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DNA oxidation photoinduced by Norharmane Rhenium(I) polypyridyl complexes: effect of the bidentate N,N' ligands on the damage profile

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Abstract: Re(I)-polypyridyl complexes show quite interesting and distinctive photochemical and photosensitizing properties. This work describes the capability to induce (or photoinduce) DNA damage of three Re(I)-complexes with a naturally occurring alkaloid called norharmane (nHo) as ligand: $[Re(CO)_3(nHo)(L)]CF_3SO_3$ where L = 2,2´-bipyridine (**ReBpy**), phenanthroline (**RePhen**) or dipyrido[3,2-*a*:2′,3′-*c*]phenazine (**ReDppz**). The interaction of the complexes with DNA was investigated by steady-state and time-resolved spectroscopy. Data show that the mode and strength of interaction depend on the chemical structure of the bidentate ligand. The complexes show a major static contribution to the overall interaction, giving rise to the formation of non-covalent adducts with DNA, and the particular trend observed was **RePhen** > **ReDppz** > **ReBpy**. Photo-oxidation at the purine bases represents the major DNA damaging mechanism. **RePhen** also induces single-strand breaks in a yield similar to that of base damage, suggesting an- additional photosensitizing pathway. Also, we performed the Ames test to evaluate the cytotoxic and mutagenic properties of both non-irradiated and photoexcited complexes. **RePhen**, but not the other complexes, turned out to be both toxic and phototoxic for the bacteria.

Introduction

Re(I)-polypyridyl complexes show quite interesting and distinctive chemical and photochemical properties, where the metal ion plays an important role due to its intrinsic reactivity and coordination geometry. Briefly, Re-complexes show relative high thermal stability, large stokes shifts and molar absorption coefficients (ε) among other peculiarities [1]. Interestingly, the photochemical and photosensitizing properties of these compounds can be fine-tuned by varying the polypyridyl ligands [2]. The use of Re^{I}-polypyridyl complexes has spread into different fields of bioinorganic and bioorganic chemistry. In particular, they have been described as promising tools for opto-electronic devices[3], cell imaging [4], cancer treatments [4b, f, 5], DNA probes [6], etc. Although conjugated Re-complexes/nucleic-acid-oligomers have recently been shown to selectively hybridize to specific DNA sequences[7], the use of Re-complexes as DNA probes is mainly based on both covalent and-or noncovalent molecular recognition between transition metal complexes and DNA [8]. The mode and extent of noncovalent interactions strongly depend on the chemical structure of the complexes (*i.e.*, ligands) [9].

The nature of the ligands has an important effect on the redox potential of the complexes, both in ground and excited states [10]. This effect can lead to changes in the photo-reactivity and/or photosensitizing properties [8b, 11]. It is worth mentioning that several metal-complexes (based on Cu(II), Fe(II/III), Ir(III), Pt(II), Rh(III), Ru(II), Re(I), Mn(II) and others as core metals) were suggested to act as artificial nucleases [12], photo-nucleases [13] and/or photocleavage [12a, 14] agents suitable, in some cases, for photodynamic therapy (PDT) upon one and/or two-photon excitation[15]. In these studies, DNA damage sensitized by photoexcited metal complexes was evaluated by the typical DNA relaxation assay, where only cleavage of the phosphodiester bonds is detected (formation of single- (SSBs) or double-strand breaks (DSBs)). To date, there is only little information on the global spectrum of DNA damage and the underlying damaging mechanisms. In the particular cases of some Ru(II)-complexes, photo-adducts with guanosine (as free nucleoside) as well as with calf thymus DNA and derivatized oligonucleotides were observed [16]. Detailed studies for Re(I)-complexes are even more scarce [17]. Since these metal complexes are able to generate reactive oxygen species as well as other radical species in the presence or absence of light, DNA damage at the nucleobases level still needs to be addressed.

The present work reports the study of the photosensitizing properties of three well characterized $[Re(CO)_3(nHo)L]CF_3SO_3$ complexes, where nHo (9*H*-pyrido[3,4-*b*]indole or β-carboline) is a naturally occurring alkaloid showing quite interesting intrinsic photochemical [18] and photosensitizing properties [19] and L represents three kinds of diimine ligands namely either 2,2'-bipyridine (bpy), 1,10-phenanthroline (phen), or dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) (Figure 1)[4a, 20]. The interaction of the three Re-complexes with DNA was investigated by means of steady-state and time-resolved spectroscopy. Moreover, the type and extent of DNA oxidative damage induced by UVA-

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photoexcited complexes were assayed with the use of DNA repair endonucleases.

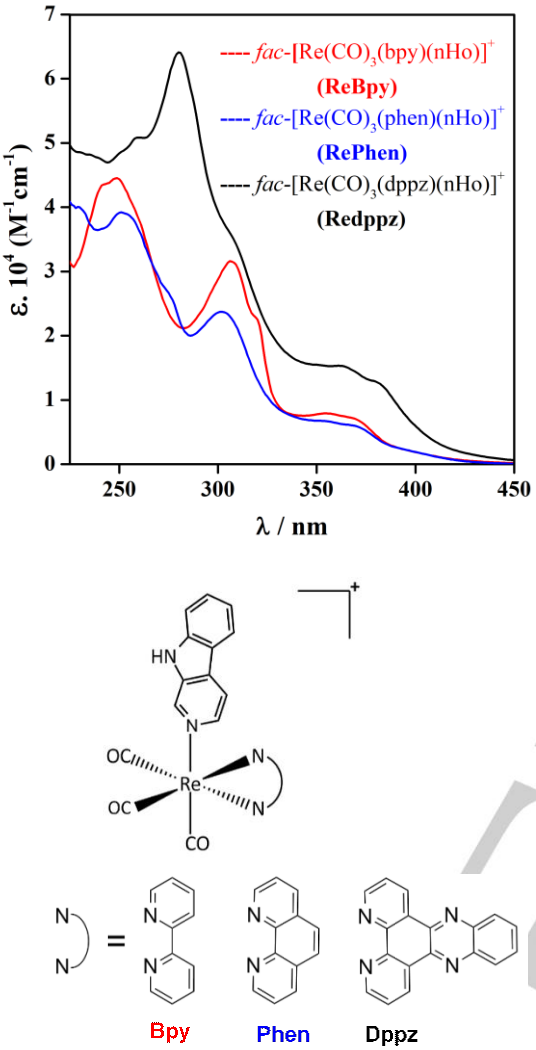

Figure 1. UV-visible absorption spectra in methanol *(top)* and chemical structure of the three Re-complexes investigated in this work (*bottom*).

Results and Discussion

Photosensitization of cell-free DNA

Dose-dependence of the DNA damage induced by photo-excited Re-complexes

Supercoiled DNA of bacteriophage PM2 was exposed to UVA irradiation (365 ± 20 nm) in the presence of increasing amounts of the investigated complexes. The number of single-strand breaks (SSB) and DNA lesions sensitive to the repair enzyme formamidopyrimidine-DNA-glycosylase (Fpg), which recognizes oxidized purines and also sites of base loss[21], were subsequently quantified by agarose gel electrophoresis and plotted against the UV^{365} absorption per cm (Figure 2).

The numbers of DNA modifications increase with the concentrations (absorbance) of the compounds investigated herein. Note that no significant DNA damage was observed when PM2 DNA solution was irradiated in the absence of photosensitizers (*control light*, y-intercept in Figure 2a). Moreover, dark incubations of PM2 DNA and Re-complexes (dark controls, Figure SI.1) showed no (in the case of **ReBpy**) and very low damage (in the cases of **RePhen** and **Redppz**). Thus, the larger part of the damage reported in Figure 2a is induced by the photoexcited Re-complexes.

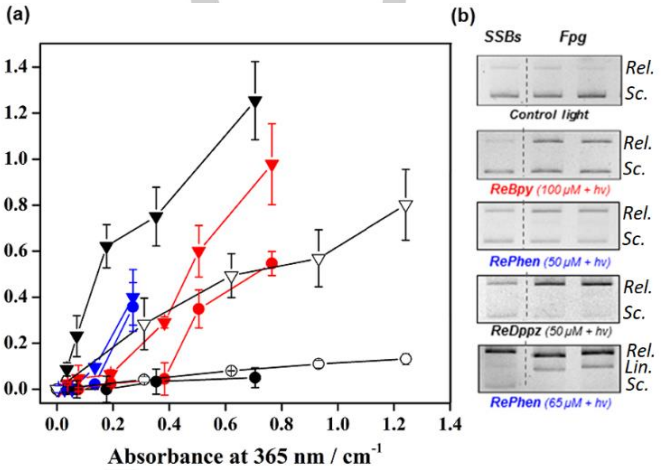

Figure 2. (a) SSBs (circles) and Fpg-sensitive modifications (down triangles) induced in PM2 DNA by exposure to UVA light (20 min) in the presence of different concentrations (indicated in units of absorption) of **nHo** free-ligand (white), **ReBpy** (red), **RePhen** (blue) and **ReDppz** (black) in in phosphate buffer solutions (pH 7.4). (b) Electrophoretic agarose gels at the maximum concentration plotted in (a): Sc., Rel. and Lin. represent supercoiled, relaxed and linear DNA. Data are the mean of 4 independent experiments (± S.D).

