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Isolation of the New Antiplasmodial Butanolide Malleastrumolide A from a *Malleastrum* sp. (Meliaceae) from Madagascar

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Abstract

An extract of a *Malleastrum* sp. (Meliaceae) collected in Madagascar by the Madagascar International Cooperative Biodiversity Group was found to have antimalarial activity, with an IC_{50} value between 2.5 and 5 μg/mL. After purification by liquid-liquid partition, chromatography on a Diaion open column, C-18 SPE and C-18 reverse phase HPLC, the new butanolide malleastrumolide A was isolated. The structure of malleastrumolide A was determined by mass spectrometry, NMR and ECD. The double bond position was determined by cross-metathesis and mass spectrometry. The compound has antiproliferative activity against the A2780 ovarian cancer cell line with an IC_{50} value of 17.4 μM and antiplasmodial activity against the drug-resistant Dd2 strain of *Plasmodium falciparum* with an IC_{50} value of 2.74 μM.

Keywords

Malleastrum sp.; Antiplasmodial activity; Butanolide

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Supplementary Material

Supporting information for this article is available on the web under https://doi.org/10.1002/cbdv.2017XXXX

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions Statement

David Kingston conceived and designed the experiments, Alexander Abedi isolated the active compound, and Yongle Du determined its structure. Vincent E. Rasamison made the extract of *Malleastrum* sp. Ana Lisa Valenciano and Maria L. Fernández-Murga carried out the antiplasmodial bioassays under the direction of Maria B. Cassera, and Wendy L. Applequist and James S. Miller provided additional details of the identity of the *Malleastrum* sp.

Introduction

Malaria is a very serious disease, causing 429,000 deaths globally in 2015.[1] The first effective class of antimalarial drugs was quinine and its analogs, with chloroquine being a low cost and widely available drug. Regrettably in the 1950's the *Plasmodium* parasite developed resistance to chloroquine, and resistant parasites to chloroquine are now widespread.[2] The best antimalarial drug class today is that of artemisinin and its analogs,[3, 4] used in combination with other antimalarials; this is known as artemisinin-based combination therapy (ACT).[5] Unfortunately the malaria parasite *P. falciparum* has developed significant resistance to artemisinin in Southeast Asia,[6] so there is a continuing need to discover new and effective antimalarial drugs. This paper reports the results of a study of a *Malleastrum sp.* (Meliaceae) for new antiplasmodial agents. The plant was collected as part of the Madagascar International Cooperative Biodiversity Group (ICBG) program; previous work on antiproliferative compounds from Madagascar ICBG collections of this genus resulted in the discovery of new limonoids and diterpenes.[7, 8]

Results and Discussion

Isolation and Structure Elucidation

An extract of the wood of a tree of the *Malleastrum* genus (Meliaceae) was found to have moderate antiplasmodial activity against the Dd2 drug-resistant strain of *P. falciparum*, and was selected for isolation studies. Liquid-liquid partition between aqueous MeOH and hexanes and then dichloromethane (DCM) indicated that all fractions had some antiplasmodial activity, with IC_{50} values of about 7.0, 1.3 and 7.0 μg/mL for the hexanes, DCM, and aqueous MeOH fractions, respectively. The major aqueous MeOH fraction was separated by chromatography on a Diaion open column, C-18 SPE, and C-18 reverse phase HPLC to yield the new long-chain butanolide malleastrumolide A (Fig. 1).

Malleastrumolide A was isolated as a yellow powder. Its positive ion HRESIMS revealed a peak for a protonated molecular ion at *m/z* 339.2897 and for a sodiated molecular ion at *m/z* 361.2714, both corresponding to a molecular formula of $C_{21}H_{38}O_3$. Its IR spectrum exhibited bands at 3398 and 1761 cm^{-1}, indicating the presence of hydroxyl and ester carbonyl groups. The presence of a hydroxylated lactone was suggested by ^{1}H NMR signals at δ_H 2.55 (*td*, ^3J(H,H) = 5.7, 8.1, H-C (2)), 3.84 (*dd*, ^3J(H,H) = 8.1, 6.4, H-C (3)), and 4.20 (*dq*, ^3J(H,H) = 6.4, 6.4, H-C (4)) and by ^{13}C NMR signals at δ_C 175.8 (C-1), 48.6 (C-2), 79.1 (C-3) and 79.8 (C-4). The hydroxylactone partial structure was confirmed by ^2J-HMBC correlations between H-2 and C-3, H-3 and C-4, and ^3J-HMBC correlations between H-4 and C-1, H-4 and C-2 (Table 1 and Fig. 2).

