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Longeracemosones A–F, Aromatase Inhibitors from *Dunbaria longeracemosa*

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Six dihydroflavonols, longeracemosones A–F (**1**–**6**), together with two known flavonoids, mundulinol (**7**) and sericetin (**8**), have been isolated from *Dunbaria longeracemosa*. The structures of the isolated compounds were elucidated by spectroscopic analysis and the absolute configurations were determined by analysis of the CD spectra and by the modified Mosher's method. A novel conversion of the linear chromene in **1** to the angular chromene in **2** was observed and the mechanism of this unique transformation is proposed. The isolated compounds were evaluated for their cytotoxic and antioxidant activity and compounds **3**, **4**, and **7** exhibited potent aromatase inhibitory activity with IC_{50} values of 0.3, 0.4, and 1.2 μM, respectively.

Introduction

Dunbaria longeracemosa (syn. *D. bella*) is a climber tree belonging to the family of Leguminosae (or Fabaceae), subfamily Papilionoideae.[1] This plant is known in Thai as Khang-Krung. The flower of this plant is edible and its leaves or roots mixed with the leaves of *Senna hirsuta* have been used in Thai folk medicine for the treatment of fever. To the best of our knowledge, *D. longeracemosa* has never been chemically investigated. In this paper we report the isolation and characterization of six new bioactive dihydroflavonols from *D. longeracemosa*.

Results and Discussion

A methanol extract of the air-dried whole plant of *D. longeracemosa* was mixed with water and then partitioned with CH_2Cl_2. The CH_2Cl_2 layer was sequentially subjected to column chromatography on silica gel, HPLC, and preparative TLC to obtain six new dihydroflavonols **1**–**6**, longeracemosones A–F, along with two known flavonoids, mundulinol (**7**) and sericetin (**8**). The spectral data of the known flavonoids **7** and **8** were identical in all respects to those reported in the literature.[2]

Longeracemosone A (**1**) was obtained as a pale-yellow oil and its molecular formula was established by HRFABMS as $C_{21}H_{18}O_6$. The ^1H NMR spectrum of **1** shows the signals of two hydroxy groups at δ_H = 11.90 (s) and 3.42 ppm (d, J = 1.4 Hz), an aldehydic proton at δ_H = 10.21 ppm (s, 1 H), a monosubstituted phenyl ring at δ_H = 7.54 (m, 2 H) and 7.35–7.43 ppm (m, 3 H), and two protons attached to oxygenated carbon at δ_H = 5.15 (d, J = 12.1 Hz) and 4.49 ppm (dd, J = 12.1, 1.4 Hz). The signals of the two protons attached to oxygenated carbon are typical of 2-H and 3-H in a dihydroflavonol skeleton.[3]

The presence of two *cis*-coupled olefinic protons (J = 10.1 Hz) at δ_H = 5.55 (3′′-H) and 6.55 ppm (4′′-H) and two methyl singlets at δ_H = 1.45 (5′′-H) and 1.46 ppm (6′′-H) suggest the presence of a 2,2-dimethylchromene ring in **1**; the HMBC correlations of 4′′-H, 5′′-H, and 6′′-H to C-2′′ confirmed the presence of a 2,2-dimethylchromene unit. The ^{13}C NMR resonance at δ_C = 196.4 ppm was assigned to a conjugated ketone carbonyl, which chelates to a hydroxy group showing a downfield signal at δ_H = 11.90 ppm (s), and thus this hydroxy was placed at C-5. The HMBC spectrum also showed a correlation between 5-OH and C-5, which confirms the position of 5-OH. The HMBC correlations observed between 2-H and C-3, C-4, and C-2′ (or C-6′), between 3-H and C-1′, and between 3-OH and C-2, C-3, and C-4 indicate that the dihydroflavonol unit in **1** is attached to a monosubstituted phenyl ring at C-2 and that the hydroxy group is located at C-3. The HMBC spectrum of **1** shows a correlation between the aldehydic proton and C-7, C-8, and C-9, which places the aldehyde group at C-8. The HMBC correlations between 3′′-H and C-6 and between 4′′-H and C-6 and C-7 indicate that the 2,2-dimethylpyran unit is fused to the C-6 and C-7 positions of ring A in **1**. Based upon these data, the gross structure of **1** was established. The coupling constant of $J_{2\text{-H},3\text{-H}}$ (12.1 Hz) suggests that the two protons at C-2 and C-3 are in *trans* diaxial positions. The absolute stereochemistry at C-2 in **1** is *R*, as revealed by the analysis of the CD spectrum in which positive and negative Cotton effects were observed at 325 and 262–300 nm, respectively.[4] Based on the spectroscopic data, longeracemosone A (**1**) was identified as a 6,7-pyrano-fused (2*R*,3*R*)-8-formyl-3,5-dihydroxyflavanone.

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The assignments of the ^1H and ^{13}C NMR resonances of **1** are presented in Tables 1 and 2.

Longeracemosone B (**2**) has a molecular formula of $C_{21}H_{18}O_6$ (by HRFABMS), the same as **1**. In general, the ^1H and ^{13}C NMR spectroscopic data of **2** are similar to those of **1** and analysis of the NMR data reveals that compound **2** is a regioisomer of **1**. The downfield shifts of an aldehydic carbon (from δ_C = 185.5 ppm for **1** to δ_C = 191.8 ppm for **2**) and a chelated hydroxy proton (from δ_H = 11.90 ppm for **1** to δ_H = 13.04 ppm for **2**) indicate that there is a hydrogen bond between the aldehydic carbonyl and 7-OH. The HMBC correlations between the 7-OH proton and C-6 and C-7 and between the aldehydic proton and C-7 and C-8 confirm the positions of the aldehyde and 7-OH in **2**. Further HMBC correlations between 3′′-H and C-6 and between 4′′-H and C-5 established the position of the 2,2-dimethylpyran unit in **2** as shown. Again, the coupling constant of $J_{2\text{-H},3\text{-H}}$ (12.2 Hz) suggests that the two protons at C-2 and C-3 are in a *trans* diaxial position. Longeracemosones A (**1**) and B (**2**) should have a similar biosynthetic pathway and they should also share the same absolute configuration. Thus, based upon these spectroscopic data, longeracemosone B (**2**) was identified as a 5,6-pyrano-fused (2*R*,3*R*)-8-formyl-3,7-dihydroxyflavanone. The protons and carbons in **2** were assigned by analysis of the spectroscopic data shown in Tables 1 and 2.

