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PERSPECTIVE

Luminescent bioactive NHC-metal complexes to bring light into cells

Catherine Hemmert[a,b] and Heinz Gornitzka[a,b]

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Organometallic complexes are mostly used in catalytic applications and nowadays biomedical applications reach more and more attention. Until the last decade, research in the later area was focused on screening of complexes against cancer cell lines, bacterias or parasites. Since a couple of years mechanistic studies help to elucidate the mode of action of such complexes, one of the applied methods consits on studying cell uptake and innercellular distribution of luminescent bioactive complexes, in order to identify the main targets. This perspective summarizes the results obtained with luminescent bioactive NHC–metal complexes in this field of research.

Introduction

The discovery of cytotoxic effects of cisplatin in 1969 by Rosenberg and co-workers led to a small revolution in coordination chemistry.[1] However, in spite of their great success, administration of platinum complexes presents important drawbacks such as severe side effects (nephrotoxicity) and development of drug resistance, which limit their domain of applicability.[2] Another important problem for the platinum drugs is the low selectivity due to their mode of action: it is generally accepted that platinum anticancer drugs coordinate DNA in both normal cells and tumor cells.[3]

A lot of metal complexes have been developed and evaluated for their biological activities until now with new challenges as selectivity, low toxicity, less side effects, activities against resistant strains.

In parallel, Wanzlick and Öfele published in 1968 the first use of *N*-heterocyclic carbenes (NHCs) as ligands to coordinate transition metals.[4] NHCs represent one of the most important classes of ligands in organometallic chemistry. The easy preparation of NHC-precursors has allowed an almost infinite access to new organometallic complexes, in which the nitrogen atoms of the azoliums rings can be functionalized by numerous organic groups, organometallic moieties and biologic entities. NHCs are extremely good σ–donors, they make strong metal-carbene bonds, giving stable complexes in biological medium. So, it is not a surprise that numerous NHC-metal complexes show promising pharmacological properties and exhibit very interesting biological activities as reflected in several review articles.[5] Varying the properties of the NHC ligands and the nature of the metals (mainly Cu, Ag, Au, Ni, Pd, Pt and Ru), different diseases have been targeted, as fungi[5b,5i], bacteria[5b-d, 5i], cancer[5a-f,5h-k] and more recently parasites[6,7]. In order to better understand the biological properties of such complexes, mechanistic studies are required. For some metals, the discussion is limited to some main modes of action as for examples DNA-target for platinum, and thioredoxin reductase-(TrxR) and/or mitochondria-target for gold(I). However, for most biological active NHC-complexes, more than one target seems to be involved. Moreover, structure-activity relationships and mode(s) of action at the molecular level of such complexes remain unclear. For these reasons, mechanistic studies should be continued and all the available tools to obtain information must be explored. Modern fluorescence confocal microscopy techniques permit to follow luminescent molecules in cells to observe the cellular uptake and the intracellular distribution. This approach helps to identify targeted organelles and to give a first idea concerning the main modes of action involved in the biological activities of these molecules. In the case of metal-NHC complexes three types of luminescent complexes could be distinguished: bioactive complexes showing native luminescence and bioactive complexes becoming luminescent after addition of organic or organometallic chromophores. In the following we will present these three types of theranostic[8] NHC-metal-complexes.

Bioactive NHC-metal complexes showing native luminescence

The first example concerning luminescence studies of the intracellular distribution of biological active NHC-metal complexes has been reported by Baker, Berners-Price and co-workers in 2006.[9] This publication deals with the distribution of a dinuclear gold complex in RAW264.7 cells (a mouse leukemic monocyte-macrophage cancer cell line). The authors designed cyclic biscarbene ligands for the stabilisation of dinuclear gold complexes showing short metal-metal distances (see ***cis*-A** and

[a] CNRS, LCC (Laboratoire de Chimie de Coordination), 205 route de Narbonne, BP 44099, F-31077 Toulouse Cedex 4, France.
[b] Université de Toulouse, UPS, INPT, F-31077 Toulouse Cedex 4, France.
e-mail: heinz.gornitzka@lcc-toulouse.fr and catherine.hemmert@lcc-toulouse.fr, fax: +33 (0)561553003, tel.: +33 (0)561333161

