

Radio- and photosensitization of DNA with compounds containing platinum and bromine atoms^{*}

Małgorzata A. Śmialek^{1,2,a}, Sylwia Ptasińska³, Jason Gow², Søren Vrønning Hoffmann⁴, and Nigel J. Mason²

¹ Gdańsk University of Technology, Faculty of Ocean Engineering and Ship Technology, Department of Control and Energy Engineering, G. Narutowicza 11/12, 80-233 Gdańsk, Poland

² Department of Physical Sciences, The Open University, MK7 6AA, Milton Keynes, UK

³ Radiation Laboratory and Department of Physics, University of Notre Dame, Notre Dame, 46556, IN, USA

⁴ ISA, Department of Physics and Astronomy, Aarhus University, 8000 Aarhus C, Denmark

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Abstract. Irradiations of plasmid DNA by both X-rays and UV light in the presence and absence of compounds containing platinum and bromine atoms were performed in order to assess the sensitization potential of these compounds. Plasmid DNA pBR322 was incubated with platinum (II) bromide, hydrogen hexabromoplatinate (IV), hydrogen hexahydroxyplatinate (IV) and sodium hexahydroxyplatinate (IV). Incubation was followed by X-ray or UV irradiations. It was found that amongst the sensitizers tested, during irradiations carried out in the presence of platinum (II) bromide, the highest levels of double strand breaks formation upon X-ray treatment were recorded. In contrast much less damage was induced by UV light. Data presented here suggests that this compound may be a promising radiosensitizer for cancer treatment.

1 Introduction

A comprehensive understanding of the mechanisms by which high- and low-energy radiation interacts with cellular components, DNA in particular, underlies advances in modern radiotherapy. For many years attempts were made to increase the levels of lethal cellular damage in a controlled manner using various types of drugs, that apart from chemical damage, may act as radiosensitizers. Such concomitant treatment has been shown to be particularly beneficial in the treatment of neck, lung, pancreas or stomach cancers [1].

However the mechanisms by which such radiosensitization occurs are still unclear. Recent research has highlighted that low-energy electrons, which are the most abundant species created during high energy irradiations, may be as efficient in causing damage to DNA [2], as the incident radiation itself. Such secondary-particle damage can be initiated by any type of ionizing radiation, such as X-rays or γ -rays. Accordingly a wide range of radiosensitizing compounds are being investigated, amongst which platinum-based ones have been found to be the most successful [3,4]. It has been shown that such compounds coordinate themselves to nitrogen atoms of purines in DNA

helix [3]. One of the most widely used cancer-therapy drugs is cisplatin, which apart from being a very efficient chemotherapeutic drug, toxic to living cells [5], can be also used as a potential source of secondary particles, mainly low energy electrons and radicals, emitted upon interaction with high-energy radiation. This property of cisplatin was investigated recently and an increase in single (SSB) and double strand breaks (DSB) formation was reported [6].

The enhancement of DNA damage upon Pt-resonant X-ray radiation in presence of platinum-derived complexes due to the Auger effects has been widely investigated [7,8], showing that DNA damage increases upon platinum adducts. Apart from platinum-containing drugs [9], brominated compounds [10,11] were also found to be efficient radio- and photosensitizers in clinical radiotherapy. Therefore, an even more efficient drug may be developed if it contains both platinum and bromine, although to our best knowledge, such studies have not yet been performed. In our previous studies we showed that one of these compounds, PtBr₂, causes increased radiosensitivity in plasmid DNA, when compared with widely-used cisplatin [12]. This enhancement can be attributed to dissociative electron attachment to PtBr₂ and bromide anion formation [13]. In the present work we investigate three other platinum (IV) compounds as possible candidates for therapeutic drugs and cross examine them against PtBr₂ (Fig. 1). The reason for choosing the compounds used in our study was both low complexity of the molecules

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^a e-mail: smialek@pg.gda.pl

