Cytochrome P3A4 Inhibitors and Other Constituents of *Fibraurea tinctoria*

Chung-Ren Su,[†] Yune-Fang Ueng,[‡] Nguyen Xuan Dung,[§] M. Vijaya Bhaskar Reddy,[†] and Tian-Shung Wu*[,†,⊥]

Department of Chemistry, National Cheng Kung University, Tainan 701, Taiwan, Republic of China, National Research Institute of Chinese Medicine, Taipei 112, Taiwan, Republic of China, Department of Chemistry, College of Natural Sciences, Hanoi National University, Hanoi 10000, Vietnam, and Department of Applied Chemistry, Providence University, Taichung 433, Taiwan, Republic of China

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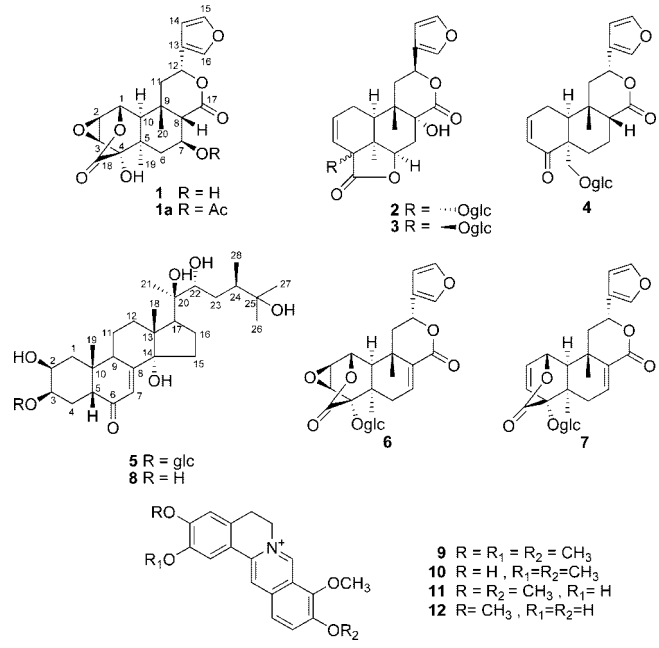
Four new furanoditerpenoids, fibrauretin A (**1**), fibrauretinoside A (**2**), *epi*-fibrauretinoside A (**3**), and *epi*-12-palmatoside G (**4**), and a new ecdysteroid glucoside, fibraurecdyside A (**5**), together with seven known compounds including two furanoditerpenoids (**6** and **7**), an ecdysteroid (**8**), and four quaternary protoberberine alkaloids (**9**–**12**) were isolated from the stems of *Fibraurea tinctoria*. The structures of **1**–**5** were established on the basis of spectroscopic evidence. Among these compounds, palmatine (**9**) and jatrorrhizine (**10**) showed inhibitory effects against cytochrome P450 3A4 (CYP3A4) with IC_{50} values of 0.9 and 2.1 μM, respectively.

The superfamily of cytochrome P450 (P450) enzymes catalyze the oxidation of a variety of xenobiotics. Among cytochrome P450 members, P450 3A4 (CYP3A4) is reported to be the most abundant P450 form in the human liver and intestines.[1] Approximately 50% of marketed drugs are its substrates, so the modulation of CYP3A4-catalyzed oxidation is a major concern in terms of drug interactions. In a search for CYP3A4 inhibitory active secondary metabolites from plants, we found a CH_3OH extract of *Fibraurea tinctoria* to inhibit CYP3A4 (IC_{50}, 5.1 μg/mL). Furthermore, the *n*-BuOH-soluble portion of the CH_3OH extract was also inhibitory against CYP3A4 activity, with an IC_{50} value of 3.4 μg/mL.