Table 1. ^1H NMR (400 MHz, $CDCl_3$) data of compounds **1**–**6**.[a]

Position	**1**	**2**	**3**	**4**	**5**	**6**
2	5.15 (d, 12.1)	5.15 (d, 12.2)	5.01 (d, 11.9)	5.05 (d, 11.9)	5.04/5.06 (d, 11.9)	5.04/5.05 (d, 11.9)
3	4.49 (dd, 12.1, 1.4)	4.48 (d, 12.2)	4.50 (d, 11.9)	4.50 (d, 11.9)	4.48/4.55 (dd, 11.9, 1.3)	4.52/4.54 (d, 11.9)
2′,6′	7.54 (m)	7.55 (m)	7.54 (m)	7.54 (m)	7.53 (m)	7.50 (m)
3′–5′	7.35–7.43 (m)	7.44–7.50 (m)	7.41–7.48 (m)	7.41–7.48 (m)	7.40–7.47 (m)	7.41–7.46 (m)
3′′	5.55 (d, 10.1)	5.59 (d, 10.1)	5.54 (d, 10.0)	5.55 (d, 10.0)	5.55 (d, 10.0)	5.57 (d, 10.1)
4′′	6.55 (d, 10.1)	6.60 (d, 10.1)	6.65 (d, 10.0)	6.66 (d, 10.0)	6.65/6.652 (d, 10.0)	6.65 (d, 10.1)
5′′,6′′	1.45, 1.46 (s)	1.52, 1.61 (s)	1.48 (s)	1.47, 1.49 (s)	1.46,1.47/1.47,1.48 (s)	1.46,1.47/1.47,1.475
1′′′	10.21 (s)	10.08 (s)	2.71 (dd, 13.7, 7.7) 2.87 (dd, 13.7, 5.2)	2.74 (dd, 13.8, 8.0) 2.87 (dd, 13.8, 4.5)	2.60/2.64 (dd, 14.8, 8.7) 2.92[b] (m)	4.47 (d, 7.1)
2′′′	–	–	4.18 (dd, 7.7, 5.2)	4.23 (dd, 8.0, 4.5)	2.92[b] (m)	3.57/3.61 (d, 7.1)
4′′′	–	–	4.75 (br. s) 4.81 (br. s)	4.77 (br. s) 4.87 (br. s)	1.23/1.25 (s)	1.09/1.15 (s)
5′′′	–	–	1.65 (s)	1.68 (s)	1.21/1.24 (s)	1.15/1.27 (s)
3-OH	3.42 (d, 1.4)	–	3.60 (br. s)	3.55 (br. s)	3.60/3.62 (d, 1.3)	3.49/3.50 (s)
5-OH	11.90 (s)	–	11.45 (s)	11.45 (s)	11.48/11.49 (s)	11.62/11.622 (s)
7-OH	–	13.04 (s)	–	–	–	–
1′′′-OCH_3	–	–	–	–	–	3.33/3.35 (s)

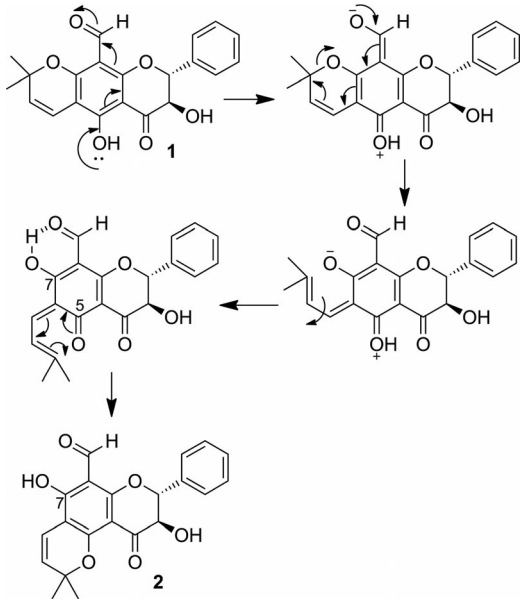
[a] Chemical shifts are given in ppm. Multiplicity and coupling constants [Hz] are presented in parentheses. [b] Overlapped signal.

Table 2. ^{13}C NMR (100 MHz, $CDCl_3$) data of compounds **1**–**6**.[a]

