SUPPORTING INFORMATION

A functional and self-assembling octyl-phosphonium-tagged esculetin as an effective siRNA delivery agent

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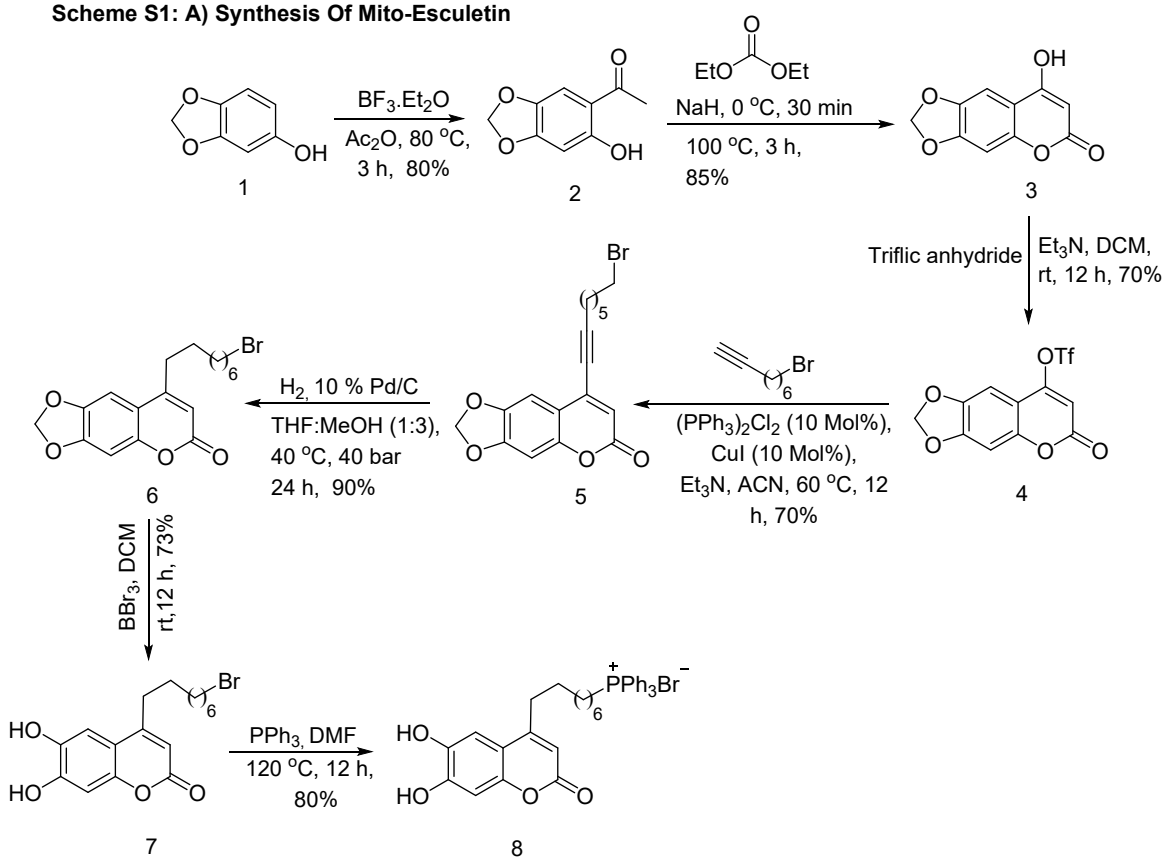
General information:

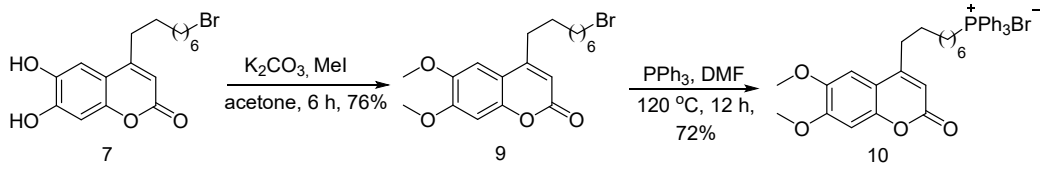
Unless otherwise mentioned, commercially available reagents were used without any further purification. The moisture and air sensitive reactions were carried out under nitrogen atmosphere using oven-dried glassware with magnetic stirring. Reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica plates with the help of UV-light, iodine and p-anisaldehyde stains. Column chromatography was performed on silica gel 60–120 mesh) using distilled EtOAc and petroleum ether as eluents. Evaporation of solvents was done under reduced pressure at temperature less than 40 °C. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 solvents using either 300 MHz or 400 MHz or 500 MHz spectrometers at ambient temperature. Chemical shifts δ and coupling constants J are given in ppm (parts per million) and Hz (hertz) respectively. Chemical shifts are reported relative to residual solvent as an internal standard (for $CDCl_3$; ^{1}H: δ = 7.26 and ^{13}C: δ = 77.16 ppm and for DMSO-d_6; ^{1}H: δ = 2.50 and ^{13}C: δ = 39.5 ppm). Peak splitting patterns are designated as follows: s = singlet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, t = triplet, q = quartet, m = multiplate, brs = broad singlet. Waters-TOF spectrometer was used to record high-resolution mass spectra (HRMS).

Synthetic scheme:

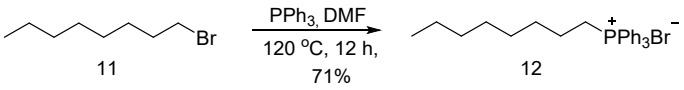
Scheme S1: A) Synthesis Of Mito-Esculetin



B) Synthesis of (8-(6,7-dimethoxy-2-oxo-2H-chromen-4-yl)octyl)triphenylphosphonium



C) synthesis of octyltriphenylphosphonium:



Synthetic procedures:

1. Procedure for the synthesis of 1-(6-hydroxybenzo[d][1,3]dioxol-5-yl)ethan-1-one (2):

A solution of sesamol (5.6 g, 40 mmol) in acetic anhydride (20 mL) was cooled to 0 °C under nitrogen atmosphere. The solution was slowly added with boron trifluoride/diethyl ether

complex (10 mL), and then the mixture was stirred at 90 °C for 3 hours. The resulting mixture was added to saturated aqueous sodium acetate (50 mL), and stirred at room temperature. The solid formed was removed by filtration, the solvent was evaporated under reduced pressure, and the residual solid was suspended in methanol, thereby washed, then collected by filtration and dried to obtain **2** (5.850 g, 80%).

2. Procedure for the synthesis of 8-hydroxy-6H-[1,3]dioxolo[4,5-g]chromen-6-one (3):

To a solution of **2** (5 g, 1 eq.) in diethyl carbonate (80 mL) under nitrogen atmosphere was added sodium hydride (2.66 g, 4 eq.), and the mixture was stirred for 30 min at 0 °C. The resulting solution was heated at 100 °C for 3 h, then cooled to 0 °C and 50% aqueous MeOH (10 mL) was cautiously added. After extraction with ether (3 x 100 mL), the reaction mixture was acidified to pH 2 with 2N hydrochloric acid, and the precipitated solid was filtered and dried under vacuum to obtain **3** (4.9 g, 85%).

3. Procedure for the synthesis of 6-oxo-6H-[1,3]dioxolo[4,5-g]chromen-8-yl trifluoromethanesulfonate (4):

Trifluoromethanesulfonic anhydride (4.3 mL, 1.3 equiv) was added dropwise over 10 min. to a mixture of **3** (4 g, 1 eq.) and triethylamine (3.5 mL, 1.3 equiv) in dry dichloromethane (30 mL) at 0 °C. Then the mixture was stirred for 12 h at room temperature. After that mixture was diluted with 50% ether:hexane and filtered through a short pad of silica, the filtrate was concentrated to a residue, which was purified by flash chromatography to give the corresponding product **4** (4.6 g, 70%).

4. Procedure for the synthesis of 8-(8-bromooct-1-yn-1-yl)-6H-[1,3]dioxolo[4,5-g]chromen-6-one (5):

A round-bottom flask was flame-dried under high vacuum. Upon cooling, coumarin **4** (1.0 g, 1 eq.), $PdCl_2(PPh_3)_2$ (207 mg, 0.1 eq.), CuI (56 mg, 0.1 eq.), acetonitrile (10 mL), triethylamine (0.61 mL, 1.5 equiv) and 8-bromooctyne (0.838 g, 1.5 eq.) were added. The reaction mixture was stirred overnight at 60 °C. Following completion of the reaction (monitored by TLC), the reaction mixture was cooled, diluted with ethyl acetate (20 mL), and filtered through a short silica gel bed. The filtrate was concentrated to a residue which was purified by flash chromatography to give the corresponding product **5** (0.790 g, 70%).

5. Procedure for the synthesis of 8-(8-bromooctyl)-6H-[1,3]dioxolo[4,5-g]chromen-6-one (6):

A well stirred mixture of coumarin **5** (0.7 g) in methanol was allowed to pass through a H-Cube reactor packed with 10% Pd/C at 1 mL/min, 40 °C, and pressure of 40 bar. After completion of the reaction, the solvent was evaporated under reduced pressure to give corresponding product **6** (0.641 g, 90%).

6. Procedure for the synthesis of 4-(8-bromooctyl)-6,7-dihydroxy-2H-chromen-2-one (7):

8-(8-bromooctyl)-6H-[1,3]dioxolo[4,5-g]chromen-6-one **6** (0.6 g, 1.0 equiv) was dissolved in dry DCM (15 mL) in a 50 mL round-bottom flask and the mixture was cooled to −78 °C. BBr_3 (1.0 M in DCM, 4 eq.) was added slowly dropwise. The reaction was allowed to warm to room temperature and stirred for 12 h. MeOH (2 mL) was added, with subsequent stirring for another 15 minute and the solvent was removed under vacuum. The crude product was purified by column chromatography on silica gel to afford **7** (0.425 g, 73%) as a yellow solid.

7. Procedure for the synthesis of (8-(6,7-dihydroxy-2-oxo-2H-chromen-4-yl)octyl) triphenylphosphonium (8):

To a stirred solution of compound **7** (0.2 g, 1 eq.) in dry DMF (6 ml) was added triphenylphosphine (0.156 g, 1.1 eq.) and the resulting mixture was heated to 120 °C for 12 h under nitrogen atmosphere. After completion of the reaction, DMF was distilled off completely under reduced pressure to obtain crude product. The crude product was washed several times with hexane and diethyl ether to afford **8** (0.240 g, 80%) as yellow solid.

8. Procedure for the synthesis of 4-(8-bromooctyl)-6,7-dimethoxy-2H-chromen-2-one (9):

To a solution of compound **7** (0.2 g, 1 eq.) in 10 ml of dry acetone, was added K_2CO_3 (0.302 g, 4 eq.) and MeI (0.308 g, 4 eq.). The above mixture was stirred at room temperature for 6 h. After completion of the reaction as indicated by TLC, the reaction mixture was filtered and the solvent was removed by evaporation at vacuum to get crude products, followed by chromatography to afford **9** (0.165 g, 76%) as yellow solid.

9. Procedure for the synthesis of (8-(6,7-dimethoxy-2-oxo-2H-chromen-4-yl)octyl)triphenylphosphonium (10):

To a solution of compound **9** (0.120 g, 1 eq.) in dry DMF (6 ml) was added triphenylphosphine (0.087 g, 1.1 eq.) and the resulting mixture was heated to 120 °C for 12 h under nitrogen atmosphere. After completion of the reaction, DMF was distilled off completely under

reduced pressure to obtain crude product. The crude product was washed several times with hexane and diethyl ether to afford **10** (0.127 g, 72%) as yellow solid.

