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In vitro inhibition of gene transcription by novel photo-activated polyazaaromatic ruthenium(II) complexes†‡

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Under visible irradiation, $[Ru(TAP)_2(phen)]^{2+}(Cl^-)_2$, $[Ru(TAP)_2(POQ\text{-}Nmet)]^{2+}(Cl^-)_2$ and $[Ru(bpy)_2(phen)]^{2+}(Cl^-)_2$ were able to dramatically reduce the *in vitro* transcription of a plasmid DNA template by a bacteriophage RNA polymerase. This photoactivity is related to two different mechanisms of reactivity towards DNA exhibited by these complexes under illumination.

Ruthenium(II) complexes undergo different photoreactions with nucleic acids.[1] For instance, it has been known for some years that $Ru(bpy)_3^{2+}$ or $Ru(phen)_3^{2+}$ can photosensitize strand breaks in DNA.[2] The photoreaction proceeds with rather low quantum yields ($1–7 \times 10^{-6}$) and has a major dioxygen-dependent pathway. In the series $Ru(bpy)_n(TAP)_{3-n}^{2+}$, it has been well established that the complexes with $n = 0$ or 1 are much more efficient at causing strand breaks than those with $n = 2$ or 3. This is due to a change in the mechanism of cleavage from Type II (which is $^1O_2$ mediated) for $n = 2$ or 3 to Type I (direct electron transfer mediated) for $n = 0$ or 1.[3] In this latter case, the photoelectron transfer between the photooxidising complexes, containing at least two π-deficient TAP or HAT ligands, and dGMP, DNA and polynucleotides has been clearly demonstrated.[3b,4] In contrast, these TAP or HAT complexes are poor dioxygen photo-sensitizers. It has also been shown that complexes with at least two TAP ligands are able, as a direct consequence of the photo-electron transfer process, to form photoadducts.[3b,5] This reaction is targeted to a guanine residue in DNA which becomes linked, *via* its exocyclic amino function, to a pyrazine ring of one of the TAP ligands in the complex. This process does not occur for the bpy or phen complexes.

These photochemical properties make the Ru(II) compounds interesting candidates for several applications such as molecular tools or probes for DNA structural studies.[6] Moreover, compounds that photoreact with DNA are also known to have dramatic effects on DNA functions and therefore disturb the expression of genes. The most likely origins of these effects are either the inhibition of the binding of enzymes involved in gene transcription and gene replication or the prevention of the progression of these enzymes along DNA.[7]

As the inhibition of transcription is a means to prevent the growth of cancerous cells, the photoactive Ru(II) complexes could thus be regarded as interesting anti-tumour drug candidates.

In the present work, we examined the inhibition of transcription by three Ru(II) complexes whose possible activity in an *in vitro* system has not yet been demonstrated until now. We also compared the activity of two types of complexes (bpy *vs.* TAP complexes) that present an important difference in the type of DNA damaging and mechanism of reaction as described above. We examined their effects on the gene expression of a target plasmid vector transcribed by a bacteriophage RNA polymerase, in a completely controlled *in vitro* transcription system (see ESI†). The two novel TAP complexes[8] exhibit the same photooxidising power, but have different binding constants with DNA,[9a] the bifunctional complex $[Ru(TAP)_2(POQ\text{-}Nmet)]^{2+}$ has a higher DNA affinity due to the quinoline moiety. The DNA interactions and photoreactions of the three complexes of Fig. 1 have been extensively examined.[9]

Prior to transcription, the target DNA was mixed to a given concentration of the tested complex and the resulting solution was irradiated during a given time at a constant temperature (see ESI†). Only afterwards the polymerase was added to the mixture for the transcription process. As a control, the same mixture was prepared and incubated in the same conditions, but in the dark.

The results show that after an irradiation of 30 min at 37 °C, with a concentration of 10^{-5} mol L^{-1} of complex, both the $[Ru(TAP)_2(phen)]^{2+}(Cl^-)_2$ and $[Ru(TAP)_2(POQ\text{-}$

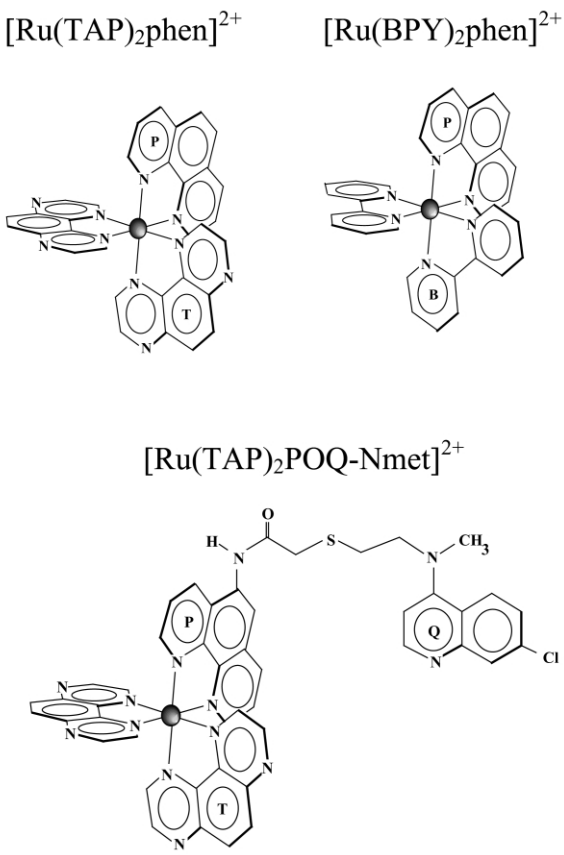

Fig. 1 Structures of polyazaaromatic ruthenium(II) complexes.

