N-heterocyclic carbene platinum complexes: a big step forward for effective anti-tumor compounds

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Abstract: The discovery of cisplatin is an important milestone in the history of successful anti-cancer drugs. This is also a notable example of the translational scientific achievement that remains a driving force in multidisciplinary research. The clinical success of cisplatin has led chemists to vary the ligands around the platinum metal to improve cisplatin's effectiveness while reducing its side effects. While there have been more failures than successes, key developments in the elucidation of mechanisms of the tumor-resistance properties have been accomplished. N-Heterocyclic carbene as ligand for organometallic chemistry is a relatively young area that offers new opportunities in many fields including bio-inorganic chemistry. These ligands fit the prerequisites for metal drug development and recent achievements have demonstrated the great potential of these compounds. The properties of these metal NHC complexes also allow for easy post-functionalization, thus enabling molecular diversity for efficient drug design. Overall, N-heterocyclic carbene ligands reveal new opportunities for Pt drug development.

1. Introduction

Cancer is the spontaneous development of genetically abnormal cells known as tumors which can affect any part of the body. These tumors can cause serious health problems and today cancer is the second leading cause of death after cardiovascular diseases. Comprehensive cure or remission of cancer still face many key challenges, although increasingly more effective and better-targeted treatments are now available.

Nevertheless, the scarcity of effective anti-cancer drugs remains evident and cancer may soon be the leading cause of death in developed countries. Cancer treatments currently have the lowest clinical trial success rate of all major diseases. Cancer presents difficult challenges for which combining existing approaches with new approaches could help address this health problem.^[1] Such objective will be achieved by intensifying research on the main forms of cancer treatment, which are surgery, chemotherapy, radiotherapy and hormone therapy, and by supporting advances in novel emerging treatments.

Chemists have an important role to play in the fight against cancer and it started with the discoveries made by German chemist, Paul Ehrlich (in the early 1900s), who was the first to use the term 'chemotherapy' and defined it as the use of chemicals to treat a disease.^[2] However, a certain pessimism concerning the ability of

chemicals to cure cancer remained until the 1960s and 1970s.^[3] The first positive results showing the ability to cure acute childhood leukemias and Hodgkin's disease with combination chemotherapy (i.e. the use of more than one chemotherapy medication) have reversed these trends.^[4] Significant successes have then been achieved by neoadjuvant chemotherapy (i.e. chemotherapy administered before surgery to reduce the size of the tumor and facilitate more a effective surgery, for example) or adjuvant chemotherapy (i.e. therapy given in addition to the primary therapy). The mortality rate started to decline in late 1980s and has continued to decline each year. With the increasing development of novel cytotoxic agents, it is expected that the list of cancers effectively treated and cured using combined therapies will continue to grow.

Since its approval 40 years ago,^[5] cisplatin has become an important drug for the chemotherapy treatment of several cancer types, in particular ovarian, lung, testicular and bladder cancers, and also lymphoma, myeloma and melanoma, mostly in combination with a wide range of other drugs.^[6,7] However, cisplatin demonstrates severe side effects which greatly limit the effectiveness of the drug. In addition, some patients show resistance to these drugs and others develop drug resistance during the treatment.^[8] This has prompted inorganic chemists to synthesize and bioassess thousands of Pt analogs with the hope of reducing the toxicity of the compound but also to overcome resistance. Overall, there has been more failures than successes and only two compounds (i.e. carboplatin and oxaliplatin) have gained international approvals.^[9,10] Therefore, recent strategies are moving towards promoting the effective release of these Pt compounds into the tumor rather than synthesizing new Pt complexes. For example, platinum(IV) analogs of cisplatin have gained interest in that sense, because of their kinetic inertness that lower reactivity and potential side reactions.^[11]

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In fact, all approved platinum drugs have an identical basic chemical structure which consists on amine ligands, chlorides, sulfates or carboxylates. It is therefore not surprising that synthesized Pt analogs behave the same way and only few came out to approval. Moreover, such compounds usually suffer from low stability under physiological conditions, interacting with various reactive functions *in vivo* and often resulting in toxic side effects. A successful strategy in improving complex stability is by incorporating strong donor ligands -such as N-heterocyclic carbene ligands (NHC)- which generate highly stable metal-ligands bonds even in the presence of reactive functions.