Position	**1**	**2**	**3**	**4**	**5**	**6**
2	83.5	84.0	83.3	83.2	83.2/83.3	83.7/83.6
3	72.0	72.5	72.5	72.5	72.2/72.5	72.4/72.5
4	196.4	189.3	196.2	196.1	196.1/196.2	196.3/196.4
5	161.0	161.7	156.4	156.4	156.5	157.8
6	103.2	103.6	103.2	103.1	103.16/103.22	103.5
7	164.4[b]	164.2	160.7	160.7	160.9/161.0	161.0
8	107.5	104.3	106.0	106.0	105.3	106.2/106.1
9	164.0[b]	166.2	159.9	159.9	159.8	159.0
10	99.7	101.4	100.3	100.3	100.28/100.34	100.2
1′	135.3	135.6	136.2	136.2	136.1/136.2	135.6/135.8
2′,6′	127.2	127.4	127.2	127.2	127.3/127.5	127.1/127.2
3′,5′	128.7	128.8	128.7	128.6	128.6	128.7
4′	129.3	129.5	129.2	129.2	129.2/129.3	129.3/129.4
2′′	80.2	80.5	79.0	79.1	78.9	79.2
3′′	127.0	126.5	126.2	126.2	126.3/126.4	126.58/126.61
4′′	114.4	114.4	115.3	115.3	115.3	115.0
5′′,6′′	28.4, 28.5	28.5, 28.7	28.4, 28.5	28.4, 28.5	28.3, 28.5/28.6	28.3, 28.4
1′′′	185.5	191.8	29.0	28.9	21.7/21.8	74.66/74.73
2′′′	–	–	75.8	75.5	63.2/63.3	65.7/65.9
3′′′	–	–	147.0	147.1	59.1/59.2	56.84
4′′′	–	–	110.4	110.1	18.9/19.0	19.2/19.5
5′′′	–	–	17.7	18.2	24.8	24.7/24.9
1′′′-OCH_3	–	–	–	–	–	56.69/56.77

[a] Chemical shifts are given in ppm. [b] Interchangeable.

Longeracemosones A (**1**) and B (**2**) are unstable and easily decomposed during purification by silica gel TLC. Interestingly, the conversion of **1** into **2** was observed at room temperature, and this can be rationalized by ring-opening of the 2,2-dimethylchromene ring assisted by the presence of the phenolic hydroxy group in *para* position to the aldehyde group, as shown in Scheme 1. Subsequent proton transfer from the protonated carbonyl group to the phenoxide and bond rotation gives rise to the quinone methide intermediate. The newly formed hydrogen bond between the phenolic hydroxy group at C-7 and the aldehydic oxygen could be the driving force for the conversion. As far as we are aware, this novel and unique transformation of a linear ring-fused chromene to an angularly fused chromene is unprecedented.



Scheme 1. Proposed mechanism for the conversion of **1** into **2**.

Longeracemosone C (**3**) was isolated as a yellow amorphous solid and its molecular formula, $C_{25}H_{26}O_6$, was established by HRFABMS. The ^1H and ^{13}C NMR spectroscopic data (Table 1 and Table 2) of **3** are similar to those of the known flavonol mundulinol (**7**)[2] except for the signals of a prenyl group at C-8. Analysis of the NMR data of **3** revealed the replacement of the prenyl group in **7** by a 2-hydroxy-3-methylbut-3-ene moiety, which shows signals typical of an *exo*-methylene (δ_H = 4.81 and 4.75 ppm), an oxygenated methine (δ_H = 4.18 ppm), and a singlet due to methyl protons (δ_H = 1.65 ppm). This was further supported by the fragment ion at *m*/*z* = 351 $[M - C_4H_7O_1]^+$ in the EI mass spectrum. The absolute stereochemistry of the secondary alcohol at C-2′′′ of **3** was determined by the modified Mosher's method.[5] Longeracemosone C (**3**) was treated with both (*R*)- and (*S*)-methoxyphenylacetic acid (MPAA) to afford MPA monoester (**3a**) and MPA diester (**3b**). Analysis of the differences in the chemical shifts of the (*R*)- and (*S*)-MPA diesters (Figure 1) indicates that the absolute stereochemistry at C-2′′′ is *S*. Although there is no proton at C-4 in **3**, the differences in the chemical shifts of the (*R*)- and (*S*)-MPA monoesters (Figure 1) imply that the absolute configuration at C-3 is *R*, which confirms the *R* configuration deduced by analysis of the CD spectra of compounds **1** and **2** mentioned earlier. On the basis of these data, longeracemosone C (**3**) was identified as a 6,7-pyrano-fused (2*R*,3*R*,2′′′*S*)-3,5-dihydroxy-8-(2-hydroxy-3-methylbut-3-enyl)flavanone.

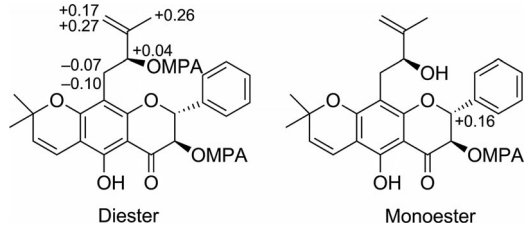


Figure 1. $\Delta\delta$ ($\delta_R - \delta_S$) values of the (*R*)- and (*S*)-MPA diesters and monoesters of **3**.

A molecular formula of $C_{25}H_{26}O_6$ was deduced from the HRFABMS of longeracemosone D (**4**). The NMR spectra of **4**, as well as the UV and IR spectra, almost superimposed those of **3**, which suggests that **4** is a stereoisomer of **3**. As expected, analysis of the differences in the chemical shifts of the (*R*)- and (*S*)-diesters of **4** indicate that C-2′′′ in **3** has the *R* configuration (Figure 2), in contrast to that of **3**. Again, analysis of the chemical shift differences between the (*R*)- and (*S*)-MPA monoesters (Figure 2) suggests the *R* configuration at C-2 in **4**. Longeracemosone D (**4**) was therefore identified as a 6,7-pyrano-fused (2*R*,3*R*,2′′′*R*)-3,5-dihydroxy-8-(2-hydroxy-3-methylbut-3-enyl)flavanone.

