Electronic Supporting Information

Synthesis and preclinical evaluation of BOLD-100 radiolabeled with ruthenium-97 and ruthenium-103

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1. Gamma spectra

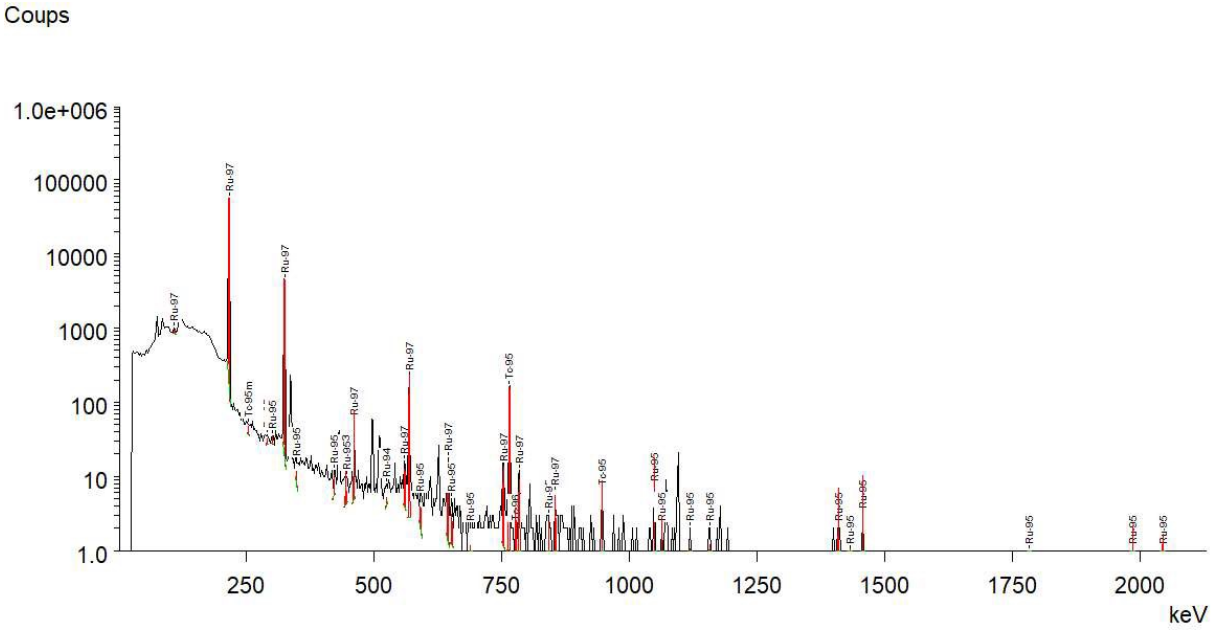

Figure S1: Representative γ-ray spectrum of ^{97}Ru 18 h after end of irradiation and after radiochemical Mo/Ru separation. Due to irradiation of ^{nat}Mo, also traces of ^{95}Ru ($t_{1/2}$ = 1.6 h), ^{95m}Tc ($t_{1/2}$ = 61 d), and ^{95}Tc ($t_{1/2}$ = 20.0 h) were observed.

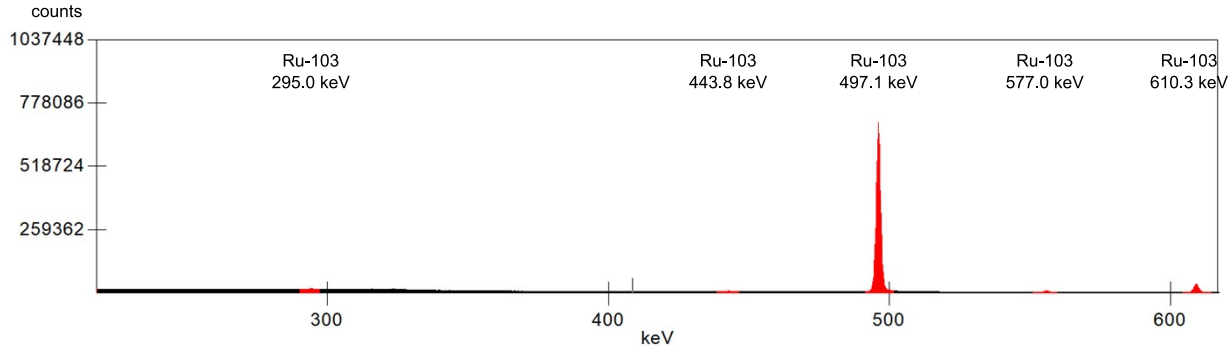

Figure S2: Representative γ-ray spectrum of ^{103}Ru, 16 days after end of irradiation and after target workup.

2. Syntheses[1]

[^{103}Ru]KP1019 ([^{103}Ru]indazolium *trans*-[tetrachloridobis(indazole)ruthenate(III)]) (1b): 3 mL of the ^{103}Ru-solution (5.5 mg Ru, in HCl > 33%) and $RuCl_3 \cdot xH_2O$ (8 mg, 39 µmol) were refluxed with ethanol (3 mL) for 3 h. The total amount of [^{103}Ru]$RuCl_3$ was 19 mg (92 µmol, 1 eq., 54.7 MBq). Indazole (72 mg, 608 µmol, 6.64 eq.), dissolved in H_2O (0.1 mL) and conc. HCl (37%, 0.4 mL), was added to the ruthenium solution. The reaction mixture was refluxed for 3 h. After cooling down to room temperature (RT), the formed suspension was centrifuged, and the supernatant was removed. The remaining solid was washed three times with 2 M HCl (1 mL). The obtained product (49 mg, 90%, 48.5 MBq, yield determined by isolated radioactivity) was analyzed using HPLC.

HPLC: t_R ($Hind^+$) = 5.37 min, t_R ($[^{103}Ru][Ru(ind)_2Cl_4]^-$) = 7.79 min (UV), 8.06 min (radiochannel)

RCY: 90%

RCP: > 99%

[^{103}Ru]Cesium *trans*-[tetrachloridobis(indazole)ruthenate(III)] (2b): Indazolium salt **1b** (49 mg, 82 µmol) was suspended in ethanol (0.75 mL), CsCl (15 mg, 90 µmol, 2.8 eq.) was added, and the mixture was stirred at RT for 2 h. The solid was separated by centrifugation, washed twice with ethanol (0.5 mL), and stirred in a 2:1 v/v ethanol water mixture (0.75 mL) for 15 min at RT. The product (44 mg, 78%, 41.7 MBq) was centrifuged, washed twice with ethanol (0.5 mL), and analyzed using HPLC.

HPLC: t_R ($[^{103}Ru][Ru(ind)_2Cl_4]^-$) = 7.66 min (UV), 7.93 min (radiochannel)

RCY: 78%

RCP: > 99%

[^{103}Ru]BOLD-100 ([^{103}Ru]sodium *trans*-[tetrachloridobis(indazole)ruthenate(III)]) (3b): Cesium salt **2b** (44 mg, 71 µmol) was suspended in 1.1 M $NaAl(SO_4)_2 \cdot 18\ H_2O$ solution (1 mL), CsCl (spatula tip, catalytic amount) was added, and the mixture was stirred at RT for 24 h. The crude product was obtained by centrifugation and washing with saturated Na_2SO_4 solution. The obtained solid was stirred in ACN (0.5 mL) for 15 min at RT. The suspension was centrifuged and washed twice with ACN (0.5 mL). The filtrates were combined, diluted with MTBE (5:1), and set aside for 2 h. The precipitated brown needles were collected by centrifugation, washed with MTBE, and dried on air to yield pure c.a. [^{103}Ru]BOLD-100 (16.1 mg, overall radiochemical yield: 18.2 MBq, 35%; overall chemical yield determined by weighing the substance: 17.2 mg, 39%).

