Cytotoxic Flavaglines and Bisamides from *Aglaia edulis*

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Two new cyclopenta[*b*]benzofurans, aglaroxin A 1-*O*-acetate (**2**) and 3′-methoxyaglaroxin A 1-*O*-acetate (**3**), a new benzo[*b*]oxepine, 19,20-dehydroedulisone A (**4**), and five new cyclopenta[*bc*]benzopyrans, edulirin A (**5**), edulirin A 10-*O*-acetate (**6**), 19,20-dehydroedulirin A (**7**), isoedulirin A (**8**), and edulirin B (**9**), were isolated from the bark of *Aglaia edulis*, along with one known cyclopenta[*b*]benzofuran, aglaroxin A (**1**). Additionally, four new amides, aglamides A−D (**10**−**13**), as well as three known compounds, aglalactone, scopoletin, and 5-hydroxy-3,6,7,4′-tetramethoxyflavone, were isolated from the leaves and/or twigs of this species. The structures of the new compounds (**2**−**13**) were elucidated by interpretation of their spectroscopic data. All isolates obtained in this study were evaluated for cytotoxicity against both several human cancer cell lines (Lu1, LNCaP, and MCF-7) and a nontumorigenic (HUVEC) cell line. Among these isolates, the cyclopenta[*b*]benzofurans (**1**−**3**) exhibited potent in vitro cytotoxic activity (ED_{50} range 0.001 to 0.8 μg/mL). Aglaroxin A 1-*O*-acetate (**2**) was further evaluated in the in vivo P388 lymphocytic leukemia model, by intraperitoneal injection, but found to be inactive in this model.

The cyclopenta[*b*]benzofurans have been isolated only from the genus *Aglaia* (Meliaceae) and occur with two structurally related groups of compounds, the benzo[*b*]oxepines and cyclopenta[*bc*]-benzopyrans.[1,2] These classes of compounds have been termed as "flavaglines" because their mutual biogenetic origin is postulated to involve a flavonoid unit linked to a cinnamic acid moiety.[3−6] Among the flavaglines, cyclopenta[*b*]benzofurans have received considerable recent attention as interesting lead compounds for cancer chemotherapy,[1,2,6−20] as a result of the cyclopenta[*b*]-benzofuran derivative, rocaglamide from *Aglaia elliptifolia*, being found to exhibit antineoplastic activity in an in vivo model.[20] As a part of a National Cooperative Drug Discovery Group (NCDDG) program to discover new antitumor agents from plants,[21,22] the leaves, twigs, and bark of *Aglaia edulis* (Roxb.) Wall. (Meliaceae) were separately collected in Indonesia. The chloroform-soluble partitions of the three methanol extracts of these three plant parts were subjected to detailed investigation due to their cytotoxic activities demonstrated against a small panel of human cancer cell lines. Bioassay-guided purification of the bark of *A. edulis* led to the isolation of two new cyclopenta[*b*]benzofurans (**2** and **3**), a new benzo[*b*]oxepine (**4**), and five new cyclopenta[*bc*]benzopyrans (**5**−**9**), along with one known compound, aglaroxin A (**1**). Additionally, four new amides (**10**−**13**), as well as three known compounds, were isolated from the leaves and/or twigs of this species. All isolates obtained in this study were evaluated for cytotoxicity against several human cancer cell lines, and the new cyclopenta[*b*]benzofuran **2** was further tested in an in vivo model. In a preliminary investigation, two new benzo[*b*]oxepines and their hydrolytic derivatives were characterized from the bark of *A. edulis*.[23]

Results and Discussion

The known cyclopenta[*b*]benzofuran aglaroxin A (**1**) was isolated from the bark of *A. edulis* and identified by comparison with literature spectroscopic data.[5,24] Three other known compounds, aglalactone,[3,25] scopoletin,[26] and 5-hydroxy-3,6,7,4′-tetramethoxy-flavone,[27] were obtained from the leaves and twigs.

Compound **2** was obtained as an amorphous powder, $[\alpha]^{22}_{D}$ −69 (*c* 0.1, EtOH). Its HRESIMS exhibited a sodiated molecular ion peak at *m*/*z* 584.1875, consistent with an elemental formula of $C_{31}H_{31}NO_9Na$ (calcd 584.1891). The ^{1}H NMR spectroscopic data of compound **2** exhibited a close similarity to those of aglaroxin A (**1**), suggesting that **2** is also a cyclopenta[*b*]benzofuran derivative.[5] Its ^{1}H NMR spectrum showed signals for two aromatic rings, constituted by a characteristic AA′BB′ system of a *para*-disubstituted benzene ring at δ_H 7.08 (2H, d, J = 8.8 Hz, H-2′, 6′) and 6.63 (2H, d, J = 8.8 Hz, H-3′, 5′) and a monosubstituted benzene ring at δ_H 7.06−7.00 (3H, m, H-3″, 4″, 5″) and 6.90 (2H, brd, J = 7.2 Hz, H-2″, 6″), as well as methylenedioxy signals at δ_H 5.87 and 5.86. The ^{1}H NMR spectrum of compound **2** further exhibited signals at δ_H 6.00 (1H, d, J = 5.3 Hz, H-1), 4.62 (1H, d, J = 13.6 Hz, H-3), and 4.19 (1H, dd, J = 13.6, 5.3 Hz, H-2), for H-1, H-3, and H-2, respectively. The downfield shift of H-1 from δ_H 4.84 in aglaroxin A (**1**) to δ_H 6.00 in compound **2**, as well as a singlet signal at δ_H 1.90 (3H, s) in the ^{1}H NMR spectrum, suggested the presence of an acetyl group at C-1. Consistent with the ^{1}H NMR spectrum of compound **2**, its ^{13}C NMR spectrum also displayed the signals for a disubstituted and a monosubstituted benzene ring, as well as for two quaternary carbons at δ_C 101.0 (C-3a) and 93.3 (C-8b). In the HMBC spectrum of compound **2**, correlations from H-1 and OAc to the signal at δ_C 169.8 indicated the acetoxy group to be located at C-1, while correlations from resonances for H-2 and the two N−Me groups to the signal at δ_C 167.5 suggested the location of the amide group at C-2. The relative configuration of compound **2** was established by analysis of the splitting patterns and coupling constants of the ^{1}H NMR signals. The vicinal coupling constant values of the methine protons at the C-1, 2, and 3 positions ($J_{1,2}$ = 5.3 Hz and $J_{2,3}$ = 13.6 Hz) were consistent with 1α, 2α, and 3β configurations, respectively, as well as a *cis*-B/C ring junction.[17] The NOESY correlations from H-2 to H-1, H-2′, 6′, and H-2″, 6″, from H-3 to H-2″, 6″, and from H-1 to H-2 confirmed the relative configurations of H-1, H-2, and H-3 as being β, β, and α, respectively. The absolute configuration of compound **2** was

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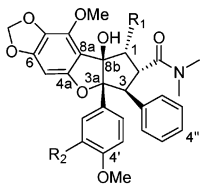
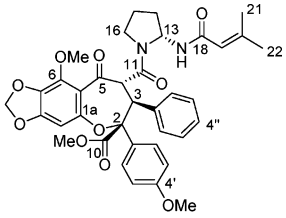


1 R_1 = OH R_2 = H

2 R_1 = OAc R_2 = H

3 R_1 = OAc R_2 = OCH_3



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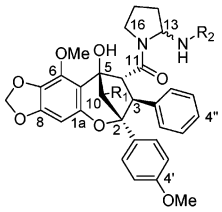
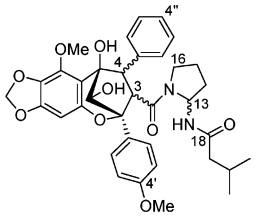
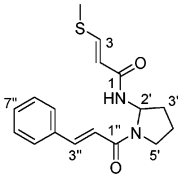


	R_1	R_2
5	OH	
6	OAc	
7	OH	



8 H-3α, H-4β

9 H-3β, H-4α



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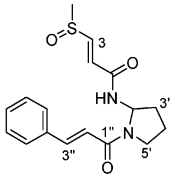

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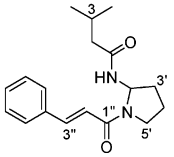

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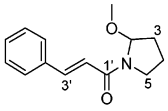

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determined as 1*R*, 2*R*, 3*S*, 3a*R*, and 8b*S* on the basis of comparison of its CD spectrum with other rocaglamide derivatives,[18,28] which showed a strong negative Cotton effect around 220 nm as the most characteristic feature. Accordingly, the structure of **2** was assigned as aglaroxin A 1-*O*-acetate.

