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Metal complex–DNA interactions: from transcription inhibition to photoactivated cleavage

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Metal ions and complexes, because of their cationic character, three-dimensional structural profiles, and propensity for performing hydrolysis, redox, or photoreactions, have a natural aptitude for interacting with DNA. Indeed, the need for cellular regulation of DNA led to the evolution of metallonucleases to catalyze and repair DNA strand breaks. Moreover, inorganic constructs such as cisplatin and bimetallic rhodium acetate exert antitumor activity by inner-sphere coordination to DNA. Because binding and cleavage of DNA is at the heart of cellular transcription and translation, it is an obvious target for therapeutic intervention and the development of diagnostic structural probes. To this end, new metal complexes have been designed that utilize or create open coordination positions for DNA binding and hydrolysis, generate reactive oxygen-containing species or other radicals for DNA oxidation, or perform direct redox reactions with DNA. The recent emerging themes are the development of bifunctional architectures containing multiple metal-binding or reactive sites, specialized ligand implementation, or incorporation of site-specific targeting substructures. This review describes their employment in novel reaction strategies that do not require bimolecular cofactors and as site-specific probes or cleavage agents.

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Introduction

Lewis acidic metal centers are chemically well suited to influence fundamental cellular processes because of their affinity for basic nitrogen and oxygen donor ligands, as well as their capacity to support larger aromatic architectures capable of π interactions with the nucleic acid building blocks. Their ability to directly hydrolyze phosphodiester linkages as well as promote redox chemistry or generate reactive oxygen-derived species further accentuates their natural aptitude for participation in cellular functions involving nucleic acids. Because binding and cleavage of nucleic acids lies at the heart of cellular transcription and translation, these substrates are obvious targets for therapeutic intervention and the development of diagnostic probes of nucleic acid structure.

The discovery that diamminedichloroplatinum(II) ($Pt(NH_3)_2Cl_2$, cisplatin) promotes cancer cell death by binding to DNA via chloride ligand exchange [1••], coupled with the steadily emerging understanding of the role of metal ions in restriction endonuclease [2], hydrolase and phosphatase activity, has ignited a firestorm of work to examine the interactions of metal complexes with both DNA [3] and RNA [4••]. Because of biological differences in DNA and RNA function and the need for high DNA stability, DNA lacks a C2′ ribose ring hydroxyl moiety that in RNA serves as a nucleophile in the transfer of a phosphate diester, leading to formation of a C5′ ribose hydroxyl leaving group and strand scission. The absence of the C2′ hydroxyl in the DNA backbone leads to enhanced kinetic stability and, thus, greater challenges for cleavage reagents.

An excellent review of metallonucleases for RNA cleavage has recently appeared [4••]. As such, the scope of this work will be restricted to new developments and emerging strategies over the past two years for transcription inhibition, thermal DNA cleavage, photochemical strand scission and site-specific DNA recognition with the ultimate goal of influencing subcellular processes.

DNA binding and transcription inhibition

Inner-sphere coordination

Cisplatin is extremely effective against testicular and other cancers. The mechanism of cellular cytotoxicity derives from the generation of a covalent 1,2-intrastrand d(GpG)cross-link that kinks the DNA structure, inhibiting transcription. Several compositional variants of cisplatin, including modification of the amine donors as well as replacement of the labile chloride ligands with carboxylates, are active and currently in clinical trials (Figure 1a). Advanced ligand designs to cisplatin frameworks, in hopes of enhancing transport properties and targeting, as well as generating antitumor function to cisplatin-resistant cell lines [5,6], are at the frontier of the field (Figure 1b). Mechanistic developments have challenged the established model of N7 coordination by guanine (versus N1, N7 of adenine) as the key mechanism by which platinum-based compounds crosslink DNA [7,8]. More recent advances in ligand design include the

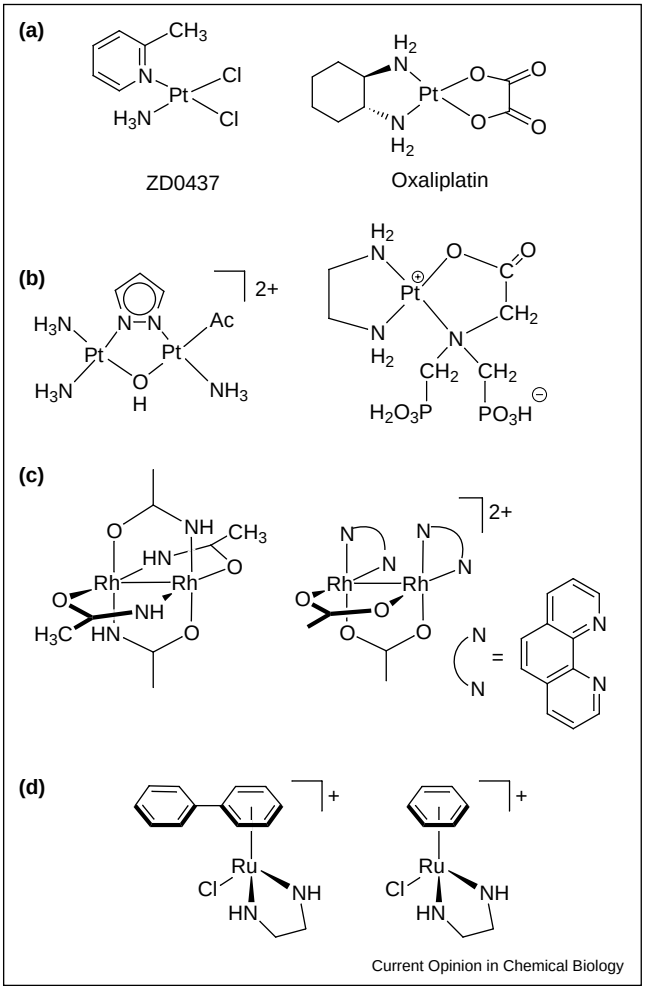
Glossary
List of abbreviations.
5-EG: 5-ethylguanine
bpm: 2,2′-bipyrimidine
bpy: 2,2′-bipyridine
dpp: 2,3-bis(2-pyridyl)pyrazine
dtdeg: bis [4′-(2,2′:6′,2″-terpyridyl)]-diethyleneglycol ether
DTPB: 1,1,4,7,7-penta(2′-benzimidazol-2-ylmethyl)-triazaheptane
EDTA: ethylenediaminetetraacetic acid
EGTB: *N*,*N*,*N*′,*N*′-tetrakis(2′-benzyimidazol-2-yl-methyl)-1,4-bis(ethylamino)-bis(ether)
en: ethylenediamine
IDB: *N*,*N*-bis(2′-benzimidazol-2-yl-methyl) amine
phen: 1,10-phenanthroline
tpy: 2,2′:6′,2″-terpyridine

