SHORT COMMUNICATION

# Tellimoside, a Flavonol Glycoside from *Brasenia schreberi*, Inhibits the Growth of Cyanobacterium (*Microcystis aeruginosa* LB 2385)

Min-Kyung Lee · Hee-Jung Park · Sung-Ho Kwon · Ye-Jin Jung · Ha-Na Lyu · Do-Gyeong Lee · Na-Young Song · Hong-Keun Choi · Sangkyu Park · Nam-In Baek · Youn-Hyung Lee

**Abstract** Principal components of aquatic plants responsible for inhibiting the growth of *Microcystis aeruginosa*, a blue-green alga (cyanobacterium) were determined. Methanol extracts of *Brasenia schreberi* showed strong inhibitory activity against the growth of the cyanobacterium. Two purified flavonoids were afforded through repeated column chromatographies. The compounds were determined as quercetin (**1**) and quercetin 3-*O*-(6''-galloyl)-β-D-glucopyranoside, tellimoside (**2**) by the interpretation of spectroscopic data including NMR, MS, and IR. Compounds **1** and **2** were first isolated from *Brasenia schreberi*. In addition, compound **2** showed very strong inhibitory activity against the growth of *M. aeruginosa*.

**Keywords** *Brasenia schreberi* · cyanobacterium · *Microcystis aeruginosa* · tellimoside

Algal bloom is an indication of rapid increase in the population of algae in an eutrophicated aquatic systems due to increases in temperature and humidity during summer. Cyanobacteria, which are found to be a major cause of algal bloom outbreaks, are one of the oldest prokaryotes. Cyanobacteria not only produce substances poisonous against aquatic life, but also decrease the dissolved oxygen (DO) levels in water ecosystems (Kim et al., 1998). Among the various types of cyanobacteria, such as *Microcystis aeruginosa*, *Anabaena affinis*, *A. macrospora*, *Oscillatoria kawamurae*, and *O. tenuis*, the genus *Microcystis* exists in most of Korean lakes (Bishop et al., 1959). Cyanobacteria produce several secondary metabolites such as alkaloids, polypeptides, and nonribosomal peptides. In particular, such fatal toxic secondary metabolites, microcystin and anatoxin, are very harmful to fish and humans (Schwimmer and Schwimmer, 1968; Kolmakov and Gladyshev, 2003).

Treatment with chemical substances such as phosphorus and potassium and ultrasonic treatment are currently used to control algal blooms. However, because these methods could also cause environmental pollution as a secondary side effect, there exists a need to develop an environmentally-friendly solution to control algal blooms (Carmichael, 1992). Some studies have identified compounds present in aquatic plants, which inhibit the growth of algal blooms (Ahn et al., 1977; Greca et al., 1989; Nakai et al., 1998). The results of these studies indicated that a high potential exists to regenerate the polluted aquatic system and to increase the amount of DO in a lake undergoing exuberant growth of aquatic plants. Based on these results, the extracts of 32 aquatic plants were prepared to evaluate their inhibitory activities against the growth of *M. aeruginosa*. The alcohol extracts of *Potamogeton malaianus, Monochoria korsakowii, Ceratophyllum demersum,* and *Phragmites japonica* were observed to have very weak inhibition activity (Kwon et al., 2012). On the other hand, *Persicaria amphibian, Trapa japonica*, and *Brasenia schreberi* showed greater than 60% inhibition activities against *M. aeruginosa*. *B. schreberi* was finally selected to identify the principal components

M.-K. Lee · H.-J. Park · Y.-J. Jung · H.-N. Lyu · D.-G. Lee · N.-Y. Song · N.-I. Baek (✉)
Graduate School of Biotechnology, Kyung Hee University, Yongin, Gyeonggi-Do 446-701, Republic of Korea
E-mail: nibaek@khu.ac.kr

S.-H. Kwon · H.-K. Choi · S. Park
Department of Biological Science, Ajou University, Suwon, Gyeonggi-Do 443-749, Republic of Korea

Y.-H. Lee (✉)
Department of Horticultural Biotechnology, Kyung Hee University, Yongin, Gyeonggi-Do 446-701, Republic of Korea
E-mail: younlee@khu.ac.kr

possessing inhibitory activity based on its maximal inhibition activity. Therefore, the present study was initiated to identify the principal component of *B. schreberi* responsible for inhibiting the growth of the cyanobacteria, *M. aeruginosa*.

