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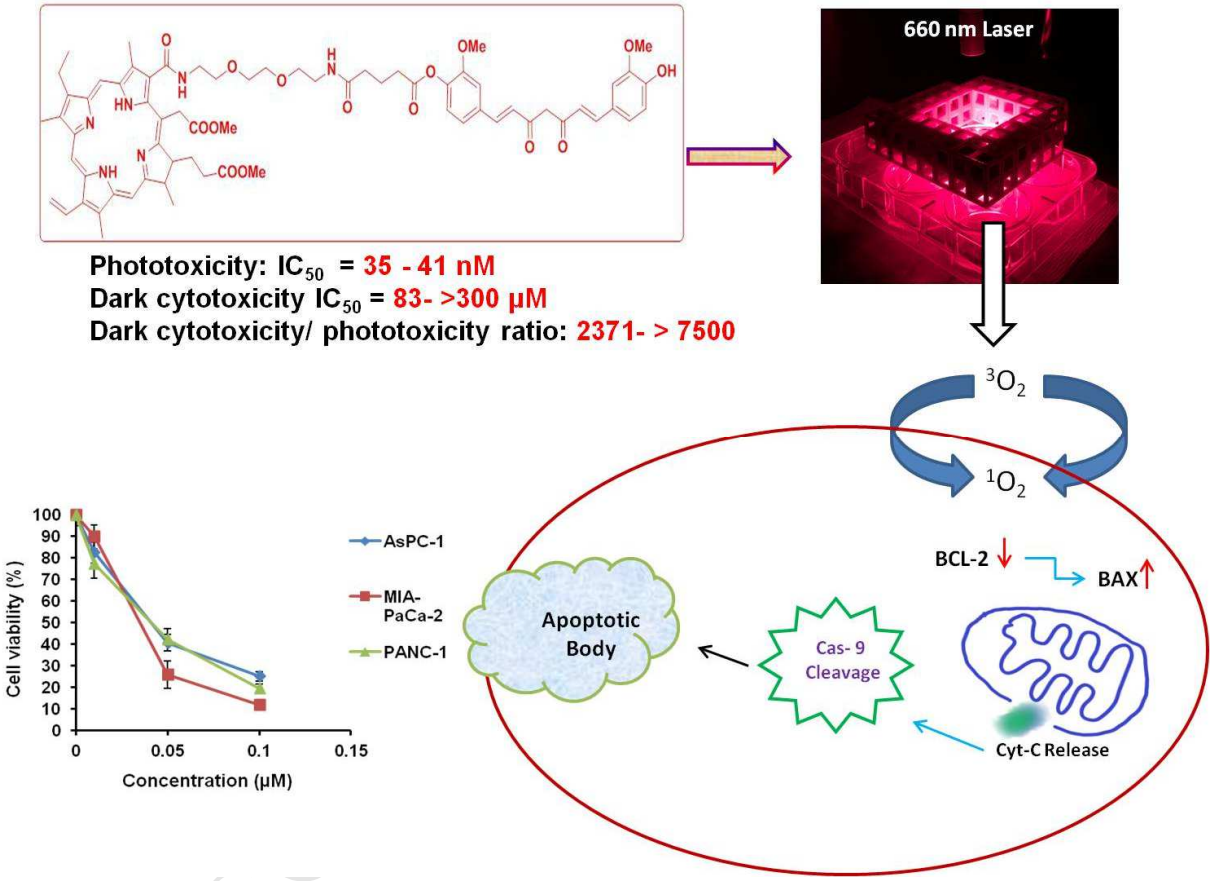

Synthesis of Novel Chlorin e6-Curcumin Conjugates as Photosensitizers for Photodynamic Therapy against Pancreatic Carcinoma

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Abstract: Curcumin (cur) has been comprehensively studied for its various biological properties, more precisely for its antitumor potential and it has shown the promising results as well. On the other hand, Chlorin e6 (Ce6) has mostly been used as a photosensitizer in photodynamic therapy (PDT) against a variety of carcinomas. In the present study, we have synthesized a series of Chlorin e6-curcumin (Ce6-cur) conjugates and investigated their photosensitizing potential against pancreatic cancer cell lines. All the synthesized compounds were characterized by UV, ^{1}H NMR, ^{13}C NMR and LC-MS. These Ce6-cur conjugates showed better physicochemical properties and higher singlet oxygen generation capability. The cellular uptake was studied in AsPC-1 cells using fluorescence-activated cell sorting (FACS). Compound **17** was rapidly internalized within 30 min and sustained for 24h. Compound **17** showed excellent PDT efficacy with IC_{50} of 40, 35 and 41 nM against AsPC-1, MIA PaCa-2 and PANC-1 respectively with exceptional dark / phototoxicity ratio in the range of 2371-7500. Moreover, the treatment of compound **17** upregulated the expression of BAX, Cytochrome-C and cleaved caspase 9 while downregulating the Bcl-2 expression an anti-apoptotic protein marker. These results demonstrate outstanding capability of compound **17** as a potent photosensitizer which could improve the PDT efficacy in pancreatic cancer patients.

Key Words; Photodynamic therapy, Photosensitizer, Ce6, Curcumin, Pancreatic Cancer.

Introduction

Photodynamic therapy (PDT) relies on the accumulation of photosensitizer in tumors, which upon irradiation of light of appropriate wavelength generates singlet oxygen and other cytotoxic reactive oxygen species (ROSs) that results in cell membrane damage and subsequent cell death

[1]. PDT uniquely stimulates cell death by directly activating apoptosis, and therefore bypasses many cell death signaling pathways. PDT has several advantages over other conventional cancer treatment modalities. It is relatively non-invasive because irradiation is limited to the tumor site, shows lower systemic toxicity and relatively selective destruction of tumors, partly due to preferential localization of photosensitizer within the tumor [2]. An ideal photosensitizing agent should be a single pure compound to allow quality control analysis with low manufacturing costs and good stability in storage. It should have a high absorption peak between 600 and 800 nm (red to deep red) as absorption of photons with wavelengths longer than 800 nm does not provide enough energy to excite oxygen to its singlet state, thus there is substantially reduced ROS generation upon irradiation [3].

Chlorins are reduced porphyrins, they absorb light in the 660 nm region, fluoresce at 670 nm and have high quantum yield of singlet oxygen generation. Ce6 possess three different carboxylic acids that can be derivatized in multiple ways. Remarkable clinical benefits have been obtained with Ce6 mediated PDT in the treatment of various cancers, including melanoma, bladder, and nasopharyngeal cancers [4]. Ce6 was conjugated to peptides [5], sugars [6], polyamines [7], mono amino acids [8] and diamino acids [9].

The Indian spice curcumin (also known as diferuloylmethane), extracted from the turmeric plant, has long held a role in Indian / Hindu rituals, traditions, customs, and cuisines [10]. It is an orange-yellow, crystalline powder remarkably insoluble in water; however, it is exceedingly soluble in ethanol and DMSO. In contrast with conventional cytotoxic drugs which often have side effects such as nausea, vomiting or fatigue curcumin has minimal toxicity. The safety of curcumin has been approved by the Food and Drug Administration and World Health Organization; In addition, its safety is strongly supported by the fact that this agent has been used

in traditional Hindu and Chinese medicine for thousands of years. Curcumin can modulate the activity of a variety of molecules that play important roles in cancer progression, with more than 30 molecular targets identified to date [11]. Of these molecules, NF-κB appears to be one of the primary targets of curcumin. Curcumin may induce apoptosis of cancer cells through blocking of NF-κB survival pathway, generation of reactive oxygen species (ROS), down-regulation of Bcl-XL, or activation of caspase-8 pathways. Curcumin has shown good anti-cancer activity against pancreatic cancer [12] and it is used as sensitizing agents in combination with Cisplatin, Doxorubicin, 5-Fluorouracil and Gemcitabine [13]. Recently, curcumin is being used as a photosensitizer for anti-microbial photodynamic therapy [14].

Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer (PC), is a kind of digestive-tract malignant tumor with highly invasive and metastatic features and has become the fourth most lethal malignancies in the US [15]. The patients with pancreatic cancer have poor survival rate with less than 5% of patients surviving 5 years after diagnosis. Chemotherapy is the most important adjuvant treatment for recurrent pancreatic cancer patients who are not indicated for resection. However, PDAC is also notoriously resistant to Gemcitabine (GEM), which is the first-line chemotherapeutic agent [16]. Therefore, it is of utmost importance to identify new molecular targets for the effective treatment of pancreatic cancer. Ce6 is an effective PS given its several advantages for clinical use, such as activation by near-infrared wavelengths, relatively deep penetration through layers of tissues and potency against a broad spectrum of cancers. Curcumin is well known for anti-cancer activity, it acts as a sensitizer for resistant cells and used as a photosensitizer in anti-microbial PDT. Herein we have conjugated Ce6 and curcumin with linkers to improve the Ce6 PDT efficacy.

Results and Discussion

Synthesis of Ce6-curcumin

The synthetic route of the Ce6-cur conjugates is depicted in **Scheme 1 & 2**. Four Ce6-cur conjugates were synthesized by incorporating hydrophobic and hydrophilic linkers. The linkers used for conjugating curcumin and Ce6 are Propane, Hexane, 2,2′-(Ethylenedioxy)bis(ethylamine) (mono PEG) and 4,9-Dioxa-1,12-dodecanediamine (diPEG). N-Boc-monoprotected Propane **1**, Hexane **2**, 2′-(Ethylenedioxy)bis(ethylamine) (mono PEG) **3**, and 4,9-Dioxa-1,12-dodecanediamine (diPEG) **4** (**Scheme 1**) were prepared with minor modifications to the preparations described in the literature [17]. The mono-carboxylic acid derivative of curcumin **6** was synthesized by reacting curcumin **5** with glutaric anhydride in the presence of DMAP and TEA [18]. Dimethyl ester of Ce6 **8** (DME Ce6) was obtained by selective esterification of Chlorin e6 **7** using 5 % of H_2SO_4 in methanol [8]. The DME Ce6 **8** was activated using 1-hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDCI) and coupled individually with **1**, **2**, **3** and **4** to give tert-butyl protected Ce6-propane **9**, Ce6-hexane **12**, Ce6-monoPEG **15**, Ce6-diPEG **18** derivatives (**Scheme 2**), which were followed by deprotection with trifluoroacetic acid (TFA) to afford Ce6-propane-NH_2 **10**, Ce6-hexane-NH_2 **13**, Ce6-monoPEG-NH_2 **16**, Ce6-diPEG-NH_2 **19** derivatives. The final coupling of curcumin monocarboxylic acid **6** with **10**, **13**, **16**, and **19** using HOBt and EDCI to give Ce6-cur conjugates **11**, **14**, **17** and **20**.

