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Bioactive Luminescent Transition-Metal Complexes for Biomedical Applications

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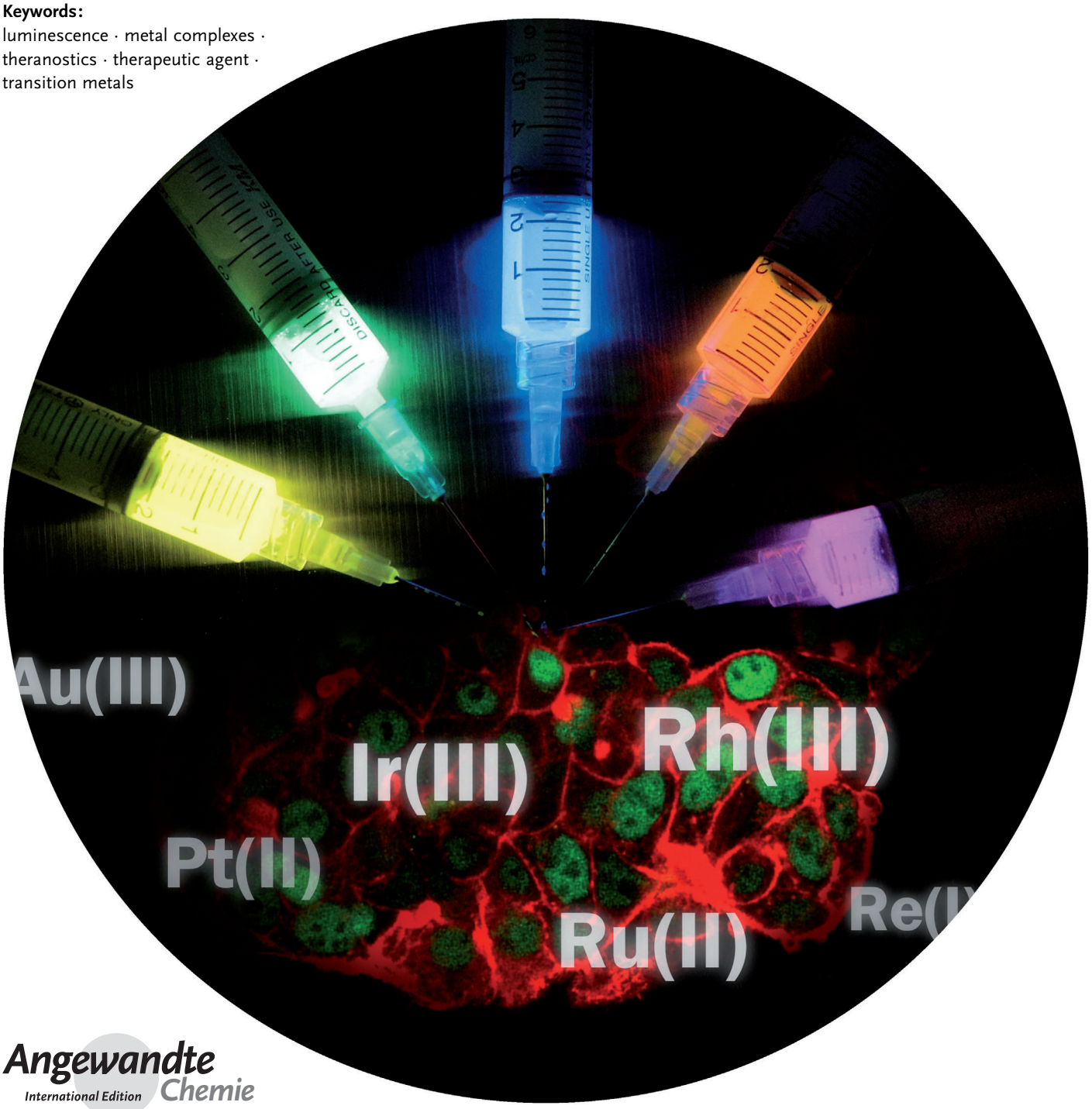

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The serendipitous discovery of the anticancer drug cisplatin cemented medicinal inorganic chemistry as an independent discipline in the 1960s. Luminescent metal complexes have subsequently been widely applied for sensing, bio-imaging, and in organic light-emitting diode applications. Transition-metal complexes possess a variety of advantages that make them suitable as therapeutics and as luminescent probes for biomolecules. It is thus highly desirable to develop new luminescent metal complexes that either interact with DNA through different binding modes or target alternative cellular machinery such as proteins as well as to provide a more effective means of monitoring disease progression. In this Review, we highlight recent examples of biologically active luminescent metal complexes that can target and probe a specific biomolecule, and offer insights into the future potential of these compounds for the investigation and treatment of human diseases.

From the Contents

1. Introduction

The clinical success of cisplatin[1] stimulated the search for new metal complexes for the treatment of various diseases. For example, gold complexes have been developed for the treatment of rheumatoid arthritis, silver complexes as antimicrobial agents, antimony complexes for the treatment of leishmaniasis, vanadium(IV) complexes as antiviral and antidiabetic agents,[2–5] arsenic trioxide (Trisenox) for the treatment of acute promyelocytic leukaemia,[6] and metal-activated bleomycin for the treatment of Hodgkin's lymphoma and testicular cancer.[7] Transition-metal-based therapeutic agents currently under clinical trials include third generation antitumor platinum complexes such as liposomal cisplatin (Lipoplatin), satraplatin, and picoplatin,[8,9] the antitumor ruthenium complexes NAMI-A and KP-1019,[10] and the antimalarial ferrocene–quinoline conjugate ferroquine.[11] These examples serve to highlight the long history of therapeutic metal complexes, and engender promise that the field of inorganic medicine will continue to develop vigorously in the future.

Metal complexes contain organic ligands bound to the metal center in a precise three-dimensional arrangement. Compared to small organic molecules, metal-based complexes offer several advantages as therapeutics. The auxiliary ligands can be readily modified to tune the steric and/or electronic properties of the complex, potentially allowing fine-control of the selectivity and reactivity of the metal complex for a particular disease target. The preparation of metal complexes is highly modular compared to the linear and sometimes lengthy and protecting-group-laden syntheses of organic molecules. Thus, transition-metal complexes can usually be prepared in fewer steps and with greater flexibility for modification during each step of the synthesis. Additionally, metal ions with variable oxidation states can coordinate ligands in different geometries and participate in various biological redox reactions. Metal complexes bearing labile ligands can also coordinate with the disease targets through ligand-exchange reactions with the biomolecules. Recently, Alessio and co-workers suggested that all anticancer metal compounds can be categorized into five classes according to their mode of action: 1) functional metal compounds that bind covalently to a biological target, 2) structural metal compounds that interact with the target noncovalently, 3) carrier metal compounds that deliver active ligands in vivo, 4) bioactive metal catalysts, and 5) photoactive metal compounds.[12] Current developments in the field of metal-based (chemo)therapeutics have been reviewed by the research groups of Sadler,[13] Che,[14] Sava,[10,15] Dyson,[16] Alessio,[12] and Gasser, Ott, and Metzler-Nolte.[17]

Meanwhile, phosphorescent metal complexes have been widely applied for sensing, bio-imaging, and organic light-emitting diode applications.[18–27] Phosphorescent metal complexes possess several advantageous features that make them suitable as sensing or imaging probes. Generally, the photophysical properties of metal complexes are sensitive to changes in the local environment. Furthermore, they usually display significant Stokes shifts which can prevent self-quenching and allow for easy resolution of the excitation and emission light. Moreover, the long phosphorescence lifetime of metal complexes compared to organic dyes allows their phosphorescence to be readily distinguished in the presence of endogenous fluorophores likely to be present in biological environments by use of time-resolved spectroscopy or fluorescence lifetime imaging microscopy. Lo et al. and Zhao et al. have recently reviewed the use of phosphorescent metal complexes for sensing and imaging applications.[28,29]

The use of metal-based complexes as therapeutic agents or biomarkers of disease status has been well-documented.

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Given that many transition-metal complexes also display interesting photophysical properties, it is not unimaginable that metal-based compounds could be developed for therapeutic activity through the simultaneous targeting and detection of a specific biomolecule. The term "theranostic", which was first postulated by Funkhouser a decade ago,[30] is defined as the combination of therapy and diagnostic imaging into a single modality. The advantage of this approach is that the therapeutic and detection abilities are combined within a single metal complex, thus allowing the progress of the disease to be monitored without the requirement for an additional labeling or imaging agent. Also, this approach could potentially overcome undesirable biodistribution and selectivity issues possessed by different imaging and therapeutic agents. In this Review, we aim to summarize the development of luminescent transition-metal complexes that display biological activity. In particular, we will describe the mode of action of the metal complexes and discuss their potential for the treatment of diseases. This Review does not intend to be exhaustive; rather, it aims to introduce the reader to recent strategies for the development of theranostic luminescent metal complexes.