*B. schreberi* belongs to the *Brasenia* genus and is distributed in the fresh water ponds and lakes of eastern Asia, Africa, and America. Round alternate leaves, which are found floating on the surface of the water and small purple flowers, characterize the plant. Stems and the underwater part of the plant are covered by mucilage (Kim et al., 2008). *B. schreberi* was extracted with aqueous methanol (MeOH), suspended in water, and then partitioned into ethyl acetate (EtOAc), *n*-butyl alcohol (*n*-BuOH), and $H_2O$ fractions, each of which was tested for inhibition of the growth of *M. aeruginosa*. The EtOAc fraction, which showed significant inhibition activity, was applied to repeated $SiO_2$ and octadecyl silica gel (ODS) column chromatographies to yield two active compounds. The structural elucidation and the activity evaluation of the isolated compounds were also performed.

The silica gel ($SiO_2$) and octadecyl silicagel (ODS) resins used for column chromatography were Kiesel gel 60 (Merck, Germany) and RP-18 (Merck), respectively. Flash column chromatography was carried out using the SNAP Cartridge KP-Sil (Biotage, Sweden). Thin layer chromatography (TLC) analysis was carried out using Kiesel gel 60 $F_{254}$ and RP-18 $F_{254S}$ (Merck), and detection was achieved using a Spectroline Model ENF-240 C/F UV lamp (Spectronics Corporation, USA) with a 10% $H_2SO_4$ solution. Deuterium solvents were purchased from Merck Co. Ltd and Sigma Aldrich Co. Ltd (USA). Optical rotation was measured on a JASCO P-1010 digital polarimeter (JASCO, Japan). The fast atom bombardment mass spectrometry (FAB-MS) spectrum was recorded on a JEOL JMS-700 (JEOL, Japan). The IR spectrum was collected on a PerkinElmer Spectrum One fourier transform (FT)-IR spectrometer (PerkinElmer, England). The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (Varian, USA).

The whole plants of *B. schreberi* were collected in Chun Jin Ho Lake, Gangwon Province, Korea in September 2008 and were identified by Prof. Sang-Gyu Park of the College of Life Science, Ajou University, Suwon, Korea. A voucher specimen (KHU20081011) is reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea. The cyanobacterium used was *M. aeruginosa* UTEX 2385, provided by Texas University, Austin, USA.

The fresh whole plants of *B. schreberi* (8 kg) were extracted in 80% aqueous methanol (MeOH, 3 L × 2) for 12 h at room temperature. The extracts were successively partitioned with water (1 L), ethyl acetate (EtOAc, 1 L × 2), and normal-butanol (*n*-BuOH, 1 L × 2).

The EtOAc extract (30 g) was subjected to flash column chromatography (c.c.) (SNAP Cartridge KP-Sil, 100 g) and eluted with a gradient of chloroform ($CHCl_3$–MeOH; 50:1→30:1→20:1→10:1→7:1→5:1→4:1→3:1→2:1→1:1 100% MeOH, 1 L of each). The eluted solutions were monitored by TLC to produce

23 fractions (BSE1 to BSE23). Fraction BSE16 [192 mg, Ve/Vt (elution volume/total volume) 0.65–0.74] was subjected to ODS c.c. (2.5 cm × 4 cm) and eluted with MeOH–$H_2O$ (1:1, 800 mL), yielding seven fractions (BSE16-1 to BSE16-7). Fraction BSE16-5 (12 mg, Ve/Vt 0.57–0.86) was subjected to ODS c.c. (1 cm × 2 cm) and eluted with MeOH–$H_2O$ (3:2, 300 mL) to yield purified compound **1** [BSE16-5-2, 5.1 mg, Ve/Vt 0.21–0.65, TLC (RP-18 $F_{254S}$) Rf 0.34 MeOH–$H_2O$=2:1]. Fraction BSE22 [2.1 g, Ve/Vt 0.91–0.96] was subjected to flash c.c. (SNAP Cartridge KP-Sil, 100 g) and eluted with $CHCl_3$–MeOH (10:1→5:1, 2.5 L of each), yielding ten fractions (BSE22-1 to BSE22-10). Fraction BSE22-4 (649 mg, Ve/Vt 0.33–0.56) was subjected to a Sephadex LH-20 c.c. (3 cm × 20 cm) and eluted with MeOH–$H_2O$ (1:1, 3 L), yielding nine fractions (BSE22-4-1 to BSE22-4-9). Fraction BSE22-4-5 (415 mg, Ve/Vt 0.43–0.66) was subjected to ODS c.c. (3 cm × 4 cm) and eluted with MeOH–$H_2O$ (1:2, 2 L), yielding nine fractions (BSE22-4-5-1 to BSE22-4-5-9), including purified compound **2** [BSE22-4-5-6, 11 mg, Ve/Vt 0.56–0.71, TLC (RP-18 $F_{254S}$) Rf 0.26, MeOH–$H_2O$=3:1].