Determination of absorbance

We recorded the absorption of compounds in DMSO in which all compounds and reference compounds are soluble as shown in **Fig. 1a**. The absorption spectra of all compounds were

typical to Ce6 derivatives with one soret band and Q-band. All the photosensitizers **7**, **11, 14, 17,** and **20** effectively absorbed the red light with the major soret peak at λ_{max} = 405-408 nm. The soret peak of Ce6 **7** was sharp while Ce6-cur conjugates have a broad peak, the broad peak was due to curcumin conjugation. All the compounds exhibit a bathochromic shift as compared to starting material **7** by 10 nm. Conjugation of curcumin to Ce6 increases the absorption to a longer wavelength.

Fluorescence

Curcumin and Ce6 are two fluorophores, herein we conjugated them together with linkers, and evaluated their fluorescence. All the compounds show fluorescence at 650-700 nm (**Fig. 1b**). Compound **17** and **11** show higher fluorescence than Ce6 **7**, while compound **14** & **20** have same fluorescence intensity as that of Ce6 **7**.

Measurement of singlet oxygen generation

Singlet oxygen generation is the key event in the photodynamic killing of cancer cells. The ability of Ce6-cur conjugates and Ce6 to produce singlet oxygen under photosensitizing condition was studied in DMSO using 1, 3-diphenylisobenzofuran (DPBF) as a singlet oxygen trap. DPBF reacts irreversibly with $^1O_2$ and the reaction can be monitored by measuring the decrease in DPBF absorption intensity at 418 nm. Irradiation of a solution of Ce6-cur conjugates, Ce6, methylene blue (MB) and DPBF with 660 nm laser led to quenching of DPBF absorption band at 418 nm as shown in **Fig. 1c**. All the compounds show higher $^1O_2$ generation than MB. The $^1O_2$ generation of all the compounds is in the order **11** (7.39 %), **7** (9.88 %), **14** (10.05%), **17** (12.13%), **20** (12.32 %) and MB (33.93 %).

Cellular Uptake Assay

There is a direct correlation of cellular uptake of PS to PDT, higher the cellular internalization, stronger the PDT efficacy of PS [19]. The cellular uptake of 5 µM Ce6-cur conjugates and Ce6 in AsPC-1 cells at various incubation time was measured by Fluorescence-activated cell sorting (FACS). All the compounds have shown time dependent uptake into the cells as shown in **Fig. 2a-e**. Interestingly compound **17** uptake was higher at 3h. The fluorescence intensity of compound **17** was nearly 47 and 29 fold higher than free Ce6 **7** after 3h and 24h respectively (**Fig. 2c**). In order to understand the uptake of compound **17**, we again studied the time dependent uptake at 30 min, 1h and 2h as shown in **Fig. 2d & e**. The compound **17** was uptaken by the cells within 30 min of incubation, reached maximum within one hour and thereafter sustained within the cells for 24h. The Compound **11** and **20** have also shown higher uptake compare to free Ce6. The difference in uptake might be due to linkers as hydrophilic linkers (**17** & **20**) have higher uptake than hydrophobic (**11** & **14**), with exception **11**.

Cytotoxicity Assessment

An ideal PS should possess minimal dark toxicity, be cytotoxic in the presence of light at a defined wavelength and have higher singlet oxygen quantum yield [20]. The dark toxicity of Ce6-cur conjugates and Ce6 was evaluated in AsPC-1, MIA PaCa-2 and PANC-1. The cells were exposed to increasing concentrations (0-300 µM) of Ce6-cur conjugate and Ce6 after 72h and then cell viability was determined by classical MTT assay (**Fig. 3a-c)**. All the compounds under study have shown less dark toxicity than the starting material Ce6 **7** except compound **17** against MIA PaCa-2, increased dark toxicity in the presence of Ce6-cur conjugate can be attributed to curcumin. However, we couldn't understand clearly why the dark toxicity was observed only in one of the cancer cell line. Interestingly, after exposure to light (660 nm, 50 mW, 9 J/cm^2), all the conjugates were found to be highly toxic to AsPC-1, MIA PaCa2 and

PANC-1 (**Fig. 4 a-d**). From the **Table-1**, it is clear that Ce6-cur conjugates are highly effective and have lower IC_{50} values. The most active compound of the series was compound **17** (**Fig. 4d**) its IC_{50} values were in the range of 40, 35, 41 nM against AsPC-1, MIA PaCa-2 and PANC-1 respectively while maintaining a higher ratio of dark/phototoxicity in the range of 2371-7500 (**Table-1**). AsPC-1 and PANC-1 cells are Gemcitabine resistant cells, thus the anti-cancer activity on these cell lines at nanomolar concentrations of conjugates might be due to the sensitization facilitated by curcumin [16]. The IC_{50} of compound **17** was nearly 488, 452, 247 folds lower than the free Ce6 **7**(19.53, 15.84, 10.14 µM against AsPC-1, MIA PaCa-2 and PANC-1 respectively). Compound **11** and **20** also shown good PDT efficacy with IC_{50} in the range of 4.2-5.74 µM and 4.64 to 15.74 µM respectively. We speculate that the higher cytotoxicity of the compound **17** might be attributed to its higher cellular uptake, higher singlet oxygen generation potential as evidenced by FACS and DPBF assay and synergistic activity of curcumin and Ce6.

Apoptosis

There are varieties of mechanism by which a photosensitizer (PS) execute the cell death process; however, apoptosis and necrosis are the most common mechanisms among them. We therefore, examined the mechanism of cell death induced by compound **17**, by using flow cytometer with the help of Annexin V-FITC and Propidium iodide staining. Annexin V dye binds with phosphatidylserine residues which are released in the outer membrane of the apoptotic cells and produces a florescence due to FITC labeling allowing the differentiation of apoptotic and necrotic cells [21]. As shown in F**ig. 5**, increasing dose of compound **17** (20 to 60 nM) induced a massive apoptotic population in AsPC-1 cells (21 to 40 %, respectively) suggesting the intense apoptotic potential of compound **17** in AsPC-1 pancreatic cells.

The mechanism of apoptosis is much complicated having two major pathways i.e. intrinsic apoptosis which is associated with mitochondrial dysfunctioning and extrinsic pathway regulated by death domain receptors [22]. We therefore, elucidated the particular mechanism of apoptosis induced by compound **17** through the immunoblot examination. The results obtained from the examination suggest that the treatment of compound **17** triggered the intrinsic apoptotic pathway as the downregulation of Bcl-2 and up regulation of Bax was observed (**Fig. 6**). Also, the Cytochrom-C release a defining feature of mitochondrial stress was observed to be over expressed along with the upregulation of cleaved caspase 9 (**Fig. 6**). Thus, compound **17** have potential to trigger the mitochondria associated intrinsic apoptotic pathway.

Conclusion

In summary, we have designed and synthesized the novel Ce6-cur conjugates and characterized by UV, ^{1}H NMR, ^{13}C NMR and LC-MS. These conjugates exhibit excellent physicochemical properties and higher singlet oxygen generation. On cellular level, the uptake of these conjugates was far better as compared to free Ce6. Compound **17** has shown outstanding cytotoxicity against three pancreatic cancer cells AsPC-1, MIA PaCa2 and PANC-1 with IC_{50} values ranging from 35-41 nM. Additionally it had highest dark/photo toxicity ratio in the range of 2371-7500. Moreover, Compound **17** has potential to trigger the intrinsic apoptotic pathway in AsPC-1 a pancreatic cancer cell line. So, in the premise of our data we propose that compound **17**, a novel Ce6-cur conjugate with highest dark/ phototoxicity ratio is a potent photosensitizer with enhanced PDT efficacy in vitro. We further plan to validate its promising potential as a photosensitizer *in vivo.*

Acknowledgements

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Materials and Methods

All air and moisture sensitive reactions were performed in dried and distilled solvents under argon atmosphere. All solvents and reagents were purchased from commercial sources, unless otherwise stated. Silica gel 60 (230 X 400 mesh, Zeochem) was used for column chromatography. Analytical thin-layer chromatography (TLC) was carried out on silica gel plates G254 (Merck). ^{1}H and ^{13}C spectra were recorded on Bruker 400 spectrometers, with TMS as internal standard and $CDCl_3$ as the solvents. Chemical shifts (δ) and coupling constant (J) are given in ppm and Hz, respectively. ESI mass spectrometric data were obtained on Shimadzu LC-MS. UV absorption spectra were measured on Thermo Scientific UV spectrophotometer.

Measurement of Singlet Oxygen Photogeneration

1, 3-Diphenylisobenzofuran (DPBF) was used as a selective $^{1}O_2$ acceptor, which was bleached upon reaction with $^{1}O_2$ [23]. Five sample solutions of DPBF in DMSO (50 μM) containing, respectively, DPBF only (50 μM, a control sample), DPBF + methylene blue (MB) (1 μM), DPBF + **7** (1 μM), DPBF + **11** (1 μM), DPBF + **14** (1 μM), DPBF + **17** (1 μM), DPBF + **20** (1 μM), were prepared in the dark. All the samples were placed in a 96-well plate and the container was covered with aluminum foil. The samples were irradiated (660 nm, 50 mW, 2 J/cm^2) for 40 seconds. After irradiation, visible spectra of the sample solutions were measured spectrophotometrically. The normalized absorbance of DPBF at 418 nm in these samples was reported. The $^{1}O_2$ photo generation activities of MB, **7, 11, 14, 17, and 20** can be compared with the different absorbance decay of each sample relative to the DPBF control sample.

Synthesis of 5-(4-((1E, 6E)-7-(4-hydroxy-3-methoxyphenyl)-3, 5-dioxohepta-1, 6-dien-1-yl)-2-methoxyphenoxy)-5-oxopentanoic acid (6)

To a solution of curcumin (2.01 g, 5.46 mmol), and DMAP (112 mg, 0.92 mmol) in THF (100 mL) was added (1.33 mL, 9.55 mmol) of Et_3N. Glutaric anhydride (95%) (0.685 g, 6 mmol) in THF (5 mL) was added slowly drop wise to the curcumin solution. The mixture was stirred and refluxed under argon overnight. THF was removed under vacuum, EtOAc (55 mL) was added, followed by the addition of 1M HCl (15 mL); the mixture was stirred for 10 minutes. The organic phase was separated and extracted with EtOAc three times; the solvent was removed and dried. The product was purified via column chromatography, and eluted with CH_2Cl_2: MeOH, 95: 5. Yield: 69 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 370 (4.7 X 10^5), 414 (4.4 X 10^5) nm. ^{1}HNMR ($CDCl_3$, 400 MHz): δ 7.65 (d, *J* = 16 Hz, 2H), 7.20-6.95 (m, 5H), 6.96 (d, 1H), 6.48-6.57 (m, 2H), 5.85 (s, 2H), 3.98 (s, 3H), 3.90 (s, 3H), 2.75-2.71 (t, *J* = 8 Hz, 2H), 2.61-2.57 (t, *J* = 8 Hz, 2H), 2.15-2.12 (t, *J* = 8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 184.56, 181.80, 178.26, 170.84, 151.28, 148.03, 146.84, 141.09, 139.40, 134.12, 127.53, 124.25, 123.07, 121.73, 120.99, 114.89, 111.37, 109.69, 101.58, 55.96, 32.82, 19.92. LC-MS: 483 [M+H].