HPLC: t_R ($[^{103}Ru][Ru(ind)_2Cl_4]^-$) = 7.80 min (UV), 8.11 min (radiochannel)

RCY: 35%, total chemical yield: 39%

RCP: > 99%

All chromatograms can be found in section 3.2 (Figure S9–S14).

3. HPLC[2]

All impurities resulted from the dissolution of the samples in the HPLC starting conditions (10% ACN + 0.1% TFA, 90% H_2O + 0.1% TFA).

3.1 UV and radio chromatograms of [^{97}Ru]BOLD-100

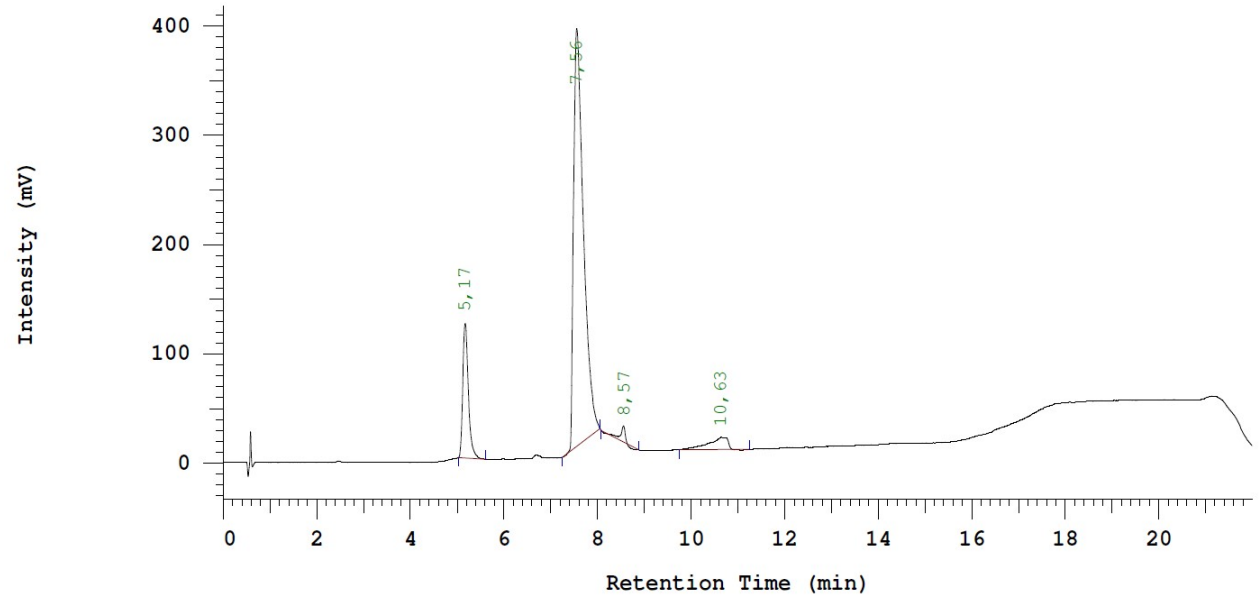

Figure S3: UV chromatogram of 1a (220 nm, 20 µL injection, manually integrated); t_R (Hind$^+$) = 5.17 min, t_R ([^{97}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.56 min, t_R ([^{97}Ru][Ru(ind)$_2$Cl$_3$(H$_2$O)]) = 8.57 min, t_R ([^{97}Ru][Ru(ind)$_2$Cl$_3$(ACN)]) = 10.63 min.

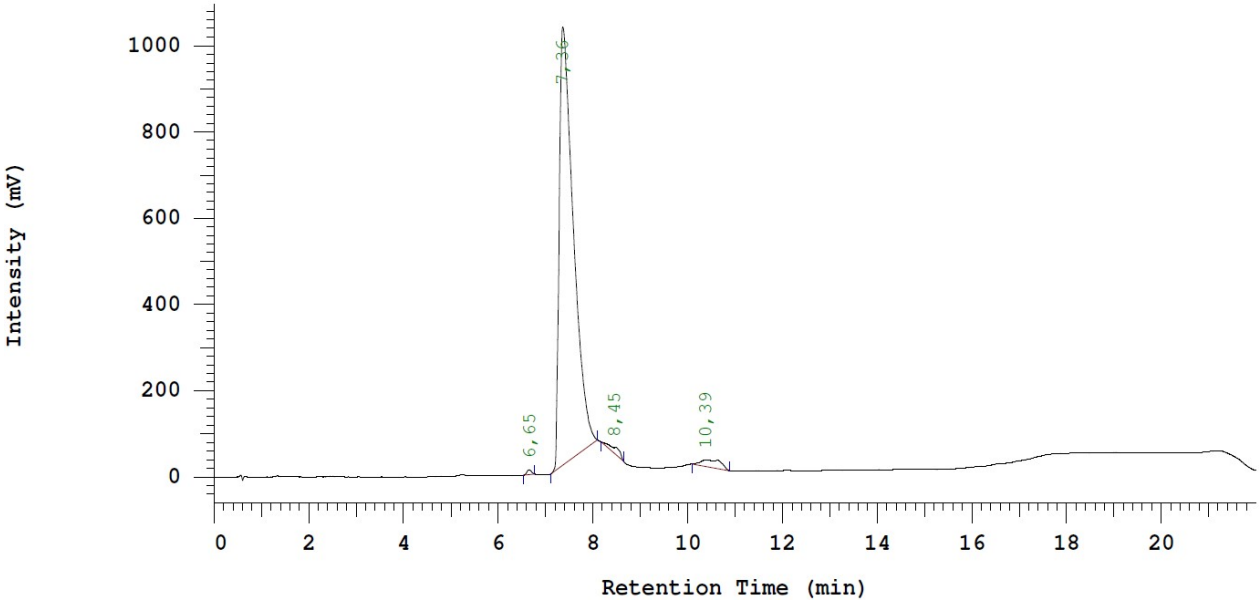

Figure S4: UV chromatogram of **2a** (220 nm, 20 µL injection, manually integrated); t_R ([^{97}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.36 min, t_R ([^{97}Ru][Ru(ind)$_2$Cl$_3$(H$_2$O)]) = 8.45 min, t_R ([^{97}Ru][Ru(ind)$_2$Cl$_3$(ACN)]) = 10.39 min.