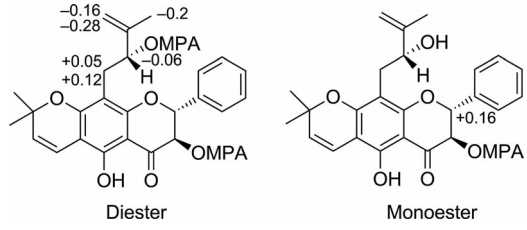
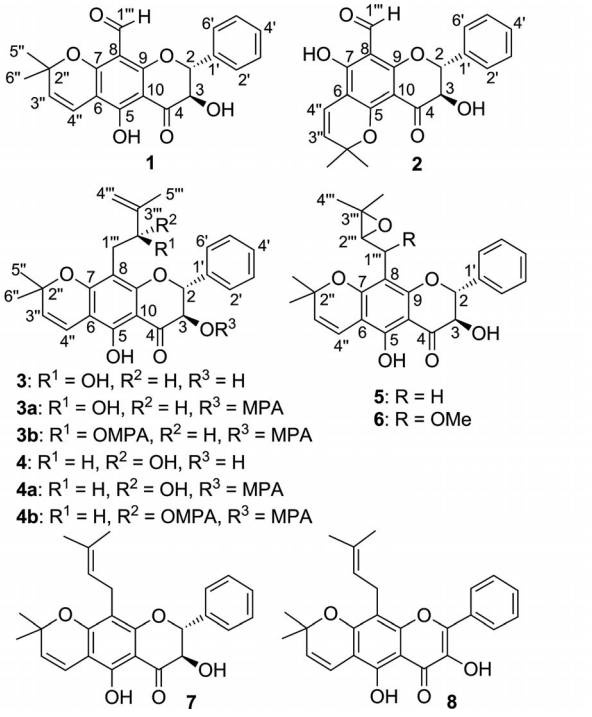


Figure 2. $\Delta\delta$ ($\delta_R - \delta_S$) values of the (*R*)- and (*S*)-MPA diesters and monoesters of **4**.

Longeracemosone E (**5**), a yellow amorphous solid, has the molecular formula $C_{25}H_{26}O_6$, determined by HRFABMS. However, the ^1H and ^{13}C NMR spectra of **5** display duplicate signals of some protons and carbons (see Tables 1 and 2), which suggests that it is a diastereomeric mixture. The NMR data of **5** are similar to those of compounds **3**, **4**, and **7**, and careful analysis of the spectroscopic data revealed that **5** possesses a 2,3-epoxy-3-methylbutane unit. Longeracemosone E (**5**) was therefore identified as 6,7-(2,2-dimethylchromene)-8-(2,3-epoxy-3-methylbutyl)-3,5-dihydroxyflavanone. As the NMR signals of particular positions (see Tables 1 and 2) are duplicated, it is possible that compound **5** contains two C-2′′′ epimers. Previously, we isolated a flavone with a 2,3-epoxy-3-methylbutane moiety from *Derris reticulata*,[6] which was obtained as a mixture of two epimers at the C-2′′′ position.

Longeracemosone F (**6**) has a molecular formula of $C_{26}H_{28}O_7$, determined by HRFABMS. Similarly to compound **5**, the ^1H and ^{13}C NMR spectra of **6** display duplicate signals of particular positions, which suggests that it is a diastereomeric mixture. The NMR data of **6** are almost identical to those of **5**, except that one of the methylene protons at the 1‴-position in **5** is replaced by a methoxy signal (δ_H = 3.33/3.35 ppm and δ_C = 56.69/56.77 ppm) in **6**. Therefore compound **6** is a methoxy derivative of **5** and is identified as a 6,7-pyrano-fused 8-(2,3-epoxy-1-methoxy-3-methylbutyl)-3,5-dihydroxyflavanone. Note that compound **6** may be an artifact arising from the use of MeOH during the extraction and purification processes. A possible pathway proceeds via the formation of the quinone methide intermediate, which subsequently reacts with MeOH to give the product **6**. Such a mechanism has previously been reported by our group.[7]



Compounds **3**, **4**, **7**, and **8** were evaluated for their cytotoxicity against five cancer cell lines: KB (human mouth epidermal carcinoma), HepG2 (human hepatocellular liver carcinoma), HuCCA-1 (human cholangiocarcinoma), A549 (human lung carcinoma), and S102 (human liver cancer). Compounds **3**, **4**, and **7** showed weak cytotoxic activity with IC_{50} values of 11.0–36.7 μM, whereas sericetin (**8**) exhibited cytotoxic activity against the KB (5.7 μM) and HepG2 (5.9 μM) cell lines, the same order of magnitude as the standard drug, etoposide (Table 3). Compounds **3**, **4**, and **7** inhibited aromatase with IC_{50} values of 0.3, 0.4, and 1.2 μM, respectively, and were found to be more potent than the reference compound ketoconazole (IC_{50} = 2.4 μM). Compound **8** was inactive towards aromatase inhibition. Inhibitors of aromatase are known to be promising candidates for the development of cancer chemoprevention agents.[8] Unfortunately, the biological activities of compounds **1** and **2** could not be assessed due to limited amounts of the materials.

Table 3. Cytotoxic activity for compounds **3**, **4**, **7**, and **8**.

Compd.	Cytotoxic activity (IC_{50}) [μM]				
	KB	HepG2	HuCCA-1	A549	S102
3	23.2	11.0	36.7	15.3	29.0
4	26.1	12.4	36.7	27.8	34.4
7	21.7	11.6	25.2	15.9	17.7
8	5.7	5.9	21.3	28.7	37.1
Etoposide	0.9	1.4	9.4	0.7	1.7

Conclusions

A chemical investigation of the whole plant extract of *Dunbaria longeracemosa* led to the isolation of six new dihydroflavonols, longeracemosones A–F (**1**–**6**), and two known flavonoids, mundulinol (**7**) and sericetin (**8**). The absolute configurations at C-2 and C-3 of the isolated compounds were determined by analysis of the CD spectra and the absolute configuration of the secondary alcohol in the 2-hydroxy-3-methylbut-3-ene moiety of **3** and **4** was established by the modified Mosher's method. Compounds **3**, **4**, and **7** showed only weak cytotoxicity against five cancer cell lines, however, they inhibited aromatase with IC_{50} values of 0.3, 0.4, and 1.2 μM, respectively. Sericetin (**8**) exhibited cytotoxic activity against the KB and HepG2 cancer cell lines, but it did not inhibit the aromatase enzyme.