10. Procedure for the synthesis of octyltriphenylphosphonium (12):

To a solution of compound **11** (0.2 g, 1 eq.) in dry DMF (6 ml) was added triphenylphosphine (0.298 g, 1.1 eq.) and the resulting mixture was heated to 120 °C for 12 h under nitrogen atmosphere. After completion of the reaction, DMF was distilled off completely under reduced pressure to obtain crude product. The crude product was washed several times with ethyl acetate and diethyl ether to afford **12** (0.277 g, 71%) as colorless liquid.

Spectral Data:

1-(6-hydroxybenzo[d][1,3]dioxol-5-yl)ethan-1-one (2):

Brown solid; R_f = 0.7 (EtOAc/Hexane, 1:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.03 (brs, 1H), 7.05 (s, 1H), 6.44 (s, 1H), 5.98 (s, 2H), 2.52 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 202.0, 162.1, 154.5, 140.5, 112.3, 107.2, 102.0, 98.7, 26.5; ESI-MS (*m*/*z*): 181 $[M+H]^+$.

8-hydroxy-6H-[1,3]dioxolo[4,5-g]chromen-6-one (3):

White solid; R_f = 0.3 (EtOAc/Hexane, 1:1); 1H NMR (500 MHz, DMSO-d_6): δ 7.17 (s, 1H), 7.04 (s, 1H), 6.15 (s, 2H), 5.59 (s, 1H).; 13C NMR (75 MHz, DMSO-d_6): δ 166.0, 162.2, 151.3, 150.6, 144.1, 109.1, 102.4, 100.3, 97.7, 88.7; ESI-MS (m/z): 207 $[M+H]^+$.

6-oxo-6H-[1,3]dioxolo[4,5-g]chromen-8-yl trifluoromethanesulfonate (4):

Yellow solid; R_f = 0.7 (EtOAc/Hexane, 1:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.01 (s, 1H), 6.89 (s, 1H), 6.35 (s, 1H), 6.14 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.2, 157.6, 153.4, 151.4, 145.9, 107.6, 103.1, 102.8, 99.9, 98.9; ESI-MS (*m*/*z*): 339 $[M+H]^+$.

8-(8-bromooct-1-yn-1-yl)-6H-[1,3]dioxolo[4,5-g]chromen-6-one (5):

Yellow solid; R_f = 0.6 (EtOAc/Hexane, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (s, 1H), 6.80 (s, 1H), 6.35 (s, 1H), 6.08 (s, 2H), 3.43 (t, J = 6.7 Hz, 2H), 2.56 (t, J = 7.0 Hz, 2H), 1.93 – 1.88 (m, 2H), 1.82 – 1.65 (m, 2H), 1.54 – 1.50 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 160.9, 151.5, 150.8, 145.0, 138.1, 115.2, 113.1, 104.2, 104.1, 102.5, 98.3, 75.4, 33.8, 32.6, 28.2, 28.1, 27.7, 19.8; ESI-MS (*m*/*z*): 377 $[M+H]^+$.

8-(8-bromooctyl)-6H-[1,3]dioxolo[4,5-g]chromen-6-one (6):

Yellow solid; R_f = 0.6 (EtOAc/Hexane, 1:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.98 (s, 1H), 6.83 (s, 1H), 6.15 (s, 1H), 6.07 (s, 2H), 3.41 (t, J = 6.8 Hz, 2H), 2.69 – 2.65 (m, 2H), 1.90 – 1.82 (m, 2H), 1.70 – 1.63 (m, 2H), 1.47 – 1.41 (m, 4H), 1.39 – 1.33 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 161.5, 156.4, 150.9, 145.0, 113.2, 111.1, 102.4, 101.9, 98.6, 34.0, 32.8, 32.3, 29.4, 29.2, 28.7, 28.2, 28.1; ESI-MS (*m*/*z*): 381 $[M+H]^+$.

4-(8-bromooctyl)-6,7-dihydroxy-2H-chromen-2-one (7):

Yellow solid; R_f = 0.3 (EtOAc/Hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 6.97 (s, 1H), 6.75 (s, 1H), 5.94 (s, 1H), 3.33 (t, *J* = 6.8 Hz, 2H), 2.60 – 2.54 (m, 2H), 1.81 – 1.72 (m, 2H), 1.63 – 1.53 (m, 2H), 1.39 – 1.22 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO d_6):) δ 161.6, 156.3, 148.8, 148.1, 141.9, 111.1, 109.4, 108.4, 102.8, 33.5, 32.1, 31.4, 31.2, 28.7, 28.0, 27.6, 27.5; ESI-MS (*m*/*z*): 369 $[M+H]^+$.

(8-(6,7-dihydroxy-2-oxo-2H-chromen-4-yl)octyl)triphenylphosphonium (8):

Yellow solid; R_f = 0.3 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.24 (brs, 1H), 9.30 (brs, 1H), 7.91 – 7.76 (m, 15H), 7.04 (s, 1H), 6.74 (s, 1H), 6.03 (s, 1H), 3.55 (t, J = 14.6 Hz, 2H), 2.65 – 2.61 (m, 2H), 1.57 – 1.43 (m, 6H), 1.26 – 1.32 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d6): δ 160.7, 156.7, 150.0, 148.0, 142.8, 134.8, 133.6, 133.5, 130.2, 130.1, 119.0, 118.1, 110.6, 109.4, 109.1, 102.8, 31.1, 29.8, 29.6, 28.4, 27.8, 21.6, 20.3, 20.1, 19.8; HRMS (ESI): calcd. for $C_{35}H_{36}O_4P^+$ $[M]^+$ 551.2351; found 551.2344.

4-(8-bromooctyl)-6,7-dimethoxy-2H-chromen-2-one (9):

Yellow solid; R_f = 0.6 (EtOAc/Hexane, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.96 (s, 1H), 6.85 (s, 1H), 6.15 (s, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.19 (t, J = 7.0 Hz, 2H), 2.72 (t, J = 7.6 Hz, 2H), 1.85 – 1.78 (m, 2H), 1.74 – 1.67 (m, 2H), 1.45 – 1.35 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.8, 156.2, 152.7, 149.7, 146.2, 111.9, 111.2, 105.1, 100.4, 56.6, 56.4, 33.5, 31.9, 30.5, 29.8, 29.4, 29.3, 28.5, 28.0; ESI-MS (*m*/*z*): 397 $[M+H]^+$.

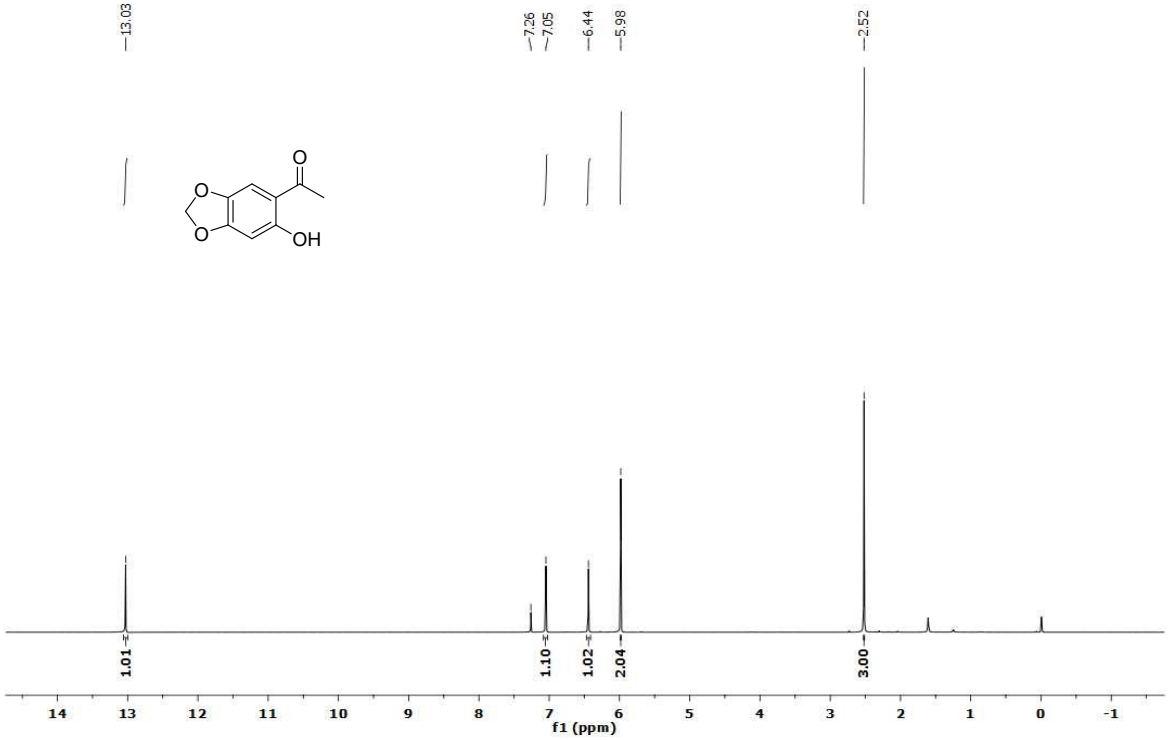
(8-(6,7-dimethoxy-2-oxo-2H-chromen-4-yl)octyl)triphenylphosphonium (10):

Yellow solid; R_f = 0.3 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.82 (m, 15H), 7.14 (s, 1H), 7.07 (s, 1H), 6.14 (s, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 3.62 – 3.51 (m, 2H), 2.80 – 2.73 (m, 2H), 1.64 – 1.44 (m, 6H), 1.35 – 1.24 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d6): δ 160.5, 157.0, 152.4, 148.9, 145.7, 134.8, 133.6, 133.5, 130.2, 130.1, 118.9, 118.1, 111.1, 110.1, 105.9, 100.3, 56.1, 56.1, 30.8, 29.8, 29.7, 28.4, 27.5, 21.7, 20.4, 19.9; HRMS (ESI): calcd. for $C_{37}H_{40}O_4P^+$ $[M]^+$ 579.2664; found 551.2665.

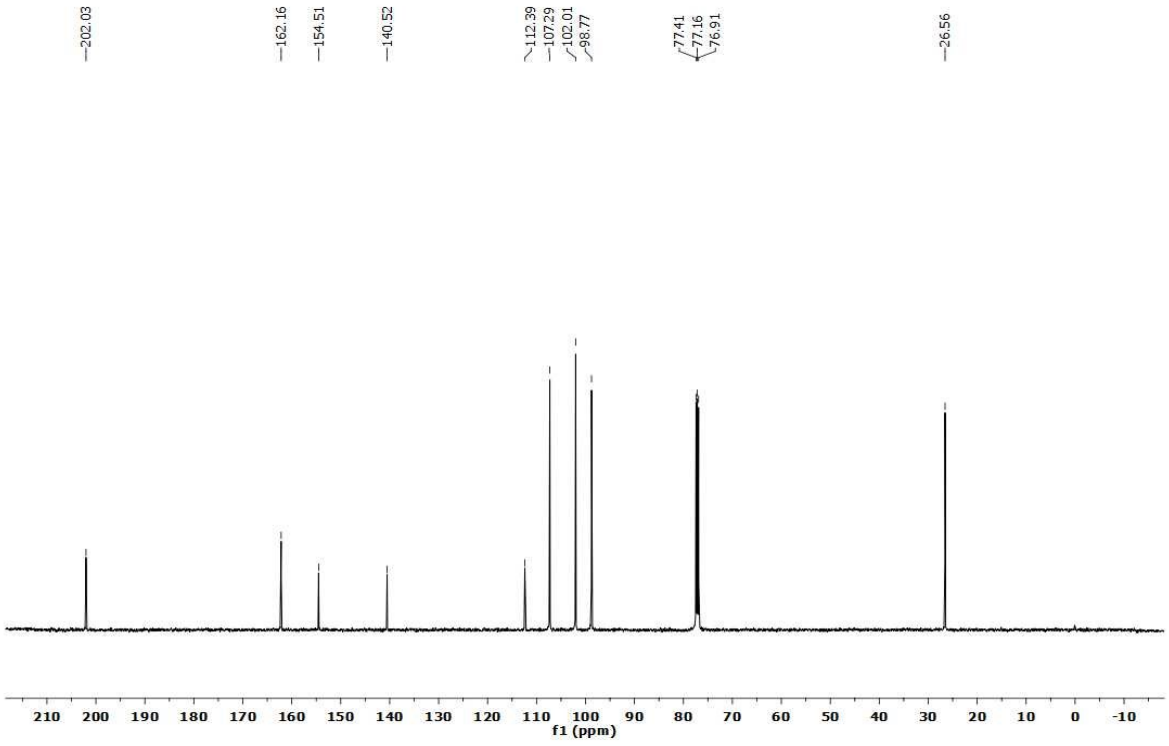
Octyltriphenylphosphonium (12):

