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ANTIANDROGENIC PHENOLIC CONSTITUENTS FROM *DALBERGIA COCHINCHINENSIS*

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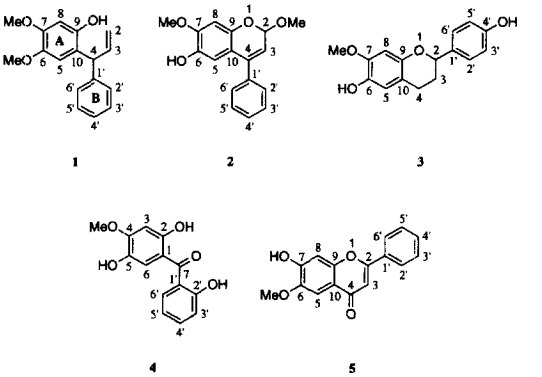
Key Word Index—*Dalbergia cochinchinensis*; Leguminosae; flavone; flavan; neoflavene; benzophenone; spectroscopic analysis; antiandrogenic activity.

Abstract—Four new compounds, 9-hydroxy-6,7-dimethoxydalbergiquinol, 6-hydroxy-2,7-dimethoxyneoflavene, 6,4′-dihydroxy-7-methoxyflavan and 2,2′,5-trihydroxy-4-methoxybenzophenone, in addition to eight known phenolic compounds including 7-hydroxy-6-methoxyflavone, have been isolated from the stems of *Dalbergia cochinchinensis*. Their structures were established by spectroscopic techniques including one- and two-dimensional NMR methods. The first two compounds showed potent inhibitory activity towards 5α-dihydrotestosterone (DHT) which binds with an androgen receptor to form a DHT-receptor complex that causes androgen-dependent diseases. © 1997 Elsevier Science Ltd

INTRODUCTION

Dalbergia cochinchinensis (Leguminosae) is a perennial tree that mainly grows in Indonesia, Iran and Vietnam. Previous study on this plant yielded 12 well-characterized phenolic compounds having antiandrogenic activity [1]. We have investigated the minor chemical constituents of the stems of this plant to obtain more active compounds. The isolation and structure elucidation of four new compounds and their activities against androgen-related bio-reactions are reported in this paper. The ethanol extract yielded a total of 13 compounds on chromatographic separation. Four new phenolic compounds were identified as 9-hydroxy-6,7-dimethoxydalbergiquinol (**1**), 6-hydroxy-2,7-dimethoxyneoflavene (**2**), 6,4′-dihydroxy-7-methoxyflavan (**3**) and 2,2′,5-trihydroxy-4-methoxybenzophenone (**4**). In addition, one compound, 7-hydroxy-6-methoxyflavone (**5**), has been isolated for the first time from this plant.



RESULTS AND DISCUSSION

The ethanolic extract of *Dalbergia cochinchinensis* on column chromatography over silica gel followed by silica gel low-pressure liquid chromatography and medium-pressure liquid chromatography on silica gel/ODS provided 13 compounds. The compounds, latifolin, 2,5-dihydroxy-4-methoxybenzophenone, 5-*O*-methyllatifolin, methoxydalbergione, 6,4′-dihydroxy-7-methoxyflavanone, liquiritigenin, calycosin and isoliquiritigenin were isolated, characterized on the basis of mass, UV, IR, ^{1}H NMR, ^{13}C NMR and finally by comparison with the literature data [1–4]. Four new phenolic compounds, **1**–**4**, and a new isolate from this plant, **5**, were characterized on the basis of spectroscopic techniques including high-field one- and two-dimensional NMR spectral analyses.

Compound **1** showed the characteristic bands of the aromatic ring, hydroxyl and methoxy groups in the IR spectrum. The molecular ion peak $[M]^+$ in EI mass spectrum appeared at *m/z* 270, which was further confirmed by the HR mass spectrum which cor-

