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석사학위 논문

Ionic Liquids for Boron Neutron Capture Therapy Agents

Synthesis, Characterization, and Biological Evaluation using 1-Methylimidazole Unit

조선대학교 대학원

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붕소-중성자 포획요법용 이온성 액체 :
1-메틸이미다졸을 이용한 합성, 특성화 및 생물학적 평가

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조선대학교 대학원

화 학 과

전 원 희

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지도교수 이 종 대

이 논문을 이학 석사학위신청 논문으로 제출함.

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화 학 과

전 원 희

전원희의 석사학위논문을 인준함

위원장 조선대학교 교수 손 홍 래 (인)

위 원 조선대학교 교수 김 호 중 (인)

위 원 조선대학교 교수 이 종 대 (인)

2022년 12월

조선대학교 대학원

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Abstract

붕소-중성자 포획요법용 이온성 액체
1-메틸이미다졸을 이용한 합성, 특성화 및 생물학적 평가

Won Hui Jeon
Advisor : Prof. Lee Jong Dae, Ph.D,
Department of Chemistry,
Graduate School of Chosun University

붕소 중성자 포획 요법(Bonoron Neutron Capture Therapy, BNCT)은 10B가 함유된 암 특이적 약물과 열 중성자 조사의 조합을 기반으로 하는 암 치료를 위한 이진 치료 방법이다. 성공적인 BNCT를 위해서는 높은 수준의 축적과 암세포로의 10B의 선택적 전달이 필요하다. 효과적인 BNCT 제제의 설계는 (1) 낮은 세포 독성, (2) 정상 조직보다 종양 조직에서 더 높은 흡수, (3) 좋은 수용성을 요구한다. 여기서는 알킬기 치환 1-알킬-3-메틸이미다졸륨 양이온과 테트라플루오로보레이트, 비스(옥살레이토)보레이트 및 비스(말로네이토)보레이트 음이온의 다양한 사슬 길이인 이온성 액체(IL)를 함유한 붕소의 설계 및 합성에 대해 보고한다. 이들의 합성 과정, 세포 독성, 암세포로의 세포 내 흡수 활성, BNCT 매개체로서의 잠재성도 보고된다. 시험관내 예비 연구는 화합물 1-9이 A549와 HCT116 세포에서 낮은 세포 독성이지만, 동일한 암세포 계열에서 BPA보다 낮은 붕소 농도가 축적되었음을 확인했다.

1. Introduction

It is known that the increase in the incidence of various types of cancer creates a constant need to develop new anticancer drugs, including synthetic compounds of different chemical classes, possessing cytotoxic properties. Boron neutron capture therapy (BNCT) is a binary treatment modality for cancer involving the selective accumulation of chemical agents containing the isotope ^{10}B in cancer cells followed by irradiation with thermal neutrons. Capture of a thermal neutron by a ^{10}B nucleus initiates a nuclear reaction in which decay of an excited ^{11}B nucleus produces a high linear energy transfer α-particle and lithium nucleus. Because of the short trajectory of these heavy particles (5–9 μm; approximately one cell diameter), radiation damage is limited to those cells containing ^{10}B. Thus, if ^{10}B agents can be selectively targeted to tumor cells, side effects typically associated with ionizing radiation can be avoided.[1–6]

For successful BNCT, a high level of accumulation and selective delivery of ^{10}B into cancer cells are required. The design of effective BNCT agents requires the following criteria: (1) low systemic toxicity and higher uptake in tumor tissue than in normal tissue [tumor to blood (T/B) ratios should be greater than 3]; (2) ^{10}B must be retained in the tumor tissue but also be rapidly cleared from blood and normal tissues; and (3) the concentration of boron inside or near tumor cells must be $\geq 10^9$ ^{10}B atoms/cell (20 −35 μg/gram of tumor tissue).[7–13] In this context, only two compounds, sodium mercaptoborate (BSH)[14] and L-4-boronophenylalanine (BPA)[15–18] have been used for the clinical treatment of cancers such as malignant glioma, malignant melanoma, and recurrent head and neck cancer, which are not enough for treatment of multiple tumor types (Chart 1).[19–22]

To date, numerous boron-containing analogues including amino acids,[23–33] biochemical precursors of nucleic acids,[34–45] carbohydrates,[46–61] amines,[62–71] porphyrins,[72–79] peptides,[80–83] liposomes,[84–88] and monoclonal antibodies have been developed.[89–91] However, most of them do not satisfy the above criteria for clinical applications. Therefore, more potent boron agents are highly required in order to improve the therapeutic effect and to apply to various tumor types.

