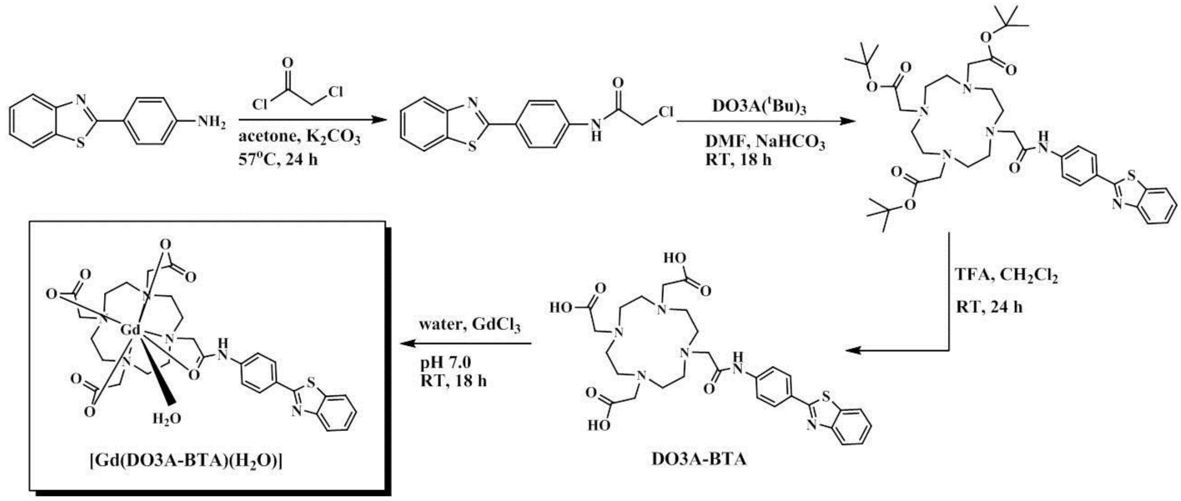
Supporting information

Gadolinium complex of DO3A-benzothiazole aniline (BTA) conjugate as a theranostic agent



Scheme S1

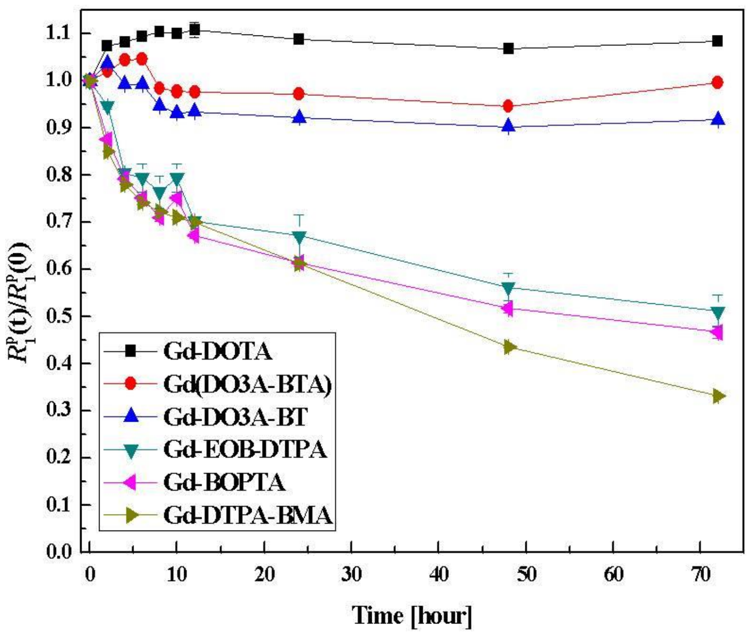


Figure S1. Evolution of longitudinal relaxation rates $R_1^{p}(t)/R_1^{p}(0)$ as a function of time for various MRI CAs ($[Gd]_0$ and $[ZnCl_2]_0$ = 2.5 mM in PBS (pH 7.4) at 128 MHz and 293 K).

