# Antimitotic and Antifungal C-3/C-3″-Biflavanones from *Stellera chamaejasme*

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**Two new C-3/C-3″ biflavanones, chamaejasmenin D (1) and isochamaejasmenin B (2), were isolated from the roots of *Stellera chamaejasme*, together with five known biflavanones, chamaejasmenin A (3), chamaejasmenin B (4), neochamaejasmin A (5), sikokianin A (6), and chamaejasmenin C (7). Their structures were elucidated by spectroscopic methods, including 2D NMR techniques. Among these compounds, 1—3 demonstrated potent antimitotic and antifungal activity with minimum inhibitory concentration (MIC) values of 6.25, 6.25, and 3.12 μg/ml, respectively.**

**Key words** *Stellera chamaejasme*; chamaejasmenin D; isochamaejasmenin B; antimitotic; antifungal; *Pyricularia oryzae*

The root of *Stellera chamaejasme* L. (Thymelaeaceae), a toxic plant widely distributed in the north and southwest of China, is used as "Langdu" in traditional Chinese medicine. It has long been used for the treatment of scabies, tinea, stubborn skin ulcers, chronic tracheitis, and tuberculosis in China.[1] In recent years, "Langdu" is also being used for the treatment of intestinal, gastric, and pulmonary cancers.[2,3] These clinical applications suggest *S. chamaejasme* roots may contain antineoplastic and antifungal components. The ethanol extract of *S. chamaejasme* roots was screened using *Pyricularia oryzae* as the indicator organism and showed potent activity. Bioactivity-directed fractionation indicated that the EtOAc fraction showed the most potent activity (Table 2). Further fractionation and purification of the EtOAc extract led to the isolation and characterization of two new C-3/C-3″ biflavanones, chamaejasmenin D (**1**) and isochamaejasmenin B (**2**), together with the five known biflavanones chamaejasmenin A (**3**),[4] chamaejasmenin B (**4**),[4] neochamaejasmin A (**5**),[5] sikokianin A (**6**)[6] and chamaejasmenin C (**7**)[4] (Fig. 1). **1**, **2** and **3** showed potent activity against *P. oryzae*. This paper reports the structure elucidation of the new compounds, and antimitotic and antifungal activities of **1**—**7**.

## Results and Discussion

Chamaejasmenin D (**1**), obtained as white amorphous powder, showed a $[M-H]^-$ peak at *m/z* 569.1449 (Calcd for $C_{32}H_{25}O_{10}$: 569.1448) in the negative HR-ESI-MS, corresponding to a molecular formula of $C_{32}H_{26}O_{10}$. The EI-MS gave two major fragments at *m/z* 299 ($[M-C_{15}H_{11}O_5]^+$) and 271 ($[M-C_{17}H_{15}O_5]^+$), indicating that the molecule was composed with two units. The color development reaction with HCl–Mg reagent showed a red color, indicating that **1** is a flavonoid. The IR spectrum showed the presence of a hydroxyl group (3480 $cm^{-1}$), conjugated carbonyl group (1642 $cm^{-1}$), and aromatic ring (1600, 1517 $cm^{-1}$). Its UV spectrum showed a maximum absorption at 298 nm (log $\varepsilon$ 4.44) and a shoulder peak at 330 nm, which are characteristic of an A-ring oxygen substituted flavanone.[1]

The $^1$H-NMR spectrum of **1** (Table 1) contained signals of two methoxyl protons [$\delta$ 3.82 (3H, s), 3.84 (3H, s)], two protons of $C_2$-H, $C_2''$-H [$\delta$ 5.52 (1H, br s), 5.44 (1H, br s)], and two protons of $C_3$-H, $C_3''$-H [$\delta$ 3.08 (1H, s), 2.99 (1H, s)] at the C and C′ rings of biflavanone, and four hydroxyl protons [$\delta$ 8.42, 9.49, 11.56, 11.56]. The twelve aromatic proton signals [$\delta$ 5.79—7.15] showed the presence of two sets of typical 5,7-dioxygenated A rings [$\delta$ 5.79 (1H, d, $J$=2.0 Hz), 5.89 (1H, d, $J$=2.2 Hz), 5.93 (1H, d, $J$=2.0 Hz ), 6.02 (1H, d, $J$=2.2 Hz)], and two sets of typical *para*-oxygenated B rings [$\delta$ 6.76 (2H, d, $J$=8.5 Hz), 6.86 (2H, d, $J$=8.6 Hz), 7.04 (2H,

