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(*Psidium guajava*)

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Supporting Information

ABSTRACT: *P* L., a species native to South America, has been widely cultivated in the tropical and subtropical areas of China for its popular fruits. The preliminary analysis by liquid chromatography-ultraviolet (LC-UV) indicated the presence of meroterpenoids in the fruits of *P.* (guava). Subsequent fractionation of the petroleum ether extract resulted in the identification of two new meroterpenoids, psiguajavadials A (**1**) and B (**2**), together with 14 previously described meroterpenoids (**3**−**16**). Their structures were fully elucidated by comprehensive spectroscopic techniques and theoretical calculations. All of the meroterpenoids showed cytotoxicities against five human cancer cell lines, with guajadial B (**12**) being the most effective having an IC_{50} value of 150 nM toward A549 cells. Furthermore, biochemical topoisomerase I (Top1) assay revealed that psiguajavadial A (**1**), psiguajavadial B (**2**), guajadial B (**12**), guajadial C (**14**), and guajadial F (**16**) acted as Top1 catalytic inhibitors and delayed Top1 poison-mediated DNA damage. The flow cytometric analysis indicated that the new meroterpenoids psiguajavadials A (**1**) and B (**2**) could induce apoptosis of HCT116 cells. These data suggest that meroterpenoids from guava fruit could be used for the development of antitumor agents.

KEYWORDS: , , , *DNA* *I* ,

It is well-known that human DNA topoisomerase I (Top1) could regulate the DNA topology through key cellular events, such as DNA replication and gene transcription.[1] Three camptothecin (CPT) derivatives, topotecan,[2] irinotecan,[3] and belotecan,[4] have been approved for the treatment of tumor so far. Some CPT derivatives are effective Top1 inhibitors, which can trap the enzyme as it cleaves the DNA by creating a Top1-DNA cleavage complex (Top1 cm^3), leading to DNA damage. Although, top1 catalytic inhibitors are currently less common,[5] these inhibitors could prevent the formation of covalent cleavage complex by blocking the nucleophilic attack of the scissile strand. In a previous work, indolizinoquinolinedione derivatives have been identified as Top1 catalytic inhibitors.[6]

P , a species of family Myrtaceae, is widely distributed throughout the tropical and subtropical regions.[7] The guava fruit is popularly consumed worldwide for its palatable taste and diverse health benefits. Moreover, guava fruit is also an important ingredient used for beverages, juice, and wine. Previous pharmacological studies indicate that the extract of guava exhibited antioxidant,[8] hypoglycemic,[9] antidiarrheal,[10] antifungal,[11] and hepatoprotective[12] properties. As a folk medicine,[13] the leaves of *P.* have been extensively studied for structurally interesting and antitumor meroterpenoids in the past decade.[14−18] However, the secondary metabolites of guava fruit are not characterized except for carotenoids.[19] In our search for bioactive meroterpenoids from *P.* , a phytochemical investigation of guava fruit by LC-UV has led to the isolation of two new meroterpenoids, psiguajavadials A (**1**) and B (**2**), together with 14 known analogues. In addition, the cytotoxicity against five tumor cell lines and Top1 inhibitory activity of these isolates were tested. In the current paper, we report the isolation, structure elucidation, Top1 inhibitory, and antitumor activities of these meroterpenoids in guava fruit.

. Optical rotations were determined on a Jasco P-1020 polarimeter. ECD spectra were recorded on an Applied Photophysics spectropolarimeter. UV spectra were taken on a Shimadzu UV2401 PC spectrophotometer. IR spectra were determined on a Bruker FT-IR Tensor-27 infrared spectrophotometer with KBr discs. HRESIMS were measured using an Agilent 1290 UPLC/6540 Q-TOF mass spectrometer. 1D and 2D NMR spectra were performed on Bruker Avance III-600 spectrometer with TMS as an internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals, and coupling constant (J) values were reported in Hz. Sephadex LH-20 (GE Chemical

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Corporation), Si gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China), and RP-18 (50 μm, Merck, Germany) were used for column chromatography (CC). Semipreparative HPLC was performed on an Agilent 1260 instrument with a ZORBAX SB-C18 column (5 μm, 9.4 × 250 mm). TLC spots were visualized under UV and then sprayed with 10% $FeCl_3$ in EtOH.