Determination of Binding constants

The binding constants of various CAs with HSA was measured according to the ligerature method.[1] The non-linear increase of the proton paramagnetic relaxation rate measured at 64 MHz on solutions containing 0.64 mM HSA and various concentrations of CAs (0-6 mM). The proton data obtained in HSA solution were fitted using Equation S1, where K_a is the binding constant characterizing the interaction with HSA, p^0 is the HSA concentration, s^0 is the concentration of the paramagnetic complex, N is the number of independent interaction sites (N was set to 1), and r_1^c and r_1^f are the relaxivities of the complex HSA-constant agent and of the free constant agent, respectively.

$$R_1^{p^{Obs}} = 1000 \times \left\{ \left(r_1^f \times s^0 \right) + \frac{1}{2} \left(r_1^c - r_1^f \right) \left(\left(N \times p^0 \right) + s^0 + K_a^{-1} - \sqrt{ \left(\left(N \times p^0 \right) + s^0 + K_a^{-1} \right)^2 - 4 \times N \times s^0 \times p^0 } \right) \right\}$$

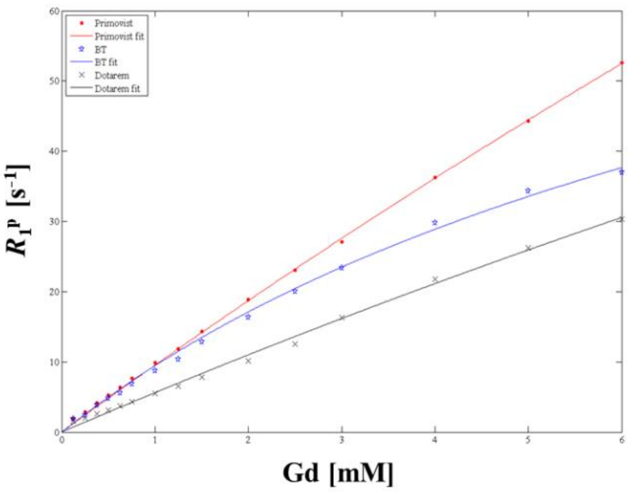


Figure S2. Proton longitudinal paramagnetic relaxation rates of [Gd(DO3A-BTA)(H_2O)], Gd-EOB-DTPA and Gd-DOTA as a function of [Gd] in PBS (pH 7.4) solutions of HSA (0.67 mM) at 64 MHz and 293 K.

Table S1. Binding constants of variable CAs with HSA

	K_a[M^{-1}]
Gd(DO3A-BTA)	160
Gd-DOTA	21
Gd-EOB-DTPA	27

Table S2. Relaxivity data of Gd-DOTA and Gd(DO3A-BTA) with HSA 0.67 mM in PBS (64MHz, 293K)

	r_1(mM^{-1}s^{-1})	r_2(mM^{-1}s^{-1})
Gd(DO3A-BTA)	7.83 ± 0.05	11.34 ± 0.92
Gd-DOTA	4.33 ± 0.03	4.08 ± 0.27
Gd-EOB-DTPA	8.52 ±	11.18 ±

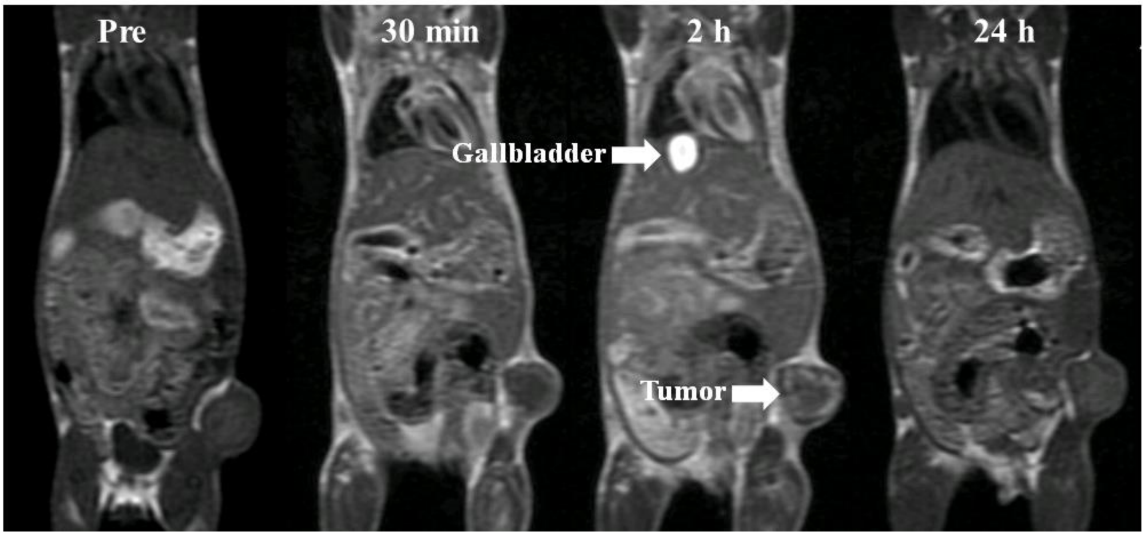


Figure S3. *In vivo* T_1 weighted MR coronal images of mice obtained by tail vein injection with Gd(DO3A-BTA) (0.1 mmol/kg).

