DO3A-benzothiazole conjugates for use as Gd-based theragnostic agents

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Introduction

Magnetic resonance imaging (MRI) has proved to be a powerful non-invasive technique. The prominent advantage of MRI is a high spatial resolution and the ability to distinguish soft tissues. The contrast of the resulting image can be enhanced by injection of paramagnetic or superparamagnetic agents. Paramagnetic gadolinium (III) complexes, for example, are most widely used in MRI as water relaxation agents to improve image contrast. In addition, the therapeutic potential of gadolinium has also been noted and has drawn a great deal of investigation for some time. Investigations have so far been focused on such areas as Gd-based neutron capture therapy (Gd-NCT) and chemotherapy, and yet no clinical applications have so far been reported. In this regard we have designed and synthesized a new family of bifunctional chelates, DO3A-benzothiazole conjugates for gadolinium complexes (Gd-DO3A-BT) as potential theragnostic agents. Benzothiazoles are known to possess potent antitumor properties in select breast, ovarian and renal cancer cell lines. In this studies are presented their synthesis, MR properties, tumor-targeting and anti-tumor activities on cultured breast cancer cell line MCF-7.

Material and Methods

All reagents were purchased from commercial sources and used as received. Characterization of new compounds have been performed by analytical and various spectroscopic techniques (MRI, MS). T_1 measurements were carried out using an inversion recovery method with variable inversion time (TI) at 1.5 T (64 MHz). T_1 relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each TI value. For *in vivo* MRI, the mice were anesthetized by 1.5% isoflurane in oxygen. MR images of anaesthetized mice (n=4) were obtained pre- and post- Gd-DO3A-BT (0.1 mmol Gd/kg) injection by tail vein with a 1.5 Tesla (T) MR unit (GE Healthcare, Milwaukee, WI, USA) with home-made small animal RF coil. The coil was of the receiver type, and the inner diameter of the coil was 50 mm. The imaging parameters for SE (Spin echo) were as follows: repetition time (TR) = 300 ms; echo time (TE) = 12 ms; 7 mm field of view (FOV); 192×128 matrix size; 1.2 mm slice thickness; number of acquisition (NEX) = 8. Images were obtained during 300 min after injection. MCF-7 breast cancer cells were plated on 35 mm corning dishes and cultured for 24 h. The medium was removed, Gd-DO3A-BT in DMEM serum-depleted media (containing 0.1 % DMSO as a co-solvent) added, and incubation continued for 18 h. The stained cells were washed once with PBS buffer (pH 7.4). The cells were harvested with a solution of trypsin-EDTA (GIBCO, 0.25 % trypsin, 1 mM EDTA·Na) after which DMEM was added. The cells were transferred to a 15 mL centrifuge tube to be centrifuged at 1000 rpm, 4℃ for 3 min. After removing the supernatant, DMEM was added and the cells transferred to a micro test tube to be centrifuged at 6200 rpm for 3 min. The tube was then filled with DMEM for MR measurement using an 8-channel knee coil. T_1-weighted MRI parameters are as follows: FSE (Fast spin echo) sequence, TR = 500 ms; TE = 11 ms; echo train length (ETL) = 4; 7 mm FOV; 192×128 matrix size; 0.8 mm slice thickness; NEX = 15.

Results and Discussion

Scheme 1 shows the synthesis of Gd-DO3A-BT. Its R_1 relaxivity is 3.84 mM^{-1}sec^{-1}, almost the same values as that of structurally related Dotarem® (R_1=3.69 mM^{-1}sec^{-1}) (Table 1). Figure 1 shows *in vivo* coronal images of mice obtained by tail vein injection. The pattern of enhancement compares well with those of liver-specific MRI CAs such as Primovist® and Multihance® in that heart and abdominal aorta are enhanced specifically and enhancement lasts as long as 1 h. More characteristically, it is to be noted that excretion is made via bile duct, confirming hepatobiliary uptake. Figure 2 shows T_1-weighted MR images of MCF-7 cells incubated with Gd-DO3A-BT (100 μM) for 18 h, revealing the tumor-specific nature of the present series. In addition, Table 2 shows antitumor activity of the present system represented as GI_{50} (the ability to inhibit cancer cell growth) and TGI (the concentration of Gd-complex needed to cause total growth inhibition) toward MCF-7 cells after exposures for 18 h.

Conclusions

We have successfully synthesized Gd-DO3A-BT as a new family of multifunctional MRI/optical imaging probes with concomitant antitumor activity as well as tumor-specificity.

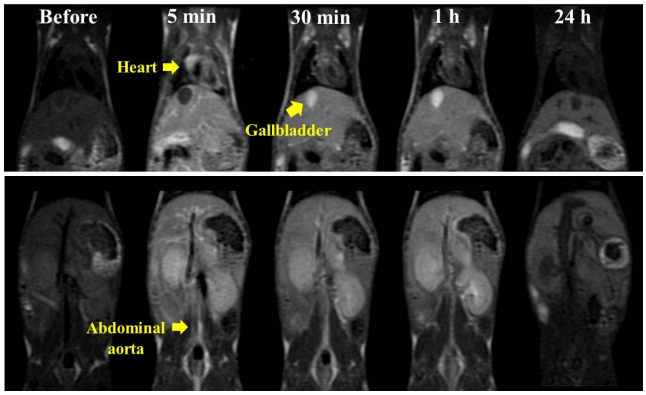
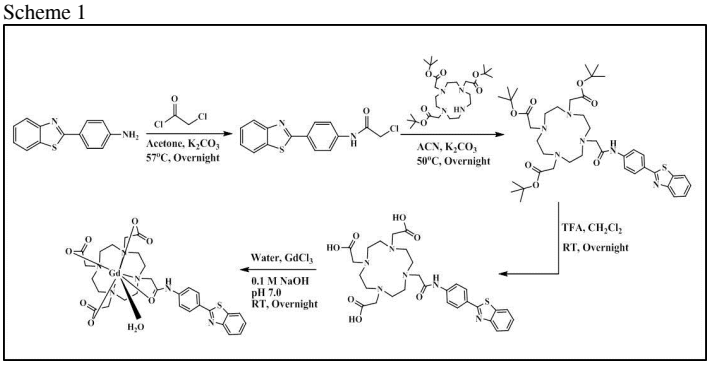


Figure 1. *In vivo* T_1 weighted MR coronal images of mice obtained by tail vein injection with Gd-DO3A-BT (0.1 mmol/kg).

Table 1. Relaxivity data of contrast agents in PBS (pH 7.4)

	R_1 [mM^{-1}s^{-1}]	R_2 [mM^{-1}s^{-1}]
Gd-DO3A-BT	3.84 ± 0.19	4.07 ± 0.19
Dotarem®	3.69 ± 0.12	3.98 ± 0.19
Primovist®	6.64 ± 0.20	7.00 ± 0.21
Multihance®	5.13 ± 0.16	5.42 ± 0.22

Scheme 1



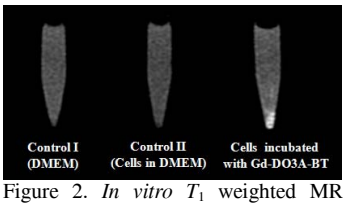


Figure 2. *In vitro* T_1 weighted MR images of MCF-7 cells incubated with Gd-DO3A-BT (100 μM) for 18 h.

Table 2. *In vitro* activity of Gd-DO3A-BT in MCF-7 cells

	Gd-DO3A-BT [μM]
GI_{50}	216.805
TGI	254.860