From the data shown in this Figure, two major points deserve to be highlighted:

(i) Re-complexes are more efficient photosensitizers than the uncoordinated βC. The extent of DNA-damage induced by nHo (free βC ligand) [19b] is lower compared with the Re-complexes. The comparison of the slopes of the concentration-dependent data (Figure 2a) indicates that Re-complexes were, in terms of Fpg-sensitive sites, at least 1.5-fold more potent as damaging agent than the free ligand nHo, and 4-fold more potent (with the exception of **ReDppz**), in terms of SSBs. This might be a consequence of a higher relative affinity of Re-complexes to the DNA molecules and/or a higher capability to generate reactive oxygen species such as singlet oxygen (see below).

(ii) the type and extend of DNA damage induced by the three Re-complexes depend on the chemical nature of the accompanying structural ligand (bpy, phen or dppz). **ReBpy** and **RePhen** induce, in a quite similar extent, both SSBs and Fpg-sensitive base modifications; whereas **ReDppz** only induces the latter type of modification. The difference observed could be a consequence either of a distinctive mode of interaction with the DNA-double helix (intercalation and/or groove-binding) or due to

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a distinctive photosensitizing pathway (i.e., type I, type II or a combination of both) (see below).

(iii) The agarose gel of a DNA sample irradiated in the presence of a high amount of **RePhen** (65 μM, Abs^{365nm} = 0.30), when treated with Fpg protein, showed the formation of the linear DNA form (Figure 2b). Thus, **RePhen** relatively often induces the formation of Fpg-sensitive modifications and SSBs at two closely opposed positions of the double-stranded DNA, giving rise to the formation of double-strand breaks (DSBs).

Quantification of endonuclease-sensitive modifications in PM2 DNA: damage profiles

As mentioned above, Fpg protein (Fpg) recognizes both oxidized purines such as 8-oxoG and sites of base loss (AP sites) [21]. To further investigate whether AP-sites contribute to the overall DNA damage, we also determined the numbers of modifications sensitive to *endonuclease IV (Endo IV)*, an enzyme that specifically recognizes AP sites [22] (Figure 3). In contrast to the free nHo ligand, photoexcited Re-complexes induce quite low numbers of AP-sites (identified by endonuclease IV). Thus, the major part of the modifications recognized by Fpg protein represent photooxidised purine nucleobases, typically 8-oxoG. In addition, **RePhen** also shows a quite efficient generation of SSBs.

In view of the relative long-lived triplet electronic excited states of Re-complexes, the formation of cyclobutane pyrimidine dimers (CPDs) via triplet energy transfer appeared possible. Therefore, the recognition of lesions by *T4 endonuclease V (T4 endo V)* was also evaluated in the irradiated samples. Having in mind that *T4 endo V* recognizes both CPDs and AP sites, lesions were also quantified using a combination of both *Endo IV* and *T4 endo V* enzymes. [23]

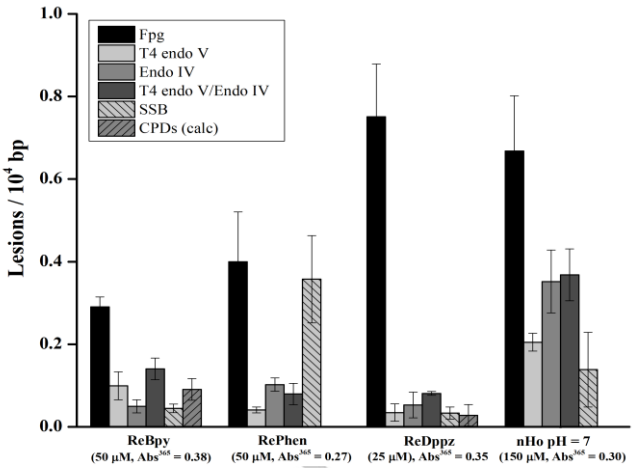

Figure 3. DNA damage profiles showing the numbers of SSBs and several types of endonuclease sensitive modifications induced in PM2 DNA by photoexcited (UVA 366 nm) $[Re(CO)_3(nHo)L]^+$ complexes and by free nHo. Data shown for nHo were taken from reference [19b]. Data are the means of 3 independent experiments (± S.D).

Data show that, as in the case of the free nHo ligand [19b, c] **RePhen** and **ReDppz** are unable to photoinduce the formation of CPDs. On the contrary, **ReBpy** induces a significant number of modifications identified as CPDs. This fact can be explained in terms of the photophysical properties of these three photosensitizers, since only **ReBpy** has the required triplet state energy (*i.e.*, E_T, ≥ 267 kJ/mol) [24] to spontaneously generate CPDs, through T-T energy transfer processes (see below).

Interaction with calf thymus DNA

As mentioned above, the different levels of DNA damage photosensitized by the three Re-complexes might be a consequence of the different binding affinity between each Re-complex and the DNA. To further investigate this, titration of Re-complex solutions (100 μM) with calf thymus DNA (ctDNA) were monitored by UV-visible absorption spectroscopy (Figure 4). The three Re-complexes follow the same qualitative trend. Briefly, the absorption bands of the complexes, placed between 300 nm and 450 nm, exhibit a hypochromic effect upon increasing [ctDNA]. The observed phenomenon suggests the presence of quite specific binding modes with the double-stranded ctDNA, where the nHo ligand would also contribute to the global interaction (*vide infra*). The extent of the interaction, quantified by eq. (1) as K_G, depends on the chemical structure of the bidentate ligands: $K_G^{RePhen} \geq K_G^{ReDppz} > K_G^{ReBpy}$ (Table 1).

When comparing these data with those previously reported for related complexes, it is evident that:

(i) Re-complexes show larger K_G values than those observed for uncoordinated nHo ligand, suggesting that the structure Re(I)-bidentate ligands (bpy, phen and dppz) have a major contribution to the overall binding.

(ii) **RePhen** and **ReDppz** showed K_G values slightly larger than **ReBpy**. This tendency correlates well with the intrinsic relative affinity of these ligands (dppz ≈ phen > bpy) to DNA and the quite poor interaction described previously for $Re(CO)_3$-complexes with bpy ligands.[25]

(iii) The calculated K_G value for **RePhen** [4.5 (± 0.5) x 10^3 M^{-1}] is of the same order of magnitude as those observed for related complexes such as $Ru(phen)_2(bpy)Cl_2$ [4.6 (± 1) x 10^3 M^{-1}) [26] showing that the phenanthroline aromatic ring is the principal responsible of the overall interaction in this complex.

(iv) Re(I)-complexes showed K_G values one order of magnitude lower than related metal complexes such as $[Pt(DMP)(DIP)]^{+2}$ (where DIP = 4,7-dipheny 1-10-phenantroline) [27] and $[Ru(phen)_2(phi)]^{+2}$ [26], with K_G values of 2.0 (± 0.2) x 10^4 M^{-1} and 4.7 (± 0.6) x 10^4 M^{-1}, respectively). While it is clear that coulombic contributions (*i.e.*, electrostatic attraction) are playing a key role in the overall interaction between these cationic complexes and the negatively charged DNA backbone, additional contributions such as steric impediment or hydrogen bonding, among others, may also be acting.

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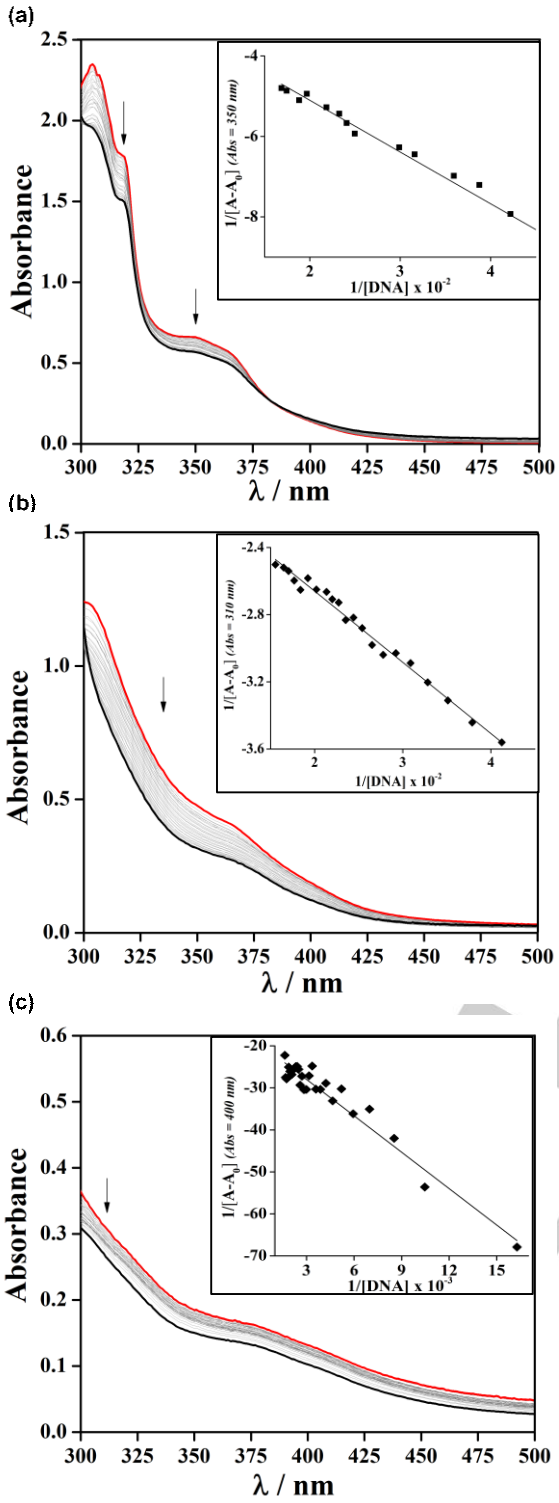

Figure 4. UV-visible absorption spectra of **(a) ReBpy** (100 µM), **(b) RePhen** (100 µM) and **(c) ReDppz** (120 µM) air-equilibrated buffered solution, recorded in the presence of increasing amounts of ctDNA. Arrows indicate the variation in the absorption spectra upon increasing [ctDNA] (initial and final concentrations are highlighted in red and black, respectively). *Insets*: representative Benesi–Hildebrand plots.

