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Photocaging of Single and Dual (Similar or Different) Carboxylic and Amino Acids by Acetyl Carbazole and its Application as Dual Drug Delivery in Cancer Therapy

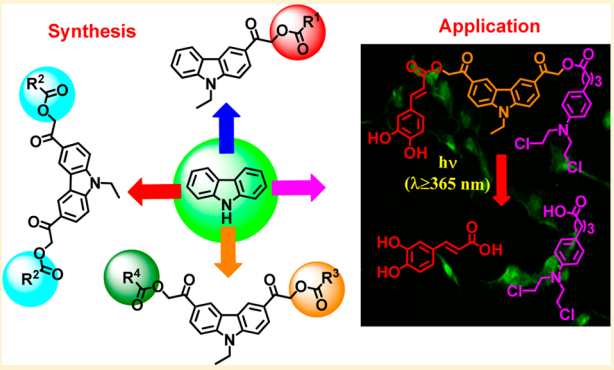
Yarra Venkatesh,[†] Y. Rajesh,[‡] S. Karthik,[†] A C Chetan,[‡] Mahitosh Mandal,[‡] Avijit Jana,[*,§,⊥] and N. D. Pradeep Singh[*,†]

[†]Department of Chemistry, [‡]School of Medical Science and Technology, Indian Institute of Technology Kharagpur, 721302 West Bengal, India

[§]Biomaterials Group, [⊥]Division of Natural Products Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad 500007, India

Supporting Information

ABSTRACT: A new fluorescent photoremovable protecting group (FPRPG) based on acetylcarbazole framework has been explored for the first time release of single and dual (similar or different) substrates from single chromophore. Mechanistic studies of the photorelease process revealed that photorelease of two (similar or different) substrates from acetyl carbazole proceeds via a stepwise pathway. Further, we constructed photoresponsive dual drug delivery system (DDS) to release two different anticancer drugs (caffeic acid and chlorambucil, 1 equiv each). In vitro study reveals that our DDS exhibit excellent properties like biocompatibility, cellular uptake, and photo-regulated dual drug release.



INTRODUCTION

In recent times, photoremovable protecting groups (PRPGs) have gained considerable importance particularly in the area of photoresponsive drug delivery systems (DDSs),[1−3] because of their ability to offer high spatiotemporal control over the release of biologically active molecules on exposure to light. Several PRPGs have been successfully utilized for the construction of photoresponsive DDSs which includes o-nitrobenzyl,[4] coumarinyl-4-ylmethyl,[5] acridin-9-ylmethyl,[6,7] perylene-3-ylmethyl,[8,9] perylene-3,4,9,10-tetrayltetramethyl,[10] 1-acetylpyrene,[11] and quinolin-2-ylmethyl[12−14] derivatives. DDSs constructed so far by the means of the aforementioned PRPGs, can deliver only one active molecule at a given time. Recently, dual DDSs have become a promising strategy particularly in cancer treatment.[15] Hence there is a real need to develop single chromophoric photoresponsive dual DDSs which will release two different drugs simultaneously on exposure to light. On account of this issue here we designed a fluorescent photoremovable protecting group (FPRPG) based on carbazole chromophore with two arms so that two different active molecules can be photocaged and followed by exposure to light will release them simultaneously.

Carbazoles are gaining considerable importance among nitrogen containing heterocyclic compounds, mainly because of their interesting properties, like (i) wide band gap and high luminescence efficiency,[16] (ii) exhibit diverse biological activities,[17] (iii) flexible functionalization on the parent skeleton, etc. The above said unique properties of carbazoles prompted us to design carbazole derived FPRPGs for the photocaging and subsequent release of either one or two equivalent of caged substrate.

Herein, we demonstrated for the first time acetyl carbazole (AC-CBZ) as a FPRPG for the release of single and dual (similar or different) carboxylic acids including amino acids on exposure to UV light ($\lambda \geq 365$ nm). Further, we synthesized a dual drug delivery system using AC-CBZ and demonstrated its ability to perform as a fluorescent imaging agent and phototrigger for delivery of caffeic acid (CA) and chlorambucil (CBL) simultaneously. Primarily CA is reported for possessing antitumor activity and antimetastatic activity.[18] Second CBL, an alkylating agent is mainly used in chronic lymphocytic leukemia.[19] By employing a natural compound like CA in the system, the anticancer activity of CBL gets potentiated in comparison to CBL individually.

RESULTS AND DISCUSSION

Single and dual arm carbazole FPRPGs (**2** and **6**) and their caged esters were synthesized following a sequence of chemical reactions as shown in Scheme 1. First, single arm FPRPG **2** was readily prepared from the commercially available 9*H*-carbazole

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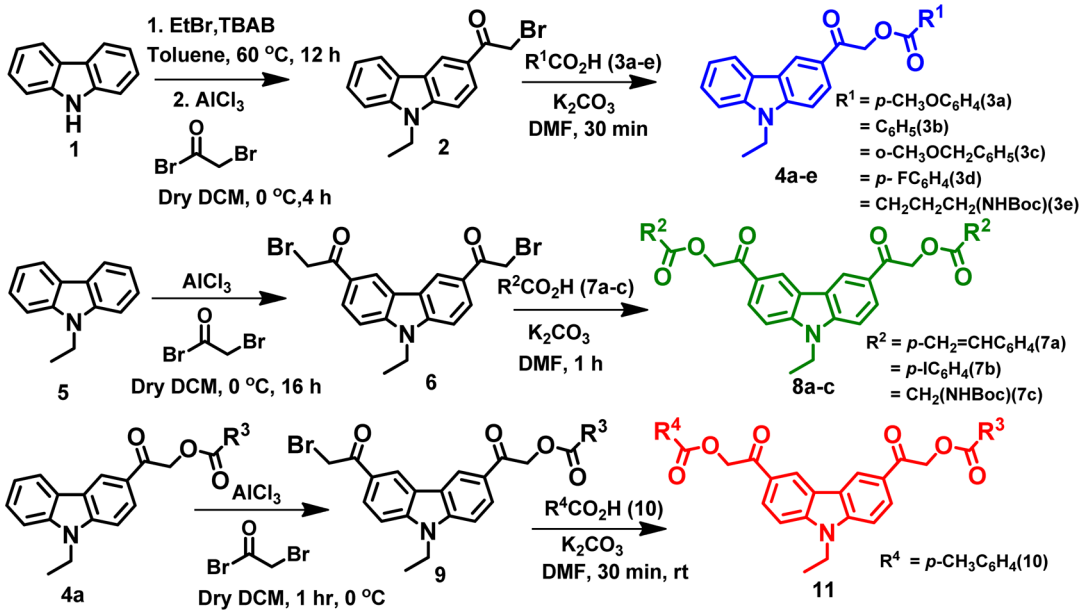
Scheme 1. Synthesis of Single, Dual (Similar or Different) Arm Caged Esters of Acetyl Carbazole



(**1**) by reacting with ethyl bromide followed by Friedel–Crafts (FC) acylation with one equiv of bromoacetyl bromide. The corresponding caged esters **4a–e** of FPRPG **2** were synthesized by carrying out esterification reaction with corresponding carboxylic acids and amino acids **3a–e** in the presence of K_2CO_3. Next, the dual arm FPRPG **6** was prepared by FC acylation of **5** with two equiv of bromoacetyl bromide. Now, the corresponding dual arm caged esters **8a–c** of similar acids were obtained by treating **6** with two equiv of carboxylic acids and amino acids **7a–c**, respectively.

