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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · JUNE 2014

Impact Factor: 3.8 · DOI: 10.1021/np500130x · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

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Antifungal and Cytotoxic 2-Acylcyclohexane-1,3-diones from *Peperomia alata* and *P. trineura*

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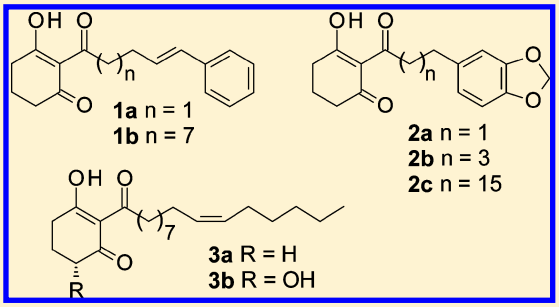
Supporting Information

ABSTRACT: Bioactivity-guided fractionation of the separate CH_2Cl_2 extracts from the aerial parts of *Peperomia alata* and *P. trineura* yielded seven polyketides: alatanone A [3-hydroxy-2-(5′-phenylpent-4′*E*-enoyl)-cyclohex-2-en-1-one, **1a**] and alatanone B [3-hydroxy-2-(3′-phenyl-6′-methylenedioxypropanoyl)cyclohex-2-en-1-one, **2a**] from *P. alata* and trineurone A [3-hydroxy-2-(11′-phenylundec-10′*E*-enoyl)cyclohex-2-en-1-one, **1b**], trineurone B [3-hydroxy-2-(15′-phenyl-18′-methylenedioxy-pentadecanoyl)cyclohex-2-en-1-one, **2b**], trineurone C [3-hydroxy-2-(17′-phenyl-20′-methylenedioxyheptadecanoyl)cyclohex-2-en-1-one, **2c**], trineurone D [3-hydroxy-2-(hexadec-10′*Z*-enoyl)cyclohex-2-en-1-one, **3a**], and trineurone E [(6*R*)-(+)-3,6-dihydroxy-2-(hexadec-10′*Z*-enoyl)cyclohex-2-en-1-one, **3b**] from *P. trineura*. The isolated compounds were evaluated for antifungal activity against *Cladosporium cladosporioides* and *C. sphaeospermum* and for cytotoxicity against the K562 and Nalm-6 leukemia cell lines.



The plant family Piperaceae comprises four genera,[1] with *Peperomia* being the second largest, with approximately 1700 species.[2] *Peperomia* species produce several classes of secondary metabolites, such as prenylated chromenes,[3] miscellaneous aromatic compounds,[4,5] prenylated chromones,[6] cyclobutane derivatives,[7] lignans,[8−11] prenylated quinones,[12] acetophenone derivatives,[13] polyketides,[14] and terpenoids.[15]

Species of *Peperomia*, together with those of *Virola*,[16] are the most important natural sources of a specific type of polyketide known as 2-acylcyclohexane-1,3-diones. Such compounds are important chemical messengers found in the secretion from mandibular glands of the moth *Ephestia kuehniella* and also in the feces of the Indian meal moth *Plodia interpunctella*. They act as ovipositing cues (kairomones) for the ichneumon wasp *Nemeritis canescens* (Gravenhorst) and parasitic wasp *Venturia canescens*.[17−19] Additional biological properties observed for these polyketides include cytotoxic activity against nasopharyngeal carcinoma and gastric adenocarcinoma and ovarian, breast, and hepatocellular carcinoma cell lines.[14,20−22]

In a continuing investigation of the Piperaceae, two crude extracts from *Peperomia* species were selected for further scrutiny based on their demonstrated antifungal and cytotoxic activities. Thus, the crude extracts from aerial parts of *P. alata* and *P. trineura* were subjected to chromatographic fractionation to afford seven new polyketide derivatives: alatanone A (**1a**) and alatanone B (**2a**) from *P. alata* and trineurone A (**1b**), trineurone B (**2b**), trineurone C (**2c**), trineurone D (**3a**), and trineurone E (**3b**) from *P. trineura*. The antifungal and cytotoxic activities of all compounds, except **3a**, were assessed. Although several species of *Peperomia* have been studied chemically, only the volatile oil of *P. alata* has been analyzed,[23] whereas no previous report exists for *P. trineura*. Thus, in the present study, the isolation, structure determination, and biological evaluation of seven novel polyketides from these two *Peperomia* species are described.

RESULTS AND DISCUSSION

The dichloromethane extracts from the aerial parts of *P. alata* and *P. trineura* were subjected to an evaluation of their antifungal activity against *Cladosporium cladosporioides* and *C.*

Received: February 11, 2014

dx.doi.org/10.1021/np500130x | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

sphaerospermum and to cytotoxic assays against the K568 and Nalm-6 leukemic cell lines; the extracts displayed activities in both cases. Thus, these extracts were separately submitted to bioguided chromatographic purification procedures to isolate the bioactive compounds.