Fibraurea tinctoria Lour. (Menispermaceae) is a common dye-producing plant that is widely distributed in mainland China, Indonesia, Malaysia, Thailand, and Vietnam.[2] The stem bark of this species is used for the treatment of dysentery and for analgesic, antipyretic, antidote, and diuretic effects.[3] A number of chemical constituents including protoberberine alkaloids and several furanoditerpenoids have been isolated from this species.[4,5] The present investigation on the CYP3A4 inhibitory compounds from this plant has led to the isolation and characterization of four new furanoditerpenoids, fibrauretin A (**1**), fibrauretinoside A (**2**), *epi*-fibrauretinoside A (**3**), and *epi*-12-palmatoside G (**4**), and a new ecdysteroid glucoside, fibraurecdyside A (**5**), together with seven known compounds including two furanoditerpenoids, one ecdysteroid, and four quaternary protoberberine alkaloids. The structures of the known compounds were identified as fibraurinoside (**6**),[5] fibleucinoside (**7**),[5] makisterone A (**8**),[6] palmatine (**9**),[4] jatrorrhizine (**10**),[4] columbamine (**11**),[4] and stepharanine (**12**),[7] by comparing their spectroscopic data with those reported in the literature. In the present paper, we report the isolation and structure elucidation of compounds **1**–**5** and the evaluation of CYP3A4 inhibitory activities of compounds **1**–**12**.

Results and Discussion

The air-dried and powdered whole plants of *F. tinctoria* were extracted with methanol under reflux. The solvent was evaporated, and then the resulting residue was partitioned with $CHCl_3$, *n*-BuOH, and H_2O, successively. The *n*-BuOH fraction showed CYP3A4 inhibitory activity with an IC_{50} value of 3.4 μg/mL. The *n*-BuOH fraction was chromatographed on reversed-phase Diaion HP-20 gel using H_2O/CH_3OH gradients to furnish two new furanoditerpenoids (**1** and **2**), a new ecdysteroid glucoside (**5**), and seven known compounds (**6**–**12**). The H_2O fraction was purified on reversed-phase Diaion HP-20 gel using H_2O/CH_3OH gradients to give two new furanoditerpenene glucosides (**3** and **4**).



Fibrauretin A (**1**) was obtained as an optically colorless powder, mp 237–238 °C. The HRFABMS of **1** showed a peak at *m*/*z* 391.1395 corresponding to the molecular formula $C_{20}H_{22}O_8$. The IR absorption bands at 1760 and 1723 cm^{-1} indicated the presence of two lactone carbonyls, which were confirmed by resonances at δ 174.3 and 171.8 in the ^{13}C NMR spectrum. The ^1H NMR spectrum displayed signals at δ 4.82 (1H, d, J = 2.5 Hz), 3.80 (1H, dd, J = 4.3, 2.5 Hz), and 3.61 (1H, d, J = 4.3 Hz), assignable to the protons of the lactone proton at C-1 and a β-epoxide ring.[8] In addition, signals at δ 6.60 (1H, dd, J = 1.6, 1.2 Hz), 7.67 (1H, dd, J = 1.6, 1.4 Hz), and 7.74 (1H, dd, J = 1.4, 1.2 Hz) were assignable to an α-substituted furan ring. Moreover, a downfield signal was observed at δ 3.54 (1H, m) due to a hydroxyl group. Acetylation of **1** yielded a monoacetylated product, **1a**, which indicated the presence of a hydroxyl group at C-7. This was confirmed by HMBC correlations of H-7 (δ 3.54) with C-8 (δ 45.0) and C-17 (δ 174.3). The position of a γ-lactone group was confirmed by the HMBC correlations from both H-3 (δ 3.61) and H-1 (δ 4.82) to C-18 (δ 171.8). The relative configuration of OH-7

* To whom correspondence should be addressed. Tel: 886-6-2757575, ext. 65333. Fax: 886-6-2740552. E-mail: tswu@mail.ncku.edu.tw.
[†] National Cheng Kung University.
[‡] National Research Institute of Chinese Medicine.
[§] Hanoi National University.
[⊥] Providence University.

