Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Targeted,Image Guided & Dually Locked Photoresponsive Drug Delivery System

S. Karthik,[a] B. N. Prashanth Kumar,[b] Moumita Gangopadhyay,[a] Mahitosh Mandal,[b] and N. D. Pradeep Singh*[a]

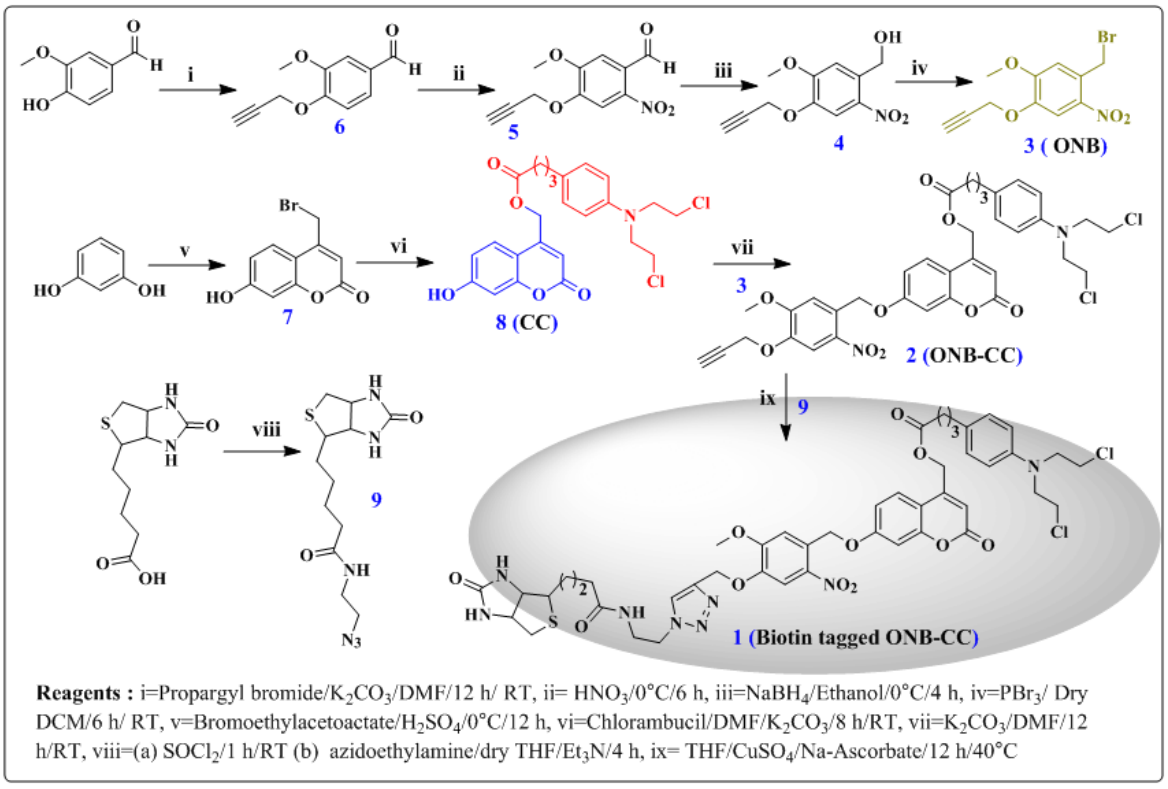
[a] Department of Chemistry, Indian Institute of Technology Kharagpur;

[b] School of Medical Science and Technology, Indian Institute of Technology Kharagpur

Supporting Information

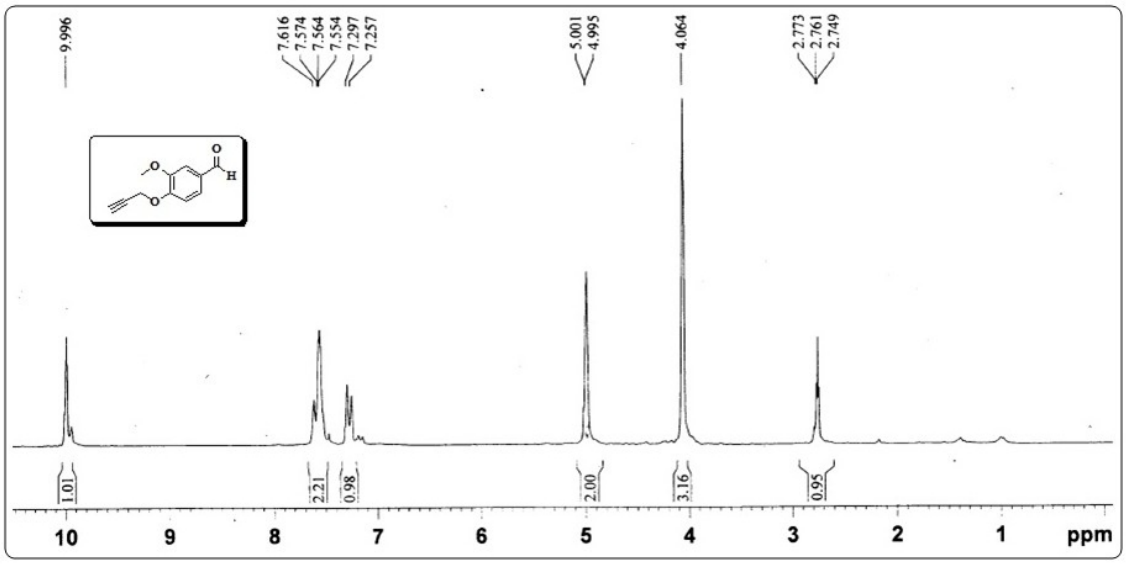
1) General Information: All reagents were purchased from Sigma Aldrich and used without further purification. Acetonitrile and dichloromethane were distilled from CaH_2 before use. ^1H NMR spectra were recorded on a BRUKER-AC 200 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). ^{13}C NMR (50 MHz) spectra were recorded on a BRUKER-AC 200 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 77.0 ppm). UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. Photolysis of the conjugates were carried out using 125 W medium pressure Hg lamp supplied by SAIC (India). Chromatographic purification was done with 60-120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. RP-HPLC was taken using mobile phase acetonitrile, at a flow rate of 0.6mL / min (detection: UV 254 nm).

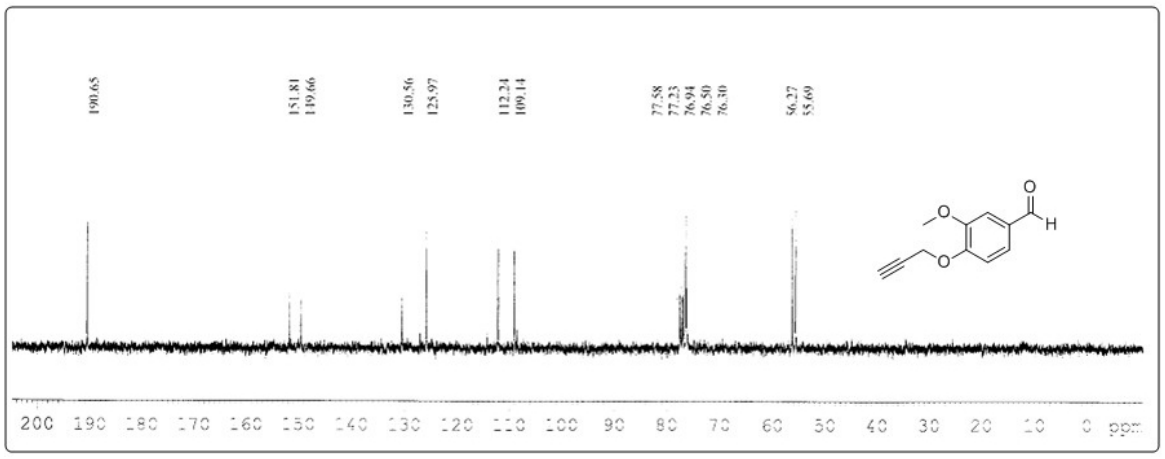
2) Synthesis of biotin tagged O-nitrobenzoyl caged Coumarin chlorambucil