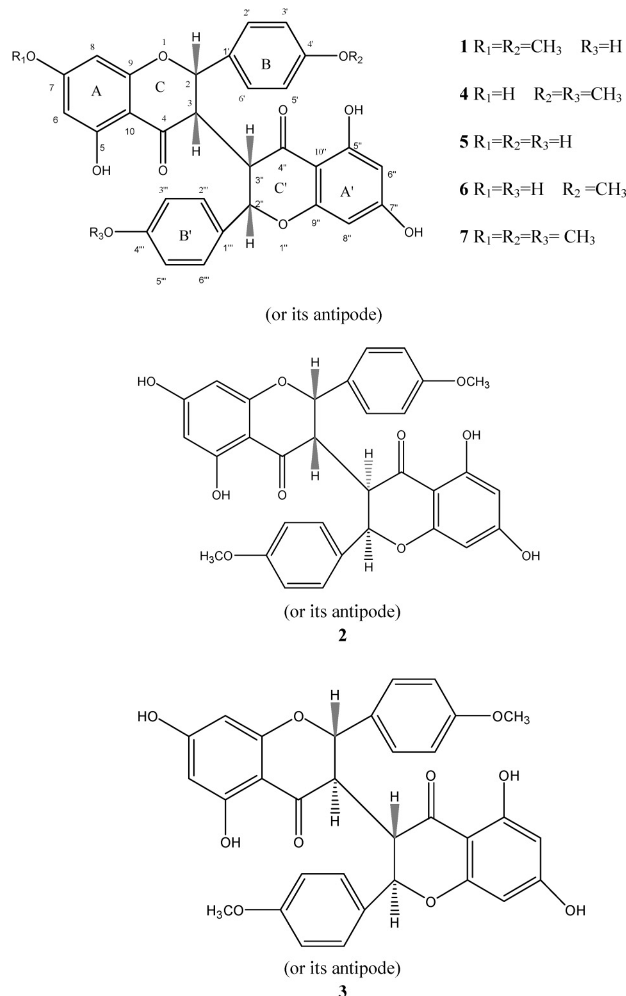


Fig. 1. Chemical Structures of **1**—**7**

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Table 1. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) Data of **1** and **2** ($\delta$ in Acetone-$d_6$)

