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Article

Lanostane Triterpenes, Flavanones and Acetogenins from the Roots of *Uvaria siamensis* and Their Cytotoxic Activity

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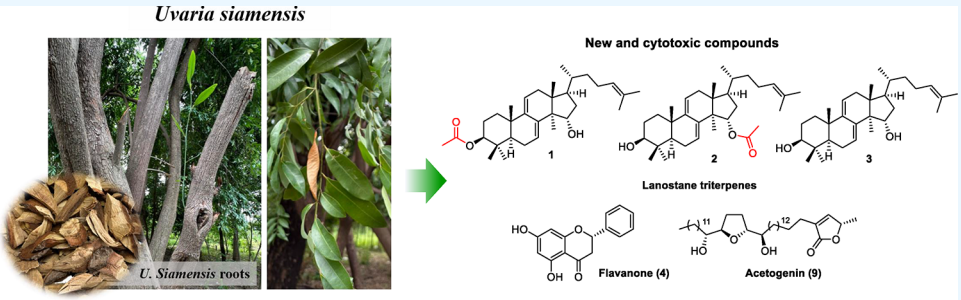

ABSTRACT: Our phytochemical investigation of the roots of *Uvaria siamensis* led to the isolation of two new lanostane triterpenes, 3-acetylpolycarpol (**1**) and 15-acetylpolycarpol (**2**), as well as 15 known compounds (**3**−**17**). The structures of the isolated compounds were elucidated by an analysis of spectroscopic data. Compounds **1**−**9** were tested against nonsmall cell lung cancer cells (A549) and human cervical carcinoma cells (HeLa) using an MTT assay. Polycarpol (**3**) and uvariamicin-II (**9**) exhibited potent cytotoxicity against A549 cancer cells, while 15-acetylpolycarpol (**2**) and pinocembrin (**4**) displayed potent cytotoxicity against HeLa cancer cells. Further analysis of the apoptosis-inducing properties revealed that uvariamicin-II (**9**) and pinocembrin (**4**) induced apoptosis in a dose-dependent manner in A549 and HeLa cells, respectively. These two compounds also showed weak cytotoxicity toward *Vero* cells.

1. INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for the highest mortality rates among both men and women. In 2020, an estimated 1.8 million people died from lung cancer, accounting for 18% of all cancer-related deaths.[1] In Thailand, 23,713 cases were reported in 2020, contributing to 12.4% of all cancer-related deaths.[2] Cervical cancer is also one of the most common cancers in Thailand, and it is the fourth most common cancer in women worldwide.[3] Current cancer treatments include immunotherapy, radiation, and targeted therapies. Chemotherapy is one of the most widely used cancer treatments. However, toxicity and side effects against healthy cells are common problems for patients.[4] Natural products have played a crucial role in the discovery of anticancer drugs. More than 80% of small-molecule cancer drugs approved by the FDA between 1981 and 2019 are natural products or their derivatives. Trabectedin, romidepsin, and ingenol mebutate are examples of such compounds.[5]

Uvaria siamensis (Scheff.) L.L.Zhou, Y.C.F.Su & R.M.K.Saunders (formerly *Melodorum fruticosum* Lour.) is a flowering tree with fragrant yellow flowers belonging to the Annonaceae family.[6] Previous phytochemical studies of *U. siamensis*, including our earlier work on the leaves and flowers, revealed the presence of heptenolides, dihydrochalcones, flavonoids, and aromatic amides in different parts of the plant such as flowers, leaves, stem, and stem bark.[6−8] As part of our continuous efforts in search of bioactive metabolites from natural sources in Thailand,[9,10] we decided to investigate the roots of *U. siamensis*. In this work, we report the isolation of two new lanostane triterpenoids (**1** and **2**) and 15 known compounds. The latter consist of polycarpol (**3**),[11] pinocembrin (**4**),[11] isochamanetin (**5**),[12] chamanetin (**6**),[12] dichamanetin (**7**),[11] 7-*O*-methyldichamanetin (**8**),[13] uvariamicin-II (**9**),[14] (4*E*)-7-benzoyloxy-6-hydroxy-2,4-heptadien-4-olide (**10**),[8] (4*E*)-6-acetoxy-7-benzoyloxy-2,4-heptadien-4-olide (acetylmelodorinol) (**11**),[6] isomelodrinol (**12**),[6] (4*Z*)-6-acetoxy-7-benzoyloxy-2,4-heptadien-4-olide (**13**),[6] benzyl benzoate (**14**),[15] 2-methoxybenzyl benzoate (**15**),[15] benzoic acid (**16**),[8] and 6α-methoxycyperene (**17**).[16] Their structures were

