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ORIGINAL ARTICLE

Synthesis and in vitro evaluation of thiododecaborated α, α-cycloalkylamino acids for the treatment of malignant brain tumors by boron neutron capture therapy

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Abstract Boron-neutron capture therapy (BNCT) is an attractive technique for cancer treatment. As such, α, α-cycloalkyl amino acids containing thiododecaborate ($[B_{12}H_{11}]^{2-}$-S-) units were designed and synthesized as novel boron delivery agents for BNCT. In the present study, new thiododecaborate α, α-cycloalkyl amino acids were synthesized, and biological evaluation of the boron compounds as boron carrier for BNCT was carried out.

Keywords Boron neutron capture therapy · Boron cluster · Boronated amino acid · Immunocytochemistry

Abbreviations

BNCT	Boron neutron capture therapy
L-10BPA	p-(10B)borono-L-phenylalanine
10BSH	Mercapto-undeca-hydro-closo-dodeca(10B) borate
ACBC	1-Aminocyclo-butane-1-carboxylic acid
PET	Positron emission tomography
ICP-OES	Inductively coupled plasma optical emission spectrometry
LAT	L-type amino acid transporter
EAATs	Excitatory amino acid transporters
ODS	Octadecylsilyl
WST	Water soluble tetrazolium
DMEM	Dulbecco’s Modified Eagle’s Medium
FBS	Fetal bovine serum
EDTA	Ethylenediaminetetraacetic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
PBS	Phosphate-buffered saline
NMR	Nuclear magnetic resonance
ESI–MS	Electrospray ionization mass spectrometry

Introduction

Boron neutron capture therapy (BNCT)is based on the nuclear fission reaction of ^{10}B with thermal/epithermal neutrons yielding high linear energy transfer (LET) α particles (^{4}He) and recoiling ^{7}Li nuclei in tumor cells, and has attracted attention as a potential treatment for malignant brain tumors (Barth et al. 2005). For a boron delivery agent to be successful in BNCT, the following criteria must be met: (1) high tumor-targeting selectivity (T/N >3); (2) low systemic toxicity; and (3) tumor concentrations of ~20 µg ^{10}B/g tumor tissues. Although many kinds of boron-containing compounds such as amino acids, nucleic acids and porphyrins have been reported as boron delivery agents (boron carriers) for BCNT (Soloway et al. 1998), only two compounds are used for the treatment of brain tumorsvia BNCT: p-borono-L-phenylalanine (BPA) and mercapto-closo-undecahydrododecaborate (BSH).

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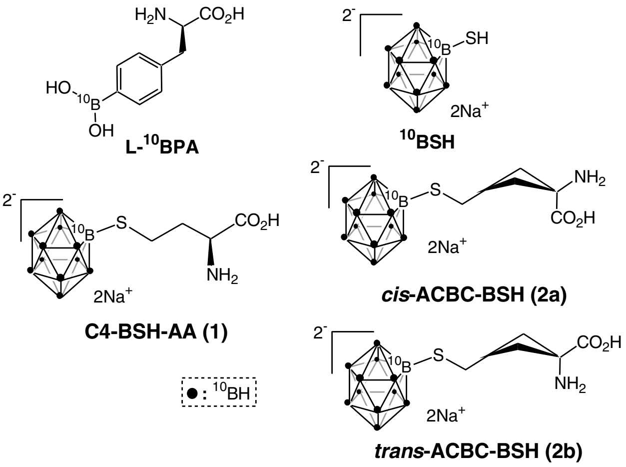


Fig. 1 Boron-containing compounds

As part of our studies on novel boron delivery agents for BNCT, we designed and synthesized thiododecaborate ($[B_{12}H_{11}]^{2-}$-S-) unit containing L-amino acids (**1**), which constitute a new class of tumor-seeking and water-soluble amino acids (Fig. 1). The in vitro evaluation of the cytotoxicity, killing effects by neutron irradiation, and micro distribution analysis performed previously by our group suggested that **1** might be a potential delivery agent for BNCT (Hattori et al. 2012).