Synthesis of Dimethylester of chlorin e6 (DME Ce6) (8)

Chlorin e6 (**7**) (3 g, 5.02 mmol) was dissolved in 5% sulfuric acid in methanol and allowed to stir in the dark, under argon overnight. The reaction was poured into cold saturated aqueous $NaHCO_3$ and extracted twice with CH_2Cl_2. The extract was washed twice with brine, dried over Na_2SO_4 and filtered. The solvent was evaporated. It was then purified on a silica gel column afford 2.8 g, Yield: 88 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 656 (9.2 X 10^5), 501 (2.9 X 10^5), 399 (3.6 X 10^6) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.62 (s, 1H), 9.49 (s, 1H), 8.73 (s, 1H), 8.03

(m, 1H), 6.32 (dd, *J* = 17.8, 1.2 Hz, 1H), 6.13 (dd, *J* = 11.5, 1.2 Hz, 1H), 5.50 (d, *J* = 18.6 Hz, 1H), 5.23 (d, *J* = 18.6 Hz, 1H), 4.45 (m, 2H), 3.82 (s, 3H), 3.76 (q, *J* = 7.6 Hz, 2H), 3.62 (s, 6H), 3.46 (s, 3H), 3.25 (s, 3H), 1.69 and 2.12 (m, 2H), 2.19 and 2.56 (m, 2H), 1.81 (d, *J* = 7.1 Hz, 3H), 1.64 (t, *J* = 7.6 Hz, 3H), -1.71 (s, 1H), -1.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.58, 169.89, 167.31, 155.01, 148.84, 145.12, 139.77, 137.17, 136.17,135.85, 135.58, 134.84, 130.67, 129.33, 121.80, 102.47, 98.63, 93.60, 52.84, 51.65, 49.53, 39.22, 30.98, 29.34, 22.75, 19.59, 17.66, 12.69, 12.15, 11.29. LC-MS: 625 [M+H].

Synthesis of *tert*-butyl (3-aminopropyl) carbamate (1)

To a stirred and cooled solution (0 ºC) of 1, 3-diaminopropane (3.64 mL, 43.5 mmol) in $CHCl_3$ (45 mL) was added a solution of di-*tert*-butyl bicarbonate (0.95 g, 4.35 mmol) in $CHCl_3$ (22 mL) drop wise over a period of 3h. The reaction mixture was allowed to warm to room temperature and stirred for additional 20h. The precipitated white solid was filtered and the $CHCl_3$ was washed with water (2 x 20 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to give compound *tert*-butyl (3-aminopropyl)carbamate **(1)** 475 mg, Yield: 63% as a clear oil which was used for the next reaction without any further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.91 (bs, 1H), 3.16 (dq, *J* = 12.7, 6.4 Hz, 2H), 2.73 (t, *J* = 6.6 Hz, 2H), 1.58 (p, *J* = 6.6 Hz, 2H), 1.41 (s, 9H).

Synthesis of Ce6-Propane-NHBoc (9)

DME Ce6 **8** (1 g, 1.60 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL). EDCI (368 mg, 1.92 mmol) and HOBt (260 mg, 1.92 mmol) were then added and allowed to stir until completely dissolved under nitrogen. After 30 min, *tert*-butyl (3-aminopropyl)carbamate (**1**) (836 mg, 4.80 mmol) and DIEA (413 mg, 3.2 mmol) were mixed in CH_2Cl_2 (20 mL) and added to the reaction

mixture. The mixture was allowed to stir at room temperature for 12h under nitrogen. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and then washed with brine and water, respectively. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated. The product was purified via column chromatography to afford 520 mg of **9**, Yield: 41 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 670 (4.3 X 10^5), 504 (2.0 X 10^5), 408 (7.6 X 10^5) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.62 (s, 1H), 9.57 (s, 1H), 8.73 (s, 1H), 8.03 (m, 1H), 6.31 (dd, *J* = 17.8, 1.2 Hz, 1H), 6.08 (dd, *J* = 11.5, 1.2 Hz, 1H), 5.48 (d, *J* = 18.6 Hz, 1H), 5.20 (d, *J* = 18.6 Hz, 1H), 4.38 and 4.27 (m, 2H), 3.76 (m, 5H), 3.58 (m, 8H), 3.40 (s, 6H), 3.25 (s, 4H), 1.69 and 2.12 (m, 2H), 1.90 and 2.49 (m, 2H), 1.90 (m, 3H), 1.68 (m, 3H) 1.50 (s, 11 H), -1.67 (s, 1H), -1.88 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.87, 168.78, 166.70, 156.56, 154.17, 149.07, 144.74, 138.86, 136.10, 13503, 134.77, 134.50, 130.14, 129.90, 129.45, 128.20, 121.61, 102.15, 101.40, 98.83, 93.67, 79.35, 53.08, 52.17, 51.65, 49.26, 37.62, 31.14, 30.37, 29.64, 28.38, 23.05, 19.69, 17.76, 12.19, 11.35. LC-MS: 781 [M+H].

Synthesis of Ce6-Propane amine (10)

The compound **9** (500 mg, 0.64 mmol) was dissolved in dry CH_2Cl_2 (20 mL) in an ice bath under argon. TFA (2 mL) was added, and the reaction mixture was stirred overnight. The reaction mixture was evaporated several times with diethyl ether to remove residual TFA. Then the precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to give a crude compound, purified by silica gel chromatography to give 350 mg of **10**, Yield: 80 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 658 (6.4 X 10^5), 501 (2.0 X 10^5), 400 (2.5 X 10^6) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.62 (s, 1H), 9.56 (s, 1H), 8.73 (s, 1H), 8.01 (m, 1H), 6.29 (dd, *J* = 16 Hz, 1H), 6.07 (dd, *J* = 16 Hz, 1H), 5.50 (d, *J* = 20 Hz, 1H), 5.20 (d, *J* = 20 Hz,

1H), 4.40 and 4.27 (m, 2H), 3.86 and 3.61 (m, 2H), 3.71 (m, 5H), 3.53 (s, 3H), 3.48 (s, 4H), 3.41 (s, 3H), 3.26 (s, 3H), 2.89 (t, *J* = 8 Hz, 2H), 2.49 (m, 2H), 1.69 and 2.12 (m, 2H), 1.85 (t, *J* = 4 & 8 Hz, 2H), 1.77 (m, 6H), -1.71 (s, 1H), -1.92 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.87, 168.78, 166.70, 154.17, 149.07, 144.74, 138.86, 136.10, 135.03, 134.77, 134.50, 130.14, 129.90, 129.45, 128.20, 121.61, 102.15, 101.40, 98.83, 93.67, 53.08, 52.17, 51.65, 49.26, 37.62, 31.14, 30.37, 29.64, 28.38, 23.05, 19.69, 17.76, 12.19, 11.35. LC-MS: 681 [M+H].

Synthesis of Chlorin e6-curcumin conjugate (11)

Compound **6** (250 mg, 0.51 mmol) was dissolved in dry CH_2Cl_2. A mixture of HOBt (83 mg, 0.62 mmol), EDCI (120 mg, 0.62 mmol), and DIEA (66 mg, 0.51 mmol) in CH_2Cl_2 was added, the mixture was then allowed to stir for 30 min. Compound **10** (352 mg, 0.51 mmol) and DIEA (66 mg, 0.51 mmol) were mixed in CH_2Cl_2 and added to this reaction mixture. The mixture was stirred overnight. It was diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was purified by silica gel column chromatography to afford 280 mg of **11**, Yield: 47 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 668 (3.3 X 10^5), 504 (1.6 X 10^5), 403 (7.6 X 10^5) nm. ^{1}H NMR ($CDCl_3$, 400MHz): δ 9.59 (s, 1H), 9.55 (s, 1H), 8.72 (s, 1H), 8.00 (m, 1H), 7.49 (d, *J* = 16 Hz, 1H), 7.40 (d, *J* = 16 Hz, 1H), 7.01 (m, 2H), 6.93 (m, 4H), 6.85-6.81 (m, 2H), 6.33-6.25 (m, 2H), 6.07 (dd, *J* = 4 Hz, 1H), 5.56 (s, 1H), 5.44 (d, *J* = 16 Hz, 1H), 5.19 (d, *J* = 20 Hz, 1H), 4.40 and 4.26 (m, 2H), 3.85 (m, 5H), 3.75-3.69 (m, 8H), 3.57-3.53 (m, 6H), 3.46-3.40 (m, 8H), 3.23 (s, 3H), 2.58 (t, *J* = 8 Hz, 2H), 2.50 (m, 1H), 2.34 (t, *J* = 8 & 4 Hz, 2H), 2.15-2.09 (m, 2H), 2.05 (t, *J* = 8 Hz, 2H), 1.67 (m, 6H), -1.71 (s, 1H), -1.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 184.43, 181.53, 173.59, 172.69, 171.19, 170.04, 168.90, 166.73, 151.17, 149.03, 147.92, 146.75, 144.81, 141.01, 140.94, 139.13, 136.17, 134.92, 134.85, 134.60, 134.56, 133.94,

130.27, 129.77, 129.34, 127.86, 127.40, 124.06, 123.20, 122.92, 121.67, 120.90, 114.79, 111.27, 109.53, 102.15, 101.43, 98.84, 93.72, 55.82, 53.07, 51.67, 49.25, 37.91, 36.32, 33.01, 31.11, 29.62, 23.05, 21.02, 19.68, 17.76, 12.17, 11.35. LC-MS: 1145 [M+H].

Synthesis of *tert*-butyl (6-aminohexyl)carbamate (2)

Di-*tert*-butyl dicarbonate (4.0 g, 18.4 mmol) was dissolved in $CHCl_3$ and added drop-wise to a solution of hexamethylenediamine (10.6 g, 91.6 mmol) in $CHCl_3$ at 0 °C. The mixture was allowed to warm to room temperature. After stirring for 12h, the reaction crude was filtered and washed with $CHCl_3$. The filtrates were collected and solvent was evaporated. The residue was re-dissolved in EtOAc and washed with water and then brine. The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford 1.68 g, Yield: 42 % of *tert*-butyl (6-aminohexyl)carbamate (**2**). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.52 (bs, 1H), 3.10 (q, *J* = 6.6 Hz, 2H), 2.68 (t, *J* = 7.0 Hz, 2H), 1.49-1.30 (m, 17H), 1.25 (t, *J* = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.1, 79.1, 42.2, 40.5, 33.8, 30.2, 28.4, 26.7, 26.6.

