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Design, synthesis, and biological evaluation of the analogues of glaziovianin A, a potent antitumor isoflavone

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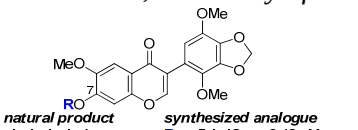

natural product
glaziovianin A
R = Me : IC_{50} = 0.59 μM

synthesized analogue
R = allyl : IC_{50} = 0.46 μM
R = Bn : IC_{50} = 0.19 μM
R = propargyl : IC_{50} = 0.17 μM

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Design, synthesis, and biological evaluation of the analogues of glaziovianin A, a potent antitumor isoflavone

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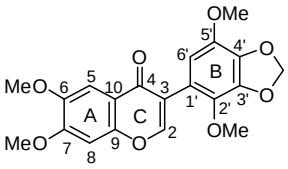
ABSTRACT

Various analogues of glaziovianin A, an antitumor isoflavone, were synthesized, and their biological activities were evaluated. O^7-Modified glaziovianin A showed strong cytotoxicity against HeLa S_3 cells. Compared to glaziovianin A, the O^7-benzyl and O^7-propargyl analogues were more cytotoxic against HeLa S_3 cells and more potent M-phase inhibitors. Furthermore, O^7-modified molecular probes of glaziovianin A were synthesized for biological studies.

1. Introduction

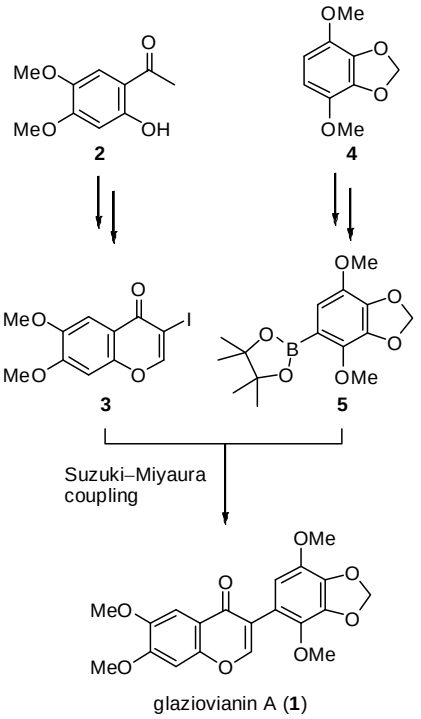
Glaziovianin A (**1**), isolated from the leaves of the Brazilian tree *Ateleia glazioviana*, exhibited a broad spectrum of cytotoxicity against a panel of 39 human cancer cell lines (termed JFCR39) at the Japanese Foundation for Cancer Research (Figure 1).[1] On the basis of COMPARE analysis, the pattern 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.



glaziovianin A (**1**)

Figure 1. Structure of glaziovianin A (**1**).

We previously synthesized glaziovianin A (**1**) by using Suzuki–Miyaura coupling as a key step (Scheme 1).[5] 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.



Scheme 1. Total synthesis of glaziovianin A (**1**) by our group.

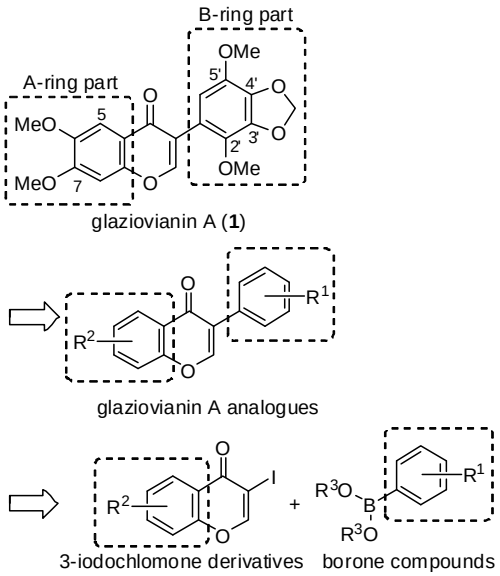
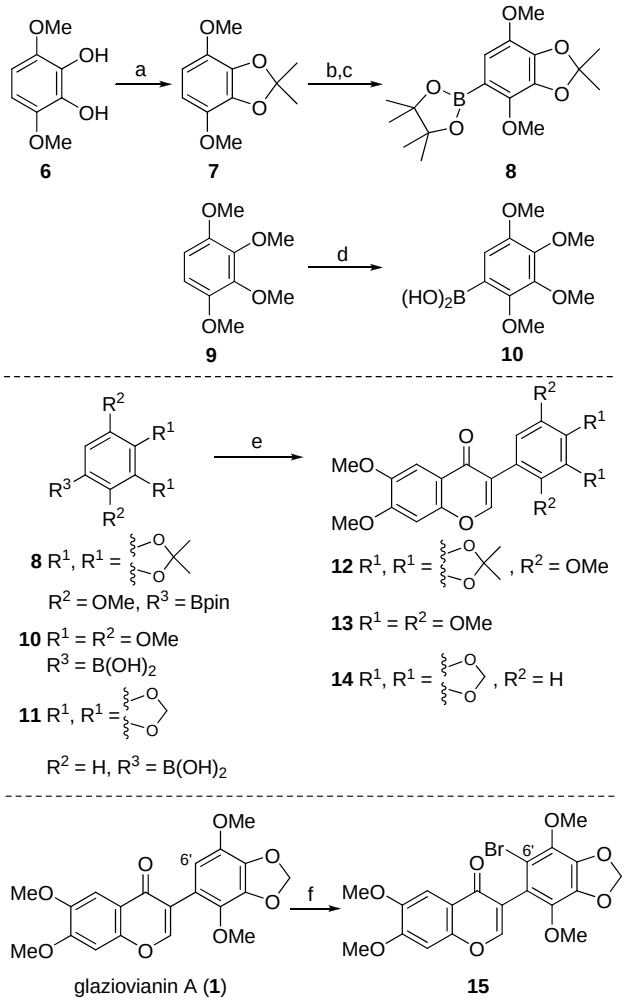


Figure 2. Key structural moieties of glaziovianin A.

2. Results and discussion

2.1. Synthesis of B-ring analogues of glaziovianin A

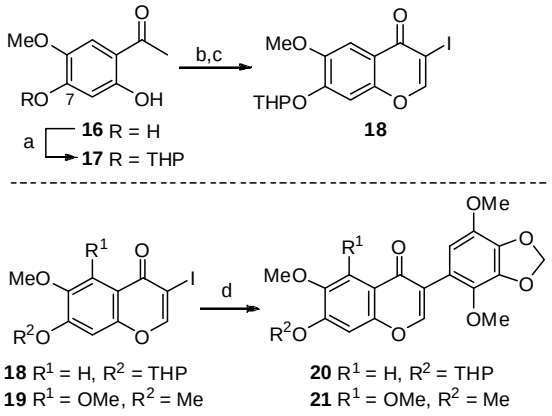
First, we prepared B-ring analogues of glaziovianin A (Scheme 2). The diol group in 3,6-dimethoxybenzene-1,2-diol (**6**)[6] was protected as an acetonide group to give compound **7**. The bromination of **7** gave a monobromo compound, which was converted into arylboronate **8** by using $PdCl_2$(dppf), bis(pinacolato)diboron, and KOAc in DMF at 150 °C.[7] The Suzuki–Miyaura coupling[8] between 3-iodo-6,7-dimethoxy-4*H*-chromen-4-one (**3**)[5] and boron compounds, such as arylboronate **8**, 2,3,4,5-tetramethoxyphenylboronic acid (**10**),[9] 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**).



Scheme 2. Synthesis of B-ring analogues of glaziovianin A. Reagents and conditions: (a) 2-methoxypropene, PPTS, benzene, reflux, 72%; (b) NBS, DMF, rt, 57%; (c) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMF, 150 °C, 28%; (d) *n*-BuLi, $B(OMe)_3$, THF, rt; (e) **3**, $PdCl_2$(dppf), 1 M Na_2CO_3 aq., 1,4-dioxane, rt {64% for **12**, 11% for **13** (from **9**), 41% for **14**}; (f) NBS, DMF, rt, 31%.