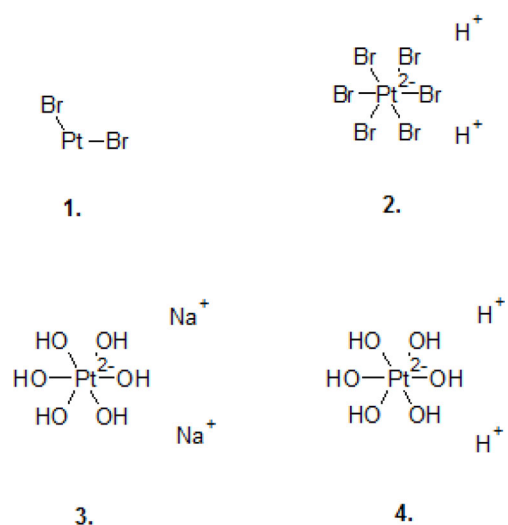


Fig. 1. Compounds investigated as potential radio- and photosensitizers: (1) PtBr₂, (2) H₂PtBr₆, (3) Na₂Pt(OH)₆ and (4) H₂Pt(OH)₆.

themselves as well as lack of large functional groups that may alter the results of simple Pt-Br compound and radiation interaction.

2 Methodology

2.1 Plasmid DNA

To model cellular DNA damage, plasmid DNA pBR322 was used. Plasmid DNA was prepared using a procedure previously described in detail [14] and suspended in ultra-high purity (UHP) H₂O with pH adjusted to 8.0 with 1 M NaOH. This plasmid has already been widely used in numerous irradiation studies [15,16]. Sodium hydroxide was chosen to stabilize plasmid DNA under vacuum conditions as it was shown that a lack of cations in the vicinity of phosphate backbone in DNA solution prior to solvent evaporation leads to DNA strand breaks and is due to constituent water removal from DNA [17].

2.2 Sample preparation and analysis

Two compounds containing both platinum and bromine atoms were chosen to be tested as potential radiosensitizers: platinum (II) bromide (PtBr₂) and hydrogen hexabromoplatinate (IV) (H₂PtBr₆). Additionally, two similar compounds, containing platinum and lacking bromine ions: hydrogen hexahydroxyplatinate (IV) (H₂Pt(OH)₆) together with sodium hexahydroxyplatinate (IV) (Na₂Pt(OH)₆) were tested. The compounds and reagents were purchased from Sigma-Aldrich, UK. H₂Pt(OH)₆ and Na₂Pt(OH)₆ were chosen to serve as a reference for H₂PtBr₆ and allowed us to quantify the damage induced upon irradiation due to addition of bromide ions. Derivatives of Pt(IV) and Pt(II) were selected to see if the radiation damage enhanced by a Pt(II) compound

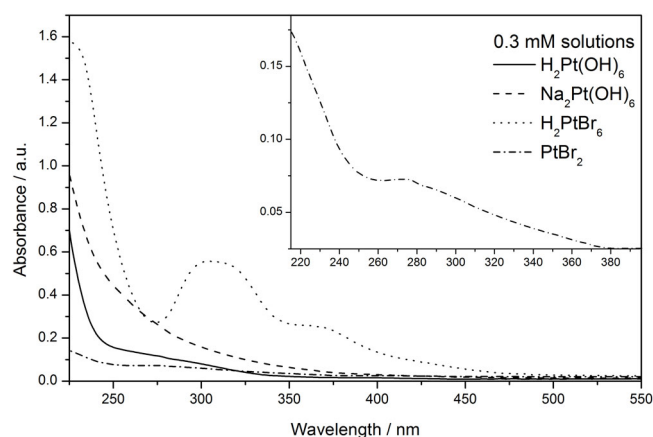


Fig. 2. The UV absorption spectra of 0.3 mM solutions of H₂Pt(OH)₆, Na₂Pt(OH)₆, H₂PtBr₆ and PtBr₂.

with associated bromide ions is any different to Pt(IV)-based compounds.

All compounds were dissolved in UHP water to obtain 0.3 mM solutions. The pH of all solutions was measured and, when necessary, adjusted to 7.5–8.5 (the working range for reactions involving DNA) with 1 M sodium hydroxide. All solutions were filtrated through a 0.2 micron syringe filter.

The absorption spectra of these solutions were recorded in order to assess any possible absorption bands in the UV region of the light used later for irradiation. These spectra are shown in Figure 2. Complexing of DNA and Pt compounds of interest was performed in the following way: 1 μ g (0.35 pmoles) of plasmid DNA, which contains 824 pmoles of guanine bases, was incubated with 3 nmoles of each compound (guanine binding site saturation conditions) in 20 μ l of the total volume of the reaction at 37 $^{\circ}$ C for 9 hours. Additionally, some pure DNA was also kept under the same conditions to serve as control samples in the irradiation experiments.