| Position | **1** | | **2** | |
|---|---|---|---|---|
| | [1]H ($\delta$) | [13]C ($\delta$) | [1]H ($\delta$) | [13]C ($\delta$) |
| 2 | 5.52, 1H, br s | 81.4, d | 4.97, 1H, br s | 81.7, d |
| 3 | 3.08, 1H, s | 47.5, d | 3.86, 1H, br s | 48.3, d |
| 4 | | 197.4, s | | 196.0, s |
| 5 | | 164.7, s | | 165.1, s |
| 6 | 5.89, 1H, d, $J$=2.2 Hz | 95.2, d | 5.97, 1H, d, $J$=1.3 Hz | 97.2, d |
| 7 | | 168.8, s | | 168.3, s |
| 8 | 6.02, 1H, d, $J$=2.2 Hz | 94.1, d | 5.82, 1H, d, $J$=1.3 Hz | 95.9, d |
| 9 | | 163.8, s | | 163.8, s |
| 10 | | 104.0, s | | 102.2, s |
| 1′ | | 129.0, s | | 130.5, s |
| 2′ | 7.15, 1H, d, $J$=8.6 Hz | 128.1, d | 7.22, 1H, d, $J$=8.6 Hz | 129.7, d |
| 3′ | 6.86, 1H, d, $J$=8.6 Hz | 114.6, d | 7.00, 1H, d, $J$=8.6 Hz | 114.9, d |
| 4′ | | 160.3, s | | 161.4, s |
| 5′ | 6.86, 1H, d, $J$=8.6 Hz | 114.6, d | 7.00, 1H, d, $J$=8.6 Hz | 114.9, d |
| 6′ | 7.15, 1H, d, $J$=8.6 Hz | 128.1, d | 7.22, 1H, d, $J$=8.6 Hz | 129.7, d |
| 2″ | 5.44, 1H, br s | 81.3, d | 4.97, 1H, br s | 81.7, d |
| 3″ | 2.99, 1H, s | 47.4, d | 3.86, 1H, br s | 48.3, d |
| 4″ | | 197.0, s | | 196.0, s |
| 5″ | | 165.0, s | | 165.1, s |
| 6″ | 5.79, 1H, d, $J$=2.0 Hz | 96.5, d | 5.97, 1H, d, $J$=1.3 Hz | 97.2, d |
| 7″ | | 167.3, s | | 168.3, s |
| 8″ | 5.93, 1H, d, $J$=2.0 Hz | 95.5, d | 5.82, 1H, d, $J$=1.3 Hz | 95.9, d |
| 9″ | | 164.0, s | | 163.8, s |
| 10″ | | 103.5, s | | 102.2, s |
| 1‴ | | 128.0, s | | 130.5, s |
| 2‴ | 7.04, 1H, d, $J$=8.5 Hz | 128.2, d | 7.22, 1H, d, $J$=8.6 Hz | 129.7, d |
| 3‴ | 6.76, 1H, d, $J$=8.5 Hz | 116.1, d | 7.00, 1H, d, $J$=8.6 Hz | 114.9, d |
| 4‴ | | 157.9, s | | 161.4, s |
| 5‴ | 6.76, 1H, d, $J$=8.5 Hz | 116.1, d | 7.00, 1H, d, $J$=8.6 Hz | 114.9, d |
| 6‴ | 7.04, 1H, d, $J$=8.5 Hz | 128.2, d | 7.22, 1H, d, $J$=8.6 Hz | 129.7, d |
| 7-OMe | 3.82, 3H, s | 55.6, q | | |
| 4′-OMe | 3.84, 3H, s | 56.2, q | 3.82, 3H, s | 55.6, q |
| 4‴-OMe | | | 3.82, 3H, s | 55.6, q |

d, $J$=8.5 Hz), 7.15 (2H, d, $J$=8.6 Hz)].[1)]

