From cisplatin to photoreactive Ru complexes: targeting DNA for biomedical applications†

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Since the discovery of cisplatin, the search for therapeutic agents based on other metallic compounds developed rapidly thanks to the versatility of coordination chemistry. This short review focuses on advances in research for new complexes based on Pt(IV), Ru(II) and Ru(III) either as anticancer drugs or as molecular (photo)reagents for biomedical applications. The DNA binding mechanisms of these compounds are highlighted alongside with the novel strategies developed to improve their reactivity and/or specificity towards DNA.

Introduction

Cisplatin [*cis*-diamminedichloridoplatinum(II)], approved for clinical use in 1978, is one of the most widely used complexes in anticancer therapy with second-generation Pt drugs such as carboplatin [*cis*-diammine(cyclobutanedicarboxylato)platinum(II)] (approved in 1993) or oxaliplatin [(1*R*,2*R*-diamminocyclohexane)-(oxalato)platinum(II)] (approved in 2002) (see Fig. 1).[1]

The activity of these anticancer platinum complexes results from the formation of adducts with cellular DNA.[2] They are indeed able to form either intrastrand or interstrand crosslinkings with DNA, involving guanine residues. Whereas the predominant adducts of Pt compounds on DNA are 1,2-GG and 1,2-AG intrastrand crosslinkings, the most common crosslinking occurs between two guanine residues on opposite strands.[3] The general mechanism for the formation of such adducts involves the hydrolysis of the Pt compound to give mono- and diaqua intermediates which are the active species towards DNA. Coordinated water is subsequently displaced by the nucleobase of the target DNA.

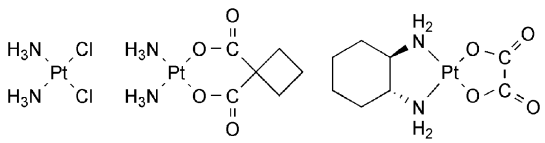

Fig. 1 Structures of cisplatin, carboplatin and oxaliplatin.

The results obtained with Cisplatin opened the way to the search for other coordination compounds either with the specific aim of improving the anticancer activity or, more largely, to be used in biomedical applications. This review aims to describe the main strategies developed in the last decades to improve/trigger the biological activity of Pt and Ru complexes related to their DNA binding. Changing the oxidation state of Pt from II to IV allows the preparation of active Pt(IV) compounds taking advantage of the possibility of using photons as reagent for triggering their activity. In parallel, the research on Ru complexes follows two approaches: (i) use of Ru(III) compounds activated by reduction and (ii) use of Ru(II) complexes binding to DNA either by substitution in the dark, or by covalent addition upon illumination. Emphasis will be given in this review on DNA binding and (photo)reactivity of those two types of metal complexes towards DNA, while other metals, medical aspects as well as protein binding will not be considered here.

I. Pt(IV) complexes

Thousands of platinum complexes have already been prepared and studied and around 40 have been examined in clinical trials.[1b,4] One strategy emerged during the past decade to avoid toxic side-effects, consists in using non-toxic Pt(IV) pro-drugs whose activity can be triggered by light directly at the site of the cancer.[5] By a judicious choice of the ligands it is thus possible to use such Pt(IV) compounds as pro-drugs which can be activated by light and release the active anti-cancer species only in the irradiated tumoural cells. Different examples of such compounds have been described in the

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† Dedicated to Professor Jean-Pierre Sauvage on the occasion of his 65th birthday.

Cécile Moucheron

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cis,trans,cis-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ *cis,trans*-$[Pt(N_3)_2(OH)_2(en)]$

Fig. 2 Structures of *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)]$.

literature.[6] Among them, *cis*,*trans*,*cis*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ and *cis*,*trans*-$[Pt(N_3)_2(OH)_2(en)_2]$, reported by Sadler *et al.*, can be photoactivated and form Pt-nucleotide crosslinkings at guanine residues, similar to the damages produced by cisplatin (Fig. 2).[7]

The photoactivation mechanism of Pt(IV)-diazido complexes involves absorption in the LMCT (ligand to metal charge transfer) band followed by a reductive elimination of the azido ligand with production of N_2.[7b,d,8] In a similar way, *trans*,*trans*,*trans*-$[Pt(N_3)_2(OH)_2(NH_3)_2]$ is inert in the dark but is cytotoxic when photoactivated by irradiation in its LMCT band.[7c] Upon UVA irradiation in the presence of GMP (guanosine-5′-monophosphate), photoreduction of the complex and photosubstitution of one ligand of the complex by the base leads very rapidly to the formation of a bis-GMP adduct *trans*-$[Pt(NH_3)_2(GMP)_2]^{2+}$ through the N7 atom of GMP.[7c]

II. Ru(III) complexes

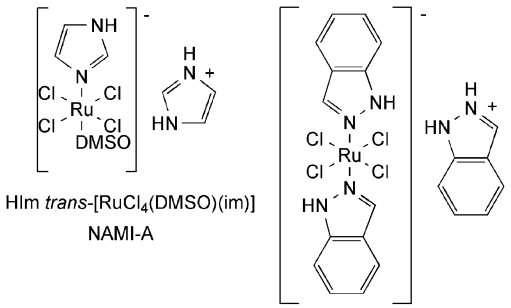
Besides the abundant studies focussing on new Pt(II) and, more recently, Pt(IV) complexes, the search for anticancer drugs based on other metallic compounds also developed rapidly. In this field, ruthenium compounds have retained much attention for several years.[9,10]

Ruthenium based drugs are generally less toxic than the platinum based derivatives, which makes them well-suited for medical applications.

