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Isolation and Characterization of Novel Cytotoxic Saponins from *Archidendron ellipticum*

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Abstract—A series of new ester saponins, elliptosides A–J, has been isolated from the tropical plant *Archidendron ellipticum* (Leguminosae). These saponins were particularly cytotoxic to certain renal and melanoma cancer cell lines in the NCI's 60-cell line human tumor screen. The structures of elliptosides A, E, and F were elucidated by spectroscopic and chemical means. Elliptoside A showed in vivo antitumor activity against the LOX melanoma cell line. Published by Elsevier Science Ltd.

Introduction

An organic extract of the tropical legume *Archidendron ellipticum* (Bl.) Nielsen (Leguminosae) showed prominent cytotoxicity toward certain renal and melanoma cancer cell lines in the NCI 60-cell line in vitro screening panel,[1,2] providing the initial basis for selection of the extract for bioassay-guided fractionation. The isolation of sterols, triterpenes and coumarins has been reported previously from this genus.[3] We report here the isolation of a series of new saponins from this plant, the structure elucidation of three members of the series, and in vitro and in vivo antitumor evaluation of the lead compound, elliptoside A.

Results

Solvent/solvent partitioning of the extract concentrated bioactivity in the polar, water-soluble fraction. Gel permeation through Sephadex LH-20 in MeOH located the most potent bioactivity in early fractions, indicating that the active molecules were of relatively high molecular weight. Preliminary NMR analysis of those fractions suggested that the active constituents were triterpene saponins. Preparative HPLC, using a wide-pore, amino-bonded phase matrix[4,5] eluted with a CH_3CN–H_2O mixture, resolved the presumed saponin mixture into several distinct fractions. Subsequent chromatography of these fractions using a wide-pore C_4 matrix, with a gradient of MeOH–H_2O–HOAc, led to isolation of elliptosides A–J. Detailed structural analyses were carried out on elliptosides A, E, and F.

Elliptoside A was characterized by NMR as having eight sugar units (anomeric carbons from 95–110 ppm), four carbonyl functionalities, and five olefinic bonds. Hydrolysis with 6 N HCl gave acacic acid lactone **1**, which was characterized by NMR and comparisons of spectroanalytical data to literature values.[6] This genin appeared to be common to the ten saponins isolated.

Hydrolysis of the saponin with 1 N HCl, followed by gas-liquid chromatography of the trimethylsilyl derivatives of the hydrolysis products, indicated the presence of the monosaccharides glucose (2 units), *N*-acetylglucosamine, rhamnose, quinovose, fucose, xylose, and arabinose. Basic hydrolysis liberated the monoterpene acid **2**, identical by ^1H NMR to that reported from Gleditsia saponin C (although the sign and magnitude of the optical rotation differ, indicating the opposite chirality at C-6).[7] In addition, **2** and **3** could be detected by NMR in hydrolysates of the mixture of saponins. Two monoterpene residues could also be readily identified in the intact saponin by 2-D NMR (HMBC, HMQC, COSY); however, establishing their connection to the rest of the molecule proved more challenging (vide infra).

With these data indicating an unsaturation number of 22, the high-resolution mass spectrum supported a molecular formula of $C_{98}H_{155}NO_{45}$ for elliptoside A. Additional information was obtained by mild base hydrolysis (0.5 M KOH) of the mixture of saponins to yield two prosaponins, **4** and **5**. Both were trisaccharides

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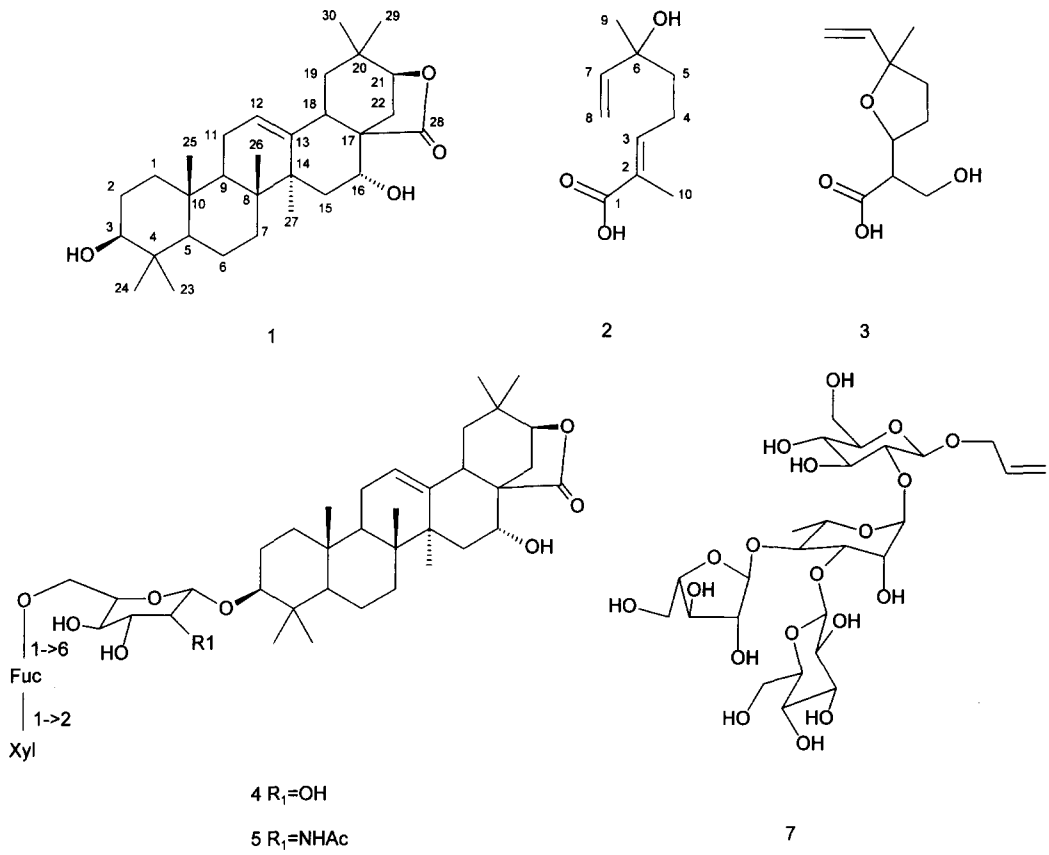