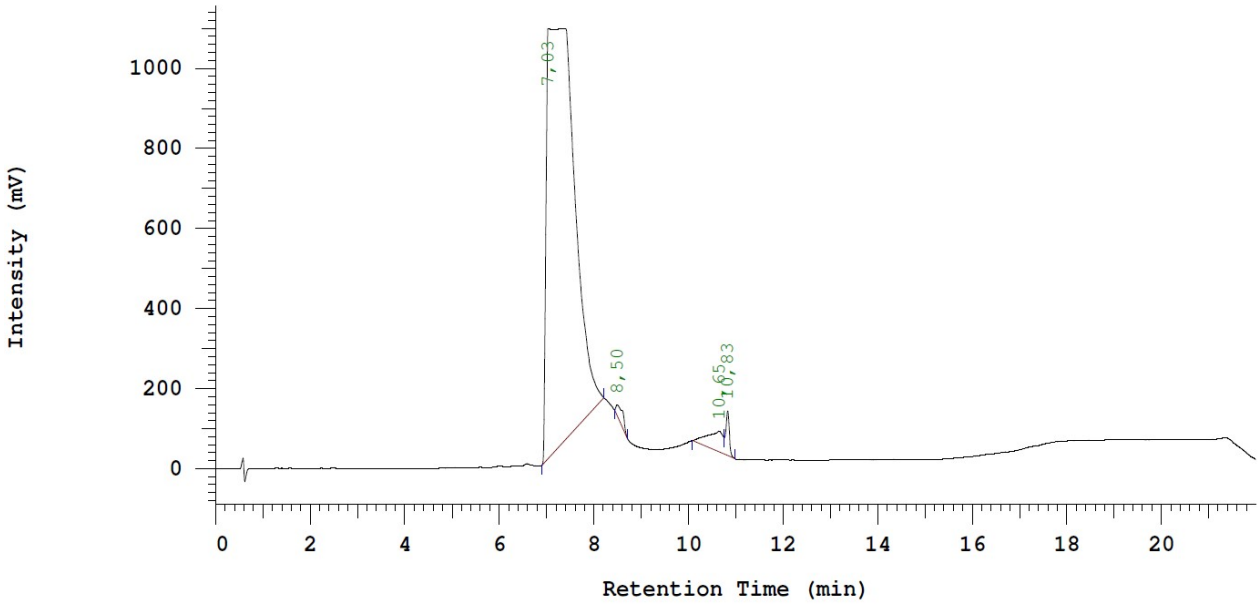

Figure S5: UV chromatogram of **3a** (220 nm, 20 µL injection, manually integrated), t_R ([^{97}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.03 min, t_R ([^{97}Ru][Ru(ind)$_2$Cl$_3$(H$_2$O)]) = 8.50 min, t_R ([^{97}Ru][Ru(ind)$_2$Cl$_3$(ACN)]) = 10.65, 10.83 min.

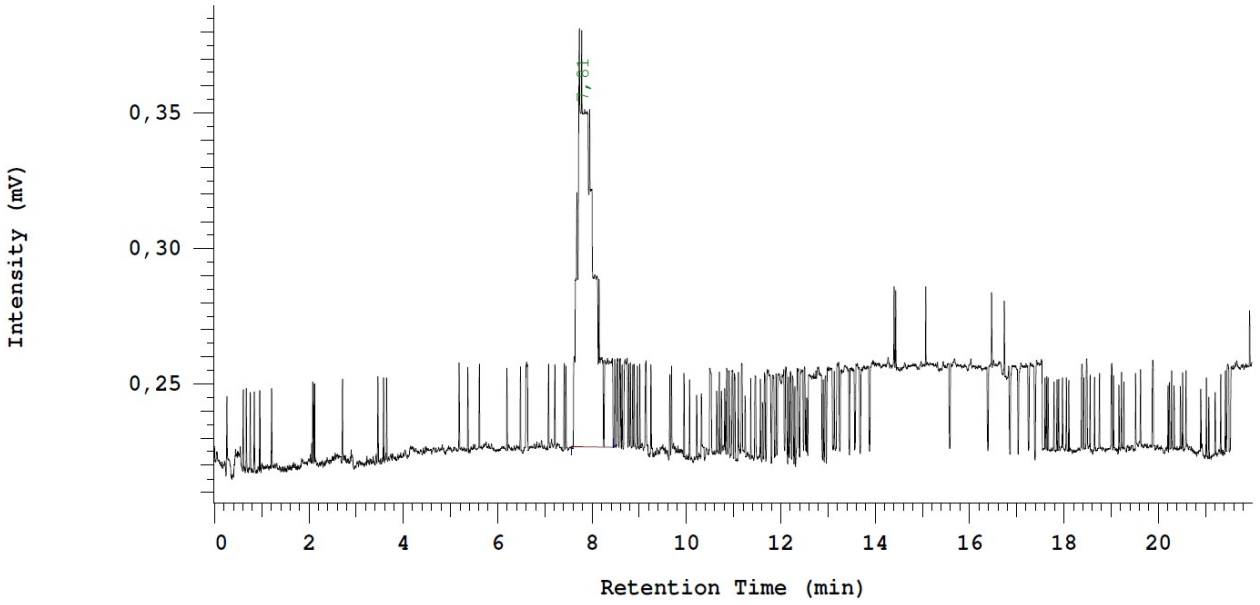

Figure S6: Radio chromatogram of **1a** (220 nm, 20 µL injection, manually integrated); t_R ([^{97}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.81 min.

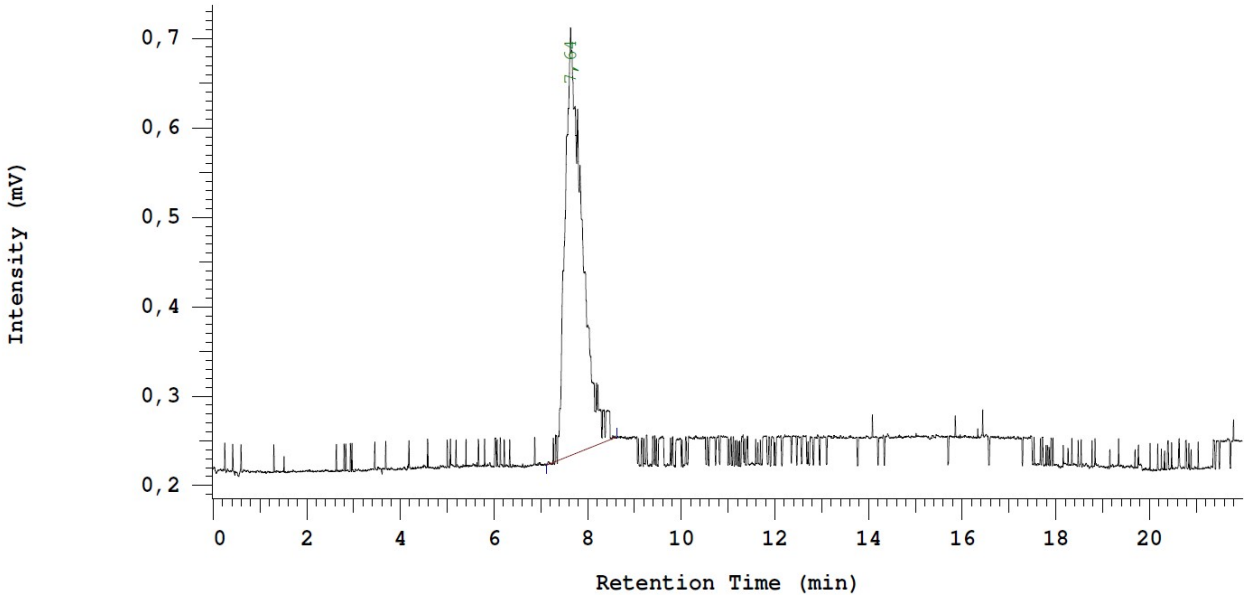

Figure S7: Radio chromatogram of **2a** (220 nm, 20 µL injection, manually integrated); t_R ([^{97}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.64 min.