Particularly, Ru(III) derivatives act as pro-drugs whose mechanism of activity involves a "activation-by-reduction" process,[10,11] that could be responsible for their low toxicity as compared to Pt(II) compounds.[12] Among those derivatives, [Him]*trans*-$[RuCl_4(DMSO\text{-}S)(im)]$ (im = imidazole), better known as NAMI-A (Fig. 3), and HInd *trans*-$[RuCl_4(ind)_2]$ (indazolium [*trans*-tetrachlorobis(1*H*-indazole)ruthenate(III)]), currently known as KP1019 (Fig. 3), have attracted much attention since they have reached the level of clinical investigation.[13,14]

By analogy with Pt compounds, DNA binding is thought to be at the origin of the activity of such compounds. KP1019 is able to induce efficient twisting and bending of DNA, likely *via* the formation of monofunctional DNA adducts.[15] It gives preferentially guanine- and adenine-adduct formation in competitive reactions with the four nucleotides. Model studies highlight that KP1019 binds to adenine derivatives through N7.[15] Formation of DNA interstrand and DNA-protein cross-linkings has been demonstrated, but to a much lower extent than with cisplatin.[16] Several studies also indicate strong interactions with proteins. This aspect, out of the scope of this paper, has been the subject of a recent review by Keppler.[17]

Whereas KP1019 exerts its apoptosis activity against primary tumours either by the intrinsic mitochondrial pathway or *via*



HIm *trans*-$[RuCl_4(DMSO)(im)]$
NAMI-A

HInd *trans*-$[RuCl_4(ind)_2]$
KP1019

Fig. 3 Structures of [Him]*trans*-$[RuCl_4(DMSO\text{-}S)(im)]$ (NAMI-A) and [HInd]*trans*-$[RuCl_4(ind)_2]$ (KP1019).

DNA binding,[18] NAMI-A mainly inhibits metastases formation and reduces metastases weight but displays much less pronounced effects on primary tumours.[19] At the molecular level, its binding to DNA is much weaker than that of Pt derivatives and its activity is likely the consequence of strong drug–protein interactions. Its behaviour in presence of nucleic bases has nevertheless been explored.[14c,19] While in physiological aqueous solutions, a mixture of species resulting from both chloride and DMSO hydrolysis after reduction is obtained, at low pH, slow DMSO hydrolysis forms a mono-aquated Ru(II) reactive species. In the presence of 9-methyladenine (used as a model of DNA) at low pH, it has been shown that NAMI-A binds to the base through N7. Other experiments suggest binding to 9-ethylguanine as well.

III. Ru(II) complexes

III.1 Ru(II) arene complexes

Whereas Ru complexes are considered as relatively inert towards ligand substitution in their +3 oxidation state, Clarke proposed that their activity is triggered by *in vivo* reduction to more reactive Ru(II) species as mentioned above.[10,20]

In that area, Ru(II)-arene complexes, active either *in vitro* or *in vivo*, look very promising.[21] These complexes display a pseudo-octahedral "piano-stool" $[(\eta^6\text{-arene})Ru(X)(Y)(Z)]$ structure, with the arene occupying three coordination sites forming the "seat" of the stool and the other ligands occupying the three remaining coordination sites forming the "legs". As observed for other Ru complexes and for Pt compounds, their cytotoxicity is correlated with DNA binding.[22]

The role of the arene moiety, as well as the influence of the other ligands on the aqueous chemistry of several complexes have been investigated by Sadler and co-workers.[23]

Bridging two monodentate ligands to have only one single ligand exchange site (Y and Z linked to form a chelate (L)) leads to higher anticancer activities (Fig. 4).[24,25] Complexes with three monodentate ligands are presumably too reactive with cells components and/or culture medium and are thus deactivated before they reach their target.

The general mechanism for monofunctional complexes $[(\eta^6\text{-arene})Ru(L)(Cl)]^+$ involves the rapid loss of the chloride ligand with formation of more reactive aquated species, followed by the substitution of the labile aqua ligand by a nucleobase. A more detailed study performed with nucleotides

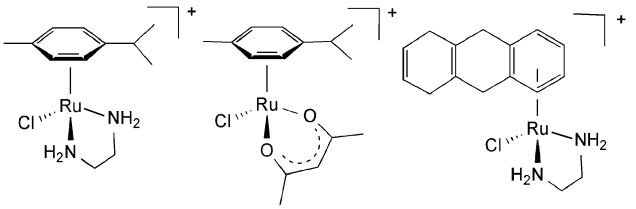


Fig. 4 Examples of Ru(II) arene complexes.

shows that the initial aquation of the complex is followed by a rapid binding to the 5′-phosphate group. A rearrangement gives then rise to the nitrogen-bound adducts.[26]

The influence of the arene ligand on the DNA binding of the complexes has been summarised in a recently published review.[27]

A few dinuclear complexes based on the same moieties have been studied. Among them, the dinuclear complex $[\{(\eta^6\text{-indan})Ru(Cl)\}_2(\mu\text{-}2,3\text{-dpp})_2]^{2+}$ also reacts and binds to DNA in the dark, *via* the same hydrolysis pathway and aquated intermediate as described above.[28] *In vitro* DNA transcription experiments suggest that the adduct formed in the dark inhibits only to a small extent the RNA synthesis by a RNA polymerase. In contrast, the adduct formed between DNA and the pre-irradiated complex inhibits more efficiently the DNA transcription, attributed to interstrand crosslinkings. Enhanced interstrand crosslinkings are also observed with subsequent irradiation of DNA modified by the complex in the dark. This increased reactivity is attributed to the indan loss upon illumination.[28] This example shows how illumination can help and activate the reactivity of a compound. Using light as a partner appears here also as a valuable strategy.

Several Ru(II)-arene complexes with the 1,3,5-triaza-7-phosphaadamantane (PTA) ligand, called RAPTA compounds, have been studied (Fig. 5).[29]

It has been demonstrated that $[Ru(\eta^6\text{-}p\text{-cymene})Cl_2(PTA)]$, RAPTA-C, induces DNA damage at pH characteristic of hypoxic cells, whereas only little damage is observed at healthy cell pH. This pH related activity is likely due to protonation of PTA moiety at low pH. ESI-MS experiments performed with RAPTA-C and a single strand oligonucleotide indicate that upon formation of the adduct, the chloride and arene ligands are lost, while the PTA moiety remains intact. The structure of the adduct has still to be determined.

The antitumour activity of RAPTA derivatives has also been examined.[30] Like the NAMI-A complex, they are not active against primary tumours but show activity against tumour metastases, slightly less effective than NAMI-A.