Compound **1**: Yellow powder, IR $v_{max}$ (KBr, cm$^{-1}$) 3446, 1666, 1518, 1496; negative FAB-MS *m/z* 301 [M–H]$^-$; ^{1}H-NMR (400 MHz, CD$_3$OD) δ 7.78 (1H, d, *J*=2.0 Hz, H-2'), 7.67 (1H, dd, *J*=2.0, 8.8 Hz, H-6'), 6.92 (1H, d, *J*=8.8 Hz, H-5'), 6.42 (1H, d, *J*=2.4 Hz, H-8), 6.22 (1H, d, *J*=2.4 Hz, H-6); ^{13}C-NMR (100 MHz, CD$_3$OD) δ 174.6 (C-4), 165.4 (C-7), 162.3 (C-5), 158.0 (C-9), 148.9 (C-4'), 146.4 (C-3'), 146.1 (C-2), 137.1 (C-3), 124.0 (C-1'), 121.5 (C-6'), 116.1 (C-2'), 115.8 (C-5'), 104.4 (C-10), 99.1 (C-6), 94.3 (C-8).

Compound **2**: Yellow powder, $[\alpha]_D^{25}$ −20.8° (*c*=0.1, MeOH); IR $v_{max}$ (KBr, cm$^{-1}$) 3350, 1700, 1658, 1610, 1567, 1506, 1449, 1360, 1270; negative FAB-MS *m/z* 615 [M–H]$^-$; ^{1}H-NMR (400 MHz, CD$_3$OD) δ 7.53 (1H, dd, *J*=2.2, 8.4 Hz, H-6'), 7.51 (1H, d, *J*=2.2 Hz, H-2'), 6.96 (2H, s, H-2''', 6'''), 6.69 (1H, d, *J*=8.4 Hz, H-5'), 6.31 (1H, d, *J*=2.0 Hz, H-8), 6.15 (1H, d, *J*=2.0 Hz, H-6), 5.23 (1H, d, *J*=7.2 Hz, H-1''), 4.38 (1H, dd, *J*=4.8, 12.0 Hz, H-6''b), 4.30 (1H, dd, *J*=1.6, 12.0 Hz, H-6''a); ^{13}C-NMR (100 MHz, CD$_3$OD) δ 168.0 (COO, C-7'''), 179.2 (C-4), 165.7 (C-7), 162.7 (C-5), 159.2 (C-2), 158.2 (C-9), 149.5 (C-4'), 146.1 (C-3''', 5'''), 145.6 (C-3'), 139.6 (C-4'''), 135.2 (C-3), 123.4 (C-6'), 121.1 (C-1''', 1'), 117.1 (C-2'), 115.8 (C-5'), 110.0 (C-2''', 6'''), 105.4 (C-10), 104.1 (C-1''), 98.8 (C-6), 93.6 (C-8), 78.0 (C-5''), 75.8 (C-3''), 75.6 (C-2''), 71.4 (C-4''), 64.3 (C-6'').

