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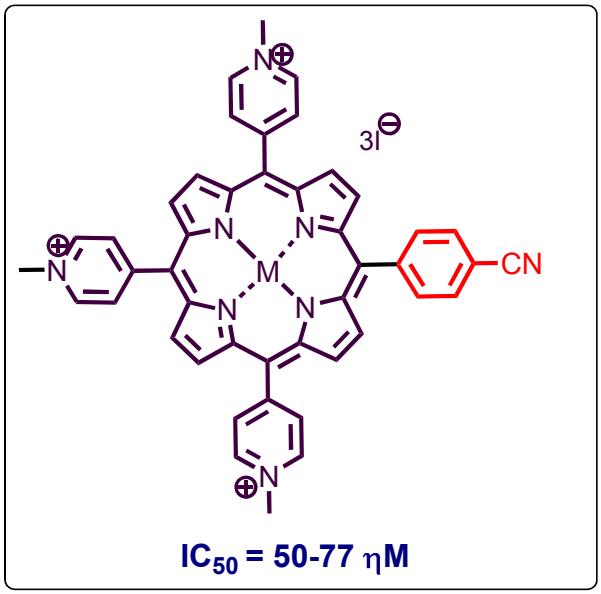
A facile I(III)-mediated synthesis of cyanoporphyrins and their significant photocytotoxicity (IC_{50} = 54 nM) against A549 cancer cell line has been described.



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COMMUNICATION

Synthesis of meso-(4′-cyanophenyl) porphyrins: efficient photocytotoxicity against A549 cancer cells and their DNA interactions

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We report a facile iodine (III)-mediated synthesis of cyanoporphyrins and their DNA photocleavage activity. Cationic-porphyrin **9a** showed intercalative binding towards DNA, whereas Zn(II)-cyanoporphyrinate **9b** showed outside electrostatic binding as indicated by their absorption and emission spectra. Porphyrin **9a** displayed significant photocytotoxicity against A549 cancer cell line with an IC_{50} value of 54 nM.

