Supporting information

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

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Materials and methods

CH₂Cl₂ and CHCl₃ were passed through basic alumina prior to use. Chromatographic purifications were performed using 70-230 mesh silica.

¹H NMR and ¹³C NMR spectra were recorded using CDCl₃ or DMSO solutions at 300, 400 and 600 MHz for ¹H and 75.46, 100.6 and 150.92 MHz for ¹³C. Chemical shifts (δ) are reported in ppm relative to CHCl₃ (δ = 7.26 for ¹H and δ = 77.0 for ¹³C). ¹³C 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) NaN₃ (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 MgSO₄ and the solvent evaporated. The obtained crude azide showed good purity at ¹H NMR analysis [CDCl₃, 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). ¹³C NMR (CDCl₃, 75 MHz) δ 173.6 (CO), 60.2 (CH₂), 51.3 (CH₂), 34.1 (CH₂), 28.6 (CH₂), 28.5 (CH₂), 26.3 (CH₂), 24.7 (CH₂), 14.2 (CH₃).] and therefore it was directly employed in the next step without further purification. The crude azide was dissolved in a 1:1 tBuOH/H₂O mixture (25 ml) and CuSO₄·5H₂O (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 NH₃ (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 MgSO₄, 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. ¹H NMR (CDCl₃, 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), 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 = 7.1 Hz, 3H). ¹³C NMR (CDCl₃, 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 (CH₂), 50.3 (CH₂), 34.0 (CH₂), 30.0 (CH₂), 28.4 (CH₂), 26.1 (CH₂), 24.6 (CH₂), 14.2 (CH₃).

**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 CH₂Cl₂/EtOH (5 mL) and the dimer [(ppy)₂Ir(µ-Cl)]₂ (0.02 g, 0.019 mmol), previously obtained following literature procedures was added. The mixture was left to stir at r.t. 72h. Then the solvent was evaporated and the crude product was washed with Et₂O to remove the excess of ligand to give 0.03 g of pure 3 (96% yields). ¹H NMR (400 MHz, CDCl₃): 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 Hz, 2H), 2.23 (t, J = 7.5 Hz, 2H), 1.95 (quint, J = 7.5 Hz, 2H), 1.51 (quint, J = 7.7 Hz, 2H), 1.32-1.14 (m, 7H). ¹³C NMR (CDCl₃, 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 (CH₂), 50.2 (CH₂), 34.1 (CH₂), 29.6 (CH₂), 28.2 (CH₂), 25.9 (CH₂), 24.7 (CH₂), 14.2 (CH₃).

**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/H₂O (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 MgSO₄, 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. ¹H NMR (400 MHz, CDCl₃): 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). ¹³C NMR (CDCl₃, 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 (CH₂), 33.3 (CH₂), 28.3 (CH₂), 26.8 (CH₂), 24.3 (CH₂), 23.5 (CH₂). 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**

![Cholesterol-PEG copolymers](image)

**Cholesterol-PEG-COOH copolymer**

H₂N-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 Et₂O (30 mL x 6) using vortex for re-dispersion and centrifuge (6000 rpm, 10 min) for precipitation, and dried under vacuum. ¹H NMR (400 MHz, DMSO (d₆)): δ = 8.80 (m, 1H), 7.00 (m, 1H), 5.30 (m, 1H), 3.61 (m, OCH₂, PEG ), 2.00-0.80 (m, 41H), 0.60 (m, 3H) ppm. ¹³C NMR (400 NMR, DMSO (d₆)): 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 (CCl₄, v = cm⁻¹): 2871, 1719, 1457, 1348, 1323, 1294, 1248, 1111, 948.

**Cholesterol-PEG-NH₂ copolymer**

H₂N-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 Et₂O X 6 using vortex for redispersion and centrifuge (6000 rpm, 10 min) for precipitation, and dried under vacuum. ¹H NMR (400 MHz, DMSO (d₆)): 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. ¹³C NMR (400 NMR, DMSO (d₆)): 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 (CCl₄, v = cm⁻¹): 2873, 1721, 1468, 1359, 1300, 1285, 1246, 1110, 947.

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