† Electronic supplementary information (ESI) available: representatve autoradiograph for the transcribed messenger RNA of expected size and experimental procedures. See http://www.rsc.org/suppdata/cc/b2/b202905g/

‡ Abbreviations: POQ-Nmet: 5-{4-[*N*-methyl-*N*-(7-chloroquinolin-4-yl)amino]-2-thiabutanecarboxamido}-1,10-phenanthroline; TAP: 1,4,5,8-tetraazaphenanthrene; HAT: 1,4,5,8,9,12-hexaazatriphenylene

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Table 1 Transcription inhibition: percentage of transcribed messenger RNA. Minimum and maximum percentage of produced RNA from 3–4 different experiments are in parenthesis. R_{inh}^{50}: ratio [Ru]/[DNA] for 50% inhibition, [DNA] in equivalent base pairs concentration

	Complex concentration/μM			
Complex	1	10	50	R_{inh}^{50}
$Ru(TAP)_2(phen)]^{2+}$	96 (94–97)	52 (48–56)	6 (4–10)	0.018
$[Ru(TAP)_2(POQ\text{-}Nmet)]^{2+}$	98 (93–100)	52 (47–57)	4 (2–5)	0.018
$[Ru(bpy)_2(phen)]^{2+}$	100 (100–100)	80 (74–87)	24 (20–27)	0.050

Nmet)]$^{2+}(Cl^-)_2$ reduced the transcription rate or the relative amount of transcribed messenger RNA to about 50%, as referred to the dark control taken as 100% (Table 1, Fig. S1 in ESI†). Under the same conditions, $[Ru(bpy)_2(phen)]^{2+}(Cl^-)_2$ reduced the relative transcribed RNA to only 20% (Table 1). An increase of the concentration of complex relative to the target DNA resulted in a further decrease of the transcription. At a concentration of 5×10^{-5} M, the TAP complexes were able to almost completely inhibit the transcription (Table 1). The ratio [Ru]/[DNA] at which 50% of the messenger RNA is transcribed, R_{inh}^{50} (Table 1), indicates that the inhibition by the TAP complexes is very efficient. Values reported recently for Rh(III) complexes were in the order of 0.13–12.5.[7b]

It is important to note that (i) the inhibition by the complex is triggered by the illumination since no inhibition occurs in the dark (Fig. S1) and the activity increases, at a constant concentration of complex, with the illumination time, (ii) the irradiation of the complexes alone in the absence of target DNA, which was added in that case after the irradiation, results in the same behaviour as the dark control. No secondary product resulting from complex photodegradation is thus responsible for the inhibition effect.

In conclusion, the three studied complexes inhibit the DNA transcription process under illumination. Even though it could be thought *a priori* that any DNA damage should induce an inhibition, such an activity had never been demonstrated before with Ru(II) compounds, even with the classical bpy complexes. The inhibition process originates from damages on the plasmid DNA, however these damages are different for the two types of complexes. For $Ru(bpy)_2(phen)^{2+}$, which cannot give rise to a photo-electron transfer with DNA, a damaging by a type II mechanism with possible DNA cleavage has to be responsible for the activity. Under the same conditions, the two TAP complexes, that are poor dioxygen photo-sensitizers, damage DNA by a photoinduced electron transfer and are more efficient inhibitors of RNA synthesis than $Ru(bpy)_2(phen)^{2+}$. As the charge transfer process leads to photoadduct formation, these produced adducts could also be responsible for the photoactivity. Previous studies have shown that the adduct formation proceeds with a much higher quantum yield than cleavages. The better activity of the TAP complexes could thus be attributed to the type I photo-oxidation, with concomitant formation of covalent adducts with DNA.

On the other hand, the comparison of the results obtained with the two TAP complexes $[Ru(TAP)_2(phen)]^{2+}$ and $[Ru(TAP)_2(POQ\text{-}Nmet)]$,$^{2+}$ seems to indicate that an increase of affinity of the complex for the target DNA by a factor of 25 due to the quinoline moiety[10] does not play an essential role in the inhibition of transcription. The results are indeed identical for both TAP complexes. The same observation has been reported for Rh(III) complexes.[7b]

Since the biological activity of both $[Ru(TAP)_2(phen)]^{2+}(Cl^-)_2$ and $[Ru(TAP)_2(POQ\text{-}Nmet)]^{2+}(Cl^-)_2$ in physiological conditions corresponds to a strong and even complete inhibition of gene transcription, we believe that these novel complexes could become potential candidates for a photodynamic therapy with implanted fiber-optic light sources.[11] The difference in the mechanism of activity of the TAP complexes (type I photo-oxidation) as compared to other organic dyes in photodynamic therapy, could represent a real advantage because the activity is no longer based on the presence of dioxygen.

In order to combine the efficient photoreactivity of the TAP complexes towards DNA with a selectivity of photoreaction with the guanines of a targeted gene, oligonucleotides derivatized with these compounds have been prepared and studied.[11]

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