. Fresh mature guava fruits were collected from Xishuangbanna region, Yunnan Province, China, in December 2015. The specimen was identified by Dr. Rong Li (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (HY0026) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

. The dried and powdered plant material (6.0 kg) was extracted with petroleum ether (20 L × 3) at room temperature, and the solvent was evaporated in vacuo. The petroleum ether extract (420 g) was subjected to column chromatography over silica gel with a petroleum ether−EtOAc mixture (gradient from 1:0 to 5:1 v/v) to give six fractions (A−F). The subfraction B (32 g) was separated by column chromatography over Sephadex LH-20 ($CHCl_3$−MeOH, 3:2 v/v) and subsequently by column chromatography over RP-18 silica gel, eluted with MeCN−H_2O (gradient from 85:15 to 100:0 v/v) to afford four subfractions B_1−B_4. Subfraction B_1 (120 mg) was further purified by semipreparative HPLC using MeCN−0.01% TFA (gradient from 85:15 to 92:8 v/v) as mobile phase to yield compounds psiguajavadial A (**1**; 3.2 mg, $_R$ = 19.66 min), guadial A (**3**; 2.8 mg, $_R$ = 18.95 min), guadial B (**4**; 4.3 mg, $_R$ = 18.76 min), and guadial C (**5**; 3.5 mg, $_R$ = 19.22 min). Guajadial (**9**; 320 mg) and psidial A (**10**; 110 mg) were respectively obtained from subfractions B_3 (2.8 g) and B_2 (0.5 g) by recrystallization, while the residue of subfraction B_3 was purified by semipreparative HPLC with a gradient of MeCN−0.01% TFA (88:12 to 95:5 v/v) to afford psiguajavadial B (**2**; 6.2 mg, $_R$ = 25.64 min), psiguadial B (**6**; 45.8 mg, $_R$ = 28.65 min), psiguadial C (**7**; 5.1 mg, $_R$ = 24.98 min), psiguadial D (**8**; 32.6 mg, $_R$ = 27.35 min), 4,5-diepipsidial A (**11**; 8.3 mg, $_R$ = 25.48 min), and guajadial B (**12**; 9.4 mg, $_R$ = 29.22 min). Likewise, subfraction B_4 (1.1 g) was further purified by semipreparative HPLC (MeCN−0.01% TFA, gradient from 90:10 to 95:5 v/v) to give guajadial C (**13**; 28.7 mg, $_R$ = 28.45 min), guajadial D (**14**; 25.4 mg, $_R$ = 29.23 min), guajadial E (**15**; 32.1 mg, $_R$ = 25.89 min), and guajadial F (**16**; 29.6 mg, $_R$ = 27.52 min).

*Psiguajavadial A (**1**).* Colorless gum; $[\alpha]_D^{24}$ + 115.3 (0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.47), 285 (4.54) nm; ECD (MeOH) 250 ($\Delta\varepsilon$ − 1.8), 278 ($\Delta\varepsilon$ + 9.6) nm; IR (KBr) ν_{max} 3441, 2957, 2929, 1633, 1442, 1384, 1301, 1221, 1179 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data, see Table 1; HRESIMS / 429.1680 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_5Na$, 429.1672).

*Psiguajavadial B (**2**).* Colorless gum; $[\alpha]_D^{22}$ − 110.3 (0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.41), 295 (4.57) nm; ECD (MeOH) 268 ($\Delta\varepsilon$ − 3.3), 308 ($\Delta\varepsilon$ + 1.5), 351 ($\Delta\varepsilon$ + 1.2); IR (KBr) ν_{max} 3440, 2969, 2924, 1630, 1443, 1384, 1302, 1270, 1181 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR data, see Table 2; HRESIMS / 475.2477 $[M + H]^+$ (calcd for $C_{30}H_{35}O_5$, 475.2479).

*Guajadial C (**13**).* ECD (MeOH) 282 ($\Delta\varepsilon$ + 12.3) nm.

*Guajadial E (**15**).* ECD (MeOH) 246 ($\Delta\varepsilon$ + 4.9), 285 ($\Delta\varepsilon$ − 13.0), 345 ($\Delta\varepsilon$ − 2.8) nm.

. The cytotoxic activities of compounds **1**−**16** were evaluated against five human cancer cell lines (HCT116, CCRF-CEM, DU145, Huh7, and A549) using the MTT method in 96-well microplates. The compounds were tested in a five-dose assay ranging from 10^{-8}∼10^{-4} M. The IC_{50} value of each compound was calculated by nonlinear regression analysis. The experiments were conducted for three independent replicates, and camptothecin (CPT) was used as a positive control.

