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Bioactive 6*S*-Styryllactone Constituents of *Polyalthia parviflora*

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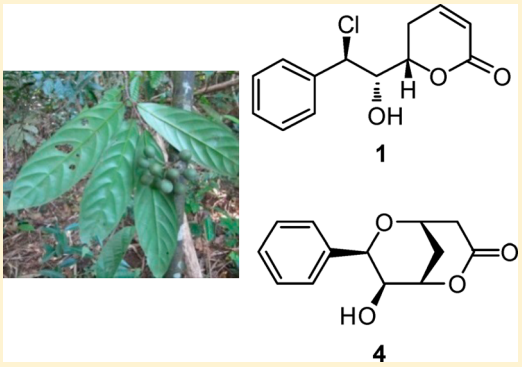
Supporting Information

ABSTRACT: Parvistones A–E (**1**–**5**), five new styryllactones possessing a rare α,β-lactone moiety and a 6*S* configuration, were isolated from a methanolic extract of *Polyalthia parviflora* leaves. The structures and the absolute configuration of the isolates were elucidated using NMR spectroscopy, specific rotation, circular dichroism, and X-ray single-crystal analysis. Compounds **8**, **9**, **11**, and **12** were isolated for the first time. The results were supported by comparing the data measured to those of 6*R*-styryllactones. Moreover, a plausible biogenetic pathway of the isolated compounds was proposed. The structure–activity relationship of the compounds in an in vitro anti-inflammatory assay revealed the 6*S*-styryllactones to be more potent than the 6*R* derivatives. However, the effect was opposite regarding their cytotoxic activity. In addition, 6*S*-styrylpyrones isolated showed more potent anti-inflammatory and cytotoxic activity when compared to the 1*S*-phenylpyranopyrones obtained.



Several classes of secondary metabolites have been isolated from the genus *Polyalthia* (Annonaceae) including acetogenins,[1] terpenoids,[2] alkaloids,[3] benzopyrans,[4] 2-substituted furans,[5] and styryllactones.[6] Some of these compounds were found to possess potent cytotoxic and anti-inflammatory activities.[2,7] In general, *Polyalthia* spp. contain a diverse group of secondary metabolites; however, not all species belonging to this genus have been thoroughly investigated. Among the little-studied species is *Polyalthia parviflora* Ridl. This plant is a shrubby tree that can be found in different Asian countries including Thailand, Malaysia, and Cambodia. It is considered a preferred food for domesticated and wild herbivores.[8] Traditional healers in Asia use a water decoction of *P. parviflora* to treat bodily discomfort.[9] Only one study has investigated the phytochemical constituents of *P. parviflora*, reporting the isolation of *p*-coumarate, *p*-hydroxyphenylethyl ferulate, dehydrodiscretamine, and (−)-discretamine.[9] Due to the scarcity of reports on *P. parviflora*, an extensive phytochemical and biological investigation on the secondary metabolite content of this species was initiated.

The chromatographic purification of the methanolic extract of *P. parviflora* leaves led to the isolation of 13 6*S*-styryllactones (**1**–**13**), which can be subdivided into 6*S*-styrylpyrones (**1**–**3** and **7**–**13**) and 1*S*-phenylpyranopyrones (**4**–**6**). Details on the isolation, structure elucidation, a plausible biogenetic route, and biological activities of these two classes of secondary metabolites are presented herein. Moreover, the preliminary structure–activity relationships of **1**–**13**, in terms of their anti-inflammatory and cytotoxic activities, are discussed.

RESULTS AND DISCUSSION

The ^{1}H NMR spectroscopic data of the 6*S*-styrylpyrones, **1**–**3**, were closely comparable to one another except for certain distinguishing features (Table 1). The HRESIMS data of **1**

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Chart 1. New Compounds Isolated from *Polyalthia parviflora* (1−5, 8, 9, 11, and 12) and Compounds Isolated Previously from *Goniothalamus* spp. (14 and 15) That Showed Biological Activity

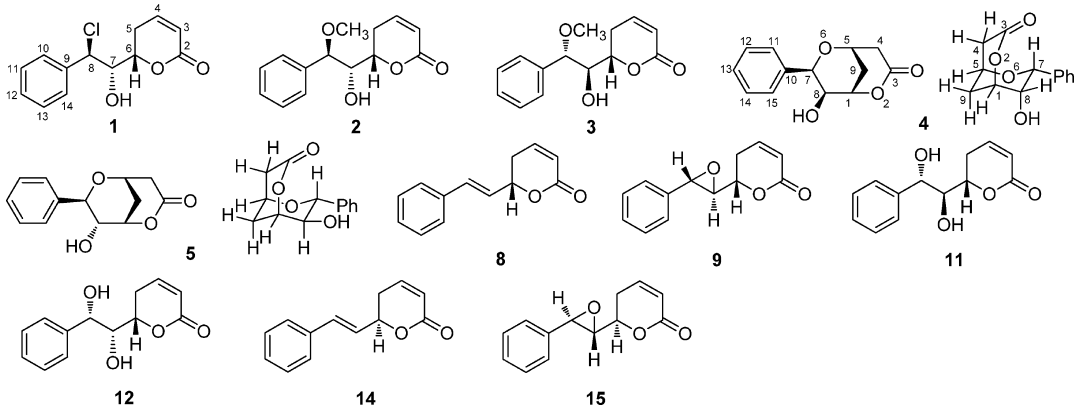

Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−6 (400 MHz, $CDCl_3$ for 1−4 and 6; 600 MHz, $CDCl_3$ in 5)