Finally, the dual arm caged ester **11** with two different caged acids (*p*-toluic acid and **3a**, respectively) was synthesized by the means of second FC acylation on **4a** and subsequent esterification reaction with *p*-toluic acid as shown in Scheme 1. All the synthesized caged esters were characterized by ^{1}H, ^{13}C, and mass spectral analysis (see Figure S1–S11 in SI).

UV absorption and emission spectra of 1×10^{-5} M solution of caged esters (**4a–e**, **8a–c**, and **11**) in absolute ethanol were recorded. Figure1a and b shows the normalized absorption and

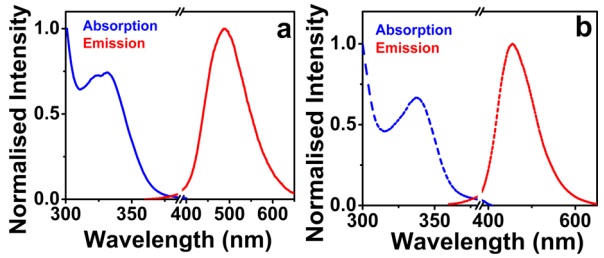


Figure 1. UV absorption and emission spectra of (a) single arm caged ester **4c** (b) dual arm caged ester **11**.

emission spectra of **4c** and **11**, respectively, in absolute ethanol. The absorption spectrum of **4c** and **11** shows intense peak centered at 328 and 338 nm, respectively, while in the emission spectrum the emission maximum of **4c** and **11** were centered at about 488 and 456 nm, respectively. The absorption, emission maxima, Stoke's shifts, and fluorescence quantum yield of all the caged esters are summarized in Table 1. The fluorescence quantum yields (Φ_f) of **4a–e** and **8a–c**, **11** in absolute ethanol (EtOH) at room temperature were in the range of $0.091 < \Phi_f < 0.097$ and $0.27 < \Phi_f < 0.28$, respectively (Table 1). Fluorescence quantum yields were calculated using 9,10-diphenylanthracene as the standard ($\Phi_f = 0.95$ in ethanol).

Considering our main interest to study the application of acetyl carbazole as a FPRPG, we irradiated degassed solution of caged esters **4a–e**, **8a–c**, and **11** (1.0×10^{-4} M) individually in acetonitrile/H_2O (ACN/H_2O) (7:3 v/v) using 125 W medium pressure Hg lamp as the UV light source ($\lambda \geq 365$ nm) and 1 M $CuSO_4$ solution as the UV cut–off filter. Irradiation of single arm caged esters **4a–e** in ACN/H_2O (7:3 v/v) for 60 min results in release of the corresponding carboxylic and amino acids **3a–e** in high chemical (93–97%) and quantum (0.100–0.104) yields as shown in Table 1.

As a representative example, we have shown the course of the photorelease of **4c** at regular intervals of irradiation time by reverse phase (RP) HPLC (Figure 2). The HPLC chart shows gradual depletion of the peak at t_R 5.76 min with an increase in irradiation time, indicating the photodecomposition of **4c**. On the other hand, we also noted a gradual increase of two new major peaks at t_R 4.42 min and t_R 2.64 min, corresponding to the photoproducts 3-(hydroxyacetyl) 9-ethyl 9*H* carbazole and 2-methoxyphenylacetic acid (**3c**), respectively. The corresponding photoproducts were confirmed by injecting authentic sample and also by isolation followed by ^{1}H NMR spectroscopy (see Figure S12 in SI) which depicts clean photocleavage of the ester linkage.

To understand whether the photorelease proceeds through a triplet or singlet excited state, the Stern–Volmer quenching experiment on the carbazole caged ester **4c** (see Figure S15 in SI) was performed by using triplet quencher potassium sorbate (PS). Photolysis of 1×10^{-4} M solution of **4c** was irradiated without addition and with addition of different (0.5×10^{-4}, and 1×10^{-4} M respectively) concentration of a triplet quencher, potassium sorbate (PS) and the course of photolysis was monitored by reverse phase HPLC and the normalized HPLC peak area was plotted against irradiation time (min). From the Figure S15 in SI, it can be seen that on addition of increasing

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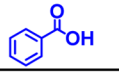
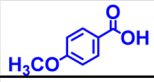
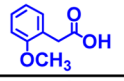
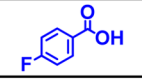
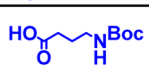
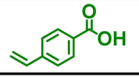
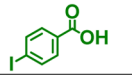
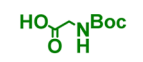
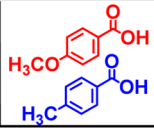
Table 1. Synthetic Yield, Photophysical and Photochemical Data for Caged Esters (4a–e, 8a–c, and 11)

Caged ester	Carboxylic Acid	Synthetic yield[a] %	Absorption	Fluorescence			Deprotection yield[f] %	Quantum yield
			λ_{max} (nm)[b]	λ_{max} (nm)[c]	Stoke's shift (nm)[d]	Φ_f[e]		Φ_p[g]
4a		88	329	487	158	0.097	96	0.103
4b		92	328	488	160	0.096	97	0.104
4c		95	328	486	158	0.094	95	0.102
4d		89	328	488	160	0.095	93	0.100
4e		80	329	486	157	0.091	94	0.101
8a		83	338	456	118	0.27	92	0.099
8b		88	338	459	121	0.28	94	0.101
8c		76	337	458	121	0.27	95	0.102
11		86	338	457	119	0.28	94 92	0.050 0.049

[a]Based on isolated yield. [b]Maximum absorption wavelength. [c]Maximum emission wavelength. [d]Difference between maximum absorption wavelength and maximum emission wavelength. [e]Fluorescence quantum yield (error limit within ±5%). [f]% of the acid released as determined by ^{1}H NMR. [g]Photochemical quantum yield for the acid release (error limit within ±5%).