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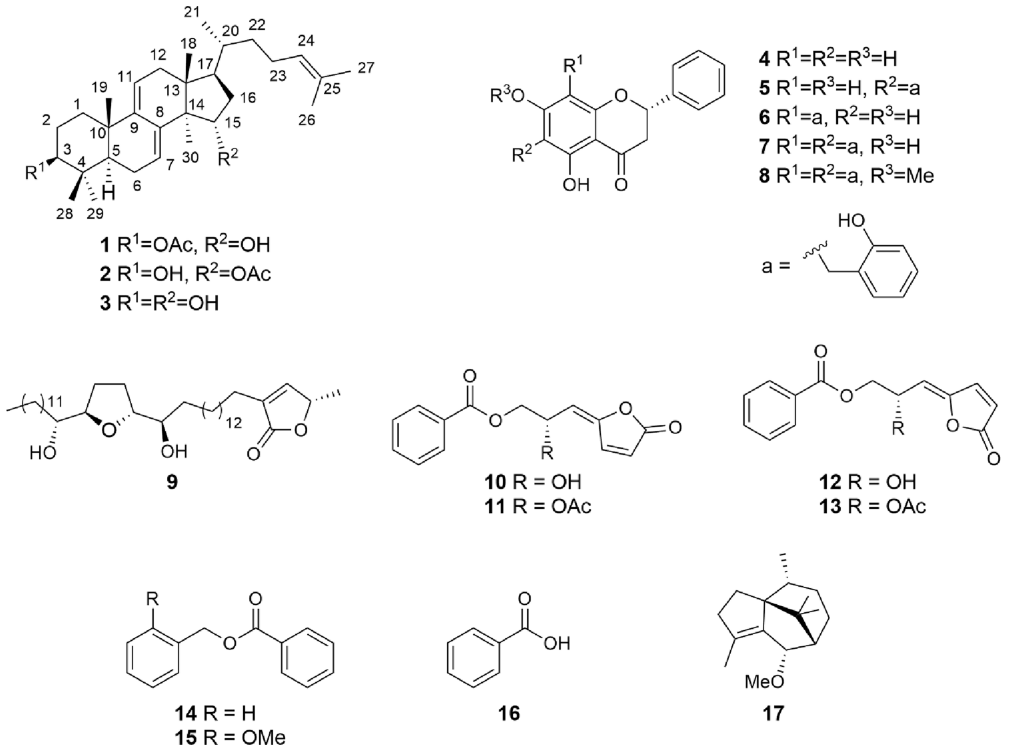

Figure 1. Skeletal (shorthand) structures of compounds **1**−**17**.

elucidated by analysis of NMR and ESI-MS data and by comparison of their spectroscopic data with those reported in the literature. The skeletal (shorthand) structures of the isolated compounds are shown in Figure 1.

2. RESULTS AND DISCUSSION

Compound **1** was obtained as a white, amorphous solid. Its molecular formula was determined as $C_{32}H_{50}O_3$ from the positive-ion ESI-TOF MS (*m*/*z* 483.3833 $[M + H]^+$, calcd 483.3833). The IR spectrum of **1** showed absorption bands of hydroxy (3371 cm^{-1}) and ester carbonyl (1730 cm^{-1}) groups. The ^1H NMR spectrum showed signals of three olefinic protons at δ_H 5.84 (d, *J* = 6.4 Hz, H-7), 5.30 (d, *J* = 6.8 Hz, H-11) and 5.08 (td, *J* = 6.8, 1.6 Hz, H-24); two oxygenated methine protons at δ_H 4.50 (dd, *J* = 11.2, 4.4 Hz, H-3) and 4.27 (dd, *J* = 9.2, 5.2 Hz, H-15); nine methyl groups at δ_H 2.05 (s, 3-COMe), 1.68 (s, H-27), 1.60 (s, H-26), 1.00 (s, H-19), 0.95 (s, H-29), 0.93 (s, H-30), 0.88 (d, *J* = 8.0 Hz, H-21), 0.88 (s, H-28), and 0.60 (s, H-18); three methine protons at δ_H 1.65 (m, H-17), 1.33 (m, H-20) and1.18 (dd, *J* = 11.6, 4.0 Hz H-5); and seven methylene protons. The ^{13}C (Table 1), DEPTQ, and HSQC NMR spectra showed 32 carbon peaks. They include six olefinic carbons at δ_C 146.0 (C-9), 141.1 (C-8), 131.3 (C-25), 121.2 (C-7), 125.1 (C-24), and 116.5 (C-11); two oxygenated methine carbons at δ_C 80.9 (C-3) and 74.9 (C-15); and acetyl carbons at δ_C 171.1 and 21.5. Moreover, eight methyl, seven methylene, three methine, and four quaternary carbon signals were detected. The NMR data are shown in Table 1.

The $^1H-^1H$ COSY and HMBC correlations (Figure 2) indicated the presence of four spin systems, enabling the assignment of fragments C-1/C-2/C-3, C-5/C-6/C-7, C-11/C-12, and C-15/C-16/C-17/C-20(C-21)/C-22/C-23/C-24. The key HMBC correlations from H_3-28(H_3-29) to C-3, C-4, and C-5 indicated the location of an oxygenated carbon at C-3, which was esterified. HMBC correlation between H-3 and the acetyl carbon at δ_C 171.1 confirmed that the acetyl group was located at the oxygen of C-3. The HMBC correlations between H_3-30 and C-8, C-13, C-14, and C-15 led to the assignment of an oxygenated carbon at C-15. The presence of a double bond at C-24(25) was deduced from HMBC correlations of H_3-26 and H_3-27 with C-24 and C-25. The NMR data of compound **1** were very similar to those of polycarpol (**3**),[11] with slight differences arising from an additional acetyl group esterifying the C-3 hydroxy group in **1**. The NOESY (Figure 3) correlations between H-3/H-5 and H-17/H_3-21, and between H-15/H_3-18 indicated that all of these hydrogens are on the same face. Comparison of the optical rotation of compound **1** ($[\alpha]_D^{25}$ = +82, *c* = 1.00 in $CHCl_3$) with that of polycarpol (**3**) ($[\alpha]_D^{29}$ = +66, *c* = 0.71 in $CHCl_3$)[17] suggested that the absolute configuration of compound **1** was identical to that of **3**. Therefore, the structure of **1** was established as shown in Figure 1.

