Design and synthesis of metal-based chemotherapeutic agents for targeted DNA interactions or DNA repair pathway modulation

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Chapter 6

Summary, Discussion, Conclusion and Outlook

6.1 Summary

Chemotherapy and DNA damage have been closely associated ever since the discovery of cisplatin and nitrogen mustard derivatives and their DNA-binding mechanisms. The primary objective of the research outlined in this thesis was to design and study metal-based chemotherapy agents and their interactions with DNA, directly or indirectly, through the photoactivation of DNA repair pathway inhibitors. Chapter two focused on direct DNA interactions via a study of the structure-activity relationship for a library of d^8 metal compounds based on the $[M(H_2bapbpy)]Cl_2$ ($H_2bapbpy$ = 6,6'-bis(2"-aminopyridyl)-2,2'-bipyridine) scaffold. The synthetized compound library contained $H_2bapbpy$-derived ligands coordinated to either a palladium(II) or a platinum(II) center. The $H_2bapbpy$-based ligands had either their terminal pyridyl groups functionalized with a methyl, methoxy, chloride, or trifluoromethyl substituent, or their amine bridges replaced by thioether, ether, methylene, or carbonyl bridges. The complexes containing NH or CH_2 bridges could easily be deprotonated in aqueous solution, allowing the determination of their acidity constants (pK_a). Calf thymus DNA titration in solution allowed the determination of DNA-binding constant, which confirmed intercalation as the prevalent binding mode for most of these complexes at pH 4.5-5.0. Titration at pH 8.0 of complexes that could be deprotonated showed that two reactions, ie first protonation, followed by intercalation, were needed for these complexes to intercalate into DNA. Furthermore, high DNA concentration samples at pH 8.0 yielded UV-vis spectra identical to those of the same samples obtained at pH 5, indicating that these metal complexes were fully protonated once fully bound to DNA, even at such basic pH. Remarkably, titrations at pH 8.0 of compounds characterized by a low pK_a showed no significant interactions with DNA, highlighting the crucial role of the protonation state of the metal complexes for DNA intercalation: only the bicationic, fully protonated, species interact significantly with double-stranded DNA through intercalation.

The crystal structures obtained in chapter two revisited the fact that upon coordination of the $H_2bapbpy$-based ligands to the metal centers, the ligand structure forms helical chiral complexes due to the steric clash between the terminal pyridines of the ligand. The formation of these chiral complexes is described in more details in chapter 3, where $H_2bapbpy$-based ligands were coordinated to ruthenium(II) instead of palladium(II) or platinum(II). The octahedral ruthenium(II) center offer two additional axial coordination sites, compared to d^8 metal centers, where enantiomerically pure ligands could be coordinated. These additional chiral ligands allowed us to determine whether the terminal pyridines of the $H_2bapbpy$ derivative can freely exchange position in solution at room temperature, and hence if the helix can reverse its chirality. The complex $[Ru(H_2bapbpy)(MTSO)Cl]^+$ where (MTSO = (*R*)-methyl p-tolylsulfoxide) showed the formation of diastereotopic aromatic protons by ^{1}H-NMR. These signals allowed the determination of a coalescence energy of 44 kJ/mol for the inversion of the helical

chirality by variable temperature ^{1}H-NMR experiments. In contrast, the increased strain induced by the larger terminal quinoline groups in complex [Ru(H_2biqbpy)(DMSO)(Cl)]Cl (H_2biqbpy = bis(aminoquinoline)bipyridine), resulted in a coalescence temperature higher than 376 K, which pointed to an absence of helical chirality inversion at room temperature. Interestingly, upon further increase of the steric strain by introducing methoxy groups *ortho* to the nitrogen atoms of the terminal pyridyl groups in H_2bapbpy, we serendipitously discovered a ring-closing reaction that took place upon trying to make [Ru(OMe-H_2bapbpy)(DMSO)Cl]$^{+}$. This reaction generated, in excellent yields, complex [Ru(L")(DMSO)Cl]Cl where L" is an asymmetric polypyridyl macrocycle coordinated in a tetradentate fashion to ruthenium. This unexpected transformation appears to be specific to ruthenium(II), as macrocyclization did not occur upon coordination of the same ligand to palladium(II) or rhodium(III).

