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Chemistry & Drug Discovery

Supporting Information

Supporting Information Available:

Description of **(1)** Materials and Analysis, (**2)** Synthesis of {2-[2-(2-aminoethoxy) ethoxy}ethyl}carbamic acid tert-butyl ester: mono-Boc-sPEG: **(compound 1)**, **(3)** Synthesis of tert-butyl 2-(2-(2-(2-(6-methoxynaphthalen-2-yl) propanamido) ethoxy) ethoxy) ethylcarbamate: (NPX-sPEG-BOC): **(compound 2a)**, **(4)** Synthesis of N- 2-(2-(2aminoethoxy)ethoxy)ethyl)-2-(6-methoxynaphthalen-2-yl) propanamide: (NPX-sPEG-NH_2): **(compound 2b)**. **(5)** Preparation of NSAID QD conjugates. ***(6)*** Enzymatic assay. **(7)** Cell culture experiments.

1) Materials and Analysis

CdTe QD synthesis was carried out in degassed millipore water (18 MΩ.cm) and standard Schlenk techniques were used in the manipulation of air-sensitive materials and solutions. All compounds were obtained from Sigma-Aldrich (Gillingham) except for Aluminium Telluride (Al_2Te_3) which was obtained from Cerac Inc. (Milwaukee) and used without further purification. QD purification was performed on size exclusion chromatography gel Sephadex G-25 (Amersham Biosciences). Syntheses of the NSAID (non-steroidal anti-inflamatory drug) derivative was performed in anhydrous solvents under dry and oxygen free argon. The purifications by column chromatography were carried out using a silica gel 60 (Merck, 60-230 mesh), dichloromethane (DCM), ethyl acetate and methanol (MeOH). The progress of reaction was followed by thin layer chromatography (TLC) on silica plates (Merck, 60F254). The following developing systems were used: UV light at 254 nm, ninhydrin and potassium permanganate. ^{1}H and ^{13}C spectra were recorded on a Bruker Advance AC-400 instrument at 400 and 100 MHz respectively. Chemical shifts were measured relative to that of $CDCl_3$ (δ 7.26) for ^{1}H, (δ 77.16) for ^{13}C and expressed indirectly in relation to TMS. The following abbreviations are used to describe the signal multiplicities: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), sp (septet) and m (multiplet). Chemical shifts are expressed in parts per million and listed as follows: shift in parts per million (multiplicity, integration, coupling and attribution). Mass spectra (MS) were recorded on a Micromass Time of Flight (oa-TOF) equipped with an electrospray ionisation (ES) interface operated in the positive mode. Conjugation reactions with QDs were carried out in borate buffer (pH 7.5) with EDC·HCl (1-ethyl-3-(3-diaminopropyl) carbodiimide hydrochloride) and naproxen

QD conjugates were purified over a G-25 sephadex column equilibrated in Millipore water and filtered with 0.2 µm filters (PAL, Life Sciences). Enzymatic tests were carried out using Trypsin 10X solution in PBS 1X buffer at pH 7.4. The UV-Vis absorption spectra were recorded using a Cary 50 UV-Vis spectrophotometer. Fluorescence measurements were performed on a Cary Eclipse spectrometer. Transmission electron microscopy (TEM) images were taken on a Hitachi H-7000 at a beam voltage of 100 kV. Samples for TEM were prepared by deposition and drying of a 10 µL drop of solution onto a formvar coated 400 mesh carbon grid. Live cell images were recorded using a Cellomics KineticScan® analyser. Luminescence decays were measured using time-correlated single photon counting (Time-Harp, PicoQuant). The samples were excited by 480 nm picosecond pulses generated by a PicoQuant, LDH-480 laser head controlled by a PDL-800B driver. The setup was operated at an overall time resolution of ~ 150 psec. Decays were measured to 3000-5000 counts in the peak and reconvoluted using non-linear least squares analysis (FluoFit, PicoQuant), using an equation of the form: $I(t) \propto \sum_i a_i \exp(-t/t_i)$, where τ_i are the PL decay times. The pre-exponential factors α_i, were taken into account by normalisation of the initial point in the decay to unity. The quality of fit was judged in terms of a χ^2 value (with a criteria of less than 1.1 for an acceptable fit) and weighted residuals. The τ_i and α_i parameters were used then to calculate the average lifetime, τ_{av}: $t_{av} = \frac{\sum a_i t_i^2}{\sum a_i t_i}$

2) Synthesis of {2-[2-(2-aminoethoxy)ethoxy}ethyl}carbamic acid tert-butyl ester: mono-BOC-sPEG: <u>compound 1</u>.