Compound **2** was obtained as a white, amorphous solid. Its molecular formula was determined as $C_{32}H_{50}O_3$ from the pseudomolecular ion at *m*/*z* 483.3829 $[M + H]^+$ (calcd 483.3833) in positive ESI-TOF MS. The IR spectrum of **2** showed absorption bands of hydroxy (3371 cm^{-1}) and ester carbonyl (1730 cm^{-1}) groups. The ^1H and ^{13}C NMR data of compound **2** (Table 1) were very similar to those of compound **1** and polycarpol (**3**). Analysis of 2D NMR spectra (Figure 2) led to the conclusion that the acetyl moiety [δ_H 2.08 (m)/ δ_C 171.4 and 21.6] was attached to the oxygen at C-15 [δ_H 5.07 (m)/ δ_C 77.7] in compound **2** instead of the oxygen at C-3 in compound **1**. This assignment was supported by the key HMBC correlation between H-15 and the 15-OAc carbonyl carbon (δ_C = 171.4). Analysis of NOESY correlations (Figure 3) indicated that compound **2** shared the same relative configuration with compound **1**. Compound **2** had a positive value of optical rotation ($[\alpha]_D^{25}$ = +86, *c* = 1.00 in $CHCl_3$),

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data of 1 and 2 in $CDCl_3$ (δ in ppm, J in Hz, $CDCl_3$)

	1		2	
position	δ_H	δ_C	δ_H	δ_C
1	1.99, m	35.6	1.97, m	35.8
	1.53, m		1.42, m	
2	1.75, m	24.4	1.75, m	27.9
	1.70, m		1.66, m	
3	4.50, dd (11.2, 4.4)	80.9	3.24, dd (11.2, 4.4)	79.0
4		37.7		38.8
5	1.18, dd (11.6, 4.0)	49.2	1.08, dd (11.6, 4.4)	49.0
6	2.15, m	22.9	2.04, m	23.0
7	5.84, d (6.4)	121.2	5.47, d (6.4)	121.4
8		141.1		140.4
9		146.0		146.0
10		37.4		37.6
11	5.30, d (6.8)	116.5	5.31, d (6.4)	116.2
12	2.30, d (19.2)	38.6	2.30, d (16.8)	38.2
	2.07, m		2.05, m	
13		44.5		44.2
14		52.1		51.4
15	4.27, dd (9.2, 5.2)	74.9	5.07, m	77.7
16	1.96, m	40.3	2.00, m	25.1
	1.73, m		1.85, m	
17	1.65, m	49.0	1.67, m	49.1
18	0.60, s	16.1	0.65, s	16.0
19	1.00, s	23.0	0.96, s	23.0
20	1.33, m	35.9	1.35, m	35.9
21	0.88, d (8.0)	18.5	0.88, d (8.0)	18.5
22	2.05, m	25.9	2.01, m	25.1
	1.87, m		1.68, m	
23	1.94, m	36.4	1.99, m	35.8
	1.65, m		1.43, m	
24	5.08, td (6.8, 1.6)	125.1	5.07, m	125.0
25		131.3		131.3
26	1.60, s	25.9	1.59, s	25.9
27	1.68, s	17.2	1.67, s	17.8
28	0.88, s	28.2	1.01, s	28.3
29	0.95, s	17.1	0.87, s	18.4
30	0.93, s	17.8	1.00, s	16.1
$-OCOCH_3$	2.05, s	21.5	2.08, s	21.6
$-OCOCH_3$		171.1		171.4

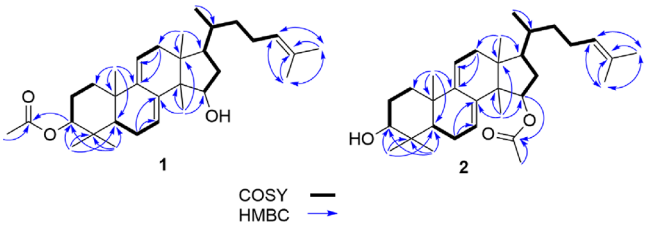

Figure 2. HMBC and $^{1}H-^{1}H$ COSY correlations of compounds 1 and 2.

which was similar to that of compound 1 and polycarpol (3). Therefore, the absolute configuration of compound 3 should be identical to that of polycarpol (3).[11]

The cytotoxicity of compounds 1−9 on nonsmall cell lung cancer cells (A549) and human cervical carcinoma cells (HeLa) was investigated using an MTT assay. Polycarpol (3) and uvariamicin-II (9) exhibited potent cytotoxic activity

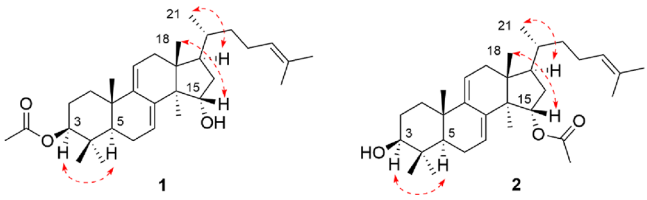

Figure 3. Selected NOESY correlations of compounds 1 and 2.

toward the A549 cell line with IC_{50} values of 6.1 and 5.0 μM, respectively, while Compound 7 demonstrated strong cytotoxic activity with an IC_{50} value of 14.9 μM (Table 2).