1- . The compounds were tested for Top1 inhibitory activities using Top1-mediated relaxation assay. Briefly, the reaction mixture (20 μL) containing 1.0 μL of supercoiled pBR322 DNA (0.5 μg/μL) with relaxation buffer (10 mM Tris-HCl, PH 7.5, 50 mM KCl, 5.0 mM $MgCl_2$, 15 μg/mL BSA, 40 μg/mL DTT) was incubated with 1 unit of calf thymus Top1 in the absence or presence of the compound for 0.5 h at 37 °C. Then to the sample was added 4.0 μL 6 × loading buffer and was analyzed using a 0.8% agarose gel in TBE buffer at 4.6 V/cm for 1.5 h. The gel was stained with 1 × gel red for 0.5 h and subsequently visualized with a UV transilluminator. Being a well-known Top1 inhibitor, CPT was used as a positive control.

1- . Top1-mediated cleavage assay was conducted as previously described. The reaction mixture (20 μL), containing 1.0 μL of supercoiled pBR322 DNA (0.5 μg/μL) and 10

Table 1. ^1H and ^{13}C NMR Spectroscopic Data of Psiguajavadial A (1) in $CDCl_3$

no.	δ_C	δ_H (mult., *J* in Hz)	no.	δ_C	δ_H (mult., *J* in Hz)
1	90.9, C		1′	34.7, CH	4.22, t (6.9)
2	30.4, CH	1.27, dd (8.5, 2.3)	2′	102.7, C	
3	13.3, CH_2	a 0.52, br t (6.7)	3′	164.3, C	
		b 0.31, br t (4.2)	4′	104.4, C	
4	34.2, C		5′	168.8, C	
5	25.3, CH_2	a 1.93, q (10.6)	6′	103.9, C	
		b 1.56, dd (12.2, 8.1)	7′	169.7, C	
6	34.1, CH_2	a 1.64, dd (14.7, 8.5)	8′	143.9, C	
		b 0.91, q (11.4)	9′	126.9, CH	7.13, d (7.6)
7	38.4, CH_2	a 2.33, dd (14.1, 7.0)	10′	128.4, CH	7.27, t (7.5)
		b 2.13, dd (14.1, 7.0)	11′	126.2, CH	7.20, t (7.2)
8	32.3, CH	1.36, m	12′	128.4, CH	7.27, t (7.5)
9	20.1, CH_3	0.94, d (7.2)	13′	126.9, CH	7.13, d (7.6)
10	19.7, CH_3	0.93, d (7.2)	14′	192.2, CH	10.10, s
5′−OH		13.57, s	15′	191.6, CH	10.16, s
7′−OH		13.22, s			

Table 2. ^1H and ^{13}C NMR Spectroscopic Data of Psiguajavadial B (2) in $CDCl_3$

no.	δ_C	δ_H (mult., *J* in Hz)	no.	δ_C	δ_H (mult., *J* in Hz)
1	127.1, CH	4.90, br d (9.2)	1′	48.6, CH	3.79, s
2	24.3, CH_2	a 1.54, br d (14.0)	2′	105.7, C	
		b 1.23, overlapped	3′	163.0, C	
3	40.0, CH_2	a 2.03, dd (15.1, 4.9)	4′	103.6, C	
		b 1.80, t (13.9)	5′	168.6, C	
4	40.9, C		6′	104.0, C	
5	80.0, CH	4.45, d (7.1)	7′	168.8, C	
6	28.5, CH	0.93, t (7.8)	8′	142.8, C	
7	32.7, CH	0.57, br t (9.9)	9′	128.9, CH	6.91, br s
8	23.3, CH_2	a 1.92, m	10′	128.2, CH	7.25, overlapped
		b 0.99, q (12.8)	11′	126.9, CH	7.25, overlapped
9	37.6, CH_2	a 1.95, t (13.9)	12′	132.1, CH	7.49, br s
		b 1.87, t (12.5)	13′	128.7, CH	7.33, br s
10	131.3, C		14′	192.7, CH	10.18, s
11	19.4, C		15′	191.4, CH	10.07, s
12	30.5, CH_3	1.17, s	5′−OH		13.74, s
13	19.9, CH_3	1.23, s	7′−OH		13.05, s
14	18.0, CH_3	1.08, s			
15	26.0, CH_3	1.13, s			

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units of Top1 in the buffer, was incubated for 0.5 h at 37 °C. Then to the sample was added 4.0 μL of proteinase K (5.5 mg/mL) at 55 °C for 15 min. The reaction was terminated by the addition of 5.0 μL 6 × loading buffer and was analyzed using a 0.8% agarose gel in TBE buffer at 3 V/cm for 0.5 h. The gel was stained with 0.125 μg/mL ethidium bromide (EB) for 0.5 h. The sample was visualized with a UV transilluminator.

