NOVEL TRIVALENT C_3-SYMMETRICAL PHENYLBORONIC ACID PINACOL ESTERS AND THEIR BIOLOGICAL EVALUATION

Makoto Furutachi,a Saho Fuchigami,a Kenta Ako,a Saho Goto,a Toshiaki Gondo,a Mai Takuse,a Moeko Yoshida,a Kazumi Yokomizo,b Jian-Rong Zhou,b Aya Matsunaga,b Nozomi Hiraga,b Nobuhiro Kashige,a Fumio Miake,a and Kunihiro Sumotoa*

aFaculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. bFaculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Nishi-ku, Kumamoto 860-0082, Japan. E-mail: kunihiro@adm.fukuoka-u.ac.jp

Abstract – We report the preparation of newly designed trivalent C_3-symmetrical cyclic phenylboronic acid derivatives constructed on a symmetrical benzene or a cyclohexane ring. The synthesis of these C_3-symmetrical molecules 4 was accomplished by an amide bond formation reaction using amino-substituted phenylboronic acid pinacol esters 2 and C_3-symmetrical benzene-1,3,5-tricarboxylic acid trichloride 3a or cyclohexane-1,3,5-tricarboxylic acid trichloride 3b in the presence of Et_3N. We confirmed that this procedure is conventionally applicable to the preparation of targeted C_3-symmetrical cyclic boronic acid derivatives 4 in good to excellent yields. We also report the results of biological evaluation of the prepared compounds.

Many host receptors that consist of homo-oligometric units (homo-multiligands) often construct symmetric macromolecule architectures such as C_2- or C_3-symmetrical geometry receptor systems. Molecular recognition of two-fold (C_2) or three-fold (C_3) geometric symmetrical macromolecules is one of the common features in several important biological processes.1,2 With reference to the molecular symmetry, small oligovalent molecules having C_2- or C_3-symmetrical geometry have frequently appeared in various biologically active substances.2-4 An oligovalent molecule is generally expected to show enhanced biological potential compared to that of the corresponding monovalent molecule.4 On the other hand, regarding lectin-like carbohydrate recognition molecules, much attention has recently been paid to the design of synthetic receptors for carbohydrates.5 We have been interested in small molecules that
interfere with such carbohydrate recognition stages in order to find new bioactive leads.\textsuperscript{6-8} Regarding carbohydrate (sugar chain) recognition molecules, we have been particularly interested in boronic acid derivatives because many boronic acid functionalities (A) have a property to react with various 1,2-diol functionalities included in carbohydrates and generate cyclic derivatives (B) formed with reversible covalent bonds (see Figure 1).\textsuperscript{9,10} From this point of view, we have recently synthesized a few C\textsubscript{2}-symmetrical cyclic phenylboronic acid esters as new targeted bivalent molecules.\textsuperscript{11,12} Among previously targeted C\textsubscript{2}-symmetrical bivalent phenylboronic acid ester derivatives in this series, we have found an interesting bivalent seed molecule 1 (Figure 1) possessing significant antibacterial and anti-herpes simplex virus type 1 (anti-HSV-1) activities. The identified bivalent C\textsubscript{2}-symmetrical molecule 1 has a C\textsubscript{7}-methylene linker and two \textit{meta}-oriented boronic acid pinacol ester functionalities in the molecule. Compound 1 showed a high level of antibacterial activity (MIC=27.1 µM) against a Gram-positive strain \textit{[Staphylococcus aureus (S. aureus)]} and anti-HSV-1 activity [50\% effective concentration (EC\textsubscript{50})=8.0 µM].\textsuperscript{11}

![Figure 1](image)

As an extension of this study, we carried out further investigations of these symmetrical classes of compounds. In this paper, we report the synthesis of new trivalent C\textsubscript{3}-symmetrical phenylboronic acid derivatives incorporating a benzene or cyclohexane ring as a symmetrical template in the molecules and we report results of biological evaluations of the obtained C\textsubscript{3}-symmetrical phenylboronic acid derivatives. The results for synthesis of new C\textsubscript{3}-symmetrical cyclic boronic acid derivatives constructed on a benzene ring or a cyclohexane ring having three amide bonds are summarized in Tables 1 and 2. The synthesis of these target trivalent C\textsubscript{3}-symmetrical molecules 4aa–4ca constructed on a benzene ring was accomplished by an amide bond formation reaction using amino-substituted phenylboronic acid pinacol esters 2 and benzene-1,3,5-tricarboxylic acid trichloride 3a in the presence of a base such as Et\textsubscript{3}N. The preparation and physical and spectroscopic data of compounds 4aa–4ca have been reported in detail as a separated paper.\textsuperscript{13} New C\textsubscript{3}-symmetrical compounds 4ab and 4bb constructed on a cyclohexane ring were also prepared by a procedure similar to that for compounds 4aa–4ca with cyclohexane-1,3,5-tricarboxylic acid trichloride in good yields (see EXPERIMENTAL). The structures of targeted trivalent C\textsubscript{3}-symmetrical products 4ab and 4bb were confirmed by both spectroscopic data and elemental analysis.\textsuperscript{14,15}
Table 1. Preparation of Trivalent \( C_3 \)-Symmetrical Phenylboronic Acid Pinacol Esters