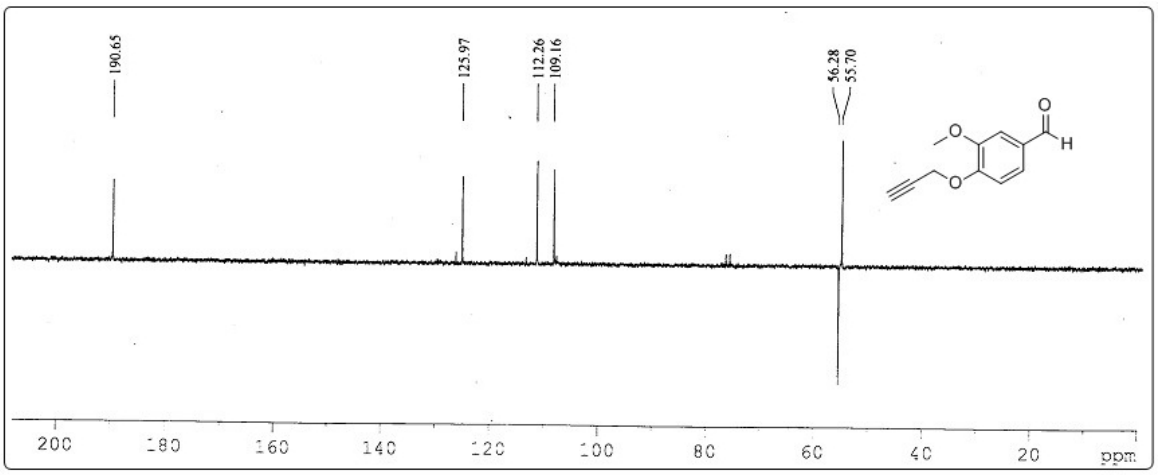


Scheme S1: Synthesis of biotin tagged O-nitrobenzoyl caged coumarin chlorambucil

3-methoxy-4-propargyloxy benzaldehyde (6): propargyl bromide (0.8 ml, 1.97 mmol) was added to a suspension of vanillin (1.5 g, 1.64 mmol) and potassium carbonate (4.93 mmol) in CH_3CN. The suspension was heated to reflux for 6h and the solvent was removed under reduced pressure, water was added and the aqueous phase was extracted with ethyl acetate. The solvent was removed under reduced pressure to give compound 6 in 90% yield as yellow crystalline solid. ^1H NMR ($CDCl_3$, 200 MHz): δ = 9.99 (1H, s), 7.61 (1H, d, J=8.2Hz), 7.56 (1H, s), 7.29 (1H, d, J=8.2Hz), 4.99 (2H, s), 4.06 (3H, s), 2.76 (1H, s) ; ^{13}C NMR ($CDCl_3$, 50 MHz): δ =190.6(1C), 151.8 (1C), 149.6 (1C), 130.5 (1C), 125.9 (1C), 112.2 (1C), 109.1 (1C), 76.3 (1C), 76.5 (1C), 56.2 (1C), 55.6(1C). HRMS cal. For $C_{11}H_{10}O_3$: 190.0630, found: 190.0651.

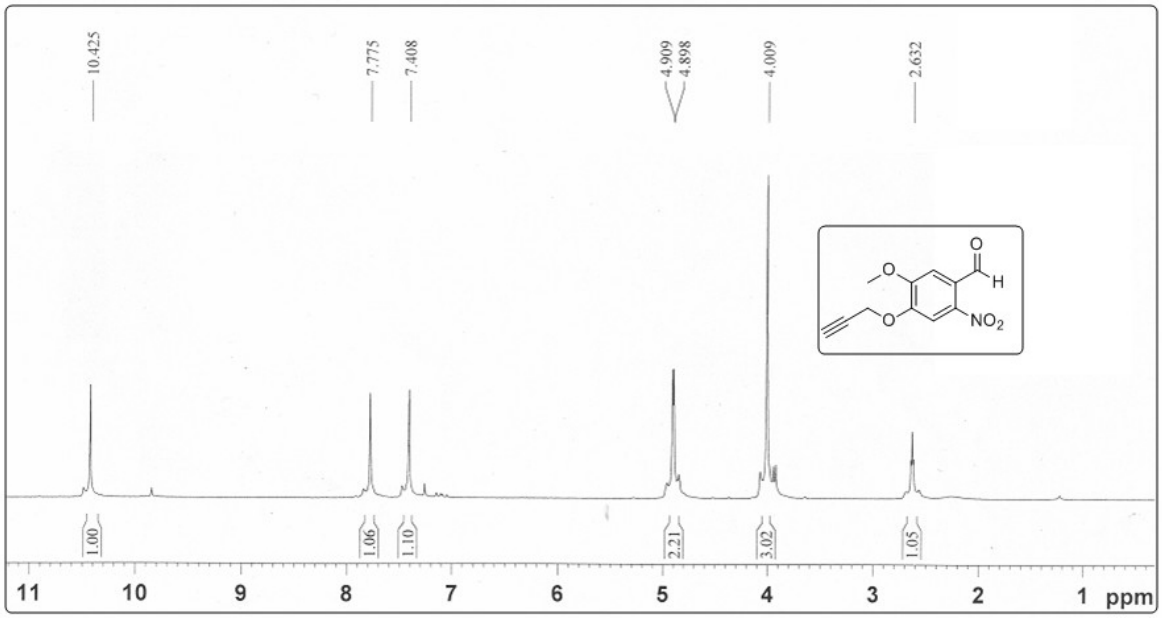


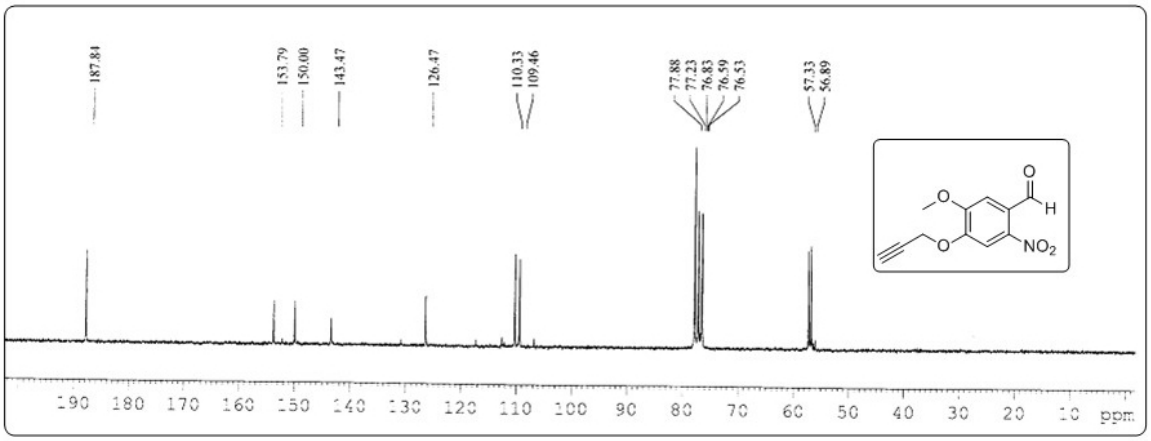




5-methoxy-4-propargyl-2-nitrobenzaldehyde(5):3-methoxy-4-propargyloxy benzaldehyde (11.5 mmol) was suspended in 60 mL of ice-cold HNO_3. The solution was slowly warmed to room temperature, and the reaction was stirred until all of the material dissolved. The solution was then poured onto ice and the precipitate was filtered off then washed with cold water. The product was recrystallized from boiling EtOH (approximately 30 mL),

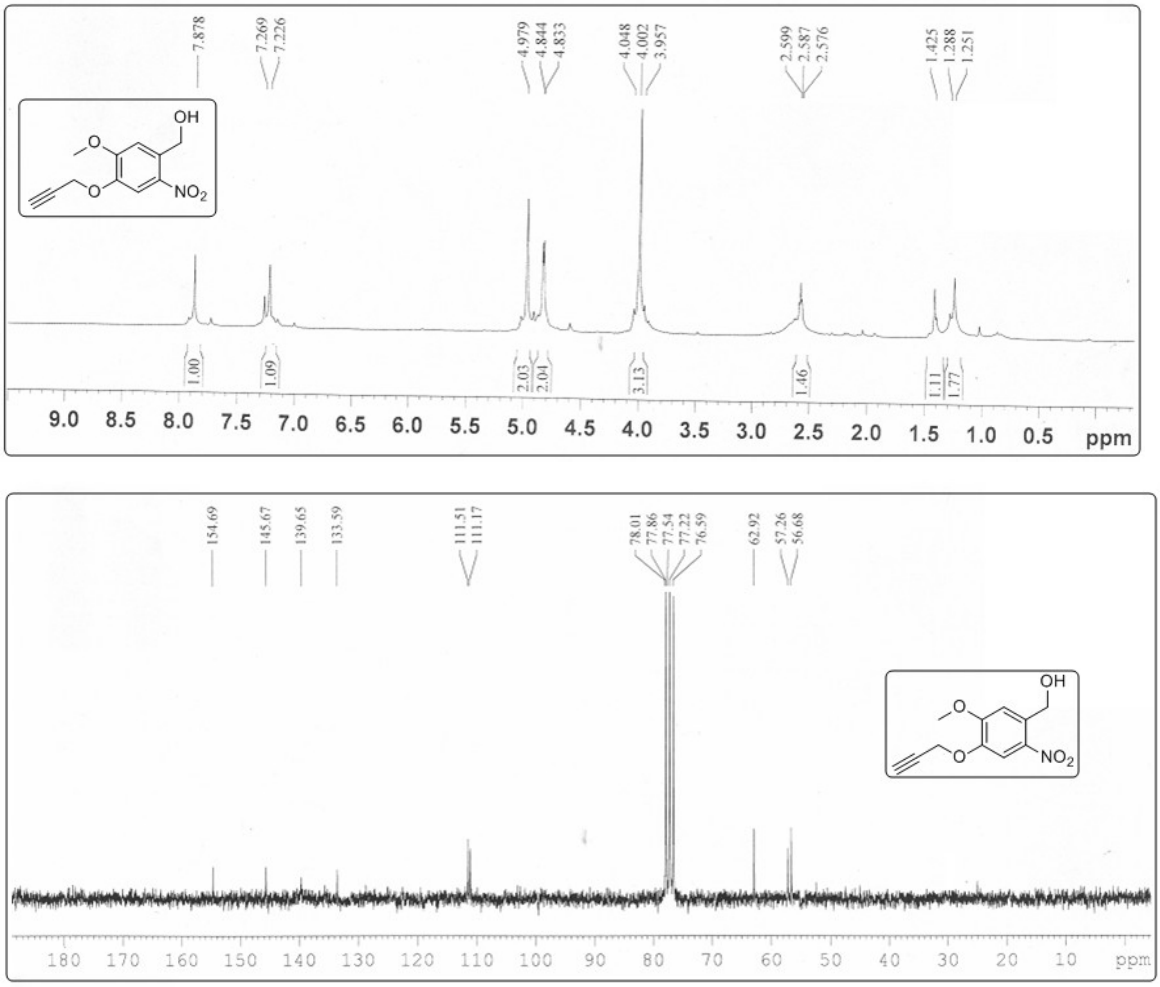
affording compound 5 in 73% yield as a yellow solid .^{1}H NMR ($CDCl_3$, 200 MHz): δ = 10.42(1H, s), 7.77 (1H, s), 7.40 (1H, s), 4.90 (2H, s), 4.00 (3H, s) 2.63 (1H, s); ^{13}C NMR ($CDCl_3$, 50 MHz): δ =187.8(1C), 153.7(1C), 150.0(1C), 143.4(1C), 126.4 (1C), 110.3 (1C), 109.4 (1C), 76.8 (1C), 76.5 (1C), 57.3(1C), 56.8(1C). HRMS cal. For $C_{11}H_9NO_5$: 235.0481, found: 235.0449.