Synthesis of Ce6-Hexane-NHBoc (12)

DME Ce6 **8** (1 g, 1.60 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL). EDCI (614 mg, 3.2 mmol) and HOBt (432 mg, 3.2 mmol) were then added and allowed to stir until completely dissolved under nitrogen. After 30 min, *tert*-butyl (6-aminohexyl)carbamate (**2**) (1.73 g, 8.0 mmol) and DIEA (620 mg, 4.8 mmol) were mixed in CH_2Cl_2 (20 mL) and added to the reaction mixture. The mixture was allowed to stir at room temperature for 12h under nitrogen. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and then washed with brine and water, respectively. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated. The product was purified via column chromatography to afford 700 mg of **12**, Yield: 53 %. UV-Vis

(DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 670 (3.57 X 10^5), 504(9.8 X 10^5), 408(9.82 X 10^5) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.63 (s, 1H), 9.58 (s, 1H), 8.74 (s, 1H), 8.03 (m, 1H), 6.31 (dd, *J* = 16 Hz, 1H), 6.09 (dd, *J* = 12 Hz, 1H), 5.49 (d, *J* = 20 Hz, 1H), 5.21 (d, *J* = 20 Hz, 1H), 4.40 and 4.26 (m, 2H), 3.85 (m, 5H), 3.58 (m, 8H), 3.42 (s, 3H), 3.25 (s, 3H), 3.10 (m, 2H), 1.69 and 2.12 (m, 2H), 1.74 (m, 2H), 1.67 (m, 6H), 1.50 (s, 17H), 1.36 (s, 2H), -1.71 (s, 1H), -1.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.56, 169.39, 168.74, 166.67, 156.05, 154.15, 149.10, 144.74, 138.82, 136.12, 134.99, 134.75, 134.49, 130.14, 129.47, 128.43, 121.63, 102.15, 101.35, 98.85, 93.68, 79.08, 52.14, 51.65, 49.25, 40.49, 37.81, 31.14, 30.95, 29.48, 28.45, 26.76, 23.07, 19.70, 17.78, 14.22, 12.20, 11.37. LC-MS: 823 [M+H].

Synthesis of Ce6-Hexane amine (13)

The compound **12** (700 mg, 0.85 mmol) was dissolved in of dry CH_2Cl_2 (30 mL) in an ice bath under argon. TFA (3 mL) was added, and the reaction mixture was stirred overnight. The reaction mixture was evaporated several times with diethyl ether to remove residual TFA. Then the precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to give a crude compound, purified by silica gel chromatography to give 350 mg of **13**, Yield: 73 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 658 (4.7 X 10^5), 501 (1.4 X 10^5), 400 (1.9 X 10^6) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.63 (s, 1H), 9.58 (s, 1H), 8.73 (s, 1H), 8.01 (m, 1H), 6.31 (dd, *J* = 16, 4 Hz, 1H), 6.09 (dd, *J* = 12 Hz, 1H), 5.50 (d, *J* = 20 Hz, 1H), 5.21 (d, *J* = 20 Hz, 1H), 4.40 and 4.27 (m, 2H), 3.75 (m, 5H), 3.53 (s, 3H), 3.49 (s, 4H), 3.42 (s, 3H), 3.25 (s, 3H), 2.62 (t, *J* = 4 & 8 Hz, 2H), 2.46 (m, 2H), 1.69 and 2.12 (m, 2H), 1.76-1.63 (m, 10H), 1.43 (m, 8H), -1.71 (s, 1H), -1.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.57, 169.40, 168.75, 166.68, 154.15, 149.10, 144.74, 138.83, 136.14, 134.98, 134.80, 134.75, 134.49, 130.14, 129.45,

128.47, 121.63, 102.15, 101.35, 98.86, 93.69, 52.14, 51.65, 49.25, 41.97, 40.52, 37.78, 31.14, 29.45, 26.92, 23.08, 19.71, 17.78, 12.20, 11.37. LC-MS: 723 [M+H].

Synthesis of Chlorin e6-curcumin conjugate (14)

Compound **6** (300 mg, 0.62 mmol) was dissolved in dry CH_2Cl_2. A mixture of HOBt (100 mg, 0.74 mmol), EDCI (143 mg, 0.74 mmol), and DIEA (66 mg, 0.51 mmol) in CH_2Cl_2 was added, the mixture was then allowed to stir for 30 min. Compound **13** (352 mg, 0.51 mmol) and DIEA (160 mg, 1.24 mmol) were mixed in CH_2Cl_2 and added to this reaction mixture. The mixture was stirred overnight. It was diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was purified by silica gel column chromatography to afford 450 mg of **14**, Yield: 61 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 668 (2.9 X 10^5), 505 (1.4 X 10^5), 406 (6.9 X 10^5) nm. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.60 (s, 1H), 9.55 (s, 1H), 8.72 (s, 1H), 8.02 (m, 1H), 7.38 (m, 2H), 6.94-6.81 (m, 8H), 6.30-6.19 (m, 3H), 6.07 (dd, *J* = 4 Hz, 1H), 5.50-5.44 (m, 2H), 5.23-5.17 (m, 2H), 4.40 and 4.26 (m, 2H), 3.86-3.78 (m, 5H), 3.73-3.71 (m, 9H), 3.54 (s, 4H), 3.46 (s, 4H), 3.41 (s, 3H), 3.27-3.24 (m, 6H), 2.54 (t, *J* = 8 Hz, 2H), 2.50 (m, 1H), 2.26 (t, *J* = 8 Hz, 2H), 2.10-1.99 (m, 5H), 1.74-1.62 (m, 9H), -1.71 (s, 1H), -1.92 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 184.38, 181.46, 173.61, 172.21, 171.20, 169.51, 168.75, 166.68, 154.15, 151.08, 149.08, 147.91, 146.71, 144.75, 140.89, 138.99, 136.15, 134.95, 134.75, 134.72, 134.45, 133.96, 130.19, 129.51, 129.42, 128.33, 127.28, 124.11, 123.13, 122.84, 121.60, 120.90, 114.75, 111.29, 109.45, 102.10, 101.41, 98.84, 93.70, 55.84, 53.11, 52.18, 51.67, 49.23, 40.31, 39.18, 38.90, 37.78, 35.18, 32.83, 29.68, 26.52, 23.09, 21.14, 19.69, 17.78, 12.19, 11.36. LC-MS: 1187 [M+H].

Synthesis of *tert*-butyl(2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (3)

Under a nitrogen atmosphere, to a solution of 2,2'-(ethylenedioxy)-bis-(ethylamine) (14.8 g, 100 mmol) in anhydrous $CHCl_3$ (100 mL) cooled to 0 ^{0}C was added dropwise di-*tert*-butyldicarbonate (2.18 g, 10 mmol) in $CHCl_3$ (50 mL). After been stirred 24h at room temperature, the solvent is evaporated under vacuum. The thick oil obtained is taken up in CH_2Cl_2 (100 mL). The organic layer is successively washed with saturated aqueous NaCl (50 mL), water (50 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford 2.20 g, Yield: 89% of crude *tert*-butyl(2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (**3**). This material was used without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.15 (br s, 1H, NH), 3.63–3.51 (m, 8H), 3.31 (td, *J* = 5.5 Hz, 2H), 2.88 (t, *J* = 4.8 Hz, 2H), 1.45 (s, 9H), 1.40 (s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): d 155.42, 78.13, 72.80, 69.63, 41.08, 39.67, 27.77.

Synthesis of Ce6-MonoPEG-NHBoc (15)

DME Ce6 **8** (1.5 g, 2.40 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL). EDCI (552 mg, 2.88 mmol) and HOBt (388 mg, 2.88 mmol) were then added and allowed to stir until completely dissolved under nitrogen. After 30 min, *tert*-butyl(2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (**3**) (2 g, 8.41 mmol) and DIEA (620 mg, 4.8 mmol) were mixed in CH_2Cl_2 (20 mL) and added to the reaction mixture. The mixture was allowed to stir at room temperature for 12h under nitrogen. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and then washed with brine and water, respectively. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated. The product was purified via column chromatography to afford 1.3 g of **15**, Yield: 63 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 667 (4.1 X 10^5), 506 (1.8 X 10^5), 410 (8.5 X 10^5) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.63 (s, 1H), 9.57 (s, 1H), 8.73 (s, 1H),

8.02 (m, 1H), 6.31 (dd, *J* = 16 Hz, 1H), 6.09 (dd, *J* = 16 Hz, 1H), 5.51 (d, *J* = 20 Hz, 1H), 5.24 (d, *J* = 20 Hz, 1H), 4.66 and 4.02 (m, 2H), 4.42 and 4.31 (m, 2H), 3.84 (m, 2H), 3.74-3.66 (m, 7H), 3.54-3.50 (m, 9H), 3.29-3.25 (m, 5H), 2.97 (m, 2H), 2.50 and 2.17 (m, 2H), 1.74 and 2.08 (m, 2H), 1.67 (m, 8H), 1.50 (s, 9H) -1.71 (s, 1H), -1.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.56, 169.51, 168.83, 166.69, 155.86, 154.24, 149.10, 144.75, 138.88, 136.09, 135.02, 134.78, 134.52, 130.16, 129.90, 128.26, 121.60, 102.32, 101.37, 98.83, 93.66, 79.12, 70.44, 69.86, 53.11, 52.19, 51.62, 49.22, 40.41, 37.89, 31.12, 29.70, 28.43, 23.09, 19.71, 17.76, 12.18, 11.36. LC-MS: 855 [M+H].

Synthesis of Ce6-MonoPEGamine (16)

The compound **15** (1.3 g, 1.52 mmol) was dissolved in dry CH_2Cl_2 (30 mL) in an ice bath under argon. TFA (3 mL) was added, and the reaction mixture was stirred overnight. The reaction mixture was evaporated several times with diethyl ether to remove residual TFA. Then the precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to give a crude compound, purified by silica gel chromatography to give 1 g of **16**, Yield: 87 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 658 (5.7 X 10^5), 501 (1.8 X 10^5), 399 (2.2 X 10^6) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.63 (s, 1H), 9.58 (s, 1H), 8.74 (s, 1H), 8.03 (m, 1H), 6.31 (dd, *J* = 16, 4 Hz, 1H), 6.09 (dd, *J* = 12 Hz, 1H), 5.54 (d, *J* = 20 Hz, 1H), 5.25 (d, *J* = 20 Hz, 1H), 4.42 and 4.00 (m, 2H), 4.28 and 4.01 (m, 2H), 3.86 (m, 2H), 3.75 (m, 6H), 3.66 (m, 2H) 3.54-3.49 (m, 9H), 3.43 (m, 2H), 3.26 (s, 3H), 3.21 (t, *J* = 8 & 4 Hz, 2H), 2.49 and 2.10 (m, 2H), 1.69 and 2.10 (m, 2H), 1.67-1.63 (m, 6H), -1.71 (s, 1H), -1.92 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.58, 169.57, 168.79, 166.78, 154.08, 149.06, 144.71, 138.80, 136.14, 135.08, 134.83, 134.73, 134.49, 130.12, 129.51, 128.60, 121.63, 102.36, 101.26, 98.84, 93.68, 72.78,

70.47, 69.98, 53.10, 52.19, 51.66, 49.22, 41.05, 40.32, 37.75, 31.11, 29.68, 23.12, 19.73, 17.78, 12.21, 11.39. LC-MS: 755 [M+H].

