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Bioassay-Guided Isolation of Constituents of *Piper sarmentosum* Using a Mitochondrial Transmembrane Potential Assay

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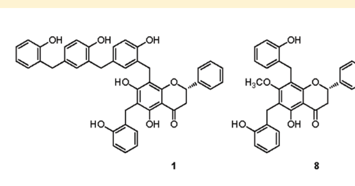
Supporting Information

ABSTRACT: Bioassay-guided fractionation was conducted on a chloroform-soluble extract of the aerial parts of *Piper sarmentosum* collected in Vietnam, monitored by a mitochondrial transmembrane potential assay using HT-29 human colon cancer cells. This led to the isolation of four new *C*-benzylated dihydroflavones, sarmentosumins A–D (**1**–**4**), as well as 14 known compounds. The structures of the new compounds were elucidated on the basis of spectroscopic data interpretation. Among these compounds, **1**–**4** as well as five known *C*-benzylated dihydroflavones (**5**–**9**) and a piperamide, pipercallosine (**11**), were found to induce apoptosis in HT-29 cells by moderately reducing the mitochondrial transmembrane potential ($\Delta\Psi m$), with ED_{50} values ranging from 1.6 to 13.6 μM. Furthermore, 7-methoxydichamanetin (**8**) and pinocembrin (**10**) exhibited proteasome inhibitory activities in a human 20S proteasome bioassay with IC_{50} values of 3.45 ± 0.18 and 2.87 ± 0.26 μM, respectively. This is the first time that *C*-benzylated dihydroflavones have been reported to demonstrate an apoptotic effect associated with disruption of the mitochondrial transmembrane potential.



Piper sarmentosum Roxb. (Piperaceae), known as "wild betel", is a terrestrial herb with long creeping stems and a characteristic pungent odor. This plant is distributed mainly in tropical and subtropical regions of Asia, from northeast India to southern mainland China and Malaysia, and the Andaman Islands.[1−3] *P. sarmentosum* is used as a folk medicine in certain countries of Southeast Asia for the treatment of various ailments including asthma, cough, dyspepsia, fever, fungal dermatitis of the feet, headache, pleurisy, and toothache.[1−4] Besides its medicinal uses, the leaves of *P. sarmentosum* are also consumed as a popular spice.[5]

Several studies have been carried out to evaluate the biological activities of extracts of different parts of *P. sarmentosum*. An aqueous extract of the whole plant was reported to show hypoglycemic effects.[6] An aqueous extract of the leaves was found to possess antinociceptive and anti-inflammatory activities.[7] Methanol extracts of the leaves exhibited neuromuscular blocking action[8] and antibacterial properties,[9] in addition to the root having antiamebic effects.[10] An ethanol extract of the whole plant showed insecticidal activity against mosquitoes,[11,12] while a chloroform extract of the leaves was reported to have antimalarial activity[13] and an antiangiogenic effect.[14] In addition, both the aqueous and organic phase extracts of the fruits and leaves were found to exhibit antioxidant activity.[15] Previous phytochemical investigations on *P. sarmentosum* have resulted in the isolation of aromatic alkene, amide, lignan, phenylpropanoid, and sterol chemical constituents.[16,17] Among these compounds, certain amides from *P. sarmentosum* exhibit antiplasmodial, antimycobacterial, antifungal, and antituberculosis properties,[16,17] and thus may be considered as a major class of active principles from this plant.

As part of an ongoing investigation on the discovery of natural anticancer agents from tropical plants, a $CHCl_3$-soluble extract of the aerial parts of *P. sarmentosum*, including the leaves, twigs, stems, and inflorescence, collected in Vietnam, exhibited mitochondrial transmembrane potential (MTP) inhibitory activity in HT-29 human colon cancer cells, as used in an initial screening procedure. This extract was not appreciably cytotoxic for HT-29 cells, when evaluated using a standard protocol.[18] In the present investigation, activity-guided fractionation of this extract using this MTP assay led to the isolation of the four new *C*-benzylated dihydroflavones (**1**–**4**), together with 13 known compounds. The structures of the four new compounds **1**–**4** were established