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Table 1. ^{1}H NMR Spectroscopic Data (δ_H, mult. J_{HH} in Hz) of **1**–**4**

position	**1** (DMSO-d_6)[a]	**1a** (CD_3OD)[b]	**2** (DMSO-d_6)[a]	**3** (DMSO-d_6)[a]	**4** (CD_3OD)[a]
1	4.82 (d, 2.5)	4.86 (d, 2.9)	2.47 (dd, 19.6, 7.6) 2.11 (dd, 19.6, 5.5)	2.46 (dd, 19.3, 7.5) 2.11 (dd, 19.3, 5.1)	2.80 (d, 20.1) 2.39 (dd, 20.1, 4.8)
2	3.80 (dd, 4.3, 2.5)	3.90 (dd, 4.2, 3.0)	6.06 (ddd, 10.4, 7.6, 5.5)	6.04 (ddd, 10.2, 7.5, 5.1)	6.96 (m)
3	3.61 (d, 4.3)	3.61 (d, 4.2)	5.83 (d, 10.4)	5.85 (d, 10.2)	5.97 (d, 10.2)
6	1.95 (dd, 13.7, 6.1) 1.32 (dd, 13.2, 13.6)	2.03 (dd, 13.6, 13.0) 1.23 (dd, 13.6, 7.0)	4.60 (s)	4.56 (s)	2.32 (m) 1.72 (m)
7	3.54 (m)	4.13 (m)	3.03 (d, 14.0) 1.83 (d, 14.0)	2.53 (d, 14.9) 1.74 (d, 14.9)	2.01 (d, 9.7) 1.71 (m)
8	2.87 (d, 3.9)	3.15 (d, 3.7)			2.68 (s)
10	1.51 (br s)	1.72 (br s)	1.51 (br s)	1.95 (d, 7.5)	2.23 (d, 6.4)
11	2.16 (d, 14.0) 1.91 (dd, 14.3, 12.0)	2.32 (d, 13.3) 1.76 (dd, 13.3, 12.3)	2.32 (dd, 12.8, 4.7) 1.93 (dd, 12.8, 11.3)	2.20 (m)	2.12 (d, 14.0) 1.88 (dd, 14.0, 12.0)
12	5.62 (d, 11.5)	5.69 (d, 12.3)	5.04 (dd, 11.3, 4.7)	4.95 (dd, 8.5, 4.1)	5.43 (d, 12.0)
14	6.60 (dd, 1.6, 1.2)	6.58 (br s)	6.56 (br s)	6.80 (br s)	6.53 (dd, 1.5, 0.5)
15	7.67 (dd, 1.6, 1.4)	7.52 (br s)	7.61 (br s)	7.57 (br s)	7.50 (dd, 1.5, 0.5)
16	7.74 (dd, 1.4, 1.2)	7.65 (br s)	7.53 (br s)	7.50 (br s)	7.59 (d, 0.5)
19	1.08 (s)	1.34 (s)	1.26 (s)	1.27 (s)	3.91 (d, 9.6) 3.51 (d, 9.6)
20	1.24 (s)	1.44 (s)	0.78 (s)	0.80 (s)	1.10 (s)
OH-4	6.30 (s)				
$COCH_3$		2.22 (s)			
1′			4.48 (d, 7.9)	4.48 (d, 7.8)	4.18 (d, 7.8)
2′			2.96 (dd, 8.3, 7.9)	2.96 (dd, 8.3, 7.9)	3.16 (dd, 8.3, 7.8)
3′			3.14 (dd, 8.8, 8.3)	3.14 (dd, 8.5, 8.3)	3.25 (dd, 9.4, 8.4)
4′			3.10 (dd, 9.2, 8.8)	3.11 (dd, 8.5, 8.4)	3.23 (m)
5′			2.93 (dd, 8.8, 5.2)	2.90 (dd, 8.5, 5.4)	3.22 (m)
6′			3.54 (d, 10.4) 3.42 (dd, 10.4, 5.2)	3.56 (d, 10.9) 3.42 (dd, 10.4, 5.4)	3.83 (d, 11.4) 3.63 (dd, 11.4, 5.4)

[a] Recorded in 500 MHz. [b] Recorded in 300 MHz.

was assigned as β, on the basis of the NOE correlation exhibited between H-7 (δ 3.54) and CH_3-19 (δ 1.08). A NOE correlation evident between H-12 (δ 5.62) and CH_3-20 (δ 1.24) indicated that both are present in a β-configuratoin and the furan ring in an α-configuration. Thus, the structure of **1** was proposed as shown, and this compound has been named fibrauretin A (**1**).

