### **Supporting information**

## **'AIE + ESIPT' Assisted Photorelease: Fluorescent Organic Nanoparticles for Dual Anticancer Drug Delivery with Real-Time Monitoring Ability**

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#### 1. General experimental information:

<sup>1</sup>H NMR (400 MHz) spectra was recorded on a BRUKER-AC 400 MHz spectrometer. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm). Data is reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). <sup>13</sup>C NMR (100 MHz) spectra were recorded on a BRUKER-AC 400 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance the internal standard (deuterochloroform: 77.0 ppm, deuterated dimethyl sulfoxide: 39.5 ppm). UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, fluorescence emission spectra were recorded on a JEOL-AccuTOF JMS-T100L mass spectrometer. Transmission Electron Microscopy (TEM) was measured on a FEI Tecnai G220S-Twin at 200 kV. The TEM sample was prepared by dispersing compounds in

water and dropping on the surface of a copper grid. DLS measurements were done using a Brookhaven 90 Plus particle size analyzer and Imaging was done using a fluorescence microscope (IX 51, Olympus) high-performance CCD camera with the appropriate filter using Image-Pro discovery 5.1 software. Photolysis of all the ester conjugates were carried out using 125 W medium pressure mercury lamp supplied by SAIC (India). Chromatographic purification was done with 60-120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) was used. Chromatographic purification was done with 60-120 mesh silica gel (Merck). RP-HPLC was taken using acetonitrile/water as mobile phase (detection: UV 300 nm).



2. Synthesis of compound 5:

Scheme S1: Synthesis procedure of the DDS (compound 5).

Synthesis of compound **3** was carried out according the procedure described in our earlier work.<sup>1</sup> **Step iii**: Treatment of compound **2** (71 mg, 0.29 mmol) with ferulic acid (56.3 mg, 0.29 mmol) in presence of potassium carbonate ( $K_2CO_3$ ) (48 mg, 0.34 mmol) in dry N,N– dimethylformamide (DMF) at room temperature for a period of 6 h afforded 4. The reaction mixture was extracted in ethyl acetate (EtOAc) (3 × 100 ml) and washed with brine. Then, the organic portion was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated in rotatory evaporator. The crude reaction mixture was purified by silica column chromatography using 20 % EtOAc in petroleum ether to give the caged conjugate 4 in 96 % yield as a white colored solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz):  $\delta = 10.32$  (s, 1H), 9.65 (s, 1H), 8.27 (s, 1H), 8.11-8.08 (d, J = 12 Hz, 1H), 7.64-7.60 (d, J = 16 Hz, 1H), 7.37 (s, 1H), 7.17-7.15(d, J = 8 Hz, 1H), 7.11-7.09 (d, J = 8Hz, 1H), 6.82-6.80 (d, J = 8 Hz, 1H), 6.63-6.59 (d, J = 16 Hz, 1H), 5.53 (s, 2H),3.83 (s, 3H). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 100 MHz):  $\delta = 190.8$ , 166.5, 165.6, 150.0, 148.42, 146.3 (2C), 135.8, 129.9, 125.9 (2C), 123.8, 122.6, 118.44, 116.0, 114.2, 111.8, 66.4, 56.1. FTIR (KBr, cm<sup>-1</sup>): 3430 (broad), 1700, 1661, 1590, 1512, 1430, 1364, 1161. Calculated mass [MH<sup>+</sup>]: 357.0974 and found mass [M+Na<sup>+</sup>]: 379.0793.

**Step iv**: Compound **5** was prepared by treating compound **3** (100 mg, 0.21 mmol) and **4** (75 mg, 0.21 mmol) with hydrazine (11 mg, 0.21 mmol) in methanol at room temperature for overnight. The precipitate was filtered and washed with methanol. The crude reaction mixture was purified by silica column chromatography using 30 % EtOAc in petroleum ether to afford compound **5** in 58 % yield as a light yellow solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 11.89$  (s, 2H), 8.83 (s, 1H), 8.82 (s, 1H), 8.10 (s, 1H), 8.6-8.01 (d, J = 20 Hz, 2H), 7.79 (s, 1H), 7.7-7.75 (D, J = 12Hz, 1H), 7.17-7.09 (m, 6H), 6.96-6.95 (d, J = 4Hz, 1H), 6.67-6.65 (d, J = 8Hz, 2H), 6.49-6.46 (d, J = 12 Hz, 1H), 5.45 (s, 2H), 5.33 (s, 3H), 3.96 (s, 3H), 3.74-3.64 (m, 8H), 2.67-2.64 (t, J = 6 Hz, 2H), 2.54-2.52 (t, J = 6 Hz, 2H), 2.04-2.01 (m, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):  $\delta = 190.24$ , 189.97, 173.04, 166.55, 164.77, 164.72, 164.70, 164.25, 148.29, 146.82, 146.41, 144.40, 133.49, 133.41,

133.28, 133.19, 130.61, 129.78 (3C), 126.85, 126.77, 123.48, 117.94, 117.10, 114.77, 114.22, 112.29 (2C), 109.46, 65.64, 65.49, 55.98, 53.66, 40.55, 33.86, 33.17, 26.73. FTIR (KBr, cm<sup>-1</sup>): 3410 (broad), 1696, 1625, 1512, 1153. Calculated mass [MH<sup>+</sup>]: 818.2247 and found mass [MH<sup>+</sup>]: 818.2237.