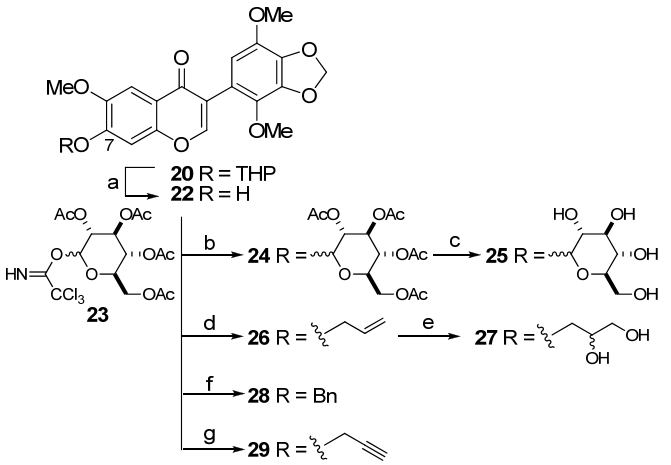
2.2. Synthesis of A-ring analogues of glaziovianin A

Next, we tried to modify the A-ring part of glaziovianin A (Scheme 3). The hydroxy group at the C7 position of **16**[10] 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**.[11] The cross coupling reaction of arylboronate **55** and iodochromone compounds **18** and **19**[12], gave glaziovianin A analogues **20** and **21**, respectively.



Scheme 3. Synthesis of glaziovianin A analogues **20** and **21**. Reagents and conditions: (a) DHP, PPTS, CH_2Cl_2, rt, 80%; (b) $Me_2NCH(OMe)_2$, 90 °C, quant; (c) I_2, py, $CHCl_3$, rt, 75%; (d) **5**, $PdCl_2$(dppf), 1 M Na_2CO_3 aq., 1,4-dioxane, rt (66% for **20**, 16% for **21**)

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·H_2O provided O^7-demethyl analogue **22**, which is a suitable precursor for the synthesis of O^7-modified glaziovianin A. Thus, we tried to synthesize glycoside **25** and diol **27** in order to improve water solubility. Glycosylation of the O^7 position in **22** with tetraacetyl glucopyranosyl trichloroacetimidate **23** by using Schmidt glycosylation[13] gave tetraacetyl glycosylisoflavone **24**. Removal of the acetyl groups of **24** by using NaOMe afforded glycoside **25**. Next, allylation of O^7-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 4. Synthesis of O^7-modified analogues of glaziovianin A. Reagents and conditions: (a) *p*-TsOH·H_2O, MeOH, $CHCl_3$, rt, 85%; (b) **23**, BF_3·Et_2O, MS3A, CH_2Cl_2, rt, 27% (α : β = 1 : 5.6); (c) NaOMe, MeOH, rt, 91%; (d) allyl bromide, K_2CO_3, MeCN, rt, 81%; (e) OsO_4, py, rt, 84%; (f) benzyl bromide, K_2CO_3, MeCN, rt, 80%; (g) propargyl bromide, K_2CO_3, MeCN, rt, 67%.

2.3. Cytotoxicity of glaziovianin A (1) and its analogues against HeLa S_3 cells

Table 1 summarizes the cytotoxicity of glaziovianin A (**1**) and its analogues against HeLa S_3 cells and their calculated Log P (cLog P) values.[14] Compound **12**, which has an acetonide 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^7-Demethyl analogue **22** exhibited no cytotoxicity, perhaps because of its instability. On the other hand, compounds **24**, **25**, and **27**, which have O^7-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^7 instead of the methyl group, showed cytotoxicity with IC_{50} values of 0.46, 0.19, and 0.17 μM, respectively.[15] Thus, some alkyl groups at O^7 had enhanced cytotoxicity against HeLa S_3 cells. In particular, O^7-propargyl analogue **29** is more active than glaziovianin A (**1**) itself. These results indicated that the hydrophobicity of the O^7-alkyl group in glaziovianin A analogues improves cytotoxicity. However, compound **20**, which has a THP group at O^7, showed no cytotoxicity even at 100 μM. This indicated that steric hindrance of the O^7-substituent reduces cytotoxicity to a large extent.

Table 1 Cytotoxicity of glaziovianin A (**1**) and its analogues against HeLa S_3 cells

		cytotoxicity	
compound	cLog P	IC_{50} (μM)	relative value
glaziovianin A (**1**)	2.19	0.59	1
12	2.79	>100	-
13	2.15	22.0	0.027
14	2.44	56.2	0.010
15	3.02	67.8	0.009
20	3.03	>100	-
21	2.06	>100	-
22	1.92	>100	-
24	1.00	>100	-
25	0.08	>100	-
26	2.88	0.46	1.3
27	1.13	>100	-
28	3.92	0.19	3.1
29	2.40	0.17	3.5

2.4. Effects of glaziovianin A (1) and O^7-alkylated analogues 26, 28, and 29 on cell cycle progression and spindle structures in HeLa S_3 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 O^7-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 O^7-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 O^7-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**.

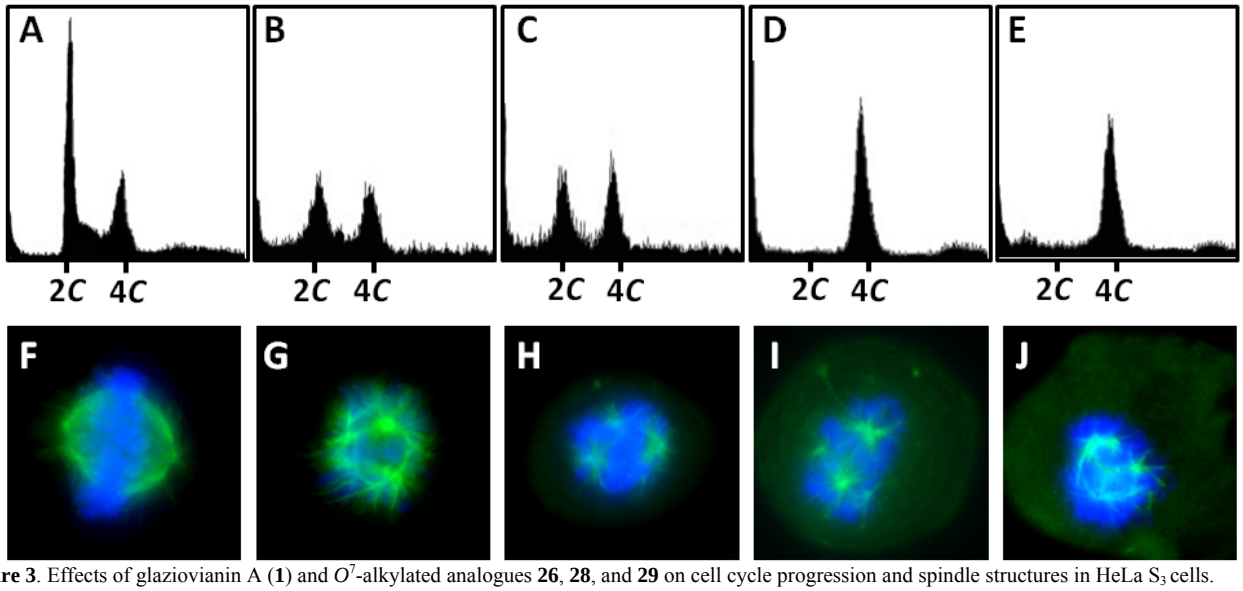


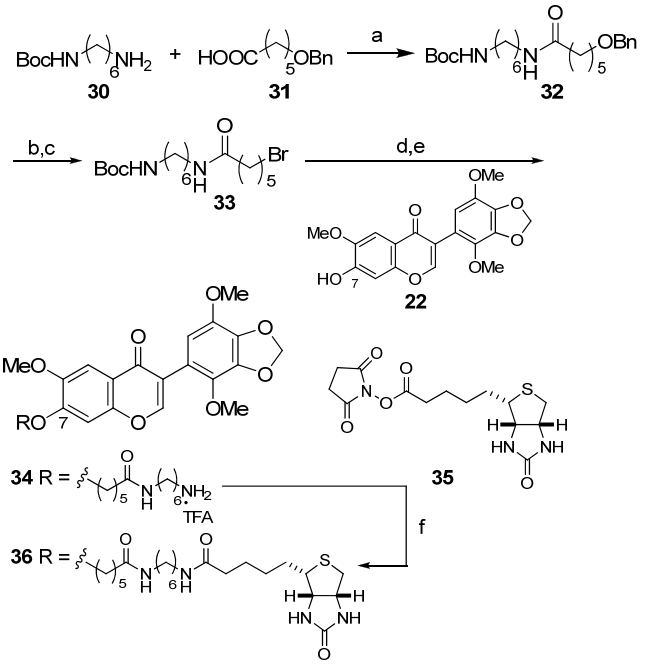
Figure 3. Effects of glaziovianin A (**1**) and O^7-alkylated analogues **26**, **28**, and **29** on cell cycle progression and spindle structures in HeLa S_3 cells.