From the ^{13}C-NMR data (Table 1), two carbonyls ($\delta_C$ 197.4, 197.0) and two methoxyl groups ($\delta_C$ 55.6, 56.2) were observed. The C-2, C-2″ ($\delta_C$ 81.4, 81.3) and C-3, C-3″ ($\delta_C$ 47.5, 47.4) signals in ^{13}C-NMR suggested **1** consisted of two units of flavanones.[4—6)] Linkage of the B ring to the C ring was established at C-2 by HMBC experiments, in which 2′-H [$\delta$ 7.15 (1H, d, $J$=8.6 Hz)] and 6′-H [$\delta$ 7.15 (1H, d, $J$=8.6 Hz)] correlated with C-2 ($\delta_C$ 81.4), 2-H ($\delta$ 5.52) coupled with C-1′ ($\delta_C$ 129.0). In the same way, linkage of the B′ ring to C-2″ of the C′ ring was deduced by the cross peaks of 2‴-H [$\delta$ 7.04 (1H, d, $J$=8.5 Hz)] and 6‴-H [$\delta$ 7.04 (1H, d, $J$=8.5 Hz)] with C-2″ ($\delta_C$ 81.3), and 2″-H ($\delta$ 5.44) with C-1‴ ($\delta_C$ 128.0). The presence of signals of 2-H [$\delta$ 5.52 (1H, br s)], 2″-H [$\delta$ 5.44 (1H, br s)], 6-H [$\delta$ 5.89 (1H, d, $J$=2.2 Hz)], 6″-H [$\delta$ 5.79 (1H, d, $J$=2.0 Hz)], 8-H [$\delta$ 6.02 (1H, d, $J$=2.2 Hz)], 8″-H [$\delta$ 5.93 (1H, d, $J$=2.0 Hz)], 2′,6′-H [$\delta$ 7.15 (2H, d, $J$=8.6 Hz)], 2‴,6‴-H [$\delta$ 7.04 (2H, d, $J$=8.5 Hz)], 3′,5′-H [$\delta$ 6.86 (2H, d, $J$=8.6 Hz)], and 3‴,5‴-H [$\delta$ 6.76 (2H, d, $J$=8.5 Hz)] suggested that the linkage of the two flavanones was only at the C-3/C-3″ positions, which was confirmed by comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of known C-3/C-3″ biflavanones.[4—6)] The HMBC correlations of one methoxyl group ($\delta$ 3.82) with C-7 ($\delta_C$ 168.8) on the A ring, and another methoxyl group ($\delta$ 3.84) with C-4′ ($\delta_C$ 160.3) on the B ring indicated that these two methoxyl groups were connected to C-7 and C-4′, respectively. The signals of C-10 ($\delta_C$ 104.0) and C-10″ ($\delta_C$ 103.5) were determined by HMBC experiments, in which protons at $\delta$ 5.98 (6-H) and 6.02 (8-H) correlated with C-10 ($\delta_C$ 104.0), and protons at $\delta$ 5.79 (6″-H) and 5.93 (8″-H) correlated with C-10″ ($\delta_C$ 103.5), respectively. The stereochemistries at the C-2/C-3 and C-3″/C-2″ positions were determined as *cis–cis* by comparison of the $J$-values (0 and 0 Hz) of the corresponding protons with the known C-3/C-3″ biflavanones.[4—6)] However, the chirality of C-3/C-3″ remains uncertain, like those biflavanones isolated from the same plant in previous studies.[1,4—6)] The structure of **1** was established to be [3,3′-Bi-4*H*-1-benzopyran]-4,4′-dione, 2,2′,3,3′-tetrahydro-5,5′,7′-trihydroxy-7-methoxy-2-(4-methoxyphenyl)-2′-(4-hydroxyphenyl)-(+), and named chamaejasmenin D.

Table 2. Antimitotic and Antifungal Activity of Different Extracts of *Stellera chamaejasme* Roots and Compounds **1**—**7** against *Pyricularia oryzae*

| Extract or compound | Concentration ($\mu$g/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 400 | 200 | 100 | 50 | 25 | 12.5 | 6.25 | 3.12 |
| EtOH | +++ | − | − | − | − | − | − | − |
| Petroleum ether | + | − | − | − | − | − | − | − |
| $CHCl_3$ | ++ | + | − | − | − | − | − | − |
| EtOAc | × | +++ | ++ | + | − | − | − | − |
| *n*-BuOH | − | − | − | − | − | − | − | − |
| **1** | × | × | × | +++ | +++ | ++ | + | − |
| **2** | × | × | × | ++ | + | + | + | − |
| **3** | × | × | × | × | ++ | ++ | + | + |
| **4** | ++ | ++ | ++ | + | − | − | − | − |
| **5** | × | +++ | + | − | − | − | − | − |
| **6** | ++ | + | − | − | − | − | − | − |
| **7** | − | − | − | − | − | − | − | − |

×, complete inhibition; +++, strong inhibition; ++, inhibition; +, weak inhibition; −, no activity, which were estimated by Kobayashi *et al.* (1996).[11)]

Isochamaejasmenin B (**2**), a brown amorphous powder, had a molecular formula of $C_{32}H_{26}O_{10}$ established by the negative HR-ESI-MS ($[M-H]^-$, $m/z$ 569.1449 (Calcd for $C_{32}H_{25}O_{10}$: 569.1448)). The ^{1}H-, ^{13}C-NMR (Table 1), IR and UV data suggested **2** to be a C-3/C-3″ biflavanone like **1**, and its structure was highly symmetric, which was also indicated by the EI-MS [$m/z$ 570 ($M^+$), 285 (1/2 $M^+$)].