The molecular formula of compound **3** was determined as $C_{32}H_{33}NO_{10}$, from the sodiated molecular ion peak at *m*/*z* 614.1999 (calcd for $C_{32}H_{33}NO_{10}Na$, 614.1997) in the HRESIMS. The ^{1}H NMR and ^{13}C NMR spectra of compound **3** were similar to those of compound **2** except for evidence of an additional methoxy group on the B ring. The symmetrical ^{1}H NMR resonance pattern for the AA′BB′ system of the *para*-substituted B ring of compound **2** changed in the case of compound **3** to an ABC pattern with one *meta*-coupled proton at δ_H 6.56 (1H, d, J = 1.8 Hz, H-2′), one *ortho*-coupled proton at δ_H 6.67 (1H, d, J = 8.5 Hz, H-5′), and one *ortho*- and *meta*-coupled proton at δ_H 6.84 (1H, dd, J = 8.5, 1.8 Hz, H-6′). The ^{1}H NMR chemical shifts and coupling patterns agreed with the presence of a 3′,4′-dimethoxyphenyl substituent. The CD spectrum of compound **3** was very similar to that of compound **2**, indicating also an absolute configuration of 1*R*, 2*R*, 3*S*, 3a*R*, and 8b*S*. Therefore, the structure of compound **3** was elucidated as 3′-methoxyaglaroxin A 1-*O*-acetate.

The HRESIMS of compound **4** provided a sodiated molecular ion peak at *m*/*z* 693.2421, corresponding to an elemental formula of $C_{37}H_{38}N_2O_{10}Na$ (calcd 693.2418). The ^{1}H NMR spectroscopic data of **4** in CD_3OD revealed characteristic signals for three methoxy groups at δ_H 3.82 (3H, s, MeO-6), 3.75 (3H, s, MeO-4′), and 3.14 (3H, s, MeO-10), for a *para*-substituted aromatic ring at δ_H 7.33 (2H, d, J = 8.8 Hz, H-2′, 6′) and 6.77 (2H, d, J = 8.8 Hz, H-3′, 5′), and for a monosubstituted aromatic ring at δ_H 7.50 (2H, brd, J = 6.9 Hz, H-2″, 6″) and 7.23 (3H, m, H-3″, 4″, 5″). In addition, resonances for a methine pair appeared at δ_H 5.20 (1H, d, J = 9.6 Hz, H-3) and 4.84 (1H, d, J = 9.6 Hz, H-4) and were mutually coupled in the ^{1}H−^{1}H COSY spectrum. On the basis of the observed HMQC correlations, these two signals were found to correspond to the ^{13}C NMR signals at δ_C 51.9 (C-3) and 65.3 (C-4), respectively. Characteristic signals of a pyrrolidine-type bisamide unit in **4** were also apparent, with two carbonyl groups at δ_C 168.9 (C-11) and 167.7 (C-18). The ^{1}H NMR spectrum exhibited a signal for a vinyl proton at δ_H 5.45 (1H, s, H-19), together with the resonances of two geminal vinyl methyls at δ_H 2.15 (3H, s, H-21) and 1.93 (3H, s, H-22), indicating the presence of a terminal dimethylvinyl group in the bisamide side chain. The ^{13}C NMR spectrum of **4** also exhibited a conjugated carbonyl signal at δ_C 194.6 (C-5). All the above-mentioned NMR observations suggested that compound **4** is a benzo[*b*]oxepine derivative possessing a pyrrolidine-type bisamide side chain.[5,6,23] HMBC correlations from two methine protons, δ_H 5.20 (H-3) and 4.84 (H-4), to δ_C 168.9 (C-11), and from the proton at δ_H 5.20 (H-3) to δ_C 131.1 (C-2″, 6″) of the monosubstituted aromatic ring, indicated that the locations of the pyrrolidine-type bisamide unit and the aromatic ring are at C-4 and C-3, respectively. NOESY correlations were used to establish the relative configurations at all chiral centers in compound **4** except for C-13. The configuration at C-13 of **4** was proposed as 13*R* on the basis of comparison of ^{1}H NMR chemical shifts of the 2-aminopyrrolidine unit with analogous data for the previously reported benzo[*b*]oxepine derivatives, edulisones A (13*R*) and B (13*S*), obtained in our laboratory.[23] In our preliminary investigation, it was found that several protons close to the epimeric site (C-13) of 13*R* and 13*S* benzo[*b*]oxepines have differential ^{1}H NMR chemical shifts.[23] The ^{1}H NMR spectrum of **4** exhibited a similar chemical shift profile and splitting pattern to edulisone A (13*R*), showing signals for H-14a and H-14b at δ_H 1.94 and 1.66 and overlapping signals for H-16a and H-16b centered at δ_H 3.38, respectively. Accordingly, the new compound **4** was assigned structurally as 19,20-dehydroedulisone A.

The ^{1}H and ^{13}C NMR spectra of compound **5** showed closely related signals to those of compound **2**, exhibiting typical resonances for a 6-methoxy-7,8-methylenedioxy-substituted aromatic ring A, a *para*-substituted aromatic ring B, a monosubstituted aromatic ring C, and three methine protons. However, the signals for three methine protons of compound **5** showed different coupling patterns from those of compound **2**. The proton resonance at δ_H 4.56 (1H, s, H-10) appeared as a sharp singlet, while the two other signals at δ_H 4.37 (1H, d, J = 9.3 Hz, H-3) and 4.22 (1H, d, J = 9.3 Hz, H-4) were coupled to one another. Moreover, two characteristic quaternary carbons of cyclopenta[*b*]benzofurans at δ_C 101.0 and 93.3, corresponding to C-3a and C-8b in compound **2**, were missing and replaced in **5** by two quaternary carbon signals at δ_C 89.7 (C-2) and 83.5 (C-5). Accordingly, the ^{1}H and ^{13}C NMR spectroscopic data suggested that compound **5** has a cyclopenta[*bc*]benzopyran skeleton instead of being a cyclopenta[*b*]benzofuran.[4,15] In addition, characteristic signals of a pyrrolidine-type bisamide unit were apparent in compound **5**, with two carbonyl group signals observed at δ_C 172.1 (C-11) and 174.1 (C-18). All of the above-mentioned NMR observations suggested that compound **5** is a cyclopenta[*bc*]-benzopyran derivative containing a bisamide side chain.[4−6,13,15,28−30] In the HMBC spectrum, correlations from the proton at δ_H 4.37 (H-3) to δ_C 172.1 (C-11) and 131.6 (C-2″, 6″) of the monosub-

stituted aromatic ring and from the proton at δ_H 4.22 (H-4) to δ_C 109.9 (C-5a) and 172.1 (C-11) suggested that the locations of the aromatic ring and the pyrrolidine-type bisamide unit are at C-3 and C-4, respectively. The vicinal coupling constant (9.3 Hz) between H-3 and H-4 was compatible only with a H-3α and a H-4*β* configuration for this class of compound.[4] In addition, NOESY correlations were observed from H-3 and H-10 to H-2′, 6′ as well as from H-4 to H-2″, 6″, indicating the relative configurations at C-3, C-4, and C-10 as shown. However, the stereochemistry at C-13 remained uncertain for **5** because relevant NOESY correlations were not observed. Therefore, the structure of compound **5** was assigned as (−)-1-[(2*R*,3*S*,4*R*,5*R*,10*S*)-2,3,4,5-tetrahydro-5,10-dihydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-3-phenyl-2,5-methano-1-benzoxepin-4-carbonyl]-2-(3-methylbutanoylamino)pyrrolidine and has been named edulirin A.

The ^{1}H and ^{13}C NMR data of compound **6** were almost identical to those of compound **5** except for signals denoting the presence of an additional acetate group at C-10. Compound **6** displayed very similar HMBC NMR spectroscopic correlations to compound **5**, supporting the attachment of the benzyl ring and the amide group to C-3 and C-4, respectively. Cross-peaks appearing between the methyl of the acetyl group and H-2″, 6″ and between H-10 and H-2′, 6′ in the NOESY spectrum, indicated the same configurations at C-3, C-4, and C-10 as in **5**. However, we were unable to determine the configuration of C-13. Thus, compound **6** was assigned as edulirin A 10-*O*-acetate.

