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Constituents of the Leaves and Stem Bark of *Aglaia foveolata*

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Abstract

The previously known potent cytotoxic agent silvestrol (**1**) (0.002% w/w yield) and five new flavagline derivatives (**2–6**) were isolated from the leaves of *Aglaia foveolata* collected in Indonesia. The new compound **5** has an unprecedented cyclic amide moiety in its cyclopenta[*b*]benzopyran skeleton, while compound **6** is a novel benzo[*b*]oxepine derivative in which the oxepine ring is cleaved. Pyramidatine (**7**), a biogenetic precursor of the new flavaglines **2–6**, was isolated from the leaf extract investigated. Silvestrol was also isolated from the stem bark of *A. foveolata* (yield of 0.02% w/w) along with a new baccharane-type triterpenoid (**8**). The structures of the new compounds were elucidated on the basis of their NMR and mass spectrometric data. All new compounds isolated were tested against a panel of cancer cell lines, but only compound **2** was cytotoxic (IC₅₀ range = 1.4–1.8 μM), and is the first member of the cyclopenta[*b*]benzopyran class found to exhibit this type of activity. Compound **2** also showed significant NF-κB inhibitory activity in an Elisa assay (IC₅₀ = 0.37 μM).

Keywords

Aglaia foveolata; Meliaceae; Flavaglines; Cyclopenta[*b*]benzofuran; Cyclopenta[*b*]benzopyrans; Benzo[*b*]oxepine; Bisamide; Cytotoxicity; NF-κB inhibitory activity

INTRODUCTION

The genus *Aglaia* (Meliaceae) is distributed mainly in the tropical rain forests of Southeast Asia,¹ and is characterized by the presence of unique secondary metabolites called “flavaglines”.² Flavaglines are a group of compounds representative of the cyclopenta[*b*]

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benzofuran-, cyclopenta[*b*]benzopyran-, and benzo[*b*]oxepine-type skeletons, all of which are biogenetically derived from flavonoid and cinnamic amide moieties.²⁻⁴ To date, about 60 naturally occurring cyclopenta[*b*]benzofuran-type compounds, many of which exhibit insecticidal and antiproliferative activities, have been isolated from over 30 *Aglaia* species. The structural variation and biological properties of the flavaglines have been reviewed recently.^{5,6}

There have been only two previous studies on *Aglaia foveolata* Pannell, which was studied initially by a collaborative French and Malaysian research group, and afforded two new dammarane-type triterpenes, foveolins A and B.⁷ In a later study, silvestrol and its isomer, episilvestrol, were identified as constituents of *A. foveolata* during a screening program for new antitumor agents from plants,⁸ as part of a multidisciplinary, collaborative natural products drug discovery project.⁹ Silvestrol is a highly potent cytotoxic agent for cancer cells, and possesses an unusual 1,4-dioxanyloxy moiety at position C-6 of the cyclopenta[*b*]benzofuran skeleton.⁸ Silvestrol was active in an *in vivo* murine hollow fiber test and also in the *in vivo* P388 murine leukemia model with a T/C value of 150% at 2.5 mg/kg/injection.⁸ Studies on its cellular mechanism of action in LNCaP (human hormone-dependent prostate cancer) cells showed that it induced apoptosis in a manner independent of p53 activity.^{10,11} Silvestrol has also shown selective and potent Bcl-2- and p53- independent antitumor activity against chronic lymphocytic leukemia cells obtained from patients, relative to normal peripheral blood mononuclear cells.¹² Although the total synthesis of silvestrol has not been reported, the 1,4-dioxanyloxy moiety has been synthesized recently.¹³ Silvestrol has also been isolated from the bark of *A. leptantha* Miq. collected in Malaysia, and was found to inhibit the growth of PC3 (human prostate tumor) cells in a murine *in vivo* xenograft study.¹⁴

In our previous study, silvestrol was obtained from fruits and twigs of *A. foveolata* with yields of 0.01% and 0.0085% w/w, respectively. In the present investigation, the leaves and stem bark of recollected samples of *A. foveolata* were examined for the presence of silvestrol (**1**) and potentially new analogs of this compound.

RESULTS AND DISCUSSION

Following bioassay-guided fractionation, silvestrol (**1**) was isolated from the CHCl₃-soluble partitions of the MeOH extracts of the leaves and stem bark of *A. foveolata*, in yields of 0.002% and 0.02% w/w, respectively. From the leaf extract, five new flavaglines (**2-6**) and a known bisamide, pyrimidatine (**7**),¹⁵ were obtained. In addition, a new baccharene-type triterpene (**8**), and a known triterpene, 17,24-epoxy-25-hydroxy-3-oxobaccharan-21-oic acid,⁸ were also isolated from the stem bark. The structures of all compounds were elucidated on the basis of their NMR and MS data, and, in the case of the known compounds, by comparison with literature spectroscopic data.

The new compound, **2**, [α]_D²⁰ -30.0 (*c* 0.16, CHCl₃), was obtained as a white amorphous powder. In the HRESIMS, a sodiated molecular ion peak at *m/z* 675.2682 was consistent with a molecular formula of C₃₈H₄₀N₂O₈ (calcd for C₃₈H₄₀N₂O₈Na, 675.2682). The presence of twelve quaternary carbons, including two amide carbons (δ_C 167.5 and 170.0), nineteen methines, four methylenes, and three methyl carbons were established from the ¹³C and DEPT NMR spectra (Table 1). In the ¹H NMR spectrum, characteristic benzene-ring signals (two monosubstituted, one *p*-disubstituted, and one 1,2,3,5-tetrasubstituted) and resonances for three methoxy groups were observed (Table 2). Furthermore, three methines at δ_H 3.22 (d, *J* = 9.0 Hz, H-3), 4.00 (d, *J* = 9.0 Hz, H-4), and 4.91 (s, H-10) were typical of a cyclopenta[*b*]benzopyran skeleton.^{5,16} The remaining methylenes and two amide protons at δ_H 5.49 (br t, *J* = 5.5 Hz, NH-12) and 6.46 (br t, *J* = 5.5 Hz, NH-17) showed correlations in the DQF-COSY spectrum, suggesting the presence of a 1,4-butanediamide chain. In the HMBC spectrum, a