Synthesis of chlorin e6-curcumin conjugate (17)

Compound **6** (700 mg, 1.45 mmol) was dissolved in dry CH_2Cl_2. A mixture of HOBt (235 mg, 1.74 mmol), EDCI (333 mg, 1.74 mmol), and DIEA (187 mg, 1.45 mmol) in CH_2Cl_2 was added, the mixture was then allowed to stir for 30 min. Compound **16** (1.09 g, 1.45 mmol) and DIPEA (187 mg, 1.45 mmol) were mixed in CH_2Cl_2 and added to this reaction mixture. The mixture was stirred overnight. It was diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was purified by silica gel column chromatography to afford 770 mg of **17**, Yield: 44 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 668 (2.9 X 10^5), 502 (1.5 X 10^5), 406 (7.3 X 10^5) nm. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.62 (s, 1H), 9.56 (s, 1H), 8.73 (s, 1H), 8.01 (m, 1H), 7.48 (d, *J* = 16 H, 1H), 7.37 (d, *J* = 16 H, 1H), 7.01-6.99 (m, 2H), 6.93-6.83 (m, 4H), 6.71 (d, *J* = 8 Hz, 1H), 6.31-6.24 (m, 2H), 6.08 (d, *J* = 12 Hz, 1H), 5.56-5.48 (m, 2H), 5.25-5.12 (m, 2H), 4.40 and 4.26 (m, 2H), 4.01 (m, 1H), 3.83 (m, 6H), 3.73 (m, 6H),3.64 (m, 2H), 3.59 (s, 3H), 3.49-3.47 (m, 6H), 3.41 (s, 3H), 3.25 (s, 3H), 3.07 (m, 2H), 2.70 (m, 2H), 2.50 (m, 2H), 2.14-2.06 (m, 6H), 1.94 (t, *J* = 4 & 8 Hz, 2H), 1.71-1.58 (m, 8H), -1.71 (s, 1H), -1.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 184.36, 181.56, 173.58, 172.01, 170.82, 169.56, 169.00, 166.67, 151.03, 147.96, 146.76, 144.78, 140.90, 140.87, 139.08, 136.21, 134.96, 134.84, 134.60, 133.79, 130.35, 129.79, 129.37, 128.27, 127.31, 124.01, 123.00, 122.87, 121.74, 120.76, 114.83, 111.16, 109.54, 102.28, 101.33, 98.84, 93.81, 70.29, 69.55, 55.76, 53.10, 52.26, 51.68, 49.22, 40.39, 38.59, 37.79, 34.26, 31.12, 29.69, 23.12, 20.47, 19.69, 17.76, 12.17, 11.36. LC-MS: 1219 [M+H].

Synthesis of tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (4)

A solution of 4,7,10-trioxa-1,13-tridecanediamine (7.5 g, 34.1 mmol) in $CHCl_3$ (100 mL) was treated with BOC-anhydride (3.7 g, 16.9 mL). The mixture was stirred at room temperature for 12h. The solvent was removed, and the resulting yellow oil was purified by silica gel flash chromatography to produce the oil 5.5 g of tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (**4**), Yield: 49 %. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.1 (s, 1H), 3.58-3.50 (m, 12H), 3.21 (d, *J* = 6.9 Hz, 2H), 2.79 (t, *J* = 8 Hz, 2H), 1.75-1.69 (m, 4H), 1.59 (s, 2H), 1.42 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.0, 69.2, 68.9, 66.7, 48.0, 37.6, 30.4, 28.6, 27.3.

Synthesis of Ce6-diPEG-NHBoc (18)

DME Ce6 **8** (1 g, 1.60 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL). EDCI (368 mg, 1.92 mmol) and HOBt (260 mg, 1.92 mmol) were then added and allowed to stir until completely dissolved under nitrogen. After 30 min, tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)carbamate (**4**) (1.28 g, 4 mmol) and DIEA (414 mg, 3.20 mmol) were mixed in CH_2Cl_2 (20 mL) and added to the reaction mixture. The mixture was allowed to stir at room temperature for 12h under nitrogen. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and then washed with brine and water, respectively. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated. The product was purified via column chromatography to afford 1.2 g of **18**, Yield: 81 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 667 (4.2 X 10^5), 506 (2.1 X 10^5), 410 (7.9 X 10^5) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.68 (s, 1H), 9.63 (s, 1H), 8.79 (s, 1H), 8.07 (m, 1H), 6.37 (dd, *J* = 20 Hz, 1H), 6.14 (dd, *J* = 14.8 Hz, 1H), 5.61 (d,

J = 20 Hz, 1H), 5.27 (d, *J* = 16 Hz, 1H), 4.44-4.34 (m, 3H), 4.05 (m, 1H), 3.82-3.72 (m, 8H), 3.63-3.48 (m, 12H), 3.31 (s, 5H), 2.70 (s, 2H), 2.60-2.49 (m, 3H), 2.35 (m, 2H), 2.20-2.09 (m, 6H), 1.78-1.70 (m, 8H), 1.37 (s, 9H) -1.64 (s, 1H), -1.94 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.55, 169.44, 168.86, 166.76, 154.15, 148.99, 144.79, 138.82, 136.18, 135.01, 134.92, 134.74, 134.62, 130.25, 129.75, 129.35, 128.31, 121.84, 102.15, 101.27, 98.87, 93.69, 79.08, 70.12, 69.67, 69.05, 68.28, 53.07, 52.45, 51.68, 49.07, 39.09, 37.60, 31.06, 29.75, 29.00, 23.14, 19.56, 17.73, 12.18, 11.29. LC-MS: 927 [M+H].

Synthesis of Ce6-diPEGamine (19)

The compound **18** (1.2 g, 1.52 mmol) was dissolved in of dry CH_2Cl_2 (30 mL) in an ice bath under argon. TFA (5 mL) was added, and the reaction mixture was stirred overnight. The reaction mixture was evaporated several times with diethyl ether to remove residual TFA. Then the precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to give a crude compound, purified by silica gel chromatography to give 1 g of **19**, Yield: 93 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 657 (5.0 X 10^5), 501 (1.5 X 10^5), 400 (2.0 X 10^6) nm. ^{1}H NMR($CDCl_3$, 400 MHz): δ 9.63 (s, 1H), 9.58 (s, 1H), 8.78 (s, 1H), 8.04 (m, 1H), 6.33 (dd, *J* = 16 Hz, 1H), 6.12 (dd, *J* = 12 Hz, 1H), 5.58 (d, *J* = 20 Hz, 1H), 5.31 (d, *J* = 16 Hz, 1H), 4.43 and 4.29 (m, 2H), 3.82-3.76 (m, 5H), 3.63-3.56 (m, 9H), 3.45-3.39 (m, 9H) 3.26-3.22 (m, 5H), 2.76 (m, 2H), 2.18-2.13 (m, 8H), 1.73-1.61 (m, 8H), -1.76 (s, 1H), -1.96 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.55, 169.44, 168.86, 166.75, 154.15, 148.99, 144.79, 138.82, 136.18, 135.01, 134.92, 134.74, 134.62, 130.25, 129.75, 129.35, 128.31, 121.84, 102.15, 101.27, 98.87, 93.69, 69.98, 69.67, 68.28, 53.07, 52.45, 51.68, 49.07, 39.09, 37.60, 31.06, 29.75, 23.14, 19.56, 17.73, 12.18, 11.29. LC-MS: 827 [M+H].

Synthesis of chlorin e6-curcumin conjugate (20)

Compound **6** (500 mg, 1.45 mmol) was dissolved in dry CH_2Cl_2. A mixture of HOBt (167 mg, 1.23 mmol), EDCI (236 mg, 1.23 mmol), and DIEA (133 mg, 1.03 mmol) in CH_2Cl_2 was added, the mixture was then allowed to stir for 30 min. Compound **19** (856 mg, 1.03 mmol) and DIEA (133 mg, 1.03 mmol) were mixed in CH_2Cl_2 and added to this reaction mixture. The mixture was stirred overnight. It was diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was purified by silica gel column chromatography to afford 770 mg of **20**, Yield: 41 %. UV-Vis (DMSO): λ_{max} (ε / M^{-1} cm^{-1}) 668 (2.7 X 10^5), 502 (1.5 X 10^5), 407 (6.1 X 10^5) nm. ^{1}H NMR (400MHz, $CDCl_3$): δ 9.67 (s, 1H), 9.63 (s, 1H), 8.80 (s, 1H), 8.09 (m, 1H), 7.53 (d, *J* = 16 H, 1H), 7.46 (d, *J* = 16 H, 1H), 7.20 (m, 1H), 7.06-7.04 (m, 2H), 6.96-6.84 (m, 4H), 6.37-6.32 (m, 3H), 6.15 (dd, *J* = 12 Hz, 1H), 5.65-5.57 (m, 2H), 5.34-5.23 (m, 2H), 4.49 and 4.37 (m, 2H), 4.01 (m, 1H), 3.92-3.89 (m, 4H), 3.82-3.69 (m, 12H), 3.62 (s, 3H), 3.55-3.51 (m, 6H), 3.47 (s, 3H), 3.38-3.36 (m, 2H), 3.31 (s, 3H), 2.89 (m, 2H), 2.64-2.52 (m, 3H), 2.43-2.33 (m, 6H), 2.25-2.09 (m, 5H), 1.84-1.69 (m, 9H), -1.66 (s, 1H), -1.89 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 184.35, 181.66, 173.54, 171.56, 170.96, 169.28, 168.76, 166.90, 154.04, 151.13, 149.07, 147.97, 146.77, 144.74, 140.99, 139.15, 138.75, 136.18, 135.09, 134.80, 134.67, 134.48, 133.87, 130.19, 129.92, 129.43, 128.74, 127.42, 124.09, 123.09, 122.96, 121.71, 121.58, 120.81, 114.81, 111.26, 109.55, 102.42, 101.44, 101.23, 98.80, 93.72, 70.30, 69.99, 69.12, 55.86, 53.10, 52.14, 51.67, 49.19, 39.11, 37.72, 34.84, 32.91, 31.16, 29.67, 29.27, 28.15, 23.09, 20.77, 19.71, 17.81, 12.20, 11.38. LC-MS: 1291 [M+H].