Ionic liquids (ILs) are a class of low melting point ionic compounds, which have attracted increasing attention for multiple applications.[92–96] The imidazolium-based ILs possess excellent and advantageous properties over the conventional organic solvents and they have been widely applied in chemical industry.[97,98] However, despite the great number of published papers, still little attention has been paid to the BNCT agents. In this study, we describe the synthesis of room temperature ionic liquids (RTILs) containing a various alkyl-substituted imidazolium cation with BF_4^-, BOB [bis(oxalate)borate], and BMB [bis(malonato)borate] anions in their structure. As is well known, one of the advantages of ionic liquids is their high solubility in polar solvents such as water while maintaining a liquid phase at room temperature. Although 1-alkyl-3-methylimidazolium cation is known to increase cytotoxicity as the number of carbon atoms in the alkyl chain increases, it may show sufficient potential as a candidate agents for BNCT if it can show low cytotoxicity by controlling the number of carbon atoms. To the best of our knowledge, this is the first time that to confirm the potential as an agents for BNCT using an ionic liquids based on 1-methylimidazole moiety. In view of their excellent water solubilities and controllable toxicities using variations in the alkyl chain length and borate anions size, we have focused our interest on boron-containing ionic liquids for use in BNCT.

Experimental

General considerations

All manipulations were performed in a dry nitrogen or argon atmosphere using standard Schlenk techniques. Acetonitrile, acetone, and tetrahydrofuran (THF) was distilled under nitrogen from sodium/benzophenone. The elemental analyses were performed using a Carlo Erba Instruments CHNS-O EA 1108 analyzer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-JNM-AL300 spectrometer at 300.1 and 75.4 MHz, respectively. ^{11}B NMR spectra were recorded on a Bruker Ascend 400 spectrometer (Billerica, MA, USA) (operating at 128.4 MHz) at the Korea Basic Science Institute (KBSI) Gwangju Center. All ^{11}B chemical shifts were referenced to $BF_3{\cdot}O(C_2H_5)_2$ (0.0 ppm), where a negative sign indicated an upfield shift. All ^{1}H and ^{13}C chemical shifts were measured relative to internal residual peaks arising from the lock solvent (99.5% $CDCl_3$). 1-Butyl-3-methylimidazolinium bromide, 1-pentyl-3-methylimidazolinium bromide, 1-hexyl-3-methylimidazolinium bromide, potassium bis(oxalato)borate,[99] and potassium bis(malonato)borate[100,101] were synthesized by literature procedure. 1-Methylimidazole, $NaBF_4$, butyl bromide, pentyl bromide, and hexyl bromide were purchased from Aldrich Chemicals.

Cell viability assay (MTT assay)

The boron compound (20 mg) was dissolved in DMSO (1.0 mL) and the resulting solution was either diluted with MEM (Modified Eagle Medium) (10% FCS) or BPA was directly dissolved in the same medium. In a Falcon 3072, 96-well culture plate, A549 and HCT116 cancer cells (1×10^3 cells/well) were cultured in five wells with the medium containing boron compounds at various concentrations (1–100 ppm) and incubated for 72 h at 37 °C in a CO_2 incubator. DMSO is typically non-toxic at the concentrations less than 0.5% and control experiments confirmed that DMSO was non-toxic at the concentrations used in the present experiments. After incubation, the

medium was removed, the cells were washed three times with phosphate-buffered saline [PBS (–)], and the CellTiter 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay [MTT, 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used for counting cells on a Microplate reader. The results are presented in Table 3 as the concentration of agent that resulted in a cell culture with 50% of the cell number of the corresponding untreated group (IC_{50}).