position	1	2	3	4	5	6
1				4.89 (quin, 4.0, 2.0)	4.97 (m)	4.93 (d, 1.6)
2						
3	6.02 (ddd, 10.4, 2.8, 1.2)	6.00 (ddd, 9.2, 2.4, 0.8)	6.05 (dd, 9.5, 2.4)			
4	6.91 (ddd, 10.4, 5.8, 2.8)	6.92 (ddd, 9.2, 6.1, 2.4)	6.97 (dt, 9.5, 6.4, 2.4)	2.88 (dd, 19.2, 5.2)	2.90 (dd, 19.7, 4.8)	2.88 (dd, 19.4, 4.8)
				2.99 (d, 19.2, 11.6)	3.01 (dd, 19.7, 1.5)	2.93 (dd, 19.4, 1.6)
5	2.54 (ddt, 19.7, 5.8, 1.2)	2.43 (ddd, 18.6, 6.1, 4.4)	2.26 (ddd, 18.2, 6.4, 4.0)	4.54 (m)	4.49 (m)	4.46 (s)
	2.62 (ddt,19.7, 10.8, 2.8)	2.66 (ddt, 18.8, 11.6, 2.4)	2.84 (ddt, 18.2, 13.2, 2.4)			
6	4.36 (ddd, 10.8, 5.8, 4.0)	4.36 (ddd, 11.6, 5.6, 4.4)	4.95 (ddd, 12.6, 4.0, 1.2)			
7	4.32 (ddd, 9.6, 5.6, 4.0)	4.15 (t, 5.6)	3.61 (dt, 8.4, 1.2)	4.97 (d, 1.2)	4.43 (d, 9.9)	4.43 (d, 9.6)
8	5.13 (d, 5.6)	4.37 (d, 5.6)	4.38 (d, 8.4)	3.96 (m)	3.64 (dt, 9.9, 2.4)	3.57 (dd, 9.6, 2.4)
9				1.86 (dd, 15.0, 4.4)	2.26 (m)	2.22 (m)
				2.61 (ddt, 15.0, 4.4, 2.0)		
10	7.48 (m)	7.40 (m)	7.41 (m)			
11	7.39 (m)	7.38 (m)	7.38 (m)	7.39 (m)	7.42 (m)	7.41 (m)
12	7.36 (m)	7.36 (m)	7.36 (m)	7.42 (m)	7.40 (m)	7.38 (m)
13	7.39 (m)	7.38 (m)	7.38 (m)	7.34 (m)	7.39 (m)	7.36 (m)
14	7.48 (m)	7.40 (m)	7.41 (m)	7.42 (m)	7.40 (m)	7.38 (m)
15				7.39 (m)	7.42 (m)	7.41 (m)
OCH_3-8		3.24 (s)	3.22 (s)			
OH-7		2.02 (brs)				
OH-8				1.60 (d, 2.8)		

suggested the presence of a chloride atom (*m*/*z* 275.0449 $[M + Na]^+$, 277.0425 $[M + Na + 2]^+$, 3:1) and a molecular formula of $C_{13}H_{13}ClO_3$. Moreover, the HRESIMS data of **2** and **3** indicated that they possess the same molecular formula ($C_{14}H_{16}O_4$) with seven degrees of unsaturation. The elemental formulas proposed for **1**−**3** were supported by the ^{13}C NMR data (Table 2), which showed one methylene, 10 methines, and two quaternary carbon atoms in **1** and one additional methyl group in **2** and **3**.

The ^{1}H NMR spectra (Table 1) of **1** indicated the presence of a monosubstituted benzene ring, two conjugated *cis* double bonds, three protons connected to electron-withdrawing groups, and two methylene groups, thus suggesting that **1** possesses a styrylpyrone skeleton.[10] The ^{1}H NMR spectra (Table 1) of **2** and **3** showed similar profiles, with one additional methoxy signal. The highly deshielded H-8 (δ_H 5.13) and the shielded C-8 (δ_C 62.1) in **1** as well as the $J_{H\text{-}6/H\text{-}7}$ value (J = 4.0 Hz in **1**, J = 5.6 Hz in **2**, and J = 1.2 Hz in **3**), the $J_{H\text{-}7/H\text{-}8}$ value (J = 5.6 Hz in **1** and **2**, while J = 8.4 Hz in **3**) (Table 1), and the key COSY, HMBC, and NOESY correlations (Figure S1, Supporting Information) revealed the relative configurations of **1**−**3**.

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−6 (100 MHz, $CDCl_3$ for 1−4 and 6; 150 MHz, $CDCl_3$ in 5)

position	1	2	3	4	5	6
1				74.7	76.6	76.8
2	163.4	163.8	164.0			
3	121.0	120.9	120.8	169.2	168.6	169.2
4	145.2	145.7	146.0	36.3	36.6	36.5
5	24.6	24.4	26.2	66.1	65.8	65.7
6	77.2	77.4	76.1			
7	75.5	74.4	74.8	70.5	74.5	74.2
8	62.1	82.6	81.9	68.2	72.6	72.5
9	136.4	136.9	138.5	24.0	29.9	29.7
10	128.5	127.9	127.8	136.7	137.7	137.9
11	128.6	128.5	128.7	126.1	127.3	127.4
12	129.0	128.4	128.5	128.9	128.7	128.5
13	128.6	128.5	128.7	128.3	128.8	128.6
14	128.5	127.9	127.8	128.9	128.7	128.5
15				126.1	127.3	127.4
OCH_3-8		56.8	56.7			

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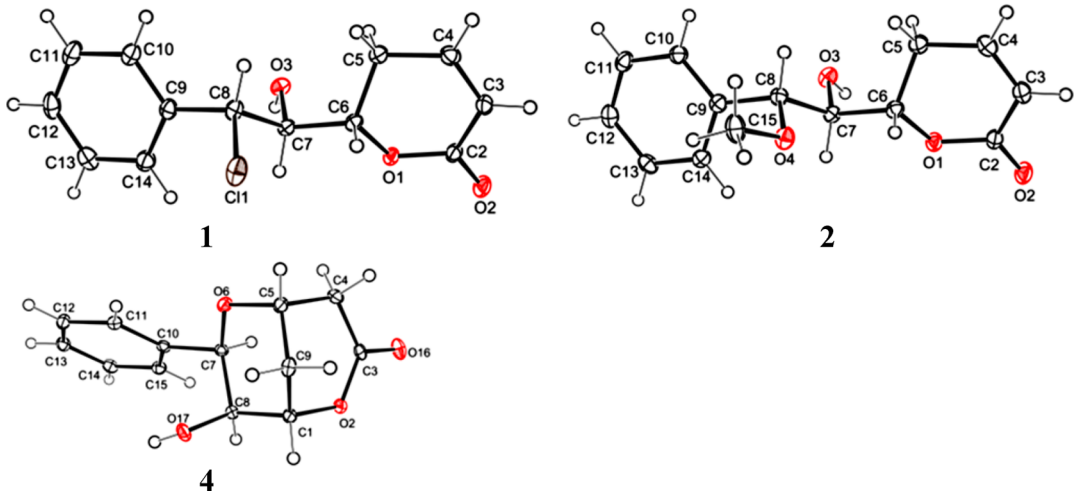

Figure 1. ORTEP plots of **1**, **2**, and **4**.