(A–E) O^7-alkylated analogues inhibited cell cycle progression in G2/M phase. HeLa S_3 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) O^7-alkylated analogues induced abnormal spindle formation. HeLa S_3 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.

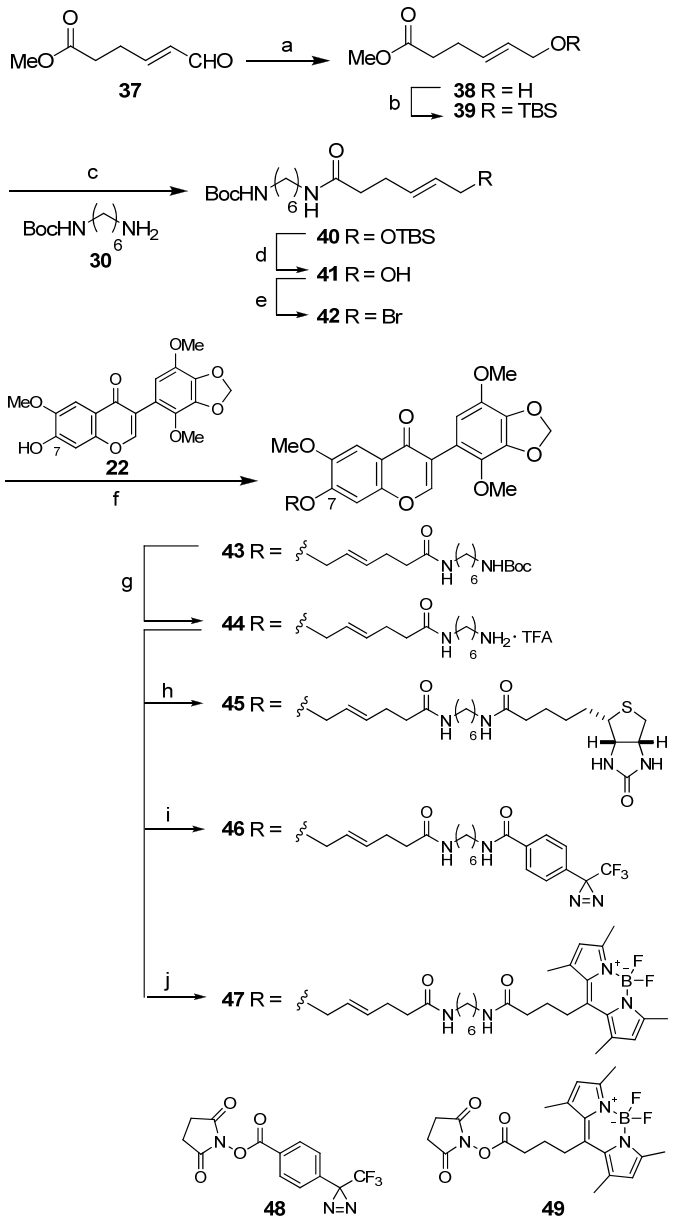
2.5 Synthesis of biotin, fluorescence, and photoaffinity probes of glaziovianin A

In section 2.3, we described that O^7-alkylated glaziovianin A analogues showed more potent cytotoxicity against HeLa S_3 cells than glaziovianin A itself. So, we next examined the synthesis of O^7-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**[18] with 6-benzyloxyhexanoic acid **31**[19] 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^7-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**[20] afforded O^7-biotinyl glaziovianin A (**36**).



Scheme 5. Synthesis of biotin probe of glaziovianin A. Reagents and conditions: (a) DCC, HOBt, DMF, rt, 81%; (b) H_2, 5% Pd/C, EtOH, rt, 89%; (c) NBS, PPh_3, CH_2Cl_2, rt, 40%; (d) **22**, K_2CO_3, MeCN, reflux, 34%, recovery of **22**: 46%; (e) TFA, CH_2Cl_2, rt, 62%; (f) **35**, Et_3N, 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^7-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**[21] 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^7-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**,[20] trifluoromethyldiazirine analogue **48**,[22] and BODIPY analogue **49**,[23] afforded O^7-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) $NaBH_4$, MeOH, 0 °C, 70%; (b) TBSCl, imidazole, DMF, rt, 91%; (c) **30**, toluene, reflux, 69%; (d) TBAF, THF, rt, 86%; (e) NBS, PPh_3, CH_2Cl_2, rt, 64%; (f) **22**, K_2CO_3, MeCN, reflux, 72%, recovery of **22**: 14%; (g) TFA, CH_2Cl_2, rt, 55%; (h) **35**, Et_3N, DMF, rt, 47% (i) **48**, Et_3N, MeCN, rt, 49%; (j) **49**, Et_3N, DMF, rt, 65%.

The cytotoxicities of biotin probes **36** and **45**, as well as that of fluorescent probe **47**, against HeLa S_3 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 Cytotoxicities of glaziovianin A (**1**) and the molecular probes of glaziovianin A against HeLa S_3 cells

	cytotoxicity	
compound	IC_{50} (μM)	relative value
glaziovianin A (**1**)	0.59	1
biotin probe **36**	15.2	0.039
biotin probe **45**	15.5	0.038
BODIPY probe **47**	17.4	0.034

3. Conclusion

In conclusion, we have investigated the structure–cytotoxicity relationships of glaziovianin A (**1**). The results revealed that O^7-modified glaziovianin A analogues have strong cytotoxicity against HeLa S_3 cells. Among them, O^7-benzyl and O^7-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^7-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

^{1}H NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz) or a Bruker AVANCE 500 (500 MHz) spectrometer. Chemical shifts for ^{1}H 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. ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 (67.8 MHz) or a Bruker AVANCE 500 (125 MHz) spectrometer. Chemical shifts for ^{13}C 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}). High resolution ESI mass spectra were recorded on an Applied Biosystems QStar/Pulsar *i* spectrometer. Fuji Silysia silica gel BW-820MH was used for column chromatography. Anhydrous benzene, CH_2Cl_2, MeOH, THF, toluene, and MeCN were used as obtained from commercial supplies for moisture-sensitive reaction. Other organic solvents for moisture-sensitive reactions were distilled by standard procedure. MS3A was heated in a microwave oven for 2 minutes.

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 (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.8 g, hexane–EtOAc = 40:1) to give acetonide **7** (48.6 mg, 72%) as a white solid: IR ($CHCl_3$) 3024, 2987, 2941, 1612, 1450, 1423, 1055 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.40 (s, 2H), 3.84 (s, 6H), 1.73 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 120.5 (2C), 118.6 (2C), 117.0 (2C), 106.5, 60.1 (2C), 20.9 (2C); HRMS (ESI) *m/z* 233.0772, calcd for $C_{11}H_{14}NaO_4$ [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 acetonide **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 CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (2.0 g, hexane–CH_2Cl_2 = 8:1) to give bromide **7a** (38.2 mg, 57%) as a white solid: IR ($CHCl_3$) 3007, 2992, 2941, 1610, 1453, 1430, 1412, 1045, 1026 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.64 (s, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 1.72 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) 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* 310.9899, calcd for $C_{11}H_{13}BrNaO_4$ $[M+Na]^+$ 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)diboron (52.5 mg, 207 μmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (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 N_2 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 (Na_2SO_4), 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 arylboronate **8** (12.9 mg, 28%) as a white solid: IR ($CHCl_3$) 3010, 2987, 2941, 1608, 1452, 1435, 1302, 1058 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.81 (s, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 1.70 (s, 6H), 1.33 (s, 12H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 142.9, 142.5, 139.1, 138.8, 137.7, 114.0, 106.6, 83.1 (2C), 61.4, 57.2, 25.0 (4C), 21.6, 21.4; HRMS (ESI) *m/z* 359.1646, calcd for $C_{17}H_{25}BNaO_6$ $[M+Na]^+$ 359.1636.

