In Vitro and *In Vivo* Evaluation of ^{89}Zr-DS-8273a as a Theranostic for Anti-Death Receptor 5 Therapy

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SUPPLEMENTARY MATERIALS AND METHODS

Determining chelator:antibody ratio by ESI-MS. Detergents are known to interfere with ESI-MS. Polysorbate 80 was removed from the antibody samples using a Pierce detergent removal spin columns (Thermo Scientific, Australia) according to manufacturer's instructions. Briefly, the 0.5 mL columns were equilibrated with 20 mM sodium acetate buffer, pH 6.5. 100 uL of samples were applied to the columns and eluted in the equilibration buffer.

Protein samples were analysed using an Agilent 6220 ESI-TOF LC/MS Mass Spectrometer coupled to an Agilent 1200 LC system (Agilent, Palo Alto, CA). All data were acquired and reference mass corrected via a dual-spray electrospray ionisation (ESI) source. Acquisition was performed using the Agilent Mass Hunter Acquisition software version B.02.01 (B2116.30). Ionisation mode: Electrospray Ionisation; Drying gas flow: 7 L/min; Nebuliser: 35 psi; Drying gas temperature: 325°C; Capillary Voltage (Vcap): 4000 V; Fragmentor: 350 V; Skimmer: 65 V; OCT RFV: 250 V; Scan range acquired: 300–3200 m/z Internal Reference ions: Positive Ion Mode = m/z = 121.050873 & 922.009798. DTPA-modified protein samples were incubated in $CuSO_4$ for 30 minutes prior to analysis. For all samples, protein desalting and chromatographic separation was performed using an Agilent Poroshell C18 2.1 x 75 mm, 5µm column using 5% (v/v) acetonitrile ported to waste (0-5min). Upon desalting of sample the flow was ported back into the ESI source for subsequent gradient elution with (5% (v/v) to 100% (v/v)) acetonitrile / 0.1% formic acid over 15 min at 0.25 mL/min. Analysis was performed using Mass Hunter version B.06.00 with BioConfirm software using the maximum entropy protein deconvolution algorithm.

SUPPLEMENTARY FIGURES

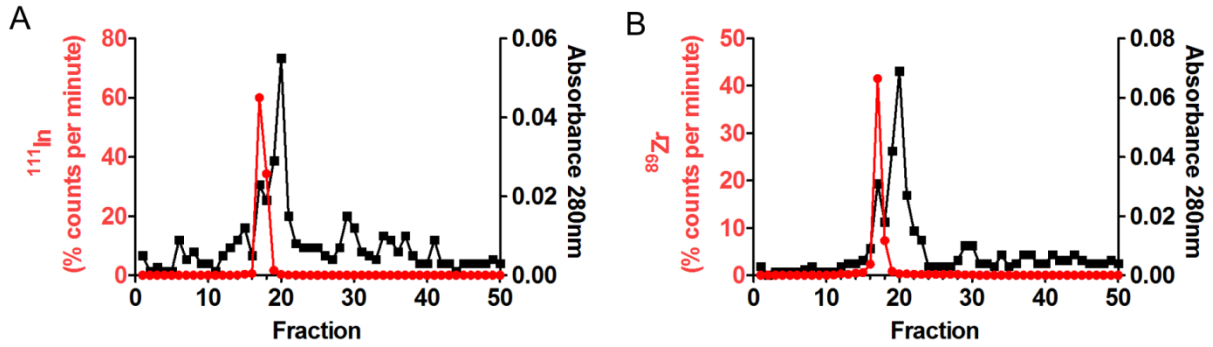


Figure S1 - Superdex 200 HR 10/30 Column Size Exclusion Chromatography (SEC) analysis of (A) ^{111}In–CHX-A"-DTPA-DS-8273a and (B) ^{89}Zr-Df-Bz-NCS DS-8273a. The radiochromatograms (red) show one single peak for both ^{89}Zr- and ^{111}In-labeled DS-8273a. The UV chromatograms (black) show two peaks representing thyroglobulin (700 kDa, fraction 16) and human serum albumin (66 kDa, fraction 20).