Based on the similar properties of NAMI-A and RAPTA complexes, a new class of Ru(II) complexes has been recently developed, which combines the structural aspects of NAMI-A (imidazole ligands) and the "piano-stool" arene-Ru(II) moiety

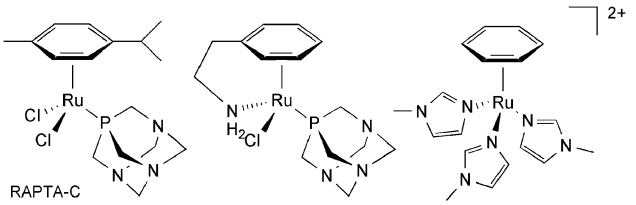


Fig. 5 Examples of RAPTA and RAPTA-NAMI complexes.

(Fig. 5).[31] Some of them are currently studied in *in vivo* experiments.

III.2 Ru(II) polyazaaromatic complexes

Another type of Ru complexes has also been considered for biological applications. Among those compounds, the recently reported $[Ru(bpy)_2(dppn)]^{2+}$ (see Fig. 6) displays interesting cytotoxic activity in the dark.[32]

On the other hand, as mentioned above in the section relating to Pt(IV) compounds, the use of a photoreactive species would be advantageous as the drug activity can be triggered at a selected site at a chosen time.

In that specific field Ru(II) polyazaaromatic complexes appear to be particularly attractive as photoprobes of genetic material or as photoreactive agents.[33] Interestingly, some Ru(II) complexes containing highly π-deficient polyazaaromatic ligands, such as TAP (1,4,5,8-tetraazaphenanthrene) or HAT (1,4,5,8,9,12-hexaazatriphenylene, Fig. 6) are able to form adducts with DNA upon visible irradiation.[34]

In order to rationalise their behaviours, photophysical and electrochemical properties of these octahedral compounds have to be considered.[34a] As proposed for $[Ru(bpy)_3]^{2+}$, the ^{1}MLCT (metal to ligand charge transfer) state reached by visible absorption rapidly deactivates by intersystem crossing to a ^{3}MLCT state with a unitary quantum efficiency. This ^{3}MLCT state can deactivate, either radiatively or nonradiatively, or populate a higher ^{3}MC state by thermal activation.

The ^{3}MC state can either deactivate nonradiatively or lead to a dechelation due to the distorted geometry of this state. Two different chemistries take place from the ^{3}MLCT and ^{3}MC states.[34a]

III.2.1 Formation of photoadduct by substitution

When irradiated in the visible, $[Ru(TAP)_3]^{2+}$ can lead to the formation of a photoadduct with AMP (adenosine-5′-monophosphate) by photosubstitution of one TAP ligand.[35]

The origin of this reactivity is easily explained based on the photophysical scheme described above. It has indeed been demonstrated that the ^{3}MC state of this complex can be efficiently populated from the ^{3}MLCT, thus leading to a high quantum yield of photodechelation (loss of a ligand under irradiation). This induces a high probability of substitution of one of the TAP ligands by a DNA base. The photoreactivity of $[Ru(TAP)_3]^{2+}$ in the presence of AMP, studied by visible absorption and laser flash photolysis, reveals the formation of $[Ru(TAP)_2(AMP)(X)]^{n+}$ ($X = Cl^-, H_2O$). This photoreactivity is similar to the chemistry reported for all the complexes described above, with transformation of the chelation sphere around the metallic center.

IIII.2.2 Formation of different photoadducts. Interestingly quite different photoadducts can also be formed with those photooxidising complexes under visible illumination. New entities attributed to the formation of a covalent adduct of $[Ru(TAP)_3]^{2+}$ on the oligonucleotide, were evidenced for the first time by denaturing polyacrylamide gel electrophoresis (PAGE) experiments performed with an illuminated solution of $[Ru(TAP)_3]^{2+}$ and ^{32}P-labeled oligonucleotides.[36] Dialysis experiments gave further evidence for photoadduct formation.

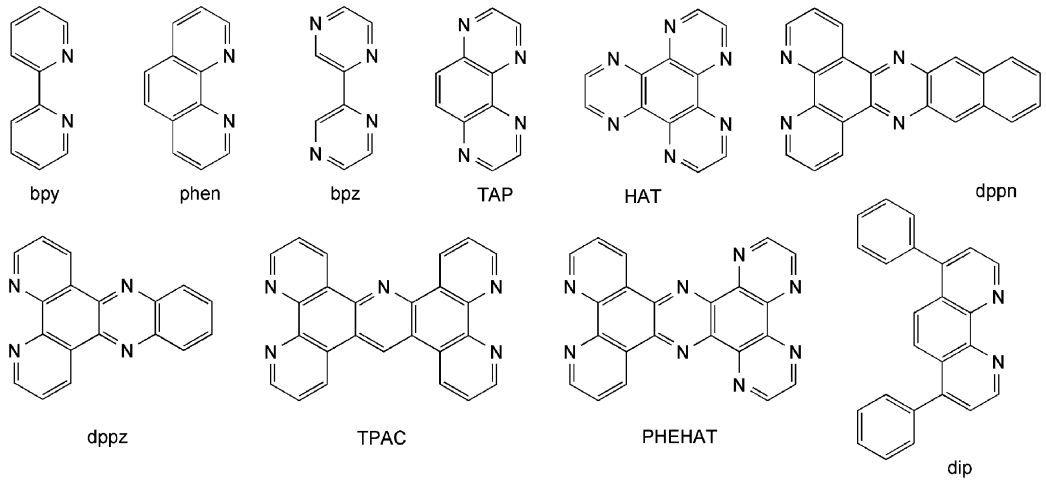


Fig. 6 Structure of the different polyazaaromatic ligands.

$$[Ru(L)_3]^{2+*} + GMP \longrightarrow [Ru(L)_2(L^{\bullet-})]^{+} + GMP^{\bullet+}$$
$$[Ru(L)_2(L^{\bullet-})]^{+} + GMP^{\bullet+} \longrightarrow [Ru(L)_3]^{2+} + GMP$$

Scheme 1

$[Ru(TAP)_2(bpy)]^{2+}$ or $[Ru(bpz)_3]^{2+}$ (bpz = −2,2′-bipyrazine) give rise to the same type of photoadducts upon illumination, but not $[Ru(bpy)_2(TAP)]^{2+}$.[34a,37]

In order to determine the primary processes responsible for the formation of these photoadducts, detailed photophysical and photochemical studies were performed, with a large number of complexes in the presence of different mono- or polynucleotides. The behaviour of those complexes can be rationalised by their redox properties.[38] Complexes with the formula $[Ru(TAP/HAT)_{3-n}(bpy/phen)_n]^{2+}$ (where n = 0 or 1), strongly oxidising in their ^{3}MLCT state, are capable of abstracting one electron from a guanine residue to generate an oxidised GMP^+ radical and a monoreduced complex $[Ru(L)_2(L^-)]^+$ (Scheme 1).