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

All solvents were degassed by freeze–thawing. To a stirred solution of arylboronate **8** (12.9 mg, 38.4 μmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (3.1 mg, 3.79 μmol) in 1,4-dioxane (0.25 mL) were added aqueous 1 M Na_2CO_3 (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 N_2 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 (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 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 $CHCl_3$ (1 mL), and SiliaBond (SILICYCLE, SiliaBond 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 ($CHCl_3$) 3016, 2937, 1634, 1605, 1444, 1278, 1160, 1037 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.5, 154.0, 153.2, 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 $C_{22}H_{22}NaO_8$ $[M+Na]^+$ 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 (5 mL) and dried ($MgSO_4$). Removal of the solvent afforded boronic acid **10** (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)-4*H*-chromen-4-one (13)

To a stirred solution of crude boronic acid **10** (41.0 mg, 152 μmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (17.8 mg, 15.4 μmol) in 1,4-dioxane (1.0 mL) were added 1 M Na_2CO_3 aq. (0.75 mL, 750 μmol) and iodochromone **3** (103 mg, 310 μmol) in 1,4-dioxane (1.0 mL) at room temperature. After being stirred at room temperature under a stream of N_2 for 17 h, the reaction mixture was diluted with water (1 mL) and extracted with CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (3 mL), dried (Na_2SO_4), 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 → 2:1) to give a brown solid (containing Pd-metal) (7.0 mg). The brown solid (7.0 mg) was dissolved in $CHCl_3$ (1 mL), and SiliaBond (SILICYCLE, SiliaBond Thiourea, 70 mg) was added. The resulting mixture was stirred at room temperature for 30 min, filtered, and concentrated to give glaziovianin A analogue **13** (7.0 mg, 11% in 2 steps) as a white solid: IR ($CHCl_3$) 3010, 2946, 1637, 1606, 1448, 1297, 1271, 1072 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.62 (s, 1H), 6.88 (s, 1H), 6.67 (s, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.0, 154.4, 153.3, 152.0, 147.4, 139.9, 139.3, 137.8, 137.4, 121.5, 119.0, 117.7, 110.6, 104.8, 99.7, 60.8, 58.7, 57.4, 57.1, 56.4, 56.2; HRMS (ESI) *m/z* 425.1201, calcd for $C_{21}H_{22}NaO_8$ $[M+Na]^+$ 425.1207.

4.1.7. 3-(Benzo[*d*][1,3]dioxol-5-yl)-6,7-dimethoxy-4*H*-chromen-4-one (14)

To a stirred solution of boronic acid **11** (50.4 mg, 152 μmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (17.1 mg, 14.8 μmol) in 1,4-dioxane (1.0 mL) were added 1 M Na_2CO_3 aq. (0.75 mL, 750 μmol) and iodochromone **3** (108 mg, 323 μmol) in 1,4-dioxane (1.0 mL) at room temperature. After being stirred at room temperature under a stream of N_2 for 12 h, the reaction mixture was diluted with water (1 mL) and extracted with CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (3 mL), dried (Na_2SO_4), filtered through a pad of Florisil, and concentrated. The residual oil was purified by column chromatography on silica gel (0.7 g, hexane–$CHCl_3$ = 1:1 → 1:2) to give a brown solid (containing Pd-metal) (21.0 mg). The brown solid (21.0 mg) was dissolved in $CHCl_3$ (3 mL), and SiliaBond (SILICYCLE, SiliaBond Thiourea, 200 mg) was added. The resulting mixture was stirred at room temperature for 30 min, filtered, and concentrated to give a glaziovianin A analogue **14** (20.2 mg, 41%) as a white solid: IR ($CHCl_3$) 3005, 2940, 1635, 1608, 1439, 1274, 1154 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.92 (s, 1H), 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.2, 154.1, 153.1, 152.2, 147.6, 143.0, 142.2, 139.9, 139.0, 121.8, 121.6, 117.6, 113.8, 104.9, 101.8, 99.7, 56.5, 56.3; HRMS (ESI) *m/z* 349.0683, calcd for $C_{18}H_{14}NaO_6$ $[M+Na]^+$ 349.0683.

4.1.8. 3-(6-Bromo-4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6,7-dimethoxy-4*H*-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 CH_2Cl_2 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (0.7 g, hexane–EtOAc = 5:1 → 2:1) to give a bromide **15** (3.8 mg, 31%) as a white solid: IR ($CHCl_3$) 3004, 2937, 1640, 1612, 1505, 1428, 1264, 1155, 1047 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.62 (s, 1H), 6.92 (s, 1H), 6.03 (s, 2H), 4.00 (s, 3H), 3.99 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.2, 154.2, 153.3, 152.0, 147.2, 139.1, 138.7, 137.2, 136.5, 121.6, 118.3, 117.6, 104.9, 101.8, 99.5, 86.4, 60.5, 57.2, 56.4, 56.2; HRMS (ESI) *m/z* 486.9985, calcd for $C_{20}H_{17}BrNaO_8$ $[M+Na]^+$ 486.9999.

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

To a stirred solution of phenol **16** (16.5 mg, 90.7 μmol) in CH_2Cl_2 (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 $NaHCO_3$ (1 mL) and extracted with CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (1.5 g, hexane–EtOAc = 20:1) to give THP ether **17** (19.3 mg, 80%) as a white solid: IR ($CHCl_3$) 3518, 3014, 2950, 1632, 1504, 1372, 1330, 1261, 1228, 1216, 1208 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 12.5 (s, 1H), 7.12 (s, 1H), 6.74 (s, 1H), 5.50 (t, J = 3.0 Hz, 1H), 3.89–3.82 (m, 1H), 3.85 (s, 3H), 3.66–3.62 (m, 1H), 2.56 (s, 3H), 2.01–1.89 (m, 3H), 1.74–1.64 (m, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 201.8, 159.3, 154.2, 142.1, 113.3, 112.3, 104.3, 96.6, 62.1, 57.1, 29.9, 26.2, 24.8, 18.5; HRMS (ESI) *m/z* 289.1051, calcd for $C_{14}H_{18}NaO_5$ $[M+Na]^+$ 289.1046.

4.1.10. (*E*)-3-(Dimethylamino)-1-(2-hydroxy-5-methoxy-4-(tetrahydro-2*H*-pyran-2-yloxy)phenyl)prop-2-en-1-one (17a)

THP ether **17** (19.3 mg, 72.6 μmol) was dissolved in *N*,*N*-dimethylformamide dimethyl acetal (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 ($CHCl_3$) 3689, 2949, 1631, 1541, 1507, 1372, 1284, 1222, 1209, 1108 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 14.0 (s, 1H), 7.84 (d, *J* = 12.4 Hz, 1H), 7.18 (s, 1H), 6.72 (s, 1H), 5.62 (d, *J* = 12.4 Hz, 1H), 5.46 (t, *J* = 3.2 Hz, 1 H), 3.89 (m, 1H), 3.85 (s, 3H), 3.63 (m, 1H), 3.22 (br s, 3H), 3.17 (br s, 3H), 2.01–1.88 (m, 3H), 1.72–1.61 (m, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 190.2, 160.0, 154.0, 152.7, 141.7, 113.6, 112.9, 105.0, 96.8, 89.8, 62.4, 58.2, 40.3 (2C), 30.2, 25.1, 19.0; HRMS (ESI) *m/z* 344.1471, calcd for $C_{17}H_{23}NNaO_5$ $[M+Na]^+$ 344.1474.

4.1.11. 3-Iodo-6-methoxy-7-(tetrahydro-2*H*-pyran-2-yloxy)-4*H*-chromen-4-one (18)

To a stirred solution of enamine **17a** (150 mg, 467 μmol) in $CHCl_3$ (4.7 mL) were added pyridine (0.19 mL, 2.36 mmol) and I_2 (250 mg, 984 μmol) at 0 °C. After being stirred at room temperature for 12 h in dark, the mixture was diluted with saturated aqueous $Na_2S_2O_3$ (5 mL), stirred at room temperature for 10 min, and extracted with EtOAc (5 mL×3). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (8 g, hexane–EtOAc = 10:1 → 4:1) to give iodochromone **18** (140 mg, 75%) as a white solid: IR ($CHCl_3$) 3024, 2999, 1640, 1605, 1541, 1503, 1350, 1219, 1203 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.55 (s, 1H), 7.20 (s, 1H), 5.55 (t, *J* = 2.7 Hz, 1H), 3.95 (s, 3H), 3.91–3.82 (m, 1H), 3.68–3.63 (m, 1H), 2.10–1.87 (m, 3H), 1.77–1.62 (m, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.2, 156.8, 156.7, 151.7, 148.5, 115.6, 105.2, 103.7, 97.1, 86.2, 62.0, 56.3, 29.9, 24.9, 18.3; HRMS (ESI) *m/z* 424.9867, calcd for $C_{15}H_{15}INaO_5$ $[M+Na]^+$ 424.9862.

