Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

ELECTRONIC SUPPLEMENTARY INFORMATION

Functionalization of multi-walled carbon nanotubes with coumarin derivatives and their biological evaluation

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Materials and techniques

Solvents and reagents were used as received from commercial sources. Sonication was carried out in a batch using Bandeling Sonorex Type RK52H equipment. Nuclear magnetic resonance spectra (^{1}H NMR recorded at 300 or 500 MHz, ^{13}C NMR recorded at 75 or 125 MHz) were obtained on Varian Instruments and are referenced in ppm relative to TMS or the solvent signal. Thin-layer chromatographic separations were performed on Merck silica gel 60-F_{254} precoated aluminum plates. Flash chromatography was accomplished on Merck silica gel (200–400 mesh). Preparative separations were carried out by MPLC Büchi C-601 using

Merck silica gel 0.040–0.063 mm and the eluting solvents were delivered by a pump at the flow-rate of 3.5–7.0 mL min^{-1}.

Transmission electron microscopy (TEM), EDX analyses, thermogravimetry (TGA), FTIR analyses and acid-base titration were carried out to characterize pristine and functionalized MWCNTs. The morphology of MWCNTs was analyzed using an HRTEM JEOL JEM 2010 analytical electron microscope (LaB_6 electron gun) operating at 200 kV and equipped with a Gatan 794 Multi-Scan CCD camera for digital imaging. HRTEM samples were prepared by placing a drop of the MWCNTs dispersed in isopropanol on holey-carbon coated copper grids. EDX analyses were carried out in a JEOL, JSM 5600 LV, scanning electron microscope using a voltage of 20 kV. Thermogravimetrical studies were performed from 30 to 1000 °C at 10 °C/min under nitrogen on a TA Instruments SDTQ600 (TA instruments). IR spectra were recorded on a Nicolet FT-IR Impact 400D spectromer. UV spectra have been performed by Thermo Nicolet mod, Evolution 500 spectrophotometer.

Fourier transform-infrared (FT-IR) spectroscopy

The functionalization of various surface-modified MWCNTs was confirmed by FT-IR spectroscopy. FT-IR spectra were recorded on a Nicolet FT-IR Impact 400D spectromer. For pristine CNT, the curve doesn't show significant absorption peaks whereas for CNT-Ox, carboxylation in nitric acid and sulfuric acid provides a typical absorption peak at 1710 cm^{-1}. For the CNT-Link-Coum-oleic and CNT-Link-Coum the IR spectra show a complex absorbance set around 1600-1730 cm^{-1} indicating the existence of carbonyl groups and the double bounds. The IR spectra of these conjugates also provide the absorbances at 3200-3500

cm^{-1} that can be assigned to the N-H and O-H stretching modes together with the absorbances at around 1050 cm^{-1} corresponding to the C-O and C-N stretching modes (figure 1).