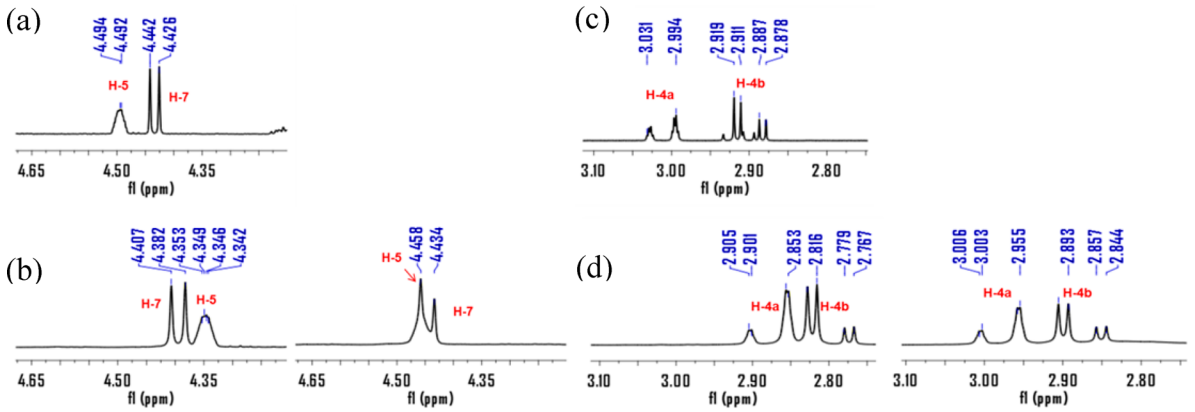

Figure 2. Differences in the chemical shift ($\Delta\delta$) values of H-5 and H-7 between 4.3 and 4.6 ppm in (a) **5** and (b) **6**. Differences in the chemical shift ($\Delta\delta$) values of H_{4a}–H_{4b} between 2.7 and 3.1 ppm in (c) **5** and (d) **6**.

The spectroscopic data indicated that the relative configurations between H-6, H-7, and H-8 are *erythro* and *threo* in **1** and **2** but *threo* and *threo* in **3**. The *S* configuration at C-6 in **1**–**3** was deduced in each case from their negative specific rotation, a negative Cotton effect at 257 or 262 nm in the CD spectrum[11] (Table S2, Supporting Information), and the X-ray single-crystal analysis of **1** and **2** (Figure 1). Literature reports revealed that almost all naturally occurring styryllactones possess the 6*R* configuration, which showed a positive Cotton effect in the CD spectrum at 250–272 nm (n–π^*).[12,13] However, a small number of compounds of this type have been found to possess a 6*S* configuration, showing opposite specific rotations to derivatives with the 6*R* configuration.[14] Thus, the structure and absolute configurations of **1**–**3** were determined as (6*S*,7*S*,8*R*)-8-chlorogoniodiol, (6*S*,7*R*,8*R*)-8-methoxygoniodiol, and (6*S*,7*S*,8*S*)-8-methoxygoniodiol, respectively. Aiming to provide a straightforward method for the differentiation of 6*S*- and 6*R*-styryllactones, the specific rotation and circular dichroism data obtained in this study were compared. It was found that changing the configuration at C-6 significantly affected the spectroscopic data of styryllactones (Table S2, Supporting Information).

HRESIMS data revealed the molecular formulas of the isolated 1*S*-phenylpyranopyrones, **4**–**6**, to be $C_{13}H_{14}O_4$ in each case, suggesting the presence of seven degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **4** were closely related to those of (+)-9-deoxygoniopypyrone.[13,15] However, the spectroscopic data of **5** and **6** were similar to those of 8-*epi*-9-deoxygoniopypyrone,[15] indicating that **4**–**6** are diastereomers with some structural differences. The data suggested that the equatorial proton (H-8) in **4** occurs in an axial position in **5** and **6**. Additionally, H-5 was shifted downfield and distinctly separated from H-7 in **5** (Figure 2a). However, the corresponding signal was shifted upfield and overlapped with H-7 in **6** (Figure 2b). Also, the $\Delta\delta$ of the H_{4a}–H_{4b} value differed between **5** (0.11 ppm) and **6** (0.05–0.08 ppm) (Figure 2c and d). Interestingly, significant differences in the specific rotation values were observed for these compounds (**4**: −49, **5**: −280, and **6**: +67) (Table S2, Supporting Information).

The absolute configurations of **4**–**6** were deduced by comparing their spectroscopic data with similar compounds reported previously. Tai et al. reported the isolation of phenylpyranopyrones with a bicyclo structure and proposed three conformations, namely, the boat–boat, chair–chair, and boat–chair conformations (Figure S2, Supporting Information).[15] Additionally, all styrylpyrones isolated from this plant were found to possess a 6*S* configuration, and their structural similarity suggested a common biogenetic pathway, which implies that the configuration of C-1 in **4**–**6** (originally C-6 in the isolated styrylpyrones) is *S* (Table S2, Supporting Information). The NOE cross-peaks (Figure 3) between H-9b and H-4b, H-4a and H-7, and H-8 and H-9a revealed a chair–chair and boat–chair conformation in **4** and **5**, and **6**,

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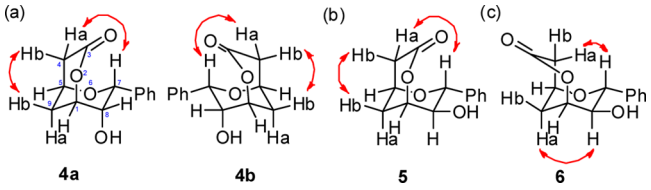

Figure 3. (a) Two possible structures of **4**, (b) structure of **5**, and (c) structure of **6**. Structures were suggested based on the NOESY spectra.

respectively. Thus, the absolute configuration of **4** was suggested as 1*S*,5*S*,7*R*,8*R* with **4a** as the proposed structure (Figure 3). Additionally, the CD profile (Table S2, Supporting Information) and the X-ray structural analysis data (Figure 1) of **4** confirmed a reversed configuration from that of (+)-9-deoxygoniopypyrone. Therefore, **4** was assigned as 1*S*,5*S*,7*R*,8*R*,3-*endo*,7-*endo*-(−)-9-deoxygoniopypyrone, and this is its first report from Nature.[16] The other diastereomers, **5** and **6**, were assigned as 1*S*,5*S*,7*R*,8*S*,3-*endo*,7-*endo*-(−)-8-*epi*-9-deoxygoniopypyrone and 1*S*,5*S*,7*R*,8*S*,3-*exo*,7-*endo*-(+)-8-*epi*-9-deoxygoniopypyrone, respectively.[15]

Interestingly, it was found that the signs of specific rotation for all isolated 1*S*-phenylpyranopyrones (**4**−**6**) (Table S2, Supporting Information) did not change upon altering the orientation of the 8-hydroxy group from axial (**4**: −49) to equatorial (**5**: −280); however, the absolute values changed dramatically. On the other hand, the data indicated that changing the direction of the 3-carbonyl group altered the sign of the specific rotation (**4**, 3-*endo*: −49 and **6**, 3-*exo*: +67).