The synthesis of mono-BOC-sPEG was conducted as previously described.[1] Briefly, Boc anhydride (3.0 g, 13.7 mmol) in dioxane (20 mL) was added dropwise to a solution of 2,2'-(ethylenedioxy)bis(ethylamine) (12.0 ml, 82.3 mmol) in dioxane (50 mL) over a 5 hour period. The reaction mixture was then concentrated under vacuum and the resulting oil was dissolved in water (40 mL) and washed with DCM (5 X 50 mL). The combined organic phases were further washed with brine (4 X 40 mL) and

dried over $MgSO_4$. Thus, **compound 1** (2.97 g, 87%) was isolated as a clear slightly yellow oil. TLC (DCM/MeOH 8/2, UV and ninhydrin) R_f = 0.36; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, Boc), 1.63 (bs, 2H, NH_2), 2.85 (t, 2H, ^{3}J = 5.0 Hz, CH_2NH_2), 3.29 (m, 2H, CH_2NH), 3.49 and 3.51 (t, 2H, ^{3}J = 5.0 Hz, CH_2O), 3.59 (bs, 4H, OCH_2CH_2O), 5.17 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 28.5 ($(CH_3)_3C$), 40.4 (CH_2NH), 41.8 (CH_2NH_2), 67.2, 70.3 and 73.4 (CH_2O), 79.2 ($(CH_3)_3C$), 156.1 (CO). ESI-MS, m/z 249.18 in agreement with the mass calculated for $[M+H]^+$.

3) Synthesis of tert-butyl 2-(2-(2-(2-(6-methoxynaphthalen-2-yl) propanamido) ethoxy) ethoxy) ethylcarbamate: (NPX-sPEG-BOC): <u>compound 2a</u>.

To a solution of naproxen ((S)-(+)-6-methoxy-2-α-methyl-2-naphthalene acetic acid, 2.06 g, 8.95 mmol) in dry DCM (30 mL) was added thionyl chloride (850 µL, 11.6 mmol). The reaction was stirred and heated to reflux for one hour and then evaporated and dried under vacuum. The yellow solid was then dissolved in dry THF (30 mL) and added dropwise to a solution of **compound 1** (2.0 g, 8.0 mmol) and NEt_3 (2.02 mL, 14.1 mmol) in dry THF (30 mL) at 0 °c. The reaction mixture was then stirred at room temperature for 3 hours, filtered and concentrated under vacuum. The residue was dissolved in DCM (30 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (3 X 15 mL) and then with water (30 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The crude reaction product was further purified by column chromatography (DCM/ethyl acetate 9/1 then DCM/MeOH 95/5) to yield **2a** (2.4 g, 65%) as a white solid. TLC (DCM/MeOH 9/1, UV and ninhydrin) R_f = 0.44; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, $(CH_3)_3C$), 1.57 (d, 3H, ^{3}J = 7.0 Hz, CH_3CH), 3.19 (bs, 2H, CH_2NHCOO), 3.33-3.60 (m, 10H, CH_2OCH_2), 3.67 (q, 1H, ^{3}J = 7.0 Hz, $CHCH_3$), 3.91 (s, 3H, OCH_3), 4.94 (bs, 1H, NHCOO), 5.92 (bs, 1H, NHCO), 7.10-7.13 (m, 2H, $CHC(OCH_3)CH$), 7.38 (d, 1H, ^{3}J = 8.0 Hz, CHC(CH)CH), 7.67-7.70 (m, 3H, CH). ^{13}C NMR ($CDCl_3$) δ 18.6 ($CHCH_3$), 28.5 ($C(CH_3)_3$), 39.4 (CH_2NHCO), 40.2 (CH_2NHCOO) 47.0 ($CHCH_3$), 55.4 (CH_3O), 69.9, 70.1, 70.3 (CH_2OCH_2), 79.3 ($C(CH_3)_3$), 105.6, 119.1, 126.1, 126.4, 127.4, 129.3 (CH), 129.0,133.8, 136.5 (C=C, $C(CHCH_3)$), 156.0 (CH_3OC), 157.7 (NHCOO), 174.4 (NHCO). ESI-MS, m/z 483.24 in agreement with the mass calculated for $[M+Na]^+$.