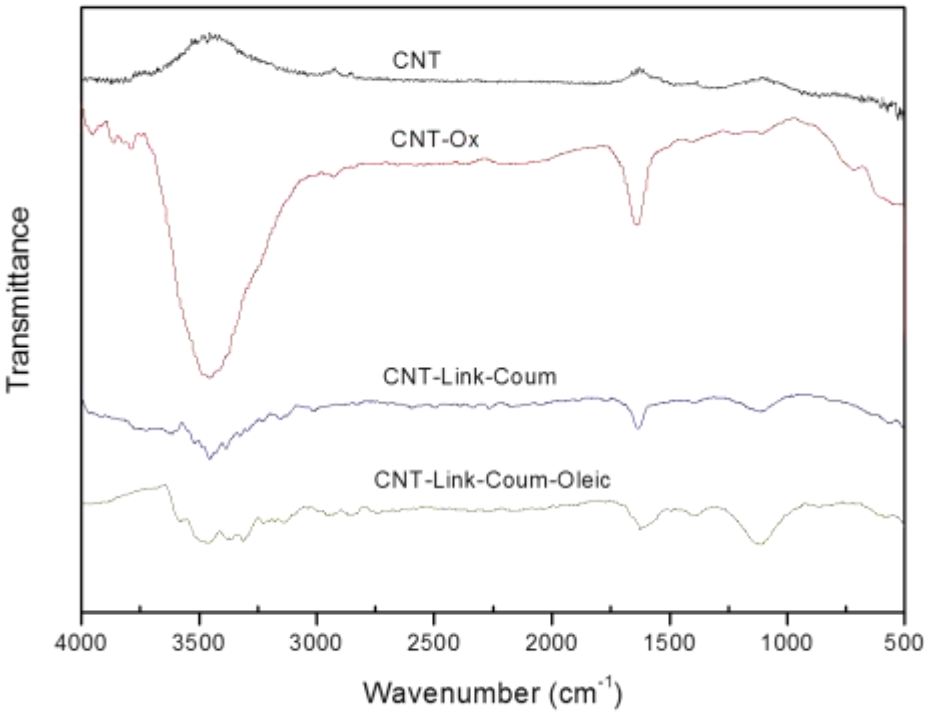


Figure 1. FTIR spectra of the samples: CNT, CNT-Ox, CNT-Link-Coum, CNT-Link-Coum-Oleic.

UV/Vis spectroscopy

UV spectra have been performed by Thermo Nicolet mod, Evolution 500 spectrophotometer. The UV spectra confirm the presence of coumarins covalently bonded to the nanotubes. MWCNTs conjugated with 7-oleate coumarin and 7-hydroxy coumarin show an absorbance at 240-300 nm, while no absorbance was detected for the MWCNTs functionalized with the linker; a different spectrum was registered for isothiocyanatoethoxy-7-oleate coumarin.

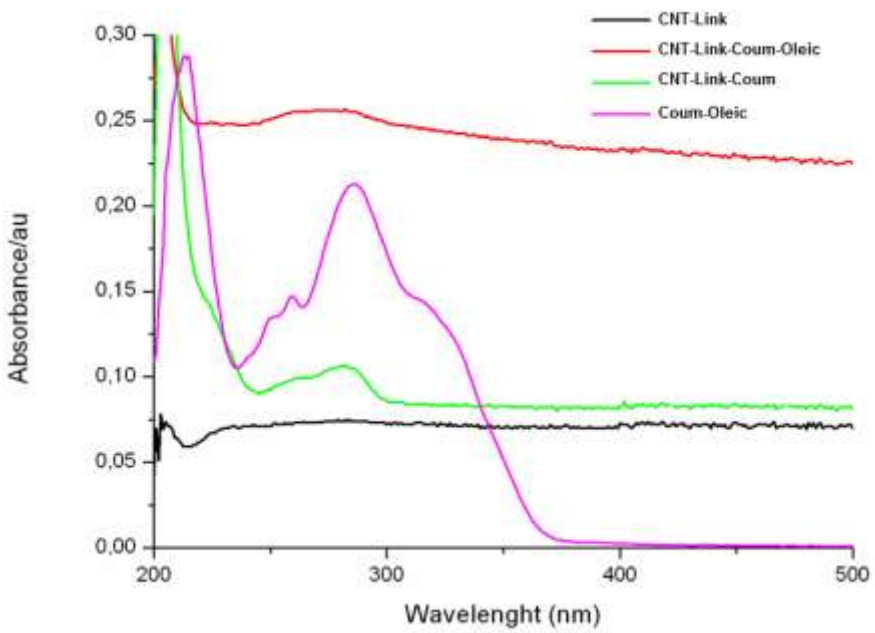


Figure 2. UV/Vis spectra of the samples: CNT-Link, CNT-Link-Coum, CNT-Link-Coum-Oleic and free Coum-Oleic.

Preparation of *CNT-LINK BLANK SAMPLE*.