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responded to molecular formula $C_{17}H_{18}O_3$. In the EI mass spectrum, the loss of a methyl group from $[M]^+$ yielded the fragment ion at *m/z* 255, and further fragmentation of this peak gave *m/z* 115 and 91 corresponding to the phenylcyclopropene fragment ion and tropylium ion, respectively. The cleavage of ring B and C_2H_4 from $[M]^+$ provided fragment ion *m/z* 165. The mass spectrum of this compound was found to be similar to *R*(+)-dalbergiphenol earlier reported from *Dalbergia paviflora* [2], but some differences were observed in the NMR spectral studies. The ^1H NMR spectrum showed two multiplet signals at δ_H 7.28 and 7.20 ppm, equivalent to 2H and 3H, respectively, which was characteristic of an unsubstituted benzene ring. The spectrum also showed two singlets at δ_H 6.68 and 6.60 ppm of one proton each for para-positioned aromatic ring protons. Three signals in the olefinic range in the spectrum were observed at δ_H 6.30, 5.25 and 5.01 ppm, equivalent to one proton each. These splitting patterns and coupling constants of geminal and vicinal protons are found to be similar to the allylic system of other similar reported compounds [1, 2]. The presence of two methoxy groups was indicated by the two singlets at δ_H 3.65 and 3.70 ppm. In the ^{13}C NMR spectrum of **1**, olefinic carbons observed at δ_C 139.5 and 116.5 ppm and the ring junction carbon (C-4) at δ_C 48.2 ppm showed down-field shift from δ_C 40.0 ppm in the case of *R*-latifolin due to the absence of a hydroxyl group at the C-2′ position [1, 2]. The locations of the two benzoyl methoxy were determined to be in ring A by HMBC spectrum as follows: the HMBC cross peaks were observed between the methoxy methyl protons at δ_H 3.70 ppm and an aromatic carbon of C-6 position at δ_C 142.5 ppm, and also between the other methoxy methyl protons at δ_H 3.65 ppm and an aromatic carbon of C-7 position at δ_C 148.1 ppm. These locations were also supported by the NOESY spectrum. This spectrum showed cross peaks between the methoxy methyl protons at δ_H 3.70 ppm and a singlet aromatic proton of the 8 position at δ_H 6.38 ppm, and also between the methoxy methyl protons at δ_H 3.65 ppm and a singlet aromatic proton of the 5 position at δ_H 6.60 ppm. Furthermore, the location of a hydroxy group was suggested to be at the 9 position by the difference in the ^{13}C chemical shift between **1** (δ_{C9} 147.5 ppm) and its methyl derivative (δ_{C9} 151.3 ppm). These two-dimensional correlations, together with the above-mentioned spectral findings, confirmed **1** as 9-hydroxy-6,7-dimethoxydalbergiquinol.

Compound **2** showed the $[M]^+$ peak at *m/z* 284 in the EI mass spectrum which confirmed the molecular formula as $C_{17}H_{15}O_4$. IR showed the presence of aromatic rings with methoxy and hydroxy substitutions. The ^1H NMR spectrum of this compound showed signals in the aromatic region at δ_H 7.30–7.39 ppm, equivalent to five protons as multiplet, indicative of an unsubstituted benzene nucleus. Two singlet peaks appearing at δ_H 6.65 and 6.71 ppm suggested the presence of a tetra-substituted benzene ring, two singlets at 3.52 and 3.90 ppm were attributed to two methoxy groups, and also one D_2O exchangeable singlet at 5.28 ppm was assigned to a hydroxyl group. In the aromatic region of the ^{13}C NMR spectrum, a total of 10 signals ($5 \times C$, $5 \times CH$) were observed. The signals at δ_C 114.5, 115.6 and 96.2 ppm suggested the presence of a pyran nucleus with an olefin bond at the C-3 position. The HMBC correlations of methoxy methyl protons at δ_H 3.52 and 3.90 ppm, with carbons at δ_H 147.2 and 96.2 ppm, respectively, fixed the position of both the methoxy groups at C-2 and C-7. Further NOE correlations in the NOESY spectrum between the protons at δ_H 3.90 and 6.65 ppm, δ_H 3.52 and 5.60 ppm and also δ_H 6.71 and 7.30 ppm suggested the complete structure of **2** as 6-hydroxy-2,7-dimethoxyneoflavene.

