Flavonoids from the Heartwood of the Thai Medicinal Plant Dalbergia parviflora and Their Effects on Estrogenic-Responsive Human Breast Cancer Cells

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Flavonoids from the Heartwood of the Thai Medicinal Plant *Dalbergia parviflora* and Their Effects on Estrogenic-Responsive Human Breast Cancer Cells

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From the heartwood of *Dalbergia parviflora*, eight new compounds, khrinones A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), isodarparvinol B (**6**), dalparvin (**7**), and (3*S*)-sativanone (**22**), along with 32 known compounds, have been isolated and characterized as 17 isoflavones, nine isoflavanones, five flavanones, six isoflavans, and three miscellaneous substances. Isolates were evaluated for their cell proliferation stimulatory activity against the MCF-7 and T47D human breast cancer cell lines, and their luciferase inductive effects using luciferase transiently transfected MCF-7/luc and T47D/luc cells were also determined. Isoflavones such as genistein (**10**), biochanin A (**11**), tectorigenin (**12**), and 2′-methoxyformononetin (**13**) stimulated the proliferation of both cells, and concentrations of lower than 1 μM of these compounds showed equivalent activity to 10 pM of estradiol (E2). The new isoflavanone (**22**) also showed activity against both cell types, although it was weaker than that of the corresponding isoflavone (2′-methoxyformononetin, **13**). Two optically active isoflavanones (**22** and **24**: (3*S*)-violanone) stimulated the proliferation of both cell lines at lower concentrations than three racemates (**21**: vestitone, **23**: 7,3′-dihydroxy-4′-methoxyisoflavanone, and **25**: 3-*O*-methylviolanone). Bowdichione (**20**), an isoflavone with a quinone structure in its B-ring, showed activity against only one cell line associated with MCF-7 in these assays.

Phytoestrogens, plant compounds that structually and biologically mimic mammalian estrogen, have been reported to possess diverse pharmacological properties.[1] Consumption of a diet rich in soy-containing foods, known to be a rich source of phytoestrogens such as isoflavones, has been proposed as being a chemopreventive factor against breast cancer in Asian populations.[2] According to a recent report, pre- and postmenopausal Asian women consuming more than 20 mg of dietary isoflavones per day showed a 29% reduction in breast cancer risk compared to low-level isoflavone Asian consumers (less than 5 mg per day), and the moderate isoflavone consumers (more than 10 mg per day) exhibited a 12% decrease in risk, thereby demonstrating a dose−response relationship.[3]

As a part of our continued screening program of phytochemicals with estrogenic activity from Thai medicinal plants, a methanol extract of the heartwood of *Dalbergia parviflora* Roxb. (Leguminosae) was investigated.[4,5] The heartwood of *D. parviflora*, which is known as "khri" in Thai, is used to normalize menstruation and as a blood tonic, expectorant, stomachic, and cardiotonic in Thai traditional medicine. We investigated the MeOH extract of the heartwood of *D. parviflora*, and eight new phenolic compounds (**1**−**7** and **22**) together with 32 known compounds were isolated. This paper deals with the isolation of these compounds, the characterization of the new compounds by spectroscopic methods, and the assessment of their estrogenic activity by measuring the proliferation of the cells and the stimulatory potency of these substances in the estrogen-responsive breast cancer cell lines MCF-7 and T47D.

Results and Discussion

A part of the methanolic extract of *D. parviflora* was chromatographed on a silica gel column using chloroform−MeOH solvent systems. Fractions of 500 mL were collected and pooled by TLC analysis to afford a total of 26 combined fractions. The proliferation of MCF-7 cells was highly stimulated following treatment with fractions 9 and 14−17. Purification of the active fractions was performed using silica gel column chromatography and HPLC to give 40 pure phenolic compounds, including eight new compounds (**1**−**7** and **22**). Thirty-two known compounds were identified as ericibenin D (**8**),[6] formononetin (**9**),[7] genistein (**10**),[8] biochanin A (**11**),[9] tectorigenin (**12**),[10] 2′-methoxyformononetin (**13**),[11] theralin (**14**),[12] 2′-methoxybiochanin A (**15**),[13] calycosin (**16**),[14] pratensein (**17**),[15] 3′-methoxydaidzein (**18**),[16] 7-demethylrobustigenin (**19**),[17] bowdichione (**20**),[18] (3*R*,3*S*)-vestitone (**21**),[19] (3*R*,3*S*)-7,3′-dihydroxy-4′-methoxyisoflavanone (**23**),[20] (3*S*)-violanone (**24**),[21] (3*R*,3*S*)- 3-*O*-methylviolanone (**25**),[22] (3*S*)-secundiflorol H (**26**),[23] (3*R*,3*S*)-onogenin (**27**),[24] (2*S*)-pinocembrin (**28**),[25] (2*S*)-liquiritigenin (**29**),[26] (2*S*)-naringenin (**30**),[27] (3*R*)-vestitol (**31**),[28] (3*R*)-mucronulatol (**32**),[29] (3*R*)-5′-methoxyvestitol (**33**),[16] hydroxyobustyrene (**34**),[30] xenognosin (**35**),[31] (2*R*,3*R*)-pinobanksin,[25] isoliquiritigenin (**36**),[32] (3*R*,3*S*)-dihydrovesticarpin,[23] (3*R*,3*S*)-3′-hydroxy-8- methoxyvestitol,[33] and (3*S*)-8-demethylduartin,[24] by comparison of their spectroscopic data with the reported values in the literature.