N-heterocyclic carbenes have emerged as a privileged class of metal ligand due to their attractive features.^[12] They allowed generating robust complexes that were employed as highly efficient catalysts for example.^[13] More recently, they have been considered with success, for other applications including medicinal chemistry.^[14] Since the first report on the anticancer properties of gold(I) NHC complexes in 2004,^[15] there has been a keen interest in NHC metal complexes for biological applications. Au, Ag, Pt, Pd, Cu, Ru or Ir NHC complexes have been investigated for the development of metallodrugs. Moreover, these organometallics are, usually kinetically stable, relatively lipophilic, usually uncharged and they fit prerequisites for easy optimization (i.e. ease of derivatization). Nowadays it is one of the most active area of bioorganometallic chemistry and several comprehensive reviews can be found in the literature.^[16,17]

However, preclinical and clinical developments for such new molecules still faces multiple challenges. In particular, a major barrier for organometallic compounds remains their poor water solubility, so that organic solvents -usually dmsO- are still needed to ensure biological activity. Greater understanding of the mechanisms of action is also required.

Such challenges may be overcome by strategies of *post-functionalization* of the molecules by enabling enhanced molecular structure diversity and also conjugation of biomolecular species. Indeed, the high stability of the metal NHC complexes and Pt in particular offers various possibilities of post-functionalization. This microreview provides a brief overview of the chemistry of N-heterocyclic carbene platinum complexes in medicinal chemistry and covers a personal account on our contribution in finding suitable NHC Pt candidates to fight cancer. In particular, we focus on the possibilities of chemical modifications using various post-functionalization strategies.

2. Platinum NHC complexes exhibit anticancer activities

The first report on the anticancer properties of metal NHC complexes was on gold metal in 2004 (Figure 1).^[15] Soon afterwards, the first group 10 metal NHC complexes was reported on palladium metal.^[18] The authors established the potent anticancer activity of a *bis*(NHC) palladium complex of general formula (NHC)₂PdCl₂ with IC₅₀ up to 0.8 μM on HCT116 cancer

cells. Surprisingly, during that period, NHC platinum complexes have been neglected. Only in 2010, Marinetti and collaborators first established the cytotoxic activity of the *trans*-configured platinum complexes (NHC)PtX₂(amine), with IC₅₀ at the micromolar range.^[19] They demonstrated their anti-cancer efficiency against both cisplatin-sensitive and cisplatin resistant cell lines by *in vitro* experiments.

Figure 1. Molecular structure of the first gold, palladium and platinum anti-cancer NHC complexes.

Marinetti's complexes, which are Pt(II) square planar compounds combining a N-heterocyclic carbene and a neutral ligand (typically neutral amine) with two halides in *trans* position soon proved to be an optimal platform for further development in medicinal chemistry (Figure 2). The N-heterocyclic carbene moiety offers easy modification at the R¹-R⁴ positions. In addition, the neutral ligand L provides diversity.

Figure 2. General molecular structure of the NHC Pt complexes.

The ease of access of these complexes and their diversity has enabled a rapid growth in the field, and the literature is now full of promising examples. As proof of their diversity, figure 3 shows selected examples of such platinum NHC complexes.^[20] These complexes usually display IC₅₀ values in the micromolar range against various cancer cell lines, even against cisplatin-resistant cell lines.^[21] Screening of the substituents on the carbene even allowed to identify complexes having biological activities with IC₅₀ below 0.01 μM.^[22]

Figure 3. Selected chemical structures of Pt(II) NHC complexes with anticancer activities.

3. Direct functionalization for diversity enhancement

However, evaluating the anti-cancer activity of these compounds by *in vitro* cell viability measurements constitutes the first step in a long way to demonstrate their efficacy *in vivo*. In particular, they must be inert with respect to their biological environment before reaching the target cancer cells. They must also exhibit a high selectivity towards cancer cells. Active targeting by binding the Pt compound to a suitable ligand that has a high affinity for a specific receptor is therefore necessary.^[23] Different optimizations are needed to address such issues. All requirements should be pre-evaluated and also modulated by means of simple and highly modular synthetic pathways.^[24] Reactions that modify biomolecules to generate bioconjugates are widely studied with various applications found in imaging, biomarkers, drug delivery, revealing enzyme functions, etc.^[25] On the other hand, the post-functionalization of coordination compounds has long been understudied because it was thought that complexes were incompatible with chemical transformations by nature.^[26] However, chemical modification of a preformed metal complex provides a divergent access to generate potentially large family of new complexes. This concept is being revisited in part because of the success of click chemistry and the copper-catalyzed azide-alkyne cycloaddition reaction. The post-functionalization of platinum N-heterocyclic carbene complexes is possible through various synthetic strategies that are listed below.