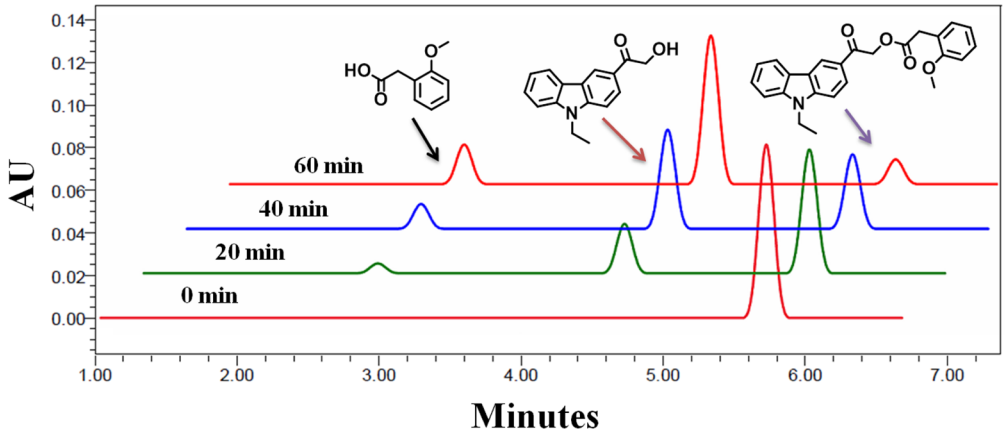


Figure 2. HPLC profile for the photolysis of the caged ester **4c** (1×10^{-4} M) in ACN/H_2O (70:30 v/v) at regular interval of time (0–60 min, time interval =20 min).

amount of PS, rate of photorelease decreases drastically, indicating photocleavage of **4c** proceeds via the triplet excited state.

Based on the Stern–Volmer quenching experiment, solvent effect studies on photorelease, along with the literature[11,13] precedence we suggested a possible mechanism for the photolysis of **4a–e**, **8a–c**, and **11** as shown in Scheme 2. Irradiation of **4c** in aqueous ACN leads to a singlet excited state, which then undergoes intersystem crossing to the triplet state. Cleavage of the ester C–O linkage in carbazole caged esters proceeds from the triplet excited state either by heterolytic or homolytic fashion followed by single electron transfer, to form an ion-pair intermediate. Trapping of the ion-pair intermediate by polar solvent yields 3-(hydroxyacetyl) 9-ethyl 9*H* carbazole along with the free carboxylic acid "2-methoxyphenylacetic acid".

Formation of the 1-(9-ethyl-9H-carbazol-3-yl) ethanoyl carbocation intermediate was supported by the means of trapping of the intermediate by performing photolysis of **4c** in MeOH system for 60 min. Formation of the photoproduct 3-

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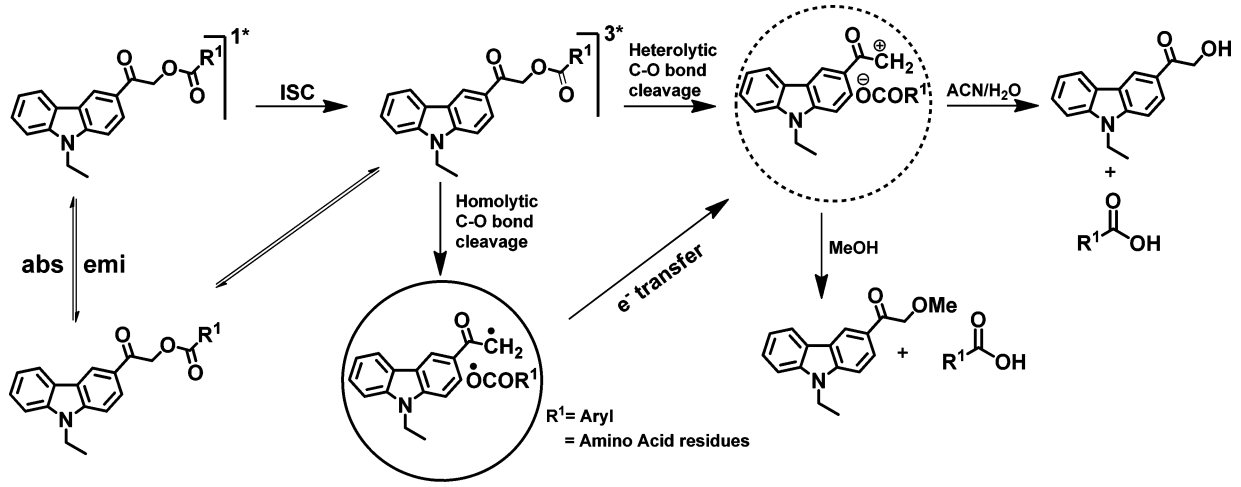
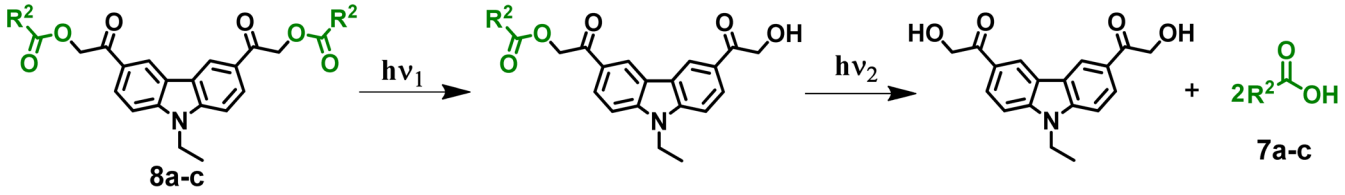
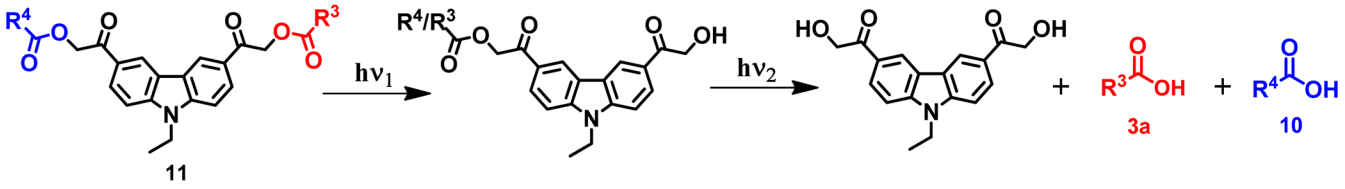
Scheme 2. Possible Photorelease Mechanism



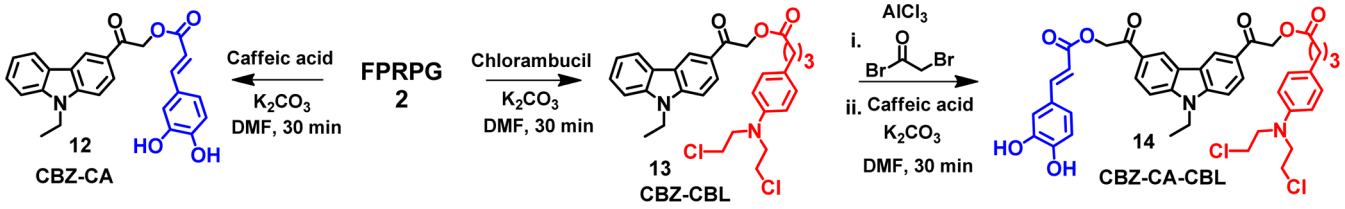
Scheme 3. Stepwise Photorelease of Dual Arm Caged Esters 8a−c



Scheme 4. Stepwise Photorelease of Dual Arm Caged Ester 11



Scheme 5. Synthesis of Photoresponsive CBZ-CA, CBZ-CBL, and CBZ-CA-CBL Conjugates



(methoxyacetyl) 9-ethyl 9*H* carbazole (see Figure S14 in SI) confirms the trapping of 1-(9-ethyl-9*H*-carbazol-3-yl) ethanoyl carbocation by the nucleophilic attack of methanol.