Fibrauretinoside A (**2**) was isolated as an optically colorless powder, mp 166–167 °C. A positive Molisch's test was indicative of compound **2** being a furanoditerpenoid glucoside.[9] The molecular formula of **2** was determined as $C_{26}H_{32}O_{12}$ from the protonated molecular ion peak at *m*/*z* 537.1970 $[M + H]^+$ in the HRFABMS. The IR spectrum displayed absorptions at 3404, 1758, and 1727 cm^{-1}, due to hydroxyl and lactone carbonyl groups. The ^{1}H NMR spectrum revealed the presence of signals at δ 6.56 (1H, br s), 7.53 (1H, br s), and 7.61 (1H, br s), assignable to a furan ring, and olefinic protons at δ 6.06 (1H, ddd, J = 10.4, 7.6, 5.5 Hz, H-2) and 5.83 (1H, d, J = 10.4 Hz, H-3). In addition, the ^{1}H NMR spectrum showed two tertiary methyl groups at δ 1.26 (3H, s) and a higher field methyl group at δ 0.78 (3H, s, CH_3-20), indicating that both CH_3-20 and the furan ring are in a β-configuration.[10] The position of the γ-lactone group was confirmed by the HMBC correlations from both H-6 (δ 4.60) and H-3 (δ 5.83) to C-18 (δ 174.9). The anomeric proton signal at δ 4.48 (1H, d, J = 7.9 Hz), which correlated to a carbon resonance at δ 98.3 in the HMQC spectrum, suggested the presence of a sugar residue with a β-D-glucosyl moiety. The glucosyl moiety in **2** was found to be linked to C-4 on the basis of the HMBC correlation observed between H-1′ (δ 4.48) and C-4 (δ 77.9), which in turn showed cross correlations with H-2 (δ 6.06), H-3 (δ 5.83), and CH_3-19 (δ 1.26), respectively. The key NOESY correlations observed between H-1′ and CH_3-19, CH_3-19 and H-6, CH_3-19 and H-10, and H-10 and H-12 indicated an α-configuration at the C-4 position. However, the configuration of OH-8 could not be determined by NOESY spectroscopy and was proposed as being α on the basis of ^{13}C NMR chemical shifts due to the steric effects and conformational changes in the molecule.[11] From the above spectroscopic data, the structure of **2** was determined, and this compound has been named fibrauretinoside A (**2**).

epi-Fibrauretinoside A (**3**) was obtained as a colorless powder, mp 152–153 °C. The HRFABMS of **3** exhibited a molecular ion peak at *m*/*z* 537.1970, consistent with the molecular formula $C_{26}H_{32}O_{12}$. The IR, ^{1}H NMR, and ^{13}C NMR spectra of **3** were similar to those of **2** and gave evidence of an opposite configuration at the C-4 position. This assignment was further supported by an absence of NOE correlation between H-1′ and CH_3-19. Thus, the above data suggested that **3** is a stereoisomer of **2**, with the only difference being a β-oriented substituent at C-4 in **3**.

epi-12-Palmatoside G (**4**) was isolated as a colorless powder, mp 132–133 °C. HRFABMS analysis established its molecular formula as $C_{25}H_{30}O_{10}$. IR absorption bands were observed at 3383, 1729, and 1666 cm^{-1} due to hydroxyl, lactone carbonyl, and α,β-unsaturated carbonyl groups, respectively. The ^{1}H NMR spectroscopic data of **4** showed the presence of a furan ring, olefinic protons, and a sugar moiety for which the signals appeared between δ 4.18 and 3.16, indicating that this compound is a furanoditerpenoid glucoside. In addition, the ^{1}H NMR spectrum showed a downfield tertiary methyl group at δ 1.10 (3H, s, CH_3-20) and a symmetrical pair of coupled protons at δ 3.91 (1H, d, J = 9.6 Hz, H-19) and 3.51 (1H, d, J = 9.6 Hz, H-19). The coupling constant of the anomeric proton δ 4.18 (1H, d, J = 7.8 Hz) suggested that a glucose substituent with a β-configuration was present. The glucose moiety in **4** was found to be linked to C-19, on the basis of the HMBC correlations from H-1′ (δ 4.18) to C-19 (δ 76.2) and from H-19 (δ 3.91 and 3.51) to C-1′ (δ 104.0) in the HMBC spectrum. The above spectroscopic data of **4** are very similar to those of palmatoside G,[12] except for the configuration at the C-12 position. The NOE correlations observed among CH_3-20 (δ 1.10),