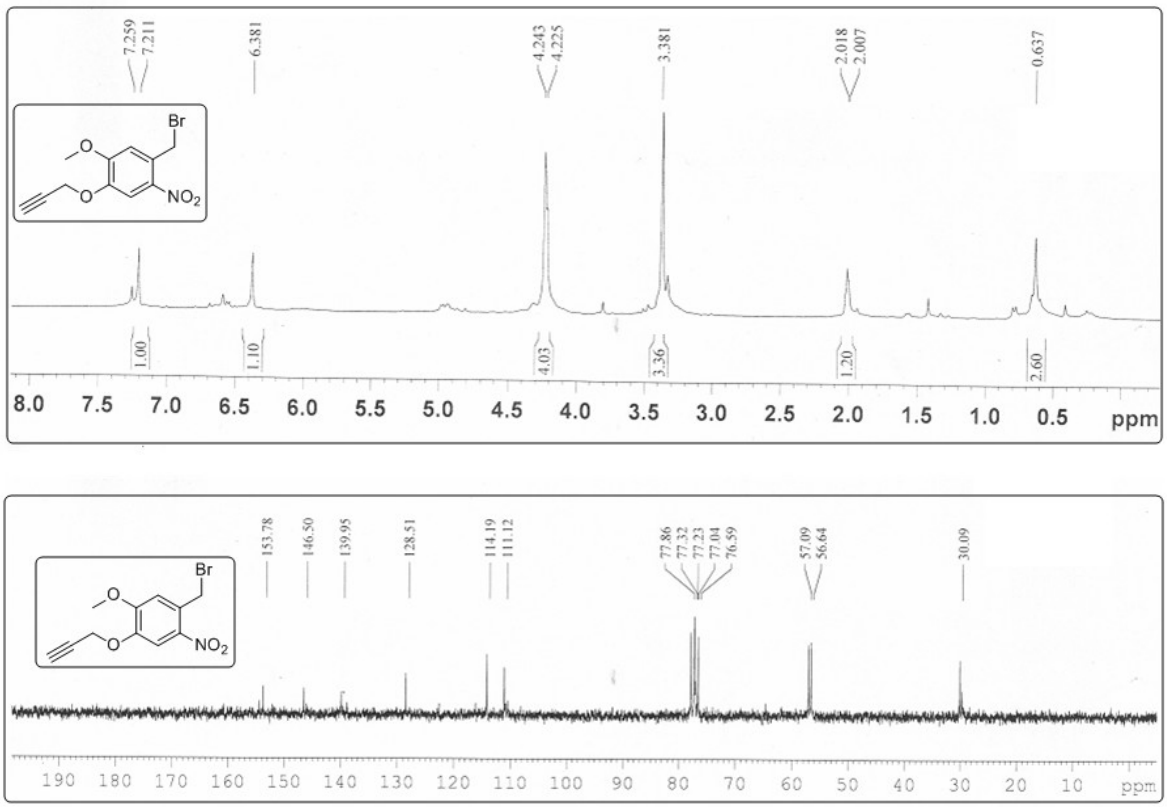


5-methoxy-4-propargyl-2-nitrobenzylalcohol (4): To a solution of 5-methoxy-4-propargyl-2-nitrobenzaldehyde (1.5 g, 47.4 mmol) in 200 mL of ethanol was slowly added $NaBH_4$ (0.9 g, 23.8 mmol). The mixture was stirred at room temperature for 30 min in 0ºC. The solvent was then evaporated, and the residue was partitioned between EtOAc and water. After extraction with EtOAc, the combined organic layers were washed with brine, dried over $NaSO_4$, filtered, and evaporated and the crude product was purified by flash column chromatography (EtOAc/hexane 4/6) (82%) as a pale yellow solid. ^{1}H NMR($CDCl_3$, 200 MHz): 7.87 (1H, s), 7.26 (1H, s), 4.97(2H, s), 4.83(2H, s) 4.00 (3H, s), 2.58(1H, s); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 154.6 (1C), 145.6 (1C), 139.6 (1C), 133.5

(1C), 111.5 (1C), 111.1 (1C), 78.0 (1C), 77.8 (1C), 62.9 (1C), 57.2 (1C), 56.6 (1C). HRMS cal. For $C_{11}H_{11}NO_5$: 237.0637, found: 237.0649.



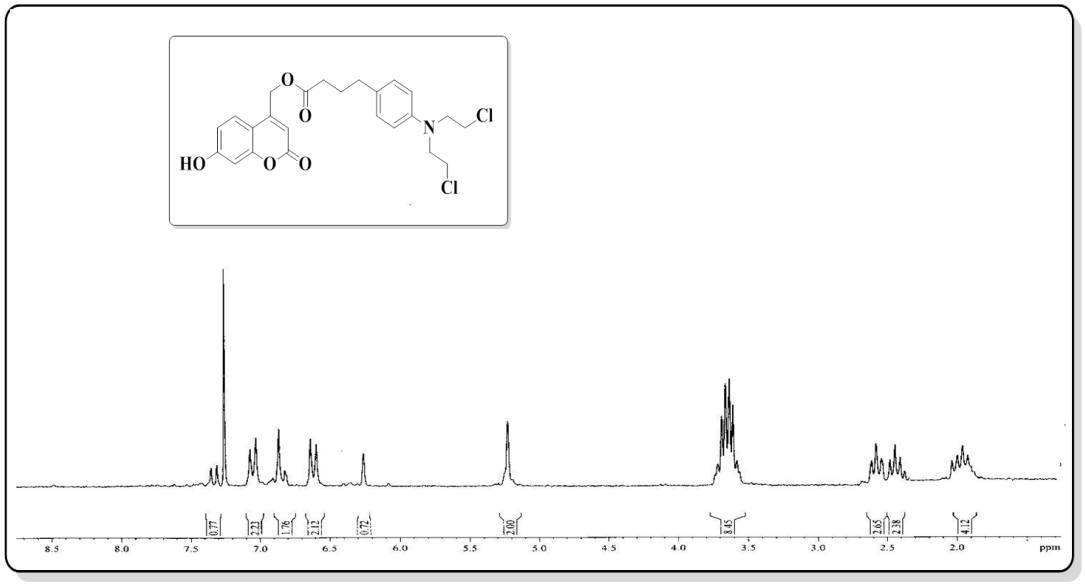
5-methoxy-4-propargyl-2-nitrobenzylbromide (3): Under an N_2 atmosphere at room temperature (RT) with external cooling, we added powder 5-methoxy-4-propargyl-2-nitrobenzyl alcohol (1.08 mmol) to a stirred solution of PBr_3 (1.11 mmol) in CH_2Cl_2 (20 mL). Stirring was continued at RT for 1 h. The reaction mixture was neutralized with a 2 N aqueous $NaHCO_3$ solution (100 mL). The organic phase was then separated, and the aqueous phase was rinsed twice with CH_2Cl_2 (50 mL). The solution was then dried over $NaSO_4$ and the solvent was removed to yield 5-methoxy-4-propargyl-2-nitrobenzylbromide (95%) as slightly yellow needles. ^1H NMR ($CDCl_3$, 200 MHz): δ = 7.21 (1H, s), 6.38 (1H, s), 4.25 (4H, s), 3.38 (3H, s), 2.01(1H, s); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 153.7(1C), 146.5(1C), 139.9 (1C), 128.5 (1C), 114.1 (1C), 111.1 (1C), 77.8 (1C), 76.5 (1C), 57.0 (1C), 56.6 (1C), 30.0 (1C). HRMS cal. For $C_{11}H_{10}BrNO_4$: 298.9793, found: 298.9790.