The extend and type of the photosensitized damage might be modulated not only by the type and extent of the interaction between the DNA and the photosensitizer in its electronic ground state (S_0), but also in its excited state. The latter interaction was further explored by steady-state and time-resolved luminescence titration experiments. Data show that significant changes in the Re-complexes' emission spectra in terms of both intensity and shape when [ctDNA] is increased (Figure 5). The behavior observed strongly depends on the chemical structure of the bidentate ligand. Briefly, **ReBpy** and **RePhen** showed a significant (~5 nm) hypsochromic shift of the maximum emission wavelengths when [ctDNA] increases. The hypsochromic shift is indicative of a change in the polarity environmental conditions of both fluorophores as is shown in Figure SI.2. Thus, results shown in Figure 5 confirm the interaction between these two Re-complexes and ctDNA, where ctDNA would provide a less polar surroundings with respect to the bulk solution. Also, a uniform decrease in the spectral region of the two emission bands, attributed to an nHo intraligand excited state (IL_{nHo}) and a Metal to Ligand Charge Transfer excited state ($MLCT_{Re\rightarrow L}$, where L is bpy or phen), was observed. Stern-Volmer plots of the steady-state emission data (*i.e.*, I_0/I vs [DNA]) showed a linear behavior with both **ReBpy** and **RePhen**. The corresponding K_{SS} values are listed in Table 1.

In the particular case of **ReDppz**, an analysis on the rather low intense and short-lived emission band assigned to the $MLCT_{Re\rightarrow dppz}$ transition could not be done. Is well known that this transition is deactivated in water by a vibrational deactivation *via* hydrogen bonding between water molecules and the nitrogen atoms from the dppz[6b, 28]. This effect was also seen in **ReDppz** (figure SI.3). Besides, the IL_{dppz} emission band overlaps with the rather intense and relatively long-lived emission band assigned to the IL_{nHo} transition (Figure SI.2). However, a decrease of the latter band with a clear bathochromic shift is observed when [ctDNA] is increased. This indicates that **ReDppz** is sensing changes in the polarity of the environment, due to its interaction with ctDNA as is shown in Figure SI.2.

To further evaluate potential dynamic contributions to the overall emission quenching described above, luminiscence lifetimes of the three Re-complexes were measured in the presence of increasing [ctDNA]. Lifetimes were recorded at the maximum of emission of the two distinctive emission bands (IL_{nHo} and $MLCT_{Re-L}$), except for **ReDppz,** which only shows one major emission band corresponding to IL_{nHo}, In all cases, small changes of the τ_0/τ vs [ctDNA] relationship were observed (Figures 5, right column and Figure SI.4). This is accounted by the rather small slope (K_D) of the corresponding Stern-Volmer representation (insets in Figure 5) that is one or two orders of magnitude lower than K_{SS} (Table 1). These results clearly indicate that Re-complexes interact with ctDNA mainly through the formation of a ground-state static complex. Note that, in the case of **RePhen**, a distinguishing dynamic contribution was observed for the $MLCT_{Re\rightarrow phen}$ band, showing a K_D value one order of magnitude higher than that observed for the IL_{nHo} band (Table 1). Thus, in this particular case, a dynamic interaction between the phen ligand and ctDNA appears to play a role as an additional deactivation pathway (see below) [29].

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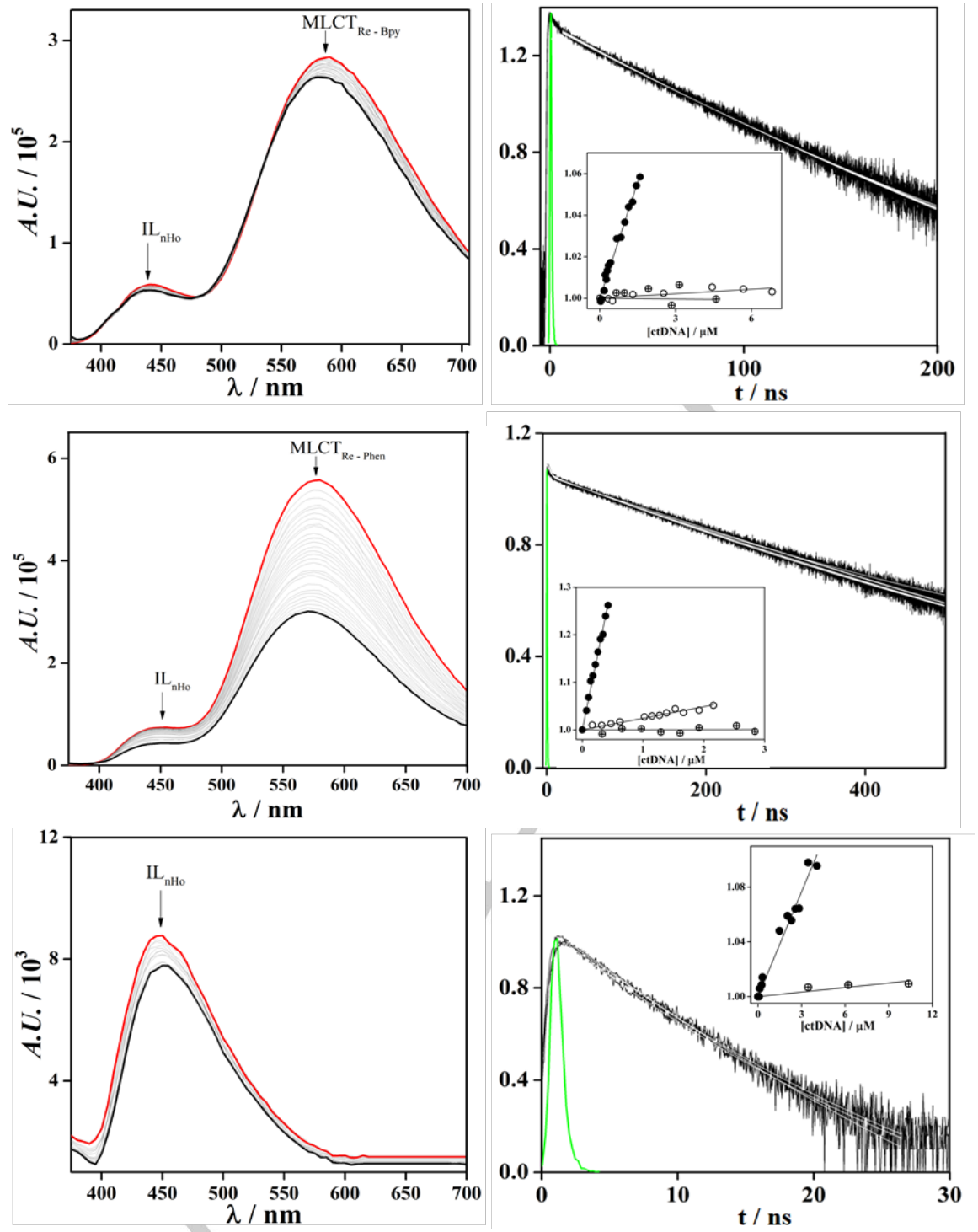

Figure 5. Steady-state and time-resolved emission of **(a) ReBpy** (20 μM), **(b) RePhen** (15 μM) and **(c) ReDppz** (15 μM) air-equilibrated buffered solution with 0.2% of DMSO, recorded in the presence of increasing amounts of ctDNA. *Graphs on the left:* corrected emission spectra. Arrows indicate the variation in the emission spectra upon increasing [ctDNA] and the corresponding transitions for each emission bands are also depicted (initial and final concentrations are highlighted in red and black, respectively. *Graphs on the right*: luminiscence decays recorded at wavelengths of the emission maximum of the corresponding $MLCT_{Re\rightarrow L}$ transition (585 nm and 575 in (a) and (b), respectively) (black lines. λ_{exc} = 341 nm), prompt signal (green line) and mono-exponential fitting curves (white lines). Insets in the graph on the right: Stern-Volmer plots of the emission intensities (I) (black circles) and lifetimes (τ) (white and cross circles for the $MLCT_{Re\rightarrow L}$ transition and IL_{nHo} respectively).