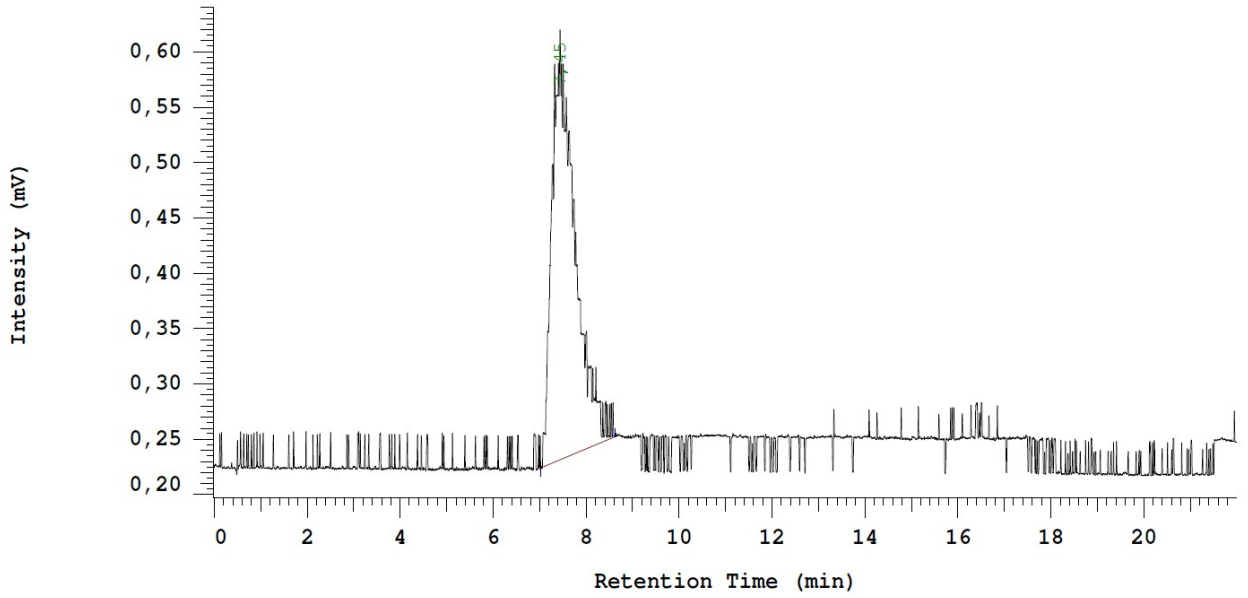

Figure S8: Radio chromatogram of **3a** (220 nm, 20 µL injection, manually integrated); t_R ([^{97}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.45 min.

3.2 UV and radio chromatograms of [^{103}Ru]BOLD-100

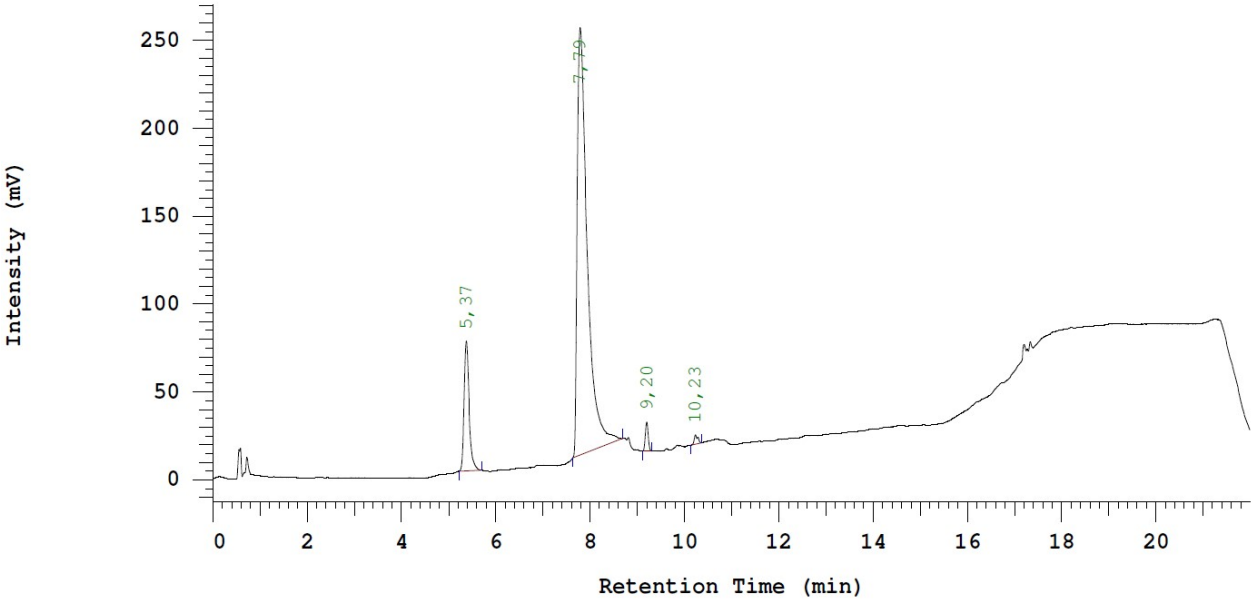

Figure S9: UV chromatogram of **1b** (220 nm, 20 µL injection, manually integrated); t_R ($Hind^+$) = 5.37 min, t_R ([^{103}Ru][$Ru(ind)_2Cl_4]^-$) =7.79 min, t_R ([^{103}Ru][$Ru(ind)_2Cl_3(H_2O)$]) = 9.20 min, t_R ([^{103}Ru][$Ru(ind)_2Cl_3(ACN)$]) = 10.23 min.

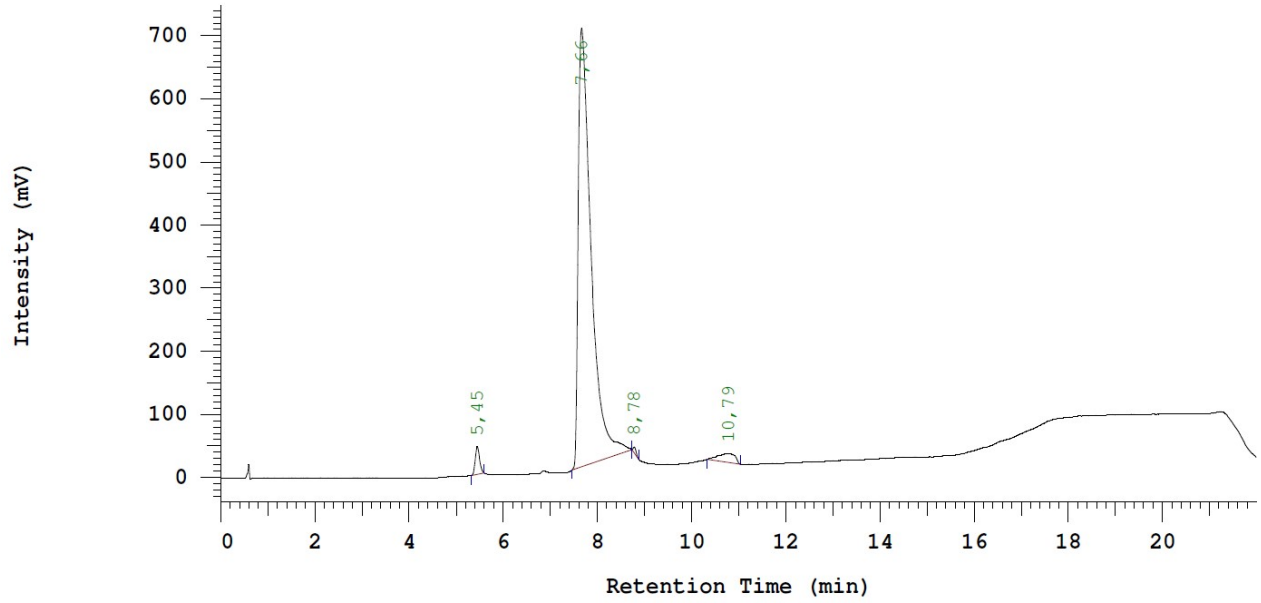

Figure S10: UV chromatogram of **2b** (220 nm, 20 µL injection, manually integrated); t_R ($Hind^+$) = 5.45 min, t_R ([^{103}Ru][$Ru(ind)_2Cl_4]^-$) = 7.66 min, t_R ([^{103}Ru][$Ru(ind)_2Cl_3(H_2O)$]) = 8.78 min, t_R ([^{103}Ru][$Ru(ind)_2Cl_3(ACN)$]) = 10.79 min.