The spectroscopic data of compounds **8** ($[\alpha]_D^{20}$ −51), **9** ($[\alpha]_D^{20}$ −241), **11** ($[\alpha]_D^{20}$ −63), and **12** ($[\alpha]_D^{20}$ −132) (Table S1, Supporting Information), with a 6*S* configuration, were similar to the reported data of (*S*)-goniothalamin,[17] (−)-*ent*-goniothalamin oxide,[18] (6*S*,7*S*,8*S*)-goniodiol,[19] and (−)-7-*epi*-goniodiol [(6*S*,7*R*,8*S*)-goniodiol],[20] respectively. These have been synthesized previously and have been isolated from Nature for the first time in the present study.

The specific rotation values and the negative Cotton effect around 250−272 nm suggested that C-6 possesses an *S* configuration in the remainder of the isolated compounds (**7**, **10**, and **13**) (Table S2, Supporting Information). These compounds were identified as dehydrogoniothalamin (**7**),[21] (−)-5-hydroxygoniothalamin (**10**),[14] and (2*E*,6*E*)-methyl-5-hydroxy-7-phenylhepta-2,6-dienoate (**13**)[22,23] by comparing their NMR data with those reported in the literature.

The relatively large number of styryllactones isolated in the current study facilitated the comparison of their spectroscopic data with similar compounds, thus establishing a feasible method for the direct determination of styryllactone configuration. The specific rotation and circular dichroism (CD) data of 13 6*S*-styryllactones (**1**−**13**) isolated in the current study from *P. parviflora* were compared to the corresponding spectroscopic data of 19 6*R*-styryllactones (**14**−**32**) isolated previously from *Goniothalamus* spp. (Figure S3, Supporting Information) (Table S2, Supporting Information).[13,24−26] 6*S*-Styryllactones showed reversed signs of specific rotation values and a negative Cotton effect between 250 and 272 nm in comparison to those of 6*R* derivatives. These data were also supported by single-crystal X-ray crystallographic analysis (Figure 1).

The structural similarity of the isolated compounds suggested a common biogenetic route proceeding through the shikimate and phenylpropanoid pathways (Scheme S1, Supporting Information). The condensation of cinnamoyl-CoA with two molecules of malonyl-CoA yields a linear triketide intermediate that undergoes either reduction, dehydration, thioester cleavage, or methylation to yield **13** or reduction, cyclization, and dehydration to render the styryllactone **7**. Hydration and hydrogenation of **7** yield compound **10** and goniothalamin (**8**), respectively. The oxidation of the double bond between C-7 and C-8 of **8** results in the formation of goniothalamin oxide (**9**). Epoxide ring opening followed by halogenation (Cl^-) at the appropriate position with the aid of haloperoxidase provides **1**. Alternatively, the hydrolysis of goniothalamin oxide (**9**) may result from the formation of goniodiol derivatives such as **11** and **12**. These goniodiol derivatives are converted further into two sets of compounds, of which one is formed via methyltransferase utilizing *S*-adenosylmethionine (SAM) as the methyl donor, leading to the formation of **2** and **3**. The other set of compounds may be formed through the attack of the hydroxy group on the lactone ring, resulting in the formation of two six-ring linkages, the pyranopyrones, such as **4**−**6**.

Following the establishment of the isolated compound structures, their in vitro biological activity was evaluated. Extracts of other *Polyalthia* species, especially *P. longifolia*, exhibited potent anti-inflammatory activity in vivo, suggesting the presence of substances with this type of activity.[27,28] Inflammation is a serious disorder linked to other debilitating diseases, especially cancer.[29,30] However, the rate of introduction of new anti-inflammatory agents has been declining in recent years. Thus, discovering safe and effective anti-inflammatory agents from natural sources is still a desirable research goal.[31] Aiming to fulfill this, the anti-inflammatory activity of the isolated compounds from *P. parviflora* was evaluated and compared for the first time to previously isolated styryllactones (Table S3 and Figure S41, Supporting Information). The results indicated that the 6*S*-styryllactones showed more potent anti-inflammatory effects than their 6*R* derivatives.[24] Among the isolates, **7**, **8**, and **10** exhibited inhibitory activity against fMLP/CB-induced superoxide anion generation and elastase release by human neutrophils (Table 3). The activity of compounds **8** and **10** was comparable to the positive control, sorafenib, in the inhibition of superoxide anion generation assay. On the other hand, compound **7** was more potent than **8** and **10** in the inhibition of elastase release. However, the 1*S*-phenylpyranopyrones did not show any discernible anti-inflammatory activity, indicating that the α,β-lactone moiety is essential for the activity (Table 3). It is worthy of mention that compounds isolated from *Piper methysticum* possessing structures closely related to the styryllactones from *P. parviflora* were found to exhibit potent anti-inflammatory activity, suggesting the possible utilization of this class of compounds as anti-inflammatory agents.[32]

Cytotoxicity against cancer cells is another important activity demonstrated by other *Polyalthia* species that have shown promising in vitro results.[33] In previous reports, compounds with a styryllactone nucleus exhibited potent cytotoxic activity.[13,34,35] Structurally similar compounds such as the kavapyrones, isolated from *Piper methysticum*, were found to possess a cytotoxic effect by inhibiting TNF-α release.[36] Compound **10** exhibited the most potent activity against the lung (A549) cancer cell line, with an IC_{50} of 7.9 μM (Table 3). In general, the activity of the styryllactones was highly affected by the substituents at C-8, showing the following order of decreasing potency: Cl > OCH_3 > epoxide > OH. The 1*S*-phenylpyranopyrones that did not show any activity in the anti-inflammatory assay were also inactive in the cytotoxic assay. Interestingly, the 6*R*-styryllactones were more active against the tested cancer cell lines than the 6*S*-derivatives.[24,25]

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Table 3. Anti-inflammatory Activity of Compounds 7, 8, and 10 from *Polyalthia* spp. on FMLP/CB-Induced Superoxide Anion Generation and Elastase Release in Human Neutrophils[a] and the Cytotoxic Activity of Compound 10 against Three Different Cancer Cell Lines (Hep G2, MDA-MB-231, and A549)[b]

	superoxide anion	elastase release	Hep G2	MDA-MB-231	A549
compound	IC_{50} (μM)[c]	IC_{50} (μM)[c]	IC_{50} (μM)[c]	IC_{50} (μM)[c]	IC_{50} (μM)[c]
7	11.6 ± 1.2	6.8 ± 0.9			
8	8.3 ± 1.4	15.4 ± 1.1			
10	8.1 ± 2.3	14.6 ± 0.7	>10	>10	7.9
sorafenib[d]	6.3 ± 0.4	1.9 ± 0.4			
doxorubicin[d]			0.74	0.88	0.87