Compound **3** showed bands at 3370, 1614 and 963 cm^{-1} in the IR spectrum that are characteristic of the hydroxyl group and aromatic nucleus. The mass spectrum (EIMS) showed a $[M]^+$ peak at *m/z* 258 and characteristic at *m/z* 120 and 107 of a hydroxy-substituted B ring of a flavan, and HRMS confirmed the molecular formula as $C_{16}H_{16}O_4$. In ^1H NMR spectrum triple doublet peak at δ_H 4.84 ppm was found to be coupled with doublets of doublets at δ_H 2.05, 2.10, 2.68 and 2.90 ppm, suggestive of the pyran nucleus of a flavan [9–11]. Two singlet peaks at δ_H 6.43 and 6.54 ppm and two doublets at δ_H 6.81 and 7.30 ppm suggested the presence of methoxy (δ_H 3.90 ppm) and hydroxyl groups *ortho* to each other in ring A and a hydroxyl or methoxy (δ_H 3.90 ppm) group at the *para* position in ring B, respectively. In the ^{13}C NMR spectrum, the signals at δ_C 25.6 (CH_2), 31.2 (CH_2) and 78.8 ppm (oxygenated methine) showed the presence of a pyran nucleus, which was further confirmed by the HMBC correlations of protons at δ_H 2.68 and 2.90 ppm with carbons at δ_C 114.4 and 78.8 ppm. The methoxy group was assigned at the C-7 position by the correlation of the proton signal at δ_H 3.90 ppm with carbon at δ_C 149.6 in the HMBC and the correlation of protons at δ_H 3.90 and 6.43 ppm in the NOESY spectrum. These data confirmed the structure of **3** as 5,4′-dihydroxy-7-methoxyflavan.

Compound **4**, which had a molecular formula of $C_{14}H_{12}O_5$, was found to possess aromatic rings: one carbonyl (1508 cm^{-1} in IR spectrum), one methoxy and three hydroxy groups. The ^1H NMR spectrum of **4** showed two singlet signals at δ_H 7.11 and 7.54 ppm and four triple doublets at δ_H 8.28, 7.81, 7.60 and 7.43 ppm assigned to ring A and ring B, respectively. The presence of a singlet signal at δ_H 4.00 ppm, equivalent to three protons, suggested the presence of one methoxy group. In ^{13}C NMR, carbonyl carbon appeared at δ 178.0 that was found to be similar to those of benzophenones [1–3, 12]. In ring A, signals of C-3 and C-6 shifted upfield to δ_C 100.7 and 109.5 ppm due to oxygenation at positions 2, 4 and 5. The chemical shifts of C-2′ at δ_C 157.6 in ring B showed a downfield shift suggesting hydroxy substitution. Also upfield shifts observed for C-3′ and C-5′ were further

attributed to the hydroxy group in the *ortho* and *para* positions, respectively [4]. The substituted positions of methoxy and hydroxy groups were confirmed by HMBC and NOESY correlations giving the structure of **4** as 2,2′,5-trihydroxy-4-methoxybenzophenone.

Compound **5**, its molecular formula $C_{16}H_{12}O_4$, showed two multiplet signals at δ_H 7.4 and 7.6 ppm, corresponding to 2H and 3H protons in the ^{1}H NMR, respectively, suggestive of an unsubstituted B ring. Whereas two singlets appeared at δ_H 7.02 and 6.88 ppm, suggesting the *ortho*-substituted A ring. The singlets at δ_H 3.99 and 6.28 ppm were assigned to a methoxy group and the H-3 olefinic methine proton. ^{13}C NMR showed aromatic signals, carbonyl at δ_C 161.4, methoxy at δ_C 55.6 and olefins at δ_C 112.6 and 155.7 ppm suggesting the compound as a flavone [4]. The methoxy and hydroxyl groups were found to be attached at the C-6 and C-7 positions, respectively, on the basis of long-range correlations observed in HMBC and finally confirmed by NOESY correlation of methoxy proton at δ_H 3.99 ppm with proton at δ_H 6.88 ppm. The structure of **5** was found to be 7-hydroxy-6-methoxyflavone on the basis of the above spectral studies; it was earlier isolated from *Trigonella foenum-graecum* [13]. This compound has been isolated for the first time from *Dalbergia cochinchinensis*.

The eight known compounds, latifolin, 2,5-dihydroxy-4-methoxybenzophenone, 5-*O*-methyllatifolin, methoxydalbergione, 6,4′-dihydroxy-7-methoxyflavanone, liquiritigenin, calycosin and isoliquiritigenin were characterized on the basis of mass, UV, IR, ^{1}H NMR, ^{13}C NMR and finally identified by comparison with the literature data [1–4].