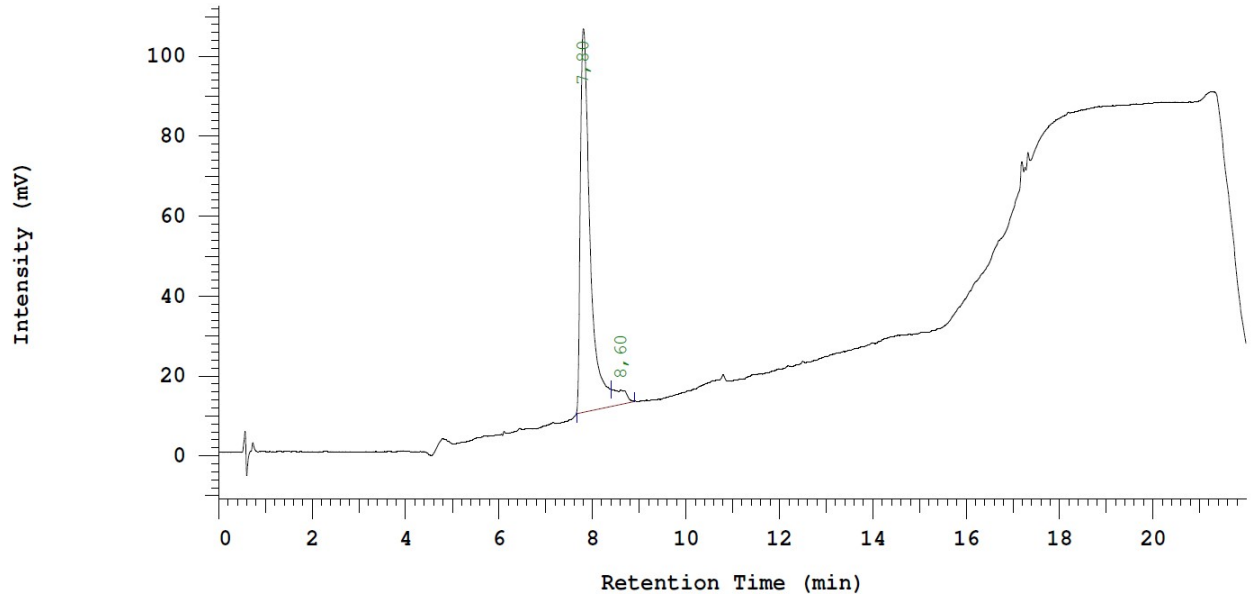

Figure S11: UV chromatogram of **3b** (220 nm, 20 µL injection, manually integrated); t_R ([^{103}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.80 min, t_R ([^{103}Ru][Ru(ind)$_2$Cl$_3$(H$_2$O)]) = 8.60 min.

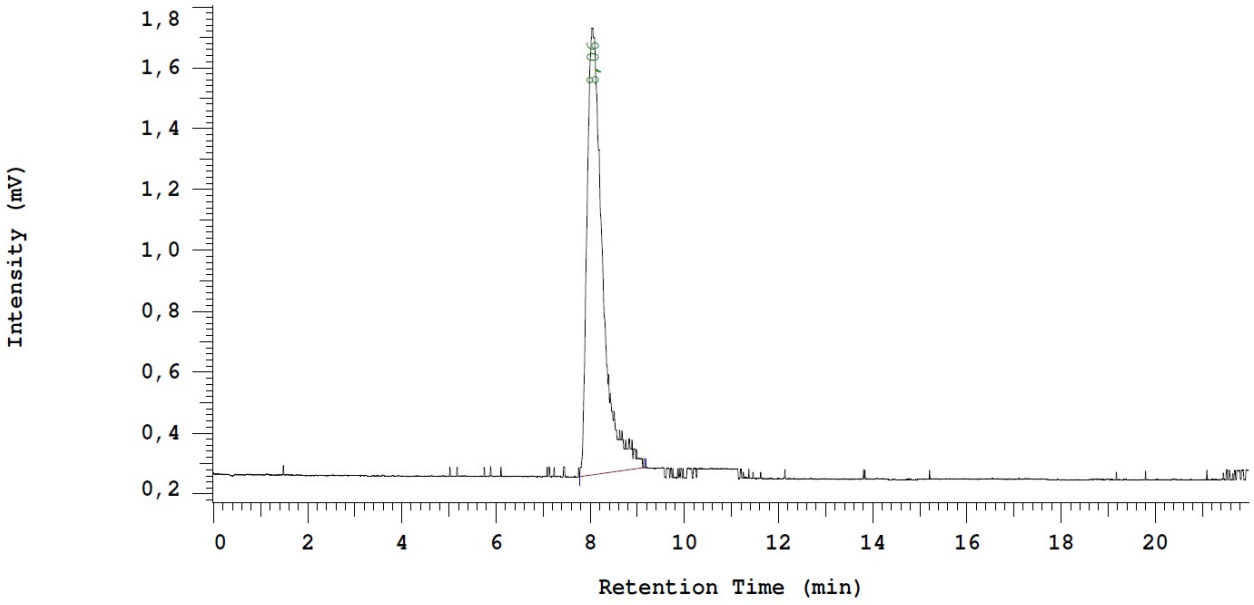

Figure S12: Radio chromatogram of **1b** (20 µL injection, manually integrated); t_R ([^{103}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 8.06 min.