3.1. Azide-alkyne cycloaddition of the N-heterocyclic carbene backbone

Azide-alkyne cycloaddition has extensively been studied in recent years as a remarkably efficient tool to assemble building blocks.²⁷ We believe that such reaction could be a solution of choice for emerging new class of cytotoxic molecules. This is supported by the high kinetic stability of platinum(II) NHC complexes. However, the copper-catalyzed alkyne-azide cycloaddition (CuCAAC) was found incompatible with the presence of group 10 transition metal complexes containing halide ligands.^[28] Alkyne-functionalized

NHC platinum complexes do not undergo azide-alkyne cycloaddition in the presence of copper but rather result in decomposition products thereof (Figure 4a). Indeed, the method for coupling terminal alkynes with aryl halides involves the formation of palladium acetylide catalyzed by copper(I) species.^[29] An investigation of the product of the reaction revealed the presence of insoluble orange/brown solids that have been assigned to metallopolymers.³⁰

Figure 4. a) Incompatibility of CuCAAC with alkyne-functionalized NHC platinum or palladium complexes. b) Post-functionalization with ruthenium catalyst $\text{Cp}^*\text{RuCl}(\text{PPh}_3)_2$.

Fortunately, ruthenium was compatible and the attempt with the $[\text{Cp}^*\text{RuCl}(\text{PPh}_3)_2]$ catalyst (originally reported by Fokin and co-workers^[31]) gave the expected 1,5-triazole product (Figure 4b). Further investigation proved the efficiency of the ruthenium catalyst to conduct RuCAAC in presence of NHC platinum complexes and also NHC palladium to some extent.^[32] Unfortunately, attempts to extend the methodology on ruthenium(II) or rhodium(I) NHC complexes were unsuccessful.^[33] Figure 5 shows examples of Pt NHC complexes that have been obtained by RuCAAC.^[30,32] The cycloaddition products **10** and **11** were isolated in 82% and 59%, respectively. The oestrogen-functionalized platinum complex **12** was designed as a possible candidate to target hormone-dependent cancers because of the overexpression of oestrogen receptors onto the cancer cell surface.^[34]

The antiproliferative activity of these functionalized Pt NHC complexes has been evaluated on a large panel of cancer cell lines (MCF7, HCT116, SKOV3, PC3, KB, OVCAR8, HL60).^[32] The functionalization resulted in lower cytotoxic activities. Whereas the alkyne-containing Pt NHC complex **9** gave IC_{50} between 0.04 and 0.17 μM , the corresponding benzyl-functionalized compound **10** provided IC_{50} between 0.35 and 1.9 μM . IC_{50} of 0.5 to 10 μM were measured with complex **11**. Nevertheless, these values were still significantly lower than cisplatin. The oestrogen-functionalised Pt complex **12** exhibited good cytotoxicity towards ovarian SKOV3 and OVCAR-8 cells (IC_{50} of 3.1 and 2.1 μM) and colon HCT-116 cells (IC_{50} of 0.3 μM)

that overexpress oestrogen receptors intracellularly.^[35] However, a lower activity was observed against the breast cancer cells MCF7 ($IC_{50} = 9.1 \mu M$), which also overexpresses oestrogen receptors.^[36]

Figure 5. Examples of post-functionalized Pt NHC complexes by RuCAAC (yield in parenthesis).