1- **.** Top1-mediated EMSA assay was performed to assess either the compounds hampered the binding of Top1 to DNA. 1.0 μL supercoiled pBR322 DNA (0.1 μg/μL) was incubated in 20 μL buffer with 4 units of Top1 in the absence or presence of the compound at 37 °C for 0.5 h. Then to the sample was added 4 μL 6 × loading buffer and was analyzed using a 0.8% agarose gel with 1% EB in TBE buffer at 0.8 V/cm for 6.5 h. The gel was stained with 1 × gel red for 0.5 h and visualized with a UV transilluminator.

1- **.** The DNA binding affinity of the compounds was tested by Top1-mediated unwinding assay. The reaction mixture (20 μL) contained 1.0 μL of supercoiled pBR322 DNA (0.2 μg/μL) or relaxed pBR322 DNA (1.0 μL) as a substrate and 20 units of Top1 in the buffer. The compound was incubated with DNA at room temperature for 10 min prior to the addition of excess Top1 for 0.5 h at 37 °C. The reaction was terminated by the addition of 2.0 μL of 5% SDS and 3.0 μL of 5 mg/mL proteinase K. The DNA intercalator ethidium bromide (EB) was used as a positive control. Then to the sample was added 5.0 μL 6 × loading buffer and was analyzed using 0.8% agarose gel in TBE buffer at 4.6 V/cm for 1.5 h. The gel was stained with 1 × gel red for 0.5 h and visualized with a UV transilluminator.

. HCT116 cells (3.0×10^5 cells/mL) were grown in culture medium on a 6-well plate treated with 10, 20, and 40 μM of each compound or untreated for 24 h. Then the cells were harvested from the medium and washed with cold PBS, resuspended in 1 × binding buffer, and then stained with 5.0 μL of FITC annexin V and 10.0 μL of propidium iodide (KeyGen Biotech, China) for 15 min in the dark. The stained cells were analyzed by using flow cytometry (BD, FACSCalibur, USA) within 1.0 h. The experiments were repeated three times.

fl **.** HCT116 cells grown on coverglass bottom dish were fixed in 4% paraformaldehyde/PBS for 15 min, then permeabilized with 0.5% triton-X100/PBS at 37 °C for 30 min, and finally blocked with 5% goat serum/PBS at 37 °C for 3 h. Immunofluorescence assay was performed using standard methods, and the slides were incubated alternately with phospho-γ

. The petroleum ether extract of the fruits of *P.* was subjected to column chromatography over silica gel, Sephadex LH-20, RP-18, and semipreparative HPLC to afford 16 compounds for the first time, including two new ones (**1** and **2**, Figure 1).

Psiguajavadial A (**1**) was isolated as a colorless gum. Its molecular formula, $C_{25}H_{26}O_5$, was determined by HRESIMS which showed a sodium cluster at / 429.1680 $[M + Na]^+$ (calcd. for $C_{25}H_{26}O_5Na$, 429.1672) and the ^{13}C NMR data (Table 1), indicating 13 degrees of unsaturation. The UV spectrum was characteristic of *P* meroterpenoids with absorption maxima at 205 and 285 nm. The IR spectrum revealed the presence of hydroxy (3441 cm^{-1}) and aromatic ring (1633 and 1442 cm^{-1}). The ^1H NMR, ^{13}C NMR, and HSQC spectra indicated the presence of two hydroxy protons [δ_H 13.57 (1H, s) and 13.22 (1H, s)], a 3,5-diformyl phloroglucinol unit [δ_H 10.16 (1H, s), δ_C 191.6; δ_H 10.10 (1H, s), δ_C 192.2; δ_C 169.7, 168.8, 164.3, 104.4, 103.9, and 102.7)], a monosubstituted aromatic ring [δ_C 143.9; δ_H 7.27 (2H, t, J = 7.5 Hz), δ_C 128.4 × 2; δ_H 7.20 (1H, t, J = 7.2 Hz), δ_C 126.2; and δ_H 7.13 (2H, d, J = 7.6 Hz), δ_C 126.9 × 2], and a methine proton [δ_H 4.22 (1H, t, J = 6.9 Hz), δ_C 34.7]. The remaining ten carbon signals of the monoterpene moiety were ascribed to two quaternary carbons (including one oxygenated at δ_C 90.9), two methines, four methylenes, and two methyls. In the ^1H NMR spectrum, the two upfield signals at δ_H 0.52 (1H, br t, J = 6.7 Hz) and 0.31 (1H, br t, J = 4.2 Hz) suggested the presence of a cyclopropane unit. By comparing the ^1H and ^{13}C NMR spectroscopic data (Table 1) of psiguajavadial A (**1**) and guadial A (**3**),[16] it was found that they shared the same planar structure, except for the difference in the configurations of chiral centers at C-1 and C-1′.