Compounds **1a**–**3a** displayed a common set of signals in their ^{1}H NMR spectra that allowed their initial identification as 2-acylcyclohexane-1,3-diones (Figure 1).[20] They displayed

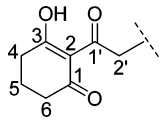


Figure 1. 2-Acylcyclohexane-1,3-dione moiety for all compounds (except **3b**) isolated from *P. alata* and *P. trineura*.

unusual singlets at δ 18.26 (1H, s, OH) assigned to the enones, which were supported by the characteristic absorption bands for hydrogen-bonded hydroxy (3026–3027 cm^{-1}), conjugated carbonyl (1665–1667 cm^{-1}), and conjugated hydrogen-bonded carbonyl (1556–1558 cm^{-1}) groups in their IR spectra. Their ^{1}H and ^{13}C NMR spectra also displayed a further common set of signals: two triplets at δ ~2.6 ($J \approx 6$ Hz, H_2-4) and ~2.4 ($J \approx 6$ Hz, H_2-6) (Table 1), which were correlated with quintuplets (H-5) at δ ~1.9 ($J \approx 6$ Hz) in COSY experiments. This fragment, C-4 through C-6, was confirmed by the chemical shifts at δ ~33, ~19, and ~38 ppm, respectively. The carbons C-1, C-2, C-3, and C-1′ displayed consistent signals at δ ~195, ~113, ~198, and ~205 ppm, respectively. The HMBC correlations also showed cross-peaks between H-5 and C-1 and C-3 and between H-6 and C-2 and C-4 and firmly established the core of 2-acylcyclohexane-1,3-diones.[20]

Compounds **1a** and **1b**, obtained as yellowish oils, exhibited $[M + H]^+$ ion peaks at m/z 271.1335 (**1a**) and 355.2274 (**1b**), in their HRESIMS, consistent with molecular formulas of $C_{17}H_{18}O_3$ and $C_{23}H_{30}O_3$, respectively. Their ^{1}H NMR spectra showed signals that corresponded to a monosubstituted aromatic ring (δ 7.16–7.36, m, 5H each) and signals assignable to the *E*-olefinic hydrogens of **1a** at δ 6.43 (dd, J = 15.8 and 1.4 Hz, H-5′) and 6.25 (dt, J = 15.8 and 7.0 Hz, H-4′) and of **1b** at

Table 1. ^{1}H NMR Spectroscopic Data for All Compounds ($CDCl_3$, 500 MHz)

	1a	1b	2a	2b	2c	3a	3b
position	δ_H (J, Hz)	δ_H (J, Hz)	δ_H (J, Hz)	δ_H (J, Hz)	δ_H (J, Hz)	δ_H (J, Hz)	δ_H (J, Hz)
1							
2							
3							
4	2.63 t (6.4)	2.66 t (6.5)	2.66 t (6.5)	2.66 t (6.5)	2.66 t (6.5)	2.66 t (6.5)	2.76 m
5	1.93 quint (6.4)	1.97 quint (6.5)	1.96 quint (6.5)	1.97 quint (6.5)	1.97 quint (6.5)	1.97 m	1.82 ddt (15.6, 13.0, 11.0, H_{ax})
							2.38 m (H_{eq})
6	2.47 t (6.4)	2.48 t (6.5)	2.48 t (6.5)	2.47 t (6.5)	2.48 t (6.5)	2.48 t (6.5)	4.08 dd (13.0, 5.5)
1′							
2′	3.22 t (7.2)	3.01 t (7.0)	3.30 t (7.6)	3.01 t (7.5)	3.01 t (7.5)	3.01 t (7.4)	3.07 ddd (15.5, 8.5, 6.5)
							2.97 ddd (15.5, 8.5, 6.5)
3′	2.53 ddd (7.2, 7.0, 1.4)	1.61 m	2.85 t (7.6)	1.53–1.64 m	1.53–1.64 m	1.61 m	1.63 m
4′	6.25 dt (15.8, 7.0)	1.27–1.41 m		1.22–1.38 m	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
5′	6.43 dd (15.8, 1.4)	1.27–1.41 m	6.75 d (1.5)	1.22–1.38 m	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
6′		1.27–1.41 m		1.22–1.38 m	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
7′	7.32 m	1.27–1.41 m		1.22–1.38 m	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
8′	7.27 m	1.46 m	6.71 d (7.8)	1.22–1.38 m	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
9′	7.17 m	2.20 ddd (7.0, 6.5, 1.5)	6.69 dd (7.8, 1.5)	1.22–1.38 m	1.22–1.38 m	2.02 m	2.02 m
10′	7.27 m	6.22 dt (16.0, 7.0)		1.22–1.38 m	1.22–1.38 m	5.36 t (4.6)	5.35 t (5.0)
11′	7.32 m	6.37 d (16.0)		1.22–1.38 m	1.22–1.38 m	5.36 t (4.6)	5.35 t (5.0)
12′				1.22–1.38 m	1.22–1.38 m	2.02 m	2.02 m
13′		7.31–7.36 m		1.22–1.38 m	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
14′		7.26–7.30 m		1.53–1.64 m	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
15′		7.16–7.19 m		2.51 t (7.5)	1.22–1.38 m	1.25–1.33 m	1.25–1.40 m
16′		7.26–7.30 m			1.53–1.64 m	0.90 t (7.5)	0.90 t (7.5)
17′		7.31–7.36 m		6.67 d (1.5)	2.51 t (7.5)		
18′							
19′					6.67 d (1.5)		
20′				6.72 d (8.0)			
21′				6.61 dd (8.0, 1.5)			
22′					6.71 d (8.0)		
23′					6.61 dd (8.0, 1.5)		
OCH_2O			5.90 s	5.91 s	5.91 s		
OH	18.26 s	18.26 s	18.09 s	18.26 s	18.26 s	18.26 s	18.26 s

