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SUPPLEMENTARY INFORMATION

The water soluble peripherally tetra-substituted zinc(II), manganese(III) and copper(II) phthalocyanines as new potential anticancer agents

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1. Materials

2-(2-Morpholin-4-ylethoxy)ethanol [1] was prepared according to the literature procedures. All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. All solvents were dried and purified as described by Perrin and Armarego [2]. Acetic acid, agarose, ascorbic acid (AA), bromophenol blue, bovine serum albumin (BSA), calf thymus deoxyribonucleic acid (CT-DNA), ethylenediaminetetraacetate (EDTA), dithiothreitol (DTT), ethidium bromide (EB), glycerol, hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), magnesium chloride ($MgCl_2$), β-mercaptoethanol (ME), potassium chloride (KCl), sodium dodecyl sulfate (SDS), spermidine, trizma-base (Tris), xylene cyanol, were obtained from Sigma-Aldrich. Supercoiled pBR322 plasmid DNA and Topoisomerase I Human Assay Kit was purchased from Fermantas and Topogen.

1.2. Equipment

The IR spectra were recorded on a Perkin Elmer 1600 FT-IR Spectrophotometer, using KBr pellets. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometers in $CDCl_3$, DMSO-d_6 and chemical shifts were reported (δ) relative to Me_4Si as internal standard. Mass spectra were measured on a Micromass Quatro LC/ULTIMA LC-MS/MS spectrometer. MALDI-MS of complexes were obtained in dihydroxybenzoic acid as MALDI matrix using nitrogen laser accumulating 50 laser shots using Bruker Microflex LT MALDI-TOF mass spectrometer Bremen, Germany). Optical spectra in the UV-vis region were recorded with a Perkin Elmer Lambda 25 spectrophotometer. The elemental analyses were performed on a Costech ECS 4010 instrument. The DNA thermal denaturation

experiments were performed using Cary 100 Bio UV-Vis Spectrophotometer containing Varian Cary Temperature Controller Unit. Viscosity experiments were measured with Ostwald Viscometer at room temperature. Photocleavage experiments were performed using a General Electric quartz line lamp (300 W). 650 and 750 glasses cut off filters (Schott) were used for DNA photocleavage. To prevent ultraviolet and infrared radiations were used a water filter. Image of the DNA photocleavage and topoisomerase I inhibition experiments were photographed using BioRad Gel Doc XR system.

1.3. DNA binding experiments

1.3.1. Electronic absorption titration experiments

The investigation of **2a, 3a** and **4a** compounds with CT-DNA were studied using electronic absorption titration experiments to calculate the intrinsic binding constants (K_b) to CT-DNA using Wolfe-Shimer equation and determine the binding modes to CT-DNA. For all of the experiments, the stock solutions of **2a**, **3a** and **4a** compounds were prepared in distilled water and stored at 25 ˚C. The stock solution of CT-DNA were prepared in buffer (5 mM Tris-HCl/50 mM NaCl (pH 7.2)) followed by stirring and kept at 4 °C for four days. UV absorbance ratio of the CT-DNA solution at 260 and 280 nm (A_{260}/A_{280}) was 1.84, showing that the DNA solution was sufficiently free of protein due to in range of 1.8-1.9 ratios. The concentration of CT-DNA was measured with UV absorbance at 260 nm using the molar extinction coefficient (ε) of 6600 M^{-1} cm^{-1}. To determine the intrinsic binding constants of **2a**, **3a** and **4a** compounds with CT-DNA, the absorption titration spectra were recorded using 1 cm quartz cuvvettes from 800 nm to 350 nm at room temperature. Absorption titration spectra of **2a**, **3a** and **4a** compounds (75 μM) without CT-DNA were recorded and increasing

concentrations of CT-DNA (0-5 μM) were added to both cuvvettes and allowed to keep for 10 min at room temperature and changes in the absorption spectrum were monitored. K_b of the **2a**, **3a** and **4a** compounds were measured using the following Wolfe-Shimer equation [3]:

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad \text{(F. 1)}$$

where [DNA] is the concentration of CT-DNA. The apparent absorption coefficient ε_a = A_{obsd}/[Compound], ε_f = the extinction coefficient of the free compound and ε_b = The extinction coefficient of the compound when fully bound to DNA, respectively. In plots of [DNA]/(ε_a–ε_f) versus [DNA], K_b is given by the ratio of slope to the intercept.

