Supporting information

Hybrid Cholesterol-based nanocarriers containing phosphorescent Ir complexes: in vitro imaging on glioblastoma cell line.

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Materials and methods

CH_2Cl_2 and $CHCl_3$ were passed through basic alumina prior to use. Chromatographic purifications were performed using 70-230 mesh silica.

^1H NMR and ^{13}C NMR spectra were recorded using $CDCl_3$ or DMSO solutions at 300, 400 and 600 MHz for ^1H and 75.46, 100.6 and 150.92 MHz for ^{13}C. Chemical shifts (δ) are reported in ppm relative to $CHCl_3$ (δ = 7.26 for ^1H and δ = 77.0 for ^{13}C). ^{13}C NMR spectra were acquired with ^1H broad band decoupled mode. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; oct, octet; m, multiplet; b, broad signal. Fourier transform infrared (FTIR) spectra were recorded on a Perkin-Elmer Spectrum 2000. Mass spectra (MS) were obtained with an electrospray ionization source (ESIMS). All the ESIMS spectra were performed using MeOH as the solvent. Elemental analyses were performed using Flash EA1112 Automatic Elemental Analyzer CE instruments. All reactions were carried out in air and using undistilled solvent without any precautions to exclude moisture, unless otherwise mentioned.

Synthesis of 2

To a solution of ethyl 7-bromoheptanoate **1** (1.19 g, 5.0 mmol) in DMSO (17 ml) NaN_3 (0.65 g, 10 mmol) was added. The mixture was left to stir at room temperature for 24h, then 75 ml of water and

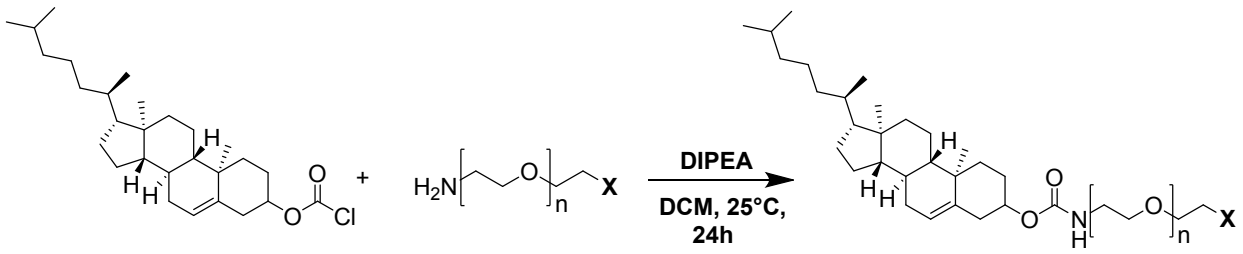
75 ml of EtOAc were added. The aqueous layer was separated and extracted with EtOAc (3 x 50 ml). The collected organic phases were dried over $MgSO_4$ and the solvent evaporated. The obtained crude azide showed good purity at ^{1}H NMR analysis [$CDCl_3$, 300 MHz, δ = 4.07 (q, J = 7.2 Hz, 2H), 3.21 (t, J = 6.9 Hz, 2H), 2.25 (t, J = 7.7 Hz, 2H), 1.64-1.50 (m, 4H), 1.38-1.28 (m, 4H), 1.21 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.6 (CO), 60.2 (CH_2), 51.3 (CH_2), 34.1 (CH_2), 28.6 (CH_2), 28.5 (CH_2), 26.3 (CH_2), 24.7 (CH_2), 14.2 (CH_3).] and therefore it was directly employed in the next step without further purification.[1] The crude azide was dissolved in a 1:1 *t*BuOH/H_2O mixture (25 ml) and $CuSO_4 \cdot 5H_2O$ (0.062 g, 0.25 mmol), Na-(L) ascorbate (0.324 g, 1.5 mmol) and ethynyl pyridine (0.56 mL, 5.5 mmol) were added. After 24h stirring at room temperature, the solvent was removed under vacuum and aqueous NH_3 (35 ml) and DCM (35 ml) were added and the mixture was left to stir overnight. Therefore, after separation of the phases, the aqueous layer was extracted with DCM (3 x 25 ml). The collected organic layers were dried over $MgSO_4$, filtered and the solvent removed under vacuum. The crude product was purified by flash chromatography (EtOAc/DCM=6/4 and DCM/MeOH=9/1) to give 1.43 g (4.7 mmol, 95% yields from 1) of **2** as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.58 (dq, J_D = 4.9 Hz, J_Q = 0.9 Hz, 1H), 8.18 (dt, J_D = 8.0 Hz, J_T = 1.1 Hz, 1H), 8.12 (s, 1H), 7.78 (td, J_T = 7.8 Hz, J_D = 1.9 Hz, 1H), 7.23 (ddd, J = 7.5 Hz, J = 4.9 Hz, J = 1.3 Hz, 1H), 4.42 (t, J = 7.1 Hz, 2H), 4.12 (q, J = 7.1 Hz, 2H), 2.28 (t, J = 7.3 Hz, 2H), 2.03-1.91 (m, 2H), 1.70-1.53 (m, 2H), 1.42 - 1.35 (m, 4H), 1.25 (t, J = 7.1Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5 (CO), 150.3 (C), 149.3 (CH), 148.3 (C), 136.8 (CH), 122.7 (CH), 121.7 (CH), 120.2 (CH), 60.2 (CH_2), 50.3 (CH_2), 34.0 (CH_2), 30.0 (CH_2), 28.4 (CH_2), 26.1 (CH_2), 24.6 (CH_2), 14.2 (CH_3).