dx.doi.org/10.1021/np500130x | *J. Nat. Prod.* XXXX, XXX, XXX–XXX

δ 6.37 (d, J = 16.0 Hz, H-11′) and 6.22 (dt, J = 16.0 and 7.0 Hz, H-10′). In addition, two α- and β-carbonyl-methylene hydrogens were observed for **1a** at δ 3.22 (t, J = 7.2 Hz, H-2′) and 2.53 (ddd, J = 7.2, 7.0, and 1.4 Hz, H-3′) and for **1b** at δ 3.01 (t, J = 7.0 Hz, H-2′) and 1.61 (m, H-3′), respectively. The ^{13}C NMR spectra of **1a** and **1b** displayed signals assignable to C-2′ at 40.4 and 40.6, respectively. Further analysis of COSY correlations allowed the complete assignments of the hydrogens of **1a** and **1b** and supported the analysis of the HMBC correlations of **1a** (Figure 3 and Table 1S, Supporting

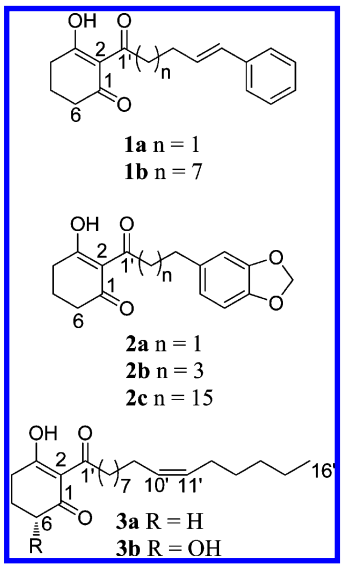


Figure 2. Structures of 2-acylcyclohexane-1,3-diones from *P. alata* (**1a**, **2a**) and *P. trineura* (**1b**, **2b**, **2c**, **3a**, **3b**).

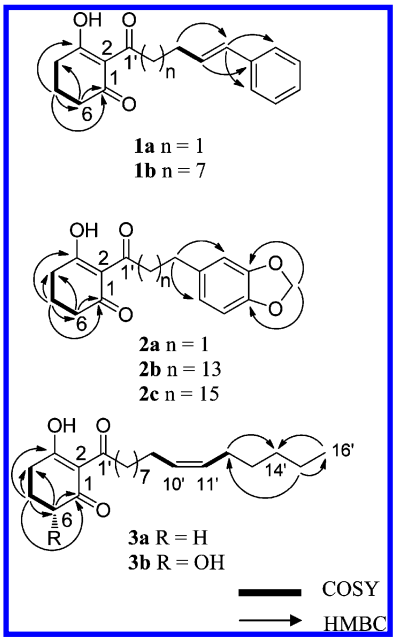


Figure 3. Main COSY and HMBC correlations observed for 2-acylcyclohexane-1,3-diones.