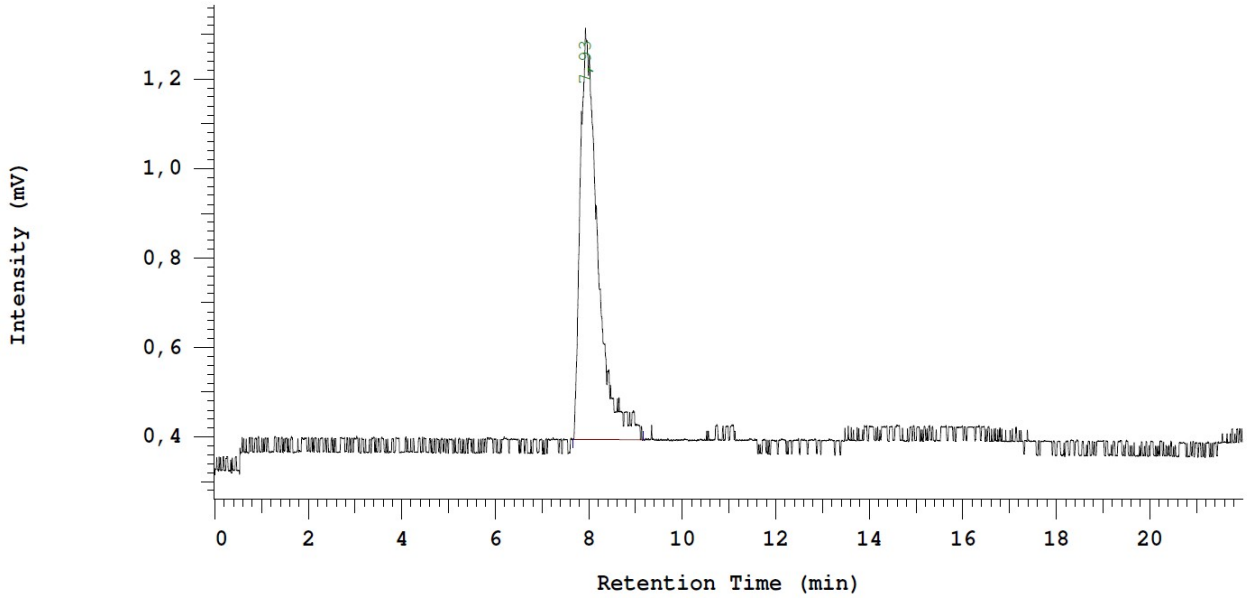

Figure S13: Radio chromatogram of **2b** (20 µL injection, manually integrated); t_R ([^{103}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 7.93 min.

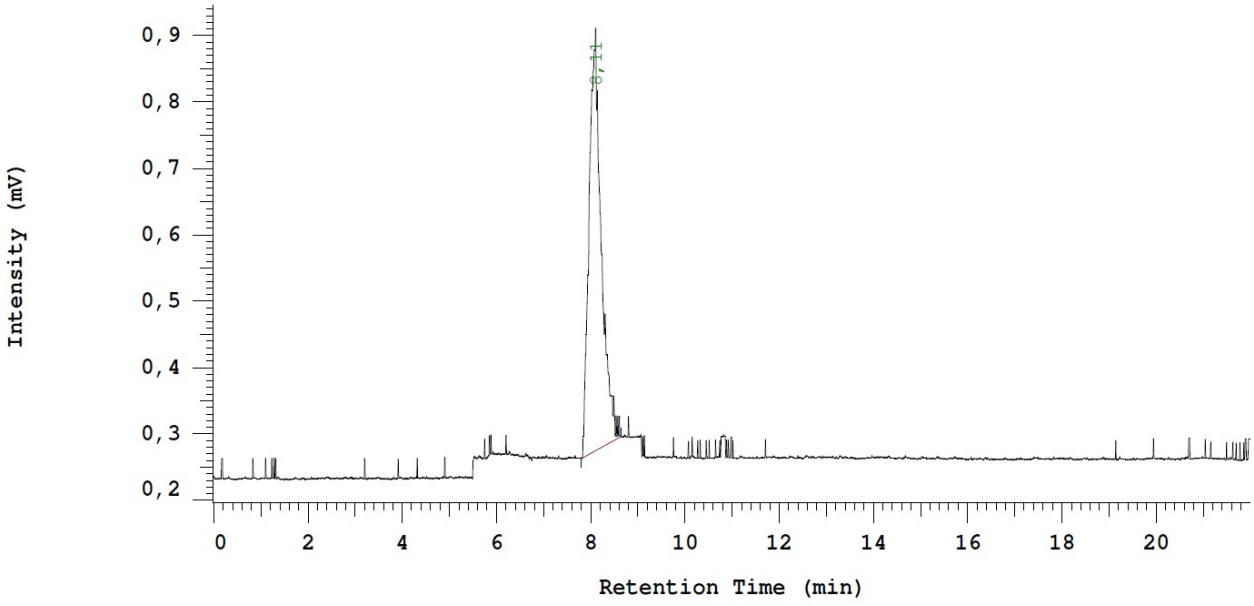

Figure S14: Radio chromatogram of **3b** (20 µL injection, manually integrated); t_R ([^{103}Ru][Ru(ind)$_2$Cl$_4$]$^-$) = 8.11 min.

4. Biodistribution data

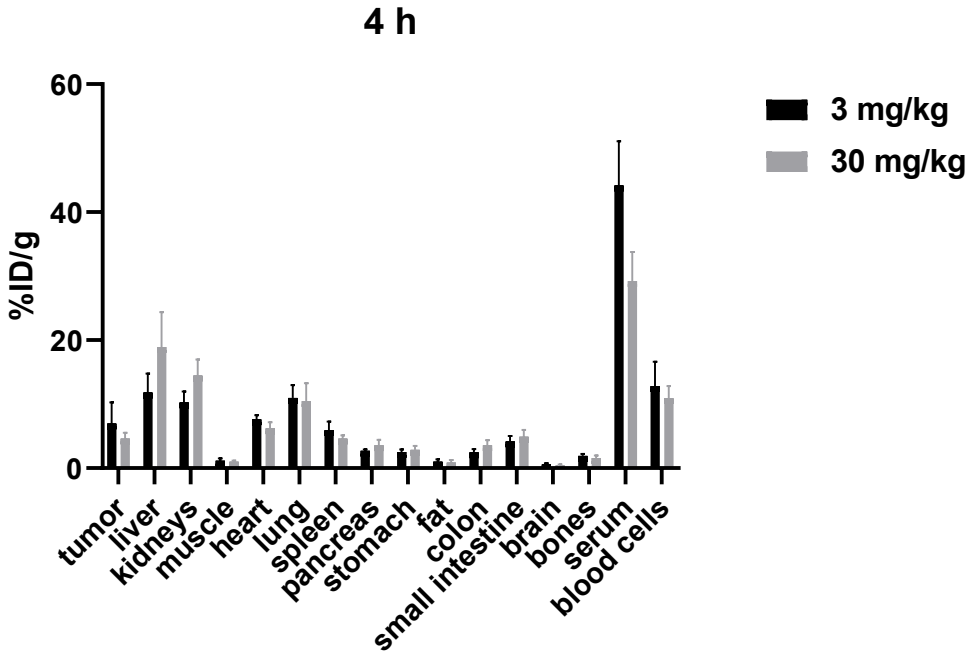

Figure S15: Tissue distribution of compound **3b** (c.a. [^{103}Ru]BOLD-100) 4 h p.i. (%ID of ^{103}Ru/g tissue). Values are means, error bars represent standard deviations of n ≥ 7 per time point. Values of 30 mg/kg are included for comparison and slight variations from previously published ones can be ascribed to additionally obtained data.[1]