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DNA competitive binding with ethidium bromide

It is well-known that some positively charged metal complexes with bidentate planar ligands, such as phen or dppz, can interact with double-stranded DNA through intercalation and/or groove-binding, among others.[6b, 8b] However, chemical structure of the bidentate ligands as well as electronic charge distribution on the complex can modulate the mode and extent of this interaction [30]. To further evaluate the contribution of intercalation of the three Re(I)-complexes investigated herein to the overall interaction with ctDNA, ethidium bromide (EtBr) fluorescence displacement experiments were employed. Results with the free ligand nHo were included for comparison.

Data depicted in Figure 6 show that increasing concentrations of the three Re-complexes decreases the fluorescence intensity of EtBr-ctDNA adduct. In all the cases, I_0/I vs [ctDNA] plots show a linear relationship (insets in Figure 6). The corresponding K'_{SS} values, obtained from the respective slopes, are listed in Table 1. When comparing the slopes of the Stern-Volmer plots obtained from the Re-complexes steady-state emission quenching (K_{SS}) and K'_{SS} obtained from EtBr displacement experiments four points rise to the surface:

(i) the extent of intercalation strongly depends on the chemical structure of the diimine ligand: $K'_{SS}{}^{\mathbf{ReDppz}} > K'_{SS}{}^{\mathbf{RePhen}} > K'_{SS}{}^{\mathbf{ReBpy}}$. This is in agreement with the fact that non-covalent interaction between DNA and classical planar intercalating agents depends on the extension of the planar fused-rings (dppz > phen > bpy).

(ii) In the cases of **ReBpy** and **RePhen**, intercalation contributes little to the overall interaction with ctDNA (*i.e.*, K'_{SS} are one order of magnitude lower than the corresponding K_{SS} values). This is in agreement with results reported for related metallic-complexes, with bpy or phen as ligands, which were shown to interact with DNA mainly by electrostatic interaction and groove binding in some cases[8b, 26]. Thus, attractive coulombic forces are playing an important role in the overall interaction of **ReBpy** and **RePhen** with ctDNA. The latter fact also explains why Re(I)-complexes sometimes have lower binding constants than related metal complexes, such as Ru(II)-complexes.

(iii) The main interaction mode of **ReDppz** with double-stranded ctDNA appears to be the intercalation into the stacked bases (*i.e.*, $K'_{SS}{}^{\mathbf{ReDppz}}$ is the same order of magnitude than $K_{SS}{}^{\mathbf{ReDppz}}$). This is in agreement with the higher intercalative ability reported for related dppz-complexes [6b, 9b, 31]

It is noteworthy that the magnitude of the binding constants obtained for **ReDppz** are rather small when comparing with related metal-complexes with dppz as bidentate ligand. The latter fact suggests that nHo moiety would be playing an important role (due to steric effect and/or coulombic repulsion) giving rise to a decrease in the overall interaction of **ReDppz**.

(iv) The last point that deserves to be highlighted is related to the observation that no change of the emission spectra was observed when nHo free ligand was used as competitive agent. This result suggests that either nHo is not an intercalative agent or its intrinsic binding constant is not high enough to promote the EtBr displacement. This result is quite relevant and shed light on the mode of interaction between βCs and DNA that has been a subject of controversy for more than 40 years. [19b, c, 32]

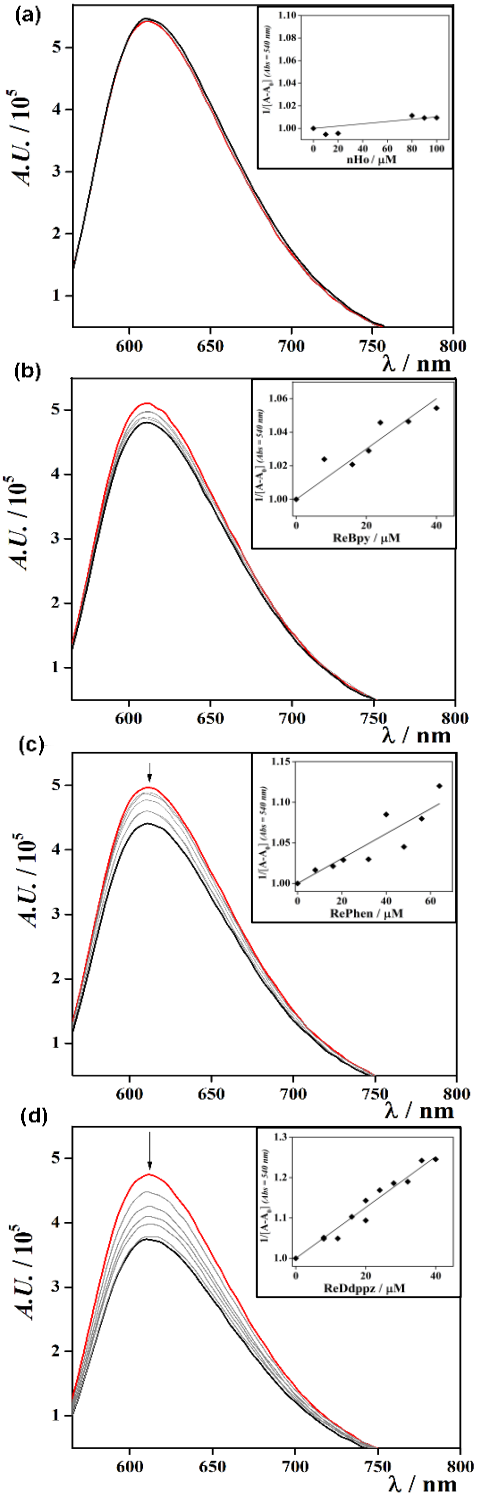

Figure 6. Displacement of EtBr (20 μM) by: **(a)** nHo, **(b) ReBpy**, **(c) RePhen** and **(d) ReDppz**. *Insets:* Stern-Volmer Plot of the fluorescence intensity. The concentration of the complexes increased from 0 to 60 μM (100 μM for nHo).

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Table 1: Summary of $Re(CO)_3$(nHo)L/ctDNA interaction parameters measured in buffer aqueous solution (pH 7.4): K_G is the Benesi-Hildebrand equilibrium constant, K_{SS} and K_D are the slope of the Stern-Volmer plot obtained from steady-state (*i.e.*, from static quenching) time-resolved (*i.e*, dynamic quenching) data.

Compound	K_G /10^3 M^{-1}	K_{ss} /10^3 M^{-1}	K'_{ss} [c] /10^3 M^{-1}	K_D /10^3 M^{-1}
nHo	0.2 ± 0.1[a]	2.8[a][b]	0	0.54 ± 0.02[a]
ReBpy	1.4 ± 0.4	4 ± 0.3	0.15 ± 0.03	0 (IL_{nHo}) 0.060 ± 0.004 ($MLCT_{Re\rightarrow Bpy}$)
RePhen	4.5 ± 0.5	31 ± 2	1.5 ± 0.4	0.055 ± 0.003 (IL_{nHo}) 2.4 ± 0.4 ($MLCT_{Re\rightarrow Phen}$)
ReDppz	3.2 ± 0.7	2.5 ± 0.7	6 ± 1	0.11 ± 0.05 (IL_{nHo}) nd ($MLCT_{Re\text{-}dppz}$)

[a] Data from Ref [19b], for nHo cationic species (pH 4.4). [b] Data calculated as the average of K_{SS} measured at pH 4.4 and 10.9. [c] Calculated from the displacement of ethidium bromide.

Role of Reactive Oxygen Species (ROS)

Differences in the type and extent of ROS production by the photoexcited Re-complexes might also contribute to the differences observed between the photo-oxidative DNA damage profiles (Figure 3). In particular, singlet oxygen ($^1O_2$) is a key oxidative agent that mainly induces purine oxidation. Therefore, we quantified the quantum yields of $^1O_2$ production (Φ_Δ) by photo-excited **ReBpy**, **RePhen** and **ReDppz**. Having in mind the solvent effect on the photophysical properties of these Re-complexes, Φ_Δ values were determined in D_2O and acetonitrile, as representative polar protic and aprotic solvents, respectively (Table 2). Briefly, Φ_Δ values determined in acetonitrile were ~4-7-folds higher than in D_2O. This strong solvent-effect can be explained in terms of the higher stability of $MLCT_{Re\text{-}L}$ (where L = bpy, phen or dppz) in acetonitrile with respect to aqueous solvents. In the cases of **ReBpy** and **RePhen**, the only dominant emitting species observed in acetonitrile is $MLCT_{Re\text{-}L}$ (Figures SI.2 a and b). In the case of **ReDppz** in acetonitrile, besides the only emission band observed in aqueous solvent assigned to IL_{nHo}, an additional emission band assigned to $^3IL_{dppz}$ is observed (Figure SI.2c). This might be responsible for the higher efficiency of $^1O_2$ production. Moreover, in all the cases, lifetime of $MLCT_{Re\text{-}L}$ is considerably longer in the aprotic solvent (Table 2). Beside this quite strong solvent effect, Φ_Δ also depends on the chemical structure of the bidentate ligand: $\Phi_\Delta^{ReDppz} \approx \Phi_\Delta^{RePhen} > \Phi_\Delta^{ReBpy}$.