Table 2. Cytotoxicity of Isolated Compounds 1−9[a]

	IC_{50} (μM)		
compound	A549	HeLa	*Vero*
1	inactive	26.2 ± 3.0	36.9 ± 2.8
2	inactive	4.6 ± 2.1	17.8 ± 10.8
3	6.1 ± 0.4	10.3 ± 0.9	7.5 ± 5.2
4	inactive	2.71 ± 0.9	103.3 ± 10.9
5	inactive	inactive	inactive
6	inactive	inactive	inactive
7	14.9 ± 0.6	inactive	48.7 ± 2.3
8	inactive	inactive	inactive
9	5.0 ± 2.6	inactive	59.6 ± 17.6
doxorubicin	0.04 ± 0.0	0.12 ± 0.0	2.1 ± 0.44

[a]Cell viability was measured using the MTT assay after 72 h of incubation. Data are expressed as the mean ± SD of three independent experiments.

For HeLa cancer cells, 15-acetylpolycarpol (2) and pinocembrin (4) displayed potent cytotoxic activity with IC_{50} values of 4.6 and 2.7 μM, respectively. Compounds 1 displayed strong cytotoxic activity with an IC_{50} value of 10.3 μM, while compound 3 exhibited moderate cytotoxic activity with an IC_{50} value of 26.2 μM. Interestingly, compounds 1−2 and 4−9 showed weak cytotoxic activity against *Vero* cells or were inactive. Polycarpol (3), on the other hand, displayed potent cytotoxic activity with an IC_{50} value of 7.5 μM. Compounds 4 and 9 were therefore selective toward cancer cell lines.

It is worth mentioning that 3-acetylpolycarpol (1), 15-acetylpolycarpol (2), and polycarpol (3) are structurally very similar (difference of an acetyl group), and yet, their cytotoxic effects were quite different. Derivatives with an acetyl group, 3-acetylpolycarpol (1) and 15-acetylpolycarpol (2), selectively exhibited cytotoxic activity against HeLa cancer cells, while that without an acetyl moiety, polycarpol (3), selectively inhibited the growth of A549 cancer cells (Table 2). Polycarpol (3) has been isolated from many plants, including *Cleistochlamys kirkii*,[18] *Xylopia pierrei*,[17] and *Sphaerocoryne gracilis*.[19] Polycarpol (3) was previously reported to have cytotoxic activity against the NCI-H187 cancer cell line with an IC_{50} value of 45.5 μM, while pinocembrin (4) was inactive against this cancer cell line.[17] Pinocembrin (4) was formerly reported to have broad biological activities including anti-inflammatory, antioxidant, antimicrobial, and anticancer activities,[20] and it was reported to exhibit cytotoxic activity against HCT 116 colorectal cancer cells.[21] However, modifications to its structure, specifically the addition of a 2-hydroxybenzyl group at either the C-6 (compound 5) or C-8 (compound 6) positions, appear to diminish its cytotoxic effects against the HeLa cell line. Despite this reduction in

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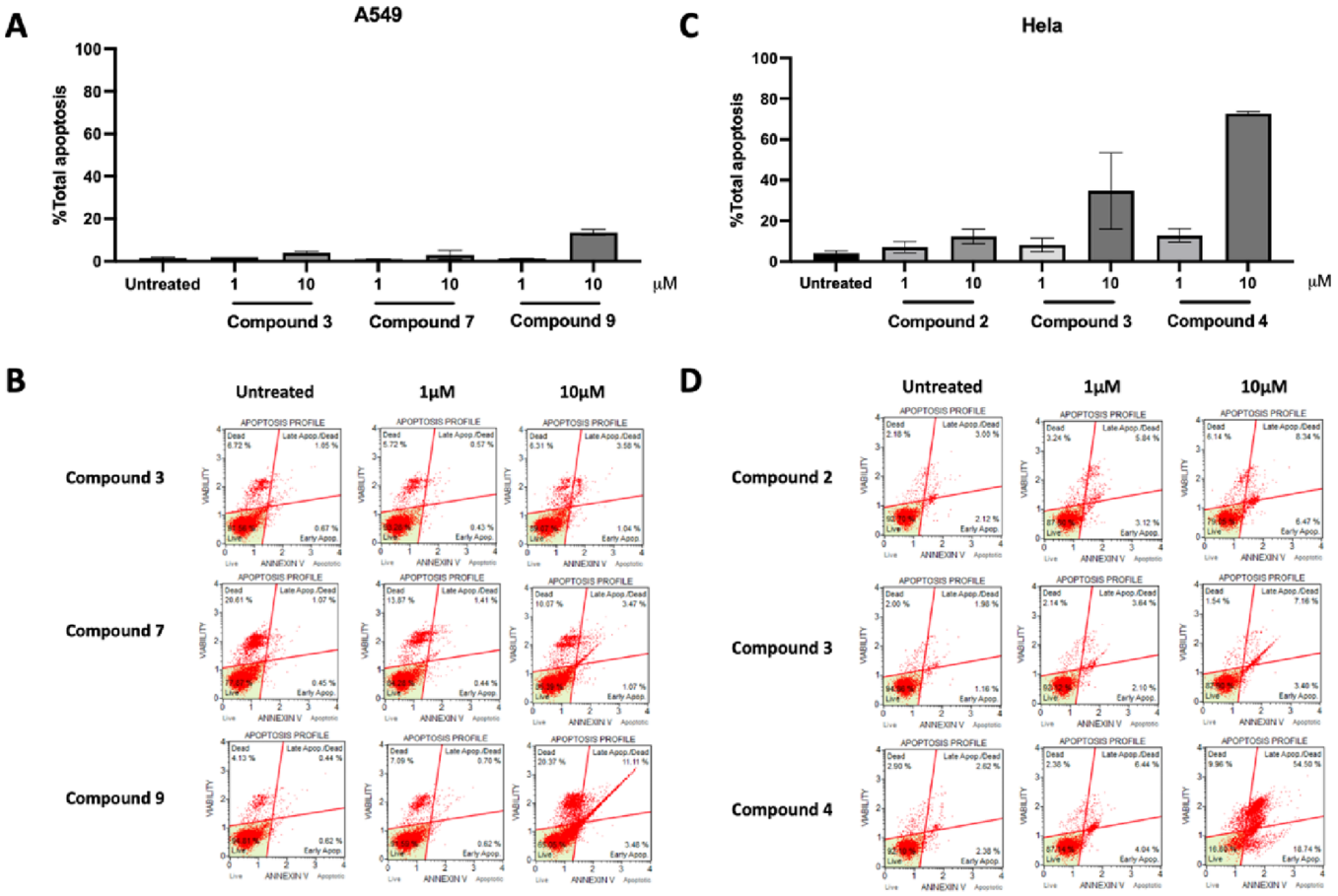

