

Supplementary material

Preclinical Characterisation of PSMA/GRPR-targeting Heterodimer [⁶⁸Ga]Ga-BQ7812 for PET Diagnostic Imaging of Prostate Cancer: a Step towards Clinical Translation

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General information

Instruments and Equipment

Analytical high performance liquid chromatography (HPLC) was performed on a Dionex UltiMate 3000 HPLC system with a Bruker amazon SL ion trap mass spectrometer and detection by UV (diode array detector, 214, 254, and 280 nm) and electrospray ionization (ESI) MS using a Penomenex Kinetex C18 column (50 × 3.0 mm, 2.6 µm particle size, 100 Å pore size) with gradients of H₂O/CH₃CN/0.05% HCOOH as mobile phase at a flow rate of 1.5 mL/min. Preparative reversed phase high-performance liquid chromatography (RP-HPLC) was performed by UV-triggered (254 nm) fraction collection with a Glison HPLC system using a Machery-nagel NUCLEODUR C18 HTec column (21 × 125 mm, particle size 5 µm) and H₂O/CH₃CN/0.1% TFA as mobile phase at a flow rate of 10 mL/min. ⁶⁸Ge/Ga-generator (Cyclotron Co., Obninsk, Russia) using 0.1 M metal-free hydrochloric acid was used to elute gallium-68. Radioactivity content was measured using an automated gamma counter (3-inch NaI(Tl) detector, 2480 Wizard2, PerkinElmer). nanoPET imaging of PC3-pip xenografted mice using a nanoScan PET/MR1 3T camera (Mediso Medical Imaging System Ltd., Budapest, Hungary) and CT scan was performed using a nanoScan SPECT/CT camera (Mediso Medical Imaging System Ltd., Budapest, Hungary). Reconstruction of the PET/CT scans was done using Nucline nanoScan 3.04.014.0000 software. The absorbed doses in the dosimetry investigation were estimated using OLINDA/EXM 1.0. Statistical analysis was performed by one-way ANOVA with Bonferroni's test corrected for multiple comparisons using GraphPad Prism 8 for Windows (GraphPad Software Inc, San Diego, CA, USA), p-values <0.05 were considered statistical significant.

Chemicals and Solvents

Starting materials and solvents were purchased from Sigma Aldrich, Fisher Scientific, and Honeywell, and used without further purification if nothing else stated. NOTA-bis(tBu)-ester was purchased from CheMatech, France; Fmoc Rink Amide MBHA resin (loading 0.69 mmol/g) and L-Glu(OBn)-O(tBu) were purchased from Iris Biotech GmbH (Marktredwitz, Germany).

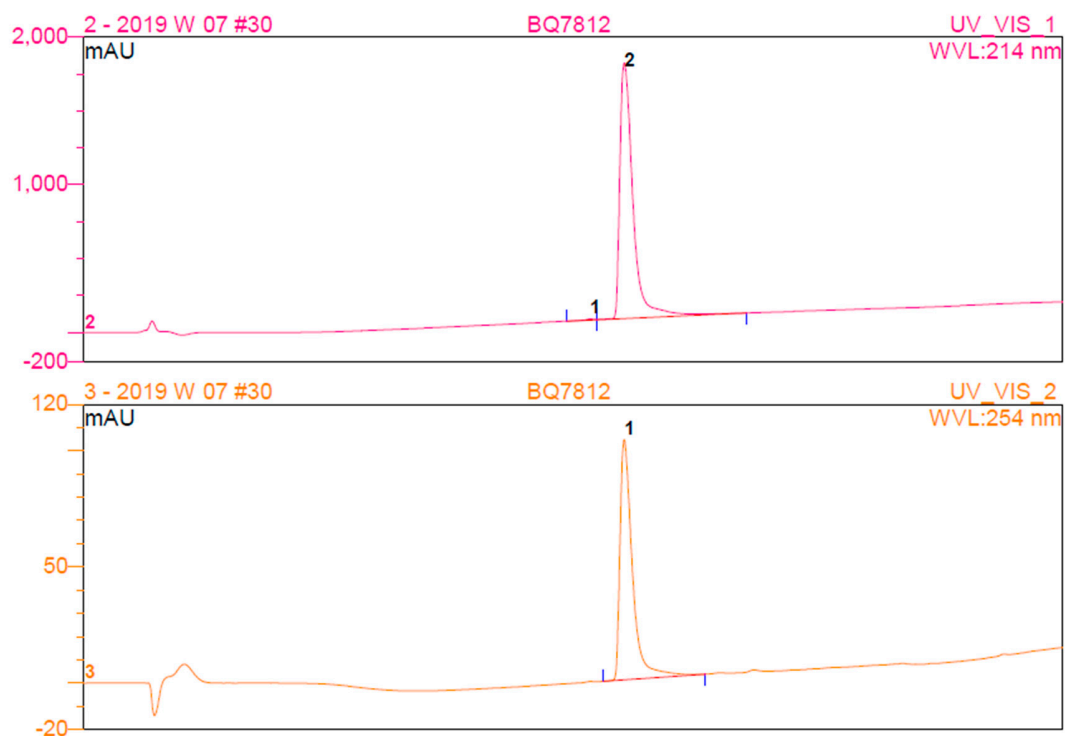


Figure S1. Analytical HPLC chromatogram of BQ7812. UV-detection at 214 and 254 nm.