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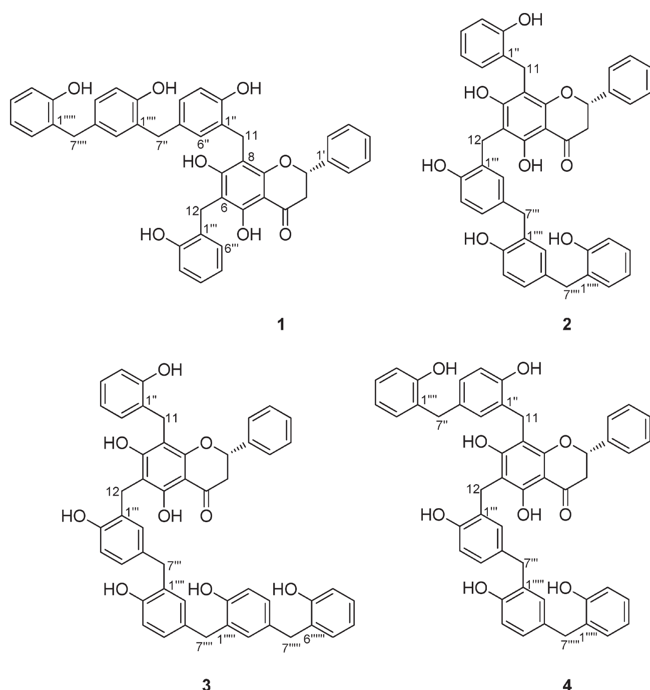
by spectroscopic data interpretation. The known compounds were identified as isochamanetin (**5**),[19] 7-methoxychamanetin (**6**),[20,21] dichamanetin (**7**),[19,22] 7-methoxydichamanetin (**8**),[21] 5″-(2‴′-hydroxybenzyl)uvarinol (2‴-hydroxy-5″-benzylisouvarinol-B) (**9**),[23] pinocembrin (**10**),[19,22] pipercallosine (**11**),[24] pellitorine,[25] 2,4-dodecadienamide,[25,26] pipercallosidine,[24] 7-methoxyisochamanetin,[20] sesamin,[27] benzoic acid,[28] and *trans*-cinnamic acid,[29] by spectroscopic analysis and comparison of the data obtained with literature values.



Induction of apoptosis, or programmed cell death, has been considered as an important mechanism of action for many antineoplastic agents, so targeting the apoptotic signaling pathway is a promising approach for anticancer drug discovery. The mitochondria play an important role in regulation of the intrinsic apoptotic pathway, and the loss of mitochondrial transmembrane potential is one of the key events that may occur during the apoptotic process. Thus, measurement of mitochondrial membrane potential ($\Delta\Psi$m) is a suitable method to study signaling mechanisms involved in the initiation of the apoptotic cascade.[30,31] In a similar fashion, inhibition of the proteasome results in accumulation of many ubiquitinated proteins that are involved with multiple cellular functions and eventually triggers apoptosis. Proteasome is the catalytic core of the complex ubiquitin-dependent protein degration pathway and plays an important role in cell-cycle regulation. Since tumor cells are more susceptible to proteasome inhibition than normal cells, targeting the proteasome pathway has been demonstrated as a strategy to investigate potential new cancer chemotherapeutic agents.[32] Accordingly, the isolates obtained in this study were evaluated for their effects in the MTP and proteasome assays.