On the other hand, α, α-cycloalkyl amino acids radiolabeled with ^{18}F, such as 1-aminocyclobutane-1-carboxylic acid (ACBC) (Soloshonok and Sorochinsky 2010), are useful positron emission tomography (PET) probes for brain cancer diagnosis, since unusual amino acids with small α, α-alkyl rings are selectively incorporated via L-type amino acid transporter (LAT)and are temporarily retained in cancer cells (Rice et al. 2011). These results led us to develop cycloalkyl amino acidsmodified with thiododecaborate units in their side chainsfor BNCT.

Here, we described the design and synthesis of two thiododecaborated α, α-cycloalkyl amino acids, namely *cis* and *trans* ACBC-BSH (**2a**, **2b**) with no asymmetric carbon atoms, by an extension of our previously reported method (Kusaka et al. 2011). The biological evaluation of ACBC-BSH is also described.

Material and method

General

L-^{10}BPA and ^{10}BSH were provided by Stella Pharma Corporation (Osaka, Japan). The synthesis of BSH-amino acid **1** was carried out according to a previously described methodby Kusaka et al. (2011). Cyclobutyl-*cis*-hydantoin benzyl ether **3a** and **3b** were prepared according to the literature method by Rammeloo and Stevens (2002). *S*-cyanoethylthio-dodecaborate (**5**) was prepared according to the previously described method by (Hattori et al. 2010). Flash ODS column chromatography was carried out using IsoleraSpektra (Biotage Sweden AB, Uppsala, Sweden) with a SNAP KP-C18-HS Cartridge (Biotage Sweden AB, Uppsala, Sweden). ^{1}H NMR and ^{13}C NMR spectra were measured on a JMTC-400/54/SS (400 MHz, JEOL Ltd., Tokyo, Japan). ESI–MS measurements were performed on a XevoQTof (Waters Corporation, Massachusetts, US). The purity of **2a** and **2b** was analyzed by capillary electrophoresis, and the analysis of **2a** and **2b** revealed the purity to be >95 %.Capillary electrophoresis analysis was carried out using Photal CAPI-3300 (Otsuka Electronics Co. Ltd., Osaka, Japan) equipped with a fused silica capillary of 75 µm i.d. and a total length of 50 cm (effective length of 38 cm). A new capillary was conditioned with 0.1 M NaOH for 30 min followed by 15 min distilled water and 15 min electrolyte buffer (5 mM sodium phosphate buffer, pH 9.0). The applied potential was 20 kV and detection at 200 nm. Inductively coupled plasma optical emission spectrometry (ICP-OES) was obtained on a VISTA-MPX ICP-OES Spectrometer (Seiko Instruments, Chiba, Japan). Fluor® 488Goat Anti Mouse IgG was purchased from Life Technologies (Carlsbad, CA, US). Permafluor was purchased from Immunotech (Marseille, France).

*Cis 5-(3-iodometylcyclobutane)hydantoine (**4a**)*

To a solution of hydantoine **3a** (807 mg, 3.1 mmol) in MeOH (200 ml) was added 20 % $Pd(OH)_2/C$ (100 mg). The resulting suspension was placed under a hydrogenator, and hydrogenated at 3 atom for 12 h. The $Pd(OH)_2/C$ catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residual solid was suspended in CH_2Cl_2 (150 mL), and PPh_3 (1.63 g, 6.2 mmol), imidazole (422 mg, 6.2 mmol) and I_2 (1.57 g, 6.2 mmol) was added to the suspension at 0 °C. After stirring for 48 h at change to room temperature, the suspension was filtered, and the resulting solid was washed with CH_2Cl_2. The solid was recrystallized from MeOH to give **4a** (625 mg, 72 %) as colorless crystals. ^{1}H-NMR(DMSO-d$_6$): $\delta = 10.6$ (1H, *s*), 8.18 (1H, *s*), 3.29 (2H, J = 7.6 Hz, *d*), 2.41–2.54 (4H, *m*), 1.83–1.88 (2H, *m*).^{13}C-NMR(D_2O): $\delta = 14.06$, 29.54,39.05, 55.28, 155.94, 178.40.MS: (pos. ESI, *m/z*) 280.0875 [M+H].