4) Synthesis of N- 2-(2-(2aminoethoxy)ethoxy)ethyl)-2-(6-methoxynaphthalen-2-yl) propanamide: (NPX-sPEG-NH2):<u>compound 2b</u>.

Trifluoroacetic acid (TFA, 10 mL, 130 mmol) was added slowly to a solution of **2a** (1.8 g, 3.9 mmol) in dry DCM (10 mL) at 0 °C. The reaction mixture was stirred until the disappearance of the protected compound (as monitored by TLC). After completion of the reaction, the mixture was concentrated and TFA removed by evaporation cycles with DCM/cyclohexane (1:1, v/v). The residue was then dried under vacuum to yield **2b**·TFA (1.70 g, 92%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.57 (d, 3H, ^{3}J = 7.0 Hz, CH_3CH), 2.96 (m, 2H, CH_2NH_2), 3.36-3.61 (m, 10H, CH_2OCH_2), 3.78 (q, 1H, ^{3}J = 7.0 Hz, $CHCH_3$), 3.90 (s, 3H, OCH_3), 6.57 (bs, 1H, NH), 7.10-7.14 (m, 2H, $CHC(OCH_3)CH$), 7.31 (m, 1H, CHC(CH)CH), 7.65-7.72 (m, 3H, CH). ^{13}C NMR ($CDCl_3$) δ 17.8 ($CHCH_3$), 39.7 (CH_2NH), 39.9 (CH_2NH_2), 46.7 ($CHCH_3$), 55.4 (OCH_3), 66.1, 69.6, 69.8, 70.2 (CH_2OCH_2), 105.7, 119.6, 126.1, 126.3, 127.7, 129.3 (CH), 128.9, 134.0, 135.0 (C=C, $C(CHCH_3)$), 158.1 (CH_3OC), 174.5 (CO). ESI-MS, m/z 361.21 in agreement with the mass calculated for $[M + H]^{+}$.

5) Preparation of NSAID-QD conjugates.

Typically, 80 µl of a solution of compound **2b** (0.1g / 5ml buffer) (25 molar excess) was added to 0.25 mL of concentrated QDs. The volume was increased to 2ml with buffer (final QD concentration of 5 x 10^{-5})[2] and allowed to stir for 10 minutes. 1 mg of EDC (N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide) was added to the reaction mixture. The QDs were allowed to react at room temperature for 1 hour and then were stored in the fridge overnight allowing the unreacted EDC to hydrolyse and lose its activity.[3] The solution was further purified over sephadex G-25, and a coloured luminescent fraction of approximately 2 mL was collected, filtered through a 0.2 µm filter and stored again at 4 °C for further characterisation and use.

6) Enzymatic assay.

Purified naproxen QD conjugates (1 mL, QD conc. ~10^{-5})[2] were mixed with trypsin 10X (5 mg / mL) in 1 mL 1X PBS buffer at pH 7.4. The solution was incubated at 37

°C for up to 24 hours and fluorescence measurements were taken at different stages in order to monitor the QD luminescence evolution and possible drug release.

7) Cell culture experiments.