#### 3. Photophysical properties of 5:

The absorption and emission spectra of a degassed solution of 5 ( $2 \times 10^{-5}$  M) in different solvents were recorded on a UV-Vis spectrophotometer and fluorescence spectrophotometer respectively.

# 4. Determination of incident photon flux $(I_0)$ of the UV lamp by potassium ferrioxalate actinometry:

Potassium ferrioxalate actinometry<sup>2, 3</sup>was used for the determination of incident photon flux ( $I_0$ ) of the UV lamp used for irradiation. Solution of potassium ferrioxalate, 1,10–phenanthroline and the buffer solution were prepared following the literature procedure.<sup>2</sup>

0.006 M solution of potassium ferrioxalate was irradiated using 125 W medium pressure Hg lamp as visible light source ( $\geq$  410 nm) and 1M NaNO<sub>2</sub> solution as UV cut–off filter. At regular interval of time (3 min), 1 ml of the aliquots was taken out and to it 3 ml of 1,10–phenanthroline solution and 2 ml of the buffer solution were added and the whole solution was kept in dark for 30 minutes. The absorbance of red phenanthroline–ferrous complex formed was then measured spectrophotometrically at 510 nm. The amount of Fe<sup>2+</sup> ion was determined from the calibration graph. The calibration graph was plotted by measuring the absorbance of phenanthroline–ferrous complex at several known concentration of Fe<sup>2+</sup> ion in dark. From the slope of the graph the molar absorptivity of the phenanthroline–ferrous complex was calculated to be 1.10 × 10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup> at 510 nm which is found to be similar to reported value.<sup>2</sup> Using the known quantum yield for potassium ferrioxalate actinometer at 406.7 nm<sup>3</sup> the number of Fe<sup>2+</sup> ion formed during photolysis and the fraction of light absorbed by the actinometer, the incident intensity (I<sub>0</sub>) of the 125 W Hg lamp was determined as 2.886×10<sup>16</sup> quanta S<sup>-1</sup>.

#### 5. Time resolved studies:

Fluorescence lifetimes were obtained from a time-correlated single photon counting (TCSPC) spectrometer using nano-LED (IBH, U.K.) as the light source at 408 nm. The experimental setup for picosecond TCSPC has been described elsewhere.<sup>[2]</sup> Briefly, the samples were excited at 408 nm using a picosecond laser diode (IBH, Nanoled), and the signals were collected at the magic angle (54.7°) using a Hamamatsu microchannel plate photomultiplier tube (3809U). The instrument response function of our setup was 90 ps. The average fluorescence lifetimes for the decay curves were calculated from the decay times and the relative contributions of the components using the following equation (1).

 $\langle \tau \rangle = \mathbf{a}_1 \tau_1 + \mathbf{a}_2 \tau_2 - \dots (1)$ 

where  $\tau_1$  and  $\tau_2$  are the first and second components of the decay time of **5** and  $a_1$  and  $a_2$  are the corresponding relative amplitudes of these components.

A 20 mL solution of 5 ( $1 \times 10^{-4}$  M) was prepared in acetonitrile HEPES buffer (1:19). Nitrogen was passed through the solution for 30 min. The time-resolved fluorescence decays were recorded at emission wavelength of 518 nm with an excitation wavelength of 408 nm. The representative time resolved fluorescence decay profiles of 5 in the above mentioned cases at 518 nm are depicted in **Figure S12**.

#### 6. Quenching studies in presence of triplet state quencher (potassium sorbate):

An experiment was performed to ensure whether the photorelease occurs from singlet or triplet excited state, in which photolysis of **5** ( $1 \times 10^{-4}$  M) was carried out in presence of different concentration (0  $\mu$ M, 30  $\mu$ M and 60  $\mu$ M) of potassium sorbate (PoS). At different time of irradiation (0, 1, 2, 3, 4 and 5 min) aliquots were collected and analyzed by RP-HPLC using acetronitrile and water as mobile phase (detection: UV 250 nm). Peak areas were determined by RP-HPLC. HPLC peaks area of caged compound (from HPLC peak area) versus irradiation time were plotted. The result showed that at a concentration of 60  $\mu$ M of PoS, the drug release by **5** was completely arrested, clearly indicating that photorelease occurs from the triplet excited state (**Figure S13**).