A mixture of 5-(2-aminoethoxy)-7-hydroxy-2H-chromen-2-one (0.15 mmol, 30 mg) and CNT-Link (100 mg) in absolute ethanol (6 mL) was stirred at room temperature for 12h. The mixture was filtered under vacuum on a 0.1 μm Millipore membrane. The solid residue was washed for three-time with ethanol (3 x 20 mL) and each time sonicated for 10 min and separated from the supernatant by filtration. The solid residue was then dried under vacuum at 50 °C to give sample CNT-Link blank**.**

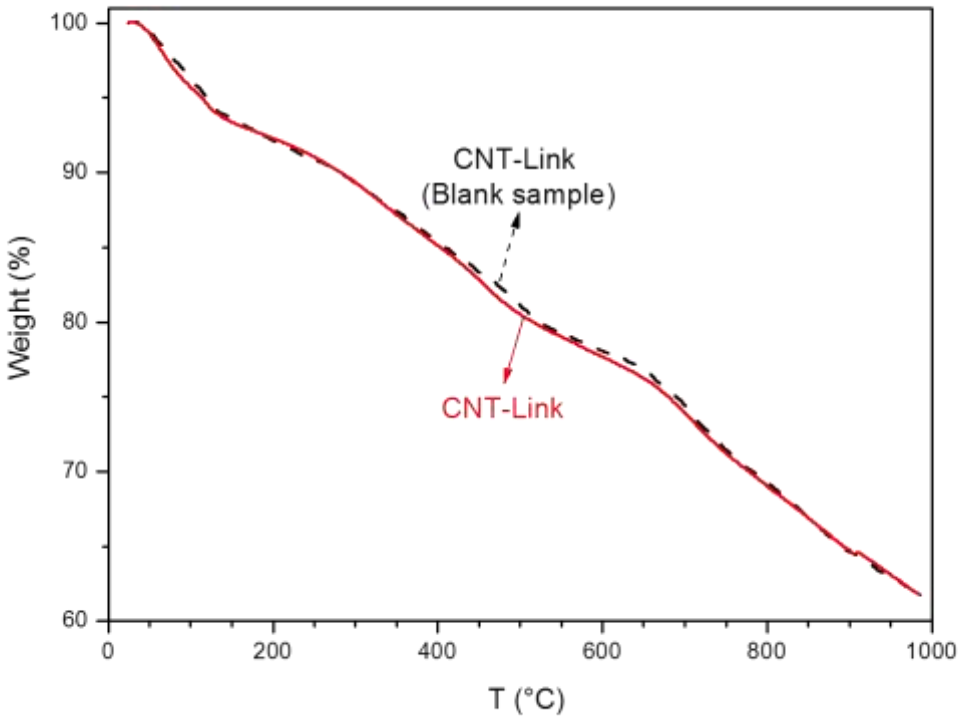
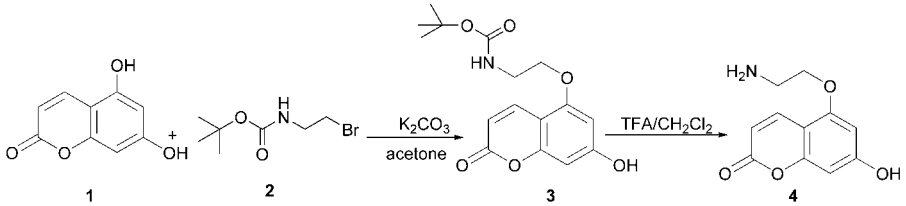


Figure 3. TGA curve of CNT-Link and CNT-Link (Blank sample)

Preparation of Coumarine Derivatives

Synthesis of 5-oxyethyl-tert-butyl carbamate-7-hydroxy coumarin ***4***

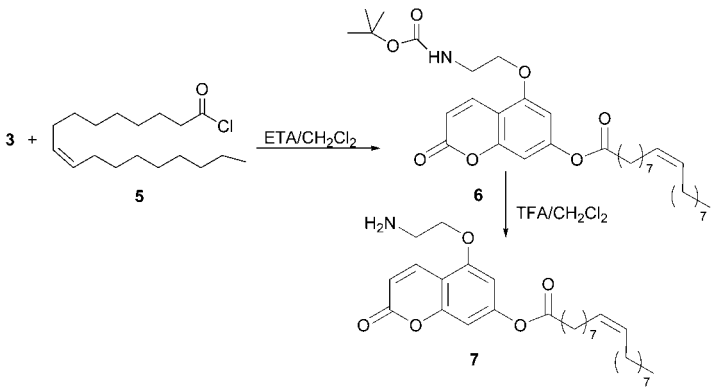


To a solution of 5,7-dihydroxy-2*H*-chromen-2-one **1** (601 mg 3.38 mmol) in acetone (30 mL) potassium carbonate (513 mg, 3.72 moles) was added and the solution was refluxed for 1