[a]Results are presented as means ± SEM (n = 3). Compounds **4**, **5**, and **13** were not tested (the amount of the compound was insufficient to be evaluated). Compounds **2**, **3**, **6**, **11**, and **12** were inactive in both of the anti-inflammatory assays. Compound **1** showed selective activity against superoxide anion with IC_{50} 30.0 ± 2.5 μM, and compound **9** exhibited anti-inflammatory activities against superoxide anion and elastase release with IC_{50} 21.2 ± 2.2 μM and 19.8 ± 2.4 μM, respectively. [b]Results are presented as means (n = 3). Compounds **4**, **5**, and **13** were not tested (the amount of the compound was insufficient to be evaluated). Compound **6** was inactive in the cytotoxicity assay, and compound **12** was inactive against the Hep G2 cancer cell line. Compounds **1**−**3**, **7**−**9**, **11**, and **12** showed a lack of cytotoxicity with IC_{50} values of >10 μM. [c]Concentration necessary for 50% inhibition (IC_{50}). [d]Sorafenib tosylate and doxorubicin liposome were used as the positive controls for the anti-inflammatory assay and cytotoxicity assay, respectively.

In conclusion, 13 unusual styryllactones including nine new 6*S*-styrylpyrones and 1*S*-phenylpyranopyrones were isolated from the methanolic extract of *P. parviflora* leaves. This work enabled the determination of the absolute configuration of this class of compounds to be made. The results indicated that the configuration at C-6 of styrylpyrones affected their spectroscopic data as well as their anti-inflammatory and cytotoxic activities.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting points were determined with a Yanaco MP-500D micromelting point apparatus (Yanaco, Kyoto, Japan). Specific rotations were measured with a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). UV spectra were obtained on a JASCO V-530 UV/vis spectrophotometer (JASCO, Tokyo, Japan) in MeOH. CD spectra were taken using a JASCO model J-810 spectrophotometer. IR spectra were recorded on a Mattson Genesis II FT-IR spectrophotometer (Mattson Instruments, Madison, WI, USA). ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were taken on a Varian Unity Inova-600, Varian Unity Plus-400, or Varian Gemini-200 NMR spectrometer (Varian Inc., Palo Alto, CA, USA). Chemical shifts are presented in parts per million (δ), and coupling constants (J) are expressed in hertz. ESIMS were recorded on a Finnigan POLARISQ mass spectrometer (Thermo Finnigan, Austin, TX, USA). HRESIMS were measured on a Bruker Daltonics APEX II mass spectrometer (Bruker Instruments, Billerica, MA, USA). Silica gel (Kieselgel 60, 70−230, and 230−400 mesh, Merck KGaA, Darmstadt, Germany) was used for column chromatography, and precoated plates with silica gel (Kieselgel 60, F_{254}, Merck KGaA) and RP-18 (F_{254s}, Merck KGaA), were employed for TLC analysis. TLC spots were detected under UV light at 254 and 365 nm and also by spraying with 50% H_2SO_4 followed by heating on a hot plate. A reversed-phase solid-phase extraction (SPE) column (2.7 × 4 cm) was purchased from Supelco (Sigma-Aldrich Co., St. Louis, MO, USA). HPLC was performed with Shimadzu LC-20AT pumps connected to a RID-10A refractive index (RI) detector (Shimadzu Inc., Kyoto, Japan). Reversed-phase columns (Thermo ODS Hypersil, 250 × 10 mm, i.d., 5 μm, C_{18}) were used for HPLC separations.

Plant Material. The leaves of *Polyalthia parviflora* were collected from Quyhop district, Nghean Province, Vietnam (19°19′32.69″ N, 105°11′36.57″ E), during March 2006, and the plant materials were identified and authenticated by Assoc. Prof. Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology. A voucher specimen (Annona DA105) was deposited in the Herbarium of the Faculty of Biology, Vinh University, Vietnam.

Extraction and Isolation. The leaves of *P. parviflora* (1.1 kg) were air-dried, powdered, and soaked with methanol (4.0 L × 3) at room temperature, and the combined extracts were concentrated under reduced pressure to give a brown syrup (19.8 g). The methanolic extract was partitioned between H_2O and CH_2Cl_2 (1:1). The CH_2Cl_2 layer was further partitioned between *n*-hexane and 80% aqueous MeOH (1:1).

The 80% aqueous MeOH layer (PM 8.83 g) was subjected to open column chromatography on silica gel (70−230 mesh, column: 5 × 32 cm), using CH_2Cl_2, and gave nine subfractions (PM1−PM9), as well as pure compounds **7** (63.2 mg) and **8** (1009.0 mg). Subfraction PM7 (1.98 g) was fractionated into 14 further subfractions using a silica gel column (230−400 mesh, column: 2 × 30 cm), with gradients of CH_2Cl_2−CH_3CN−MeOH (90:10:0, 0.2 L; 80:20:0, 0.4 L; 60:0:40, 0.3 L; 0:0:100, 0.3 L). Subfraction PM7-3 (149.7 mg) was purified by silica gel chromatography (230−400 mesh, column: 1 × 40 cm; CH_2Cl_2−*n*-hexane, 85:15) to give 16 subfractions and to obtain **8** (62.2 mg) and **9** (6.1 mg). Subfraction PM7-5 (302.2 mg) was separated by silica gel chromatography (230−400 mesh, column: 2 × 32 cm; CH_2Cl_2−*n*-hexane−THF, 40:55:5) to give 15 subfractions. Subfraction PM7-5-8 (88.0 mg) was further separated by RP-SPE (column: 2.7 × 4.0 cm; MeOH−H_2O, 50:50, 15.0 mL; 75:25, 15.0 mL; 90:10, 15.0 mL; 100:0, 6.0 mL) to obtain seven subfractions. Subfraction PM7-5-8-SP4 (33.6 mg) was further subjected to RP-HPLC (MeOH−H_2O, 50:50, flow rate: 3.0 mL/min) to give **5** (0.91 mg, t_R = 18.7 min). Subfraction PM7-5-8-SP5 (5.91 mg) was purified using RP-HPLC (MeOH−H_2O, 50:50, flow rate: 3.0 mL/min) to furnish **13** (0.35 mg, t_R = 38.5 min). Subfraction PM7-7 (261.1 mg) and subfraction PM7-8 (510.6 mg) were combined and were further separated by silica gel chromatography (230−400 mesh, column: 2 × 22.5 cm; CH_2Cl_2−*n*-hexane−THF, 75:15:10) to give eight subfractions and a precipitate (**1**) (38.2 mg). Subfraction PM7-7-2 (58.1 mg) was purified by preparative silica TLC (*n*-hexane−THF, 80:20) to give **2** (12.7 mg). Subfraction PM7-7-3 (191.3 mg) was subjected to RP-SPE (column: 2.7 × 4 cm; MeOH−H_2O, 50:50, 15.0 mL; 75:25, 15.0 mL; 90:10, 15.0 mL; 100:0, 6.0 mL) to obtain five subfractions. Subfraction PM7-7-3-SP2 (142.2 mg) was purified by RP-HPLC (MeOH−H_2O, 50:50, flow rate: 3.0 mL/min) to give **3** (11.2 mg, t_R = 49.6 min). Subfraction PM7-7-4 (68.5 mg) was separated by RP-SPE (column: 2.7 × 4 cm; MeOH−H_2O, 50:50, 15.0 mL; 75:25, 15.0 mL; 90:10, 15.0 mL; 100:0, 4.5 mL) to give five subfractions. Subfraction PM7-7-4-1 (11.1 mg) was purified using RP-HPLC (MeOH−H_2O, 40:60, flow rate: 3.0 mL/min) to give **4** (1.3 mg, t_R = 15.9 min). Subfraction PM8 (4.74 g) was subjected to silica gel chromatography (230−400 mesh, column: 5 × 31 cm; CH_2Cl_2−MeOH, 90:10, 0.4 L; 80:20, 0.6 L) to afford 16 subfractions and pure compound **6** (331.0 mg). Subfraction PM 8-13 (67.1 mg) was separated by a reversed-phase preparative TLC (CH_3CN−H_2O, 50:50) to give **10** (18.7 mg). Subfractions PM8-16 to PM8-19 (1.43 g) were combined and subjected to silica gel chromatography (230−400 mesh, column: 2 × 34 cm, CH_2Cl_2−THF, 90:10) to afford 16 subfractions and pure compound **6** (383.7 mg). Subfraction PM8-16-7 (63.7 mg) was purified by reversed-phase preparative TLC (CH_3CN−H_2O, 50:50) to obtain **11** (28.2 mg). Subfraction PM8-16-10 (55.5 mg) was purified by reversed-phase preparative TLC (CH_3CN−H_2O, 50:50) to give **12** (20.0 mg).