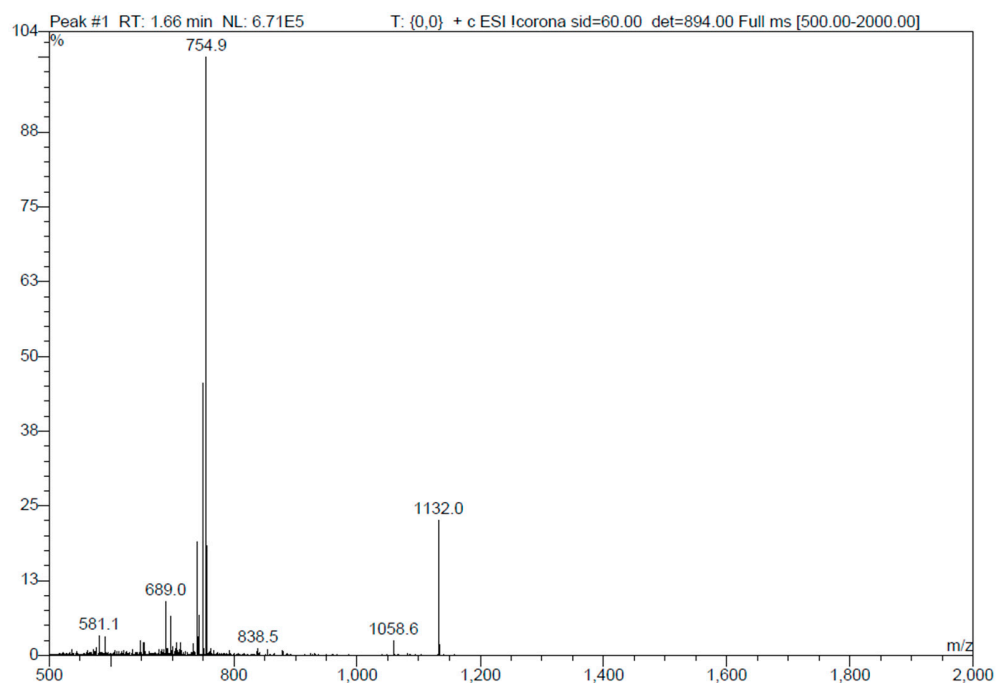


Figure S2. MS-chromatogram of BQ7812 with observed $[M+2H]^{2+} = 1132.0$ and $[M+3H]^{3+} = 754.9$.

Table S1. Ex vivo biodistribution of [⁶⁸Ga]Ga-BQ7812 (40 pmol/animal, 30 kBq) in BALB/c nu/nu PC3-pip xenografted tumour bearing mice at 1 and 3 h pi. Data are presented as average ± standard deviation. To the left; Uptake of activity was calculated as percent injected dose per tissue weight (% ID/g), To the right; tumour-to-organ ratios. Statistical analysis was done using one-way ANOVA with Bonferroni's test corrected for multiple comparisons.

	Uptake of [⁶⁸ Ga]Ga-BQ7812 (%IA/g)				Tumour-to-organ ratio (T/O)	
	1 h pi			3 h pi	1 h pi	3 h pi
	Non-blocked	GRPR blocked	PSMA blocked	Non-blocked	Non-blocked	Non-blocked
Blood	0.86 ± 0.16	0.69 ± 0.28	0.80 ± 0.24	0.16 ± 0.02 ^c	13 ± 3	35 ± 4 ^c
Salivary Glands	0.44 ± 0.07	0.29 ± 0.07 ^a	0.38 ± 0.12	0.14 ± 0.03 ^c	24 ± 3	40 ± 8 ^c
Lungs	0.86 ± 0.15	-----	-----	0.20 ± 0.05 ^c	12 ± 3	29 ± 5 ^c
Liver	0.89 ± 0.10	-----	-----	0.57 ± 0.03 ^c	11.8 ± 1.0	9.9 ± 0.8
Spleen	0.92 ± 0.16	-----	-----	0.33 ± 0.03 ^c	12 ± 3	18 ± 3
Pancreas	5.63 ± 0.58	0.76 ± 0.14 ^a	6.33 ± 1.86	0.60 ± 0.16 ^c	1.9 ± 0.2	10 ± 4 ^c
Small Intestine	1.33 ± 0.31	-----	-----	0.20 ± 0.02 ^c	8 ± 2	28 ± 4 ^c
Kidneys	45.26 ± 16.55	34.50 ± 8.74	13.78 ± 0.45 ^b	10.55 ± 2.13 ^c	0.3 ± 0.1	0.53 ± 0.07 ^c
Tumour (PC3-pip)	10.44 ± 0.97	5.73 ± 0.72 ^a	8.26 ± 1.03 ^b	5.68 ± 0.62 ^c	-----	-----
Muscle	0.21 ± 0.04	-----	-----	0.05 ± 0.01 ^c	52 ± 12	116 ± 22 ^c
Bone	0.31 ± 0.06	-----	-----	0.13 ± 0.03 ^c	35 ± 8	47 ± 13

a Value in GRPR-blocked group is significantly different than in Non-blocked group;

b Value in PSMA-blocked group is significantly different than in Non-blocked group;

c Value in Non-blocked group 3 h pi is significantly different than in Non-blocked group 1 h pi.