*Trans 5-(3-iodometylcyclobutane)hydantoine (**4b**)*

The title compound was prepared following the same procedure described above for **4a**, starting from benzyloxycyclobutane **3b** (598 mg, 2.3 mmol). Compound **4b** (465 mg, 63 %) was obtained as colorless crystals.

^{1}H-NMR(DMSO-d_6): δ = 10.6 (1H, *s*), 8.04 (1H, *s*), 3.48 (2H, J = 8.4 Hz), 2.61–2.69 (1H, *m*), 2.25–2.30 (2H, *m*), 2.08–2.13 (2H, *m*). ^{13}C-NMR(D_2O): δ = 13.20, 30.13,37.73, 57.26, 156.14, 177.57.MS: (pos. ESI, m/z) 280.0923 [M+H].

Cis S-((5,7-diazaspiro[3.4]octane-6,8-dione-1-yl)-methyl)thioundecahydro-closo-dodecaborate (2-) ditetramethylammonium Salt (**6a**).

S-cyanoethylthio-dodecaborate (**5**) (354 mg, 0.97 mmol) was added to a solution of compound **4a** (325 mg, 1.2 mmol) in MeCN (40 ml), and the mixture was refluxed for 24 h. The mixture was concentrated in vacuo and the resulting residue was subsequently suspended in acetone (100 mL). The suspension was filtered and 25 w % NMe_4OH (354 mg, 0.97 mmol) was added to the filtered solution. The precipitate was recrystallized from H_2O to give **6a** (410 mg, 91 %) as colorless crystals. ^{1}H-NMR(D_2O): δ = 3.00 (24H, *s*), 2.40–2.49 (4H, *m*), 2.28–2.38 (1H, *m*), 1.94 (2H, J = 10.7, *t*), 0.65–1.60 (11H, *m*,). ^{13}C-NMR(D_2O): δ = 28.38, 37.71,39.41, 55.89, 59.73, 159.79, 179.85.MS: (neg. ESI, *m/z*) 158.1452 $[A]^{2-}$.

Trans S-((5,7-diazaspiro[3.4]octane-6,8-dione-1-yl)-methyl)thioundecahydro-closo-dodecaborate (2-) ditetramethylammonium Salt (**6b**).

The title compound was prepared following the same procedure described above for **6a**, starting from dodecaborate **5** (207 mg, 0.56 mmol) and iodocyclobutane **4b** (250 mg, 0.68 mmol). Compound **6b** (292 mg, 92 %) was obtained as colorless crystals. ^{1}H-NMR(D_2O): δ = 3.00 (24H, *s*), 2.55–2.62 (4H, *m*), 2.20–2.30 (1H, *m*), 2.08–2.14 (2H, *m*), 0.65–1.60 (11H, *m*).^{13}C-NMR(D_2O): δ = 29.46, 37.15,38.06, 55.87, 61.11, 160.78, 182.30. MS: (neg. ESI, *m/z*) 158.1452 $[A]^{2-}$.

Cis S-((3-amino-3-hydroxycarbonylcyclobutyl)-methyl)thioundecahydro-closo-dodecaborate (2-) disodium Salt (**2a**).

A solution of precursor **5a** (300 mg, 0.65 mmol) in 0.5 N NaOH (30 mL) was stirred for 18 h at 100 °C, and the mixture was acidified with Amberlite IR-120(H^+). After filtration, the solution was neutralized with 1 N NaOH. The mixture was concentrated in vacuo and the residual oil was purified by flash ODS column chromatography to give **2a** (210 mg, 96 %) as a colorless oil. ^{1}H-NMR(D_2O): δ = 2.45–2.51 (6H, *m*), 1.68 (1H, *m*), 0.65–1.60 (11H, br). ^{13}C-NMR(D_2O): δ = 29.88, 36.22,37.95, 55.33, 177.86. MS: (neg. ESI, *m/z*) 145.6298 $[A]^{2-}$.