Khrinone A (**1**) was assigned the molecular formula $C_{16}H_{12}O_6$, as determined from its molecular ion $[M]^+$ peak at *m*/*z* 300.0647 in the HRFABMS. The ^{1}H NMR spectrum of **1** showed a methoxy group signal [δ 3.74 (s)], two singlet aromatic proton signals [δ 6.49 (s) and 6.66 (s)], and ABX-type aromatic proton signals [δ 6.86 (d, J = 2 Hz), 6.94 (dd, J = 8, 2 Hz), and 7.95 (d, J = 8 Hz)], and it was suggested to be an isoflavone from the characteristic signal for H-2 observed at δ 8.17 (s). A nuclear Overhauser effect (NOE) was observed for one of the singlet signals [δ 6.49 (s)], which was assigned to H-3′ by irradiation of the methoxy group signal [δ 3.74 (s)]. The HMBC spectrum supported the NMR assignments since another singlet aromatic proton signal showed a correlation with C-3 (δ 121.4). Furthermore, a correlation of the hydroxy proton signal at δ 8.70 (OH-2′) with the carbon signal at δ 110.4 (C-1′) was observed, and H-2 [δ 8.17 (s)] also showed a correlation with the carbon signal. These results indicated the

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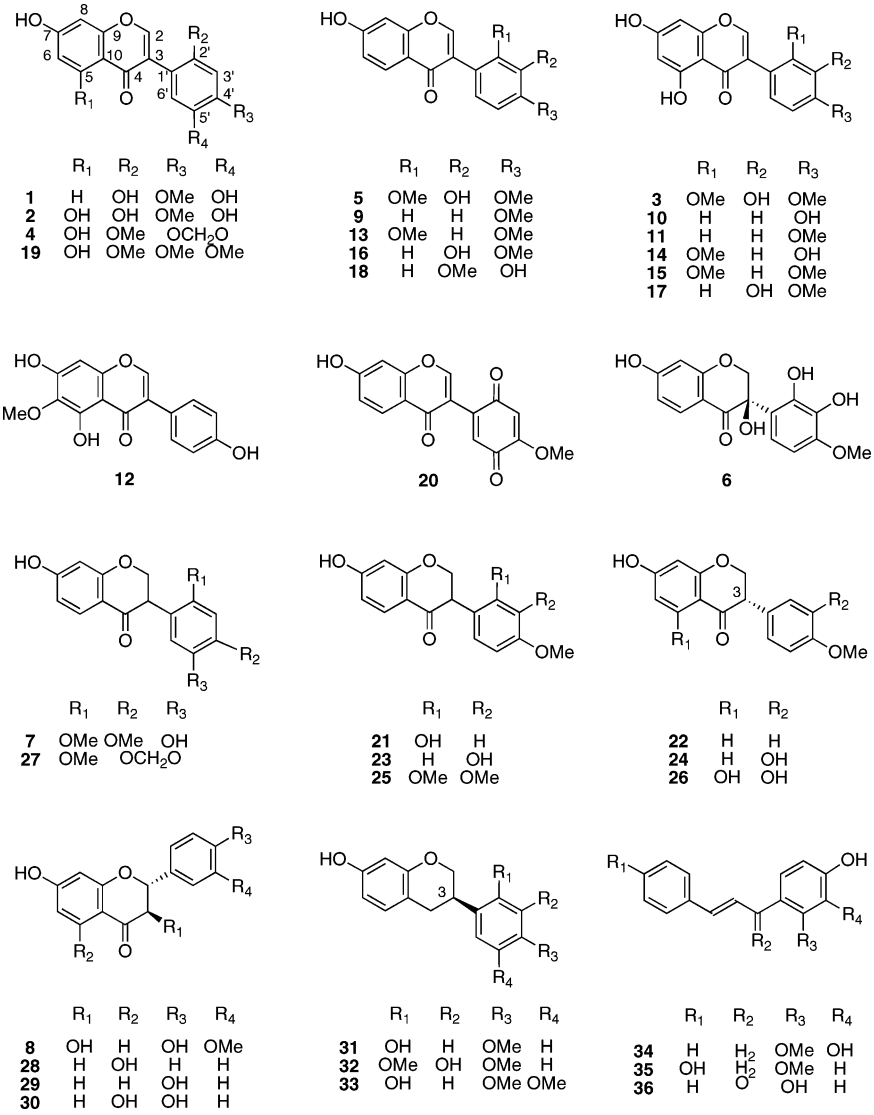
Chart 1



attachment position of the methoxy group as C-4′. Although the acetate gave identical NMR data to the compound 2′-methoxy-7,4′,5′-triacetyloxyisoflavone,[34] the structure of khrinone A (**1**) was assigned as 4′-methoxy-7,2′,5′-trihydroxyisoflavone, since Bekker et al. concluded the position of the methoxy group in the former compound erroneously, by using its NOE spectrum.

Khrinone B (**2**) was assigned the molecular formula $C_{16}H_{12}O_7$, as determined from its molecular ion $[M]^+$ peak at *m/z* 316.0609 in the HRFABMS. The ^{1}H NMR spectrum of **2** showed a characteristic singlet proton signal [δ 8.15 (1H, s)] for an isoflavone skeleton with a chelated hydroxy group [δ 12.9 (s)]. The ^{1}H NMR spectrum also exhibited a methoxy signal [δ 3.74 (s)], two singlet aromatic proton signals [δ 6.49 (s) and 6.64 (s)], and *meta*-coupled aromatic proton signals [δ 6.21 (d, $J = 2$ Hz) and 6.37 (d, $J = 2$ Hz)]. A NOE was observed at H-3′ [δ 6.49 (s)] by irradiation of the methoxy group [δ 3.74 (s)] but was not observed at H-2 [δ 8.15 (s)]. The HMBC spectrum indicated that the methoxy group is attached to C-4′ from the correlations across three bonds from both H-2 and the hydroxy proton signals [δ 8.70 (s)] to C-1′ (δ 108.7). From these results, the structure of khrinone B (**2**) was confirmed as 4′-methoxy- 5,7,2′,5′- tetrahydroxyisoflavone.

Khrinone C (**3**) was assigned the molecular formula $C_{17}H_{14}O_7$, as determined from its molecular ion $[M]^+$ peak at *m/z* 330.0746 in the HRFABMS. The ^{1}H NMR spectrum of **3** showed singlet signals at δ 7.98 (1H, s) and 12.98 (1H, s), indicating the compound to be a 5-hydroxyisoflavone. Besides the two methoxy group signals at δ 3.77 and 3.87 (each 3H, s), *meta*-coupled proton signals at δ 6.28 (1H, d, $J = 2$ Hz, H-6) and 6.41 (1H, d, $J = 2$ Hz, H-8) were observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum showed the presence of two methoxy signals, and one of them was indicated to have substitutent groups at both *ortho* positions from the chemical shift of δ 60.4. In the difference NOE spectrum of **3**, NOEs were observed for H-2 (δ 7.98) by irradiation at δ 3.77 (OC$\underline{H}_3$-2′) and for H-5′ (δ 6.76) by irradiation at δ 3.87 (OC$\underline{H}_3$-4′). From these results, the structure of khrinone C (**3**) was determined as 2′,4′-dimethoxy-5,7,3′-trihydroxyisoflavone, and this was supported by the HMQC and HMBC spectra.