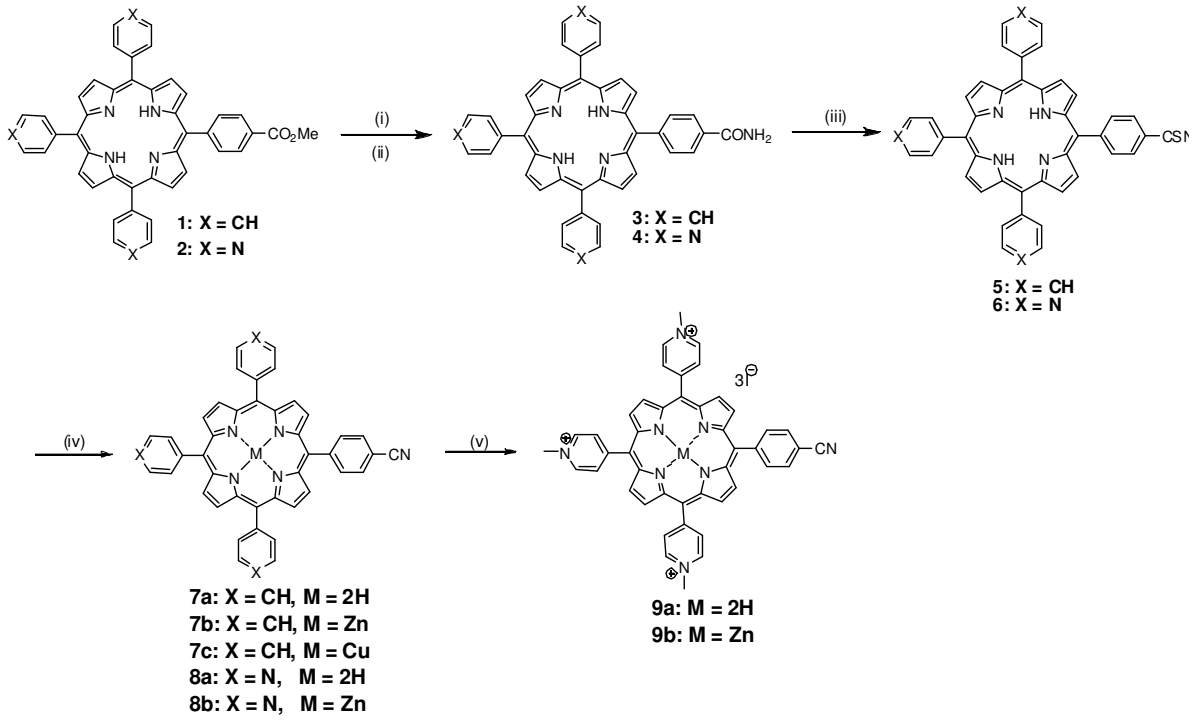
Introduction

Porphyrins have been widely used for their applications in the fields of materials chemistry,[1] biosensors,[2] fluorescence imaging,[3] medicine[4] and photodynamic therapy (PDT)[5, 6] in particular. Cationic porphyrins have been exhaustively studied for their interactions with biomolecules like deoxyribonucleic acid (DNA), RNA and proteins.[7, 8] Among the cationic porphyrins reported so far, meso-tetrakis-(*N*-methyl-4-pyridyl)porphyrin (TMPyP) and its metallated analogues are thoroughly studied for their strong binding to G-quadruplex, triplex, duplex DNA and causing single strand or double strand cleavage in DNA upon irradiation with appropriate light in presence/absence of a reducing or oxidizing agent.[9-11] Positively charged TMPyP exhibits distinct mode of binding towards DNA, such as (i) intercalation into the base pairs (ii) outside binding (iii) outside binding with self-stacking along the surface of the DNA apart from strong electrostatic attraction for the negatively charged base pairs.[12] These abilities have earned these porphyrins applications in photodynamic therapy (PDT) as anti-cancer and anti-infective chemotherapeutic agents.[13] Peripheral substituents at meso- and *β*-positions play a crucial role in determining the physico-chemical properties and hence, DNA binding modes of the porphyrin core.[14, 15] For the modulation of steric and electronic properties of porphyrins it is imperative to study the role of peripheral substituents. Substituting electron-withdrawing and donating groups on porphyrin macrocycles are expected to significantly affect their electronic and biological properties.[16, 17] Therefore, in recent years, immense efforts have been directed towards the synthesis of functionalized porphyrin derivatives.[18, 19] Hypervalent iodine reagents have been widely used as versatile and eco-friendly reagents with numerous applications in the synthesis of biologically important heterocycles. The increasing significance of hypervalent iodine reagents may be attributed to their low toxicity, ready availability, high efficiency and ease of experimentations.

Results and Discussion

Based on our earlier report to prepare 3,5-bis(indolyl)-1,2,4-thiadiazoles[20] in a single step and broaden its synthetic scope, we attempted IBD-mediated oxidative dimerization of porphyrin thioamide **5** in an effort to prepare bis(porphyrinyl)-1,2,4-thiadiazoles.[21, 22] However, oxidative dimerization of porphyrin thioamide **5** led to the exclusive formation of 5-(4′-cyanophenyl)-10,15,20-triarylporphyrin **7**

To our knowledge there is no report for the direct conversion of porphyrin thioamide to cyanoporphyrins, although the reports for oxidative dimerization of arylthioamides to 3,5-diarylthiadiazoles using *o*-iodoxybenzoic acid discloses the formation of nitriles as side-products.[23, 24] In this manuscript, we report a facile protocol for the synthesis of 5-(4′-cyanophenyl)-10,15,20-triarylporphyrins **7** and **8** from readily available porphyrin thioamides **5** and **6** using iodobenzene diacetate (IBD). The porphyrin ester[25] **1** was hydrolyzed to give (4-carboxylphenyl)porphyrin, which upon treatment with thionyl chloride followed by purging of ammonia afforded the desired (carboxamidophenyl)porphyrin **3** (Scheme 1).[22] Further reaction of (carboxamidophenyl)porphyrin **3** with Lawesson's reagent (LR) in toluene at 60 °C resulted in the formation of porphyrin thioamide **5**.[21] The IR spectrum of **5** exhibited a characteristic peak at 1618 cm^{-1} for C=S stretching whereas, the C=O stretching for carboxamide **3** appeared at 1666 cm^{-1}. In similar steps, porphyrin thioamide **6**, was prepared from porphyrin ester **2** as outlined in scheme 1. The reaction of porphyrin thioamide **5** with equimolar quantity of IBD was initially attempted in acetonitrile, but the reaction was slow and did not complete owing to poor solubility of the starting material. However, the reaction proceeded smoothly in dimethylformamide (DMF) and dichloromethane (DCM) but we preferred the latter due to ease in isolation of cyanoporphyrins **7-8**. For the formation of cyanoporphyrins **7-8**, precursor porphyrin thioamide **6** is essential as IBD-mediated oxidations of arylamides have been described to generate corresponding arylamines.[26] The resulting cyanoporphyrins **7** could be readily purified by washing with methanol, and **8** by a mixture of DCM-hexane.