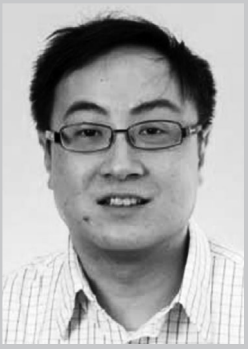
2. Luminescent Properties of Transition-Metal Complexes

The photophysical properties of heavy-metal complexes are remarkably different from those of organic fluorophores. The excited states of luminescent metal complexes are usually complicated and may be dominated by the following types of charge-transfer reactions: metal-to-ligand charge transfer (MLCT), ligand-to-metal charge transfer (LMCT), and intraligand charge transfer (ILCT). Some less-common electronic transitions include ligand-to-ligand charge transfer (LLCT), metal-metal-to-ligand charge transfer (MMLCT), and metal-to-ligand-ligand charge transfer (MLLCT). The emission properties of metal complexes depend heavily on the nature of the metal center and auxiliary ligands as well as features of the local environment.[29]

The photophysical properties of d^6, d^8, and d^{10} complexes have aroused particular interest. These complexes generally possess a strong spin–orbit coupling between the singlet and triplet spin state because of the heavy atom effect, thus facilitating the intersystem crossing of the excited electron from the ^{1}MLCT to the ^{3}MLCT state.[31] The relaxation of the ^{3}MLCT excited state produces a strong and sufficiently long-lived phosphorescence emission that allows the imaging of biological events. Since the ^{3}MLCT excited state is strongly affected by nonradiative processes, whereby the energy of the excite state is released as heat instead of light because of small energy differences between the energy levels, metal complexes can be judiciously designed to yield selective interactions with biomolecules which can be detected through their photophysical properties. Such complexes could act simultaneously as selective probes and inhibitors against a biological target.

3. Luminescent Metal Complexes That Target DNA

The interactions between luminescent metal complexes and double-helical DNA have long been studied by inorganic chemists to elucidate the mechanisms of anticancer drugs and



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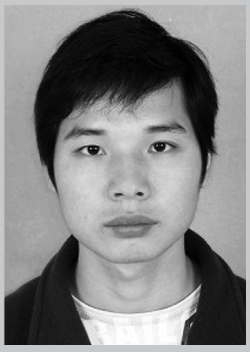

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augment the arsenal of metal-based therapeutics. However, noncanonical DNA structures such as G-quadruplexes[32,33] have also been explored over the last decade as potential targets for chemotherapeutic treatment. The design of metal complexes that bind or interact with specific DNA topologies has emerged as paramount to avoid interactions with "off-targets". In this section, we describe current developments in luminescent metal complexes that target DNA for therapeutic applications, focusing particularly on those complexes that offer alternative modes of actions compared to the archetypical chemotherapeutic metal complex cisplatin.

3.1. Complexes That Bind Noncovalently to Duplex DNA

The most abundant conformation of DNA found under cellular conditions is B-form double-helical DNA, which contains a large major groove and a small minor groove. Metal complexes can interact with DNA through either covalent (irreversible) or noncovalent (reversible) interactions. The mechanism of action of covalent complexes such as cisplatin involves rapid aquation followed by inner-sphere coordination with the DNA target. The DNA–metal complex adducts disrupt the conformation of the DNA and hinder the unwinding of the DNA by polymerase, thus impairing DNA transcription or replication processes. However, many cancers have developed resistance to cisplatin and its analogues, which has stimulated the development of noncovalent complexes that offer a different mode of action.

The realm of noncoordinative interactions can be further subdivided in two main types: 1) intercalation and 2) groove binding. In intercalation, metal complexes directly insert between adjacent base pairs of the DNA, stabilized by the π-π stacking interactions between a planar, aromatic ligand of the metal complex and the adjacent base pairs. On the other hand, groove binding is mainly achieved through favorable van der Waals and electrostatic interactions between the functional groups of the metal complex with the groove walls and floor constructed by the negative phosphate backbone. However, groove-binding complexes tend to display a limited switch-on response to nucleic acids because of insufficient protection of the metal center within the hydrophobic environment of the DNA. Thus, most DNA-interacting luminescent metal complexes are metallointercalators.

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The intercalation of small molecules, including metal complexes, into DNA is a classical strategy for the treatment of cancer. The insertion of the metal complex into DNA hinders DNA replication, which promotes cell death. The DNA and the metal complex can be considered to exhibit a host–guest relationship, with the DNA acting as the host to accept all or part of the metal complex. In general, metal complexes bearing a large heterocyclic ligand can intercalate into the double helix through stacking interactions with adjacent DNA bases. In the following section, we describe biologically active ruthenium, platinum, rhenium, and gold metallointercalators that bind to duplex DNA. An interesting alternative strategy based on a ruthenium triple-stranded helicate will also be discussed.

3.1.1. Platinum(II) Complexes

Although chemotherapeutic Pt^{II} complexes have historically been studied most for their ability to cross-link DNA, other Pt^{II} complexes have been reported that exert their cytotoxic effects through reversible interactions with DNA. In particular, some of these complexes exhibit a luminescent switch-on effect upon binding, thereby allowing them to be used as emissive probes for DNA.

Che and co-workers have reported a number of luminescent Pt^{II} complexes of general form $[Pt^{II}(C\hat{}N\hat{}N)L]^{n+}$ (CNN = 2-phenyl-6,6′-bipyridine, or terpyridine), and have evaluated them for their DNA-binding and cytotoxic properties (Figure 1).[34–37] Most of the Pt^{II} complexes were demonstrated to bind to DNA through intercalation, presumably

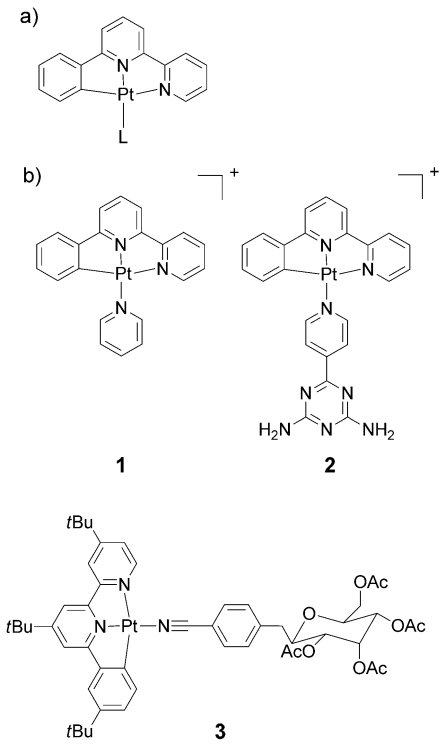

Figure 1. a) General structure of luminescent Pt(C^N^N) complexes. b) Some examples of Pt^{II} complexes that reversibly target duplex DNA.

though insertion of the planar aromatic C^N^N chromophore between adjacent base pairs. However, complex **3** was instead found to interact with duplex DNA through groove binding, presumably as a consequence of steric hindrance caused by the bulky tBu$_3$terpy ligand. In general, these complexes are non-emissive or only slightly emissive in the aqueous buffer solution, but display an intense luminescence when bound to duplex DNA. This effect is presumably due to the protection of the [Pt(C^N^N)] moiety from the aqueous buffer solution upon intercalation into duplex DNA, which suppresses nonradiative decay of the excited state through complex–solvent interactions. The enhancement of the luminescence of these complexes upon DNA binding was presumed to originate from emission from the ^{3}MLCT [Pt→π*(C^N^N)] excited state.

Comparisons between series of related complexes suggested that the DNA-binding and biological properties of these complexes were highly dependent upon the nature of the ancillary ligand L. For example, introduction of a cationic minor-groove-binding motif into the $[Pt^{II}(C^N^N)L]^{n+}$ system improved the binding affinity of second-generation Pt^{II} complex **2** for duplex DNA compared to parent compound **1**. Similarly, the glycosylated aryl acetylide motif of complex **3** was found to be essential for high cytoxicity (100-fold higher cytotoxicity than cisplatin) among a series of terpyridine-based complexes.