Khrinone D (**4**) was assigned the molecular formula $C_{17}H_{12}O_7$, as determined from its molecular ion $[M]^+$ peak at *m/z* 328.0557 in the HRFABMS. The ^{1}H NMR spectrum of **4** showed a singlet signal at δ 8.03 (1H, s) for an isoflavone skeleton, with a chelated proton at δ 13.0 (1H, s), indicating the compound to be a 5-hydroxyisoflavone. The ^{1}H NMR spectrum also exhibited two singlet proton signals at δ 6.76 and 6.84 (each 1H, s), *meta*-coupled proton signals at δ 6.27 (1H, d, $J = 2$ Hz, H-6) and 6.40 (1H, d, $J = 2$ Hz, H-8), a methoxy group signal at δ 3.74 (3H, s), and a methylenedioxy signal (δ 5.99). The methoxy group was found to be attached to C-2′ from the difference NOE observed at H-3′ (δ 6.76) by irradiation at δ 3.74. From these results, the structure of khrinone D (**4**) was established as 5,7-dihydroxy-2′-methoxy- 4′,5′-methylenedioxyisoflavone, and this was supported by the HMQC and HMBC spectra.

Table 1. ^{1}H NMR Data for Compounds **1**−**7** and **22** (400 MHz)

position	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[c]	**6**[c]	**7**[b]	**22**[a]
2	8.17 s	8.15 s	7.98 s	8.03 s	8.01 s	4.89 d (12)	4.54 dd (11,11)	4.51 dd (11,11)
						4.19 d (12)	4.13 dd (11,5)	4.15 dd (11,5)
3							4.44 dd (11,5)	4.41 dd (11,5)
5	7.95 d (8)				8.03 d (9)	7.76 d (9)	7.77 d (9)	7.67 d (9)
6	6.94 d (8,2)	6.21 d (2)	6.28 d (2)	6.27 d (2)	6.94 dd (9,2)	6.51 d (9,2)	6.57 d (9,2)	6.51 dd (9,2)
7								
8	6.86 d (2)	6.37 d (2)	6.40 d (2)	6.40 d (2)	6.85 d (2)	6.30 d (2)	6.40 d (2)	6.33 d (2)
2′								
3′	6.49 s	6.49 s		6.76 s			6.72 s	6.57 d (2)
4′								
5′			6.76 s		6.77 d (9)	6.47 d (9)		6.47 dd (9,2)
6′	6.66 s	6.64 s	6.76 s	6.84 s	6.72 d (9)	6.90 d (9)	6.62 s	6.97 d (9)
MeO	3.74 s	3.74 s	3.77 s	3.74 s	3.70 s	3.88 s	3.75 s	3.71 s
			3.87 s		3.88 s		3.85 s	3.74 s
OH-5		12.9 s	13.0 s	13.0 s				
OH-7	10.8 s	10.8 s						10.5 s
OH-2′	8.70 s	8.70 s						
OH-5′	8.28 s	8.28 s						
OCH_2O				5.99 s				

[a] Recorded in DMSO-d_6. [b] Recorded in acetone-d_6. [c] Recorded in MeOH-d_4.

Khrinone E (**5**) was assigned the molecular formula $C_{17}H_{14}O_6$, as determined from its molecular ion $[MH]^+$ peak at *m*/*z* 315.0872 in the HRFABMS. The ^{1}H NMR spectrum suggested its structure to be a 7-hydroxyisoflavone from a singlet proton signal at δ 8.01 (1H, s, H-3) and the characteristic H-5 proton in low field at δ 8.03 (1H, d, J = 9 Hz, H-5), which showed an ABX coupling pattern with proton signals at δ 6.94 (1H, dd, J = 9, 2 Hz, H-6) and 6.85 (1H, d, J = 2 Hz, H-8). The ^{13}C NMR spectrum showed the presence of two methoxy signals, and one of them was indicated to have substituent groups at both its *ortho* positions from the chemical shift of δ 60.8. Additionally, another partial structure of a trioxygenated B ring was suggested from signals observed at δ 147.9, 140.8, and 150.8. A NOE was seen at δ 6.77 (1H, d, J = 9 Hz), in which one of the methoxy signals (δ 3.88) was irradiated in the difference NOE spectrum, and it indicated the methoxy group to be OMe-4′ of the 2′,3′,4′-trioxygenated B-ring. In the HMBC spectrum, the methoxy signals (δ 3.70 and 3.88) showed correlations with oxygenated aromatic carbons (δ 147.9 and 150.8, respectively). On the basis of the above spectroscopic evidence, khrinone E (**5**) was proposed as 7,3′-dihydroxy-2′,4′-dimethoxyisoflavone.

Isodalparvinol B (**6**), $[\alpha]_D$ −407.8, a colorless amorphous powder, was assigned the molecular formula $C_{16}H_{14}O_7$, as determined from its molecular ion $[M]^+$ peak at *m*/*z* 318.0755 in the HRFABMS. Analysis of the ^{1}H NMR spectrum indicated a pair of *ortho*-coupled proton signals [δ 6.47 (1H, d, J = 9 Hz, H-5′) and 6.90 (1H, d, J = 9 Hz, H-6′)] and ABX-type coupled protons [δ 6.30 (1H, d, J = 2 Hz, H-8), 6.51 (1H, dd, J = 9, 2 Hz, H-6), and 7.76 (1H, d, J = 9 Hz, H-5)], in addition to a methoxy proton resonance [δ 3.88 (3H, s)]. Two proton signals at δ 4.89 (1H, d, J = 12 Hz) and 4.19 (1H, d, J = 12 Hz) also showed correlations with carbon resonances at δ 75.7 (C-2) in the HMQC spectrum, and **6** was thus considered to be an isoflavanonol derivative. Moreover, a NOE was observed at δ 6.47 (H-5′) on irradiating the methoxy group signal at δ 3.88, and the methoxy group was assigned to C-4′ after analysis of the HMQC and HMBC spectra. The absolute configuration at C-3 of **6** was determined as 3*S* due to a negative Cotton effect ($[\theta]_{325}$ −6560) in the CD spectrum.[35] On the basis of the above spectroscopic evidence, isodalparvinol B (**6**) was defined as (3*S*)-4′-methoxy-3,7,2′,3′-tetrahydroxyisoflavanonol.