RuCAAC offers an easy way to functionalize platinum NHC complexes. However, *in vitro* studies have demonstrated that functionalization of the complexes usually resulted in lowering the biological activity of the native Pt compounds. One potential drawback of functionalization by azide-alkyne cycloaddition reaction is the irreversible nature of the chemical bond. Indeed, the functionalization of the native molecule aims at improving the solubility or to provide selectivity towards cancer cells. However, this derivatization can also lead to a decrease in anti-cancer activity due to reduced cellular internalization. Alternatively, once in the cytosol it can also prevent the drug to go further in the organelles. In this respect, the use of cleavable linkers may be helpful for an efficient release of the drug into the cancer cell and an improved efficiency of the active ingredient.^[37]

3.2. Post-functionalization by oxime ligation

Reactions that allow for covalent and reversible tagging of molecules with high selectivity for biological applications are of particular interest. In particular, a *ligation and release* strategy that responds to change in cellular conditions is useful for efficient release of active drug for example. Oxime / hydrazone chemistry is one of the most proficient strategies used in bioconjugation owing to its simplicity and efficiency.^[38, 39] Interestingly, the functionalization of bioorganometallic complexes by carbonyl condensation remains relatively rare although it appeared as a

promising method.^[40] With respect to the metal NHC chemistry, carbonyl condensation such as oxime, imine or hydrazone-bond formation of an aldehyde-functionalized NHC Pt complex has been successfully performed. Figure 6 displays the molecular structure of NHC Pt(II) complex in which an aldehyde moiety has been introduced for post-functionalization.

Figure 6. Post-functionalization by imine/oxime ligation.

In preliminary experiments, the clean formation of the expected condensed products were observed by direct addition of a primary amine, hydrazine or hydroxylamine to the aldehyde-containing Pt compound, as deduced from *in situ* NMR experiments.^[41] This simple and straightforward strategy was then extended to generate and isolate various post-functionalized complexes, starting from complex **13**. Figure 7 shows selected examples of such post-functionalized Pt compounds. Complex **14** containing *o*-benzylhydroxylamine was isolated in the 92% yield. The complex **15** containing a metronidazole derivative has been isolated in the 41% yield. Metronidazole can target hypoxic cells which are known to be overproduced in the closed tumor microenvironment.^[42] Complex **16** contains a pyridinium moiety which may target proteoglycans. Proteoglycans are known to be overexpressed in many forms of cancer.^[43] The urea derivative **17** may be of interest in targeting and inhibiting glutamate carboxypeptidase II (also known as PSMA Prostate Specific Membrane Antigen), a protein that is overexpressed in prostate cancer.^[44] It should be noted that all complexes were found to be stable in solution in the presence of water, as deduced from NMR studies.

Figure 7. Examples of post-functionalized Pt NHC complexes by oxime ligation (yield in parenthesis).

Although imine ligation displays lower stability than oxime (or hydrazone), it can be used as an intermediate to generate secondary amine and thus to create a covalent and irreversible post-functionalization.^[45] Figure 8 shows a selected example. Treatment of Pt NHC complex **13** with L-Phe-OBn generated the corresponding imine complex which was directly reacted with NaHB(OAc)₃ to give product **18** in the 65% yield (two steps).

Figure 8. Post-modification of NHC platinum complex **13** by tandem imine ligation/reduction (yield in parenthesis).

The antiproliferative activity of these complexes has been evaluated on various cancer cell lines. Table 1 shows some representative results on three different cancer cells (namely MCF7, HCT116, and PC3).^[41] The native complex **13** with aldehyde-functionalized moiety displays a high cytotoxic activity, with values significantly lower than cisplatin. Post-functionalization of the complex with a neutral fragment such as for complexes **14** and **15** usually did not change the biological activity of the platinum. Clear differences with the pyridinium or

urea-functionalized complexes **16** and **17** were observed. Much lower activities were measured with these complexes containing pendant ionic fragments, which may be due to the low aptitude of these positively or negatively charged complexes to cross the membrane. Further surveys confirmed this feature.^[46] Nevertheless, these examples validate the oxime methodology for post-functionalization of Pt NHC complexes.

Table 1. Selected *in vitro* cytotoxic activities (IC₅₀ values in μ M) after 72h of incubation.^[a]

	13	14	15	16	17
MCF7	0.78 $\pm$ 0.05	0.35 $\pm$ 0.03	0.94 $\pm$ 0.03	75.5 $\pm$ 4.1	78.2 $\pm$ 6.3
HCT116	0.17 $\pm$ 0.07	0.13 $\pm$ 0.02	0.11 $\pm$ 0.01	17.1 $\pm$ 2.4	23.7 $\pm$ 3.9
PC3	0.79 $\pm$ 0.06	0.28 $\pm$ 0.02	5.13 $\pm$ 0.24	60.4 $\pm$ 2.3	52.2 $\pm$ 9.0

[a] MCF7 breast carcinoma; HCT116 colon cancer cells; PC3 prostate adenocarcinoma. Stock solutions in DMSO. IC₅₀ for cisplatin (used as reference in water): MCF7: 19.8 $\pm$ 0.6, HCT116: 6.4 $\pm$ 0.2, PC3: 10 $\pm$ 1 μ M.