***trans*-A** in Figure 1). The native luminescence of these complexes is probably due to aurophilic interactions.

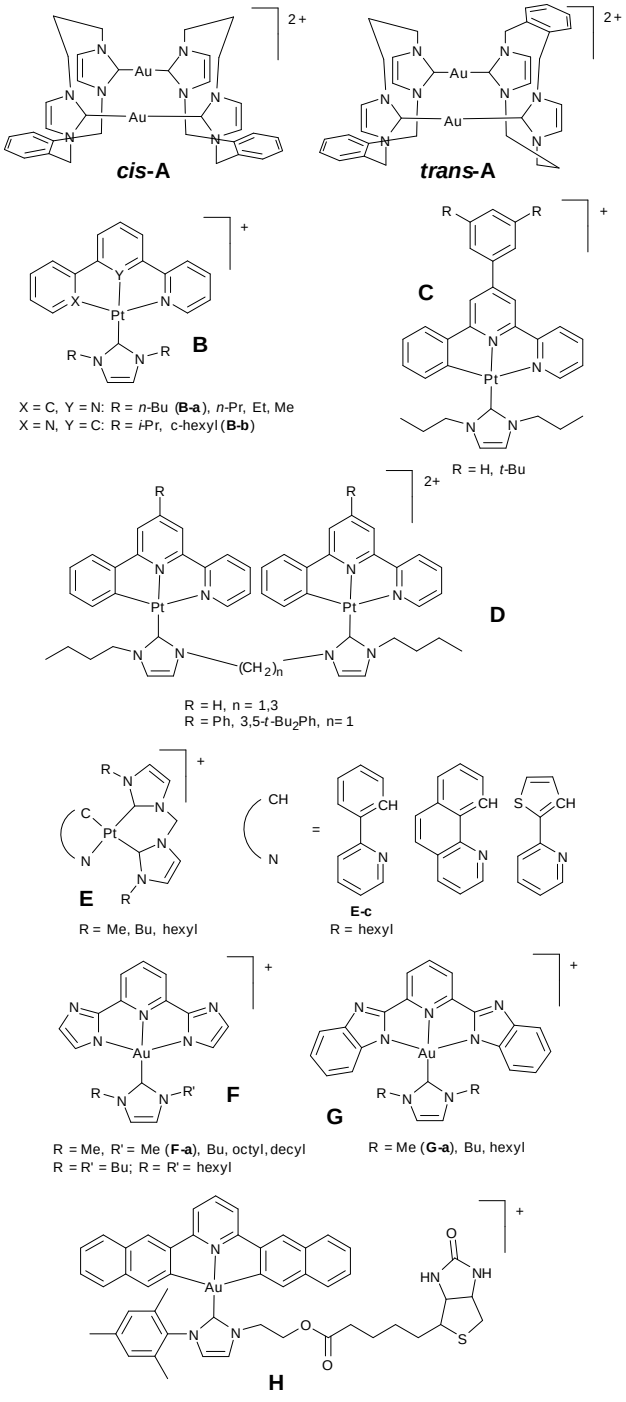

Figure 1: Examples **A** to **H** concerning bioactive NHC-metal complexes showing native luminescence.

Interestingly, two emission bands could be observed in aqueous solution with relative intensities depending on the excitation wavelengths used. One of the excitation and emission bands could be used for cellular distribution studies. Cells were incubated for 15 h with 200 µM (four times the IC_{50} value, 52 µM, 48 h incubation) at 37 °C. Excitation at 351 nm gave an emission at 496 nm and a punctuated pattern of luminescence indicated the presence of the complexes in cell organelles. Comparison with brightfield images permitted to exclude cell nuclei as targets. Moreover, co-localization studies using LysoTracker red and MitoTracker green provided evidence for localization of the gold complexes within lysosomes rather than mitochondria (see Figure 2).

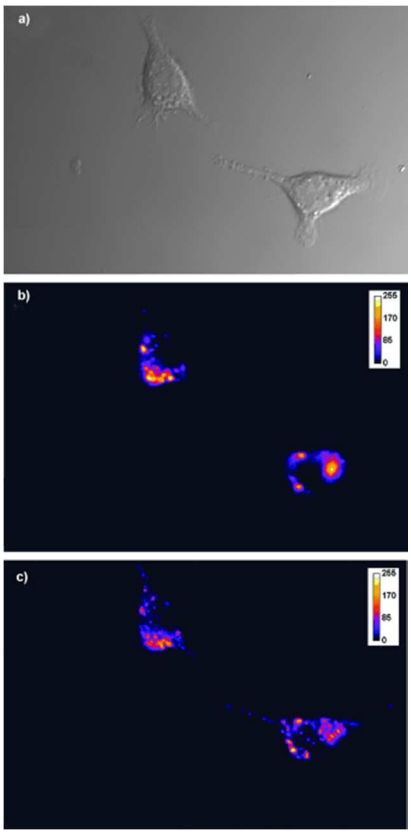

Figure 2: a) Brightfield image, b) luminescence image showing ***cis*-A** distribution and c) luminescence image showing LysoTracker red distribution. Reproduced from Ref. 9 with permission from John Wiley and Sons.