4.1.12. 3-(4,7-Dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-7-(tetrahydro-2*H*-pyran-2-yloxy)-4*H*-chromen-4-one (20)

All solvents were degassed by freeze–thawing. To a stirred solution of arylboronate **5** (10.1 mg, 32.8 μmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (2.2 mg, 2.69 μmol) in 1,4-dioxane (0.10 mL) were added aqueous 1 M Na_2CO_3 (0.13 mL) and iodochromone **18** (10.4 mg, 25.9 μmol) in 1,4-dioxane (0.22 mL) at room temperature. After being stirred at room temperature under a stream of N_2 for 24 h, the reaction mixture was diluted with water (1 mL) and extracted with CH_2Cl_2 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), filtered through a pad of Florisil, and concentrated. The residual oil was purified by column chromatography on silica gel (0.7 g, hexane–EtOAc = 4:1 → 2:1) to give a brown oil (7.9 mg). The brown oil (7.9 mg) was dissolved in $CHCl_3$ (1 mL), and SiliaBond (SILICYCLE, SiliaBond Thiourea, 80 mg) was added. The resulting mixture was stirred at room temperature for 30 min, filtered, and concentrated to give a glaziovianin A analogue **20** (7.8 mg, 66%) as a white solid: IR ($CHCl_3$) 3007, 2948, 1639, 1607, 1500, 1468, 1430, 1352, 1297 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.62 (s, 1H), 7.22 (s, 1H), 6.52 (s, 1H), 6.01 (s, 2H), 5.56 (s, 1H), 3.96 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 2.06–1.97 (m, 4H), 1.72–1.68 (m, 4H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.4, 153.5, 151.8, 151.4, 148.2, 139.0, 138.8, 136.9, 136.6, 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, calcd for $C_{24}H_{24}NaO_9$ $[M+Na]^+$ 479.1313.

4.1.13. 3-(4,7-Dimethoxybenzo[*d*][1,3]dioxol-5-yl)-5,6,7-trimethoxy-4*H*-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 $PdCl_2(dppf)\cdot CH_2Cl_2$ (5.8 mg, 7.10 μmol) in 1,4-dioxane (0.45 mL) were added aqueous 1 M Na_2CO_3 (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 N_2 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 (Na_2SO_4), 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 ($CHCl_3$) 3013, 2998, 2933, 1646, 1611, 1508, 1428, 1231, 1157, 1068 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ7.88 (s, 1H), 6.71 (s, 1H), 6.52 (s, 1H), 6.01 (s, 2H), 3.96 (s, 3H), 3.95 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.2, 154.7, 153.3, 151.6, 148.9, 142.8, 139.0, 138.7, 136.9, 136.6, 121.4, 117.9, 113.1, 109.8, 101.7, 92.5, 60.3, 56.9, 56.6, 56.4, 56.3; HRMS (ESI) *m/z* 439.0999, calcd for $C_{21}H_{20}NaO_9$ $[M+Na]^+$ 439.1000.

4.1.14. 3-(4,7-Dimethoxybenzo[*d*][1,3]dioxol-5-yl)-7-hydroxy-6-methoxy-4*H*-chromen-4-one (22)

To a stirred solution of glaziovianin A analogue **20** (24.0 mg, 52.6 μmol) in $CHCl_3$ (0.50 mL) and MeOH (0.10 mL) was added *p*-TsOH·H_2O (1.1 mg, 5.79 μmol) at room temperature. After the mixture was stirred at room temperature for 3 h, Et_3N (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 = 1:1 → 1:3) to give O^7-demethyl analogue **22** (16.7 mg, 85%) as a white solid: IR ($CHCl_3$) 3610, 3015, 2945, 1635, 1599, 1502, 1461, 1288 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.3, 153.8, 151.6, 151.8, 148.2, 139.1, 138.8, 137.1, 136.6, 121.2, 118.5, 117.6, 109.7, 105.3, 104.0, 102.0, 60.1, 56.9, 56.6; HRMS (ESI) *m/z* 395.0745, calcd for $C_{19}H_{16}NaO_8$ $[M+Na]^+$ 395.0737.

4.1.15. (3*R*,4*S*,5*R*)-2-(Acetoxymethyl)-6-(3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4-oxo-4*H*-chromen-7-yloxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (24)

To a stirred solution of O^7-demethyl analogue **22** (24.7 mg, 66.4 μmol) and MS3A (244 mg) in CH_2Cl_2 (0.50 mL) were added a solution of imidate **23** (65.1 mg, 133 μmol) in CH_2Cl_2 (0.50 mL) and $BF_3\cdot Et_2O$ (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 CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.7 g, hexane–EtOAc = 4:1 → 2:1) to give tetraacetyl glycosylisoflavone **24** (mixture of anomeric isomers, α-isomer: 1.9 mg, 4.1%; β-isomer: 10.7 mg,

23%) as white solids: **24** (β-isomer): IR ($CHCl_3$) 3009, 2940, 1672, 1667, 1646, 1522, 1434, 1245 cm^{-1}; ^1H NMR (270 MHz, pyridine-d_5) δ 8.19 (s, 1H), 7.84 (s, 1H), 7.57 (s, 1H), 6.77 (s, 1H), 5.99 (s, 2H), 5.92 (d, J = 8.4 Hz, 1H), 4.67–4.45 (m, 6H), 4.00 (s, 3H), 3.80 (s, 3H), 3.70 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (67.8 MHz, pyridine-d_5) δ 173.8, 170.9, 170.4, 169.8, 169.3, 157.8, 153.9, 151.8, 151.4, 140.0, 138.9, 137.8, 136.7, 126.5, 120.7, 118.9, 109.4, 107.1, 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 $C_{33}H_{34}NaO_{17}$ $[M+Na]^+$ 725.1688.

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

To a stirred solution of tetraacetyl 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 $CHCl_3$ (1 mL×6). The combined extracts were dried (Na_2SO_4) and concentrated to give mixture of glycoside **25** (α-isomer: 0.4 mg, 12%; β-isomer 2.7mg, 79%) as white solids: **25** (β-isomer): IR ($CHCl_3$) 3540, 3015, 2944, 1646, 1530, 1456, 1435, 1246 cm^{-1}; ^1H NMR (270 MHz, pyridine-d_5) δ 8.07 (s, 1H), 7.86 (s, 1H), 7.64 (s, 1H), 6.74 (s, 1H), 5.99 (s, 2H), 5.89 (d, J = 7.0 Hz, 1H), 4.67–4.61 (m, 1H), 4.44–4.26 (m, 5H), 3.99 (s, 3H), 3.79 (s, 3H), 3.63 (s, 3H), (Signals due to four protons (OH) were not observed); ^{13}C NMR (67.8 MHz, pyridine-d_5) δ 173.7, 157.5, 153.4, 150.7, 150.4, 141.1, 139.3, 137.7, 136.6, 127.7, 120.9, 115.6, 109.2, 107.1, 106.3, 103.5, 100.9, 74.8, 71.8, 71.4, 68.5, 64.4, 61.9, 58.4, 58.1; HRMS (ESI) *m/z* 557.1253, calcd for $C_{25}H_{26}NaO_{13}$ $[M+Na]^+$ 557.1266.

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

To a stirred solution of O^7-demethyl analogue **22** (6.1 mg, 16.4 μmol) in MeCN (0.16 mL) were added K_2CO_3 (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 $NaHCO_3$ (1 mL) and extracted with CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, hexane–EtOAc = 5:1 → 3:1) to give allyl ether **26** (5.5 mg, 81%) as a white solid: IR ($CHCl_3$) 3008, 2938, 1639, 1607, 1503, 1469, 1430, 1267, 1099, 1063 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) 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, 1.4, 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) 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 $C_{22}H_{20}NaO_8$ $[M+Na]^+$ 435.1050.