In vitro boron incorporation into A549 and HCT116 cancer cells

A549 and HCT116 cancer cells were cultured in Falcon 3025 dishes (150 mm). When the cell population had increased to fill the dish (3.6×10^7 cells/dish), the boron compounds and BPA (10 μM) were added. The cells were incubated for 3 h at 37 °C in a medium of MEM and 10% FBS (20 mL). The cells were washed thrice with Ca/Mg-free PBS (–), collected by a rubber policeman, digested with a mixture of 60% $HClO_4$–30% H_2O_2 (1:2) solution (2 mL), and then decomposed for 1 h at 75 °C. After filtration through a membrane filter (Millipore, 0.22 mm), the boron concentration was determined by ICP-OES (Perkin Elmer. Ltd, Optima 7300). Each experiment was performed in triplicate. The average boron concentration of each fraction is indicated in Figure *.

Synthesis of 1-alkyl-3-methylimidazolinium borates (1–9).

General procedure 1-Alkyl-3-methylimidazolinium bromide (20.0 mmol) was dissolved in 30 mL of acetone and 1.2 equiv. of $NaBF_4$, KBOB, or KBMB, in 10 mL of acetone and 1 mL of water, were added. The mixture was stirred at 25 °C for 24 h, then the precipitate was filtered off with aluminum oxide, washed with cold acetone (10 mL × 3) and dried under reduced pressure. The target compounds were obtained as colorless liquid.

1-Butyl-3-methylimidazolinium tetrafluoroborate (1): Yield: 81% (3.7 g). ^{1}H NMR (DMSO-d_6) δ 1.25 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH$_2$–C*H*$_3$), 1.61 (sextet, $^{3}J_{H-H}$ = 6.0 Hz, –CH$_2$–C*H*$_2$–CH$_3$), 2.12 (quintet, $^{3}J_{H-H}$ = 6 Hz, –C*H*$_2$–CH$_2$–CH$_3$), 4.21 (s, N–C*H*$_3$), 4.52 (t, $^{3}J_{H-H}$ = 6 Hz, N–C*H*$_2$), 8.05, 8.12 (s, *H*–C=C–*H*), 9.46 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.23 (–CH$_2$–*C*H$_3$), 18.78 (–*C*H$_2$–CH$_3$), 31.38 (–*C*H$_2$–CH$_2$–CH$_3$), 35.75 (N–*C*H$_3$), 48.56 (N–*C*H$_2$), 122.43, 123.78 (H–*C*=*C*–H), 136.68 (N=*C*–H). ^{11}B NMR (DMSO-d_6) δ –1.30.

1-Pentyl-3-methylimidazolinium tetrafluoroborate (2): Yield: 78% (3.7 g). ^{1}H NMR (DMSO-d_6) δ 1.58 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH$_2$–C*H*$_3$), 1.93 (sextet, $^{3}J_{H-H}$ = 6.0 Hz, –CH$_2$–C*H*$_2$–CH$_3$), 2.01 (quintet, $^{3}J_{H-H}$ = 6 Hz, –C*H*$_2$–CH$_2$–CH$_3$), 4.56 (s, N–C*H*$_3$), 4.86 (t, $^{3}J_{H-H}$ = 6 Hz, N–C*H*$_2$), 8.38, 8.45 (d, $^{3}J_{H-H}$ = 3 Hz, *H*–C=C–*H*), 9.77 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.66 (–CH$_2$–*C*H$_3$), 21.48 (–*C*H$_2$–CH$_3$), 27.61 (–*C*H$_2$–CH$_2$–CH$_3$), 29.05 (–*C*H$_2$–CH$_2$–CH$_2$), 35.70 (N–*C*H$_3$), 48.81 (N–*C*H$_2$), 122.38, 123.73 (H–*C*=*C*–H), 136.63 (N=*C*–H). ^{11}B NMR (DMSO-d_6) δ –1.29.

1-Hexyl-3-methylimidazolinium tetrafluoroborate (3): Yield: 87% (4.4 g). ^{1}H NMR (DMSO-d_6) δ 1.58 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH$_2$–C*H*$_3$), 1.93 (m, –C*H*$_2$–C*H*$_2$–CH$_3$), 2.87 (quintet, $^{3}J_{H-H}$ = 6 Hz, –C*H*$_2$–CH$_2$–CH$_2$), 4.57 (s, N–C*H*$_3$), 4.88 (t, $^{3}J_{H-H}$ = 6 Hz, N–C*H*$_2$), 8.40, 8.48 (d, $^{3}J_{H-H}$ = 3 Hz, *H*–C=C–*H*), 9.79 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.81 (–CH$_2$–*C*H$_3$), 21.92 (–*C*H$_2$–CH$_3$), 25.17 (–*C*H$_2$–CH$_2$–CH$_2$–CH$_3$), 29.38 (–*C*H$_2$–CH$_2$–CH$_2$–CH$_2$), 30.60 (–*C*H$_2$–CH$_2$–CH$_3$), 35.74 (N–*C*H$_3$), 48.80 (N–*C*H$_2$), 122.42, 123.77 (H–*C*=*C*–H), 136.66 (N=*C*–H). ^{11}B NMR (DMSO-d_6) δ –1.29.