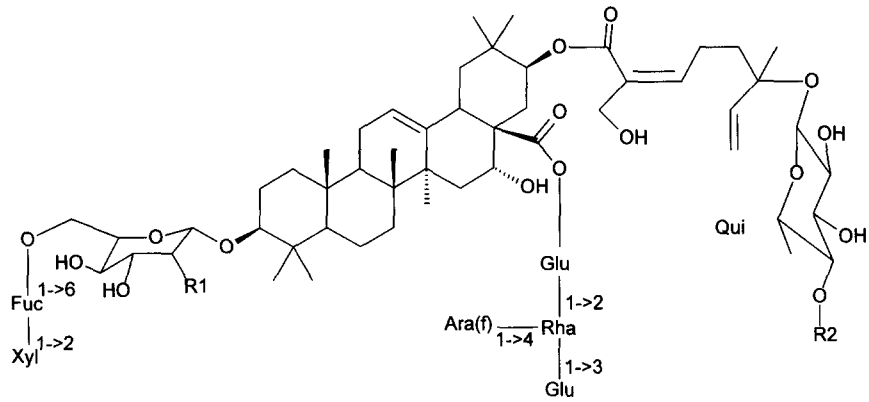
of acacic acid lactone and differed only in whether the internal sugar at C-3 of the triterpene was glucose or *N*-acetylglucosamine. This indicated that the fourth carbonyl of elliptoside A (δ174.0) was due to the acetate unit of glcNAc. The prosaponins were also analyzed by NMR as their peracetates. Compounds **4** and **5** were identical to the prosaponins julibrosides A_2 and A_3, obtained from *Albizia julibrissin*.[8]

The C-21 substituent in elliptoside A was defined by HMBC correlations between (a) one monoterpene ester carbonyl (δ 169.5) and the H-21 proton (δ 5.43), (b) the other monoterpene carbonyl (δ 169.0) and a quinovose H-4 proton (δ 4.62), and (c) the correlation between the 81.2 quaternary carbon in one monoterpene and the quinovose anomeric proton (δ 4.41). FABMS fragmentation data also supported these assignments. 1-D TOCSY experiments, in which either the quinovose anomeric proton or the C-6 Me was irradiated and mixing times were varied, permitted the assignment of proton resonances around the quinovose sugar residue. The large coupling constants (6–8 Hz) observed in this experiment clearly distinguished this sugar as quinovose. However, similar experiments on the rhamnose and fucose units were not completely successful in traversing the saccharide rings. HSQC-TOCSY gave results for the quinovose moiety concordant with the 1-D TOCSY experiments. Milder basic hydrolysis conditions (1% KOH) with elliptoside A yielded **6**, which lacked the terminal monoterpene ester, as evidenced by FABMS data and the loss of one set of monoterpene ^{1}H NMR resonances.

The C-28 substituent group was cleaved using LiI, 2,6-lutidine, and allyl alcohol[9,10] to yield the branched tetrasaccharide allyl ether **7**, identical by ^{1}H NMR and FABMS to that obtained from julibroside I.[10] The C-28 carbonyl resonance (δ 175.7) and the anomeric proton of a glucose residue of the tetrasaccharide (δ 5.33) were correlated by HMBC to establish the linkage in elliptoside A between the triterpene and the tetrasaccharide moieties. Thus, the complete structure of elliptoside A was deduced as **8**.

Elliptoside E was identified by comparison with elliptoside A. High-resolution FABMS of elliptoside E and the unsaturation number of 22 yielded the formula $C_{98}H_{155}NO_{46}$. Elliptoside E lacked the prominent methyl resonance at δ 12.5 (C-MT_2-10, see Table 2) seen in the ^{13}C NMR spectrum of elliptoside A, and instead possessed an extra hydroxymethylene resonance at δ 56, indicating hydroxylation in one of the monoterpene units. All other ^{1}H, ^{13}C NMR, and MS data were similar to those of elliptoside A (Tables 1–3), supporting **9** as the structure of elliptoside E.

Elliptoside F (**10**) was elucidated in similar fashion. The signals for the NHAc moiety were lacking in the ^{13}C NMR, as was the δ 12 signal for the monoterpene methyl group. Mass spectral analysis indicated a formula of $C_{96}H_{152}O_{46}$, which was consistent with the presence of a glucose as the internal sugar at C-3 and hydroxylation of both monoterpene units. Detailed structural characterizations were not attempted for elliptosides B, C, D, G, H, I, and J; however, the

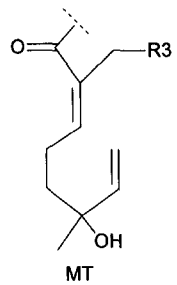


6 R_1 = NHAc, R_2 = H

8 R_1 = NHAc, R_2 = MT, R_3 = H

9 R_1 = NHAc, R_2 = MT, R_3 = OH

10 R_1 = OH, R_2 = MT, R_3 = OH



MT

Table 1. ^{13}C NMR data, triterpene part (125 MHz, CD_3OD:D_2O 4:1 v/v)

Carbon no.	1[a]	5a[b,c]	6	8	9	10	11[d]	12[b]
C-1	38.5	39.8	36.1	36	36	36	38.5	39.6
C-2	27.2	27.1	27.2	26.9	27	27.1	26.5	27.2
C-3	78.9	90	89.8	90.3	90.3	90	89.3	90.2
C-4	38.8	40	39.9	39.9	39.9	40.2	39.2	40
C-5	55.4	57.1	57.1	56.8	56.9	56.9	56.2	57.1
C-6	18.2	19.4	19.6	19.4	19.4	19.4	18.5	19.2
C-7	32.1	34.4	34.6	35	34.4	34.4	32.5	34.5
C-8	40.2	40.6	40.6	40.7	40.7	40.7	40.5	41.4
C-9	46.9	48.2	48.1	47.9	48	47.9	47.5	48.4
C-10	37	37.9	37.7	37.8	37.8	37.8	37	37.9
C-11	23.5	24.5	24.3	24.4	24.4	24.4	23.7	24.6
C-12	125.5	122.7	124	124.2	124.2	124.2	125.7	126.7
C-13	139.1	145.6	143.7	143.3	143.3	143.4	138.5	139.7
C-14	43.3	42.6	42.7	42.5	42.5	42.4	43.4	44.6
C-15	36.2	36	36.4	36.2	36.2	36.2	34.1	34.8
C-16	67.5	76.2	76.3	74.1	74.1	74.1	70.8	71.1
C-17	49.7	53.6	52.3	52.2	52.2	52.2	47.5	49.3
C-18	40.5	41.9	41.7	41.4	41.4	41.4	41.2	42.5
C-19	43.6	49.5	48.7	48.4	48.6	48.4	42.6	43.7
C-20	33.8	36.8	36.4	35.8	35.8	35.8	33.8	33.3
C-21	83.8	75	75	78.6	78.6	78.6	82.6	85.3
C-22	26.5	41.7	41.7	39.7	39.7	39.8	28.3	29
C-23	28	28.6	28.6	28.5	28.5	28.4	28.4	28.5
C-24	15.7	17	17.1	17.1	17.1	17.1	16.1	16.3
C-25	15.7	16	16.2	16.1	16.1	16.2	15.6	16.1
C-26	16	18.2	17.7	17.6	17.8	17.6	16	16.4
C-27	29	27.4	27.4	27.3	27.3	27.3	28.7	29.2
C-28	181	183.8	175.3	175.7	175.6	175.7	178.2	181.6
C-29	29	30	29.4	29.3	29.3	29.3	28	28.8
C-30	23.8	19.3	19.4	19.3	19.4	19.4	24.3	24.3

[a]$CDCl_3$.
[b]CD_3OD.
[c]NMR data for ring-opened lactone, cf shifts for C-21 and C-22.
[d]C_6D_6.