3.3. Post-functionalization by ligand substitution

Transition metal complexes comprised of coordinative bonds which are considered as the strongest of non-covalent interactions.^[47] Coordination bonds are highly directional and they constitute reversible interaction used to obtain diversity in molecular structures. Therefore, it seems obvious to functionalize metal NHC complexes by ligand exchange. N-Heterocyclic carbenes are known to have a strong σ -donation that is central to high catalyst activity.^[48] NHC donation will influence bonding interactions of other ligands present around the metal. Since N-heterocyclic carbene ligand has a strong *trans*-effect, it easily labilizes the ligand located in opposite side. The ligand exchange is in equilibrium, it is driven by thermodynamics.

Diversity in Pt NHC complexes can be directly introduced from a Pt(0) NHC complex as first demonstrated by Marinetti in 2010 (Figure 9).^[19] Indeed, the first synthesis of complexes of type (NHC)PtX₂(amine) involved the use of the platinum(0) complex (NHC)Pt(dvtms) (dvtms = 1,3-divinyl-1,1,3,3-tetra-methyldisiloxane). Oxidation of the platinum(0) complex with iodine (I₂) followed by *in situ* olefin-to-ligand exchange with the desired amine generated the mentioned complex in good yield.^[49] Various amines were used (cyclohexylamine **19**, morpholine, benzylamine, etc.). Pyridine was also found suitable to generate the corresponding complex, such as **20**. The strategy was then extended to access dinuclear Pt NHC complexes such as **21**,^[50] or to functionalize NHC Pt with a G-quadruplex DNA ligand, such as **23**.^[51]

Figure 9. Synthetic approach to the (NHC)PtX₂(amine) complexes from Pt(0) NHC (yield of the two steps in parenthesis).

Pyridine NHC platinum complexes (NHC)PtX₂(py), which are highly stable complexes prepared in one step from the corresponding imidazolium salt,^[52] were found as very versatile precursors for post-functionalization through ligand substitution (Figure 10). The labile pyridine is easily displaced by various nitrogen-based ligand such as amine, *o*-hydroxylamine, hydrazine derivatives.

Figure 10. Non-covalent post-functionalization by ligand substitution.

The method was applied to various amines, amino acids or peptides. Figure 11 displays selected examples of Pt NHC complexes obtained by pyridine-amine substitution (**24–30**).^[53,54] The substitutions were highly selective and no by-products were observed, thus indicating that the somewhat low yields observed may rather result from the low reactivity of the ligand itself. Pt NHC complexes with pnictogen ligands were also obtained in good to excellent yield such as compounds **31** and **32**.^[55] Noteworthy, the *cis*-configuration was observed for all these complexes.

The biological effect of the ligand variations has been studied by *in vitro* studies on various cancer cell lines. Most of these complexes were found to be significantly more active than cisplatin, with IC₅₀ usually at the micromolar range. The nature of the ligand was also found to influence the biological activity. For example, Pt NHC complex **24** (which could be studied from stock solution in ethanol) displayed IC₅₀ of 0.59 μM for MCF7 cells, 0.39 μM for HCT116 cells and 0.57 μM for cisplatin-resistant SK-OV3 cells. Arsine-containing NHC Pt complex **31** displayed an IC₅₀ activity of 1.0 μM for MCF7 cells and 7.40 μM for HCT116 cells, whereas the cationic complex **32** gave IC₅₀'s of 1.58 μM and 0.75 μM, respectively. Nevertheless, the cationic complexes with pnictogen ligands displayed an average improved activity compared to their neutral monopnictogen analogues, as also noted by Muenzner.^[20]

Bioconjugation of Pt NHC with a G-quadruplex DNA ligand such as compound **23** (Figure 9) gave a conjugate with a lower activity of one order of magnitude compared to the native complex **19** (IC₅₀ = 8 μM for **23** vs. 0.5 μM for **19** on A2780 cells).^[51] However, the conjugate **23** can recognize the G-quadruplex structures from telomeric structures, thereby directing the Pt fragment towards these structures that induce metal coordination.

