Supplementary Information

Triphenylarsonium-functionalised gold nanoparticles: potential nanocarriers for intracellular therapeutics

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Materials

All reagents and solvents were purchased from Sigma-Aldrich or Fisher Scientific and used as received.

Characterisation

^{1}H and NMR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dichloromethane (CD_2Cl_2) on a Brucker AVANCE III (400 MHz) NMR spectrometer. Electrospray Ionization Mass Spectrometry Samples were dissolved in EtOH: DIH_2O (50:50) to a concentration of approximately 1 mg/mL for molecular ion determination. Electrospray mass spectra were recorded using a Thermo Finnigan MAT LCQ classic electrospray ionisation mass spectrometer (ESI-MS) (Thermo Scientific, Sanjose, California, USA) in positive ion mode. Samples were introduced by direct infusion using the syringe pump on the instrument at a flow rate of 5 μL/min with an acquisition time of one minute. IR spectra were recorded using a Bruker Alpha Platinum ATR FTIR spectrometer. Elemental analyses were carried out by MEDAC Ltd (Chobham, Surrey, UK). Ultraviolet-visible absorption spectra of aqueous colloidal solutions were recorded at room temperature on a Jenway 6715 UV/Vis spectrophotometer. Transmission electron micrographs of AuNPs were obtained on a Jeol 2000 FX transmission electron microscope (TEM) (ETC, Brunel University, Middlesex, UK), set at 100 KV. Freeze-dried AuNP samples were re-suspended in ethanol, and placed on a Holey coated copper grid, and the grids were left to dry in air at room temperature. Particle

size distribution was obtained over 1000 AuNPs using Abel imaging software. XPS measurements were made on a VG Escalab 210 Photoelectron Spectrometer. The X-ray source was a non-monochromated Al Ka source (1486.6 eV), operated with an X-ray emission current of 20 mA and an anode high tension (acceleration voltage) of 12 kV. The freeze-dried sample was placed on a standard sample stud employing double sided adhesive tape and the take-off angle was fixed at 90^{o} relative to the sample plane. The area corresponding to each acquisition was of 0.79mm^2. Each analysis consisted of a wide survey scan (pass energy 50 eV, 1.0 eV step size) and high-resolution scans (pass energy 50 eV, 0.05 eV step size) for component speciation. The binding energy scale of the instrument was calibrated using the Au 4f5/2 (83.9 eV), Cu 2p3/2 (932.7 eV) and Ag 3d5/2 (368.27 eV) lines of cleaned gold, copper and silver standards from the National Physical Laboratory (NPL), UK. The software CasaXPS 2.3.15 was used to fit the XPS spectra peaks, and the binding energies obtained in the XPS spectra were corrected for specimen charging by referencing the C 1s to 284.6 eV.

Synthesis of the 3-triphenylarsoniopropylthiosulfate zwitterion (3) and triphenylarsoniumpropylthioacetate bromide (4).

Compounds **3** and **4** were prepared from the bromopropyltriphenylarsonium bromide, which was obtained in high yield using the method of Moorhoff.[1]

Triphenylarsoniopropylthiosulfate zwitterion 3

Bromopropyltriphenylarsonium bromide (0.250 g, 4.9 x 10^{-4} mol) was refluxed overnight with $Na_2S_2O_3$ (0.212 g, 8.56 x 10^{-4} mol) in aqueous EtOH. Zwitterion **3** was isolated from this mixture by extraction with dichloromethane (3 x 10 mL) and purified by triturating with diethyl ether to yield **3** as a white solid in 59% yield,. m.p. 168^{o}C. Elemental Analysis: found: C, 54.46; H, 4.40; S 13.94. $C_{21}H_{21}AsO_3S_2$ requires: C, 54.78; H, 4.56; S 13.91. IR υ_{max}/cm^{-1} 3020 and 2912 (CH), 1483, 1438, 1209 (SO), 1082 1010 (SO), 744, 688, 623, 523, 466. ^{1}H NMR**:** δ 2.3 (2H, m), 3.2 (2H, t), 3.6 (2H,m), 7.6–7.9 (15H, m) ppm . ESI-MS**:** 460.89 [M]$^+$, 461.97 [M+H$^+$], 482.90 [M+Na$^+$].

Triphenylarsoniumpropylthioacetate bromide 4

Bromopropyltriphenylarsonium bromide (0.250 g, x 4.9 10^{-4} mol) was refluxed overnight under nitrogen with $KSC(O)CH_3$ (0.098 g, 8.56 x 10^{-4} mol) in aqueous EtOH. Thioacetate

salt **4** was isolated from this mixture by extraction with dichloromethane (3 X 10 mL) and purified by triturating with diethyl ether as a white solid in 44% yield. m.p. 234 - 238°C. Elemental Analysis: found: C, 54.97; H, 4.81; S 6.42. $C_{22}H_{24}AsOS$ requires: C, 54.87; H, 4.77; S 6.36. IR υ_{max}/cm^{-1} 3020 and 2910 (CH), 1683 (CO), 1482, 1436, 1136 1084, 995, 950, 760, 740, 625, 494. ^{1}H NMR**:** δ 1.6 (3H, s), 2.1 (2H, m), 3.2 (2H, t), 3.8 (2H,m), 7.6–7.9 (15H, m) ppm . ESI-MS**:** 423.13 $[M]^+$, 424.13 $[M+H^+]$.