■ RESULTS AND DISCUSSION

Compound **1** was obtained as a pale yellow gum. The HRESIMS showed a sodiated molecular ion peak at m/z 703.2297 $[M + Na]^+$, corresponding to a molecular formula of $C_{43}H_{36}O_8Na$, which indicated 26 degrees of unsaturation. The UV spectrum displayed characteristic absorptions of a dihydroflavone nucleus at 331 and 281 nm. The IR spectrum exhibited absorptions for hydroxy (3370 cm^{-1}), carbonyl (1631 cm^{-1}), and aromatic moieties (1605 and 1501 cm^{-1}). In the ^{1}H NMR spectrum, the signals for an ABX system at δ_H 2.74 (1H, dd, J = 17.2, 2.8 Hz, H-3β), 3.03 (1H, dd, J = 17.2, 13.2 Hz, H-3α), and 5.32 (1H, dd, J = 13.2, 2.8 Hz, H-2) were used to characterize the C ring of the dihydroflavone moiety. Signals of five protons at δ_H 7.40−7.46 were assigned to the aromatic protons of ring B. A singlet at δ_H 12.56 indicated the presence of an OH group at C-5, which formed an intramolecular hydrogen bond with the oxygen atom at C-4. These ^{1}H NMR signals were indicative of a typical skeleton of dihydroflavanone such as pinocembrin (5,7-dihydroxyflavanone, **10**), except for the absence of two aromatic protons around δ_H 6.10 ascribed to H-6 and H-8, which implied that C-6 and C-8 of compound **1** are substituted. Besides these proton signals assigned to the dihydroflavone nucleus, singlets at δ_H 3.69 (2H, s), 3.75 (2H, s), 3.86 (2H, s), and 3.87 (2H, s) attributed to four methylene groups and signals for 14 aromatic protons from δ_H 6.70 to 7.40 could be recognized in the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum, a total of 43 carbon signals were observed, which were sorted from the DEPT and HSQC spectra into five methylenes, an oxygenated methine, a carbonyl, and 36 carbon signals in the aromatic region from δ_C 102 to 162, including 19 tertiary carbons and 17 quaternary carbons, consistent with the presence of six benzene rings in the whole molecule. The NMR spectra of **1** were very similar to those of dichamanetin (**7**), a 5,7-dihydroxyflavanone with two (2-hydroxyphenyl)methyl groups attached to C-6 and C-8, respectively,[19,22] which was also obtained in the present investigation. On comparison of the NMR data of these two compounds, two additional *p*-substituted-2-hydroxyphenyl methyl groups were recognized in compound **1**, based on the signals of two more methylene protons at δ_H 3.69 (2H, s, 7″) and 3.86 (2H, s, H-7‴′), and six further aromatic protons resonating between δ_H 6.6 to 7.2 in the ^{1}H NMR spectrum. In addition, extra carbon signals appeared in pairs at δ_C 35.6/35.8 (C, C-7″ and C-7‴′), 126.2/127.5 (C, C-1″ and C-1‴′), 151.4/152.2 (C, C-2″ and C-2‴′), 115.7/116.1 (CH, C-3″ and C-3‴′), 127.9/127.8 (CH, C-4″ and C-4‴′), 131.9/132.0 (C, C-5″ and 5‴′), and 132.1/131.0 (CH, C-6″ and 6‴′) in the ^{13}C NMR spectrum. In the ESIMS/MS of compound **1**, fragment peaks from the parent ion at m/z 703 $[M + Na]^+$ were observed at m/z 609 $[M - C_6H_5OH + Na]^+$, 597 $[M - C_7H_6O + Na]^+$, 397 $[M - C_6H_4(OH)CH_2C_6H_3(OH)CH_2C_6H_4(OH) + Na]^+$, 385 $[M - C_6H_4(OH)CH_2C_6H_3(OH)CH_2C_7H_5O + Na]^+$, and 303 $[M - C_6H_4(OH)CH_2C_6H_3(OH)CH_2C_6H_4(OH) - C_6H_5OH + Na]^+$, which supported the presence of four hydroxyphenyl groups. When the ^{1}H NMR ($CDCl_3$) spectrum of dichamanetin (**7**) is considered, the H-6″ and H-6‴ proton signals appeared downfield at δ_H 7.26 and 7.55, respectively, and both resonated as a double doublet with J values of 8.0 and 1.8 Hz. While the H-6‴ signal in the ^{1}H NMR spectrum of compound **1** did not change when compared with that of **7**, the H-6″ resonance shifted upfield to δ_H 7.18 and appeared as a doublet with only a small J value of 2.0 Hz. This difference suggested that H-5″, the proton adjacent to H-6″, is substituted in **1**. The connectivites of three hydroxyphenyl methyl units of the substituent group on C-8 were established by HMBC correlations of H-6″ with C-11 and C-7″, H-6‴′ with C-7″ and C-7‴″, H-6‴″ with C-7‴′, H-7″ with C-4″, C-6″, C-2‴′, and C-6‴′, and H-7‴′ with C-4‴′, C-6‴″, C-2‴″, and C-6‴″. The attachments of the benzylated groups on C-6 and C-8 of the flavanone nucleus of **1** were also established by correlations between the methylene protons of H-12 with C-6, C-5, C-7, and C-1‴, as well as H-11

with C-7, C-8, C-9, and C-1″ in the HMBC spectrum. The absolute configuration at C-2 of compound **1** was assigned as *S*, based on the negative CD absorption maximum at 292 nm, by comparison of the curve with those of known dihydroflavone analogues.[32,33] Thus, the structure of compound **1** was elucidated as (2*S*)-5,7-dihydroxy-8-(2″-hydroxy-5″-(2⁗-hydroxy-5⁗-(2‴″-hydroxybenzyl)benzyl)-benzyl)-6-(2‴-hydroxybenzyl)flavanone, with this substance accorded the trivial name sarmentosumin A.