Figure 11. Non-covalent functionalization by ligand substitution (yield of the substitution step in parenthesis).

Ligand exchange reaction has also been used to probe the platinum interaction with the nucleobases of the DNA.^[56,57] A dye pair has been incorporated at both ends of the NHC Pt molecule for fluorescence resonance energy transfer FRET (Complex **33**, Figure 12).^[54] A DABSYL moiety has been used as a 'dark quencher'. Upon excitation of the anthracene moiety, the fluorophore's emission is suppressed by FRET resonance. After addition of DNA in a solution of complex **33**, the luminescence properties of the anthracene was recovered because of ligand exchange. These results were also corroborated by optical tweezers techniques and liquid phase atomic force microscopy (AFM).^[56]

Figure 12. Molecular structure of Pt NHC dabsyl derivative **33** and interaction with DNA.

Water solubility is one of the important parameters for a pharmacological response and still remains a challenge for all of these Pt complexes, since the vast majority so far must be presolubilized in DMSO.

Functionalization by ligand substitution allows for ease of synthesis of cationic complexes that display high water solubility. Reaction of a pyridine NHC Pt complex with ethylenediamine or diethylenetriamine gave complexes **34** and **35**, respectively (Figure 13).

Figure 13. Non-covalent functionalization by ligand substitution. Example of cationic highly stable water-soluble Pt NHC complexes.

Both complexes display high water solubility and stability. However, *in vitro* studies revealed that they are completely inactive ($IC_{50} > 100 \mu M$). This observation may indicate the cellular transport mode of such NHC metallodrug family. The mechanism by which cisplatin enters is still not completely understood.^[58] Experimental studies have shown that two pathways are involved, which are i) passive diffusion uptake and (ii) transporter-mediated uptake,^[59] such as copper transporter Ctr1 for example.^[60] Moreover, these transporters have been suggested to have a role in the development of drug resistance.^[61] For example, Ctr1 mutations have been associated with cisplatin resistance.^[62] On the other hand, Clarke and coll. recently demonstrated the major role of passive membrane diffusion in the uptake of cisplatin.^[63] They showed that the neutral charge of cisplatin facilitates diffusion of the drug through the membrane, whereas positively charged complexes significantly lower the rate of permeation. We assumed that our Pt NHC complexes could behave in the same way, explaining the low biological activity of cationic Pt NHC complexes **34** and **35**.

Liposomal formulations are successful drug delivery systems through a mechanism of endocytosis and thus improve the efficacy of therapeutic drugs particularly cisplatin and derivatives.^[10,64] As representative examples, LipoplatinTM or AroplatinTM, which are cisplatin-loaded liposomes, show promising therapeutic results in clinical studies.^[65,66] Although the use of liposomes to increase the efficiency of Pt NHC complexes would be an axis of relevant development in the field, we instead decided to take advantage of ligand exchange post-functionalization to develop simple systems able to cross the membrane. We thus turned our attention to poly(ethylenimine) which is a well-established transfection agent that condenses DNA into positively charged particles and enter into the cell by endocytosis.^[67,68,69] Since drug delivery is believed to occur through cell endocytosis of the drug-PEI assembly, we have considered using this pathway for platinum. Direct reaction of pyridine NHC Pt with PEI polymer in ethanol gave the expected Pt-NHC-PEI conjugates in high yield (compound **36**, Figure 13).^[70] These multivalent cationic platinum complexes are soluble in water and cryo-electron microscopy experiments (cryo-EM) revealed that they self-organize into spherical particles with a mean diameter of 150 nm (Figure 14, a).

Figure 14. a) Cryo-EM observation of **36** in water (Scale bar 100 nm); b) effect on HCT 116 cell metabolic activity of **36**, oxaliplatin and cisplatin.