Experimental Section

General Methods: ^1H and ^{13}C NMR spectra were recorded with Bruker AM 400 (400 MHz) and Varian GEMINI 2000 (200 MHz) spectrometers in $CDCl_3$ with $Si(CH_3)_4$ as the internal standard. Infrared spectra (IR) were obtained with Perkin–Elmer System 2000 FT-IR and 1760 X FT-IR spectrometers. Mass spectra were recorded with a Finnigan Mat GCQ spectrometer for EIMS and a Finnigan MAT 90 spectrometer for HRFABMS. ESI-TOF MS spectra were recorded with a Bruker MicroTOFLC mass spectrometer. UV spectra were recorded with a Shimadzu UV/Vis 2001s spectrophotometer. Optical rotations were measured by using the sodium D line (590 nm) with a JASCO DIP3-70 digital polarimeter. CD spectra were recorded in MeOH with a JASCO J-810 spectropolarimeter. High-performance liquid chromatography (HPLC) was performed by using a C18 reversed-phase column as well as an Exsil 100–10ODS HICHROM stainless-steel column (250 × 21.20 mm).

Plant Material: Whole plants of *Dunbaria longeracemosa* were collected from Ubon Ratchathani province in May 2002 and were identified by Dr. Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. A voucher specimen has been deposited at the Laboratory of Natural Products, Chulabhorn Research Institute.

Extraction and Isolation: Air-dried whole plants of *D. longeracemosa* (0.4 kg) were ground and extracted with MeOH three times at room temperature. Evaporation of the MeOH extract gave a residue, which was suspended with water and then partitioned with CH_2Cl_2. The CH_2Cl_2 layer was evaporated to give a dark solid

(48 g), which was subjected to column chromatography on silica gel using hexane as eluent with increasing proportions of EtOAc, EtOAc as eluent with increasing proportions of MeOH, and finally with MeOH to provide 12 fractions. Fraction 4 (6.8 g), which was eluted by 8% EtOAc in hexane, was further separated by silica gel column chromatography using a gradient mixture of hexane and acetone and finally with acetone to afford fractions A1–A11. Fraction A2 was purified by preparative TLC using 2% acetone in hexane as eluent to provide sericetin (**8**; 118 mg). Fraction A4 was separated by reversed-phase HPLC and eluted with MeOH/H_2O (9:1) to give mundulinol (**7**; 510 mg). Fraction A6 was purified by HPLC and eluted with MeOH/H_2O (7:3) to give **2** (8 mg) and **5** (25 mg). Fraction A8 was separated by HPLC using MeOH/H_2O (7:3) as eluent to furnish compounds **1** (15 mg), **3** (40 mg), **4** (45 mg), and **6** (5 mg).

Longeracemosone A (1): Pale-yellow oil. $[\alpha]_D^{28}$ = –17.8 (*c* = 0.74, $CHCl_3$). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 211 [4.01], 268 nm [4.41]. CD (MeOH): λ (Δε) = 221 (+3.03), 262 (–2.34), 276 (–1.94), 300 (–1.74), 325 nm (+0.34 M^{-1} cm^{-1}). For the ^{1}H and ^{13}C NMR data, see Table 1 and Table 2. MS (EI): *m*/*z* (%) = 366 (28) $[M]^+$, 351 (100), 247 (16), 245 (13), 231 (71), 229 (25), 205 (58), 203 (26), 175 (7), 159 (12), 91 (24), 77 (17). HRMS (FAB): calcd. for $C_{21}H_{19}O_6$ $[M + H]^+$ 367.1182; found 367.1180.

Longeracemosone B (2): Pale-yellow oil. $[\alpha]_D^{23}$ = –63.0 (*c* = 0.19, $CHCl_3$). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 217 [4.06], 275 nm [3.99]. CD (MeOH): λ (Δε) = 221 (+2.00), 270 (–1.50), 279 (–1.50), 304 (–1.20), 347 nm (+0.28 M^{-1} cm^{-1}). For the ^{1}H and ^{13}C NMR data, see Table 1 and Table 2. MS (EI): *m*/*z* (%) = 366 (23) $[M]^+$, 351 (80), 333 (9), 309 (13), 247 (60), 231 (100), 229 (24), 218 (12), 205 (23), 203 (27). HRMS (FAB): calcd. for $C_{21}H_{19}O_6$ $[M + H]^+$ 367.1182; found 367.1175.

Longeracemosone C (3): Yellow amorphous solid. $[\alpha]_D^{26}$ = +25.7 (*c* = 0.61, $CHCl_3$). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 204 [4.21], 267 [4.37], 275 [4.41], 314 nm [3.92]. CD (MeOH): λ (Δε) = 233 (+4.59), 272 (+6.25), 302 (–6.93), 325 (+1.75), 353 nm (+2.34 M^{-1} cm^{-1}). FTIR (KBr): ṽ = 3436, 2967, 1627, 1457, 1287, 1193, 1127, 1025, 900 cm^{-1}. For the ^{1}H and ^{13}C NMR data, see Tables 1 and 2. MS (EI): *m*/*z* (%) = 422 (14) $[M]^+$, 352 (29), 351 (100), 261 (8), 231 (55), 205 (34), 189 (33), 163 (12), 135 (9). HRMS (FAB): calcd. for $C_{25}H_{27}O_6$ $[M + H]^+$ 423.1808; found 423.1804.