Table 2. ^{13}C NMR Spectroscopic Data of **1**–**4**

position	**1**[a,c]	**2**[a,d]	**3**[a,c]	**4**[b,d]
1	71.0	22.4	22.3	24.7
2	49.0	132.2	132.1	149.6
3	52.0	122.1	122.1	128.9
4	80.1	77.9	78.2	202.3
5	41.7	42.5	42.5	50.7
6	28.1	80.1	81.2	23.9
7	49.6	31.8	32.5	18.1
8	45.0	86.5	88.0	44.8
9	36.1	49.3	48.5	36.8
10	53.5	40.8	41.1	46.4
11	48.9	48.0	48.4	46.9
12	69.5	71.7	70.7	70.5
13	124.3	126.5	128.3	124.6
14	109.5	110.4	111.3	108.7
15	144.1	140.4	140.2	143.9
16	140.8	143.6	142.6	140.4
17	174.3	174.7	174.9	175.9
18	171.8	174.9	176.4	
19	23.8	21.1	21.5	76.2
20	29.0	19.6	19.5	25.6
1′		98.3	98.3	104.0
2′		73.6	73.6	73.9
3′		77.4	77.5	77.0
4′		70.1	70.2	70.5
5′		76.8	76.7	77.0
6′		60.9	61.0	61.7

[a] Recorded in DMSO-d_6. [b] Recorded in CD_3OD. [c] Recorded in 125 MHz. [d] Recorded in 75 MHz.

H-12 (δ 5.43), and H-8 (δ 2.68) indicated the furan ring to be in the α-configuration and H-8 and H-12 to be in the β-configuration. Thus, the structure of **4** was determined as *epi*-12-palmatoside G.

Fibraurecdyside A (**5**) was obtained as colorless needles. The molecular formula was assigned as $C_{34}H_{56}O_{12}$ from the molecular ion peak at *m*/*z* 657.3853 in the HRFABMS. The IR spectrum displayed absorptions at 3380 and 1648 cm^{-1}, due to the presence of hydroxyl and α,β-unsaturated carbonyl groups. The NMR data recorded in **5** were similar to those of makisterone A (**12**),[6] except for the presence of ^1H NMR signals at δ 4.85 (1H, d, $J = 7.8$ Hz) and δ 3.86 to 4.48, in conjunction with ^{13}C NMR resonances at δ 104.2, 78.6, 78.5, 74.7, 71.6, and 62.6, corresponding to a glucose moiety in **5**. The coupling constant of the anomeric proton (7.8 Hz) suggested the presence of a sugar residue with a β-configuration. The glucose moiety in **5** was found to be linked to C-3, on the basis of the key HMBC correlation observed from H-1′ (δ 4.85) to C-3 (δ 77.7). Thus, the structure of fibraurecdyside A was deduced as **5**.

CYP3A4 activity was monitored by nifedipine oxidation with a expressed human form of this enzyme. All isolated compounds (**1**–**12**) were evaluated for their inhibitory effects against CYP3A4, and the results are shown in Table 4. Among them, the quaternary protoberberine alkaloids showed potent inhibitory activity against CYP3A4. In particular, palmatine (**9**) and jatrorrhizine (**10**) exhibited potent inhibition of CYP3A4 with IC_{50} values of 0.9 and 2.1 μM, respectively, while columbamine (**11**) possessed moderate inhibitory activity with an IC_{50} value of 30.6 μM. Stepharanine (**12**) was not evaluated above 2.5 μM due to its low solubility. Interestingly, compound **9**, possessing four methoxy groups, showed more potent inhibition than **10** and **11**, with three methoxy groups.