The signals of two methoxyl protons [$\delta$ 3.82 (6H, s)], two sets of typical 5,7-dioxygenated A rings [$\delta$ 5.97 (2H, d, $J$=1.3 Hz), 5.82 (2H, d, $J$=1.3 Hz)], and two sets of typical *para*-oxygenated B rings [$\delta$ 7.00 (4H, d, $J$=8.6 Hz), $\delta$ 7.22 (4H, d, $J$=8.6 Hz)] in the ^{1}H-NMR, together with the presence of two carbonyls ($\delta_C$ 196.0), two methoxyl groups ($\delta_C$ 55.6), and C-2, C-2″ ($\delta_C$ 81.7) and C-3, C-3″ ($\delta_C$ 48.3) signals in the ^{13}C-NMR indicated that **2** was similar to the known C-3/C-3″ biflavanone chamaejasmenin B.[4)] Further, the HMBC correlations of two methoxyl groups ($\delta$ 3.82) with C-4′, C-4‴ ($\delta_C$ 161.4) on the B, B′ rings revealed that these two methoxyl groups were also connected to C-4′ and C-4‴ as chamaejasmenin B, indicating that **2** and chamaejasmenin B had the same plane structure. Comparison of the ^{1}H-NMR spectral data of **2** with those of chamaejasmenin B revealed that the chemical shift values and splitting patterns of **2** agreed well with those of chamaejasmenin B, except that the signals of 2,2″-H [$\delta$ 5.32 (2H, s)] and 3,3″-H [$\delta$ 2.98 (2H,

s)] in chamaejasmenin B were different from those of 2,2″-H [$\delta$ 4.97 (2H, br s)] and 3,3″-H [$\delta$ 3.86 (2H, br s)] in **2**, which suggested that the stereochemistries of **2** and chamaejasmenin B were different.[4,7—9] The $[\alpha]_D^{20}$ values of **2** and chamaejasmenin B were 0° ($c$=0.1, MeOH) and +150° ($c$=1.0, EtOH), respectively, indicating that **2** was the *meso*-form and chamaejasmine B was the *d*-form. There are four chirality carbons at the C-2, C-3, C-2″, and C-3″ positions in **2** and chamaejasmenin B, respectively. The *J*-values (0 and 0 Hz) of 2,3-H and 2″,3″-H in **2** indicated that **2** has *cis–cis* geometry at the C-2/C-3 and C-3″/C-2″ positions, same as that of chamaejasmenin B. Therefore, the differences in their stereochemistries should be only at the C-3/C-3″ positions. The stereochemistry of **2** at the C-3/C-3″ position was exactly adverse to that of chamaejasmenin B (Fig. 1), which had *cis* or *trans* geometry at the C-3/C-3″ positions.[4] However, the chirality of C-3/C-3″ remains uncertain, like those known biflavanones.[1,4—6] In previous studies, the *meso*-form and *d*- or *l*-form biflavanones as **2** and chamaejasmenin B were commonly reported, such as isochamaejasmin (*meso*-form)[7] and chamaejasmenin (*l*-form),[8] isoneochamaejasmin A (*meso*-form)[9] and neochamaejasmin A (*d*-form)[5] isolated from the same plant. The structure of **2** was thus established to be [3,3′-Bi-4*H*-1-benzopyran]-4,4′-dione, 2,2′,3,3′-tetrahydro-5,5′,7′,7-tetrahydroxy-2,2′-bis(4-methoxyphenyl)-(*meso*), and named isochamaejasmenin B.

Compounds **3**—**7** were identified as chamaejasmenin A (**3**),[4] chamaejasmenin B (**4**),[4] neochamaejasmin A (**5**),[5] sikokianin A (**6**),[6] and chamaejasmenin C (**7**)[4] by comparison of their $^{1}$H-, $^{13}$C-NMR, MS, and $[\alpha]_D$ data with those reported previously.

Morphological deformations of mycelia or conidia of microorganisms, such as curling, swelling, hyperdivergence, bead formation, and inhibition of germination, are often induced in the presence of bioactive substances.[10] A new screening bioassay detecting deformation of mycelia germinated from conidia of *Pyricularia oryzae*, a phytopathogenic fungus, was developed for quantitative application in screening antimitotic and antifungal agents by Kobayashi *et al.*[11] This bioassay method is quick, easy to perform, and has been efficiently used in the screening of antimitotic, antineoplastic, and antifungal agents,[12] such as rhizoxin[13] and fusarielin A[14] from fungal metabolites.

