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Ichiro Hayakawa,* Akiyuki Ikedo, Takumi Chinen, Takeo Usui, and Hideo Kigoshi*
Department of Chemistry, Graduate School of Pure and Applied Sciences, University of Tsukuba
Graduate School of Life and Environmental Sciences, University of Tsukuba

natural product
glaziovianin A

synthesized analogue
R = \text{Me}: K_{\text{D}} = 0.89 \mu M
R = \text{Br}: K_{\text{D}} = 0.69 \mu M
R = \text{propargyl}: K_{\text{D}} = 0.17 \mu M
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Design, synthesis, and biological evaluation of the analogues of glaziovianin A, a potent antitumor isoflavone

Ichiro Hayakawa,a* Akiyuki Ikedo,a Takumi Chinen,b Takeo Usui,b and Hideo Kigoshi*a*

aDepartment of Chemistry, Graduate School of Pure and Applied Sciences, University of Tsukuba, Tennodai, Tsukuba, 305-8571, Japan
bGraduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba, 305-8572, Japan

1. Introduction

Glaziovianin A (1), isolated from the leaves of the Brazilian tree Ateleia glazioviana, exhibited a broad spectrum of cytotoxicity against a panel of 39 human cancer cell lines (termed JFCR39) at the Japanese Foundation for Cancer Research (Figure 1).1 On the basis of COMPARE analysis, the pattern of the differential cytotoxicities of glaziovianin A (1) suggested that glaziovianin A (1) inhibits cancer cell proliferation by inhibiting tubulin polymerization.2 Indeed, we previously reported that glaziovianin A (1) arrested the cell cycle progression in M phase as do tubulin inhibitors.1 Interestingly, glaziovianin A (1) induced abnormal spindle structures with unaligned chromosomes in mitotic cells, but did not influence on the microtubule network in interphase cells. Although the target molecule(s) or inhibitory mechanism remained to be revealed, these results suggest that glaziovianin A (1) is a novel mitotic inhibitor. Mitotic inhibitors including tubulin polymerization inhibitors have become clinically important drugs, especially against breast cancer.3 Also, glaziovianin A (1) showed antitumor activities in a mouse xenograft model (unpublished data). These results encouraged us to develop novel anticancer drugs based on glaziovianin A (1). A structure–cytotoxicity relationship study of glaziovianin A (1) was preliminarily reported by our group.4 In the present paper, we describe in detail the structure–activity relationships of glaziovianin A, specifically concerning its cytotoxicity and its effects on cell cycle progression and spindle structure. We also describe the synthesis of molecular probes of glaziovianin A for biological studies and target identification.

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ABSTRACT

Various analogues of glaziovianin A, an antitumor isoflavone, were synthesized, and their biological activities were evaluated. O7-Modified glaziovianin A showed strong cytotoxicity against HeLa S3 cells. Compared to glaziovianin A, the O7-benzyl and O7-propargyl analogues were more cytotoxic against HeLa S3 cells and more potent M-phase inhibitors. Furthermore, O7-modified molecular probes of glaziovianin A were synthesized for biological studies.

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Figure 1. Structure of glaziovianin A (1).

We previously synthesized glaziovianin A (1) by using Suzuki–Miyaura coupling as a key step (Scheme 1).1 We used a similar strategy to synthesize glaziovianin A analogues. To develop these analogues, the structure of glaziovianin A (1) can be divided into two structural moieties: an A-ring and a B-ring (Figure 2). Therefore, we synthesized 3-iodochromone analogues as an A-ring and boron compounds as a B-ring.
Results and discussion

2.1. Synthesis of B-ring analogues of glaziovianin A

First, we prepared B-ring analogues of glaziovianin A (Scheme 2). The diol group in 3,6-dimethoxybenzene-1,2-diol (6) was protected as an acetonide group to give compound 7. The bromination of 7 gave a monobromo compound, which was converted into arylboronate 8 by using PdCl₂(dppf), bis(pinacolato)diboron, and KOAc in DMF at 150 °C. The Suzuki–Miyaura coupling of 3-iodo-6,7-dimethoxy-4H-chromen-4-one (3) and boron compounds, such as arylboronate 8, 2,3,4,5-tetramethoxyphenylboronic acid (10), or commercially available 3,4-(methylenedioxy)phenylboronic acid (11), gave glaziovianin A analogues 12–14, respectively. On the other hand, bromide 15 was synthesized from selective bromination at the C6′ position of glaziovianin A (1).

2.2. Synthesis of A-ring analogues of glaziovianin A

Next, we tried to modify the A-ring part of glaziovianin A (Scheme 3). The hydroxy group at the C7 position of 16 was protected as a THP group to afford compound 17. Condensation of 17 with N,N-dimethylformamide dimethyl acetal gave an enamine in quantitative yield. Iodinative cyclization of the enamine afforded iodochromone 18. The cross coupling reaction of arylboronate 55 and iodochromone compounds 18 and 19, gave glaziovianin A analogues 20 and 21, respectively.
Next, we attempted to synthesize glaziovianin A analogues that have various functional groups at the C7 position (Scheme 4). Treatment of compound 20 with p-TsOH·H2O provided O'-demethyl analogue 21, which is a suitable precursor for the synthesis of O'-modified glaziovianin A. Thus, we tried to synthesize glycoside 25 and diol 27 in order to improve water solubility. Glycosylation of the O' position in 22 with tetracetyl glucopyranosyl trichloroacetimidate 23 by using Schmidt glycosylation13 gave tetraacetyl glycosylisoflavone 24. Removal of the acetyl groups of 24 by using NaOMe afforded glycoside 25. Next, allylation of O'-demethyl analogue 22 with allyl bromide gave allyl ether 26. Dihydroxylation of the terminal olefin of 26 afforded diol 27. On the other hand, the hydroxy group at the C7 position in 22 was alkylated into benzyl ether 28 and propargyl ether 29 in order to increase lipid solubility.

**Scheme 3.** Synthesis of glaziovianin A analogues 20 and 21. Reagents and conditions: (a) DHP, PPTS, CH2Cl2, rt, 80%; (b) Me2NCH(OMe)2, 90 °C, quant; (c) I2, py, CHCl3, rt, 75%; (d) NaOMe, MeOH, rt, 91%; (e) allyl bromide, K2CO3, MeCN, rt, 85%; (f) OsO4, py, rt, 84%; (g) benzyl bromide, K2CO3, MeCN, rt, 80%; (h) propargyl bromide, K2CO3, MeCN, rt, 67%.

**Scheme 4.** Synthesis of O'-modified analogues of glaziovianin A. Reagents and conditions: (a) p-TsOH·H2O, MeOH, CHCl3, rt, 85%; (b) 23, BF3·Et2O, MS3A, CH2Cl2, rt, 27% (α : β = 1 : 5.6); (c) NaOMe, MeOH, rt, 91%; (d) allyl bromide, K2CO3, MeCN, rt, 81%; (e) OsO4, py, rt, 84%; (f) benzyl bromide, K2CO3, MeCN, rt, 80%; (g) propargyl bromide, K2CO3, MeCN, rt, 67%.