\[
\begin{align*}
\text{Entry} & \quad \text{Product 4} & \quad \text{Yield (%)}^a & \quad \text{MIC [\( \mu \text{M} \) (\( \mu \text{g/mL} \))] S. aureus} & \quad \text{EC}_{50} \quad (\text{\( \mu \text{M} \))} & \quad \text{CC}_{50} \quad (\text{\( \mu \text{M} \))} & \quad \text{Cytotoxic activity} \\
1 & \quad 4\text{aa} & \quad 91^b & \quad >157.4 \quad (>128) & \quad 157.4 \quad (128) & \quad >100 & \quad 101.0 \\
2 & \quad 4\text{ba} & \quad 81^b & \quad 157.4 \quad (128) & \quad >157.4 \quad (>128) & \quad >100 & \quad >200 \\
3 & \quad 4\text{ca} & \quad 36^b \quad (76^c) & \quad 157.4 \quad (128) & \quad >157.4 \quad (>128) & \quad >100 & \quad >200 \\
\end{align*}
\]

\( ^a \) Isolated yield. \( ^b \) Data were taken from ref.13. \( ^c \) Estimated crude yield.

\( C_3 \)-symmetrical structures of synthesized boronic acid derivatives 4\text{ab} and 4\text{bb} were confirmed by \( ^{13}\text{C} \)-NMR spectroscopic analysis. All \( C_3 \)-symmetrical cyclic phenylboronic acid pinacol esters 4 showed magnetically equivalent carbon signals assignable to a third of the molecules that indicated \( C_3 \)-symmetrical molecular structures in solution, except for the carbon in a phenyl ring connected to a substituent (boronic acid esters). \( ^{14}\) The structures of the \( C_3 \)-symmetrical phenylboronic acid derivatives 4 and the results of evaluation of biological activities (antibacterial and anti-HSV-1 activities) are summarized in Tables 1 and 2. All \( C_3 \)-symmetrical molecules 4\text{aa}–4\text{ca} constructed on a benzene ring were inactive by assays for antibacterial activities [against \( S. \text{ aureus} \) and \( E. \text{ coli} \)] and anti-HSV-1 activities. Only \textit{para}-substituted compound 4\text{aa} showed a low level of cytotoxic activity [50\%
Table 2. Preparation of Trivalent C₃-Symmetrical Phenylboronic Acid Pinacol Esters

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product 4</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MIC [µM (µg/mL)]</th>
<th>Anti-HSV-1 activity</th>
<th>Cytotoxic activity</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td>S. aureus</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4ab</td>
<td>46</td>
<td>156.2 (128)</td>
<td>&gt;156.2 (&gt;128)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2</td>
<td>4bb</td>
<td>77</td>
<td>156.2 (128)</td>
<td>&gt;156.2 (&gt;128)</td>
<td>65.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolated yield.

cytotoxic concentration (CC₅₀)=101.0 µM].

On the other hand, among the C₃-symmetrical compounds constructed on a cyclohexane ring, meta-substituted compound 4bb showed a moderate level of anti-HSV-1 activity (EC₅₀=65.2 µM) with a low level of cytotoxic activity (CC₅₀=>200 µM). The results of biological evaluation of previously prepared compounds 1 with a flexible methylene linker indicated that the length of a linker is important for the expression of biological activities.<sup>11</sup> Regarding the C₃-type molecules described in this paper, a saturated flexible cyclohexane core (compound 4bb) seems to be a preferable structure rather than a rigid unsaturated benzene core for the expression of anti-HSV-1 activity. Trivalent C₃-symmetrical phenylboronic acid pinacol esters 4bb in this study also had an meta-substituted cyclic boronic acid ester group on the phenyl rings. Regarding C₃-type molecules in this study, it is noteworthy that meta-substituted cyclic boronic acid ester (4bb) was again biologically active compound and that compound 4bb with a flexible cyclohexane ring as the C₃-template showed remarkable anti-HSV-1 activity (EC₅₀=65.2 µM) compared to that of compound 4ba with a rigid benzene ring as the C₃-template.
On the basis of the structural information obtained by the above-described modifications for biological activities in the \( C_2 \)- and \( C_3 \)-symmetrical phenylboronic acid ester series together with recent information on other receptor-type molecule series, further molecular modifications to other new types of molecules are now under investigation in order to develop new promising bioactive leads.