3.1.2. Ruthenium(II) Complexes

As a consequence of their rich photophysical properties, Ru^{II}-diimine complexes have been studied extensively as luminescent probes over the past few decades.[29] The propeller-shaped Ru^{II} complexes can exist in two enantiomeric forms that interact selectively with chiral DNA molecules. Early studies by Barton et al. revealed that the Λ form of $[Ru(phen)_3]^{2+}$ interacts preferentially with B-form DNA.[38] Furthermore, it was found that Δ-$[Ru(phen)_3]^{2+}$ preferred to intercalate into DNA, while Λ-$[Ru(phen)_3]^{2+}$ bound in the minor groove of the DNA. However, Kim and Norden later suggested that the intercalation observed for $[Ru(phen)_3]^{2+}$ may have been caused by an experimental artifact.[39]

Extending the aromatic system of the Ru^{II}-diimine complexes from $[Ru(bpy)_3]^{2+}$ (where bpy = bipyridine), which has no enhancement of its luminescence in the presence of duplex DNA, to the more extended $[Ru(bpy)_2(dppz)]^{2+}$ (**4**)[40] (where phen = 1,10-phenanthroline) and $[Ru(phen)_2(dppz)]^{2+}$ (**5**),[41] (where dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine (Figure 2) complexes generated avid DNA-binding "molecular-light switches". The intense enhancement in the luminescence displayed by the complexes was attributed to protection of the dppz moiety from solvent quenching upon intercalation into DNA, thereby leading to an effective emission from the ^{3}MLCT excited state. Photophysical studies of the resolved enantiomers of $[Ru(phen)_2(dppz)]^{2+}$ conducted by Hiort, Lincoln, and Norden confirmed that although both isomeric forms were able to intercalate into DNA, the luminescence quantum yield of Δ-$[Ru(phen)_2(dppz)]^{2+}$ was about 10 times higher than its Λ counterpart.[42] Recently, Lincoln and co-workers applied fluorescence lifetime imaging microscopy (FLIM) to the Δ and Λ isomers of Ru^{II}-dppz complexes in live and fixed cells, thus indicating that these complexes can potentially be used as probes for biomolecular binding and cellular microenvironments.[43]

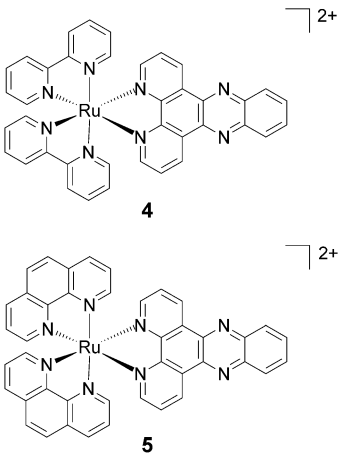

Figure 2. Classical molecular light switches $[Ru(bpy)_2(dppz)]^{2+}$ (**4**) and $[Ru(phen)_2(dppz)]^{2+}$ (**5**).

The development of $[Ru(NN)_2(dppz)]^{2+}$ analogues or related systems has recently been reviewed by Tuite and co-workers.[44] Although most such polypyridyl complexes show only low to moderate cytoxicity towards cancer cells, with the exception of $[Ru(bpy)_2(dppn)]^{2+}$ (where dppn = benzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine),[45] the success of other cytotoxic ruthenium complexes such as NAMI-A and KP1019 suggest that these luminescent metallointercalators could potentially be developed as effective chemotherapeutics. Furthermore, recent studies by Sheldrick and co-workers have demonstrated the anticancer potential of both substitutionally inert and labile rhodium(III) complexes bearing dppz and related polypyridyl ligands; however, these complexes do not show useful luminescent properties.[46]

A novel strategy that circumvents the issue of cross-resistance to anticancer drugs was reported by Hannon and co-workers.[47] Unlike most anticancer drugs, which bind to DNA through intercalation or covalent linkages, the synthetic triple-stranded ruthenium(II) helicate $[Ru_2(\mathbf{6})_3]^{4+}$ (see Figure 3 for the ligand) forms a metallosupramolecular cylinder that binds noncovalently in the major groove of DNA. The DNA-binding mode of the ruthenium helicate was monitored by linear dichroism spectroscopy, which showed that the cylinder interacted specifically with calf thymus DNA (ctDNA) through bending or coiling of the DNA. Interest-

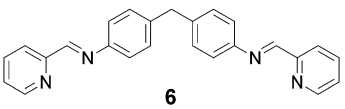

Figure 3. Ligand **6** of the diruthenium(II) triple-stranded helicates.

ingly, the helicate displayed a modest luminescence response to ctDNA that was comparable with that of the ruthenium intercalator $[Ru(phen)_3]^{2+}$. Although the helicate exhibited lower cytotoxicity towards cancer cells compared to cisplatin, its unique binding mode may avoid the most common mechanisms of drug resistance, thus suggesting that it could potentially be developed as an effective chemotherapeutic against resistant tumors.

The Das and Thomas research group recently reported a groove-binding mononuclear Ru^{II} complex with a catechol pendant (**7**, Figure 4) that displays a large affinity for specific

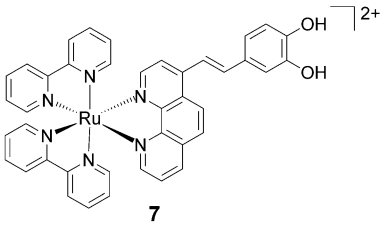

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Figure 4. Ruthenium(II) complex **7** with a groove-binding cataechol moiety.

sequences.[48] It was postulated that the binding of the catechol moiety in the minor groove of duplex DNA was accompanied by the interaction of the Ru^{II} core with alternating purine-pyrmidine sequences in the minor groove, with a special preference for AT·TA steps ($K > 10^7\,M^{-1}$). Interestingly, the ^{3}MLCT luminescence of this complex was initially reduced upon addition of small quantities of ctDNA, as a result of the formation of hydrogen-bonding interactions with the catechol group. After reaching the emission minimum, the further addition of ctDNA led to a gradual increase in the luminescence response through saturation of the groove site by the catechol moiety and the substantial movement of the Ru^{II} core from the bulk solvent environment into the hydrophobic interior of the DNA.

3.1.3. Rhenium(I) Complexes

The DNA-binding properties of rhenium complexes have been comparatively less explored than their platinum and ruthenium counterparts. Pioneering studies on the DNA-binding abilities of rhenium complexes were reported by the research groups of Schanze and Yam. Schanze and co-workers reported that the rhenium(I)-dipyridophenazine complex *fac*-$[Re(dppz)(CO)_3(4\text{-Mepy})]^+$ could function as a luminescent "switch-on" probe for duplex DNA.[49] Meanwhile, Yam et al. developed $[Re(dppz)(CO)_3(py)]^+$ and $[Re(dppn)(CO)_3(py)]^+$ as luminescent metallointercalators (Figure 5).[50] Similar to the Ru^{II} and Pt^{II} complexes described previously, the large polyaromatic moiety in the Re^{I} complexes allows them to intercalate effectively into double-stranded DNA. Interestingly, the lowest lying excited state of *fac*-$[Re(dppz)(CO)_3(4\text{-Mepy})]^+$ is the phosphorescent interligand triplet state (^{3}IL) originating from the dppz ligand, instead of the expected ^{3}MLCT. The intercalative binding mode of these complexes

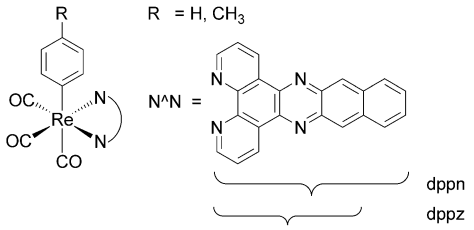

Figure 5. General structures of luminescent rhenium(I) metallointercalators bearing a dppn or dppz moiety.

suggests that these may potentially be developed as luminescent anticancer therapeutics through targeting of duplex DNA.

3.1.4. Gold Complexes

Au^{III} is an isoelectronic congener of Pt^{II}, and its metal complexes have also been studied for their DNA-binding and biological properties. Recently, Yan et al. reported a series of cyclometalated gold(III) complexes bearing N-heterocyclic carbene ligands, including complex **8** (Figure 6) which exhibited anticancer activity.[51] Complex **8** targets DNA and inhibits topoisomerase I (TopoI) activity. It stabilizes the cleavable TopoI–DNA complexes and prevents the religation step, thereby resulting in DNA nicking and inducing apoptosis. Complex **8** displayed superior activity against cancer cell lines than cisplatin, and was significantly less cytotoxic against normal lung fibroblast cells. Consistent with its proposed mode of action, minor cross-resistance was observed with the well-known topoisomerase poison camptothecin. In an in vivo model, complex **8** significantly suppressed tumor growth without inducing toxic side effects in nude mice. Gel shift mobility experiments revealed that complex **8** interacted with duplex DNA through intercalation, and a fivefold enhancement in the luminescence intensity was observed upon interaction of **8** with duplex DNA. This result suggests that this luminescent gold(III) metallointercalator could potentially be developed as an effective therapeutic.

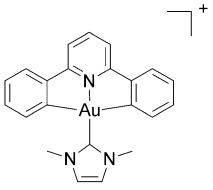

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Figure 6. Gold(III)–NHC complex that targets DNA and inhibits TopoI activity.