To evaluate the inhibition activity on the growth of cyanobacterium, 10 mL of *M. aeruginosa* including a 0.500–0.600 mg/mL density of chlorophyll a was inoculated in 90 mL of L16 liquid medium supplemented with $NaNO_2$ in a 250 mL Erlenmeyer flask. The cells were treated with 1 mg of sample (the extract, fractions or the isolated compounds) dissolved in MeOH and incubated at 25°C, 150 rpm, over 2000 lux, with a light/dark cycle (16.8) in an air circulation system. Inhibition activity was measured by the estimation of absorbance at 630, 647, and 664 nm. The sampling for measurement was performed every two days during continuous incubation. The amount of chlorophyll a was estimated
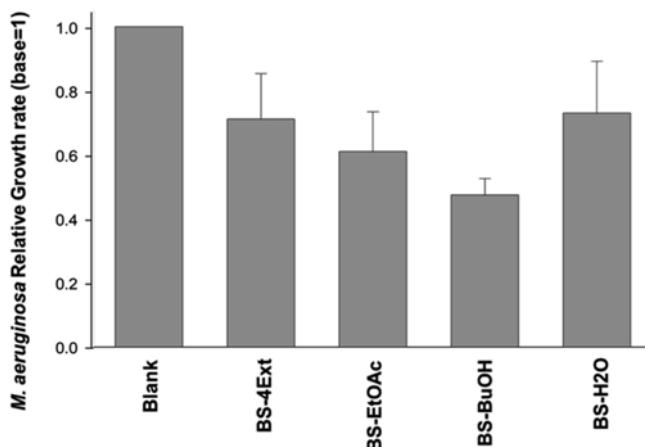
Fig. 1 The methanol extract and solvent fractions obtained from *B. schreberi* inhibit the growth of cyanobacterium, *M. aeruginosa*, compared to the blank; blank, methanol; BS-4Ext, methanol extract of *B. schreberi*; BS-EtOAc, EtOAc fraction; BS-BuOH, *n*-BuOH fraction; BS-H₂O, H₂O fraction obtained from the methanol extract of *B. schreberi*.



Fig. 2 Chemical structures of the flavonoids from *Brasenia schreberi*.

by the following proportional arithmetic formula using the absorbances at 630, 647, and 664 nm: Chlorophyll a =11.85× O.D. 664−1.54×O.D. 647−0.08×O.D. 630, where O.D. is an optical density at each nm wavelength. The relative growth of the cells was calculated as a percentage of viable cells in the treated groups versus that in the control groups according to the following estimation: Growth rate = (St-S0/time), that is, the growth rate at time t (the ratio of relative growth) is equal to the growth at time t (the ratio of blank growth) divided by the growth at time 0 (the ratio of sample growth).

The MeOH extracts of *B. schreberi* were partitioned into EtOAc, *n*-BuOH, and H₂O fractions through solvent fractionation. Among these three fractions, the EtOAc and *n*-BuOH fractions showed very significant inhibition activities on the growth of *M. aeruginosa* (Fig. 1). Isolation of the principal components from the *n*-BuOH fraction responsible for inhibiting the growth of *M. aeruginosa* was unsuccessful, because too many active components were contained in the fraction in very low concentrations. On the other hand, repeated column chromatography with the EtOAc fraction was carried out to produce the purified active components and finally afforded two flavonoids as the principal components capable of inhibiting the growth of *M. aeruginosa*.

