Structure–activity relationship study of glaziovianin A against cell cycle progression and spindle formation of HeLa S_3 cells

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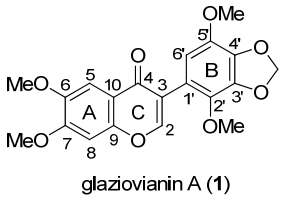
Abstract—Various derivatives of glaziovianin A, an antitumor isoflavone, were synthesized, and the cytotoxicity of each against HeLa S_3 cells was investigated. Compared to glaziovianin A, the O^7-allyl derivative was found to be more cytotoxic against HeLa S_3 cells and a more potent M-phase inhibitor.

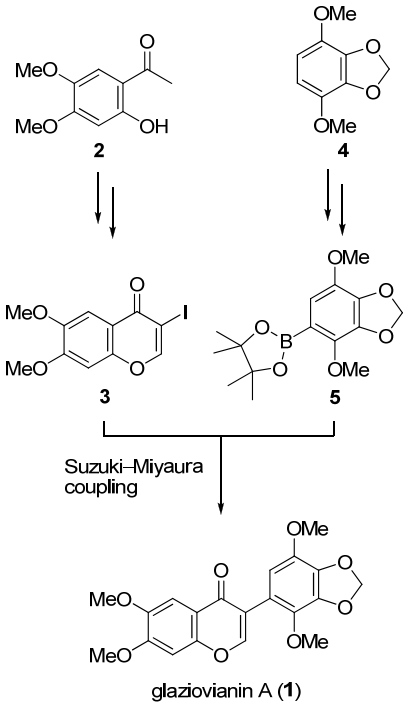
In 2007, glaziovianin A (**1**) was isolated from the leaves of the Brazilian tree *Astelia glazioviana* by Yokosuka et al. (Fig. 1).[1] Glaziovianin A (**1**) exhibited cytotoxicity against HL-60 cells with an IC_{50} value of 0.29 μM. Also, glaziovianin A (**1**) was evaluated against a panel of 39 human cancer cell lines (termed JFCR39) at the Japanese Foundation for Cancer Research. The pattern of the differential cytotoxicities of glaziovianin A (**1**) has suggested that the activity of glaziovianin A (**1**) involves the inhibition of tubulin polymerization as a mechanism of action.[2] Inhibitors of tubulin polymerization have become clinically important drugs against breast cancer. Because glaziovianin A showed antitumor activities in a mouse xenograft model (unpublished data), we think that modification of glaziovianin A (**1**) can lead to the discovery of novel compounds that possess antitumor activity and that inhibit tubulin polymerization. In this paper, we report the structure–activity relationship study of glaziovianin A (**1**).



glaziovianin A (**1**)

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

We previously reported the synthesis of glaziovianin A (**1**) by using Suzuki–Miyaura coupling as a key step (Scheme 1).[3] The method of synthesizing glaziovianin A analogues was based on our previous strategy. To develop analogues of glaziovianin A (**1**), its structure can be divided into two structural moieties: an A-ring and a B-ring (Fig. 2). Therefore, we synthesized 3-iodochromone derivatives as an A-ring and borone compounds as a B-ring.