Parvistone A (1): colorless plate crystals (CH_2Cl_2−MeOH); mp 127−129 °C; $[\alpha]_D^{22}$ −124 (*c* 0.22, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 207 (4.09), 251 (2.80) nm; CD (*c* 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 212 (+3.45), 257 (−0.83) nm; IR (KBr) ν_{max} 3405 (OH), 2359, 1706 (C═O), 1253 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2;

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HRESIMS m/z 275.0449 $[M + Na]^+$, 277.0425 $[M + Na + 2]^+$ (3:1) (calcd for $C_{13}H_{13}ClO_3Na$, 275.0451).

X-ray Crystallographic Data for 1. A colorless plate crystal was grown in 1:1 CH_2Cl_2–MeOH. Crystal data for **1**: $C_{13}H_{13}ClO_3$; M_r 252.68; T = 100(2) K; wavelength 1.541 78 Å; crystal system orthorhombic; space group, $P2_12_12_1$ (no. 19); unit cell dimensions a = 7.4066(2) Å, b = 9.4215(3) Å, c = 17.4684(5) Å; volume 1218.97(6) Å^3; Z = 4; D_{calc} = 1.377 mg/m^3; absorption coefficient 2.733 mm^{-1}; $F(000)$ = 528; approximate crystal size, 0.40 × 0.20 × 0.05 mm; reflection collected 17 171; independent reflections 2064 [R(int) = 0.0272]. The absolute configuration of **1** was determined with Flack's parameter of 0.043(4). Crystallographic data for the structural analysis were deposited with the Cambridge Crystallographic Data Centre (CCDC 972834). These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).

*Parvistone B (**2**):* colorless crystals (EtOAc–MeOH); mp 100–102 °C; $[\alpha]_D^{21}$ −80 (c 0.6, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 207 (3.94), 256 (2.50) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 208 (+4.84), 262 (−0.87) nm; IR (KBr) ν_{max} 3457 (OH), 2357, 1718 (C=O), 1246 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS m/z 271.0947 $[M + Na]^+$ (calcd for $C_{14}H_{16}O_4Na$, 271.0946).

X-ray Crystallographic Data for 2. The colorless plate crystal was grown in 1:1 EtOAc–MeOH. Crystal data for **2**: $C_{14}H_{16}O_4$; M_r 248.27; T = 100(2) K; wavelength 1.541 78 Å; crystal system orthorhombic; space group $P2_12_12_1$ (no. 19); unit cell dimensions a = 8.3051(2) Å, b = 9.8097(2) Å, c = 15.5103(3) Å; volume 1263.63(5) Å^3; Z = 4; D_{calc} = 1.305 mg/m^3; absorption coefficient 0.787 mm$^{-1;}$ $F(000)$ = 528; approximate crystal size, 0.40 × 0.40 × 0.10 mm; reflections collected 8379; independent reflections 2156 [R(int) = 0.0287]. The absolute configuration of **2** was determined with Flack's parameter of 0.03(6). Crystallographic data for the structural analysis were deposited with the Cambridge Crystallographic Data Centre (CCDC 972833). These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).

*Parvistone C (**3**):* colorless crystals (CH_2Cl_2–MeOH); mp 103–105 °C; $[\alpha]_D^{22}$ −216 (c 0.20, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 208 (4.12), 251 (3.06) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 208 (+4.06), 262 (−1.23) nm; IR (KBr) ν_{max} 3430 (OH), 2359, 1719 (C=O), 1247 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS m/z 271.0941 $[M + Na]^+$ (calcd for $C_{14}H_{16}O_4Na$, 271.0941).

*Parvistone D (**4**):* colorless crystals (EtOAc–MeOH); mp 192–194 °C; $[\alpha]_D^{21}$ −49 (c 0.26, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 206 (3.91), 257 (2.15) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 208 (+4.02), 255 (−0.45), 283 (−0.26) nm; IR (KBr) ν_{max} 3451, 1719, 1186, 1057 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS m/z 257.0787 $[M + Na]^+$ (calcd for $C_{13}H_{14}O_4Na$, 257.0784).

X-ray Crystallographic Data for 4. The colorless plate crystal was grown in 1:1 EtOAc–MeOH. Crystal data for **4**: $C_{13}H_{14}O_4$; M_r 234.24; T = 100(2) K; wavelength, 1.541 78 Å; crystal system orthorhombic; space group, $P2_1$ (no. 4), unit cell dimensions a = 7.8491(4) Å, b = 6.2050(4) Å, c = 11.2346(6) Å; β = 95.747(3)°; volume 544.42(5) Å^3; Z = 2; D_{calc}= 1.429 mg/m^3; absorption coefficient 0.879 mm^{-1}; $F(000)$ = 248; approximate crystal size, 0.30 × 0.12 × 0.11 mm; reflection collected 3857; independent reflections 1337 [R(int) = 0.0165]. The absolute configuration of **4** was determined with Flack's parameter of 0.05(8). Crystallographic data for the structural analysis were deposited with the Cambridge Crystallographic Data Centre (CCDC 988476). These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).