Synthesis of complex 3. In a 25 ml round-bottom flask equipped with a stirring bar, the ligand **2** (0.014 g, 2.5 equiv) was dissolved in a 3:1 mixture of CH_2Cl_2/EtOH (5 mL) and the dimer $[(ppy)_2Ir(\mu\text{-}Cl)]_2$ (0.02 g, 0.019 mmol), previously obtained following literature procedures[2] was added. The mixture was left to stir at r.t. 72h. Then the solvent was evaporated and the crude product was washed with Et_2O to remove the excess of ligand to give 0.03 g of pure **3** (96% yields). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.9 (s, 1H), 9.19 (d, J = 7.9 Hz, 1H), 7.99 (t, J = 7.8 Hz, 1H), 7.91 (dd, J = 8.2 Hz, J =4.1 Hz, 2H), 7.79-7.71 (m, 3H), 7.69-7.63 (m, 3H),7.45 (d, J = 6.2 Hz, 1H), 7.21 (t, J = 6.9 1H), 7.05-6.83 (m, 6H), 6.32 (dd, J = 12.2 Hz, J = 7.6 Hz, 2H), 4.46 (oct, J = 7.1 Hz, 2H), 4.12 (q, J = 7.5 2H), 2.23 (t, J = 7.5 2H), 1.95 (quint, J = 7.5 Hz, 2H), 1.51 (quint, J = 7.7 Hz, 2H), 1.32-1.14 (m, 7H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6 (CO), 168.4 (C), 167.7 (C), 150.2 (C), 150.1 (C), 149.4 (CH), 149.3 (CH), 148.5 (C), 148.3 (CH), 146.6 (C), 143.7 (C), 143.6 (C), 139.7 (CH), 137.9 (CH), 137.8 (CH), 131.8 (CH), 131.7 (CH), 130.6 (CH), 130.0 (CH), 129.3 (CH), 125.8 (CH), 125.0 (CH), 124.6 (CH), 124.3 (CH), 123.1 (CH), 122.7 (CH), 122.6 (CH),

122.1 (CH), 119.4 (CH), 119.3 (CH), 60.2 (CH_2), 50.2 (CH_2), 34.1 (CH_2), 29.6 (CH_2), 28.2 (CH_2), 25.9 (CH_2), 24.7 (CH_2), 14.2 (CH_3).