Scheme 1 Synthesis of 5-(4′-cyanophenyl)porphyrins (**7-9**)[a]

[a]Reagents and conditions: (i) KOH, MeOH:H_2O, 90 °C, 4 h; (ii) $SOCl_2$, Toluene, 110 °C, 1 h, then $NH_{3(g)}$, CH_2Cl_2, 0-25 °C, 45 min; (iii) 2,4-Bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-dithione (Lawesson's reagent), Toluene/THF (**6**), 60 °C, 1 h; (iv) $PhI(OAc)_2$, CH_2Cl_2, 25 °C, 45 min, then $Zn(OAc)_2$ or $Cu(OAc)_2$, $CHCl_3$:MeOH, 65 °C, 1-2 h; (v) **8a**-**b**, CH_3I, $CHCl_3$, 65 °C, 72 h.

The pyridyl groups in **8a-b** were quaternized with methyl iodide in chloroform to obtain cationic porphyrins **9a-b**. The porphyrins **7a-c** and **9a-b** were characterized by IR, ESI-MS and ^{1}H NMR spectral data. The FT-IR spectra showed C≡N stretching band at about 2227-2229 cm^{-1} for all the synthesized porphyrins **7-9**. In the ESI-MS spectra, $[M+H]^+$ ion peak was observed for porphyrin **7a-c** and **9a-b**. The β-pyrrolic and aromatic protons were also in agreement with the proposed structures as observed in ^{1}H NMR. (for spectral data see ESI). The ^{13}C NMR of cyanoporphyrin **8a** showed a characteristic signal at δ 114.9 ppm due to nitrile carbon, whereas; precursor porphyrin thioamide **6** exhibited a characteristic signal at δ 168.3 ppm for thioamide carbon. For further confirmation, we synthesized (4-cyano-phenyl)-tripyridylporphyrin **8a** from the reaction of pyrrole with appropriate 4-cyanoarylaldehydes[27-29] and the product obtained was found to be identical in all respects to porphyrin **8a**. A probable pathway for the formation of cyanoporphyrins is shown in the figure 1. Nucleophilic attack of the sulphur atom of thioamide **5/6** on the electron-deficient iodine of IBD may form adduct [**A**] which rearranges to cyanoporphyrin **7/8** by the loss of sulphur, iodobenzene and acetic acid. Formation of expected bis(porphyrinyl)-1,2,4-thiadiazole would require the nucleophilic attack of another molecule of porphyrin thioamide on adduct [**A**], which would be sterically demanding due to the bulkier size of

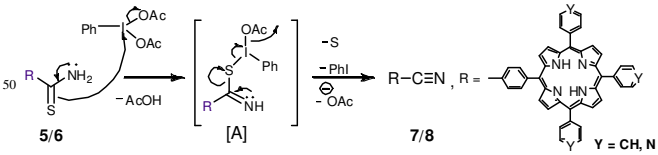


Fig. 1 A plausible mechanism for the formation of porphyrin **7/8**.

porphyrin thioamide. The interactions of porphyrins with Calf thymus DNA (ctDNA) were studied using UV-vis and fluorescence spectroscopy. The spectral measurements were performed at 25 °C in Milli-Q water. Stock solutions for porphyrins **7-9** were prepared in dimethylformamide and ctDNA stock solution was prepared in buffer (5 mM Tris-HCl, pH 7.4). The absorption spectra of cyanoporphyrins **7** (5 μM) and **9** (2 μM) were recorded with increasing amounts of ctDNA (0-500 μM). The photophysical parameters did not exhibit any typical changes in absence of DNA for cyanoporphyrins **7a-c** and **9a-b** as compared to their metallated or free base counterparts, also they did not show aggregation in the Tris-HCl buffer at given concentrations (Fig. 2a, 3a and 4a). Absorption spectra of free base cyanoporphyrin **7a** showed a bathochromic shift of 14 nm for the Soret band at λ_{max} = 409 nm and hypochromicity of 16% (hypochromicity, $H\% = [(A_0 - A_s)/ A_0] \times 100$, where A_0 and A_s are absorbances at λ_{max} of soret bands for free and bound porphyrins, respectively) with increasing ctDNA (0-500 μM)