Measurement of Cellular uptake

To examine the cellular permeability of the synthesized compounds AsPC-1 cells were used. Briefly, exponentially growing 2 x 10^5 cells were seeded in six well plates and incubated in dark at 37 ^oC in a CO_2 incubator. After 24h, media was removed and cells were incubated with various synthesized compounds for a different time intervals. Once the incubation was completed, the cells were collected by trypsinization and washed thrice with phosphate buffered saline (PBS). Thereafter, the cells were centrifuged and the pellets were dissolved in 500 µl of PBS to carry out FACS.

Cell Viability Assay

AsPC-1 pancreatic cancer cells were grown in RPMI-1640 medium (life technologies corporation, USA) supplemented with 10% fetal bovine serum (life technologies corporation, USA) and 1% Penicillin & Streptomycin (life technologies corporation, USA), whereas MIA-PaCa2 and PANC-1 cells were grown in DMEM supplemented with 10% fetal bovine serum (life technologies corporation, USA) and 1% Penicillin & Streptomycin (life technologies corporation, USA). Exponentially growing 5.0 x 10^3 cells were seeded in 96 well plates and incubated overnight at 37 ^oC in a CO_2 incubator. Next morning the cells were treated with different concentrations of the synthesized photosensitizers for 3h. After 3h, the cells were irradiated with laser light 660 nm, 50 mW, 9 J/cm^2. The cells were then incubated for 72h in dark at 37 ^0C in a 5% CO_2 incubator. For the evaluation of dark toxicity of the synthesized compounds, the cells were kept under similar conditions without laser irradiation. After 72h, MTT assay was performed for both the groups to determine cell viability.

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Annexin V and Propidium Iodide Staining

To determine the mechanism of cell death annexin v and propidium iodide staining was carried out. Briefly, 2 x 10^5 cells were seeded in six well plates and incubated at 37 ^oC in a CO_2 incubator. After 24h, media was removed and the cells were treated with compound **17**. After 3h cells were irradiated with the light (660 nm, 50 mW, 9 J/cm^2) and incubated for 24h at 37 ^oC in a CO_2 incubator. Thereafter, cells were collected, washed three times with PBS and dissolved in the FACS buffer. Annexin V and PI staining was then carried out with help of FACS assay kit (BD Pharmigen) as per the manufacturer's protocol.

Western Blot

For western blot cells were seeded in six well plates and treated with compound **17**. After 3h, cells were irradiated with the laser light (660 nm, 50 mW, 9 J/cm^2) and incubated for additional 24h. Then the cells were washed with PBS, lysed on ice in modified RIPA buffer (50 mM Tris–HCl pH 7.4, 1% NP-40, 0.25% Sodium Deoxycholate, 150 mM NaCl, 1 mM Na_3VO_4, and 1 mM NaF) containing protease inhibitors (100 µM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, 10 µg/ml pepstatin, and 2 mM EDTA). The lysates were centrifuged at 10,000g for 10 min at 4 ^oC, and the supernatant fractions were collected. The proteins were separated on 10 % SDS-PAGE and transferred to Immobilon P membranes (Millipore Corp., Bedford, MA, USA). The specific proteins were detected using an enhanced chemiluminescence (ECL) Western blotting kit (Amersham, GE Healthcare) according to the manufacturer's instructions.

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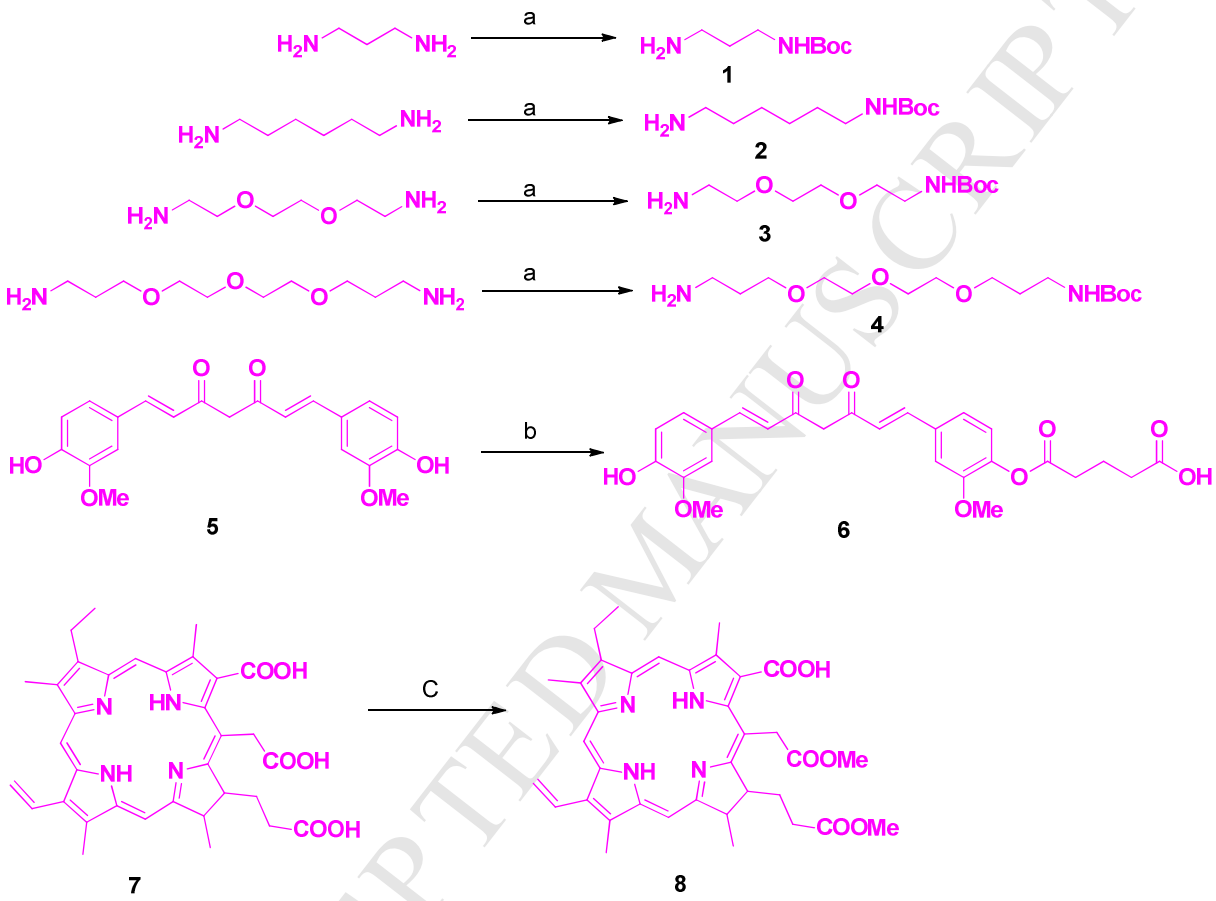

Scheme 1. Reagents and condition: (a) $(Boc)_2O$, $CHCl_3$, rt, 24h; (b) Glutaric anhydride, TEA, DMAP, THF, 12h reflux; (C) 5 % H_2SO_4, MeOH, rt, 12h.

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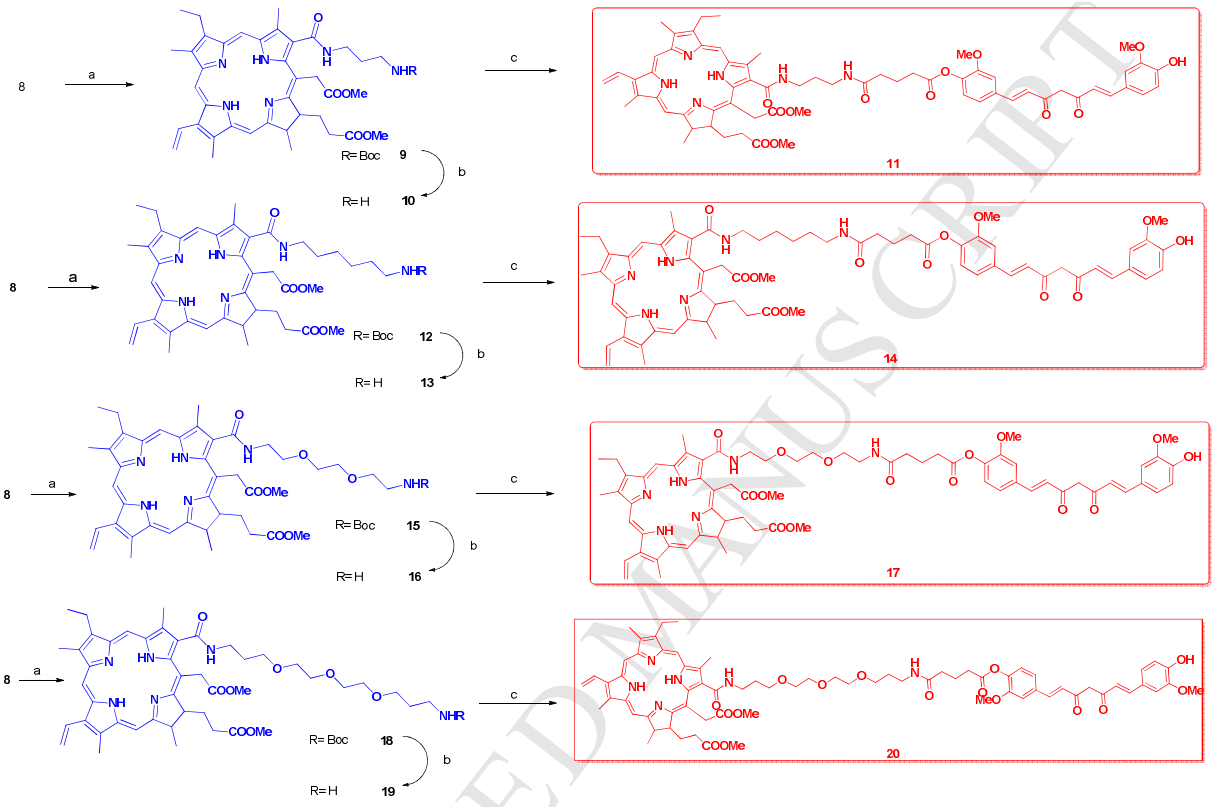

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637 **Scheme 2.** Reagents and condition: (a) EDCI, HOBt, DIEA, **1**, **2**, **3**, **4**, CH_2Cl_2, rt, 12h; (b) TFA,