This latter trend correlates well with the DNA damage extent described above (Figures 2 and 3), *i.e.*, the higher the Φ_Δ, the higher the overall photoinduced DNA damage. Although oxidized DNA base modifications sensitive to Fpg-endonuclease can be photoinduced via type I and/or type II mechanism, our results suggest that $^1O_2$ plays a key role. Note that ctDNA provides a less protic environment to the three investigated Re-complexes (Figures 5 and SI.2). Thus, for the three investigated Re-complexes a considerable increase in the efficiency of $^1O_2$ production (*i.e.*, in Φ_Δ) is expected in the presence of ctDNA in comparison to pure buffer solution.

Recently, it was demonstrated that electron transfer processes between ctDNA and photoexcited Re-complexes also take place[6b, 33]. Thus, type I mechanisms might well contribute to the described overall DNA damage.

Table 2: Summary of the most relevant photophysical parameters of $[Re(CO)_3(nHo)L]^+$ complexes discussed along the text.

Compound	ϕ_Δ (ACN)	ϕ_Δ (D_2O)	$\tau_{MLCTRe\rightarrow L}$ (ACN) / ns	$\tau_{MLCTRe\rightarrow L}$ (buffer) / ns	E_T /kJ mol^{-1}
nHo	0.33 ± 0.03	0.08[c]	3.0 (N*)	< 0.5 (N*)[c] 20.8 (C*)[c]	[a][b]261
ReBpy	0.28 ± 0.03	0.06 ± 0.01	148.2	48.8	269[d]
RePhen	0.70 ± 0.04	0.10 ± 0.01	260.6	155.3	247[d]
ReDppz	0.66 ± 0.03	nd	27.6[e]	nd[e]	nd[e]

[a] Data from Ref [19b], for nHo cationic species (pH 4.4). [b] Calculated from the phosphorescence emission maximum. [c] Data from Ref [18]. [d] Calculated from the $E_{0,0}$ value [34]. [e] $MLCT_{Re\text{-}dppz}$ state is not formed in aqueous solution, whereas $^3IL_{dppz}$ is largely observed in acetonitrile (ACN). N* and C*, represent the photoexcited neutral and cationic species of nHo, respectively.

Ames test

Cytotoxic and mutagenic properties of both non-irradiated and photoexcited **ReBpy**, **RePhen** and **ReDppz** were investigated for bacterial mutagenicity in the *Salmonella typhimurium* strain TA100. According to data depicted in Figure 7, **ReBpy** and **ReDppz** do not show any toxic and/or mutagenic effects neither in the absence nor in the presence of light (UVA, 365 ± 20 nm). The negative result can be a consequence either of a lack of drug uptake by the bacteria or a negligible photoreactivity of these two particular Re-complexes in a polar-protic environment.

On the contrary, **RePhen** was found to be cytotoxic and this effect is clearly enhanced when subject to UVA irradiation. The results indicate that **RePhen** is taken up by the bacteria. The complex is non-mutagenic in TA100 in the dark. It may be, however, weekly mutagenic under irradiation at toxic concentrations, as evident if the number of mutants is calculated per survivors (see Figure SI.5). It is noteworthy that **RePhen** showed the highest Φ_Δ among the diimine ligands, which is even enhanced when the complex is placed in a non-protic environment. Such a non-protic environment could be represented by the bacterial cell wall. Singlet oxygen, produced upon **RePhen** photoexcitation in the cell wall might reach the bacterial DNA by diffusion and give rise to photomutagenicity.

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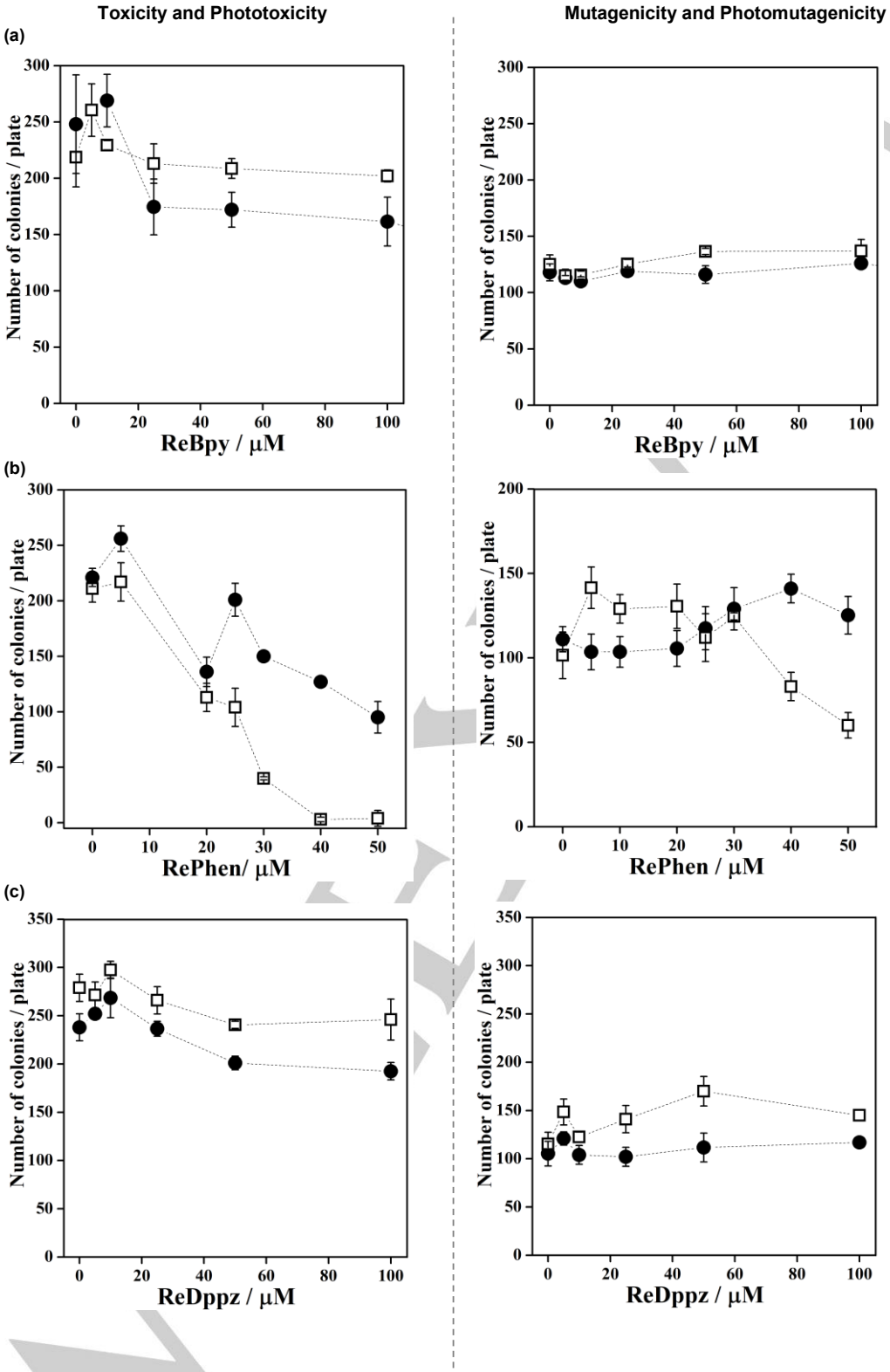

Figure 7: Toxicity and phototoxicity (left panels) and mutagenicity and photomutagenicity (right panels) of **(a) ReBpy**, **(b) RePhen** and **(c) ReDppz in *Salmonella typhimurium* TA100**. Black circles and empty squares depict data obtained in dark and upon photoexcitation, respectively.

Conclusions

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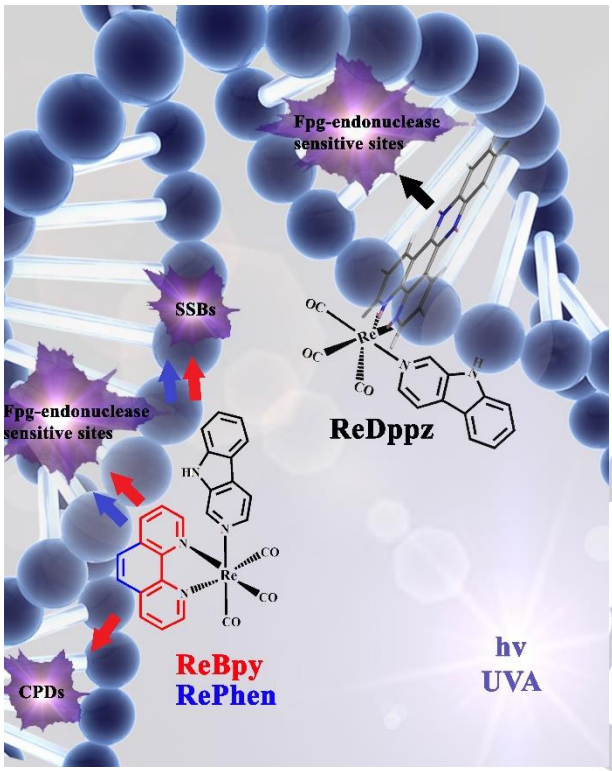
The current work provides insight into the interactions of three novel Re(I)-polypyridyl complexes, in both electronic ground and excited states, with cell-free DNA. Data reported herein in this work provide unambiguous evidence that the chemical structure of the rhenium ligands strongly modulates the type and extent of both the interaction (intercalation and/or groove binding) and DNA damage profile (photooxidation of purine nucleobases, and/or generation of SSBs and/or CPDs formation). The major conclusions can be summarized in Scheme 1.