#### 7. Photochemical rate constant determination for compound 5:

A 10 mL solution of 5 ( $1 \times 10^{-3}$  M) was prepared acetonitrile-HEPES buffer. Nitrogen was passed throughout the solution for 30 min and irradiated using 125 W medium pressure Hg lamp as light source ( $\geq 410$  nm) and 1M NaNO<sub>2</sub> solution as UV cut-off filter. At regular interval of time, 20µL of the aliquots was taken and dissolved the particles by adding 20µL of acetonitrile. Then it was analyzed by RP-HPLC using mobile phase acetonitrile (detection: UV 254 nm). Peak areas were determined by RP-HPLC, which indicated gradual decrease of the caged compound with time, and the average of three runs. The reaction was followed until the consumption of the ester is less than 5% of the initial area. Based on HPLC data for caged compound, the natural logarithm of the concentration of caged compound (lnC) (from HPLC peak area) versus irradiation time was plotted. We observed an exponential correlation for the disappearance of the caged compounds which suggested a first order reaction. Further, the quantum yield for the photolysis of caged compound was calculated using equation (2)

$$(\Phi)_{\rm CG} = (\Phi)_{\rm act} \times [(k_p)_{\rm CG}/(k_p)_{\rm act}] \times [F_{\rm act}/F_{\rm CG}] \dots (2)$$

Where, the subscript 'CG' and 'act' denote caged compound and actinometer, respectively.  $\Phi$  is the relative photolysis quantum yield,  $k_p$  is the photolysis rate constant and F is the fraction of light absorbed. Potassium ferrioxalate was used as an actinometer.

pН	Rate constant
5	$9.35 \times 10^{6}  {\rm s}^{-1}$
7	$2.10 \times 10^8  { m s}^{-1}$
9	$2.23 \times 10^8  { m s}^{-1}$

Table S1: Photorelease rate constants of 5 in different pH

#### 8. Preparation of NDDS-5 nanoparticles:

Photoresponsive NDDS-5 nanoparticles were prepared by reprecipitation technique. To 25 mL water, 5  $\mu$ L THF solution of compound 5 (3 mM) was added slowly for 30 min with constant sonication. The size of NDDS-5 nanoparticles determined using TEM and DLS analysis.



Scheme S2: Synthesis of NDDS-5 nanoparticles.

#### 9. Antiproliferative activity assays

#### 9.1. Cell lines:

HeLa cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India, and maintained in dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Cells were cultured at 37 °C in a CO<sub>2</sub> incubator (Thermo Fisher Scientific, USA).

#### 9.2. Real time cellular uptake and localization study:

To study the cellular uptake and localization of NDDS-5 nanoparticles, HeLa cells were first seeded at a density of  $1 \times 10^5$  cells/well of a 6-well plate and then incubated with 10 µg mL<sup>-1</sup> of NDDS-5 nanoparticles for 4 h at 37 °C in a CO<sub>2</sub> incubator. After incubation, the cells were irradiated by UV-visible light ( $\geq$  410 nm) using UV-visible lamp (Bangalore GeneiPvt. Ltd.) for 0–5 min. Thereafter cells were fixed using 4% paraformaldehyde for 10 min and washed twice with phosphate-buffer saline (PBS). Imaging was done using confocal microscopy (CLSM; Nikon Eclips Ti-E inverted microscope).

In Vitro Cytotoxicity assay:

#### 9.3. Before Irradiation:

The cytotoxicity of NDDS-5, **6** and chlorambucil (Cbl) were determined with or without UV irradiation on HeLa cells using MTT assay. cells  $(1 \times 10^5 \text{ cells/well of a 96-well plate})$  were treated for 72 h with different concentrations  $(0.1-10 \ \mu \text{g mL}^{-1})$  and cell viability was determined by MTT assay (Mossman, 1983), measuring absorbance at 595 nm using a micro plate reader (Bio-Rad 550). Cell viability was calculated using the formula, Viability (%) =  $100 \times \text{A2/A1}$ ; [Where A2 = Absorbance of the treated cell; A1 = Absorbance of the control cells]).

#### 9.4. After Irradiation:

Cancer cell line  $(1 \times 10^5 \text{ cells/well of a 96-well plate})$  was treated with different concentrations of NDDS-5 and Cbl (0.1–10 µg mL<sup>-1</sup>) and incubated for 4 h at 37 °C in CO<sub>2</sub> incubator. Thereafter, the cells were irradiated by UV-visible light,  $\lambda \ge 410$  nm for 15 min (keeping the culture plate 6 cm away from the light source) using UV-Visible lamp (Lelesil innovative systems) under aseptic condition. After irradiation, the cells were again incubated for 48 h and cell viability was measured by MTT assay (Mossman, 1983). Cell viability was calculated as described above.