A large contribution in this field of research has been given by the group of Che. In 2011, this group published luminescent cyclometalated platinum(II) complexes containing NHC ligands.[10] Three families of mono- and dinuclear $[(C^N^N)Pt(II)(NHC)]^+$ complexes (HC^N^N = 6-phenyl-2,2'-bipyridine, see **B** (R = *n*-Bu, *n*-Pr, Et and Me), **C** and **D** in Figure 1) were synthesized and their photopysical properties have been studied. Among them, complex **B-a** shows very potent *in vitro* (with IC_{50} ranging from 0.057 to 0.77 µM on three different tumoral cell lines) and *in vivo* anticancer activity, and could significantly inhibit tumor growth in the nude mice model. Furthermore, these complexes show a high selectivity towards cancer cells in comparison to normal cells (IC_{50} value is 232 folds higher for normal cell derived human lung fibroblast cell line CCD-19Lu (IC_{50} value 11.6 µM, 48 h) than for cervical epithelioid carcinoma HeLa cells (IC_{50} value 0.057 µM, 48 h). For the studies on the fluorescence microscope HeLa cells were treated with 1 µM of **B-a** for 1 h. After excitation at 350 nm, the complex emitted at 545 nm and majority of **B-a** was found to co-localize

with MitoTracker (for mitochondria and cytoplasmic structure), no co-localization with DNA binder Hoechst 33342 or LysoTracker (for lysosomes) have been observed (see Figure 3). Thus, the authors suppose that DNA is unlikely to be the primary target of **B-a**.

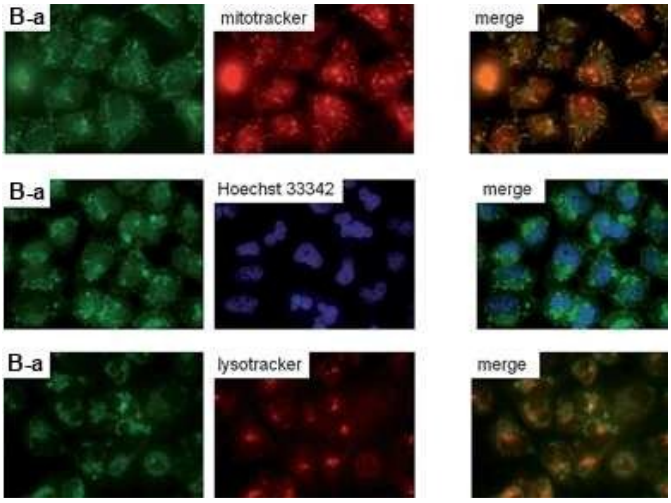

Figure 3: Fluorescent microscopic examination of B-a in the same batch of HeLa cells either co-incubated with MitoTracker (top), Hoechst 33342 (middle) or LysoTracker (bottom), showing that the majority of **B-**a can be co-localized with MitoTracker. Adapted from Ref. 10 with permission from The Royal Society of Chemistry.

Very recently, Che and co-workers published some new cationic NHC-Pt(II) complexes of type (N^C^N)Pt(II)(NHC) (for some examples see **B**, R = *i*-Pr, c-hexyl in Figure 1), which show rich photophysical properties combined with interesting cytotoxic activities against HeLa cells.[11] The most active one of this series, namely **B-b**, gave an IC_{50} value of 0.46 µM for 72 h. The intense emission of the $[(N^C^N)Pt(II)(NHC)]^+$ complexes allowed monitoring of cellular uptake using fluorescence microscopy. For this, HeLa cells were treated with 2 µM of **B-b** and green emission (λ_{ex} = 340 nm, λ_{em} = 540 nm) could be observed in the cytoplasm after 10 min.. This observation was very close to the results obtained in 2011 by the same group. In both cases, localization in the nucleus could be excluded using the nuclei specific dye Hoechst 33342. However, experiments with mitochondria specific MitoTracker revealed the absence of accumulation of **B-b** in mitochondria. Nevertheless, significant mitochondria disruption/swelling was identified. In 2013, the same group described a panel of luminescent Pt(II) complexes bearing bis(NHC) ligands on one side and anionic C^N ligands (HC^N = 2-phenylpyridine, benzo[*h*]quinolone and 2-(thiophen-2-yl)pyridine) on the other side (see **E** in Figure 1).[12] Cytotoxicity has been tested *in vitro* on seven human carcinoma cell lines. In order to examine the subcellular localization, HeLa cells were treated with 5 µM of **E-c** (IC_{50} = 0.68 µM, 72 h) and after 10 min. the sample was irradiated at 340 nm and a strong green emission around 510 nm could be observed in the cytoplasm. Localization in the endoplasmic reticulum (ER) domain has been evidenced using an ER-Tracker. No significant co-localization could be found with lysosome-specific LysoTracker and mitochondria-specific MitoTracker. Supplementary experiments permitted to conclude that the cytotoxicity of this kind of complexes is due to apoptosis induced by ER-stress and mitochondrial dysfunctions. Also in this case a DNA independent cell death pathway is favoured.