Scheme 1. Main photochemical pathways involved in the DNA damage photosensitized by Re-complexes (**ReBpy**, **RePhen** and **ReDppz**). Red, blue and black arrows indicate the type of DNA-damage induced by **ReBpy**, **RePhen** and **ReDppz**, respectively.

Also, we show the photomutagenic and phototoxic potential of these complexes against S*almonella typhimurium* through the classical Ames test.

Experimental Section

General

Norharmane, bpy, phen and ethidium bromide (EtBr) were purchased from Sigma-Aldrich in the highest purity available (> 99.9 %) and were used without further purification. $[Re(CO)_3(nHo)L]^+$ complexes showed on Figure 1 and dppz ligand were synthesized according to the procedures described elsewhere. [4a, 20]

DNA material: DNA from bacteriophage PM2 (PM2 DNA, 10^4 bp) was prepared according to the method of Salditt *et. al.*[35] Calf thymus DNA (Sigma-Aldrich), ctDNA, was dissolved in Tris (10 mM) with EDTA (1 mM) at pH 7.4.

Enzymes: Formamidopyrimidine-DNA glycosylase (Fpg protein) was obtained from *E. coli* strain JM105 harboring plasmid pFPG230. [36] Endonuclease IV and T4 endonuclease V were partially purified from an inducible overproducer (*E. coli* strain A 32480 carrying the plasmid ptac-denV) provided by L. Mullenders, Leiden. All repair endonucleases were tested following the procedures described elsewhere.[37]

Bacterial culture media: (i) Nutrient Broth, 2.5 g of Nutrient Broth No. 2 (Oxoid CM67, Thermo Fisher Scientific Inc.) dissolved in 100 ml of distilled water and 100 µg/ml of Ampicillin. *(ii)* Resuspension Medium, 1.6 g of Bacto Nutrient Broth (Difco 0003, VWR) with 5.0 g of NaCl dissolved in 1000 ml of distilled water. *(iii)* Top Agar, 12 g of Bacto-Agar (Difco 1040) and 12 g of NaCl dissolved in 2000 ml of distilled water. *(iv)* HBT buffer, 192 mg of L-Histidin-HCl, 250 mg of D-Biotin and 100 ml of L-Tryptophan dissolved in 1200 ml of 250 mM Phosphate Buffer (pH 7,4). *(v)* Minimal Agar, 30 g of Bacto-Agar (Difco 0140) dissolved in 1600 ml of distilled water.

Binding Studies

The room-temperature interaction of the Re complexes with ctDNA, in phosphate buffer (0.1 mM, pH 7.4) aqueous solutions, was studied by *(i)* UV-visible absorption and *(ii)* emission spectroscopy. Due to the poor solubility of the complexes in buffer, 0.2% of DMSO was added to the solution. A molar absorption coefficient at 260 nm (ε^{260nm}) of 6600 (M, in bases)$^{-1}$ cm^{-1} was used to calculate the [DNA] of the stock solutions. Briefly:

(i) UV-visible spectrophotometric titration: measurements were made on a Cary 60 spectrophotometer, in quartz cells of 1 cm path length (105.250-QS Hellma). Spectra of the complexes were recorded in the presence of increasing amounts of ctDNA and data analyzed by the Benesi–Hildebrand approach (eq. (1)).[38] Experimental difference (ED) spectra were obtained by subtracting the spectrum at 0 mM of DNA from the subsequent spectra recorded at different DNA concentration.

$$\frac{1}{\Delta A} = \frac{1}{(\varepsilon\mathbb{C}\text{-ctDNA} - \varepsilon\mathbb{C})}\frac{1}{[\mathbb{C}]_0} + \frac{1}{K_G\,(\varepsilon\mathbb{C}\text{-ctDNA} - \varepsilon\mathbb{C})\,[\mathbb{C}]_0}\frac{1}{[\text{ctDNA}]} \qquad (1)$$

where $\varepsilon_{\mathbb{C}\text{-ctDNA}}$ and $\varepsilon_{\mathbb{C}}$ are the molar absorption coefficients of the binding complex between $[Re(CO)_3(nHo)L]^+$ complex and ctDNA ($\mathbb{C}$-ctDNA) and free $[Re(CO)_3(nHo)L]^+$ complex ($\mathbb{C}$), respectively, at the titration wavelength. ΔA is the change of absorbance, at a concentration of ctDNA, relative to the completely free $[Re(CO)_3(nHo)L]^+$ complex, $\mathbb{C}$, ([ctDNA] = 0 M) at the same wavelength. K_G values were obtained from the slopes and intercepts of eq. (1).

ii) Spectrofluorometric titration: steady-state and time-resolved emission measurements were made on a Fluoromax4 (HORIBA Jobin Yvon) and a single-photon-counting equipment FL3 TCSPC-SP (HORIBA Jobin Yvon), respectively. Measurements were made in quartz cells of 1 x 1 cm path length. Emission spectra and luminescence decays of $[Re(CO)_3(nHo)L]^+$ complexes were recorded in the presence of increasing amounts of ctDNA. Data were analyzed according the Stern-Volmer equations (2) and (3):[19a, d, 34]

$$I_0/I = 1 + K_{SV}[ctDNA] \qquad (2)$$

where I_0 and I are the intensity of luminescence calculated as the integral under the entire emission spectra in the absence and in the presence of ctDNA and K_{SV} is the Stern-Volmer constant. When a dynamic process is operating, the Stern Volmer constant K_{SV} is called K_D, being $K_D = k_q\tau_0$,

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where k_q represents the quenching or deactivation constant rate and τ_0 is the luminescence lifetime of the $[Re(CO)_3(nHo)L]^+$ complex recorded in the absence of ctDNA. K_D was calculated from the slope of the relationship between τ_0 and τ, as shown in eq. (3), where τ are the luminescence lifetimes of the complexes at a given ctDNA concentration.

$$\tau_0/\tau = 1 + K_D[ctDNA] \quad (3)$$

If a dynamic process is operating eq (2) changes to eq. (3), where K_D is the dynamic Stern-Volmer constant, and τ_0 and τ are the lifetimes of the $[Re(CO)_3(nHo)L]^+$ complexes, recorded in the absence and in the presence of ctDNA, respectively. When the nature of the quenching process is static, K_{SV} is equal to the equilibrium constant for ground-state complex formation, now defined as K_{SS} (for steady-state quenching), and can be calculated from eq. (2).

iii) Ethidium bromide fluorescence displacement experiments: EtBr is a typical DNA intercalating agent. Upon intercalation into the stacked bases in double-stranded DNA, EtBr fluorescence yield is greatly enhanced, ~25 times, with respect to the bulk solution [39]. In the presence of an additional DNA intercalating compound, that might compete (displace) with EtBr for the DNA intercalating sites, fluorescence of the EtBr-DNA complex is quenched.[39a, 40] Upon photoexcitation at EtBr lower energy excitation band (λ_{ex} = 540 nm), fluorescence emission spectra were recorded from 550 nm to 800 nm in the presence of increasing concentration of **ReBpy**, **RePhen** or **ReDppz**.

Singlet oxygen production

Details of the system used have been published previously.[41] Briefly, quantum yields of photosensitized singlet oxygen production, Φ_Δ, were obtained using the third harmonic of a Q-switched Nd-YAG laser as the excitation source (λ_{exc} = 355 nm, Surelite II- Continuum), looking at the 1270 nm $^1O_2$ phosphorescence with a Ge-photodiode (Applied Detector Corporation, resolution time of 1 µs). Measurements were performed in both air-equilibrated acetonitrile and D_2O solutions. The average of signals generated by 64 laser shots were recorded to improve the signal-to-noise ratio. Single exponential analysis of emission decays was performed with the exclusion of the initial part of the signal. Φ_Δ was determined by measuring its phosphorescence intensity using an optically matched solution of a reference sensitizer. In acetonitrile, the reference used was phenalenone (Φ_Δ = 0.975) [42], whereas sulphonated perinaphthenone (PNS) (Φ_Δ = 0.97 ± 0.06) was used in experiments performed in D_2O [43].

DNA photoproducts characterization

Irradiation set-up. A mixture of $[Re(CO)_3(nHo)L]^+$ complex buffered aqueous solutions (10 mM KH_2PO_4, 50 mM NaCl, pH 7.4) containing 2% ethanol (to further ensure total complex dissolution) and PM2 DNA (at 10 µg/ml) were placed in a 96 well-plate on ice and then irradiated with a Philips HPW 125W lamp (365 ± 20 nm), set at a distance of 10 cm (dose of 30 kJ/m^2). Treated DNA was precipitated in ethanol/sodium acetate solution and, then, dissolved in BE1 buffer (20 mM Tris–HCl, pH 7.5; 100 mM NaCl and 1 mM EDTA) for damage analysis.

Quantification of endonuclease-sensitive modifications in PM2 plasmid. The DNA relaxation assay used to quantify endonuclease-sensitive sites (ESS) and single-strand breaks (SSB) in PM2 DNA has been previously described.[19a, 22b, 44] Briefly, it makes use of the fact that supercoiled PM2 DNA is converted by either a SSBs or the incision of a repair enzyme into a relaxed (nicked) form, which migrates separately from the supercoiled form in agarose gel electrophoresis. In particular, enzymes used were Fpg protein (*Fpg*), T4 endonuclease V (*T4 endo V*) and endonuclease IV (*Endo IV*).