use of 'bone-seeking' [(bis(phosphonomethyl)amino-κN)-acetato-κO]$^{2-}$ ligands that have a high affinity for the hydroxylapatite bone matrix to combat osteosarcoma and bone metastases [9•] (e.g. Figure 1b).

Much like cisplatin, simple dirhodium carboxylate lantern complexes (e.g. $Rh_2(\mu\text{-}O_2CR)_4L_2$ R = Me, Et, Pr, CF_3; L = solvent) are known to possess antitumor activity, which is proposed to derive from inhibition of DNA transcription and translation [10,11] (Figure 1c). Several structural variants (e.g. formamidinate complexes $Rh_2(\mu\text{-}HNCOCH_3)_4$ [12]) also show transcription inhibition, some of which are cationic (e.g. *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2]^{2+}$) and bind to the anionic biopolymer scaffold with association constants on the order of 10^2–10^4 M^{-1}. Detailed, 2D NMR studies reveal equatorial N7/O6 coordination spanning the Rh—Rh core to form $Rh_2(OAc)_2$[d(pGpG)] in a similar manner to the structure of the *cis*-$[Pt(NH_3)_2]$[d(pGpG)] adduct [13]. Gel shift assays support formation of stable interstrand cross-links indicating that DNA is a potential biological target for these compounds [14••].

Finally, antitumor monodentate Ru(II) arene compounds of the general formula $[(\eta^6\text{-arene})Ru(en)Cl]^+$ have been shown to bind preferentially to N7 of guanine residues in double-stranded DNA [15–17] (Figure 1d). DNA binding has also been proposed to include arene intercalation and minor groove binding for the larger arene systems, but not for those with single hydrocarbon rings. This may affect differently later events in damaged DNA biochemistry, and consequently may result in different biological responses.

Figure 1



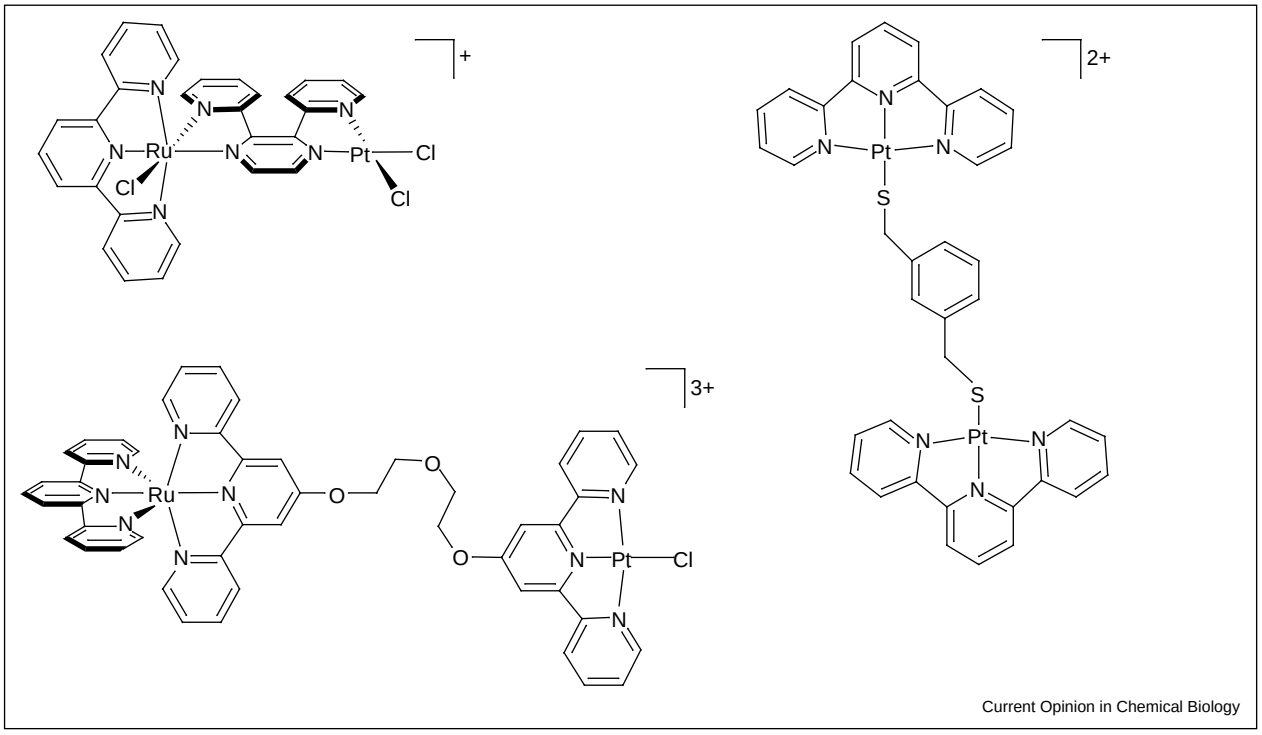
Proposed metal complex transcription inhibitors. **(a)** Cisplatin antitumor analogues in clinical trials. **(b)** Platinum compounds designed for cisplatin-resistant cell lines (left), and as bone-seeking antitumor agents (right). **(c)** Rhodium carboxylate and formamidinate d^7—d^7 lantern structures as transcription inhibitors. **(d)** Ruthenium arene complexes with binding affinity for N7 of guanine.