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

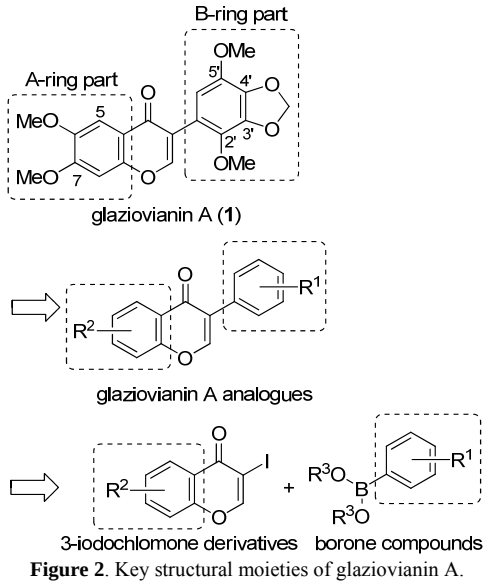
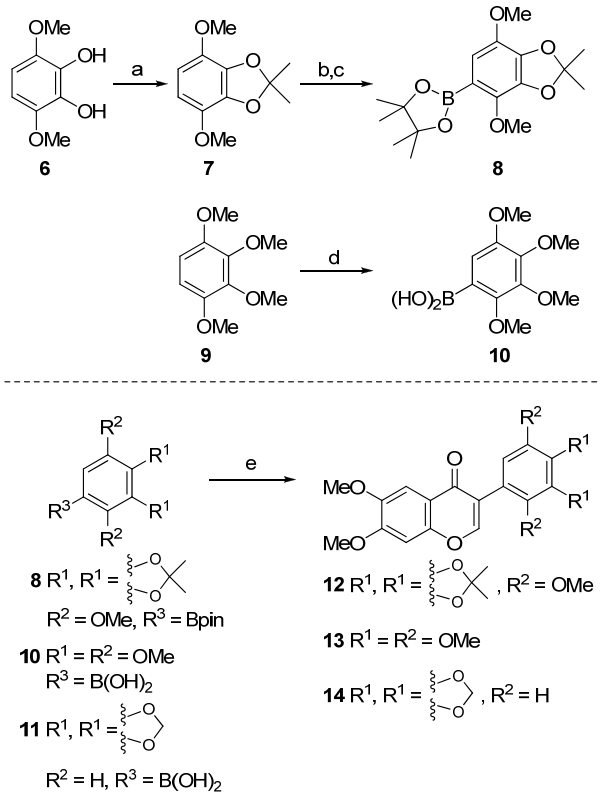


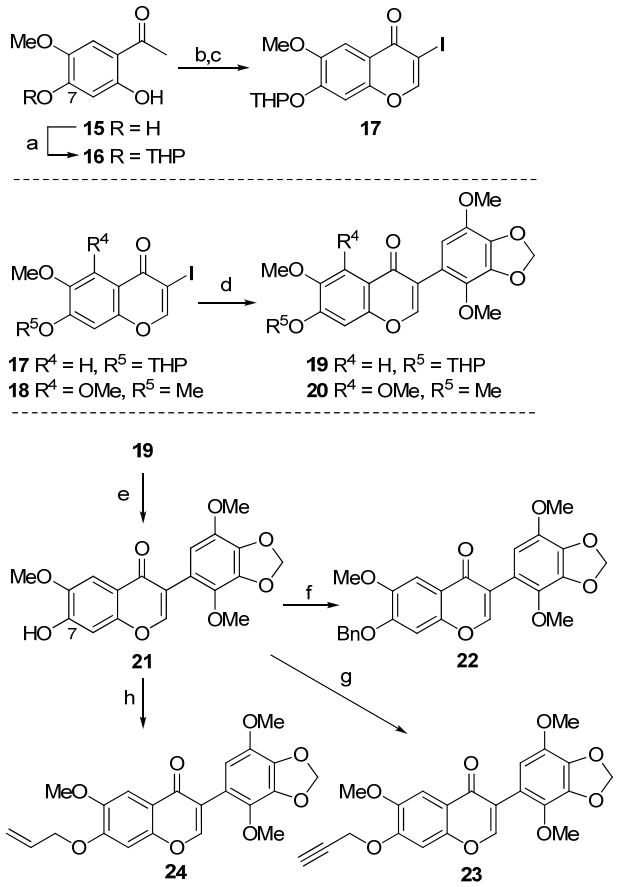
Figure 2. Key structural moieties of glaziovianin A.