1-Butyl-3-methylimidazolinium bis(oxalato)borate (4): Yield: 67% (4.4 g). ^{1}H NMR (DMSO-d_6) δ 1.61 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH$_2$–C*H*$_3$), 1.97 (sextet, $^{3}J_{H-H}$ = 6.0 Hz, –CH$_2$–C*H*$_2$–CH$_3$), 2.84 (quintet, $^{3}J_{H-H}$ = 6 Hz, –C*H*$_2$–CH$_2$–CH$_3$), 4.63 (s, N–C*H*$_3$), 4.95 (t, $^{3}J_{H-H}$ = 6 Hz, N–C*H*$_2$), 8.56, 8.65 (d, $^{3}J_{H-H}$ = 6 Hz, *H*–C=C–*H*), 10.17 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.17 (–CH$_2$–*C*H$_3$), 18.64 (–*C*H$_2$–CH$_3$), 29.46 (oxalate ring), 31.32 (–*C*H$_2$–CH$_2$–CH$_3$), 35.74 (N–*C*H$_3$), 48.37 (N–*C*H$_2$), 122.33, 123.58 (H–*C*=*C*–H), 136.63 (N=*C*–H), 208.71 (oxalate ring *C*=O). ^{11}B NMR (DMSO-d_6) δ 6.35.

1-Pentyl-3-methylimidazolinium bis(oxalato)borate (5): Yield: 61% (4.1 g). ^{1}H NMR (DMSO-d_6) δ 1.55 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH_2–CH_3), 1.94 (m, –CH_2–CH_2–CH_3), 2.82 (quintet, $^{3}J_{H-H}$ = 6 Hz, –CH_2–CH_2–CH_2), 4.60 (s, N–CH_3), 4.91 (t, $^{3}J_{H-H}$ = 6 Hz, N–CH_2), 8.51, 8.59 (s, *H*–C=C–*H*), 10.08 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.69 (–CH_2–CH_3), 21.47 (–CH_2–CH_3), 27.56 (–CH_2–CH_2–CH_3), 29.08 (–CH_2–CH_2–CH_2), 29.51 (oxalate ring), 35.76 (N–CH_3), 48.66 (N–CH_2), 122.37, 123.65 (H–*C*=*C*–H), 136.66 (N=*C*–H), 208.70 (oxalate ring *C*=O). ^{11}B NMR (DMSO-d_6) δ 6.89.

1-Hexyl-3-methylimidazolinium bis(oxalato)borate (6): Yield: 67% (4.7 g). ^{1}H NMR (DMSO-d_6) δ 1.55 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH_2–CH_3), 1.96 (m, –CH_2–CH_2–CH_3), 2.83 (m, –CH_2–CH_2–CH_2), 4.61 (s, N–CH_3), 4.92 (t, $^{3}J_{H-H}$ = 6 Hz, N–CH_2), 8.51, 8.61 (d, $^{3}J_{H-H}$ = 3 Hz, *H*–C=C–*H*), 10.16 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.68 (–CH_2–CH_3), 21.77 (–CH_2–CH_3), 25.03 (–CH_2–CH_2–CH_2–CH_3), 29.32 (–CH_2–CH_2–CH_2–CH_2), 29.44 (oxalate ring), 30.46 (–CH_2–CH_2–CH_3), 35.73 (N–CH_3), 48.64 (N–CH_2), 122.32, 123.57 (H–*C*=*C*–H), 136.63 (N=*C*–H), 208.62 (oxalate ring *C*=O). ^{11}B NMR (DMSO-d_6) δ 7.30.