Colorless liqiud; R_f = 0.3 ($CHCl_3$/MeOH, 1:9); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 – 7.67 (m, 15H), 3.84 – 3.75 (m, 2H), 1.65 – 1.58 (m, 4H), 1.26 – 1.16 (m, 8H), 0.82 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.1, 133.7, 133.7, 130.6, 130.5, 118.7, 118.0, 31.7, 30.5, 30.4, 29.2, 28.9, 23.0, 22.6, 14.1; HRMS (ESI): calcd. for $C_{26}H_{32}P^+$ $[M]^+$ 375.2242; found 375.2242

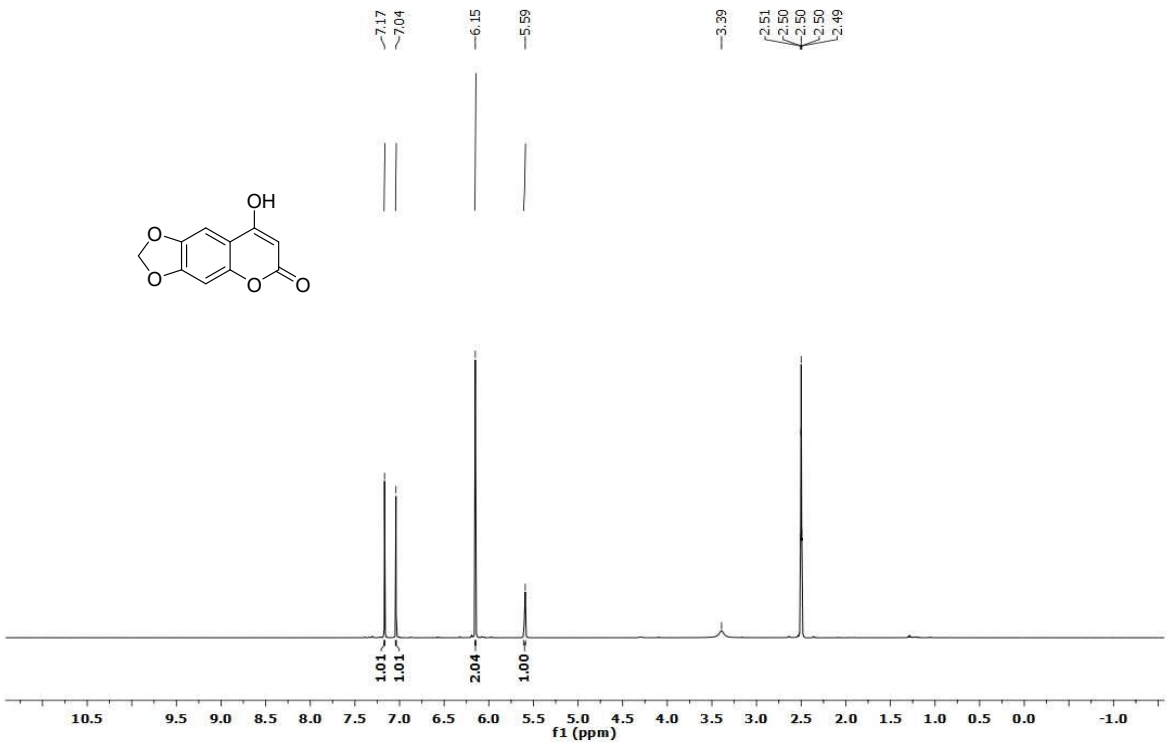
^{1}H and ^{13}C NMR spectra :



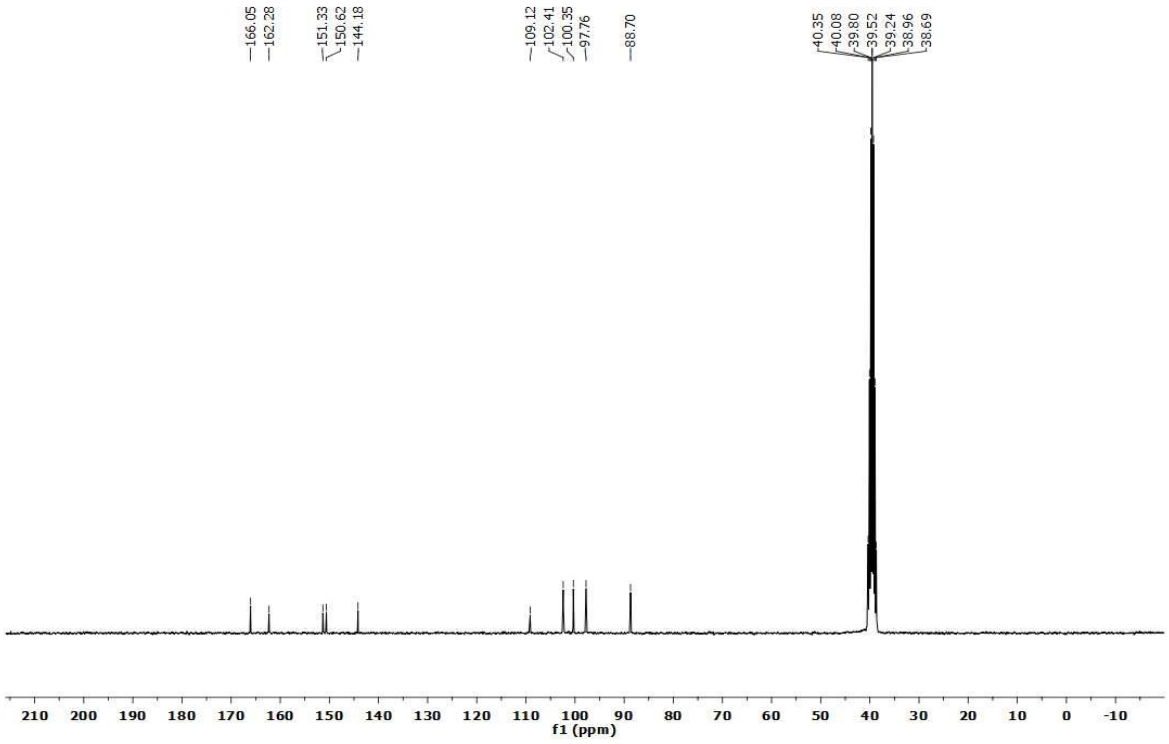
^{1}H-NMR spectrum of **2** (300 MHz, $CDCl_3$)



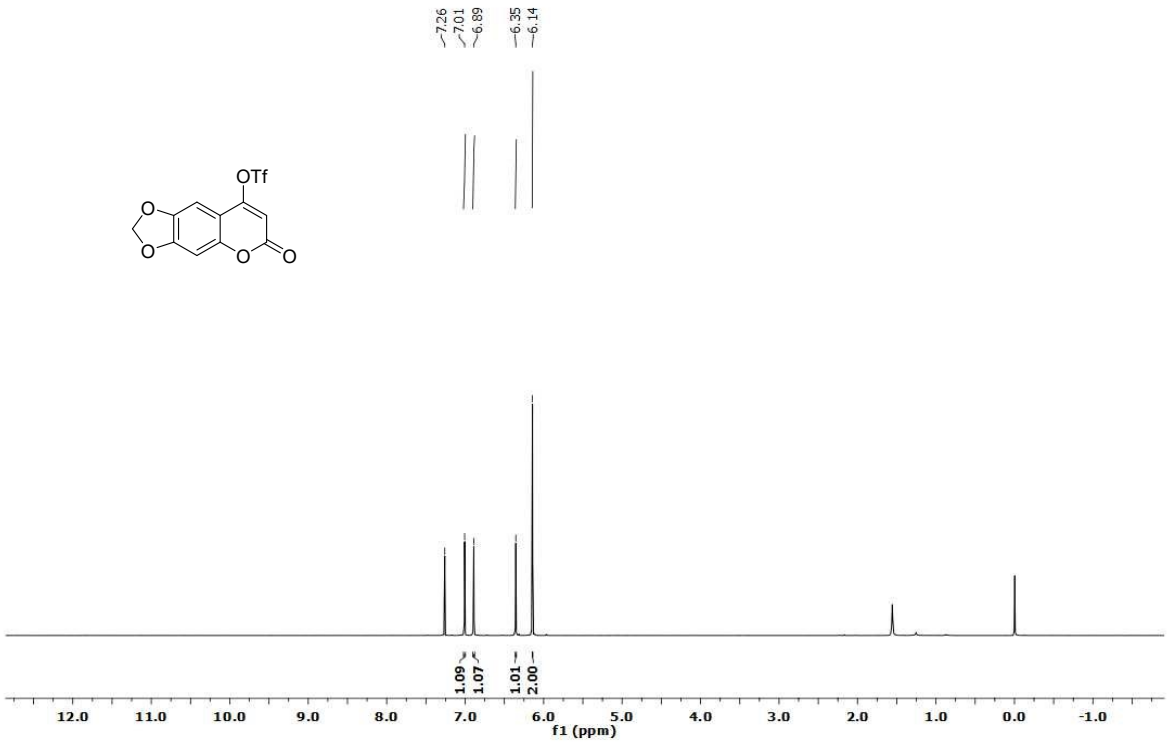
^{13}C-NMR spectrum of **2** (126 MHz, $CDCl_3$)



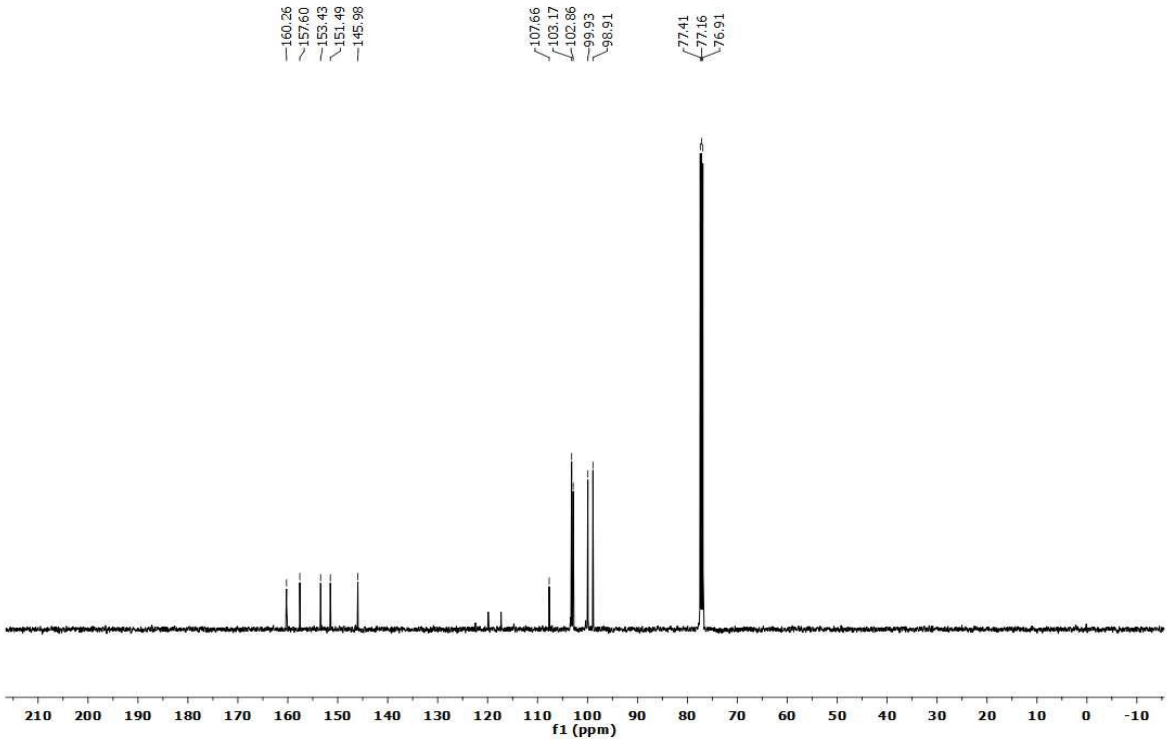
^{1}H-NMR spectrum of **3** (500 MHz, DMSO-d6)



