Photoinduced DNA Damage Efficiency and Cytotoxicity of Novel Viologen Linked Pyrene Conjugates

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EXPERIMENTAL SECTION

Materials. Antibiotics were purchased from PAA (Cölbe, Germany) and all other chemicals were obtained from Sigma Chemie (Deisenhofen, Germany). Mouse lymphoma L1210 cells were obtained from W. J. Caspary, Research Triangle Park, NC, USA. The synthesis of the viologen linked pyrene derivatives **1-3** has been achieved as per the reported procedures.[1] A stock solution of 1 mM in phosphate buffer was used for the studies.

DNA damage analysis. The exposure of PM2 DNA (40 μg mL^{-1}) to near UV radiation (360 nm) in the presence and absence of the viologen linked pyrene derivatives was carried out on ice in phosphate buffer (5 mM KH_2PO_4, 50 mM Nacl, pH 7.4) by means of a black light lamp (Osram HQV; 5 min at 10 cm distance). The modified DNA was precipitated by ethanol/sodium acetate and redissolved in BE1 buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA), and the DNA damage was quanitified by means of various endonucleases and DNA relaxation assay, as reported earlier.

Cell Culture. Murine leukemia cells (L1210) cells were cultured in suspension in Rosewell Park Memorial Institute-1640 supplemented with antibiotics 10 % foetal calf serum, glutamine (2 mM), penicillin (100 U/mL) and streptomycin (50 μg/mL). Cell cultures were grown in a humidified atmosphere with 5 % CO_2 in air at 37 ^{0}C.

Cytotoxicity testing in L1210 cells. L1210 were irradiated with violgoen linked derivatives **1** and **2** in Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) (140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4, 0.1% glucose, pH 7.4) and then irradiated on ice (2 x 10^6 cells/mL) with UV lamp at a distance of 10 cm for 22.5 min (360 nm, 90 kJm^{-2}). The viologen linked derivatives were removed by two centrifugation steps and resuspended in full medium at 0.5 x 10^6 cells/mL which is then counted after 48 hours.

Quantum yields for singlet oxygen generation. Irradiation was carried out with a light source 200 W xenon lamp (model 3767) on an Oriel optical bench (model 11200) with a 345 nm band pass filter. Quantum yields for singlet oxygen generation in air-saturated methanol were calculated by plotting the depletion in absorbance of the diphenylisobenzofuran (DPBF) against the irradiation time and using standard 1-acetonaphthone [$\Phi(^1O_2) = 0.71$].[2]

REFERENCE

1. M. Hariharan, J. Joseph, D. Ramaiah, *J. Phys. Chem. B* 2006, **110**, 24678.
2. R. G. Zepp, P. F. Schlotzhauer, R. M. Sink, *Environ. Sci. Technol.* 1985, **19**, 74.

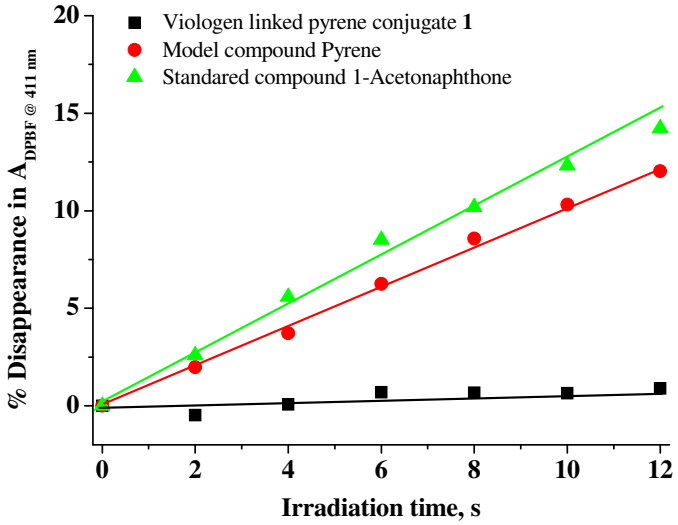

Figure S1. Percentage disappearance of 1,3-diphenylisobenzofuran (DPBF) monitored at 411 nm with increasing time of UV irradiation with 345 nm band pass filter in the presence of (■) viologen linked pyrene conjugate **1**; (●) the model compound, pyrene and (▲) standard compound, 1-acetonaphthone under similar conditions.

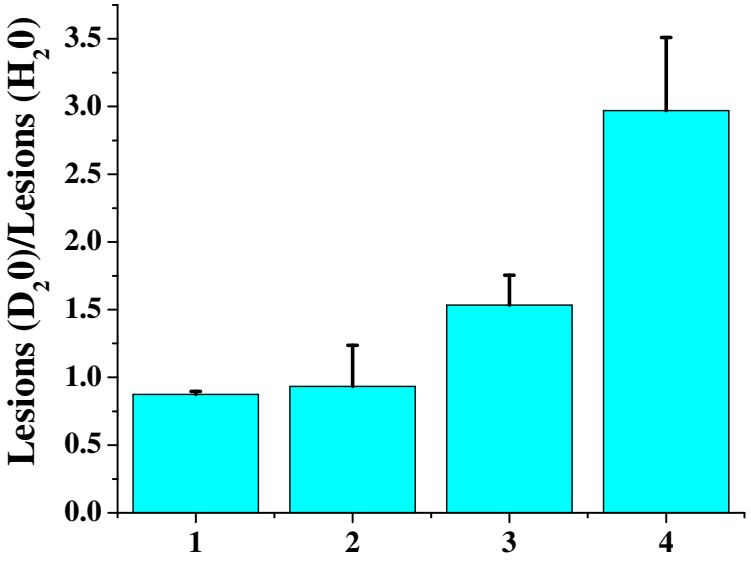

Figure S2. Effect of D_2O on Fpg-sensitive modifications induced in PM2 DNA by the conjugates **1-3** and the model compound pyrene (**4**) on UV irradiation with 360 nm light (90 kJm^{-2}) under similar conditions.

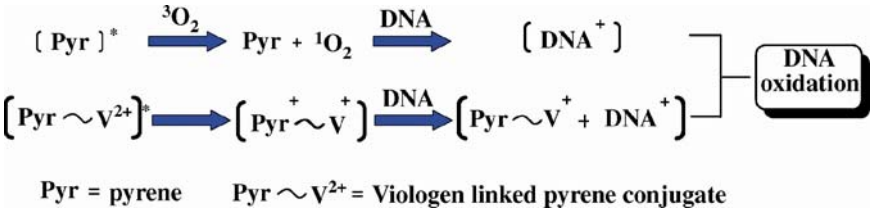

Figure S3. Schematic representation of the DNA oxidation pathways induced by the photoactivated viologen linked pyrene conjugates **1**-**3** and pyrene