concomitant with a moderate increase in its fluorescent intensity (Fig. 2a-b), indicative of outside binding or stacking along the ctDNA helix.[20] Compounds **7b** (λ_{max} = 408 nm) and **7c** (λ_{max} = 409 nm) did not show significant changes in their UV-visible as well as emission spectra (see ESI).

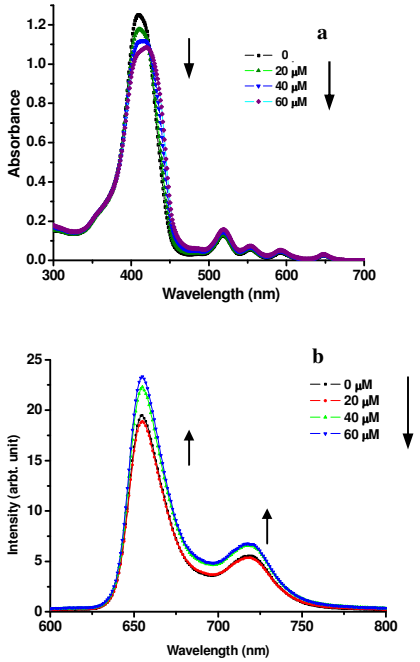


Fig. 2 (a) Absorption spectra of **7a** (5 μM). (b) Emission spectra of **7a** (5 μM). Arrows show the absorbance changes upon increasing the ctDNA concentration (pH 7.4).

In the case of free base cationic porphyrin **9a** the Soret band (λ_{max} 421 nm) showed a red shift of 12 nm and hypochromicity of 36% and further at higher DNA/porphyrin ratios changed to a sharp peak at 433 nm (Fig. 3a).[30, 31,32, 33] The fluorescence intensity increased gradually at lower DNA concentrations (0-8 μM) probably due to decrease in self-association of porphyrin molecules. At higher DNA concentrations (10-500 μM), the porphyrin self-assembly was disrupted and then monomeric forms got intercalated into DNA, thereby showing enhanced (14 times) fluorescent intensity (Fig. 3b). On the other hand, the absorption spectra of Zn(II)-cyanoporphyrinate **9b** displayed lesser red shift (5 nm) and hypochromicity in the Soret band (λ_{max} 434 nm), indicating binding through non-intercalating electrostatic interactions with the negatively charged DNA double helix.[12,34, 35] The possible presence of axial ligands blocked intercalative binding of **9b**. This trend has been reported for Mn^{+3}, Fe^{+3}, Zn^{+2} and Co^{+2} complexes of porphyrins.[12] The emission spectra of **9b** (Fig. 4b) in a DNA-free environment exhibited an emission peak at 631 nm which upon increasing DNA concentration showed a blue shift with a moderate increase in intensity resembling outside binding with self-stacking along the DNA surface.[36, 37] The apparent binding constants (K_{app}) were calculated based upon the changes in the UV-vis. absorption spectra of **7a**, **9a** and **9b** upon addition of ctDNA in Tris-HCl buffer (pH 7.4). The corresponding binding constants were determined using the following equation.

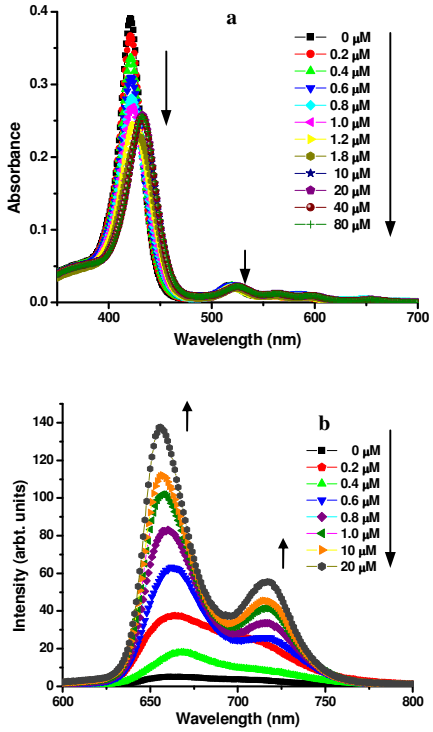


Fig. 3 (a) UV-vis spectra of **9a** (2 μM). (b) Emission spectra of **9a** (2 μM). Arrows show the absorbance changes upon increasing ctDNA concentration (pH 7.4).