**EXPERIMENTAL**

IR spectra were measured on a Shimadzu FT/IR-8100 spectrometer. \(^1\)H- and \(^{13}\)C-NMR spectra were obtained on a JEOL JNM ECZ600R at 25 °C. Chemical shifts are expressed in \( \delta \) ppm relative to the solvent peaks for \(^1\)H-NMR [dimethyl sulfoxide-\( d_6 \) (DMSO-\( d_6 \) (2.50 ppm)] and \(^{13}\)C-NMR [DMSO-\( d_6 \) (39.50 ppm)]. The signal assignments were confirmed by \(^1\)H-\(^1\)H two-dimensional (2D) correlation spectroscopy (COSY), \(^1\)H-\(^{13}\)C heteronuclear multiple-quantum coherence (HMQC), and \(^1\)H-\(^{13}\)C heteronuclear multiple-bond connectivity (HMBC) spectra. High-resolution FAB-MS spectra [HRMS (FAB)] were obtained by a JEOL JMS-700T mass spectrometer.

**Preparation of \( C_3 \)-Symmetrical Cyclic Phenylboronic Acid Pinacol Esters.**

The preparation of and spectroscopic data for the three compounds 4aa–4ca in Table 1 have already been reported.\(^{13}\) New \( C_3 \)-symmetrical compounds 4ab and 4bb constructed on a cyclohexane ring were also prepared by a procedure similar to that for compounds 4aa–4ca with (1s,3s,5s)-cyclohexane-1,3,5-tricarboxylic acid 5 in good yields (46-77%).

\((1s,3s,5s)\)-\( N^1, N^3, N^5 \)-Tris(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclohexane-1,3,5-tricarboxamide (4ab). To a solution of \((1s,3s,5s)\)-cyclohexane-1,3,5-tricarboxylic acid (5) (216.2 mg, 1.00 mmol) in \( CH_2Cl_2 \) (6.00 mL) were added oxalyl chloride (6) (514.1 µL, 6.00 mmol) and DMF (2 drops). The resulting mixture was stirred at reflux for 1 h. After evaporation, \( CH_2Cl_2 \) (16.00 mL), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (2a) (657.3 mg, 3.00 mmol), and Et\( _3N \) (457.5 µL, 3.30 mmol) were successively added to the mixture. The resulting solution was stirred for 18 h at room temperature and then water (ca. 100 mL) was added. The mixture was extracted with \( CH_2Cl_2 \) (x3) and the combined organic extract was dried over \( Na_2SO_4 \). After filtration, the solvents that were used were evaporated under reduced pressure. The obtained crude material was washed with \( CH_2Cl_2/n \)-hexane to give the desired product (4ab) (377.1 mg, 46% yield) as a white solid. Mp >250 °C; IR (KBr) 3450 (NH), 1655 cm\(^{-1}\) (C=O); FAB-MS (positive) \( m/z \) 820 (M+H)\(^+\). HRMS (FAB) Calcd for C\(_{45}H_{61}B_3N_3O_9\)\(^+\): \( m/z \) 820.4681 (M+H)\(^+\). Found: 820.4696; \(^1\)H-NMR (DMSO-\( d_6 \)) \( \delta \) 1.27 (36H, s, CH\(_3\)), 1.66 [3H, dd, \( J = 13.2 \), 25.8 Hz, C(=O)-CH-CH\(_2\)], 1.97-2.05 [3H, m, C(=O)-CH-CH\(_2\)], 2.51-2.60 [3H, m, C(=O)-CH-CH\(_2\)], 7.60 (6H, d, \( J = 8.4 \) Hz, Ar H-3, H-5 in B-C\(_6H_4\)-N), 7.63 (6H, d, \( J = 8.4 \) Hz, Ar H-2, H-6 in B-C\(_6H_4\)-N), 10.01 (3H, s, NH); \(^{13}\)C-NMR (DMSO-\( d_6 \)) \( \delta \) 24.7 (CH\(_3\)), 31.1 [C(=O)-CH-CH\(_2\)], 43.5 [C(=O)-CH-CH\(_2\)], 83.4 (B-O-C-C-O-B), 118.3 (Ar C-2, C-6 in B-C\(_6H_4\)-N), 135.2 (Ar C-3, C-5 in B-C\(_6H_4\)-N), 142.0 (Ar C-1 in