The relative configuration of psiguajavadial A (**1**) was then assigned by its ROESY spectrum (Figure 2). The ROE

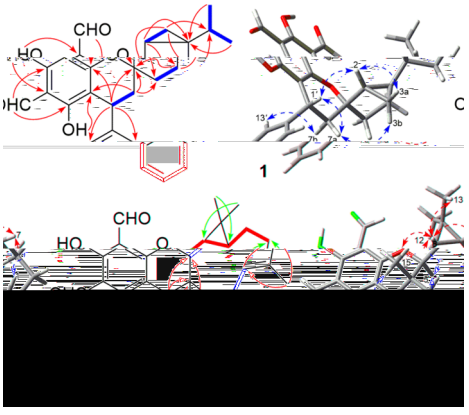

Figure 2. Key 2D NMR correlations of **1** and **2**.

correlations of H-1′ with H-2/H-7a, of H-2 with H-3a, of H-7a with H-3b, and of H-7b with H-13′ inferred that these were cofacial. To determine the absolute configuration, the electronic circular dichroism spectra of psiguajavadial A (**1**) were calculated and compared with the experimental ECD spectrum. The calculated ECD curve well matched the experimental one for the 1*R*,2 ,4*R*,1′*R*-configuration (Figure 3). Therefore, the structure of psiguajavadial A (**1**) was established as shown.

Psiguajavadial B (**2**) was obtained as a colorless gum. Its molecular formula, $C_{30}H_{34}O_5$, was established from a pseudo-molecular ion peak in the HRESIMS at / 475.2477 $[M + H]^+$

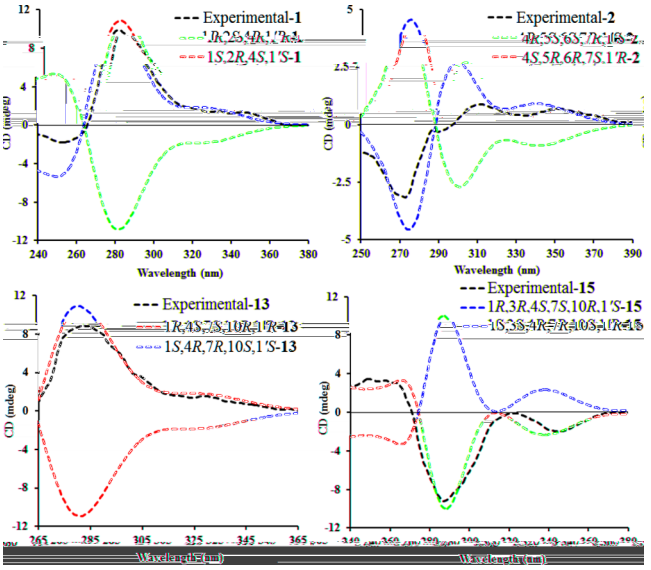

Figure 3. Calculated and experimental ECD spectra of **1**, **2**, **13**, and **15**.

(calcd. for $C_{30}H_{35}O_5$, 475.2479) and the ^{13}C NMR data. As for psiguajavadial A (**1**), the 3,5-diformylbenzyl phloroglucinol moiety was readily identified using diagnostic 2D NMR techniques (Figure 2). Moreover, a double bond (δ_H 4.90, br d, J = 9.2 Hz, δ_C 127.1; δ_C 131.3) was identified in the terpene part by the ^1H and ^{13}C NMR data. These groups accounted for 11 out of the 14 indices of hydrogen deficiency, and the remaining indices required the construction of three additional rings. With the aid of $^1H-^1H$ COSY, HSQC, HMBC, and ROESY experiments, all of the ^1H and ^{13}C NMR signals of psiguajavadial B (**2**) were assigned as shown in Table 2. Overall, the NMR data of psiguajavadial B (**2**) exhibited similarities to a known meroterpenoid, psiguadial D (**8**)[16] that was also obtained in this study, with significant differences for C-5 (δ_C 85.3→80.0) and C-1′ (δ_C 43.9→48.6), indicating that psiguajavadial B (**2**) was an epimer of psiguadial D (**8**) at C-1′.

The relative configuration of psiguajavadial B (**2**) was determined by ROESY experiment (Figure 2). The H-6 showed ROESY correlations with H-7/H_3-13/H_3-15, while the H_3-15 correlated with H-1′ indicated that all of these were cofacial. Besides, the H_3-12 showed correlations only with H-5 indicating these both to be cofacial. However, ROESY correlations of H_3-14 with H-5 and of H-1 with H-7 substantiated the double bond between C-1 and C-10 was *E* configuration rather than that of Z configuration in psiguadial D (**8**). Moreover, the absolute configuration of psiguajavadial B (**2**) was determined as 4*R*,5 ,6 ,7*R*,1′ by comparing its experimental ECD spectrum with the calculated ones (Figure 3). Thus, the structure of psiguajavadial B (**2**) was established as depicted.