The photocaging of biologically active compounds is a well-established method to increase the tissue selectivity of anticancer compounds, as photocaged compounds remain inert in the dark and become active by local activation using visible light. DNA repair inhibitors used in chemotherapy are especially interesting compounds to photocage: on the one hand, a wide variety of cancers show mutations in the DNA repair pathways, but on the other hand, one does not want to impede DNA repair in the whole body. Chapter 4 describes the photocaging of the RAD51 inhibitor B0Cl, an improved analog of the well-known RAD51 inhibitor B02, which blocks double strand break repair *via* homologuous recombination. The inhibitor was caged by the ruthenium scaffold [Ru(tpy)(NN)(L)]$^{2+}$ where tpy=2,2':6',2''-terpyridine, NN is either biq (biq=2,2'-biquinoline), dppz (dppz=dipyrido[3,2-a:2',3'-c]phenazine), or dppn (dppn= (benzo[i]dipyrido[3,2-a,2',3'-c]phenazine), and L is the pyridine-based RAD51 inhibitor. The biq ligand induces steric strain on the ruthenium complex, thereby increasing the quantum yield of photosubstitution, compared to dppz or dppn derivatives. The dppz moiety bound to ruthenium can intercalate into DNA, resulting in DNA damage. The most extended ligand, dppn, is a known generator of $^{1}O_2$ when hit by UV or visible light. The combination of these ligands with a RAD51 inhibitor could result in synergy between DNA damage induced by the ruthenium bidentate species and the photoactivated inhibition of HR. However, the ruthenium compound bearing the dppz or dppn ligand demonstrated abysmal quantum yields of photosubstitution, which probably prevents B0Cl to be released. On the other hand, the complex [Ru(tpy)(biq)(B0Cl)]$^{2+}$ had a good quantum yield for light-induced uncaging of B0Cl (0.063 in MeCN) upon green light irradiation (505 nm), while the compound remained inert in the dark. Further biological testing was therefore performed with this complex. The light-activated cytotoxicity (EC_{50}) of the compound, determined in two uveal melanoma cell lines, was 2.7 µM and 3.3 µM for the cell lines OMM2.5 and MM66, respectively. We note here that the significant dark toxicity of this compound suggested another

(unknown) mode of cytotoxic action for this compound. Combination treatment with both the compound and doxorubicin resulted in an increase in double-stranded breaks in the dark, while RAD51 foci decreased upon light activation, which is consistent with the successful photorelease of the B0Cl inhibitor. These initial results demonstrate that the B0Cl inhibitor was successfully caged and successfully photoreleased in cells to reduce homologous recombination activity. However, the uveal melanoma cell lines used in this study are not reported to be exceptionally responsive to RAD51 inhibitors and other cell lines should be considered, such as those coming from triple-negative breast tumors.

Chapter 4 demonstrated the potential of photocaging one inhibitor, while the ruthenium complexes studied in chapter 3 $[Ru(H_2bapbpy)(X)(Y)]^{2+}$ offered two axial coordination sites. The combination of these ideas are reported in chapter 5, where we describe the conjugation of two identical or different inhibitors to the $[Ru(H_2bapbpy)(X)(Y)]^{2+}$ photocage. In this series of compounds, two identical RAD51 inhibitors, either B0Cl or B02, were photocaged to one ruthenium center (X=Y), or a single X=RAD51 inhibitor was combined with another inhibitor Y for either STF31, NAMPT, or DNA-PK. Synthetic lethality is based upon the exploitation of a genetic mutation in a tumor, where disruption of one gene does not impact the cell viability but disruption of two results in cell death. This technique is skillfully employed through the treatment of PARP inhibitors against BRCA-mutated cancer cells. However, synthetic lethality necessitates genetic mutations and its success relies on identifying specific genetic mutations in the tumor that can be exploited while sparing healthy cells, which requires extensive and time-consuming research. Nonetheless, synthetic lethality demonstrated the effectiveness of targeting two genes in generating cytotoxicity in cancer cells. The combination treatment of two different inhibitors would not offer selectivity for the cancer cells. However, the combination of two inhibitors with one photocage scaffold, would result in a tumor selectivity that relies on localized prodrug photoactivation. In chapter 5, we report blue light-induced photosubstitution for the $[Ru(H_2bapbpy)(X)(Y)]^{2+}$ scaffold bearing two identical or different inhibitors X and Y. All compounds showed similar photosubstitution quantum yields: ~0.06 for the first substitution and ~0.003 for the second. By sufficient light irradiation of the tumor it would be possible to use such photocaged compounds to release two different inhibitors in the irradiated tumor and generate selective and local synthetic lethality.