Table 2. ^{13}C NMR data, monoterpene part

Carbon no.	2[a]	6	8	9	10
MT_1-1	—	168.6	169	169	169
MT_1-2	—	132.9	132.5	132.5	132.5
MT_1-3	—	148.1	148.6	148.6	148.6
MT_1-4	—	24.5	24.1	24.1	24.1
MT_1-5	—	39.6	39.5	39.5	39.5
MT_1-6	—	82.3	81.2	81.2	81.2
MT_1-7	—	144.3	143.6	143.7	143.7
MT_1-8	—	115.3	116	116	116
MT_1-9	—	24.2	24	24	24
MT_1-10	—	56.7	56.4	56.5	56.4
MT_2-1	n.d.[b]	—	169.5	168.5	168.5
MT_2-2	n.d.	—	128.3	131.9	131.9
MT_2-3	144.8	—	145.1	145.5	145.5
MT_2-4	23.7	—	24.6	24.5	24.5
MT_2-5	40.4	—	41.4	41.7	41.7
MT_2-6	n.d.	—	73.8	73.9	73.9
MT_2-7	144.3	—	145.5	149.3	149.3
MT_2-8	112.3	—	112.8	112.9	112.9
MT_2-9	28.1	—	16.7	16.8	16.7
MT_2-10	12	—	12.5	56.3	56.3

[a] $CDCl_3$.
[b] Insufficient S/N for detection.

available spectroanalytical data confirmed their close structural relationship to **8–10**.

The purified saponins gave essentially indistinguishable 'mean-graph'[1] differential cytotoxicity profiles in the NCI 60-cell line screen.[1,2] A representative 'mean graph' profile of elliptoside A (**1**) is provided in alphanumeric format in the Experimental section. The most potent of the elliptosides were I and J (mean panel GI_{50} 0.5 μg/mL), which were about twofold more potent than A (mean panel GI_{50} 1 μg/mL) and sixfold more potent than E.

Pure elliptoside A and the parent saponin mixture were screened for in vivo antitumor activity using a human melanoma (LOX) xenograft model. Athymic mice were implanted ip with LOX melanoma cells and then treated with saponin mixture or purified compound by intravenous or intraperitoneal routes. As indicated in Table 4, intraperitoneal administration of both preparations resulted in dose-related increases in lifespan. Ninety per cent 'long-term survivors' were obtained with purified elliptoside A. However, only very modest indications of activity were observed when treatment was administered intravenously, or when the tumor was implanted subcutaneously and treatment was administered intraperitoneally (data not shown). The failure to obtain strong antitumor activity with the latter systemic chemotherapy models discouraged further pursuit of these particular saponins as antitumor drug development candidates.

Discussion

The structures of elliptosides A, E, and F are novel, although similar to the saponins reported from other genera of legumes. The most closely related saponins appear to be the julibrosides, recently reported from *Albizia julibrissin*.[8,11] Other ester saponins from the Leguminosae include the *Gleditsia japonica* saponins,[6,12–18] in which the monoterpene esters were found pendant on a C-28 substituent rhamnose; the acaciasides[19] from *Acacia auriculiformis*, in which a single monoterpene moiety was esterified at C-21; and the saponins from *Calliandra anomala*,[20,21] in which the monoterpene groups are located on the C-28 oligosaccharide. Simple monoterpene ester glycosides of 2,6-dimethyloctadienoic acid have also been found in *Gymnocladus chinensis*.[22]

While many of the above leguminous plants were studied because of folk-medicinal uses, few legume ester saponins have been demonstrated to have biological activity in the pure state. Cytotoxic saponins, on the other hand, have been reported occasionally in the phytochemical literature. A notable example is the saponin of *Acer negundo* reported by Kupchan's group.[23,24] Testing (data not shown) of the latter saponin, as well as eight other unrelated cytotoxic saponins from the NCI pure chemicals repository, did not yield a differential cytotoxicity profile in the NCI 60-cell line screen comparable to that of the elliptosides. However, a number of crude legume extracts from the NCI natural products repository yielded in vitro patterns of cytotoxicity which correlated with that of *Archidendron ellipticum*; the highest COMPARE[1] correlation coefficients were obtained from extracts of different species of *Albizia*. The extracts of *Albizia zygia* and *Albizia altissima* were preliminarily fractionated and found to have the characteristic ^1H NMR spectra, HPLC profiles, and cytotoxicity fingerprints of the saponins from *Archidendron ellipticum*. Evaluations of