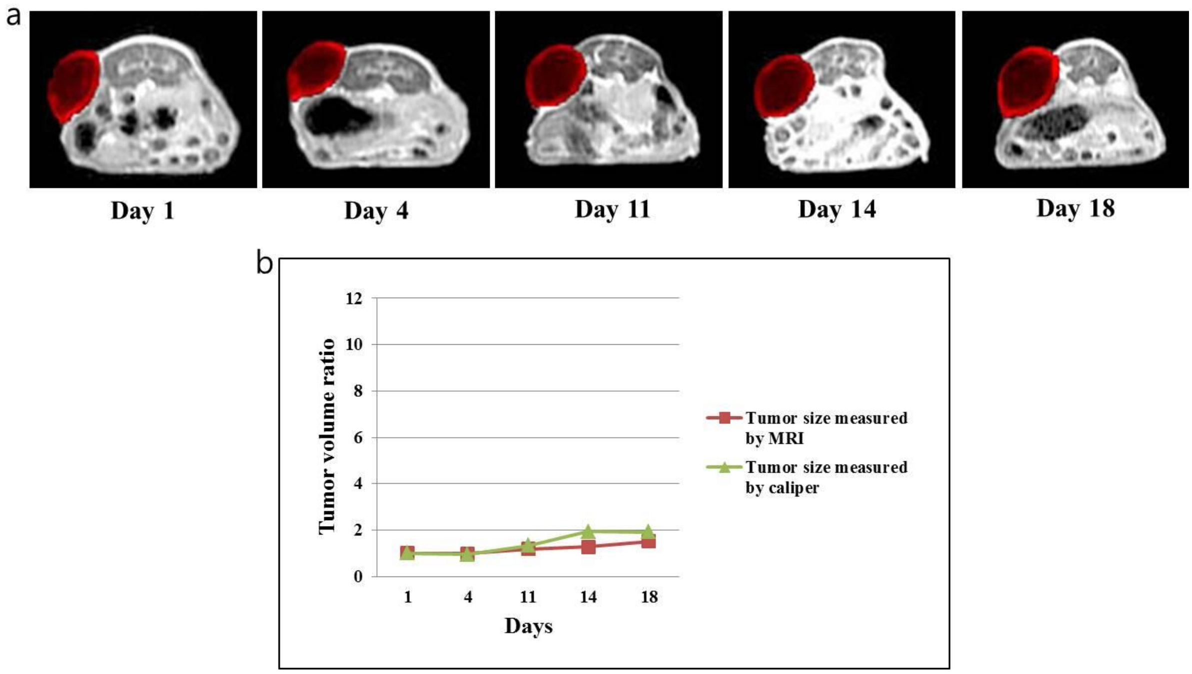


Figure S4. (a) MR monitoring of tumor size (days 1 to 18). (b) Tumor volume ratios measured by MRI (red square) and a caliper (green triangle). Mice were injected each day with a new dose of Gd(DO3A-BT) at 0.1 mmol/kg.

Table S3. Gd concentration in tumors as a function of time

	1 h	6 h	24 h
Gd concentration in tumors (μg Gd/g tumor)	40.41	221.02	55.78

Table S4. Amounts of Gd in cell fractions (μg/cell fractions) in 2 × 10^5 cells

	Nucleus	Cytosol	Membrane
SK-HEP-1	2.55	5.40	0.93
MCF-7	9.66	18.35	1.19
MDA-MB-231	2.21	11.98	0.74