Dalparvin (**7**), a colorless amorphous powder, was assigned the molecular formula $C_{17}H_{16}O_6$, as determined from its molecular ion $[M]^+$ peak at *m*/*z* 316.0947 in the HRFABMS. The ^{1}H NMR spectrum of **7** showed the characteristic three-spin system of an isoflavanone skeleton at δ 4.44 (1H, dd, J = 11, 5 Hz, H-2), 4.54 (1H, dd, J = 11, 11 Hz, H-2), and 4.13 (1H, dd, J = 11, 5 Hz, H-3). The ABX spin system at δ 6.40 (1H, d, J = 2 Hz, H-8), 6.57 (1H, dd, J = 9, 2 Hz, H-6), and 7.77 (1H, d, J = 9, H-5) and two singlet protons at δ 6.62 (H-6′) and 6.72 (H-3′) indicated the presence of a 7-oxygenated A-ring and a 2′,4′,5′-trisubstituted B-ring. NOEs were observed at H-3′ [δ 6.72 (s)] on irradiation of the methoxy groups at δ 3.75 and 3.85. From this spectroscopic evidence, the structure of dalparvin (**7**) was established as 7,5′-dihydroxy-2′,4′-dimethoxyisoflavanone. The CD spectrum and $[\alpha]_D$ value indicated that **7** was obtained as a racemate.

(3*S*)-Sativanone (**22**), $[\alpha]_D$ +9.7, a colorless amorphous powder, was assigned the molecular formula $C_{17}H_{16}O_5$, as determined from its molecular ion $[MH]^+$ peak at *m*/*z* 301.1006 in the HRFABMS. From the ^{1}H NMR spectrum, **22** was indicated as being an isoflavanone derivative from its characteristic signals [δ 4.15 (1H, dd, J = 11, 5 Hz, H-2), 4.51 (1H, dd, J = 11, 11 Hz, H-2), and 4.41 (1H, dd, J = 11, 5 Hz, H-3)] with two sets of ABX-type coupled protons [δ 6.33 (1H, d, J = 2 Hz, H-8), 6.51 (1H, dd, J = 9, 2 Hz, H-6), and 7.67 (1H, d, J = 9 Hz, H-5) and δ 6.47 (1H, dd, J = 9, 2 Hz, H-5′), 6.57 (1H, d, J = 2 Hz, H-3′), and 6.97 (1H, d, J = 9 Hz, H-6′)]. Moreover, NOEs were observed at δ 6.57 (H-3′) on irradiating both methoxy group signals at δ 3.71 and 3.74, and the methoxy groups were assigned to C-2′ and C-4′, respectively. Although the NMR data of **22** showed good agreement with (*R*)-sativanone reported by Chan et al., the specific rotation value ($[\alpha]_D$ −35) is reversed.[36] The absolute configuration at C-3 of **22** was determined as 3*S* due to a negative Cotton effect ($[\theta]_{325}$ −10 600) in the CD spectrum. Sativanone was first isolated from the heartwood of *Dalbergia stevensonii* as a racemate, and only the *R*-form has been isolated previously.[23] On the basis of the above spectroscopic evidence, **22** was assigned as a new compound with the structure (3*S*)-2′,4′-dimethoxy-7-hydroxyisoflavanone, or (3*S*)-sativanone.

The isolates tested in this study are polyoxygenated flavonoids, and most have a 7,4′-dioxygenated skeleton with some exceptions. Genistein (**10**), 5,7,4′-trihydroxyisoflavone, showed the most potent cell proliferation stimulatory activity against both cell lines of all compounds tested, but the activity was almost 1000 times less than that of estradiol (Table 3). Its 4′-methoxylated derivative, biochanin A (**11**), exhibited activity 1/10 that of the parent compound. Isoflavones having an additional methoxy group compared to genistein (**10**), such as tectorigenin (**12**) and theralin (**14**), also showed low activity, 1/10 the level of genistein. Other isoflavones with more than two oxygenated groups in their B-ring were 100 times less active than genistein. Among them, compounds with an *ortho*-dioxygenated B-ring (**1**−**3**, **16**−**19**) were indicated for their low induction of cell proliferation by evaluating their EqE_{100} values.

The new isoflavanone (3*S*)-sativanone (**22**) has the same substituent groups at the same positions as 2′-methoxyformononetin

Table 2. ^{13}C NMR Data for Compounds **1**−**7** and **22** (100.4 MHz)

position	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[c]	**6**[c]	**7**[b]	**22**[a]
2	154.8	155.6	155.3	156.0	155.7	75.7	71.9	70.3
3	121.4	119.9	118.6	119.0	123.7	75.1	47.9	46.6
4	175.2	180.3	181.6	181.3	178.3	192.2	191.1	190.2
5	127.1	161.8	163.8	163.8	128.5	130.9	130.0	128.9
6	115.1	98.8	99.9	100.0	116.5	112.0	111.2	110.5
7	162.5	164.1	165.0	165.5	164.8	166.5	165.0	164.2
8	102.0	93.6	94.6	94.6	103.4	103.6	103.5	102.3
9	157.4	157.5	159.1	159.1	159.9	164.8	164.5	163.2
10	116.4	104.5	106.1	106.0	118.1	114.0	116.0	114.0
1′	110.4	108.7	122.3	112.0	120.0	119.8	117.5	116.1
2′	148.1	148.2	147.2	141.9	147.9	144.5	152.0	158.1
3′	101.5	101.1	140.3	102.4	140.8	135.4	99.4	98.8
4′	148.1	148.1	150.0	154.3	150.8	149.9	148.1	159.9
5′	138.8	138.7	107.4	149.5	108.2	103.9	141.3	104.9
6′	117.9	118.1	121.9	115.9	122.3	118.3	117.5	130.5
MeO	55.5	55.5	60.4	57.2	60.8	56.6	56.6	55.6
			56.6		56.8		57.0	55.2
OCH_2O				96.2				

[a] Recorded in DMSO-d_6. [b] Recorded in acetone-d_6. [c] Recorded in MeOH-d_4.