It has been shown for some complexes that the electron transfer is coupled to a proton transfer in water.[39]

Electrospray mass spectrometry (ESMS) experiments further evidenced formation of a covalent adduct. The results showed that a covalent bond is formed between one of the Ru(II) complex ligands and guanine with the simultaneous loss of two hydrogen atoms.[40]

Thus in this new type of photoadduct, the chelation sphere around the metal remains intact (see Fig. 7). Determination of the photoadduct structure was achieved mainly by NMR spectroscopy, first with the system $[Ru(TAP)_3]^{2+}$ and GMP (Fig. 7).[40] This structure can be rationalised in terms of the primary processes already described. The photo-induced electron transfer, coupled or followed by a proton transfer, gives rise to two radicals. Those two species can combine to form an intermediate which, after rearomatisation, produces the photoadduct (Scheme 2).

The structure of the photoadduct formed between $[Ru(TAP)_2(bpy)]^{2+}$ and DNA upon illumination, after acidic hydrolysis and enzymes digestion, has been determined in the same way. Two geometric isomers were detected by NMR (see Fig. 8). In that case also, the covalent bond forms with the exocyclic N of the guanine residue.

More recently, the structure of the photoadducts between $[Ru(HAT)_2(phen)]^{2+}$ and GMP was determined. In this case also two geometric isomers were detected but, more surprisingly, the data suggest that the covalent bond with the HAT ligand is formed with the O6 atom of the guanine unit instead of the exocyclic nitrogen (see Fig. 8).[41]

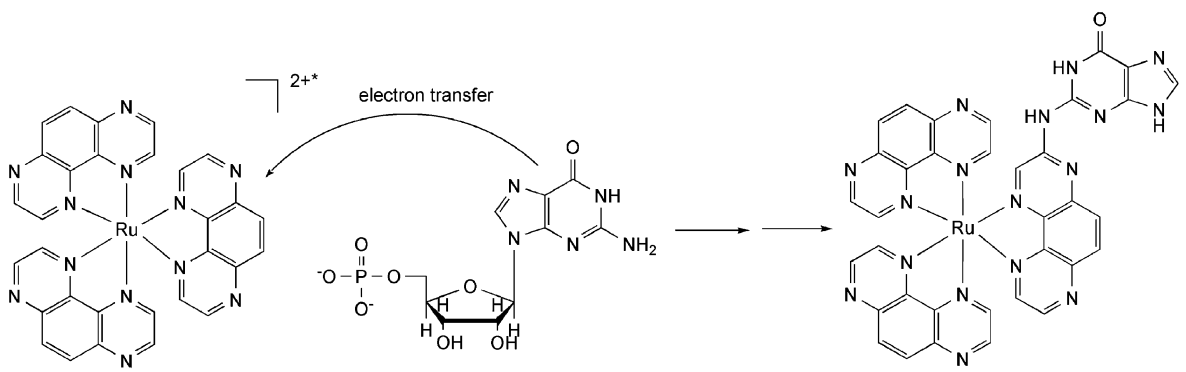


Fig. 7 Structure of the photoadduct formed after irradiation of $[Ru(TAP)_3]^{2+}$ in the presence of guanosine-5′-monophosphate (GMP), after acidic treatment with HCl to remove the sugar-phosphate.

$$\{[Ru(TAP)_2(TAPH^{\bullet})]^{2+}, GMP(-H)^{\bullet}\} \longrightarrow [Ru(TAP)_2(TAPH\text{-}GMP(-H))]^{2+}$$

$$[Ru(TAP)_2(TAPH\text{-}GMP(-H))]^{2+} \xrightarrow{-2H} [Ru(TAP)_2(TAP(-H)\text{-}GMP(-H))]^{2+}$$

Scheme 2

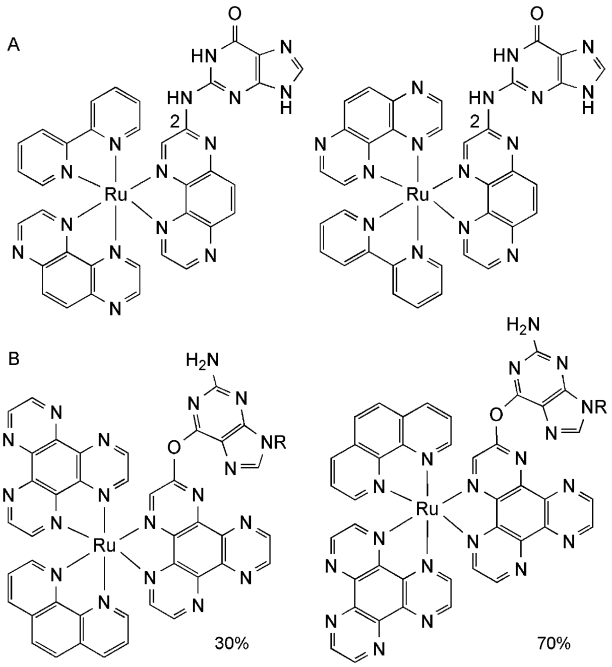


Fig. 8 Structure of the two photoadduct isomers between (a) $[Ru(TAP)_2(bpy)]^{2+}$ and DNA after excision by enzymatic and hydrolytic procedures, and (b) $[Ru(HAT)_2(phen)]^{2+}$ and GMP; R represents the sugar-phosphate group.

The difference in the structures of the photoadducts with the HAT and TAP complexes is not yet explained. Moreover with that complex, two other photoadducts were detected. One of them contains one more oxygen and could correspond to the addition of a 8-oxoGMP to the HAT ligand of the complex.

The other adduct is much more interesting and corresponds to the formation of a bisadduct, thus the addition of two GMP moieties on the same Ru(II) complex.

The formation of photoadducts with DNA and polynucleotides is of course possible only if the two partners interact with each other. The geometry of interaction of the complexes with nucleic acids could be crucial for the photoadduct formation.

