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Synthesis and biological evaluation of new boron-containing chlorin derivatives as agents for both photodynamic therapy and boron neutron capture therapy of cancer

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ABSTRACT

New boron-containing chlorin derivatives **9** and **13** as agents for both photodynamic therapy (PDT) and boron neutron capture therapy (BNCT) of cancer were synthesized from photoprotoporphyrin IX dimethyl ester (**2**) and L-4-boronophenylalanine-related compounds. The in vivo biodistribution and clearance of **9** and **13** were investigated in tumor-bearing mice. The time to maximum accumulation of compound **13** in tumor tissue was one-fourth of that of compound **9**, and compound **13** showed rapid clearance from normal tissues within 24 h after injection. The in vivo therapeutic efficacy of PDT using **13** was evaluated by measuring tumor growth rates in tumor-bearing mice with 660 nm light-emitting diode irradiation at 3 h after injection of **13**. Tumor growth was significantly inhibited by PDT using **13**. These results suggested that **13** might be a good candidate for both PDT and BNCT of cancer.

Photodynamic therapy (PDT) is a medical treatment that utilizes a cancer-selective photosensitizing agent and visible light in the presence of oxygen to selectively destroy cancer cells by generating singlet oxygen ($^1O_2$) and free radicals that have a strong cytocidal effect through photochemical reactions.[1–3] Porphyrin derivatives such as Photofrin®[4,5] have been most frequently used as cancer-selective photosensitizing agents in PDT. Recently, chlorin derivatives represented by Laserphyrin® (mono-L-aspartyl chlorin e6, Npe6, Talaporfin sodium),[6,7] which was approved in Japan in 2004 for treatment of lung cancer, have been applied to PDT as cancer-selective photosensitizing agents, since the excitation efficiency of chlorin derivatives is higher than that of porphyrin derivatives.

On the other hand, boron neutron capture therapy (BNCT) has attracted attention as a bimodal therapy for cancer as is PDT. BNCT is a cancer treatment that selectively destroys cancer cells by producing high linear energy transfer α particles and lithium-7 nuclei through nuclear reaction between boron-10 (^{10}B)-containing agents selectively incorporated into cancer cells and low-energy thermal neutrons.[8–11] In this treatment, accumulation in and selectivity to cancer cells of boron-containing agents are the most important factors for reducing damage to normal cells. Although L-4-boronophenylalanine (BPA)[12,13] and disodium mercapto-closo-dodecaborate (BSH)[14,15] (Fig. 1), which are boron-containing agents used in current clinical trials for BNCT, are very safe, accumulation in and selectivity to cancer cells of BPA and BSH are insufficient, and improvement of these drugs is required. A number of candidate boron-containing agents for BNCT, such as boron-containing nucleic acids,[16–18] amino acids,[19,20] peptides,[21] carbohydrates[22,23] and liposomes,[24] have been synthesized and evaluated over the past few decades. Boron-containing porphyrin[25–28] and chlorin[29] derivatives have also been synthesized, and dual applications with boron-containing porphyrin and chlorin derivatives in PDT and BNCT have been attempted.

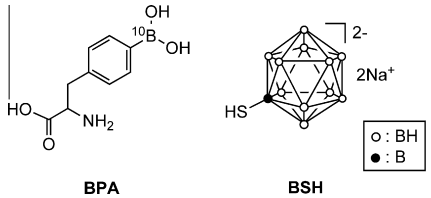

Figure 1. Chemical structures of BPA and BSH.

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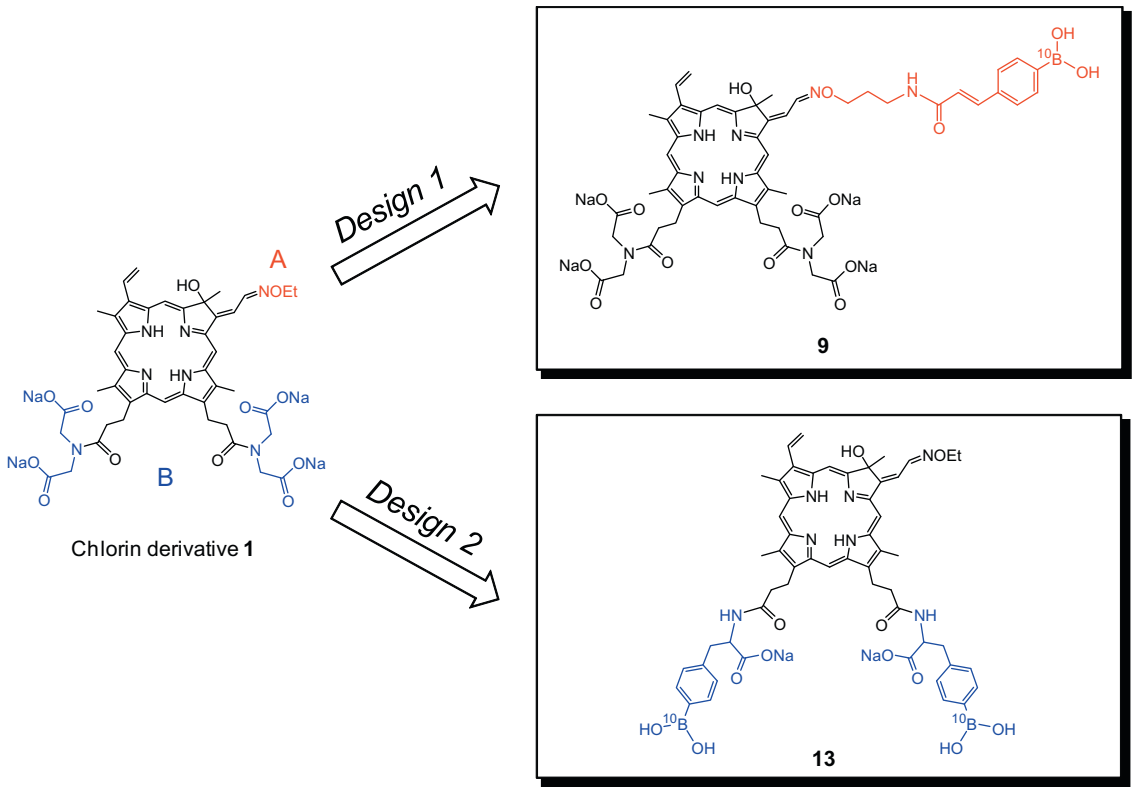