Information). In addition, the HMBC correlations also showed cross-peaks between H-3′ and C-1′ and C-5′ and between H-5′ and C-7′ and C-11′ for **1a** (Figure 3). In the case of compound **1b**, additional HMBC correlations were observed between H-10′ and C-12′ and between H-11′ and C-13′ and C-17′, which indicated an E double bond conjugated to the monosubstituted aromatic ring. Therefore, the structure of **1a** was established as 3-hydroxy-2-(5′-phenylpent-4′E-enoyl)cyclohex-2-en-1-one (alatanone A), whereas the structure of **1b** was established as 3-hydroxy-2-(11′-phenylundec-10′E-enoyl)cyclohex-2-en-1-one (trineurone A) (Figure 2).

Compounds **2a**–**2c**, yellowish, amorphous solids, had their molecular formulas determined as $C_{16}H_{16}O_5$ (m/z 289.1073, **2a**), $C_{28}H_{40}O_5$ (m/z 457.2947, **2b**), and $C_{30}H_{44}O_5$ (m/z 485.3264, **2c**), respectively. The ^{1}H NMR spectra of **2a**–**2c** were also similar to those of **1a** and **1b**, except for the signals absent for double-bond protons. A second important difference between these two sets of compounds was the singlet at δ ~5.9 (2H) for **2a**–**2c**, which together with the splitting pattern observed for the aromatic hydrogens at δ 6.69 (dd, J = 7.8 and 1.5 Hz), 6.71 (d, J = 7.8 Hz), and 6.75 (d, J = 1.5 Hz) for **2a** accounted for the 3,4-methylenedioxyphenyl moiety of **2a** (Table 1). Compounds **2b** and **2c** also displayed similar signals for the 3,4-methylenedioxyphenyl group, and the ^{13}C NMR spectra of these three compounds confirmed the methylenedioxy group (δ ~101) (Table 2). The saturated aliphatic chain of **2a** was characterized by the signals assigned to protons and carbons of H-2′ (δ 3.30, t, J = 7.6, Hz) and C-2′ (δ 42.7) and of H-3′ (δ 2.85, t, J = 7.6 Hz) and C-3′ (δ 30.3), whereas for compounds **2b** and **2c** the H-2′ (δ 3.01, t, J = 7.5 Hz) and C-2′ (δ 40.6), and H-3′ (δ 1.53–1.64, m, 2H) and C-3′ (24.7), signals had precisely the same chemical shifts. The long aliphatic chains of **2b** and **2c** were characterized by broad multiplets centered at δ 1.3 (20H and 24H, respectively).

Confirmatory NMR data based on the COSY and HMBC correlations were obtained, and complete assignments were made (Tables 1 and 2; Table 1S, Supporting Information). Therefore, the structures of **2a**–**2c** were established as 3-hydroxy-2-(3′-phenyl-6′-methylenedioxypropanoyl)cyclohex-2-en-1-one (alatanone B, **2a**), 3-hydroxy-2-(15′-phenyl-18′-methylenedioxypentadecanoyl)cyclohex-2-en-1-one (trineurone B, **2b**), and 3-hydroxy-2-(17′-phenyl-20′-methylenedioxyheptadecanoyl)cyclohex-2-en-1-one (trineurone C, **2c**), respectively.

Compounds **3a** and **3b** were obtained as yellowish oils. The IR spectrum of **3b** showed additionally a sharp absorption band typical of a free hydroxy group at 3466 cm^{-1}. The HRESIMS of **3a** indicated a molecular formula of $C_{22}H_{36}O_3$, because of the molecular ion peak at 349.2751 $[M + H]^+$.

The ^{1}H NMR spectra of compounds **3a** and **3a** exhibited triplets at δ ~5.3 (J = 4.6 and 4.8 Hz, H-10′, H-11′, respectively), characteristic of a double bond (Table 1). The Z double bond was confirmed on the basis of the typical shielded signals of α-olefinic carbons at δ ~27 as compared to an E system at δ ~32.[24] For these two compounds, a triplet at δ 0.90 (J = 7.5 Hz, H-16′) indicated a terminal methyl group in a monounsaturated aliphatic side chain.

The COSY spectrum displayed the connectivities H-4 through H-6 and H-9′ through H-12′, whereas the HMBC data showed correlations between H-14′ and C-12′ and C-16′ (Figure 3). Thus, on the basis of the evidence obtained, compound **3a** was established as 3-hydroxy-2-(hexadec-10′Z-enoyl)cyclohex-2-en-1-one (trineurone D).