The same year, this group presented cyclometalated gold(III) NHC complexes of type $[(N^N^N)Au(III)(NHC)]^+$ ($H_2N^N^N$ = 2,6-bis(imidazo-2-yl)pyridine (H_2IPI) and 2,6-bis(benzimidazo-2-yl)pyridine (H_2BPB), see **F** and **G**, respectively in Figure 1).[13] The protonated forms of the two ligands are strongly luminescent. The corresponding Au(III)-NHC complexes are sensitive towards thiols, which leads to their reduction to cytotoxic Au(I)-NHC complexes. While the complexes are not luminescent, their degradation leads to the release of the fluorescent $H_2N^N^N$ proligands. Thus, this system could be used as "switch-on" luminescent probe. For intracellular fluorescence studies, HeLa cells have been incubated with 20 µM solution of **F-a** (IC_{50} = 14.4 µM, 72 h). After 10 min., significant blue fluorescence at 420 nm (λ_{ex} = 365 nm) was detected in the cytoplasm, but not in the nucleus or extracellular environment. A significant portion of the fluorescence signal was localized in the mitochondria using MitoTracker red. These results are in good agreement with the often discussed target mitochondrial TrxR for Au(I)-NHC complexes. Indeed, with respect to the possible mechanisms of action, the antiproliferative effects of gold NHC complexes have been shown to be mediated by strong antimitochondrial effects *via* inhibition of the selenoenzymes TrxRs, involved in maintaining the redox homeostasis of cells.[14] In order to significantly decrease the high sensitivity of these gold complexes by oxygen quenching in biological medium and consequently the lifetime of phosphorescence, the group of Che designed a luminescent cyclometalated gold(III) complex forming a conjugate with avidin (a tetrameric or dimeric biotin-binding protein) that possesses anti-proliferation capacity.[15] For this, a biotin moiety was incorporated in a NHC ligand taking part of a $[C^N^C)Au(III)(NHC)]^+$ complex **H** (see Figure 1). The biotin unit permitted the formation of an **H-avidin** conjugate showing 4 fold increased emission in open air compared to **H**. Both complexes, **H** and **H-avidin**, show cytotoxicity with IC_{50} values in the low micromolar range after 48 h against HeLa, human liver cancer cells HepG2 and human breast cancer cells MDA-MB-231 cell lines. In order to study intracellular distribution, HeLa cells have been treated for 4 h with 10 µM of **H-avidin** (IC_{50} = 1.1 µM, 48 h). Excitation at 488 nm led to emission at >505 nm and the conjugate was observed to be located in the cytoplasm with negligible nuclear uptake. At last, Che and collaborators reported a family of luminescent cyclometalated gold(III) complexes containing a *cis*-chelating bis-NHC and a bidentate C-deprotonated C^N ligand. These complexes display emission in acetonitrile solutions with λ_{em} ranging from 498 to 633 nm. Moreover, one water-soluble complex of this series (see **I** in Figure 4), shows a significant *in vitro* inhibitory activity (IC_{50} = 0.15 µM) towards deubiquitinase (DUB) UCHL3, which constitutes a potential anti-cancer target for gold(III) complexes.[16]

In 2013 Veige and co-workers developed a chiral dinuclear gold(I) 18 membered metallamacrocycle involving bis(NHC) ligands (**J(+/-)** in Figure 4), in order to determine if cell-selectivity could be improved by employing a chiral system.[17]

The cytotoxicity of both chiral **J(+)** and **J(-)** and racemic **J(+/-)** complexes has been tested against healthy human bronchial epithelial cells HBE 135-E6E7 and human embryonic kidney cells HEK 293 and two cancerous cell lines, NCI-H23 and HeLa. The study revealed moderate activity for all complexes with IC_{50} ranging from 2.28 to 14.35 µM and no significant difference in cytotoxicity between the chiral and the corresponding racemic systems, and no selectivity between cancerous and normal cells. The native luminescence of these complexes (λ_{ex} = 488 nm, λ_{em} = 615 nm) permitted to determine if the molecules penetrates into the cells. After 3 h incubation time on HeLa cells, **J(+/-)** remained near the cell membrane and after 20 h it was visibly observed near the nucleus.