First, we tried to modify a methylene acetal part at the B-ring of glaziovianin A (Scheme 2). The diol group in 3,6-dimethoxybenzene-1,2-diol (**6**) was converted to compound **7**. The bromination of compound **7** gave a monobromo compound, which was converted into arylboronate **8**.[4] 2,3,4,5-Tetramethoxyphenylboronic acid (**10**) was prepared by the lithiation of 1,2,3,4-tetramethoxybenzene (**9**) followed by treatment with trimethyl borate.[5] The Suzuki–Miyaura coupling[6] of 3-iodo-6,7-dimethoxy-4*H*-chromen-4-one (**3**)[3] with boron compounds, such as arylboronate **8**, 2,3,4,5-tetramethoxyphenylboronic acid (**10**), or commercially available 3,4-(methylenedioxy)phenylboronic acid (**11**), afforded glaziovianin A analogues **12**–**14**, respectively.



Scheme 2. Synthesis of B-ring analogues of glaziovianin A. Reagents and conditions: (a) 2-methoxypropene, PPTS, benzene, rt, 72%; (b) NBS, DMF, rt, 69%; (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**}.

Next, we prepared A-ring analogues. Selective protection of the hydroxy group at the C7 position of **15**[7] afforded compound **16** (Scheme 3). Condensation of **16** with *N*,*N*-dimethylformamide dimethyl acetal gave an enamine, which was converted to iodochromone **17**.[8] We tried a cross coupling reaction with arylboronate **5**[3] and iodochromone compounds, such as **17** or **18**[9], to provide compounds **19** and **20**, respectively. The THP group in **19** was removed by using *p*-TsOH·H_2O to give a 7-hydroxy derivative (**21**), which is a suitable precursor for the synthesis of glaziovianin derivatives. Conversion of the hydroxy group at C7 in **21** into various ethers afforded benzyl ether **22**, propargyl ether **23**, and allyl ether **24**.



Scheme 3. Synthesis of A-ring analogues of glaziovianin A. Reagents and conditions: (a) DHP, PPTS, CH_2Cl_2, rt, 80%; (b) $Me_2NCH(OMe)_2$, 90 °C, quant; (c) I_2, pyr, $CHCl_3$, rt, 70%; (d) **5**, $PdCl_2$(dppf), 1 M Na_2CO_3 aq., 1,4-dioxane, rt (66% for **19**, 16% for **20**); (e) *p*-TsOH·H_2O, MeOH, $CHCl_3$, rt, 85%; (f) benzyl bromide, K_2CO_3, MeCN, rt, 80%; (g) allyl bromide, K_2CO_3, MeCN, rt, 78%; (h) propargyl bromide, K_2CO_3, MeCN, rt, 70%.

Table 1 summarizes the cytotoxicity of glaziovianin A (**1**) and its analogues against HeLa S_3 cells.[10] Compound **12**, which has an acetonide group instead of the methylene acetal group, showed no cytotoxicity even at 100 μM. Also, compound **13**, which has four methoxy groups at the B-ring, was about 40-fold less cytotoxic than glaziovianin A (**1**). These results indicated that steric hindrance of C3' and C4' at the B-ring part was shown to reduce cytotoxicity to a large extent. Compound **14**, which lacks methoxy groups at C2' and C5', was less cytotoxic than glaziovianin A (**1**), which indicated that the electron density of the B-ring might be essential for cytotoxicity. On the other hand, compound **20**, which has an extra methoxy group at C5 of the A-ring, showed no cytotoxicity at 100 μM. This result showed that the steric hindrance and electron density of the A-ring reduced cytotoxicity to a large extent. While the 7-demethyl derivative **21** exhibited no cytotoxicity, compounds **22**–**24**, which each have an alkyl group at O^7 instead of the methyl group, showed cytotoxicity with IC_{50} values of 0.75, 0.74, and 0.19 μM, respectively. Furthermore, compound **19**, which has a THP group at O^7, showed no cytotoxicity even at 100 μM. These results indicated that the hydrophobicity of the O^7-alkyl group in glaziovianin derivatives is important for cytotoxicity. However, the THP group seems to be too large. It is worth noting that allyl ether **24**[11] is more active than glaziovianin A (**1**) itself.