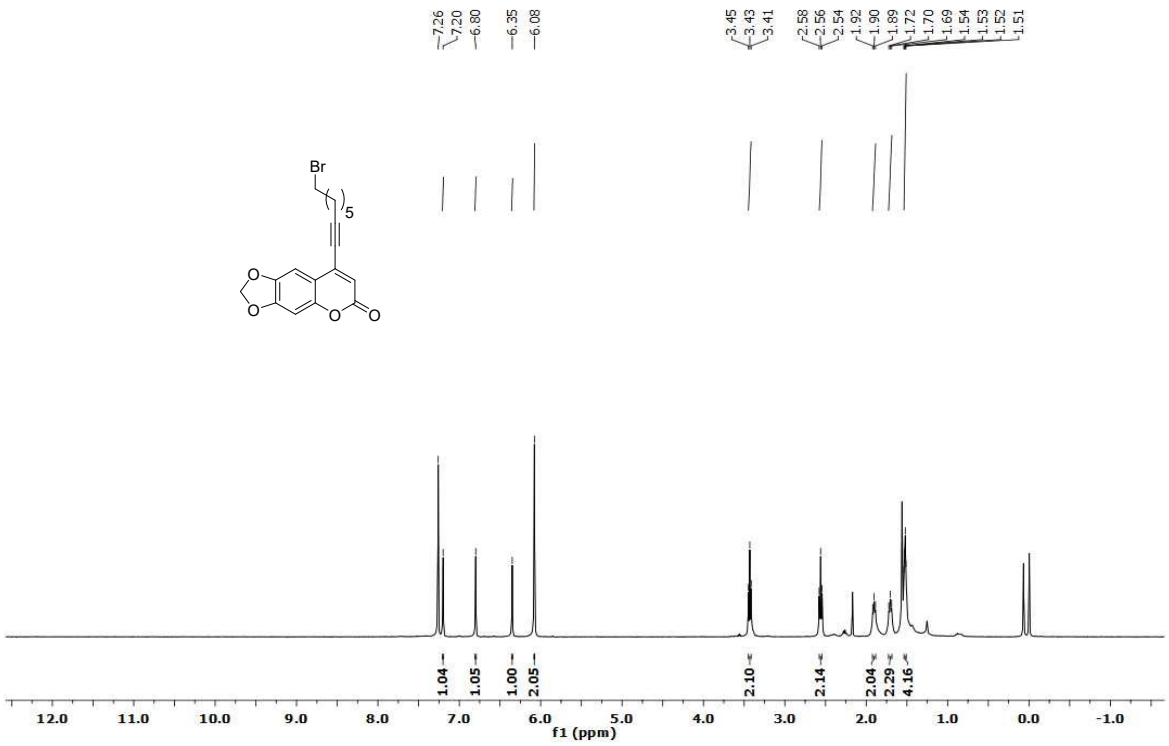
^{13}C-NMR spectrum of **3** (75 MHz, DMSO-d6)



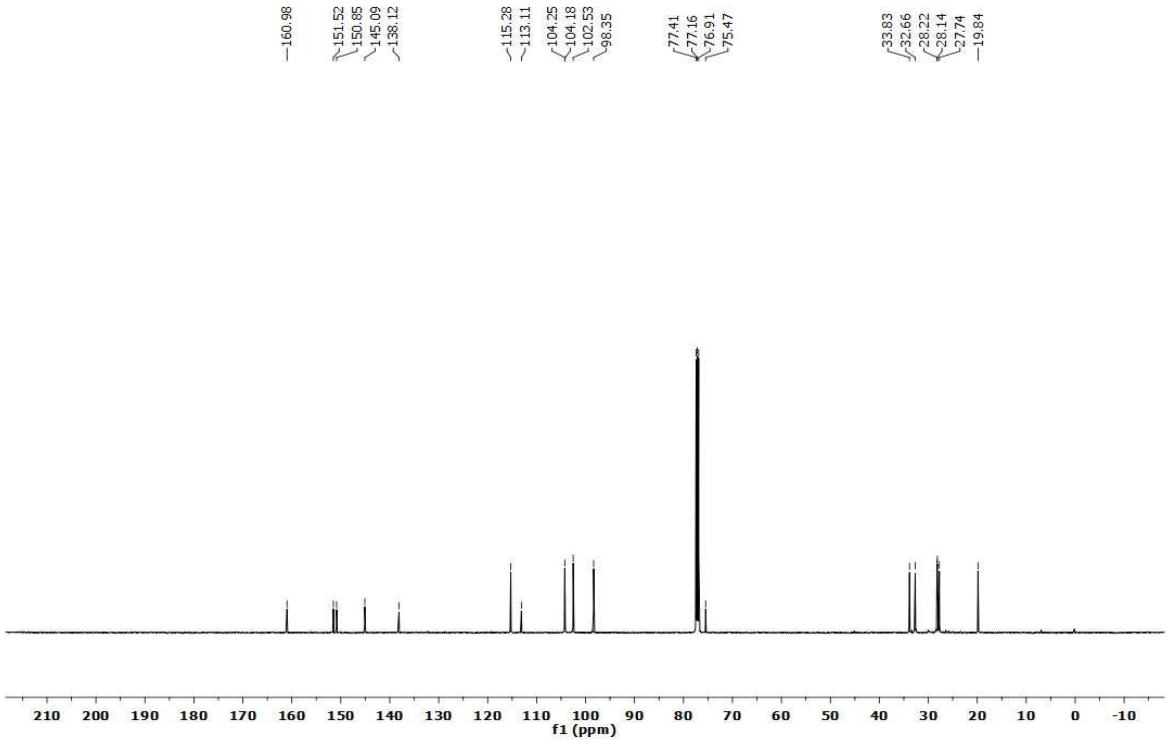
^{1}H-NMR spectrum of **4** (500 MHz, $CDCl_3$)



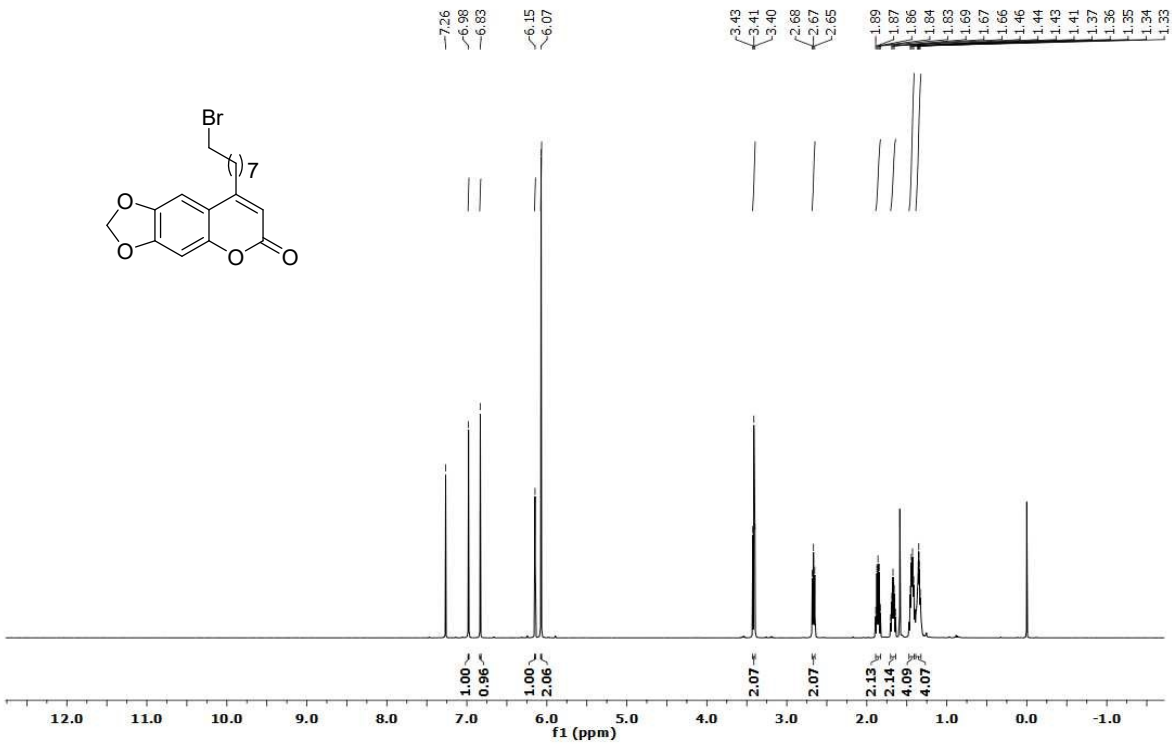
^{13}C-NMR spectrum of **4** (126 MHz, $CDCl_3$)



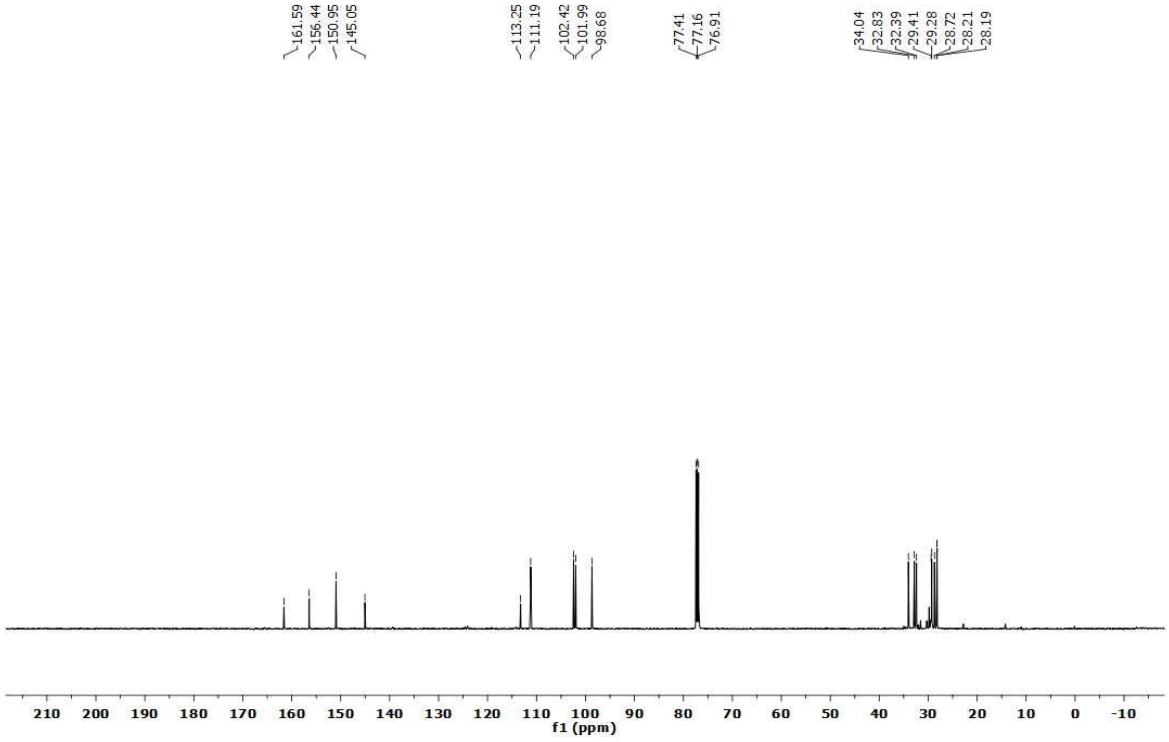
^{1}H-NMR spectrum of **5** (400 MHz, $CDCl_3$)