hour, then *tert*-butyl 2-bromoethylcarbamate **2** (832 mg, 3.72 mmol) was added and the solution was refluxed for 18 h. The reaction was stopped and filtered after cooling to room temperature. The ***tert*-butyl 2-(7-hydroxy-2-oxo-2H-chromen-5-yloxy)ethylcarbamate 3** was obtained in 45% yields after MPLC purification using 0.5% methanol:chloroform solvent mixture. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.62 (s, 9H), 3.65 (t, 2H, *J* = 5.5 Hz), 4.22 (t, 2H, *J* = 5.5 Hz), 5. 20 (bs, 1H), 5.80 (bs, 1H), 6.25 (d, 1H, *J* = 8.5 Hz), 6.50 (s, 1H), 6.60 (s, 1H), 8.18 (d, 1H, *J* = 8.5). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 161.1, 160.0, 155.1, 152.49, 125.6, 113.8, 112.1, 101.7, 79.5, 67.7, 39.8, 28.3. HRMS (ES) calcd for $C_{16}H_{19}NO_6$: 321.1212 $[M+H]^+$; Found**:** 321.1208. EA calcd (%) for $C_{16}H_{19}NO_6$: calcd. C 59.81, H 5.96, N 4.36; found C 59.78, H 5.92, N 4.31.

To a solution of *tert*-butyl 2-(7-hydroxy-2-oxo-2H-chromen-5-yloxy)ethylcarbamate **3** (400 mg, 1.25 mmol) in dichloromethane (50 mL), 8 ml of trifluoroacetic acid (TFA) was added dropwise and the reaction mixture was stirred at room temperature overnight. After stirring overnight, the solvent was removed and the residue was subjected to flash chromatography using a 9 :1 mixture of $CHCl_3$/MeOH to afford **5-(2-aminoethoxy)-7-hydroxy-2H-chromen-2-one 4** , yellow oil, 80% yield. ^{1}H NMR (CD_3OD , 300 MHz) δ 3.75 (t, 2H, *J* = 5.5 Hz), 4.62 (t, 2H, *J* = 5.5 Hz), 6.25 (d, 1H, *J* = 8.5 Hz), 6.60 (s, 1H), 6.62 (s, 1H), 8.45 (d, 1H, *J* = 8.5). HRMS (ES) calcd for $C_{11}H_{11}NO_4$: 221.0688 $[M+H]^+$; Found**:** 221.0682. EA calcd (%) for $C_{11}H_{11}NO_4$: calcd. C 59.73, H 5.01, N 6.33; found C 59.70, H 4.98, N 6.30.

Synthesis of (9Z)-5-(2-aminoethoxy)-2-oxo-2H-chromen-7-yl octadec-9-enoate 7



To a solution of **3** in CH_2Cl_2 (20 mL) 1.2 eq. of Oleoyl chloride and 1.2 eq. of triethylamine were added and the mixture was then stirred for 12h at r.t. The solvent was, then removed and the residue was subjected to purification by MPLC using a 98/2 mixture of $CHCl_3$/MeOH to obtain **(9Z)-5-(2-aminoethoxy)-2-oxo-2H-chromen-7-yl octadec-9-enoate** *(N*-Protected with tert-butyl carbamate), yellow oil, 70% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (t, 3H, *J* = 7.5 Hz), 1.27 (m, 20H), 1.45 (s, 9H), 1.73 (t, 2H, *J* = 7.2 Hz), 2.01 (m, 4H), 2.18 (t, 2H, *J* = 7.2 Hz), 3.56 (t, 2H, *J* = 4.2 Hz), 4.10 (t, 2H, *J* = 4.2 Hz), 5.01 (bs, 1H), 5.35 (m, 2H), 6.29 (d, 1H, *J* = 9.5 Hz), 6.48 (s, 1H), 6.69 (s, 1H), 8.04 (d, 1H, *J* = 9.5 Hz). HRMS (ES) calcd for $C_{34}H_{51}NO_7$: 585.3666 $[M+H]^+$; Found**:** 585.3662. EA calcd (%) for $C_{34}H_{51}NO_7$: calcd. C 69.71, H 8.78, N 2.39; found C 69.68, H 8.80, N 2.35.