Testosterone, a hormone essential for the growth of secondary male sexual characteristics, is also responsible for androgen-dependent diseases, such as prostatomegaly, prostate cancer, male pattern baldness, hirsutism and acne [7, 8]. Testosterone is converted to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase, located in the cytoplasm of the prostate cell [5]. The DHT binds with an androgen receptor to form the DHT-receptor complex that causes the diseases [6]. To investigate the possibility of the isolated compounds as antiandrogen agents, inhibitory assays for the 5α-reductase and the DHT-receptor complex were carried out. As a result (Table 2), 9-hydroxy-6,7-dimethoxydalbergiquinol (**1**) and 6-hydroxy-2,7-dimethoxyneoflavene (**2**) showed effective action against the formation of the DHT-receptor binding complex, whereas no noticeable activity was shown against the 5α-reductase.

EXPERIMENTAL

General methods. Mps are uncorrected. One- and two-dimensional NMR were recorded at 300 K using standard pulse sequences with TMS as int. standard. Chemical shifts were reported in δ, and coupling constants (J) are given in Hz. TLC was performed on precoated Merck Kiesel gel $60F_{254}$, and spots were visualized by heating with 10% H_2SO_4.

Plant material. Stems of *Dalbergia cochinchinensis* were collected from Vietnam in 1993. The plant material was air dried, and a voucher specimen has been deposited at the National Institute of Health Sciences, Japan and at the Biological Science Research Center, Lion Corporation, Japan.

Extraction and isolation. Dried stems (1.5 kg) of *D. cochinchinensis* were extracted with hot EtOH, and evaporation *in vacuo* gave 172 g of EtOH extract, which was then fractionated over a silica gel (1 kg, Merck 60–120 mesh) open column with *n*-hexane–EtOAc and EtOAc–MeOH gradients. The frs thus obtained were subjected either to direct crystallization or to further purification on silica gel or ODS columns using various chromatographic techniques, such as low-pressure liquid chromatography and medium-pressure liquid chromatography. Finally, all compounds were purified by HPLC on an ODS column using MeCN–H_2O solvent systems and yielded 13 compounds. The data of four new compounds **1**–**4** and one first isolate, compound **5**, from this plant are as follows:

2-Hydroxy-4,5-dimethoxydalbergiquinol (**1**) was obtained as a pale yellow viscous solid (165 mg): mp 45–50°; $[\alpha]_D$ −6.7° ($CHCl_3$, *c* 2.86); UV λ_{max}^{MeOH} nm (log ε): 381.5 (2.88), 292.0 (3.92), 210.0 (4.44); IR ν_{max}^{KBr} cm^{-1}: 3437, 2935, 1614, 1518, 1450, 1199, 999; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.28, 7.20 (2H and 3H, respectively, *m*, H-2′, H-3′, H-4′, H-5′ and H-6′), 6.60 (1H, *s*, H-5), 6.38 (1H, *s*, H-8), 6.30 (1H, *ddd*, J = 6.0, 8.7, 16.2 Hz, H-3), 5.25 (1H, *dt*, J = 1.5, 10.2 Hz, H-2a), 5.01 (1H, *dt*, J = 1.5, 16.2 Hz, H-2b), 4.94 (1H, *dt*, J = 1.0, 6.0 Hz, H-4), 3.70 (3H, *s*, 6-OMe), 3.65 (3H, *s*, 7-OMe); ^{13}C NMR (100 MHz, $CDCl_3$): listed in Table 1; EIMS *m/z* (rel. int.): 270 ([M]$^+$, 100), 255 (75), 223 (56), 195 (63), 165 (58), 128 (61), 115 (85), 77 (84), 69 (85); HRMS *m/z*: [M]$^+$ 270.124946 ($C_{17}H_{18}O_3$ requires 270.125595).