6.2 Discussion

Chapter 2 and 3 describe the coordination of the H_2bapbpy ligand to palladium(II), platinum(II) and ruthenium(II). Even though these complexes share the same tetradentate ligand, their chemical properties vary a lot depending on the metal. The column 10 metals palladium and platinum showed limited differences. The large

number of crystal structures allowed to identify clear trends. Table 1 shows a smaller distortion for the platinum compounds in comparison with their palladium analogues, as measured by the N1N3N4N6 dihedral angle. The ruthenium compounds demonstrated the least distortion of the tetradentate ligand, although measurements also depend on the identity of the axial ligands present. Irrespective of the metal center, the molecular structures of ruthenium, palladium, and platinum H_2bapbpy complexes clearly exhibit chirality induced by steric clashes between their terminal pyridines. This steric clash prevents the tetrapyridyl ligand from adopting a flat conformation, resulting in a helical conformation of the ligand structure upon coordination to a metal center. In chapter 3 we reported that the terminal pyridines of $[Ru(H_2bapbpy)(DMSO)(Cl)]^+$ can freely exchange at room temperature which is not true for the more sterically strained $[Ru(H_2biqbpy)(DMSO)(Cl)]^+$. These trends are expected to be similar for palladium and platinum compounds. Nevertheless, due to the saturated square planar d^8 coordination sphere, it was not feasible to accommodate an additional chiral ligand or chiral auxiliary with these metals, which hindered the determination of the coalescence energy and enantiomeric resolution of the complex. In the literature, methods have been documented where the inclusion of a chiral counterion enabled chiral resolution without coordination.[1,2] However, counterion metathesis of $[Pt(H_2biqbpy)]Cl_2$, using enantiomerically pure compounds like L-tartaric acid, antimonyl L-tartrate and TRISPHATtetrabutylammonium (tris(tetrachlorocatecholato)phosphate), did not result in the formation of distinct signals for diastereoisomers or diastereomeric protons evident by ^{1}H-NMR. The absence of observation of diastereoisomers or diastereomeric protons in the ^{1}H-NMR spectra does not necessarily imply that they did not form. It is possible that the signals of diastereoisomers overlapped due to similarities in their chemical environments, making them indistinguishable by NMR analysis. As stated previously, the observed chirality trends are likely applicable to platinum and palladium H_2bapbpy compounds as well. However, additional techniques (chiral HPLC) and research would be necessary to confirm this hypothesis.

Table 1. The measured distortion of the compounds, where the angle N1N3N4N6 taken as a measure of distortion.

H_2bapbpy complex	$[Ru(H_2bapbpy)(DMSO)(Cl)]^{1+}$	$[Pd(4Me\text{-}H_2bapbpy)]^{2+}$	$[Pd(4Me\text{-}H_2bapbpy)]^{2+}$
Distortion	12.5(4)[3]	14.5(8)	16.6(4)
H_2biqbpy complex	$[Ru(H_2biqpy)(EtOHPy)_2)]^{2+}$	$[Pd(H_2biqbpy)]^2$	$[Pd(H_2biqbpy)]^{2+}$
Distortion	16.6(4)	17.9(1)	20.3(1)

Another interesting trend between the three previously mentioned metals is their acidity. In general, the pK_a values found for the palladium complexes were 1 pK_a unit higher than those found for their platinum analogues, while the deprotonation of ruthenium-coordinated H_2bapbpy compounds was not observed. These results

highlight the striking electronic differences between metal centers that look *a priory* quite similar. The deprotonation of a ligand coordinated to an electron-withdrawing metal is common and important for various catalytic and synthetic transformations.[4–6] Furthermore, several ruthenium compounds have been reported where ligands coordinated to ruthenium(II) could be deprotonated. Examples include the compound $[Ru(4\text{-}COOH\text{-}tpy)(4\text{-}NH_2\text{-}tpy)]^{2+}$ where both the carboxylic acid and the amine could be deprotonated,[7] and several pincer-based ligands coordinated to ruthenium reported by Fogler *et al.*.[8] The salient differences in this thesis are striking as the three metal centers are all coordinated to the same H_2bapbpy ligand. However, the deprotonation of a ligand coordinated to a metal center is a highly intricate process influenced by a range of factors, including electronic properties, steric hindrance, the presence and nature of axial ligand, and the nature of the metal-ligand coordination bond.