Compound **1** was obtained as a yellow powder and showed a pseudomolecular ion peak [M–H]⁻ at *m/z* 301 in the negative FAB/MS spectrum, thus indicating a molecular weight of 302. The IR spectrum (KBr) showed absorbance bands due to hydroxyl (3446 cm⁻¹), conjugated ketone (1666 cm⁻¹), and double bonds (1518 cm⁻¹). In the ¹H-NMR spectrum, three olefin methine signals [(δ_H 7.78, d, *J*=2.0 Hz, H-2'), (δ_H 7.67, dd, *J*=2.0, 8.8 Hz, H-6'), (δ_H 6.92, d, *J*=8.8 Hz, H-5')] due to a 1,2,4-trisubstituted benzene ring and two aromatic methine signals [(δ_H 6.42, d, *J*=2.4 Hz, H-8), (δ_H 6.22, d, *J*=2.4 Hz, H-6)] due to a 1,2,3,5-tetrasubstituted benzene ring were observed. Accordingly, compound **1** was assumed
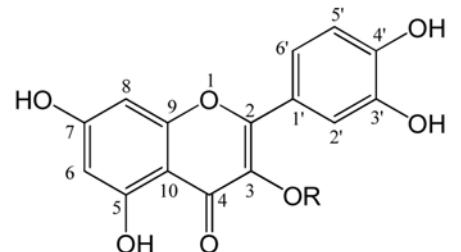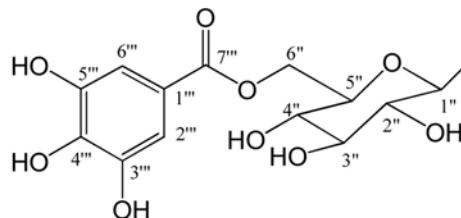
to be a quercetin-type flavonoid. The multiplicities of 15 carbon signals observed in the ¹³C-NMR spectrum were determined through distortionless enhancement by polarization transfer (DEPT) experiments. The NMR data indicated the presence of one conjugated ketone (δ_C 174.6, C-4), seven oxygenated olefin quaternary carbons [δ_C 165.4 (C-7), 162.3 (C-5), 158.0 (C-9), 148.9 (C-4'), 146.4 (C-3'), 146.1 (C-2), 137.1 (C-3)], two olefin quaternary carbons [δ_C 124.0 (C-1'), 104.4 (C-10)], and five olefin methines [δ_C 121.5 (C-6'), 116.1 (C-2'), 115.8 (C-5'), 99.1 (C-6), 94.3 (C-8)]. Compound **1** was finally identified as quercetin, the most well-known flavonol, through comparison of the spectroscopic data with those of existing literature (Arima and Danno, 2002) (Fig. 2).

Compound **2** was obtained as a yellow amorphous powder and showed a pseudomolecular ion peak [M–H] at *m/z* 615 in the negative FAB/MS spectrum, indicating a molecular weight of 616. The IR spectrum (KBr) showed absorptions at 3350, 1700, 1658, 1610, 1567, 1449 and 1360 cm⁻¹, typical of hydroxyl, conjugated ketone, and double bonds. The NMR data of compound **2** were almost identical to those of compound **1** with the exception of monosaccharide and phenyl moieties. In the ¹H-NMR spectrum, the two singlet olefine proton signals at δ_H 6.96 (2H, H-2''', 6''') were assigned to the two equivalent protons of a gallic acid. An anomeric proton signal of compound **2** appeared at δ_H 5.23 (1H, d, *J*=7.2 Hz, H-1''), and other oxygenated methines and methylene signals of the hexose moiety appeared at δ_H 3.28-4.21 (m, H-2'', 3'', 4'', 5''), δ_H 4.30 (1H, dd, *J*=1.6, 12.0 Hz, H-6''a), and δ_H 4.38 (1H, dd, *J*=4.8, 12.0 Hz, H-6''b). In the ¹³C-NMR spectrum, 28 carbon signals were shown to indicate the presence of a hexose with a galloyl moiety. The multiplicities of the carbon signals were determined through DEPT experiments. The signals observed in

absorbance
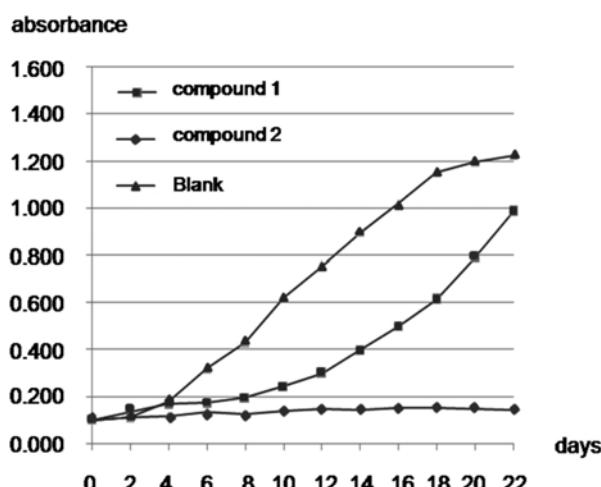


**Fig. 3** Chlorophyll a concentration of media cultured by *M. aeruginosa* UTEX 2385 treated with compounds **1** and **2** from *B. schreberi*. Ten milliliters of cyanobacterium including chlorophyll a was inoculated into 90 mL of L16 liquid medium. Next, the samples were treated and incubated. The inhibition activity was expressed as the concentration of the amount of chlorophyll a, which was estimated by the following proportional arithmetic formula using the absorbances at 630, 647, and 664 nm: Chlorophyll a =11.85×O.D.664−1.54×O.D.647−0.08×O.D.630.