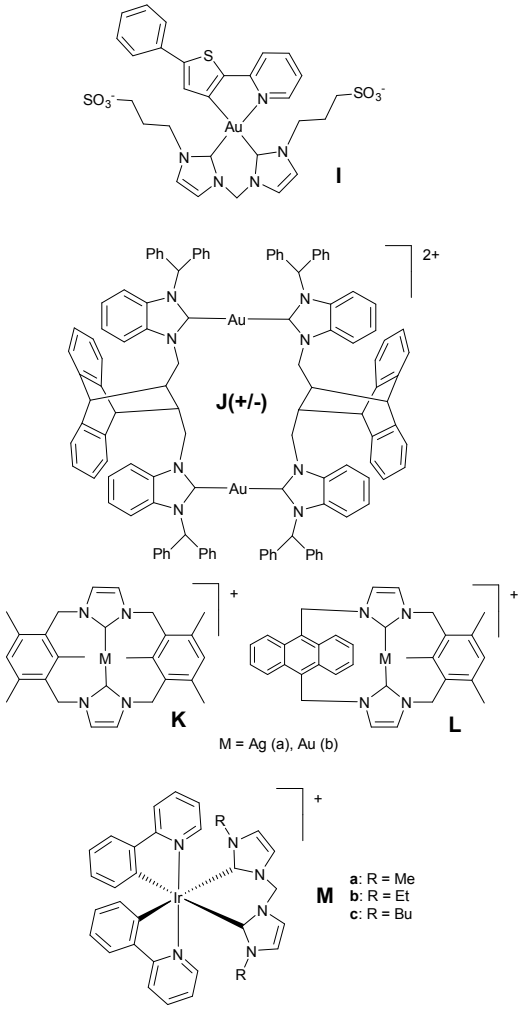

Figure 4: Examples **I** to **M** concerning bioactive NHC-metal complexes showing native luminescence.

In 2014 Liu, Mao and co-workers published four Ag(I)- and Au(I)-NHC complexes containing NHC ligands derived from imidazolium-linked cyclophanes (**K** and **L** in Figure 4).[18] In two of these complexes fluorescent anthracene groups are incorporated in the cyclic bis(NHC) ligands (**L**). Proligands and complexes have been tested *in vitro* against HeLa, human lung adenocarcinoma epithelial A549, the corresponding cisplatin-resistant strain A549R, human breast carcinoma MDA-MB-231 and human normal liver LO2 cell lines. The gold complexes exhibit higher activities than the silver complexes and all complexes show moderate selectivity between cancerous and normal cells. For mechanistic studies HeLa cells were incubated during 1 h at 37 °C with 50 µM of **L-a** (IC_{50} = 12.4 µM, 48 h) or with 15 µM of **L-b** (IC_{50} = 4.5 µM, 48 h). The samples have been excited at 405 nm and blue emission between 400 and 450 nm has been observed on the confocal microscope. Co-localization analysis with organelle-specific stains, including MitoTracker green and LysoTracker green, showed that **L-a** and **L-b** were localized in mitochondria and not in lysosomes. Based on these results, deeper ingoing mechanistic studies focused on a mitochondrial pathway were performed and permitted to evidence differences in the modes of action between the silver and the gold comlexes. Silver complexes mainly induce early apoptosis *via* a caspase and ROS (reactive oxygen species) independent pathway while the most aqueous stable gold complexes induce both early and late apoptosis through a caspase dependent pathway (activation of caspase-3/7).

In 2015 Mao and co-workers presented a rare example of Ir(III) complexes in this domain. Three cyclometalated Ir(III) complexes (**M** in Figure 4) containing bis(NHC) ligands have been explored as theranostic and photodynamic agents.[19] The complexes have been tested *in vitro* against five human cancerous (HeLa, A549, A549R, HepG2 and mammary carcinoma MCF-7) and normal human liver (LO2) cell lines. All complexes show activities with IC_{50} values in the micromolar range and selectivity up to 10:1 between cancerous and normal cells. In **M** one 2-phenylpyridine (ppy) from the strongly luminescent $Ir(ppy)_3$ complex is replaced by a neutral bis(NHC) ligand, resulting in a cationic Ir(III) complex displaying also rich photophysical properties, perfectly adapted for the study of their intracellular fate (λ_{ex} = 405 nm, λ_{em} = 520 nm). HeLa and A549 cells have been incubated for 20 min. with MitoTracker red and then for 10 min. with 20 µM of **M-a** to **M–c**. This study shows clearly localization of complexes **M** in mitochondria. A real-time tracking of mitochondria in HeLa cells stained with **M-c** at 37 °C for different time intervals illustrated the quick (**M–c** is effectively taken up by HeLa cells after 1 min. incubation) and efficient (swollen mitochondria can be observed after 25 min. treatment) cell uptake of these complexes. Moreover, these complexes can act as efficient photosensitizers; the cytotoxicity of **M** against cisplatin-resistant A549R cells is increased by 279- to 3488-fold upon irradiation at 365 nm. At this stage, we can also mentioned the work of Zhou and collaborators who reported luminescent non-cytotoxic (IC_{50} values ranging from 50 to > 200 µM towards HeLa and A549 tumoral cell lines) iridium(III) complexes, containing two NHC and an N^O or N^N ancillary ligand, as living cell imaging reagents.[20]