$$\frac{[DNA]}{(\varepsilon_A - \varepsilon_F)} = \frac{[DNA]}{(\varepsilon_B - \varepsilon_F)} + \frac{1}{K_b(\varepsilon_B - \varepsilon_F)}$$

The molar absorption coefficients for the given solution, free porphyrin and for the porphyrin complex in fully bound form are ε_A, ε_F and ε_B, respectively. A plot of [DNA] / (ε_A- ε_F) *vs* [DNA] will have a slope of 1 / (ε_B - ε_F) and a y-axis intercept equal to 1 / K_b (ε_B - ε_F), K_b is binding constant. The binding constants of porphyrins **9a**: 8.2 × 10^5 M^{-1} is comparable to H_2TMPyP (7.7 × 10^5 M^{-1}) and for zinc metallated **9b**: 2.1 × 10^5 M^{-1} is less as compared to free base, presumably because zinc metallated complexes can accommodate axial ligands which sterically hinders association with DNA. Further, we studied the DNA cleavage activity of porphyrins **7a-b** and **9a-b** using agarose gel electrophoretic mobility assay. The photocleavage experiments were performed with high pressure Xe-arc through a band-path filter (□ =300-390 nm, 4 mW, UV-A) or a white LED light source (□ = 400-800 nm, 2 mW, visible). Typically, a solution of *Φ*X174 DNA (0.5 μg) and an appropriate porphyrin in 20 mM Tris-HCl buffer (pH 7.2) containing 20 mM NaCl and 2.5 vol% DMSO (total volume 20 μL) was exposed to UV-A light at ambient temperature. The resultant mixtures were then analyzed by gel electrophoresis (1% agarose gel) with ethidium bromide staining. DNA cleavage was determined by the formation of relaxed circular DNA (Form II). The DNA photocleavage studies of porphyrins **7a-c** (1-20 μM) showed no visible photocleavage of circular DNA. However, cationic porphyrin **9a** efficiently converted more than 95% of plasmid DNA from form I to form II

upon 30 min of UV exposure (310-390 nm), on the other hand, **9b** up to ~85% (Fig. 5a). The DNA cleavage efficiency of porphyrins **9** (**9a** and **9b**, 82% and 74% conversion, respectively) was almost comparable under visible light (>400 nm) and UV light (Fig. 5b).

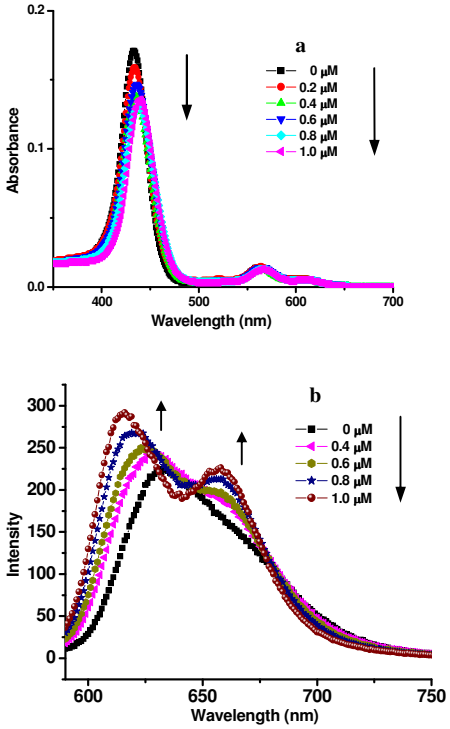


Fig. 4 (a) UV-vis spectra of **9b** (2 μM). (b) Fluorescence spectra of **9b** (2 μM). Arrows show the absorbance changes upon increasing ctDNA concentration (pH 7.4).