Table 3. NMR Spectroscopic Data of **5** in Pyridine-d_5

position	δ_H	δ_C
1	2.04 (dd, 12.9, 3.7) 1.71 (dd, 12.9, 12.8)	39.0
2	4.07 (dd, 12.8, 3.7)	67.5
3	4.26 (s)	77.7
4	2.16 (m) 1.68 (m)	30.6
5	2.90 (m)	51.4
6	6.18 (d, 3.9)	203.0
7	6.18 (s)	121.6
8		166.3
9	3.53 (m)	34.2
10		38.7
11	1.78 (m) 1.64 (m)	21.1
12	2.50 (ddd, 12.7, 12.6, 4.6) 2.13 (m)	32.0
13		48.1
14		84.2
15	1.93 (s)	31.9
16	2.41 (dd, 9.4, 9.2) 2.09 (m)	21.4
17	2.89 (m)	50.0
18	1.16 (s)	18.0
19	0.85 (s)	24.1
20		77.0
21	1.54 (s)	21.6
22	3.94 (d, 10.8)	74.7
23	1.55 (m)	34.6
24	2.25 (m)	41.9
25		72.1
26	1.28 (s)	26.5
27	1.31 (s)	28.2
28	1.05 (d, 6.8)	15.5
OH	6.29 (br s)	
1′	4.85 (d, 7.8)	104.2
2′	3.99 (dd, 8.3, 7.8)	74.7
3′	4.16 (dd, 9.1, 8.3)	78.6
4′	4.12 (dd, 9.1, 9.1)	71.6
5′	3.86 (dd, 9.1, 5.4)	78.5
6′	4.48 (dd, 11.6, 1.8) 4.24 (dd, 11.6, 5.4)	62.6

Table 4. Inhibition of Human CYP3A4-Catalyzed Nifedipine Oxidation Activity by Compounds **1**–**12**[a]

compound	IC_{50}, μM[b]
9	0.9 ± 0.3
10	2.1 ± 0.2
11	30.6 ± 6.6
12	>2.5[c]
ketoconazole	0.21 ± 0.03

[a] Compounds **1**–**8** were inactive ($IC_{50} > 100$ μM). [b] Estimates of variance (denoted by ±) are presented from the analysis of individual sets of data with duplicates. [c] Due to the low solubility of **12** in DMSO, the highest concentration studied was 2.5 μM. The DMSO concentration in the assay was 0.4%.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto MP-S3 micro melting point apparatus and were uncorrected. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer, and IR spectra were recorded on a Shimadzu FT-IR Prestige-21 spectrophotometer. ^1H and ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on Bruker AVANCE-300, -500, and AMX-400 spectrometers, using tetramethylsilane (TMS) as internal standard. Standard pulse sequences and parameters were used for the NMR experiments, and all chemical shifts are reported in parts per million (ppm, δ). FABMS were obtained on a JEOL JMS-700 spectrometer, and EIMS were obtained on a VG-70-250S spectrometer. Column chromatography was performed on silica gel (70–230 mesh, 230–400 mesh). Fractions were monitored by TLC (Merck precoated Si gel 60 F254 plates), using UV light. TLC was conducted on precoated Kieselgel 60 F 254 plates (Merck), and the spots were detected either by examining the plates under a UV lamp or by treating the plates with a 10% methanolic solution of *p*-anisaldehyde acid followed by heating at 110 °C.