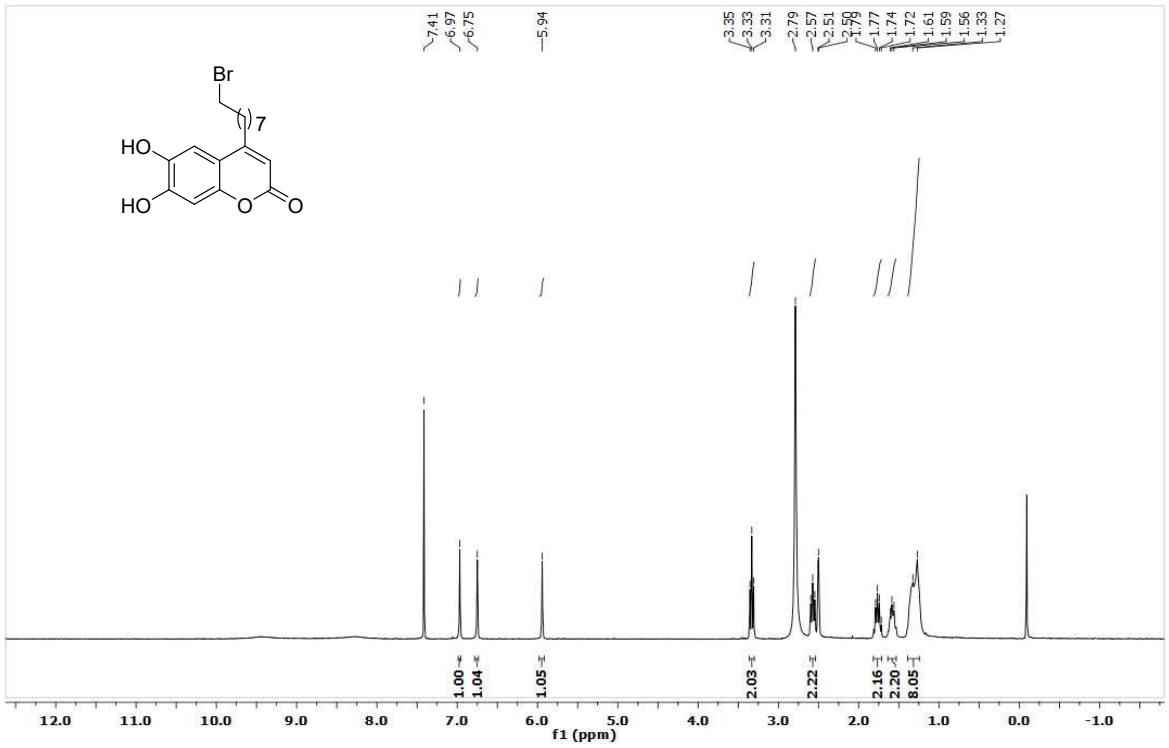
^{13}C-NMR spectrum of **5** (126 MHz, $CDCl_3$)



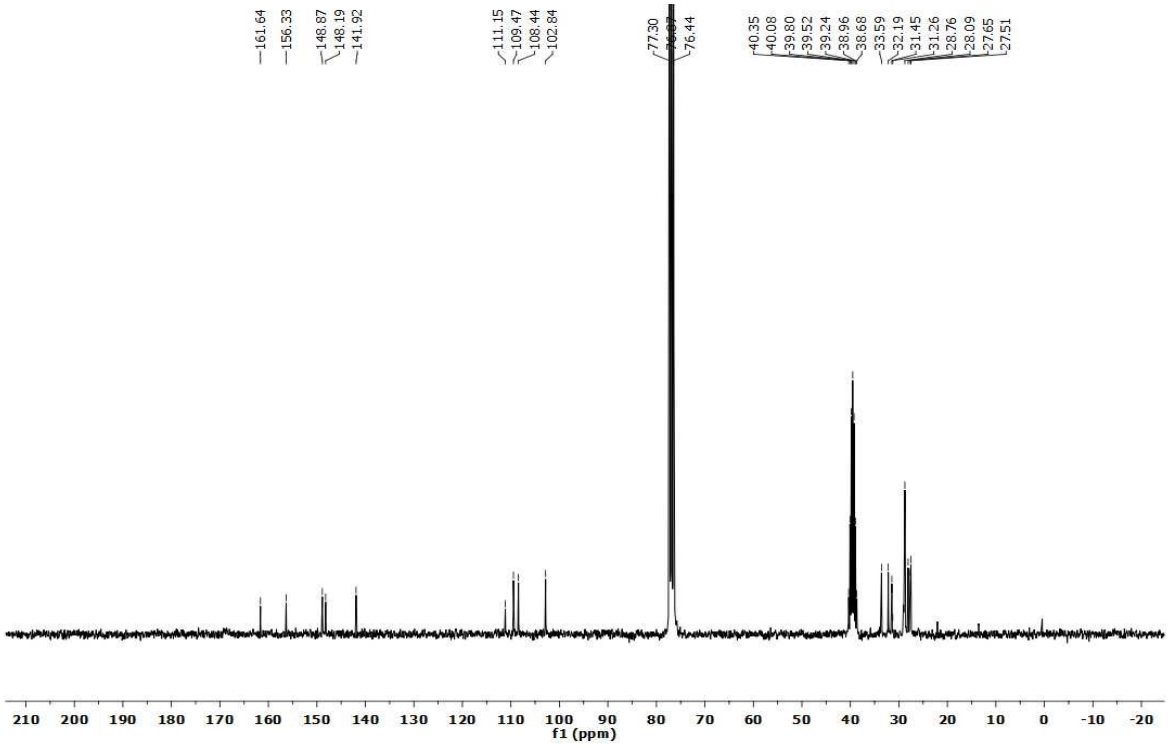
^{1}H-NMR spectrum of **6** (500 MHz, $CDCl_3$)



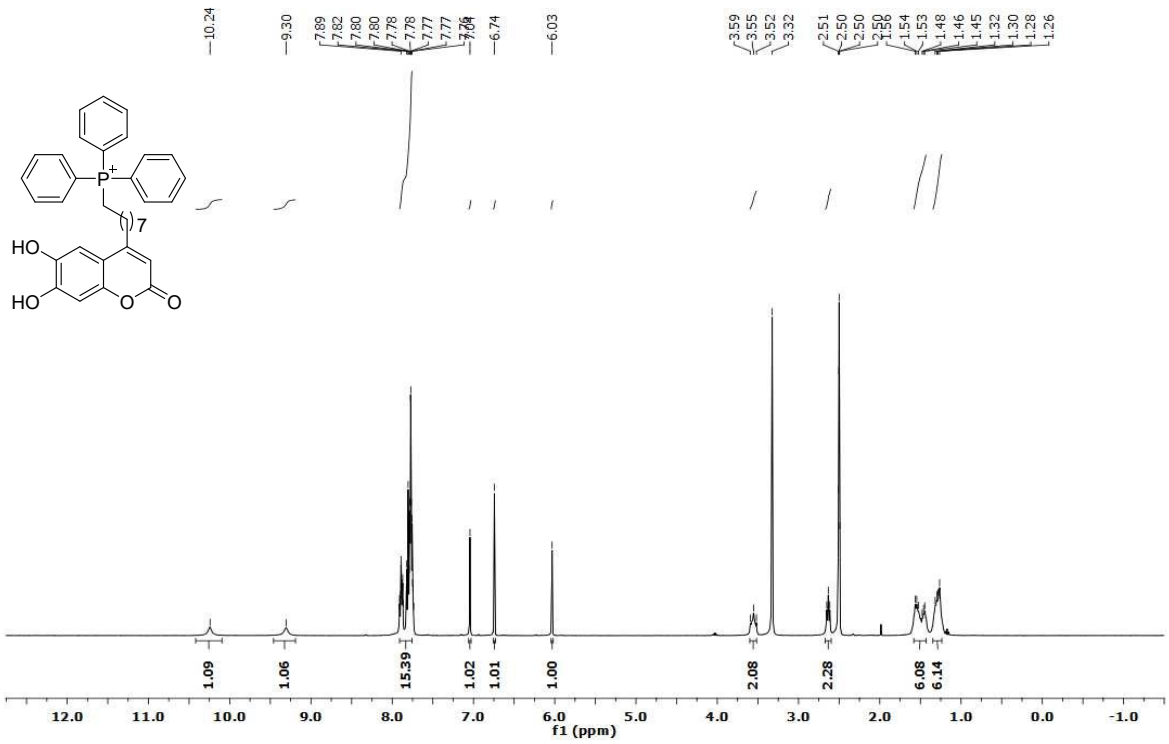
^{13}C-NMR spectrum of **6** (126 MHz, $CDCl_3$)



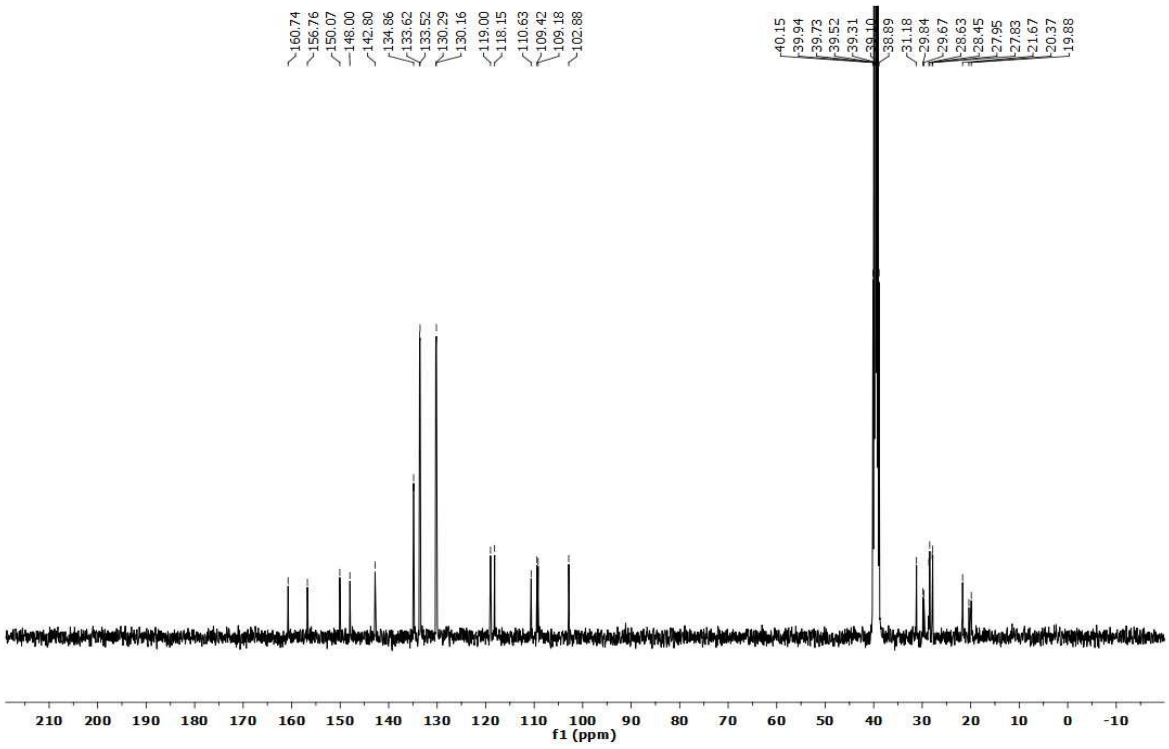
^{1}H-NMR spectrum of **7** (300 MHz, $CDCl_3$+DMSO-d6)