The size of the polymer and the overall density of metal ions onto polymer chains modulate the *in vitro* activity of the systems.^[71]

The studies showed that the best conjugate (i.e., linear PEI of 25 kDa and 20 Pt per. polymer chain) is highly active against various cancer cell lines, such as HCT 116 and displayed higher potencies than the two clinically used cisplatin and oxaliplatin ($IC_{50} = 2.8 \mu M$, Figure 13, b). Importantly, no change of *in vitro* activity was observed while keeping in solution the NHC-Pt-PEI conjugate **36** for 3 months.

Similar trends were observed *in vivo* on nude mice bearing HCT116 xenografts. Indeed, greater tumor volume control and lower side effects have been observed compared to mice treated with oxaliplatin as shown on Figure 15.

Figure 15. Antitumor effect on nude mice bearing HCT116 xenografts. **36** and oxaliplatin were injected in vehicle at $5 \mu mol/kg$. Results are expressed as the mean of 2 independent experiments $\pm$ SEM *** p-value <0.001 difference between 2PEI30 and vehicle, # p-value = 0.5 between 2PEI30 and oxaliplatin.

A drug biodistribution study also confirmed the successful drug internalization, further suggesting greater uptake of platinum in the mitochondria for **36** rather than in the nucleus which is the main target for oxaliplatin.^[70] Finally, the formation of ROS in the mitochondria of cancer cell death due to treatment with NHC-Pt-PEI complexes has been evidenced, thus confirming the occurrence of a parallel mechanism of cytotoxicity induced by **36**.

4. NHC Platinum (IV) complexes

Amongst all strategies to develop new platinum-based drugs with the aim to overcome the shortcomings of the current platinum(II) drugs, the development and use of platinum(IV) complexes is of particular interest.^[11,72] Unlike platinum (II) complexes, platinum (IV) complexes are almost always six-coordinates and thus adopt octahedral geometries. Such saturated compounds usually show high stability in solution and are also prompt to minimize unwanted side reactions with biomolecules such as proteins in bloodstream, prior to bind DNA. In addition, the two extra ligands provide a mean for adjusting the biological properties of the pro-drug, such as lipophilicity, redox stability, cancer-cell targeting or improved cellular uptake.

In vivo reduction of the Pt(IV) center to Pt(II) -and loss of two ligands- is believed to be the mode of activation of these agents. The resulting Pt(II), usually cisplatin or a related derivative then exerts its antitumor effect by DNA binding and inducing

apoptosis.^[73] As a representative example, Satraplatin, which was the first platinum complex reported to have oral activity, is undergoing various phase I to III clinical trials.^[74,75] Considering these promising results, we decided to investigate the biological potential of platinum(IV) compounds stabilized by NHC ligands.

The synthesis of platinum(IV) complexes by the direct oxidation of previous platinum(II) NHC complexes [(NHC)PtX₂L] has been investigated based on recent reports on forming platinum(IV) complexes.^[76] Exposure of [(NHC)PtI₂L] to Br₂ in dichloromethane induced an oxidative bromine addition to the Pt center.^[77] The reaction proceeded very quickly (5 minutes at 0°C) and cleanly to give the corresponding *trans* Pt(IV) complexes [(NHC)PtBr₂L] (Figure 16). The complexes were easily isolated by a precipitation with pentane.

Figure 16. Synthesis of NHC Pt(IV) complexes by oxidation of NHC Pt(II) complexes.

The chloride Pt(IV) counterparts were successfully obtained by oxidation with PhICl₂ as reagent (Figure 17). The reaction was complete after 1 hour at 0°C and again the Pt(IV) complexes were easily isolated by precipitation and yields were usually very good. Figure 18 displays the molecular structure of a representative complex **43**.

Figure 17. Synthesis of NHC Pt(IV) complexes by oxidation of NHC Pt(II) complexes (yield in parenthesis).

Figure 18. Molecular structure of Pt(IV) NHC chloride complex **43** featuring 1-methyl-3-benzylimidazol-2-ylidene NHC and pyridine ligand (97% yield).