The obtained compound was deprotected with trifluoroacetic acid to give **(9Z)-5-(2-aminoethoxy)-2-oxo-2H-chromen-7-yl octadec-9-enoate**. ^{1}H NMR (CD_3OD, 500 MHz) δ 0.89 (t, 3H, *J* = 6.9 Hz), 1.27 (m, 20H), 1.75 (qt, 2H, *J* = 7.2 Hz), 2.05 (m, 4H), 2.60 (t, 2H, *J* = 7.2 Hz), 3.55 (t, 2H, *J* = 4.2 Hz), 4.20 (t, 2H, *J* = 4.2 Hz), 5.35 (m, 2H), 6.34 (d, 1H, *J* = 10.0 Hz), 6.77 (s, 1H), 6.81 (s, 1H), 8.32 (d, 1H, *J* = 10.0 Hz). ^{13}C NMR (CD_3OD, 125 MHz) δ 36.3, 38.1, 40.7, 63.3, 67.9, 70.5, 71.3, 71.4, 124.1, 124.3, 129.2, 130.1, 130.2, 130.8, 130.9, 133.3, 135.4, 135.5, 146.6, 167.8, 169.7. HRMS (ES) calcd for $C_{29}H_{43}NO_5$: 485.3141 $[M+H]^+$; Found**:** 485.3139. EA calcd (%) for $C_{29}H_{43}NO_5$: calcd. C 71.72, H 8.92, N 2.88; found C 71.69, H 8.90, N 2.85.

Identification of coumarin isothiocyanate derivatives

To a solution of 5-(2-aminoethoxy)-7-hydroxy-2H-chromen-2-one (0.15 mmol, 30 mg) in absolute ethanol (5 mL), CS_2 (1.5 mmol, 114 mg) and triethylamine (0.15 mmol, 0.02 mL) were added. The reaction mixture was stirred for 30 min at room temperature and then cooled on an ice bath. Di-tert-butyl dicarbonate (Boc_2O, 0.15 mmol, 33 mg), dissolved in absolute ethanol, was added followed by the immediate addition of a catalytic amount of dimethylaminopyridine (DMAP, 1-3 mol%) in absolute ethanol (1 mL). The reaction mixture was kept in the ice bath for 5 min and then allowed to reach the room temperature. After that evolution of gas from the reaction mixture had ceased (approximately 15 min), before the CNT-Link and triethylamine addition the reaction mixture was evaporated in vacuo. The the residue was taken up in diethyl ether and triethylammonium hydrochloride was filtered off, and the filtrate was evaporated in vacuo to afford**, 5-(2-isothiocyanatoethoxy)-7-hydroxy-2H-chromen-2-one,** red solid, m.p. 78-80 °C, 95% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.65 (t, 2H, *J* = 7.5 Hz), 4.66 (t, 2H, *J* = 7.5 Hz), 6.34 (d, 1H, *J* = 9.5 Hz), 6.60 (s, 1H), 6.62 (s, 1H), 8.10 (d, 1H, *J* = 9.5), 8.15(bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 160.4, 155.1, 152.5, 149.1, 137.8, 119.4, 114.5, 114.0, 100.6, 99.3, 66.9, 44.5. HRMS (ES) calcd for $C_{12}H_9NO_4S$: 263.2692 $[M+H]^+$; Found**:** 263.2690. EA calcd (%) for $C_{12}H_9NO_4S$: calcd. C 54.75, H 3.45, N 5.32; found C 54.78, H 3.47, N 5.35.