Compound **7** was obtained as an amorphous powder. Its HRESIMS exhibited a sodiated molecular ion peak at *m*/*z* 665.2452, consistent with an elemental formula of $C_{36}H_{38}N_2O_9Na$ (calcd 665.2469). The structure of compound **7** was found to be very similar to that of the compound **5**. However, the ^{1}H NMR spectrum of compound **7** exhibited a signal for a vinyl proton at δ_H 5.26 (1H, s, H-19), together with the resonances of two geminal vinyl methyls at δ_H 2.19 and 1.84, indicating the presence of a terminal dimethylvinyl group in a bisamide side chain. Its HMBC and NOESY NMR spectra were similar to those of compound **5**. A combination of its 2D ^{1}H−^{1}H COSY, HMQC, and HMBC spectra and the NOESY correlations from H-3 and H-10 to H-2′, 6′ and from H-4 to H-2″, 6″ were used to establish the same H-3α, H-4*β*, and C-10 configurations as in edulirin A (**5**), with the C-13 configuration again unresolved. Therefore, compound **7** was assigned as 19,20-dehydroedulirin A.

A sodiated molecular ion of compound **8** was observed at *m*/*z* 667.2608 (calcd for $C_{36}H_{40}N_2O_9Na$, 667.2626) in the HRESIMS, which was used to determine the molecular formula. The ^{1}H and ^{13}C NMR spectroscopic data of compound **8** were closely comparable to those of edulirin A (**5**), suggesting this to be also a cyclopenta[*bc*]benzopyran derivative possessing a pyrrolidine-type bisamide side chain.[6,28] However, the HMBC experiment clearly indicated that the substituents at C-3 and C-4 were mutually exchanged in these compounds. HMBC correlations from H-4 to C-5a and C-2″, 6″ and from H-3 to C-2 and C-11 suggested that the locations of the pyrrolidine-type bisamide unit and the monosubstituted aromatic ring are at C-3 and C-4, respectively, in compound **8**. The relative configurations at C-3 and C-4 were determined, in turn, as H-3α and H-4*β*, on the basis of the NOESY correlations between H-3 and H-2′, 6′ and between H-4 and H-2″, 6″, as well as the vicinal coupling constant value (7.0 Hz) between H-3 and H-4. We were unable to assign the configuration at C-13 for this compound. Therefore, the structure of compound **8** was elucidated as (−)-1-[(2*R*,3*S*,4*R*,5*R*,10*S*)-2,3,4,5-tetrahydro-5,10-dihydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-4-phenyl-2,5-methano-1-benzoxepin-3-carbonyl]-2-(3-methylbutanoylamino)pyrrolidine and has been named isoedulirin A.

Compound **9** was assigned the same molecular formula as compound **8** based on its observed sodiated molecular ion peak at *m*/*z* 667.2608 in the HRESIMS. Correlations from H-4 to C-5a and C-2″, 6″ and from H-3 to C-2 and C-11 were apparent in its HMBC NMR spectrum, indicating that the locations of the pyrrolidine-type bisamide unit and the aromatic ring were the same as in compound **8**. However, the stereochemistry at C-3 and C-4 of compound **9** was reversed as compared with compound **8**. In addition to the coupling constant of 11.0 Hz between H-3 and H-4, NOESY correlations between OCH_3-6 and H-2″, 6″, between H-3 and H-2″, 6″, and between H-10 and H-2′, 6′ supported the assignments at C-3 and C-4 of compound **9** as being H-3*β* and H-4α, respectively.[6,28] The configuration at C-10 of **9** was identical to that of **8** on the basis of the observed NOESY correlation between H-10 and H-2′, 6′, while no NOE effect was observed between H-10 and H-3 or H-4. Therefore, the structure of compound **9** was elucidated as (−)-1-[(2*R*,3*R*,4*S*,5*R*,10*S*)-2,3,4,5-tetrahydro-5,10-dihydroxy-2-(4-methoxyphenyl)-6-methoxy-7,8-methylenedioxy-4-phenyl-2,5-methano-1-benzoxepin-3-carbonyl]-2-(3-methylbutanoylamino)pyrrolidine and has been named edulirin B. Once again, the C-13 configuration was unresolved.

Compound **10** was obtained as an amorphous powder, $[\alpha]^{22}_D$ 0 (*c* 0.1, MeOH). Its HREIMS exhibited a molecular ion peak at *m*/*z* 316.1225, consistent with a molecular formula of $C_{17}H_{20}N_2O_2S$. The presence of a cinnamoyl moiety was suggested by the characteristic signals at δ_H 7.68 (1H, d, J = 15.4 Hz, H-3″), 7.53 (2H, m, H-5″, 9″), 7.36 (3H, m, H-6″, 7″, 8″), and 6.97 (1H, d, J = 15.4 Hz, H-2″) in the ^{1}H NMR spectrum.[31,32] The coupling constant (J = 15.4 Hz) of H-2″ and H-3″ indicated an *E*-configuration of the cinnamoyl moiety.[31,32] Characteristic signals of a 2-aminopyrrolidine unit in compound **10** were apparent from signals at δ_H 6.19 (1H, brt, J = 7.2 Hz, H-2′), 3.60 (1H, m, H-5′a), 3.43 (1H, m, H-5′b), 2.20 (1H, m, H-3′a), 2.00 (1H, m, H-3′b), and 1.96 (2H, m, H-4′). The chemical shift at δ_H 2.31 of a sharp methyl singlet was consistent with the presence of a S−CH_3 group,[33] which was found to correspond to the ^{13}C NMR signal at δ_C 15.0 on the basis of the observed HMQC correlation. This functionality was linked to an olefinic group [δ_H 7.77 (1H, d, J = 14.6 Hz, H-3/δ_C 144.1 (C-3); δ_H 5.78 (1H, d, J = 14.6 Hz, H-2/δ_C 116.0 (C-2)], which was connected in turn to a carbonyl functionality (δ_C 163.9, C-1). Accordingly, the structure of compound **10** was elucidated as (*E*,*E*)-*N*-cinnamoyl-2-[3-(methylthio)propenoylamino]pyrrolidine and has been named aglamide A.

In the HRESIMS of compound **11**, a sodiated molecular ion peak was observed at *m*/*z* 355.1079, consistent with an elemental formula of $C_{17}H_{20}N_2O_3SNa$. The ^{1}H and ^{13}C NMR spectra of **11** were closely comparable to those of **10**. The only differences in the 1D NMR spectra were that methyl signals migrated to δ_H 2.45 from δ_H 2.31 in the ^{1}H NMR spectrum, and to δ_C 34.7 from δ_C 15.0 in the ^{13}C NMR spectrum, when compared with **10**. This agrees with the resonance of a O═S−CH_3 unit, suggesting that in the structure of compound **11** a O═S−CH_3 group replaced the S−CH_3 group in compound **10**. Therefore, compound **11** was assigned as (*E*,*E*)-*N*-cinnamoyl-2-[3-(methylsulfoxide)propenoylamino]pyrrolidine and has been named aglamide B.

Compound **12** was obtained as an amorphous powder, $[\alpha]^{22}_D$ 0 (*c* 0.1, MeOH). Its HRESIMS exhibited a sodiated molecular ion peak at *m*/*z* 323.1732, consistent with a molecular formula of $C_{18}H_{24}N_2O_2Na$ (calcd 323.1729), and the absence of sulfur from the molecule. When compared to the ^{1}H NMR spectrum of aglamide A (**10**), compound **12** showed very similar data as far as ^{1}H NMR resonances of the cinnamic acid and the pyrrolidine moieties were concerned. However, signals for two methyl doublets were observed at δ_H 0.96 (3H, d, J = 6.0 Hz, H-4) and 0.88 (3H, J = 6.0 Hz, H-5) instead of the singlet signal for a S−CH_3 group in **12**. On comparing the remaining ^{1}H and ^{13}C NMR data, compound **12** was assigned as (*E*,*E*)-*N*-cinnamoyl-2-(3-methylbutanoylamino)pyrrolidine and has been named aglamide C.