This bioassay method was also successfully applied to screen for antineoplastic and antifungal agents from traditional Chinese medicines.[15] In our study, the activities of extracts or compounds were screened using *P. oryzae* as the indicator organism in a 96-well micro-plate assay for inhibition of hyphal growth or for deformation of conidia; minimum inhibitory concentration (MIC) values were calculated based on weight per volume ($\mu$g/ml).[11] The positive control, rhizoxin, had a MIC value of 1.25 $\mu$g/ml.

Compounds **1**—**7** were screened using *P. oryzae* as the indicator organism. Chamaejasmenin A (**3**), chamaejasmenin D (**1**), and isochamaejasmenin B (**2**) demonstrated potent antimitotic and antifungal activity with MIC values of 3.12, 6.25, and 6.25 $\mu$g/ml, respectively, chamaejasmenin B (**4**), neochamaejasmin A (**5**) and sikokianin A (**6**) showed weak activity, and chamaejasmenin C (**7**) was inactive (Table 2).

Previous chemical and pharmacological studies on *S. chamaejasme* indicated that the biflavanones from this plant exhibited antibacterial and immunomodulating activity,[1] while some of the biflavanones also showed anti-HIV activity.[16] In our study, the biflavanones isolated from *S. chamaejasme* root showed potent antimitotic and antifungal activity against *P. oryzae*, which suggested that the biflavanones might be the principal antimitotic and antifungal components in *S. chamaejasme*.

## Experimental

**General Experimental Procedures** Optical rotations were measured on a P-1020 polarimeter (JASCO, Japan). UV spectra were obtained with a UV-260 spectrophotometer (Shimadzu, Japan). IR spectroscopic data were recorded on a Avatar 360 E.S.R. FT-IR spectroscopy (Thermo Nicolet, U.S.A.) with KBr pellets. NMR spectra were obtained on a Bruker DRX-400 NMR, chemical shifts were reported with respect to acetone-$d_6$ ($\delta_H$ 2.04, $\delta_C$ 206.0 ppm). EI-MS were recorded on an Agilent 5973N mass spectrometer. HR-ESI-MS data were obtained on a Q-Tof Micro spectrometer (Micro Mass, England). Column chromatography was performed with silica gel (200—300 mesh, Yantai, China), silica gel H (10—40 $\mu$m, Yantai, China), Lichroprep $RP_{18}$ gel (40—63 $\mu$m, Merck, Darmstadt, Germany), and Sephadex LH-20 (Pharmacia). TLC analysis was run on $GF_{254}$ precoated silica gel plates (10—40 $\mu$m, Yantai, China). An inverted microscope (37×B, Shanghai Sixth Optic Instrument Factory, Shanghai, China) was used for observing the shape of the mycelia that germinated from conidia.

**Plant Material** The roots of *Stellera chamaejasme* L. were collected in Ganzi, Sichuan Province, China, in September of 2003. The identity of the plant material was verified by Professor Daofeng Chen, and a voucher specimen (DFC-RXLD0309) is deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, China.