Table 3. ^{13}C NMR data, sugar residues

Carbon no.	5	6	7	8	9	10[a]	11	12[a,b]
2AG-1	104.8	104.8	—	104.6	104.6	106.3	104.1	103.2
2AG-2	57.8	57.9	—	57.5	57.6	77.5	55.9	71.9
2AG-3	75.7	75.7	—	76.7	76.7	77.9	74.1	73.6
2AG-4	72.1	72.2	—	70.9	70.8	70.8	74.3	69.9
2AG-5	77	77.1	—	76.6	76.6	76.6	74.8	73.8
2AG-6	69.8	69.9	—	69.6	69.6	69.6	68.9	68.2
MeC=O	173.4	173.3	—	174	174	—	173.2	—
MeC=O	23.1	23.2	—	23.2	23.2	—	22.9	—
Fuc-1	103.7	103.8	—	103.5	103.5	103.5	103.5	102.8
Fuc-2	82	82.3	—	82	82	82	76.9	75.5
Fuc-3	72.7	72.7	—	72.5	72.5	72.5	72	73.4
Fuc-4	74.9	74.3	—	74.8	74.8	74.8	70	70.9
Fuc-5	71.7	71.7	—	71.6	71.6	70.8	70.1	68.8
Fuc-6	27.3	27.4	—	27.6	27.8	27.7	28	28
Xyl-1	106.8	106.9	—	106.5	106.5	106.6	102.5	101.7
Xyl-2	75.9	76	—	75.9	75.9	75.9	73.8	73.2
Xyl-3	71.1	71.1	—	70.8	70.8	70.8	73.8	68.7
Xyl-4	77.4	77.5	—	77.5	77.5	77.5	71.9	72.9
Xyl-5	67.2	67.3	—	67	67	67	63.4	62.7
Qui-1	—	99.2	—	98.9	98.9	98.9	—	—
Qui-2	—	75.4	—	75.9	75.3	75.2	—	—
Qui-3	—	75.3	(Allyl)	75.2	75.1	75.1	—	—
Qui-4	—	77.1	69.3	77.2	76.7	76.8	—	—
Qui-5	—	71.1	135.2	70.9	70.8	70.9	—	—
Qui-6	—	18.4	118.2	18.1	18.2	18.1	—	—
Glu_A-1	—	95.3	98.6	95.2	95.2	95.2	—	—
Glu_A-2	—	82.6	84.3	82.1	82	82	—	—
Glu_A-3	—	71.5	73.9	71.4	71.4	71.4	—	—
Glu_A-4	—	78.2	71.8	78.3	78.3	78.3	—	—
Glu_A-5	—	75.4	75.4	75.3	75.3	75.4	—	—
Glu_A-6	—	62.7	62.6	62	62	62	—	—
Rha-1	—	101.3	104	101.3	101.3	101.3	—	—
Rha-2	—	71.1	71.3	70.9	70.9	71.2	—	—
Rha-3	—	82.6	82.8	82.1	82.1	82.1	—	—
Rha-4	—	78.7	78.6	78.2	78.2	78.3	—	—
Rha-5	—	69.1	68.8	69.2	69.2	69.1	—	—
Rha-6	—	18.7	18.5	18.6	18.6	18.6	—	—
Glu_B-1	—	105.8	105.4	105.5	105.5	105.5	—	—
Glu_B-2	—	77.6	77.6	75	75	75	—	—
Glu_B-3	—	77.9	77.9	77.8	77.8	77.8	—	—
Glu_B-4	—	71.2	71	71.8	71.8	71.8	—	—
Glu_B-5	—	77.7	73.6	77.2	77.2	77.2	—	—
Glu_B-6	—	62.3	62.6	62	62	62	—	—
Ara-1	—	111	110.5	110.5	110.5	110.5	—	—
Ara-2	—	85.6	84.3	85.1	85.1	85.2	—	—
Ara-3	—	78.7	78.1	78.7	78.7	78.7	—	—
Ara-4	—	84	81.7	83.7	83.7	83.7	—	—
Ara-5	—	63.1	62.3	62.6	62.7	62.7	—	—

[a]First sugar is Glu, not 2-AG.
[b]C_6D_6.

extracts of *Gleditsia japonica* and *Gleditsia triacanthos*, however, revealed no evidence of differential cytotoxicity.

To ascertain whether the triterpene/monoterpene moieties of the elliptosides could produce the observed biological activity, we tested anatoliosides A–E,[25,26] as well as desmonoterpenyl elliptoside A (**6**) in the NCI 60-cell line screen. Neither **6** nor any of these monoterpene ester glycosides produced the distinctive cytotoxicity of the elliptosides, supporting the requirement for both the terminal monoterpene and the

Table 4. Effect of treatment with (a) saponin mixture and (b) elliptoside A (**8**) on survival of athymic mice xenografted with human LOX melanoma[a]

Treatment	Surviving mice		
	Day 30	Day 60	Day 100
Control	1/20	1/20	1/20
Saponin mixture 2.0 mg/kg	1/10	1/10	1/10
1	7/10	5/10	5/10
0.5	4/10	2/10	2/10
0.25	0/10	0/10	0/10
0.13	1/10	0/10	0/10
Control	1/20	1/20	1/20
Elliptosides A 1.0 mg/kg	3/10	3/10	3/10
0.5	10/10	9/10	9/10
0.25	4/10	3/10	3/10
0.13	6/10	0/10	0/10
0.06	0/10	0/10	0/10
0.03	0/10	0/10	0/10

[a]Mice were inoculated intraperitoneally with 1×10^7 tumor cells. The treatment was administered on days 1, 5, and 9, in the case of the saponin mixture, and on days 1 and 5 for purified elliptoside A.

triterpene portion of the active molecules. Interestingly, Ideka et al.[10] attributed the cytotoxicity of julibrosides to the C-28 oligosaccharide moiety.

Experimental

General

All solvents used were of HPLC grade. NMR was performed on a Varian VXR-500 spectrometer equipped with a Nalorac inverse detection probe. ^{13}C NMR spectra were acquired using an aquisition time of 0.4–0.6 s. Pulse sequences used included COSY, HMQC, HMBC, TOCSY,[27] 1-D TOCSY,[28] difference NOE, 2-D HETJ, and HSQC-TOCSY.[29]

Plant material

The plant extracts used in the present study were obtained from the NCI natural products repository. *Archidendron ellipticum* (Bl.) Nielsen was collected by Meijer, van Balgooy and Ridsdale (voucher no. 122546) in October 1987, in secondary forest along the Mamut River, Ranau District, Sabah, Malaysia, and dried in the field. Leaf material (824 g) was ground and extracted with CH_2Cl_2:MeOH (1:1 v/v), then rinsed with MeOH; the combined organic phases were evaporated to yield 97.2 g of crude organic extract. A bulk recollection of 7 kg of leaves was made in August 1994 in the Sepilok Forest Reserve, Sandakan, Malaysia.

Gleditsia japonica var. *koraiensis* (Nakai) Nakai (fruits and leaves) was collected from a specimen in the Arnold Arboretum, dried and extracted by the same protocol. *Gleditsia triacanthos* L. (seed pods) was collected near Frederick, Maryland and identified by one of the authors (J.A.B). *Albizia altissima* Hook. f. was collected by J. M. Fay in February 1988, along the Sangha River in Cameroon. *Albizia zygia* (D.C.) J.F. Macbr. was collected by Nemba, Mambo, and Thomas in February 1988, in Southwest Province, Cameroon. Voucher specimens are deposited in the Smithsonian Sorting Center, U.S.A.