The doublet methyl group at 1.46 ppm was connected to C-4 as indicated by ^2J-HMBC correlations between H-5 and C-4, and ^3J-HMBC correlations between H-5 and C-3 (Fig. 2). The presence of an unsaturated C_{16} chain, with a terminal methyl group at δ_H 0.88 (*t* 6.9, H-16') and δ_C 14.1 (C-16'), was inferred from the ^{1}H NMR and ^{13}C NMR spectra. The vinyl protons appeared at δ_H 5.34 (*m*, 2H) and δ_C 129.9 and 130.0. The CH_2 groups of the side chain resonated at δ_H 2.01 (*m*, 4 H), 1.86 (*m*, 1 H), 1.60 (*m*, 1 H), 1.48 (*m*, 1 H), 1.27–1.34 (*m*, 19 H) and at δ_C 22.6, 26.8–31.9 (13 C). The long side chain was attached to C-2, based

on the ^{2}J-HMBC correlations between H-1' and C-2, and the ^{3}J-HMBC correlations between H-1' and C-1, H-1' and C-3, and H-2' and C-2 (Figure 1).

The position of the double bond was determined by the cross-metathesis method.[9] Malleastrumolide A was reacted with methyl acrylate and second-generation Hoveyda-Grubbs catalyst, and the crude product was analyzed by positive ion HRESIMS. The major peak at *m/z* 307 corresponded to $C_{15}H_{24}O_5Na^+$, and peaks at *m/z* 285 and 591 corresponded to $C_{15}H_{24}O_5+H^+$ and to $(C_{15}H_{24}O_5)_2Na^+$, respectively. This analysis confirmed the position of the double bond as between C-7' and C-8' (Fig. 3).

The relative configuration of the double bond was assigned as *Z* based on the ^{13}C NMR signals at δ_C 27.2 and 27.2 (C-6' and C-9'), since carbons adjacent to a *E* double bond in an alkenyl chain normally resonate at 27–28 ppm,[10] while carbons adjacent to a *Z* double bond normally resonate at 32–34 ppm.[11] The relative configuration of the lactone was assigned as 2*R**,3*R**,4*S** by the ^{13}C NMR signals at δ_C 79.1 (C-3) and 79.8 (C-4),[12] and by the NOESY crosspeaks between H-1' and H-3, H-2 and H-4, and H-3 and H-5, as shown in Fig. 4.

The absolute configuration of malleastrumolide A was assigned by its ECD spectrum (Fig. 5). The $n \rightarrow \pi^*$ transition band at 216 nm showed a negative Cotton effect, defining the absolute configuration of C-2 as *R*.[13, 14] The structure and absolute stereochemistry of malleastrumolide A were thus assigned as (2*R*,3*R*,4*S*)-2-((*Z*)-hexadec-7-en-1-yl)-3-hydroxy-4-methyl-butanolide.[10]

Bioassays

Malleastrumolide A was tested for antiplasmodial activity against the Dd2 strain of *P. falciparum* and for antiproliferative activity to A2780 ovarian cancer cells. It displayed moderate antiplasmodial activity (Table 2), with an IC_{50} value of 2.74 μM, and weak antiproliferative activity, with an IC_{50} value of 17.44 μM.

Experimental Section

General Experimental Procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter. UV and IR spectroscopic data were measured on a Shimadzu UV-1201 spectrophotometer and a MIDAC M-series FTIR spectrophotometer, respectively. CD spectra were obtained on a JASCO J-815 circular dichroism spectrometer. NMR spectra were recorded in $CDCl_3$ on Bruker Avance 500 or 600 spectrometers. The chemical shifts are given in δ (ppm), and coupling constants (*J*) are reported in Hz. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS in the positive ion mode.

Antiplasmodial Bioassays

The effect of each fraction and pure compound on parasite growth of the *P. falciparum* Dd2 strain was measured in a 72 h growth assay in the presence of drug as described previously with minor modifications. Briefly, ring stage parasite cultures (100 μL per well, with 1% hematocrit and 1% parasitemia) were then grown for 72 h in the presence of increasing

concentrations of the drug in a 5% $CO_2$5% O_2, and 90% N_2 gas mixture at 37 °C. After 72 h in culture, parasite viability was determined by DNA quantitation using SYBR Green I.[15] The half-maximum inhibitory concentration (IC_{50}) calculation was performed with GraphPad Prism software using nonlinear regression curve fitting. IC_{50} values are the average of three independent determinations with each determination in duplicate and are expressed ± SD and artemisinin was used as positive control.[16]

In vitro antiproliferative activity against A2780 cells

The A2780 ovarian cancer cell line antiproliferative bioassay was performed at Virginia Tech as previously reported.[17, 18] The A2780 cell line is a drug-sensitive ovarian cancer cell line.[19] Paclitaxel was used as the positive control.