Figure 4. Effect of the selected anticancer compounds on apoptosis induction on cancer cell lines. The compound-treated A549 (A, B) and HeLa (C, D) cells were analyzed using the MUSE cell analyzer. Bar graph represents total apoptotic cells (Annexin-V+/7-AAD-and Annexin-V+/7-AAD +). Representative scattering plots represent stained cells under the desired treatment condition.

activity against HeLa cells, these structural changes do not render the compounds entirely inactive. Interestingly, the concurrent addition of the 2-hydroxybenzyl group at both the C-6 and C-8 positions enhances cytotoxic effects against the A549 lung cancer cell line, as observed in compound **7**. Moreover, compound **8**, which contains a methoxy group at the C-7 position, exhibits a complete loss of cytotoxic activity. This suggests that the incorporation of a 2-hydroxybenzyl group in pinocembrin derivatives plays a critical role in modulating their bioactivity. Uvariamicin-II (**9**) is known to exhibit cytotoxic activity against A549, HeLa, MCF-7 (human breast carcinoma), MKN-45 (human gastric adenocarcinoma), and SMMC-7721 and HepG2 (human hepatoma carcinoma) cancer cell lines.[22]

Many natural products isolated from various biosources exhibit cytotoxic activity by modulating apoptosis.[5] Programmed cell death, or apoptosis, is a multifactorial process that eliminates unwanted cells without any inflammatory response in an energy-dependent manner.[23] Acetogenins, specifically uvamicranins A−E, which were isolated from the stems of *Uvaria micrantha*, exhibited cytotoxic activity through apoptosis induction, leading to cell cycle arrest in the human hepatocellular carcinoma cell line.[24]

In the present work, we selected the most potent anticancer compounds, e.g., compounds **2**−**4**, **7**, and **9** for further investigation on A549 and HeLa cell lines. As shown in Figure 4, uvariamicin-II (**9**) exhibited the highest level of apoptosis in A549 cells (15%) in a dose-dependent manner (Figure 4A,B). Polycarpol (**3**) and dichamanetin (**7**) displayed a moderate level of apoptosis when compared to the untreated condition; cells treated with both **3** and **7** maintained a normal morphology and density. As mentioned above, 15-acetylpolycarpol (**2**) and pinocembrin (**4**) showed promising anticancer activity on HeLa cells with IC_{50} values below 10 μM (Table 2). Pinocembrin (**4**) increased apoptotic cells in a dose-dependent manner (Figure 4C,D), similarly, polycarpol (**3**) also induced apoptosis in HeLa cells in a dose-dependent manner, as evidenced by an increase in apoptotic cell populations at both tested concentrations (Figure 4C,D). Although the effect was less pronounced compared with pinocembrin (**4**), polycarpol still demonstrated notable apoptotic activity. However, 15-acetylpolycarpol (**2**) did not induce apoptosis in HeLa cells, even at the concentration of 10 μM, suggesting an alternative cell death pathway for **2**.

3. CONCLUSIONS

Seventeen compounds, including two new polycarpol derivatives (**1**−**2**) and 15 known compounds (**3**−**17**), were isolated from the roots of *U. siamensis*. Among the isolated compounds, polycarpol (**3**), and uvariamicin-II (**9**) displayed cytotoxic activity toward the A549 cell line, while the new lanostane triterpenes **1** and **2** exhibited cytotoxic activity against the HeLa cell line. Pinocembrin (**4**) and uvariamicin-II (**9**) were selective toward cancer cell lines. Additionally, this work

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demonstrated that compounds **4** and **9** could induce the apoptosis of HeLa and A549, respectively.