Isolation

The crude organic extract (23.5 g) was partitioned in a mixture of $CHCl_3$:MeOH:H_2O (10:9:4, v/v) to give 15.7 g of an inactive nonpolar fraction and 6.3 g of a cytotoxic polar fraction. The cytotoxic fraction was permeated through a Sephadex LH-20 column (7×38 cm) in MeOH to yield three fractions of 1.74 g, 1.40 g and 3.13 g, respectively. The first fraction was potently cytotoxic and gave ^1H NMR signals indicative of triterpene saponins. The second fraction was less cytotoxic but also appeared to consist largely of saponins, while the third fraction was noncytotoxic and composed largely of flavonoid glycosides. The cytotoxic first Sephadex LH-20 fraction was further separated in 100 mg aliquots on a 2×30 cm wide-pore polymeric amino HPLC column (5 μ spherical, 300Å, YMC) using 80% CH_3CN at 25 mL/min, and rinsing with 60% CH_3CN at 18 mL/min. Ten fractions were collected. Elliptosides A–D were found in fraction 7 (152 mg), elliptosides E–H in fraction 9 (181 mg), and elliptosides I–J in fraction 6 (107 mg). Each of these three active fractions was separately chromatographed on a 2.1×25 cm wide-pore C4 column (5 μ spherical, 300 Å, Rainin) using a gradient of MeOH–H_2O/40 mM HOAc from 50 to 90% MeOH at 10 mL/min over 100 min. Yields of elliptosides were A: 19 mg, B: 14 mg, C:13 mg, D: 5 mg, E: 38 mg, F: 18 mg, G: 22 mg, H: 14 mg. The elliptoside I–J fraction was further chromatographed on a wide-pore phenyl column (5 μ spherical, 300 Å, YMC) with 65% MeOH to give elliptoside I (1 mg) and elliptoside J (2 mg).

Elliptoside A (8). $[\alpha]_D$ −21.9° (*c* 0.74, MeOH); FABMS (positive ion mode, magic bullet) *m/z* 2089 $[M+Na]^+$, 2067 $[MH]^+$, 1503, 975, 674, 482, 350, 204 (confirmed as daughter ions by linked scan); HRFABMS *m/z* 2198.8870 $[M+Cs]^+$(calcd for $C_{98}H_{155}NO_{45}Cs$, 2198.8925), 674.4242 (calcd for $C_{38}H_{60}NO_9$, 674.4268), 482.1869 (calcd for $C_{19}H_{32}NO_{13}$, 482.1874), 368.1555 (calcd for $C_{14}H_{26}NO_{10}$, 368.1557), 350.1435 (calcd for $C_{14}H_{24}NO_9$, 350.1451), 313.1662 (calcd for $C_{16}H_{25}O_6$, 313.1651), 204.0879 (calcd for $C_8H_{14}NO_5$, 204.0872); Sugar composition: Ara 1.0, Rha 1.5, Fuc 0.9, Xyl 1.1, Qui 0.6, Glu 2.0, GlcNAc 0.4; Similar results were obtained from alditol acetate analysis;[30] ^1H NMR (MeOH-d_4:D_2O, 4:1 v/v) 6.92 (1H, t, J = 7.8 Hz, MT_1-3), 6.81 (1H, dt, 7.6, 1.5, MT_2-3), 6.01 (1H, dd, 10.7, 17.8, MT_1-7), 5.90 (1H, dd, 10.9, 17.8, MT_2-7), 5.43 (1H, dd, 5.3, 8.5, H-21), 5.35 (2H, d, 2.1, H-12, Ara-1), 5.33 (2H, d, 7.8, Glu_A-1, Rha-1), 5.31 (1H, d, 2.8), 5.26 (1H, dd, 1.8, 18.1, MT_1-8b), 5.21 (1H, dd, 1.4, 16.0, MT_2-8b), 5.15 (1H, dd, 1.4, 17.7, MT_1-8a), 5.07 (1H, dd,

1.4, 10.6, MT_2-8a), 4.62 (1H, t, 9.6, Qui-4), 4.53 (1H, d, 7.8, Xyl-1), 4.52 (1H, d, 7.4, Glu_B-1), 4.51 (1H, d, 8.2, Fuc-1), 4.47 (1H, d, 7.8, GlcNAc-1), 4.41 (1H, d, 7.8, Qui-1), 4.33 (2H, s, MT_1-10), 4.23 (1H, d, 5.4, Glu_A-3), 4.09 (1H, dd, 3.6, 2.1, Ara-4), 4.04 (1H, m, Ara-2), 3.98 (2H, dd, 5.3, 11.7, Xyl-5), 3.90 (1H, dd, 3.9, 6.7, Glu_A-2), 3.83 (1H, d, 9.9), 3.82 (1H, Glu_A-6), 3.79 (1H, d, 9.9, Rha-5), 3.76 (2H, dd, 2.8, 12, Glu_B-6), 3.73 (1H, dd, 5, 12, Ara-5a), 3.68 (1H, d, Fuc-4), 3.66 (2H, m, Fuc-2, Fuc-3), 3.63 (2H, m, Ara-5b, Ara-3), 3.55 (3H, m, GlcNAc-5, Glu_A-4, Rha-2) 3.54 (1H, m, Xyl-3), 3.47 (4H, m, Qui-3, Qui-5, GlcNAc-3, GlcNAc-4), 3.41 (1H, d, 9.2, Fuc-5), 3.40 (1H, t, 8.9, Xyl-2), 3.35 (1H, m, Glu_B-2), 3.28 (2H, m, Qui-2, Xyl-4), 2.97 (1H, dd, 3.6, 12.7), 2.48 (1H, d, 6.4, MT_1-4), 2.46 (m), 2.44 (m), 2.24 (m, MT_2-4), 2.14 (2H, d, 8.5, H-15, H-22), 1.97 (3H, s, NHAc), 1.90 (2H, brs, H-11), 1.82 (1H, d, 2.1, MT_2-10), 1.78 (2H, m, MT_1-5, H-22), 1.62 (1H, t, 6.6, MT_2-5), 1.40 (4H, brs, H-18, H-27), 1.35 (3H, s, MT_1-9), 1.32 (3H, d, 5.6, Rha-6), 1.27 (3H, s, MT_2-9), 1.26 (3H, d, 6.8, Fuc-6), 1.22 (1H, s), 1.14 (1H, d, 7.1), 1.08 (3H, d, 6.4, Qui-6), 1.03 (4H, s, H-6, H-30), 0.96 (3H, s, H-23), 0.94 (3H, s, H-25), 0.86 (3H, s, H-29), 0.75 (3H, s, H-26), 0.74 (3H, s, H-24); ^{13}C NMR, see Tables 1–3.

Elliptoside B. $C_{96}H_{152}O_{45}$ FABMS (glycerol) *m/z* 2047 [M+Na].

Elliptoside C. FABMS (mb) *m/z* 2089.

Elliptoside D. FABMS (glyc) *m/z* 2089.