Table 3. Cell Proliferation Stimulatory Activities against MCF-7 and T47D Cells[a]

	MCF-7		T47D	
compound	EqE_{10} (μM)	EqE_{100} (μM)	EqE_{10} (μM)	EqE_{100} (μM)
1	>100	>100	>100	>100
2	>100	>100	>100	>100
3	4.5	93.3	0.2	8.6
4	1.8	4.2	0.01	>100
5	4.7	>100	>100	>100
6	>100	>100	>100	>100
7	2.1	4.0	0.6	11.6
8	>100	>100	5.7	>100
9	>100	>100	0.03	>100
10 (genistein)	0.01	0.11	0.01	0.08
11	0.3	3.8	0.3	0.5
12	0.5	2.7	0.3	6.9
13	0.3	5.2	0.01	1.7
14	0.6	>100	1.7	>100
15	3.2	19.6	0.4	>100
16	>100	>100	4.1	>100
17	4.5	50.4	6.4	>100
18	4.7	>100	>100	>100
19	>100	>100	0.1	>100
20	0.01	1.4	>100	>100
21	5.7	45.3	1.5	33.7
22	2.7	8.6	2.0	9.2
23	24.5	>100	>100	>100
24	2.1	9.4	2.1	8.9
25	2.5	46.3	6.1	>100
26	2.9	>100	0.5	5.7
27	4.2	9.4	1.0	8.1
28	17.0	>100	7.6	>100
29	0.1	0.65	0.64	>100
30	0.2	0.7	0.09	1.6
31	>100	>100	0.02	0.2
32	>100	>100	0.4	6.4
33	1.1	0.2	0.02	>100
34	>100	>100	5.1	>100
35	0.1	4.6	0.7	>100
36	1.1	4.0	1.0	>100

[a] EqE_{10} and EqE_{100} represent the concentration of the compound that stimulated cell proliferation equivalent to that induced by 10 and 100 pM estradiol, respectively. These values were determined by linear regression analysis using at least five different concentrations in quadruplicate. (2*R*,3*R*)-Pinobanksin, (±)-dihydrovesticarpin, (3*R*,3*S*)-3′-hydroxy-8-methoxyvestitol, and (3*S*)-8-demethylduartin were inactive.

(**13**) and showed moderate activity (EqE_{10} and EqE_{100}: 2.7 and 8.6 μM for MCF-7 cells; 2.0 and 9.2 μM for T47D cells, respectively), and the activity was less stimulatory than the corresponding planar compound. Another chiral isoflavanone, (3*S*)-violanone (**24**), was also recognized for its moderate activity and was more potent than the corresponding isoflavone khrinone E (**5**). The activity of **24** was the highest of all the isoflavanones tested and has an *ortho*-dioxygenated B-ring. Bowdichione (**20**) was unusual in being active only against MCF-7 cells; two isoflavans, (3*R*)-vestitol (**31**) and (3*R*)-mucronulatol (**32**), were estrogenic to T47D cells.

To determine the estrogenic characteristics of the constituents further, a luciferase reporter assay was carried out at concentrations ranging from 0.01 to 10 μM (Table 4). Some compounds possessing cell proliferation stimulatory effects showed luciferase induction. Genistein (**10**) and naringenin (**30**), the most active isolates, with low EqE_{100} values for both MCF-7 and T47D cell proliferation, were observed to have luciferase-inducing activity in both luciferase-transfected MCF-7 (MCF-7/luc) and luciferase-transfected T47D (T47D/luc) cells at low concentrations, and the values correspond to the reported activity.[37] Compounds **11**−**13** showed luciferase induction at low concentrations in T47D/luc cells, while they were observed to stimulate cell proliferation in both MCF-7 and T47D cells. 3′-Methoxydaidzein (**18**) showed luciferase induction at low concentrations for both MCF-7/luc and T47D/luc cells, whereas no stimulating effect on T47D cell proliferation was observed, and **20** exhibited activity against one cell line associated with MCF-7.

Table 4. Luciferase Reporter Assay Using Luciferase-Transfected Human Breast Cancer Cells[a]

	MCF-7/Luc		T47D/Luc	
compound	EqE_{10} (μM)	EqE_{100} (μM)	EqE_{10} (μM)	EqE_{100} (μM)
1	>100	>100	5.6	8.4
2	>100	>100	>100	>100
3	>100	>100	0.2	2.0
4	0.01	0.6	5.3	>100
5	5.9	>100	>100	>100
6	1.2	3.4	>100	>100
7	0.7	1.6	2.6	>100
8	>100	>100	0.3	>100
9	0.01	0.01	0.01	0.01
10 (genistein)	0.01	0.01	0.01	0.6
11	>100	>100	0.01	0.01
12	>100	>100	1.4	2.2
13	>100	>100	1.8	6.2
14	0.01	0.4	>100	>100
15	>100	>100	1.8	3.1
16	0.6	4.7	8.8	>100
17	>100	>100	>100	>100
18	1.1	2.2	1.2	5.8
19	5.4	7.8	6.3	>100
20	0.7	0.9	>100	>100
21	>100	>100	7.3	>100
22	>100	>100	0.7	1.1
23	>100	>100	6.0	>100
24	>100	>100	0.6	>100
25	>100	>100	3.3	>100
26	0.6	2.7	1.3	>100
27	>100	>100	1.9	5.6
28	>100	>100	>100	>100
29	0.01	0.01	0.01	5.9
30	1.7	2.6	0.01	5.8
31	>100	>100	>100	>100
32	0.9	7.8	6.3	>100
33	>100	>100	>100	>100
34	>100	>100	>100	>100
35	>100	>100	0.01	5.7
36	0.01	>100	>100	>100

[a] EqE_{10} and EqE_{100} represent the concentration of the compound that stimulated cell proliferation equivalent to that induced by 10 and 100 pM estradiol, respectively. These values were determined by linear regression analysis using at least five different concentrations in quadruplicate. (2*R*,3*R*)-Pinobanksin, (±)-dihydrovesticarpin, (3*R*,3*S*)-3′-hydroxy-8-methoxyvestitol, and (3*S*)-8-demethylduartin were inactive.