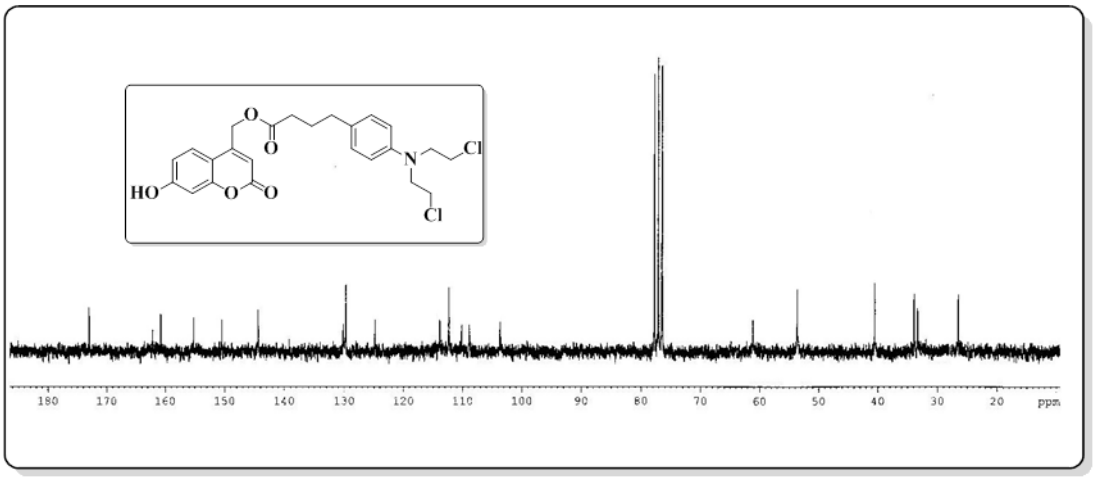


4-Bromomethyl-7-hydroxy-coumarin (7)[1]***:*** A mixture containing 1,3-dihydroxy-benzene (2 mmol), ethyl 4-bromoacetoacetate 5 (2 mmol), and a catalytic amount of conc. H_2SO_4 was stirred at 0ºC for 8 h. After addition of water (50 ml) the resulting precipitate was filtered off and dried under vacuum, providing 4-Bromomethyl-7-hydroxy-coumarin as a white solid. (70% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.94 (1H, d, J= 8.2 Hz), 7.45 (1H, s), 7.41 (1H, d , J=8.2 Hz), 7.19 (1H, d, J=8.2 Hz), 7.09 (2H, d, J=8.8 Hz),6.63 (2H, d, J=8.8 Hz), 6.89 (1H, d, J=8.2 Hz), 5.37(2H, s), 4.24(2H, t, J=6), 3.69–3.52 (8H ,m) 3.48 (2H, t, J=6), 2.56–2.49 (2H, t, J = 7.2 Hz), 2.43–2.36 (2H, t, J = 7.4 Hz), 1.96–1.85 (2H, m); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 173.4 (1C), 161.9(1C), 156.5(1C), 149.6 (1C), 14.5 (1C), 136.4 (1C), 128.5 (1C), 126.7 (2C), 121.2 (1C), 119.9 (1C), 118.9 (1C), 112.2 (2C), 107.2 (1C), 67.6 (1C), 66.2(1C), 53.6 (2C), 40.6 (2C), 33.9 (1C), 33.7 (1C), 32.9 (1C), 31.2 (1C), 26.8 (1C), 23.9 (1C).

7-hydroxy-Coumarin Chlorambucil Conjugate (8): Treatment of 7-hydroxy-4-bromomethyl coumarin (0.29mmol) with chlorambucil (0.29 mmol) in the presence of potassium carbonate (0.34 mmol) in dry N,N-dimethylformamide (DMF) at room temperature for a period of 6 h afforded the conjugate as a yellow solid. The crude conjugate was purified by column chromatography using 35% EtOAc in pet ether to give the 7-hydroxy-Coumarin Chlorambucil Conjugate (70%). Yellow solid. ^{1}H NMR ($CDCl_3$,

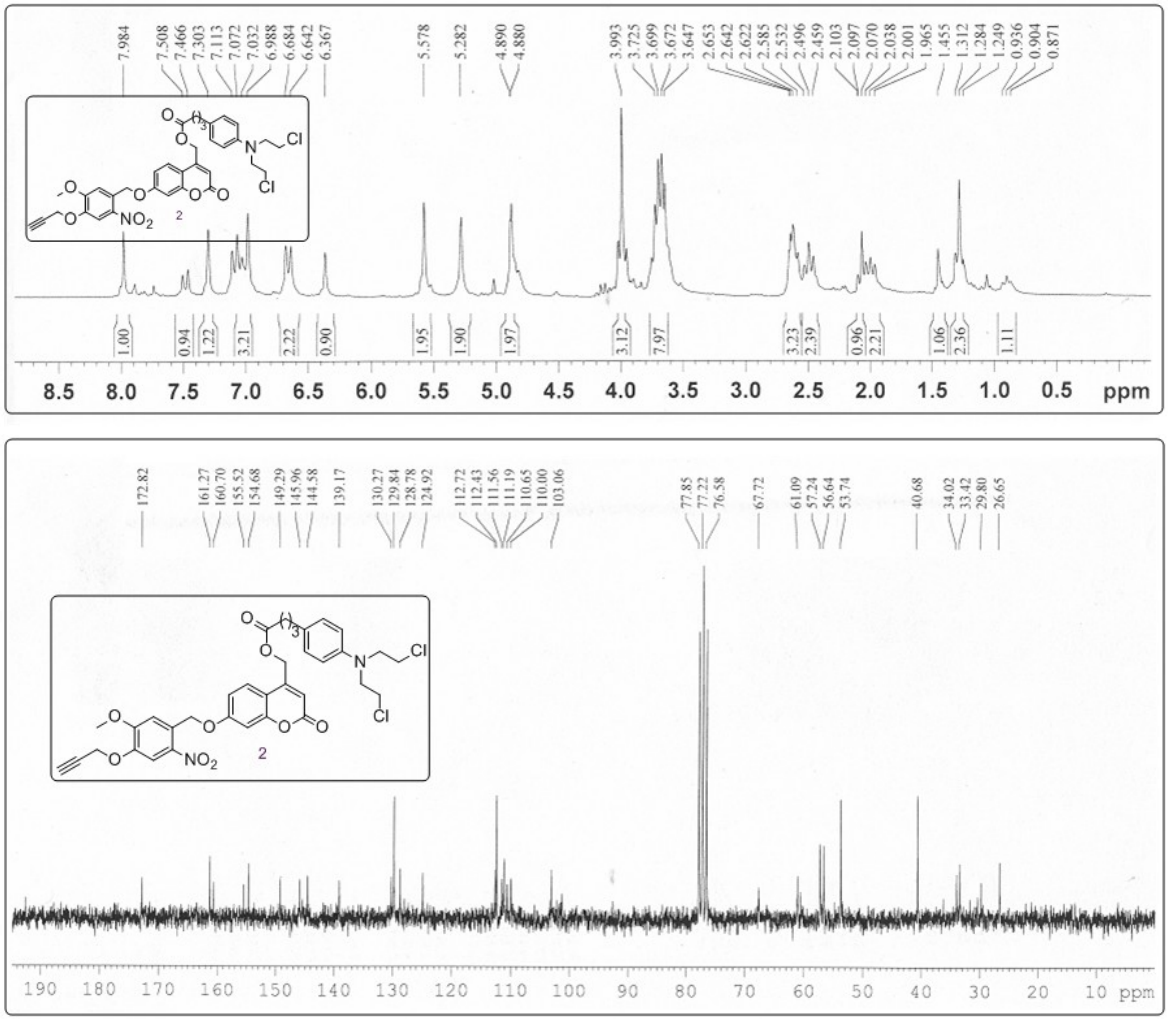
200 MHz):δ= 7.39 (1H,d,J= 8.2 Hz), 7.09(2H ,d,J= 8.4 Hz), 6.85 (2H ,s), 6.64 (2H,d,J= 8.4Hz), 6.35(1H,s), 5.25 (2H,s), 3.69−3.52 (8H,m), 2.56−2.49 (2H ,t,J= 7.2 Hz), 2.43−2.36 (2H ,t,J= 7.4 Hz), 1.96−1.85 (2H ,m).^{13}C NMR ($CDCl_3$, 50 MHz):δ= 173.6, 162.3, 155.3, 151.2, 145.3,129.3, 126.3, 114.3, 113.7, 111.2, 109.7, 104.6, 64.6, 53.6, 40.6, 33.9,33.7, 26.8. FTIR (KBr, cm^{-1}): 1715, 1619, 3200. HRMS cal. For $C_{24}H_{25}Cl_2NO_5$: 478.1190, found: 478.1186.





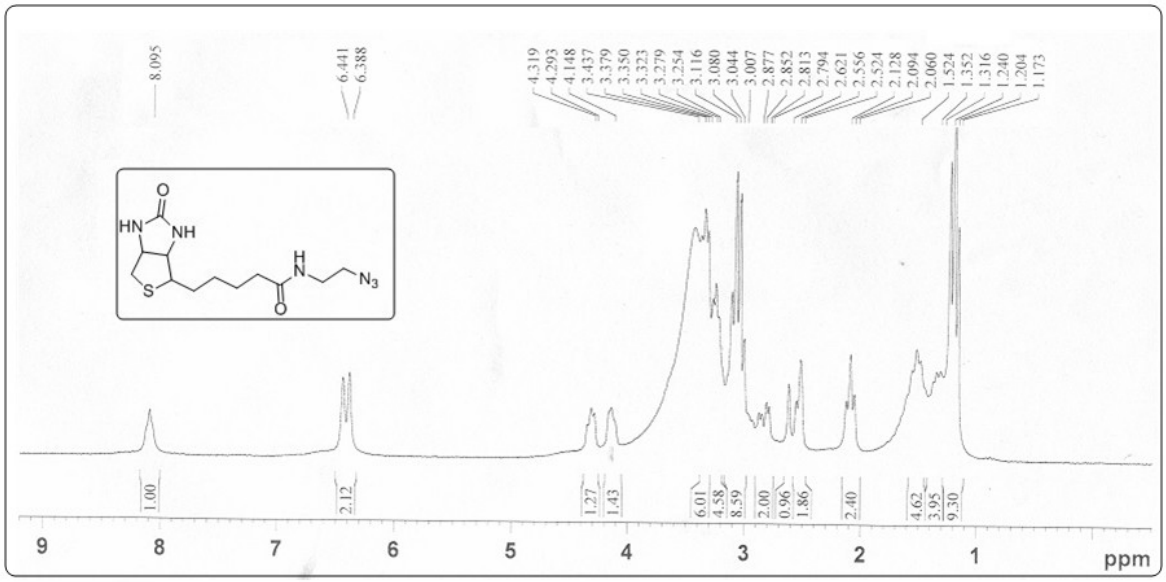
5-methoxy-4-propargyl-2-nitrobenzyloxycoumarin-chlorambucil (2) Treatment of 5-methoxy-4-propargyl-2-nitrobenzylbromide (3) (0.30mmol) with 7-hydroxy-Coumarin Chlorambucil (8) (0.30 mmol) in the presence of potassium carbonate (0.34 mmol) in dry N,N-dimethylformamide (DMF) at room temperature for a period of 12 h afforded the conjugate as a yellow solid. The crude conjugate was purified by column chromatography using 40% EtOAc in pet ether to give the compound **2** (70%) as a light Yellow solid. ^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.98 (1H,s), 7.48 (1H,d,J= 8.4 Hz), 7.30 (1H,s), 7.09