#### 9.5. Real-time in-vitro drug release and cytotoxicity

Cytotoxicity of time-dependent control-released drug with irradiation on HeLa cell line was determined by conventional MTT assay (Mossman, 1983). Briefly, cells in exponential growth phase were trypsinised and seeded in 96-well culture plates ( $1 \times 10^5$  cells/well). After 12 h of cell seeding, the medium was replaced with a fresh complete medium (DMEM) containing 10 µg mL<sup>-1</sup> of NDDS-5 and was kept for 4 h at 37 °C in a CO<sub>2</sub> incubator. Drug-treated cells were irradiated with UV-visible light ( $\geq$  410 nm) for 0–5 min. The assay was performed in triplicate

for each time frame (0, 1, 3, and 5 min). Then the plate was further incubated for 72 h at 37 °C in a  $CO_2$  incubator. Real-time in-vitro drug release was measured spectrophotometrically in accordance with measuring cell viability by MTT assay. All Experiments were repeated in triplicate and the values shown are the mean with s.d.

#### 9.6. Confocal laser scanning microscopy (CLSM)

Real-time drug release from NDDS-5 after UV-visible light ( $\geq$  410 nm) irradiation was also studied by CLSM imaging. HeLa cells (1 × 10<sup>5</sup> cells/6-well) were treated with 10 µg mL<sup>-1</sup> of NDDS-5 and put aside for 4 h at 37 °C. Then, cells were irradiated with UV-visible light ( $\geq$  410 nm) for 0–15 min. Thereafter, the plate was incubated for 48 h at 37 °C in a CO<sub>2</sub> incubator. The effect of released drug on the cell was visually observed by CLSM (Nikon Eclipse Ti-E inverted microscope).



Figure S1: <sup>1</sup>H NMR of compound 4.



**Figure S2:** <sup>13</sup>C NMR of compound **4**.



Figure S3: IR spectra of compound 4.



Figure S4: HRMS of compound 4.



Figure S5: <sup>1</sup>H NMR of compound 5.



Figure S6: <sup>13</sup>C NMR of compound 5.



Figure S7: IR spectra of compound 5.



Figure S8: HRMS of compound 5.



Figure S9: Normalized absorption and emission spectra of 5 ( $1 \times 10^{-5}$  M in THF-water binary mixture with 99% water).



Figure S10: ESI-MS spectra recorded during the photolysis (at partial conversions) of 5.



**Figure S11:** <sup>1</sup>H NMR of compound **6**.



Figure S12: <sup>13</sup>C NMR of compound 6.



**Figure S13:** (a) Release of chlorambucil under bright and dark conditions. "On" and "Off" implies the switching on and off of the visible light source, respectively. (b) The amount of chlorambucil released from compound **5** on photolysis ( $\geq$  410 nm) at different time intervals.



Figure S14: HPLC chromatogram recorded during the photolysis ( $\geq$  410 nm) of 5 in pure acetonitrile.



Figure S15: Time resolved florescence decay curve for 5.



**Figure S16**: Time course of photolysis of **5** under irradiation of visible light ( $\geq$  410 nm) in presence of different concentration triplet quencher (potassium sorbate (PoS)).



Figure S17: Amount of 5 photodecomposed at different interval of visible light ( $\geq$  410 nm) irradiation in different pH.



Figure S18: Percentage of chlorambucil released by 5 as a function of fluorescence intensity change.



Figure S19: TEM (a,b,c) images and DLS (d) data of NDDS-5 nanoparticles.



**Figure S20**: confocal images of cellular internalization of NDDS-5; (a) fluorescence field 575 nm channel, (b) fluorescence field 460 nm channel and (c) merged (575 nm + 460 nm).



**Figure S21**: Subcellular localization of NDDS-5 in HeLa cells. 1a, 1b & 1c represent the nuclear staining experiment. 2a, 2b, 2c & 2d represent the lyso tracker experiment (Yellow emission of monomer and dimer were changed to green for better visualization). 3a, 3b, 3c & 3d represent the mito tracker experiment.



**Figure S22:** Confocal images captured at different interval of time during photoirradiation ( $\geq$  410 nm) of NDDS-5 treated HeLa cells. i, ii, iii and iv indicate 0 min, 1 min, 3 min and 5 min of visible light irradiation. a) fluorescence field 575 nm channel, (b) fluorescence field 460 nm channel and (c) merged (575 nm + 460 nm).



**Figure S23:** Cell cytotoxicity analysis for HeLa cells: treated with NDDS-5, **6** and chlorambucil (a) before photolysis, (b) after photolysis and (c) NDDS-5 treated cells at different time intervals of light irradiation. Experiments were repeated in triplicate and the values shown are the mean with s.d.

#### **References**:

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