1-Butyl-3-methylimidazolinium bis(malonato)borate (7): Yield: 94% (6.7 g). ^{1}H NMR (DMSO-d_6) δ 0.82 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH_2–CH_3), 1.20 (sextet, $^{3}J_{H-H}$ = 6.0 Hz, –CH_2–CH_2–CH_3), 1.73 (quintet, $^{3}J_{H-H}$ = 6 Hz, –CH_2–CH_2–CH_3), 3.47 (s, malonate ring –CH_2 –), 3.88 (s, N–CH_3), 4.21 (t, $^{3}J_{H-H}$ = 6 Hz, N–CH_2), 7.84, 7.93 (d, $^{3}J_{H-H}$ = 3 Hz, *H*–C=C–*H*), 9.47 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.19 (–CH_2–CH_3), 18.64 (–CH_2–CH_3), 31.34 (–CH_2–CH_2–CH_3), 35.76 (N–CH_3), 48.34 (N–CH_2), 49.41 (malonate ring C–H), 122.23, 123.57 (H–*C*=*C*–H), 136.63 (N=*C*–H), 171.4 (oxalate ring *C*=O). ^{11}B NMR (DMSO-d_6) δ. 2.93

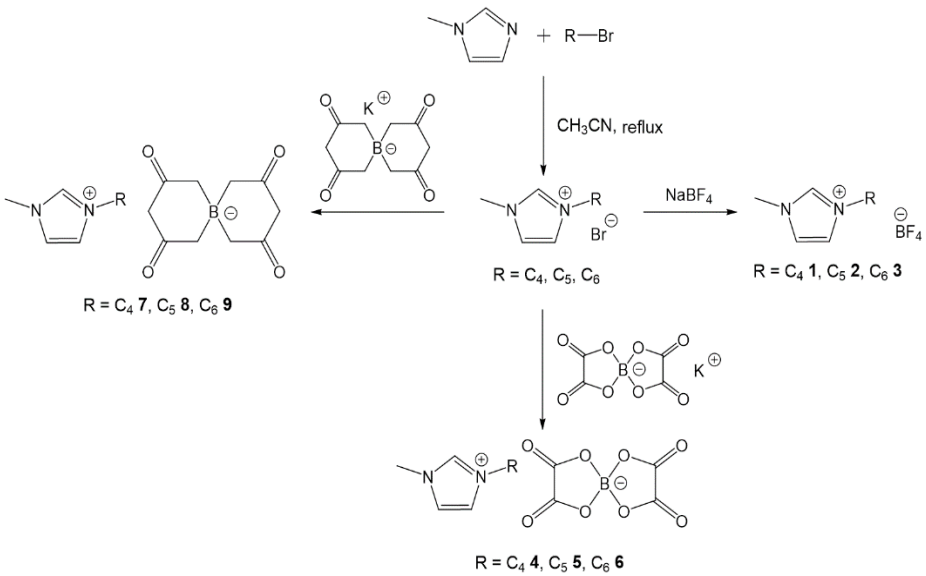
1-Pentyl-3-methylimidazolinium bis(malonato)borate (8): Yield: 91% (6.7 g). ^{1}H NMR (DMSO-d_6) δ 0.83 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH_2–CH_3), 1.18 (m, –CH_2–CH_2–CH_3), 1.27 (m, –CH_2–CH_2–CH_3), 1.77 (quintet, $^{3}J_{H-H}$ = 6 Hz, –CH_2–CH_2–CH_2), 3.39 (s, malonate ring –CH_2–), 3.87 (s, N–CH_3), 4.19 (t, $^{3}J_{H-H}$ = 6 Hz, N–CH_2), 7.79, 7.88 (d, $^{3}J_{H-H}$ = 3.0 Hz, *H*–C=C–*H*), 9.37 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.69 (–CH_2–CH_3), 21.47 (–CH_2–CH_3), 27.55 (–CH_2–CH_2–CH_3), 29.07 (–CH_2–CH_2–CH_2), 35.76 (N–CH_3), 48.65 (N–

CH_2), 49.39 (malonate ring), 122.36, 123.65 (H–*C*=*C*–H), 136.65 (N=*C*–H), 171.2 (malnate ring *C*=O). ^{11}B NMR (DMSO-d_6) δ. 2.84