The known compounds (**3**−**16**) were identified as guadial A (**3**),[16] guadial B (**4**),[20] guadial C (**5**),[20] psiguadial B (**6**),[15] psiguadial C (**7**),[16] psiguadial D (**8**),[16] guajadial (**9**),[21] psidial A (**10**),[22] 4,5-diepipsidial A (**11**),[23] guajadial B (**12**),[24] and guajadials C−F (**13**−**16**),[25] by comparing the spectroscopic data with those reported in the literature. To the best of our knowledge, these known meroterpenoids, which were previously reported from the leaves of *P.* , are obtained for the first time from its fruits. In addition, the absolute configurations of guajadials C (**13**) and E (**15**) were, respectively, determined as 1*R*,4 ,7 ,10*R*,1′*R* and 1*R*,3*R*,4 ,7 ,10*R*,1′ (Figure 3) by ECD evidence.

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Table 3. Top1 Inhibitory (%) and Cytotoxic (μM) Effects of Meroterpenoids 1−16

		cytotoxicity (IC_{50} ± SD)				
compd	relaxation assay	HCT116	CCRF-CEM	DU145	Huh7	A549
1	++++	7.60 ± 1.0	25.2 ± 4.0	20.2 ± 4.7	48.8 ± 5.3	2.99 ± 0.1
2	++++	21.6 ± 2.8	9.63 ± 3.4	26.3 ± 4.1	13.7 ± 1.7	0.90 ± 0.8
3	++++	5.74 ± 1.4	2.95 ± 0.6	5.35 ± 0.7	28.0 ± 2.6	9.62 ± 3.8
4	++++	26.5 ± 2.1	6.72 ± 3.2	18.0 ± 2.5	55.3 ± 6.3	13.4 ± 4.4
5	++++	13.0 ± 3.1	12.9 ± 3.8	14.5 ± 3.2	29.6 ± 2.7	5.70 ± 1.2
6	++	15.5 ± 2.6	18.2 ± 0.1	43.3 ± 5.4	47.0 ± 4.3	8.73 ± 2.1
7	+++	14.4 ± 1.2	9.30 ± 1.0	49.1 ± 5.1	10.8 ± 2.5	3.06 ± 0.9
8	++++	7.00 ± 1.9	2.59 ± 0.4	6.08 ± 3.9	5.20 ± 2.0	1.07 ± 0.1
9	+++	20.4 ± 1.5	0.87 ± 0.5	11.8 ± 3.9	20.7 ± 2.2	2.42 ± 1.2
10	++	11.5 ± 1.9	17.4 ± 5.8	11.9 ± 0.1	47.0 ± 6.8	14.1 ± 0.7
11	+++	9.13 ± 4.1	7.00 ± 2.2	4.79 ± 2.7	2.82 ± 0.6	0.16 ± 0.03
12	++++	3.54 ± 2.3	7.58 ± 0.7	16.4 ± 1.8	2.93 ± 0.5	0.15 ± 0.05
13	++++	4.42 ± 0.3	6.9 ± 1.6	42.8 ± 9.6	55.4 ± 1.1	33.6 ± 2.9
14	++++	0.61 ± 0.1	16.0 ± 8.9	30.3 ± 5.3	44.09 ± 8.3	36.2 ± 7.1
15	+++	4.69 ± 1.8	12.7 ± 2.8	23.2 ± 3.5	51.5 ± 3.0	18.4 ± 5.2
16	++++	3.66 ± 0.3	7.80 ± 1.9	27.7 ± 6.7	11.1 ± 3.0	13.8 ± 1.0
CPT	++++	0.012 ± 0.009	0.0019 ± 0.0003	0.015 ± 0.003	0.0022 ± 0.0003	0.040 ± 0.001

Top1 inhibitory activity was semiquantitatively expressed relative to CPT at 50 μM as follows: ++++, more than 90% of the activity; +++, between 60% and 89% of the activity; ++, between 30% and 59% of the activity; + , less than 29% of the activity.

. Based on the fact that meroterpenoids from the leaves of *P.* have demonstrated potential antitumor activities, all of the isolated meroterpenoids were evaluated for their inhibitory effects on the growth of five human tumor cells (HCT116, CCRF-CEM, DU145, Huh7, and A549). A primary screening showed that all of these meroterpenoids exhibited cytotoxicity with the cell viability rates less than 50% at 60 μM concentration. Then, a further assay was carried out to obtain the IC_{50} values (Table 3). The results demonstrated that all of these isolates showed cytotoxicity against the selected cancer cells. The compounds 4,5-diepipsidial A (**11**) and guajadial B (**12**) displayed significant cytotoxicity against A549 cells with IC_{50} values of 160 and 150 nM, respectively.