Elliptoside E (9). $[\alpha]_D$ −24.3° (*c* 1.03, MeOH); FABMS (positive ion mode, magic bullet) *m/z* 2105 $[M+Na]^+$, 2083 $[MH]^+$, 1924, 1503, 1457, 1159, 993, 975, 482, 350, 204. HRFABMS *m/z* 2214.8818 $[M+Cs]^+$ (calcd for $C_{98}H_{155}NO_{46}Cs$, 2214.8875); ^1H NMR (partial, MeOH-d_4:D_2O, 4:1 v/v) 6.97 (1H, t, J = 7.8 Hz), 6.92 (1H, t, 7.6), 6.01 (1H, dd, 11.4, 17.4), 5.91 (1H, dd, 10.8, 17.4) 5.43 (1H, dd, 5.9, 10.7, H-21), 5.07 (1H, d, 10.8), 4.64 (1H, t, 9.5), 4.41 (1H, d, 7.9), 3.98 (1H, dd, 5.4, 11.7), 2.47 (1H, t, 7.9), 2.14 (2H, dd), 1.97 (3H, s, NHAc) 1.40 (3H, s), 1.35 (3H, s), 1.32 (3H, d, 5.9 Hz), 1.28 (3H, s), 1.27 (3H, d, 6.8), 1.11 (3H, d, 6.2), 1.04 (3H, s), 0.96 (3H, s), 0.93, (3H, s), 0.86 (3H, s), 0.75 (3H, s), 0.74 (3H, s); ^{13}C NMR, see Tables 1–3.

Elliptoside F (10). HRFABMS *m/z* 2173.8613 [M+Cs] (calcd for $C_{96}H_{152}O_{46}Cs$, 2173.8609); ^1H NMR (partial, MeOH-d_4:D_2O, 4:1 v/v) δ 6.97 (1H, t, J = 7.8 Hz), 6.92 (1H, t, 7.8), 6.01 (1H, dd, 11.6, 17.9), 5.91 (1H, dd, 11.1, 17.6), 5.44 (1H, dd, 5.7, 11.3), 5.07 (1H, dd, 10.7, 1.5), 4.64 (1H, t, 9.4), 4.42 (1H, d, 7.8), 4.36 (1H, d, 7.8), 3.95 (1H, dd, 5.4, 11.2), 2.97 (1H, dd, 4, 14.7), 1.78 (1H, t, 8.1), 1.41 (3H, s), 1.35 (3H, s), 1.32 (3H, d, 5.9), 1.28 (3H, s), 1.26 (3H, d, 6.9), 1.11 (3H, d, 6.3), 1.05 (3H, s), 1.04 (3H, s), 0.95 (3H, s), 0.86 (3H, s), 0.84 (3H, s), 0.80 (1H, d, 12), 0.75 (3H, s); ^{13}C NMR see Tables 1–3.

Elliptoside G. $C_{98}H_{155}NO_{46}$ FABMS (glycerol) *m/z* 2104; HRFABMS (mb) 2214.8860 [M+Cs] (calcd for $C_{98}H_{155}NO_{46}Cs$, 2214.8875).

Elliptoside H. FABMS (mb) *m/z* 2104.

Elliptoside I. FABMS (mb) *m/z* 2061.

Elliptoside J. FABMS (mb) *m/z* 2065; ^1H NMR (partial, MeOH-d_4) 7.73 (1H, d, J = 8.5 Hz), 7.67 (1H, d, 15.9), 7.56 (2H, d, 8.4), 6.95 (2H, d, 8.6), 6.91 (4H, m), 6.41 (1H, d, 15.8), 6.04 (2H, m), 5.85 (1H, d, 12.1), 5.50 (1H, dd, 5.6, 10.8).

Sugar determination. Carbohydrate composition was determined by the method of York et al.[31] The samples were treated with anhydrous methanolic HCl (1 M, 80 °C, 16 h), then *N*-acetylated (MeOH–pyridine, Ac_2O, 6 h, rt). The resulting methyl glycosides were derivatized with Tri-Sil (Pierce) and analyzed by GC-MS using a 30 m DB-1 column using *myo*-inositol as an internal standard.

Hydrolysis of saponin mixture with 3 N HCl. Saponin mixture (50 mg) in 2 mL MeOH was hydrolyzed with 0.5 mL concd HCl for 6 h at reflux. Acacic acid lactone **1** (2 mg) was purified by VLC (hexane:EtOAc, 4:1) on silica and cyano-bonded phase HPLC (hexane-*i*PrOH), and characterized by ^1H and ^{13}C NMR,[6,8] HMQC, HMBC, COSY, TOCSY, and difference NOE spectra. ^{13}C NMR see Table 1.

Hydrolysis of saponin mixture with KOH. Hydrolysis of 90 mg of saponin mixture was performed with 3 mL of 0.5 M KOH for 3 h at 100 °C. The reaction mixture was adjusted to pH 5 with 1 N HCl and extracted with *n*-BuOH. The BuOH layer was evaporated to give 65 mg of residue and purified by vacuum-liquid chromatography using a $CHCl_3$–MeOH step gradient to yield 11 mg of a mixture of monoterpenes. The mixture was chromatographed by cyano-bonded phase HPLC (Rainin Dynamax, hexane-*i*PrOH gradient, 5–50%, 6 mL/min) to yield **2** (1.2 mg) and a mixture of **2** and **3** (2.8 mg) which were identified by comparison to literature spectral values.[7] The trisaccharide prosaponins **4** (7 mg) and **5** (12 mg) were eluted from the silica VLC column with more polar $CHCl_3$–MeOH mixtures. Each compound was further purified by diol HPLC (YMC 2 × 30 cm, eluting with a gradient of MeOH in EtOAc from 20–100%).

Monoterpene free acid 2. $[\alpha]_D$ −9.1° (*c* 0.35, $CHCl_3$), (lit.[7] +17.8°); ^1H NMR ($CDCl_3$) 6.90 (1H, t, J = 7.5 Hz), 5.91 (1H, dd, 11, 17), 5.24 (1H, dd, 18, 1), 5.10 (1H, dd, 11, 1), 2.26 (2H, m), 1.83 (3H, s), 1.55–1.75 (2H, m), 1.30 (3H, s).