1-Hexyl-3-methylimidazolinium bis(malonato)borate (9): Yield: 93% (7.1 g). ^{1}H NMR (DMSO-d_6) δ 0.79 (t, $^{3}J_{H-H}$ = 6.0 Hz, –CH_2–CH_3), 1.20 (m, –CH_2–CH_2–CH_3), 1.75 (quintet, $^{3}J_{H-H}$ = 6 Hz, –CH_2–CH_2–CH_2), 3.47 (s, malonate ring –CH_2–), 3.88 (s, N–CH_3), 4.20 (t, $^{3}J_{H-H}$ = 6 Hz, N–CH_2), 7.84, 7.93 (d, $^{3}J_{H-H}$ = 3 Hz, *H*–C=C–*H*), 9.48 (s, N=C–*H*). ^{13}C NMR (DMSO-d_6) δ 13.71 (–CH_2–CH_3), 21.79 (–CH_2–CH_3), 25.03 (–CH_2–CH_2–CH_2–CH_3), 29.35 (–CH_2–CH_2–CH_2–CH_2), 30.48 (–CH_2–CH_2–CH_3), 35.74 (N–CH_3), 48.60 (N–CH_2), 49.22 (malonate ring), 122.31, 123.56 (H–*C*=*C*–H), 136.63 (N=*C*–H), 170.62 (malonate ring *C*=O). ^{11}B NMR (DMSO-d_6) δ. 2.73

Results and Discussion

The synthetic route used to prepare 1-alkyl-3-methylimidazolium salts is depicted in Scheme 1. The imidazolium bromides [C_nMIM]Br (C_n: n = 4 butyl, n = 5 pentyl, n = 6 hexyl) are prepared in high yield from 1-methylimidazole and the appropriate alkyl bromide in a modification to the literature procedure for the related 1-alkyl-3-methylimidazolium halides. The synthesis of [C_nMIM]Br has been describe previously using a somewhat more complicated method. The relatively electron withdrawing effect of the bromide activates alkyl bromide, $CH_3(CH_2)_nBr$, to such an extent that it reacts smoothly with 1-methylimidazole in the acetonitrile solvent to give target compound. However, as the alkyl chain in the alkyl bromide precursor increases in length, the temperature and reaction time required to complete the reaction also increases.



Scheme 1. Preparation of 1-alkyl-3-methylimidazolium borates (**1**–**9**).

Treatment of 1-alkyl-3-methylimidazolium bromides with sodium tetrafluoroborate ($NaBF_4$), potassium bis(oxalate)borate (KBOB), and bis(malonate)borate (KBMB) in acetone gave the target compounds **1**–**9** in good yields (**1** 81%, **2** 78%, **3** 87%, **4** 67%, **5** 61%, **6** 67%, **7** 94%, **8** 91%, **9** 93%). For imidazolium borates were filtered off with aluminum oxide (basic) in order to remove the NaBr or KBr salts formed during the anion exchange reaction, then washed with cold acetone solvent. Unfortunately, in the cases of K[bis(oxalate)borate] and K[bis(malonato)borate], the solubility in acetone was so low that the reaction did not proceed unless a solvent mixed with water was used. When the reaction was carried out using a mixed solvent, the bis(malonato)borate coordinated 1-alkyl-3-methylimidazolium ionic liquids could be obtained with a very high yields. The imidazolium borate ionic liquids synthesized in this way were then dried under vacuum for 2 days using Schlenk technique. The salts **1**–**9** are liquid form at room temperature and were further purified by filtration through silica and left at 60 °C for 2 days. They are stable in air and showed no signs of decomposition up to 100 °C. Compounds **1**–**9** showed the characteristic vibrational absorption bands of the C–H unit and C=O unit in their infrared (IR) spectra between 3150 ~ 2950 cm^{-1} and between 1708 ~ 1734 cm^{-1}, respectively. Diagnostic signals for compounds **1**–**9** were observed at around δ 0.79 ~ 1.61 and 3.87 ~ 4.63 in the ^1H NMR spectra and at around δ 13.17 ~ 13.81 and 35.70 ~ 35.76 in the ^{13}C NMR spectra of the terminal –CH_3 and N–CH_3 units and at around δ 4.19 ~ 4.95 in the ^1H NMR spectra and at around δ 48.37 ~ 48.81 in the ^{13}C NMR spectra of the ^+N–CH_2 unit of the alkyl chain.