Compound **2** was obtained as a colorless gum. Its molecular formula was determined to be $C_{43}H_{36}O_8$, the same as that of compound **1**, from the sodiated molecular ion peak at *m/z* 703.2292 in the HRESIMS. The UV, IR, and ^{1}H and ^{13}C NMR spectra of **2** displayed a very close resemblance to those of compound **1**. In the ^{1}H NMR spectrum of compound **2**, the H-6″ signal appeared as a broad doublet with a *J* value of 7.6 Hz at δ_H 7.22, while the H-6‴ resonance was shifted upfield from δ_H 7.55 to δ_H 7.38 and appeared as a broad singlet, which indicated H-5‴ to be substituted. In the ^{13}C NMR spectrum of **2**, subtle differences from that of **1**, such as downfield shifts of 0.8 ppm for C-8 and 0.8 ppm for C-9, as well as an upfield shift of 1.0 ppm for C-5, were observed. These data suggested that the substituents include three benzyl functionalities attached to C-6, which was confirmed by key HMBC correlations of H-12 with C-6, C-5, C-7, and C-1‴, H-6‴ with C-12 and C-7‴, H-6⁗ with C-7‴ and C-7⁗, and H-6‴″ with C-7⁗. It is notable that the observed diagnostic fragmentation ions of *m/z* 609, 597, 397, 385, and 303 from the parent ion at *m/z* 703 [M + Na]$^+$ of compound **2** were similar to those of compound **1**, except for the relative abundance of the ion peaks at *m/z* 609 and 597. In the tandem mass spectrum of compound **1**, the ion peak at *m/z* 609 [M − C_6H_5OH + Na]$^+$ was the predominant daughter ion, while in that of compound **2**, the ion peak at *m/z* 597 [M − C_7H_6O + Na]$^+$ occurred as the most abundant fragment ion. This difference may be rationalized in that when a monohydroxybenzyl group is substituted on C-6 such as in compound **1**, the molecular ion tends to lose a phenol group (C_6H_5OH) to give a stable fragment at *m/z* 609, while when a monohydroxybenzyl group is located on C-8 as in compound **2**, the predominant peak occurs at *m/z* 597 from the loss of a moiety of C_7H_6O. This might be caused by the steric interference of the OH groups substituted on C-5 and C-7 or the stability of the obtained fragments. According to the above analysis, the structure of compound **2** (sarmentosumin B) was elucidated as (2*S*)-5,7-dihydroxy-6-(2‴-hydroxy-5‴-(2⁗-hydroxy-5⁗-(2‴″-hydroxybenzyl)benzyl)-benzyl)-8-(2″-hydroxybenzyl)flavanone.

The HRESIMS of compound **3** showed a sodiated molecular ion peak at *m/z* 809.2693, corresponding to the elemental formula $C_{50}H_{42}O_9Na$, indicating the presence of an extra C_7H_6O moiety when compared to compounds **1** and **2**. In the NMR spectrum of **3**, signals corresponding to an additional *p*-substituted *o*-hydroxyphenyl group could be recognized. In addition, the chemical shifts and splitting patterns of the diagnostic signals of H-6″ and H-6‴ were almost identical to those in compound **2**, which suggested that a moiety containing four hydroxybenzyl functionalities was located on C-6, with the monohydroxybenzyl group therefore positioned at C-8. The major fragment peaks at *m/z* 703 [M − C_7H_6O + Na]$^+$ and 397 [M − $C_6H_4(OH)CH_2C_6H_3(OH)CH_2C_6H_3(OH)$-$CH_2C_6H_4(OH)$ + Na]$^+$ in the tandem mass spectrum of **3** supported the structure proposed. The connectivities between these hydroxyphenyl groups were established using their HMBC correlations, which were comparable with those of compound **2**. Thus, the structure of compound **3** (sarmentosumin C) was determined to be 5,7-dihydroxy-6-(2‴-hydroxy-5‴-(2⁗-hydroxy-5⁗-(2‴″-hydroxy-5‴″-(2‴‴-hydroxybenzyl)benzyl)benzyl)-benzyl)-8-(2″-hydroxybenzyl)flavanone.

The molecular formula of compound **4** was established as $C_{50}H_{42}O_9Na$, on the basis of the sodiated molecular ion peak observed at *m/z* 809.2722 in the HRESIMS, the same as that of compound **3**. By comparison with the ^{1}H NMR data of compounds **4** and **3**, the H-6″ signal of **4** was shifted downfield from δ_H 7.22 to δ_H 7.17 and appeared as a doublet with a small *J* value of 1.7 Hz. These observations were consistent with both H-5″ and H-5‴ being substituted. The most abundant fragment ion peaks in the tandem mass spectrum of compound **4** from the parent ion at *m/z* 809 were observed at *m/z* 597 [M − C_6H_4-$(OH)CH_2C_7H_5O$ + Na]$^+$ and 503 [M − $C_6H_4(OH)CH_2C_6H_3$-$(OH)CH_2C_6H_4$ (OH) + Na]$^+$ and suggested the location of a di(hydroxybenzyl) functionality and a tri(hydroxybenzyl) functionality at C-8 and C-6, respectively. This inference was confirmed by the HMBC correlations of H-6″ with C-11 and C-7″, H-6⁗ with C-7″, H-6‴ with C-12 and C-7‴, H-6‴″ with C-7‴ and C-7‴″, and H-6‴‴ with C-7‴″. Thus, the structure of compound **4** (sarmentosumin D) was determined as (2*S*)-5,7-dihydroxy-6-(2‴-hydroxy-5‴-(2‴″-hydroxy-5‴″-(2‴‴-hydroxybenzyl)benzyl)benzyl)-8-(2″-hydroxy-5⁗-(2⁗-hydroxybenzyl)-benzyl)flavanone.