In case of dual arm caged esters **8a−c**, it was observed that photoirradiation results in release of two equivalent of caged carboxylic acids **7a−c** simultaneously (Scheme 3) in high chemical (92−95%) and quantum (0.099−0.102) yields (Table 1). The photoproducts were isolated and analyzed by spectroscopy and in every case we found the released carboxylic and amino acids were the only significant photoproduct in addition to the FPRPGs (see Figure S13 in SI). The photochemical quantum yield (Φ_p) was calculated using potassium ferrioxalate as an actinometer[8] (see pages 23, 24 in the SI).

After successful demonstration of acetyl carbazole as a FPRPG for two similar carboxylic and amino acids. Further, we intended to explore the same FPRPG for the release of two different carboxylic acids simultaneously. We irradiated dual arm caged ester **11** in ACN/H_2O (7:3 v/v) for 60 min and recorded ^{1}H NMR (see Figure S16 in SI) and we found that it releases carboxylic acids **3a** in 94% (Φ_p = 0.05) and **10** in 92% (Φ_p = 0.049).

A stepwise photocleavage of ester linkage for the release of two different carboxylic acids from **11** was proposed. Photoirradiation of **11** results in release of two different carboxylic acids **3a** and **10** along with the production of the photoproduct 3,6-bis(hydroxyacetyl) 9-ethyl 9*H* carbazole through the two probable intermediates as shown in Scheme 4. The above proposed mechanism was further validating by analyzing the photolysate of **11** by the means of high-resolution mass spectrometry (HRMS). After 30 min of photoirradiation of the caged ester **11**, the reaction mixture was subjected to

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HRMS analysis, and it was found that all the possible intermediates (see Scheme S1 in SI) for the stepwise mechanism were present in the reaction mixture along with released carboxylic acids (see Figure S17 in SI).

After successful demonstration of bis-acetyl carbazole as a FPRPG for the simultaneous release two different carboxylic acids, we were interested to explore its ability as a phototrigger for the construction of a DDS with the potency of simultaneous delivery of two different anticancer drugs. For this purpose, we synthesized singly protected CBZ-CA, CBZ-CBL, and dual CBZ-CA-CBL conjugate **14** by caging CA on one arm and CBL on another arm of the FPRPG AC-CBZ as depicted in Scheme 5. The conjugates were characterized by ^{1}H, ^{13}C, and mass spectral analysis (see Figure S18−S20 in SI).

Photorelease of the corresponding drug molecule from the dual caged CBZ-CA-CBL conjugate was performed by photoirradiation of CBZ-CA-CBL conjugate (**14**) in ACN/H_2O (7:3 v/v). After photolysis, the aliquot was evaporated under vacuum and the photolysate was redissolved in $CDCl_3$ with mesitylene as the internal standard and the ^{1}H NMR was recorded (see Figure S21 in SI). The % of CA (91% and Φ_p = 0.046) and CBL (94% and Φ_p = 0.051) released was calculated by ^{1}H NMR spectroscopy.

To investigate the cellular uptake property of the designed dual DDS CBZ-CA-CBL conjugate (**14**), the cell imaging studies were performed by treating the glial cancer cells (U87MG) with 10 μM of DDS **14**. Confocal microscopy study confirms the cellular internalization of DDS **14** into U87MG cells within 3 h of treatment (green coloration, Figure 3ib and ic).

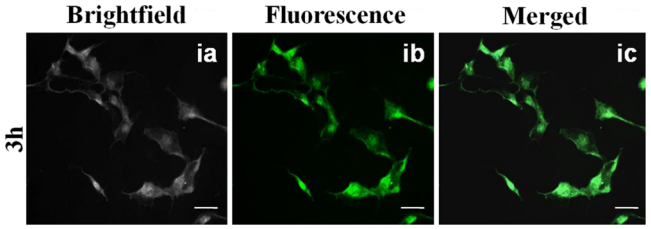

Figure 3. Confocal fluorescence and brightfield images of U87MG cells after 3 h of incubation with DDS **14** exhibited green fluorescence indicating successful internalization (ib and ic) (scale bar = 20 μm).

The in vitro cytotoxicity assay of CBZ-CA-CBL conjugate (**14**) in normal (HaCaT) cells reveals 80% cell viability before photolysis and 40% cell viability after photolysis at higher concentration (80 μM) of DDS **14** (see Figure 4). Henceforth, it could be said that our DDS **14** is biocompatible in nature.

In order to assess the anticancer activity, MTT assay[20] was performed for CBZ-CA, CBZ-CBL, and CBZ-CA-CBL in the cancer cells (U87MG). It was observed that before photolysis cell viability remains more than 70% at highest concentration (25 μM) of CBZ-CA, CBZ-CBL, and CBZ-CA-CBL (Figure 5a). After photolysis, CBZ-CA exhibited above 75% viability (IC_{50} at 15 μM) and CBZ-CBL 60% viability (IC_{50} at 15 μM) individually. But our CBZ-CA-CBL expressed appreciably lower cell viability of 45% (IC_{50} at 9 μM) of CBZ-CA-CBL (Figure 5b). Thus, the efficient anticancer activity of the CBZ-CA-CBL could be attributed to dual effect of released CA and CBL from the CBZ-CA-CBL.

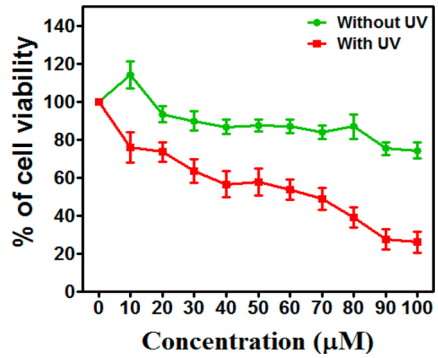

Figure 4. Cell viability assay of DDS **14** in HaCaT cells with and without UV. Values are presented as mean ± SD.

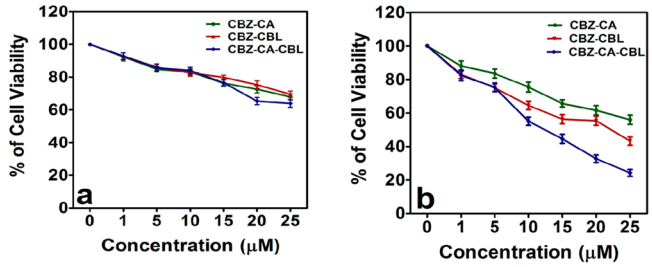

Figure 5. (a,b) Cell viability assay of CBZ-CA, CBZ-CBL, and CBZ-CA-CBL in U87MG cell line: (a) before photolysis, (b) after photolysis. Values are presented as mean ± SD.

