Organic & Biomolecular Chemistry

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Detection of Boronic Acid Derivatives in Cells Using Fluorescent Sensor

Cite this: DOI: 10.1039/xoxxoooooox

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Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

The detection of boron-containing compounds requires very expensive facilities and/or tedious pretreatments. In an effort to develop a convenient detection method for boronic acid derivatives, boron chelating-ligands were synthesized for use in fluorescent sensor. In this paper, the synthesis and properties of the fluorescent sensor for boronic acid derivatives are reported.

Studies regarding the interaction between boric acid containing compounds and biomolecules have increased in recent years. Thus, boronic acid containing compounds, including *p*-boronophenylalanine (L-BPA, for boron-neutron capture therapy)[1] and Bortezomib (for the treatment of multiple myeloma)[2] have been touted as a new class of pharmaceuticals[3] (Fig. 1).

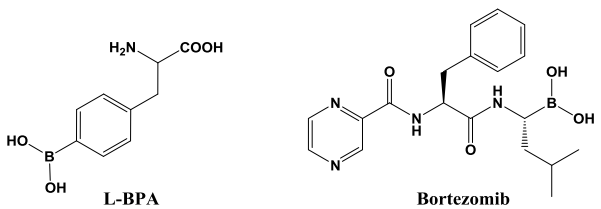


Fig. 1. Boron containing pharmaceuticals.

To develop novel medicines, it is necessary to elucidate the distribution of medicine. For example, boron accumulation in the cell nucleus kills cells more efficiently during boron neutron capture therapy (BNCT)[4], grasp of the exact intracellular localization, distribution, and tumor/normal tissue ratio of boron pahrmaceuticals are very important. The distribution of boron pharmaceuticals in cells and biological tissues can be evaluated *via* detection of the boron atom, because biological tissues contain little or no boron. However, most boron-detection methods, such as immune-staining[5] and α-autoradiography[6] require very expensive facilities and/or tedious pretreatments. Therefore, a convenient and affordable method for boron-detection is required.

Boron(III)-containing fluorescent dyes, such as BODIPY are used in various fields, including chemical biology, analytical chemistry and material science[7]. Generally, boron(III)-containing fluorescent dyes have heterocyclic structures that consist of boron(III) and *N*,*N*- or *N*,*O*-type chelating-ligands. To facilitate coordination to the nitrogen and/or oxygen moieties, boron(III) stabilizes the ligand and renders the π system planar. This complexation proceeds selectively and rapidly, and the resulting boron(III) complex exhibits strong fluorescence, due to the conjugated π system. Recently, a detection method based on the complexation of an *N*,*O*-type chelating-ligand (10-hydroxybenzo[h]quinolone, HQB) with boronic acids and thier derivatives was reported[8]. This method is convenient, selective, and useful for detecting boronic acid and protected boronic acid containing compounds on a solid support. As such, we were motivated to develop a method to detect boronic acid derivatives in biological environments using chelating-ligands as fluorescent boron-sensors. Accordingly, we designed and synthesized fluorescent sensors for boronic acid derivatives, and elucidated the properties of the sensor-boronic acid complexes.

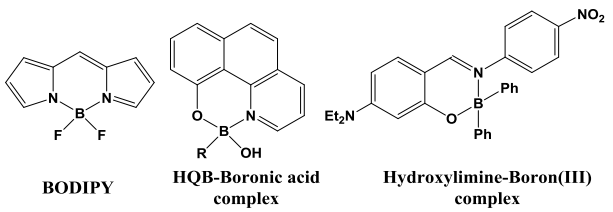
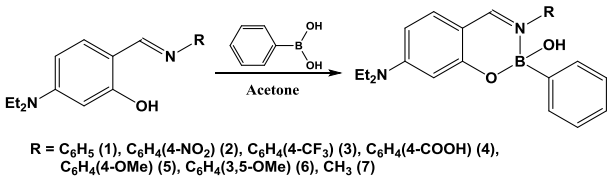


Fig. 2. Boron(III) fluorescent dyes.

In order for a fluorescent sensor to detect boronic acid derivatives in biological environments, the following criteria must be met: i) rapid reaction with boronic acid at room temperature; ii) change in emission

upon complexation with boronic acid; iii) fluoresce in water; iv) stability in water. Based on the aforementioned criteria, we set out to design viable fluorescent boron-sensors. To develop a water-soluble fluorescent boron-sensor, we investigated various *N*,*O*-type chelating-ligands used as precursors for boron(III) fluorescent dyes[9]. In this study, we synthesized *o*-iminophenol type boron-sensors **1**-**7** which were based on a hydroxylimine-boron(III) complex[10].