The HRESIMS of **3b** exhibited a molecular ion peak at m/z 365.2687, supportive of a molecular formula of $C_{22}H_{36}O_4$. The ^{1}H NMR spectrum of **3b** indicated a typical 3,6-dihydroxycyclohexane-1,3-dione[25,26] because of the signals that occurred at δ 4.08 (dd, J = 13.0 and 5.5 Hz, H-6), 1.82 (ddt, J = 15.6,

dx.doi.org/10.1021/np500130x | J. Nat. Prod. XXXX, XXX, XXX–XXX

Table 2. ^{13}C NMR Spectroscopic Data for All Compounds ($CDCl_3$, 125 MHz)

	1a	1b	2a	2b	2c	3a	3b
position	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C
1	195.2, C	195.3, C	195.2, C	195.3, C	195.3, C	195.3, C	195.6, C
2	113.2, C	113.0, C	113.2, C	113.0, C	113.0, C	113.0, C	110.3, C
3	198.1, C	198.6, C	198.3, C	198.6, C	198.6, C	198.6, C	197.9, C
4	33.0, CH_2	33.3, CH_2	33.1, CH_2	33.3, CH_2	33.3, CH_2	33.3, CH_2	32.0, CH_2
5	19.0, CH_2	19.1, CH_2	19.1, CH_2	19.1, CH_2	19.1, CH_2	19.1, CH_2	27.1, CH_2
6	38.7, CH_2	38.9, CH_2	38.7, CH_2	38.9, CH_2	38.9, CH_2	38.8, CH_2	71.6, CH
1′	205.2, C	206.3, C	204.9, C	206.4, C	206.3, C	206.3, C	206.1, C
2′	40.4, CH_2	40.6, CH_2	42.7, CH_2	40.6, CH_2	40.6, CH_2	40.6, CH_2	40.3, CH_2
3′	27.8, CH_2	24.7, CH_2	30.3, CH_2	24.7, CH_2	24.7, CH_2	24.7, CH_2	24.6,CH_2
4′	129.1, CH	29.2−29.4, CH_2	134.8, C	29.2−29.7, CH_2	29.2−29.7, CH_2	29.4−29.8, CH_2	29.3−29.7, CH_2
5′	130.7, CH	29.2−29.4, CH_2	109.1, CH	29.2−29.7, CH_2	29.2−29.7, CH_2	29.4−29.8, CH_2	29.3−29.7, CH_2
6′	137.5, C	29.2−29.4, CH_2	147.6, C	29.2−29.7, CH_2	29.2−29.7, CH_2	29.4−29.8, CH_2	29.3−29.7, CH_2
7′	126.0, CH	29.2−29.4, CH_2	145.8, C	29.2−29.7, CH_2	29.2−29.7, CH_2	29.4−29.8, CH_2	29.3−29.7, CH_2
8′	128.4, CH	29.2−29.4, CH_2	108.2, CH	29.2−29.7, CH_2	29.2−29.7, CH_2	29.4−29.8, CH_2	29.3−29.7, CH_2
9′	126.9, CH	33.0, CH_2	121.3, CH	29.2−29.7, CH_2	29.2−29.7, CH_2	27.2, CH_2	27.2, CH_2
10′	128.4, CH	131.2, CH		29.2−29.7, CH_2	29.2−29.7, CH_2	129.8, CH	129.8, CH
11′	126.0, CH	129.7, CH		29.2−29.7, CH_2	29.2−29.7, CH_2	129.9, CH	129.9, CH
12′		137.9, C		29.2−29.7, CH_2	29.2−29.7, CH_2	26.9, CH_2	26.9, CH_2
13′		125.9, CH		29.2−29.7, CH_2	29.2−29.7, CH_2	29.4−29.8, CH_2	29.8, CH_2
14′		128.4, CH		31.8, CH_2	29.2−29.7, CH_2	32.0, CH_2	31.3, CH_2
15′		126.7, CH		35.7, CH_2	29.2−29.7, CH_2	22.3, CH_2	22.3, CH_2
16′		128.4, CH		136.9, C	31.8, CH_2	14.0, CH_3	14.0, CH_3
17′		125.9, CH		108.9, CH	35.7, CH_2		
18′				145.3, C	136.9, C		
19′				147.4, C	108.9, CH		
20′				108.0, CH	145.3, C		
21′				121.0, CH	147.4, C		
22′					108.0, CH		
23′					121.0,CH		
OCH_2O			100.8, CH_2	100.7, CH_2	100.7, CH_2		

13.0, and 11.0 Hz, H-5_{ax}), 2.38 (m, H-5_{eq}), and 2.76 (m, H-4) (Table 1). The equatorial orientation of the hydroxy group at C-6 of **3b** was determined on the basis of the large diaxial coupling constant between H-5_{ax} and H-6_{ax} (J = 13.0 Hz).[20,25,26] Its positive optical rotation, $[\alpha]_D$ +21 (MeOH), contrasted with the negative values observed for several surinones isolated from *P. sui*.[26] Compound **3b** displayed an ECD curve matching that of (*R*)-2-acyl-3,6-dihydroxycyclohex-2-en-1-one[27] and accordingly was determined as (6*R*)-(+)-3,6-dihydroxy-2-(hexadec-10′*Z*-enoyl)cyclohex-2-en-1-one (trineurone E).