3.2. Complexes That Stabilize the Noncanonical DNA Secondary Structures

Guanine-rich DNA sequences have the ability to adopt four-stranded structures, termed G-quadruplexes, which are comprised of planar stacks of four guanine moieties stabilized by Hoogsteen hydrogen bonding and monovalent cations (Figure 7).[32,52–55] G-quadruplex-forming sequences have been found to be over-represented in various areas of the human genome,[33,56,57] including telomeres and the promoter regions of oncogenes such as c-*myc*,[58] c-*kit*,[59] *bcl*-2,[60] and *K*-

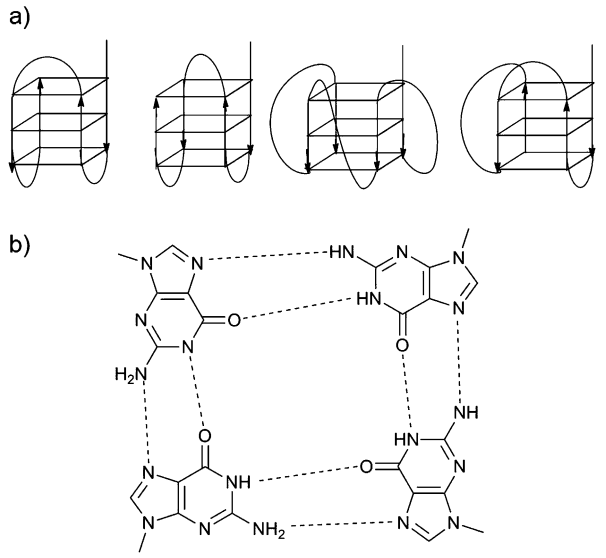

Figure 7. a) Some common topologies of unimolecular G-quadruplexes. b) Coplanar arrangement of a guanine tetrad stabilized by Hoogsteen hydrogen bonding.

RAS.[61] As a consequence of their putative roles in various biological processes such as the regulation of gene expression and modulation of telomere function,[62–65] G-quadruplex DNA has emerged as a potential target for anticancer therapy.[66] Studies from the leading research groups of Balasubramanian,[67,68] Hurley,[69,70] Neidle,[71,72] and others[73–75] have uncovered organic small molecules capable of interacting strongly with G-quadruplex DNA and thereby inhibiting telomerase activity and/or regulating oncogenic transcription. Certain metal complexes have also been reported to exhibit G-quadruplex-binding activities, as recently reviewed by Vilar and co-workers.[76] Several interesting examples in recent years have shown the G-quadruplex structures can also be selectively recognized by luminescent metal complexes with a "light switch" effect and potential biological activity.

3.2.1. *Complexes That Target the Human Telomeric G-Quadruplex*

Human telomeric overhangs are comprised of TTAGGG repeats, and investigations into the molecular structure and the putative in vivo presence of the human telomeric G-quadruplex have made the telomeric G-quadruplex arguably the most-studied of all the quadruplexes to date.[77] As telomeric DNA shortening occurs after every successful cell division, normal cells undergo programmed cell death after a finite number of divisions because of excessive telomeric shortening.[78] Telomerase, which is over-expressed in almost all cancer cell lines, maintains the length of the telomeres and thus delays apoptosis.[79] However, since telomerase only accepts single-stranded overhangs as substrates, stabilization of the human telomeric G-quadruplex structure has been proposed to represent a potential anticancer strategy.[80,81]

Ma et al. synthesized a series of dppz-based Pt^{II} complexes. The square-planar geometry offered by the Pt^{II} complex in conjunction with the heterocyclic aromatic dppz scaffold enabled the complexes to effectively interact with the terminal face of the human telomeric G-quadruplex through end-stacking interactions. Upon binding to the telomeric G-quadruplex, some of the complexes exhibited a strong luminescence, which could be attributed to the protection of the Pt(dppz) moiety by the G-quadruplex. A 293-fold increase in the luminescence intensity was found when complex **9** (Figure 8) bound to telomeric G-quadruplex

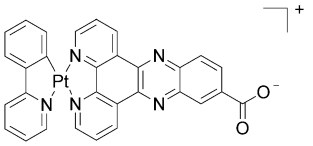

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Figure 8. A selected example of a luminescent dppz-based Pt^{II} G-quadruplex binder.

DNA, and was 10-fold selective for the G-quadruplex over duplex DNA. Complex **9** displayed sub-micromolar efficacy against telomerase activity in vitro and exhibited cytotoxicity towards multidrug- and cisplatin-resistant cell lines. This pivotal study showed that the dppz ligand, the key component of the octahedral Ru^{II} "molecular light switch" DNA metallointercalators, could be harnessed as a G-quadruplex-binding motif by incorporating it within the square-planar geometry of a Pt^{II} complex.

Thomas and co-workers reported two dinuclear polypyridylruthenium complexes $[(Ru(bpy)_2)_2(tpphz)]^{4+}$ (**10**) and $[(Ru(phen)_2)_2(tpphz)]^{4+}$ (**11**, where tpphz = tetrapyridophenazine; Figure 9) that display strong binding affinities to both duplex DNA and the antiparallel G-quadruplex formed from the human telomeric sequence (HTS).[82] Interestingly, the interaction of these complexes with HTS was accompanied by a 150-fold increase in the luminescence signal, with a blue shift of approximately 30 nm of the emission maximum. Further experiments revealed that the luminescence response of these complexes was selective for only those G-quadruplex topologies containing diagonal loops.[83]

Yao and co-workers reported that the archetypical molecular light-switch metallointercalator $[Ru(bpy)_2(dppz)]^{2+}$ was also able to bind the HTS G-quadruplex with high affinity and a significant luminescence response.[84] Furthermore, it was found that the complex could induce the formation of the G-quadruplex motif even in the absence of stabilizing cations. Following on from this study, Mao, Ji, and co-workers recently examined the binding of Ru^{II} complexes containing two dppz moieties with HTS.[85] The luminescence emission of these complexes was enhanced with increasing concentrations of the HTS G-quadruplex, and binding constant values of 9×10^7 and 4.5×10^7 were reported for **12a** and **12b** (Figure 9), respectively.

The telomerase-inhibition activity of luminescent Ru^{II} complexes has only recently been investigated. Liu and co-workers reported a series of $[Ru(phen)_2(N^N)]$ complexes

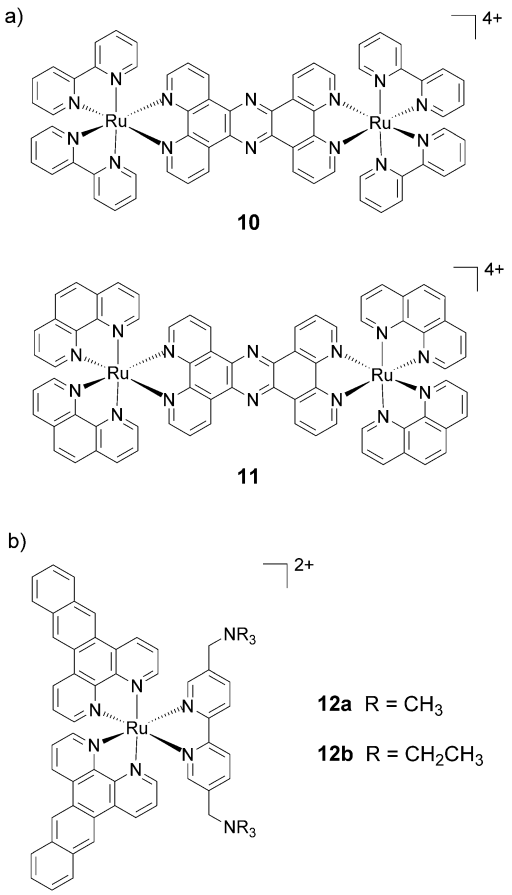

Figure 9. a) Dinuclear polypyridyl complexes that display high affinity for the HTS G-quadruplex and duplex DNA. b) An HTS-selective G-quadruplex binder bearing two dppz motifs.

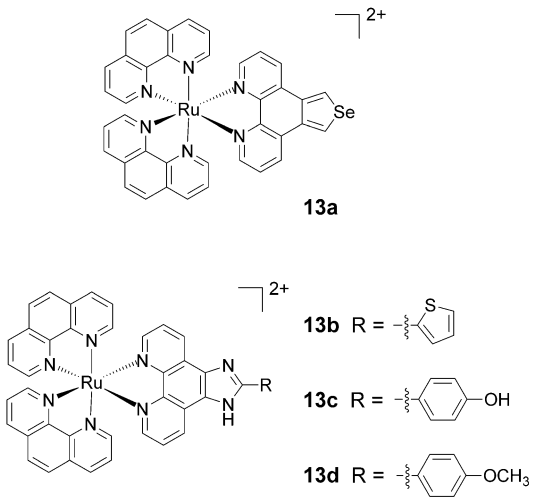

Figure 10. Ruthenium(II) complexes that recognize telomeric DNA and inhibit telomerase activity.