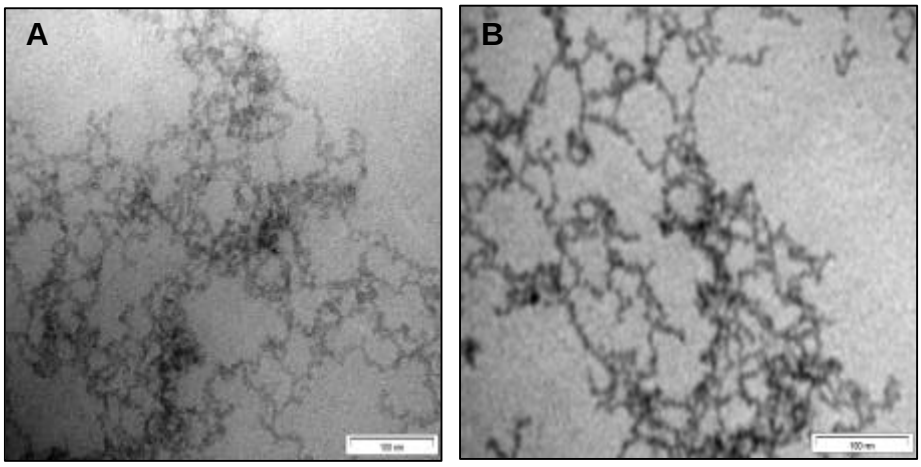
THP-1 cells (ECACC, Salisbury, England) were seeded out onto 96 well microtitre plates at a concentration $2x10^5$ cells / mL. The THP-1 cells, cultured with 100 ng / mL PMA (to enable monocyte to macrophage differentiation) were incubated for 72 hours at 37 °C, 5 % CO_2 in supplemented (10 % foetal bovine serum; 2 mM / L-glutamine; 100 µg / mL penicillin; 100 mg / mL streptomycin) RPMI 1640 media. Twelve hours prior to the experiment, the medium was exchanged with serum free medium. Lipopolysaccharide (LPS) (Alexis Corporation, Switzerland) was then added to the culture one hour before the addition of the QDs, to stimulate the expression of the COX-2 receptor, which also is the receptor for Naproxen. The QDs were pipetted directly into the wells at a concentration of 10 µL / 200 µL and incubated for three hours at 37 °C and 5 % CO_2. The cells were counterstained with Hoescht 33342 dye and fixed with 3 % paraformaldehyde. The images were acquired and analysed using a Cellomics KineticScan®.

[1] Braun, M.; Camps, X.; Vostrowsky, O.; Hirsch, A.; Endress, E.; Bayerl, T. M.; Birkert, O.; Gauglitz, G. European J. Org. Chem. **2000**, 7, 1173-1181.

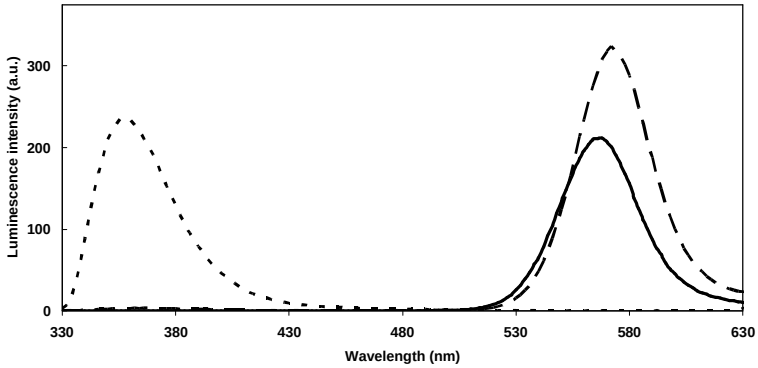
[2] Yu, W. W.; Qu, L.; Guo, W.; Peng, X. Chem. Mater. **2003**, 15, 2854-2860.

[3] Wang, S.; Mamedova, N.; Kotov, N. A.; Chen, W.; Studer, J. Nano. Lett. **2002**, 2, 817-822.

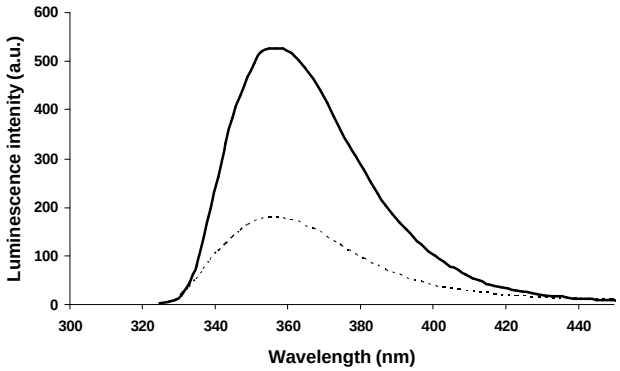
Figures referred to in text.



TEM images of original CdTe QDs (**A**) and their naproxen QD conjugates (**B**) (scale bar = 100 nm).



UV-vis absorption and normalised luminescence spectra ($?_{ex}$ 450nm) of solid line: original QDs, dashed line: purified naproxen – QD conjugates following storage overnight, dotted line: naproxen derivative in water.



Luminescence emission spectra ($?_{ex}$ 317 nm) of initial quantities (solid line) of the naproxen derivative added and of the unreacted moiety flushed from the sephadex column following purification (dashed line).