1 . As shown in Figure 4, psiguajavadial A (**1**), guajadial C (**13**), guajadial D (**14**), and guajadial F (**16**) exhibited an equipotent inhibitory activity to CPT at 50 μM, whereas psiguajavadial A (**1**), psiguajavadial B (**2**), guajadial B (**12**), guajadial D (**14**), and guajadial F (**16**) showed a dose-dependent inhibition of Top1 activity at the concentrations of 0.2, 1, 5, 25, and 125 μM, respectively. The new compounds (**1** and **2**) were selected as lead for further studies. The new compounds could not induce Top1-mediated DNA cleavage up to 50 μM concentration, which means the new compounds are not the Top1 poisons. Top1-mediated EMSA (Figure 5B) indicated that the new compounds did not hamper the binding of Top1 to DNA. It is well established whether the meroterpenoids produce DNA unwinding in the presence of excess Top1. EB was used as a positive control for Top1-mediated unwinding assay. The reaction was carried out with supercoiled pBR322 DNA as substrate or with relaxed pBR322 DNA as substrate (Figure 5C). The Top1 assays indicate that meroterpenoids are Top1 catalytic inhibitors, which prevent the formation of covalent cleavage complex by blocking the nucleophilic attack of the scissile strand.

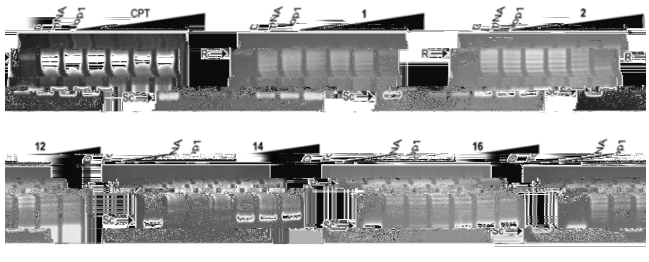

Figure 4. Top1-mediated relaxation assay. lane 1, pBR322 DNA alone; lane 2, pBR322 DNA and Top1 without compound; lanes 3−7, pBR322 DNA, Top1 and CPT/tested compounds at 0.2, 1, 5, 25, and 125 μM, respectively. R, relaxed DNA; Sc, supercoiled DNA.

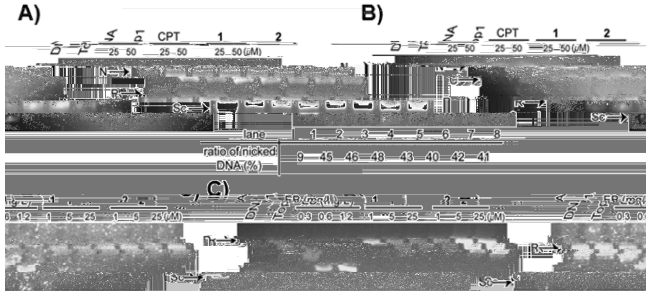

Figure 5. (A) Top1-mediated DNA cleavage assay. Lane 1, pBR322 DNA alone; lane 2, pBR322 DNA and Top1 without compound; lanes 3−8, pBR322 DNA, Top1 and drugs at 25 and 50 μM. respectively. (B) DNA mobility shift analysis of Top1-DNA binding. Lane 1, pBR322 DNA alone; lane 2, pBR322 DNA and Top1 without compound; lanes 3−8, pBR322 DNA, Top1 and drugs at 5 and 25 μM, respectively. (C) Top1-mediated unwinding assay. Lane 1, DNA alone; lane 2, DNA and Top1 without compound; lanes 3−5, DNA, Top1 and EB at 0.3, 0.6, and 1.2 mg/L; lanes 6−11, DNA, Top1 and tested compounds at 1, 5, and 25 μM, respectively. R, relaxed DNA; Sc, supercoiled DNA; N, nicked DNA; C, Top1-bound DNA.