Cytotoxicity for HT-29 cells and activities in the mechanism-based MTP assay and in a proteasome cell-based inhibition assay were determined for the compounds isolated from *P. sarmentosum*. In cytotoxicity testing against HT-29 cells, using standard protocols, all compounds were considered inactive, with ED_{50} values of >20 μM. However, sarmentosumins A−D (**1**−**4**), together with five known *C*-benzylated dihydroflavones, isochamanetin (**5**), 7-methoxychamanetin (**6**), dichamanetin (**7**), 7-methoxydichamanetin (**8**), and 5″-(2⁗-hydroxyphenyl)-methyluvarinol (2‴-hydroxy-5″-benzylisouvarinol-B) (**9**), and the known amide pipercallosine (**11**) were found to induce apoptosis in HT-29 cells by moderately reducing the mitochondrial transmembrane potential ($\Delta\Psi$m), with ED_{50} values ranges from 1.6 to 13.6 μM (Table 3). It was noticeable that all of the *C*-benzylated dihydroflavones tested exhibited similar effects on the disruption of mitochondrial transmembrane potential, while pinocembrin (**10**), a known dihydroflavone that does not possess a benzylated group on ring B, was inactive in this assay (ED_{50} >20 μg/mL). These results suggested strongly that the *C*-benzylated dihydroflavones are the major active principles of *P. sarmentosum* responsible for MTP reduction. Also, it was observed that the substitution of hydroxybenzyl groups at C-6 or/and C-8 in these compounds is essential for activity in this mechanism-based assay. A *C*-benzylated dihydroflavone, 7-methoxydichamanetin (**8**), and pinocembrin (**10**) exhibited proteasome inhibitory activities in a human 20S proteasome bioassay with IC_{50} values of 3.45 ± 0.18 and 2.87 ± 0.26 μM, respectively (Table 3). Since dichamanetin (**7**), with an OH group at C-7, did not show any inhibitory effect in this assay, it may be implied that the OCH_3 group at C-7 of 7-methoxydichamanetin plays an important role in mediating proteasome inhibitory activity.

C-Benzylated dihydroflavones are rare plant-derived natural products and have only been isolated from several species of the family Annonaceae thus far.[19,20,34−40] Accordingly, this is the first time that *C*-benzylated dihydroflavones have been isolated and characterized from a plant in the family Piperaceae. To date, evaluation of the bioactivity of *C*-benzylated dihydroflavones has

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Table 1. ^{1}H NMR Chemical Shifts (δ_H) of Compounds 1−4[a]

position	1	2	3	4
2	5.32, dd (13.2, 2.8)	5.36, dd (13.2, 2.8)	5.38, dd (13.2, 2.8)	5.34, dd (13.2, 2.8)
3	2.74, dd (17.2, 2.8)	2.76, dd (17.2, 2.8)	2.76, dd (17.2, 2.8)	2.76, dd (17.2, 2.8)
	3.03, dd (17.2, 13.2)	3.03, dd (17.2, 13.2)	3.04, dd (17.2, 13.2)	3.03, dd (17.2, 13.2)
11	3.87, s	3.79, s	3.78, s	3.81, s
12	3.75,s	3.80,s	3.81,s	3.75,s
2′, 6′	7.46, m	7.49, m	7.50, m	7.45, m
3′, 5′	7.44, m	7.48, m	7.48, m	7.45, m
4′	7.40, m	7.45, m	7.45, m	7.45, m
3″	6.64, brd (8.0)	6.74[b]	6.74[b]	6.64,brd (8.0)
4″	6.82[b]	7.03[b]	7.04[b]	6.84[b]
5″		6.78[b]	6.77[b]	
6″	7.18, d (2.0)	7.22, brd (7.6)	7.22, brd (7.8)	7.17, d (1.7)
7″	3.69, s			3.72, s
3‴	6.75, brd (8.0)	6.70[b]	6.70[b]	6.72[b]
4‴	7.05[b]	6.87[b]	6.87, brd (8.0)	6.90[b]
5‴	6.84[b]			
6‴	7.55, dd (8.0, 1.2)	7.38, brs	7.36, brs	7.38, d (1.8)
7‴		3.84, s	3.86, s	3.86, s
3⁗	6.65, d (8.0)	6.78[b]	6.77[b]	6.80[b]
4⁗	6.92, brd (8.0)	6.96, brd (7.2)	6.95, brd (8.0)	7.14[b]
5⁗				6.88[b]
6⁗	6.88, brs	7.04, brs	7.04, brs	7.11[b]
7⁗	3.86, s	3.91, s	3.90[b]	
3′′′′′	6.76[b]	6.80[b]	6.77[b]	6.77[b]
4′′′′′	7.10[b]	7.12[b]	6.99, brd (8.0)	6.98, brd (8.0)
5′′′′′	6.86[b]	6.88[b]		
6′′′′′	7.06[b]	7.10[b]	7.06, brs	7.07, brs
7′′′′′			3.90[b]	3.93[b]
3′′′′′′			6.79[b]	6.77[b]
4′′′′′′			7.13[b]	7.11[b]
5′′′′′′			6.87, brt (7.6, 7.2)	6.81[b]
6′′′′′′			7.11[b]	6.90[b]
OH-5	12.56, s	12.70, s	12.84, s	12.81, s