Figure 2. Molecular design strategy for efficient PDT/BNCT agents.

Recently, we synthesized a water-soluble chlorin derivative **1** (Fig. 2) that had tumor selectivity and rapid clearance from photoporphyrin IX dimethyl ester (**2**),[30,31] and we confirmed the usefulness of PDT using the water-soluble chlorin derivative **1**.[32] By using chlorin derivatives based on the chemical structure of **1** as boron delivery agents, it is expected that more effective agents for both PDT and BNCT can be developed.

In this study, we synthesized two kinds of new boron-containing chlorin derivatives bonded with BPA-related compounds and chlorin derivatives prepared from photoprotoporphyrin IX dimethyl ester (**2**). We investigated the cancer-selective accumulation and clearance from normal tissues of boron-containing chlorin derivatives, and then evaluated the therapeutic efficacy of PDT using a candidate agent for both PDT and BNCT.

We designed two kinds of new boron-containing chlorin derivatives that combined BPA-related compounds and chlorin derivative **1** (Fig. 2). One is a derivative **9** that combined a BPA-related compound in the A-position of the chlorin derivative **1** to leave iminodiacetic acid groups because iminodiacetic acid groups of the chlorin derivative **1** were shown in our previous study to be important for superior tumor accumulation and clearance characteristics.[32] The other is a derivative **13** in which one carboxyl group of the iminodiacetic acid group (B-position) was replaced by another polar group, namely, a 4-boronophenyl group.

The synthesis of boron-containing chlorin derivative **9** is shown in Scheme 1. Reaction of aldehyde **2** with hydroxylamine hydrochloride in pyridine gave oxime **3**. Diamide **4** was prepared by hydrolysis of methyl esters of **3** with aqueous sodium hydroxide in tetrahydrofuran and condensation with dimethyl iminodiacetate in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in *N*,*N*-dimethylacetamide, followed by reoxime formation of aldehyde produced by removal of the oxime group during condensation (94% yield for three steps). *O*-Alkylation of **4** with sodium hydride and 3-(*tert*-butoxycarbonylamino)propyl bromide in *N*,*N*-dimethylformamide afforded *tert*-butyloxycarbonyl (BOC) derivative **5**, which was then *N*-deprotected with trifluoroacetic acid in dichloromethane to give aminopropyl derivative **6** (41% yield for two steps). In addition, aminopropyl derivative **6** was synthesized directly from **4** by *O*-alkylation using non-protected 3-aminopropylbromide in 69% yield, because decomposition of **5** was confirmed under the acidic condition to remove the BOC group. Condensation of **6** with 4-boronocinnamic acid **7** (^{10}B-enriched) in the presence of 4-dimethylaminopyridine and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in *N*,*N*-dimethylacetamide afforded amide **8** (60%), which was then hydrolyzed under basic conditions to give boronocinnamic acid derivative **9** in 83% yield. On the other hand, boronophenylalanine derivative **13** was synthesized by condensation of **10** with L-4-boronophenylalanine ethyl ester hydrochloride **11** (^{10}B-enriched) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in *N*,*N*-dimethylacetamide, followed by hydrolysis of ethyl esters **12** with aqueous sodium hydroxide in *N*,*N*-dimethylformamide in 42% yield for two steps (Scheme 2). The newly synthesized boron-containing chlorin derivatives **9** and **13** were fully characterized by ^{1}H NMR spectroscopy, UV–visible absorption spectroscopy and liquid chromatography mass spectrometry analysis, and they showed good water solubility (100 mg/mL).

The in vivo biodistribution and clearance of synthesized boron-containing chlorin derivatives **9** and **13** were evaluated according to our previous procedure.[32] Figure 3 shows the distributions of boron-containing chlorin derivatives **9** and **13** in mouse tissues and serum for periods ranging from 1 h to 24 h after intravenous injection of 10 μmol/kg of **9** and **13**. Both compounds **9** and **13** showed good tumor-selective accumulation and rapid clearance from normal tissues. These results suggest that the molecular designs of the boron-containing chlorin derivatives **9** and **13** based on the chemical structure of compound **1** are successful. The