Determination of IC_{50} and Incorporation of Boron into A549 and HCT116 Cells

A549 adenocarcinomic human alveolar basal epithelial and HCT116 human colorectal carcinoma cells were treated with compounds **1–9** and BPA for 3 days, after which the cell viability was determined by the MTT [3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. As can be seen from Table 1, compounds **1–9** showed higher cytotoxicity than BPA, with IC_{50} (the half maximal inhibitory concentration) values in the range of 39.01–838.8 μM. Interestingly, the BOB and BMB anion coordinated compounds (**4–9**) showed higher cytotoxicity than the BF_4 anion coordinated 1-alkyl-3-methylimidazolium ionic liquids (**1–3**) in A549 and HCT116 cells. As expected, as the number of carbons in alkyl chain and the size of borates increased, the cytotoxicity increased.

Table 1. Cytotoxicity (IC_{50}) of A549 and HCT116 cells.

	Cell Viability IC_{50} (μM)[a]	
Comp. No.	A549	HCT116
1	231.98 ± 3.6	838.83 ± 78.3
2	89.16 ± 12.4	270.27 ± 12.4
3	44.81 ± 1.3	106.06 ± 13.4
4	169.18 ± 1.8	709.33 ± 89.3
5	89.36 ± 5.7	154.57 ± 25.5
6	< 39.0625	77.57 ± 5.6
7	169.28 ± 11.4	458.71 ± 100.1
8	64.58 ± 0.6	167.49 ± 26.1
9	< 39.0625	56.24 ± 12.5
BPA	1180.1 ± 57.3	964.6 ± 269.6

[a]A549 and HCT116 cells were incubated for 72 h in the presence of compounds **1–9** and BPA, and then the percentages of viable cells were determined by MTT assay. The drug concentrations required to inhibit cell viability by 50% (IC_{50}) were determined from semi-logarithmic concentration-response plots, and the results represent the means ± s.d. of triplicate samples.

We next examined the level of intracellular accumulation of the compounds **1–9** by determining their boron concentrations via inductively coupled plasma optical emission spectroscopy (ICP-OES). As shown in Table 2, although the toxicity increases as the number of carbon atoms in the alkyl substituent increases, it can be seen that the intracellular accumulation becomes better as the lipophilicity increases. On the other hand, it was found that as the size of the boron anion increased, the concentration of boron accumulated in cells decreased.

Table 2. Intracellular boron uptake of compounds **1** – **9**.

Comp. No.	Boron Concentration (ppm/3 × 10^5 cells/mL)[a]	
	A549	HCT116
1	0.784 ± 0.005	0.643 ± 0.002
2	0.850 ± 0.005	0.680 ± 0.001
3	0.974 ± 0.004	0.797 ± 0.003
4	0.358 ± 0.002	0.231 ± 0.001
5	0.411 ± 0.001	0.354 ± 0.001
6	0.545 ± 0.002	0.551 ± 0.001
7	0.331 ± 0.001	0.201 ± 0.001
8	0.382 ± 0.003	0.330 ± 0.005
9	0.477 ± 0.003	0.463 ± 0.001
BPA	4.47 ± 0.005	4.37 ± 0.002

[a]HCT116 cells (3 ×10^5 cells) were incubated for 3 h in the presence of compounds **1–9** or BPA (10 ppm). After three washes, the accumulated boron concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). The values are the mean ± SD from three samples.

Conclusions

In this study, we have described the synthesis, characterization, and biological activities of a series of butyl-, pentyl-, and hexylimidazolium cation with various borate anions for BNCT agents. We have developed a general and versatile method for the preparation of imidazolium-based ionic liquids with bromide anion. 1-Alkyl-3-methylimidazolium borate ILs were prepared by simple anion exchange and were used to inhibit the growth of tumor cells. As above mentioned, the ionic liquid was expected to show low toxicity due to its high solubility in polar solvents such as water, but it showed higher toxicity than BPA due to the characteristics of the alkylated imidazole cation. In addition, as the size of boron anion increased in the order of BF_4, BOB, and BMLB, toxicity increased, and intracellular boron concentration decreased. Through the results of this study, it was confirmed that the composition and structure of boron anion affect the toxicity and accumulation of boron concentration, and the possibility of developing an effective ionic liquid for BNCT using various boron anions was confirmed.

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