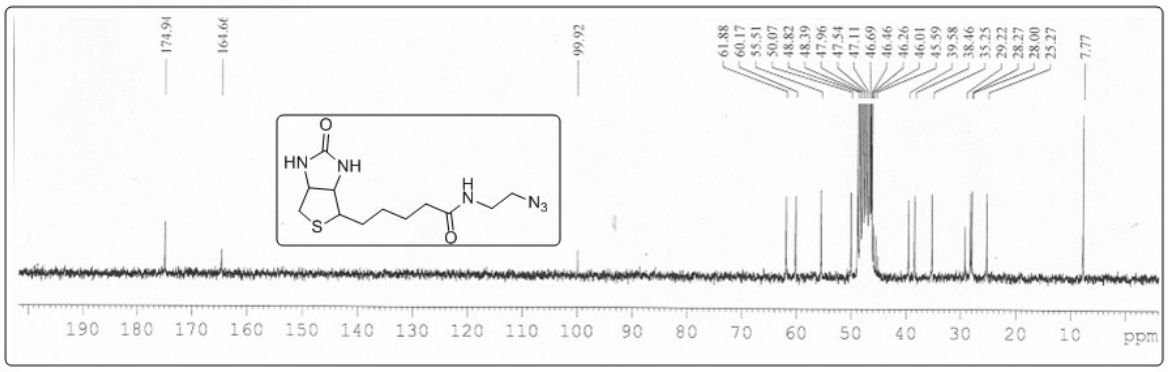
(2H,d,J= 8.4 Hz), 7.03 (1H,s), 6.66 (2H,d,J= 8.2), 636 (1H,s), 5.57 (2H,s), 5.28 (1H, s), 4.89 (2H ,s), 4.99 (2H ,s), 3.69−3.52 (8H ,m), 2.56−2.49 (2H ,t,J= 7.2 Hz), 2.43−2.36 (2H ,t,J= 7.4 Hz), 2.01(1H, s), 1.96−1.85 (2H, m).^{13}C NMR (CDCl3, 50 MHz):δ= 172.8, 161.2, 160.7, 155.5, 154.6, 149.2, 145.9, 144.5, 139.1, 130.2, 129.8, 128.7, 124.9, 112.7, 112.4, 111.5, 111.9, 110.6, 110.0, 103.0, 67.7, 61.0, 57.2, 56.6, 53.7, 40.6, 34.2, 33.4, 29.8, 26.6. HRMS cal. For $C_{35}H_{34}Cl_2N_2O_9$: 697.0870, found: 697.0858.

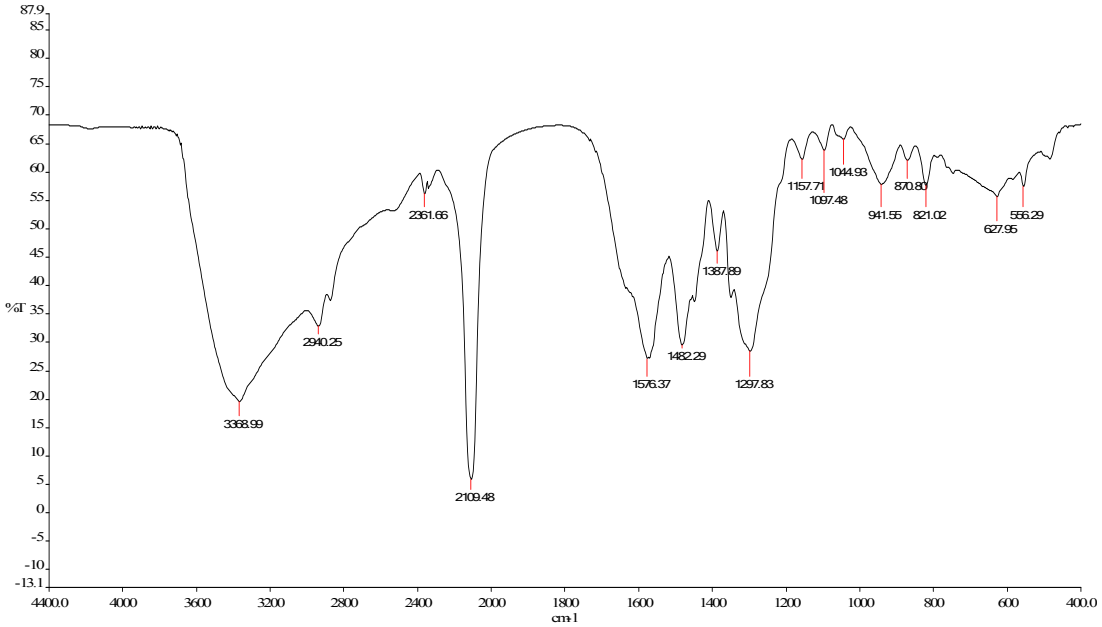


Azido- biotin (9): A Biotin (200 mg, 0.410 mmol) was placed in 50 ml reaction flask and cooled to 0°C. 4.5 ml $SOCl_2$ was added to the flask and the mixture was stirred for 1 h at room temperature and excess $SOCl_2$ was evaporated. The crude acid chloride was dissolved in 5 ml dry THF to this azido-aminoethane[2] and catalytic amount of triethyl amine was added. The solution was stirred for 4 h at room temperature, and then concentrated in vacco. and the residue was purified by column chromatography using chloroform/methanol (20/1) to give the colourless waxy stuff. The analytical data was in accordance with the reported characterization data.[2,3] ^{1}H NMR (DMSO-d_6, 200 MHz): δ=

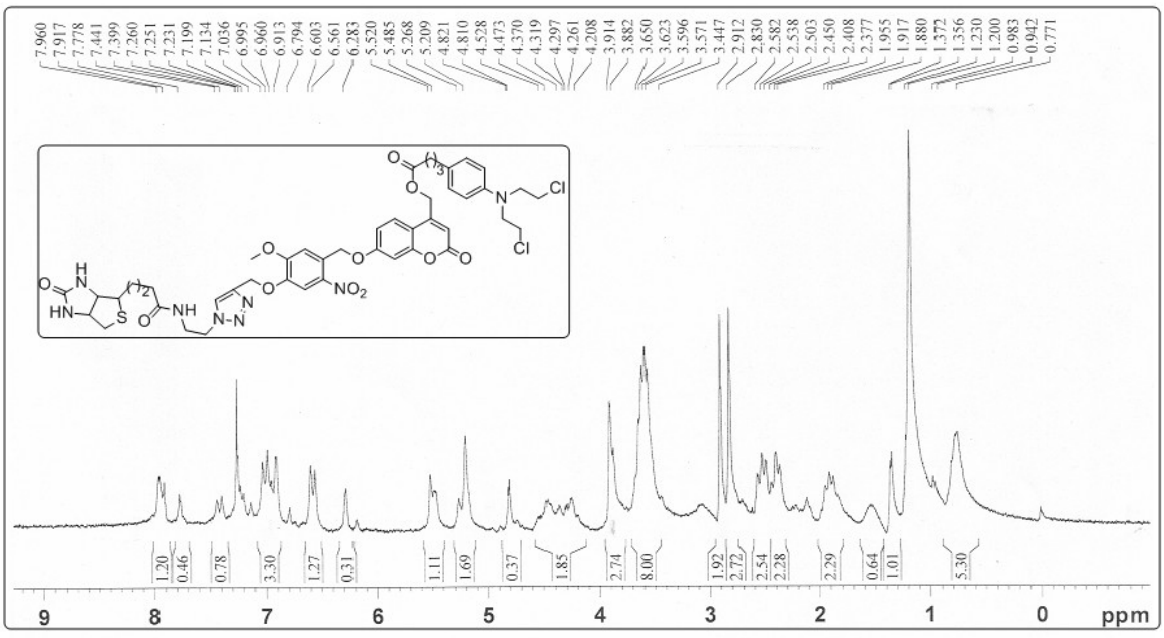
8.09 (1H), 6.44 (1H,s), 6.38 (1H,s), 4.31-4.29 (1H,m), 4.14 (1H,m), 3.43-3.11 (6H,m), 2.87 (1H,d,J=12.4 Hz),2.62 (1H,d,J=12.9), 2.09 (2H,t,J=7.4 Hz),1.55-1.20 (6H,m)^{13}C NMR (DMSO-d_6, 50 MHz):δ= 174.9, 164.6, 99.9, 61.8, 60.1, 55.5, 50.0, 39.5, 38.4, 35.2, 29.2, 28.2. FTIR (KBr, cm^{-1}): 2109,1676,1433,1297.