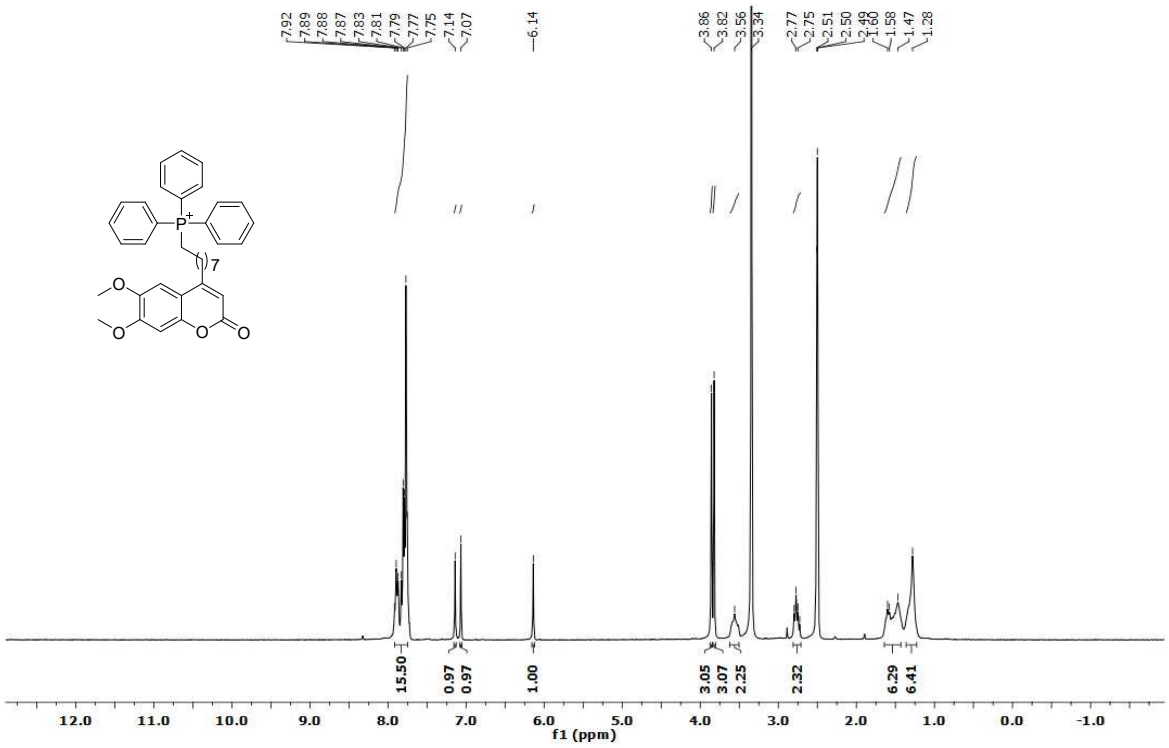
^{13}C-NMR spectrum of **7** (75 MHz, $CDCl_3$+DMSO-d6)



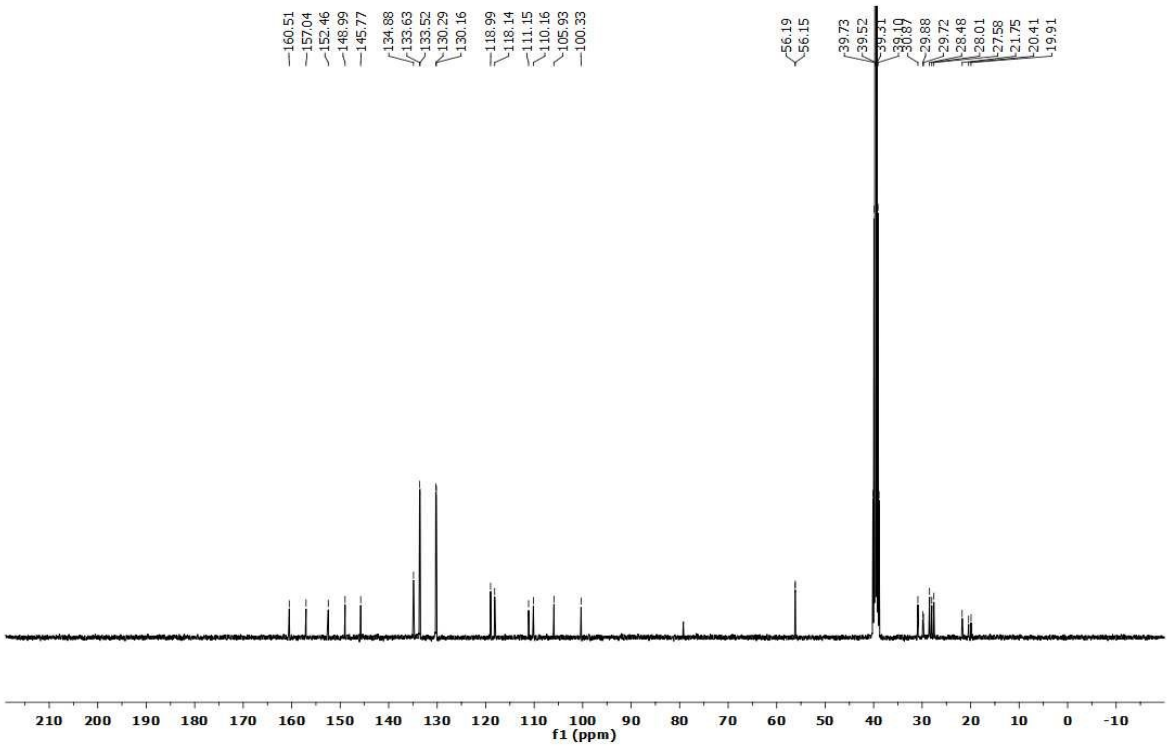
^{1}H-NMR spectrum of **8** (400 MHz, DMSO-d6)



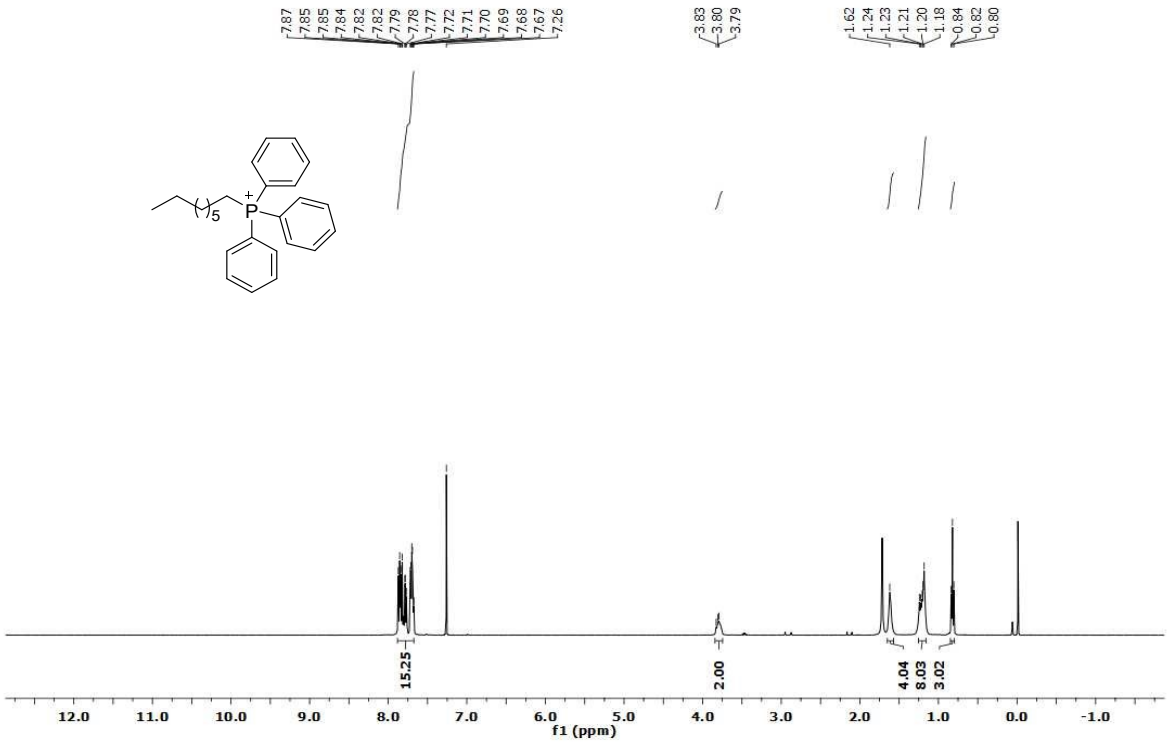
^{13}C-NMR spectrum of **8** (101 MHz, DMSO-d6)



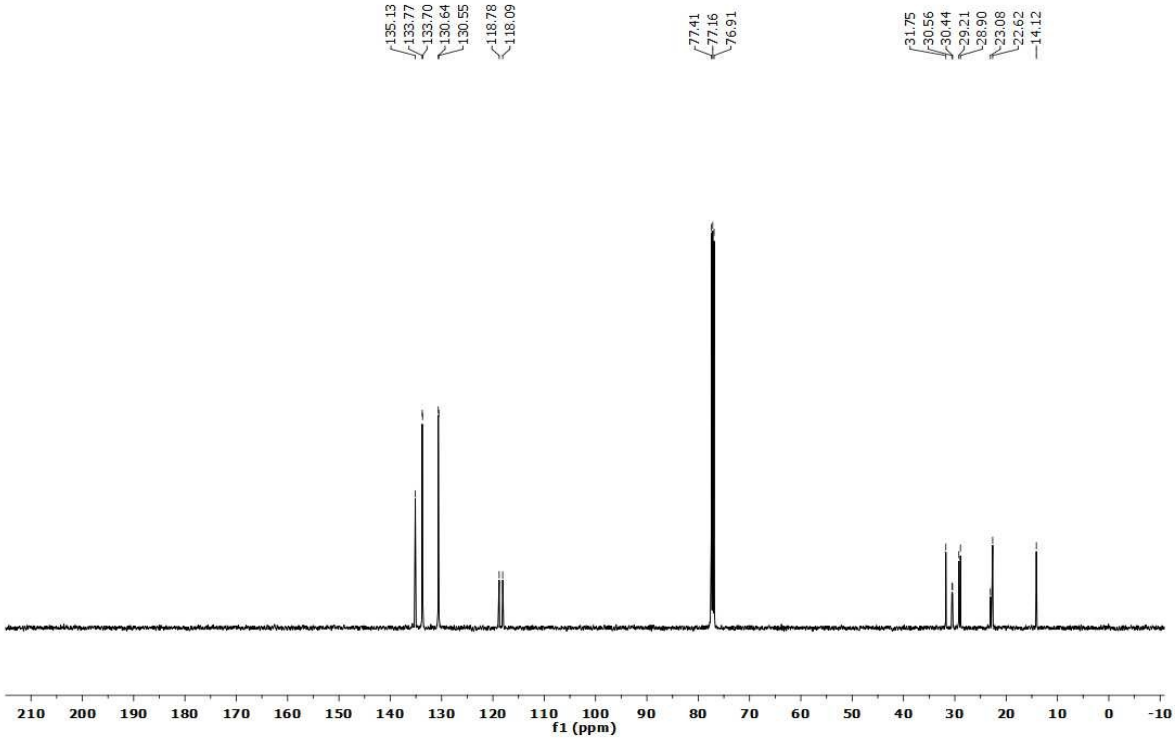
^{1}H-NMR spectrum of **10** (300 MHz, DMSO-d6)