A large library of NHC Pt(IV) complexes with bromide or chloride ligands could be easily generated by using the oxidation reaction.^[78] In addition, akin to platinum(II) NHC complexes, the pyridine ligand located *trans* to the carbene was found sufficiently labile for ligand substitution thus increasing chemical diversity. All complexes were found stable under air in the solid state or in chlorinated solvents. However, they were quite unstable in DMSO/water, which can be problematic for biological studies. Further investigations in pure DMSO revealed that the Pt(IV) complexes containing bromide ligands quickly transformed into Pt(II) species within half a day at room temperature. The same reduction process occurred with Pt(IV) chloride species albeit much slowly (1 month for complete conversion). In both cases, the reduction led to the exclusive formation of *cis*-[(NHC)PtX₂(DMSO)] species as shown on Figure 19. This difference in Br/Cl reactivity may be related to a reductive elimination process that is more difficult with chloride than bromide.

Figure 19. Synthesis of NHC Pt(IV) complexes by oxidation of NHC Pt(II) complexes.

Table 2 shows results on *in vitro* antiproliferative activities of the Pt(IV) complexes **37–42**. Chloride complexes were found significantly more active than bromide complexes (compare entries 1 with 4, entries 2 with 5 and entries 3 with 6). This noticeable difference in activity could be correlated with the

intrinsic stability of these compounds when in solution in DMSO/water.

Table 2. Selected *in vitro* cytotoxic activities (IC₅₀ values in μ M) after 72h of incubation.^[a]

Entry	Complex	MCF7	HCT116	PC3
1	37	4.0 $\pm$ 0.2	5.0 $\pm$ 1.0	5.0 $\pm$ 1.0
2	38	5 $\pm$ 1	14 $\pm$ 2	5 $\pm$ 1
3	39	23 $\pm$ 5	7.5 $\pm$ 0.3	10 $\pm$ 1
4	40	1.8 $\pm$ 0.6	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2
5	41	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	1.0 $\pm$ 0.1
6	42	9.5 $\pm$ 0.3	4.6 $\pm$ 0.3	3.8 $\pm$ 0.1

[a] MCF7 breast carcinoma; HCT116 colon cancer cells; PC3 prostate adenocarcinoma. Stock solutions in DMSO.

Indeed, investigation of the *in vitro* activities of the reduced species *cis*-[(NHC)PtX₂(DMSO)] revealed very high IC₅₀ values (> 65 μ M) so that the observed biological activities of the NHC Pt(IV) species are most likely correlated with respect to their stability. Nevertheless, the redox activation of the NHC Pt(IV) compounds was ascertained in presence of glutathione GSH as a model for the bioreducing agent by means of Cyclic Voltammetry. These experiments support the efficient release of cytotoxic NHC Pt(II) analogs in biological media. Further biological mechanistic investigations also showed that the cytotoxic activity was associated with a mitochondrial dysfunction and the production of Reactive Oxygen Species (ROS). The occurrence of a cytotoxicity mechanism different from that of cisplatin drug was also confirmed by evaluating the anticancer activity of Pt(IV) NHC compounds on chemoresistant cell lines such as cisplatin-resistant A2780R.^[78]

5. conclusion

Although the field of metal N-heterocyclic carbene complexes as anticancer drug is relatively young, their potential has already been established by numerous studies. This is particularly true for platinum NHC complexes that showed higher cytotoxic activities than cisplatin or related compounds. They also demonstrated high activities against cisplatin-resistant cell lines. Mechanistic investigations suggest that these compounds do not have a single target but rather an action on different organelles such as the nucleus and mitochondria. This makes it possible to increase the effectiveness of the compound and especially to circumvent the mechanism of chemoresistance centered on the nucleus. The rapid successes of these complexes derive from the richness of N-heterocyclic carbene metal chemistry and the favorable biocompatibility of these complexes. Moreover, they fit

prerequisites for metal drug development and allow easy post-functionalization thus enabling molecular diversity for efficient drug design. They are now subject to more intensive biological mechanistic investigations and *in vivo* studies. There is no doubt that such organometallic compounds will bring out new drugs to fight cancer disease.

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Keywords: N-heterocyclic carbene • Platinum • Bioinorganic chemistry • Functionalization • Cancer

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Entry for the Table of Contents

MINIREVIEW

N-Heterocyclic carbene as a ligand for organometallic chemistry is a relatively young area that offers new opportunities in bio-inorganic chemistry. The properties of these metal NHC complexes allow for easy post-functionalization, thus enabling molecular diversity for efficient drug design.

Bioinorganic N-heterocyclic carbene chemistry

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N-heterocyclic carbene platinum complexes: a big step forward for effective anti-tumor compounds