III.2.3 Interaction of Ru(II) complexes with DNA. The main criteria to be fulfilled for preparation of potential photoactive anti-cancer Ru(II) complexes include good affinity for DNA, interaction with targeted base sequences (section III.2.4) and excellent photoreactivity.

Except for $[Ru(HAT)_2(phen)]^{2+}$ ($K_{aff} = 3.8 \times 10^5\ M^{-1}$), most of the complexes already discussed, although photoreactive, display only a moderate affinity for DNA (10^3–$10^4\ M^{-1}$).[36,42] Two approaches have thus been followed in order to increase the DNA binding constant of this type of complexes.

Complexes with an extended planar ligand. The well-known $[Ru(bpy/phen)_2(dppz)]^{2+}$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine, Fig. 6) complex[43] has been extensively studied as an intercalator towards nucleic acids.[44] Despite its very high affinity for DNA, this complex displays no photoreactivity towards DNA since it is not sufficiently photooxidising to produce the guanine radical cation. Thus $[Ru(TAP)_2(dppz)]^{2+}$ has been prepared and studied with nucleic acids.[39,45] This compound binds with a high affinity to DNA and is able to photo-induce an electron transfer process from guanine residues of DNA or $[poly(dG\text{-}dC)]_2$ in the picosecond timescale. It has been shown that the electron transfer observed with these polynucleotides or with GMP is coupled with a proton transfer.

Ru(II) complexes containing another planar DNA intercalating ligand, PHEHAT (PHEHAT = 1,10-phenanthrolino-[5,6-*b*]1,4,5,8,9,12-hexaazatriphenylene, Fig. 6), with two possible chelation sites, have been prepared and studied.[46] The $[Ru(phen)_2(PHEHAT)]^{2+}$ which acts as a light switch strongly binds to DNA *via* intercalation of the PHEHAT moiety between the stacking of bases. Its excited state is able to abstract an electron from GMP with a rather low efficiency, but this process cannot be detected with DNA.[46a] In contrast, the luminescent $[Ru(TAP)_2(PHEHAT)]^{2+}$ combines a good affinity for DNA, thanks to the intercalative properties of the PHEHAT, with a high photoreactivity towards guanine moieties, either in GMP or DNA. This latter compound is thus a very good candidate as a photoreagent for DNA.[46b]

Bifunctional Ru(II) complexes. These compounds contain two subunits: a metallic moiety and an organic entity which can also interact with DNA, or two metallic subunits.

The addition of an organic subunit to the metallic complex enhances the affinity of the resulting system for DNA as compared to that of the monofunctional complex.

$[Ru(TAP)_2(POQ)]^{2+}$ (POQ = 5-{4-[*N*-(7-chloroquinolin-4-yl)-amino]-2-thiabutylcarboxamido}-1,10-phenanthroline) and $[Ru(TAP)_2(POQ\text{-}Nmet)]^{2+}$ (POQ-Nmet = 5-{4-[*N*-methyl-*N*-(7-chloroquinolin-4-yl)amino]-2-thiabutylcarboxamido}-1,10-phenanthroline) are examples of this type of bifunctional complexes which combine a high affinity for DNA to the photoreactivity characteristic of TAP complexes.[42,47]

The case of two metallic subunits linked by an aliphatic chain has also been studied. The detailed behaviours of those complexes have been discussed in a recent review paper.[48]

More recently the dinuclear $[(TAP)_2Ru(TPAC)Ru(TAP)_2]^{4+}$ (TPAC = tetrapyrido[3,2-*a*:2′,3′-*c*:3″,2″-*h*:2‴,3‴-*j*] acridine, Fig. 6) complex[49] containing four TAP ligands was examined in presence of nucleic acids and appears to be a very efficient photoreactive agent.[50]

III.2.4 Applications of DNA photoadducts

Photoadduct formation with free complexes. The DNA damage caused by the photoreactive Ru(II) complexes under illumination could be exploited to induce dramatic perturbations on the DNA functions. In this field, the ability to photo-induce transcription inhibition *in vitro* was examined with different complexes. Under visible irradiation $[Ru(TAP)_2(phen)]^{2+}$ and $[Ru(TAP)_2(POQ\text{-}Nmet)]^{2+}$ reduce the *in vitro* transcription rate of a plasmid DNA template by a bacteriophage RNA polymerase to about 50% whereas no inhibition is observed in the dark.[51]

This strong photoactivity is attributed to DNA damage by the photo-induced electron transfer and photoadduct formation.

Those photoreactive systems can thus be regarded as DNA photoprobes able to mark the nucleic acids irreversibly or as photoreagents for biomedical applications.

Photoadduct formation with complexes tethered to oligonucleotides. One major disadvantage of metallic compounds which react with DNA in the dark, such as Pt(II) or Ru(III) compounds, or which photoreact with DNA, such as Pt(IV) or TAP and HAT Ru(II) complexes, is the lack of specificity of the reaction, induced or not by illumination. They are unable to target a specific gene, thus a specific base sequence.

Antisense or antigene strategies provide means to inhibit targeted base sequences of a particular gene.[52] One antisense oligonucleotide is already in clinical use in that context, while some others are under clinical investigation.[53] Unfortunately the double (antisense strategy) or triple (antigene strategy) helix formed after hybridisation of the target with the synthetic oligonucleotide (ODN) is recognised and destroyed by enzymes *in vivo*. The use of synthetic oligonucleotides derivatised by photoreactive Ru(II) complexes looks promising in this field. One can indeed take advantage of the photoadduct formation to increase the stability of the "synthetic ODN-target" system *via* the photo-induced irreversible attachment of the synthetic oligonucleotide.

With the aim of possible biomedical applications, several Ru(II) labeled sequences have been prepared by tethering $[Ru(TAP)_2(dip)]^{2+}$ (dip = 4,7-diphenyl-1,10-phenanthroline, Fig. 6) to different 17-mer oligonucleotides *via* a modified thymidine at the middle of the synthetic sequence or to the 5′-end terminal phosphate group.[54] The so-called probe-sequence can hybridise to its complementary sequence, called the target sequence. If the target sequence contains at least one guanine residue in the vicinity of the Ru(II) complex after hybridisation with the probe sequence, under visible illumination, the photo-induced electron transfer followed by the formation of the photoadduct, will produce a photocrosslinking of the probe and target strands (Fig. 9).