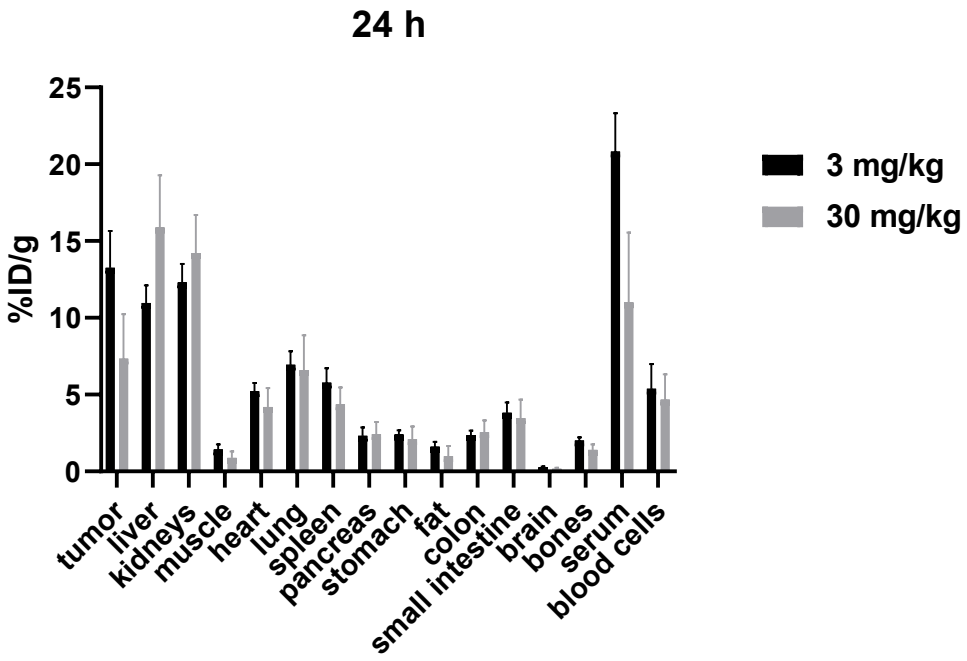

Figure S16: Tissue distribution of compound **3b** (c.a. [^{103}Ru]BOLD-100) 24 h p.i. (%ID of ^{103}Ru/g tissue). Values are means, error bars represent standard deviations of $n \geq 7$ per time point. Values of 30 mg/kg are included for comparison and slight variations from previously published ones can be ascribed to additionally obtained data.[1]

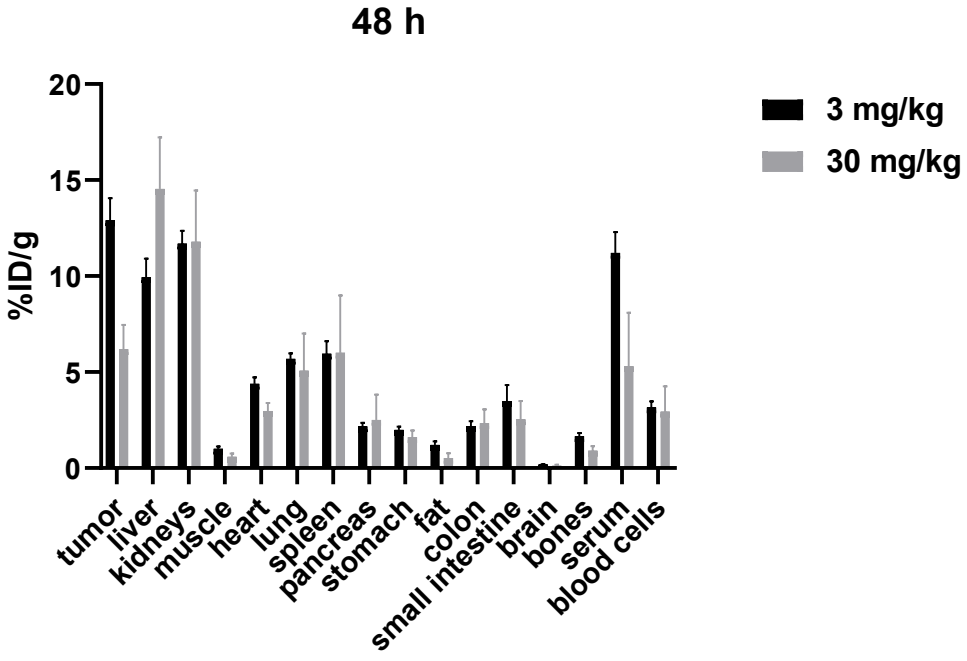

Figure S17: Tissue distribution of compound **3b** (c.a. [^{103}Ru]BOLD-100) 48 h p.i. (%ID of ^{103}Ru/g tissue). Values are means, error bars represent standard deviations of $n \geq 3$ per time point. Values of 30 mg/kg are included for comparison.[1]

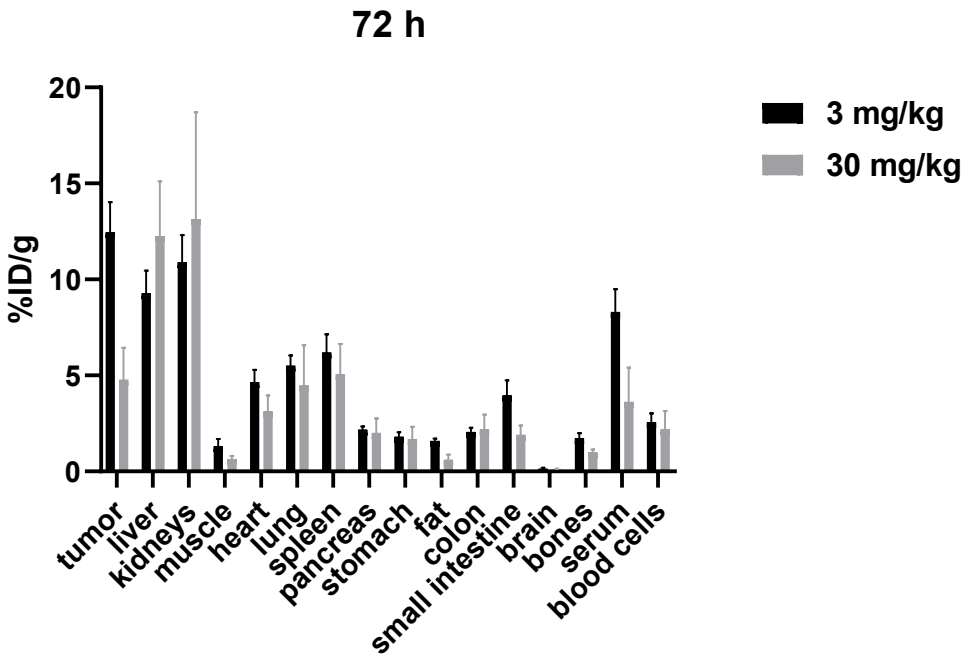

Figure S18: Tissue distribution of compound **3b** (c.a. [^{103}Ru]BOLD-100) 72 h p.i. (%ID of ^{103}Ru/g tissue). Values are means, error bars represent standard deviations of $n \geq 4$ per time point. Values of 30 mg/kg are included for comparison.[1]

Table S1: Tissue distribution data of **3b** (c.a. [^{103}Ru]BOLD-100) in CT26 allograft bearing Balb/c mice over 72 h with injected amounts of 3 mg/kg and 30 mg/kg Values are means ± standard deviations of n ≥ 3. %ID/g = percent injected dose of ^{103}Ru per gram tissue. Values of 30 mg/kg are included for comparison and slight variations from previously published ones can be ascribed to additionally obtained data.[1]