the low magnetic field region indicated the presence of one ester carbon ($\delta_C$ 168.0), three oxygenated quaternary carbons [$\delta_C$ 146.1 (C-3''', 5'''), 139.6 (C-4''')], two olefine methine carbons [$\delta_C$ 110.0 (C-2''', 6''')], and one olefine quaternary carbon ($\delta_C$ 121.1) due to a galloyl moiety. The chemical shifts of the hexose carbon signals [$\delta_C$ 104.1 (C-1''), 71.4 (C-4''), 75.6 (C-2''), 75.8 (C-3''), 78.0 (C-5'') 64.3 (C-6'')] and the coupling constant of the anomer proton ($\delta_H$ 5.23, $J$=7.2 Hz) suggested the presence of a β-glucopyranosyl group. The signals of H-6'' ($\delta_H$ 4.30, 4.38) and C-6'' ($\delta_C$ 64.3) were shifted about 0.4–0.6 ppm downfield in the ^{1}H-NMR spectrum and about 4.2 ppm in the ^{13}C-NMR spectrum, respectively, due to an esterification effect, indicating that the galloyl group was linked to C-6''. The gradient heteronuclear multiple bonding connectivity (gHMBC) spectrum enabled us to establish the locations to be connected among the aglycone, glucopyranose, and gallic acid moieties of compound **2**. The anomer proton signal of the glucose ($\delta_H$ 5.23) was correlated with an oxygenated olefin quaternary carbon signal ($\delta_C$ 135.2. C-3) of the aglycone quercetin and a cross peak between the oxygenated methylene proton signals of the glucose [$\delta_H$ 4.30 (H-6''a), 4.38 (H-6''b)], and the ester carbon of the galloyl moiety ($\delta_C$ 168.0) was observed. By comparing these data with those reported in the literature (Collins et al., 1975), compound **2** was finally identified as quercetin 3-*O*-(6''-galloyl)-β-D-glucopyranoside, which was previously isolated from *Tellima grandijbra* and named as tellimoside (Masuda et al., 2001; Lee et al., 2004) (Fig. 2). The present study marks the first isolation of compounds **1** and **2** from *B. schreberi*.

The isolated flavonoids **1** and **2** were evaluated for their inhibition activities on the growth of *M. aeruginosa*. Compound **1**

showed weak inhibition activity compared to that of the negative control, whereas compound **2** showed very strong inhibition activity (Fig. 3). Although compound **2** has the quercetin moiety as the aglycone of the glucoside, it inhibited the growth of *M. aeruginosa* far beyond the inhibition shown in compound **1**, quercetin. The sugar and galloyl groups of compound **2** increase the polarity of the compound such that the glycoside moves more easily to *M. aeruginosa* in the aquatic system. Quercetin (**1**), which is the most frequently observed flavonoid in nature, has been known to have a variety pharmacological activities such as antioxidant, anti-tumor, anti-virus, and inhibition of leukemia cell growth (Yoshida et al., 1990; Kang and Liang, 1997; Bachmetov et al., 2012; Duchnowicz et al., 2012). However, the inhibition effect on the growth of the cyanobacterium has not yet been reported. Tellimoside (**2**) is the very rarely occurring flavonoid glycoside, and no activity including inhibition effect on the living organisms has been reported. The present study proved the potent inhibitory effect of compound **2** from *B. schreberi* against the proliferation of alga including cyanobacteria. Therefore, an environmentally-friendly approach using this compound to inhibit blue-green alga is feasible.

The MeOH extract of *B. schreberi* and the EtOAc fraction showed strong inhibitory activity against the growth of *M. aeruginosa*, a blue-green alga (cyanobacterium). The repeated column chromatography for the EtOAc fraction led to isolation of two flavonoids, which were identified as quercetin (**1**) and quercetin 3-*O*-(6''-galloyl)-β-D-glucopyranoside, tellimoside (**2**) by the interpretation of spectroscopic data including NMR, MS, and IR. Compound **2** showed very strong inhibitory activity against the growth of *M. aeruginosa*.

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