638 CH_2Cl_2, rt, 12h; (c) EDCI, HOBt, DIPEA, **6**, CH_2Cl_2, rt, 12h.

	AsPC-1			MIA PaCa-2			PANC-1		
Comp	Dark toxicity[a]	Photo toxicity[a]	Ratio	Dark toxicity[a]	Photo toxicity[a]	Ratio	Dark toxicity[a]	Photo toxicity[a]	Ratio
7	126	19.53	6.45	89	15.84	5.61	67	10.14	6.60
11	>300	5.74	>52.26	200	4.2	47.61	>300	4.58	>65.50
14	285	10.07	28.3	265	47.32	5.6	>300	45.56	>6.58
17	186	0.04	4650	83	0.035	2371	>300	0.04	>7500
20	193	5.93	32.54	>300	4.64	>64.65	>300	15.74	>19.05

Table 1. Dark toxicity and Phototoxicity of Ce6-cur conjugates, [a]IC_{50} (μM)

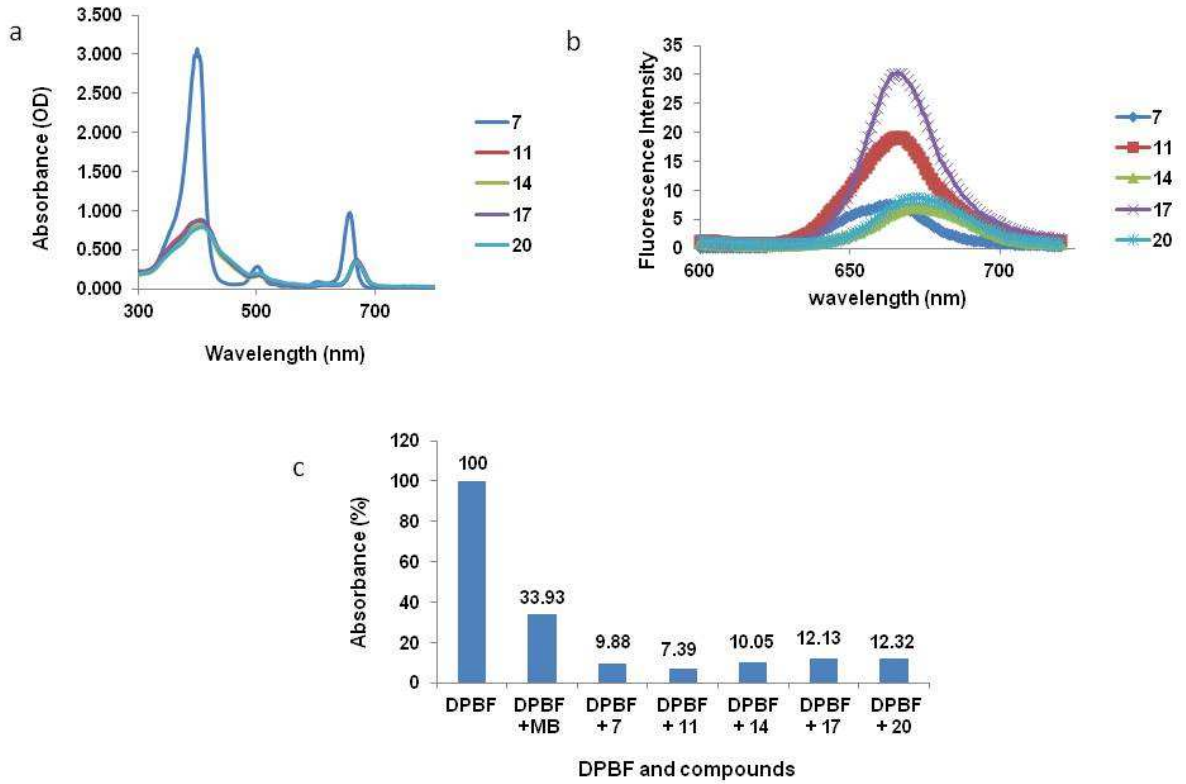

Figure 1. Photophysical properties of Ce6-cur conjugate: (a) absorption spectra of Ce6-cur conjugates; **7** (16 µM, 2 % DMSO, and 98 % DI water), **11**(8.7 µM, 2 % DMSO, and 98 % DI water), **14** (8.4 µM, 2 % DMSO, and 98 % DI water), 1**7** (8.2 µM, 2 % DMSO, and 98 % DI water), **20** (7.7 µM, 2 % DMSO, and 98 % DI water), (b) fluorescence spectra of Ce6-cur conjugates; **7** (16 µM, 2 % DMSO, and 98 % DI water), **11**(8.7 µM, 2 % DMSO, and 98 % DI water), **14** (8.4 µM, 2 % DMSO, and 98 % DI water), 1**7** (8.2 µM, 2 % DMSO, and 98 % DI water), **20** (7.7 µM, 2 % DMSO, and 98 % DI water) (c) Singlet oxygen generation of Ce6-cur conjugates.

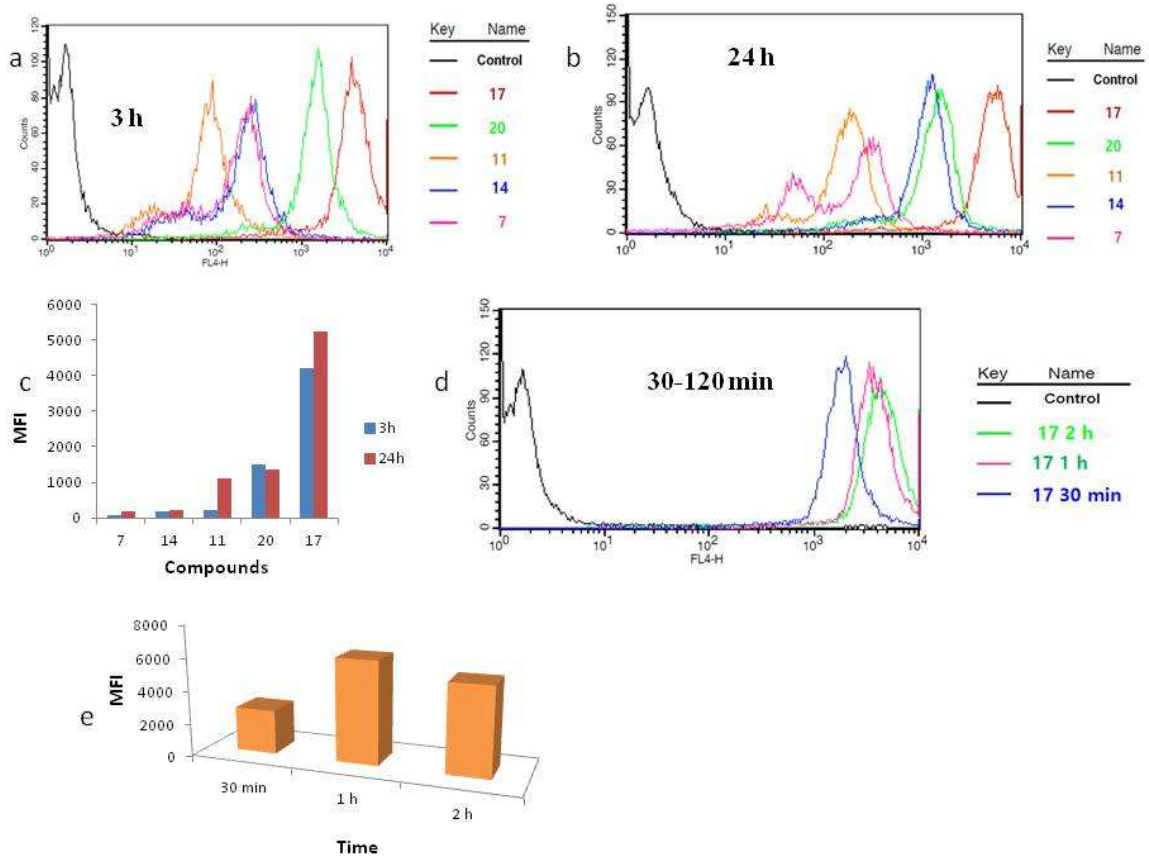

Figure 2. The cellular uptake in AsPC-1 cells at 5µM concentration: (a) after 3h; (b) after 24h; (c) mean fluorescence intensities of Ce6-cur conjugates; (d) uptake of **17** 30-120 min; (e) mean fluorescence intensities of compound **17.**

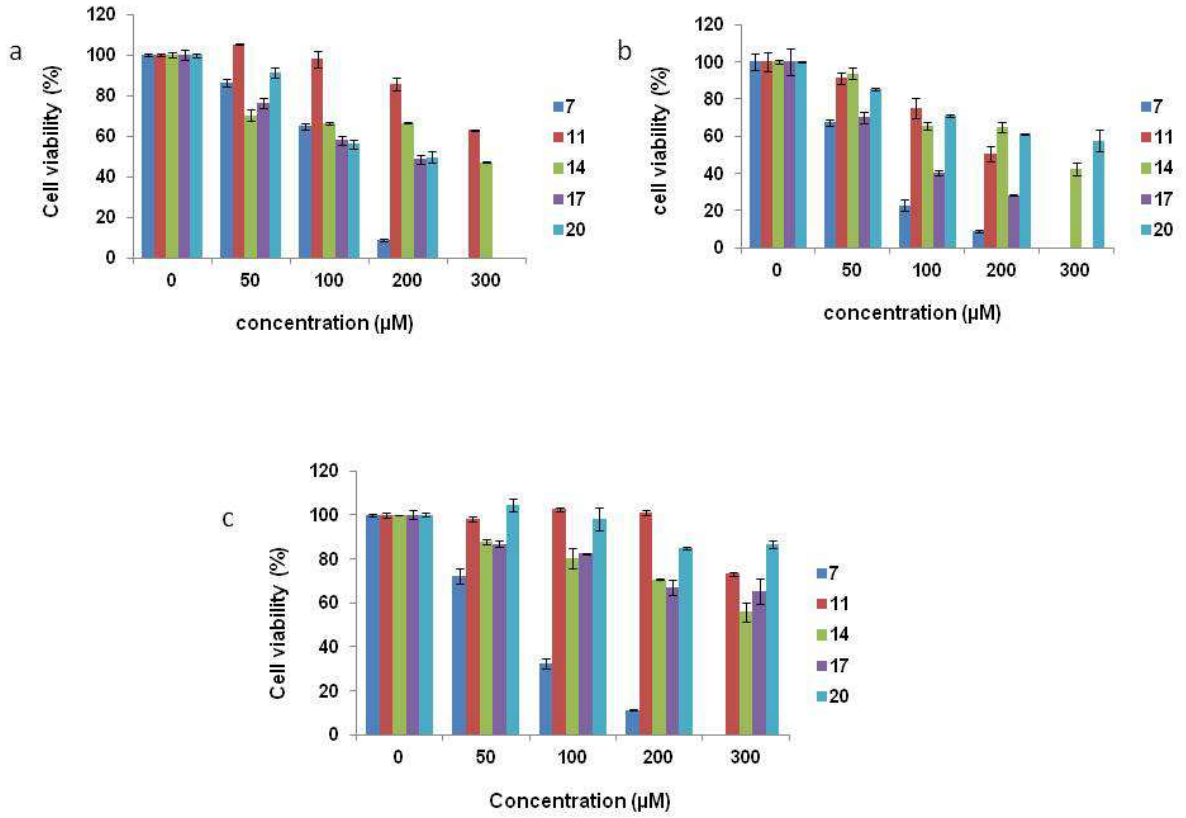

Figure 3. Dark toxicity of Ce6-cur conjugates (a) AsPC-1 cells; (b) MIA PaCa-2 cells; (c) PANC-1cells.