In chapter 5, we investigated the feasibility of employing a ruthenium scaffold as a platform for photoactivated dual drug delivery. We describe there the successful synthesis of two ruthenium-based compounds, each incorporating two distinct pharmaceutical agents. Both compounds underwent photodissociation upon blue light irradiation. This therapeutic approach holds promise due to its potential advantages, including the induction of synergistic effects that surpass treatments involving independent single agents,[9] or single photocaged agents as discussed in chapter 4. However, it is important to note that this photoactivated dual drug delivery platform is not without its own set of strengths and weaknesses when compared to the conventional treatment utilizing two independent pharmaceutical agents. First and foremost, photoactivation offers improved selectivity over conventional administration techniques, which is due to the localized activation occurring exclusively at the intended site, thereby mitigating potential side effects.[10] The integration of both agents within a singular delivery system, allows reduced side effects for both agents through a unified approach, thereby also simplifying treatment regimens.[11] Furthermore, the increased spatiotemporal control in delivery facilitates the delivery of the two agents at the exact same site. While combination treatments are frequently employed in clinical settings, achieving success has often proven challenging due to variations in pharmacokinetics and tissue distribution among individual components within these combinations.[12] Currently, individual medicinal agents undergo comprehensive research, leading to well-defined safety and efficacy profiles. However, when contemplating the combination of two agents, a rigorous evaluation of safety and efficacy becomes imperative, regardless of whether the two inhibitors are administered independently or combined on one scaffold.

The co-localization of the two agents with pharmacokinetics of a single compound can be particularly advantageous and lead to stronger synergistic effects. For example,

the combined delivery of a DNA damaging agent and a DNA repair inhibitor, ensures the presence of the DNA repair inhibitor at the newly formed DNA damage site. On the other hand, the challenges of light-activated therapy, specifically with blue light irradiation, is the limited tissue penetration depth,[13] resulting in viable treatment only for small or thin tumors allowing direct exposure to the light source (skin, bladder, etc.). On the other hand, progress in the field of fiber optics has dramatically improved the performances of non-invasive internal irradiation devices, thereby opening possibilities for light-activated treatments with light of different color and across a broad spectrum of tumors, including those situated within challenging anatomical locations such as the brain.[14–16] Specific challenges of the compounds described in chapter 5 are the requirements of a metal-coordinating functional group on the pharmaceutical agents, and the synergy induced by a 1:1 stoichiometry between both inhibitors. Typically, efforts to optimize concentrations for independently administered agents may indeed result in achieving maximal synergy at varying concentrations for each agent during testing in simplified *in vitro* lab conditions. However, differences in pharmacokinetics between these two agents may require higher dosage levels to observe synergistic effects in mice and ultimately in real patients.[17] The nature of the scaffold discussed in chapter 5 chemically restricts concentrations of both agents to a 1:1 ratio.

While the potential supply of pharmaceutical agents is vast, the availability of chemotherapy agents capable of chemoselective coordination to the ruthenium center remains more constrained. Though in theory a substantial number of pharmaceutical agents carries chemical groups capable of coordination to a metal center, a considerable portion of these agents is, in fact, unsuitable for such interactions. Several factors should be considered here, such as steric hindrance, electronic effects, and differing degrees of hardness and softness of the metal-binding atoms (S, N, O). Additionally, instances where multiple coordinating groups are present in a given chemotherapy agent further complicate the isolation and purification of a single and chemically pure ruthenium-based PACT prodrug. Moreover, inhibitors are often readily available for approximately 50 €/mg, however, their availability often comes with limitations in terms of quantity. Although free inhibitors find their utility in small amounts (1-5 mg) for running biological assays, the successful coordination and characterization of novel ruthenium-coordinated prodrugs, as well as other critical biological assays, demand sizable quantities of these inhibitors (50-100 mg) before the efficacy of the newly formulated ruthenium-caged compounds can even be determined. The synthesis of these organic inhibitors in principle may offer higher quantities compared to commercial offers, but such syntheses are often protected by patents. Therefore, the photocaging of enzyme inhibitors with ruthenium requires substantial preliminary investments, encompassing both financial commitments and, in the case of synthesis, a considerable amount of time. These factors, combined with the 1:1 stoichiometry

imposed by the [Ru(H_2bapbpy)(X)(Y)]$^{2+}$ scaffold, somewhat limits the possibility of dual drug delivery combination therapies with a ruthenium-based PACT compound.