cross peak was observed between signals at δ_C 167.5 (C-18) and δ_H 7.82 (2H, d, $J = 8.0$ Hz, H-20,24), and indicated that one of the monosubstituted benzene rings is connected to this butanediamide chain. Further HMBC correlations from H-3 to C-2, C-10, C-11, and C-1', and from H-4 to C-5, C-5a, C-11, C-1', and C-2',6', established the connectivity of the 1,4-butanediamide moiety and the second monosubstituted benzene ring to C-3 and C-4, respectively. The relative configuration of compound **2** was determined from the analysis of 3J coupling constants and 2D NOESY correlations. The vicinal coupling constant value between H-3 and H-4 was 9.0 Hz, which was compatible with a H-3 α and H-4 β configuration.¹⁷ This configuration was further supported by the upfield 1H NMR signal of OMe-6 (δ_H 3.11), which was placed within the shielding zone of the phenyl ring at C-4 α . In the NOESY spectrum, cross peaks between H-10/H-2',6', H-4/H-10, H-4/H-2',6', H-3/H-2',6', and H-3/NH-12 established an endo relationship between H-4 and H-10, and further confirm the H-3 α , H-4 β relative configuration. The new compound **2** has been named foveoglin A, and was assigned structurally as shown. To the best of our knowledge, there is only one previous report of the isolation of two other cyclopenta[*b*]benzopyran-type compounds possessing a benzoyl-1,4-butanediamide moiety, which were obtained from the leaves of *A. andamanica* Hiern collected in Thailand.¹⁸ The structures of these known compounds, pyramidalglains A and B, are similar to foveoglin A (**2**), except for the absence of a 10-acetoxy group in the latter compound and differences in the substituents affixed to positions 3 and 4.

The HRESIMS of compound **3** [white amorphous powder, $[a]^{22}_D +170$ (c 0.20, $CHCl_3$)] was used to establish a molecular formula of $C_{38}H_{40}N_2O_8$, the same as that obtained for compound **2**. The 1H and ^{13}C NMR spectra of compounds **2** and **3** were comparable, suggesting that compound **3** also possesses a cyclopenta[*b*]benzopyran skeleton with a benzoyl-1,4-butanediamide moiety. In the HMBC spectrum, the same correlations were also observed for both compounds, indicating in **3** the same connectivity of the diamide moiety and the unsubstituted phenyl ring, as determined for compound **2**. NOESY correlations and the $^3J_{(H-3,H-4)}$ coupling constant (8.7 Hz) of **3** revealed that these two compounds also have the same relative configuration at positions C-3 and C-4. However, the lack of any NOE interaction between H-4 and H-10 in compound **3** (foveoglin B) suggested that this compound is the C-10 epimer of compound **2**. Due to the availability of foveoglin B, its monoacetate (**3a**) was also prepared in order to investigate the effect of a different substituent at position C-10 on its cytotoxicity. The structure of **3a** was confirmed from its NMR and MS data. In the 1H NMR spectrum of **3a**, proton H-10 was shifted downfield to δ_H 5.96 ppm due to the adjacent OAc group in position C-10.

Compound **4**, obtained as amorphous white powder with an $[a]^{22}_D$ value of +10 (c 0.30, $CHCl_3$), also gave the same molecular formula as compounds **2** and **3**, as determined by HRESIMS. Both the 1H and ^{13}C NMR spectra of compound **4** were similar to those of **3**, suggesting that they have the same gross structures. However, HMBC correlations from both δ_H 4.67 (H-3) and δ_H 3.44 (H-4) to δ_C 137.4 (C-1') and 173.8 (C-11); from H-3 to δ_C 89.9 (C-2), 129.8 (C-1'), and 129.2 (C-2',6'); and from H-4 to δ_C 79.6 (C-5) and 110.4 (C-5a), clearly indicated that the substituents at C-3 and C-4 were mutually exchanged in these compounds. In the 1H NMR spectrum of **4**, the OMe-6 resonance was observed at δ_H 3.86, which confirmed that this methoxy group was no longer in the shielding region of the aromatic ring. The configurations at positions C-3 and C-4 were determined as H-3 β and H-4 α , respectively, based on the vicinal coupling constant ($J = 5.8$ Hz),¹⁷ and was confirmed by NOESY correlations between H-3/H-2',6', H-3/H-2',6', H-4/H-2',6', and H-4/NH-12. A NOE cross peak was also observed between OH-10 (δ_H 6.03, d, $J = 9.6$ Hz) and H-3 (δ_H 4.67, d, $J = 5.8$ Hz), which confirmed the endo relationship between this hydroxyl group and H-3 proton. Therefore, the new compound **4** (isofoveoglin) was proposed as shown. As for compound **3**, a monoacetate derivative (**4a**) was prepared, and its structure was confirmed by NMR and mass spectrometry data. The presence of an acetyl at position C-10 caused the downfield shift of

proton H-10 to δ_{H} 6.10 ppm. In addition, H-4 was also shifted to δ_{H} 5.20 ppm, which confirmed the endo relationship between this proton with the acetyl group at position C-10 in **4a**.