The different tested sequences have been chosen to examine the influence of different parameters (i) on the luminescence quenching due to the photo-induced electron transfer and (ii) on the photocrosslinking formation. It has been shown that high percentages of photocrosslinking are obtained, even when there is only one guanine residue in the target strand. The ionisation potential (IP) of the guanine bases, their distance from the site of anchoring of the complex on the probe sequence, and geometric factors originating from the linker, influence dramatically the luminescence quenching and the photocrosslinking formation (Fig. 10).[55]

The luminescence quenching caused by the photo-induced electron transfer from the G residue to the excited complex, is directly correlated with the IP of the guanine residue in the target sequence.[55a] In contrast, the crosslinking originating from the recombination of the two radicals formed by the photo-induced electron transfer, cannot be directly related to the IP but is much more dependent on geometrical constraints. It has been shown that the tethered complex reaches more easily the guanine residues in the 3′ direction on the complementary sequence, as compared to the guanines directed to the 5′ end (compare sequences Ru2, Ru8 and Ru9 with sequences Ru3, Ru10 and Ru11 in Fig. 10).[56]

Increasing the distance between the guanine residue and the anchoring site results in decreased photocrosslinking formation as well as lowered extent of luminescence quenching. The stretching of the linker towards the 3′ end of the complementary strand allows the complex to abstract an electron from a GG located up to five base pairs from the anchoring site, but leads to photoadduct with a base separated by a maximum of three base pairs from the anchoring site.

The maximum distance required for photocrosslinking formation is thus shorter than for luminescence quenching. Indeed, recombination of the two radicals formed by the photo-induced electron transfer requires a more defined geometry than the photo-induced electron transfer at the origin of the luminescence quenching.

When the linker stretches towards the 5′ end, the complex can give rise to a photoadduct formation with a guanine residue separated by four base pairs from the anchoring site.[56]

When the complex is tethered at the 5′ end of the probe sequence as compared to the anchoring in the middle of the sequence, the trends are the same but the yields of photocrosslinking are lower than expected based on the IP values. This is again related to the influence of the linker in directing the complex more towards the 3′ end of the complementary sequence relative to the 5′ end.[56]

With the aim of biomedical applications, those photocrosslinkings could be used to perturb the function of the enzymes involved in gene expression. To validate this approach, the influence of the presence of a Ru-derivatised oligonucleotide photoadduct in a DNA matrix on the elongation of a primer by two different polymerases (Klenow fragment, exo^- and polymerase β) has been examined.[56] A 17-mer Ru-derivatised oligonucleotide was hybridised to its complementary sequence at the 5′ end of a 40-mer matrix used as template for the enzyme (Fig. 11). After hybridisation with the 13-mer primer, the mixture was illuminated and incubated in the presence of the four triphosphate nucleotides and each polymerase. The elongation of the primer by the enzyme was then analysed by PAGE experiments in denaturing conditions. It has been demonstrated that the activity of both polymerases is blocked with 100% efficiency at the level of the photocrosslinking. This result is particularly promising as DNA polymerases are responsible for DNA replication.

In order to apply these Ru-derivatised oligonucleotides as sequence-specific DNA photoreagents, photodamages must be stable enough in biological medium to inhibit the activity of nucleases responsible for DNA repair. Photocrosslinked duplexes were incubated in the presence of a typical exonuclease enzyme (exonuclease III from *Escherichia coli*) and the digested samples were analysed by PAGE to evaluate their resistance to 3′-exonucleolytic activity.[55d] It turns out that the enzyme activity is also blocked with 100% efficiency at the level of the photocrosslinking. The fact that the photocrosslinkings are able to block the activity of nucleases is also very promising in view of inhibiting a targeted gene.

While these results are promising, the development of the systems based on $[Ru(TAP)_2(dip)]^{2+}$ and described in Fig. 9 is

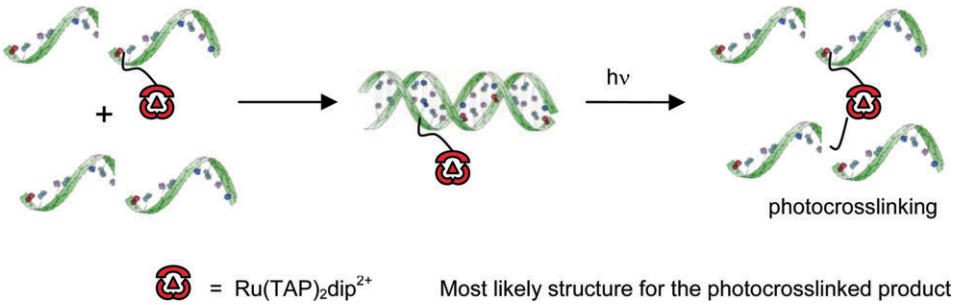


Fig. 9 Photocrosslinking formation.