Intercalative binding

Although inner-sphere coordination is a prominent mechanism in metal–DNA interactions, the documented intercalative ability of inert chiral transition metal complexes (e.g. $[Ru(diimine)_3]^{2+}$) and their variable luminescent 'lightswitch' behavior in the presence and absence of DNA [18] once again demonstrates the aptitude of transition metal complexes for modulating and probing DNA structure through intercalation [19].

The combined advantages of inner-sphere DNA binding and optical properties of an intercalative inert chiral framework have recently been grafted into a single molecular construct toward the development of multifunctional supramolecular complexes [20]. These Ru(II)/Pt(II) bimetallic polyazine bridging ligand (BL) structures of the form $[(tpy)RuCl(BL)PtCl_2]PF_6$ (Figure 2; see Glossary for definition of tpy and other ligands) possess optical properties associated with the Ru(II)-polyazine scaffold and labile chloride ligands at the Pt(II) center analogous to cisplatin. Conceptually similar bimetallic

Figure 2



Bifunctional intercalating and inner sphere coordinating Pt(II) and Ru(II)-terpyridine derivatives.

$[Pt_2(terpyridine)_2SR]^{2+}$ complexes with variable linker lengths and conformations are proposed to bind to DNA as a two-point intercalative biopolymer chelate [21]. In this theme, a highly flexible heterodinuclear Ru(II)–Pt(II) terpyridine complex [(tpy)Ru(dtdeg)PtCl]Cl_3 possessing an intramolecular Ru–Pt distance of 14.5 Å allows cooperative electrostatic/intercalative binding of the Ru(II) unit in the minor groove and coordination of the platinum moiety in the major groove [22]. Constructs of this type will undoubtedly become more prominent as DNA binding approaches evolve toward synthetic metallo-restriction enzymes.

Thermal DNA cleavage

Hydrolysis

The need for cellular regulation of DNA led to the evolution of metallonucleases and topoisomerases to catalyze and repair DNA strand breaks. Lewis acidic metal ions such as Mg(II), Ca(II) and Zn(II) have been recruited to cleave the phosphodiester bonds due in part to their ability to lower the pK_a of coordinated water, creating the active hydroxyl nucleophile. In some cases, with a second equivalent of metal ion, the leaving group is simultaneously polarized for nucleophilic attack [3,23]. In accordance with the key criteria for effective hydrolytic activity (Lewis acidity, affinity for hard oxygen ligands, substitutional lability), the most effective hydrolytic metal ions have been shown to be lanthanide ions (Ln(III), Eu(III), Dy(III), Ce(IV)), as well Zn(II), Ca(II), Cu(II) and, to some extent, Fe(III). Although many mononuclear systems involving azacrown, Schiff-base, diimine, and aminocarboxylate ligands are hydrolytically effective [3,4,23], the current synthetic trend is the evelopment of dinuclear and multinuclear metal assemblies that better position metal ions at the cleavage site in an analogous manner to type II restriction endonucleases [24].

Of these second generation strategies, the trivalent diiron complex $[Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}OAc)]Cl(BF_4)_2$ has been shown to not only cleave DNA in the absence of external agents, but also exhibits one of the largest known rate enhancements, $\sim 10^{10}$ vs DNA [25]. Inhibition by added phosphate suggests that inner-sphere phosphate coordination to Fe(III) is a key mechanistic step. Comparison of cleavage activity to other active diiron complexes reveals that the oxo-bridge, presence of fewer anionic ligands, and open coordination positions are all important structural aspects for enhancing the cleaving activity of these compounds. Several of these compositional features are in common with the purple acid phosphates, providing insight into the key structural components required for biological activity.