(Figure 10; N^N = 1,10-phenanthrolineselenazole: **13a**; 2-thiophenimidazo[4,5-*f*][1,10]phenanthroline: **13b**; 2-(4-methoxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline: **13c**; and 2-(4-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline: **13d**) as luminescent light switches of telomeric DNA with promising telomerase-inhibition activities.[86–89] These complexes were able to bind to the HTS G-quadruplex with strong affinities and a significant enhancement of the luminescence. Furthermore, some of complexes exhibited low micromolar EC_{50} values against telomerase activity in the telomerase repeat amplification protocol (TRAP) assay. Significantly, some complexes displayed a broad spectrum of cytotoxicity against human cancer cells, while remaining inactive ($IC_{50} > 100$ μM) towards a normal cell line.[86,87] The authors also demonstrated the application of **13c** and **13d** in cellular imaging.[87] The complexes were taken up in HepG2 cells and accumulated in the nuclei, a feature that has been noted to depend on the chirality of the complex.[86]

3.2.2. *Complexes That Target the c-myc G-Quadruplex*

The c-*myc* proto-oncogene plays an important role in many cellular processes, including those related to the cell cycle and apoptosis, and the over-expression of c-*myc* has been associated with a variety of cancers.[90–92] The transcriptional activity of c-*myc* is modulated by the nuclease hypersensitive element III_1 (NHE III_1), a guanine-rich sequence located upstream of the P1 promoter.[93–95] The induction or stabilization of this G-quadruplex by small molecules could potentially downregulate c-*myc* transcription and inhibit cancer growth. This has stimulated the development of luminescent metal complexes that could function as probes for the c-*myc* G-quadruplex and/or downregulate c-*myc* gene expression.

Wu et al. reported Pt^{II} Schiff-base complexes including **14** (Figure 11) that exhibited high selectivity towards the c-*myc*

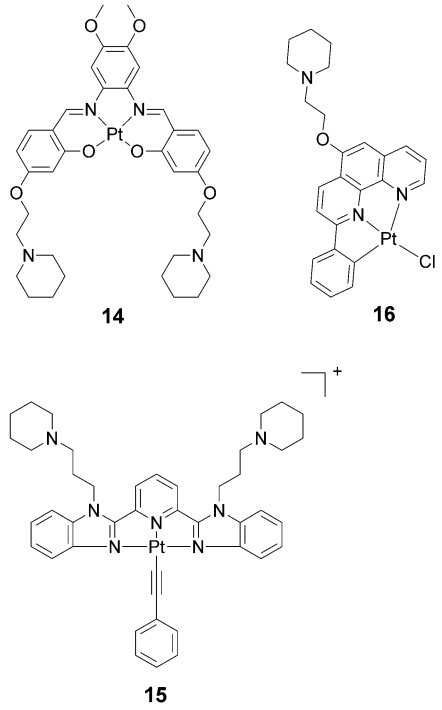

Figure 11. Luminescent Pt^{II}-based c-*myc* G-quadruplex stabilizers.

G-quadruplex over duplex DNA.[96] The square-planar arrangement of the Pt^{II} center arranges the Schiff base ligands into a planar scaffold, which can bind to the terminal G-quadruplex quartets through stacking interactions. Furthermore, the pendant cationic piperidine side chains of **14** after protonation could improve the water solubility and interact with the grooves or loops of the G-quadruplex through electrostatic interactions. The end-stacking binding mode of this complex to the c-*myc* G-quadruplex was confirmed through NMR spectroscopy and molecular modeling experiments. Additionally, complex **14** exhibited a marked enhancement of phosphorescence in the presence of c-*myc* G-quadruplex, which allows it to function as a useful luminescent probe for the c-*myc* G-quadruplex. The complex showed significant cytotoxicity towards cancer cell lines, with comparable potency to cisplatin, and was approximately tenfold less cytotoxic towards normal cells. Complex **14** was found to stabilize the c-*myc* G-quadruplex at micromolar concentrations in a cell-free system and could reduce c-*myc* RNA levels in human cells. Later, Wang et al. reported 2,6-bis(benzimidazol-2-yl)pyridine (bzimpy) Pt^{II} complexes including **15** as biologically active luminescent G-quadruplex probes.[97] Similar to Schiff base complex **14**, the bzimpy-based Pt^{II} complex also contains two pendant piperidine side chains that can contribute additional binding interactions with the G-quadruplex structure. The emission response of **15** was approximately tenfold more selective for c-*myc* G-quadruplex over duplex DNA, thus suggesting that this complex could be utilized as a selective luminescent probe for the c-*myc* G-quadruplex. These complexes were also able to stabilize the c-*myc* G-quadruplex in vitro and downregulate c-*myc* expression in cancer cells. Together, these studies by Che and co-workers demonstrate that Pt^{II} complexes bearing a central aromatic scaffold with pendant amine side chains could be developed as selective luminescent probes for G-quadruplex DNA and downregulators of c-*myc* expression in human cells.

By utilizing the phosphorescent properties conferred by the phenyl-substituted phenanthroline ligand, Vilar and co-workers synthesized a Pt(C^N^N) complex **16** that acts as a luminescent light switch for c-*myc* G-quadruplex DNA.[98] This complex bound strongly to the c-*myc* G-quadruplex ($K =$ ca. 7×10^7) with a 1000-fold selectively over duplex DNA. Furthermore, the complex displayed a strong enhancement of luminescence upon binding to c-*myc* G-quadruplex. After encapsulation of the complex in a hexaruthenium cage, the complex could successfully penetrate cell nuclei and was found to accumulate in the nucleoli. Significantly, co-staining experiments revealed that the complex did not overlap with the duplex DNA probe DAPI, thus suggesting it may target alternative DNA topologies in the cells, such as G-quadruplex DNA.

Alzeer et al. reported a zinc-containing guanidinium-modified phthalocyanine, tetrakis(diisopropylguanidinio)zinc(II) phthalocyanine (Zn-digp, **17**) as a luminescent probe for the c-*myc* G-quadruplex and a downregulator of c-*myc* expression.[99] Zn-digp exhibited a strong "light-switch" effect in the presence of nucleic acids, with a 200-fold luminescence enhancement observed. Significantly, the compound was selective for the c-*myc* G-quadruplex over the human telomeric G-quadruplex, and also displayed a 5000-fold affinity for the c-*myc* G-quadruplex compared to ctDNA. The authors also demonstrated the application of Zn-digp for cellular imaging, and co-staining experiments revealed little overlap with the duplex-binding dye Hoechst 33342 (Figure 12). Zn-digp (**17**) was also shown to lower c-*myc*

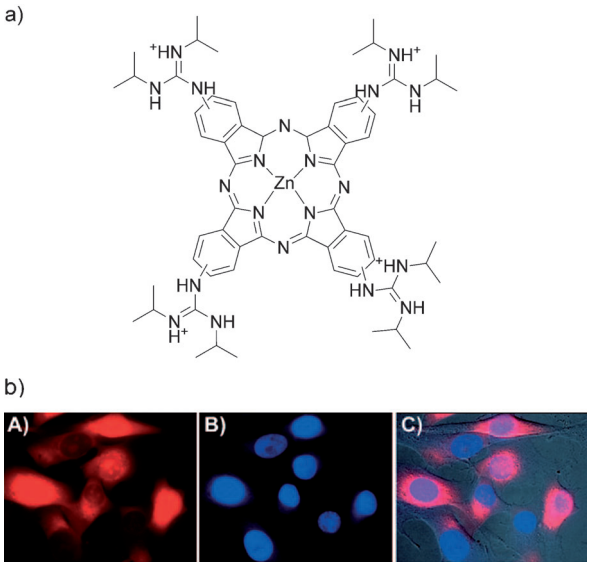

Figure 12. a) Luminescent zinc-containing guanidinium-modified phthalocyanine Zn-digp as a selective c-*myc* G-quadruplex stabilizer. b) SK-Mel-28 cells stained with A) 3 μM Zn-digp and B) 8 μM Hoechst 33342. C) Overlay of (A) and (B) with white-light absorbance under the confocal microscope. Reproduced from Ref. [99].

RNA levels in a cellular system. This study suggests that metal-containing phthalocyanine compounds could be developed as highly selective luminescent therapeutics that specifically target c-*myc* expression because of their enhanced selectivity for the c-*myc* G-quadruplex compared to duplex DNA, the predominant nucleic acid conformation in the cellular nucleus.