6-Hydroxy-2,7-dimethoxyneoflavene (**2**) was obtained as a pale yellow amorphous solid (40 mg): mp 106–108°; $[\alpha]_D$ +1.4° ($CHCl_3$, *c* 0.57); UV λ_{max}^{MeOH} nm (log ε): 381 (2.60), 319 (4.30), 234.5 (4.78), 213 (4.84); IR ν_{max}^{KBr} cm^{-1}: 3439, 2982, 1628, 1577, 1508, 1448, 1284, 987. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.39–7.30 (5H, *m*, H-2′, H-3′, H-4′, H-5′ and H-6′), 6.71 (1H, *s*, H-5), 6.65 (1H, *s*, H-8), 5.73 (1H, *d*, J = 4.2 Hz, H-3), 5.60 (1H, *d*, J = 4.2 Hz, H-2), 5.28 (1H, *s*, 6-OH), 3.90 (3H, *s*, 7-OMe), 3.52 (3H, *s*, 2-OMe); ^{13}C NMR (100 MHz, $CDCl_3$): listed in Table 1; EIMS *m/z* (rel. int.): 284 ([M]$^+$, 42), 253 (100), 237 (10), 152 (8), 69 (6).

5,4′-Dihydroxy-7-methoxyflavan (**3**) was obtained as colourless crystals (300 mg): mp 190–192°; $[\alpha]_D$ +6.5° (MeOH, *c* 0.52); UV λ_{max}^{MeOH} nm (log ε): 296 (3.60), 223.5 (4.11), 205 (4.35); IR ν_{max}^{KBr} cm^{-1}: 3337, 2926, 2310, 1634, 1615, 1594, 1442, 1339, 1232, 1193, 963; ^{1}H NMR (400 MHz, CD_3OD): δ_H 7.30 (2H, *d*, J = 8.3 Hz, H-2′ and H-6′), 6.81 (2H, *d*, J = 8.3 Hz,

Table 1. ^{13}C NMR assignments of compounds **1–5**

Carbon No.	**1***	**2***	**3†**	**5***	Carbon No.	**4†**
2	116.5 (CH_2)	96.2 (CH)	78.8 (CH)	155.7 (C)	1	115.9 (C)
3	139.5 (CH)	115.6 (CH)	31.2 (CH_2)	112.6 (CH)	2	153.3 (C)
4	48.2 (CH)	114.5 (C)	25.6 (CH_2)	161.4 (C)	3	100.7 (CH)
5	113.4 (CH)	111.6 (CH)	116.1 (CH)	99.6 (CH)	4	156.7 (C)
6	142.5 (C)	139.9 (C)	141.0 (C)	150.0 (C)	5	145.8 (C)
7	148.1 (C)	147.4 (C)	149.6 (C)	142.4 (C)	6	109.5 (CH)
8	101.3 (CH)	100.4 (CH)	101.7 (CH)	110.5 (CH)	7	178.0 (C)
9	147.5 (C)	145.4 (C)	148.1 (C)	149.4 (C)	1′	122.1 (C)
10	120.1 (C)	138.6 (C)	114.4 (C)	111.8 (C)	2′	157.6 (C)
1′	141.9 (C)	137.7 (C)	134.3 (C)	135.6 (C)	3′	118.9 (CH)
2′	128.3 (CH)	128.7 (CH)	128.4 (CH)	128.3 (CH)	4′	135.5 (CH)
3′	128.2 (CH)	128.3 (CH)	116.0 (CH)	129.5 (CH)	5′	124.9 (CH)
4′	126.3 (CH)	128.0 (CH)	158.0 (C)	129.6 (CH)	6′	126.9 (CH)
5′	128.2 (CH)	128.3 (CH)	116.0 (CH)	129.5 (CH)	4-OCH_3	56.9 (CH_3)
6′	128.3 (CH)	128.7 (CH)	128.4 (CH)	128.3 (CH)		
2-OCH_3		54.9 (CH_3)				
6-OCH_3	55.4 (CH_3)			55.6 (CH_3)		
7-OCH_3	56.4 (CH_3)	56.0 (CH_3)	56.3 (CH_3)			

* in $CDCl_3$.
† in CD_3OD.