The fourth new compound isolated from *A. foveolata* leaves, **5**, $[\alpha]_{\text{D}}^{20}$ -51.7 (*c* 0.41, CHCl_3), displayed a sodiated molecular ion peak in the HRESIMS at m/z 673.2528, corresponding to a molecular formula of $\text{C}_{38}\text{H}_{38}\text{N}_2\text{O}_8$ (calcd for $\text{C}_{38}\text{H}_{38}\text{N}_2\text{O}_8\text{Na}$, 673.2526). Characteristic signals in the ^1H and ^{13}C NMR spectra confirmed the presence of a cyclopenta[*b*]benzopyran skeleton and a benzoyl-1,4-butanedi- amide moiety. The ^1H NMR spectrum of **5** was similar to that of compound **4**, except for the absence of signals belonging to H-10 and one amide proton. The molecular formula also confirmed that compound **5** has two less hydrogens than compound **4**. As evident from the ^{13}C and DEPT spectra, the signal for a hydroxymethine carbon at position C-10 was replaced by a quaternary carbon at δ_{C} 90.6. In the HMBC spectrum, cross peaks between this quaternary carbon with H-4 and H-13 were observed, indicating that N-12 formed a bond with C-10 to create a five-membered cyclic amide, an unprecedented moiety among the *Aglaia* cyclopenta[*b*]benzopyran derivatives^{5,6} (Figure 1). Key HMBC correlations from H-3 to C-1', C-1?, and C-2?,6? confirmed the position of the unsubstituted phenyl group at C-3. Both protons H-3 (δ_{H} 4.04) and H-4 (δ_{H} 3.77) appeared as singlets in the ^1H NMR spectrum, which would only be possible if their dihedral angle was close to 90°. Insight II molecular modeling was used to generate the 3D structure of compound **5**, and the optimized model showed a H-3 and H-4 dihedral angle of 87.5° (Figure 2). Furthermore, NOESY correlations between H-3/H-4, H-3/H-2',6', H-3/H-2?,6?, H-4/H-2?,6?, H-13/H-2',6', and H-2',6'/H-2?,6? are also compatible with this model. Thus, the new compound **5** (cyclofoveoglin) was assigned as indicated.

Compound **6**, $[\alpha]_{\text{D}}^{20}$ -16.1 (*c* 0.18, CHCl_3), was obtained as a white amorphous solid with a molecular formula of $\text{C}_{37}\text{H}_{38}\text{N}_2\text{O}_8$, as determined from the sodiated molecular ion peak at m/z 661.2529 observed in the HRESIMS (calcd for $\text{C}_{37}\text{H}_{38}\text{N}_2\text{O}_8\text{Na}$, 661.2526). The presence of three aromatic rings and a benzoyl-1,4-butanedi- amide moiety, as in the previous four compounds (**2** - **5**), was evident from the characteristic signals in the ^1H and ^{13}C NMR spectra of **6**. However, relative to these earlier described compounds, the proton signals of H-3 and H-4 were shifted downfield in **6** by more than 1 ppm, and appeared at δ_{H} 5.63 and 5.30, respectively. In addition, the ^{13}C NMR spectrum also showed the presence of two conjugated keto groups at δ_{C} 198.5 and 196.9. The carbon resonances of **6** were similar to those of known benzo[*b*]oxepine-type compounds,^{5,16,19} except for the presence of an extra conjugated keto signal, and the absence of three resonances, one belonging to a quaternary carbon at position C-2 and two representative of the COOMe moiety at position C-10. In the HMBC spectrum, correlations were observed from the signals at δ_{H} 5.63 (d, J = 10.9 Hz, H-3) to C-2, C-1', C-1?, and C-2?,6?; from δ_{H} 5.30 (d, J = 10.9 Hz, H-4) to C-5 and C-11; and from δ_{H} 7.97 (d, J = 8.9 Hz, H-2',6') to C-2, establishing the structure proposed for **6** (Figure 3). The relative configurations at positions C-3 and C-4 were determined from the H-3 and H-4 vicinal coupling constant, 2D NOESY data, and molecular modeling. The two possible relative configurations at stereocenters C-3 and C-4, 3*R**,4*R** and 3*R**,4*S**, were modeled using Insight II. The 3*R**,4*S** model (Figure 4) showed all the observed NOESY correlations and was consistent with the observed $^3J_{(\text{H-3,H-4})}$ coupling constant (10.9 Hz). This result is also in agreement with previous studies on other *Aglaia* benzo[*b*]oxepine derivatives, in which only the 3*R**,4*S** and 3*S**,4*R** isomers have been isolated so far.^{4,16,19,20} Compound **6**, which was named secofoveoglin, is a novel benzo[*b*]oxepine derivative of a type unprecedented in nature, in which the oxepine ring is cleaved.

A known bisamide, pyramidatine (**7**), was isolated as the major constituent from the leaf extract. Pyramidatine has been isolated previously from *Aglaia andamanica*,¹⁸ and *Aglaia pyramidata* Hance [syn. *A. silvestris* (M. Roemer) Merrill].¹⁵ The isolation of pyramidatine from *A. foveolata* leaves further supports the proposed biogenetic origin of the flavaglines.³

⁴ The benzoyl-1,4-butanedi-amine part of pyridatone constitutes the bisamide side chain of flavaglines isolated in the present study, while the second aromatic ring can be found as the unsubstituted aryl ring of the flavaglines.