In keeping with this trend, the dinuclear zinc complex Zn_2(L2O) based on a bis(1,4,7,-triazacyclononane) ligand containing a 2-hydroxypropyl linker [26] shows enhanced

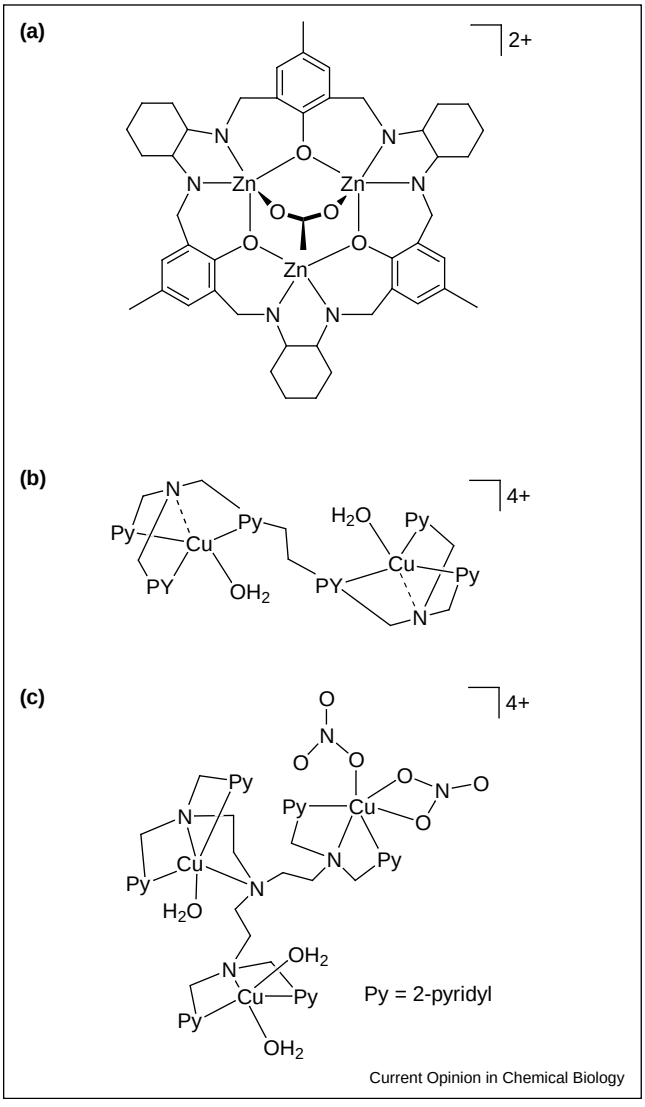
cleavage activity against the model substrate 2-hydroxypropyl-4-nitrophenyl phosphate (HPNP) over its mononuclear analogue. The differential cleaving activity is ascribed to a cooperative effect of the two metal centers, serving to both deprotonate coordinated water and stabilize the rate-limiting transition state for phosphodiester bond cleavage. Similarly, the cationic trinuclear zinc complex $[Zn_3L1(\mu\text{-OAc})](ClO_4)(PF_6)$, where L1 is a hexaazatriphenolic macrocycle, is able to cleave calf thymus DNA under ambient conditions [27] (Figure 3a). Finally, a binuclear Cu(II)Cd(II)hexaaza macrocyclic species hydrolyzes the model substrate bis(*p*-nitrophenyl) phosphate upon binding to the coordinatively unsaturated Cu(II) and concomitant attack at phosphorous by the basic hydroxide ligand bound to Cd(II). The synergy between the basic nucleophile at the Cd(II) site and stabilization of the leaving group intermediate at the Cu(II) center closely mimics biological nuclease activity [28].

Oxidative cleavage

The reactivity of reduced metal inorganic compounds (e.g. Fe(II), Cu(I)) with oxidants such as O_2 or H_2O_2, coupled with identification of Fe(II) bleomycin as an antitumor agent (C4′ H-atom abstraction) and the development of $[Cu(phen)_2]^+$ as an active DNA oxidant (recently confirmed C1′ H-atom abstraction [29]) paved the way for the design of new synthetic architectures for metallonuclease activity. Although mononuclear Cu(I) [30–32], Fe(II) [33] species that react with O_2 or H_2O_2 to generate O_2^- or $^{\bullet}OH$ continue to contribute to our understanding of metal complex modulated DNA degradation, synthetic trends, as in hydrolytic metallonuclease design, are moving toward higher nuclearity systems. The advantage of this approach for oxidative cleavage is the potential for reactivity at two spatially distinct DNA sites in an effort to generate genuine double strand lesions in a selective manner. One of the simplest of these designs is the tethering of two phenanthroline units by a serinol and other diol bridges linked through their C2 or C3 carbon atoms [34]. Here, the chemical nature of the bridge appears to play an intimate role in influencing DNA cleavage efficiency upon reaction with ascorbate and O_2. Within this theme, the compound $[Cu^{II}_2(L)(H_2O)_2](ClO_4)_4$ (Figure 3b) selectively cleaves DNA strands that extend from the 3′ side of frayed duplex structures at a site two residues displaced from the junction [35]. Effective cleavage requires guanine in the first position of the 3′ overhang adjacent to the central duplex and adenine in the same position on the 5′ overhang. The reactive dioxygen derived species is even capable of mediating efficient specific strand scission at concentrations where $[Cu(phen)_2]^{2+}$ does not detectably modify DNA.