2.3. Cytotoxicity of glaziovianin A (1) and its analogues against HeLa S3 cells

Table 1 summarizes the cytotoxicity of glaziovianin A (1) and its analogues against HeLa S3 cells and their calculated Log P (cLog P) values.14 Compound 12, which has an acetamide group instead of the methylene acetal group of glaziovianin A (1), showed no cytotoxicity even at 100 μM. Also, compound 13, which has methoxy groups at C3' and C4', instead of the methylene acetal group, was much less cytotoxic than glaziovianin A (1). These results indicated that the steric hindrance of C3' and C4' at the B-ring part reduced the cytotoxicity of glaziovianin A (1) to a large extent. Compound 14, which lacks methoxy groups at C2' and C5', was about 100-fold less cytotoxic than glaziovianin A (1). Also, compound 15, which has a bromo group at C6' of the B-ring, was less cytotoxic than glaziovianin A (1). These results suggested that the electron density and/or steric hindrance of the B-ring might be responsible for cytotoxicity. On the other hand, compound 21, which has an extra methoxy group at C5 of the A-ring, showed no cytotoxicity even at 100 μM, indicating that the steric hindrance and electron density of the A-ring extinguished cytotoxicity. O'-Demethyl analogue 22 exhibited no cytotoxicity, perhaps because of its instability. On the other hand, compounds 24, 25, and 27, which have O'-hydrophilic functional groups, showed no cytotoxicity at 100 μM, and the cLog P values of these compounds, 1.00, 0.08, and 1.13, are much smaller than that of glaziovianin A (1). These results showed that the improvement of water solubility diminishes cytotoxicity. In contrast, compounds 26, 28, and 29, which have, respectively, an allyl, a benzyl, and a propargyl group at O' instead of the methyl group, showed cytotoxicity with IC50 values of 0.46, 0.19, and 0.17 μM, respectively.15 Thus, some alkyl groups at O' had enhanced cytotoxicity against HeLa S3 cells. In particular, O'-propargyl analogue 29 is more active than glaziovianin A (1) itself. These results indicated that the hydrophobicity of the O'-alkyl group in glaziovianin A analogues improves cytotoxicity. However, compound 20, which has a THP group at O', showed no cytotoxicity even at 100 μM. This indicated that steric hindrance of the O'-substituent reduces cytotoxicity to a large extent.
Table 1  Cytotoxicity of glaziovianin A (1) and its analogues against HeLa S3 cells

<table>
<thead>
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<th>compound</th>
<th>cLog P</th>
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<th>relative value</th>
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<td>12</td>
<td>2.79</td>
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<td>24</td>
<td>1.00</td>
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<td>27</td>
<td>1.13</td>
<td>&gt;100</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
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<td>29</td>
<td>2.40</td>
<td>0.17</td>
<td>3.5</td>
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</table>

2.4. Effects of glaziovianin A (1) and O7-alkylated analogues 26, 28, and 29 on cell cycle progression and spindle structures in HeLa S3 cells

We previously reported that glaziovianin A (1) inhibited the cell cycle progression in the M-phase with abnormal spindle structures.1 Here, we investigated the effects of the cytotoxic O7-alkylated analogues 26, 28, and 29 on both cell cycle progression and spindle structures.16,17 As shown in Figures 3A–E, all the analogues inhibited the cell cycle progression in the G2/M phase at a concentration of 1 μM for 24 h treatment. To confirm whether or not these compounds also induce abnormal spindle structures with unaligned chromosomes, as does 1, microtubules and chromosomes in mitotic cells treated with 1 μM O7-alkylated analogues for 6 h were observed (Figures 3F–J). Normal bipolar spindles were observed in DMSO-treated cells, but abnormal multipolar spindles were formed in most of the cells treated with 1 (Figures 3F and 3G, respectively). As expected, analogues 26, 28, and 29 also induced abnormal multipolar spindles, and these structures were resembled with the spindles induced by 1 (Figures 3H–J). Because it is well known that the disruption of a mitotic spindle inhibits mitosis by activating the spindle checkpoint, these results strongly suggested that O7-alkylated analogues inhibited cell cycle progression in the M phase by inducing abnormal spindle formation. Especially, benzyl (28) and propargyl (29) analogues completely arrested cell cycle progression, indicating that these compounds are more potent cell cycle inhibitors than 1 and 26.

Figure 3. Effects of glaziovianin A (1) and O7-alkylated analogues 26, 28, and 29 on cell cycle progression and spindle structures in HeLa S3 cells.

(A–E) O7-alkylated analogues inhibited cell cycle progression in G2/M phase. HeLa S3 cells were treated with DMSO (A), 1 μM of glaziovianin A (1) (B), compound 26 (C), 28 (D), or 29 (E) for 24 h, and DNA contents were determined with flow cytometric analysis.

(F–J) O7-alkylated analogues induced abnormal spindle formation. HeLa S3 cells were treated with DMSO (F), 1 μM of glaziovianin A (1) (G), compound 26 (H), 28 (I), or 29 (J) for 6 h, and microtubules (green) and chromosomes (blue) were stained with anti-α-tubulin antibody (DM1A, Santa Cruz Biotechnology) and DAPI, respectively.
2.5 Synthesis of biotin, fluorescence, and photoaffinity probes of glaziovianin A

In section 2.3, we described that O'-alkylated glaziovianin A analogues showed more potent cytotoxicity against HeLa S3 cells than glaziovianin A itself. So, we next examined the synthesis of O'-modified molecular probes of glaziovianin A for biological studies and target identification. First, we synthesized a biotin probe of glaziovianin A (1) to confirm the target biomolecule, tubulin (Scheme 5). Condensation of mono-Boc-1,6-diaminohexane 30 with 6-benzyloxyhexanoic acid 31 gave amide 32. Removal of the benzyl group of 32 afforded an alcohol, which was converted into bromide 33. The coupling reaction between bromide 33 and O'-demethyl analogue 22, followed by removal of the Boc group gave amine 34 as a TFA salt. Amidation of amine 34 with (+)-biotin N-hydroxysuccinimide ester 35 afforded O'-biotinyl glaziovianin A (36).

Scheme 5. Synthesis of biotin probe of glaziovianin A. Reagents and conditions: (a) DCC, HOBt, DMF, rt, 81%; (b) H2, 5% Pd/C, EtOH, rt, 89%; (c) NBS, PPh3, CH2Cl2, rt, 40%; (d) 22, K2CO3, MeCN, reflux, 34%, recovery of 22: 46%; (e) TFA, CH2Cl2, rt, 62%; (f) 35, Et3N, DMF, rt, 41%.

Next, we synthesized a fluorescent probe for a dynamic analysis of glaziovianin A in living cells, as well as a photoaffinity probe for an analysis of the binding site between glaziovianin A and the target protein. In Scheme 5, the coupling reaction between bromide 33 and O'-demethyl analogue 22 was in low yield, perhaps because of the low reactivity of bromide 33. Therefore, we next tried to employ allylic bromide 42 as a linker (Scheme 6). Reduction of known aldehyde 37 gave allylic alcohol 38, which was protected by a TBS group, thus affording TBS ether 39. Condensation of TBS ether 39 and amine 30 gave amide 40. Removal of the TBS group in 40 afforded allylic alcohol 41, which was converted into allylic bromide 42. The coupling reaction between O'-demethyl analogue 22 and allylic bromide 42 gave the coupling compound 43 in good (72%) yield. Removal of the Boc group in coupling compound 43 gave amine 44 as a TFA salt. Amidation of compound 44 with N-hydroxysuccinimide esters, such as (+)-biotin analogue 35, trifluoromethylidiazirine analogue 48, 22 and BODIPY analogue 49 afforded O'-modified biotin probe 45, photoaffinity probe 46, and fluorescent probe 47, respectively.

Scheme 6. Synthesis of biotin, photoaffinity, and fluorescent probes of glaziovianin A. Reagents and conditions: (a) NaBH4, MeOH, 0 °C, 70%; (b) TBSCl, imidazole, DMF, rt, 91%; (c) 30, toluene, reflux, 69%; (d) TBAF, THF, rt, 86%; (e) NBS, PPh3, CH2Cl2, rt, 64%; (f) 22, K2CO3, MeCN, reflux, 72%, recovery of 22: 14%; (g) TFA, CH2Cl2, rt, 55%; (h) 35, Et3N, DMF, rt, 47% (i) 48, Et3N, MeCN, rt, 49%; (j) 49, Et3N, DMF, rt, 65%.