Compound **8** was obtained as white amorphous powder, $[\alpha]_D^{20} +33.2$ (c 0.46, CHCl_3), and showed a sodiated molecular ion peak at m/z 559.3606 $[\text{M} + \text{Na}]^+$ in the HRESIMS, supporting a molecular formula of $\text{C}_{31}\text{H}_{52}\text{O}_7$ (calcd for $\text{C}_{31}\text{H}_{52}\text{O}_7\text{Na}$, 559.3611). The ^1H NMR spectrum of **8** exhibited signals from seven tertiary methyl groups at δ_{H} 0.93 (s), 0.99 (s), 1.00 (s), 1.17 (s), 1.23 (s), 1.24 (s), and 1.29 (s), two characteristic oxymethine groups at δ_{H} 3.54 (t, $J = 5.7$ Hz, H-24) and 3.66 (d, $J = 11.0$ Hz, H-17), and one methoxy group at δ_{H} 3.72 (s). The ^{13}C and DEPT NMR spectra exhibited the presence of 31 signals (eight CH_3 , ten CH_2 , five CH , and eight C), including two carbonyls (δ_{C} 175.6, C-21 and 178.9, C-3), two oxygenated quaternary carbons (δ_{C} 75.0, C-25 and 76.4, C-4), and two oxygenated methines (δ_{C} 75.7, C-17 and 77.8, C-24). The characteristic signals in the ^1H and ^{13}C NMR spectra of **1** were comparable to those of 17,24-epoxy-25-hydroxy-3-oxobaccharan-21-oic acid, a baccharane-type triterpene previously isolated in our earlier work on *A. foveolata*.⁸ The main differences observed in the ^{13}C NMR spectrum of these two compounds were the absence of a saturated ketone resonance at position C-3, which was replaced by a carbonyl signal in **8**, and the presence of an oxygenated quaternary carbon instead of a non-oxygen-bearing carbon at position 4. In addition, an extra methoxy signal at δ_{H} 3.72 was observed for compound **8**, which showed a HMBC correlation to C-21 (δ_{C} 175.6), and in turn exhibited HMBC cross peaks with H-16, H-17, and H-22. In the HMBC spectrum of **8**, correlations were observed between the carbonyl at δ_{C} 178.9 (C-3) and H-2 (δ_{H} 2.21, m, and 2.49, m) and between the oxygenated quaternary carbon at δ_{C} 76.4 (C-4) and δ_{H} 1.23 (s, H₃-29), 1.29 (s, H₃-28), and 1.37 (m, H-5), confirming that the A ring of this new compound has been cleaved between positions C-3 and C-4. The relative stereochemistry of compound **8** was determined using a 2D NOESY experiment. The observed NOE correlations between H-17/ CH_3 -26, H-17/ CH_3 -27, H-17/ CH_3 -30, and CH_3 -30/H-9 established the α - and β -orientations of H-17 and H-24, respectively. In addition, significant NOE enhancements were also observed between CH_3 -19/ CH_3 -29 and CH_3 -18/H-13, and were consistent with the relative stereochemistry of other known baccharane-type triterpenes isolated previously.⁸ The new compound **8** was elucidated as 17,24-epoxy-25-hydroxy-21-methoxy-3,4-seco-baccharane.

All of the compounds isolated were tested in a small panel of cancer cell lines, and only silvestrol (**1**) and foveoglin A (**2**) were cytotoxic for the cell lines tested (Table 3). Isofoveogline (**4**) and cyclofoveoglin (**5**) showed weak cytotoxicity, while the remaining compounds were inactive. Interestingly, foveoglin B (**3**), the C-10 epimer of foveoglin A (**2**), did not exhibit any cytotoxic activity, which suggests that an endo orientation between H-10 and H-4 is necessary for cytotoxicity. Structural modification at position C-10, from a hydroxyl to an acetoxyl, caused the loss of activity in compound **4**. The cyclization of the amide groups as in compound **5** did not affect the cytotoxicity of these compounds. Previously, only a few of cyclopenta[*b*]benzopyran-type flavaglines have been tested for cancer cell cytotoxicity, and none were shown to exhibit cytotoxic activity.^{16,20-23} It was thought previously that the cyclopenta[*b*]benzopyran-type compounds are inactive, with only the cyclopenta[*b*]benzofuran representatives of the flavaglines exerting this type of biological activity. However, this present investigation has shown that some cyclopenta[*b*]benzopyran derivatives may be cytotoxic, depending on the type of the amide moiety, the positions of substituents at C-3 and C-4, and the orientation of the OH-10. Pyridatone (**7**), the precursor of compounds **2** - **6**, was not cytotoxic in the cell lines tested. Pyridatone has been tested previously in a different panel of cancer cell lines, and was found to be inactive.¹⁵

All new compounds were also tested for NF- κ B inhibitory activity in an enzyme-based Elisa assay. It was found that only foveoglin A (**2**) was active, with an IC_{50} value of 0.37 μM . All

other compounds exhibited $IC_{50} > 5 \mu M$, and were considered inactive. Previously, only a limited number of cyclopenta[*b*]benzopyran-type has been tested for the NF- κ B inhibitory activity,^{23,24} and only one showed potent NF- κ B inhibitory activity.²³

The isolation of silvestrol (**1**) in this investigation is significant, since the leaves of *A. foveolata* potentially could be used as a renewable resource for the future supply of this promising antileukemic agent, in the event of its further development.

EXPERIMENTAL

General

Optical rotations were measured using a Perkin-Elmer 241 automatic polarimeter (Waltham, MA). UV spectra were obtained with a Spectramax plus 384 spectrometer (Molecular Devices Corporation, Sunnyvale, CA). IR spectra were run on a Nicolet Protégé 460 FTIR spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA). NMR spectroscopic data

were recorded in CD₃OD at room temperature using a Bruker DRX-400 spectrometer. Electrospray ionization (ESI) mass spectrometric analyses were performed with a 3-tesla Finnigan FTMS-2000 Fourier transform mass spectrometer. HPLC were run on a SunFire Semiprep (5 μ m, 150 $\times$ 10 mm i.d.) or SunFire Preparative (5 μ m, 150 $\times$ 19 mm i.d.) C₁₈ OBD column (Waters, Milford, MA). All solvents used from chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ). Molecular modeling was performed using the InsightII program (Accelrys Software, Inc., Burlington, MA) with Discover® minimization energy.

Plant material

The leaves and stem bark of *Aglaia foveolata* Pannell were collected at Timpah village, Kapuas Regency, Central Kalimantan, Indonesia, in July 2006. The plant was identified by S. R. and I. R. Voucher specimens (collection number SR-IS.2) have been deposited at the Herbarium Bogoriense, Bogor, Indonesia. *A. foveolata* is a small tree *ca.* 9 m height and *ca.* 14 cm diameter breast height, with free branches at *ca.* 7 m height.