The estrogenic activity of phytoestrogens is thought to be mediated through the estrogen receptors (ERs). The crystallographic analyses for these receptors complexed with ligands have revealed the receptor cavity and the manner of ligand binding.[38] The typical phytoestrogen genistein was demonstrated to bind to the receptors in the same manner as E_2; that is, the interactions between the OH-4′ group and Glu, Arg, and water and another interaction between the OH-7 group and His were recognized.[39] The estrogenic effects of isolated flavonoids from *D. parviflora* are supported by this analysis, and the results help to bridge the gap between common uses and the current lack of studies on their effectiveness to normalize menstruation. In addition, some compounds exhibited cell-specific stimulatory activity on its proliferation and/or assay-specific activity. These results may lead to new selective estrogen receptor modulators (SERMs), though it must be kept in mind that ligand-bound ERs activate intracellular signaling pathways as well as target gene transcription in the nucleus.[40]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. CD spectra were recorded on a JASCO J-20A spectropolarimeter. UV spectra were recorded on a Hitachi U3410 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-α 400 instrument, and chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as internal standard at 35 °C. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H} = 145$ Hz) and HMBC (optimized for $^nJ_{C-H} = 8$ Hz) pulse sequences with a pulsed field gradient. HRFABMS data were obtained using JEOL JMS-SX 102 and JMS-700 mass spectrometers using a *m*-nitrobenzyl alcohol matrix. HPLC was carried out with a JASCO model 887-PU pump and an 875-UV variable-wavelength detector with a reversed-phase column (Capcell Pak ODS, 5 μM, 2 × 25 cm, Shiseido Fine Chemicals Co. Ltd., at 6 mL/min with detection at 205 nm, and a Develosil-Lop-ODS column, 10−20 μM, 5 × 100 cm, Nomura Chemical Co., Ltd.)

Chemicals. Eagle's MEM and RPMI media were purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Fetal bovine serum (FBS) was obtained from Gibco (Grand Island, NY), antibiotics from Meiji Seika Kaisha Ltd. (Tokyo, Japan), L-glutamine from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and 17β-estradiol and dextran-coated charcoal (DCC) from Sigma Chemicals (St. Louis, MO).

Plant Material. Commercially available heartwood of *Dalbergia parviflora* was purchased from a Thai medicinal herb store in Bangkok, in July 2004. The plant was identified by Dr. C. Niyomdham of The Forest Herbarium, Bangkok. A voucher specimen was deposited at The Forest Herbarium (68143).

Extraction and Isolation. The dried heartwood of *D. parviflora* (2 kg) was extracted three times with MeOH (3 × 20 L) at room temperature. The extracts were combined and concentrated under reduced pressure at 60 °C to yield 910 g of a viscous mass. A part of this concentrated extract (150 g) was chromatographed on a silica gel column (12 × 40 cm) and fractionated using chloroform−MeOH (98:2, 96:4, 94:6, 90:10, 15 L each). Fractions of 500 mL were collected and pooled by TLC analysis to afford a total of 26 combined fractions. Purification of fraction 9 (8.5 g) using HPLC on a Develosil-Lop-ODS column (5 × 100 cm, flow rate, 45 mL/min with detection at 205 nm), with MeCN−H_2O (35:65) as the eluent, gave **4** (30 mg), **11** (320 mg), **15** (45 mg), **19** (40 mg), **22** (3.8 g), **25** (310 mg), **26** (2000 mg), **27** (130 mg), **28** (85 mg), **32** (870 mg), **34** (55 mg), (3*R*,3*S*)-3′-hydroxy-8-methoxyvestitol (270 mg), (2*R*,3*R*)-pinobanksin (350 mg), and (3*S*)-8-demethylduartin (250 mg) (t_R = 268, 328, 488, 188, 240, 210, 220, 84, 307, 167, 340, 130, 80, and 66 min, respectively). Purification of fraction 14 (8.9 g) using HPLC on a Develosil- Lop-ODS column (5 × 100 cm, flow rate, 45 mL/min with detection at 205 nm), with MeCN−H_2O (30:70) as the eluent, gave **3** (150 mg), **9** (300 mg), **12** (610 mg), **17** (715 mg), **21** (25 mg), **24** (3170 mg), **35** (15 mg), and (3*R*,3*S*)-dihydrovesticarpin (45 mg) (t_R = 228, 435, 220, 371, 444, 204, 542, and 197 min, respectively). Purification of fraction 15 (4.7 g) using HPLC on a Develosil-Lop-ODS column (5 × 100 cm, flow rate, 45 mL/min with detection at 205 nm), with MeCN−H_2O (30:70) as the eluent, gave **2** (980 mg), **7** (520 mg), **8** (22 mg), **23** (125 mg), **30** (315 mg), **31** (300 mg), and **33** (140 mg) (t_R = 106, 188, 74, 150, 250, 450, and 238 min, respectively). Purification of fraction 16 (1.28 g) using HPLC on a Develosil-Lop-ODS column (5 × 100 cm, flow rate, 45 mL/min with detection at 205 nm), with MeCN−H_2O (35:65) as the eluent, gave **1** (100 mg) (t_R = 80 min). Purification of fraction 17 (5.25 g), using HPLC on a Develosil-Lop-ODS column (5 × 100 cm, flow rate, 45 mL/min with detection at 205 nm), with MeCN−H_2O (30:70) as the eluent, gave **5** (2 mg), **6** (6 mg), **10** (200 mg), **14** (20 mg), **16** (500 mg), **18** (2 mg), **20** (3 mg), **29** (30 mg), and **36** (115 mg) (t_R = 120, 112, 210, 188, 130, 126, 83, 124, and 385 min, respectively). Compounds **8**−**21**, **23**−**36**, (3*R*,3*S*)- 3′-hydroxy-8-methoxyvestitol, (2*R*,3*R*)-pinobanksin, (3*S*)-8-demethylduartin, and (3*R*,3*S*)-dihydrovesticarpin were identified by comparison of their spectroscopic data with published values.[6−33]

Khrinone A (1): amorphous powder; UV (MeOH) λ_{max} (log ε) 248 (4.43), 268 (4.32), 300 (4.37) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 300.0647 [M]$^+$ (calcd for $C_{16}H_{12}O_6$, 300.0634).