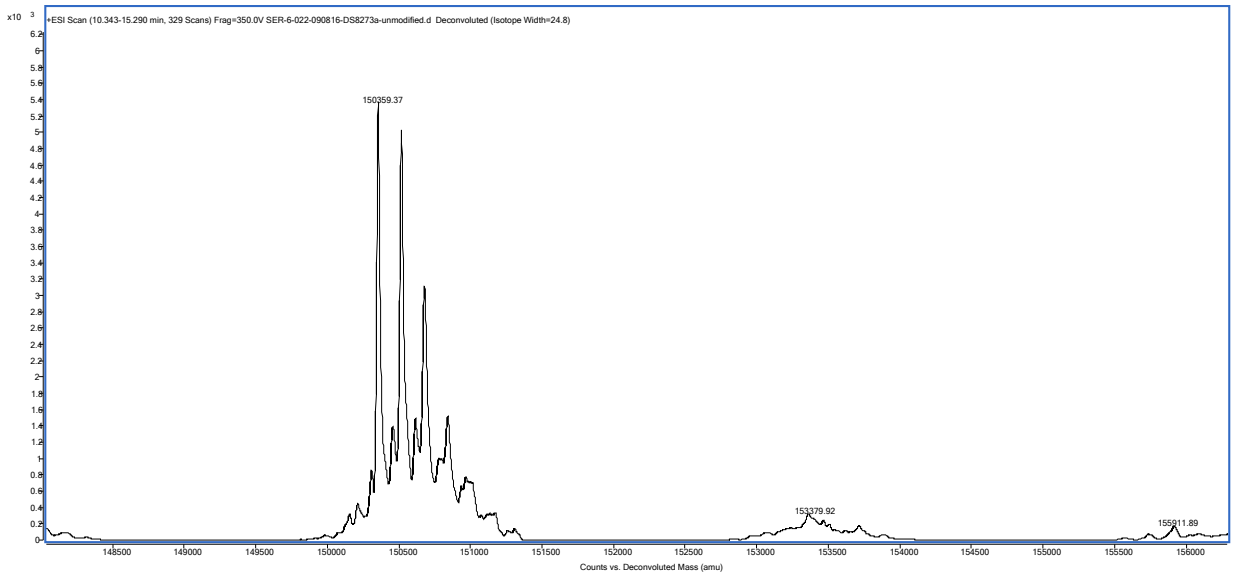
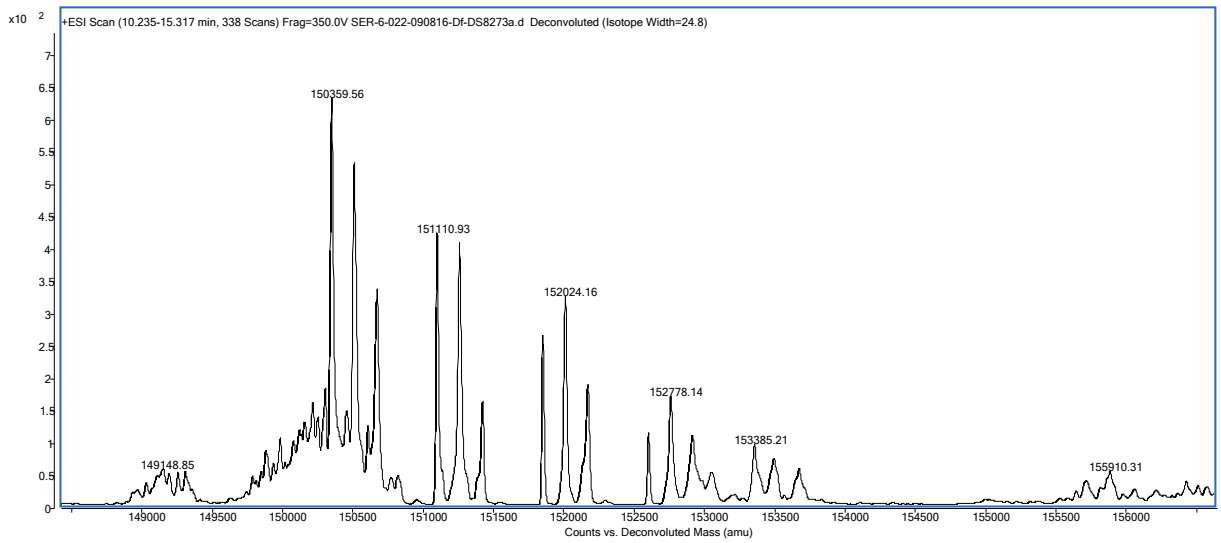
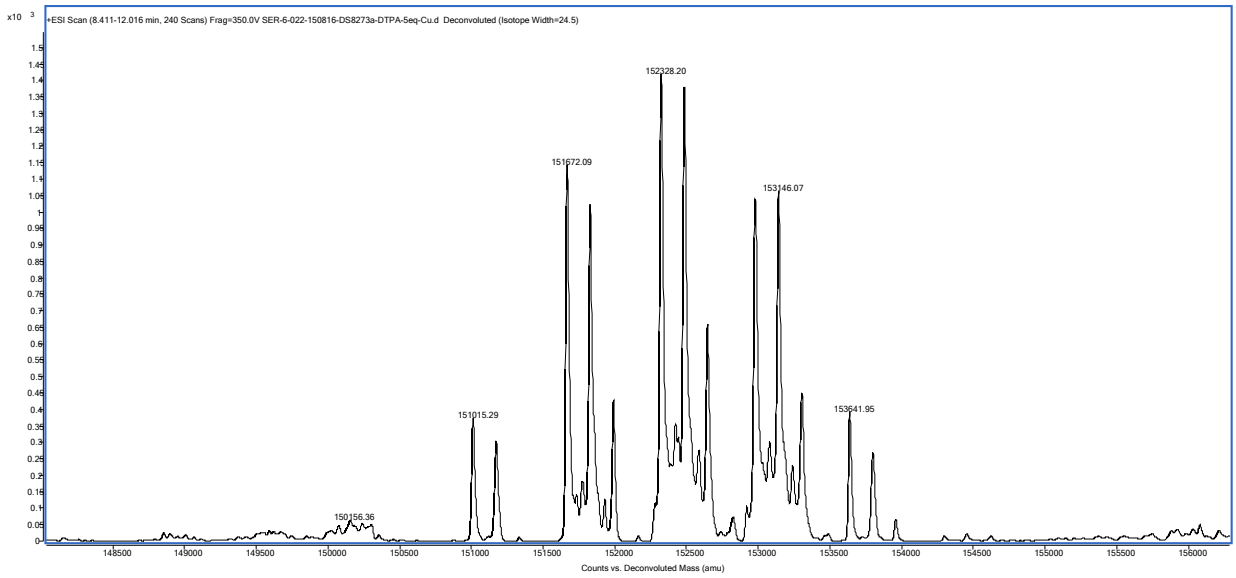