From studies of this type, trinuclear copper complexes have emerged as plausible agents for DNA modification due to their ability to perform two- or three-electron reduction of dioxygen to form peroxo intermediates or the equivalent of a hydroxy radical. For example, the trinuclear copper complex, $[Cu_3^{II}(L)(H_2O)_3(NO_3)_2](NO_3)_4{\cdot}5H_2O$ (L = 2,2′,2″-tris(dipicolylamino)triethylamine) (Figure 3c), exhibits a remarkable ability to promote specific strand scission at junctions between single- and double-stranded DNA [36••]. Once again, strand scission occurs on the 3′ overhang at the junction of a hairpin or frayed duplex structure; recognition is dependent on metal nuclearity and requires only a purine at the first unpaired position and a guanine at the second unpaired position on the 5′ strand. Similarly, the trinuclear Cu(II) complex, Cu_3-L (L = *N*,*N*,*N*′,*N*′,*N*″,*N*″-

Figure 3



Multinuclear metal complexes for DNA hydrolysis or oxidation. **(a)** Trinuclear Zn(II) hexaazatriphenolic complex that effectively hydrolyzes DNA. **(b)** Dimetallic and **(c)** Trimetallic Cu(II) picoylamine complexes that site-specifically oxidize hairpin or frayed DNA structures.

hexakis(2-pyridyl)-1,3,5-tris(aminomethyl)benzene), exhibits efficient oxidative strand scission of plasmid DNA [37]. The complex appears to be more efficient than its mononuclear analogue at the same [Cu^{2+}] concentration, suggesting a possible synergy between at least two of the three Cu(II) centers. Finally, the novel linear tricopper complex $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ (HL = (*N*-pyrid-2-ylmethyl)benzenesulfonylamide) has been shown to cleave DNA efficiently in the presence of hydrogen peroxide/sodium ascorbate, but unlike the amine and imine systems above, $^{\bullet}OH$ and $^1O_2$ are implicated in DNA degradation [38].

Photo-induced DNA modification

Bimolecular oxygen-derived intermediates

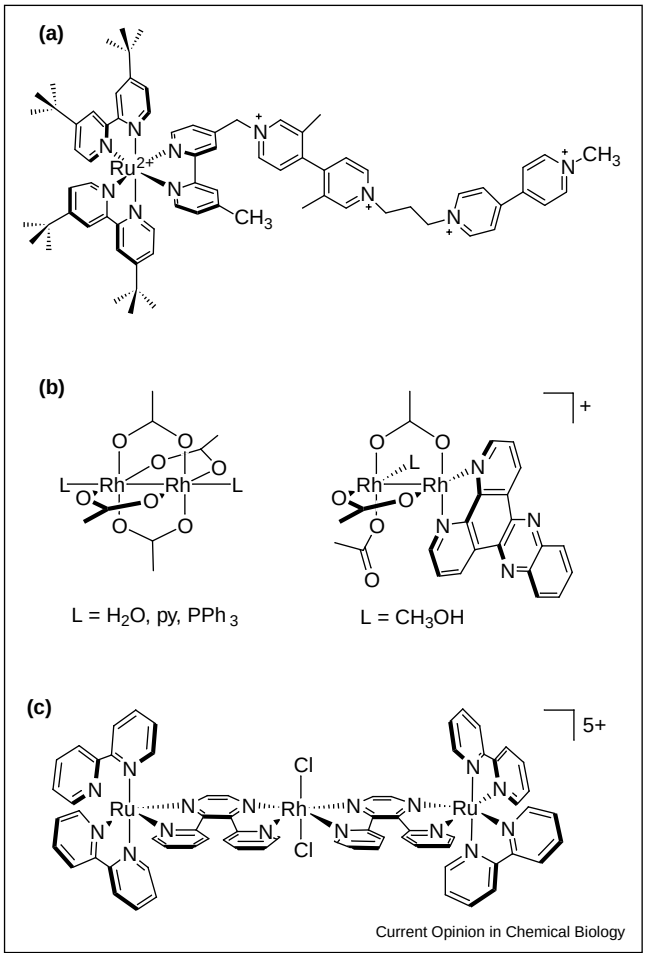
Photodynamic therapy (PDT) [39] has enjoyed considerable clinical success with large porphyrin macrocycles that often localize in lipophilic environments and convert $^3O_2$ to the toxic $^1O_2$ from tissue-transparent, near-infrared photo-preparation of the $^3\pi\pi^*$ state. Although DNA is not the biological target for $^1O_2$ oxidation in PDT, the DNA toxicity of oxygen-derived intermediates ($^1O_2$, O_2^-, $^{\bullet}OH$) is well established. Metal complexes, by virtue of their d-electron orbital angular momentum and facile redox properties, are predisposed to the photochemical generation of such toxic oxygen species. One of the challenges in achieving the success of PDT using a small metallonuclease is the need for absorption at long wavelengths. Porphyrins typically have extinction coefficients of $\sim$20 000 $M^{-1}cm^{-1}$ between 500 and 800 nm, whereas ligand field bands have values usually $< 500\ M^{-1}cm^{-1}$. However, metal–ligand charge transfer bands in this region can be of the order $\sim$2000–15 000 $M^{-1}cm^{-1}$ and thus are viable for photoinduced processes. Some of the more recent molecular designs in this area involve the use of Cu(II) and good donor ligands such as amine, imine or sulfur.