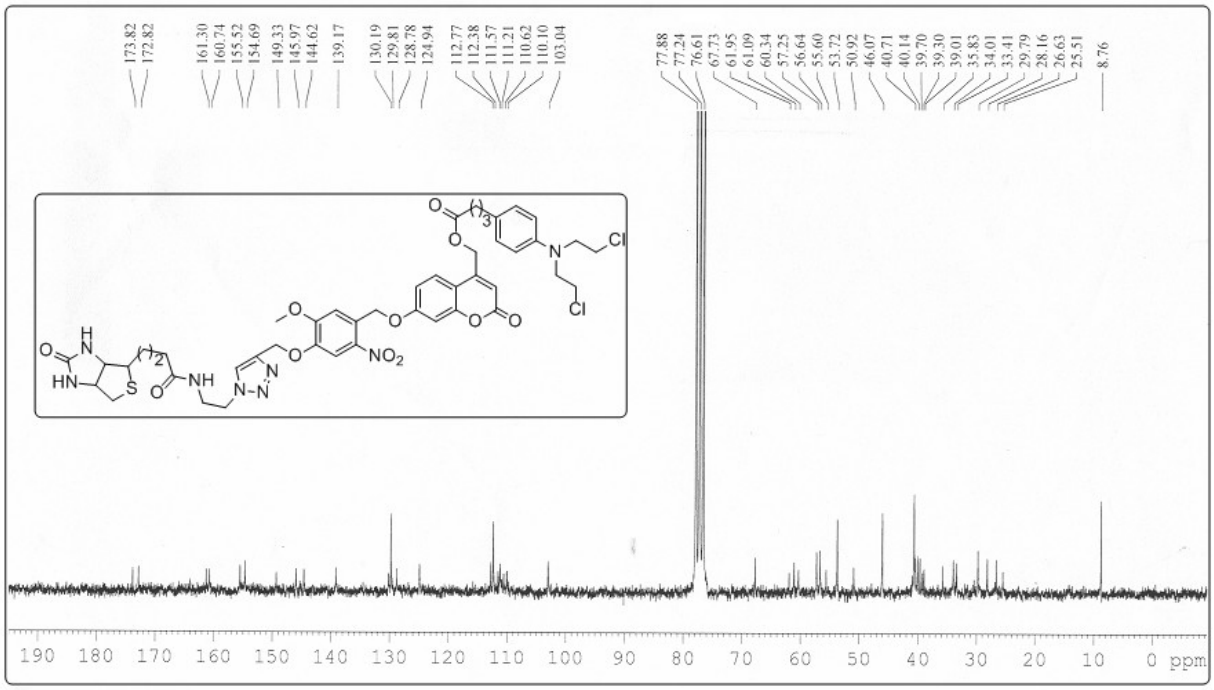


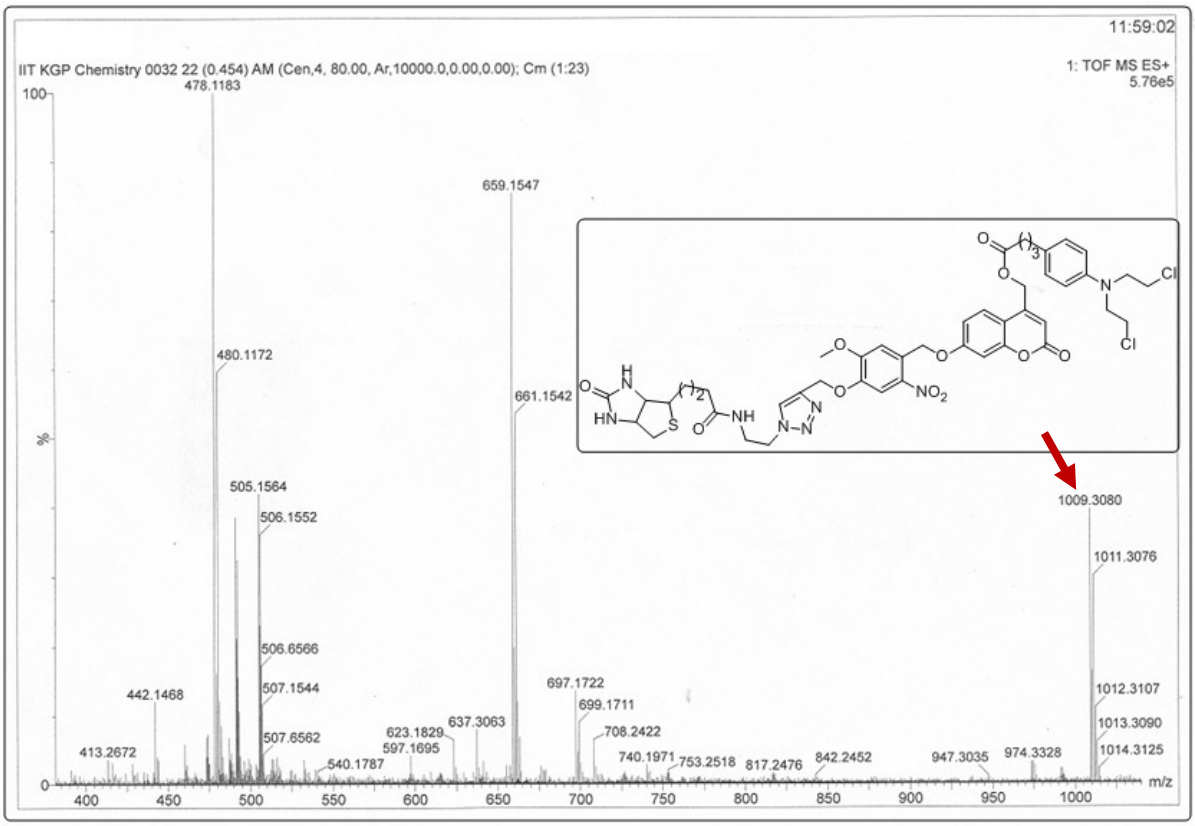


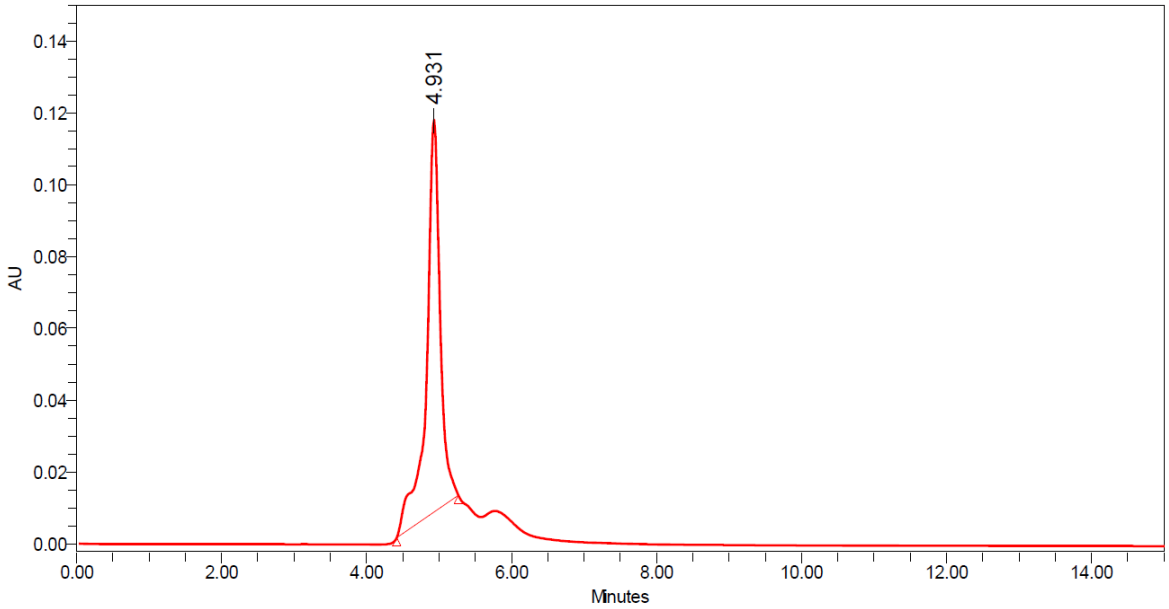


Biotin tagged O-nitrobenzoyl caged Coumarin chlorambucil (1): To a THF (2.0 mL) solution of compound **8** (103.5 mg, 0.01 mmol) were added sodium ascorbate (10 mol%) and **9** (40 mg, 0.01 mmol). The reaction mixture as degassed for 15min by purging argon gas. Then 2.0 mg (0.002 mmol) of $CuSO_4$ in 0.5 mL water was added to the reaction mixture. The reaction was continued for 6 h. Then the crude reaction mixture was directly passed through silica column chromatography using DCM/MeOH (8:2) as eluent to afford a (50%) yellow waxy solid. Further, the purity of biotin tagged ONB-CC were determined by RP-HPLC to be 96% using mobile phase acetonitrile/water (50/50 v/v), at a flow rate of 1mL/min (detection: UV 254 nm) ^{1}H NMR ($CDCl_3$, 200 MHz):δ= 7.98 (1H,s), 7.48 (1H,d,J= 8.4 Hz), 7.30 (1H,s), 7.09 (2H,d,J= 8.4 Hz), 7.03 (1H,s), 6.66 (2H,d,J= 8.2), 636 (1H,s), 5.57 (2H,s), 5.28 (1H, s), 4.89 (2H ,s), 4.99 (2H ,s), 3.69−3.52 (8H ,m), 2.56−2.49 (2H ,t,J= 7.2 Hz), 2.43−2.36 (2H ,t,J= 7.4 Hz), 2.01(1H, s), 1.96−1.85 (m, 2H).^{13}C NMR (CDCl3, 50 MHz):δ= 173.8,172.8, 161.3, 160.7, 155.5, 154.6, 149.3, 145.9, 144.6, 139.1, 130.2, 129.8, 128.7, 124.9, 112.7, 112.3, 111.5, 111.2, 110.6, 110.0, 103.0, 67.7, 61.9, 61.0,60.3, 57.2, 56.6, 55.6, 53.7, 46.0, 40.7, 39.7, 39.3, 39.0, 34.0, 33.4, 29.7,28.1, 26.6, 25.5. HRMS cal. For $C_{47}H_{54}Cl_2N_8O_{11}S$: 1009.3069, found: 1009.3060.