4. EXPERIMENTAL SECTION

4.1. General Procedures. Optical rotations were measured by using a JASCODIP-1000 digital polarimeter (JASCO Inc., Japan). UV spectra were obtained by using a JASCO J-810 apparatus. IR spectra were obtained using a Bruker Tenser 27 spectrophotometer (Bruker, Germany). NMR spectra were acquired on a Bruker Avance 400 NMR spectrometer (Bruker, Germany) using $CDCl_3$ and CD_3OD as solvents. Chemical shifts were recorded in δ (ppm) using the solvent's residual peak (^{1}H NMR) or the solvent's peak (^{13}C NMR) as internal standards. The HRESITOFMS were carried out on a Bruker micrOTOF mass spectrometer (Bruker, Germany). Column chromatography (CC) was carried out on MERCK silica gel 60 (230−400 mesh) (Merck, Darmstadt, Germany), and Sephadex LH-20 was used for size-exclusion chromatography. Thin-layer chromatography was carried out with precoated MERCK silica gel 60 PF254 (Merck, Darmstadt, Germany); the spots were visualized under UV light (254 and 365 nm) and further stained by spraying *p*-anisaldehyde and then heated until charred. Unless otherwise noted, all chemicals were obtained from commercially available sources and were used without further purification. Solvent ratios are based on volumes.

4.2. Plant Material. The roots of *U. siamensis* (Annonaceae) were collected from Amphoe Chum Phae, Khon Kaen Province, Thailand (coordinates: N 16°39′08.1″ E 102°04′18.5″). Prof. Dr. Pranom Chantaranothai from the Faculty of Science at Khon Kaen University identified the plant. A voucher specimen, identified as AVS-NPR001, was deposited to the Faculty of Applied Science at King Mongkut's University of Technology North Bangkok.

4.3. Extraction and Isolation. The air-dried powdered roots of *U. siamensis* (1.5 kg) were extracted separately with *n*-hexane and EtOAc (each 3 × 10 L) by maceration in each solvent at room temperature for 3 days. Removal of solvents under reduced pressure gave the crude hexane (5.00 g) and EtOAc extracts (25.0 g).

Five grams of the crude hexane extract were fractionated over silica gel column chromatography (CC), eluting with a gradient of *n*-hexane:EtOAc by increasing polarity. Every fraction (200 mL) was analyzed by TLC. Fractions with similar TLC patterns were combined to give four pooled fractions, URH_1−URH_4. Fraction URH_3 was purified by silica gel CC, eluting with an isocratic system of *n*-hexane:CH_2Cl_2 followed by Sephadex LH-20 CC with 100% MeOH to give compounds **1** (4.0 mg), **2** (10.5 mg) and **3** (20.5 mg) as white amorphous powders. Fraction URH_4 was separated by silica gel CC, eluting with pure CH_2Cl_2 to yield compounds **14** (10.0 mg), **15** (5.2 mg), and **17** (25.3 mg) as colorless oils.

Twenty-five grams of the crude EtOAc extract were fractionated over silica gel, eluting with a gradient system of *n*-hexane:EtOAc and then EtOAc:MeOH, by increasing polarity, to give seven fractions. Fractions with similar TLC patterns were combined to provide ten pooled fractions, URE_1−URE_7. Fraction URE_3 was washed with *n*-hexane and then crystallized in EtOAc to afford an additional amount of compound **3** (3.26 g) as a white crystal. Fraction URE_4 was fractionated over Sephadex LH-20 with 100% MeOH to yield five subfractions, $URE_{4.1}$−$URE_{4.5}$. Subfraction $URE_{4.2}$ was purified by CC, eluting with *n*-hexane:CH_2Cl_2 (7:3) to afford compounds **10** (13.0 mg) and **13** (5.6 mg) as brown, viscous oils. Subfraction $URE_{4.3}$ was purified over Sephadex LH-20 with 100% MeOH to give compound **9** (30.0 mg) as a pale-yellow oil, and compounds **11** (9.0 mg) and **12** (7.5 mg) as brown viscous oils. Fraction URE_7 was separated by silica gel CC, eluting with a gradient system of *n*-hexane:EtOAc to give three subfractions, namely, $URE_{7.1}$−$URE_{7.3}$. Subfraction $URE_{7.1}$ was further purified by silica gel CC, eluting with an isocratic system of *n*-hexane:EtOAc (4:1), to give compound **4** as a pale-yellow solid (13.0 mg), compound **5** as a yellow viscous oil (20.3 mg), and compound **8** as a pale yellow viscous oil (35.2 mg). Subfraction $URE_{7.3}$ was purified by silica gel CC, eluting with an isocratic system of *n*-hexane:EtOAc (7:3) to provide compounds **6** (16.2 mg) and **7** (15.4 mg) as a pale yellow, viscous oil.

*4.3.1. 3-Acetylpolycarpol (**1**).* White amorphous solid; IR (neat) ν_{max}: 3302, 2922, 1711, 1459, 1373, 1263, 1024, 820, 750 cm^{-1}; see Table 1 for ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz); HRESIMS *m*/*z*: 483.3833 [M + H]$^+$, calcd for $C_{32}H_{50}O_3^+$, 483.3833).