Figure S2 – ESI-MS profile of unconjugated DS-8273a



mass observed (amu)	counts	no of chelators	counts x chelators
150359	633.09	0	0
151110	423.01	1	423.01
151864	263.81	2	527.62
152619	112.29	3	336.87
153385	94.15	4	376.6
Total counts:	1526.35	Total chelators:	1664.1
		Average chelators:mAb	**1.1:1**

Figure S3 – ESI-MS profile of Df-Bz-NCS-DS-8273a with calculated average chelator:antibody ratio of 1.1:1



mass observed (amu)	counts	no. of chelators	counts x chelators
151015	375.31	1	375.31
151672	1143.45	2	2286.9
152328	1420.66	3	4261.98
152985	1034.79	4	4139.16
153641	393.83	5	1969.15
Total counts:	4368.04	Total chelators:	13032.5
		Average chelators:mAb	**3.0:1**

Figure S4 – ESI-MS profile of CuDTPA-CHX-A″-DS-8273a with calculated average chelator:antibody ratio of 3.0:1

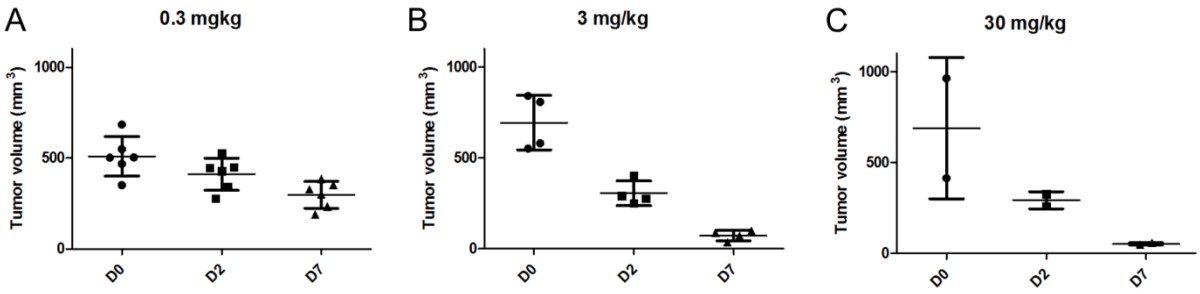


Figure S5 – Tumor growth inhibition in COLO205 tumor-bearing mice at different doses of DS-8273a observed in biodistribution and imaging studies. Bars, SD; 0.3mg/kg, *n* = 6; 3 mg/kg, *n* = 4; 30 mg/kg, *n* = 2