In all the cases described in this section, the native luminescent properties of the bioactive complexes have been exploited using fluorescence microscopy experiments.

In the following paragraph, organic dyes have been added to bioactive NHC-metal complexes in order to drive them luminescent.

NHC-metal complexes with organic chromophores

In 2012 Cresteil, Roland and coll. synthetized and studied the anticancer activity of 14 NHC-silver(I) complexes on several types of human cancer cell lines (MCF-7, colon adenocarcinoma HCT116 and HCT15, promyelocytic leukemia HL60, 72 h) and drug-resistant cell lines (MCF-7R and HL60R, 72 h).[21] For the most active ones with IC_{50} values in the nanomolar range, further mechanistic investigations have been performed. It has been shown that these complexes induce cell death *via* an apoptotic process. A modification of mitochondrial membrane potential has been evidenced, no overproduction of ROS and no effect on cell cycle could be observed. Furthermore, caspases 3 and 7 were not activated, while a translocation of Apoptosis Inducing Factor (AIF) and caspase-12 from mitochondria to the nucleus has been shown. Unfortunately, none of these complexes are luminescent and thereby the authors chose a silver complex involving a pyrene substituted NHC (IC_{50} = 0.42 µM on MCF-7; see **N** in Figure 5), very similar to the active ones for intracellular localization studies. For this, HL60 cells have been treated with 1 µM of **N** for 24 h. Excitation of **N** at 360 nm led to emission at 500 nm and co-localization with MitoTracker red demonstrated that mitochondria are effectively targeted by the silver-NHC complex **N**.

In 2013 Mohr, Casini, Rigobello and co-workers attached a fluorescent anthracenyl group at N1 position of a NHC ligand (see **O** in Figure 5).[22] The neutral NHC-AgCl and NHC-AuCl complexes have been studied for their anticancer activities, their selectivity and their intracellular distribution. Both complexes have been tested against cisplatin sensitive A2780S and resistant A2780R human ovarian cancer cell lines and on normal human embryonic kidney cells HEK-293T with IC_{50} values ranging from 3 to 7 µM after 24 h for both complexes. While the silver complex shows a very moderate selectivity between cancer and non-tumorigenic cells, the gold complex gives no selectivity. Mechanistic studies revealed that both complexes inhibit the two major isoforms of the selenoenzyme TrxR, namely the cytosolic TrxR1 and the mitochondrial TrxR2, the silver complex being more effective than the gold one. Furthermore, dimerization of peroxiredoxin 3 (Prx3) by formation of disulfide bonds was observed, proving the ability of these complexes to reach the mitochondrial target. Indeed, one of the major antioxidant role of Trx is to reduce Prxs. In order to understand the subcellular distribution of theses NHC-metal complexes and to gain further mechanistic insights, all three tested cell lines were treated with the luminescent silver or gold complexes (10 µM for Ag or 20 µM for Au) for 1 h. Moreover, propidium iodide was used as nuclear marker. Fluorescence experiments (λ_{ex} = 405 nm, λ_{em} = 425 nm) on the microscope showed that both complexes accumulate at the nuclear level and the authors conclude that DNA is not the expected target for silver(I) and gold(I) complexes, which most likely interact with proteins such as TrxR1 and TrxR2, also present in the nuclear compartment.

In 2014 Bodio, Casini and co-workers have synthetized three *N*-heterocyclic carbene gold(I) complexes involving a coumarin unit, two of them bearing 1-thio-β-D-glucose-type ligands, and assessed their *in vitro* antiproliferative activity against three human tumoral cell lines (ovarian cancer A2780, mammary carcinoma MCF-7, and lung cancer A549) along with non cancerous human embryonic kidney HEK-293 cells, by using the classical MTT test.[23] The complexes show moderate activity with IC_{50} ranging from 11.6 to > 100 µM and no selectivity. Preliminary mechanistic studies demonstrated that the complexes are able to inhibit the cytosolic and mitochondrial TrxRs, probably by binding the selenocysteine residue in the active site. Using fluorescence confocal microscopy, the uptake of the most active complex **P** (see Figure 5) was evaluated by treating A2780 cells with 20 µM of the complex at 37 °C for 2 h. Fluorescence images obtained at 461 nm after excitation at 358 nm show that cell viability is maintained despite the high concentration of the complex, but it accumulates in the nucleus (co-localization with propidium iodide staining), which seems to be very similar to previously results discussed above.