[a] Measured at 400 MHz and obtained in $CDCl_3$ with TMS as internal standard; *J* values (Hz) are given in parentheses. Assignments supported with ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. [b] Multiplicity patterns unclear due to signal overlapping.

been limited. Several analogues have been reported to exhibit moderate to marginal cytotoxicity against the KB and PS cell lines[34,35] and to possess antimicrobial activity.[36] *C*-Benzylated dihydroflavones have not been evaluated using the MTP and proteasome assays previously. Finally, as polyphenol derivatives, the *C*-benzylated dihydroflavones may be responsible for the previously reported antioxidant effects of the water and organic solvent extracts of *P. sarmentosum*.[15]

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 343 automatic polarimeter. UV spectra were obtained with a Perkin-Elmer Lambda 10 UV/vis spectrometer. CD spectra were recorded on a JASCO J-810 spectrometer. IR spectra were measured on a Thermo Scientific Nicolet 6700 FT-IR spectrometer. NMR spectroscopic data were run on a Bruker Avance DRX-400 or 600 MHz spectrometer, and the data were processed using MestReNova 6.0 software. Accurate mass values were obtained on a Micromass LCT ESI spectrometer. Sodium iodide was used for mass calibration for a calibration range of m/z 100−2000. The tandem mass spectrometric analysis was performed on a Micromass ESI-Tof II (Micromass, Wythenshawe, UK) mass spectrometer equipped with an orthogonal electrospray source (Z-spray) operated in the positive-ion mode. Silica gel (230−400 Mesh; Sorbent Technologies, Atlanta, GA) was used for column chromatography. Analytical TLC was performed on precoated 250 μm thickness silica gel plates (UV_{254}, glass backed; Sorbent Technologies). HPLC was conducted using a Waters system comprised of a 600 controller, a 717 Plus autosampler, and a 2487 dual-wavelength absorbance detector, with Waters Sunfire analytical (4.6 × 150 mm), semipreparative (10 × 150 mm), and preparative (19 × 150 mm) PrepC_{18} (5 μm) columns.

Plant Material. The aerial parts, including the leaves, twigs, stems, and inflorescence of *P. sarmentosum*, were collected in Kego Nature Reserve, Hatinh Province, Vietnam, by T.N.N., Vuong Tan Tu, and D.D.S. in December 2008, who also identified this plant. A voucher specimen (collection number DDS 14301) has been deposited in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, Illinois.

Extraction and Isolation. The dried aerial plant parts (311 g), including the leaves, twigs, stems, and inflorescence of *P. sarmentosum*,

Table 2. ^{13}C NMR Chemical Shifts (δ_C) of Compounds 1−4[a]