3.3. Photoactive Complexes That Cause DNA Damage

Photocleavage of cellular DNA is an alternative strategy for the development of anticancer metal complexes. These complexes are able to interact with DNA and to cleave DNA upon irradiation with UV/Vis light by virtue of their unique photophysical and photochemical properties. DNA cleavage can be classified as either oxidative or hydrolytic.[100,101] In the oxidative pathway, DNA nucleobases are oxidized by reactive oxygen species (ROS), including $^1O_2$ and ·OH, generated by the light-activated redox-active metal complexes. By comparison, the hydrolytic cleavage of DNA involves the fragmentation of the phosphodiester sugar backbone of oligonucleotides, which leads to disruption of the overall DNA architecture.

After the first report of the DNA-cleaving 4d Ru^{II} polypyridyl complexes by Barton, Turro, and co-workers,[102]

a number of other photoactivated metal complexes have been developed. The isoelectronic rhodium(II) analogues have also been reported to display DNA-cleaving properties. Comprehensive reviews on the mechanistic features of these complexes have been reported by the research groups of Schatzschneider and Barton.[103–105] Recently, the research group of Chakravarty played a pivotal role in pioneering the development of 3d transition-metal complexes as photocleavage agents (Figure 13).[106–113] Compared to their second-row 4d counterparts, these 3d photocleavage complexes are generally photoactivated by longer excitation wavelengths, which may offer deeper penetrative power into biological tissues. A recent review on photoactivated metallopharmaceuticals has been written by Schiller and co-workers.[104]

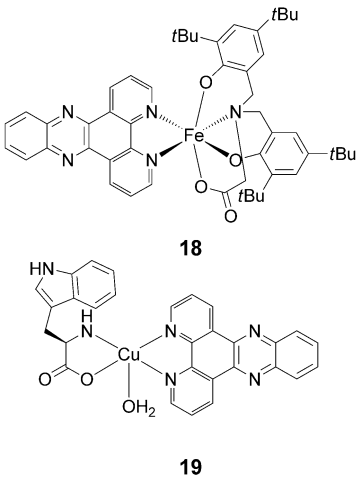

Figure 13. Examples of 3d transition-metal complexes that photocleave DNA.

4. Luminescent Complexes That Target Proteins

Recent progress in molecular biology has greatly expanded the repertoire of protein (and enzyme) targets for therapeutic intervention. Although most reported protein inhibitors have been small organic molecules, inorganic metal complexes have received increasing attention because of their modular and facile synthesis and precise three-dimensional shape.[115–117] Dyson and co-workers have stressed the importance of finding new protein targets for anticancer drugs in the postgenomic era.[15] Metal-based inhibitors can be broadly classified into two types: 1) complexes with labile ligands that can engage in substitution reactions with key residues in the target protein or enzyme targets, and 2) substitution-inert metal complexes that interact noncovalently with binding sites in the target protein or enzyme.

In this section, we will describe recent examples of biologically active luminescent metal complexes that target proteins. As a consequence of the role of particular proteins as markers and causative agents of diseases, such dual-function metal complexes could potentially occupy a niche position in allowing the simultaneous treatment and monitoring of pathological progression. These studies will be described in more detail compared to the preceding examples, as we focus on the ability of these complexes to interact with protein targets with a switch-on luminescence response.

A luminescent Au^{I}-phosphine complex containing a naphthalimide ligand (**20**) was reported by Ott et al. in 2009 (Figure 14).[118] Gold complexes had previously been reported

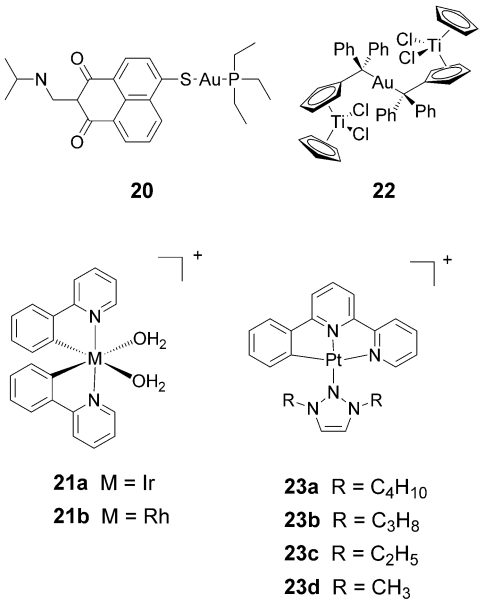

Figure 14. Examples of protein-targeting luminescent metal complexes.

to display strong anticancer activities through inhibition of thioredoxin reductase (TrxR), a disulfide reductase that promotes tumor growth and development, by ligand dissociation and formation of a covalent linkage to the cysteine or selenocysteine residues of the active site. Bagowski et al. combined the anti-TrxR effects of the gold ion with the *N*-(*N*′,*N*′-dimethylaminoethyl)-4-mercapto-1,8-naphthalimide ligand,[119] which contains the desirable pharmacophoric elements of the naphthalimide class of anticancer agents: a heterocyclic scaffold for DNA intercalation and a potentially cationic side chain for electrostatic interactions with the phosphate DNA backbone. In vitro assays revealed that complex **20** inhibited TrxR at a sub-micromolar concentration, and the covalent formation of the gold-enzyme adduct was confirmed by mass spectrometry experiments, which revealed that the naphthalimide ligand was lost upon binding. Treatment of cancer cells with complex **20** resulted in strong antiproliferative and pro-apoptotic effects, mediated through the mitochondrial apoptotic pathway.

Complex **20** exhibited strong fluorescence in nonpolar solvents, but its emission was quenched in aqueous solution, presumably through solvent quenching of the photoinduced electron transfer (PET). Biodistribution experiments showed that the gold complex was effectively internalized into specific cellular compartments, including the nucleus. By comparison, the [AuCl(PEt_3)] complex without any naphtha-

limide groups showed a much lower rate of nuclear uptake, thus suggesting that the intercalating aromatic ligand played an important role as a vector to facilitate the transport of metals into the nucleus. In an in vivo model, treatment of zebrafish embryos with complex **20** inhibited angiogenesis, a process which is stimulated by TrxR, whereas $[AuCl(PEt_3)]$ was almost inactive. The complex pharmacological profile of **20** suggests the presence of multiple biological targets, including nuclear DNA and mitochondrial biomolecules. This study by Ott et al. demonstrated that multifunctional luminescent metal complexes can be developed by combining an intercalating ligand (naphthalimide) with an active metal ion (gold).

The aggregation of β-amyloid (Aβ) peptide is a characteristic hallmark of Alzheimer's disease (AD). One potential approach for halting the pathological progression of AD is through inhibiting the aggregation of monomeric Aβ peptides into neurotoxic fibrils by small molecules or peptide agents. The research groups of Ma and Li reported a series of cyclometalated rhodium(III) and iridium(III) solvato complexes as inhibitors of $A\beta_{1-40}$ peptide aggregation.[120] These complexes contain aromatic coligands to interact with the hydrophobic residues around the N-terminal domain of the Aβ peptide, and labile solvato ligands that allow covalent attachment of the metal center to the imidazole N donor of histidine residues of the Aβ binding site. Rhodium(III) complex **21b** displayed nearly complete inhibition of amyloid fibrillogenesis at 5 μM, as observed by reduced fibril lengths and densities in fluorescence and TEM images. Mass spectrometry experiments confirmed the covalent attachment of the metal complex to the Aβ peptide, which suggests that the complexes could effectively bind seed fibrils and block the active elongation site. Cytotoxicity experiments revealed that antiproliferative effects were only observed at concentrations higher than those required for complete inhibition of Aβ aggregation, thus indicating the presence of a therapeutic window where Aβ fibrillogenesis can be limited without significant damage to brain cells. The iridium(III) complex **21a** showed a strong enhancement of its luminescence in the presence of histidine or Aβ peptides, and the luminescence response to aggregated Aβ was nearly three times as high as that for an equal mass concentration of monomeric Aβ peptides. This finding suggests that the complex could be used to differentiate between the monomeric and fibrillar forms of Aβ, or to monitor Aβ fibrillogenesis.

Heteronuclear metal complexes have attracted attention because of their distinct redox properties and interactions with biomolecules as well as their remarkably different kinetics of hydrolysis arising from an increased overall charge of the complexes. Recently, the research group of Picquet and Casini reported a series of Au-Ti complexes as potential anticancer agents.[121] Interestingly, complex **22** displayed two intense emissions in the blue and red regions under solid-state conditions. Complex **22** was found to be tenfold more cytotoxic towards ovarian cancer cell lines than cisplatin. Further analysis revealed that this complex does not primarily interact with DNA, but may target cellular proteins or enzymes through coordination of the dissociated Au^{III} ion with amino acid residues.