Plant Material. The whole plant of *F. tinctoria* (Menispermaceae) was collected near Hanoi in Vietnam on July 12, 2004. The plant material was identified and authenticated by Assoc. Prof. Dr. Vu Xuan Phuong, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen (FT04011) has been deposited in the herbarium of the Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

Extraction and Isolation. The air-dried and powdered whole plant of *F. tinctoria* (10 kg) was extracted with MeOH (6 × 20 L) under reflux for 8 h. The filtrate was concentrated under reduced pressure to

obtain a dark crude extract (1.6 kg), which was suspended in H_2O, then partitioned with $CHCl_3$ and *n*-BuOH to afford $CHCl_3$- (340 g), *n*-BuOH- (840 g), and H_2O-soluble portions (335 g), respectively. The CH_3OH extract and the $CHCl_3$- and *n*-BuOH-soluble partitions of *F. tinctoria* were shown to inhibit CYP3A4 activity with IC_{50} values of 5.1, 13.7, and 3.4 μg/mL, respectively.

The *n*-BuOH-soluble residue (840 g) was chromatographed over reversed-phase Diaion HP-20 gel using H_2O-CH_3OH gradients and afforded six fractions. Fraction 1 formed a yellow precipitate with CH_3OH to yield **9** (140.3 g). Fraction 2 was subjected to silica gel column chromatography using $CHCl_3-CH_3OH$ (5:1 to 0:1) as step gradient mixtures as eluents to afford five fractions (2.1–2.5). Purification of fraction 2.1 by silica gel with $CHCl_3-CH_3OH$ (3:1) afforded five subfractions (2.1.1–2.1.5). Subfraction 2.1.5 was further separated by aluminum oxide with $CHCl_3-CH_3OH$ (4:1) as eluent to obtain **10** (120.5 mg) and **11** (2.1 mg). Fraction 2.3 was chromatographed on a RP-18 column eluted with H_2O and CH_3OH to give five subfractions (2.3.1–2.3.5). Subfraction 2.3.2 was purified using a silica gel column with $CHCl_3-CH_3OH$ (4:1) as solvent to give **12** (10.3 mg). Separation of subfraction 2.3.4 with EtOAc$-CH_3OH$ (8:1) as eluent yielded **5** (30.2 mg). Fraction 3 produced a white precipitate with CH_3OH to yield pure **8** (20.3 g). In turn, the filtrate of fraction 3 was separated on a silica gel column eluted with $CHCl_3$ and CH_3OH (9:1 to 0:1), as step gradient mixtures, to afford eight fractions (3.1–3.8). Fraction 3.7 was rechromatographed over a RP-18 column eluted with a mixture of H_2O and CH_3OH to yield **6** (223 mg) and **7** (120 mg). Fraction 3.8 was further purified using a Sephadex LH-20 column, eluted with H_2O and CH_3OH as eluents, to yield **2** (8.2 mg). Fraction 4 was separated using a silica gel column, with diisopropyl ether$-CH_3OH$ (5:1) as eluent, to afford seven fractions (4.1–4.7). Purification of fraction 4.6 with a silica gel column, using EtOAc$-CH_3OH$ (15:1) as eluent, yielded **1** (13.2 mg).

The H_2O-soluble residue (335 g) was chromatographed over reversed-phase Diaion HP-20 gel, using H_2O-CH_3OH step gradient mixtures as eluents, and afforded five fractions. Fraction 3 was further chromatographed on a Sephadex LH-20 column, eluted with H_2O and CH_3OH, and then purified by preparative TLC with $CHCl_3-CH_3OH$ (4:1, $R_f = 0.4$) to yield **3** (3.6 mg). Fraction 4 was chromatographed on a RP-18 column, eluted with H_2O and CH_3OH, and then further purified by preparative TLC with $CHCl_3-CH_3OH$ (5:1, $R_f = 0.5$) to obtain **4** (3.1 mg).

Fibrauretin A (1): colorless powder; mp 237–238 °C; $[\alpha]^{25}_D$ +62.6 (*c* 0.08, CH_3OH); UV (MeOH) λ_{max} (log ε) 259 (2.41), 207 (3.64) nm; IR (KBr) ν_{max} 3472, 2976, 1760, 1723, 1642, 1506 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; FABMS *m/z* 391 $[M + H]^+$; HRFABMS *m/z* 391.1395 $[M + H]^+$ (calcd for $C_{20}H_{23}O_8$, 391.1393).