Trans S-((3-amino-3-hydroxycarbonyl cyclobutyl)-methyl)thioundecahydro-closo-dodecaborate (2-) disodium Salt (**2b**).

The title compound was prepared following the same procedure described for **2a**, starting from hydantoine **5b** (206 mg, 0.44 mmol). Compound **2b** (134 mg, 91 %) was obtained as a colorless oil. ^{1}H-NMR(D_2O): δ = 2.61–2.68 (1H, *m*) 2.51 (2H, J = 7.6 Hz, *d*), 2.17–2.20 (4H, *m*), 0.65–1.60 (11H, br).^{13}C-NMR(D_2O): δ = 30.86, 37.32,38.92, 56.73, 181.12.MS: (neg. ESI, m/z) 145.6298 $[A]^{2-}$.

Cells and cell culture

U251 (human astrocytoma), A172 (human glioblastoma), U81 (human glioblastoma), and C6 (rat glioma) cell lines used in cytotoxicity analyses, boron incorporation, tumor cell killing studies, and immunostaining, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 2 mM glutamine, and 24 mM sodium hydrogen carbonate at 37 °C in a 5 % CO_2 atmosphere. Cells in the mono-layer were harvested with 0.25 % trypsin/0.02 % ethylenediamine-tetraacetic acid (EDTA) in Ca^{2+}-free phosphate-buffered saline (PBS). Matrigel (growth factor reduced type) was purchased from BD Science (SanJose, CA, US).

Water soluble tetrazolium (WST)-8 assay

The cytotoxicity of each amino acid was assayed using the WST-8 assay with a cell counting kit (Wako Pure Chemicals, Osaka, Japan) following a modified version of the manufacturer provided protocols. Briefly, a 96-well microplate was seeded with 5×10^3 cells suspended in 100 μL of cell growth media, and allowed to settle for 16 h at 37 °C. The medium was removed by aspiration, and 100 μL of growth medium (DMEM with 10 % FBS containing each compound at different concentrations) was added to each well. After incubation for 48 h at 37 °C, the medium was removed by aspiration, and 100 μL of the WST-8 solution [5 mM WST-8, 20 mM HEPES (pH 7.4) and 0.2 mM 1-methoxy PMS dissolved in PBS] was added to each well, followed by incubation for 4 h at 37 °C.The absorbance at 450 nm (reference: 655 nm) was read on a microwell plate reader. Well without cells was used as the blank. Relative cell survival was calculated as follows;

Relative cell survival (%) = (Absorbance value of compound treated well/Absorbance value of untreated well) × 100.

The relative cell survival was plotted against the compound concentration, and IC_{50} values for each compound were graphically determined.

Boron incorporation into cultured tumor cells

Cultures were inoculated with 1.0×10^6 cells/dish, and cells were grown for 24 h in DMEM. The medium was replaced with an equivalent amount of medium containing each boron-containing amino acid (the final concentration was 2.0 mM in each case). The cells were cultured for 24 h and then the medium was removed by aspiration. The cells were washed thrice with PBS, harvested by trypsinization, and then counted. Each sample, containing 1 × 10^6 cells, was added to a mixture of $HClO_4$ (60 %, 0.3 mL) and H_2O_2 (31 %, 0.6 mL), and then heated at 75°Cfor 1 h. The mixture was filtered through a membrane filter (Millipore, 0.45 μm), and the boron concentration was measured by ICP-OES.