Extraction and Isolation of the Leaves

The dried and milled leaves (1.5 kg) of *A. foveolata* were extracted with MeOH (3 $\times$ 2.0 L) at room temperature for two days each. The combined MeOH extracts were concentrated *in vacuo* (300 mL) and water was added (300 mL) to give an aqueous MeOH solution. This aqueous extract was partitioned in turn with hexane (3 $\times$ 400 mL) and CHCl₃ (3 $\times$ 400 mL), to afford dried hexane- (greenish gum, 40 g) and CHCl₃-soluble (dark greenish gum, 70 g) residues. The CHCl₃ soluble extract was subjected to a silica gel column chromatography (11 $\times$ 31 cm, 70-230 mesh) eluted with CHCl₂-MeOH (100:1? 1:1) to give eight fractions (F1 - F8). Fraction F3 (MCF-7 cell line, ED₅₀ = 2.2 μ g/mL, 40 g) was further subjected to another silica gel column (7 $\times$ 33 cm, 230-400 mesh) eluted with hexane-EtOAc-MeOH (1:1:0? 0:1:1) to furnish nine sub-fractions (F301-309). Pyramidine¹⁵ (7) precipitated from sub-fraction F306 (hexane-EtOAc-MeOH 10:10:1.5 $\rightarrow$ 10:10:2) as a white amorphous powder (1.0 g), and was filtered off. The filtrate of F306 (MCF-7 cells, ED₅₀ <0.16 μ g/mL, 2.1 g) was chromatographed on Diaion HP-20 gel (eluted with 90% MeOH) to remove the chlorophylls, followed by reversed-phase HPLC using semipreparative C₁₈ column (CH₃CN-H₂O, 45? 52% in 30 min, 3 mL/min), to afford foveoglin B (3) (6 mg, *t*_R 19.1 min), cyclofoveoglin (5) (4 mg, *t*_R 24.5 min), isofoveoglin (2) (4 mg, *t*_R 26.2 min), and secofoveoglin (6) (2 mg, *t*_R 28.0 min). Sub-fraction F308 (ED₅₀ < 0.16 μ g/mL, 2.3 g) was chromatographed on Diaion HP-20 gel (eluted with 90% MeOH), followed by Sephadex LH-20 gel (2.5 $\times$ 75 cm, in 100% MeOH), and was finally separated by RP-HPLC (CH₃CN-H₂O, 45? 50% in 20 min, then 50? 60% in 10 min, 3 mL/min) to furnish silvestrol⁸ (1, 11 mg, *t*_R 11.8 min), foveoglin A (3) (3.0 mg, *t*_R 15.9 min), and additional quantity of foveoglin B (2) (6.5 mg, *t*_R 29.4 min).

Extraction and Isolation of the Stem Bark

The dried and milled stem bark (1.5 kg) of *A. foveolata* was extracted with MeOH (4 $\times$ 3.0 L) at room temperature for up to three days each. The combined extracts were concentrated *in vacuo*, and water was added to make a 90% aqueous MeOH solution (400 mL). The aqueous solution was partitioned in turn with hexane (3 $\times$ 400 mL) and CHCl₃ (3 $\times$ 400 mL) to give a light greenish gum (40 g) and a dark greenish gum (70 g), respectively. The CHCl₃-soluble extract was partitioned on a silica gel column (7.4 $\times$ 32 cm, 70-230 mesh) eluted with CHCl₂-MeOH (100:1? 1:1) to give ten fractions (F01 - F010). Fraction F04 (MCF-7 cells, ED₅₀ = 0.8 μ g/mL, 20 g) was chromatographed on silica gel (5 $\times$ 36 cm, 230-400 mesh) eluted with hexane-EtOAc-MeOH (1:1:0? 0:1:1) to furnish nine subfractions (F0401 - F0409). An amorphous white powder precipitated from subfraction F0403 (hexane-EtOAc-MeOH 1:1:0.02), which was identified as 17,24-epoxy-25-hydroxy-3-oxobaccharan-21-oic acid⁸ (3.0 g). Subfraction

F0409 (1.0 g) were purified on a Sephadex LH-20 column (2.5×68 cm) eluted with MeOH to give the new compound **8** (80 mg) and impure silvestrol (300 mg). Compound **8** was further purified on a reversed-phase HPLC using preparative C₁₈ column (CH₃CN-H₂O 1:1, 7 mL/min, UV detector at 210 nm, *t_R* 14.7 min). Fraction F05 (MCF-7 cells, ED₅₀ = <0.16 µg/mL, 4.35 g) was purified in the same manner as fraction F04 to give an additional quantity of impure silvestrol (150 mg). Silvestrol obtained from both fractions was combined and was finally purified on a C₁₈ reverse-phase column (2.5×17 cm) to afford pure silvestrol (**1**) (300 mg).

Foveoglin A (2)

white amorphous powder; $[\alpha]_D^{20}$ -30.0 (*c* 0.16, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 203 (4.64), 213 (4.57) (br shoulder) nm; IR (film) $\nu_{\max}$ 3330, 2946, 1641, 1618, 1516, 1494, 1456, 1305, 1254, 1216, 1147, 1099, 931 cm⁻¹; ¹H NMR data, see Table 2; ¹³C NMR data, see Table 1; HRESIMS *m/z* 675.2682 [M + Na]⁺ (calcd for C₃₈H₄₀N₂O₈Na, 675.2682).

Foveoglin B (3)

white amorphous powder; $[\alpha]_D^{20}$ +170 (*c* 0.20, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 203 (4.76), 214 (4.71) (br shoulder) nm; IR (film) $\nu_{\max}$ 3316, 2936, 1637, 1620, 1587, 1520, 1456, 1305, 1254, 1214, 1149 cm⁻¹; ¹H NMR data, see Table 2; ¹³C NMR data, see Table 1; HRESIMS *m/z* 675.2673 [M + Na]⁺ (calcd for C₃₈H₄₀N₂O₈Na, 675.2682).