1.3.2. Competetive binding experiments

Competetive binding experiments of **2a**, **3a** and **4a** compounds with EB were performed using UV-Vis spectroscopy to determine whether the intercalating effect of the compounds and their ability to displace EB from DNA-EB complex [4]. (EB-(CT-DNA)) complex was formed by adding 50 μM EB to 50 μM CT-DNA solution. Increasing the concentration of **2a**, **3a** and **4a** compounds (0-20 μM) were added to these complex and measured as the changes in the absorption spectra in the range of 435-520 nm.

1.3.3. Thermal denaturation experiments

The DNA thermal denaturation experiments were performed by monitoring the absorbance of the CT-DNA (50 μM) and **2a**, **3a** and **4a** compounds (50 μM) in a 1:1 ratio in

buffer (5 mM Tris-HCl/50 mM NaCl at pH 7.2) heating from 50 to 100 °C at rate of 1 °C per 1 min, recording the UV absorbance at 260 nm every 0.5 °C. Consequently, thermal melting temperature (*Tm*) values of CT-DNA and **2a**, **3a** and **4a** compounds were calculated automatically using thermal melting program [5].

1.3.4. Viscosity experiments

Viscosity experiments were carried out using Ostwald Viscometer at room temperature. The concentration of CT-DNA was 50 μM, **2a**, **3a** and **4a** compounds and EB were ranging from 0 to 100 μM in buffer (5 mM Tris-HCl/50 mM NaCl at pH 7.2). The flow times were measured three times with a digital timer and average flow time was calculated. The results were presented as $(\eta/\eta_o)^{1/3}$ vs [compounds]/[DNA], where η is the viscosity of DNA in the presence of the **2a**, **3a** and **4a** compounds and EB, and η_o is the viscosity of DNA alone in buffer. Viscosity values were calculated from $\eta = (t - t_0)$, where t is the observed flow time, and t_0 is the flow time of buffer [6].

1.4. DNA-photocleavage experiments

The photocleavage experiments of compounds were performed by agarose gel electrophoresis using supercoiled pBR322 plasmid DNA in the presence of irradiation at 650 nm for **2a**, **4a** compounds and 750 nm for **3a** compound. In this study, total volume of reaction mixture contained Tris-HCl buffer (pH 7.0), supercoiled plasmid pBR322 DNA (250 ng) and different concentrations (12.5 μM, 25 μM and 50 μM) of **2a**, **3a** and **4a** compounds. Then irradiated 15 min at 650 and 750 nm using a General Electric quartz line lamp (300 W)

and various filters. The mixtures were incubated at 37 ºC for 1 hour. After incubated, loading buffer (containing bromophenol blue, glycerol, SDS, xylene cyanol) was added the reaction mixture. The mixture was loaded on 0.8% agarose gel (1 mg/mL in TAE (Tris-acetate-EDTA)) with ethidium bromide staining and electrophoresis was performed at 100 V for 90 min and resulting image was photographed using BioRad Gel Doc XR system [7]. Also, DNA photocleavage experiments of compounds (25 μM) were carried out by adding oxidizing agents such as H_2O_2, 0.4 M; AA, 2.5 μM and ME, 0.4 M; using the agarose gel electrophoresis method as described above at irradiation of 650 and 750 nm [8].

1.5. Topoisomerase I inhibiton

The topoisomerase I inhibitory effects of **2a**, **3a** and **4a** compounds were carried out by using agarose gel electrophoresis. Total volume of reaction mixture contained 35 mM Tris-HCl (pH 8.0), 0.1 mg/mL BSA, 5 mM DTT, 72 mM KCl, 5 mM $MgCl_2$, 2 mM spermidine, supercoiled pBR322 plasmid DNA (250 ng), 1 Unit topoisomerase I and different concentrations of **2a**, **3a** and **4a** compounds (12.5 μM, 25 μM, 50 μM). The reaction mixture was incubated at 37 ºC for 30 min. After incubated, loading buffer (containing bromophenol blue, glycerol, sodium dodecyl sulfate, xylene cyanol) was added to the reaction mixture. These reaction mixture was loaded on 0.8% agarose gel (1 mg/mL in TAE (Tris-acetate-EDTA)) with ethidium bromide staining and electrophoresed at 45 V for 180 min and the image was photographed using BioRad Gel Doc XR system [9].

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