Ternary Schiff base copper(II) phenathroline complexes show a CuN_3OS coordination with the sulfur as an equatorial ligand. The complex binds strongly to the minor groove of bacterial DNA and exhibits both photonuclease (312 and 532 nm) and chemical nuclease activity under aerobic conditions without additional oxidant [40,41]. Extending this approach, excitation into sulfur-to-Cu(II) charge transfer transitions, and remarkably, weak Cu(II) ligand-field bands ($\sim$600–750 nm), results in the formation of $^1O_2$ and generation of relaxed circular DNA product [41]. Analogous compounds containing planar heterocyclic bases also show hydrolytic and $^{\bullet}OH$ cleavage activity under dark conditions [42,43], indicating that the continued development of such constructs may lead to multifunctional metallonuclease models.

Photoredox reactions

The development of $[Ru(diimine)_3]^{2+}$ complexes that intercalate into DNA and perform photoredox reactions with DNA bases, either directly or through reduction of an electron acceptor upon metal–ligand charge transfer excitation, led to the genesis of photoreagents that do not require reactive bimolecular cofactors. While metal–ligand charge-transfer excitation of $[Ru(bpy)_3]^{2+}$ in the absence of electron acceptor leads to formation of $^1O_2$ and O_2^-, the presence of tethered *bis*(4,4′-methylviologen) tetracation (Figure 4a) promotes formation of the Ru(III) species with sufficient lifetime to oxidize guanine [44]. Efforts have also focused on developing novel photoreagents based on bimetallic photoredox reactions and metal–metal charge transfer excited states to promote DNA cleavage. The d^7–d^7 Rh(II)–Rh(II) core of $Rh_2(\mu\text{-}O_2CCH_3)_4L_2$ (L = solvent, Figure 4b) that binds to DNA, promotes photoinduced cation formation in the presence of bimolecular electron acceptors and subsequent DNA nicking ($\lambda > 450$ nm, 15 min) [45,46•]. Intercalative mono-dppz derivatives (Figure 4b) also show DNA nicking upon photolysis ($\lambda > 395$ nm, 15 min) and cytotoxicity toward human skin cell lines with $LC_{50} \sim$15–30 μM [47].

Figure 4



DNA-binding Ru(II) and Rh(II) photoredox agents.

Other bimetallic cores such as $[((bpy)_2Ru(dpp))_2RhCl_2]^{5+}$ (Figure 4c) exhibit a low energy Ru→Rh metal-to-metal charge transfer (MMCT) excited state that is capable of direct DNA photo-nicking ($\lambda \geq 475$ nm, 10 min) in the absence of oxidants [48•]. Structurally analogous complexes (e.g. $[((bpy)_2Ru(bpm))_2RhCl_2]^{5+}$) that do not exhibit a low-lying MMCT excited state do not show DNA photocleaving activity [49], verifying the importance of metal complex electronic structure in these photoreactions.