Isofoveoglin (4)

white amorphous powder; $[\alpha]_D^{20}$ +10 (*c* 0.30, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 203 (4.72), 214 (4.63) (br shoulder) nm; IR (film) $\nu_{\max}$ 3263, 1618, 1596, 1518, 1439, 1305, 1254, 1151, 1099, 931 cm⁻¹; ¹H NMR data, see Table 2; ¹³C NMR data, see Table 1; HRESIMS *m/z* 675.2683 [M + Na]⁺ (calcd for C₃₈H₄₀N₂O₈Na, 675.2682).

Cyclofoveoglin (5)

light yellow amorphous powder; $[\alpha]_D^{20}$ -51.7 (*c* 0.41, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 203 (4.74), 214 (4.64) (br shoulder) nm; IR (film) $\nu_{\max}$ 3332, 2936, 1694, 1622, 1515, 1456, 1257, 1150, 1101, 866 cm⁻¹; ¹H NMR data, see Table 2; ¹³C NMR data, see Table 1; HRESIMS *m/z* 673.2528 [M + Na]⁺ (calcd for C₃₈H₃₈N₂O₈Na, 673.2526).

Secofoveogline (6)

white amorphous powder; $[\alpha]_D^{20}$ -16.1 (*c* 0.18, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 203 (4.80), 215 (4.65) (br shoulder), 294 (4.55) nm; IR (film) $\nu_{\max}$ 3332, 2936, 2854, 1644, 1621, 1600, 1512, 1491, 1455, 1418, 1292, 1219, 1162 cm⁻¹; ¹H NMR data, see Table 2; ¹³C NMR data, see Table 1; HRESIMS *m/z* 661.2529 [M + Na]⁺ (calcd for C₃₇H₃₈N₂O₈Na, 661.2526).

Compound 8

white amorphous powder; $[\alpha]_D^{20}$ +33.2 (*c* 0.46, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 205 (3.50) nm; IR (film) $\nu_{\max}$ 3454, 2956, 1717, 1457, 1387, 919 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 3.72 (3H, s, COOCH₃), 3.66 (1H, d, *J* = 11.0 Hz, H-17), 3.54 (1H, d, *J* = 5.7 Hz, H-24), 2.49 (1H, m, H-2a), 2.43 (1H, m, H-1a), 2.40 (1H, m, H-13), 2.21 (1H, m, H-2b), 2.12 (1H, m, H-22a), 2.06 (1H, m, H-12a), 1.89 (1H, m, H-16a), 1.85 (1H, m, H-23a), 1.79 (1H, m, H-1b), 1.69 (2H, m, H-22b and H-23b), 1.56 (2H, m, H₂-6), 1.50 (1H, m, H-9), 1.48 (1H, m, H-15a), 1.44 (2H, m, H₂-11), 1.37 (2H, m, H-5 and H-16b), 1.29 (5H, m, H₂-7 and H₃-28), 1.23 (3H, s, H₃-29), 1.24 (3H, s, H₃-26), 1.17 (3H, s, H₃-27), 1.09 (1H, m, H-12b), 1.05 (1H, m, H-15b), 1.00 (3H, s, H₃-19), 0.99 (3H, s, H₃-18), 0.93 (3H, s, H₃-30); ¹³C NMR (CDCl₃, 100 MHz) δ 178.9 (s, C-3), 175.6 (s, C-21), 77.8 (d, C-24), 76.4 (s, C-4), 75.7 (d, C-17), 75.0 (s, C-25), 51.6 (q, COOCH₃), 51.4 (d, C-5), 47.3 (s, C-20), 42.6 (s, C-14), 42.3 (d, C-9), 41.3 (s, C-10),

40.5 (s, C-8), 38.1 (d, C-13), 34.3 (q, C-28), 34.1 (t, C-1), 32.9 (t, C-7), 32.4 (t, C-22), 32.3 (t, C-16), 29.1 (t, C-2), 28.0 (t, C-15), 27.3 (q, C-29), 27.1 (q, C-27), 26.9 (q, C-26), 24.1 (t, C-12), 22.4 (t, C-11), 20.7 (t, C-23), 20.6 (q, C-19), 20.3 (t, C-6), 15.7 (q, C-18), 15.1 (q, C-30); HRESIMS m/z 559.3606 $[M + Na]^+$ (calcd for $C_{31}H_{52}O_7Na$, 559.3611).

Preparation of foveoglin B-10-O-acetate (3a)

Foveoglin B (**3**) (2 mg) was acetylated with acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature for 24 h. The reaction product was purified by preparative TLC developed using hexane-EtOAc-MeOH (1:1:0.2) to give compound **3a** (1.5 mg) (75% yield).

Compound 3a

white amorphous powder; 1H NMR ($CDCl_3$, 400 MHz) δ 7.77 (2H, td, J = 7.0, 1.5 Hz, H-20 and H-24), 7.56 (2H, td, J = 8.9, 2.0 Hz, H-2' and H-6'), 7.52 (1H, tt, J = 7.2, 2.2 Hz, H-22), 7.45 (2H, tt, J = 7.0, 1.5 Hz, H-21 and H-23), 7.16 (3H, m, H-3'' - H-5''), 6.99 (2H, m, H-2'' and H-6''), 6.89 (2H, td, J = 8.9, 2.0 Hz, H-3' and H-5'), 6.25 (1H, br t, J = 5.3 Hz, NH-17), 6.15 (1H, d, J = 2.2 Hz, H-9), 5.96 (1H, s, H-10), 5.82 (1H, d, J = 2.2 Hz, H-7), 5.66 (1H, br t, J = 5.6 Hz, NH-12), 5.27 (1H, s, OH), 4.35 (1H, d, J = 10.2 Hz, H-4), 3.77 (3H, s, OCH₃-8), 3.76 (3H, s, OCH₃-4'), 3.75 (1H, m, H-3, partly overlapped with OCH₃-4'), 3.21 (2H, br q, J = 6.3 Hz, H₂-16), 3.08 (3H, s, OCH₃-6), 2.99 (1H, dd, J = 13.1, 6.3 Hz, H-13a), 2.91 (1H, dd, J = 13.1, 6.3, H-13b), 2.28 (3H, s, COCH₃), 1.13 (4H, m, H₂-14 and H₂-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4 (C-11), 169.3 (OCOCH₃), 167.4 (C-18), 161.1 (C-8), 159.6 (C-4'), 158.8 (C-6), 152.3 (C-9a), 136.1 (C-1''), 134.5 (C-19), 131.4 (C-22), 128.7 (C-1'), 128.6 (2C, C-2' and C-6'), 128.5 (2C, C-21 and C-23), 128.3 (2C, C-2'' and C-6''), 127.8 (2C, C-3' and C-5'), 127.2 (C-4''), 126.9 (2C, C-20 and C-24), 113.7 (2C, C-3' and C-5'), 106.2 (C-5a), 94.0 (C-9), 92.8 (C-7), 86.1 (C-2), 81.6 (C-5), 79.6 (C-10), 60.4 (C-3), 60.1 (C-4), 55.5 (OCH₃-4'), 55.2 (2C, OCH₃-6 and OCH₃-8), 39.5 (C-16), 39.2 (C-13), 26.5 (C-15), 26.3 (C-14), 21.4 (OCOCH₃); HRESIMS m/z 717.2789 $[M + Na]^+$ (calcd for $C_{40}H_{42}N_2O_9Na$, 717, 2788).