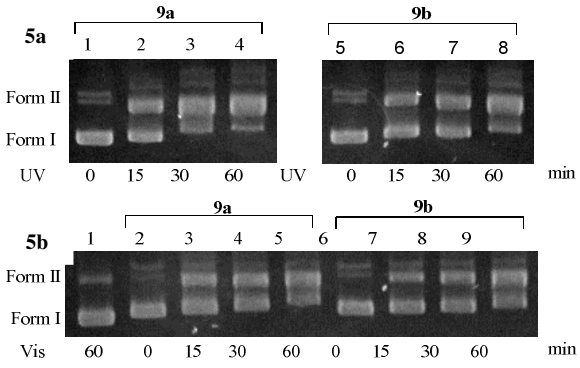


Fig. 5 Photoinduced DNA cleavage by **9a** and **9b**. ΦX174 supercoiled DNA (1.0 μg) was incubated with the porphyrins (1 μM) in 20 ml of Tris-HCl (20 mM, pH 7.2) containing NaCl (20 mM), DMSO (2.5 vol%) at ambient temperature in the dark for 30 min, and then (a) UV-irradiated (310~390 nm) for the indicated periods. Lane 1: DNA alone. (b) Exposed to visible light (>400 nm) for the indicated periods. Lane 1: DNA alone

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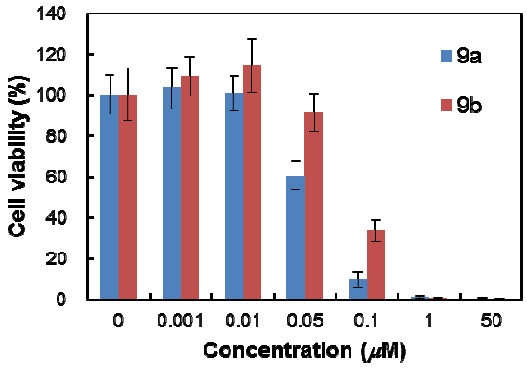

Fig. 6 Cell viability data of **9a** and **9b** in visible light

The DNA photocleavage studies of H_2TMPyP (1 μM) under same conditions gave 38% conversion of form I to form II (see ESI). Very recently, Spingler et al have also disclosed the cytotoxicity of tricationic cyanoporphyrins with nitrate counter anion towards A2780 and MCF-7 cancer cells (IC_{50} ~ 0.4 μM). In this study, they have demonstrated that the excitation of tricationic cyanoporphyrins with red light leads to the generation of singlet oxygen, although we have not identified the reactive intermediates which induce DNA cleavage.[38] WST method was used to evaluate the photocytotoxic potential of the cyanoporphyrins **9a-b** upon photoexcitation on A549 lung cancer cells. The cytotoxicities were expressed as concentration of the compounds to kill 50% of the cells (IC_{50} μM). The cyanoporphyrins **9a-b** upon incubation for 24 h at 37 °C, washed with PBS and subsequent LED irradiation led to significant decrease in the cell viability giving IC_{50} values of 54 nM for **9a** and 77 nM for **9b**, and > 50 μM for both the porphyrins in dark. Both the porphyrins were non-toxic in dark but exhibited significant cytotoxicity upon photoactivation. Further, the cyanoporphyrins **9a-b** showed better efficacy (6 folds) than that of standard cationic photosensitizer H_2TMPyP (IC_{50} 0.3-1.24 μM).

Conclusions

In summary, we have developed a facile protocol for conversion of readily available porphyrin thioamides to cyanoporphyrins by utilizing a non-metallic and stable reagent, iodobenezene diacetate. Based on absorption studies it was found that more lipophilic cyanoporhyrin **7a** bind towards ctDNA with moderate binding constant and has no photocleavage activity. However, both cationic cyanoporphyrins **9a-b** bounds tightly to DNA and they exhibited significant photocytoxicity at 1 μM concentration. At this stage of our research, it is still not clear whether the cyanoporphyrins target nuclear DNA in the living cells, but, the cationic cyanoporphyrins **9a-b** showed efficient in vitro photocytotoxicity (54-77 nM) on A549 cells upon photo-activation. These preliminary results are quite promising and further structure-activity relationship studies of cyanoporphyrins with varying metals and peripheral substituents are likely to presage developing of novel cationic porphyrins as PDT agents.

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