Immunostaining of C6 cells

Immunostaining was performed to determine the incorporation of boron-containing amino acids into C6 cells, according to a previously described method by Ogawa et al. (2005) with some modifications. Glass coverslips coated with Matrigel (3.5 μg/cm^2 protein) were seeded with C6 cells (0.8 × 10^5 cells suspended in 3 mL of DMEM), and allowed to settle for 1 h at 37 °C. The medium was replaced with an equivalent amount of medium containing compound **1a** (the final concentration was 2.0 mM in each case), and the cells were cultured for 24 h at 37 °C. After washing with DMEM, C6 cells were fixed with 10 % paraformaldehyde in PBS for 10 min at room temperature. The cells were rinsed with PBS and treated with 0.05 % Triton X-100 for 10 min at room temperature. Next, the cells were washed with PBS, and preincubated in a humid chamber with 1.0 % BSA/0.02 % NaN_3 in PBS at room temperature, followed by incubation with the anti-BSH monoclonal antibody A9H3 in PBS containing 1.0 % BSA/0.02 % NaN_3 (0.2 μg/mL) for 60 min at 32 °C. The cells were rinsed with PBS, and then incubated with Alexa-Fluor® 488Goat Anti-Mouse IgG in PBS containing 1.0 % BSA/0.02 % NaN_3 (0.2 μg/mL) for 30 min at 32 °C. After washing with PBS, the cells were mounted with Permafluor and then photographed with a microscope (IX-70, Olympus, Tokyo) equipped with a cooled charge-coupled device camera (UIC-QE; Molecular Devices Co., Sunnyvale, CA, US) controlled by MetaMorph software (Molecular Devices Co.).

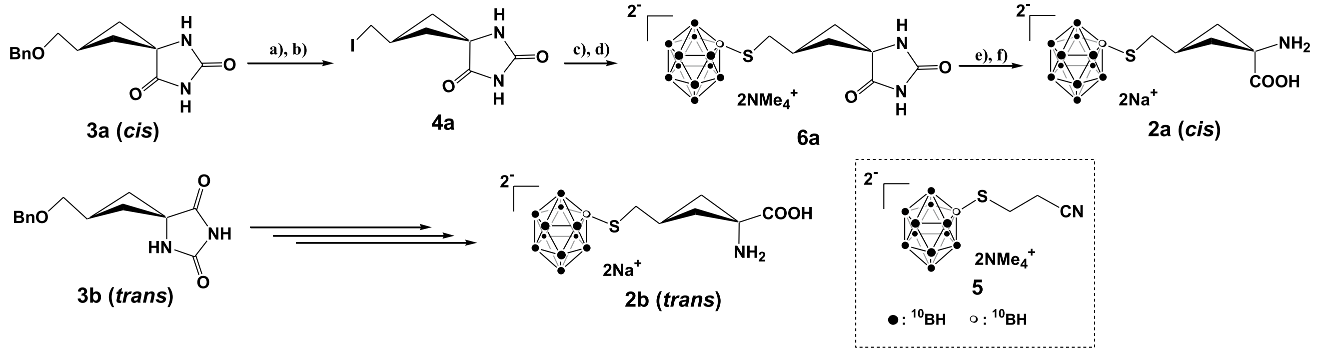
Cell-killing studies

Cultures were inoculated with 1.0 × 10^6 cells/dish, and cells were grown for 24 h in DMEM. The medium was replaced with an equivalent amount of medium containing the boron-containing amino acid (the final concentration was 2.0 mM in each case). The cells were cultured for 24 h, and the medium was removed by aspiration. The cells were washed with PBS, harvested by trypsinization and subsequently counted. After centrifugation, trypsin was removed by aspiration, and DMEM was added to the residual cells. The cell suspension in DMEM (5.0 × 10^3 cells/mL, 1 mL) was irradiated with thermal neutrons for 0–90 min in a column-shaped tube. The thermal neutron fluence was determined by averaging two gold foils, symmetrically attached to the surface of the column-shaped tube along the direction of incidence of the thermal neutrons. After thermal neutron exposure, 600 cells were placed in three Corning 60 mm tissue culture dishes containing 3 mL of DMEM to examine colony formation. Seven days later, the colonies were fixed with ethanol and stained with 0.1 % crystal violet for quantitative visualization by the naked eye.

Results and Discussion

The syntheses of *cis*/*trans*-ACBC-BSH (**2**) viahydantoin intermediates (**3**) were carried out as shown in Scheme 1.