*4.3.2. 15-Acetylpolycarpol (**2**).* White amorphous solid; IR (neat) ν_{max}: 3271, 2962, 2924, 1730, 1449, 1372, 1247, 1085, 1037, 988, 907, 820, 737 cm^{-1}; see Table 1 for ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz); HRESIMS *m*/*z*: 483.3829 [M + H]$^+$, calcd for $C_{32}H_{51}O_3^+$, 483.3833).

4.4. Cell Culture. A549 (CCL-185), HeLa (CCL-2), and *Vero* (CCL-81) cells were purchased from the American Type Culture Collection. The cells were routinely grown in Dulbecco's modified Eagle medium (DMEM) (Gibco) supplemented with 10% FBS (Cytiva), and 1% penicillin-streptomycin solution (Gibco) at 37 °C, 5% CO_2 in a humidified atmosphere in anincubator. The cells were passaged every 3 days.

4.5. Cytotoxicity Assay. The cytotoxic activity of the isolated compounds was evaluated by MTT assay as previously described.[25] A549 (5 × 10^3), HeLa (1 × 10^3), and *Vero* (2 × 10^3) cells were plated into a 96-well plate. After 16−18 h, the attached cells were replenished with a fresh culture medium containing various concentrations of each compound and incubated for another 72 h. At that time, the treated cells were replaced with fresh culture medium supplemented with 0.5 mg/mL MTT solution. After 3 h of incubation, the medium was discarded, and the formed purple formazan crystals were completely dissolved by adding 50 μL of DMSO. Absorbance was measured at 570 and 630 nm wavelengths as a measurement and reference wavelength, respectively. The data were calculated and represented as half-inhibitory concentration (IC_{50}). The IC_{50} values were determined using nonlinear regression analysis of the dose−response curves, performed with Prism 10 software. Doxorubicin was used as a positive control drug.

4.6. Apoptosis Analysis. A549 and HeLa cells were treated under the desired conditions. After 72 h, both the floating cells and attached cells were harvested. The harvested cells were resuspended with fresh medium before staining. MUSE AnnexinV and Dead Kit were used to assess the apoptotic cell population. The staining procedure was performed following the kit's instructions. The stained cells were analyzed using a MUSE Cell Analyzer. Four distinct cell populations were identified using Annexin-V/7-AAD staining: viable cells (Annexin-V-/7-AAD-), early apoptotic cells (Annexin-V+/7-AAD-), late apoptotic cells (Annexin-V+/7-

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AAD+), and necrotic cells (Annexin-V-/7-AAD+). Cell percentage was plotted on a bar graph. The experiments were performed in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c09267.

HRESIMS spectrogram, ^{1}H NMR, ^{13}C NMR, DEPTQ, COSY, HSQC, HMBC, and NOESY spectral of compounds **1** and **2** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) World Health Organization *Cervical cancer*.

(2) Somsunun, K.; Prapamontol, T.; Pothirat, C.; Liwsrisakun, C.; Pongnikorn, D.; Fongmoon, D.; Chantara, S.; Wongpoomchai, R.; Naksen, W.; Autsavapromporn, N.; Tokonami, S. Estimation of Lung Cancer Deaths Attributable to Indoor Radon Exposure in Upper Northern Thailand. *Sci. Rep* **2022**, *12* (1), 5169.

(3) World Health Organization *Lung cancer*.

(4) van den Boogaard, W. M. C.; Komninos, D. S. J.; Vermeij, W. P. Chemotherapy Side-Effects: Not All DNA Damage Is Equal. *Cancers (Basel)* **2022**, *14* (3), 627.

(5) Newman, D. J.; Cragg, G. M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod* **2020**, *83* (3), 770−803.

(6) Schevenels, F. T.; Jadsadajerm, S.; Lekphrom, R.; Yodsin, N.; Suebrasri, T.; Senawong, T.; Wisetsai, A. Siamfuranones A−C, Three Novel Furanone Derivatives from the Flowers of *Uvaria Siamensis* and Their Biological Activities. *Nat. Prod Res.* **2024**, 1−10.

(7) Nguyen, H. T. M.; Ngo, D. T. T.; Nguyen, P. D. N.; Pham, T. N. K.; Do, L. T. M.; Sichaem, J. New Prenyl Flavanone and Diarylbutanol from *Uvaria Siamensis* Stem Bark and Their *α* -Glucosidase Inhibitory Activity. *Nat. Prod. Res.* **2023**, *38*, 3982−3988.

(8) Wongwad, E.; Jadsadajerm, S.; Mungmai, L.; Wisetsai, A. Antioxidant, Cytotoxic, Anti-Glycation, and Anti-Tyrosinase Compounds from the Leaves of *Uvaria Siamensis*. *Chem. Biodiversity* **2024**, *21* (4), No. e202400319.

(9) Wisetsai, A.; Jadsadajerm, S.; Bua-art, S. Antiphytopathogenic Activity of the Bioluminescent Mushroom *Neonothopanus Nambi* against Root-Rot Disease. *Nat. Prod Res.* **2024**, *38* (6), 1085−1088.