*Parvistone E (**5**):* white powder; $[\alpha]_D^{21}$ −280 (c 0.18, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 207 (3.56) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 208 (+4.62), 265 (−0.30) nm; IR (KBr) ν_{max} 3413, 2918, 1729, 1080 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS m/z 257.0786 $[M + Na]^+$ (calcd for $C_{13}H_{14}O_4Na$, 257.0784).

*(S)-Goniothalamin (**8**):* white powder; $[\alpha]_D^{20}$ −51 (c 0.20, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 208 (4.27), 250 (4.04) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 211 (+3.89), 269 (−1.57) nm; IR (KBr) ν_{max} 2918, 2355, 1721, 1382, 1244 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table S1, Supporting Information; HRESIMS m/z 223.0730 $[M + Na]^+$ (calcd for $C_{13}H_{12}O_2Na$, 223.0730).

*(6S,7R,8R)-(−)-ent-Goniothalamin oxide (**9**):* white powder; $[\alpha]_D^{20}$ −241 (c 0.18, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 205 (3.92) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 211 (+3.89), 269 (−0.47) nm; IR (KBr) ν_{max} 3451, 2924, 2849, 1724, 1242 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table S1, Supporting Information; HRESIMS m/z 239.0681 $[M + Na]^+$ (calcd for $C_{13}H_{12}O_3Na$, 239.0679).

*(6S,7S,8S)-(−)-Goniodiol (**11**):* white powder; $[\alpha]_D^{20}$ −63 (c 0.30, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 208 (4.16) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 211 (+4.11), 256 (−1.00) nm; IR (KBr) ν_{max} 3397, 2918, 1701, 1254 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table S1, Supporting Information; HRESIMS m/z 257.0787 $[M + Na]^+$ (calcd for $C_{13}H_{14}O_4Na$, 257.0784).

*(6S,7R,8S)-(−)-7-epi-Goniodiol (**12**):* white powder; $[\alpha]_D^{20}$ −132 (c 0.30, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 207 (4.09) nm; CD (c 0.5 mM, CH_2Cl_2) λ_{max} ($\Delta\varepsilon_{max}$) 211 (+3.78), 269 (0.87) nm; IR (KBr) ν_{max} 3416, 2918, 1711, 1387, 1253, 1073 cm^{-1}; ^{1}H and ^{13}C NMR spectroscopic data, see Table S1, Supporting Information; HRESIMS m/z 257.0784 $[M + Na]^+$ (calcd for $C_{13}H_{14}O_4Na$, 257.0784).

Determination of the Cytotoxic Activity for the Isolated Compounds. Three cancer cell lines, liver (Hep G2), lung (A549), and breast (MDA-MB-231), were purchased from the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan). Cell inocula, 1 × 10^4 cells [liver (Hep G2) and breast (MDA-MB-231)] or 5 × 10^3 cells [lung (A549)], were plated in triplicate onto 96-well plates and treated with various concentrations of the isolated compounds at different time intervals. After 72 h, the growth medium was removed and 100 μL of MTT solution (0.5 mg/mL) was added to each well. The plates were then incubated at 37 °C for 1 h. The MTT crystals in each well were solubilized with 100 μL of DMSO. Doxorubicin was used as the positive control. The absorbance in individual wells was measured at 550 nm using a Multiskan Ascent microplate reader (Thermo Labsystems, Waltham, MA, USA). All experiments were performed in triplicate, and the results of the assays are presented as means ± SEM.

Preparation of Human Neutrophils. The human neutrophils were prepared and isolated by a standard method as previously described.[37,38] This study was approved by the Institutional Review Board at Chang Gung Memorial Hospital (IRB 102-1595A3).

Measurement of Superoxide Generation. To examine the superoxide anion production, the superoxide dismutase-inhibitable reduction of ferricytochrome c was assayed according to a previously reported merthod.[37,38] Neutrophils, 0.5 mg/mL ferricytochrome c, and 1 mM Ca^{2+} were equilibrated at 37 °C for 2 min and treated with the isolated compounds for 5 min. Cells were incubated with cytochalasin B (CB, 1 μg/mL) for 3 min, before activation by formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, 100 nM) for 10 min. FMLP/CB, a stimulant, was applied to activate neutrophils to produce superoxide anions. The absorption change under the reduction of ferricytochrome c at 550 nm was continuously monitored in a double-beam, six-cell positioned spectrophotometer (Hitachi U-3010, Tokyo, Japan) under constant stirring. Calculations were based on differences in the reactions with and without superoxide dismutase (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome c (ε = 21.1/mM/10 mm). Sorafenib tosylate, a tyrosine protein kinase inhibitor, was used as the positive control.

Measurement of Elastase Release. Degranulation of azurophilic granules in human neutrophils was calculated by elastase release as previously reported.[37,38] Neutrophils were equilibrated with MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide (100 μM), an elastase substrate, at 37 °C for 2 min and treated with the isolated compounds for 5 min. Cells were activated using CB (0.5 μg/mL) and FMLP (100 nM), and the change in absorbance at 405 nm was examined to determine the elastase release. The results are expressed as the percentages of elastase

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release in the FMLP/CB-activated, drug-free control system. Sorafenib tosylate was used as the positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The 1D and 2D NMR and HRESIMS spectra of all new compounds are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Panthama, N.; Kanokmedhakul, S.; Kanokmedhakul, K. *J. Nat. Prod.* **2010**, *73*, 1366−1369.

(2) Chang, F. R.; Hwang, T. L.; Yang, Y. L.; Li, C. E.; Wu, C. C.; Issa, H. H.; Hsieh, W. B.; Wu, Y. C. *Planta Med.* **2006**, *72*, 1344−1347.

(3) Lee, T. H.; Wang, M. J.; Chen, P. Y.; Wu, T. Y.; Wen, W. C.; Tsai, F. Y.; Lee, C. K. *J. Nat. Prod.* **2009**, *72*, 1960−1963.

(4) González, M. C.; Sentandreu, M. A.; Rao, K. S.; Zafra-Polo, C.; Cortes, D. *Phytochemistry* **1996**, *43*, 1361−1364.

(5) Tuchinda, P.; Pohmakotr, M.; Reutrakul, V.; Thanyachareon, W.; Sophasan, S.; Yoosook, C.; Santisuk, T.; Pezzuto, J. M. *Planta Med.* **2001**, *67*, 572−575.