The antifungal activity of the compounds was determined by direct bioautography on TLC plates[28] (Table 3). Compounds **1a** and **2a**, both isolated from *P. alata*, exhibited antifungal activities more potent than the controls and those of compounds **1b**, **2b**, **2c**, **3a**, and **3b**, which were isolated from *P. trineura*. The cytotoxic activities of all compounds were determined against the leukemic cell lines K562 and Nalm-6, and compound **3b**, the C-6 hydroxylated analogue of **3a**, was found to be the most active substance tested, with IC_{50} values of 26.0 and 14.3 μM, respectively.

Table 3. Antifungal Activities for All Compounds

	antifungal activity[a] (μg)	
compound	*C. cladosporioides*	*C. sphaerospermum*
1a	0.25	0.25
1b	5.00	25.0
2a	0.50	0.50
2b	5.00	5.00
2c	5.00	5.00
3a	10.0	25.0
3b	1.00	10.0
nystatin	1.00	1.00
miconazole	1.00	1.00

[a]Minimum amount required for the inhibition of fungal growth on thin-layer chromatographic plates (TLC).

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were recorded on a UV/visible Shimadzu 1650-PC spectrophotometer. Optical rotation values were obtained with a PerkinElmer 343 polarimeter, and the ECD was recorded using a JASCO J-720 spectropolarimeter. IR spectra were obtained on a Bomen MB-100 spectrometer. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured in $CDCl_3$ on a Bruker DRX 500 spectrometer using TMS as an internal standard. EIMS (70 eV) were measured on a Shimadzu 14B/QP5050A apparatus, and HRESIMS were measured on a Bruker Daltonics MicrOTOF QII spectrometer. Semipreparative HPLC chromatography was performed on a Shimadzu AD-VP equipped with an LC-10AD binary pump system, an SPD-10A UV detector (278 nm), and a Phenomenex reversed-phase C_{18} column (250 × 10.0 mm, 5 μm). Silica gel (Merck, 230−400 mesh) and Sephadex LH-20 (Pharmacia) were used for column chromatographic separations, and silica gel 60 PF_{254} (Merck) was used for preparative TLC purification (1.0 mm). All solvents used for column chromatography were of

dx.doi.org/10.1021/np500130x | J. Nat. Prod. XXXX, XXX, XXX−XXX

analytical grade (Labsynth Ltd.), and all solvents used for HPLC were of HPLC grade (Tedia).

Plant Material. *Peperomia alata* Ruiz & Pav. (Kato-0291) was collected in April 2004 in Ubatuba City, São Paulo, Brazil, and *Peperomia trineura* (Miq.) (Kato-0831) was collected in December 2006 in Encantado, Rio Grande do Sul, Brazil; both samples were identified by one of the authors (E.F.G.). Voucher specimens (Kato-0291 and Kato-0837) have been deposited in the Herbarium of Instituto de Botânica (SEMA/SP), São Paulo, SP, Brazil, and in the Herbarium of the Jardim Botânico do Rio de Janeiro, RJ, Brazil, respectively.

Extraction and Isolation. Dried and powdered aerial parts of *P. alata* (18.0 g) were exhaustively extracted by maceration with CH_2Cl_2 at room temperature. The resulting CH_2Cl_2 extract was filtered and concentrated in vacuo to yield 2.68 g of a crude extract, which was subjected to a silica gel column chromatography (gradient elution of EtOAc in hexanes and of MeOH in EtOAc), to afford 12 fractions. The antifungal and cytotoxic activities of fractions 5 and 6 were demonstrated, and thus, fraction 5 (790.0 mg) was chromatographed on a Sephadex LH-20 column eluted with 1:4 hexanes−CH_2Cl_2 followed by 3:2 CH_2Cl_2−Me_2CO and 1:4 CH_2Cl_2−Me_2CO, which yielded eight fractions (I−VIII). Bioactive subfractions III, IV, and V were combined according to their TLC and ^{1}H NMR spectroscopic similarities. The resulting fraction (720.0 mg) was applied to a silica gel chromatographic column (gradient elution of CH_2Cl_2 in hexanes, EtOAc in CH_2Cl_2, and MeOH in EtOAc), which yielded eight fractions (A−H). The antifungal fraction A (421.0 mg) consisted of pure **1a** (2.3% on dry weight basis). Fraction 6 (163.0 mg) was subjected to silica gel column chromatography (gradient elution of EtOAc in hexanes and of MeOH in EtOAc) to afford eight fractions (I−VIII). Compound **2a** (52.0 mg, 0.3%) was isolated in a pure form from bioactive subfraction V.