(Photo)toxicity and (photo)mutagenicity (Ames tests)

A 1/10 aliquot of *Salmonella typhimurium* (TA100 [45]) strains, grown in 20 ml of Nutrient Broth at 37° for 24 h under constant shaking, was diluted into fresh medium to ensure exponential growth. After 2 h, the culture was centrifugated and placed in resuspension medium. 200 µl of the freshly grown bacterial culture (1-2x10^9 CFU/ml) were added to a falcon tube containing 1 ml of top Agar and 10% of HBT buffer. After mixing, 200 µl of the desired complex at a calculated concentration dissolved in KCl buffer was added. For mutagenicity and toxicity assays, falcon tubes were kept in the dark for 20 minutes; whereas for photomutagenicity and phototoxicity assays, mixtures were placed into 6 well plaques and then irradiated (20 min) with a Philips HPW 125W lamp (365 ± 20 nm, 30 kJ/m^2). For the analysis of photomutageniciy or mutagenicity, 700 µl of the irradiated or unirradiated culture were mixed with 2 ml of Top agar and poured into Petri dishes containing minimal agar and incubated for 48 h at 37°C (photomutagenicity). For the analysis of phototoxicity or toxicity, the bacteria were further diluted 1:200000 before plating as described above but using regular (fully supplemented) agar.

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Keywords: rhenium tricarbonyl complexes • alkaloids • photosensitization • DNA cleavage • electron transfer

[1] a) A. Kumar, S.-S. Sun and J. A. Lees, *Top. Organomet. Chem.* **2010**, *29*, 1 - 35; b) R. Ziessel, A. Juris and M. Venturi, *Inorg. Chem.* **1998**, *37*, 5061 - 5069; c) M. R. Féliz, J. F. Rodriguez-Nieto, G. T. Ruiz and E. Wolcan, *J. Photochem. Photobiol.* **1998**, *117*, 185 - 192.
[2] M. Schutte, G. Kemp, H. G. Visser and A. Roodt, *Inorg. Chem.* **2011**, *50*, 12486 - 12498.
[3] P. K. Ng, X. Gong, S. H. Chan, L. S. M. Lam and W. K. Chan, *Chem. - Eur. J.* **2001**, *7*, 4358 - 4367.
[4] a) I. Maisuls, E. Wolcan, O. E. Piro, E. E. Castellano, G. Petroselli, R. Erra-Balsells, F. M. Cabrerizo and G. T. Ruiz, *ChemistrySelect* **2017**, *2*, 8666-8672; b) A. Kastl, S. Dieckmann, K. Wahler, T. Volker, L. Kastl, A. L. Merkel, A. Vultur, B. Shannan, K. Harlms, M. Ocker, W. J. Parak, M. Herlyn and E. Meggers, *ChemMedChem* **2013**, *8*, 924 - 927; c) K. K.-W. Lo, K. Y. Zhang and S. Li Po-Yam, *Eur. J. Inorg. Chem.* **2011**, *2011*, 3551 - 3658; d) V. Fernández-Moreira, M. L. Ortego, F. C. Williams, P. M. Coogan, M. D. Villacampa and M. C. Gimeno, *Organometallics* **2012**, *31*, 5950 - 5957; e) R. G. Balasingham, P. M. Coogan and F. L. Thorp-Greenwood, *Dalton Trans.* **2011**, *40*, 11663 - 11674; f) L. C.-C. Lee, K.-K. Leung and K. K.-W. Lo, *Dalton Trans.* **2017**, *46*, 16357 - 16380.
[5] A. Leonidova and G. Gasser, *ACS Chem. Biol* **2014**, *9*, 2180 - 2193.
[6] a) M. Kaplanis, G. Stamatakis, D. V. Papakonstantinou, M. Paravatou-Petsotas, A. C. Demopoulos and A. M. Christiana, *J. Inorg. Biochem.* **2014**, *135*, 1 - 9; b) G. T. Ruiz, M. P. Juliarena, R. O. Lezna, E. Wolcan, M. R. Féliz and G. J. Ferrraudi, *Dalton Trans* **2007**, *20*, 2020 - 2029; c) A. J. Smith, W. M. George and M. J. Kelly, *Coord. Chem. Rev.* **2011**, *255*, 2666 - 2675.
[7] G. Gasser, A. Pinto, S. Neumann, A. M. Sosniak, M. Seitz, K. Merz, R. Heumann and N. Metzler-Nolte, *Dalton Trans.* **2012**, *41*, 2304 - 2313.
[8] a) F. Zobi, O. Blacque, K. O. R. Sigel and R. Alberto, *Inorg. Chem.* **2007**, *46*, 10458 - 10460; b) E. K. Erkkila, T. D. Odom and K. J. Barton, *Chem. Rev.* **1999**, *99*, 2777 - 2795.