Sun et al. reported a series of luminescent cyclometalated Pt^{II} complexes (**23**) with N-heterocyclic carbene ligands as anticancer agents.[122] Unlike most metallointercalators, **23a** was found to bind only weakly to ctDNA, with a relatively low binding constant ($4.8 \times 10^3\ dm^3\,mol^{-1}$). It displayed nanomolar cytotoxicities towards cancer cell lines, but was approximately 200-fold less cytotoxic towards normal cells. Complex **23a** was approximately 300-fold more potent against HeLa cells than cisplatin, and was additionally found to display a synergistic anticancer effect with cisplatin. Moreover, this complex could significantly inhibit tumor growth in a nude mice xenograft model without causing reduction of the body weight. Interestingly, **23a** was found to be accumulated in the cytoplasm of cancer cells rather than in the nucleus, as revealed by fluorescence microscopy. The cytotoxic effect of this complex was attributed to inhibition of the survivin signaling pathway. Survivin is an inhibitor of apoptosis, and is more highly expressed in carcinoma cells than in normal cells.[123] Western blotting experiments indicated that complex **23a** could inhibit survivin and activate caspase-3 and poly-(ADP-ribose) polymerase in a dose-dependent manner in HeLa cells, thereby inducing apoptosis. These results suggest an alternative mode of action for Pt^{II} therapeutics with other biological targets instead of DNA, and could be an effective means of overcoming anticancer drug resistance and/or achieving synergistic effects with covalent or noncovalent DNA-binding platinum drugs. Given the luminescence properties of the complexes, it would have been interesting for the authors to also investigate whether these complexes could be used as selective probes for survivin. As an isoelectronic analogue of **23d**, the previously described gold(III) metallointercalator **8** could potentially display similar activity against the survivin pathway. In our view, the ability of complex **8** to exert cytotoxic effects against cancer cells through alternative mechanisms thus deserves further investigation.

5. Cellular Uptake and Localization of the Complexes

The avid and selective interactions achieved by luminescent metal complexes for DNA or proteins as described in this Review suggest that such theranostic complexes could potentially be developed as biological imaging or therapeutic agents. Therefore, these compounds must be able to successfully enter the cell and reach their desired location in order to exert their function(s). General methods by which molecules can penetrate the phospholipid bilayer of cells include passive diffusion, transport by membrane proteins, and endocytosis. In this section, we provide an overview of the strategies that have been employed to improve cellular uptake efficiency and to achieve localization or specific targeting within the cells.

5.1. Hydrophobicity and Cellular Uptake

Puckett and Barton have investigated the cellular uptake and localization properties of the $[Ru(N\hat{}N)(dppz)]^{2+}$ system

in HeLa cells by flow cytometry and confocal microscopy, respectively.[124] It was observed that the efficiency of the cellular uptake correlated with the hydrophobicity of the N^N ligand. The more hydrophobic complex $[Ru(dip)_2(dppz)]^{2+}$ (**24**, Figure 15; dip = 4,7-diphenyl-1,10-phenanthroline) showed an increased uptake efficiency compared to other complexes. Furthermore, the complex was found to localize in the cytosol and not in the nucleus.[124] This observation is in agreement with studies by Sheldrick and co-workers[46] as well as Lo and Zhang,[125] who reported a correlation between the hydrophobicity of the Ir^{III}/Rh^{III} complexes and their cytotoxicity. In other studies, Svensson and co-workers demonstrated that incorporating lipophilic alkyl side chains to the Ru^{II}-dppz complexes alters the cellular localization of the complexes and may lead to a decrease in the nuclear targeting efficiency:[126,127] It was found that complexes with shorter alkyl chains localized in the nuclei while complexes with longer chains were entrapped within hydrophobic membranes in the cytoplasm.

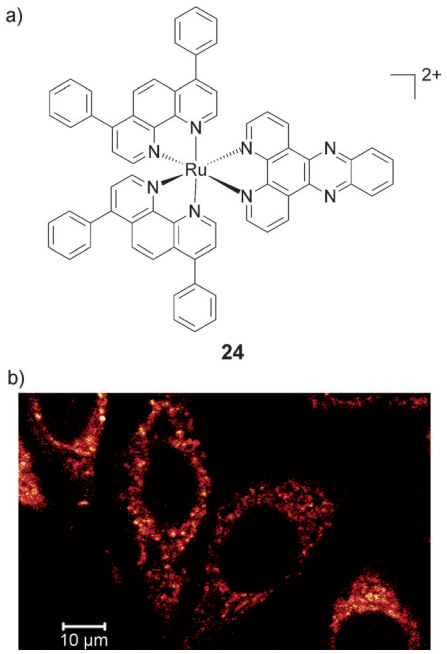

Figure 15. a) Chemical structure of $[Ru(dip)_2dppz]^{2+}$ and b) its localization in HeLa cells. Reproduced from Ref. [124].

Extensive studies by Thomas and co-workers have shown that the dinuclear $[(Ru(phen)_2)_2(tpphz)]^{4+}$[128] and mononuclear $[Ru(phen)_2(tpphz)]^{2+}$[129] complexes are internalized by cells through an active, non-endocytotic mechanism, and rapidly enter the nuclei of MCF-7 cancer cells. These complexes also possess high cytotoxicity against a panel of human cancer cell lines, including cisplatin-resistant ovarian cancer cells, thus suggesting that these luminescent Ru^{II}-polypyridyl complexes could potentially be developed as cellular DNA imaging agents and/or therapeutics.

5.2. Bioconjugation

The conjugation of metal complexes with bioactive organic moieties has been shown to be a viable strategy to generate novel anticancer agents with enhanced cytotoxicity, improved delivery, and specific localization. Specific localization or targeting may be achieved by linking different biomolecules to the metal complex. A variety of substances such as short peptides, carbohydrates, hormones, and small molecules can be covalently attached to metal complexes. As an example, Dyson and co-workers synthesized a hybrid agent comprised of a Ru^{II}-arene complex and the bioactive enthacrynic acid moiety, which was shown to exhibit an enhanced antiproliferative property against a panel of cancer cells.[130] Furthermore, Puckett and Barton have demonstrated that nuclear accumulation of an octaarginine–ruthenium(II) conjugate can be achieved by fluorescent tagging with an organic fluorophore.[131] In this section, we highlight some strategies to improve the cytotoxicity or to achieve the target-specific recognition of the luminescent metal complexes.

Studies by Lo et al. have demonstrated that improved cellular uptake and localization could be achieved by conjugating a variety of biomolecules such as indole,[132,133] biotin,[134,135] and estradiol[136] to metal complexes through an alkyl linker. In a recent study, Lo and co-workers prepared a series of luminescent Re^{I}-polypyridine-PEG-amine complexes (**25**, Figure 16) as targeted cellular imaging agents with comparable cytotoxicity to cisplatin.[137] The attachment of the PEG unit to the complexes not only confers increased water solubility, but also allows specific labeling of *n*-butylamine, bovine serum albumin (BSA), and polyethyleneimine (PEI) after activation by thiophosgene. Interestingly, however, the conjugation of PEG to Ir^{III} complexes significantly reduced their cytotoxicity against cancer cells.[138] Similarly, the Doyle research group synthesized luminescent Re^{I}-

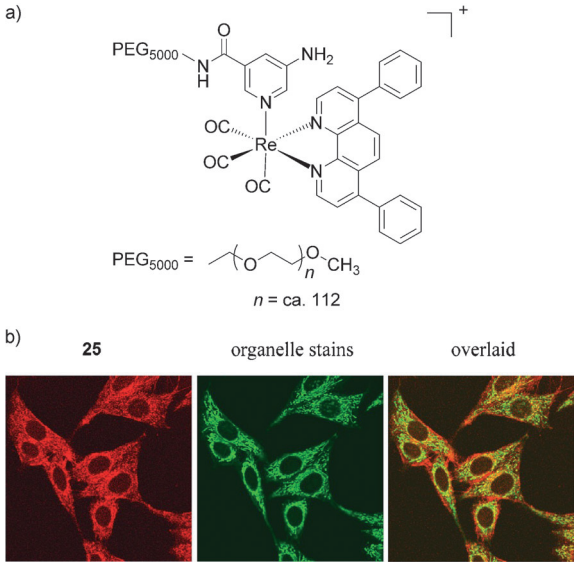

Figure 16. a) Chemical structure of the rhenium(I)-polypyridine-PEG-amine complex **25**. b) Laser-scanning confocal microscopy images of HeLa cells treated with **25** and organelle stains. Reproduced from Ref. [137].

tricarbonyl complexes functionalized with folate,[139] which were found to be active against the cisplatin-resistant folate receptor (FR) overexpressing cancer cell line, but was noncytotoxic towards the FR-negative cells. The high cytotoxicity of this complex may be attributed to the ability of the complex to interact with DNA through minor-groove binding and its subsequent inhibition of TopoI activity.