Table 2. Inhibitory effect of the compounds **1–5** on the activity of testosterone 5α-reductase and the formation of 5α-dihydrotestosterone (DHT)-receptor binding complex at various concentration

Compounds	5α-Reductase (inhibitory rate; %)			DHT-Receptor binding (inhibitory rate; %)		
	50 μg ml^{-1}	100 μg ml^{-1}	200 μg ml^{-1}	50 μg ml^{-1}	100 μg ml^{-1}	200 μg ml^{-1}
1	14.1	25.3	52.6	45.0	75.1	98.8
2	3.0	5.1	19.5	47.8	68.7	84.3
3	31.0	37.1	42.5	44.7	49.7	49.3
4	9.1	14.3	22.6	10.0	10.3	31.7
5	13.8	20.8	34.1	29.5	30.4	25.5

H-3′ and H-5′), 6.54 (1H, *s*, H-5), 6.43 (1H, *s*, H-8), 4.84 (1H, *ddd*, $J = 0.8, 2.1, 10.2$ Hz, H-2), 3.90 (3H, *s*, 7-OMe), 2.90 (1H, *dddd*, $J = 0.8, 6.1, 11.4, 16.4$ Hz, H-4a), 2.68 (1H, *ddd*, $J = 2.9, 5.4, 16.4$ Hz, H-4b), 2.10 (1H, *dddd*, $J = 2.1, 2.9, 6.1, 13.6$ Hz, H-3a), 2.05 (1H, *dddd*, $J = 5.4, 10.2, 11.4, 13.6$ Hz, H-3b), ^{13}C NMR (100 MHz, CD_3OD): listed in Table 1; EIMS *m/z* (rel. int.): 272 ($[M]^+$, 78), 166 (18), 153 (100), 120 (60), 107 (24), 91 (13), 65 (11); HRMS: $[M]^+$ 272.104065 ($C_{16}H_{16}O_4$ requires 272.104859).

2,2′,5-*Trihydroxy*-4-*methoxybenzophenone* (**4**) was obtained as a pale yellow amorphous solid (35 mg): mp 162–166°; $[\alpha]_D$ +6.01° (MeOH, *c* 0.31); UV λ_{max}^{MeOH} nm (log ε): 354 (3.79), 307 (3.88), 274.5 (3.76), 241.4 (4.34), 216 (4.21); IR ν_{max}^{KBr} cm^{-1}: 3256, 1616, 1508, 1466, 1319, 1277, 1236, 1132, 1016, 877, 756, 644, 601; ^1H NMR (400 MHz, CD_3OD): δ_H 8.28 (1H, *ddd*, $J = 0.5, 1.7, 8.0$ Hz, H-6′), 7.81 (1H, *ddd*, $J = 1.6, 7.0, 8.5$ Hz, H-4′), 7.60 (1H, *ddd*, $J = 0.5, 1.0, 8.5$ Hz, H-3′), 7.54 (1H, *s*, H-6), 7.43 (1H, *ddd*, $J = 1.0, 7.1, 8.1$ Hz, H-5′), 7.11 (1H, *s*, H-3), 4.00 (3H, *s*, 4-OMe); ^{13}C NMR (100 MHz, CD_3OD): listed in Table 1; EIMS *m/z* (rel. int.): 242 ($[M-H_2O]^+$, 100), 227 (24), 199 (20), 171 (17), 115 (12), 69 (6); HRMS: $[M-H_2O]^+$ 242.057262 ($C_{14}H_{10}O_4$ requires 242.057909).

7-*Hydroxy*-6-*methoxyflavone* (**5**) was obtained as a pale yellow amorphous solid (65 mg): mp 149–151°; $[\alpha]_D$ +8.1° ($CHCl_3$, *c* 0.09); UV λ_{max}^{MeOH} nm (log ε): 354 (3.83), 300.5 (3.65), 258 (3.90), 236 (4.08), 207 (4.45); ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.4 and 7.6 (2H and 3H, respectively, *m*, H-2′, H-3′, H-4′, H-5′ and H-6′), 7.20 (1H, *s*, H-8), 6.88 (1H, *s*, H-5), 6.28 (1H, *s*, H-3), 3.99 (3H, *s*, 6-OMe); ^{13}C NMR (100 MHz, CD_3OD): listed in Table 1; EIMS *m/z* (rel. int.): 268 ($[M]^+$, 100), 240 (35), 225 (55), 155 (8), 127 (13), 69 (16); HRMS: $[M]^+$ 268.074478 ($C_{16}H_{12}O_4$ requires 268.073559).

Inhibitory assays for 5α-reductase and the formation of DHT-receptor binding complex. The newly isolated compounds were examined for their inhibitory activities against 5α-reductase and the formation of the DHT-receptor binding complex in the same manner as previously reported [1]. The results of the assays are listed in Table 2.

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