4.1.18. 7-(2,3-Dihydroxypropoxy)-3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4*H*-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 OsO_4 (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 $NaHSO_3$ (1 mL), stirred at room temperature for 1 h, and extracted with CH_2Cl_2 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, hexane–EtOAc = 1:1 → 1:4) to give diol **27** (4.5 mg, 84%) as a white solid: IR ($CHCl_3$) 3508, 2968, 1637, 1608, 1505, 1458, 1267, 1101, 1060 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) 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, 73.3, 62.1, 57.0, 56.4; HRMS (ESI) *m/z* 435.1089, calcd for $C_{22}H_{22}NaO_{10}$ $[M+Na]^+$ 469.1105.

4.1.19. 7-(Benzyloxy)-3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4*H*-chromen-4-one (28)

To a stirred solution of O^7-demethyl analogue **22** (6.8 mg, 18.3 μmol) in MeCN (0.18 mL) were added K_2CO_3 (5.2 mg, 37.7 μmol) and benzyl bromide (3.2 μL, 26.9 μmol). After being stirred at room temperature for 5 h, the mixture was diluted with saturated aqueous $NaHCO_3$ (1 mL) and extracted with CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, hexane–EtOAc = 5:1 → 3:1) to give benzyl ether **28** (6.8 mg, 80%) as a white solid: IR ($CHCl_3$) 3007, 2939, 1606, 1470, 1298, 1227, 1153, 1064 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.63 (s, 1H), 7.49–7.19 (m, 5H), 6.90 (s, 1H), 6.51 (s, 1H), 6.02 (s, 1H), 5.27 (s, 2H), 3.99 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) 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 $C_{26}H_{23}O_8$ $[M+H]^+$ 463.1387.

4.1.20. 3-(4,7-Dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-7-(prop-2-ynyloxy)-4*H*-chromen-4-one (29)

To a stirred solution of O^7-demethyl analogue **22** (6.1 mg, 16.4 μmol) in MeCN (0.16 mL) were added K_2CO_3 (4.5 mg, 32.6 μmol) and propargyl bromide (2.1 μL, 23.6 μmol). After being stirred at room temperature for 5 h, the mixture was diluted with saturated aqueous $NaHCO_3$ (1 mL) and extracted with CH_2Cl_2 (2 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, hexane–EtOAc = 5:1 → 3:1) to give propargyl ether **29** (4.5 mg, 67%) as a white solid: IR ($CHCl_3$) 3306, 3008, 2934, 1638, 1609, 1502, 1470, 1431, 1398, 1267, 1231, 1209, 1099 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.64 (s, 1H), 7.08 (s, 1H), 6.52 (s, 1H), 6.03 (s, 2H), 4.90 (d, J = 2.2 Hz, 2H), 3.99 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 2.61 (s, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 175.2, 153.5, 151.7, 147.8, 139.0, 138.9, 137.0, 136.7, 132.0, 121.7, 118.5, 117.9, 110.0, 105.4, 101.8, 101.6, 84.1, 77.2, 60.2, 60.1,

56.9, 56.4; HRMS (ESI) *m/z* 411.1068, calcd for $C_{22}H_{18}NaO_8$ $[M+H]^+$ 411.1074.

4.1.21. *tert*-Butyl 6-(6-(benzyloxy)hexanamido)hexylcarbamate (32)

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-diaminohexane **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 $NaHCO_3$ (5 mL), water (5 mL), saturated aqueous NH_4Cl (5 mL), and brine (5 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The residual oil was purified by column chromatography on silica gel (3.0 g, $CHCl_3$–MeOH = 1:0 → 20:1) to give amide **32** (153 mg, 81%) as a white solid: IR ($CHCl_3$) 3321, 3031, 2981, 1661, 1562, 1559, 1243 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.37–7.26 (m, 5H), 5.76 (br s, 1H), 4.61 (br s, 1H), 4.49 (s, 2H), 3.47 (t, *J* = 6.5 Hz, 2H), 3.21 (dt, *J* = 6.8, 6.5 Hz, 2H), 3.09 (dt, *J* = 6.8, 6.5 Hz, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.71–1.58 (m, 6H), 1.48–1.31 (m, 8H), 1.44 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) 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* 443.2880, calcd for $C_{24}H_{40}N_2NaO_4$ $[M+Na]^+$ 443.2880.

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 H_2, 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, $CHCl_3$–MeOH = 100:1 → 20:1) to give alcohol **32a** (107 mg, 89%) as a white solid: IR ($CHCl_3$) 3342, 2975, 1655, 1558 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) 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), 1.65 (quin, *J* = 7.2 Hz, 2H), 1.57 (quin, *J* = 6.5 Hz, 2H), 1.50–1.41 (m, 6H), 1.44 (s, 9H), 1.37–1.29 (m, 4H), (A signal due to one proton (OH) was not observed); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 156.1, 79.1, 62.5, 40.1, 39.1, 36.6, 32.2, 29.9, 29.4, 28.4 (3C), 26.1, 26.0, 25.3, 25.1; HRMS (ESI) *m/z* 353.2423, calcd for $C_{17}H_{34}N_2NaO_4$ $[M+Na]^+$ 353.2411.

4.1.23. *tert*-Butyl 6-(6-bromohexanamido)hexylcarbamate (33)

To a stirred solution of alcohol **32a** (107 mg, 324 μmol) in CH_2Cl_2 (3.2 mL) were added NBS (69.4 mg, 390 μmol) and Ph_3P (102 mg, 389 μmol) at room temperature. After being stirred at room temperature for 14 h, the mixture was diluted with water (5 mL) and extracted with CH_2Cl_2 (5 mL×3). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (5.0 g, $CHCl_3$–MeOH = 100:1) to give bromide **33** (51.4 mg, 40%) as a white solid: IR ($CHCl_3$) 3327, 2970, 1649, 1553, 676 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 5.88 (br s, 1H), 4.61 (br s, 1H), 3.39 (t, *J* = 7.0 Hz, 2H), 3.21 (q, *J* = 6.5 Hz, 2H), 3.09 (q, *J* = 6.5 Hz, 2 H), 2.17 (t, *J* = 7.3 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) 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* 415.1569 calcd for $C_{17}H_{33}BrN_2NaO_3$ $[M+Na]^+$ 415.1567.

4.1.24. *tert*-Butyl 6-(6-(3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4-oxo-4*H*-chromen-7-yloxy)hexanamido)hexylcarbamate (33a)

To a stirred solution of O^7-demethyl analogue **22** (6.9 mg, 18.5 μmol) and bromide **33** (21.1 mg, 53.8 μmol) in CH_2Cl_2 (0.27 mL) was added K_2CO_3 (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 CH_2Cl_2 (1 mL×3). The combined extracts were washed with brine (1 mL), dried (Na_2SO_4), 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^7-demethyl analogue **22** (3.2 mg, 46%) as a white solid: IR ($CHCl_3$) 3344, 3020, 2967, 1657, 1600, 1568, 1502, 1288 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) 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, calcd for $C_{36}H_{48}N_2NaO_{11}$ $[M+Na]^+$ 707.3150.

4.1.25. *N*-(6-Aminohexyl)-6-(3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4-oxo-4*H*-chromen-7-yloxy)hexanamide (34)

To a stirred solution of coupling compound **33a** (4.3 mg, 6.29 μmol) in CH_2Cl_2 (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, $CHCl_3$–MeOH = 20:1 → 10:1) to give amine **34** (TFA salt; 2.7 mg, 62%) as a white solid: IR ($CHCl_3$) 3326, 3024, 2980, 1648, 1591, 1554, 1510, 1285 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) 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); ^{13}C NMR (67.8 MHz, $CDCl_3$) 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, 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, calcd for $C_{31}H_{40}N_2NaO_9$ $[M+Na]^+$ 607.2626.