Khrinone B (2): amorphous powder; UV (MeOH) λ_{max} (log ε) 260 (4.49), 298 (4.21) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 316.0609 [M]$^+$ (calcd for $C_{16}H_{12}O_7$ 316.0583).

Khrinone C (3): amorphous powder; UV (MeOH) λ_{max} (log ε) 259 (4.53), 292 (4.04), 330 (3.68) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 330.0746 [M]$^+$ (calcd for $C_{17}H_{14}O_7$ 330.0740).

Khrinone D (4): amorphous powder; UV (MeOH) λ_{max} (log ε) 225 (sh) (4.02), 260 (4.26), 300 (4.02) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 328.0557 [M]$^+$ (calcd for $C_{17}H_{12}O_7$ 328.0583).

Khrinone E (5): amorphous powder; UV (MeOH) λ_{max} (log ε) 239 (sh) (4.40), 248 (4.38), 298 (4.04) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 315.0872 [MH]$^+$ (calcd for $C_{17}H_{15}O_6$ 315.0869).

(2*R*,3*R*)-Isodalparvinol B (6): amorphous powder; $[\alpha]^{25}_D$ −407.8 (*c* 0.7, MeOH); CD (*c* 0.002, MeOH) $[\theta]_{325}$ −6560, $[\theta]_{280}$ −11000, $[\theta]_{230}$ −22800; UV (MeOH) λ_{max} (log ε) 215 (sh) (4.21), 231(4.25), 278 (4.11), 310 (3.91) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 318.0755 [M]$^+$ (calcd for $C_{16}H_{14}O_7$ 318.0740).

Dalparvin (7): amorphous powder; UV (MeOH) λ_{max} (log ε) 215 (sh) (4.46), 230 (4.26), 278 (4.19), 312 (3.98) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 316.0947 [M]$^+$ (calcd for $C_{17}H_{16}O_6$, 316.0947).

(3*S*)-Sativanone (22): amorphous powder; $[\alpha]^{25}_D$ +9.7 (*c* 1.0, MeOH); CD (*c* 0.001, MeOH) $[\theta]_{325}$ −10 600, $[\theta]_{280}$ −5220, $[\theta]_{235}$ +12 500; UV (MeOH) λ_{max} (log ε) 225 (sh) (4.48), 310 (3.97), 381 (2.16) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z* 301.1106 [MH]$^+$ (calcd for $C_{17}H_{17}O_5$, 301.1076).

Cell Culture. MCF-7 and T47D human breast cancer cells were purchased from the American Type Culture Collection (Manassas, VA). The MCF-7 cells were grown in MEM supplemented with 6 ng/mL insulin, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 2 mM glutamine, 10% FBS, and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin), under a 5% CO_2 humidified atmosphere at 37 °C. The T47D cells were grown in RPMI-1640 supplemented with 1 mM sodium pyruvate, 1 mM nonessential amino acids, 2 mM glutamine, 10% FBS, and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin), under a 5% CO_2 humidified atmosphere at 37 °C.

Cell Proliferation Assay. Cells were seeded into 96-well tissue culture plates in 5% DCC-treated, FBS-supplemented RPMI phenol red-free medium at a density of 10^4 cells/well. The test compounds were added as DMSO solution (the control contained 1% DMSO) and incubated at 37 °C with 5% CO_2 for 96 h. In all experiments, serial dilutions of estradiol were added as a positive control.[13] To evaluate relative cell concentrations, Alamar Blue reagent was used. After 3 h, fluorescence was measured at 590 nm with excitation at 530 nm using a Wallac 1420 ARVOsx multilabel counter (Perkin-Elmer Inc., Wellesley, MA).[35]

Luciferase Reporter Gene Assay. The luciferase reporter gene assay was conducted according to a previously reported procedure.[5]

Data and Statistical Analysis. Statistical differences were determined by analysis of variance followed by Dunnett's multiple comparison test. Statistical significance was established at the $p < 0.05$ level.

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Supporting Information Available: ^{1}H and ^{13}C spectra of compounds **1**−**7** and **22**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Cornwell, T.; Cohick, W.; Raskin, I. *Phytochemistry* **2004**, *65*, 995–1016. (b) Zhao, L; Brinton, R. D. *Expert Rev. Neurother.* **2007**, *7*, 1549–1564. (c) Mense, S. M.; Hei, T. K.; Ganju, R. K.; Bhat, H. K. *Env. Health Perspect.* **2008**, *116*, 426–433.

(2) Adlercreutz, H. *Lancet Oncol.* **2002**, *3*, 364–373.

(3) Wu, A. H.; Yu, M. C.; Tseng, C. C.; Pike, M. C. *Br. J. Cancer* **2008**, *98*, 9–14.

(4) Monthakantirat, O.; De-Eknamkul, W.; Umehara, K.; Yoshinaga, Y.; Miyase, T.; Warashina, T.; Noguchi, H. *J. Nat. Prod.* **2005**, *68*, 361–364.

(5) Umehara, K.; Nemoto, K.; Kimijima, K.; Matsushita, A.; Terada, E.; Monthakantirat, O.; De-Eknamkul, W.; Miyase, T.; Warashina, T.; Degawa, M.; Noguchi, H. *Phytochemistry* **2008**, *69*, 546–552.

(6) Morikawa, T.; Xu, F.; Matsuda, H.; Yoshikawa, M. *Chem. Pharm. Bull.* **2006**, *54*, 1530–1534.