Compound **13** was obtained as an amorphous powder, $[\alpha]^{22}_D$ 0 (*c* 0.1, EtOH). Its HRESIMS exhibited a sodiated molecular ion

Table 1. Cytotoxic Activity of Compounds Isolated from *A. edulis*[a]

compound	cell line[b]			
	Lu1	LNCaP	MCF-7	HUVEC
1	0.04	0.02	0.06	0.1
2	0.001	0.01	0.02	0.5
3	0.5	0.3	0.8	0.5

[a] Compounds **4**−**13**, aglalactone, scopoletin, and 5-hydroxy-3,6,7,4′-tetramethoxyflavone were all inactive for all cell lines (ED_{50} > 5 μg/mL). [b] Results are expressed as ED_{50} values (μg/mL). Key to cell lines used: Lu1 = human lung cancer; LNCaP = hormone-dependent human prostate cancer; MCF-7 = human breast cancer; HUVEC = human umbilical vein endothelial cells.

peak at *m*/*z* 254.1151, consistent with a molecular formula of $C_{14}H_{17}NO_2Na$ (calcd 254.1151). The ^1H and ^{13}C NMR data of the cinnamic acid and the pyrrolidine units were very similar to the corresponding data of aglamide C (**12**), but only a methoxy group was affixed to C-2, instead of a more complex amide substituent. Therefore, compound **13** was assigned as (*E*)-*N*-cinnamoyl-2-methoxypyrrolidine and has been named aglamide D.

In the present study, the stereochemistry at C-13 of 19,20-dehydroedulisone A (**4**) could be determined as 13*R* by comparison of its ^1H NMR spectroscopic data with those of edulisones A and B, which were previously reported on the basis of X-ray crystallography and their hydrolysis products.[23] However, the stereochemistry at C-13 in the cyclopenta[*bc*]benzopyrans (**5**−**9**) remains uncertain because the determination of the configuration of the 2-aminopyrrolidine moiety of these compounds is challenging due to the free rotation of their respective bisamide side chain. Even though certain NOESY correlations have been suggested as being useful to determine the configuration at C-13 based on Dreiding models,[6] there were insignificant NOESY correlations evident to determine the stereochemistry at C-13 in aglaxiflorin A, which is the only *Aglaia* cyclopenta[*bc*]benzopyran so far elucidated structurally by X-ray crystallography.[13] While flavaglines (**1**−**7**) were isolated from the bark of *A. edulis*, amides (**10**−**13**) were isolated from the leaves and/or twigs of this plant. Among the new bisamides, aglamide A (**10**) and aglamide B (**11**) were identified as sulfur-containing compounds, and aglamide B (**11**) represents the first sulfoxide-containing compound from the genus *Aglaia*. All four new amides (**10**−**13**) were isolated as racemates in the present study. Even though many bisamides are known from the genus *Aglaia*,[3,29−35] only a few sulfur-containing bisamides have been reported from *A. edulis* and *A. leptantha*.[33,34] Sulfur-containing bisamides may represent a chemotaxonomic characteristic of certain species in the genus *Aglaia*.

As summarized in Table 1, the cytotoxic activities of the 16 compounds isolated in the present investigation were evaluated against a small panel of human cancer cell lines. Among these isolates, three cyclopenta[*b*]benzofurans, compounds **1**−**3**, were found to be cytotoxic against the tested cell lines. Aglaroxin A (**1**) and aglaroxin A 1-*O*-acetate (**2**) showed a more potent response for three cancer cell lines in the tumor panel (Lu1, LNCaP, MCF-7 cells), as compared to the nontumorigenic HUVEC cell line. However, consistent with earlier observations,[1,2,11,12] two structurally related groups of constituents isolated from *A. edulis*, the benzo[*b*]oxepine (**4**) and cyclopenta[*bc*]benzopyrans (**5**−**9**), did not show any significant cytotoxic activity against the cancer cell panel, showing that the replacement of the furan ring of cyclopenta[*b*]-benzofuran derivatives by a pyran ring or an oxepine ring leads to the total loss of such activity.

Since aglaroxin A 1-*O*-acetate (**2**) was the most active compound of all isolates and isolated in a relatively large quantity, this compound was selected for follow-up evaluation in an in vivo P388 lymphocytic leukemia test system, a stable and reliable model for the preliminary evaluation of the antitumor activity of natural products, administered by intraperitoneal injection according to a previously published protocol.[17] However, this compound was inactive in this model [% T/C (LCK) 110 at 4.5 mg/kg/inj., ip].

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 automatic polarimeter. UV spectra were obtained with a Perkin-Elmer UV/vis lambda 10 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrophotometer. NMR spectroscopic data were recorded at room temperature on Bruker Avance DPX-300 and DRX-400 spectrometers with tetramethylsilane (TMS) as internal standard. Electrospray ionization (ESI) mass spectrometric analyses were performed with a 3-tesla Finnigan FTMS-2000 Fourier transform mass spectrometer, and electron impact (EI) mass spectra were obtained with a Kratos MS-25 mass spectrometer, using a 70 eV ionization source. A SunFire PrepC_{18}OBD column (5 μm, 150 × 19 mm i.d., Waters, Milford, MA) and a SunFire PrepC_{18} guard column (5 μm, 10 × 19 mm i.d., Waters) were used for preparative HPLC, along with two Waters 515 HPLC pumps and a Waters 2487 dual λ absorbance detector. Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thickness Partisil K6F (Whatman, Clifton, NJ) glass plates, while preparative TLC was conducted on precoated 20 × 20 cm, 500 μm Partisil K6F (Whatman) glass plates. All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ).

Plant Material. The leaves, twigs, and bark of *A. edulis* (Roxb.) Wall. were collected separately in October 2001 from Senaru Village, Bayan District, West Lombok Island, Indonesia. The plant was identified by S.R. and J.J.A. Voucher specimens have been deposited at the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Science, Bogor, Indonesia (collection number SR-022), and at the University of Illinois Pharmacognosy Field Station [accession numbers A5240 (leaves), A5241 (twigs), and A5242 (bark)].

Extraction and Isolation. The milled bark (723 g) of *A. edulis* was percolated overnight with 90% methanol (3 × 3 L), followed by solvent draining. The extract was combined and concentrated in vacuo at <40 °C. The concentrated extract was suspended in 90% MeOH and then defatted with an equal volume of hexanes saturated with 90% MeOH. The aqueous methanol extract was concentrated and suspended in H_2O and partitioned again with chloroform to give a chloroform-soluble residue. The combined chloroform extract from these partitions was washed with 1% NaCl, then evaporated to dryness.[36] This extract of *A. edulis* exhibited potent cytotoxic activity against the small human cancer cell panel used (ED_{50} range 0.2−0.6 μg/mL). Accordingly, the chloroform-soluble extract was chromatographed using a glass column (5.0 × 40 cm) packed with a slurry of silica gel (200 g, 230−400 mesh). The column was then eluted with $CHCl_3$−acetone (100:0 → 1:1, gradient mixtures of increasing polarity) and washed with MeOH. Thirteen fractions (A5242-F001−A5242-F013) were collected and tested for cytotoxic activity against the LNCaP cell line. Fraction A5242-F010 was subjected to preparative HPLC with isocratic elution by MeOH−H_2O (4:1) (4 mL/min), leading to the isolation of aglaroxin A (**1**, 2.1 mg, t_R 25.4 min, 0.00029%). The most active fractions, A5242-F006 (80 mg) and A5242-F007 (92.5 mg), were combined and subjected to preparative HPLC by gradient elution with MeOH−H_2O mixtures (60:40 → 85:15, 4 mL/min), leading to the isolation of aglaroxin A 1-*O*-acetate (**2**, 48.2 mg, t_R 32.3 min, 0.0067%), 3′-methoxyaglaroxin A 1-*O*-acetate (**3**, 3.3 mg, t_R 24.8 min, 0.00046%), and isoedulirin A (**8**, 5.0 mg, t_R 16.8 min, 0.00069%). Fraction A5242-F009 was subjected to preparative HPLC by isocratic elution by MeOH−H_2O (73:27) (4 mL/min), resulting in the purification of 19,20-dehydroedulisone A (**4**, 6.3 mg, t_R 17.6 min, 0.00087%). Fraction A5242-F008 was subjected to preparative HPLC with isocratic elution by MeOH−H_2O (75:25) (4 mL/min), leading to the isolation of edulirin A (**5**, 6.4 mg, t_R 27.1 min, 0.00089%), edulirin A 10-*O*-acetate (**6**, 17.7 mg, t_R 34.6 min, 0.0025%), 19,20-dehydroedulirin A (**7**, 4.1 mg, t_R 35.3 min, 0.00057%), and edulirin B (**9**, 6.7 mg, t_R 32.6 min, 0.00093%).