Each complex was irradiated and analyzed in triplicate and the error bars shown in all figures represent the standard deviation of the population. It was observed that, in most cases, the long-term incubation of DNA at 37 $^{\circ}$ C introduced at most 2% loss of supercoiled form of DNA but in the case of PtBr₂ 5% damage was noted (Fig. 3). For the X-ray study all samples were also vacuum-dried onto freshly cleaved mica at room temperature [18] with a small diaphragm pump and then transferred into a vacuum chamber. Loss of initial form of plasmid DNA due to that procedure is also shown in Figure 3 for PtBr₂, since in this case the overall loss was similar for all adducts.

Additionally, three reference samples were kept under high vacuum (HV) conditions (10⁻⁶ mbar) for the period of time corresponding to the largest irradiation dose during the experiment. Figure 3 shows 13% loss of the initial DNA: 8% due to placing samples on mica surface and additional 5% coming from the HV conditions.

After irradiation all the DNA complex samples were recovered from mica with 5 μ l of TE buffer and analyzed via agarose gel electrophoresis (AGE, 1.2% gels, 1 \times TBE,

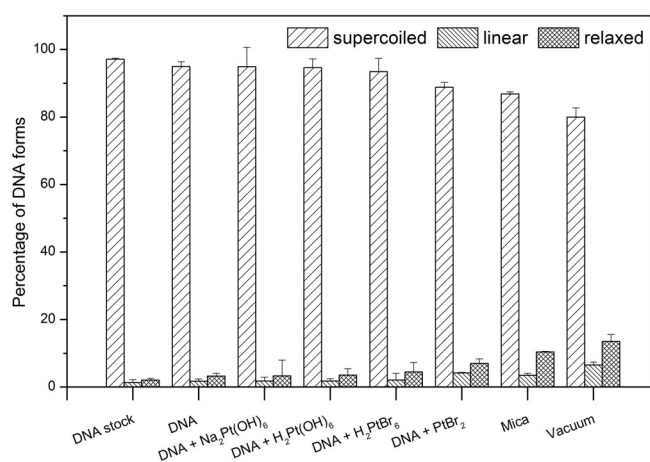


Fig. 3. Damage induced to plasmid DNA through the preparation procedure; quantities of main plasmid forms detected (from left) in: the stock solution; stock solution incubated at 37 °C for 9 h; stock solution mixed with Na₂Pt(OH)₆, inc. for 9 h at 37 °C; stock solution mixed with H₂Pt(OH)₆, inc. for 9 h at 37 °C; stock solution mixed with H₂PtBr₆, inc. for 9 h at 37 °C; stock solution mixed with PtBr₂, inc. for 9 h at 37 °C; vacuum-dried on mica and stock solution mixed with PtBr₂, inc. for 9 h at 37 °C, vacuum-dried on mica and kept under irradiation conditions for 20 min (no irradiation).

2 V/cm, stained later with a SYBR Green I dye and destained with a running buffer).

In the UV light studies, 2 μl of DNA solution with a sensitizing compound were irradiated as a liquid and collected after irradiation for further analysis.

2.3 DNA irradiations

The DNA samples were irradiated at various doses in triplicate using both X-rays and UV light. UV studies were performed on the UV1 beamline at ISA, Centre for Storage Ring Facilities at Aarhus University, Denmark, using a wet cell setup described previously [14]. Samples were irradiated as liquid with the maximum dose up to 25 Gy using various wavelengths. Wavelengths of irradiations for all compounds were chosen predominantly based on the photoabsorption spectra, shown in Figure 2. DNA mixed with Na₂Pt(OH)₆, irradiated with UV light at 215, 260, 320 and 370 nm. DNA mixed with H₂Pt(OH)₆ was irradiated at 215, 280 and 370 nm, while DNA mixed with H₂PtBr₆ – at 225, 305, 370 and 425 nm. For DNA mixed with PtBr₂, irradiations were carried at 208, 215, 260 and 370 nm, whereas pure DNA was irradiated at 208, 215, 260 and 370 nm.

For the X-ray study, an X-ray tube with a tungsten anode was used, with a tube potential of 25 kV and a current of 0.50 mA to provide a continuous X-ray spectrum. The X-ray tube was capable of delivering a dose rate of ~11.5 mGy/s. Such set-up allowed irradiation of the samples with low energy X-rays, knowing that predominant lines in the spectrum will be L α , L β and M α with energies approximately equal to 8.36 keV, 9.8 keV and 1.8 keV, respectively.