The cytotoxicities of biotin probes 36 and 45, as well as that of fluorescent probe 47, against HeLa S3 cells are shown in Table 2. Each of these probes was about 30-fold less cytotoxic than glaziovianin A (1). However, they all maintained sufficient cytotoxicities to be used as probes.
Table 2 Cytoxicities of glaziovianin A (1) and the molecular probes of glaziovianin A against HeLa S3 cells

<table>
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<th>compound</th>
<th>IC50 (µM)</th>
<th>relative value</th>
</tr>
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<tbody>
<tr>
<td>glaziovianin A (1)</td>
<td>0.59</td>
<td>1</td>
</tr>
<tr>
<td>biotin probe 36</td>
<td>15.2</td>
<td>0.039</td>
</tr>
<tr>
<td>biotin probe 45</td>
<td>15.5</td>
<td>0.038</td>
</tr>
<tr>
<td>BODIPY probe 47</td>
<td>17.4</td>
<td>0.034</td>
</tr>
</tbody>
</table>

3. Conclusion

In conclusion, we have investigated the structure–cytotoxicity relationships of glaziovianin A (1). The results revealed that O'-modified glaziovianin A analogues have strong cytotoxicity against HeLa S3 cells. Among them, O'-benzyl and O'-propargyl analogues 28 and 29 completely arrested cell cycle progression, indicating that these compounds are more potent cell cycle inhibitors than glaziovianin A (1). Furthermore, we have synthesized O'-modified molecular probes of glaziovianin A for biological studies. Further searches for target biomolecules of glaziovianin A (1) by using these probes are in progress.

4. Experimental section

4.1. Chemistry

General method

1H NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz) or a Bruker AVANCE 500 (500 MHz) spectrometer. Chemical shifts for 1H NMR are reported in parts per million (ppm) downfield from tetramethylsilane as the internal standard, and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. 13C NMR spectra were recorded on a JEOL JNM-EX270 (67.8 MHz) or a Bruker AVANCE 500 (125 MHz) spectrometer. Chemical shifts for 13C NMR are reported in ppm, relative to the central line of a triplet at 77.0 ppm for deuteriochloroform. IR spectra were recorded on a JASCO FT/IR-300 instrument and are reported in wavenumbers (cm⁻¹). IR abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. 1H and 13C NMR spectra were recorded in CDCl₃ or a Bruker AVANCE 500 (500 MHz) spectrometer. Chemical shifts for 1H NMR are reported in parts per million (ppm) downfield from tetramethylsilane as the internal standard, and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. 13C NMR spectra were recorded on a JEOL JNM-EX270 (67.8 MHz) or a Bruker AVANCE 500 (125 MHz) spectrometer. Chemical shifts for 13C NMR are reported in ppm, relative to the central line of a triplet at 77.0 ppm for deuteriochloroform. IR spectra were recorded on a JASCO FT/IR-300 instrument and are reported in wavenumbers (cm⁻¹). IR abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad.

4.1.1. 4,7-Dimethoxy-2,2-dimethylbenzo[d][1,3]dioxole (7)

To a stirred solution of catechol 6 (54.3 mg, 319 µmol) in benzene (2.5 mL) were added PPTS (5.5 mg, 21.9 µmol) and 2-methoxypropene (0.10 mL, 1.04 mmol) at room temperature. After being stirred at reflux for 48 h, the mixture was cooled to room temperature, diluted with EtOAc (5 mL), and washed with 1 M aqueous NaOH (3 mL). The EtOAc solution was washed with brine (3 mL), dried (Na₂SO₄), and concentrated. The residual solid was purified by column chromatography on silica gel (0.8 g, hexane–EtOAc = 40:1) to give acetone 7 (48.6 mg, 72%) as a white solid: IR (CHCl₃) 3010, 2987, 2941, 1612, 1450, 1423, 1055 cm⁻¹; 1H NMR (270 MHz, CDCl₃) δ 6.40 (s, 2H), 3.84 (s, 6H), 1.73 (s, 6H); 13C NMR (67.8 MHz, CDCl₃) δ 120.5 (2C), 118.6 (2C), 117.0 (2C), 106.5, 60.1 (2C), 20.9 (2C); HRMS (ESI) m/z 233.0772, calcld for C₁₁H₁₄NaO₄ [M+Na⁺]+ 233.0784.

4.1.2. 5-Bromo-4,7-dimethoxy-2,2-dimethylbenzo[d][1,3]dioxole (7a)

To a stirred solution of acetone 7 (48.6 mg, 231 µmol) in DMF (2.6 mL) was added NBS (34.9 mg, 196 µmol) at 0 °C. After being stirred at room temperature for 18 h in dark, the mixture was diluted with water (3 mL) and extracted with CH₂Cl₂ (2 mL×3). The combined extracts were washed with brine (5 mL), dried (Na₂SO₄), and concentrated. The residual solid was purified by column chromatography on silica gel (2.0 g, hexane–CH₂Cl₂ = 10:1) to give bromide 7a (38.2 mg, 57%) as a white solid: IR (CHCl₃) 3010, 2987, 2941, 1608, 1450, 1423, 1055 cm⁻¹; 1H NMR (270 MHz, CDCl₃) δ 6.40 (s, 2H), 3.84 (s, 6H), 1.73 (s, 6H); 13C NMR (67.8 MHz, CDCl₃) δ 131.2, 130.7, 128.1, 126.9, 126.4, 115.2, 105.8, 61.5, 59.7, 21.3, 21.2; HRMS (ESI) m/z 310.9889, calcld for C₁₆H₁₂BrNaO₂ [M+Na⁺]+ 310.9889.

4.1.3. 2-(4,7-Dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (8)

To a stirred solution of bis(pinacolato)diraboron (52.5 mg, 207 µmol), PdCl₂(dppf)·CH₂Cl₂ (11.3 mg, 13.8 µmol), and KOAc (54.5 mg, 556 µmol) in DMF (0.60 mL) was added a solution of bromide 7a (39.7 mg, 138 µmol) in DMF (0.50 mL) at room temperature. After being stirred at 150 °C under a stream of N₂ for 3 h, the mixture was cooled to room temperature, diluted with EtOAc (4 mL), and washed with brine (2 mL). The EtOAc solution was dried (Na₂SO₄), filtered through a pad of Florisil, and concentrated. The residual oil was purified by column chromatography on silica gel (0.8 g, hexane–EtOAc = 100:1 → 20:1) to give aryloborane 8 (12.9 mg, 28%) as a white solid: IR (CHCl₃) 3010, 2987, 2941, 1608, 1452, 1435, 1302, 1058 cm⁻¹; 1H NMR (270 MHz, CDCl₃) δ 6.81 (s, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 1.72 (s, 6H); 13C NMR (67.8 MHz, CDCl₃) δ 131.2, 130.7, 128.1, 126.9, 126.4, 115.2, 105.8, 61.5, 59.7, 21.3, 21.2; HRMS (ESI) m/z 359.1646, calcld for C₁₆H₁₂BrNaO₂ [M+Na⁺]+ 359.1636.

4.1.4. 3-(4,7-Dimethoxy-2,2-dimethylbenzo[d][1,3]dioxol-5-yl)-6,7-dimethoxy-4H-chromen-4-one (12)

All solvents were degassed by freeze-thawing. To a stirred solution of aryloborane 8 (12.9 mg, 38.4 µmol) and PdCl₂(dppf)·CH₂Cl₂ (3.1 mg, 3.79 µmol) in 1,4-dioxane (0.25 mL) were added aqueous 1 M Na₂CO₃ (0.19 mL, 190 µmol) and iodochromone 3 (19.1 mg, 57.5 µmol) in 1,4-dioxane (0.30 mL) at room temperature. After being stirred at room temperature under a stream of N₂ for 36 h, the mixture was diluted with EtOAc (2 mL) and filtered through a pad of Florisil. The filtrate was washed with brine (4 mL), dried (Na₂SO₄), and concentrated. The residual solid was purified by column chromatography on silica gel (0.8 g, hexane–EtOAc = 10:1 → 1:1) to give a brown solid (containing Pd-metal) (10.1 mg, 64%). The brown solid (10.1 mg) was dissolved in CHCl₃ (1 mL), and Siliabond (SILICYCLE, SiliBond Thiourea, 100 mg) was added. The...
resulting mixture was stirred at room temperature for 30 min, filtered, and concentrated to give glaziovianin A analogue 12 (10.1 mg, 64%) as a white solid: IR (CHCl3) 3016, 2937, 1634, 1605, 1444, 1278, 1160, 1037 cm⁻¹; 1H NMR (270 MHz, CDCl3) δ 7.92 (s, 1H), 7.62 (s, 1H), 6.87 (s, 1H), 6.49 (s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 1.67 (s, 3H), 1.65 (s, 3H); 13C NMR (67.8 MHz, CDCl3) δ 175.5, 154.0, 150.2, 152.1, 147.4, 139.0, 138.5, 137.0, 136.5, 121.5, 117.9, 117.7, 110.3, 106.4, 104.7, 99.4, 60.2, 56.9, 56.2, 56.0, 20.9, 20.7; HRMS (ESI) m/z 437.1213, calcd for C23H13NaO4 [M+Na⁺] 437.1207.