4.1.26. 6-(3-(4,7-Dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4-oxo-4*H*-chromen-7-yloxy)-*N*-(6-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexyl)hexanamide (36)

To a stirred solution of amine **34** (2.7 mg, 3.87 μmol) and (+)-biotin *N*-hydroxysuccinimide ester **35** (2.0 mg, 5.87 μmol) in DMF (0.08 mL) was added Et_3N (3.0 μL, 21.6 μmol) at room

temperature. After being stirred at room temperature for 14 h, the mixture was diluted with brine (0.5 mL) and extracted with CH_2Cl_2 (1 mL×3). The combined extracts were dried (Na_2SO_4) and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, $CHCl_3$–MeOH = 50:1 → 20:1) and by recycle HPLC [JAIGEL-1H-40 (600×20 mm) and JAIGEL-2H-40 (600×20 mm), flow rate 3.8 mL/min; detection, UV 265 nm; solvent $CHCl_3$] to give O^7-biotinyl glaziovianin A (**36**) (2.7 mg, 41%) as a white solid: IR ($CHCl_3$) 3340, 3031, 2974, 1719, 1643, 1562, 1510, 1286, 1202 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.56 (s, 1H), 6.85 (s, 1H), 6.52 (s, 1H), 6.31 (br s, 1H), 6.16 (br s, 1H), 6.00 (s, 2H), 5.27 (s, 1H), 5.02 (s, 1H), 4.60–4.53 (m, 1H), 4.39–4.31 (m, 1H), 4.10 (t, *J* = 5.9 Hz, 2H), 3.95 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.25–3.09 (m, 5H), 2.94–2.89 (m, 2H), 2.31 (t, *J* = 7.3 Hz, 2H), 2.22 (t, *J* = 7.3 Hz, 2H), 1.84–1.33 (m, 20H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.8, 175.3, 171.4, 170.0, 153.8, 151.8, 151.5, 148.1, 139.1, 138.6, 137.0, 136.8, 121.2, 118.4, 117.5, 109.7, 105.3, 103.9, 102.1, 67.4, 60.0, 56.9, 56.7, 55.6, 53.9, 45.4, 40.7, 40.5, 39.1, 37.5, 37.0, 36.4, 35.9, 35.5, 32.3, 29.8, 29.5, 27.8, 26.7, 26.4, 25.5; HRMS (ESI) *m/z* 833.3385, calcd for $C_{41}H_{54}N_4NaO_{11}S$ $[M+Na]^+$ 833.3402.

4.1.27. (*E*)-Methyl 6-hydroxyhex-4-enoate (38)

To a stirred solution of aldehyde **37** (535 mg, 3.77 mmol) in MeOH (15 mL) was added $NaBH_4$ (171 mg, 4.52 mmol) at 0 °C. After being stirred at 0 °C for 1 h, the mixture was diluted with saturated aqueous NH_4Cl (8 mL) and water (30 mL) and extracted with CH_2Cl_2 (20 mL×3). The combined extracts were dried (Na_2SO_4) and concentrated. The residual oil was purified by column chromatography on silica gel (12 g, EtOAc–hexane = 3:1) to give allylic alcohol **38** (380 mg, 70%) as a white solid: IR ($CHCl_3$) 3340, 2986, 1739, 1670 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 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); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 132.5, 129.6, 74.2, 53.0, 33.5, 29.9; HRMS (ESI) *m/z* 167.0698, calcd for $C_7H_{12}NaO_3$ $[M+Na]^+$ 167.0679.

4.1.28. (*E*)-Methyl 6-(*tert*-butyldimethylsilyloxy)hex-4-enoate (39)

To a stirred solution of allylic alcohol **38** (370 mg, 2.57 mmol) in DMF (26 mL) were added imidazole (385 mg, 5.65 mmol) and TBSCl (427 mg, 2.83 mmol) at room temperature. After being stirred at room temperature for 2 h, the mixture was diluted with water (10 mL) and extracted with EtOAc (15 mL×3). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (17 g, EtOAc–hexane = 30:1 → 10:1) to give TBS ether **39** (603 mg, 91%) as a white solid: IR ($CHCl_3$) 2978, 1743, 1661 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.72–5.64 (m, 2H), 4.21 (dt, *J* = 6.0, 1.4 Hz, 2H), 3.60 (s, 3H), 2.30 (t, *J* = 7.6 Hz, 2H), 2.16 (dt, *J* = 7.6, 5.2 Hz, 2H), 0.89 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 131.9, 128.5, 74.4, 53.2, 33.0, 29.6, 25.2 (3C), 18.4, –4.8 (2C); HRMS (ESI) *m/z* 281.1537, calcd for $C_{13}H_{26}NaO_3Si$ $[M+Na]^+$ 281.1543.

4.1.29. (*E*)-*tert*-Butyl 6-(6-(*tert*-butyldimethylsilyloxy)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 reflux for 24 h, the mixture was cooled to room temperature, diluted with water (5 mL), and extracted with CH_2Cl_2 (5 mL×3). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (18 g, $CHCl_3$–MeOH = 1:0 → 100:1) to give amide **40** (371 mg, 69%) as a white solid: IR ($CHCl_3$) 3375, 2968, 1661, 1652, 1568 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 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); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7, 157.0, 132.0, 128.5, 78.6, 74.6, 41.1, 40.2, 34.0, 29.9, 29.3, 29.1, 28.3 (3C), 25.7, 25.5, 25.2 (3C), 18.4, –4.8 (2C); HRMS (ESI) *m/z* 465.3117, calcd for $C_{23}H_{46}N_2NaO_4Si$ $[M+Na]^+$ 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 NH_4Cl (7 mL) and extracted with CH_2Cl_2 (8 mL×3). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, $CHCl_3$–MeOH = 50:1 → 20:1) to give allylic alcohol **41** (230 mg, 86%) as a white solid: IR ($CHCl_3$) 3362, 2972, 1669, 1649, 1551 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 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); ^{13}C NMR (125 MHz, $CDCl_3$) δ 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 $C_{17}H_{32}N_2NaO_4$ $[M+Na]^+$ 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 CH_2Cl_2 (6.8 mL) were added Ph_3P (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 CH_2Cl_2 (7 mL×3). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (13 g, $CHCl_3$–MeOH = 1:0 → 100:1) to give allylic bromide **42** (169 mg, 64%) as a white solid: IR ($CHCl_3$) 3368, 2976, 1660, 1642, 1539, 691 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 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 Hz, 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); ^{13}C NMR (125 MHz, $CDCl_3$) δ 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 $C_{17}H_{31}BrN_2NaO_3$ $[M+Na]^+$ 413.1410.

4.1.32. (*E*)-*tert*-Butyl 6-(6-(3-(4,7-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4-oxo-4*H*-chromen-7-yloxy)hex-4-enamido)hexylcarbamate (43)

To a stirred solution of O^7-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 K_2CO_3 (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 NH_4Cl (0.5 mL), and extracted with CH_2Cl_2 (1 mL×3). The combined extracts were washed with brine (2 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, $CHCl_3$–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 ($CHCl_3$) 3356, 3024, 2985, 1676, 1660, 1611, 1575, 1499, 1282 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 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), 2.25 (t, *J* = 7.3 Hz, 2H), 2.02 (dt, *J* = 7.3, 5.2 Hz, 2H), 1.58–1.50 (m, 4H), 1.44 (s, 9H), 1.35–1.29 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.5, 171.4, 156.4, 153.8, 151.8, 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, 102.2, 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, calcd for $C_{36}H_{46}N_2NaO_{11}$ $[M+Na]^+$ 705.2994.

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

To a stirred solution of coupling compound **43** (8.0 mg, 11.7 μmol) in CH_2Cl_2 (0.20 mL) was added TFA (0.010 mL, 135 μmol) at 0 °C. The mixture was stirred at room temperature for 3 h and concentrated. The residual solid was purified by column chromatography on silica gel (0.6 g, $CHCl_3$–MeOH = 20:1 → 10:1) to give amine **44** (TFA salt; 4.4 mg, 55%) as a white solid: IR ($CHCl_3$) 3339, 3030, 2978, 1672, 1654, 1607, 1562, 1510, 1293 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.61 (s, 1H), 6.85 (s, 1H), 6.55 (s, 1H), 6.13 (br s, 1H), 6.01 (s, 2H), 5.82–5.76 (m, 2H), 4.47 (dt, *J* = 7.2, 1.6 Hz, 2H), 3.95 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.27–3.21 (m, 2H), 2.95–2.90 (m, 2H), 2.29 (t, *J* = 7.4 Hz, 2H), 2.07 (dt, *J* = 7.4, 5.4 Hz, 2H), 1.63–1.55 (m, 4H), 1.36–1.30 (m, 4H), (Signals due to two protons (NH) were not observed); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 171.4, 153.8, 152.1, 151.5, 147.3, 138.9, 138.7, 137.1, 136.4, 136.1, 132.7, 121.1, 118.2, 117.1, 109.5, 105.0, 104.0, 102.0, 77.5, 60.3, 56.9, 56.5, 40.1, 39.4, 36.6, 35.8, 31.7, 31.4, 29.4, 25.2; HRMS (ESI) *m/z* 605.2480, calcd for $C_{31}H_{38}N_2NaO_9$ $[M+Na]^+$ 605.2470.