3 Results

3.1 UV irradiations

The results of UV irradiation of DNA samples mixed with Na₂Pt(OH)₆, PtBr₂, H₂PtBr₆, H₂Pt(OH)₆ and pure DNA are shown in Figure 4. In all cases low levels of loss of supercoiled DNA form was observed, resulting in formation of only the relaxed DNA form (SSBs). No DSBs were detected upon UV irradiation. For pure DNA, damage of less than 8% was observed at all UV wavelengths used for irradiation. Higher levels of damage, obtained for irradiation of all samples at 370 nm were attributed to second order synchrotron radiation. In addition, no dimer formation was detected in samples irradiated in presence and absence of sensitizing compounds.

3.2 X-ray irradiations

Figure 5 shows the loss of supercoiled form of DNA with increasing dose of radiation, whereas in Figures 6 and 7 the appearance of linear DNA and relaxed forms is shown, respectively. In all cases the addition of platinum increases the radiation damage when compared to pure DNA, but the presence of bromine groups can enhance the damage even more.

The highest levels of damage are obtained for DNA samples incubated in presence of PtBr₂. Also the exchange of hydroxyl groups in H₂Pt(OH)₆ to bromine ions (H₂PtBr₆) resulted in some enhancement in damage levels, mostly converted to DSBs (Figs. 6 and 7).

Relatively high levels of damage were observed for samples incubated with sodium hexahydroxyplatinate (IV). The levels of supercoiled DNA loss were twice as high at 7 Gy as in case where a corresponding acid was used as a sensitizer.

4 Discussion and conclusions

In case of all compounds tested the amount of damage induced by UV light was small if not negligible, not exceeding 10% of supercoiled DNA loss and no DSB were measured.

In contrast considerable damage was observed when these complexes were irradiated with similar doses of X-rays. Levels of supercoiled DNA loss reach 15% with 7 Gy for hydrogen hexahydroxyplatinate (IV). When hydroxyl groups in this compound were substituted with bromine atoms, interestingly this damage was observed even after lowest radiation dose of 2 Gy was applied, nonetheless at 7 Gy hardly any increase with respect to unbrominated analogue can be observed.

A vast increase in damage levels can be observed when sodium hexahydroxyplatinate (IV) was used. At radiation dose of 7 Gy over 15% increase in loss of supercoiled DNA was seen with respect to samples incubated with hydrogen hexahydroxyplatinate (IV). As in case of other platinum hydroxyl compound, loss of supercoiled DNA form arises more steadily with applied radiation dose, than in case of