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

	cytotoxicity	
compound	IC_{50} (μM)	relative value
glaziovianin A (**1**)	0.59	1
12	>100	-
13	22.0	0.027
14	56.2	0.010
19	>100	-
20	>100	-
21	>100	-
22	0.75	0.79
23	0.74	0.80
24	0.19	3.1

We previously reported that glaziovianin A (**1**) inhibited the cell cycle progression in the M-phase with abnormal spindle structures.[1] Therefore, we next investigated the effects of the most cytotoxic compound, **24**, on both cell cycle progression[12] and spindle structures[13] (Figure 3). As with glaziovianin A (**1**), compound **24** inhibited cell cycle progression in M-phase, and **24**-treated cells showed abnormal spindle structures with unaligned chromosomes at the concentration of 1 μM after 18 h treatment: these phenotypes were stronger than those of 1 μM glaziovianin A (**1**) treatment, suggesting that compound **24** is a more potent M-phase inhibitor than the original compound glaziovianin A (**1**).

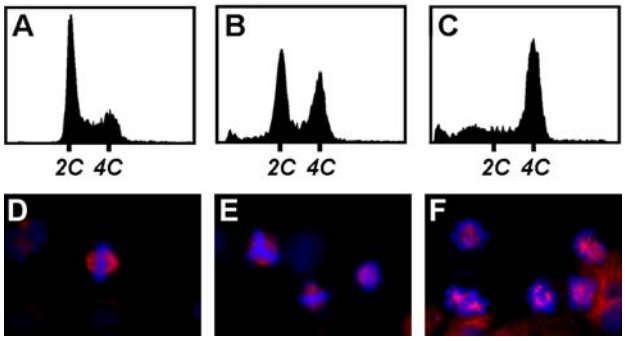


Figure 3. Effects of glaziovianin A (**1**) and compound **24** on cell cycle progression and spindle structures in HeLa S_3 cells. Effects of **1** and **24** on cell cycle progression (A–C) and spindle structures (D–F) in HeLa S_3 cells. HeLa S_3 cells were treated with DMSO (A and D), 1 μM of glaziovianin A (**1**) (B and E), or compound **24** (C and F) for 18 h. Microtubules (red) and chromosomes (blue) are shown in D–F. Microtubules and chromosomes were stained with anti-α-tubulin antibody (DM1A, Sigma) and Hoechst 33258, respectively.

In conclusion, we have investigated the structure–cytotoxicity relationships of glaziovianin A (**1**). From this work, we developed the O^7-allyl compound **24** as much more cytotoxic than glaziovianin A (**1**) against HeLa S_3 cells. Further studies on the synthesis of O^7-

modified probe molecules of glaziovianin A (**1**) for searching target biomolecules are currently in progress.

Acknowledgments

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10. 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 compounds. 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×100.
11. Chemical data for compound **24**: ^{1}H NMR (400 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; IR ($CHCl_3$) 3008, 2938, 1639, 1607, 1503, 1469, 1430, 1399, 1349, 1298, 1267, 1231, 1195, 1153, 1099, 1063, 1035, 995, 833, 697 cm^{-1}; ESIMS *m/z* 435.1057, calcd for $C_{22}H_{20}NaO_8$ $[M+Na]^+$ 435.1056.
12. Flow cytometry was used to analyses the distribution of DNA content in the cell populations. The cells were fixed with cold (–20 °C) 70% EtOH (v/v) and stained with propidium iodide (Sigma). Total fluorescence intensities were determined by quantitative flow cytometry with CyFlow PA (Partec GmbH, Munster, Germany).
13. Immunofluorescence observation of tubulin was performed as described in previous paper.[14] The DNA and microtubules were photographed with Leica AF6000 (Leica Microsystems GmbH, Wetzlar, Germany).
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