Preparation of the (*R*)-MPA Monoester (*R*)-3a and (*R*)-MPA Diester (*R*)-3b: Compound **3** (5.3 mg, 0.0126 mmol) was treated with (*R*)-(–)-α-methoxyphenylacetic acid (8.3 mg, 0.050 mmol) and *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC; 9.55 mg, 0.050 mmol) in the presence of a catalytic amount of 4-(dimethylamino)pyridine in CH_2Cl_2 at room temperature for 24 h. The reaction mixture was dried under vacuum and purified by preparative TLC (hexane/acetone, 8:2) to give (*R*)-MPA monoester (***R*)-3a** (5 mg) and (*R*)-MPA diester **(*R*)-3b** (2 mg).

(*R*)-MPA Monoester (*R*)-3a: Pale-yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.40 (s, 6 H, 5′′-Me, 6′′-Me), 1.54 (s, 3 H, 5′′′-Me), 2.61 (dd, *J* = 14.0, 8.0 Hz, 1 H, 1a′′′-H), 2.78 (dd, *J* = 14.0, 6.0 Hz, 1 H, 1b′′′-H), 3.31 (3 H, OCH_3), 4.09 (t, *J* = 6.0 Hz, 1 H, 2′′′-H), 4.66 and 4.69 (s, each 1 H, 4′′′-H), 5.25 (d, *J* = 12.0 Hz, 1 H, 2-H), 5.46 (d, *J* = 10.0 Hz, 1 H, 3′′-H), 5.75 (d, *J* = 12.0 Hz, 1 H, 3-H), 6.56 (d, *J* = 10.0 Hz, 1 H, 4′′-H), 7.10–7.40 (overlapped, B ring), 11.61 (s, 1 H, 5-OH) ppm; the α-methoxyphenylacetic acid part exhibited δ_H = 3.37 (OCH_3, 3 H), 4.69 (s, 1 H), 7.10–7.40 (overlapped, aromatic signal) ppm. HRMS (FAB): calcd. for $C_{34}H_{35}O_8$ $[M + H]^+$ 571.2332; found 571.2333.

(*R*)-MPA Diester (*R*)-3b: Pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.39 (s, 6 H, 5′′-Me, 6′′-Me), 1.52 (s, 3 H, 5′′′-Me), 2.57 (dd, *J* = 13.6, 6.1 Hz, 1 H, 1a′′′-H), 2.72 (dd, *J* = 13.6, 8.6 Hz, 1 H, 1b′′′-H), 4.69 (m, 1 H, 4a′′′-H), 4.70 (t, *J* = 1.4 Hz, 1 H, 4b′′′-H), 5.06 (d, *J* = 12.0 Hz, 1 H, 2-H), 5.41 (dd, *J* = 6.1, 8.7 Hz, 1 H, 2′′′-H), 5.44 (d, *J* = 10.0 Hz, 1 H, 3′′-H), 5.52 (d, *J* = 12.0 Hz, 1 H, 3-H), 6.51 (d, *J* = 10.0 Hz, 1 H, 4′′-H), 7.16–7.27 (overlapped, B ring), 11.55 (s, 1 H, 5-OH) ppm; the α-methoxyphenylacetic acid part exhibited δ = 7.16–7.27 (overlapped, aromatic signal), 4.61 and 4.69 (s, each 1 H), 3.25 and 3.31 (s, each 3 H, OCH_3) ppm. MS (ESI-TOF): calcd. for $C_{43}H_{43}O_{10}$ $[M + H]^+$ 719.2851; found 719.2827.

Preparation of the (*S*)-MPA Monoester (*S*)-3a and (*S*)-MPA Diester (*S*)-3b: Compound **3** (5.3 mg, 0.0126 mmol) was treated with (*S*)-(+)-α-methoxyphenylacetic acid (8.3 mg, 0.050 mmol) and *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC; 9.55 mg, 0.050 mmol) in the presence of a catalytic amount of 4-(dimethylamino)pyridine in CH_2Cl_2 at room temperature for 24 h. The reaction mixture was dried under vacuum and purified by TLC (hexane/acetone, 8:2) to give (*S*)-MPA monoester (***S*)-3a** (6 mg) and (*S*)-MPA diester (***S*)-3b** (2 mg).

(*S*)-MPA Monoester (*S*)-3a: Pale-yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.40 (s, 6 H, 5′′-Me, 6′′-Me), 1.52 (s, 3 H, 5′′′-Me), 2.60 (dd, *J* = 14.0, 8.0 Hz, 1 H, 1a′′′-H), 2.77 (dd, *J* = 14.0, 6.0 Hz, 1 H, 1b′′′-H), 4.08 (t, *J* = 6.0 Hz, 1 H, 2′′′-H), 4.64 and 4.69 (s, each 1 H, 4′′′-H), 5.09 (d, *J* = 12.0 Hz, 1 H, 2-H), 5.47 (d, *J* = 10.0 Hz, 1 H, 3′′-H), 5.76 (d, *J* = 12.0 Hz, 1 H, 3-H), 6.57 (d, *J* = 10.0 Hz, 1 H, 4′′-H), 11.63 (s, 1 H, 5-OH), 7.00–7.40 (overlapped, B ring) ppm; the α-methoxyphenylacetic acid part exhibited δ = 3.31 (3 H, OCH_3), 4.74 (s, 1 H), 7.00–7.40 (overlapped, aromatic signal) ppm. HRMS (FAB): calcd. for $C_{34}H_{35}O_8$ $[M + H]^+$ 571.2332; found 571.2338.