Plant Material

Plant specimens of *Malleastrum* (Baill.) J.-F. Leroy were collected on July 16, 2000 in the Province of Toamasina in the Alaotra-Mangoro region in Zahamena National Park in secondary forest with *Lantana camara*, on a ridge at an elevation of 880 m at coordinates 17°28'28"S, 048°44'12"E. The plant was a tree 8 m tall with a diameter at breast height of 12 cm. It is unifoliolate, with the petiole winged at the apex; it has a few small flowers and is pubescent on the leaves and inflorescences, but not densely so on the twigs. Voucher specimens are deposited at the herbarium of the Missouri Botanical Garden Herbarium and were collected by L.M. Randrianjanaka, S. Rakotonandrasana, J. Randriamanarivo & L.P. Rakotosoa 564 (MO).

The genus *Malleastrum* (Baill.) J.-F. Leroy is endemic to Madagascar and comprises 20 currently accepted species. However, there appear to be at least 4 additional species new to science among recently collected specimens, and the plant material in this study is almost certainly from one of those species that is new to science and still waiting to be named and described.

Extraction and Isolation

Dried and powdered the wood of *Malleastrum sp.* (350 g) was exhaustively extracted with ethanol in two 24-hour percolation steps to yield 10.1 g of extract. For purposes of fractionation and purification, a portion of this extract (1.95 g) designated MG0433 was shipped to Virginia Tech for bioassay and chemical studies. A total of 1.3 g of this sample (IC_{50} 2.5~5 μg/mL) was suspended in aqueous MeOH (MeOH-H_2O 9:1, 100 mL), and extracted with hexane (3 × 100 mL portions). The aqueous MeOH layer was diluted to 60% MeOH (v/v) with H_2O and extracted with DCM (3 × 100 mL portions). The hexane fraction was evaporated in vacuo to leave 180 mg of material with IC_{50} 7.0 μg/mL. The residue from the DCM fraction (20.7 mg) was the most active fraction with an IC_{50} value of l.3 μg/mL. The remaining aqueous MeOH fraction was centrifuged to give a supernatant (800 mg) with an IC_{50} of 7.0 μg/mL.

The aqueous MeOH fraction was directly applied on Diaion open column to yield 40% aqueous MeOH fraction (280 mg) with no activity, 70% aqueous MeOH fraction (188 mg) with no activity, 100% MeOH fraction (115 mg) with an IC_{50} of ~10 μg/mL and 100%

acetone fraction (47 mg) with an IC_{50} of < 1.25 μg/mL. The DCM fraction and acetone fraction were combined due to the similarity of their TLC patterns. The combined fraction was directly applied on C_{18} HPLC, and eluted by 60% to 100% acetonitrile in water gradient in 30 min to yield malleastrumolide A (**1**) (7.0 mg), with retention time of 41.7 minutes.

Malleastrumolide A (1)—yellow powder; $[\alpha]_D^{21}$ −11.3 (c 4×10^{-4} g/mL, MeOH); UV (c 0.296 mM, MeOH) λ_{max} (ε) 203 nm (1464); IR υ_{max} 3398, 2927, 2858,1781, 1761, 1665, 1461 cm^{-1}; ECD (c 0.296 mM, MeOH) $\Delta\varepsilon_{216}$ −13.4; HRESIMS m/z 361.2714 $[M+Na]^+$ (calcd. for $C_{21}H_{38}NaO_3^+$, 361.2713) and 339.2897 $[M+H]^+$ (calcd. for $C_{21}H_{39}O_3^+$, 339.2894); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see Table 1.