R = C_6H_5 (1), C_6H_4(4-NO_2) (2), C_6H_4(4-CF_3) (3), C_6H_4(4-COOH) (4), C_6H_4(4-OMe) (5), C_6H_4(3,5-OMe) (6), CH_3 (7)

Fig. 3. Synthesis of fluorescent boron-sensor.

o-Iminophenol type compounds **1**-**7** were synthesized from commercially available compounds in moderate yields, and these compounds reacted rapidly with boron compounds at room temperature (Fig. 3). To confirm the properties of these compounds as boron sensor, we measured the fluorescent spectra of compounds 1-7 and their PhB(OH)2 complexes in acetone (Table 1). Compared to *N*-phenyl type compounds **1**-**6**, the fluorescence intensity of PhB(OH)2 complexes increased (Figure S1a-6b). However, the excitation and fluorescent wavelength of these complexes were not shifted from the corresponding compounds 1-6. Furthermore, the water-solubility of 1-6 was very low, and these compounds did not react with boron compounds in water media.

Table 1. Fluorescence properties of boron-sensors and boron-sensor-PhB(OH)$_2$ complexes[a].

Compd.	Boron-Sensor Only		PhB(OH)$_2$ Complex	
	Ex max	Em max	Ex max	Em max
1	433 nm	559 nm	436 nm	469 nm
2	350 nm	668 nm	350 nm	663 nm
3	397 nm	465 nm	446 nm	474 nm
4	455 nm	479 nm	456 nm	481 nm
5	430 nm	533 nm	434 nm	500 nm
6	437 nm	556 nm	439 nm	473 nm
7	420 nm	481 nm	397 nm	431 nm
7[b]	413 nm	551 nm	408 nm	430 nm

[a] Measured at a concentration of 0.5mM in acetone at 25°C. [b] Measured in 10%DMSO/PBS at 25°C.

In contrast, the fluorescence of *N*-methyl derivative **7** differed significantly from that of the **7**-PhB(OH)$_2$ complex. The excitation and emission wavelengths of **7** were shifted by complexation with the boronic acid compound, and the fluorescence intensity of the **7**-boron complex was 20-fold higher than that of compound **7** alone (Figure S7b). This complex was prepared by only mixing the solution of **7** with the solution of PhB(OH)2 (1.0 eq.) at room temperature, and the fluorescent intensity maximised after 20 min (Fig.4). Furthermore, compound **7** formed a complex with L-BPA or Bortezomib which are clinically used for the treatment of cancer in 10%DMSO/PBS that

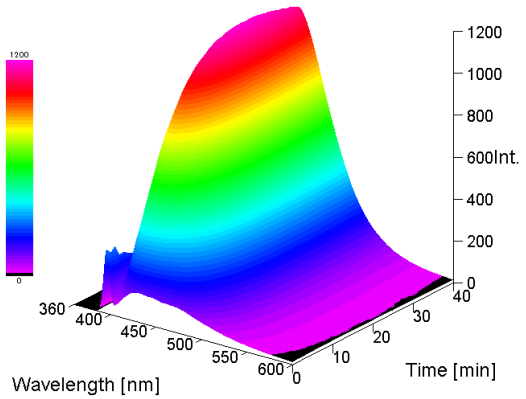


Fig. 4. Time couse of fluorescent spectra of **7**-PhB(OH)$_2$ complex in acetone (1.0mM, excitation wavelength: 413 nm) at 25°C..

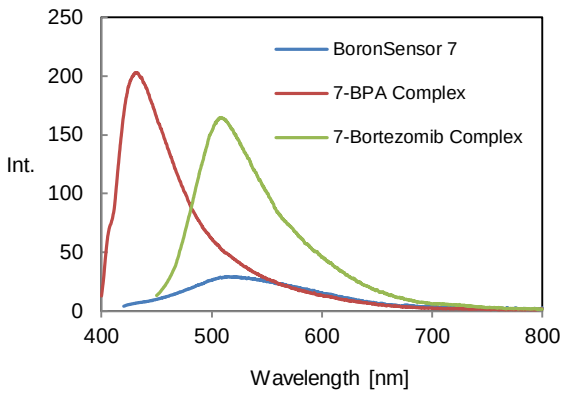


Fig.5. Fluorescent spectra of boron sensor **7** (blue line, ex 413 nm), 7-BPA complex (red line, ex 408 nm) 7-Bortezomib complex (green line, ex 432 nm) in 10% DMSO/PBS (1.0mM, pH 7.4) at 25°C..

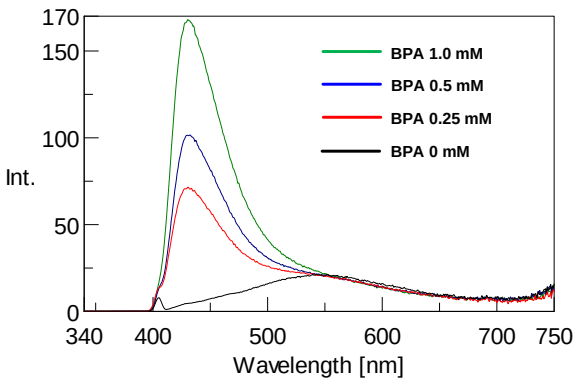


Fig. 6. Fluorescence spectra of L-BPA (0-1.0 mM) stained with the boron sensor **7** (1.0 mM) in 10%DMSO/PBS (pH 7.4, excitation wavelength: 408 nm) at 25°C.