The separated cyclobutyl-cis-hydantoin benzyl ether (**3a**) was transformed into iodocyclobutyl-cis-hydantoin (**4a**) through the hydrogenolysis of **3a** over Pd/C in MeOH followed by iodination of the hydroxyl group with I_2/PPh_3/imidazole to furnish **4a**. Condensation of **4a** with *S*-cyanoethylthiododecaborate (**5**) (Gabel et al. 1993) and subsequent deprotection were performed according to a previously reported method(Kusaka et al. 2011) to give thiododecaborated cis-cyclobutyl-hydantoin (**6a**). Hydrolysis of **6a** with



Scheme 1 Synthesis of *cis*/*trans*-ACBC-BSH (**2a** and **2b**). Reagents and conditions: **a** H_2 (3 atom)/ 20 % $Pd(OH)_2$ / MeOH, r.t. 12 h, **b** PPh_3, imidazole, I_2 / CH_2Cl_2, r.t., 48 h, **c** *S*-cyanoethylthiododecaborate (5) / MeCN, reflux, 24 h, **d** NMe_4OH / acetone, **e** 0.5 N NaOH, 100 °C, 18 h, f) amberlite IR-120(H^+), then 1 N NaOH

Table 1 Cytotoxicity of boron-containing amino acids against tumor cells

Compound	IC_{50} (mM)		
	C6	A172	U251
L-^{10}BPA	>10	>10	>10
C4-BSH-AA (**1**)	6.6	5.55	8.8
Cis-ACBC-BSH (**2a**)	2.5	2.0	>10
Trans-ACBC-BSH (**2b**)	2.1	1.9	>10

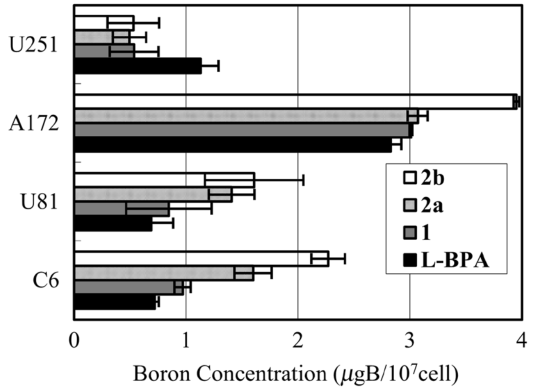


Fig. 2 Incorporated amount of boron-containing amino acids

sodium hydroxide and subsequent successive treatment with ion-exchange resin furnished the *cis*-cyclobutyl amino acid (**2a**) in high yield. The same reaction sequence was applied for the conversion of the *trans*-isomer (**3b**) into the *trans*-cyclobutyl amino acid (**2b**) via intermediate **6b**.

The cytotoxicities of the ACBC-BSH (**2a, b**), BSH-amino acid (**1**) and L-BPA toward malignant brain tumor cell lines such as U251 (human astrocytoma), A172 (human glioblastoma) and C6 (rat glioma), were determined using the WST-8 test (Table 1). As shown in Table 1, the cytotoxicity of each compound was very low. However, the cytotoxicities of the three BSH-containing amino acids were higher than that of L-BPA.

To elucidate the amounts of ACBC-BSH incorporated into the malignant brain tumor cells, we measured the boron concentrations in several cancer cells by inductively coupled plasma optical emission spectrometry (ICP-OES) (Fig. 2). As compared with L-BPA and C4-BSH-AA (**1**), **2a** and **2b** delivered a large amount of boron to C6, U81 and A172 cells (all lines express LAT) (Kanai et al. 1998; Yoshimoto et al. 2013). In particular, the intracellular boron concentration of **2b** in C6 cells was four times greater than that of L-^{10}BPA. However, the intracellular boron concentrations of **2a** and **2b**were less than that of L-^{10}BPA in U251 human astrocytoma cells which express the excitatory amino acid transporters (EAATs) (Guo et al. 2002; Kim et al. 2004). These results suggested the possibility that the *trans*-ACBC-BSH **2b** was predominantly transported by system L in glioma cells in analogy with ACBC(Oka et al. 2012), and **2b** might be useful as ^{10}B carrier for the treatment of glioblastoma.