Acetylation of Fibrauretin A (1a). Compound **1** (1.0 mg) on acetylation with acetic anhydride (0.5 mL) and pyridine (1 mL) yielded a monoacetylated product, **1a** (0.7 mg): IR (KBr) ν_{max} 3466, 2920, 1776, 1738 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz), see Table 1; EIMS *m/z* 432; HREIMS *m/z* 432.1420 (calcd for $C_{22}H_{24}O_9$, 432.1423).

Fibrauretinoside A (2): colorless powder; mp 166–167 °C; $[\alpha]^{25}_D$ +120.8 (*c* 0.05, CH_3OH); UV (MeOH) λ_{max} (log ε) 282 (3.31), 204 (4.23) nm; IR (KBr) ν_{max} 3404, 2935, 1758, 1727, 1640, 1631 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 75 MHz), see Table 2; FABMS *m/z* 559 $[M + Na]^+$, 537 $[M + H]^+$; HRFABMS *m/z* 537.1970 $[M + H]^+$ (calcd for $C_{26}H_{33}O_{12}$, 537.1972).

***epi*-Fibrauretinoside A (3):** colorless powder; mp 152–153 °C; $[\alpha]^{25}_D$ +35.4 (*c* 0.20, CH_3OH); UV (MeOH) λ_{max} (log ε) 283 (2.22), 204 (3.93) nm; IR (KBr) ν_{max} 3406, 2931, 1757, 1727, 1592 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; FABMS *m/z* 559 $[M + Na]^+$, 537 $[M + H]^+$; HRFABMS *m/z* 537.1970 $[M + H]^+$ (calcd for $C_{26}H_{33}O_{12}$, 537.1972).

***epi*-12-Palmatoside G (4):** colorless powder; mp 132–133 °C; $[\alpha]^{25}_D$ +40.5 (*c* 0.20, CH_3OH); UV (MeOH) λ_{max} (log ε) 234 (3.70), 207 (4.01) nm; IR (KBr) ν_{max} 3383, 2927, 1729, 1666, 1502 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 75 MHz), see Table 2; FABMS *m/z* 491 $[M + H]^+$; HRFABMS *m/z* 491.1918 $[M + H]^+$ (calcd for $C_{25}H_{31}O_{10}$, 491.1917).

Fibraurecdyside A (5): colorless needles; mp >300 °C; $[\alpha]^{25}_D$ +128.8 (*c* 0.07, CH_3OH); UV (MeOH) λ_{max} (log ε) 243 (3.32) nm; IR (KBr) ν_{max} 3380, 2962, 2882, 1648, 1442 cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz), see Table 3; ^{13}C NMR (pyridine-d_5, 75 MHz), see Table 3; FABMS *m/z* 657 $[M + H]^+$; HRFABMS *m/z* 657.3853 $[M + H]^+$ (calcd for $C_{34}H_{57}O_{12}$, 657.3850).

Preparation of *Escherichia coli* Membrane Fractions Expressing Human CYP3A4. The plasmids of P450 constructs were kindly provided by Dr. F. Peter Guengerich (Vanderbilt University, Nashville, TN). These constructs were transformed to *E. coli* DH5 by electroporation (Gene Pulser II, BioRad, Hercules, CA). Bacterial membrane fractions of *E. coli* expressing bicistronic human CYP3A4 were prepared by sonication and differential centrifugation following the method of Parikh et al.[13] These bacterial membrane fractions were stored at −75 °C. Membrane P450 content was determined following the CO-difference spectroscopic method of Omura and Sato.[14] Nifedipine oxidation activity was determined using 100 pmol of P450 in a 1 mL incubation mixture. The reaction was performed in a 37 °C waterbath with shaking, and the formation of the pyridine metabolite was determined by HPLC.[15] A known CYP3A4 inhibitor, ketoconazole, was used for comparison of the inhibitory effect.

Data Analysis. The concentration of a chemical required for 50% inhibition (IC_{50}) of nifedipine oxidation activity was calculated by curve fitting (Grafit, Erithacus Software Ltd., Staines, UK).[16]

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