Dried and powdered aerial parts of *P. trineura* (270.0 g) were extracted twice for 2 days by maceration with CH_2Cl_2 at room temperature. The resulting solution was filtered and concentrated in vacuo to yield 9.0 g of a crude extract, of which 6.0 g was subjected to a silica gel column chromatography (gradient of EtOAc in hexanes and MeOH in EtOAc), to afford 12 fractions. Antifungal and cytotoxic activities were detected in fractions 5 and 10.

Fraction 5 (595.0 mg) was subjected to a silica gel column chromatography (gradient elution of EtOAc in hexanes and of MeOH in EtOAc), to afford 12 fractions (I−XII), of which antifungal activity was detected in subfractions V and VI. Subfraction V (112.0 mg) was subjected to preparative TLC (hexanes−EtOAc, 7:3) to afford a mixture of compounds **1b**, **2b**, and **2c**. This subfraction was subjected to semipreparative HPLC separation using a gradient mixture of MeCN−H_2O (9:1, increased to 100% over a period of 4 min and maintained at 100% for 30 min) as the mobile phase, which yielded the pure compounds **1b** (10.4 mg, 0.006%), **2b** (12.2 mg, 0.007%), and **2c** (15.2 mg, 0.008%).

Subfraction VI (65.0 mg) and fraction 10 (250.0 mg) were subjected to preparative TLC (hexanes−EtOAc, 7:3) to afford the pure compounds **3a** (7.2 mg, 0.004%) and **3b** (25.3 mg, 0.014%).

*3-Hydroxy-2-(5′-phenylpent-4′E-enoyl)cyclohex-2-en-1-one (**1a**, alatanone A):* yellowish oil; UV (MeOH) λ_{max} (log ε) 256 (4.37), 273 (4.58) nm; IR (KBr) ν_{max} 3026, 2952, 1665, 1558, 1439, 1326, 1188, 966, 746, 695 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 270 [M$^+$] (25), 252 (40), 165 (48), 139 (73), 117 (100), 91 (47), 69 (50); HRESIMS *m*/*z* 271.1335 [M + H]$^+$ (calcd for $C_{17}H_{18}O_3$, 271.1329).

*3-Hydroxy-2-(11′-phenylundec-10′E-enoyl)cyclohex-2-en-1-one (**1b**, trineurone A):* yellowish oil; UV (MeOH) λ_{max} (log ε) 254 (4.70), 272 (4.90) nm; IR (KBr) ν_{max} 3027, 2925, 1667, 1556 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 354 [M$^+$] (11), 167 (71), 154 (58), 139 (65), 126 (17), 117 (100), 115 (63), 104 (77), 91 (74), 69 (64), 55 (79), 41 (44); HRESIMS *m*/*z* 355.2274 [M + H]$^+$ (calcd for $C_{23}H_{30}O_3$, 355.2265).

*3-Hydroxy-2-(3′-phenyl-6′-methylenedioxypropanoyl)cyclohex-2-en-1-one (**2a**, alatanone B):* yellowish, amorphous solid; UV (MeOH) λ_{max} (log ε) 234 (4.95), 278 (4.85) nm; IR (KBr) ν_{max} 3057, 2952, 2930, 1664, 1558, 1503, 1489, 1443, 1245, 1190, 1039, 928, 813 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 288 [M$^+$] (30), 270 (5), 135 (100), 91 (10), 77 (24); HRESIMS *m*/*z*: 289.1073 [M + H]$^+$ (calcd for $C_{16}H_{16}O_5$, 289.1071).

*3-Hydroxy-2-(15′-phenyl-18′-methylenedioxypentadecanoyl)-cyclohex-2-en-1-one (**2b**, trineurone B):* yellowish, amorphous solid; UV (MeOH) λ_{max} (log ε) 232 (5.02), 279 (5.10) nm; IR (KBr) ν_{max} 1665, 1559, 1490, 1470, 1442, 1032, 935 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 456 [M$^+$] (12), 167 (27), 136 (23), 135 (100), 69 (17), 55 (28), 43 (13), 41 (15); HRESIMS *m*/*z* 457.2947 [M + H]$^+$ (calcd for $C_{28}H_{40}O_5$, 457.2943).

*3-Hydroxy-2-(17′-phenyl-20′-methylenedioxyheptadecanoyl)-cyclohex-2-en-1-one (**2c**, trineurone C):* yellowish, amorphous solid; UV (MeOH) λ_{max} (log ε) 232 (3.90), 275 (4.45) nm; IR (KBr) ν_{max} 1686, 1559, 1490, 1470, 1442, 1032, 935 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 484 [M$^+$] (4), 167 (21), 139 (15), 136 (20), 135 (100), 69 (16), 55 (29), 43 (17), 41 (18); HRESIMS *m*/*z* 485.3264 [M + H]$^+$ (calcd for $C_{30}H_{44}O_5$, 485.3255).