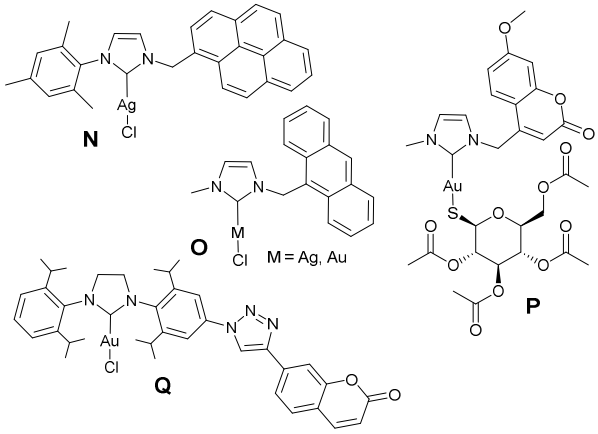

Figure 5: Examples **N** to **Q** concerning bioactive NHC-metal complexes with organic chromophores.

Gautier, Cisnetti and coll. recently reported an "auto-click" process leading to a family of Cu(I)- and Au(I)-NHC complexes functionalized by 1,2,3-triazoles bearing diverse substituents, including in particular a fluorescent coumarin moiety (see **Q** in Figure 5).[24] The uptake and intracellular distribution of the fluorescent gold(I) complex was investigated in human prostate cancer PC3 cell line by incubating for 18 h at a complex concentration of 10 µM. After excitation at 365 nm, a significant blue fluorescence at 397 nm was detected in the cytoplasm but not in the nucleus. The use of a MitoTracker red allows to show co-localization of the complex in mitochondria. Although, no antiproliferative activity was related for these complexes, this example demonstrates an interesting and easy method to attach a dye to active complexes.

NHC-metal complexes with organometallic chromophores

Another way to drive a bioactive molecule luminescent consists in the attachment of an organometallic dye. For this type of complexes only one example is reported for NHC-metal complexes. Hemmert, Gornitzka and co-workers published in 2015 a family of heterobimetallic complexes, based on the fusion of a bioactive gold(I)-NHC unit and a luminescent Ru(II)(bipy)$_3$ moiety (see **R** in Figure 6).[25] These complexes show no or low activity against parasitic diseases, namely *Leishmania infantum* and *Plasmodium falciparum* and moderate antiproliferative activity against human hepatocellular carcinoma cancerous cells Hep3B with IC_{50} comprised between 17 and 31 µM at 48 h (IC_{50} value of the reference molecule Sorafenib = 7.2 µM). It has to be pointed out that the gold-free ruthenium-imidazolium precursor show no activity against Hep3B cells with IC_{50} values higher than 100 µM, demonstrating the biological role of the gold moiety. On the other hand, the cationic bis(NHC)-gold(I) complex, involving the bipyridine-NHC ligand with R = methyl, has shown a good antimalarial activity against the *P. falciparum* strain FcM29-Cameroon with an IC_{50} = 0.33 µM.[7]

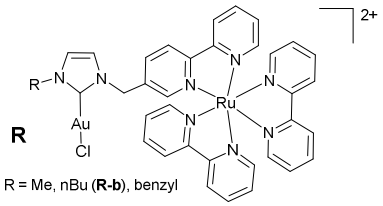

Figure 6: Example **R** concerning bioactive NHC-metal complexes with an organometallic chromophore.

The most active complex **R-b** has been used to undertake fluorescence microscope experiments. For this, Hep3B cells have been treated with 10 µM of **R-b** and the luminescence (λ_{ex} = 458 nm, λ_{em} = 620 nm) has been studied directly after treatment in order to get some information about the cell uptake, and after 24 h, 48 h and 72 h to see the intracellular distribution. The series of images taken directly after cell treatment show cell-membrane labelling followed by fast cell uptake. The images at 24, 48 and 72 h gave very similar information concerning the inner cell distribution: **R-b** was localized in the cytoplasm. Experiments using MitoTracker green for mitochondria labelling and DAPI for nuclei labelling showed that no co-localization could be observed in mitochondria or in the nucleus (see Figure 7).