CONCLUSION

We have developed single and dual arm fluorescent PRPGs based on Acetyl carbazole for the photocontrolled release of carboxylic and amino acids. Acetyl carbazole showed unique ability to release two (similar or different) carboxylic acids simultaneously in high chemical and quantum yield on exposure to UV light. Further, the photoresponsive dual CBZ-CA-CBL conjugate has been explored for the in vitro cellular imaging and showed good biocompatibility as well as precise drug release of both CBL and CA simultaneously and exhibited enhanced anticancer activity in comparison to singly protected CBZ-CA, CBZ-CBL conjugates. In future, using our newly developed FPRPG we will try to cage two different functional groups like carboxylic acids and alcohols which will open up a new area in the design of photoresponsive DDSs.

EXPERIMENTAL SECTION

General Information. All commercially available anhydrous solvents dimethylformamide (DMF), dichloromethane (DCM), petroleum ether (PE), and ethyl acetate (EA) and other chemicals were used without further purification. Acetonitrile and dichloromethane were distilled from CaH_2 before use. NMR spectra were recorded on a 600 and 400 MHz instrument. ^{1}H NMR chemical shifts were referenced to the tetramethylsilane signal (0 ppm), ^{13}C NMR chemical shifts were referenced to the solvent resonance (77.23 ppm, $CDCl_3$). Chemical shifts (δ) are reported in ppm, and spin−spin coupling constants (J) are given in Hz. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. UV/vis absorption spectra were recorded on UV/vis spectrophotometer and fluorescence spectra were recorded on fluorescence spectrophotometer. High-resolution mass spectra (HRMS) were recorded on ESI-TOF (electrospray ionization-time-of-flight). Photolysis of all the ester conjugates was carried out using a 125 W medium pressure mercury lamp. Chromatographic

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purification was done with 60−120 mesh silica gel. For reaction monitoring, precoated silica gel 60 F254 TLC sheets were used.

*9-Ethyl-9H-carbazole (**5**).* According to a previously reported procedure,[21] a mixture of carbazole (0.21 g, 1.25 mmol), bromoethane (0.54 g, 5.02 mmol), NaOH (aq., 12.5 mol L^{-1}, 5 mL, 62.5 mmol), and a catalytic amount of tetrabutyl ammonium bromide (0.05 g, 0.14 mmol) were charged in a flask. The flask was heated at 70 °C continuously for 12 h. After the completion of the reaction monitored by thin-layer chromatography, the reaction mixture was cooled to room temperature, and extracted with dichloromethane. The organic layer was washed with water and dried over anhydrous sodium sulfate (Na_2SO_4). The solvent was removed under reduced pressure and the crude product was chromatographed on a silica gel column with 20% EtOAc in pet ether as an eluent. White solid (0.20 g, 83%). mp: 68−69 °C (Lit.[22] mp 67−68 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, J = 8.0 Hz, 2H), 7.52 (t, J = 15.2 Hz, 2H), 7.44 (d, J = 8 Hz, 2H), 7.28 (t, J = 14.8 Hz, 2H), 4.43−4.37 (m, 2H), 1.47 (t, J = 14.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.0, 125.6, 122.9, 120.4, 118.8, 108.4, 37.5, 13.8. MALDI-TOF, m/z calcd for $C_{14}H_{13}N$, 196.0; found, 196.7. Physical and NMR spectral data are in accordance with those previously reported.[21]

*2-Bromo-1-(9-ethyl-9H-carbazol-3-yl)ethanone (**2**).* 9-Ethyl carbazole (0.300 g, 1.53 mmol) was dissolved in DCM (20 mL), followed by aluminum chloride ($AlCl_3$) (0.205 g, 1.53 mmol) was added and the solution of bromoacetyl bromide (0.310 g, 1.53 mmol) in DCM (10 mL) was added over 30 min while stirring at 0 °C. After completion of addition, stirring was done at 25 °C for additional 4 h. The reaction mixture was poured in 800 g of ice water and extracted with DCM (100 mL). The organic layer was dried over magnesium sulfate ($MgSO_4$), the solvent was evaporated by rotary evaporator, the residue was purified by column chromatography (eluting agent: chloroform), recrystallized from ethyl acetate−hexane to obtain the product **2**. Light green crystals (0.349 g, 72%). mp: 99−100 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.74 (s, 1H), 8.23−8.02 (m, 2H), 7.53 (q, J = 6.2, 4.6 Hz, 1H), 7.44 (dd, J = 8.9, 3.6 Hz, 1H), 7.41−7.36 (m, 1H), 7.32 (t, J = 7.4 Hz, 1H), 4.57 (s, 2H), 4.36 (q, J = 7.1 Hz, 2H), 1.45 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.9, 143.2, 140.9, 127.1, 126.9, 125.4, 123.3, 123.1, 122.8, 120.9, 120.5, 109.3, 108.5, 38.1, 31.4, 14.0. HRMS (ESI^+) calcd for $C_{16}H_{14}BrNO$ $[M+Na]^+$, 338.0156; found: 338.0160.

*1,1′-(9-Ethyl-9H-carbazole-3, 6-diyl)bis(2-bromoethanone) (**6**).* A sample of 9-ethyl carbazole (0.3 g, 1.53 mmol) was taken in 10 mL of dry DCM. To this solution, $AlCl_3$ (0.43 g, 3.22 mmol) was added and the suspension was stirred for 5 min at room temperature. The reaction mixture was cooled to 0 °C, and bromoacetyl bromide (0.28 mL, 3.22 mmol), in 10 mL of dry DCM, was added dropwise through dropping funnel over a period of 1 h. After addition, the reaction mixture was stirred at room temperature for 16 h followed by the mixture was poured on crushed ice. After melting of ice, the mixture was extracted with DCM (2 × 25 mL), washed with water (20 mL), sat. $NaHCO_3$ (20 mL) and then the organic layer was dried over anhydrous $MgSO_4$. The solvent was removed in vacuo and the crude was purified by column chromatography to obtain the product. Yellowish green crystal (0.604 g, 90%). mp: 184−186 °C (lit.[23] mp 186−190 °C). ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.81 (s, 2H), 8.21 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 4.59 (s, 4H), 4.43 (q, J = 7.4 Hz, 2H), 1.50 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.9, 144.1, 128.1, 126.7, 123.3, 123.0, 109.4, 38.6, 31.1, 14.1. HRMS (ESI^+) calcd for $C_{18}H_{15}Br_2NO_2$ $[M+Na]^+$, 457.9367; found: 457.9368.

General Procedure for the Synthesis of the Ester Conjugates (4a−e). 2-Bromo-1-(9-ethyl-9H-carbazol-3-yl)ethanone(1 equiv) was dissolved in dry *N*,*N*-dimethylformamide (DMF) (2 mL), potassium carbonate (1 equiv), and the corresponding carboxylic and amino acids **3a−e** (1 equiv) were added. The reaction mixture was stirred at room temperature for 30 min. After completion of the reaction it was extracted with ethyl acetate and washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate (EtOAC) in pet ether.