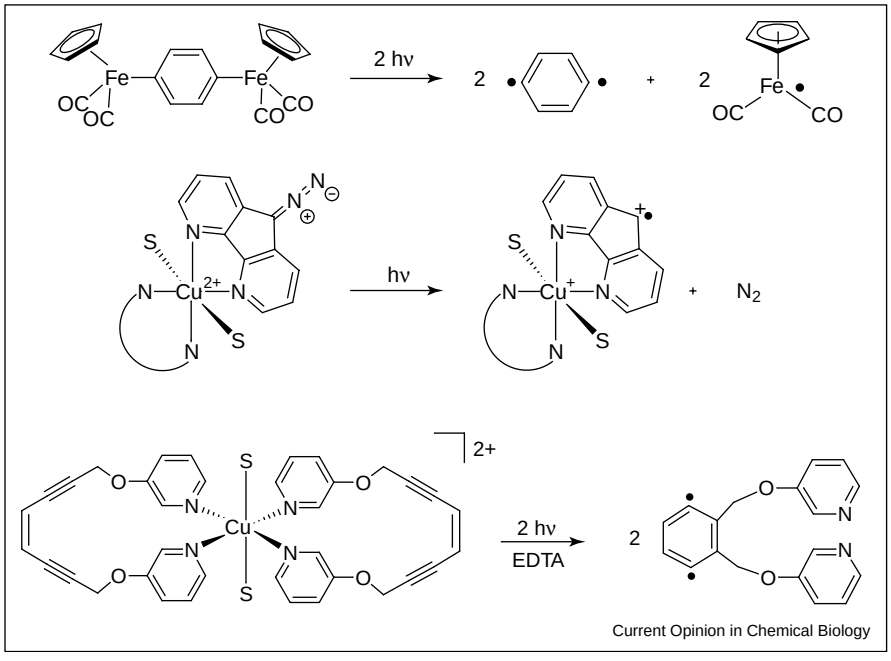
Ligand photodissociation

One of the advantages of metal complexes for binding to DNA is the ability to possess or generate *in situ* open coordination positions. To this end, ligand-field excitation of transition metal complexes is well known to foster ligand substitution reactions. In the context of the cisplatin mode of action, photochemical cisplatin-type reagents were conceptualized in the 1980s. More recently, the generation of Rh(III)-acridine intercalator conjugates that photochemically release two amines upon ligand-field photolysis (λ_{max} = 378 nm) showed DNA photocleaving activity, but no correlation to light enhanced binding was observed [50]. More strikingly, the Pt(IV) azide complex *cis*,*trans*-$[Pt(en)(N_3)_2(OH)_2]$ is unreactive with DNA until photolysis with visible light leads to dediazotation and generation of open coordination positions [51,52••]. Transcription mapping and HPLC reveal that platination sites are mainly localized to GG sequences, which contribute to formation of GG cross-links. Similarly, photoexcitation ($\lambda > 378$ nm) of *cis*-$[Ru(bpy)_2(NH_3)_2]^{2+}$ leads to loss of ammonia and covalent binding to 5-EG, as well as single and double-stranded DNA [53]. Thus, employment of photolabile ligands that can be irreversibly eliminated leads to opportunities to use open metal site coordination positions for subsequent *thermal* DNA modification mechanisms.

Unimolecular photochemical radical species

The third strategy to bypass bimolecular cofactors for DNA modification is the photoinduced generation of unimolecular radical intermediates on metal-bound ligands. Conceptually, this approach mimics the enediyne [54,55] and kinamycin [56] natural products that both contain diradical generating functional groups (*Z*-1,5-diyne-3-ene unit and N_2, respectively), and have potency against DNA substrates. Both enediyne and diazo units are susceptible to photochemical activation. In the former, Bergman cyclization generates the reactive 1,4-diradical intermediate, while UV excitation and photochemical loss of N_2 forms a carbene intermediate. Construction of ligands containing these reactive entities, and judicious choice of coordinating functional groups, results in transition metal complexes (e.g. Cu(II), Figure 5) possessing metal–ligand charge-transfer absorption bands that can lead to photogeneration of unimolecular radical intermediates [57,58]. Indeed, excitation of the metal–ligand charge-transfer bands leads to formation of Bergman cyclized product [59] and loss of dinitrogen [56], both signatures of unimolecular radical formation. While the diazo ligand and Cu(II) complex both show propensity for DNA degradation, variances in their con-

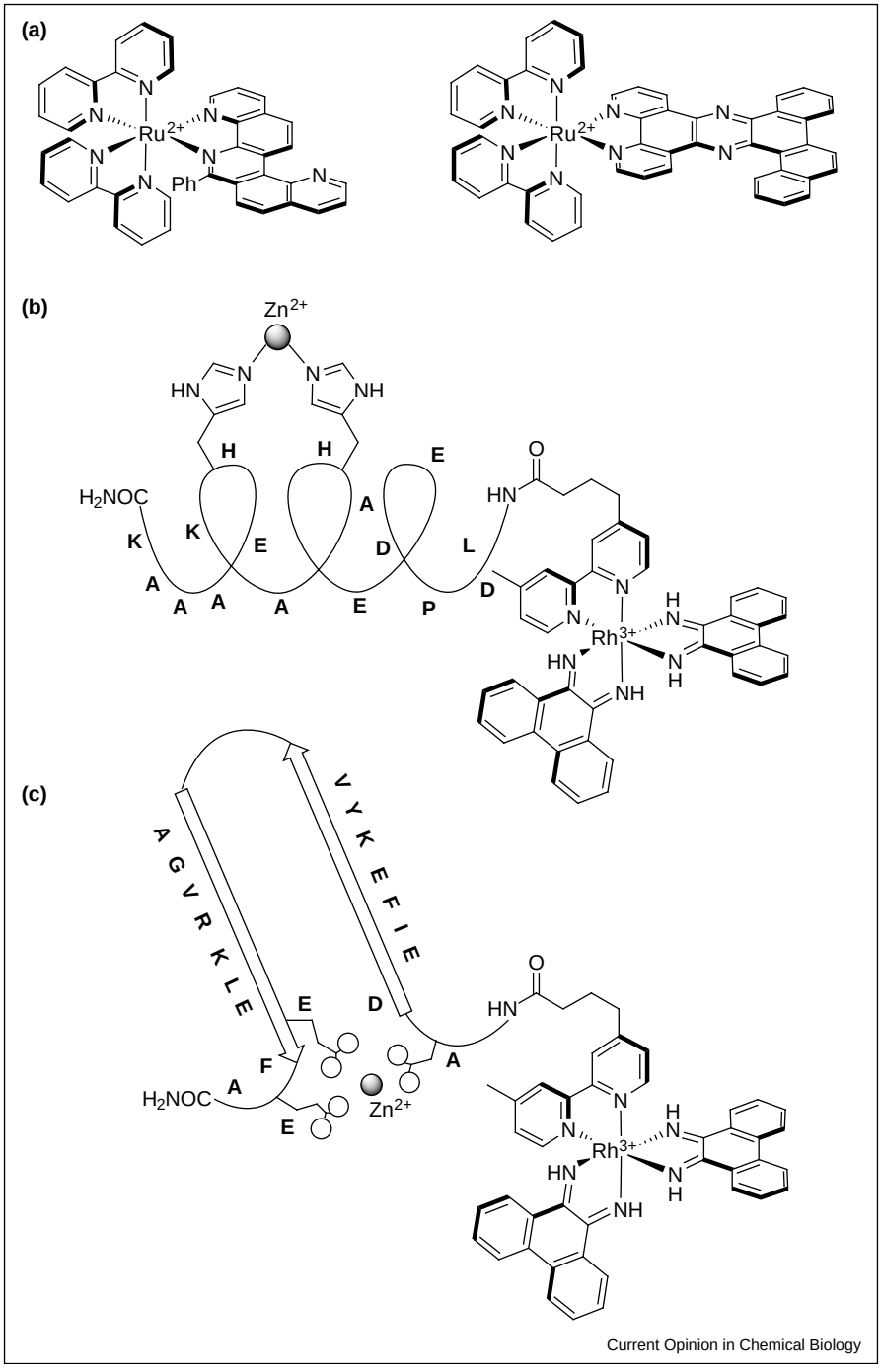
Figure 5



Approaches for photogeneration of unimolecular radical species for DNA degradation.