Preparation of isofoveoglin-10-O-acetate (4a)

Isofoveoglin (**4**) (2 mg) was acetylated following the above procedure to give compound **4a** (1.4 mg) (70% yield).

Compound 4a

white amorphous powder; 1H NMR ($CDCl_3$, 400 MHz) δ 7.82 (2H, td, J = 7.2, 1.4 Hz, H-20 and H-24), 7.61 (2H, td, J = 9.0, 2.0 Hz, H-2' and H-6'), 7.48 (1H, tt, J = 7.3, 1.9 Hz, H-22), 7.39 (2H, br t, J = 7.3 Hz, H-21 and H-23), 7.06 (3H, m, H-3'' - H-5''), 6.86 (4H, d, J = 8.5 Hz, H-3', H-5' and H-2'', H-6''), 6.67 (1H, br t, J = 5.5 Hz, NH), 6.17 (1H, d, J = 2.2 Hz, H-7), 6.10 (1H, s, H-10), 6.02 (1H, d, J = 2.2 Hz, H-9), 5.53 (1H, s, OH), 5.20 (1H, d, J = 5.3 Hz, H-3), 3.94 (3H, s, OCH₃-6), 3.81 (3H, s, OCH₃-4'), 3.74 (3H, s, OCH₃-8), 3.53 (2H, p, J = 5.5 Hz, H₂-16), 3.44 (1H, d, J = 5.3 Hz, H-4), 3.41 (2H, m, H₂-13, partly overlapped with H-3), 1.95 (3H, s, COCH₃), 1.70 (4H, m, H₂-14 and H₂-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.9 (C-11), 169.2 (OCOCH₃), 167.5 (C-18), 161.2 (C-8), 159.2 (C-4'), 156.5 (C-6), 153.7 (C-9a), 137.9 (C-1''), 134.6 (C-19), 131.3 (C-22), 128.9 (C-1'), 128.8 (2C, C-2'' and C-6''), 128.5 (2C, C-2' and C-6'), 128.3 (2C, C-21 and C-23), 127.8 (2C, C-3' and C-5'), 127.0 (2C, C-20 and C-24), 126.5 (C-4''), 113.3 (2C, C-3' and C-5'), 109.1 (C-5a), 93.9 (C-9), 92.8 (C-7), 87.1 (C-2), 81.8 (C-5), 79.2 (C-10), 59.4 (C-4), 56.2 (C-3), 55.5 (OCH₃-6), 55.2 (OCH₃-8), 53.3 (OCH₃-4'), 39.5 (C-16), 39.1 (C-13), 27.4 (C-15), 26.7 (C-14), 20.8 (OCOCH₃); HRESIMS m/z 717.2785 $[M + Na]^+$ (calcd for $C_{40}H_{42}N_2O_9Na$, 717, 2788).

Cytotoxicity evaluation

Compounds isolated from the leaves and stem bark of *A. foveolata* were tested against the Lu1 (human lung carcinoma), LNCaP (hormone-dependent human prostate carcinoma), and MCF-7 (human breast carcinoma), according to an established protocol.²⁵

NF-κB Elisa assay

The NF-κB inhibitory assay was carried out according to the published protocol.²³ Rocaglamide was used as a positive control, and exhibited IC₅₀ values of 2.0 μM in this assay.

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Figure 1.
Selected HMBC correlations for compound **5**.

Figure 2.
Preferred configuration and selected NOE interactions for compound **5**

Figure 3.
Selected HMBC correlations for compound **6**

Figure 4.
Preferred configuration and selected NOE interactions for compound **6**