To determine the distribution of each BSH-amino acid in tumor cells, we stained C6 cells that incorporated compound **2b** with the anti-BSH antibody A9H3 (Kirihata et al. 2007) (Fig. 3). ACBC-BSH **2b** was incorporated into the cell membrane of the C6 cells and aggregated on the fringe of the cell nuclei.

To confirm the usefulness of the ACBC-BSH for BNCT, we examined the tumor cell killing effects of L-^{10}BPA, C4-BSH-AA (**1**), and *cis*/*trans*-ACBC-BSH (**2a, 2b**) against C6 glioma cells by using neutron irradiation (Fig. 4). The result of this test was correlated with the result of incorporated test. ACBC-BSH showed higher killing effects than L-^{10}BPA and BSH-amino acid **1**for glioma cells. Furthermore, the trans-isomer **2b** showed higher killing effect than that of the *cis* isomer **2a**.

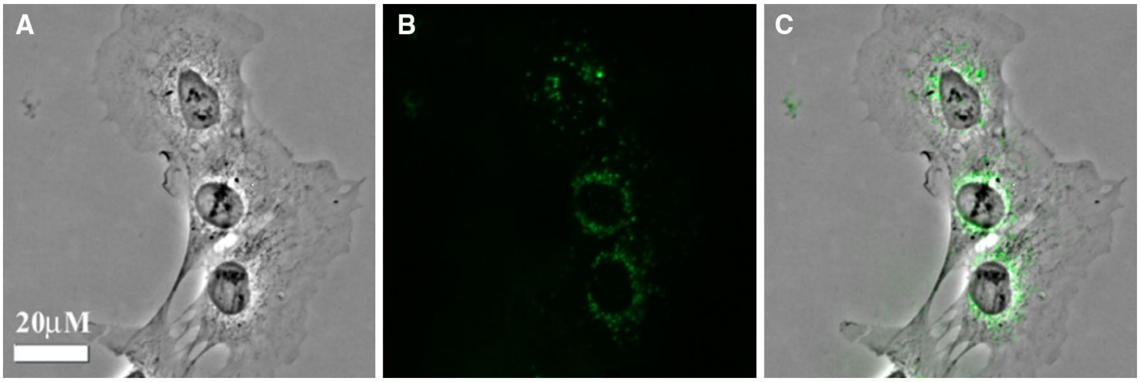


Fig. 3 The micro-distribution of compound **2b** in C6 cells. **a** A phase-contrast micrograph of C6 cells cultured in DMEM containing compound **2b**. **b** A fluorescence micrograph of C6 cells that were cultured in DMEM containing compound **2b** stained with the anti-BSH antibody A9H3. **c** A merge image of **a** and **b**

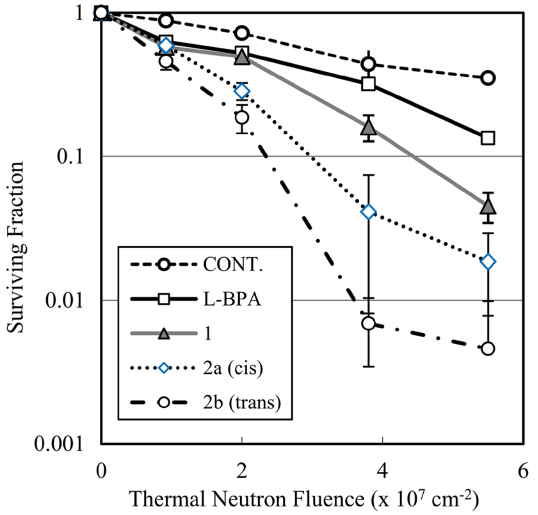


Fig. 4 Cell-killing effects of boron-containing amino acids against C6 cells

Conclusion

We synthesized novel undecahydro-*closo*-dodeca-boranylthio-containing α, α-cycloalkyl amino acids,cis-ACBC-BSH **(2a)** and trans-ACBC-BSH **(2b)**. The results of the in vitro evaluation suggested that these compounds were useful ^{10}B carriers for gliomas. Specifically, *trans*-ACBC-BSH**2b**exhibiteda high cell-killing effect against several glioma cells and was significantly accumulated within cells. The in vivo evaluation of **2b** is ongoing, and the results will be reported in the near future.