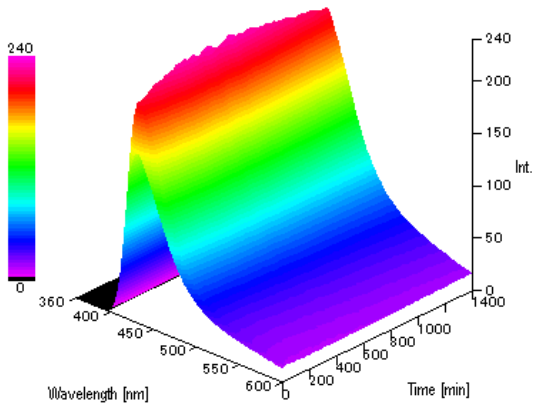


Fig. 7. Time couse of fluorescent spectra of **7**-BPA complex in 10%DMSO/PBS (1.0mM, excitation wavelength: 408 nm) at 25°C..

emitted fluorescence (Fig. 5). Importantly, the emission intensity of the BPA-**7** complex correlated with the concentration of the L-BPA (Fig. 6), and the complex was stable in 10% DMSO/PBS for 24 hour at room temperature (Fig. 7). These results suggest that compound **7** is a useful tool for the detection of boron compounds as a fluorescent sensor.

To estimate the structure of **7**-PhB(OH)$_2$ complex, ^{1}H NMR spectra of **7**, PhB(OH)$_2$ and **7**-PhB(OH)$_2$ complex were compared (Table S1). The chemical shift of all peaks were shifted, especially, *N*-CH$_3$ (boron sensor **7**), *N*-CH (boron sensor **7**) and the proton of PhB(OH)$_2$ were significantly shifted to high magnetic field. This result suggests that fluorescent sensor **7** formed the N-B-O six membered ring structure by the complexation with PhB(OH)$_2$.

Finally, in order to investigate the potential of fluorescent sensor **7** in the detection of boron pharmaceuticals, we stained glioma cells that incorporated L-BPA with **7**. The untreated cells did not emit staining with **7** (Fig. 8a-c); however, in cells treated with L-BPA, the whole of cell fluoresced with a blue colour following by the staining with fluorescent sensor **7** (Fig. 8d-f). L-BPA was widely distributed in the cyto-plasm and cell nuclei, although no regions in the cell exhibited high L-BPA concentrations[11]. These results indicate that fluorescent sensor **7** could be visualized the distribution of L-BPA in tumor cells.

In conclusion, we developed o-iminophenol type fluorescent sensors for boronic acid derivatives **1**-**7** synthesized from commercially available compounds. Fluorescent sensor **7** reacted with boron-pharmaceuticals in 10% DMSO/PBS, and could visualize the L-BPA distribution in tumor cells. These results suggest that boron-sensors may serve as a useful tool for the detection of boron-pharmaceuticals in biological tissues.

Notes and references

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Electronic Supplementary Information (ESI) available: Synthesis, fluorescent spectra, cell culture and cell staining method.. See DOI: 10.1039/c000000x/

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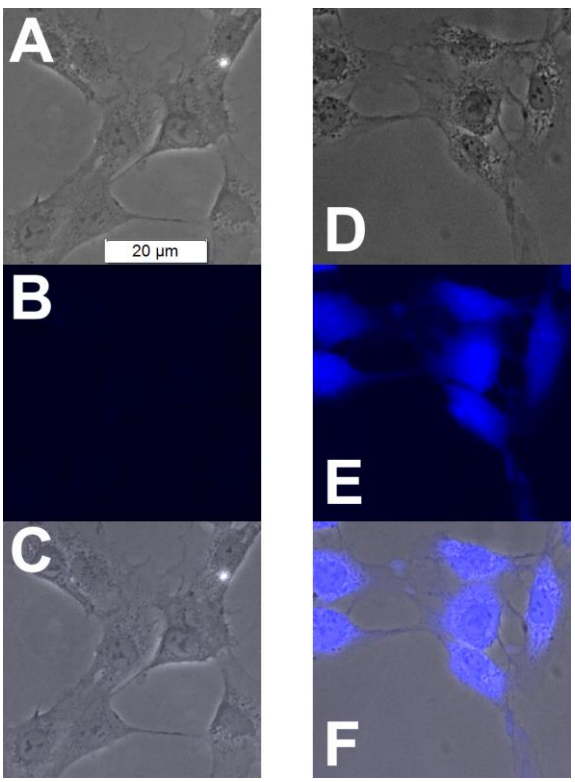

Fig. 8. The micro-distribution of L-BPA in C6 cells. (A) A phase-contrast micrograph of C6 cells that were cultured in DMEM. (B) A fluorescence micrograph of C6 cells that were cultured in DMEM stained with compound **7**. (C) A merged image of A and B. (D) A phase-contrast micrograph of C6 cells that were cultured in DMEM containing L-BPA. (E) A fluorescence micrograph of C6 cells that were cultured in DMEM containing L-BPA stained with the compound **7**. (F) A merged image of D and E.

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