*2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl Benzoate (**4a**).* Treatment of **2** (0.100 g, 0.31 mmol) with benzoic acid (0.038 g, 0.31 mmol) in the presence of potassium carbonate (K_2CO_3) (0.052 g, 0.37 mmol) in dry *N*,*N*-dimethylformamide (DMF) at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product. White solid (0.099 g, 88%). mp: 142−143 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.79 (s, 1H), 8.21 (d, J = 7.5 Hz, 2H), 8.17 (dd, J = 19.8, 8.2 Hz, 2H), 7.65−7.60 (m, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.52−7.48 (m, 3H), 7.35 (t, J = 7.4 Hz, 1H), 5.76 (s, 2H), 4.43 (m, 2H), 1.50 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 166.5, 143.3, 140.9, 133.5, 130.2, 129.9, 128.6, 126.9, 126.0, 123.3, 123.1, 121.6, 121.0, 120.4, 109.3, 108.7, 66.7, 38.1, 14.0. HRMS (ESI^+) calcd for $C_{23}H_{19}NO_3$ $[M+H]^+$, 358.1443; found: 358.1452.

*2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-Methoxybenzoate (**4b**).* Treatment of **2** (0.100 g, 0.31 mmol) with *p*-anisic acid (0.048 g, 0.31 mmol) in the presence of K_2CO_3 (0.052 g, 0.37 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product. Pale yellow solid (0.112 g, 92%). mp: 150−151 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.76 (s, 1H), 8.21−8.08 (m, 4H), 7.54 (t, J = 7.7 Hz, 1H), 7.45 (t, J = 8.7 Hz, 2H), 7.32 (t, J = 7.5 Hz, 1H), 6.96 (d, J = 8.5 Hz, 2H), 5.70 (s, 2H), 4.40 (q, J = 7.3 Hz, 2H), 3.88 (s, 3H), 1.47 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.8, 166.2, 163.8, 143.2, 140.9, 132.3, 126.9, 126.1, 126.0, 123.3, 123.1, 122.3, 121.6, 121.0, 120.4, 113.9, 109.3, 108.6, 66.5, 55.7, 38.1, 14.0. HRMS (ESI^+) calcd for $C_{24}H_{21}NO_4$ $[M+H]^+$, 388.1549; found: 388.1544.

*2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl 2-(2-Methoxyphenyl)-acetate (**4c**).* Treatment of **2** (0.100 g, 0.31 mmol) with *o*-methoxyphenylacetic acid (0.052 g, 0.31 mmol) in the presence of K_2CO_3 (0.052 g, 0.37 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product. White solid (0.120 g, 95%). mp: 116−117 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.63 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 8.01 (dd, J = 8.5, 1.9 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.40 (d, J = 8.3 Hz, 1H), 7.36 (d, J = 8.7 Hz, 1H), 7.28−7.24 (m, 2H), 7.23−7.20 (m, 1H), 6.89 (t, J = 7.4 Hz, 1H), 6.83 (d, J = 8.3 Hz, 1H), 5.45 (s, 2H), 4.34 (q, J = 7.4 Hz, 2H), 3.82 (s, 2H), 3.79 (s, 3H), 1.40 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.7, 171.7, 157.8, 143.2, 140.8, 131.3, 128.8, 126.9, 126.0, 125.9, 123.3, 123.0, 123.0, 121.6, 121.0, 120.8, 120.4, 110.7, 109.3, 108.6, 66.5, 55.7, 38.1, 35.7, 14.0. HRMS (ESI^+) calcd for $C_{25}H_{23}NO_4$ $[M+H]^+$, 402.1705; found: 402.1710.

*2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-Fluorobenzoate (**4d**).* Treatment of **2** (0.100 g, 0.31 mmol) with 4-fluorobenzoic acid (0.048 g, 0.31 mmol) in the presence of K_2CO_3 (0.052 g, 0.37 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product. White solid (0.105 g, 89%). mp: 149−150 °C. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.75 (s, 1H), 8.21 (dd, J = 8.9, 5.4 Hz, 2H), 8.17−8.10 (m, 2H), 7.54 (t, J = 7.1 Hz, 1H), 7.49−7.43 (m, 2H), 7.33 (t, J = 7.4 Hz, 1H), 7.15 (t, J = 8.7 Hz, 2H), 5.73 (s, 2H), 4.41 (q, J = 7.3 Hz, 2H), 1.47 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.4, 166.0 (d, $^1J_{C-F}$ = 252.7 Hz), 165.5, 143.3, 140.9, 132.9, 132.8, 126.9, 126.2, 126.1, 126.0, 125.8, 123.3, 123.1, 121.6, 121.0, 120.5, 115.6 (d, $^2J_{C-F}$ = 21.9 Hz), 109.3, 108.7, 66.8, 38.1, 14.0. HRMS (ESI^+) calcd for $C_{23}H_{18}FNO_3$ $[M+H]^+$, 376.1349; found: 376.1374.

*2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-((tert-Butoxycarbonyl)-amino)butanoate (**4e**).* Treatment of **2** (0.100 g, 0.31 mmol) with 4-((tert-butoxycarbonyl) amino) butanoic acid (0.048 g, 0.31 mmol) in the presence of K_2CO_3 (0.052 g, 0.37 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product. White solid (0.110 g, 80%). mp: 128−129 °C. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.70 (s, 1H), 8.15 (d, J = 7.7 Hz, 1H), 8.08 (s, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.49−7.41 (m, 2H), 7.32 (t, J = 7.4 Hz,

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1H), 5.52 (s, 2H), 4.82 (s, 1H), 4.37 (s, 2H), 3.26 (t, J = 6.6 Hz, 2H), 2.59 (t, J = 7.2 Hz, 2H), 1.95 (m, J = 7.0 Hz, 2H), 1.48 (t, J = 7.2 Hz, 3H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.6, 173.1, 156.3, 143.3, 140.9, 126.9, 125.9, 125.7, 123.3, 123.1, 121.5, 121.0, 120.5, 109.3, 108.6, 66.2, 38.1, 31.5, 28.6, 25.5, 14.0. HRMS (ESI$^+$) calcd for $C_{25}H_{30}N_2O_5$ $[M+H]^+$, 439.2233; found: 439.2224.

General Procedure for the Synthesis of the Ester Conjugates (8a−c). 1,1′-(9-Ethyl-9H-carbazole-3,6-diyl)bis(2-bromoethanone) (1 equiv) was dissolved in dry *N*,*N*-dimethylformamide (DMF) (2 mL), potassium carbonate (2 equiv), and the corresponding carboxylic and amino acids **7a−c** (2 equiv) were added. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction it was extracted with ethyl acetate and washed with saturated NaCl solution and dried over Na_2SO_4. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate (EtOAC) in pet ether.