4.1.5. 2,3,4,5-Tetramethoxyphenylboronic acid (10)

To a stirred solution of tetramethoxybenzene 9 (101 mg, 510 μmol) in THF (1.0 mL) was added n-BuLi (1.63 M solution in hexane, 0.34 mL, 554 μmol) slowly at room temperature. After the mixture was stirred at room temperature for 15 min, B(OMe)3 (0.34 mL, 2.99 mmol) was added into the mixture at −78 °C. The reaction mixture was stirred at room temperature for 3 h, diluted with aqueous 1 M HCl (2.5 mL), stirred at room temperature for 3 h, and extracted with ether (2 mL×3). The combined extracts were washed with brine (2 mL), and concentrated to give a glaziovianin A analogue 11 (138 mg) as a white solid, which was used for the next reaction without further purification.

4.1.6. 6,7-Dimethoxy-3-(2,3,4,5-tetramethoxyphenyl)-4H-chromen-4-one (13)

To a stirred solution of crude boronic acid 10 (41.0 mg, 152 μmol) and PdCl2(dppf)·CH2Cl2 (17.8 mg, 15.4 μmol) in 1,4-dioxane (1.0 mL) were added DHP (102 mg, 513 μmol) and PPTS (3.2 mg, 12.7 μmol) at 0 °C. After being stirred at room temperature for 6 h, the reaction mixture was diluted with water (0.5 mL) and extracted with CH2Cl2 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na2SO4), filtered through a pad of Florisil, and concentrated. The residual oil was purified by column chromatography on silica gel (3.0 g, hexane–EtOAc = 4:1 by volume) to give THP ether 11 (19.3 mg, 80%) as a white solid: IR (CHCl3) 3518, 3014, 2950, 1764, 1610, 1554, 1457, 1375, 1228, 1176, 1138, 1049, 1018, 99.7, 86.4, 60.5, 57.2, 56.4, 52.6; HRMS (ESI) m/z 486.9985, calcd for C30H18BrNaO6 [M+Na⁺] 486.9999.

4.1.8. 3-(6-Bromo-4,7-dimethoxybenzo[d][1,3]dioxol-5-yl)-6,7-dimethoxy-4H-chromen-4-one (15)

To a stirred solution of glaziovianin A 1 (10.2 mg, 26.4 μmol) in DMF (0.25 mL) was added NBS (4.7 mg, 26.6 μmol) at room temperature. After being stirred at room temperature for 6 h, the reaction mixture was diluted with water (0.5 mL) and extracted with CH2Cl2 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na2SO4), and concentrated. The residual oil was purified by column chromatography on silica gel (0.7 g, hexane–EtOAc = 5:1 to 1:1) to give a bromide 15 (3.8 mg, 31%) as a white solid: IR (CHCl3) 3005, 2940, 1635, 1608, 1439, 1274, 1154 cm⁻¹; 1H NMR (270 MHz, CDCl3) δ 7.92, 7.62 (s, 1H), 7.11 (d, J = 1.9 Hz, 1H), 6.98 (dd, J = 8.1, 1.9 Hz, 1H), 6.88 (s, 1H), 6.87 (d, J = 8.1 Hz, 1H), 5.99 (s, 2H), 3.99 (s, 3H), 3.99 (s, 3H); 13C NMR (67.8 MHz, CDCl3) δ 175.2, 154.1, 151.1, 152.2, 147.6, 143.0, 142.2, 139.9, 139.0, 121.8, 121.6, 117.6, 113.8, 104.9, 101.8, 99.5, 86.4, 60.5, 57.2, 56.4, 52.6; HRMS (ESI) m/z 349.0683, calcd for C18H12NaO6 [M+Na⁺] 349.0683.

4.1.9. 1-(2-Hydroxy-5-methoxy-4-(tetrahydro-2H-pyran-2-yl)oxy)phenyl)ethanone (17)

To a stirred solution of phenol 16 (16.5 mg, 90.7 μmol) in CH2Cl2 (0.90 mL) were added DHP (102 μL, 1.12 μmol) and PPTS (3.2 mg, 12.7 μmol) at 0 °C. After being stirred at room temperature for 18 h, the reaction mixture was diluted with saturated aqueous NaHCO3 (1 mL) and extracted with CH2Cl2 (2 mL×3). The combined extracts were washed with brine (2 mL), dried (Na2SO4), and concentrated. The residual solid was purified by column chromatography on silica gel (1.5 g, hexane–EtOAc = 1:1) to give THP ether 17 (19.3 mg, 80%) as a white solid: IR (CHCl3) 3518, 3014, 2950, 1632, 1504, 1372, 1326, 1216, 1183, 117.6, 113.6, 104.9, 101.8, 99.5, 86.4, 60.5, 57.2, 56.4, 52.6; HRMS (ESI) m/z 289.1051, calcd for C16H10BrNaO6 [M+Na⁺] 289.1046.
11.10. (E)-3-(Dimethylamino)-1-(2-hydroxy-5-methoxy-4-(tetrahydro-2H-pyran-2-yl)oxy)prop-2-en-1-one (17a)

THF ether 17 (19.3 mg, 72.6 μmol) was dissolved in N,N-dimethylformamide dimethyl acetate (0.10 mL, 754 μmol). The reaction mixture was stirred at 90 °C for 12 h and concentrated to afford enamine 17a (23.3 mg, quant.) as a yellow solid. Enamine 17a was used for next reaction without further purification: IR (CHCl3) 3689, 2949, 1631, 1541, 1507, 1500, 1496, 1440, 1429, 1387, 1369, 1361, 1237, 1221 cm−1; 1H NMR (270 MHz, CDCl3) δ 7.89 (s, 1H), 7.62 (s, 1H), 6.01 (s, 2H), 5.65 (s, 3H), 4.67 (s, 3H), 3.96 (m, 1H), 3.86 (m, 1H), 3.83 (s, 3H), 2.26–1.97 (m, 4H), 1.72–1.68 (m, 4H); 13C NMR (67.8 MHz, CDCl3) δ 172.2, 156.8, 156.7, 151.7, 148.5, 139.0, 138.8, 136.4, 121.3, 118.4, 118.0, 109.9, 105.3, 104.2, 101.9, 97.1, 62.1, 60.2, 56.9, 56.4, 30.1, 25.1, 18.5; HRMS (ESI) m/z 479.1310, calcld for C21H22NaO3[M+N+]+ 479.1313.

11.11. 3-[(4,7-Dimethoxybenzylidene)[1,3]dioxol-5-yl)-5,6,7-trimethoxy-4H-chromen-4-one (21)

All solvents were degassed by freeze–thawing. To a stirred solution of arylboronate 5 (20.6 mg, 66.9 μmol) and PdCl2(dppf)-CH2Cl2 (5.8 mg, 7.10 μmol) in 1,4-dioxane (0.45 mL) were added aqueous 1 M Na2CO3 (0.32 mL, 320 μmol) and iodochromone 19 (36.5 mg, 101 μmol) in 1,4-dioxane (0.48 mL) at room temperature. After being stirred at room temperature under a stream of N2 for 48 h, the reaction mixture was diluted with EtOAc (2 mL) and filtered through a pad of Florisil. The filtrate was washed with brine (4 mL), dried (Na2SO4), and concentrated. The residual solid was purified by column chromatography on silica gel (1.5 g, hexane–EtOAc = 5:1 → 2:1) to give glaziovianin A analogue 21 (4.5 mg, 16%) as a white solid: IR (CHCl3) 3093, 2933, 1641, 1611, 1508, 1428, 1321, 1157, 1068 cm−1; 1H NMR (270 MHz, CDCl3) 87.88 (s, 1H), 6.71 (s, 1H), 6.52 (s, 1H), 6.01 (s, 2H), 3.56 (s, 3H), 3.95 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H); 13C NMR (67.8 MHz, CDCl3) δ 175.2, 154.7, 153.4, 151.7, 149.7, 148.5, 139.3, 138.9, 136.7, 136.6, 136.1, 121.4, 117.9, 113.1, 109.8, 101.7, 92.5, 60.3, 56.9, 56.6, 56, 54.3; HRMS (ESI) m/z 439.0999, calcld for C21H20NaO5[M+N+]+ 439.1000.