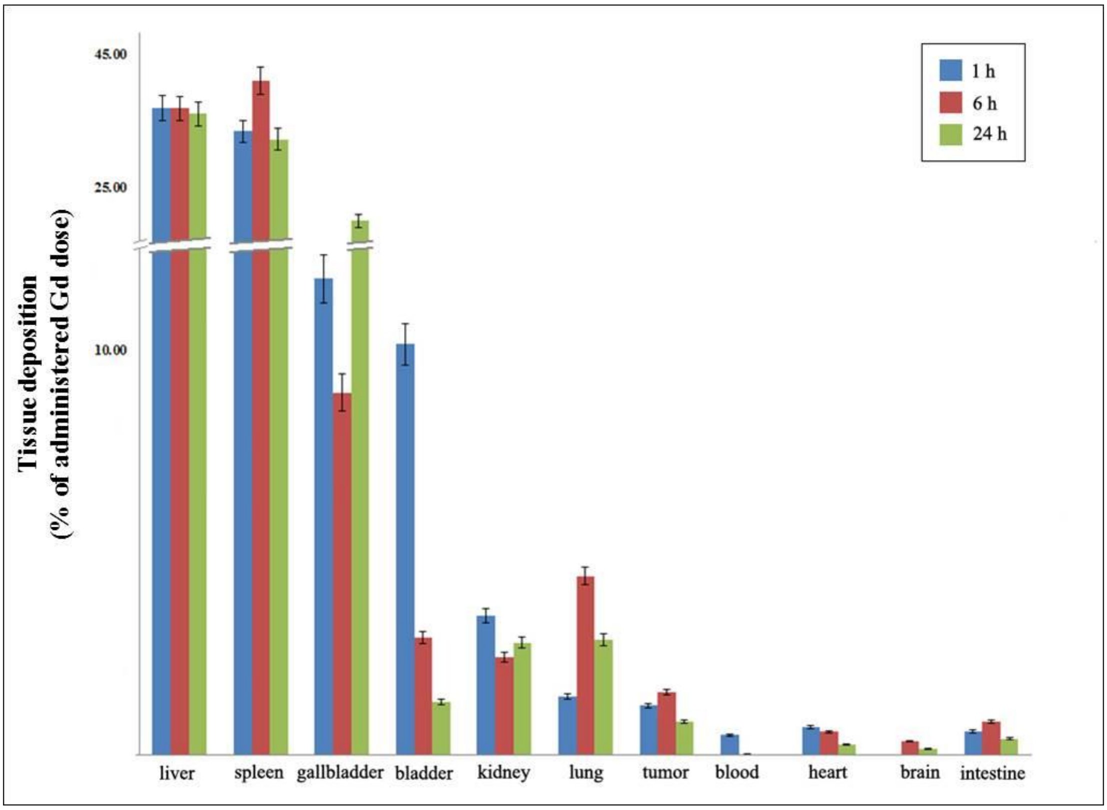


Figure S5. Biodistribution of Gd(DO3A-BTA) (0.1 mmol Gd/kg body weight) in balb/c nude mice bearing MDA-MB-231 tumor. Groups of mice (*n* = 5) were sacrificed at 1, 6 and 24 h.

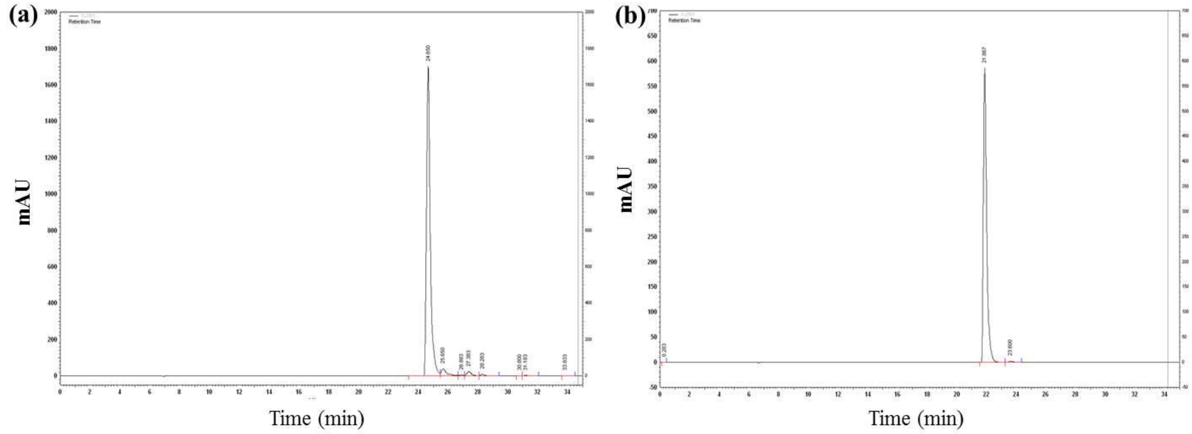


Figure S6. HPLC spectra of (a) DO3A-BT and (b) Gd(DO3A-BT)

References

(1) Muller, R. N.; Raduchel, B.; Laurent, S.; Platzek, J.; Pierart, C.; Mareski, P.; Vander Elst, L. Physicochemical characterization of MS-325, a new gadolinium complex, by multinuclear relaxometry. *Eur. J. Inorg. Chem.* **1999**, 1949-1955.