- / . Cells were collected after 24 h treatment, and the apoptotic cells were evaluated by flow cytometry. Figure 6 shows the different quadrants indicating cells at various stages: A1, necrotic cells; A2, late apoptosis cells; A3, early apoptosis cells; and A4, live cells. Psiguajavadials A (**1**) and B (**2**) both

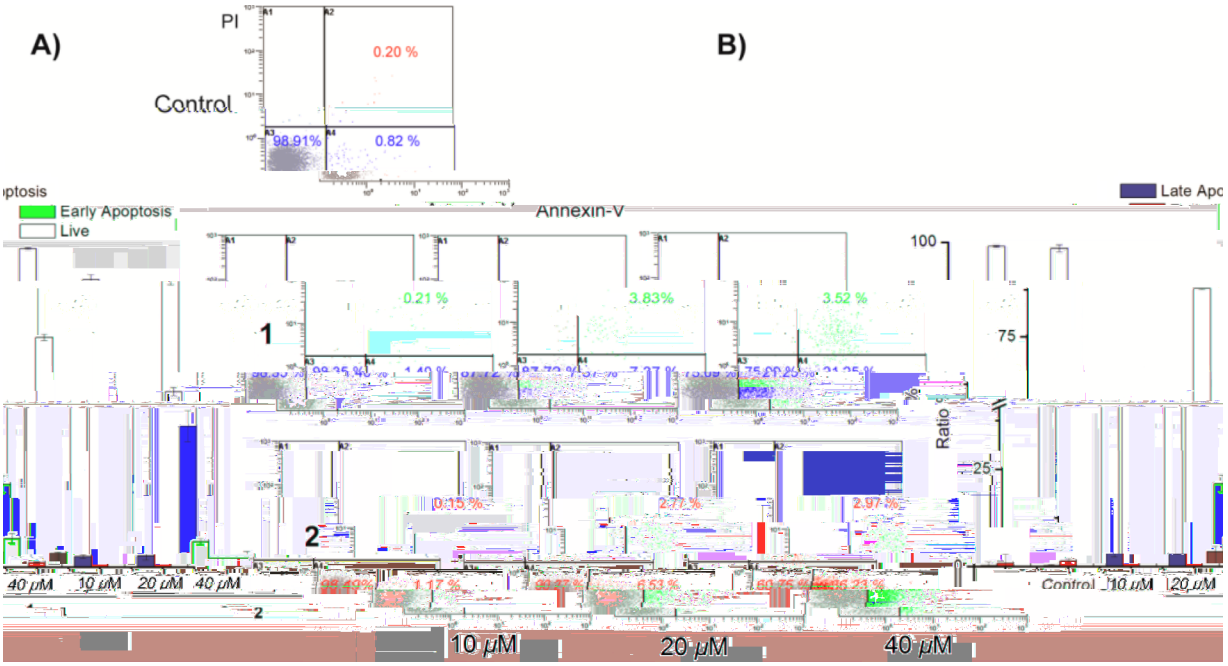

Figure 6. (A) Flow cytometry histograms and (B) the quantitation of the apoptosis of HCT116 cells untreated or treated with tested compounds at 10, 20, and 40 μM, respectively for 24 h (= 3).

displayed potent antiproliferative effects against HCT-116 cells by inducing apoptosis in a dose-dependent manner.

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A hallmark of DNA double-strand breaks termed as γH2AX was measured by immunofluorescence analysis in HCT116 cells (Figure 7). To show the DNA damage produced by positive control CPT at 0.5 μM for 3 h, γH2AX induction was assessed. On the contrary, HCT-116 cells treated with psiguajavadial A (**1**) or psiguajavadial B (**2**) up to concentration of 14 μM for 15 h could not show clear γH2AX formation, suggesting that **1** and **2** antagonized Top1-mediated DNA break. This finding is also consistent with the results obtained from the Top-mediated cleavage assay.

Figure 7. Fluorescence microscopy detection of γH2AX foci formation in HCT116 cells. Cells were treated with CPT (0.5 μM) for 3 h or treated with tested compounds (14 μM) for 15 h.

It is well-known that the edible and palatable fruits of *P.* are commonly used in daily consumption. In the current study, 16 meroterpenoids including two new ones, namely psiguajavadials A (**1**) and B (**2**), were obtained from guava fruits by an LC-UV guidance. All of these meroterpenoids showed a good cytotoxicity against five human tumor cell lines (HCT116, CCRF-CEM, DU145, Huh7, and A549) and a strong Top1 inhibitory activity. The compounds were bound to Top1 to prevent the formation of covalent cleavage complex as Top1 catalytic inhibitors. Interestingly, the 14 known meroterpenoids (**3**−**16**) were also isolated from the leaves of *P.* previously, which implies that the favorability of guava may be not only due to its pleasant taste but also hidden healthcare functions. In other words, as a resource of edible fruits and meroterpenoids that have multiple health functions, guava fruits will draw increasingly more attention from food scientists because it is consumed worldwide and has abundant nutritional and pharmaceutical values.

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Original NMR and HRESIMS spectra of psiguajavadials A (**1**) and B (**2**). (PDF)

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