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Conflict of interest The authors have declared no conflict of interest.

References

Barth RF, Coderre JA, Vicente MG, Blue TE (2005) Boron neutron capture therapy of cancer: current status and future prospects. Clin Canc Res 11:3987–4002

Gabel D, Moller D, Harfst S, Rosler J, Ketz H (1993) Synthesis of S-alkyl and S-acyl derivatives of mercaptoundeca-hydrododecaborate, a possible boron carrier for neutron capture therapy. Inorg Chem 32:2276–2278

Guo H, Lai L, Butchbach ME, Lin CL (2002) Human glioma cells and undifferentiated primary astrocytes that express aberrant EAAT2 mRNA inhibit normal EAAT2 protein expression and prevent cell death. Mol Cell Neurosci 21:546–560

Hattori Y, Uehara K, Asano T, Tanimori S, Kirihata M (2010) Synthesis of new derivatives by hetero-Michael addition in water. Proceedings of 14th International Congress on Neutron Capture Therapy: 343–344

Hattori Y, Kusaka S, Mukumoto M, Uehara K, Asano T, Suzuki M, Masunaga S, Ono K, Tanimori S, Kirihata M (2012) Biological evaluation of dodecaborate-containing L-amino acids for boron neutron capture therapy. J Med Chem 55:6980–6984

Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H (1998) Expression cloning and characterization of a transporter for large neutral amino acids activated by heavy chain of 4F2 antigen (CD98). J Biol. Chem. 273:23629–23632

Kim DK, Kim IJ, Hwang S, Kook JH, Lee MC, Shin BA, Bae CS, Yoon JH, Ahn SG, Kim SA, Kanai Y, Endou H, Kim JK (2004) System L-amino acid transporters are differently expressed in rat astrocyte and C6 glioma cells. Neurosci Res 50:437–446

Kirihata M, Uehara K, Asano T (2007) Preparation of borocaptate (10B) (BSH) derivatives as hapten compound for production of antibody against BSH. WO2007097065, 30 Aug 2007

Kusaka S, Hattori Y, Uehara K, Asano T, Tanimori S, Kirihata M (2011) Synthesis of optically active dodecaborate-containing L-amino acids for BNCT. Appl Radiat Isot 69:1768–1770

Ogawa K, Wada H, Okada N, Harada I, Nakajima T, Pasquale BE, Tsuyama S (2005) EphB2 and ephrin-B1 expressed in the adult kidney regulate the cytoarchitecture of medullary tubule cells through Rho family GTPases. J Cell Sci 119:559–570

Oka S, Okudaira H, Yoshida Y, Schuster DM, Goodman MM, Shirakami Y (2012) Transport mechanisms of trans-1-amino-3-fluoro[1-(14)C]cyclobutanecarboxylic acid in prostate cancer cells. Nucl Med Biol 39:109–119

Rammeloo T, Stevens CV (2002) A new and short method for the synthesis of 2, 4-methanoproline. Chem. Commun. 3:250–251

Rice SL, Roney CA, Daumar P, Lewis JS (2011) The next generation of positron emission tomography radiopharmaceuticals in oncology. Semin Nucl Med 41:265–282

Soloshonok AV, Sorochinsky EA (2010) Practical methods for the synthesis of symmetrically α, α-disubstituted α-amino acids. Synthesis 14:2319–2344

Soloway AH, Tjarks W, Barnum BA, Rong FG, Barth RF, Codogni IM, Wilson JG (1998) The chemistry of neutron capture therapy. Chem Rev 98:1515–1562

Yoshimoto M, Kurihara H, Honda N, Kawai K, Ohe K, Fujii H, Itami J, Arai Y (2013) Predominant contribution of L-type amino acid transporter to 4-borono-2-18F-floro-phenylalanine uptake in human glioblastoma cells. Nucl Med Biol 40:625–629