Another strategy to enhance cytotoxic effects is to tether a cancer-directing peptide to metal complexes. Recently, the research group of Lippard reported a Pt^{IV}-chlorotoxin (CTX) conjugate which is able to selectively target gliomas and related tumors.[140] The selective targeting of these cancer cells was achieved through the specific interaction between CTX and matrix metalloproteinase-2 (MMP2), a protein significantly upregulated in these tumor cells.[141] Ravera and coworkers have reported a similar approach by tethering analogues of neurotensin (NT) or somatostatin to the Pt^{IV} motif.[142] The conjugated Pt^{IV} complexes showed enhanced cytotoxicity against cancer cell lines overexpressing the NT or somatostatin receptors compared to the nonconjugated parent complex. These recent examples are exciting developments that may enhance the delivery, selectivity, and potency of these complexes. We envisage that similar approaches could be applied to luminescent metal complexes to generate bioimaging or therapeutic agents with improved selectivity.

An alternative strategy to achieve cellular localization and a cytotoxic effect is to functionalize the metal complexes with artificial peptide nucleic acids (PNAs). The research group of D'Alfonso and Licandro have established a reliable solid-phase synthetic method for the synthesis of a luminescent dinuclear Re^{I}-PNA conjugate **26** (Figure 17) for two-photon absorption imaging in cells.[143] Importantly, complex **26** is photostable and noncytotoxic, readily permeates living cells, and stains the cytoplasm and nucleus with different colors. Very recently, the research group of Gasser and Metzler-Nolte synthesized a luminescent noncytotoxic mononuclear rhenium(I) complex functionalized with PNA (**27**) by using the copper(I)-catalyzed Huisgen azide–alkyne cycloaddition reaction. The rhenium(I) complex **27** exhibited a large Stokes shift, good quantum yields, and a long fluorescence lifetime,

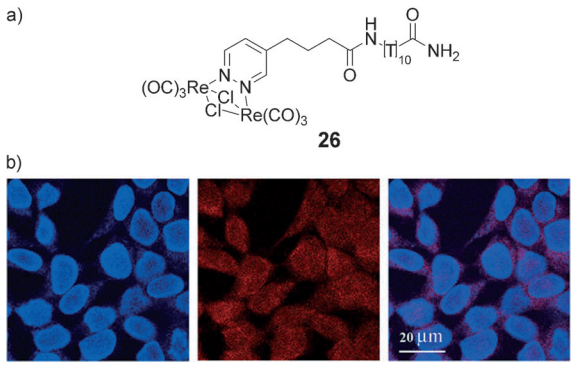

Figure 17. a) Chemical structure of the dinuclear rhenium(I)-PNA conjugate **26**. b) Laser-scanning confocal microscopy images recorded through a 485/30 (left) and a 600/40 (center) band pass filter of HEK-293 cells treated with **26** for about 10 min. The picture on the right shows an overlay of the two images. Reproduced from Ref. [143].

thus making it suitable for cellular staining (Figure 18).[144] Furthermore, preliminary data using genetically modified HeLa cells suggest that the metal–PNA conjugates could be used as antisense agents. In our view, this relatively recent strategy to improve cellular/nuclear absorption by using

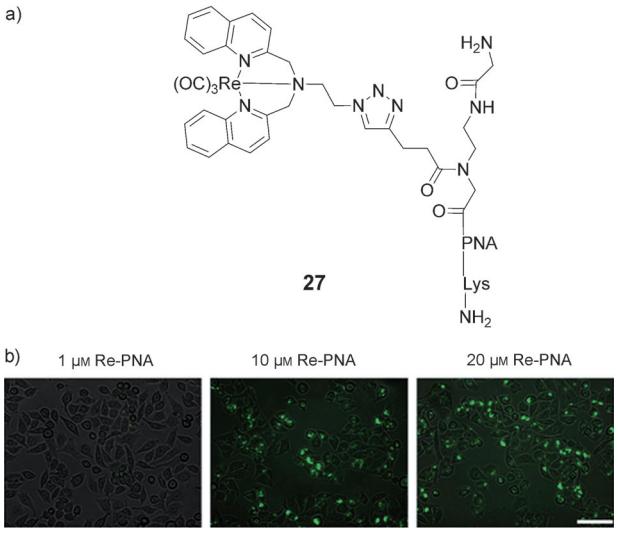

Figure 18. a) Chemical structure of rhenium(I)-PNA conjugate (**27**). b) Fluorescence micrographs of HeLa cells 24 h after delivery of three different concentrations of Re-PNA by electroporation. Reproduced from Ref. [144].

peptide or PNA-functionalized complexes deserves to be further explored as a means of improving the biological and/or staining properties of luminescent transition-metal complexes. Comprehensive reviews from the research groups of Barton,[145] Coogan,[146] Thomas,[147] and Gasser and Metzler-Nolte[148] describe the strategies that have been developed to improve cellular uptake and localization as well as the principles involved in targeted therapeutic or imaging inorganic agents.

6. Conclusion and Outlook

With the clinical success of cisplatin and its derivates, inorganic medicinal chemistry has rightfully established itself as an independent discipline. The advantages of luminescent transition-metal complexes over small organic molecules include: 1) facile and modular synthesis, 2) the ability to fine-tune the steric and electronic properties of the complex through optimization of the auxiliary ligands, 3) a precise three-dimensional arrangement of ligands dictated by the geometry of the coordination sphere, 4) the ability to undergo ligand exchange reactions with biomolecular targets, 5) photophysical properties that are highly sensitive to changes in the local environment, and 6) a long phosphorescence lifetime that can be readily distinguished from fluorescent background by use of time-resolved spectroscopy. Despite these advantages, however, organic molecules are still most widely employed for biological and sensing applications. To

further advance the development of inorganic medicinal chemistry, researchers should continue to focus their efforts on the discovery of luminescent metal complexes that could probe and/or treat the underlying mechanism of diseases, through specific modes of interactions against defined biomolecular targets. Combining the therapeutic and detection functions into a single "theranostic" metal complex could allow the simultaneous treatment and monitoring of a disease state without requiring the addition of an external labeling or imaging agent, potentially avoiding drug–probe interactions.

In this Review, we have highlighted recent examples of luminescent transition-metal complexes that display useful biological activities. We have broadly classified the metal complexes into those targeting DNA and those targeting proteins, and have discussed the mode of action of each complex. Additionally, we have discussed strategies to improve cellular uptake and/or localization of the metal complexes. In our opinion, finding and investigating new modes of action for existing biomolecular targets is one of the most important challenges for future inorganic medicinal chemistry. Opening new avenues for therapeutic intervention can combat drug resistance and offer possibilities for synergistic effects. Furthermore, it is known that the vast majority of luminescent probes are unsuitable for in vivo imaging because of the poor penentration of tissue by visible light. Looking ahead, luminescent metal complexes with an emission in the near-infrared (NIR) range would be especially attractive so as to exploit the "optical window" (700–900 nm) where the absorption of light by tissues is minimal. To this end, Prasad and co-workers demonstrated the use of a Pt^{II} complex with an emission maximum at 903 nm encapsulated inside a PEG-modified phospholipid micelle for the in vivo luminescence imaging of tumor tissues in live mice.[149]

Given the long history of the interaction of metal compounds with DNA, it is not surprising that most of the complexes presented here have been investigated for their anticancer properties. Most conventional metal-based anticancer therapeutics are not molecularly targeted but exhibit "shotgun" cytotoxicity against a broad range of tumor types. While this rather nonselective mode of action has been shared by many of the DNA-binding agents described in this Review, certain complexes have shown selective recognition of non-canonical DNA structures such as the G-quadruplex motif, thereby potentially allowing for specific inhibition of telomerase activity or oncogenic transcription.

Modern trends in inorganic medicinal chemistry have focused in the direction of targeted imaging and therapy. Pioneering studies by Meggers and co-workers have shown that inert organometallic scaffolds can be developed as kinase inhibitors with superb affinity and selectivity, although most of these complexes have been nonluminescent.[150–152] Therefore, theranostic metal complexes that can exert their biological effects through specific targets other than DNA deserve particular attention. The Pt^{II}-NHC complexes reported by Sun et al. targeted the survivin signaling pathway, and achieved a synergistic anticancer effect with cisplatin.[122] The Au^{I}-phosphine complex bearing a naphthalimide ligand reported by Ott et al. had a dual mechanism of action: the gold ion targeted thioredoxin reductase through coordination to cysteine, while the naphthalimide moiety promoted nuclear uptake through DNA intercalation.[118] Besides anticancer activity, luminescent metal complexes have also been employed to inhibit amyloid aggregation for the treatment of Alzheimer's disease, as reported recently by our research group.[120] In our view, these recent findings are exciting and should encourage medicinal inorganic chemists to explore new protein targets that can be targeted by luminescent metal complexes. We envisage that theranostic metal complexes could, and should, play a larger role for the investigation and treatment of human diseases in the future.

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