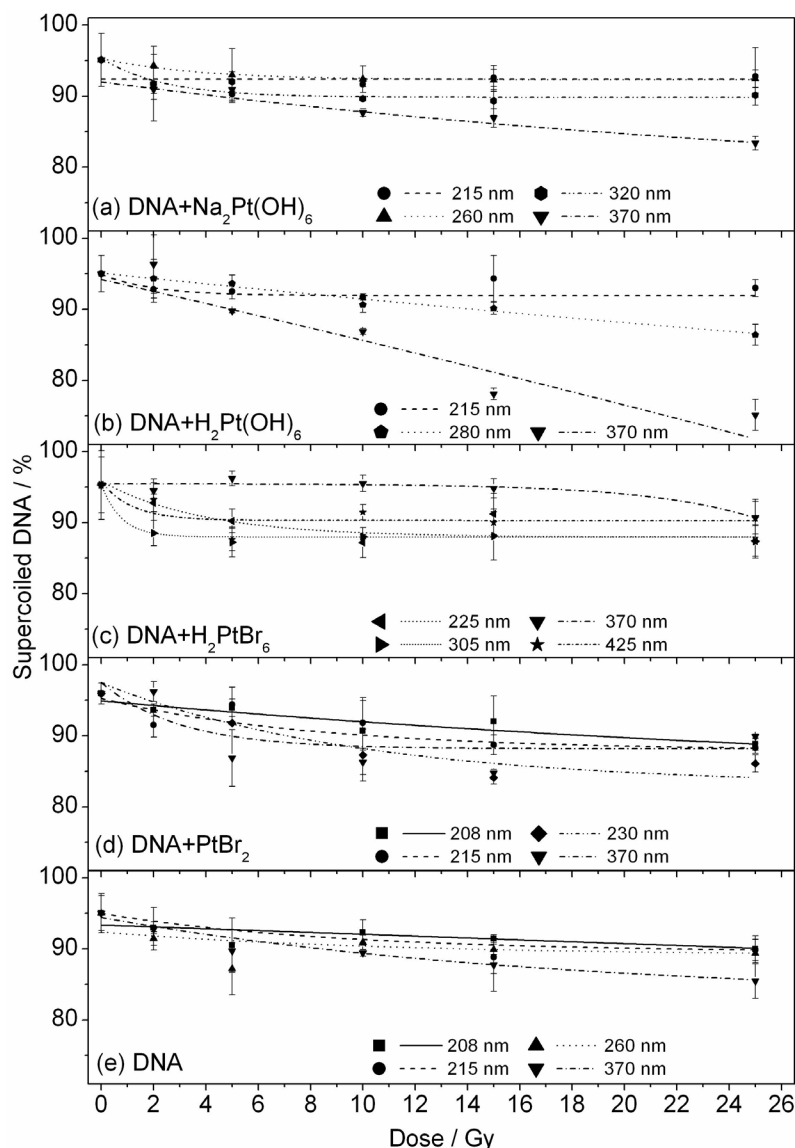


Fig. 4. Supercoiled DNA loss as a function of UV radiation dose at various wavelengths for DNA samples incubated for 9 h with $300 \mu\text{M}$ solutions of (a) $\text{Na}_2\text{Pt}(\text{OH})_6$, (b) $\text{H}_2\text{Pt}(\text{OH})_6$, (c) H_2PtBr_6 and (d) PtBr_2 and for pure DNA (e). Curves represent exponential decay function fit to experimental data.

brominated derivative and even up to 30% loss of supercoiled DNA (7 Gy) can be obtained without employing bromine.

Moreover, irradiation carried out in the presence of PtBr_2 shows even greater damage induced with respect to pure plasmid molecules (60% at 7 Gy). Damage levels observed in this case are substantial even for low doses of radiation, like in case of hydrogen hexahydroxyplatinatate (IV).

All of the dose-response curves could be fitted with simple exponential decay functions, thus it can be assumed that the DNA films were penetrated by the incident radiation with no loss of photon intensity through the film.

The irradiated brominated compounds also reveal a considerable increase in levels of the DSBs. Interestingly, the brominated derivative of Pt (IV) seemed to cause

hardly any relaxation, i.e., SSBs, in DNA molecules; however, up to 25% linear form was detected.

Our results show that a combination of Pt and Br atoms is much more efficient in radiosensitizing DNA to X-ray radiation than Pt alone-based compounds. Comparing our results with the ones obtained for cisplatin and its brominated analog [12], it can be seen that for compounds irradiated in the presence of PtBr_2 , radiation damage is significant also at lower radiation doses. Apart from an increase of SSBs levels, a substantial increase in DSBs levels was observed for compounds with Pt and Br atoms used here, which is an important feature for a potential radiosensitizer. For the investigated PtBr_2 compound this increase was almost 7-fold higher than the initial 5% of damage, which had not change over the irradiation time period, whereas for H_2PtBr_6 the damage increased 4 times

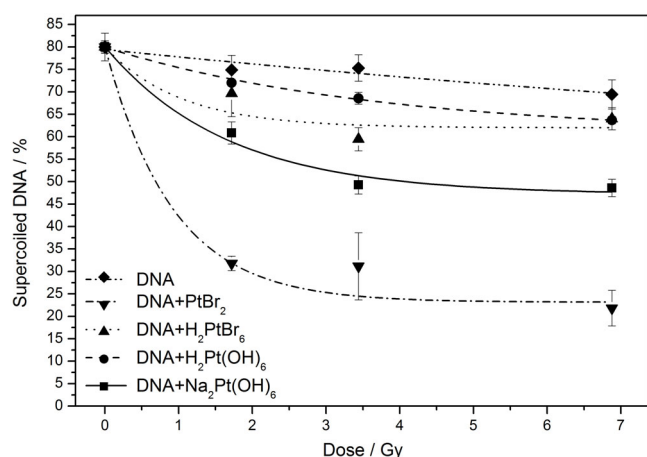


Fig. 5. Supercoiled DNA loss as a function of X-ray radiation dose for pure DNA and DNA with platinum compounds attached. Curves represent exponential decay functions fit to the experimental data.