^{13}C-NMR spectrum of **10** (101 MHz, DMSO-d6)



^{1}H-NMR spectrum of **12** (400 MHz, $CDCl_3$)



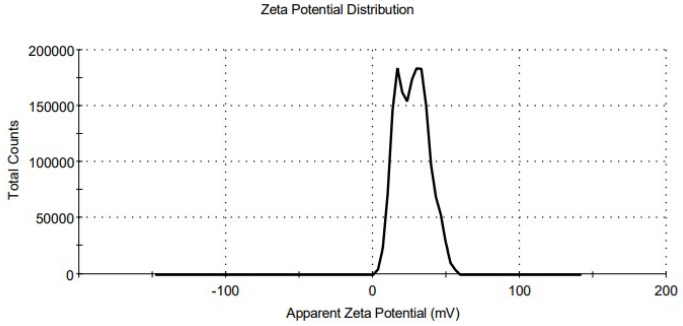
^{13}C-NMR spectrum of **12** (126 MHz, $CDCl_3$)

Cell culture:

MDA-MB-231 (a Triple negative breast cancer cell line, ATCC) and MCF-10A cells (normal mammary epithelial cells, ATCC) were grown in Dulbecco's Modified Eagle's medium (DMEM) containing 10% FBS, 1% (v/v) Sodium Pyruvate (100 mM), Sodium bicarbonate (26 mM), L-glutamine (4 mM), penicillin (100 units/ml), and streptomycin (100 µg/ml). Cells were maintained in an incubator at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

Size and surface charge measurements of Mito-Esc nanoparticles:

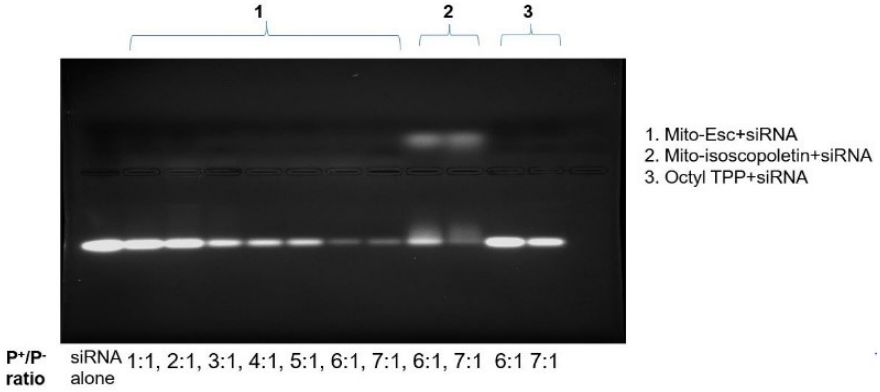
The size (hydrodynamic diameter) and the surface charge (zeta potentials) of Mito-Esc nanoparticle was measured by photon correlation spectroscopy and the electrophoretic mobility on a Zetasizer 3000HSA (Malvern, UK). The size was measured in deionized water with a sample refractive index of 1.33, a viscosity of 0.88 cP and temperature, 25 °C. The size was measured in triplicate. The zeta potential was measured using the following parameters: viscosity, 0.88 cP; dielectric constant, 78.5, and temperature, 25 °C.



Supplementary Fig. S1A. DLS: A) Size distribution Mito-Esc nanoparticles (166±30) B)Surface charge of Mito-Esc Nanoparticles (33±0.4 mV).

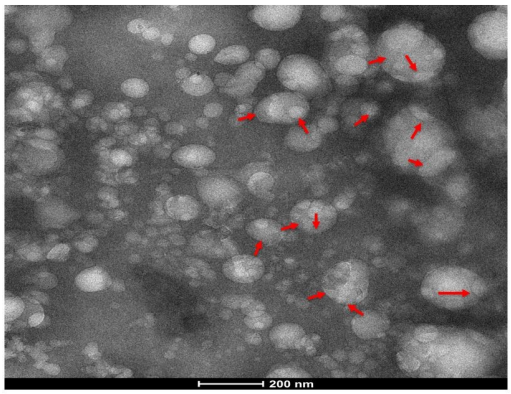
Agarose gel electrophoresis retardation assay:

Gel electrophoresis was performed with agarose gel (1.5% w/v) in tris-acetate-EDTA buffer (40 mM) with one drop of ethidium bromide added (concentration of EtBr stock solution: 0.625 mg/ml in H_2O), at 100 V for 30 min. The siRNA lipoplexes were prepared by complexing siRNA with Mito-Esc, Mito-isoscopoletin and octyl TPP cation at described P^+/P^- ratios. The samples were incubated for 30 min at room temperature prior to addition into well. The siRNA bands were visualized under UV illumination at 365 nm.



Supplementary Fig. S1B. Binding ability of Mito-Esc: Agarose Gel electrophoresis assay of siRNA lipoplexes formed with Mito-Esc, and Mito-isoscopoletin and octyl TPP at different P+/P- ratio.

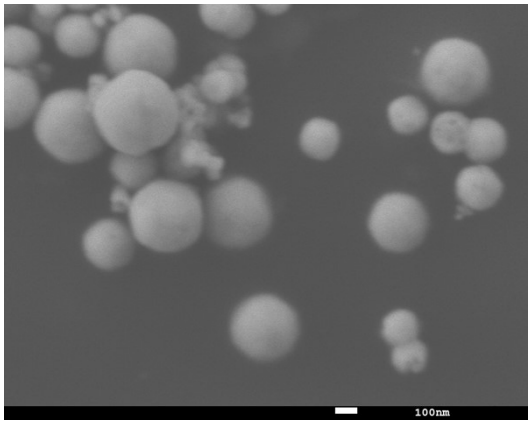
Transmission electron microscopy (TEM):



Supplementary Fig. S2. Transmission electron microscopy (TEM) image of Mito-Esc nanoaggregates.
Transmission electron microscopy studies were performed on a Tecnai T12 microscope (FEI) at 120 kV, and images were taken using SIS CCD camera; Samples were negatively stained with ammonium molybdate on 200 or 400 mesh carbon-coated copper grids (Ted Pella, Inc.). Before recording micrographs, the grids were air dried.

Going by the structure of the molecule, esculetin (coumarin) moiety and the C8 aliphatic chain contributes to hydrophobic component in the aggregate, whereas, TPP with positive charge is logically expected to contribute to provide hydrophilic balance, thus making an aggregate in nanometric size. Hence, unlike that in liposome, the Mito-Esc in aqueous media will form a predominantly micellar aggregate having hydrophobic core. Particles in micelle are generally in dynamic equilibrium and hence are expected to break, make and coalesce with each other under hydrophobic interaction over a period of time. Hence, we consistently observed an increased size and heterogeneity with time. So, initially the molecules in aggregate may exhibit a typically micellar structure, which will co-interact with other particles and may form bigger molecules (as shown by arrow head).

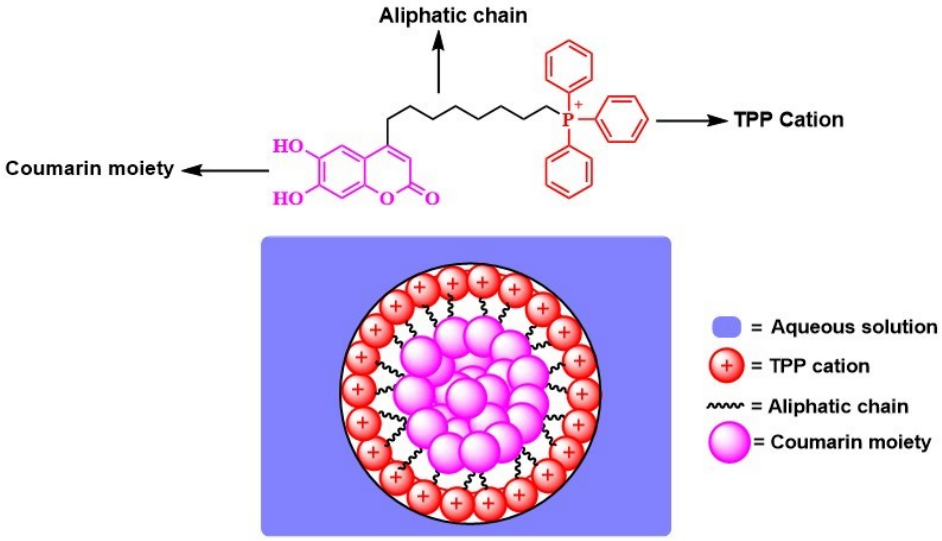
Scanning electron Microscopy (SEM):



Supplementary Fig. S3. Scanning electron microscopy (SEM) of Mito-Esc nanoaggregates

Field emission scanning electron microscopic (FESEM) analysis of Mito-Esc nanoparticle was performed on a Carl Zeiss SIGMA HD field-emission scanning electron microscope.

Schematic representation of Mito-Esc nanoaggregates:



Supplementary Fig. S4. Hypothetical representation of micellar structure of Mito-Esc nanoaggregates

Cytotoxicity of Mito-Esc and Mito-Esc/siRNA complexes:

To evaluate cytotoxicity of the Mito-Esc or Mito-Esc lipoplexes with siMnSOD, we used trypan blue dye exclusion assay. Briefly, cells were seeded in 12-well plate at a density of $3x10^4$ cells per well and cultured overnight before transfection. Medium was replaced with 0.5 mL fresh serum free DMEM. siMnSOD (40 nM) was complexed either with lipofectamine-2000 for control or with 2.5 µM Mito-Esc in serum free DMEM medium for 30 min before addition into the plates. The cells were incubated for 48 h, and at the end of the experiments, cells were trypsinized, spun at 800*g* for 2 min and resuspended in 1 mL fresh medium. The cell suspension (10 µl) was mixed with an equal amount of trypan blue and counted using an automated cell counter (Countess, Life Technologies).

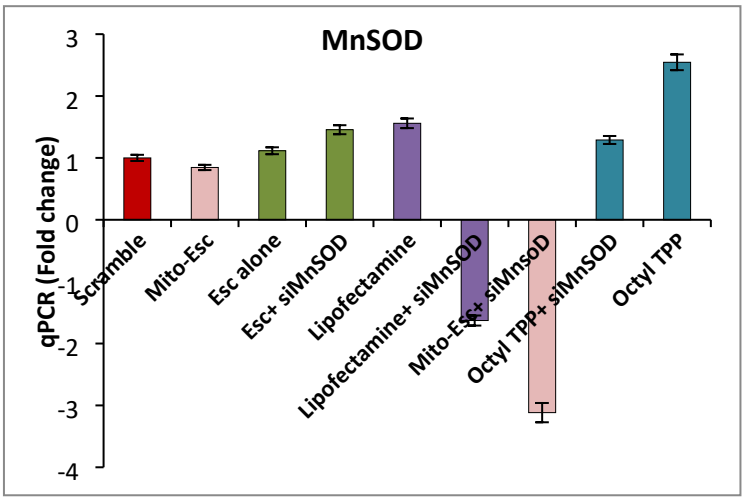
Western blotting:

At the end of the treatments, cell pellets were lysed in RIPA buffer containing protease Inhibitor cocktail, phosphatase inhibitor cocktail-2, 3. Proteins were resolved by SDS-PAGE and blotted onto nitrocellulose membrane and blocked with 5% bovine serum albumin, washed, and incubated with primary antibodies (1:1000) over night at 4 ^{0}C. Membranes were then washed and incubated for 1 h with anti-rabbit/mouse IgG horseradish peroxidase linked secondary antibodies (1:5000). Applied ECL reagent (Amersham GE) on membrane prior to developing with a chemiluminescent system (Bio-Rad).