(7) Jun, M.; Fu, H. Y.; Hong, J.; Wan, X.; Yang, C. S.; Ho, C. T. *J. Food Sci.* **2003**, *68*, 2117–2122.

(8) Kinjo, J.; Furusawa, J.; Baba, J.; Takeshita, T.; Yamasaki, M.; Nohara, T. *Chem. Pharm. Bull.* **1987**, *35*, 4846–4850.

(9) Osawa, K.; Yasuda, H.; Maruyama, T.; Morita, H.; Takeya, K.; Itokawa, H. *Chem. Pharm. Bull.* **1992**, *40*, 2970–2974.

(10) Oh, K. B.; Kang, H. J.; Matsuoka, H. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 939–942.

(11) Jain, L.; Tripathi, M.; Pandey, V. B.; Rucker, G. *Phytochemistry* **1996**, *41*, 661–662.

(12) Ko, H.; Weng, J.; Tsao, L.; Yen, M.; Wang, J.; Lin, C. *Bioorg. Med. Chem.* **2004**, *14*, 1011–1014.

(13) Reynaud, J.; Guilet, D.; Terreux, R.; Lussignol, M.; Walchshofer, W. *Nat. Prod. Rep.* **2005**, *22*, 504–515.

(14) Hirakura, K.; Morita, M.; Nakajima, K.; Sugama, K.; Takagi, K.; Niitsu, K.; Ikeya, Y.; Maruno, M.; Okada, M. *Phytochemistry* **1997**, *46*, 921–928.

(15) Matsuura, S.; Tanaka, T.; Iinuma, M.; Tanaka, T.; Himuro, N. *Yakugaku Zasshi* **1983**, *103*, 907–1000.

(16) Yahara, S.; Emura, S.; Feng, H.; Nohara, T. *Chem. Pharm. Bull.* **1989**, *37*, 2136–2138.

(17) Yenesew, A.; Midiwo, J. O.; Heydenreich, M.; Peter, M. G. *Phytochemistry* **1998**, *49*, 247–249.

(18) Brown, P. M.; Thomson, R. H.; Hausen, B. M.; Simatupang, M. H. *Liebigs Ann. Chem.* **1974**, 1295–1300.

(19) Macias, F. A.; Simonet, A. M.; Galindo, J. C. G.; Castellano, D. *Phytochemistry* **1999**, *50*, 35–46.

(20) De Oliveira, A. B.; Iracema, M.; Madruga, L. M.; Gottilieb, O. R. *Phytochemistry* **1978**, *17*, 593–595.

(21) Donnelly, D. M. X.; Kavanagh, P. J. *Phytochemistry* **1974**, *13*, 2587–2591.

(22) Guimraes, I. S. D. S.; Gottlieb, O. R.; Andrade, C. H. S.; Magalhaes, M. T. *Phytochemistry* **1978**, *17*, 1375–1377.

(23) Tanaka, T.; Ohyama, M.; Iinuma, M.; Shirataki, Y.; Komatsu, M.; Burandt, C. *Phytochemistry* **1998**, *48*, 1187–1193.

(24) Donnelly, D. M. X.; Thompson, J. C.; Whalley, W. B.; Ahmad, S. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1737–1745.

(25) Komoda, Y. *Chem. Pharm. Bull.* **1989**, *37*, 3128–3130.

(26) Kong, L. D.; Zhang, Y.; Pan, X.; Tan, R. X.; Cheng, C. H. K. *Cell. Mol. Life Sci.* **2000**, *57*, 500–505.

(27) Ibrahim, A. S. *Phytochemistry* **2000**, *53*, 209–212.

(28) Kurosawa, K.; Ollis, W. D.; Redman, B. T.; Sutherland, I. O.; Alves, H. M.; Gottlieb, O. R. *Phytochemistry* **1978**, *17*, 1423–1426.

(29) Kurosawa, K.; Ollis, W. D.; Redman, B. T.; Sutherland, I. O. *Chem. Commun.* **1968**, 1263–1267.

(30) Goda, Y.; Kiuchi, F.; Shibuya, M.; Sankawa, U. *Chem. Pharm. Bull.* **1992**, *40*, 2452–2457.

(31) El-Feraly, F. S.; Hufford, C. D. *J. Org. Chem.* **1982**, *47*, 1527–1530.

(32) Smith, B. E. C.; Swain, T. *J. Chem. Soc.* **1953**, 2185–2187.

(33) Lambert, M.; Stark, D.; Hansen, S. H.; Sairafianpour, M.; Jaroszewski, J. W. *J. Nat. Prod.* **2005**, *68*, 1500–1509.

(34) Bekker, M.; Malan, E.; Steenkamp, J. A.; Brandt, E. V. *Phytochemistry* **2002**, *59*, 415–418.

(35) Galeffi, C.; Rasoanaivo, P.; Federici, E.; Palazzino, G.; Nicoletti, M.; Rasolondratovo, B. *Phytochemistry* **1997**, *45*, 189–192.

(36) Chan, S. C.; Chang, Y. S.; Wang, J. P.; Chen, S. C.; Kuo, S. C. *Planta Med.* **1998**, *64*, 153–158.

(37) (a) Zierau, O.; Gester, S.; Schwab, P.; Metz, P.; Kolba, S.; Wulf, M.; Vollmer, G. *Planta Med.* **2002**, *68*, 449–451. (b) Harris, D. M.; Besselink, E.; Henning, S. M.; Go, V. L. W.; Heber, D. *Exp. Biol. Med.* **2005**, *230*, 58–568.

(38) Brzozowski, A. J.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J.; Carlquist, M. *Nature* **1997**, *389*, 753–758.

(39) Pike, A. C. W.; Brzozowski, A. J.; Hubbard, R. E.; Bonn, T.; Thorsell, A.; Engstroem, O.; Ljunggren, J.; Gustafsson, J.; Carlquist, M. *EMBO J.* **1999**, *18*, 4608–4618.

(40) Fox, E. M.; Andrade, J.; Shupnik, M. A. *Steroids* **2009**, *74*, 622–627.

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