**Extraction and Isolation** The roots (10 kg) of *S. chamaejasme* were air-dried, ground, and extracted with 95% EtOH at room temperature. The EtOH extract was evaporated *in vacuo* to yield a semisolid (1500 g), 1490 g of which was suspended in $H_2O$ (5000 ml) and partitioned successively with petroleum ether, $CHCl_3$, EtOAc, *n*-BuOH to yield 120 g, 30 g, 430 g, and 125 g, respectively. Three hundred grams of the bioactive EtOAc extract was subjected to column chromatography on Si gel eluted with $CHCl_3$–MeOH (99 : 1, 49 : 1, 19 : 1, 9 : 1, 4 : 1, 1 : 1) and MeOH to yield fractions 1—10. Fraction 3 (1.77 g) was chromatographed over Si gel (petroleum ether–EtOAc, 6.5 : 3.5) to give fractions 3a—e. Fraction 3a was crystallized repeatedly with petroleum ether–acetone to provide **7** (71 mg). Fraction 4 (7.82 g) was purified by column chromatography over Si gel (petroleum ether–EtOAc, 6.5 : 3.5) to afford fractions 4a—f. Fraction 4b was eluted with $CHCl_3$–MeOH–$H_2O$ (98 : 2 : 0.2) over Si gel, followed by purification with $RP_{18}$ ($CH_3CN$–$H_2O$, 3 : 2) to give **2** (12 mg) and **3** (21 mg). Fraction 4c was chromatographed over Si gel eluting with $CHCl_3$–MeOH–$H_2O$ (98 : 2 : 0.2) to provide **1** (32 mg) and **4** (271 mg). Fraction 6 (9 g) was fractioned over Si gel ($CHCl_3$–MeOH–$H_2O$, 95 : 5 : 0.2) to afford fractions 6a—f. Fraction 6c was purified by column chromatography over Sephadex LH-20 (40% EtOH) to give **6** (50 mg). Fraction 6f was isolated over Si gel with $CHCl_3$–MeOH–$H_2O$ (95 : 5 : 0.2) and purified successively with 50% MeOH over Sephadex LH-20 to afford **5** (11 mg).

Chamaejasmenin D ([3,3′-Bi-4*H*-1-benzopyran]-4,4′-dione, 2,2′,3,3′-tetrahydro-5,5′,7′-trihydroxy-7-methoxy-2-(4-methoxyphenyl)-2′-(4-hydroxyphenyl-(+)) (**1**): White amorphous powder; $[\alpha]_D^{20}$ +307° ($c$=0.01, MeOH); UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 217 (4.63), 298 (4.44), 330 (sh); IR (KBr) $cm^{-1}$: 3480, 1642, 1600, 1517; $^{1}$H- and $^{13}$C-NMR data, see Table 1; EI-MS *m/z*: 570 ($M^+$), 299, 271, 167, 153, 121, 107, 95, 69, and 57; Negative HR-ESI-MS *m/z* 569.1449 $[M-H]^-$ (Calcd for $C_{32}H_{25}O_{10}$: 569.1448).

Isochamaejasmenin B ([3,3′-Bi-4*H*-1-benzopyran]-4,4′-dione, 2,2′,3,3′-tetrahydro-5,5′,7′,7-tetrahydroxy-2,2′-bis(4-methoxyphenyl)-(*meso*) (**2**): Brown amorphous powder; $[\alpha]_D^{20}$ 0° ($c$=0.01, MeOH); UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$) 217 (4.63), 298 (4.44), 330 (sh); IR (KBr) $cm^{-1}$: 3383, 1636, 1600, 1516, 1461; $^{1}$H- and $^{13}$C-NMR data, see Table 1; EI-MS *m/z*: 570 ($M^+$), 285 (1/2$M^+$); Negative HR-ESI-MS *m/z* 569.1449 $[M-H]^-$ (Calcd for $C_{32}H_{25}O_{10}$: 569.1448).

***Pyricularia oryzae* Bioassay** The assay was performed by the method of Kobayashi *et al.*[11] *P. oryzae* was grown on a slant culture medium consisting of yeast extract (0.2%), soluble starch (1%) and agar (2%) at 27 °C. The conidia were collected after 12 to 14 d of inoculation by suspending in sterilized water. The suspension was filtered to separate conidia from mycelia. The filtrate was added to a 2% solution of yeast extract, which was adjusted

to a final concentration of 0.02% with sterilized water. For quantitative estimation, an aliquot of the conidia suspension was taken on a microscope to count the number of conidia before adding yeast extract. The suspension was adjusted to $4 \times 10^4$ conidia/ml by adding sterilized water. A 96-well micro-plate was used for the bioassay. Rhizoxin was used as the positive control. One column of the 96-well micro-plate was used for one test material with eight different concentrations. The assay plates were incubated at 27 °C for 16 h and the shape of the mycelia that germinated from conidia was observed and compared with controls under an inverted microscope.

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