(10) Wisetsai, A.; Lekphrom, R.; Suebrasri, T.; Tontapha, S.; Senawong, T.; Pudhom, K.; Choodej, S.; Schevenels, F. T. Acrotrione B, a Prenylated and Highly Oxidized Xanthenoid with Antibacterial and Anti-Proliferative Activities from the Roots of *Acronychia Pedunculata*. *Planta Med.* **2023**, *89* (04), 416−422.

(11) Jung, J. H.; Pummangura, S.; Chaichantipyuth, C.; Patarapanich, C.; McLaughlin, J. L. Bioactive Constituents of *Melodorum Fruticosum*. *Phytochemistry* **1990**, *29* (5), 1667−1670.

(12) Achenbach, H.; Höhn, M.; Waibel, R.; Nkunya, M. H. H.; Jonker, S. A.; Muhie, S. Oxygenated Pyrenes, Their Potential Biosynthetic Precursor and Benzylated Dihydroflavones from Two African *Uvaria* Species. *Phytochemistry* **1997**, *44* (2), 359−364.

(13) El-Sohly, H. N.; Lasswell, W. L.; Hufford, C. D. Two New C-Benzylated Flavanones From *Uvaria Chamae* and ^{13} C NMR Analysis of Flavanone Methyl Ethers. *J. Nat. Prod* **1979**, *42* (3), 264−270.

(14) Hisham, A.; Pieters, L. A. C.; Claeys, M.; Esmans, E.; Dommisse, R.; Vlietinck, A. J. Uvariamicin-I, II and III: Three Novel Acetogenins from *Uvaria Narum*. *Tetrahedron Lett.* **1990**, *31* (32), 4649−4652.

(15) Kodpinid, M.; Thebtaranonth, C.; Thebtaranonth, Y. Benzyl Benzoates and O-Hydroxybenzyl Flavanones from *Uvaria Ferruginea*. *Phytochemistry* **1985**, *24* (12), 3071−3072.

(16) Lekphrom, R.; Kanokmedhakul, K.; Schevenels, F.; Kanokmedhakul, S. Antimalarial Polyoxygenated Cyclohexene Derivatives from the Roots of *Uvaria Cherrevensis*. *Fitoterapia* **2018**, *127*, 420−424.

(17) Chokchaisiri, R.; Kunkaewom, S.; Chokchaisiri, S.; Ganranoo, L.; Chalermglin, R.; Suksamrarn, A. Potent Cytotoxicity against Human Small Cell Lung Cancer Cells of the Heptenes from the Stem Bark of *Xylopia Pierrei* Hance. *Med. Chem. Res.* **2017**, *26* (6), 1291−1296.

(18) Samwel, S.; Mdachi, S. J. M.; Nkunya, M. H. H.; Irungu, B. N.; Moshi, M. J.; Moulton, B.; Luisi, B. S. Cleistenolide and Cleistodienol: Novel Bioactive Constituents of *Cleistochlamys Kirkii*. *Nat. Prod. Commun.* **2007**, *2* (7), 737.

(19) Maeda, G.; Munissi, J. J. E.; Lindblad, S.; Duffy, S.; Pelletier, J.; Avery, V. M.; Nyandoro, S. S.; Erdélyi, M. A Meroisoprenoid, Heptenolides, and *C* -Benzylated Flavonoids from *Sphaerocoryne Gracilis* Ssp. *Gracilis*. *J. Nat. Prod* **2020**, *83* (2), 316−322.

(20) Rasul, A.; Millimouno, F. M.; Ali Eltayb, W.; Ali, M.; Li, J.; Li, X. Pinocembrin: A Novel Natural Compound with Versatile Pharmacological and Biological Activities. *Biomed Res. Int.* **2013**, *2013*, 1−9.

(21) Kumar, N.; Biswas, S.; Hosur Shrungeswara, A.; Basu Mallik, S.; Hipolith Viji, M.; Elizabeth Mathew, J.; Mathew, J.; Nandakumar, K.; Lobo, R. Pinocembrin Enriched Fraction of *Elytranthe Parasitica* (L.) Danser Induces Apoptosis in HCT 116 Colorectal Cancer Cells. *J. Infect Chemother* **2017**, *23* (6), 354−359.
(22) Chen, Y.; Qiu, Y.; Miao, Y.; Yuan, F.; Chen, J.; Li, X. SARs of ACGs against Diverse Human Tumor Cells. *Medicinal Chemistry Research* **2015**, *24* (8), 3212−3217.
(23) Elmore, S. Apoptosis: A Review of Programmed Cell Death. *Toxicol Pathol* **2007**, *35* (4), 495−516.
(24) Thongnest, S.; Boonsombat, J.; Keeratichamroen, S.; Lirdprapamongkol, K.; Kaweetripob, W.; Kheawchaum, S.; Mahidol, C.; Svasti, J.; Ruchirawat, S.; Prawat, H. Acetogenins from the Stems of *Uvaria Micrantha* Showing Antiproliferative Effects on HepG2 Liver Cancer Cells. *Phytochemistry* **2022**, *204*, No. 113450.
(25) Sae-Lim, P.; Seetaha, S.; Tabtimmai, L.; Suphakun, P.; Kiriwan, D.; Panichayupakaranant, P.; Choowongkomon, K. Chamuangone from *Garcinia Cowa* Leaves Inhibits Cell Proliferation and Migration and Induces Cell Apoptosis in Human Cervical Cancer *in Vitro*. *J. Pharm. Pharmacol* **2020**, *72* (3), 470−480.

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