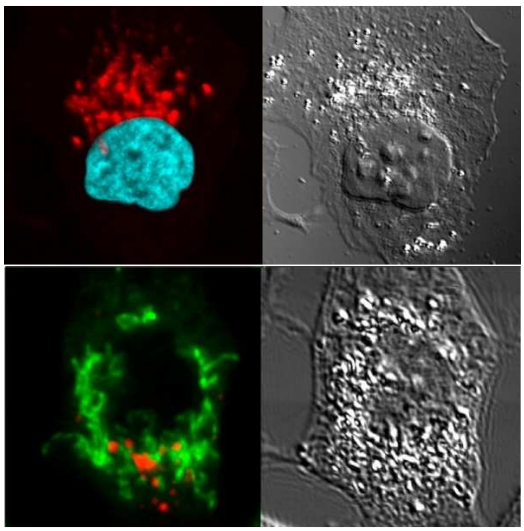

Figure 7: Fluorescence and transmission images of the cells treated with **R-b** (in red) and labeled with MitoTracker (in green, bottom) or DAPI (cyan, top). Reprinted with permission from Ref. 25. Copyright 2015 American Chemical Society.

Similar results have been obtained with the corresponding gold free Ru-imidazolium precursor of **R-b**. Furthermore, the difference of 20 nm between the emission spectra of the mixed metal Au-Ru complex and the gold-free ruthenium precursor (λ_{em} = 640 nm) has been used to evidence the slow degradation of the gold complex in the cells.

Conclusion and perspectives

The combination of biological activity and photophysical properties became a helpful tool to better understand the modes of action of bioactive molecules. Concerning the photophysical properties Baker and Berners-Price stated "importantly, the lower energy excitation and emission bands are of suitable wavelengths for cellular distribution studies".[9] In the field of NHC-metal complexes, studies of such theranostic agents have started only ten years ago with antitumoral complexes. Some copper, silver, gold, platinum and iridium complexes have been used to elucidate their cellular uptake and intracellular distribution, providing important information concerning modes of action. Some problems must be overcome: interactions of the active molecules with biomolecules (as DNA, enzymes, peptides, etc…) or other added analytic molecules (MitoTracker, LysoTracker, DAPI, …) could deactivate the luminescence leading to false conclusions; attached dyes cannot only change the biological activities, they can also have an influence regarding the target(s). One approach consists in the development of systems containing both, luminescent complexes and luminescent ligands emitting at different wavelengths after excitation. In this case the active complexes and the free ligands formed after release of the metals could be localized in cells, which could help to identify without doubt the real place of action and to detect the biological cellular targets. Another approach could be the use of a luminescent carrier. Sun, Li and collaborators reported very

recently mono and dinuclear gold(I) complexes (see **S** in Figure 8) with IC_{50} values ranging from 0.83 to 22 µM at 72 h on sensitive A2780S, resistant A2780R, HepG2, and human glioblastoma U-87 MG cancer cell lines, and a normal Madin-Darby canine kidney (MDCK) epithelial cell line.[26] The most active gold complex **S-b** is not luminescent in solution but it has been shown that it forms a host-guest adduct with a luminescent zinc(II)-based metal-organic framework (Zn-MOF); the biodegradable blue-fluorescent Zn-MOF becomes green-luminescent by inserting the gold-complex. The Zn-MOF carrier is too large to penetrate into the cancer cells but shows a slow release of the cytotoxic complex. This idea of using a luminescent carrier without changing the active complex could also be a promising approach to follow molecules in cells.

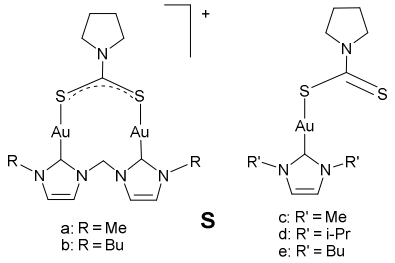

Figure 8: Example **S** concerning bioactive NHC-metal complexes used with a luminescent carrier.

Another problem is based on the fact that, currently, we localize active molecules by using luminescence in living cells. This is used to gain information about the modes of action leading to cell death. It would be better to do the same by real time monitoring on dying cells, starting with living cells and observing the inner cell distribution of the active complexes and free ligands (or proligands) during cell death.

Acknowledgements

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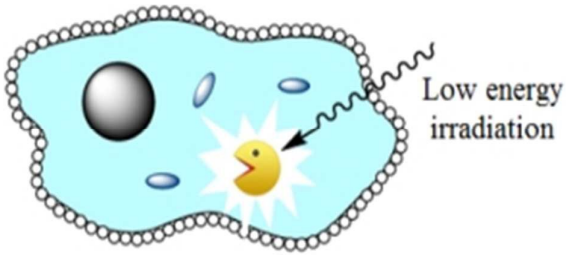

This review provides an overview of the state of the art in the field of theranostic anticancer luminescent organometallic complexes.