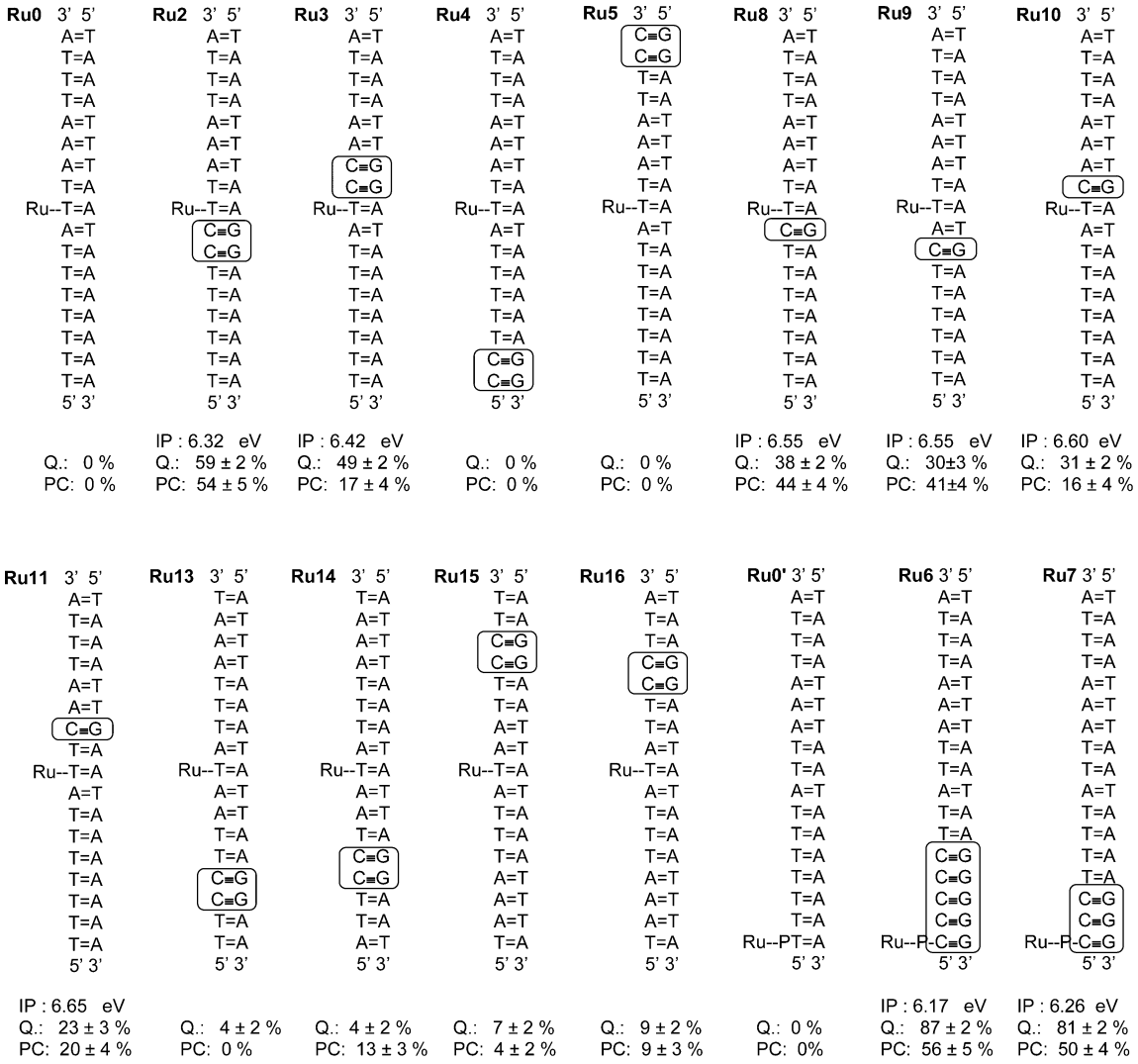


Fig. 10 Different Ru(II) labeled duplexes sequences for the study of photocrosslinking: Ru = $Ru(TAP)_2dip^{2+}$, IP = calculated ionisation potential (eV), Q = percentage of quenching, PC = percentage of ODN adduct formation.

partly prevented by the inefficiency of their synthesis. Very recently a novel tethering strategy, initially developed for the chemoselective attachment of ODNs to carbohydrates, peptides, and glycopeptides[57] has been adapted to derivatised Ru(II) complexes.[58] The anchoring of the aminooxy derivatised complexes $[Ru(TAP)_2(phen'')]^{2+}$ and $[Ru(TAP)_2(TAP'')]^{2+}$ *via* condensation with aldehyde containing ODNs allowed to examine the influence of the nature of the complexes and the anchoring site (5′ *vs.* 3′) on the photoreactivity of the conjugates (Fig. 12).[59]

The results of this study show that the excited $[Ru(TAP)_2(phen'')]^{2+}$ and $[Ru(TAP)_2(TAP'')]^{2+}$ interact slightly better with the duplex and form more photoproduct when attached at the 3′ extremity than at the 5′ position (Fig. 13).

The particular properties of $[Ru(TAP)_3]^{2+}$ play different roles in the different duplexes. Despite its very high photooxidising

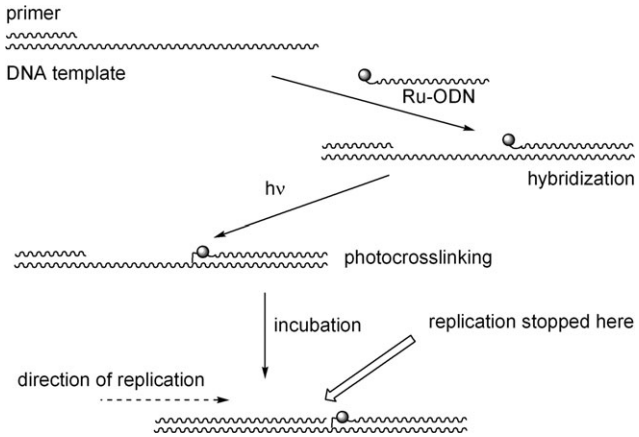


Fig. 11 Scheme of the inhibition of the primer elongation by a DNA polymerase due to photocrosslinking.

power, a photodechelation process competes with the photocrosslinking when the complex is tethered at the 5′ end, thus with a long linker, which decreases the photocrosslinking efficiency. In contrast, with a shorter linker, when the complex is attached at the 3′ end, the photodechelation is suppressed and the photocrosslinking reflects the high oxidising power of the compound.[59] This better photoreactivity of the complexes attached to the 3′ extremity of the ODNs is very interesting for biological applications as 3′-modified ODNs present the advantage over their 5′-analogues to show greater nuclease stability.[60]

This promising strategy was thought to be restricted to probe sequences without guanine residues which would be unfavourable for the photocrosslinking process. Interestingly a very recent study shows in contrast that this possibility makes the system unique and very powerful.[61] Indeed, when $[Ru(TAP)_2(phen'')]^{2+}$ is attached at the 3′ end of a G-containing oligonucleotide, the visible illumination of the resulting single strand gives rise to the formation of a cyclic photoadduct which arises from an intramolecular photoreaction. When the same photoreactive probe is hybridised with its complementary target, then illuminated, the photoreaction leads to the exclusive

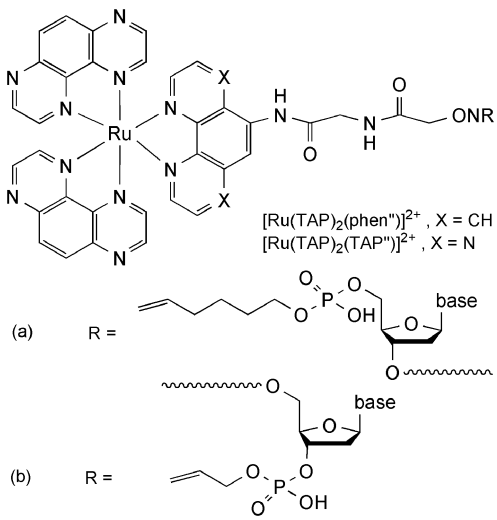


Fig. 12 Derivatised complexes and attachment at the 5′ (a) and 3′ (b) end of the oligonucleotide. Base stands for the first nucleic residue.