(1s,3s,5s)-N¹,N³,N⁵-Tris(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclohexane-1,3,5-tricarboxamide (4bb). To a solution of (1s,3s,5s)-cyclohexane-1,3,5-tricarboxylic acid (5) (216.2 mg, 1.00 mmol) in CH₂Cl₂ (6.000 mL) were added oxalyl chloride (6) (514.1 µL, 6.00 mmol) and DMF (2 drops). The resulting mixture was stirred at reflux for 1 h. After evaporation, CH₂Cl₂ (16.00 mL), 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (2b) (657.3 mg, 3.00 mmol), and Et₃N (457.5 µL, 3.30 mmol) were successively added to the mixture. The resulting solution was stirred for 18 h at room temperature and then water (ca. 100 mL) was added. The mixture was extracted with CH₂Cl₂ (x3) and the combined organic extract was dried over Na₂SO₄. After filtration, the solvents that were used were evaporated under reduced pressure. The obtained crude material was washed with CH₂Cl₂/n-hexane to give the desired product (4bb) (629.2 mg, 77% yield) as a white solid.

The structure of the product was easily established by spectroscopic data shown below.

Mp >250 °C; IR (KBr) 3444 (NH), 1658 cm⁻¹ (C=O); FAB-MS (positive) m/z 820 (M+H)⁺. HRMS (FAB) Calcd for C₄₅H₆₁B₃N₃O₉⁺: m/z 820.4681 (M+H)⁺. Found: 820.4682; ¹H-NMR (DMSO-d₆) δ 1.27 (36H, s, CH₃), 1.65 [3H, dd, J = 12.6, 25.8 Hz, C(=O)-CH-CH₂], 1.91-2.03 [3H, m, C(=O)-CH-CH₂], 2.43-2.56 [3H, m, C(=O)-CH-CH₂], 7.26-7.35 (6H, m, Ar H-4, H-5 in B-C₆H₄-N), 7.75 (3H, d, J = 6.6 Hz, Ar H-6 in B-C₆H₄-N), 7.91 (3H, s, Ar H-2 in B-C₆H₄-N), 9.87 (3H, s, NH); ¹³C-NMR (DMSO-d₆) δ 24.7 (CH₃), 31.2 [C(=O)-CH-CH₂], 43.6 [C(=O)-CH-CH₂], 83.6 (B-O-C-C-O-B), 122.3 (Ar C-6 in B-C₆H₄-N), 125.4 (Ar C-2 in B-C₆H₄-N), 128.3 (Ar C-5 in B-C₆H₄-N), 129.1 (Ar C-4 in B-C₆H₄-N), 138.8 (Ar C-1 in B-C₆H₄-N), 173.0 (C=O).

Assays for Antibacterial Activity

We used S. aureus ATCC6538P and E. coli NBRC14237 (NIHJ) (Gram-positive and Gram-negative bacteria, respectively) as target organisms. Synthesized compounds were dissolved in DMSO or dimethylformamide (DMF) to a concentration of 1.280 µg/mL. The MIC of a standard strain was measured by the authentic microdilution method to monitor bacterial growth turbidity in Müller-Hinton broth according to the Japanese Society of Chemotherapy. The values of MIC are expressed as molar concentrations (µM) for discussion of structure-activity relations.

Antiviral Activity Assay and Cytotoxicity

We used S. aureus ATCC6538P and E. coli NBRC14237 (NIHJ) (Gram-positive and Gram-negative bacteria, respectively) as target organisms. Synthesized compounds were dissolved in DMSO or dimethylformamide (DMF) to a concentration of 1.280 µg/mL. The MIC of a standard strain was measured by the authentic microdilution method to monitor bacterial growth turbidity in Müller-Hinton broth according to the Japanese Society of Chemotherapy. The values of MIC are expressed as molar concentrations (µM) for discussion of structure-activity relations.
ACKNOWLEDGEMENTS
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REFERENCES AND NOTES
14. In 13C-NMR spectra of compounds 4, we consider that the difficulty in observing the corresponding signal of this quaternary aromatic carbon linked with a boronic acid ester functionality is attributable...
to the quadrupolar relaxation of $^{11}$B.\textsuperscript{16}

15. In the reaction in which ortho-amino-substituted phenylboronic acid pinacol ester was used, the target ortho-substituted C$_3$-type compound 4cb could not be isolated and the reaction provided a complex mixture including a few unknown compounds. Our attempts to isolate the target C$_3$-type ortho-substituted 4cb were unsuccessful. However, the observation of a product showing an amide absorption [3444 (NH) and 1633 cm$^{-1}$ (C=O)] and an ion peak [$m/z$ 820 (M+H)$^+$] indicate formation of the C$_3$-type target compound 4cb.