173.82
172.82
161.30
160.74
155.52
154.69
149.33
145.97
144.62
139.17
130.19
129.81
128.78
124.94
112.77
112.38
111.57
111.21
110.62
110.10
103.04
77.88
77.24
76.61
67.73
61.95
61.09
60.34
57.25
56.64
55.60
53.72
50.92
46.07
40.71
40.14
39.70
39.30
39.01
35.83
34.01
33.41
29.79
28.16
26.63
25.51
8.76
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


11:59:02
IIT KGP Chemistry 0032 22 (0.454) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (1:23)
1: TOF MS ES+
5.76e5
100
%
0
478.1183
659.1547
480.1172
661.1542
505.1564
506.1552
506.6566
507.1544
507.6562
442.1468
413.2672
623.1829
637.3063
597.1695
540.1787
697.1722
699.1711
708.2422
740.1971
753.2518
817.2476
842.2452
947.3035
974.3328
1009.3080
1011.3076
1012.3107
1013.3090
1014.3125
400 450 500 550 600 650 700 750 800 850 900 950 1000
m/z



RP-HPLC profile of biotin tagged ONB-CC using mobile phase acetonitrile/water (50/50 v/v), at a flow rate of 1mL/min (detection: UV 254 nm)

3. Measurement of fluorescence quantum yields

The quantum yield of the O-nitrobenzyl coumarin chlorambucil (ONB-CC) and coumarin chlorambucil (CC) was determined by reference point method. Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield: 54%) was used as a standard sample to calculate the QY of ONB-CC and CC. which were dissolved in acetonitrile/water. The absorbance values of the solutions at the excitation wavelength were measured with UV–Vis spectrophotometer. Photoluminescence (PL) emission spectra of all the sample solutions were recorded by Hitachi F-7000 fluorescence spectrophotometer at an excitation wavelength of 360 nm.

$$\frac{\phi_S}{\phi_R} = \frac{A_S}{A_R}\,\frac{(Abs)_R}{(Abs)_S}\,\frac{\eta_S^2}{\eta_R^2}$$

Where **Φ** represents quantum yield, **Abs** represents absorbance, **A** represents area under the fluorescence curve, and **η** is refractive index of the medium. The subscripts **S** and **R** denote the corresponding parameters for the sample and reference, respectively.

4. Photolysis of O-nitrobenzyl coumarin chlorambucil(ONB-CC)using soft UV irradiation (≥ 365 nm) and diode laser (730 nm):

a) Photolysis of ONB-CC using soft UV irradiation (≥ 365 nm):

5 mL of 1.0×10^{-5} M aqueous acetonitrile solution (80/20 v/v) mixture of ONB-CC in quartz cuvettes, individually. They were irradiated under UV light by 125 W medium

pressure Hg vapor lamp using a suitable filter 1 M $CuSO_4$ solution in 0.1 N H_2SO_4, the transmittance for the above filter = 365 to 500 nm). At regular interval of time, 20 µL of the aliquots was taken and analyzed by RP-HPLC using mobile phase acetonitrile/water (90/10 v/v), at a flow rate of 0.4 mL/min (detection: UV 254 nm). Peak areas were determined by RP-HPLC, which indicated gradual decrease of the caged compound with time, and the average of three runs. The reaction was followed until the consumption of the caged compound is less than 5% of the initial area.

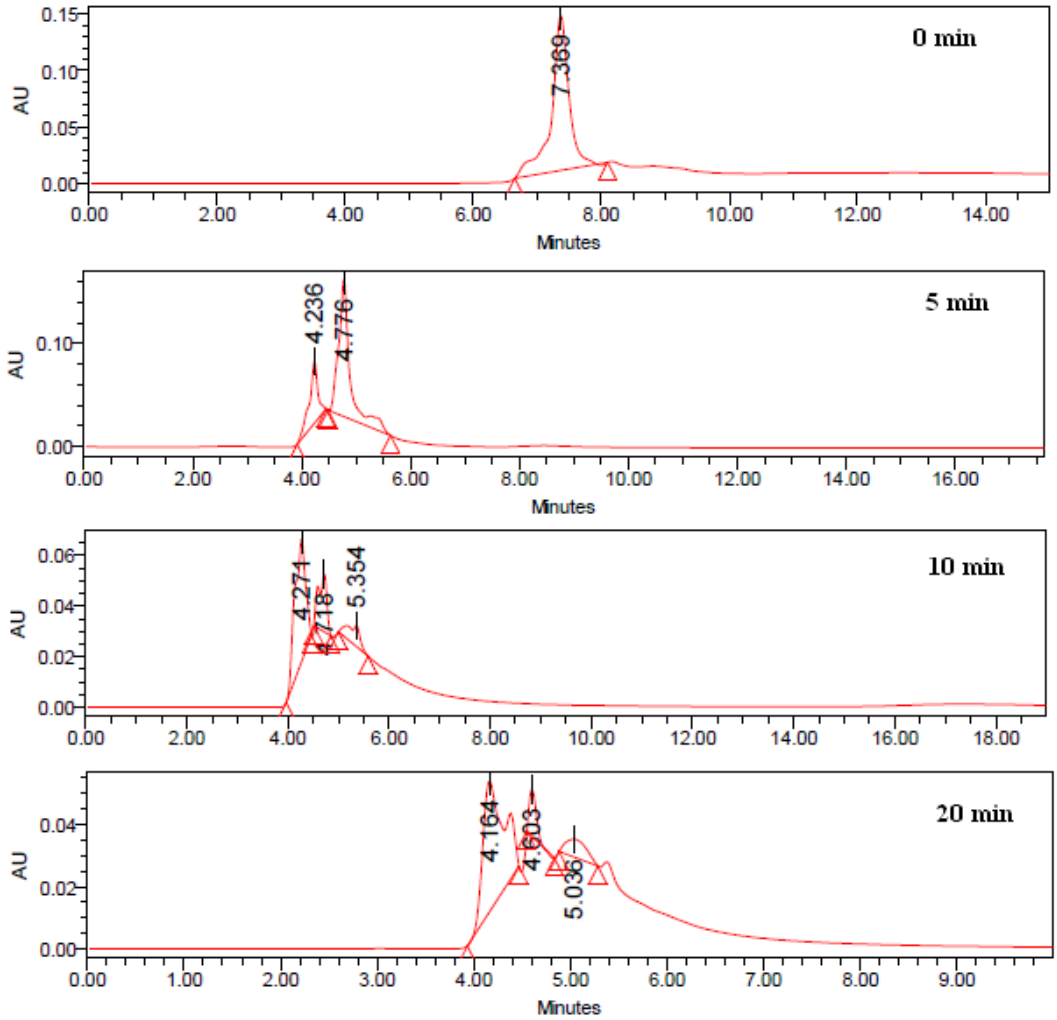


Figure S1: HPLC profile of ONB-CC under soft UV irradiation (≥365 nm) .

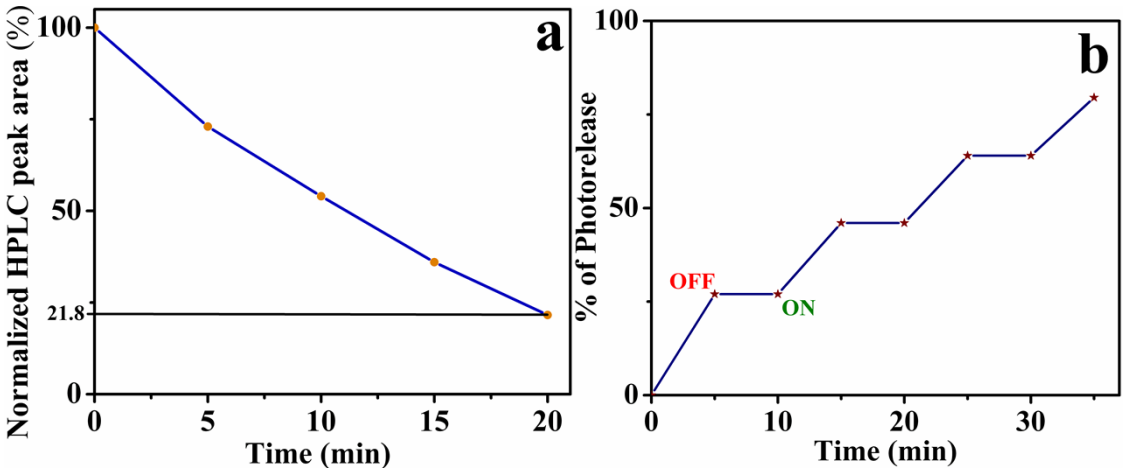


Figure S2: (a) Time course for the photorelease of ONB-CC soft UV irradiation (≥365 nm) (b) Control over the release under bright and dark conditions. "On" indicates the beginning of light irradiation and the "OFF" indicates the ending of light irradiation

b) Photolysis of ONB-CC using Red laser: 5 mL of 1.0×10^{-5} M aqueous acetonitrile solution (80/20 v/v) mixture of ONB-CC . Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated using 730 nm laser diode (30 mW/ cm^2). At regular interval of time, 20 µL of the aliquots was taken and analyzed by RP-HPLC using mobile phase acetonitrile/water (80/20 v/v), at a flow rate of 0.6 mL/min (detection: UV 254 nm).