(6) Popsavin, V.; Benedeković, G.; Popsavina, M.; Kojićb, V.; Bogdanović, G. *Tetrahedron Lett.* **2010**, *51*, 3426−3429.

(7) Chen, C. Y.; Chang, F. R.; Shih, Y. C.; Hsieh, T. J.; Chia, Y. C.; Tseng, H. Y.; Chen, H. C.; Chen, S. J.; Hsu, M. C.; Wu, Y. C. *J. Nat. Prod.* **2000**, *63*, 1475−1478.

(8) Chuakul, W.; Boonpleng, A. *Thai. J. Phytopharm.* **2004**, *11*, 33−54.

(9) Kaewamatawong, R.; Ruangrungsi, N.; Likhitwitayawuid, K. *J. Nat. Med.* **2007**, *61*, 349−350.

(10) Tuchinda, P.; Munyoo, B.; Pohmakotr, M.; Thinapong, P.; Sophasan, S.; Santisuk, S.; Reutrakul, V. *J. Nat. Prod.* **2006**, *69*, 1728−1733.

(11) Abdullah, N.; Sahibul-Anwar, H.; Ideris, S.; Hasuda, T.; Hitotsuyanagi, Y.; Takeya, K.; Diederich, M.; Choo, C. *Fitoterapia* **2013**, *88C*, 1−6.

(12) Blázquez, M. A.; Bermejo, A.; Zafra-Polo, M. C.; Cortes, D. *Phytochem. Anal.* **1999**, *10*, 161−170.

(13) Lan, Y. H.; Chang, F. R.; Yu, J. H.; Yang, Y. L.; Chang, Y. L.; Lee, S. J.; Wu, Y. C. *J. Nat. Prod.* **2003**, *66*, 487−490.

(14) Mahiwan, C.; Buayairaksa, M.; Nuntasaen, N.; Meepowpan, P.; Pompimon, W. *Am. J. Appl. Sci.* **2013**, *10*, 112−116.

(15) Tai, B. H.; Huyen, V. T.; Huong, T. T.; Nhiem, N. X.; Choi, E. M.; Kim, J. A.; Long, P. Q.; Cuong, N. M.; Kim, Y. H. *Chem. Pharm. Bull.* **2010**, *58*, 521−525.

(16) Prasad, K. R.; Dhaware, M. G. *Synlett* **2007**, *7*, 1112−1114.

(17) Fatima, A.; Kohn, L. K.; Carvalho, J. E.; Pilli, R. A. *Bioorg. Med. Chem.* **2006**, *14*, 622−631.

(18) Marquissolo, C.; de Fatima, A.; Kohn, L. K.; Ruiz, A. L.; de Carvalho, J. E.; Pilli, R. A. *Bioorg. Chem.* **2009**, *37*, 52−56.

(19) Nakashima, K.; Kikuchi, N.; Shirayama, D.; Miki, T.; Ando, K.; Sono, M.; Suzuki, S.; Kawase, M.; Kondoh, M.; Sato, M.; Tori, M. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 387−394.

(20) Yoshida, T.; Yamauchi, S.; Tago, R.; Maruyama, M.; Akiyama, K.; Sugahara, T.; Kishida, T.; Koba, Y. *Biosci. Biotechnol. Biochem.* **2008**, *72*, 2342−2352.

(21) Mukhtar, M. P.; Awang, K.; Mustafa, M. R.; Norhanom, A. W.; Ashril, Y.; Hadi, A. H. A. *Malays. J. Sci.* **2000**, *19*, 7−12.

(22) Simsek, S.; Horzella, M.; Kalesse, M. *Org. Lett.* **2007**, *9*, 5637−5639.

(23) Gazaille, J. A.; Sammakia, T. *Org. Lett.* **2012**, *14*, 2678−2681.

(24) Lan, Y. H. Studies on the Chemical Constituents and Biological Activities of *Goniothalamus* and *Fissistigma* species. Ph.D. Thesis, Kaohsiung Medical University, Kaohsiung, Taiwan, 2006.

(25) Lan, Y. H.; Chang, F. R.; Liaw, C. C.; Wu, C. C.; Chiang, M. Y.; Wu, Y. C. *Planta Med.* **2005**, *71*, 153−159.

(26) Lan, Y. H.; Chang, F. R.; Yang, Y. L.; Wu, Y. C. *Chem. Pharm. Bull.* **2006**, *54*, 1040−1043.

(27) Tanna, A.; Nair, R.; Chanda, S. *J. Nat. Med.* **2009**, *63*, 80−85.

(28) Katkar, K. V.; Suthar, A. C.; Chauhan, V. S. *Pharmacogn. Rev.* **2010**, *4*, 62−68.

(29) Coussens, L. M.; Werb, Z. *Nature* **2002**, *420*, 860−867.

(30) Karin, M.; Greten, F. R. *Nat. Rev. Immunol.* **2005**, *5*, 749−759.

(31) Tabas, I.; Glass, C. K. *Science* **2013**, *339*, 166−172.

(32) Folmer, F.; Blasius, R.; Morceau, F.; Tabudravu, J.; Dicato, M.; Jaspars, M.; Diederich, M. *Biochem. Pharmacol.* **2006**, *71*, 1206−1218.

(33) Machana, S.; Weerapreeyakul, N.; Barusrux, S. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, 368−374.

(34) Yen, C. Y.; Chiu, C. C.; Haung, R. W.; Yeh, C. C.; Huang, K. J.; Chang, K. F.; Hseu, Y. C.; Chang, F. R.; Chang, H. W.; Wu, Y. C. *Mutat. Res.* **2012**, *747*, 253−258.

(35) Chen, W. Y.; Wu, C. C.; Lan, Y. H.; Chang, F. R.; Teng, C. M.; Wu, Y. C. *Eur. J. Pharmacol.* **2005**, *522*, 20−29.

(36) Hashimoto, T.; Suganuma, M.; Fujiki, H.; Yamada, M.; Kohno, T.; Asakawa, Y. *Phytomedicine* **2003**, *10*, 309−317.

(37) Liou, J. R.; El-Shazly, M.; Du, Y. C.; Tseng, C. N.; Hwang, T. L.; Chuang, Y. L.; Hsu, Y. M.; Hsieh, P. W.; Wu, C. C.; Chen, S. L.; Hou, M. F.; Chang, F. R.; Wu, Y. C. *Phytochemistry* **2013**, *88*, 67−73.

(38) Lee, C. L.; Hwang, T. L.; Yang, J. C.; Cheng, H. T.; He, W. J.; Yen, C. T.; Kuo, C. L.; Chen, C. J.; Chang, W. Y.; Wu, Y. C. *J. Nat. Prod.* **2014**, *77*, 1770−1783.