The milled leaves (800 g) of *A. edulis* were extracted, partitioned, and tested against several cancer cell lines in the same manner as described for the bark. The chloroform-soluble extract of the leaves of *A. edulis* (A5240), which exhibited weak cytotoxicity against the panel of human cancer cell lines used (ED_{50} range 2.1−6.7 μg/mL), was chromatographed using a glass column (10 × 40 cm) packed with a slurry of silica gel (450 g, 230−400 mesh). The column was then eluted

Table 2. ^{1}H and ^{13}C NMR Chemical Shifts of Compounds **5**−**7** in CD_3OD[a]

	5		**6**		**7**	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1a	148.9, qC		148.5, qC		148.8, qC	
2	89.7, qC		89.0, qC		89.6, qC	
3	58.7, CH	4.37, d (9.3)	58.1, CH	4.50, d (9.6)	58.8, CH	4.32, d (9.5)
4	63.6, CH	4.22, d (9.3)	63.8, CH	4.25, d (9.6)	63.2, CH	4.25, d (9.5)
5	83.5, qC		82.9, qC		83.5, qC	
5a	109.9, qC		109.3, qC		109.9, qC	
6	142.4, qC		142.3, qC		142.5, qC	
7	132.1, qC		131.7, qC		131.6, qC	
8	151.0, qC		151.3, qC		151.0, qC	
9	93.6, CH	6.07, s	93.6, CH	6.21, s	93.6, CH	6.08, s
10	81.0, CH	4.56, s	81.2, CH	5.88, s	81.2, CH	4.53, s
11	172.1, qC		171.0, qC		167.8, qC	
13	64.7, CH	6.59, d (6.0)	64.8, CH	7.02, m	64.4, CH	6.61, d (5.8)
14	35.1, CH_2	2.18, 1.82, m	35.1, CH_2	2.11, 1.82, m	35.2, CH_2	2.11, 1.86 m
15	21.9, CH_2	1.89, m	21.9, CH_2	1.94, m	22.1, CH_2	1.93, m
16	47.1, CH_2	3.45, 3.30, m	47.0, CH_2	3.50, 3.32, m	47.1, CH_2	3.44, 3.28, m
18	174.1, qC		173.7, qC		172..2, qC	
19	46.0, CH_2	1.86, m, 1.71	46.1, CH_2	1.90, 1.82, m	119.4, CH	5.26, s
20	27.2, CH	1.96, m	27.2, CH	1.97, m	152.9, qC	
21	23.1, CH_3	0.85, d (6.4)	23.2, CH_3	0.92, d (6.5)	27.4, CH_3	2.19, s
22	22.4, CH_3	0.88, d (6.6)	22.4, CH_3	0.88, d (6.5)	20.2, CH_3	1.84, s
1′	131.5, qC		130.3, qC		132.1, qC	
2′,6′	131.4, CH	7.37, d (8.9)	130.8, CH	6.62, d (8.9)	131.4, CH	7.38, d (8.9)
3′,5′	113.3, CH	6.57, d (8.9)	113.8, CH	7.02, d (8.9)	113.2, CH	6.56, d (8.9)
4′	159.9, qC		160.3, qC		159.8, qC	
1″	143.2, qC		142.3, qC		142.8, qC	
2″,6″	131.6, CH	7.17−7.12, m	131.1, CH	7.18−7.04, m	131.6, CH	7.08, m
3″,5″	128.8, CH	7.00−6.92, m	129.2, CH	7.18−7.04, m	128.7, CH	6.89, m
4″	127.0, CH	7.00−6.92, m	127.4, CH	7.18−7.04, m	126.8, CH	6.89, m
MeO-6	60.8, CH_3	4.03, s	60.9, CH_3	4.03, s	60.8, CH_3	4.00, s
MeO-10						
OAc			171.9, qC			
OAc			21.5, CH_3	2.43, s		
MeO-4′	55.4, CH_3	3.65, s	55.5, CH_3	3.68, s	55.4, CH_3	3.64, s
OCH_2O	102.3, CH_2	5.86, s	102.4, CH_2	5.95, s	102.3, CH_2	5.86, s
		5.83, s		5.92, s		5.84, s

[a] ^{1}H and ^{13}C NMR spectra were acquired at 400 and 100 MHz, respectively; TMS was used as internal standard; assignments are based on ^{1}H−^{1}H COSY, DEPT, HMQC, HMBC, and NOESY spectra.

with $CHCl_3$−acetone (100:0 → 1:1, gradient mixtures of increasing polarity) and washed with MeOH. Ten fractions (A5240-F001−A5240-F010) were collected and tested for cytotoxic activity against the LNCaP cell line. Aglamide A (**10**, 573 mg, 0.058%) precipitated from fraction A5240-F002 as a white, amorphous powder. The supernatant of A5240-F005 was chromatographed over Diaion HP-20 gel, using 90% MeOH in H_2O, resulting in the purification of aglamide B (**11**, 12 mg, 0.0012%) and aglamide D (**13**, 5.1 mg, 0.000515%).

The milled twigs (994 g) of *A. edulis* were also extracted, partitioned, and evaluated for cytotoxicity in the same manner as described for the bark. This chloroform-soluble extract of the twigs of *A. edulis* (A5241), with weak cytotoxic activity against the human cancer cell panel used (ED_{50} range 6.8 to >20 μg/mL), was chromatographed using a glass column (10 × 40 cm) packed with a slurry of silica gel (450 g, 230−400 mesh). The column was then eluted with $CHCl_3$−acetone (100:0 → 1:1, gradient mixtures of increasing polarity) and washed with MeOH. Nine fractions (A5241-F001−A5241-F009) were collected and tested for cytotoxic activity against the LNCaP cell line. Aglamide C (**12**, 612.5 mg, 0.062%) precipitated from fraction A5241-F005 as a white, amorphous powder.

Aglaroxin A 1-*O*-acetate (2): white, amorphous powder; $[\alpha]^{22}_D$ −69 (*c* 0.1, EtOH); UV (EtOH) λ_{max} (log ϵ) 205 (4.84), 297 (3.73) nm; CD (EtOH) nm $\Delta\epsilon_{221}$ −8.5; IR (film) ν_{max} 3423, 2924, 1558, 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.08 (2H, d, J = 8.8 Hz, H-2′, H-6′), 7.06−7.00 (3H, m, H-3″, H-4″, H-5″), 6.90 (2H, brd, J = 7.2 Hz, H-2″, H-6″), 6.63 (2H, d, J = 8.8 Hz, H-3′, H-5′), 6.28 (1H, s, H-5), 6.00 (1H, d, J = 5.3 Hz, H-1), 5.87, 5.86 (each 1H, s, OCH_2O), 4.62 (1H, d, J = 13.6 Hz, H-3), 4.19 (1H, dd, J = 13.6, 5.3 Hz, H-2), 3.96 (3H, s, OCH_3-8), 3.67 (3H, s, OCH_3-4′), 3.32 (3H, s, NCH_3), 2.85 (3H, s, NCH_3), 1.90 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.8 (C, $COCH_3$), 167.5 (C, *C*ON), 158.6 (C, C-4′), 154.5 (C, C-4a), 152.0 (C, C-6), 140.4 (C, C-8), 137.5 (C, C-1″), 129.4 (C, C-7), 128.8 (CH, C-2′, C-6′), 127.7 (C, C-1′), 127.6 (CH, C-2″, C-6″), 127.2 (CH, C-3″, C-5″), 126.2 (CH, C-4″), 112.7 (CH, C-3′, C-5′), 109.0 (C, C-8a), 101.5 (CH_2, OCH_2O), 101.0 (C, C-3a), 93.3 (C, C-8b), 87.5 (CH, C-5), 77.6 (CH, C-1), 59.5 (CH_3, OCH_3-8), 56.4 (CH, C-3), 55.1 (CH_3, OCH_3-4′), 47.6 (CH, C-2), 37.2 (CH_3, NCH_3), 35.7 (CH_3, NCH_3), 21.0 (CH_3, CH_3CO); HRESIMS *m*/*z* 584.1875 $[M + Na]^+$ (calcd for $C_{31}H_{31}NO_9$-Na, 584.1891).