*(9-Ethyl-9H-carbazole-3,6-diyl)bis(2-oxoethane-2,1-diyl)bis(4-vinylbenzoate) (**8a**).* Treatment of **6** (0.100 g, 0.22 mmol) with 4-vinylbenzoic acid (0.067 g, 0.44 mmol) in the presence of K_2CO_3 (0.052 g, 0.44 mmol) in dry DMF at room temperature for a period of 1 h. The crude conjugate was purified by column chromatography using 40% EtOAc in pet ether to give the product. Pale yellow solid (0.108 g, 83%). mp: 178−179 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.73 (s, 2H), 8.16 (d, J = 8.5 Hz, 2H), 8.12 (d, J = 8.0 Hz, 4H), 7.47 (t, J = 9.0 Hz, 6H), 6.76 (dd, J = 17.6, 10.9 Hz, 2H), 5.88 (d, J = 17.6 Hz, 2H), 5.72 (s, 4H), 5.39 (d, J = 10.9 Hz, 2H), 4.38 (q, J = 7.2 Hz, 2H), 1.47 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.5, 166.1, 143.9, 142.5, 136.2, 130.5, 128.8, 127.0, 126.9, 126.4, 123.1, 121.7, 116.9, 109.4, 66.7, 38.4, 29.9, 14.1. HRMS (ESI$^+$) calcd for $C_{36}H_{29}NO_6$ $[M+H]^+$, 572.2073; found: 572.2073.

*(9-Ethyl-9H-carbazole-3,6-diyl)bis(2-oxoethane-2,1-diyl)bis(4-iodobenzoate) (**8b**).* Treatment of **6** (0.100 g, 0.22 mmol) with 4-iodobenzoic acid (0.113 g, 0.44 mmol) in the presence of K_2CO_3 (0.052 g, 0.44 mmol) in dry DMF at room temperature for a period of 1 h. The crude conjugate was purified by column chromatography using 40% EtOAc in pet ether to give the product. Pale yellow solid (0.155 g, 88%). mp: 183−184 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.74 (s, 2H), 8.16 (d, J = 8.5 Hz, 2H), 7.86 (d, J = 7.9 Hz, 4H), 7.82 (d, J = 8.2 Hz, 4H), 7.49 (d, J = 8.5 Hz, 2H), 5.72 (s, 4H), 4.41 (q, J = 7.2 Hz, 2H), 1.48 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.1, 165.9, 144.0, 138.0, 131.6, 129.2, 126.9, 126.9, 123.2, 121.7, 109.5, 101.5, 66.8, 38.5, 14.1. HRMS (ESI$^+$) calcd for $C_{32}H_{23}I_2NO_6$ $[M+H]^+$, 771.9693; found: 771.9679.

*(9-Ethyl-9H-carbazole-3,6-diyl)bis(2-oxoethane-2,1-diyl)bis(2-((tert-butoxycarbonyl)amino)acetate) (**8c**).* Treatment of **6** (0.100 g, 0.22 mmol) with 2-((tert-butoxycarbonyl) amino) acetic acid (0.080g, 0.44 mmol) in the presence of K_2CO_3 (0.052 g, 0.44 mmol) in dry DMF at room temperature for a period of 1 h. The crude conjugate was purified by column chromatography using 40% EtOAc in pet ether to give the product. Dark yellow solid (0.108 g, 76%). mp: 148−149 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.64 (s, 2H), 8.10 (s, 2H), 7.46 (d, J = 8.7 Hz, 2H), 5.57 (s, 4H), 5.19 (s, 2H), 4.39 (q, J = 7.0 Hz, 2H), 4.20 (d, J = 5.3 Hz, 4H), 1.48 (t, 3H)1.48 (s, 18H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.9, 170.5, 156.0, 144.0, 126.8, 123.1, 121.6, 109.5, 80.3, 66.8, 42.6, 38.5, 28.6, 14.1. HRMS (ESI$^+$) calcd for $C_{32}H_{39}N_3O_{10}$ $[M+H]^+$, 626.2714; found: 626.2663.

*2-(9-Ethyl-6-(2-((4-methoxybenzoyl)oxy)acetyl)-9H-carbazol-3-yl)-2-oxoethyl 4-methylbenzoate (**11**).* Compound **4a** (0.100 g, 0.25 mmol) was dissolved in DCM (20 mL), followed by $AlCl_3$ (0.041 g, 0.25 mmol) was added and the solution of bromoacetyl bromide (0.057 g, 0.25 mmol) in DCM (10 mL) was added over 30 min while stirring at 0 °C. After the completion of addition, stirring was done at 25 °C for 1 h. The reaction mixture was poured in 100 g of ice water and extracted with DCM (100 mL). The organic layer was dried over $MgSO_4$, the solvent was evaporated to give the product **9** (white solid (0.111 g, 85%)) and in situ reaction of **9** (0.100 g, 0.19 mmol) with *p*-toluic acid (10) (0.040 g, 0.19 mmol) in the presence of K_2CO_3 (0.052 g, 0.39 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 40% EtOAc in pet ether to give the product. White solid (0.095 g, 86%). mp: 185−186 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.79 (s, 2H), 8.23−8.18 (m, 2H), 8.18−8.13 (m, 2H), 8.09 (d, J = 7.9 Hz, 2H), 7.52 (d, J = 8.7 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 7.03−6.94 (m, 2H), 5.73 (s, 2H), 5.72 (s, 2H), 4.44 (q, J = 7.3 Hz, 2H), 3.89 (s, 3H), 2.45 (s, 3H), 1.51 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.6, 191.4, 166.3, 165.9, 163.7, 144.1, 143.7, 132.1, 130.1, 129.2, 126.9, 126.9, 126.8, 126.7, 123.0, 121.9, 121.5, 113.7, 109.2, 66.4, 66.3, 55.5, 38.3, 21.7, 13.9. HRMS (ESI$^+$) calcd for $C_{34}H_{29}NO_7$ $[M+H]^+$, 564.2022; found: 564.2014.

*(E)-2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl 3-(3,4-dihydroxyphenyl)acrylate (**12**).* Treatment of **2** (0.100 g, 0.31 mmol) with caffeic acid (0.096 g, 0.31 mmol) in the presence of K_2CO_3 (0.052 g, 0.37 mmol) in dry DMF at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product **12**. Dark yellow solid (0.105 g, 80%). mp: 157−158 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.32 (d, J = 7.7 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.57−7.52 (m, 2H), 7.30 (t, J = 7.5 Hz, 1H), 7.09 (s, 1H), 7.01 (d, J = 7.5 Hz, 1H), 6.72 (d, J = 7.6 Hz, 1H), 6.38 (d, J = 15.8 Hz, 1H), 5.69 (s, 2H), 4.52 (q, J = 7.2 Hz, 2H), 1.34 (t, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 192.4, 166.7, 146.7, 145.0, 142.9, 140.7, 127.1, 126.6, 126.0, 125.8, 123.2, 122.9, 122.4, 122.2, 122.0, 121.4, 120.4, 116.4, 115.1, 113.0, 110.3, 109.7, 66.6, 37.8, 14.2. HRMS (ESI$^+$) calcd for $C_{25}H_{21}NO_5$ $[M+H]^+$, 416.1498; found: 416.1520.