5. ***Cell Imaging and Cytotoxicity of biotin tagged O-nitrobenzyl coumarin chlorambucil (biotin tagged ONB-CC) on MDA-MB-231 cell line*****:**

a) biotin tagged ONB-CC for cell imaging studies using MDA-MB-231 cell line: To study the cellular uptake of biotin tagged ONB-CC, briefly MDA-MB-231 cell (1.5×10^4) were were seeded on each coverslips placed in 60 mm petri plate and allowed to adhere and attain the spreaded morphology. Cells were then incubated with 50 µM of biotin tagged ONB-CC in cell culture medium for 4 h at 37 °C and 5 % CO_2. Then the cells were irradiated (keeping the cell-culture plate 5 cm apart from the light source) using 125 W medium pressure Hg lamp as irradiation source (≥ 365nm) and 1M $CuSO_4$ solution as UV cut-off filter for 20 min with regular time interval (5,10,and 20 min). Thereafter, cells were fixed in 3.7 % paraformaldehyde for 15 min and washed two times with PBS. The cells were washed twice with PBS and then the nuclei were counterstained by DAPI for 20 min. Subsequent dehydration steps were performed in 50, 70, 95, 100% ethanol for 5 min each followed by permanent mounting with DPX for confocal microscopic imaging (CLSM, Olympus FluoView FV1000)[2] . The sample was then focused and observed on confocal microscope with imaging software, FV10-ASW2.0 Viewer.

b) Cytotoxicity of biotin tagged ONB-CC on MDA-MB-231 cell line[3-6]

5.i. Cytotoxicity before photolysis: The cytotoxicity in vitro was measured using the MTT (3–(4,5–dimethylthiazol–2–yl)–2,5–diphenyltetrazolium bromide, a yellow tetrazole) assay on MDA-MB-231 cell line. Briefly, cells growing in log phase were seeded into 96–well cell–culture plate at 5×10^3 cells/well. Different concentration of biotin tagged ONB-CC (0-100 µM) were added in the wells with an equal volume of PBS in the control wells. The cells were then incubated for 72 h at 37 °C in 5% CO_2. Thereafter, fresh media containing 50 µL MTT (1 mg/mL) were added to the 95 well plates and incubated for 4 h at 37 °C in 5% CO_2. Formazan crystals thus formed were dissolved in DMSO after decanting the earlier media and absorbance recorded at 595 nm.

5.ii. Cytotoxicity after photolysis: MDA-MB-231 cells maintained in minimum essential medium (in 96-well cell-culture plate at concentration of 1×10^4 cells/mL) containing 10% fetal bovine serum (FBS) and different concentration (0-100 µM) of biotin tagged ONB-CC was incubated for 4 h at 37 °C and 5 % CO_2. Then the cells were irradiated (keeping the cell-culture plate 5 cm apart from the light source) using 125 W medium pressure Hg lamp as irradiation source (≥ 365nm) and 1M $CuSO_4$ solution as UV cut-off filter. After irradiation the cells were again incubated for 72 h. Then cytotoxicity was measured using the MTT assay as described in the section 5.i.

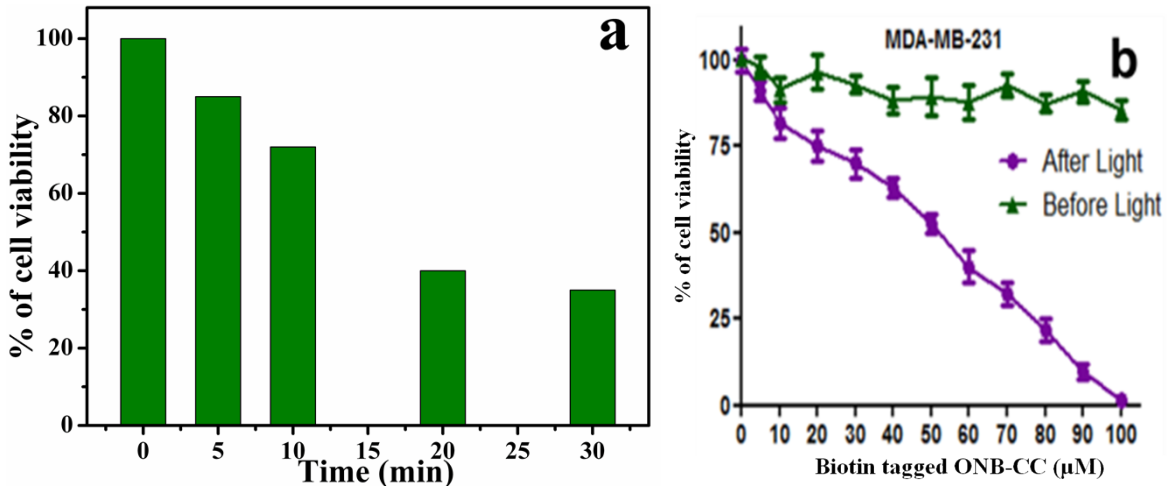


Figure S3. Cell Cytotoxicity studies of biotin tagged ONB-CC on MDA-MB-231 cells. (a) Cell viability vs different irradiation time (b)Cellviability vs different conc of biotin taggeg ONB-CC with before and after irradiation

***5.iii. Cell cycle analysis*:** MDA-MB-231 breast cancer cells were cultured in 60 mm petridishes for 24 h at 37 °C in 5% CO_2 a density of 1×10^5 and then treated with IC_{50} concentration of chlorambucil, ONB-CC and biotin tagged ONB-CC and the cells were

incubated for 12 h at 37 °C under 5% CO_2. Chemically treated cells were irradiated for 30 min by UV light (≥ 365 nm) for 20 min (keeping the cell-culture plate 5 cm apart from the light source) using 125 W medium pressure Hg lamp with 1M $CuSO_4$ solution as UV cut-off filter. After irradiation the cells were again incubated for 24 h at 37 °C under 5% CO_2. After incubation, cells were trypsinized and centrifuged at 1200 rpm for 5 min at 4 °C. The pellet was suspended in 10 ml of PBS and then centrifuged at 1300 rpm for 10 min at 4 °C. The supernatant was discarded and the pellet was fixed with 2 ml of ice-cold ethanol solution (70% v/v in PBS) at 4 °C overnight. Fixed cells were centrifuged at 1300 rpm for 10 min at 4 °C and the pellet was incubated with PI mixture (10 mg/ml RNase, 20 mg/ml propidium iodide dissolved in cold PBS) for 30 min at 37 °C. DNA content analysis was carried out on a FACS Calibur (BD Bioscience, USA) flow cytometer (10,000 events were acquired for each sample). The data obtained were processed for cell cycle analysis with the cell quest pro software package. The amount of propidium iodide intercalating to DNA was used as the parameter to determine the cell cycle distribution phases. Apoptosis fraction was considered as DNA loss resulting in a sub-G1 peak.

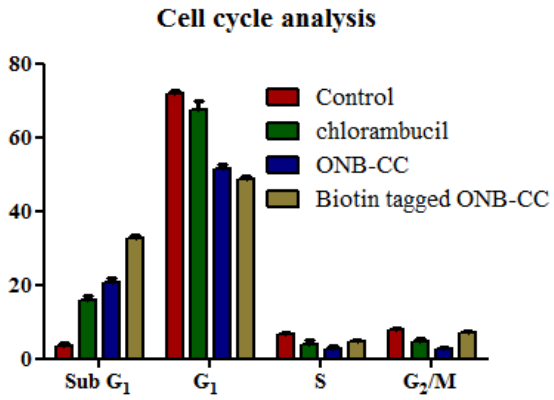


Figure S4. Cell cycle analysis by FACS calibur for 24 h after treatment to UV light for 20 min. Percentage of apoptotic cells are indicated as the proportion of cells that contained sub-G1 phase

References

1. Sanghamitra Atta , Avijit Jana , Rajakumar Ananthakirshnan ,Pradeep Singh Narayana Dhuleep. *J. Agric. Food Chem.*, 2010, *58*, 11844–11851.
2. Sheng Wang, Junlin Yin, Duozhi Chen, Fen Nie, Xiaomin Song, Cong Fei, Haofei Miao,Changbin Jing, Wenjing Ma, Lei Wang, Sichun Xie, Chen Li, Rong Zeng, Weijun Pan,Xiaojiang Hao Lin Li, *Nature Chemical Biology*, 2013,9, 579–585.

3. Yu, W.-l.; Guizzunti, G.; Foley, T. L.; Burkart, M. D.; La Clair, J. J. An optimized immunoaffinity fluorescent method for natural product target elucidation. J. Nat. Prod. 2010, 73, 1659−1666.
4. Venkatesan P, Puvvada N, Dash R, Prashanth Kumar BN, Sarkar D, Azab B, Pathak A, Kundu SC, Fisher PB, Mandal M: T. *Biomaterials* 2011, 32,3794-3806.
5. Kumar BN, Rajput S, Dey KK, Parekh A, Das S, Mazumdar A, Mandal M: *BMC Cancer* 2013, 13, 273.
6. Rajput S, Kumar BN, Sarkar S, Das S, Azab B, Santhekadur PK, Das SK, Emdad L, Sarkar D, Fisher PB, Mandal M: *PLoS One* 2013, 8, 61342.