11.12. 3-[(4,7-Dimethoxybenzylidene)[1,3]dioxol-5-yl)-7-hydroxy-6-methoxy-4H-chromen-4-one (22)

To a stirred solution of glaziovianin A analogue 21 (24.0 mg, 52.6 μmol) in CHCl3 (0.50 mL) and MeOH (0.10 mL) was added p-TsOH·H2O (1.1 mg, 5.79 μmol) at 0 °C. After being stirred at room temperature for 3 h, Et3N (0.01 mL) was added. Removal of solvent gave a solid, which was purified by column chromatography on silica gel (0.6 g, hexane–EtOAc = 5:1 → 2:1) to give O′-demethyl analogue 22 (16.7 mg, 85%) as a white solid: IR (CHCl3) 3160, 2915, 2845, 1653, 1599, 1502, 1461, 1288 cm−1; 1H NMR (270 MHz, CDCl3) δ 7.89 (s, 1H), 7.64 (s, 1H), 6.98 (s, 1H), 6.52 (s, 1H), 6.02 (s, 2H), 4.02 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H); (OH proton was not observed); 13C NMR (67.8 MHz, CDCl3) δ 175.3, 153.8, 151.6, 151.8, 148.2, 139.1, 138.8, 137.1, 136.6, 136.1, 121.2, 118.5, 117.6, 109.7, 105.3, 104.0, 102.0, 60.1, 56.9, 56.6, 56.4; HRMS (ESI) m/z 395.0745, calcld for C21H22NaO6[M+N+]+ 395.0737.

11.13. 3,4,5-Tris(methoxy)-2-(Acetoxyethyl)-6-(3-(4,7-dimethoxybenzylidene)[1,3]dioxol-5-yl)-6-methoxy-4-oxo-4H-chromen-7-yl tetrahydro-2H-pyran-3,4,5-triyl triacetate (24)

To a stirred solution of O′-demethyl analogue 22 (24.7 mg, 66.4 μmol) and MS3A (244 mg) in CH2Cl2 (0.50 mL) were added a solution of imidate 23 (65.1 mg, 133 μmol) in CH2Cl2 (0.50 mL) and BF3·Et2O (0.03 mL, 243 μmol) at 0 °C. After being stirred at room temperature for 12 h, the mixture was diluted with water (2 mL) and extracted with CH2Cl2 (2 mL×3). The combined extracts were washed with brine (5 mL), dried (Na2SO4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.7 g, hexane–EtOAc = 4:1 → 2:1) to give tetaacetyl glycosylisoflavone 24 (mixture of anomeric isomers, α-isomer: 1.9 mg, 4.1%; β-isomer: 10.7 mg, 66%).

725.1694, m/z 106.9, 103.4, 101.5, 72.9, 71.8, 71.3, 68.5, 64.2, 62.0, 58.4, 58.2, 21.8, 21.3, 20.8, 20.5; HRMS (ESI) m/z 725.1694, calcd for C33H34NaO17 [M+Na]+ 725.1688.

4.1.16. 3-(4,7-Dimethoxybenzoyl)[1,13]dioxol-5-yl)-6-methoxy-7-(3(R,4S,5S),3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)-4H-chromen-4-one (25)

To a stirred solution of tetracetyl glycosylisoflavone 24 (mixture of anomeric isomers, 4.5 mg, 6.41 μmol) in MeOH (0.09 mL) was added NaOMe (3.5 mg, 69.4 μmol) at 0 °C. After being stirred at room temperature for 1 h, the mixture was diluted with water (1 mL) and extracted with CHCl3 (1 mL×6). The combined extracts were dried (Na2SO4) and concentrated. The residual solid was purified by column chromatography on silica gel (1.0 g) to give diol 26 (4.5 mg, 80%) as a white solid: IR (CHCl3) 3007, 2934, 1638, 1609, 1530, 1470, 1298, 1227, 1153, 1106 cm⁻¹; 1H NMR (270 MHz, CDCl3) δ 7.87 (s, 1H), 7.63 (s, 1H), 7.49–7.19 (m, 5H), 7.08 (s, 1H), 6.52 (s, 1H), 6.03 (s, 2H), 4.28–4.19 (m, 5H), 3.96 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H); 13C NMR (67.8 MHz, CDCl3) δ 175.3, 153.3, 153.1, 151.9, 147.9, 139.0, 138.9, 137.0, 136.7, 135.4, 128.7 (2C), 128.3, 127.2 (2C), 121.6, 118.0, 117.9, 110.0, 105.1, 101.8, 101.3, 71.1, 60.2, 56.9, 56.4; HRMS (ESI) m/z 463.1379, calcd for C26H23O8 [M+Na]+ 463.1387.

4.1.17. 7-(Allyloxy)-3-(4,7-dimethoxybenzoyl)[1,13]dioxol-5-yl)-6-methoxy-4H-chromen-4-one (26)

To a stirred solution of 7'-demethyl analogue 22 (6.1 mg, 16.4 μmol) in MeCN (0.16 mL) were added K2CO3 (4.5 mg, 32.6 μmol) and allyl bromide (2.1 μL, 23.6 μmol) at room temperature. After being stirred at room temperature for 5 h, the mixture was diluted with saturated aqueous NaHCO3 (1 mL) and extracted with CHCl3 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na2SO4) and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, hexane:EtOAc = 1:1→3:1) to give allyl ether 26 (5.5 mg, 81%) as a white solid: IR (CHCl3) 3008, 2938, 1639, 1607, 1036 cm⁻¹; 1H NMR (270 MHz, CDCl3) δ 7.89 (s, 1H), 7.62 (s, 1H), 6.89 (s, 1H), 6.52 (s, 1H), 6.11 (ddt, J = 17.6, 10.5, 5.4 Hz, 1H), 6.02 (s, 2H), 5.48 (ddt, J = 17.6, 14.4, 1.4 Hz, 1H), 5.38 (ddt, J = 10.5, 1.4, 1.4 Hz, 1H), 4.72 (dt, J = 5.4, 1.4 Hz, 2H), 3.98 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H); 13C NMR (67.8 MHz, CDCl3) δ 175.2, 154.4, 153.4, 152.0, 147.8, 139.0, 138.9, 137.0, 136.6, 135.8, 121.5, 118.0, 117.7, 116.5, 110.0, 105.2, 101.8, 100.9, 73.3, 62.1, 57.0, 56.4; HRMS (ESI) m/z 435.1057, calcd for C22H23NaO8 [M+Na]+ 435.1050.