Prosaponin 4. FABMS: *m/z* 951 $[M+Na]^+$, 929 $[MH]^+$ HRFABMS *m/z* 929.5143 $[MH]^+$ (calcd for $C_{47}H_{77}O_{18}$, 929.5110); ^1H NMR (MeOH-d_4) 5.29 (1H, t, J = 3.7 Hz), 4.47 (1H, d, 7.3), 4.31 (1H, d, 7.8), 4.13 (1H, dd, 5.3, 11.6), 4.05 (1H, dd, 1.7, 11.6), 3.95 (1H, dd, 5.3, 11.2), 3.74 (1H, dd, 6.2, 11.8), 3.62 (1H, t, 3.5), 3.58 (1H, d, 7.3), 3.35 (1H, t, 9), 3.02 (1H, dd, 4.5, 14.5), 2.38 (1H, t, 13.9), 1.98 (1H, dd, 5.3, 8.3), 1.39 (3H, s), 1.27 (3H, s), 1.25 (3H, d, 6.4), 1.04 (3H, s), 0.95 (3H, s), 0.92 (3H, s),

0.91 (3H, s), 0.84 (3H, s), 0.77 (3H, s); ^{13}C NMR (d_4-MeOH) 180.1 (C-28), 144.4 (C-13), 123.7 (C-12), 106.9, 106.5, 103.8, 89.7 (C-3), 82.2, 78.1, 77.9, 77.4, 77.1, 75.9, 75.7, 75.0, 74.8, 74.7, 72.7, 71.7 (2C), 71.1, 69.8 (Glu-6), 67.3 (Xyl-5), 57.1 (C-5), 52.1, 48.1, 42.5, 41.4, 41.0, 40.6, 40.3, 39.8, 37.9, 36.7, 35.9, 34.3, 29.7, 28.4, 27.4, 27.3, 24.5, 19.3, 18.1, 17.8, 17.0, 16.7, 16.1.

Prosaponin 5. FABMS: *m/z* 992 $[M+Na]^+$, 970 $[MH]^+$, 692, 482; HRFABMS *m/z* 970.5381 $[MH]^+$ (calcd for $C_{49}H_{80}NO_{18}$, 970.5375); ^{1}H NMR (MeOH-d_4) 5.26 (1H, brt, J = 3.5 Hz), 4.54 (1H, brt, 2.5, H-16), 4.52 (1H, dd, 2.5, 4.5), 4.47 (1H, d, 7.5), 4.42 (1H, d, 8), 4.10 (1H, dd, 12, 2), 4.05 (1H, dd, 12, 2), 3.97 (1H, dd, 12, 4.5, 2AG-6a), 3.75 (1H, dd, 13, 6.5, 2AG-6b), 1.36 (3H, s, H-27), 1.27 (3H, d, 6), 0.96 (3H, s, H-23), 0.93 (6H, s, H-25, H-30), 0.90 (3H, s, H-29), 0.82 (3H, s, H-26), 0.75 (3H, s, H-24); ^{13}C NMR see Tables 1 and 3 (note: NMR data reported are for ring-opened lactone **5a**).

Peracetylation of prosaponins. The peracetates of **4** and **5** were prepared using Ac_2O and DMAP in pyridine at room temperature for 20 h. Acetylation of 15 mg of **5** gave a mixture purified by vacuum liquid chromatography with hexane–EtOAc on silica to give 6 mg of the peracetate **12**, which was characterized by ^{1}H NMR and ^{13}C NMR. Compound **11** was prepared in a similar fashion.

Peracetate 11. ^{1}H NMR (C_6D_6) 5.59 (1H, dd, J = 9, 9 Hz, Glu-3), 5.47 (1H, dd, 11.5, 4, C-16), 5.45 (1H, m, Glu-4), 5.40 (1H, m, Glu-2), 5.37 (1H, m, Xyl-3), 5.35 (2H, m, Xyl-2, Xyl-4), 5.23 (1H, dd, 3.5, 1, Fuc-4), 5.10 (1H, brs, H-12), 5.09 (1H, dd, 3.5, 10.5, Fuc-3), 4.82 (1H, d, 6.5, Xyl-1), 4.50 (d, 8, Glu-1), 4.37 (1H, d, 7.5, Fuc-1), 4.24 (dd, 11.5, 5, Xyl-5a), 4.08 (1H, dd, 7.5, 10.5, Fuc-2), 3.98 (1H, m, Glu-6a), 3.84 (1H, d, 5.5, H-21), 3.65 (2H, m, Glu-5, Glu-6b), 3.13 (1H, dd, 12, 5.5, H-3), 3.10 (1H, dq, 1.5, 6, Fuc-5), 3.03 (1H, dd, 11.5, , Xyl-5b), 2.66 (1H, ddd, 1.5, 6.7, 11, H-18), 2.34 (1H, dd, 14, 5, H-15a), 2.21 (1H, dd, 5.5, 11.5, H-22a), 2.02 (1H, s), 1.89 (3H, s), 1.83 (3H, s), 1.75 (3H, s), 1.71 (3H, s), 1.66 (3H, d, 6), 1.59 (3H, s), 1.20 (3H, s), 1.02 (3H, s), 1.01 (3H, s, H-23), 0.94 (3H, s, H-29), 0.93 (3H, d, 6.5), 0.89 (3H, s, H-24), 0.79 (3H, s, H-25), 0.61 (3H, s, H-30); ^{13}C NMR see Tables 1 and 3.

Peracetate 12. FABMS (noba) *m/z* 1289, 777, 307, 154, 136, HRFABMS *m/z* 1420.5092 $[M+Cs]^+$ (calcd for $C_{65}H_{93}NO_{25}Cs$, 1420.5091); ^{1}H NMR (MeOH-d_4) 5.48 (1H, t, J = 3 Hz), 5.23 (1H, dd, 8.5, 10.5), 5.16 (1H, t, 9), 5.13 (1H, dd, 3.5, 1), 5.02 (1H, ddd, 5, 9, 10), 4.97 (1H, dd, 5.5, 11), 4.91 (1H, t, 10), 4.87 (1H, dd, 3, 10), 4.84 (1H, d, 7, Xyl-1), 4.64 (1H, d, 8.5, 2AG-1), 4.61 (1H, d, 8, Fuc-1), 4.30 (1H, dd, 2, 4), 4.20 (1H, dd, 5.5, 11.5), 3.83 (3H, m), 3.74 (3H, m), 3.52 (1H, dd, 8.5, 11.5), 3.30 (1H, m), 2.58 (1H, dd, 6.5, 11.5), 2.35 (2H, br s), 2.12 (3H, s), 1.99 (12H, s, 4 × OAc), 1.97 (6H, s), 1.91 (3H, s), 1.87 (3H, s), 1.29 (3H, s), 1.15 (3H, d, 6.5), 1.02 (3H, s), 1.00 (6H, s), 0.97 (3H, s), 0.95 (3H, s), 0.78 (3H, s); ^{13}C NMR see Tables 1 and 3.

LiI cleavage of semipurified elliptoside A and saponin mixture. Saponin mixture (1.5 g) was dissolved in 20 mL 2,6-lutidine followed by addition of 4 mL allyl alcohol and 1.04 g LiI and refluxing with stirring for 16 h.[9,10] The reaction mixture was cooled, diluted with 70 mL 50% aq. MeOH, and passed through 75 mL of MB3 mixed-bed ion exchange resin using vacuum filtration. The eluate was evaporated to dryness, taken up in H_2O and eluted from 60 mL Amberlite XAD-2 resin with 30% MeOH. The residue (152 mg) was flash chromatographed on a 2 × 20 cm silica column in $CHCl_3$:MeOH:-H_2O (6:4:1 v/v). A portion of the major fraction (53 mg) was further purified by C_4 HPLC using CH_3CN:H_2O (1:1 v/v) to give **7**. A separate reaction, using a fraction containing elliptosides A–D (311 mg) was conducted under the same conditions and gave similar results.