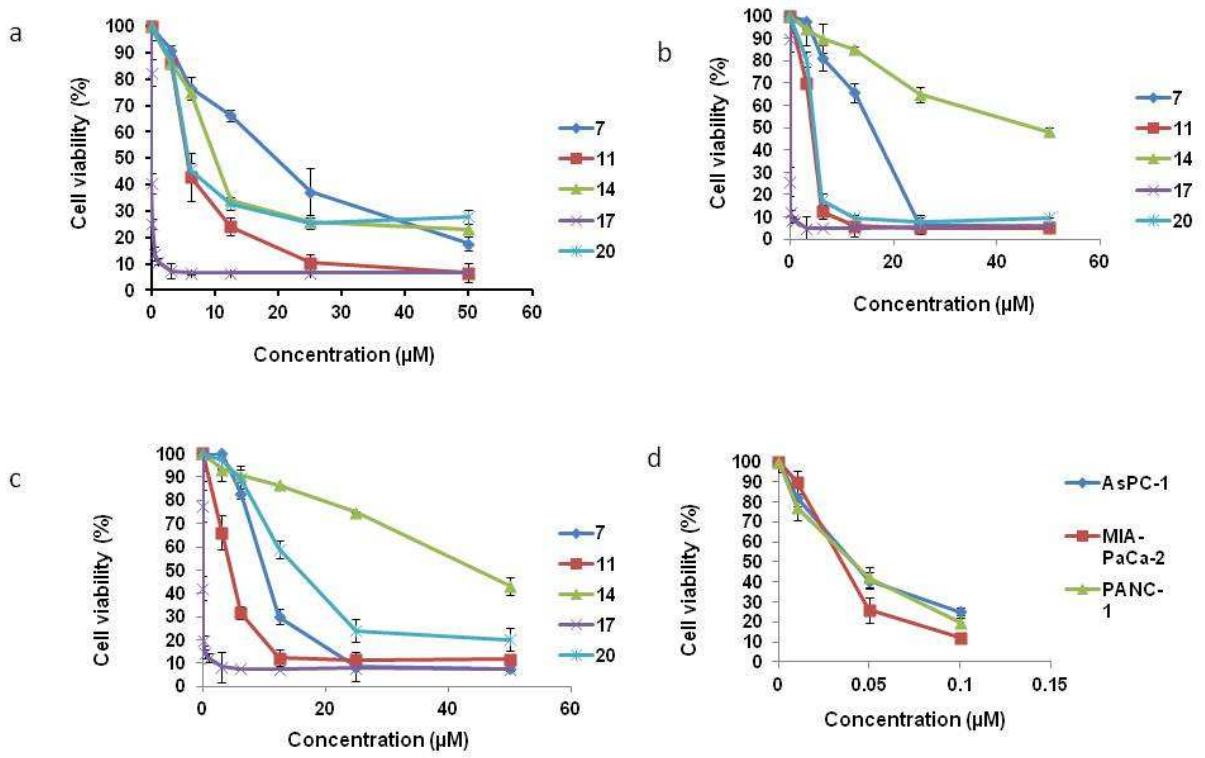

Figure 4. Phototoxicity of Ce6-cur conjugates (a) AsPC-1 cells; (b) MIA PaCa-2 cells; (c) PANC-1cells (d) phototoxicity of **17**.

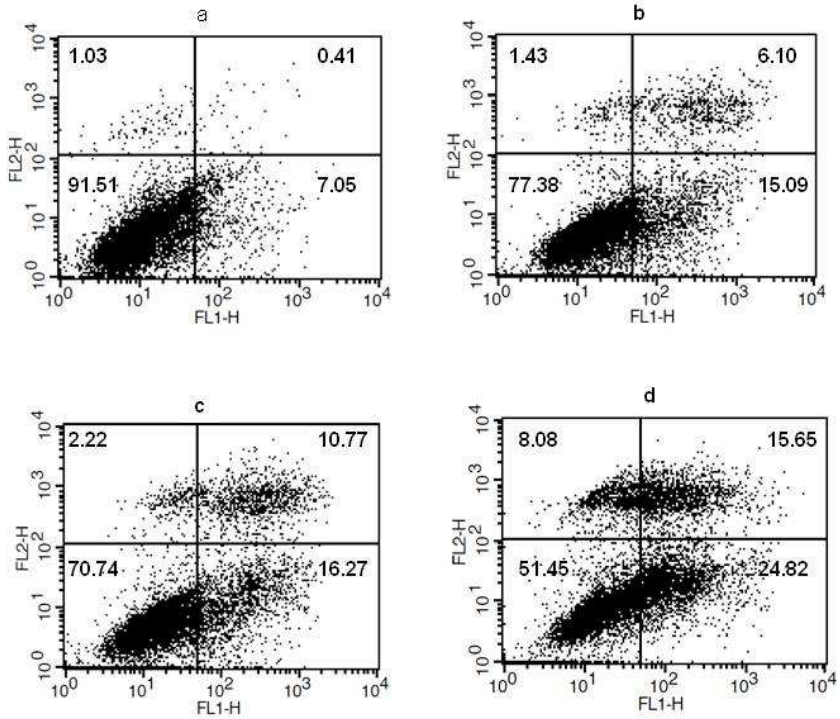

Figure 5. Flow cytometry analysis of AsPC-1 cells with Annexin V/PI double staining after PDT: (A) control; (B) treated with 20 nM of **17** at a light dose of 9 J/cm^2; (C) treated with 40 nM of **17** at a light dose of 9 J/cm^2 (d) treated with 60 nM of **17** at a light dose of 9 J/cm^2.

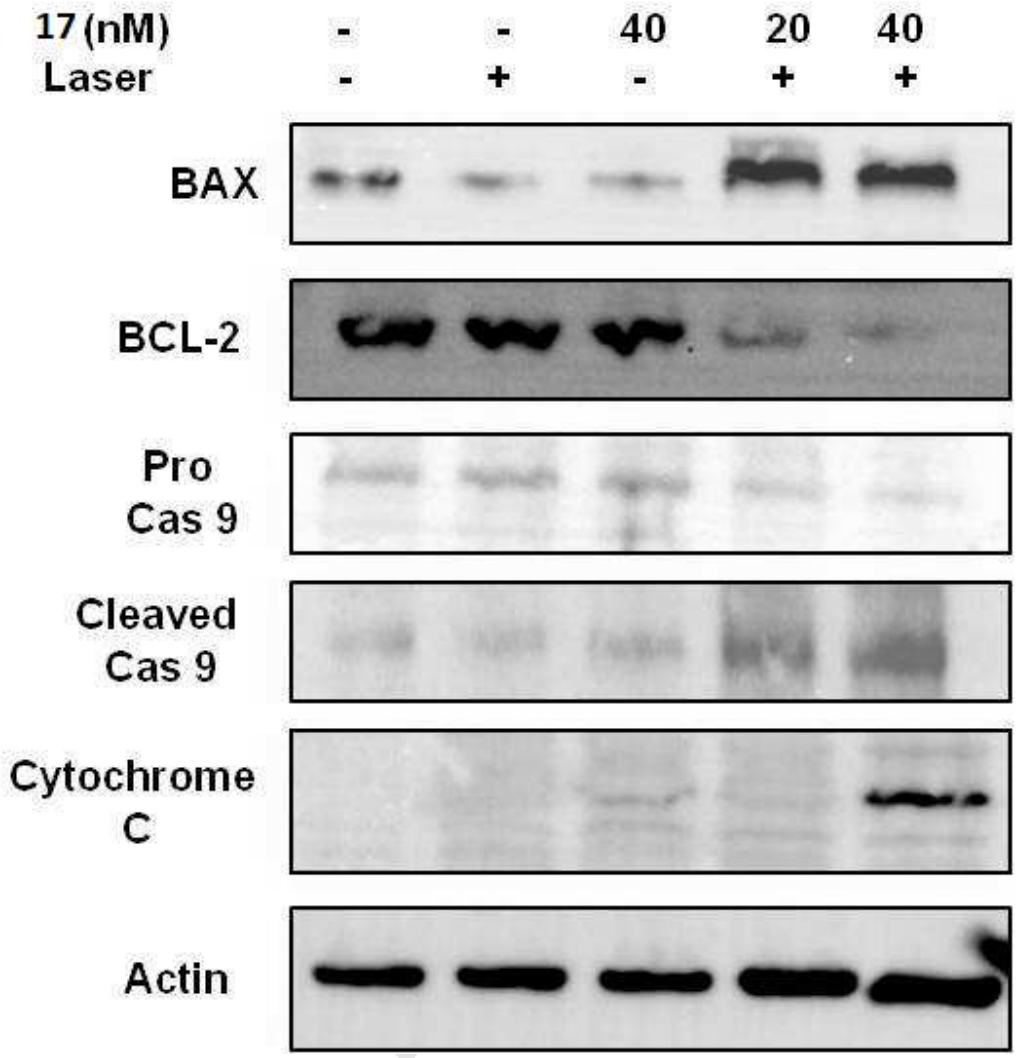

Figure 6. Expression of Apoptotic anti-apoptic markers.The total proteins (80 μM) were extracted and separated by 10% SDS-PAGE. The proteins were subjected to immunoblotting using anti-caspase, BAX, BCL-2, antibodies and anti-cytochrome-C antibody.

Research Highlights

- Four novel Chlorin e6-curcumin conjugates were designed and synthesized
- Compound **17** exhibited excellent PDT efficacy with IC_{50} values in nanomolar range.
- Compound **17** showed exceptional dark/phototoxicity ratio in the range of 2371-7500.
- Compound **17** rapidly internalized into AsPC-1 cells within 30 min and sustained for 24h.
- Compound **17** induced apoptosis in a dose-dependent manner.