4.1.18. 7-(2,3-Dihydroxypropoxy)-3-(4,7-dimethoxybenzoyl)[1,13]dioxol-5-yl)-6-methoxy-4H-chromen-4-one (27)

To a stirred solution of allyl ether 26 (5.0 mg, 12.1 μmol) in pyridine (0.12 mL) was added OsO4 (0.4 M solution in THF, 0.04 mL, 16.0 μmol) at room temperature. After being stirred at room temperature for 1.5 h, the reaction mixture was diluted with saturated aqueous NaHSO3 (1 mL), stirred at room temperature for 1 h, and extracted with CH2Cl2 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na2SO4) and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, hexane:EtOAc = 1:1→3:1) to give diol 27 (4.5 mg, 84%) as a white solid: IR (CHCl3) 3508, 2968, 1637, 1608, 1505, 1458, 1267, 1101, 1006 cm⁻¹; 1H NMR (270 MHz, CDCl3) δ 7.91 (s, 1H), 7.62 (s, 1H), 6.92 (s, 1H), 6.52 (s, 1H), 6.03 (s, 2H), 4.28–4.19 (m, 5H), 3.96 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), (Signals due to two protons (OH) was not observed); 13C NMR (67.8 MHz, CDCl3) δ 175.3, 154.4, 153.4, 151.8, 147.6, 139.1, 138.9, 137.0, 136.6, 135.8, 121.5, 118.0, 117.7, 116.5, 110.0, 105.2, 101.8, 100.9, 73.2, 62.1, 57.0, 56.4; HRMS (ESI) m/z 435.1089, calcd for C25H22NaO8 [M+Na]+ 469.1105.
To a stirred solution of 6-benzyloxyhexanoic acid 31 (97.3 mg, 450 μmol) in DMF (3.5 mL) were added HOBt (67.3 mg, 499 μmol) and DCC (103 mg, 500 μmol) at 0 °C. After the mixture was stirred at 0 °C for 15 min, mono-Boc-1,6-diaminohexaone 30 (107 mg, 461 μmol) was added at 0 °C. After being stirred at room temperature for 12 h, the mixture was filtered and concentrated. The crude product was diluted with EtOAc (10 mL), washed with saturated aqueous NaHCO3 (5 mL), water (5 mL), saturated aqueous NH4Cl (5 mL), and brine (5 mL). The combined extracts were dried (Na2SO4) and concentrated. The residual oil was purified by column chromatography on silica gel (3.0 g, CHCl3–MeOH = 1:100) to give amide 32 (153 mg, 81%) as a white solid: IR (CHCl3) 3231, 3031, 2981, 1661, 1562, 1529, 1543 cm−1; 1H NMR (270 MHz, CDCl3) δ 7.37–7.26 (m, 5H), 5.76 cm−1; 13C NMR (125 MHz, CDCl3) δ 172.9, 156.1, 138.6, 128.3 (2C), 127.6 (2C), 127.5, 79.0, 72.9, 70.2, 40.2, 39.1, 36.7, 33.9, 30.0, 29.5, 28.4 (3C), 26.2, 26.0, 25.6, 25.5; HRMS (ESI) m/z 443.8280, calculated for C33H53NO4SNa [M+Na]+ 443.8280.

4.1.22. tert-Butyl 6-(6-hydroxyhexanamido)hexylcarbamate (32a)

To a stirred solution of amide 32 (153 mg, 364 μmol) in EtOH (4.0 mL) was added 5% Pd/C (50% water wet, 80.0 mg) at room temperature. After being stirred at room temperature for 12 h under H2, the mixture was filtered through a pad of Celite and concentrated. The residual solid was purified by column chromatography on silica gel (2.5 g, CHCl3–MeOH = 1:100 → 20:1) to give alcohol 32a (107 mg, 89%) as a white solid: IR (CHCl3) 3432, 2975, 1655, 1538 cm−1; 1H NMR (270 MHz, CDCl3) δ 5.98 (br s, 1H), 4.67 (br s, 1H), 3.60 (t, J = 6.5 Hz, 2H), 3.19 (q, J = 6.5 Hz, 2H), 3.07 (q, J = 6.5 Hz, 2H), 2.16 (t, J = 7.2 Hz, 2H), 1.85 (quin, J = 7.0 Hz, 2H), 1.65 (quin, J = 7.3 Hz, 2H), 1.52–1.29 (m, 10H), 1.43 (s, 9H); 13C NMR (67.8 MHz, CDCl3) δ 171.8, 156.2, 79.4, 67.4, 40.5, 39.2, 36.6, 32.0, 29.7, 29.5, 28.9 (3C), 27.5, 26.7, 26.0, 25.5; HRMS (ESI) m/z 415.1659 calculated for C31H33BrN2NaO4 [M+Na]+ 415.1657.

4.1.24. tert-Butyl 6-(3-(4,7-dimethoxybenzoyl)hexanamido)hexylcarbamate (33a)

To a stirred solution of O-demethyl analogue 22 (6.9 mg, 18.5 μmol) and bromide 33 (21.1 mg, 53.8 μmol) in CH2Cl2 (0.27 mL) was added K2CO3 (7.4 mg, 53.6 μmol) at 0 °C. After being stirred at reflux for 18 h, the mixture was diluted with water (1 mL) and extracted with CH2Cl2 (1 mL×3). The combined extracts were washed with brine (1 mL), dried (Na2SO4), and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, hexane–EtOAc = 1:4) to give coupling compound 33a (4.3 mg, 34%) as a white solid, and recovery of O-demethyl analogue 22 (3.2 mg, 46%) as a white solid: IR (CHCl3) 3344, 3020, 2967, 1657, 1600, 1586, 1502, 1288 cm−1; 1H NMR (270 MHz, CDCl3) δ 7.89 (s, 1H), 7.60 (s, 1H), 6.86 (s, 1H), 6.52 (s, 1H), 6.02 (s, 2H), 5.64 (br s, 1H), 4.55 (br s, 1H), 4.10 (t, J = 7.0 Hz, 2H), 3.96 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.24 (q, J = 6.5 Hz, 2H), 3.10 (q, J = 6.5 Hz, 2H), 2.22 (t, J = 7.3 Hz, 2H), 1.95 (quin, J = 7.0 Hz, 2H), 1.80–1.70 (m, 2H), 1.57–1.23 (m, 10H), 1.44 (s, 9H); 13C NMR (67.8 MHz, CDCl3) δ 175.3, 171.6, 156.2, 153.8, 151.8, 151.6, 148.2, 139.1, 138.8, 137.1, 136.6, 121.2, 118.5, 117.6, 109.7, 105.3, 104.0, 102.0, 79.4, 67.4, 60.1, 56.9, 56.6, 40.5, 39.2, 37.5, 36.9, 36.6, 36.0, 32.0, 29.7, 29.5, 28.9 (3C), 25.5; HRMS (ESI) m/z 707.3139, calculated for C39H44N2O4SNa [M+Na]+ 707.3150.

4.1.25. N-(6-Aminoheptyl)-6-(3-(4,7-dimethoxybenzoyl)hexanamido)hexylcarbamate (34)

To a stirred solution of coupling compound 33a (4.3 mg, 6.29 μmol) in CH2Cl2 (0.10 mL) was added TFA (0.05 mL, 67.5 μmol) at 0 °C. The mixture was stirred at room temperature for 4 h and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, CHCl3–MeOH = 10:1 → 1:10) to give amine 34 (2.7 mg, 62%) as a white solid: IR (CHCl3) 3326, 3024, 2980, 1648, 1591, 1554, 1510, 1285 cm−1; 1H NMR (270 MHz, CDCl3) δ 7.89 (s, 1H), 7.57 (s, 1H), 6.85 (s, 1H), 6.50 (s, 1H), 6.20 (br s, 1H), 6.01 (s, 2H), 4.09 (t, J = 5.9 Hz, 2H), 3.94 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.20–3.13 (m, 2H), 2.96–2.90 (m, 2H), 2.20 (t, J = 7.3 Hz, 2H), 1.95–1.89 (m, 2H), 1.74–1.25 (m, 12H), (Signals due to two protons (NH) were not observed); 13C NMR (67.8 MHz, CDCl3) δ 175.2, 171.6, 153.8, 151.8, 151.6, 148.2, 139.1, 138.8, 137.1, 136.6, 121.2, 118.5, 117.6, 109.7, 105.3, 104.0, 102.0, 79.4, 67.4, 60.1, 56.9, 56.6, 40.5, 39.2, 37.5, 36.7, 36.6, 36.0, 32.0, 29.7, 29.5, 25.5; HRMS (ESI) m/z 607.2638 calculated for C31H43N2O4SNa [M+Na]+ 607.2627.