(*S*)-MPA Diester (*S*)-3b: Pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (s, 3 H, 5′′′-Me), 1.40 and 1.44 (s, each 3 H, 5′′-Me, 6′′-Me), 2.67 (dd, *J* = 13.5, 6.2 Hz, 1 H, 1a′′′-H), 2.79 (dd, *J* = 13.5, 8.0 Hz, 1 H, 1b′′′-H), 4.42 and 4.53 (m, each 1 H, 4′′′-H), 5.08 (d, *J* = 12.0 Hz, 1 H, 2-H), 5.37 (dd, *J* = 6.2, 8.0 Hz, 1 H, 2′′′-H), 5.47 (d, *J* = 10.0 Hz, 1 H, 3′′-H), 5.71 (d, *J* = 12.0 Hz, 1 H, 3-H), 6.56 (d, *J* = 10.0 Hz, 1 H, 4′′-H), 11.55 (s, 1 H, 5-OH), 7.06–7.26 (overlapped, B ring) ppm; the α-methoxyphenylacetic acid part exhibited δ = 3.24 and 3.32 (s, each 3 H, OCH_3), 4.57 and 4.75 (s, each 1 H), 7.06–7.26 (overlapped, aromatic signal) ppm. MS (ESI-TOF): calcd. for $C_{43}H_{43}O_{10}$ $[M + H]^+$ 719.2851; found 719.2862.

Longeracemosone D (4): Yellow amorphous solid. $[\alpha]_D^{26}$ = +18.2 (*c* = 0.63, $CHCl_3$). UV (MeOH): λ_{max} [log(ε/M^{-1} cm^{-1})] = 203 [4.31], 267 [4.44], 275 [4.47], 314 nm [3.98]. CD (MeOH): λ (Δε) = 233 (+7.88), 299 (–4.85), 325 (+1.79), 351 nm (+2.43 M^{-1} cm^{-1}). FTIR (KBr): ṽ = 3428, 2978, 1626, 1457, 1284, 1193, 1126, 1025, 901, 768, 698 cm^{-1}. For the ^{1}H and ^{13}C NMR data, see Table 1 and Table 2. MS (EI): *m*/*z* (%) = 423 (10) $[M + H]^+$, 422 (13) $[M]^+$, 352 (27), 351 (100), 231 (32), 205 (27), 189 (14), 163 (7), 135 (6). HRMS (FAB): calcd. for $C_{25}H_{27}O_6$ $[M + H]^+$ 423.1808; found 423.1807.

Preparation of the (*R*)-MPA Monoester (*R*)-4a and (*R*)-MPA Diester (*R*)-4b: Compound **4** (6.3 mg, 0.015 mmol) was treated with (*R*)-(–)-α-methoxyphenylacetic acid (10 mg, 0.06 mmol) and *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC; 10 mg, 0.052 mmol) in the presence of a catalytic amount of 4-(dimethylamino)pyridine in CH_2Cl_2 at room temperature for 24 h. The reaction mixture was dried under vacuum and purified by TLC (hexane/acetone, 8:2) to give (*R*)-MPA monoester (***R*)-4a** (6 mg) and (*R*)-MPA diester (***R*)-4b** (3 mg).

(*R*)-MPA Monoester (*R*)-4a: Pale-yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.47 and 1.48 (s, each 3 H, 5′′-Me, 6′′-Me), 1.63 (s, 3

H, 5′′′-Me), 2.71 (dd, J = 13.9, 7.3 Hz, 1 H, 1a′′′-H), 2.86 (dd, J = 13.9, 5.1 Hz, 1 H, 1b′′′-H), 4.21 (t, J = 6.0 Hz, 1 H, 2′′′-H), 4.75 and 4.82 (s, each 1 H, 4′′′-H), 5.34 (d, J = 12.5 Hz, 1 H, 2-H), 5.53 (d, J = 10.3 Hz, 1 H, 3′′-H), 5.81 (d, J = 12.5 Hz, 1 H, 3-H), 6.64 (d, J = 10.3 Hz, 1 H, 4′′-H), 7.22–7.32 (overlapped, B ring), 11.67 (s, 1 H, 5-OH) ppm; the α-methoxyphenylacetic acid part exhibited δ = 3.37 (3 H, OCH_3), 4.75 (s, 1 H), 7.22–7.32 (overlapped, aromatic signal) ppm. HRMS (FAB): calcd. for $C_{34}H_{35}O_8$ $[M + H]^+$ 571.2332; found 571.2333.

(*R*)-MPA Diester (*R*)-4b: Pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.51 (s, 3 H, 5′′′-Me), 1.43 and 1.46 (s, each 3 H, 5′′-Me, 6′′-Me), 2.75 (dd, J = 13.6, 5.8 Hz, 1 H, 1a′′′-H), 2.87 (dd, J = 13.6, 8.3 Hz, 1 H, 1b′′′-H), 4.47 (br. s, 1 H, 4a′′′-H), 4.62 (t, J = 1.4 Hz, 1 H, 4b′′′-H), 5.28 (d, J = 11.9 Hz, 1 H, 2-H), 5.44 (dd, J = 8.3, 5.8 Hz, 1 H, 2′′′-H), 5.54 (d, J = 10.0 Hz, 1 H, 3′′-H), 5.76 (d, J = 11.9 Hz, 1 H, 3-H), 6.63 (d, J = 10.0 Hz, 1 H, 4′′-H), 11.67 (s, 1 H, 5-OH) ppm; the α-methoxyphenylacetic acid part exhibited δ = 4.60 and 4.76 (s, each 1 H), 3.30 and 3.39 (s, each 3 H, OCH_3) ppm; the signals of the aromatic groups of the B ring and α-methoxyphenylacetic acid were observed at δ = 6.98 (m), 7.20–7.38 (m), 7.75 (m) ppm. MS (ESI-TOF): calcd. for $C_{43}H_{43}O_{10}$ $[M + H]^+$ 719.2851; found 719.2837.