*3-Hydroxy-2-(hexadec-10′Z-enoyl)cyclohex-2-en-1-one (**3a**, trineurone D):* yellowish oil; UV (MeOH) λ_{max} (log ε) 232 (4.91), 279 (4.99) nm; IR (KBr) ν_{max} 3004, 1669, 1560 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 348 [M$^+$] (6), 221 (9), 203 (10), 189 (14), 168 (11), 167 (100), 154 (65), 139 (56), 126 (12), 125 (6), 111 (13), 97 (9), 81 (7), 69 (36), 57 (5), 55 (75), 43 (30), 41 (58); HRESIMS *m*/*z* 349.2751 [M + H]$^+$ (calcd for $C_{22}H_{36}O_3$, 349.2733).

*(6R)-(+)-3,6-Dihydroxy-2-(hexadec-10′Z-enoyl)cyclohex-2-en-1-one (**3b**, trineurone E):* yellowish oil; $[\alpha]^{25}_D$ +21.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.93), 278 (5.00) nm; ECD (MeOH) λ ($\Delta\varepsilon$) 220 (+108), 239 (−0.05), 258 (+32), 288 (−35); IR (KBr) ν_{max} 3466, 1667, 1562 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 364 [M$^+$] (17), 183 (20), 181 (6), 168 (26), 155 (5), 153 (8), 137 (14), 126 (11), 125 (5), 111 (5), 109 (7), 95 (10), 85 (16), 81 (15), 69 (36), 67 (21), 57 (14), 55 (100), 43 (42), 41 (78), 39 (12); HRESIMS *m*/*z* 365.2687 [M + H]$^+$ (calcd for $C_{22}H_{36}O_4$, 365.2682).

Antifungal Assay. For the antifungal assays, solutions of the crude extracts, fractions, and pure compounds were prepared at different concentrations, corresponding to 100.0 μg of crude extract and 10.0, 5.0, 1.0, 0.5, 0.25, and 0.10 μg of fractions and pure compounds. The samples were applied to TLC plates, which were eluted with 9:1 $CHCl_3$−MeOH and subsequently dried to completely remove the solvents. The chromatograms were sprayed with a spore suspension of phytopathogenic fungi *C. cladosporioides* or *C. sphaerospermum* in a nutritive medium and incubated for 48 h in the dark in a moist chamber at 25 °C.[28] After incubation, clear inhibition zones appeared against a dark background. The microorganisms used in this antifungal assay were maintained at the Instituto de Botânica (SEMA/SP), Brazil. Nystatin and miconazole were used as positive controls, whereas ampicillin and chloramphenicol were used as negative controls.[28]

Cytotoxicity Assay. For the cytotoxicity assays against the K562[29] and Nalm-6[30] cell lines (myeloid *leukemia/erythroleukemia* Ph+ and acute lymphoid leukemia B), seven solutions of compounds **1a**, **1b**, **2a**, **2b**, **2c**, and **3a** were prepared, and solutions for the controls vincristine (Sigma, V8879) and imantinib mesylate (Gleevec, Novartis) with concentrations of 120−0.0012 μM and 170−0.0017 μM, respectively, were prepared in the culture medium. Thirty thousand cells/well were added to a 96-well plate. The solutions of test compounds were added to the cells, which were subsequently incubated for 48 h. After this incubation period, the plate was centrifuged, the medium was removed, the cells were resuspended with 100 μL of PBS, with 10 μL of MTT (M2128 Sigma) at a concentration of 5 mg/mL in PBS added, and the cells were incubated for another 4 h. The plate was then centrifuged, the supernatant was removed, and 150 μL of isopropyl alcohol was added and homogenized. The readings were conducted in an Elisa reader with a filter set at 570 nm. To test the temporal kinetics, six 96-well plates, or 576 wells divided into 288 for each cell type (K562 and Nalm-6), were used. Vincristine exhibited an IC_{50} value of 0.57 μM against Nalm-6 cells, and imantinib mesylate exhibited an IC_{50} value of 0.25 μM against K562 cells.

dx.doi.org/10.1021/np500130x | *J. Nat. Prod.* XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by grants from CNPq and FAPESP. E.A.F. and J.B.R. thank CNPq and FAPESP for providing a scholarship. The authors acknowledge CNPq for the research fellowships awarded to J.H.G.L., M.C.M.Y., and M.J.K.

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