The above reported procedure was applied for detected **5-(2-isothiocyanatoethoxy)-2-oxo-2H-chromen-7-yl oleate,** red solid, m.p 68-69 °C, 93% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.90 (t, 3H, *J* = 6.5 Hz), 1.25 (m, 20H), 1.75 (m, 2H), 2.0 (m, 4H), 2.60 (t, 2H, *J* = 7.2 Hz), 3.95 (t, 2H, *J* = 4.5 Hz), 4.23 (t, 2H, *J* = 4.5 Hz), 5.38 (m, 2H), 6.35 (d, 1H, *J* = 10.0 Hz), 6.55 (s, 1H), 6.78 (s, 1H), 8.10 (d, 1H, *J* = 10.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.0, 22.6,

27.1, 29.1, 29.3, 29.7, 31.9, 44.5, 45.6, 60.3, 99.8, 101.1, 103.8, 106.6, 114.5, 129.7, 130.0, 137.8, 155.1, 155.4, 155.5, 160.5, 170.2. HRMS (ES) calcd for $C_{30}H_{41}NO_5S$: 527.2705 $[M+H]^+$; Found**:** 527.2707. EA calcd (%) for $C_{30}H_{41}NO_5S$: calcd. C 68.28, H 7.83, N 2.65; found C 68.31, H 7.85, N 2.69.

Titration Analyses

The NH_2 loading of compound **2** was found to be 1.11 mmol g^{-1} by Kaiser test assay and 1.12 mmol g^{-1} by potentiometric argentometric titration.

The NH_2 loading of compound **3** was found to be 1.10 mmol g^{-1} by Kaiser test assay.

Quantitative Kaiser test protocol

Three solutions were prepared separately:

- (I): 10 g of phenol in 20 mL of absolute ethanol.

- (II): 2 mL of potassium cyanide 1 mM (aqueous solution) dissolved in 98 mL of pyridine.

- (III): 1 g of ninhydrin in 20 mL of absolute ethanol.

A mass of approximately 20 mg of carbon nanotubes was carefully weighted in a haemolysis test tube. Then, 75 µL of solution (I), 100 µL of solution (II), and 75 µL of solution (III) were successively added to the CNTs. The resulting dispersion was sonicated in a water bath for several minutes until disaggregation of the CNTs, heating at 120 °C for 5 min and diluted with 4.75 mL of 60% ethanol. After centrifugation, the supernatant was analyzed by UV-Vis spectroscopy. The absorbance at 570 nm was correlated to the amount of free amine functions on the CNT surface using the equation:

$$NH_2 \text{ loading } (\mu mol/g) = \frac{[Abs_{sample} - Abs_{blank}] \times \text{dilution (mL)} \times 10^6}{\text{Extinction coefficient} \times \text{sample weight (mg)}}$$

Dilution is 5 mL and extinction coefficient is 15000 $m^{-1}cm^{-1}$.

The blank was prepared exactly the same way but without CNTs.

The result is expressed as micromole of amino groups per gram of material.

The Kaiser test is repeated at least three times for each sample to ensure reproducibility.

Potentiometric argentometric titration

The titration was performed on carefully weighted 20 mg of **2** with an aqueous solution of $AgNO_3$ 0.01M (previously standardized with NaCl 0.02 M standard solution) using as electrode an INGOLD Ag 4805-s7/120 combination silver and conducted on a Mettler Toledo Seven Multi. The titration was carried out in high ionic strength condition by adding 25 mL of 1.0 M KNO_3 aqueous solution to the mixture and adjusting the pH to 1 with H_2SO_4. The NH_2 loading was calculated from the equation reported below, where V is the volume of $AgNO_3$ titration solution at the equivalent point, [$AgNO_3$] is the molarity of $AgNO_3$ while "sample weight" is the weight of the sample that is titrated.

$$\text{Loading } NH_2 \text{ (mmol/g)} = \frac{V(AgNO_3, \text{mL}) \cdot [AgNO_3] \text{ (mmol/ml)}}{\text{sample weight (g)}}$$

The loading value is an average of three titrations performed for each batch.