4.1.26. 6-(3-(4,7-Dimethoxybenzoyl)hexanamido)hexylcarbamate (35)

To a stirred solution of amine 34 (2.7 mg, 3.87 μmol) and (+)-bionin N-hydroxysuccinimide ester 35 (2.0 mg, 5.87 μmol) in DMF (0.08 mL) was added Et3N (3.0 μL, 21.6 μmol) at room
To a stirred solution of allylic alcohol 37 (353 mg, 3.77 mmol) in MeOH (15 mL) was added NaBH₄ (171 mg, 4.52 mmol) at 0 °C. After being stirred at 0 °C for 1 h, the mixture was diluted with saturated aqueous NH₄Cl (8 mL) and water (30 mL) and extracted with CH₂Cl₂ (20 mL×3). The combined extracts were washed with brine (5 mL), dried (Na₂SO₄), and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, CHCl₃–MeOH = 1:0 → 10:1) to give allylic alcohol 38 (380 mg, 70%) as a white solid: IR (CHCl₃) 3375, 2968, 1661, 1652, 1568 cm⁻¹; ¹H NMR (270 MHz, CDCl₃) â 5.71–5.68 (m, 2H), 4.34 (dt, J = 5.4, 1.6 Hz, 2H), 3.68 (s, 3H), 2.38 (t, J = 7.6 Hz, 2H), 2.18 (dt, J = 7.6, 5.1 Hz, 2H). (A signal due to one proton (OH) was not observed); ¹³C NMR (125 MHz, CDCl₃) â 173.1, 132.5, 129.6, 74.2, 53.0, 33.5, 29.9; HRMS (ESI) m/z 167.0698, calcd for C₅H₉NaO₃ [M+Na]+ 167.0679.

4.1.29. (E)-tert-Butyl 6-(6-(tert-butyldimethylsiloxy)hex-4-enamido)hexylcarbamate (40)

To a stirred solution of TBS compound 39 (314 mg, 1.22 mmol) in toluene (4.1 mL) was added amine 30 (394 mg, 1.82 mmol) at room temperature. After being stirred at room temperature for 4 h, the mixture was diluted with water (5 mL) and extracted with CH₂Cl₂ (5 mL×3). The combined extracts were washed with brine (5 mL), dried (Na₂SO₄), and concentrated. The residual oil was purified by column chromatography on silica gel (18 g, CHCl₃–MeOH = 1:0 → 100:1) to give amide 40 (371 mg, 69%) as a white solid: IR (CHCl₃) 3375, 2968, 1661, 1652, 1568 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) â 5.92 (br s, 1H), 5.75–5.65 (m, 2H), 4.88 (br s, 1H), 4.21 (dt, J = 6.3, 1.5 Hz, 2H), 3.27 (q, J = 6.0 Hz, 2H), 3.16 (q, J = 6.0 Hz, 2H), 2.22 (t, J = 7.8 Hz, 2H), 2.16 (dt, J = 7.8, 5.8 Hz, 2H), 1.51–1.46 (m, 4H), 1.40 (s, 9H), 1.34–1.30 (m, 4H), 0.89 (s, 9H), 0.07 (s, 6H); ¹³C NMR (125 MHz, CDCl₃) â 173.7, 157.0, 132.0, 128.5, 78.6, 74.6, 41.1, 40.2, 34.0, 29.9, 29.3, 28.1, 28.3 (3C), 25.7, 25.5, 25.2 (3C), 18.4, −4.8 (2C); HRMS (ESI) m/z 465.3117, calcd for C₂₃H₃₇N₂O₄Si [M+Na]+ 465.3119.

4.1.30. (E)-tert-Butyl 6-(6-hydroxyhex-4-enamido)hexylcarbamate (41)

To a stirred solution of TBS ether 40 (360 mg, 814 μmol) in THF (8.1 mL) was added TBAF (276 mg, 1.06 mmol) at room temperature. After being stirred at room temperature for 4 h, the mixture was diluted with saturated NH₄Cl (7 mL) and extracted with CH₂Cl₂ (8 mL×3). The combined extracts were washed with brine (10 mL), dried (Na₂SO₄), and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, CHCl₃–MeOH = 50:1 → 20:1) to give allylic alcohol 41 (230 mg, 86%) as a white solid: IR (CHCl₃) 3362, 2972, 1669, 1649, 1551 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) â 5.81–5.70 (m, 3H), 4.79 (br s, 1H), 4.25 (dt, J = 5.2, 1.9 Hz, 2H), 3.27 (q, J = 6.0 Hz, 2H), 3.19 (q, J = 6.0 Hz, 2H), 2.20 (t, J = 7.5 Hz, 2H), 2.15 (dt, J = 7.5, 5.4 Hz, 2H), 1.51–1.47 (m, 4H), 1.43 (s, 9H), 1.36–1.30 (m, 4H), (A signal due to one proton (OH) was not observed); ¹³C NMR (125 MHz, CDCl₃) â 173.5, 156.9, 132.3, 128.5, 78.4, 74.6, 41.1, 40.0, 33.7, 29.6, 29.3, 29.0, 28.2 (3C), 25.9, 25.6; HRMS (ESI) m/z 351.2281, calcd for C₁₇H₂₃BrN₂O₄Si [M+Na]+ 351.2254.

4.1.31. (E)-tert-Butyl 6-(6-bromohex-4-enamido)hexylcarbamate (42)

To a stirred solution of allylic alcohol 41 (222 mg, 677 μmol) in CH₂Cl₂ (6.8 mL) were added Ph₃P (213 mg, 813 μmol) and NBS (145 mg, 815 μmol) at room temperature. After being stirred at room temperature for 13 h, the mixture was diluted with water (5 mL) and extracted with CH₂Cl₂ (7 mL×3). The combined extracts were washed with brine (10 mL), dried (Na₂SO₄), and concentrated. The residual oil was purified by column chromatography on silica gel (13 g, CHCl₃–MeOH = 1:0 → 100:1) to give allylic bromide 42 (169 mg, 64%) as a white solid: IR (CHCl₃) 3368, 2976, 1660, 1642, 1539, 691 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) â 5.82 (br s, 1H), 5.69–5.65 (m, 2H), 4.69 (br s, 1H), 4.03 (dt, J = 5.7, 1.6 Hz, 2H), 3.26 (q, J = 5.9 Hz, 2H), 3.20 (q, J = 5.6 Hz, 2H), 2.20 (t, J = 7.7, 2H), 2.12 (dt, J = 7.7, 5.5 Hz, 2H), 1.55–1.48 (m, 4H), 1.45 (s, 9H), 1.37–1.29 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) â 173.6, 157.1, 129.7, 126.9, 78.5, 69.2, 41.1, 40.6, 33.8, 29.5, 29.1, 28.7, 28.2 (3C), 26.0, 25.5; HRMS (ESI) m/z 413.1404, calcd for C₁₇H₁₇BrN₂O₄ [M+Na]+ 413.1410.
4.1.32. (E)-tert-Butyl 6-(6-(3-(4,7-dimethoxybenzo[d]1,3)dioxol-5-yl)-6-methoxy-4-oxo-4H-chromen-7-yloxy)hex-4-enamido)hexylcarbamate (43)

To a stirred solution of O'-demethyl analogue 22 (10.3 mg, 27.7 µmol) and allylic bromide 42 (19.2 mg, 58.5 µmol) in MeCN (0.28 mL) was added K₂CO₃ (9.4 mg, 68.1 µmol) at room temperature. After being stirred at reflux for 13 h, the mixture was cooled to room temperature, diluted with saturated NH₄Cl (0.5 mL), and extracted with CH₂Cl₂ (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na₂SO₄), and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, CHCl₃–MeOH = 400:1 → 100:1) to give coupling compound 43 (11.0 mg, 72%) as a white solid, and recovery of phenol 22 (1.4 mg, 14%) as a white solid: IR (CHCl₃) 3356, 3024, 2985, 1676, 1660, 1611, 1575, 1499, 1282 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.90 (s, 1H), 7.60 (s, 1H), 6.85 (s, 1H), 6.52 (s, 1H), 6.00 (s, 2H), 5.86–5.80 (m, 3H), 4.62 (br s, 1H), 4.48 (dt, J = 7.0, 1.8 Hz, 2H), 3.96 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.27 (q, J = 6.3 Hz, 2H), 3.19 (q, J = 6.3 Hz, 2H), 2.22 (t, J = 7.3 Hz, 2H), 2.02 (dt, J = 7.3, 5.2 Hz, 2H), 1.58–1.50 (m, 4H), 1.44 (dt, J = 9.0, 7.8 Hz, 2H), 1.39–1.35 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 175.8, 171.4, 171.1, 153.8, 151.2, 147.7, 138.9, 138.7, 137.1, 136.5, 136.3, 132.5, 121.2, 118.4, 117.1, 109.5, 105.0, 104.0, 79.6, 77.6, 60.0, 56.9, 56.7, 39.8, 39.2, 36.9, 36.0, 31.8, 31.4, 29.4, 28.7 (3C), 25.3; HRMS (ESI) m/z 705.2996, cale[d for C₃₉H₃₃N₂O₃Na [M+Na]⁺ 705.2994.