centration dependences are observed due to binding constant differences. By contrast, the copper-bound enediyne form is the only active species; photochemical activation with visible light in the presence of a 25 bp double stranded 5′-labeled oligonucleotide leads to nonspecific strand breaks but products consistent with C4′ H-atom abstraction. In a similar approach, organometallic compounds of the form $[CpM(CO)_nR]_x$ (R = phenyl or methyl; n = 2 or 3, x = 1 or 2] can be photoexcited to dissociate the R group leading to either a metal-bound organic monoradical (x = 1) or a freely diffusable 1,4-phenyl diradical (x = 2) that is structurally analogous

Figure 6



Metal diimine recognition strategies. **(a)** Site-specific Ru(II) metallointercalator complexes. **(b,c)** Rh(III) complex metallointercalator–peptide conjugates with additional metal binding site for hydrolytic (Zn^{2+}) or oxidative DNA (Cu^{2+}) cleavage.

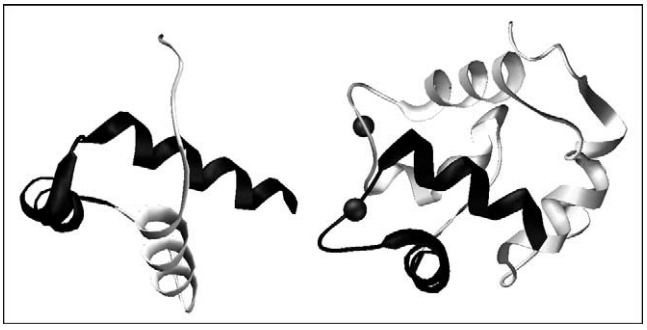
to that generated by the enediynes [60,61•] (Figure 5). These radical species are effective at generating both open circular and, under certain conditions, linear cleavage products.

Conclusions and perspective

The preceding sections document the need to satisfy two key criteria to impart effective DNA modification: first, control and yield of the reactive species performing the modification; and second, DNA sequence or structural specificity. Approaches toward the first of these criteria have been outlined above. Advancement toward the second of these goals and true therapeutic or biochemical utility requires incorporation of specific targeting strategies to affect selective DNA modification. The examples below clearly show the future direction of this field.

Barton *et al.* have developed Ru(II) [62••] (Figure 6a) and Rh(III) [63] complexes of the sterically bulky heterocyclic aromatic imine ligands that preferentially bind to the enhanced intercalation volume and preferential base stacking provided by CC (or CA, CT for Rh(III)) mismatch sites. Traditional luminescent and photochemical reactivities of these metal complexes confirm DNA cleavage at the mismatch location. Analogously, Ru(II) [64] and Rh(III) [65••] metallointercalator–peptide complexes have been prepared as DNA recognition and site-specific delivery vehicles. In the Ru(II) case, photooxidation of guanine and reaction of the peptide with the cationic hole leads to formation of covalent cross-links. The Rh(III) peptide conjugates (Figure 6b) contain a secondary metal-binding site that delivers hydrolytically active Zn^{2+} or redox active Cu^{2+}, in addition to traditional binding and photocleaving reactivity of the intercalator. The peptide is also chosen to optimize reactivity; the peptide sequence from *Bam*HI is known to bind to DNA and cleave 5′-GGATCC-3′ palindromic sites. Furthermore, using a Ce(IV)EDTA hydrolytic cleaving mechanism, Komiyama *et al.* have demonstrated site-selective DNA cleavage by addition of monophosphate oligonucleotides of variable linker length as DNA substrate additives to create gap structures and deliver a metal coordinating phosphate to either the 5′- or 3′-gap edge [66]. Finally, in a bio-inspired approach, Franklin *et al.* cleverly replaced the turn sequence of the helix-turn-helix (HTH) DNA-binding protein with the metal-binding loop of the Ca^{2+} EF-hand (Figure 7) to build chimeras that bind lanthanide ions and promote sequence selective DNA hydrolysis [67••,68]. These biological superstructures are superimposable; replacement of the turn sequence of the HTH peptide generates a catalytically active EF-hand leading to regioselective, sequence-dependent DNA hydrolysis products in the presence of Eu^{3+} or Ce^{4+}. The cleavage products are exclusively 3′-OPO_3, indicating a regioselective or multistep mechanism. Modest sequence discrimination suggests that the HTH-domain binds DNA in a folded conformation, thus enhancing selectivity. Biomimetic designs of this type are an important step toward development of truly viable artificial metallonucleases.

Figure 7



Ribbon representation of the X-ray structure of engrailed homeodomain and oncomodulin with two calcium ions (black spheres).

Acknowledgements

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- • of special interest
- •• of outstanding interest

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