MnSOD siRNA transfection:

Cells were cultured in 12-well plates at a density of $3x10^4$ cells per well (containing a glass coverslip the day before use). Briefly, florescent siRNA or siMnSOD (40 nM) was complexed either with lipofectamine-2000, as a positive control or with Mito-Esc (2.5 µM) in serum free DMEM medium for 30 min before addition into the plates. After incubation of the cells with siRNA complexes for 6 h, the medium was removed and replaced with 1 mL fresh DMEM medium containing 10% FBS and the cells were further incubated for 24 h.

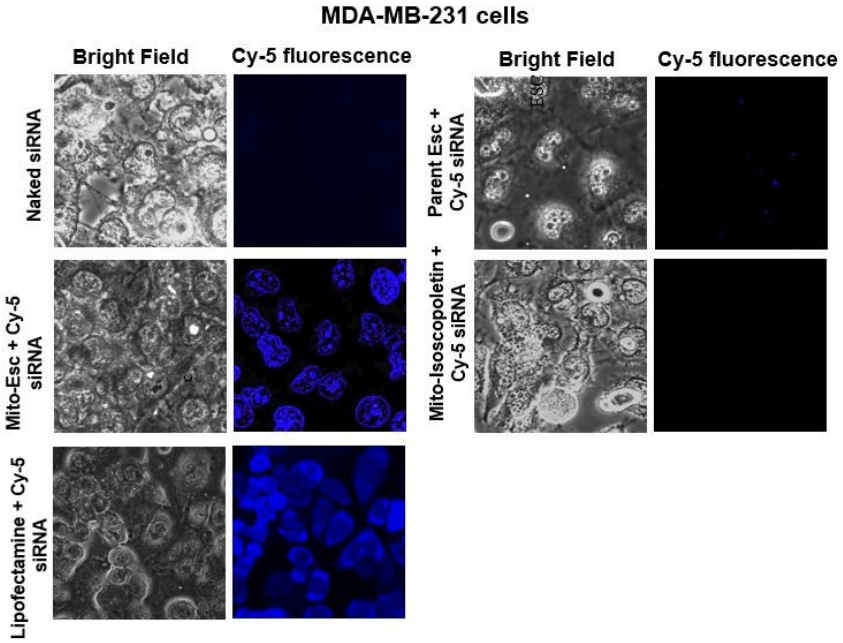
Measurement of MnSOD mRNA levels by qPCR:
Total RNA was isolated using TRIzol reagent. Subsequently, cDNA was prepared using 1 µg RNA according to the manufacturer's instructions (Thermo Fisher Scientific cDNA synthesis kit, Waltham, MA, USA). The mRNA levels were analyzed by Dynamo Color Flash SYBR Green qRT-PCR kit (Thermo Scientific, Waltham, MA, USA) with human MnSOD gene specific forward and reverse primers respectively (CGTGACTTTGGTTCCTTTGACA;ACTGAAGGTAGTAAGCGTGCTC).



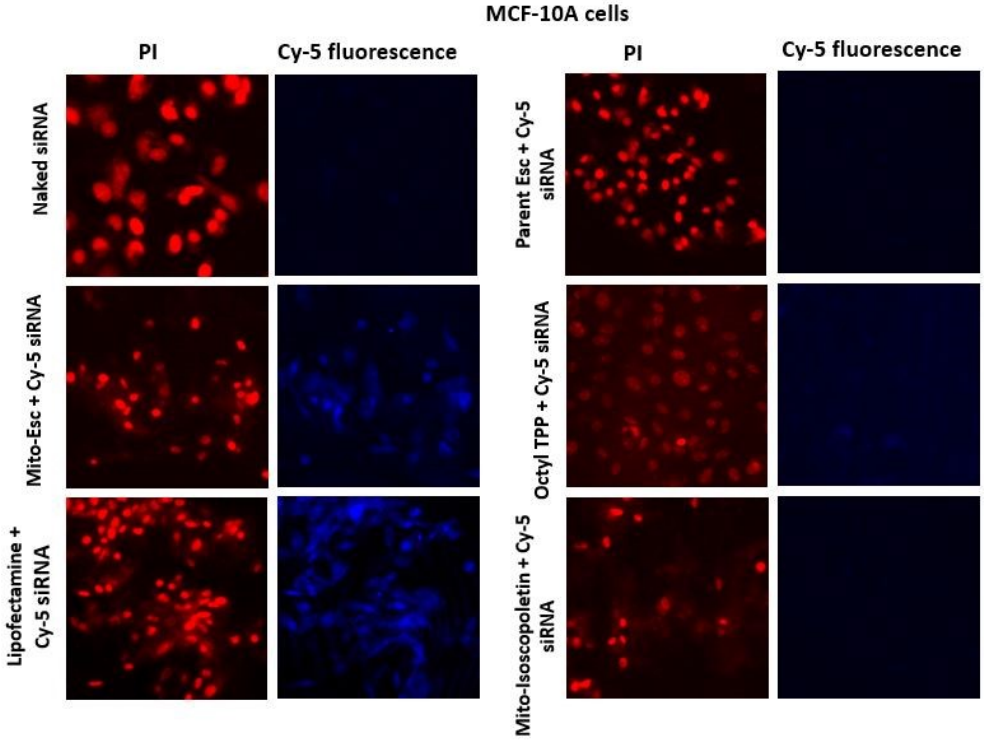
Supplementary Fig. S5. qPCR analysis of MnSOD gene in MDA-MB-231 cell line

Confocal microscopy imaging:

Briefly, MDA-MB-231 cells were seeded on coverslip in 12-well plates at a density of 3 x 10^4 cells per well in 1 mL complete DMEM and cultured for 12 h. Fluorescent (Cy-5) siRNA was complexed either with lipofectamine-2000 (positive control) or with Mito-Esc (2.5 µM) or parent esculetin (2.5 µM) or with different cationic lipids in serum free DMEM medium for 30 min before addition into the plates. These lipoplexes were added to the cells and incubated for 6 h. After that, cells were washed twice with PBS and fixed with 4% paraformaldehyde for 15 min. Finally, slides were mounted and the cells were imaged using a confocal microscope. Florescent labeled siRNA with Mito-Esc lipoplexes were prepared as described above and incubated with MDA-MB-231 cells for 24 h. The cells were stained by DAPI to stain the nucleus. The cells were mounted and observed under confocal microscope (Olympus, Tokyo, Japan).

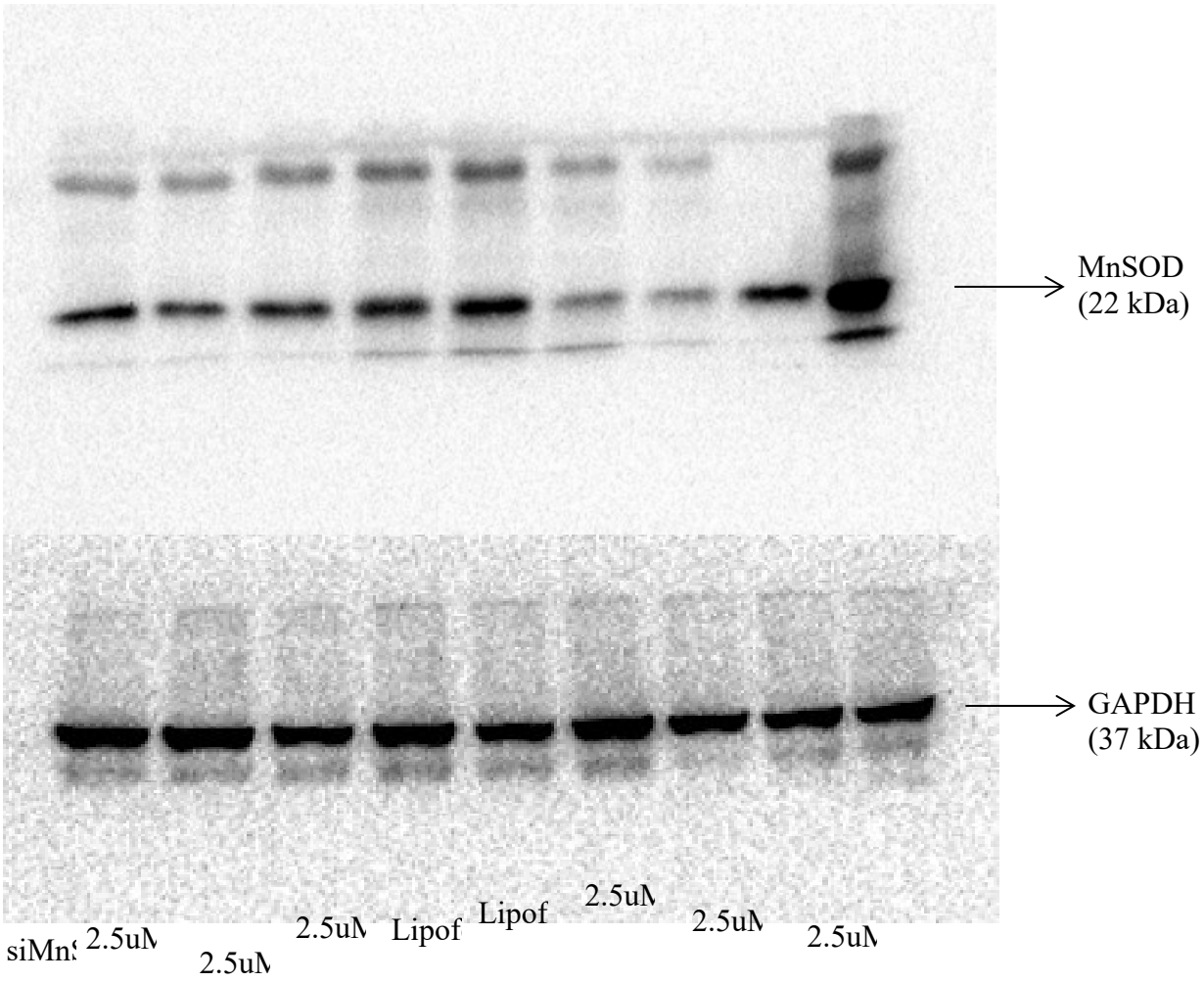


Supplementary Fig. S6. Detection of intracellular delivery of Cy-5 scrambled siRNA(Cell Signalling Technology Cat #86921) by Mito-Esc by confocal imaging. MDA-MB-231 cells were transfected with Cy-5 labeled siRNA (40 nM) with indicated delivery systems for 24 h. Transfection efficacy was evaluated using Confocal Microscopy. Cy-5 fluorescence in the cytosol is shown in blue color (Scale bar 60X).



Supplementary Fig. S7. Detection of intracellular delivery of Cy-5 siRNA by Mito-Esc using confocal imaging: MCF-10A cells were transfected with Cy-5 labeled siRNA (40 nM) with indicated delivery systems for 24 h. Transfection efficacy was evaluated using Confocal Microscopy. Cy-5 fluorescence in the cytosol is shown in blue

color. Briefly, cells were seeded on to a coverslip in 6-well plates at a density of 3 x 10^4 cells per well in 1 mL complete DMEM and cultured for 24 h. Fluorescent Cy-5 siRNA was complexed with Mito-Esc, parent esculetin, Mito-isoscopoletin, octyl TPP cation and lipofectamine-2000 in DMEM medium. Cells were then incubated with the prepared lipoplexes for 24 h, washed twice with PBS and fixed with 4% paraformaldehyde for 15 min and then treated with DAPI to stain the nucleus. Finally, slides were mounted and the cells were imaged using a confocal microscope.



Supplementary Fig. S8. Unprocessed raw blots of MnSOD and GAPDH (loading control) of the western blots represented in Fig. 3b