4.1.33. (E)-N-(6-Amino-2H-thieno[3,4-d]1,3dioxol-5-yl)-6-methoxy-4-oxo-4H-chromen-7-yloxy)hex-4-enamide (44)

To a stirred solution of coupling compound 43 (8.0 mg, 11.7 µmol) in CH₂Cl₂ (0.20 mL) was added TFA (0.010 mL, 135 mol) in MeCN (0.20 mL) was added. The combined extracts were washed with brine (2 mL), dried (Na₂SO₄), and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, CHCl₃–MeOH = 20:1) and by recycle HPLC [JAIGEL-1H-40 (600×20 mm)] to give compound 44 (1.5 mg, 47%) as a white solid: IR (CHCl₃) 3355, 3201, 2985, 1676, 1660, 1611, 1575, 1499, 1282 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 175.8, 171.4, 171.1, 153.8, 151.2, 147.7, 138.9, 138.7, 137.1, 136.5, 136.3, 132.5, 121.2, 118.4, 117.1, 109.5, 105.0, 104.0, 79.6, 77.6, 60.0, 56.9, 56.7, 39.8, 39.2, 36.9, 36.0, 31.8, 31.4, 29.4, 28.7 (3C), 25.3; HRMS (ESI) m/z 831.3245, cale[d for C₄₁H₅₂N₄NaO₁₁S [M+Na]⁺ 831.3245.

4.1.34. (E)-N-(6-(3-(4,7-Dimethoxybenzo[d]1,3dioxol-5-yl)-6-methoxy-4-oxo-4H-chromen-7-yloxy)hex-4-enamido)hexylcarbamate (45)

To a stirred solution of amine 44 (2.1 mg, 3.02 µmol) and 3-trifluoromethyl-3-phenyldiazirine succinimide ester 48 (1.5 mg, 4.59 µmol) in MeCN (0.06 mL) was added Et₂N (20 µL, 0.14 µmol) at room temperature. After being stirred at room temperature for 24 h, the mixture was diluted with brine (0.5 mL) and extracted with CH₂Cl₂ (1 mL×3). The combined extracts were dried (Na₂SO₄) and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, CHCl₃–MeOH = 300:1 → 100:1) to give O'-modified photoaffinity probe 46 (1.2 mg, 49%) as a white solid: IR (CHCl₃) 3355, 3201, 2980, 1672, 1658, 1602, 1578, 1514, 1288 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.98 (d, J = 8.0, 2H), 7.89 (s, 1H), 7.63 (s, 1H), 7.19 (d, J = 8.0 Hz, 2H), 6.86 (s, 1H), 6.51 (s, 1H), 6.30 (br s, 1H), 6.13 (br s, 1H), 6.02 (s, 2H), 5.85–5.79 (m, 2H), 4.47 (dt, J = 7.0, 1.8 Hz, 2H), 3.98 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.37–3.25 (m, 4H), 2.26 (t, J = 7.5 Hz, 2H), 2.05 (dt, J = 7.5, 5.0 Hz, 2H), 1.64–1.55 (m, 4H), 1.38–1.30 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 175.0, 174.4, 171.2, 171.8, 153.8, 152.5, 151.7, 147.3, 139.0, 138.6, 137.2, 136.4, 135.9, 134.9, 132.7, 130.2 (2C), 130.0, 126.7 (2C), 122.9 (q, J = 275 Hz), 121.1, 118.4, 117.2, 107.9, 104.9, 104.1, 102.0, 76.3, 57.0, 56.0, 39.6, 39.3, 36.5, 35.9, 31.7, 31.4, 27.9, 28.4 (q, J = 300 Hz), 25.2; HRMS (ESI) m/z 817.2675, cale[d for C₄₉H₄₇F₃N₃NaO₁₁S [M+Na]⁺ 817.2667.

4.1.35. (E)-N-(6-(3-(4,7-Dimethoxybenzo[d]1,3dioxol-5-yl)-6-methoxy-4-oxo-4H-chromen-7-yloxy)hex-4-enamido)hexylcarbamate (46)

To a stirred solution of amine 44 (1.4 mg, 2.05 µmol) and BODIPY succinimide ester 49 (1.4 mg, 3.25 µmol) in MeCN (0.05 mL) was added Et₂N (1.5 µL, 10.8 µmol) at room temperature. After being stirred at room temperature for 24 h, the mixture was diluted with brine (0.5 mL) and extracted with CH₂Cl₂ (1 mL×3). The combined extracts were dried (Na₂SO₄) and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, CHCl₃–MeOH = 100:1 → 40:1) and by recycle HPLC [JAIGEL-1H-40 (600×20 mm) and
JAIGEL-2H·4O (600×20 mm), flow rate 3.8 mL/min; detection, UV 265 nm; solvent CHCl₃ to give O-modified fluorescent probe 47 (1.2 mg, 65%) as a white solid: IR (CHCl₃) 3358, 3025, 3019, 2982, 2850, 1665, 1653, 1607, 1555, 1294 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.90 (s, 1H), 7.61 (s, 1H), 6.84 (s, 1H), 6.55 (s, 1H), 6.22 (br s, 1H), 6.11 (br s, 1H), 6.06 (s, 2H), 6.02 (s, 2H), 5.80–5.74 (m, 2H), 4.45 (dt, 3J = 7.2, 1.6 Hz, 2H), 3.96 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.28–3.20 (m, 4H), 3.05 (t, 3J = 7.90 Hz, 2H), 2.52 (s, 6H), 2.45 (s, 6H), 2.41 (t, 3J = 7.2, 1.6 Hz, 2H), 2.50 (s, 6H), 2.48 (s, 6H), 2.41 (t, 3J = 7.2, 1.6 Hz, 2H), 2.48 (s, 6H), 2.41 (t, 3J = 7.2, 1.6 Hz, 2H). After washing twice with PBS-B, coverslips were overlaid with Alexa488-conjugated anti-mouse IgG antibody in PBS-B, incubated for 1 h, washed with PBS and mounted with 0.1 µg/mL DAPI solution. The morphology of microtubule and nuclei were observed under a Leica LAS AF 6000 fluorescent microscope (Leica Microsystems GmbH, Wetzlar, Germany). Dilutions of antibodies were 1:250 (antibodies specific for α-tubulin) and 1:2000 (Alexa488-conjugated anti-mouse IgG antibody).

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**Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2011.xxx.xxxx.

**References and notes**

15. Although we previously reported the IC₅₀ values of compounds 26, 28, and 29 to be 0.19, 0.75 and 0.74 µM, respectively, we have reevaluated their cytotoxicities against HeLa S3 cells and found that those IC₅₀ values should be corrected as described in this paper.
17. Although we previously reported the effects of glaziovianin A (1) and compound 26 on cell cycle progression and spindle...
structures, we have reevaluated theirs effects along with compounds 28 and 29 and found that those effects should be corrected as described in this paper.