3′-Methoxyaglaroxin A 1-*O*-acetate (3): white, amorphous power; $[\alpha]^{22}_D$ −132 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.52), 284 (3.56) nm; CD (MeOH) nm $\Delta\epsilon_{224}$ −15.8; IR (film) ν_{max} 3422, 1742, 1637, 1517, 1455, 1257, 1057 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.07−7.00 (3H, m, H-3″, H-4″, H-5″), 6.89 (2H, d, J = 6.9 Hz, H-2″, H-6″), 6.84 (1H, dd, J = 8.5, 1.8 Hz, H-6′), 6.67 (1H, d, J = 8.5 Hz, H-5′), 6.56 (1H, J = 1.8 Hz, H-2′), 6.30 (1H, s, H-5), 6.03 (1H, d, J = 5.5 Hz, H-1), 5.89 (2H, s, OCH_2O), 4.59 (1H, d, J = 13.6 Hz, H-3), 4.16 (1H, dd, J = 13.6 Hz, J = 5.5 Hz, H-2), 4.00 (3H, s, OCH_3-8), 3.78 (3H, s, OCH_3-4′), 3.61 (3H, s, OCH_3-3′), 3.31 (3H, s, NCH_3), 2.86 (3H, s, NCH_3), 1.93 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.8 (C, $COCH_3$), 167.5 (C, *C*ON), 154.4 (C, C-4a), 152.0 (C, C-6), 148.1 (C, C-4′), 147.9 (C, C-3′), 140.4 (C, C-8), 137.5 (C, C-1″), 129.5 (C, C-7), 127.8 (CH, C-3″, C-5″), 127.6 (CH, C-2″, C-6″), 127.6 (C, C-1′), 126.4 (CH, C-4″), 120.0 (CH, C-6′), 111.8 (CH, C-2′), 109.8 (CH, C-5′), 109.2 (C, C-8a), 101.3 (C, C-3a), 101.0 (CH_2, OCH_2O), 93.3 (C, C-8b), 87.5 (CH, C-5), 77.6 (CH, C-1), 59.5 (CH_3, OCH_3-8), 56.3 (CH, C-3), 55.8 (CH_3, OCH_3-3′), 55.7 (CH_3, OCH_3-4′), 47.4 (CH, C-2), 37.1 (CH_3, NCH_3), 35.7 (CH_3, NCH_3), 21.0 (CH_3, CH_3CO); HRESIMS *m*/*z* 614.1999 $[M + Na]^+$ (calcd for $C_{32}H_{33}NO_{10}Na$, 614.1997).

19,20-Dehydroedulisone A (4): amorphous powder; $[\alpha]^{22}_D$ +95 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.88), 278 (4.09) nm; IR (film) ν_{max} 3343, 2956, 1758, 1673, 1471, 1256, 1105 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50 (2H, d, J = 6.9 Hz, H-2″, H-6″), 7.33 (2H, d, J = 8.8 Hz, H-2′, H-6′), 7.23 (3H, m, H-3″, H-4″, H-5″), 6.77 (2H, d, J = 8.8 Hz, H-3′, H-5′), 6.70 (1H, s, H-9), 6.03, 6.00 (each 1H, s, OCH_2O), 5.45 (1H, s, H-19), 5.41 (1H, d, J = 5.8 Hz, H-13), 5.20 (1H, d, J = 9.6 Hz, H-3), 4.84 (1H, d, J = 9.6 Hz, H-4), 3.82 (3H, s,

Table 3. ^{1}H and ^{13}C NMR Chemical Shifts of Compounds **8** and **9** in CD_3OD[a]

	8		**9**	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1a	148.8, qC		147.4, qC	
2	87.8, qC		87.4, qC	
3	62.3, CH	4.40, d (7.0)	57.7, CH	4.02, d (10.5)
4	62.2, CH	4.22, d (7.0)	58.2, CH	4.71, d (10.5)
5	81.9, qC		84.1, qC	
5a	116.8, qC		111.2, qC	
6	140.1, qC		141.0, qC	
7	132.2, qC		132.2, qC	
8	150.0, qC		150.9, qC	
9	94.2, CH	6.27, s	95.2, CH	6.46, s
10	83.4, CH	4.20, s	81.7, CH	4.29, s
11	171.6, qC		171.3, qC	
13	64.5, CH	5.05, d (5.6)	64.2, CH	5.49, d (5.6)
14	34.9, CH_2	1.34, 1.23, m	34.2, CH_2	1.50, m
15	21.5, CH_2	1.68, m	21.4, CH_2	1.50, m
16	46.8, CH_2	3.43, 3.13, m	46.6, CH_2	3.27, 2.76, m
18	173.9, qC		175.0, qC	
19	45.9, CH_2	1.68, 1.42, m	45.9, CH_2	1.94, 1.83, m
20	27.1, CH	1.86, m	27.2, CH	2.03, m
21	22.9, CH_3	0.83, d (6.7)	23.1, CH_3	0.91, d (6.5)
22	22.2, CH_3	0.77, d (6.6)	22.6, CH_3	0.90, d (6.5)
1′	131.8, qC		130.2, qC	
2′,6′	129.7, CH	8.08, d (8.9)	129.5, CH	7.83, d (9.0)
3′,5′	114.2, CH	7.02, d (8.9)	113.7, CH	6.96, d (9.0)
4′	160.8, qC		160.7, qC	
1″	141.8, qC		138.9, qC	
2″,6″	131.8, CH	7.51, d (7.5)	130.1, CH	7.09, m
3″,5″	129.1, CH	7.30, m	129.1, CH	7.26, m
4″	127.5, CH	7.22, m	128.2, CH	7.26, m
MeO-6	61.1, CH_3	4.04, s	59.8, CH_3	3.07, s
MeO-4′	55.7, CH_3	3.86, s	55.7, CH_3	3.82, s
OCH_2O	102.4, CH_2	5.93, s	102.5, CH_2	5.87, s
		5.90, s		5.85, s

[a] ^{1}H and ^{13}C NMR spectra were acquired at 400 and 100 MHz, respectively; TMS was used as internal standard; assignments are based on ^{1}H−^{1}H COSY, DEPT, HMQC, HMBC, and NOESY spectra.

OC*H*$_3$-6), 3.75 (3H, s, OC*H*$_3$-4′), 3.44−3.32 (2H, m, H-16), 3.14 (3H, s, OC*H*$_3$-10), 2.15 (3H, s, H-21), 1.94 (1H, m, H-14a), 1.93 (3H, s, H-22), 1.66 (1H, m, H-14b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.6 (C, C-5), 172.0 (C, C-10), 168.9 (C, C-11), 167.7 (C, C-18), 161.5 (C, C-4′), 155.7 (C, C-1a), 154.4 (C, C-8), 153.5 (C, C-20), 142.4 (C, C-6), 141.0 (C, C-1″), 134.6 (C, C-7), 131.1 (CH, C-2″, C-3″), 130.7 (CH, C-2′, C-6′), 129.2 (CH, C-3″, C-5″), 128.7 (CH, C-4″), 128.3 (C, C-1′), 119.1 (CH, C-19), 118.9 (C, C-5a), 114.7 (CH, C-3′, C-5′), 103.5 (CH_2, OCH_2O), 98.8 (CH, C-9), 92.1 (C, C-2), 65.3 (CH, C-4), 63.4 (CH, C-13), 61.0 (CH_3, OCH_3-6), 55.7 (CH_3, OCH_3-4′), 52.6 (CH_3, OCH_3-10), 51.9 (CH, C-3), 47.0 (CH_2, C-16), 34.7 (CH_2, C-14), 27.4 (CH_3, C-22), 22.3 (C, C-15), 20.3 (CH_3, C-21); HRESIMS *m*/*z* 693.2421 [M + Na]$^+$ (calcd for $C_{37}H_{38}N_2O_{10}Na$, 693.2418).

Edulirin A (5): amorphous powder; $[\alpha]^{22}_D$ −148 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (4.61), 269 (3.27), 297 (3.52) nm; IR (film) ν_{max} 3343, 2956, 1758, 1673, 1471, 1256, 1105 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 667.2608 [M + Na]$^+$ (calcd for $C_{36}H_{40}N_2O_9Na$, 667.2626).

Edulirin A 10-*O*-acetate (6): amorphous powder; $[\alpha]^{22}_D$ −71 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.59), 268 (3.31), 297 (3.55) nm; IR (film) ν_{max} 3473, 2956, 1749, 1632, 1250, 1517 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 709.2716 [M + Na]$^+$ (calcd for $C_{37}H_{40}N_2O_{10}Na$, 709.2732).

19,20-Dehydroedulirin A (7): amorphous powder; $[\alpha]^{22}_D$ −46 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.57), 269 (3.39), 297 (3.53) nm; IR (film) ν_{max} 3446, 1635, 1517, 1474, 1251, 1059 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* 665.2452 [M + Na]$^+$ (calcd for $C_{36}H_{38}N_2O_9Na$, 665.2469).