Tissue	%ID/g tissue of c.a. [^{103}Ru]BOLD-100 (3 mg/kg)			
	4 h	**24 h**	**48 h**	**72 h**
Tumor	7.00 ± 3.26	13.26 ± 2.40	12.90 ± 1.15	12.47 ± 1.56
Liver	11.83 ± 2.96	10.96 ± 1.15	9.95 ± 0.96	9.27 ± 1.19
Kidneys	10.28 ± 1.68	12.29 ± 1.23	11.70 ± 0.65	10.90 ± 1.40
Muscle	1.16 ± 0.38	1.43 ± 0.32	1.01 ± 0.12	1.32 ± 0.37
Heart	7.62 ± 0.66	5.22 ± 0.55	4.39 ± 0.34	4.63 ± 0.65
Lung	10.95 ± 2.04	6.94 ± 0.87	5.70 ± 0.28	5.51 ± 0.54
Spleen	5.91 ± 1.37	5.79 ± 0.92	5.96 ± 0.64	6.20 ± 0.94
Pancreas	2.71 ± 0.24	2.32 ± 0.54	2.18 ± 0.19	2.18 ± 0.16
Stomach	2.51 ± 0.42	2.40 ± 0.26	2.00 ± 0.16	1.82 ± 0.23
Fat	1.03 ± 0.35	1.62 ± 0.30	1.21 ± 0.18	1.59 ± 0.12
Colon	2.49 ± 0.47	2.36 ± 0.29	2.20 ± 0.24	2.06 ± 0.21
Small intestine	4.16 ± 0.84	3.83 ± 0.66	3.50 ± 0.83	3.96 ± 0.78
Brain	0.58 ± 0.17	0.28 ± 0.06	0.19 ± 0.03	0.15 ± 0.05
Bones	1.90 ± 0.31	2.01 ± 0.20	1.65 ± 0.17	1.73 ± 0.27
Serum	44.17 ± 6.90	20.82 ± 2.51	11.21 ± 1.08	8.30 ± 1.19
Blood cells	12.82 ± 3.81	5.39 ± 1.61	3.15 ± 0.32	2.58 ± 0.45

Tissue	%ID/g tissue of c.a. [^{103}Ru]BOLD-100 (30 mg/kg)			
	4 h	**24 h**	**48 h**	**72 h**
Tumor	4.64 ± 0.89	7.34 ± 2.90	6.19 ± 1.26	4.77 ± 1.68
Liver	18.94 ± 5.42	15.88 ± 3.39	14.54 ± 2.69	12.26 ± 2.85
Kidneys	14.45 ± 2.51	14.21 ± 2.49	11.79 ± 2.66	13.14 ± 5.56
Muscle	1.01 ± 0.17	0.89 ± 0.42	0.60 ± 0.16	0.65 ± 0.17
Heart	6.23 ± 0.97	4.19 ± 1.24	2.98 ± 0.41	3.12 ± 0.84
Lung	10.46 ± 2.81	6.59 ± 2.27	5.08 ± 1.93	4.47 ± 2.10
Spleen	4.60 ± 0.51	4.37 ± 1.09	6.01 ± 2.98	5.08 ± 1.56
Pancreas	3.56 ± 0.85	2.43 ± 0.79	2.50 ± 1.33	2.01 ± 0.75
Stomach	2.89 ± 0.61	2.09 ± 0.83	1.60 ± 0.36	1.69 ± 0.64
Fat	0.85 ± 0.40	1.00 ± 0.66	0.53 ± 0.25	0.62 ± 0.27
Colon	3.60 ± 0.76	2.54 ± 0.79	2.34 ± 0.72	2.19 ± 0.77
Small intestine	4.94 ± 1.05	3.47 ± 1.21	2.54 ± 0.95	1.92 ± 0.47
Brain	0.43 ± 0.14	0.17 ± 0.07	0.13 ± 0.05	0.09 ± 0.05
Bones	1.57 ± 0.40	1.42 ± 0.34	0.90 ± 0.24	1.00 ± 0.15
Serum	29.22 ± 4.56	11.01 ± 4.54	5.31 ± 2.78	3.63 ± 1.78
Blood cells	10.90 ± 1.94	4.67 ± 1.66	2.94 ± 1.32	2.19 ± 0.96

Table S2: Tumor-to-organ ratio of **3b** (c.a. [^{103}Ru]BOLD-100) for injected amounts of 3 mg/kg and 30 mg/kg Values are means ± standard deviations. Values of 30 mg/kg are included for comparison and slight variations from previously published ones can be ascribed to additionally obtained data.[1]

Organ	Tumor/organ ratio of c.a. [^{103}Ru]BOLD-100 (3 mg/kg)			
	4 h	**24 h**	**48 h**	**72 h**
Liver	0.6 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.1
Kidneys	0.9 ± 0.3	1.2 ± 0.2	1.1 ± 0.1	1.1 ± 0.0
Muscle	6.9 ± 3.3	11.5 ± 1.3	12.9 ± 2.2	9.8 ± 1.6
Heart	1.2 ± 0.4	2.7 ± 0.6	3.0 ± 0.4	2.7 ± 0.2
Lung	0.9 ± 0.3	1.9 ± 0.3	2.3 ± 0.2	2.3 ± 0.2
Spleen	1.3 ± 0.3	2.2 ± 0.3	2.2 ± 0.2	2.0 ± 0.1
Pancreas	3.3 ± 0.9	6.6 ± 2.2	5.9 ± 0.6	5.7 ± 0.4
Stomach	3.6 ± 0.9	5.9 ± 0.6	6.5 ± 0.1	6.9 ± 0.4
Fat	8.4 ± 5.2	9.2 ± 1.5	10.7 ± 0.8	7.8 ± 0.7
Colon	3.5 ± 1.1	6.1 ± 1.1	5.9 ± 0.8	6.1 ± 0.6
Small intestine	2.2 ± 0.9	3.7 ± 1.0	3.8 ± 0.7	3.2 ± 0.5
Brain	17.0 ± 9.8	55.1 ± 16.6	70.1 ± 6.9	90.3 ± 21.0
Bones	4.9 ± 2.1	6.9 ± 1.1	7.9 ± 0.8	7.2 ± 0.4
Serum	0.2 ± 0.1	0.7 ± 0.1	1.2 ± 0.1	1.5 ± 0.1
Blood cells	0.6 ± 0.4	2.9 ± 1.2	4.1 ± 0.6	4.9 ± 0.6

Organ	Tumor/organ ratio of c.a. [^{103}Ru]BOLD-100 (30 mg/kg)			
	4 h	**24 h**	**48 h**	**72 h**
Liver	0.2 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Kidneys	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.2
Muscle	4.4 ± 1.4	11.4 ± 1.8	10.6 ± 1.4	7.6 ± 2.4
Heart	0.8 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	1.5 ± 0.4
Lung	0.5 ± 0.2	1.3 ± 0.5	1.3 ± 0.3	1.2 ± 0.4
Spleen	1.0 ± 0.2	1.7 ± 0.3	1.1 ± 0.3	1.0 ± 0.3
Pancreas	1.2 ± 0.3	3.7 ± 0.7	2.7 ± 0.7	2.6 ± 0.9
Stomach	1.7 ± 0.7	4.8 ± 1.2	3.9 ± 0.3	3.0 ± 1.0
Fat	5.1 ± 2.1	10.3 ± 2.1	13.3 ± 5.0	8.6 ± 3.5
Colon	1.2 ± 0.6	3.4 ± 0.7	2.8 ± 0.8	2.3 ± 0.8
Small intestine	0.9 ± 0.3	2.8 ± 1.1	2.6 ± 0.8	2.6 ± 0.9
Brain	10.7 ± 3.3	51.9 ± 4.4	53.3 ± 14.3	54.3 ± 15.1
Bones	3.4 ± 0.5	5.4 ± 0.6	7.0 ± 0.8	4.9 ± 1.7
Serum	0.2 ± 0.0	0.8 ± 0.1	1.3 ± 0.4	1.4 ± 0.3
Blood cells	0.4 ± 0.1	2.0 ± 0.6	2.3 ± 0.6	2.3 ± 0.8

5. References

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