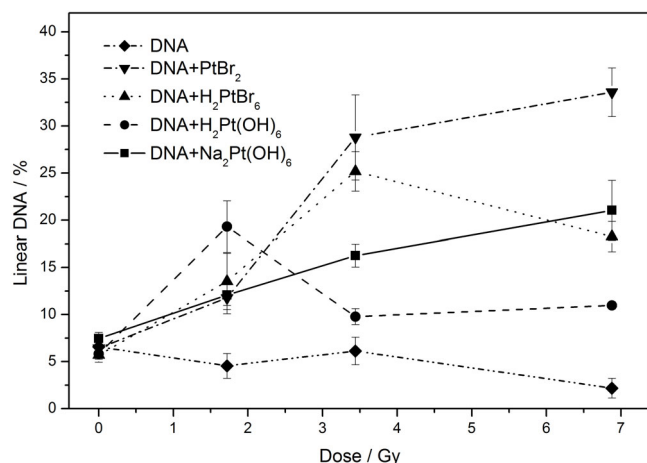


Fig. 6. Percentage amount of linear DNA form as a function of X-ray radiation dose for pure DNA and DNA with platinum compounds attached. Curves are guides to the eye.

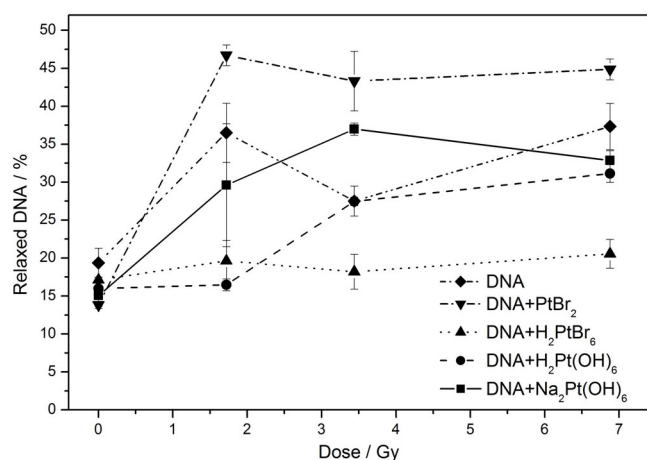


Fig. 7. Percentage amount of relaxed DNA form as a function of X-ray radiation dose for pure DNA and DNA with platinum compounds attached. Curves are guides to the eye.

at 7 Gy radiation dose with respect to unmodified irradiated plasmid DNA.

The mechanism by which DNA damage is induced by radiation in the presence of investigated compounds needs to be explored. Since the highest levels of damage were obtained for PtBr_2 , the authors focused on the mode of action of this compound. The most probable pathway, due to a positive electron affinity of bromine (3.363 eV) is dissociative electron attachment to PtBr_2 . The electron attachment studies have already been reported for electron energy range from 0 to 10 eV [13] and Br^- ion formation has been reported. The predominant channels for the dissociation of PtBr_2 molecule were found at 1.2 eV and assigned to $\text{Br}^- + \text{PtBr}$ formation and at 7.0 eV, where a $\text{Br}^- + \text{Pt} + \text{Br}$ dissociation mechanism was attributed.

In case of the anhydrous films irradiated with X-ray, although the possible damage pathway was attributed to the low energy electron impact, another mechanisms may be predominant in aqueous environment. Water surrounding would allow also for hydroxyl radical formation with assist of the investigated compounds that may act as catalysts in the radical formation process. Nonetheless, anhydrous conditions correspond very well to the environment in the close proximity of DNA in the cell, where the interactions take place at the molecular level.

Although the mechanism is not yet fully understood, and thermodynamical studies of interactions of these compounds (PtBr_2 in particular) with DNA are planned, the present data implies that there is a potential for suggested compounds to be used as radiosensitizers in radiation therapy. Based on the data presented here, it is possible that when compounds of interest are introduced into cellular environment, i.e. as surface modification of carriers, such as nanoparticles, even higher levels of damage may be obtained.

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M.A.Š. performed sample preparation and data analysis, experiments with UV and X-rays and wrote the manuscript. S.P. performed experiments with X-rays and participated in editing and revising of the manuscript. J.G. performed X-ray irradiations. S.V.H. performed experiments with UV and participated in editing and revising of the manuscript. N.J.M. participated in editing and revising of the manuscript.

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