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[9] a) W. S. Sheldrick, R. Gust, I. Ott and S. Schäfer, *Eur. J. Inorg. Chem.* **2007**, *2007*, 3034 - 3046; b) K. J. Barton, T. J. Kaiser and H. Song, *Nat. Chem.* **2012**, *4*, 615 - 120; c) L. J. Boerner and J. M. Zaleski, *Curr. Opin. Chem. Biol.* **2005**, *9*, 135 - 144.
[10] a) G. T. Ruiz, J. G. Ferraudi, E. Wolcan and R. M. Féliz, *Inorg. Chim. Acta.* **2010**, *363*, 1615 - 1618; b) G. T. Ruiz, M. P. Juliarena, R. O. Lezna, M. R. Féliz and J. G. Ferraudi, *J.Photochem. Photobiol.* **2005**, *179*, 289 - 297.
[11] a) B. M. Zeglis, J. A. Boland and K. J. Barton, *Biochemistry* **2009**, *48*, 839 - 849; b) D. A. E. Stemp, R. M. Arkin and K. J. Barton, *J. Am. Chem. Soc.* **1995**, *117*, 2375 - 2376.
[12] a) K. D. Copeland, M. P. Fitzsimons, R. P. Houser and J. K. Barton, *Biochemistry* **2002**, *41*, 343-356; b) X. Dong, X. Wang, M. Lin, H. Sun, X. Yang and Z. Guo, *Inorg. Chem.* **2010**, *49*, 2541 - 2549.
[13] a) M. H. Kaulage, B. Maji, S. Pasadi, S. Bhattacharya and K. Muniyappa, *Eur. J. Med. Chem.* **2017**, *139*, 1016 - 1019; b) Y. Sun, Y.-J. Hou, Q.-X. Zhou, W.-H. Lei, J.-R. Chen, X.-S. Wang and B.-W. Zhang, *Inorg. Chem.* **2010**, *49*, 10108 - 10116; c) B. de Souza, A. J. Bortoluzzi, T. Bortolotto, F. L. Fischer, H. Terenzi, D. E. C. Ferreira, W. R. Rocha and A. Neves, *Dalton Trans.* **2010**, *39*, 2027 - 2035; d) D. Crespy, K. Landfester, U. S. Schubert and A. Schiller, *Chem. Commun.* **2010**.
[14] a) R. Bonett, *CRC Press* **2000**, 324; b) U. Schatzschneider and J. K. Barton, *J. Am. Chem. Soc.* **2004**, *126*, 8630 -8631.
[15] a) C. Mari, V. Pierroz, R. Rubbiani, M. Patra, J. Hess, B. Spingler, L. Oehninger, J. Schur, I. Ott, L. Salassa, S. Ferrari and G. Gasser, *Chem. - Eur. J.* **2014**, *20*, 14421 - 14436; b) L. K. McKenzie, I. V. Sazanovich, E. Baggaley, M. Bonneau, V. Guerchais, J. A. G. Williams, J. A. Weinstein and H. E. Bryant, *Chem. - Eur. J.* **2017**, *23*, 234 - 238; c) J. Niesel, A. Pinto, H. W. N'Dongo Peindy, K. Merz, I. Ott, R. Gust and U. Schatzschneider, *Chem. Commun.* **2008**, *0*, 1798 - 1800.
[16] a) R. Blasius, H. Nierengarten, M. Luhmer, J.-F. Constant, E. Defrancq, P. Dumy, A. van Dorsselaer, C. Moucheron and A. Kirsch-DeMesmaeker, *Chem. - Eur. J.* **2005**, *11*, 1507 - 1517; b) J. Ghesquière, N. Gauthier, J. De Winter, P. Gerbaux, C. Moucheron, E. Defrancq and A. Kirsch-De Mesmaeker, *Chem. - Eur. J.* **2012**, *18*, 355 - 364.
[17] a) S. C. Marker, N. S. MacMillan, W. R. Zipfel, Z. Li, P. C. Ford and J. J. Wilson, *Inorg. Chem.* **2018**, *57*, 1311 - 1331; b) M. K. Knopf, B. L. Murphy, N. S. MacMillan, J. M. Baskin, M. P. Barr, E. Boros and J. J. Wilson, *J. Am. Chem. Soc.* **2017**, *139*, 14302 - 14314.
[18] a) M. M. Gonzalez, L. Salum, Y. Gholipour, M. F. Cabrerizo and R. Erra-Balsells, *Photochem. Photobiol. Sci.* **2009**, *8*, 1139 - 1149; b) M. M. Gonzalez, J. Arnbjerg, P. Denofrio, R. Erra-Balsells, P. R. Ogilby and M. F. Cabrerizo, *J. Phys. Chem. A* **2009**, *113*, 6648 - 6656; c) O. A. F. Rasse-Suriani, F. S. Garcia Einschlag, M. Rafti, T. Schmidt De Leon, P. M. David-Gara, R. Erra-Balsells and M. F. Cabrerizo, *Photochem. Photobiol.* **2018**, *94*, 36 - 51.
[19] a) M. M. Gonzalez, M. Pellon-Maison, A. M. Ales-Gandolfo, R. M. Gonzalez-Baro, R. Erra-Balsells and F. M. Cabrerizo, *Org. Biomol. Chem.* **2010**, *8*, 2543 - 2552; b) M. M. Gonzalez, M. Vignoni, M. Pellon-Maison, A. M. Ales-Gandolfo, R. M. Gonzalez-Baro, R. Erra-Balsells, B. Epe and M. F. Cabrerizo, *Org. Biomol. Chem.* **2012**, *10*, 1807 - 1819; c) M. Vignoni, R. Erra-Balsells, B. Epe and F. M. Cabrerizo, *J. Photochem. Photobiol. B* **2014**, 66 - 71; d) M. M. Gonzalez, M. P. Denofrio, F. S. Garcia Einschlag, C. A. Franca, R. Pis Diez, R. Erra-Balsells and F. M. Cabrerizo, *Phys. Chem. Chem. Phys.* **2014**, *16*, 16547 - 16562; e) M. M. Gonzalez, O. A. F. Rasse-Suriani, C. A. Franca, R. Pis Diez, Y. Gholipour, H. Nonami, R. Erra-Balsells and F. M. Cabrerizo, *Org. Biomol. Chem.* **2012**, *10*, 9359 - 9372.
[20] I. Maisuls, E. Wolcan, E. O. Piro, A. G. Etcheverria, G. Petroselli, R. Erra-Balsells, M. F. Cabrerizo and T. G. Ruiz, *Dalton Trans* **2015**, *44*, 17064 - 17074.
[21] H. E. Krokan, R. Standal and G. Slupphaug, *Biochem. J.* **1997**, *325*, 1 - 16.
[22] a) J. M. Daley, C. Zakaria and D. Ramotar, *Mutat. Res.* **2010**, *705*, 217 - 227; b) B. Epe, *Photochem. Photobiol. Sci.* **2012**, *11*, 98 - 106.
[23] The amount of CPDs produced upon photoexcitation therefore is calculated as the difference between number of sites recognized in the incubation with both endonuclease IV and T4 endonuclease V and the number of AP sites recognized by endo IV alone. We refer to this difference as "CPDscalc".
[24] V. Lhiaubet-Vallet, M. C. Cuquerella, J. V. Castell, F. Bosca and M. A. Miranda, *J. Phys. Chem. B.* **2007**, *111*, 7409 - 7414.
[25] B. N. Thornton and S. K. Schanze, *Inorg. Chem.* **1993**, *32*, 4994 - 4995.
[26] A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, J. N. Turro and K. J. Barton, *J. Am. Chem. Soc.* **1989**, *111*, 3051 - 3058.
[27] N. Shahabadi, S. Mohammadi and R. Alizadeh, *Bioinorg. Chem. Appl.* **2011**, *2011*, 8.
[28] a) W. A. McKinley, P. Lincoln and M. E. Tuite, *Coord. Chem. Rev.* **2011**, *255*, 2676 - 2692; b) H. Stoeffler, B. N. Thornton, L. S. Temkin and S. K. Schanze, *J. Am. Chem. Soc.* **1995**, *117*, 7119 - 7128.
[29] D. Z. M. Coggan, I. S. Haworth, J. P. Bates, A. Robinson and A. Rodger, *Inorg. Chem.* **1999**, *38*, 4486 - 4497.
[30] H. Ahmad, A. Wragg, W. Cullen, C. Wombwell, A. J. H. M. Meijer and A. J. Thomas, *Chem. Eur. J.* **2014**, *20*, 3089 - 3096.
[31] E. A. Friedman, J.-C. Chambron, J.-P. Sauvage, J. N. Turro and K. J. Barton, *J. Am. Chem. Soc.* **1990**, *112*, 4960 - 4962.
[32] a) K. Hayashi, M. Nagao and T. Sugimura, *Nucleic Acid Res.* **1977**, *4*; b) Z. Taira, S. Kanzawa, C. Dohara, S. Ishida and M. Matsumoto, *Jap. J. Toxicol. Envirom. Health* **1997**, *43*; c) B. K. Paul and N. Guchhait, *J. Phys. Chem. B.* **2011**, *115*, 11938 - 11949; d) T. Etienne, H. Gattuso, A. Morani and X. Assfeld, *Comput. Theor. Chem.* **2014**, *1040-1041*, 367 - 372; e) M. Balon, M. A. Muñoz, C. C., P. Guardado and M. Galán, *Biophys. Chem.* **1999**, *80*, 41 - 52; f) R. Cao, W. Peng, H. Chen, Y. Ma, X. Liu, X. Hou, H. Guan and A. Xu, *Biochem. Biophys. Res. Commun.* **2005**, *338*, 1557 - 1563.
[33] I. Maisuls, M. F. Cabrerizo, G. A. Lappin, G. T. Ruiz and J. G. Ferraudi, *J. Photochem. Photobiol.* **2018**, *Accepted*.
[34] J. R. Lakowicz, *Kluwer Academic/Plenum Publishers, New York,* **1999**.
[35] M. Salditt, S. N. Braunstein, R. D. Camerini-Otero and R. M. Franklin, *Virology* **1972**, *48*, 259-262.
[36] S. Boiteux, T. R. O'Connor, F. Lederer, A. Gouyette and J. Laval, *J. Biol. Chem.* **1990**, *265*, 3916 - 3922.
[37] E. Müller, S. Boiteux, R. P. Cunningham and B. Epe, *Nucleic Acid Res.* **1990**, *18*, 5969 - 5973.
[38] A. Wolfe, H. G. Shimer and T. Meehan, *Biochemistry* **1987**, *26*, 6392 - 6396.
[39] a) D. W. Wilson, L. Ratmeyer, M. Zhao, L. Strekowski and D. Boykin, *Biochemistry* **1993**, *32*, 4098 - 4104; b) M. F. Pohl, M. T. Jovin, W. Baehr and J. J. Holbrook, *Proc. Nac. Acad. Sci. USA* **1972**, *69*, 3805 - 3809.
[40] a) S. Dhar, M. Nethaji and R. A. Chakravarty, *J. Inorg. Biochem* **2005**, *99*, 805 - 812; b) T. E. Kydonaki, E. Tsoukas, F. Mendes, A. G. Hatzidimitriou, A. Paulo, L. C. Papadopolou, D. Papagiannopoulou and G. Psomas, *J. Inorg. Biochem.* **2015**, *160*, 94 - 105.
[41] F. Ragone, H. H. Martinez Saavedra, P. M. David-Gara, G. T. Ruiz and E. Wolcan, *J. Phys. Chem. A* **2013**, *117*, 4428 - 4435.
[42] R. Schmidt, C. Tanielian, R. Dunsbach and C. Womb, *J. Photochem. Photobiol.* **1994**, *79*, 11 - 17.
[43] C. Martí, O. Jurgens, O. Cuenca, M. Casals and S. Nonell, *J. Photochem. Photobiol.* **1996**, *97*, 11 - 18.
[44] a) B. Epe and J. Hegler, *In Methods in Enzymology, Academic Press* **1994**, *234*, 122 - 131; b) M. Häring, H. Rüdiger, B. Demple, S. Boiteux and B. Epe, *Nucleic Acid Res.* **1994**, *22*, 2010 - 2015.
[45] M. D. Maron and B. N. Ames, *Mutat. Res.* **1983**, *113*, 173 - 215.

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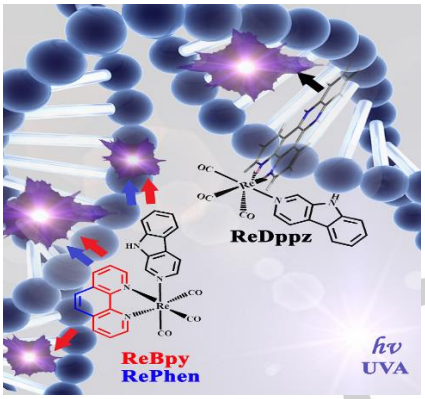
This work describes the photosensitizing properties of three nHo-Re(I) complexes with N,N' ligands. The interaction of the complexes with DNA was investigated by different spectroscopies. Quantitative DNA damage analysis shows that Re-complexes are more efficient photosensitizers than nHo. Photo-oxidation represents the major DNA damaging mechanism. Ames Test showed the phototoxic and photomutagenic properties of the compounds



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DNA oxidation photoinduced by Norharmane Rhenium(I) polypyridyl complexes: effect of the bidentate N,N' ligands on the damage profile

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