*2-(9-Ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-(4-(bis(2-chloroethyl)-amino)phenyl)butanoate (**13**).* Treatment of **2** (0.100 g, 0.31 mmol) with chlorambucil (0.096 g, 0.31 mmol) in the presence of K_2CO_3 (0.052 g, 0.37 mmol) in dry *N*,*N*-dimethylformamide (DMF) at room temperature for a period of 30 min. The crude conjugate was purified by column chromatography using 20% EtOAc in pet ether to give the product **12**. Light yellow solid (0.150 g, 88%). mp: 150−152 °C. ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.70 (s, 1H), 8.16 (d, J = 7.7 Hz, 1H), 8.08 (dd, J = 8.8, 1.7 Hz, 1H), 7.62−7.44 (m, 2H), 7.42 (d, J = 8.6 Hz, 1H), 7.35 (t, J = 7.3 Hz, 1H), 7.21−7.10 (m, 2H), 6.71−6.63 (m, 2H), 5.52 (s, 2H), 4.37 (q, J = 7.3 Hz, 2H), 3.72 (t, J = 7.4 Hz, 4H), 3.65 (t, J = 6.7 Hz, 4H), 2.69 (t, J = 7.6 Hz, 2H), 2.58 (t, J = 7.4 Hz, 2H), 2.07 (p, J = 7.5 Hz, 2H), 1.46 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.5, 173.2, 144.3, 143.0, 140.6, 130.8, 129.8, 126.7, 125.7, 125.6, 123.1, 122.8, 121.3, 120.8, 120.2, 112.2, 109.1, 108.4, 66.0, 53.6, 40.6, 37.9, 33.9, 33.3, 26.9, 13.8. HRMS (ESI$^+$) calcd for $C_{30}H_{32}Cl_2N_2O_3$ $[M+Na]^+$, 561.1688; found: 561.1688.

*(E)-2-(6-(2-((3-(3,4-Dihydroxyphenyl)acryloyl)oxy)acetyl)-9-ethyl-9H-carbazol-3-yl)-2-oxoethyl 4-(4-(Bis(2-chloroethyl)amino)-phenyl)butanoate (**14**).* Compound **12** (0.100 g, 0.18 mmol) was dissolved in DCM (20 mL). $AlCl_3$ (0.29 g, 0.18 mmol) was added and the solution of bromoacetyl bromide (0.037 g, 0.18 mmol) dissolved in dichloromethane 10 mL was added over 30 min while stirring at 0 °C. After the completion of reaction, stirring was done at 25 °C for 1 h. The reaction solution was poured in 400 g of ice water and extracted with dichloromethane 100 mL. The organic layer was dried over $MgSO_4$, the solvent was evaporated to give the product (0.105 g, 86%) and in situ reaction of **13** (0.100 g, 0.15 mmol) with caffeic acid (0.027 g, 0.15 mmol) in the presence of K_2CO_3 (0.020 g, 0.18 mmol) in dry DMF at room temperature for a period of 30 min afforded the conjugate **14**. The crude conjugate was purified by column chromatography using 40% EtOAc in pet ether to give the product. Dark yellow solid (0.089 g, 78%). mp: 180−182 °C. ^{1}H NMR (600 MHz,) δ 8.86 (s, 1H), 8.77 (s, 1H), 8.20 (d, J = 8.6 Hz, 1H), 8.13 (d, J = 8.2 Hz, 1H), 7.69 (d, J = 16.0 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.19 (s, 1H) 7.15 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 7.6 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.71 (d, J = 7.4 Hz, 2H), 6.40 (d, J = 15.8 Hz, 1H), 5.57 (s, 2H), 5.54 (s, 2H), 4.44 (q, J = 7.2 Hz, 2H), 3.70 (t, J = 7.4 Hz, 4H), 3.63 (t, J = 6.8 Hz, 4H), 2.66 (t, J = 7.5 Hz, 2H), 2.55 (t, J = 7.4 Hz, 2H), 2.04 (q, J = 7.5 Hz, 2H), 1.49 (t, J = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.6, 191.0, 173.3, 167.9, 148.5, 146.1, 145.9, 144.02, 144.0, 134.4, 132.4, 130.1, 127.1, 127.0, 126.9, 126.9, 126.3, 123.2, 123.2, 121.7, 115.7, 114.7, 114.3, 112.9, 109.5, 66.7, 66.1, 54.1, 40.5, 38.5, 34.1, 33.5, 27.0, 14.1. HRMS (ESI$^+$) calcd for $C_{41}H_{40}Cl_2N_2O_8$ $[M+H]^+$, 759.2240; found: 759.2220.

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Characterization of Photoproducts. *3-(Hydroxyacetyl) 9-Ethyl 9H Carbazole.* ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.65 (s, 1H), 8.13 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 7.59−7.50 (m, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 7.32 (t, J = 7.5 Hz, 1H), 4.99 (s, 2H), 4.35 (t, J = 7.2 Hz, 2H), 3.45 (s, 1H), 1.44 (t, J = 7.3 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 197.5, 143.4, 140.8, 126.9, 125.7, 124.7, 123.1, 123.1, 121.4, 120.9, 120.4, 109.3, 108.6, 77.5, 77.2, 76.9, 65.3, 38.0, 14.0. HRMS (ESI$^+$) calcd for $C_{16}H_{15}NO_2$ $[M+H]^+$, 254.1181; found: 254.1169.

3-(Methoxyacetyl) 9-Ethyl 9H Carbazole. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.73 (s, 1H), 8.16 (d, J = 7.7 Hz, 1H), 8.10 (d, J = 8.6 Hz, 1H), 7.52 (t, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 4.85 (s, 2H), 4.38 (t, J = 7.2 Hz, 2H), 3.57 (s, 1H), 1.45 (t, J = 7.3 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 197.5, 143.4, 140.8, 126.9, 125.7, 124.7, 123.1, 123.1, 121.4, 120.9, 120.4, 109.3, 108.6, 75.6, 59.6, 38.0, 14.0. HRMS (ESI$^+$) calcd for $C_{17}H_{17}NO_2$ $[M+H]^+$, 268.1338; found: 268.1324.

3,6-Bis(hydroxyacetyl) 9-Ethyl 9H Carbazole. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.74 (s, 2H), 8.14 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 5.03 (s, 4H), 4.45 (q, J = 7.2 Hz, 2H), 2.75 (s, 1H), 1.49 (d, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.6, 144.3, 126.8, 126.2, 123.3, 121.6, 109.5, 65.5, 38.6, 14.0. HRMS (ESI$^+$) calcd for $C_{18}H_{17}NO_4$ $[M+H]^+$, 312.1236; found: 312.1245.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02152.

Synthesis details, characterization data, and other experimental details (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*janaavijit2@gmail.com; avijit@iict.res.in.

*ndpradeep@chem.iitkgp.ernet.in.

Notes

The authors declare no competing financial interest.

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