Synthesis of complex 4. In a 25 ml round-bottom flask equipped with a stirring bar, the complex **3** (0.03 g, 0.036 mmol) was dissolved in 2 mL of DCM. A solution of KOH (0.033 g, 0.582 mmol) in a 3:1 mixture of EtOH/H_2O (1 mL) was added and mixture was left to stir at r.t. overnight. Then the solvent was evaporated and the crude product was neutralized with diluted HCl (1M). The aqueous layer was extracted with DCM (3 x 10 ml). The collected organic layers were dried over $MgSO_4$, filtered and the solvent removed under vacuum to give 0.028 g (0.0346 mmol, 96% yields) of pure **4** as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.41 (s, 1H), 9.08 (d, J = 6.1 Hz, 1H), 8.02 (t, J = 7.8 Hz, 1H), 7.91 (dd, J = 8.2 Hz, J =4.1 Hz, 2H), 7.81-7.71 (m, 3H), 7.70-7.62 (m, 3H), 7.48 (d, J = 6.2 Hz, 1H), 7.21 (t, J = 6.9 1H), 7.10-6.83 (m, 6H), 6.32 (dd, J = 12.2 Hz, J = 7.6 Hz, 2H), 4.46 (oct, J = 7.1 Hz, 2H), 2.44 (t, J = 7.5 2H), 1.95 (quint, J = 7.5 Hz, 2H), 1.67 (quint, J = 7.7 Hz, 2H), 1.42-1.21 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.9 (CO), 167.3 (C), 166.6 (C), 149.0 (2C), 148.6 (CH), 148.3 (CH), 147.5 (C), 147.3 (CH), 145.5 (C), 142.7 (C), 146.6 (C), 138.8 (CH), 137.1 (CH), 136.9 (CH), 130.8 (CH), 130.7 (CH), 129.6 (CH), 129.0 (CH), 127.5 (CH), 125.0 (CH), 123.6 (2CH), 123.3 (CH), 122.3 (CH), 121.8 (CH), 121.6 (CH), 121.2 (CH), 118.4 (2CH), 51.0 (CH_2), 33.3 (CH_2), 28.3 (CH_2), 26.8 (CH_2), 24.3 (CH_2), 23.5 (CH_2). ESI-MS : 775 [M^+]. Elemental Analysis: C, 53.28; H, 4.21; Cl, 4.39; Ir, 23.68; N, 10.35; O, 3.92.

Synthesis of Cholesterol-PEG copolymers



Cholesteryl Chloroformiate **H_2N-PEG-X** **Chol-PEG-X**

X = COOH/NH_2

Cholesterol-PEG-COOH copolymer

H_2N-PEG-COOH (3 kDa, 450 mg, 0.15 mmol) was dissolved in anhydrous dichlorometane (DCM, 10 mL) under nitrogen and cooled to 0°C with an ice bath. Then a solution of cholesteryl chloroformate (67 mg, 0.15 mmol) in anhydrous DCM (10 mL) was added dropwise. Finally,

diisopropylethylamine (DIPEA, 78.5 µl, 0.46 mmol) was added and the reaction mixture was left to react at room temperature overnight. The solvent was evaporated, the product was washed with Et_2O (30 mL x 6) using vortex for re-dispersion and centrifuge (6000 rpm, 10 min) for precipitation, and dried under vacuum. ^{1}H NMR (400 MHz, DMSO (d_6)): δ = 8.80 (m, 1H), 7.00 (m, 1H), 5.30 (m, 1H), 3.61 (m, OCH_2, PEG), 2.00-0.80 (m, 41H), 0.60 (m, 3H) ppm. ^{13}C NMR (400 NMR, DMSO (d_6)): δ = 177.80, 176.18, 161.03, 147.95, 145.33, 133.27, 127.28, 78.51, 71.98, 64.96, 54.66, 37.10, 36.52, 35.51, 32.98, 31.69, 30.59, 28.36, 27.45, 25.76, 24.19, 23.74, 19.28, 16.86 ppm. IR (CCl_4, v = cm^{-1}): 2871, 1719, 1457, 1348, 1323, 1294, 1248, 1111, 948.

Cholesterol-PEG-NH$_2$ copolymer

H_2N-PEG-NH2 (3kDa, 450 mg, 0.15 mmol) was dissolved in anhydrous DCM (10mL) under nitrogen and cooled to 0°C with an ice bath. Then a solution of cholesteryl chloroformate (15 mg, 0.033 mmol) in anhydrous DCM (10mL) was added dropwise. Finally, DIPEA (11.4 µl, 0.067 mmol) was added and the reaction mixture was left to react at room temperature overnight. The solvent was evaporated, the product was washed with 30 mL of Et_2O X 6 using vortex for redispersion and centrifuge (6000 rpm, 10 min) for precipitation, and dried under vacuum. ^{1}H NMR (400 MHz, DMSO (d_6)): δ = 8.76 (m, 1H), 7.03 (m, 1H), 5.4 (m, 1H), 3.65 (m, 2H), 2.00-0.80 (m, 41H), 0.65 (m, 3H) ppm. ^{13}C NMR (400 NMR, DMSO (d_6)): δ = 177.95, 176.23, 161.11, 147.90, 145.28, 133.16, 127.15, 78.49, 72.03, 64.95, 54.50, 36.98, 36.42, 35.491, 33.08, 31.55, 30.48, 28.31, 27.46, 25.84, 24.08, 23.71, 19.23, 16.99 ppm. IR (CCl_4, v = cm^{-1}): 2873, 1721, 1468, 1359, 1300, 1285, 1246, 1110, 947.

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