position	1	2	3	4
2	79.6	79.5	79.6	79.7
3	43.3	43.3	43.3	43.3
4	196.5	196.5	196.5	196.4
5	159.7	158.7	158.8	158.5
6	108.5	108.3	108.9	108.4
7	160.5	161.0	161.4	161.6
8	106.9	107.7	107.7	107.5
9	157.9	158.7	158.0	158.5
10	102.8	102.8	102.8	102.7
11	22.6	23.0	23.1	23.1
12	23.5	23.3	23.3	23.5
1′	138.2	138.4	138.4	138.4
2′, 6′	126.4	126.3	126.3	126.4
3′, 5′	129.0	128.9	128.9	129.0
4′	129.1	129.0	129.0	129.1
1″	126.2	126.4	126.3	126.2
2″	151.4	152.3	152.3	150.8
3″	115.7	115.3	115.3	115.7
4″	127.9	127.9	127.8	127.8
5″	131.9	120.9	120.9	132.2
6″	132.1	131.9	132.0	132.4
7″	35.6			35.6
1‴	126.4	126.3	126.3	126.4
2‴	152.4	152.1	152.0	152.3
3‴	115.4	116.3	116.4	116.4
4‴	127.8	127.8	127.8	127.7
5‴	121.3	131.1	130.3	132.3
6‴	132.1	132.0	131.9	132.0
7‴		36.1	36.4	36.5
1⁗	127.5	127.3	127.4	127.5
2⁗	152.2	152.3	152.3	153.7
3⁗	116.1	116.3	116.1	115.7
4⁗	127.8	127.8	128.0	128.2
5⁗	132.0	132.0	132.3	120.9
6⁗	131.0	131.0	131.2	130.6
7⁗	35.8	35.6	35.7	
1′′′′′	127.1	127.6	127.3	127.4
2′′′′′	153.7	153.8	152.7	152.6
3′′′′′	115.9	115.8	116.8	116.7
4′′′′′	127.8	128.0	128.5	128.0
5′′′′′	121.3	121.1	132.1	132.3
6′′′′′	130.8	130.9	131.2	131.3
7′′′′′			35.7	35.6
1′′′′′′			127.6	127.2
2′′′′′′			153.8	153.6
3′′′′′′			115.8	115.8
4′′′′′′			127.9	128.0
5′′′′′′			121.4	121.0
6′′′′′′			130.8	130.9

[a] Measured at 100 MHz and obtained in $CDCl_3$ with TMS as internal standard. Assignments were supported with HSQC and HMBC NMR spectra.

were ground and extracted with MeOH. The MeOH extract was concentrated in vacuo and partitioned to afford a hexane extract (5 g) and a $CHCl_3$ extract (10 g). The $CHCl_3$-soluble extract exhibited 69.7% inhibition in the MTP assay at a concentration of 100 μg/mL and was subjected to separation over a silica gel column using a CH_2Cl_2−acetone gradient of increasing polarity for elution to yield five fractions (F1−F5). Fractions F01 (3.0 g) and F03 (800 mg) were active in the mitochondrial transmembrane potential assay, with observed inhibitions of 77.1% and 72.5%, respectively, at a concentration of 100 μg/mL. F1 was chromatographed using silica gel (230−400 mesh, 3.0 × 30 cm) with a hexane−acetone gradient to afford 13 subfractions (F101−F113). Dichamanetin (**7**, 900 mg) was obtained as a precipitate from F112. F103 was fractionated using a HPLC semipreparative RP-18 column with MeOH−H_2O (70:30, 5 mL/min) as solvent, to give sesamin (4.0 mg), pellitorine (10 mg), and 2,4-dodecadienamide (1.0 mg). F108 was chromatographed by HPLC on a semipreparative RP-18 column, using MeOH−H_2O (80:20, 5 mL/min) as solvent, to afford pinocembrin (**10**, 10 mg), 7-methoxychamanetin (**6**, 3.0 mg), and 7-methoxyisochamanetin (0.8 mg). F110 was subjected to separation over a preparative RP-18 column, using a gradient MeOH−H_2O (0.1% TFA) solvent system (1−16 min, 75:25; 16−28 min, 80:20; 28−40 min, 90:10) (10 mL/min), to obtain pipercallosidine (2.5 mg), isochamanetin (**5**, 80 mg), pipercallosine (**11**, 13 mg), and 7-methoxydichamanetin (**8**, 2.0 mg). Active fraction F03 was chromatographed over a silica gel column, using CH_2Cl_2−acetone mixtures for elution, to yield seven subfractions (F301−F307). F303 was chromatographed on a preparative RP-18 column, using MeOH−H_2O (0.2% TFA) (55:45, 8 mL/min) as solvent, to afford benzoic acid (10 mg) and *trans*-cinnamic acid (3.0 mg). F305 was purified on a silica gel column to yield three subfractions (F305A−F305C). Further purification of F305A on a semipreparative RP-18 HPLC column using CH_3CN−H_2O (0.3% formic acid) (48:52, 6 mL/min) as solvent afforded sarmentosumin A (**1**, 6.0 mg), 5″-(2⁗-hydroxyphenyl)methyluvarinol (**9**, 6.0 mg), and sarmentosumin B (**2**, 10 mg). F305C was subjected to semipreparative RP-18 HPLC using CH_3CN−H_2O (0.25% formic acid) (50:50, 6 mL/min) and yielded sarmentosumin D (**4**, 2.5 mg) and sarmentosumin C (**3**, 2.8 mg).