Isoedulirin A (8): colorless gum; $[\alpha]^{22}_D$ +16 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.21), 268 (2.97), 295 (3.07) nm; IR (film) ν_{max} 3483, 2956, 1630, 1513, 1463, 1251, 1176, 1130, 895 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 667.2608 [M + Na]$^+$ (calcd for $C_{36}H_{40}N_2O_9Na$, 667.2626).

Edulirin B (9): amorphous powder; $[\alpha]^{22}_D$ +28 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (4.60), 281 (3.58), 294 (3.59) nm; IR (film) ν_{max} 2956, 1627, 1514, 1477, 1129, 1060 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 667.2608 [M + Na]$^+$ (calcd for $C_{36}H_{40}N_2O_9Na$, 667.2626).

Aglamide A (10): amorphous powder; $[\alpha]^{22}_D$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (4.32), 273 (4.60) nm; IR (film) ν_{max} 3264, 2978, 1652, 1578, 1418, 1191 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (1H, d, *J* = 14.6 Hz, H-3), 7.68 (1H, d, *J* = 15.4 Hz, H-3″), 7.53 (2H, m, H-5″, H-9″), 7.36 (3H, m, H-6″, H-7″, H-8″), 6.97 (1H, d, *J* = 15.4 Hz, H-2″), 6.19 (1H, brt, *J* = 7.2 Hz, H-2′), 5.78 (1H, d, *J* = 14.6 Hz, H-2), 3.60 (1H, m, H-5′a), 3.43 (1H, m, H-5′b), 2.31 (3H, s, SC*H*$_3$), 2.20 (1H, m, H-3′a), 2.00 (1H, m, H-3′b), 1.96 (2H, m, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.5 (C, C-1″), 163.9 (C, C-1), 144.1 (CH, C-3), 143.5 (CH, C-3″), 135.1 (C, C-4″), 130.4 (CH, C-7″), 129.2 (CH, C-6″, C-8″), 128.7 (CH, C-5″, C-9″), 118.4 (CH, C-2″), 116.0 (CH, C-2), 63.5 (CH, C-2′), 46.5 (CH_2, C-5′), 34.8 (CH_2, C-3′), 22.0 (CH_2, C-4′), 15.0 (CH_3, S*C*H$_3$); HREIMS *m*/*z* 316.1225 [M]$^+$ (calcd for $C_{17}H_{20}N_2O_2S$, 316.1245).

Aglamide B (11): amorphous powder; $[\alpha]^{22}_D$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.49), 275 (4.43) nm; IR (film) ν_{max} 3384, 1647, 1418 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 8.14 (1H, d, *J* = 14.5 Hz, H-3), 8.06 (1H, d, *J* = 15.5 Hz, H-3″), 7.74 (2H, m, H-5″, 9″), 7.59 (1H, d, *J* = 15.5 Hz, H-2″), 7.27 (3H, m, H-6″, H-7″, H-8″), 7.16 (1H, d, *J* = 14.5 Hz, H-2), 6.52 (1H, brt, *J* = 6.9 Hz, H-2′), 3.70 (1H, m, H-5′a), 3.57 (1H, m, H-5′b), 2.45 (3H, s, O═SC*H*$_3$), 2.12 (1H, m, H-3′a), 1.97 (1H, m, H-3′b), 1.80 (1H, m, H-4′a), 1.65 (1H, m, H-4′b); ^{13}C NMR (pyridine-d_5, 100 MHz) δ 165.5 (C, C-1″), 162.6 (C, C-1), 149.5 (CH, C-3), 142.1 (CH, C-3″), 136.2 (C, C-4″), 130.0 (CH, C-7″), 129.4 (CH, C-6″, C-8″), 128.6 (CH, C-5″, C-9″), 128.3 (CH, C-2), 120.5 (CH, C-2″), 63.7 (CH, C-2′), 46.5 (CH_2, C-5′), 39.8 (CH_2, C-3′), 34.7 (CH_3, O═S*C*H$_3$), 22.2 (CH_2, C-4′); HRESIMS *m*/*z* 355.1079 [M + Na]$^+$ (calcd for $C_{17}H_{20}N_2O_3SNa$, 355.1045).

Aglamide C (12): amorphous powder; $[\alpha]^{22}_D$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.16), 219 (4.21), 283 (4.33) nm; IR (film) ν_{max} 3278, 3059, 2957, 2869, 1648, 1600, 1533, 1416, 1197, 988 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (1H, d, *J* = 15.4 Hz, H-3″), 7.55 (2H, m, H-5″, H-9″), 7.36 (3H, m, H-6″, H-7″, H-8″), 6.97 (1H, d, *J* = 15.4 Hz, H-2″), 6.14 (1H, brt, *J* = 7.6 Hz, H-2′), 3.55 (1H, m, H-5′a), 3.38 (1H, m, H-5′b), 2.24−2.11 (4H, m, H-2, H-3, H-3′a), 1.96 (1H, m, H-3′b), 1.45 (2H, m, H-4′), 0.96 (3H, d, *J* = 6.0 Hz, H-4), 0.88 (3H, d, *J* = 6.0 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.8 (C, C-1), 165.8 (C, C-1″), 143.0 (CH, C-3″), 134.7 (C, C-4″), 130.0 (CH, C-7″), 128.9 (CH, C-6″, C-8″), 128.3 (CH, C-5″, C-9″), 117.9 (CH, C-2″), 62.8 (CH, C-2′), 46.1 (CH_2, C-5′), 45.7 (CH_2, C-2), 34.4 (CH_2, C-3′), 26.0 (CH, C-3), 22.5 (CH_3, C-4), 22.4 (CH_3, C-5), 21.4 (CH_2, C-4′); HRESIMS *m*/*z* 323.1732 [M + Na]$^+$ (calcd for $C_{18}H_{24}N_2O_2Na$, 323.1729).

Aglamide D (13): amorphous powder; $[\alpha]^{22}_D$ 0 (*c* 0.1, EtOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.20), 218 (4.22), 280 (4.35) nm; IR (film) ν_{max} 3422, 2937, 1654, 1612, 1450, 1410, 1258, 1176, 1083 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.62 (2H, m, H-5′, H-9′), 7.49 (1H, d, *J* = 15.6 Hz, H-3′), 7.39 (3H, m, H-6′, H-7′, H-8′), 6.87 (1H, d, *J* = 15.5 Hz, H-2′), 5.34 (1H, brs, H-2), 3.50 (1H, m, H-5′a), 3.29 (1H, m, H-5′b), 3.26 (3H, s, OCH_3-2), 2.04 (1H, m, H-3′a), 1.94 (1H, m, H-4′a), 1.83 (2H, m, H-3′b, 4′b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.6 (C, C-1′), 142.4 (CH, C-3′), 135.2 (C, C-4′), 131.0 (CH, C-7′), 129.9 (CH, C-6′, C-8′), 128.7 (CH, C-5′, C-9′), 119.6 (CH, C-2′), 88.8 (CH, C-2), 54.4 (CH_3, OCH_3-2), 46.5 (CH_2, C-5), 31.1 (CH_2, C-3), 21.2 (CH_2, C-4); HRESIMS *m*/*z* 254.1151 [M + Na]$^+$ (calcd for $C_{14}H_{17}NO_2Na$, 254.1151).