X-Ray Crystallography

Table 1 Crystallographic data summary

Compound reference	**3**	**4**
Chemical formula	$C_{21}H_{21}AsO_3S_2$	$C_{23}H_{24}AsBrOS$
Formula Mass	460.42	503.31
λ/Å	0.71075	0.71075
T/K	100(2)	100(2)
Space group	$P2_1/n$	$P2_1/n$
a/Å	11.687(5)	11.1700(3)
b/Å	14.366(6)	13.6075(4)
c/Å	12.127(5)	14.4757(10)
α/°	90	90
β/°	106.254(5)	91.738(7)
γ/°	90	90
V/Å^3	1954.7(14)	2199.23(18)
Z	4	4
μ/mm^{-1}	1.972	3.467
F(000)	944	1016
2θ/°	55.0	55.0
No. of reflections measured	10357	13491
No. of independent reflections	3852	4973
R_{int}	0.0510	0.0274
GOOF	1.143	0.960
Final R_1 values ($I > 2\sigma(I)$)	0.0730	0.0267
Final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.1111	0.0530
Final R_1 values (all data)	0.0858	0.0398
Final $wR(F^2)$ values (all data)	0.1173	0.0641

Single-crystal X-ray diffraction analyses of **3** and **4** were performed using a Rigaku Saturn 724+ area detector mounted at the window of an FR-E+ rotating anode generator with a Mo anode (λ=0.71075Å) and equipped with an Oxford Cryosystems cryostream device. The crystals were mounted on a MiTeGen MicroLoops and the data were collected at 100K. Data were processed and empirical absorption corrections were carried out using CrystalClear SM-Expert.[2] The structures were solved by charge-flipping using SUPERFLIP[3] and refined on F_o^2 by full-matrix least squares refinement using SHELXL-97[4]. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were added at

calculated positions to carbon atoms with isotropic displacement parameters based on the equivalent isotropic displacement parameter (Ueq) of the parent atom. Detailed crystallographic parameters are given in Table 1 below. The CIF files for the crystal structures have been deposited with the CCDC and been given the deposition numbers 1020083 (**3**) and 1020084 (**4**).

Synthesis of Triphenylarsonium-Functionalised Gold Nanoparticles

Triphenylarsonium-functionalised AuNPs were synthesised based on methods described previously.[5,6] In brief, a solution of the triphenylarsonium compound (0.25 mmol) was prepared in $HAuCl_4$ (0.12 mmol) and dichloromethane (15 mL). The solution was stirred vigorously under nitrogen for 6 hours; subsequently an aqueous solution of $NaBH_4$ (3 mL, 400 mmol L^{-1}) was added drop wise followed by the addition of deionised H_2O (15 mL) and stirred under nitrogen overnight. After 24 hours the colloidal solution was purified by dichloromethane extraction (3 x 10 mL), freeze dried, flushed with nitrogen and retained for further analysis. UV/vis $\lambda max(H_2O)$/nm 520. IR υ_{max}/cm^{-1} 2262 (CH), 1639, 1340(br),1064(br), 868, 808 680.

Cell Biology

Human prostate cancer cells (PC3) were maintained in complete media (cDMEM) which contains Dulbecco's modified Eagle medium with GlutaMAX, 4.5 g/L D-Glucose and sodium pyruvate (Invitrogen Life Technologies, Paisley, Renfrewshire, UK) containing 10% heat-inactivated fetal bovine serum (Biosera, East Sussex, Sussex, UK) and 1% penicillin-streptomycin (Invitrogen Life Technologies, Paisley, Renfrewshire, UK) at 37°C in 5% CO_2 and 95% air. Cells were routinely sub-cultured every 3 days, and routinely screened for mycoplasma.

Cytotoxicity Assay

Cytotoxicity was assessed using CellTitre-Glo luminescent cell viability assay kit (Promega Corporation, Southampton, Hampshire, UK). PC3 cells were seeded in opaque-walled 96-well plates at a density of 10,000 cells/well and allowed to adhere overnight. Cells were subsequently treated with the corresponding arsonium ligand (0-1,000 μM) for 24, 48 and 72 hours. After each incubation period, cell viability was measured according to the manufacturer's instructions. Plates were equilibrated at room temperature for 30 minutes, 100 μL of assay reagent was added to each well, placed on an orbital shaker for 2 minutes, left to

stand at room temperature for 10 minutes and read on a Wallac Victor2 1420 multilabel counter (PerkinElmer, Cambridge, Cambridgeshire, UK). All plates contain control wells and all measurements were performed in quadruplicates and three independent experiments were conducted (n = 12). Data are expressed as a percentage of live cells normalised to control, and the average, standard deviation and IC_{50} values were plotted and calculated using GraphPad Prism (GraphPad software, La Jolla, California, USA).

Cytotoxicity studies were also investigated using MTT assay to determine the cellular uptake of these compounds on PC3 cells. The MTT solutions were prepared (5mg of MTT/ml), filter sterilized and stored at 4°C for use within 2 weeks. The cells (PC3) were grown overnight in 96 well plates at a density of 10,000 cells/well. After 24 hours of incubation the plates were inverted, flicking off the medium and were replaced with required drug concentration of corresponding ligands **3** and **4** (0-1000μM) for 24, 48 and 72 hours. After the respective incubation periods, plates were removed from the incubator for the addition of 20μL of MTT solution to each well. The plates were then again incubated for a further 4 hours at 37°C. After 4 hours, the medium was removed and 100μL of acid-isopropanol was added to each well for the development of formazan crystals in the cell layer to dissolve. The absorbance was then measured at 570nm on a Wallac Victor2 1420 multilabel counter. The IC_{50} was then determined for each compound (**3** and **4**) from a plot of log drug concentration versus percentage of cell normalised to control. The MTT assay study for **3** showed that (Fig 3c) the IC_{50} values were found to 89.99, 51.99, 71.86μM for 24, 48 and 72hours respectively. The MTT assay study for **4** showed that (Fig 3d) the IC_{50} values were found to 74.65, 43.69, 74.94μM for 24, 48 and 72hours respectively. IC_{50} values were plotted and calculated using GraphPad Prism.

References

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