*Sarmentosumin A (**1**):* pale yellow gum; $[\alpha]^{20}_D$ −45.0 (c 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.64), 281 (4.09), 331 (4.00) nm; CD (c 3.68 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 212 (+4.46), 242.5 (−1.56), 292 (−4.90), 328 (−0.59) nm; IR (film) ν_{max} 3370,

Table 3. Bioactivity Evaluation of Compounds Isolated from *P. sarmentosum*[a,b]

compound	MTP[c]	proteasome[c]
1	3.7	>20
2	6.8	>20
3	2.5	>20
4	3.9	>20
5	7.7	>20
6	13.6	>20
7	3.6	>20
8	1.6	3.45 ± 0.18
9	2.3	>20
10	>20	2.87 ± 0.26
11	4.6	>20
staurosporine[d]	0.0026	
bortezomib[e]		0.0025

[a] Compounds **1**−**11** and all other known compounds evaluated were not cytotoxic against HT-29 cells (ED_{50} > 20 μM), using a standard protocol.[18] [b] 7-Methoxyisochamanetin was not tested in any of the assays used due to the limited amount isolated. [c] Results are expressed as ED_{50} values (μM). [d] Used as a positive control in the MTP assay. [e] Used as a positive control in the proteasome inhibition assay.

1631, 1605, 1501, 1455, 1215, 1122, 756 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; ESIMS/MS *m/z* 703 (36), 609 (100), 597 (62), 505 (37), 493 (5), 397 (6), 385 (6), 303 (12), 291 (5), 281 (10); HRESIMS *m/z* 703.2297 $[M + Na]^+$ (calcd for $C_{43}H_{36}O_8Na$, 703.2308).

*Sarmentosumin B (**2**):* colorless gum; $[\alpha]^{20}_D$ −18.0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.65), 280 (4.05), 331 (4.16) nm; CD (*c* 3.68 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+4.04), 286 (−2.09), 316 (+0.52), 363 (+0.56) nm; IR (film) ν_{max} 3370, 1631, 1606, 1501, 1455, 1215, 1122, 756 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; ESIMS/MS *m/z* 703 (72), 609 (20), 597 (100), 505 (51), 493 (15), 397 (70), 385 (24), 303 (38), 291 (22), 278 (15); HRESIMS *m/z* 703.2292 $[M + Na]^+$ (calcd for $C_{43}H_{36}O_8Na$, 703.2308).

*Sarmentosumin C (**3**):* colorless gum; $[\alpha]^{20}_D$ −26.0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.65), 281 (4.01), 331 (4.05) nm; CD (*c* 3.18 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+3.85), 286 (−1.82), 314 (+0.19), 359 (+0.36) nm; IR (film) ν_{max} 3399, 1631, 1605, 1503, 1463, 1217, 1121, 755 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Tables 2 and 3; ESIMS/MS *m/z* 809 (100), 715 (15), 703 (75), 611 (22), 599 (7), 503 (4), 447 (20), 397 (33), 385 (8), 303 (10), 291 (10), 278 (5); HRESIMS *m/z* 809.2693 $[M + Na]^+$ (calcd for $C_{50}H_{42}O_9Na$, 809.2727).

*Sarmentosumin D (**4**):* colorless gum; $[\alpha]^{20}_D$ −9.0 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.35), 281 (3.86), 332 (3.72) nm; CD (*c* 3.18 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 219 (+1.00), 249 (−0.79), 285.5 (−2.10), 327 (−0.19) nm; IR (film) ν_{max} 3395, 1631, 1605, 1502, 1464, 1218, 1120, 754 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Tables 2 and 3; ESIMS/MS *m/z* 809 (53), 715 (5), 703 (7), 609 (23), 597 (83), 505 (85), 503 (100), 491 (40), 399 (42), 387 (23), 303 (53), 291 (24), 278 (30); HRESIMS *m/z* 809.2722 $[M + Na]^+$ (calcd for $C_{50}H_{42}O_9Na$, 809.2727).

Mitochondrial Transmembrane Potential Assay (MTP Assay). In the present study, a JC-1 mitochondrial membrane potential assay kit obtained from Cayman Chemicals was used to detect the $\Delta\Psi$. Measurements were performed according to the protocols established previously.[41]

Cytotoxicity Assay. Compounds **1**−**16** were evaluated against human colon cancer cells (HT-29), according to a previously described protocol.[18] Paclitaxel was used as a positive control with an ED_{50} value of 0.006 μM.

Proteasome Inhibition Assay. A proteasome fraction was prepared from a HL-60 human leukemic cell line as described previously.[42,43] Isolates were tested in a proteasome inhibition assay according to the established protocols.[41,44]

■ ASSOCIATED CONTENT

Supporting Information. Structures of the known active compounds (**5**−**11**); ^{1}H, ^{13}C, and 2D NMR spectra and ESIMS/MS data of the new compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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