targeting mitochondria with ROS production and inhibition of TrxR as well as the neutral complex **6**, which is an effective and selective inhibitor of TrxR1 and 2 (TrxR1 > TrxR 2), which are localized in the cytosol and the mitochondria, respectively.[7c]

Thus, recent reports indicate that the anticancer activity of metal-NHC is not limited to cationic structures.

The efficiency of some metal-NHCs has been proven by in vivo tests. Although fragmentary, these important reports give a preliminary evaluation of their potential in cancer therapy. The Au^{I}-NHC **3** tested in nude mice (10 mg kg^{-1} week^{-1}), bearing hepatocellular carcinoma tumor (PLC), induces a significant 47% suppression of the tumor growth.[5] A silver complex showed no toxic effect to major organs at high doses (> 300 mg kg^{-1}), and cancerous-cell death was observed with OVCAR-3 xenografted mice.[8] Injected at 3 mg kg^{-1} in nude mice, the Pt^{II}-NHC **4** (R = Bu) significantly inhibits the non-small cells lung carcinoma (NCI-H460) tumor growth by 55% with no observed toxic side effects on healthy tissues.[6]

These recent investigations show that metal-NHCs affect mitochondria by inhibiting TrxT and other proteins. The possibility of using novel azolium precursors and various metals should offer interesting perspectives for the rapid and selective construction of libraries of high molecular diversity and complexity, and could be achieved by pre-[9a] or post-functionalization[9b–e] synthetic paths or by using the auto functionalization of Cu^{I}-NHCs bearing azides,[9f] thus providing a basis for the design and development of metal-based drugs associated with unprecedented biological targets.

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[1] a) C. G. Hartinger, N. Metzler-Nolte, P. J. Dyson, *Organometallics* **2012**, *31*, 5677 – 5685; b) A. Gautier, F. Cisnetti, *Metallomics* **2012**, *4*, 23 – 32; c) L. Oehninger, R. Rubbiani, I. Ott, *Dalton Trans.* **2013**, *42*, 3269 – 3264; d) I. Romero-Canelón, P. J. Sadler, *Inorg. Chem.* **2013**, DOI: 10.1021/ic400835n.
[2] S. J. Berners-Price, A. Filipovska, *Metallomics* **2011**, *3*, 863 – 873.
[3] a) E. A. Liberman, V. P. Topaly, L. M. Tsofina, A. A. Jasaitis, V. P. Skulachev, *Nature* **1969**, *222*, 1076 – 1078; b) S. Chalmers, S. T. Caldwell, C. Quin, T. A. Prime, A. M. James, A. G. Cairns, M. P. Murphy, J. G. McCarron, R. C. Hartley, *J. Am. Chem. Soc.* **2012**, *134*, 758 – 761.
[4] a) J. L. Hickey, R. A. Ruhayel, P. J. Barnard, M. V. Baker, S. J. Berners-Price, A. Filipovska, *J. Am. Chem. Soc.* **2008**, *130*, 12570 – 12571; b) R. Rubbiani, I. Kitanovic, H. Alborzinia, S. Can, A. Kitanovic, L. A. Onambele, M. Stefanopoulou, Y. Geldmacher, W. S. Sheldrick, G. Wolber, A. Prokop, S. Wölfl, I. Ott, *J. Med. Chem.* **2010**, *53*, 8608 – 8618; c) R. Rubbiani, S. Can, I. Kitanovic, H. Alborzinia, M. Stefanopoulou, M. Kokoschka, S. Mönchgesang, W. S. Sheldrick, S. Wölfl, I. Ott, *J. Med. Chem.* **2011**, *54*, 8646 – 8657.
[5] T. Zou, C. T. Lum, S.-Y. Chui, C.-M. Che, *Angew. Chem.* **2013**, *125*, 3002 – 3005; *Angew. Chem. Int. Ed.* **2013**, *52*, 2930 – 2933.
[6] R. Wai-Yin Sun, A. Lok-Fung Chow, X.-H. Li, J. J. Yan, S. Sin-Yin Chui, C.-M. Che, *Chem. Sci.* **2011**, *2*, 728 – 736.
[7] a) L. Eloy, A.-S. Jarrousse, M.-L. Teyssot, A. Gautier, L. Morel, C. Jolivalt, T. Cresteil, S. Roland, *ChemMedChem* **2012**, *7*, 805 – 814; b) A. Citta, E. Schuh, F. Mohr, A. Folda, M. L. Massimino, A. Bindoli, A. Casini, M. Pia Rigobello, *Metallomics* **2013**, *5*, 1006 – 1015; c) E. Schuh, C. Pflüger, A. Citta, A. Folda, M. P. Rigobello, A. Bindoli, A. Casini, F. Mohr, *J. Med. Chem.* **2012**, *55*, 5518 – 5528.

[8] D. A. Medvetz, K. M. Hindi, M. J. Panzner, A. J. Ditto, Y. H. Yun, W. J. Youngs, *Metal-Based Drugs* **2008**, 384010.
[9] a) M. Chtchigrovsky, L. Eloy, H. Jullien, L. Saker, E. Ségal-Bendirdjian, J. Poupon, S. Bombard, T. Cresteil, P. Retailleau, A. Marinetti, *J. Med. Chem.* **2013**, *56*, 2074 – 2086; b) J. Weaver, S. Gaillard, C. Toye, S. Macpherson, S. P. Nolan, A. Riches, *Chem. Eur. J.* **2011**, *17*, 6620 – 6624; c) E. Chardon, G.-L. Puleo, G. Dahm, G. Guichard, S. Bellemin-Laponnaz, *Chem. Commun.* **2011**, *47*, 5864 – 5866; d) E. Chardon, G.-L. Puleo, G. Dahm, S. Fournel, G. Guichard, S. Bellemin-Laponnaz, *ChemPlusChem* **2012**, *77*, 1028 – 1038; e) S. D. Köster, H. Alborzinia, S. Can, I. Kitanovic, S. Wölfl, R. Rubbiani, I. Ott, P. Riesterer, A. Prokop, K. Merz, N. Metzler-Nolte, *Chem. Sci.* **2012**, *3*, 2062 – 2072; f) C. Gibard, D. Avignant, F. Cisnetti, A. Gautier, *Organometallics* **2012**, *31*, 7902 – 7908.

