Gd-Complexes of DO3A-benzothiazole Conjugate for MRI Theragnostic Agents

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Introduction

Benzothiazoles are fused bicyclic systems possessing diverse biological properties such as anti-inflammatory, antimicrobial, and anticancer effects. As such, a great deal of research activities has been carried out in the past two decades in an effort to develop various benzothiazole derivatives with high antitumor activity. We have recently reported the synthesis of DO3A-(*p*-aniline benzothiazole) conjugate and its Gd complex of the type [Gd(**L**^{1})(H_2O)] (Gd**L**^{1}) and demonstrated that Gd**L**^{1} is truly a single molecule theranostic agent. Motivated by such intriguing properties of benzothiazoles and by our recent observations cited above in connection with Gd**L**^{1}, we have decided to pursue further studies on the structure activity relationship for the theranostic application of benzothiazole derivatives. Thus, we would like to pursue further the therapeutic propensity of the closely related Gd(III) complex of the type [Gd(DO3A-benzothiazole aniline)(H_2O)] (**2**) in the hope that it may serve as a single molecule theranostic agent. Herein, we report the design and synthesis of **2a** and **2b** for use as a theranostic agent.

Material and Methods

All reagents were purchased from commercial sources and used as received. DO3A(^{t}Bu)$_3$, benzothiazole, were synthesized according to literature method. FAB-mass spectra were obtained by using a JMS-700 model (Jeol, Japan) mass spectrophotometer. 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.

MCF-7, MDA-MB-231, HeLa, Caki-2, and SK-HEP-1 cells were plated 2×10^5 in 35 mm corning dishes. The medium was removed, **2a** and **2b** in DMEM serum-depleted media (containing 0.1 % DMSO as a co-solvent) added, and incubation continued for 24 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; 12 mm FOV; 192×128 matrix size; 1.5 mm slice thickness; NEX = 15.

Results and Discussion

A low molecular weight cyclic Gd(III) complex GdL (scheme), was synthesized and characterized by spectroscopic techniques. The relaxivities of r_1 and r_2 of **2a** and **2b** in PBS and HP-β-CD solutions are collected in Table 1. In PBS solutions, the complexes show significantly lower relaxivities. Yet, it is known that a significant increase in relaxivity is established when "host-guest' inclusion complexes are formed between the hydrophobic β-cyclodextrin cavity of hydroxylpropyl- β-cyclodextrin (HP- β-CD) and Gd(III) complexes. Figure 1 shows antitumor characteristics against three cell lines such as SK-HEP-1, MDA-MB-231, and MCF-7. Figure 2 shows T_1-weighted MR images of SK-HEP-1, MCF-7, HeLa, Caki-2, and MDA-MB-231 cells incubated with **2a** and **2b** (100 μM) for 24 h, revealing the tumor-specific nature of the present series.

Conclusions

We have synthesized a bifunctional chelate DO3A-BTA and its gadolinium complex of the type [Gd-(DO3A-BTA)(H_2O)] (**2**) to put into a new entry as a single theranostic agent. The Gd (III) complexes are intracellular as well as tumor-specific, as confirmed by MR images of cytosols and nuclei of SK-HEP-1, MCF-7, HeLa, Caki-2, and MDA-MB-231 cells. The antiproliferative activities of **2a** and **2b** were demonstrated by GI_{50} and TGI values obtained from the CCK-8 assays performed on the above cell lines.

Scheme

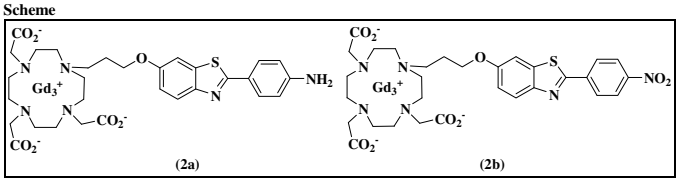

Table 1. Relaxivity Data of **2a** and **2b**(64 MHz, 293 K)[a]

	r_1 ($mM^{-1}s^{-1}$)		r_2 ($mM^{-1}s^{-1}$)	
	PBS[b]	β-CD[c]	PBS[b]	β-CD[c]
2a	1.1	7.4	2.2	10.3
2b	1.4	5.1	2.5	7.8

[a]Concentrations are given in [Gd]. [b]PBS: pH 7.4. [c] β-CD = 50 mM in PBS.

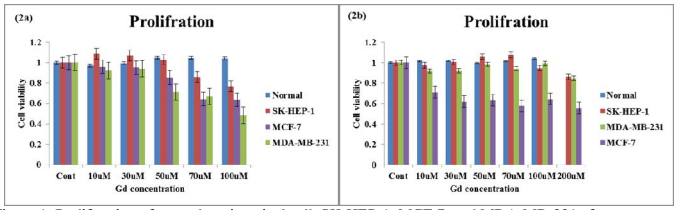

Figure 1. Proliferation of normal conjunctival cell, SK-HEP-1, MCF-7, and MDA-MB-231 after treatment with various concentrations of **2a** and **2b**.

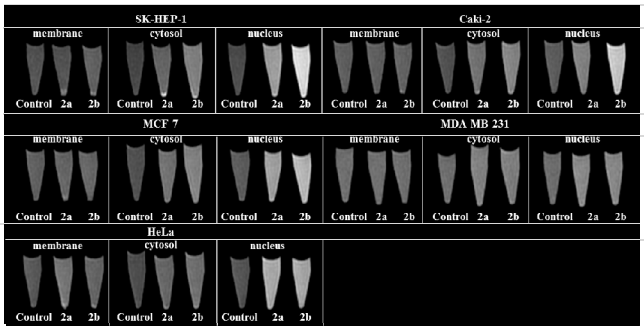

Figure 2. T_1-weighted MR images of SK-HEP-1, MCF-7, HeLa, Caki-2, and MDA-MB-231 cell fractions incubated with **2a** and **2b**.

Table 2. In Vitro Activities of **2a**, **2b** and GdL^{1} in Cancer Cell Lines

		MCF-7	MDA-MB-231
2a	GI_{50} [μM]	88.99	99.66
	TGI [μM]	50.96	197.98
2b	GI_{50} [μM]	134.36	775.30
	TGI [μM]	879.20	3.54.79
GdL^{1}	GI_{50} [μM]	258.48	237.47
	TGI [μM]	555.89	637.82