Overall, given the successful proof-of-concept of dual delivery of two inhibitors by blue light irradiation, as discussed in chapter 5, the utilization of costly inhibitors can become justified, despite the associated costs, if they have proven synergy in the literature. It is important to note, though, that this technique is not a universal panacea: its efficacy is constrained by the necessary presence of coordinating groups on both inhibitors that allow selective coordination to the ruthenium scaffold. In addition, it will be necessary to find ruthenium scaffold that allow dual delivery by higher wavelength light (green, red, or even near-infrared). Once this is done, strategic pairing of appropriate inhibitors bears strong potential for significant enhancements of existing chemotherapy regimens.

6.3 Conclusion and Outlook

The research described in this thesis discusses advancements in the development of metal complexes as chemotherapy drugs, characterized by their interactions with DNA. These interactions occur through direct pathways, such as intercalation, or indirect pathways, involving the photoactivation of DNA repair inhibitors. Chapter 2 offers a comprehensive characterization of the platinum- and palladium-based H_2bapbpy compound, shedding light on the pivotal role of protonation in their interactions with DNA. However, a noteworthy aspect remains unexplored, as these compounds have yet to undergo *in vitro* biological assessments to determine their cytotoxicity and assess their potential as viable candidates for chemotherapy. These efforts should also encompass the elucidation of the cellular uptake mechanisms and intracellular localization of these compounds. Furthermore, it is interesting to investigate whether the chemical modifications introduced to the H_2bapbpy ligand yield distinct outcomes in terms of biological behavior.

The targeting of uveal melanoma by photocaging of the RAD51 inhibitor B0Cl, described in chapter 4, was justified as metastatic uveal melanoma is a severe form of cancer and the development of an effective treatment is crucial.[18,19] The results presented in the study, however, reveal that the photocaged B0Cl inhibitor is not optimally suited for a proof-of-concept treatment approach in the context of this specific cancer type. In this context, triple-negative breast cancer (TNBC) might be a more suitable target and is characterized by low levels of estrogen steroid receptor, progesterone steroid receptor and human epidermal growth factor receptor 2. Due to its aggressive nature, no effective treatment is currently available for TNBC despite observations of defective DNA repair pathways and “BRCAness” of sporadic TNBC.[20,21] Increased RAD51 expression was correlated to breast cancer progression and metastasis and suppression of RAD51

led to decreased cancer cell migration and diminished tumor growth.[22] Furthermore, inhibition of RAD51 led to the sensitization for treatment with other DNA damagers agents such as cisplatin.[23] RAD51 emerges as a potential biomarker and promising drug target for TNBC. Triple-negative breast cancer (TNBC) cell lines, characterized by mutations within the DNA repair pathways, present a potentially more suitable candidate for targeted RAD51 inhibition, either as a standalone intervention or in synergy with a DNA damaging agent.

Chapter 5 introduced the chemical concept of dual drug delivery through photoactivation, a novel approach with promising potential in cancer treatment. However, these compounds require further research, and particularly biological testing, to confirm their efficacy. Notably, the inhibitor combinations explored in chapter 5 were selected based on their compatibility with ruthenium and synthetic availability, rather than their synergetic action in a specific cancer type. Nevertheless, this chemically innovative combination of inhibitors introduces the intriguing possibility of uncovering previously unreported synergies. The shift in cellular localization induced by the new molecular scaffold may foster novel and enhanced interactions between both inhibitors, potentially amplifying their combined therapeutic impact. This approach capitalizes on the established efficacy of the inhibitors, while introducing an unpredictable element of co-localization that could lead to exciting new avenues in cancer treatment. In such molecular prodrugs, the role of the ruthenium photoproduct will also need to be investigated, as metal complexes based on the H_2bapbpy fragment are known to possibly be biologically active. In essence, the herein presented multifaceted strategy represents a promising frontier in the search for more effective and tailored anticancer therapies, where the interplay between established inhibitors and innovative metal-based scaffolds has the potential to revolutionize the treatment of specific cancer subtypes.

6.4 Literature

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