Preparation of the (*S*)-MPA Monoester (*S*)-4a and (*S*)-MPA Diester (*S*)-4b: Compound **4** (6.3 mg, 0.015 mmol) was treated with (*S*)-(+)-α-methoxyphenylacetic acid (10 mg, 0.06 mmol) and *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC; 10 mg, 0.052 mmol) in the presence of a catalytic amount of 4-(dimethylamino)pyridine in CH_2Cl_2 at room temperature for 24 h. The reaction mixture was dried under vacuum and purified by TLC (hexane/acetone, 8:2) to give (*S*)-MPA monoester (***S*)-4a** (6 mg) and (*S*)-MPA diester (***S*)-4b** (3 mg).

(*S*)-MPA Monoester (*S*)-4a: Pale-yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.46 and 1.48 (s, each 3 H, 5′′-Me, 6′′-Me), 1.62 (s, 3 H, 5′′′-Me), 2.69 (dd, J = 13.9, 7.3 Hz, 1 H, 1a′′′-H), 2.84 (dd, J = 13.9, 5.1 Hz, 1 H, 1b′′′-H), 4.20 (t, J = 6.0 Hz, 1 H, 2′′′-H), 4.74 and 4.81 (s, each 1 H, 4′′′-H), 5.18 (d, J = 12.5 Hz, 1 H, 2-H), 5.53 (d, J = 10.3 Hz, 1 H, 3′′-H), 5.82 (d, J = 12.5 Hz, 1 H, 3-H), 6.65 (d, J = 10.3 Hz, 1 H, 4′′-H), 11.70 (s, 1 H, 5-OH), 7.05–7.35 (overlapped, B ring) ppm; the α-methoxyphenylacetic acid part exhibited δ = 3.38 (3 H, OCH_3), 4.81 (s, 1 H), 7.05–7.35 (overlapped, aromatic signal) ppm. HRMS (FAB): calcd. for $C_{34}H_{35}O_8$ $[M + H]^+$ 571.2332; found 571.2333.

(*S*)-MPA Diester (*S*)-4b: Pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.45 and 1.49 (s, each 3 H, 5′′-Me, 6′′-Me), 1.63 (s, 3 H, 5′′′-Me), 2.63 (dd, J = 13.6, 5.6 Hz, 1 H, 1a′′′-H), 2.82 (dd, J = 13.6, 9.2 Hz, 1 H, 1b′′′-H), 4.75 and 4.78 (m, each 1 H, 4′′′-H), 4.79 (d, J = 12.0 Hz, 1 H, 2-H), 5.50 (dd, J = 5.6, 9.2 Hz, 1 H, 2′′′-H), 5.52 (d, J = 10.0 Hz, 1 H, 3′′-H), 5.66 (d, J = 12.0 Hz, 1 H, 3-H), 6.61 (d, J = 10.0 Hz, 1 H, 4′′-H), 11.67 (s, 1 H, 5-OH), 7.00–7.32 (overlapped, B ring) ppm; the α-methoxyphenylacetic acid part exhibited δ = 3.28 and 3.38 (s, each 3 H, OCH_3), 4.61 and 4.82 (s, each 1 H), 7.00–7.32 (overlapped, aromatic signal) ppm. MS (ESI-TOF): calcd. for $C_{43}H_{43}O_{10}$ $[M + H]^+$ 719.2851; found 719.2855.

Longeracemosone E (5): Yellow amorphous solid. $[\alpha]_D^{27}$ = +16.86 (c = 0.49, $CHCl_3$). UV (MeOH): λ_{max} [log (ε/M^{-1}cm^{-1})] = 267 [4.59], 274 [4.64], 313 nm [4.13]. CD (MeOH): λ ($\Delta\varepsilon$) = 233 (+6.22), 300 (–3.30), 330 (+1.20), 362 nm (+1.79 M^{-1}cm^{-1}). FTIR (KBr): $\tilde{\nu}$ = 3433, 2971, 1629, 1587, 1463, 1380, 1286, 1128 cm^{-1}. For the ^{1}H and ^{13}C NMR data, see Tables 1 and 2. MS (EI): m/z (%) = 423 (6) $[M + H]^+$, 422 (13) $[M]^+$, 408 (25), 407 (100), 335 (10), 287 (12), 261 (18), 243 (11), 229 (10), 215 (27), 189 (20). HRMS (FAB): calcd. for $C_{25}H_{27}O_6$ $[M + H]^+$ 423.1808; found 423.1808.

Longeracemosone F (6): Yellow amorphous solid. $[\alpha]_D^{27}$ = + 23.57 (c = 0.52, $CHCl_3$). CD (MeOH): λ ($\Delta\varepsilon$) = 235 (+5.35), 297 (–2.73), 353 nm (+1.70 M^{-1}cm^{-1}). For the ^{1}H and ^{13}C NMR data, see Tables 1 and 2. MS (EI): m/z (%) = 452 (7) $[M]^+$, 438 (8), 437 (30), 382 (23), 381 (100), 261 (25), 235 (15), 201 (13). HRMS (FAB): calcd. for $C_{26}H_{29}O_7$ $[M + H]^+$ 453.1913; found 453.1916.

Bioassays: The cytotoxic assays were performed following the method described previously.[9] In brief, cell lines suspended in RPMI 1640 containing 10% FBS were seeded at 1×10^4 cells (100 μL) per well in a 96-well plate and incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. After 24 h, additional medium (100 μL) was added containing the test compound and vehicle, giving a final concentration of 50 μg/mL, and 0.2% DMSO and the solutions were further incubated for 3 d. Cells were subsequently fixed with 95% EtOH, stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH, after which the absorbance was measured at 550 nm. The number of surviving cells was determined from the absorbance. The results are expressed as percentage survival compared with the control. Etoposide was used as the reference compound (Table 3). The aromatase inhibitory assay was performed using the method reported by Stresser et al.[10] The reference compound, ketoconazole, exhibited an IC_{50} value of 2.4 μM.

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