4.1.34. (*E*)-6-(3-(4,7-Dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4-oxo-4*H*-chromen-7-yloxy)-*N*-(6-(5-((3a*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexyl)hex-4-enamide (45)

To a stirred solution of amine **44** (2.8 mg, 4.02 μmol) and (+)-biotin *N*-hydroxysuccinimide ester **35** (2.1 mg, 6.16 μmol) in DMF (0.08 mL) was added Et_3N (2.8 μL, 20.2 μmol) at room temperature. After being stirred at room temperature for 16 h, the mixture was diluted with brine (0.5 mL) and extracted with CH_2Cl_2 (1 mL×3). The combined extracts were dried (Na_2SO_4) and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, $CHCl_3$–MeOH = 50:1 → 20:1) and by recycle HPLC [JAIGEL-1H-40 (600×20 mm) and JAIGEL-2H-40 (600×20 mm), flow rate 3.8 mL/min; detection, UV 265 nm; solvent $CHCl_3$] to give O^7-modified biotin probe **45** (1.5 mg, 47%) as a white solid: IR ($CHCl_3$) 3350, 3020, 2982, 1723, 1673, 1650, 1607, 1574, 1286, 1199 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.60 (s, 1H), 6.82 (s, 1H), 6.56 (s, 1H), 6.15 (br s, 1H), 6.04 (br s, 1H), 6.02 (s, 2H), 5.81–5.76 (m, 2H), 5.23 (s, 1H), 4.97 (s, 1H), 4.62–4.57 (m, 1H), 4.44 (dt, *J* = 7.0, 1.5 Hz, 2H), 4.36–4.32 (m, 1H), 3.96 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.27–3.13 (m, 5H), 2.93–2.88 (m, 2H), 2.33 (t, *J* = 7.2 Hz, 2H), 2.24 (t, *J* = 7.6 Hz, 2H), 2.07 (dt, *J* = 7.6, 5.2 Hz, 2H), 1.87–1.70 (m, 4H), 1.65–1.50 (m, 6H), 1.35–1.29 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.0, 175.8, 171.2, 169.7, 154.0, 152.1, 151.5, 147.5, 139.0, 138.8, 137.1, 136.1, 135.7, 132.8, 121.0, 118.4, 117.1, 109.5, 105.4, 104.1, 101.8, 77.9, 60.1, 56.9, 56.6, 55.4, 54.0, 45.4, 40.5, 39.4, 37.2, 36.6, 35.8, 35.3, 32.0, 31.4, 29.6, 27.7, 26.7, 26.1, 25.5; HRMS (ESI) *m/z* 831.3241, calcd for $C_{41}H_{52}N_4NaO_{11}S$ $[M+Na]^+$ 831.3245.

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

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 Et_3N (2.0 μL, 14.4 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 CH_2Cl_2 (1 mL×3). The combined extracts were dried (Na_2SO_4) and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, $CHCl_3$–MeOH = 300:1 → 100:1) to give O^7-modified photoaffinity probe **46** (1.2 mg, 49%) as a white solid: IR ($CHCl_3$) 3345, 3028, 2980, 1672, 1658, 1602, 1578, 1559, 1514, 1288 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.0 Hz, 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); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 172.4, 171.1, 153.8, 152.5, 151.7, 147.3, 139.0, 138.6, 137.2, 136.4, 135.9, 134.8, 132.7, 130.2 (2C), 130.0, 126.7 (2C), 122.9 (q, $^1J_{C,F}$ = 275 Hz), 121.1, 118.4, 117.2, 109.7, 104.9, 104.1, 102.0, 77.2, 60.3, 57.0, 56.6, 39.6, 39.3, 36.5, 35.9, 31.7, 31.4, 29.5, 28.4 (q, $^2J_{C,F}$ = 50.0 Hz), 25.2; HRMS (ESI) *m/z* 817.2675, calcd for $C_{40}H_{41}F_3N_4NaO_{10}$ $[M+Na]^+$ 817.2667.

4.1.36. (*E*)-10-(4-(6-(6-(3-(4,7-Dimethoxybenzo[*d*][1,3]dioxol-5-yl)-6-methoxy-4-oxo-4*H*-chromen-7-yloxy)hex-4-enamido)hexylamino)-4-oxobutyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:1',2'-*f*][1,3,2]diazaborinin-4-ium-5-uide (47)

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 Et_3N (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 CH_2Cl_2 (1 mL×3). The combined extracts were dried (Na_2SO_4) and concentrated. The residual oil was purified by column chromatography on silica gel (0.6 g, $CHCl_3$–MeOH = 100:1 → 40:1) and by recycle HPLC [JAIGEL-1H-40 (600×20 mm) and

JAIGEL-2H-40 (600×20 mm), flow rate 3.8 mL/min; detection, UV 265 nm; solvent $CHCl_3$] to give O^7-modified fluorescent probe **47** (1.2 mg, 65%) as a white solid: IR ($CHCl_3$) 3358, 3025, 3019, 2982, 2850, 1665, 1653, 1607, 1555, 1294 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 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, *J* = 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, *J* = 8.4 Hz, 2H), 2.52 (s, 6H), 2.45 (s, 6H), 2.41 (t, *J* = 7.0 Hz, 2H), 2.28 (t, *J* = 7.4 Hz, 2H), 2.05–1.98 (m, 4H), 1.65–1.59 (m, 4H), 1.36–1.29 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 173.9, 171.7, 154.3, 153.9, 152.0, 151.2, 147.3, 144.7 (2C), 140.4 (2C), 139.1, 138.7, 137.4, 136.4, 136.0, 132.6, 131.5 (2C), 121.9 (2C), 120.9, 118.2, 117.1, 109.4, 105.1, 104.3, 101.8, 77.8, 60.0, 56.8, 56.5, 40.1, 39.5, 36.4, 35.8, 31.5, 31.2, 30.5, 29.4, 27.4, 26.5, 25.1, 16.3 (2C), 14,4 (2C); HRMS (ESI) *m/z* 921.4025, calcd for $C_{48}H_{57}BF_2N_4NaO_{10}$ $[M+Na]^+$ 921.4028.

4.2. Bioassay

Cell survival was determined by a WST-8 assay kit (Dojindo Laboratories, Kumamoto, Japan). HeLa S_3 cells (3×10^3 cells/well) in 96 well plates were incubated overnight. Then, cells were treated with various concentrations of each compound. After 48 h incubation, 10 μL of WST-8 reagents were added to the culture. After 2 h incubation, the absorbance at 450 nm was measured with iMark microplate reader (BioRad Laboratories, Inc). Absorbance correlates with the number of living cells. The number of living cells (% control) was calculated with the following formula: (each absorbance - absorbance of blank well) / (absorbance of 0 μM well - absorbance of blank well) × 100.

4.3. Cell culture and reagents

Human cervix epidermoid carcinoma HeLa S_3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal-calf serum (FCS, Cell Culture Bioscience). Cells were grown at 37 ºC in a 5% CO_2 atmosphere. Antibodies specific for α-tubulin (DM1A, #sc-32293) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Alexa488-conjugated anti-mouse IgG antibody (#A11001) was purchased from Invitrogen. DAPI solution was purchased from Wako Pure Chemical Industry (Osaka, Japan).

4.3.1. Analysis of cell cycle progression

3×10^4 cells/mL HeLa S_3 cells were treated with various concentrations of each compound for 24 h, then harvested and fixed with 70% EtOH (–20 ºC). After staining with propidium iodide solution, DNA contents were measured by flow cytometric analysis (Attune® Acoustic Focusing Cytometer; Applied Biosystems).

4.3.2. Immunofluorescence procedures

3×10^4 cells/mL HeLa S_3 cells were seeded on sterile coverslips. After treatment of each compound for 6 h, coverslips were fixed with –20 ºC MeOH for 5 min. After being washed with PBS-B (PBS containing 0.5% (w/v) BSA), coverslips were overlaid with anti-α-tubulin antibody in PBS-B then placed in a humidified container at 37 ºC and incubated for 1 h. After being washed 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-mouce 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.XXXX.XX.XXX.

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structures,[4] we have reevaluated theirs effects along with compounds **28** and **29** and found that those effects should be corrected as described in this paper.

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