7: HRFABMS (noba) *m/z* 660.2556 $[MH]^+$ (calcd for $C_{26}H_{45}O_{19}$, 661.2555); ^{1}H NMR (d_4-MeOH) 5.94 (1H, ddt, J = 16.5, 5.2, 1.5 Hz), 5.36 (1H, dd, 1.5, 17), 5.30 (1H, d, 2.3 Hz, Ara-1), 5.19 (dt, 10.5, 1.4), 4.92 (1H, d, 3.6, Glu_A-1), 4.88 (1H, d, 1.8, Rha-1), 4.53 (1H, d, 7.7, Glu_B-1), 1.27 (3H, d, 6.5, Rha-6); ^{13}C NMR, see Table 3; Sugar composition Ara 0.8, Rha 1.6, Xyl 0.1, Qui 0.1, Glu 2.0.

Hydrolysis of 9 with 1% KOH. Elliptoside A (**9**, 15 mg) was hydrolyzed in 3 mL dioxane and 3 mL 1% KOH at 0 °C for 3 h, then neutralized with 5% HCl. The reaction mixture was extracted with $CHCl_3$. The monoterpene **2** was identified from the organic layer. The aqueous layer was chromatographed on wide-pore C_4 HPLC in a MeOH–H_2O–HOAc gradient to yield 5.8 mg of the desmonoterpenyl elliptoside A **6**.

Monodesmonoterpenyl elliptoside A, 6. $[\alpha]_D$ −14.2° (*c* 0.48, MeOH); HRFABMS *m/z* 2032.7975 (calcd for $C_{88}H_{141}NO_{43}Cs$, 2032.7932); ^{1}H NMR (MeOH-d_4:D_2O, 5:1 v/v) 6.92 (1H, t, J = 7.5 Hz), 6.01 (1H, dd, 11, 18), 5.44 (1H, dd, 5.5, 11), 5.36 (1H, d, 2.8), 5.33 (1H, d, 8), 5.32 (1H, d, 2), 5.25 (1H, dd, 1.6, 18), 5.19 (1H, dd, 1.6, 11), 4.53 (1H, d, 8), 4.50 (1H, d, 8), 4.46 (1H, d, 8.7), 4.34 (1H, d, 8), 4.32 (brd, 1), 4.22 (1H, dt, 5, 1.6), 4.09 (1H, dd, 2, 3.5), 1.96 (3H, s), 1.75 (2H, t, 8.2), 1.40 (2H, s), 1.33 (3H, s), 1.33 (3H, s), 1.26 (3H, d, 6.3), 1.22 (3H, d, 6.3), 1.04 (3H, s), 0.96 (3H, s), 0.93 (3H, s), 0.86 (3H, s), 0.74 (3H, s); ^{13}C NMR see Tables 1–3.

Antitumor testing (NCI in vitro screen).[1,2] The tumor cell line subpanels are identified as follows: I (leukemia); II (lung, non-small-cell); III (colon); IV (CNS); V (melanoma); VI (ovarian); VII (renal); VIII (prostate); IX (breast). The subpanel and individual cell line identifiers are given, along with the corresponding $\log_{10}$ GI_{50}, TGI and LC_{50} values (μg/mL), respectively, for **1**:

{I} CCRF-CEM (−0.35, 0.79, >1.18), HL-60 (−0.50, −0.15, 0.48), K-562 (0.83, >1.18, >1.18), MOLT-4 (−0.20, 0.33, 1.09), RPMI-8226 (>1.18, >1.18, >1.18), SR (−1.18), HOP-62 (0.48, 0.82, 1.03), HOP-92 (−0.60, −0.43, −0.15), NCI-H226 (−0.12, 0.08, 0.55), NCI-H23 (0.55, 1.05, >1.18), NCI-H322M (0.18, 0.77, >1.18),

HCI-H460 (1.04, >1.18, >1.18), NCI-H522 (−0.56, −0.20, 0.34), {III} COLO205 (−0.13, 0.25, 0.60), HCC-2998 (0.01, 0.88, >1.18), HCT-116 (−0.03, >1.18, >1.18), HCT-15 (1.15, >1.18, >1.18), HT-29 (0.87, >1.18, >1.18), KM-12 (0.00, 0.38, 0.76), SW-620 (0.00, >1.18, >1.18), {IV} SF-268 (0.01, 0.82, >1.18), SF-295 (−0.28, −0.08, 0.12), SF-539 (0.78, 0.96, 1.13), SNB-19 (−0.23, 0.01, 0.47), SNB-75 (0.11, 0.77, 1.11), U251 (−0.03, 0.47, 0.87), {V} LOX IMVI (−0.42, 0.26, 0.80), MALME-3M (−0.18, 0.01, 0.26), M14 (−0.09, 0.24, 0.56), SK-MEL-2 (−0.19, 0.05, 0.53), SK-MEL-28 (−0.31, −0.12, 0.08), SK-MEL-5 (0.06, 0.58, 0.92), UACC-257 (−0.12, 0.19, 0.66), UACC-62 (0.52, 1.07, >1.18), {VI} IGROV1 (0.61, >1.18, >1.18), OVCAR-3 (−0.41, −0.15, 0.08), OVCAR-4 (−0.26, −0.08, 0.10), OVCAR-5 (0.36, 0.88, >1.18), OVCAR-8 (−0.06, 0.28, 0.63), SK-OV-3 (0.13, 0.51, 0.89), {VII} 786-0 (−0.25, 0.18, 0.62), ACHN (−0.47, −0.19, 0.02), CAKI-1 (−0.13, 0.31, 0.68), RXF-393 (−0.65, −0.34, 0.44), SN12C (−0.36, 0.09, 0.57), TK-10 (−0.17, 0.06, 0.48), UO-31 (0.00, 0.42, 0.80), {VIII} PC-3 (−0.44, −0.19, 0.01), DU-145 (0.15, 0.75, 1.05), {IX}MCF7/ADR-RES (0.67, 0.99, >1.18), MDA-MB-231/ATCC (−0.25, −0.01, 0.33), HS 578T (−0.09, 0.77, 1.16), MDA-MB-435 (−0.31, −0.03, 0.32), MDA-N (−0.32, 0.03, 0.43), BT-549 (0.74, 1.06, >1.18), T-47D (0.05, 0.85, >1.18).

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