$[Ru(TAP)_2phen'']^{2+}$-5'-TCC TTT TAT TAA ATT TA-3'
3'-**AGG** AAA ATA ATT TAA AT-5'

$[Ru(TAP)_2phen'']^{2+}$-3'-TCC TTT TAT TAA ATT TA-5'
5'-**AGG** AAA ATA ATT TAA AT-3'

$[Ru(TAP)_2TAP'']^{2+}$-5'-TCC TTT TAT TAA ATT TA-3'
3'-**AGG** AAA ATA ATT TAA AT-5'

$[Ru(TAP)_2TAP'']^{2+}$-3'-TCC TTT TAT TAA ATT TA-5'
5'-**AGG** AAA ATA ATT TAA AT-3'

Fig. 13 Different Ru(II) labeled duplexes for the study of photocrosslinking. The anchored complex, the ADN extremity of tethering, and the sequence are shown.

formation of the inter-strand photoaddition, with no trace of the intramolecular photoadduct. This highly selective inter- over intra-molecular reaction is attributed to the more rigid conformation of the duplex in which the G units of the probe sequence cannot be reached by the complex (Fig. 14).

When non-specific G-containing potential targets are used instead of the complementary sequence, only cyclic intramolecular photoadduct is formed and no photocrosslinking is observed. Thus the photoreactive probe "kills itself" in the presence of a "wrong" target (Fig. 14).

This unique behaviour, called the "seppuku process", is of particular interest for *in vivo* applications since it should avoid secondary reactions with non-specific targets.

Those Ru(II) polyazaaromatic complex–oligonucleotide conjugates, and more specifically the "seppuku molecules", are currently examined in gene-silencing studies.

The anti-sense strategy has also been used with another type of Ru(II) polyazaaromatic complex. In that study,[62] $[Ru(tpy)(dppz)(CH_3CN)]^{2+}$ is tethered *via* its dppz ligand to a oligonucleotide and the resulting conjugate is hybridised to its complementary G-rich sequence. Upon irradiation, the $[Ru(tpy)(dppz)(CH_3CN)]^{2+}$-conjugate undergoes hydrolysis and loss of CH_3CN. The corresponding aqua complex readily reacts with a G residue located on the complementary sequence to give a photocrosslinking. In that case the mechanism is different from the mechanism described above for the photo-oxidising Ru(II) polyazaaromatic complexes. The photoadduct is formed by substitution of a ligand and the chelation sphere no longer remains intact.

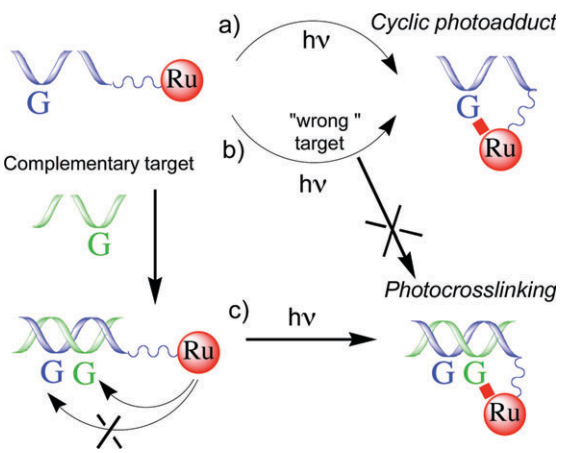


Fig. 14 Schematic representation of the "seppuku process".

Conclusions

In this perspective, we have reviewed recent advances in research for new Pt and Ru complexes which could be used for anticancer or biomedical applications. Besides new molecule synthesis, mechanistic understanding of the DNA-binding, including DNA interaction and (photo)reaction is key for further developments.

Starting from Pt(II) derivatives, several authors changed the oxidation state of platinum and studied Pt(IV) derivatives. The advantage of this strategy results from the use of Pt(IV) non-toxic pro-drugs whose activity can be triggered by light at a chosen site and time.

Another strategy consists in the use of Ru(III) complexes as pro-drugs the activity of which is triggered by *in vivo* reduction process. NAMI-A and KP1019 look very promising in that context.

As Ru(III) complexes require a reduction process to be reactive towards DNA, different types of Ru(II) compounds have been designed in another approach.

Ru(II)-arene compounds have been extensively studied and the influence of the arene moiety as well as the effect of the other ligands, chelating or not, on their binding to the nucleobases have been examined.

Ru(II) polyazaaromatic complexes are also revealed as attractive potential drugs activated by visible light.

In this latter case, DNA binding is of a different type. Indeed the chelation sphere around the metal remains intact in the photoadduct formed after the photo-induced electron transfer from the G residue to the excited complex. The interaction of the complexes with DNA can be tuned by a judicious choice of the ligands and their photoreactivity is dependent on the use of good π-acceptor subunits.

Adducts on a DNA template block the RNA synthesis by RNA polymerase. When tethered to an oligonucleotide, a photooxidising Ru complex induces photocrosslinking upon visible irradiation in the presence of a complementary sequence containing a guanine residue. This type of photo-crosslinking inhibits with 100% efficiency the function of DNA polymerase, responsible for DNA replication during cell division, as well as exonuclease responsible for DNA digestion. Such photocrosslinkings make possible the use of Ru-ODN photoreagents as candidates for DNA sequence-specific damaging agents, useful either for diagnostic purposes or for therapeutic applications based on gene-silencing.

Moreover, in this latter case, the use of intelligent "seppuku molecules" able to kill themselves if they do not find their target, allows the preparation of a new generation of highly specific systems with reduced side-effects.

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