Biological Evaluation. Extracts and solvent partitions of the bark, leaves, and twigs of *A. edulis* were tested against the Lu1 (human lung carcinoma), LNCaP (hormone-dependent human prostate carcinoma), and HUVEC (human umbilical vein endothelial cells) cell lines, using established protocols.[37,38] Chromatographic fractions were monitored using the LNCaP cell line, and all isolates were evaluated with the Lu1, LNCaP, and MCF-7 (human breast carcinoma) and HUVEC cell lines. Aglaroxin 1-*O*-acetate (**2**) was tested in an in vivo P388 lymphocytic leukemia model, by intraperitoneal injection [4.5 mg/kg/inj., ip], according to a previously published protocol.[17]

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References and Notes

(1) Proksch, P.; Edrada, R.; Ebel, R.; Bohnenstengel, F. I.; Nugroho, B. W. *Curr. Org. Chem.* **2001**, *5*, 923−938.
(2) Kim, S.; Salim, A. A.; Swanson, S. M.; Kinghorn, A. D. *Anti-cancer Agents Med. Chem.* **2006**, *6*, 319−345.
(3) Brader, G.; Vajrodaya, S.; Greger, H.; Bacher, M.; Kalchhauser, H.; Hofer, O. *J. Nat. Prod.* **1998**, *61*, 1482−1490.
(4) Nugroho, B. W.; Edrada, R. A.; Wray, V.; Witte, L.; Bringmann, G.; Gehling, M.; Proksch, P. *Phytochemistry* **1999**, *51*, 367−376.
(5) Bacher, M.; Hofer, O.; Brader, G.; Vajrodaya, S.; Greger, H. *Phytochemistry* **1999**, *52*, 253−263.
(6) Dumontet, V.; Thoison, O.; Omobuwajo, O. R.; Martin, M.-T.; Perromat, G.; Chiaroni, A.; Riche, C.; Pais, M.; Sevenet, T.; Hadi, A. H. *Tetrahedron* **1996**, *52*, 6931−6942.
(7) Ohse, T.; Ohba, S.; Yamamoto, T.; Koyano, T.; Umezawa, K. *J. Nat. Prod.* **1996**, *59*, 650−652.
(8) Wu, T. S.; Liou, M. J.; Kuoh, C. S.; Teng, C. M.; Nagao, T.; Lee, K. H. *J. Nat. Prod.* **1997**, *60*, 606−608.
(9) Cui, B.; Chai, H.; Santisuk, T.; Reutrakul, V.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron* **1997**, *53*, 17625−17632.
(10) Lee, S. K.; Cui, B.; Mehta, R. R.; Kinghorn, A. D.; Pezzuto, J. M. *Chem. Biol. Interact.* **1998**, *115*, 215−218.
(11) Bohnenstengel, F. I.; Steube, K. G.; Meyer, C.; Nugroho, B. W.; Hung, P. D.; Kiet, L. C.; Proksch, P. *Z. Naturforsch., C* **1999**, *54*, 55−60.
(12) Bohnenstengel, F. I.; Steube, K. G.; Meyer, C.; Quentmeier, H.; Nugroho, B. W.; Proksch, P. *Z. Naturforsch., C* **1999**, *54*, 1075−1083.
(13) Xu, Y. J.; Wu, X. H.; Tan, B. K.; Lai, Y. H.; Vittal, J. J.; Imiyabir, Z.; Madani, L.; Khozirah, K. S.; Goh, S. H. *J. Nat. Prod.* **2000**, *63*, 473−476.
(14) Wang, S. K.; Duh, C. Y. *Planta Med.* **2001**, *67*, 555−557.
(15) Wang, S. K.; Cheng, Y. J.; Duh, C. Y. *J. Nat. Prod.* **2001**, *64*, 92−94.
(16) Hausott, B.; Greger, H.; Marian, B. *Int. J. Cancer* **2004**, *109*, 933−940.
(17) Hwang, B. Y.; Su, B.-N.; Chai, H.; Mi, Q.; Kardono, L. B. S.; Afriastini, J. J.; Riswan, S.; Santarsiero, B. D.; Mesecar, A. D.; Wild, R.; Fairchild, C. R.; Vite, G. D.; Rose, W. C.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Kinghorn, A. D. *J. Org. Chem.* **2004**, *69*, 3350−3358.
(18) Rivero-Cruz, J. F.; Chai, H. B.; Kardono, L. B. S.; Setyowati, F. M.; Afriastini, J. J.; Riswan, S.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2004**, *67*, 343−347.
(19) Su, B. N.; Chai, H.; Mi, Q.; Riswan, S.; Kardono, L. B. S.; Afriastini, J. J.; Santarsiero, B. D.; Mesecar, A. D.; Farnsworth, N. R.; Cordell, G. A.; Swanson, S. M.; Kinghorn, A. D. *Bioorg. Med. Chem.* **2006**, *14*, 960−972.
(20) King, M. L.; Chiang, C. C.; Ling, H. C.; Fujita, E.; Ochiai, M.; McPhail, A. T. *J. Chem. Soc., Chem. Commun.* **1982**, 1150−1151.
(21) Kinghorn, A. D.; Farnsworth, N. R.; Soejarto, D. D.; Cordell, G. A.; Pezzuto, J. M.; Udeani, G. O.; Wani, M. C.; Wall, M. E.; Navarro, H. A.; Kramer, R. A.; Menendez, A. T.; Fairchild, C. R.; Lane, K. E.; Forenza, S.; Vyas, D. M.; Lam, K. S.; Shu, Y.-Z. *Pure Appl. Chem.* **1999**, *71*, 1611−1618.
(22) Kinghorn, A. D.; Farnsworth, N. R.; Soejarto, D. D.; Cordell, G. A.; Swanson, S. M.; Pezzuto, J. M.; Wani, M. C.; Wall, M. E.; Oberlies, N. H.; Kroll, D. J.; Kramer, R. A.; Rose, W. C.; Vite, G. D.; Fairchild, C. R.; Peterson, R. W.; Wild, R. *Pharm. Biol.* **2003**, *41* (Suppl.), 53−67.
(23) Kim, S.; Su, B. N.; Riswan, S.; Kardono, L. B. S.; Afriastini, J. J.; Gallucci, J.; Chai, H.; Farnsworth, N. R.; Cordell, G. A.; Swanson, S. M.; Kinghorn, A. D. *Tetrahedron Lett.* **2005**, *46*, 9021−9024.
(24) Molleyres, L.-P.; Rindlisbacher, A.; Winkler, T.; Kumar, V. *Pestic. Sci.* **1999**, *55*, 494−497.
(25) Seger, C.; Hofer, O.; Greger, H. *Monatsh. Chem.* **2000**, *131*, 1161−1165.
(26) Mohamed, M. A.; Marzouk, M. S. A.; Moharram, F. A.; El-Sayed, M. M.; Baiuomy, A. R. *Phytochemistry* **2005**, *66*, 2780−2786.
(27) Horie, T.; Ohtsuru, Y.; Shibata, K.; Yamashita, K.; Tsukayama, M.; Kawamura, Y. *Phytochemistry* **1998**, *47*, 865−874.
(28) Dreyer, M.; Nugroho, B. W.; Bohnenstengel, F. I.; Ebel, R.; Wray, V.; Witte, L.; Bringmann, G.; Muhlbacher, J.; Herold, M.; Hung, P. D.; Kiet, L. C.; Proksch, P. *J. Nat. Prod.* **2001**, *64*, 415−420.
(29) Greger, H.; Pacher, T.; Brem, B.; Bacher, M.; Hofer, O. *Phytochemistry* **2001**, *57*, 57−64.
(30) Inada, A.; Sorano, T.; Murata, H.; Inatomi, Y.; Darnaedi, D.; Nakanishi, T. *Chem. Pharm. Bull.* **2001**, *49*, 1226−1228.
(31) Saifah, E.; Jongbunprasert, V.; Kelley, C. J. *J. Nat. Prod.* **1988**, *51*, 80−82.
(32) Saifah, E.; Puripattanavong, J.; Likhitwitayawuid, K.; Cordell, G. A.; Chai, H.; Pezzuto, J. M. *J. Nat. Prod.* **1993**, *56*, 473−477.
(33) Saifah, E.; Suttisri, R.; Shamsub, S.; Pengsuparp, T.; Lipipun, V. *Phytochemistry* **1999**, *52*, 1085−1088.
(34) Greger, H.; Pacher, T.; Vajrodaya, S.; Bacher, M.; Hofer, O. *J. Nat. Prod.* **2000**, *63*, 616−620.
(35) Chaidir; Lin, W. H.; Ebel, R.; Edrada, R. A.; Wray, V.; Nimtz, M.; Sumaryono, W.; Proksch, P. *J. Nat. Prod.* **2001**, *64*, 1216−1220.
(36) Wall, M. E.; Wani, M. C.; Brown, D. M.; Fullas, F.; Oswald, J. B.; Josephson, F. F.; Thornton, N. M.; Pezzuto, J. M.; Beecher. C. W. W.; Farnsworth, N. R.; Cordell, G. A.; Kinghorn, A. D. *Phytomedicine* **1996**, *3*, 281−285.
(37) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M.; Ruangrungsi, N. *J. Nat. Prod.* **1993**, *56*, 30−38.
(38) Seo, E.-K.; Kim, N.-C.; Mi, Q.; Chai, H.; Wall, M. E.; Wani, M. C.; Navarro, H. A.; Burgess, J. P.; Graham, J. G.; Cabieses, F.; Tan, G. T.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2001**, *64*, 1483−1485.

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