Table 1

¹³C NMR spectroscopic data for compounds **2** - **6** acquired in CDCl₃^{a,b}

Position	2	3	4	5	6
2	85.6 s	86.8 s	89.9 s	86.3 s	196.9 s
3	59.0 d	61.6 d	56.0 d	47.8 d	53.8 d
4	57.1 d	61.6 d	56.0 d	56.3 d	63.6 d
5	81.8 s	83.3 s	79.6 s	83.7 s	198.5 s
5a	104.2 s	106.0 s	110.4 s	104.4 s	105.8 s
6	160.3 s	158.8 s	156.3 s	158.3 s	162.0 s
7	93.0 d	92.7 d	92.5 d	92.7 d	91.7 d
8	160.8 s	160.9 s	160.4 s	161.3 s	166.4 s
9	93.9 d	93.7 d	93.8 d	93.4 d	94.3 d
9a	152.8 s	152.8 s	153.8 s	154.0 s	168.1 s
10	73.5 d	78.7 d	82.6 d	90.6 s	-
11	170.0 s	173.4 s	173.8 s	174.0 s	168.1 s
13	39.0 t	39.0 t	39.3 t	38.6 t	38.9 t
14	26.2 t	26.0 t	26.2 t	25.6 t	26.3 t
15	26.3 t	26.5 t	26.9 t	27.0 t	26.9 t
16	39.4 t	39.5 t	39.7 t	39.7 t	39.3 t
18	167.5 s	167.6 s	167.8 s	167.7 s	167.4 s
19	134.5 s	134.5 s	134.4 s	134.7 s	134.5 s
20, 24	127.0 d	126.9 d	126.9 d	127.0 d	126.9 d
21, 23	128.6 d	128.6 d	128.6 d	128.4 d	128.6 d
22	131.5 d	131.5 d	131.5 d	131.2 d	131.4 d
1'	129.2 s	130.2 s	129.8 s	128.6 s	129.0 s
2', 6'	128.0 d	129.3 d	128.6 d	128.7 d	131.3 d
3', 5'	113.6 d	113.7 d	113.1 d	113.7 d	113.7 d
4'	159.3 s	159.5 s	158.9 s	159.9 s	163.4 s
1''	136.8 s	136.7 s	137.4 s	135.3 s	136.9 s
2'', 6''	128.6 d	128.5 d	129.2 d	127.1 d	128.9 d
3'', 5''	127.7 d	127.7 d	128.0 d	127.1 d	129.1 d
4''	127.0 d	127.0 d	126.8 d	125.8 d	127.6 d
OMe-6	55.5 q	55.7 q	56.2 q	56.1 q	56.0 q
OMe-8	55.4 q	55.3 q	55.2 q	55.3 q	55.6 q
OMe-4'	55.4 q	55.4 q	55.4 q	55.4 q	55.4 q

^a Spectra were acquired at 100 MHz, TMS was used as internal standard .

^b Multiplicity was obtained from DEPT spectra.

Table 2

¹H NMR spectroscopic data for compounds **2** - **6** acquired in CDCl₃^{a,b}

	2	3	4	5	6
3	3.22 d (9.0)	3.92 d (8.7)	4.67 d (5.8)	4.04 s	5.63 d (10.9)
4	4.00 d (9.0)	4.12 d (8.7)	3.44 d (5.8)	3.77 s	5.30 d (10.9)
7	5.86 d (2.2)	5.77 d (2.2)	6.12 d (2.1)	6.00 d (2.2)	5.95 d (2.4)
9	6.26 d (2.2)	6.04 d (2.2)	6.01 d (2.1)	5.47 d (2.2)	6.05 d (2.4)
10	4.91 s	4.89 s	4.28 d (9.6)	-	-
13	2.93 m (6.5)	2.97 dd (13.3, 6.1)	3.47 m	3.30 m	3.02 dd (12.1, 6.0)
14	2.61 m (6.5)	2.88 dd (13.3, 6.1)	1.26 m	2.32 td (14.3, 7.0)	1.31-1.21 m
	0.98 m	1.13 m		1.51 m	
15	1.56 p (7.7)	1.20 m	1.26 m	1.80 m	1.31-1.21 m
16	3.29 m (6.5)	3.22 dq (6.4, 2.2)	3.47 m	1.63 m	3.30 p (6.1)
	3.16 m (6.5)		3.31 m	3.30 m	
20,24	7.82 d (8.0)	7.78 d (7.0)	7.77 d (7.5)	7.77 d (7.3)	7.77 d (7.0)
21, 23	7.47 t (7.7)	7.45 t (7.3)	7.42 d (7.2)	7.38 d (7.5)	7.44 t (7.0)
22	7.53 t (7.2)	7.51 t (7.3)	7.49 t (7.5)	7.46 t (7.2)	7.51 t (7.2)
2', 6'	7.62 d (8.9)	7.74 d (8.7)	7.64 d (8.9)	7.62 d (8.7)	7.97 d (8.9)
3', 5'	6.89 d (8.7)	6.89 d (8.7)	6.86 d (9.0)	6.99 d (8.7)	6.84 d (8.9)
2'', 6''	6.93 m	6.98 m	6.89 m	6.70 m	7.36 d (7.3)
3'', 5''	7.16 m	7.15 m	7.10 m	6.93 m	7.24 d (7.5)
4''	7.16 m	7.15 m	7.10 m	6.93 m	7.16 t (7.3)
OMe-6	3.11 s	3.07 s	3.86 s	3.88 s	3.91 s
OMe-8	3.79 s	3.71 s	3.77 s	3.51 s	3.78 s
OMe-4'	3.77 s	3.76 s	3.79 s	3.87 s	3.81 s
NH-12	5.49 br t (5.5)	6.89 m	6.56 br t (5.5)	-	5.55 br t (5.8)
NH-17	6.46 br t (5.5)	6.37 br t (5.5)	6.61 br t (5.5)	6.61 br t (5.0)	6.34 br t (5.6)
OH-5	5.44 s		5.80 s	5.50 s	
OH-10			6.03 d (9.6)		

^aSpectra were acquired at 400 MHz, TMS was used as internal standard .

^bCoupling constants were measured in Hz.

Table 3Cytotoxic Activity of Compounds **1**, **2**, **4**, and **5**^a

Compound	Cell line ^b		
	Lu1	LNCaP	MCF-7
1 ^c	0.0012	0.0015	0.0015
2	1.8	1.4	1.8
4	17.5	21.0	16.1
5	18.1	16.0	13.5
Paclitaxel ^d	0.0023	0.0047	0.0007

^aCompounds **3**, **3a**, **4a**, **6** - **8** were considered to be inactive, since their ED₅₀ values were >20 μ M against the tested cell lines. Results are expressed as ED₅₀ values (μ M)

^bKey to cell lines used: Lu1 = human lung cancer; LNCaP = hormone-dependent human prostate cancer; MCF-7 = human breast cancer.

^cData obtained from ref. ⁸.

^dUsed as a positive control.