Determination of Double-Bond Position

Butanolide **1** (0.5 mg) was dissolved in a 10:1 mixture of $CHCl_3$ and methyl acrylate (0.5 mL), and 50 μg of second generation Hoveyda–Grubbs catalyst was added. The mixture was stirred at room temperature for 3 h, and a 10 μL aliquot was injected into a positive ion HRESIMS (Fig. 3)[9]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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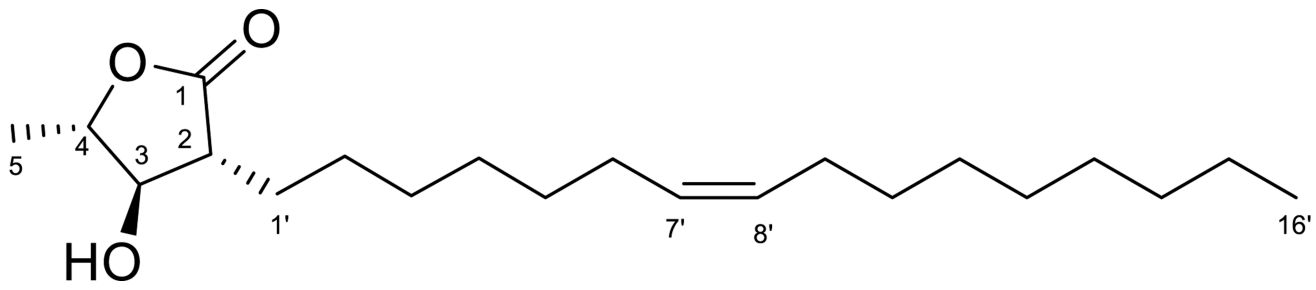


Figure 1.
Chemical structure of malleastrumolide A.

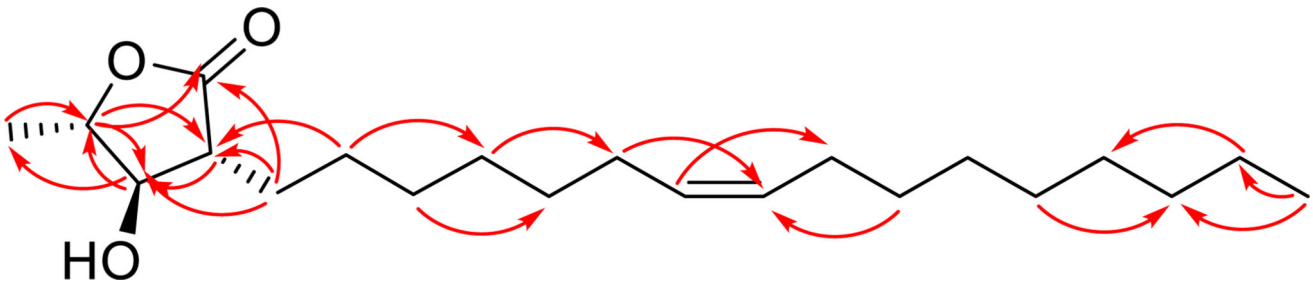
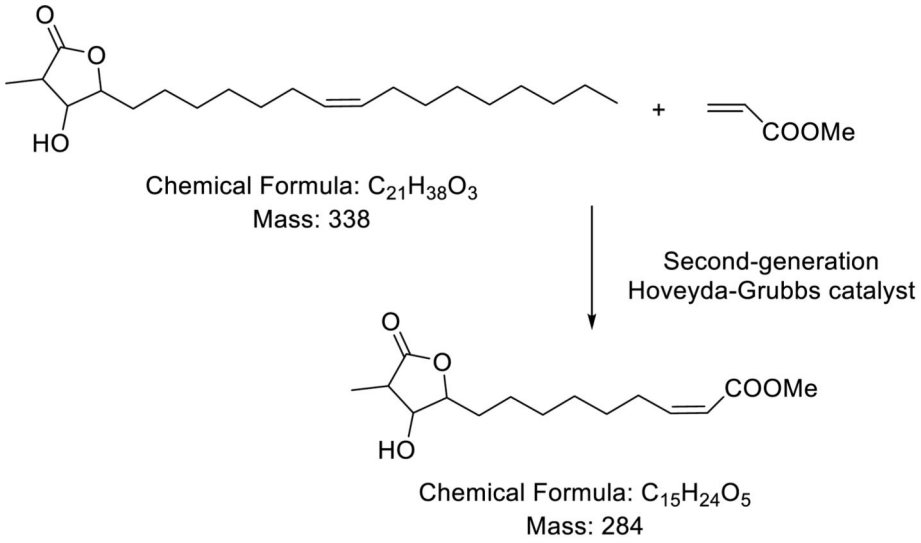


Figure 2.
HMBC correlations of malleastrumolide A



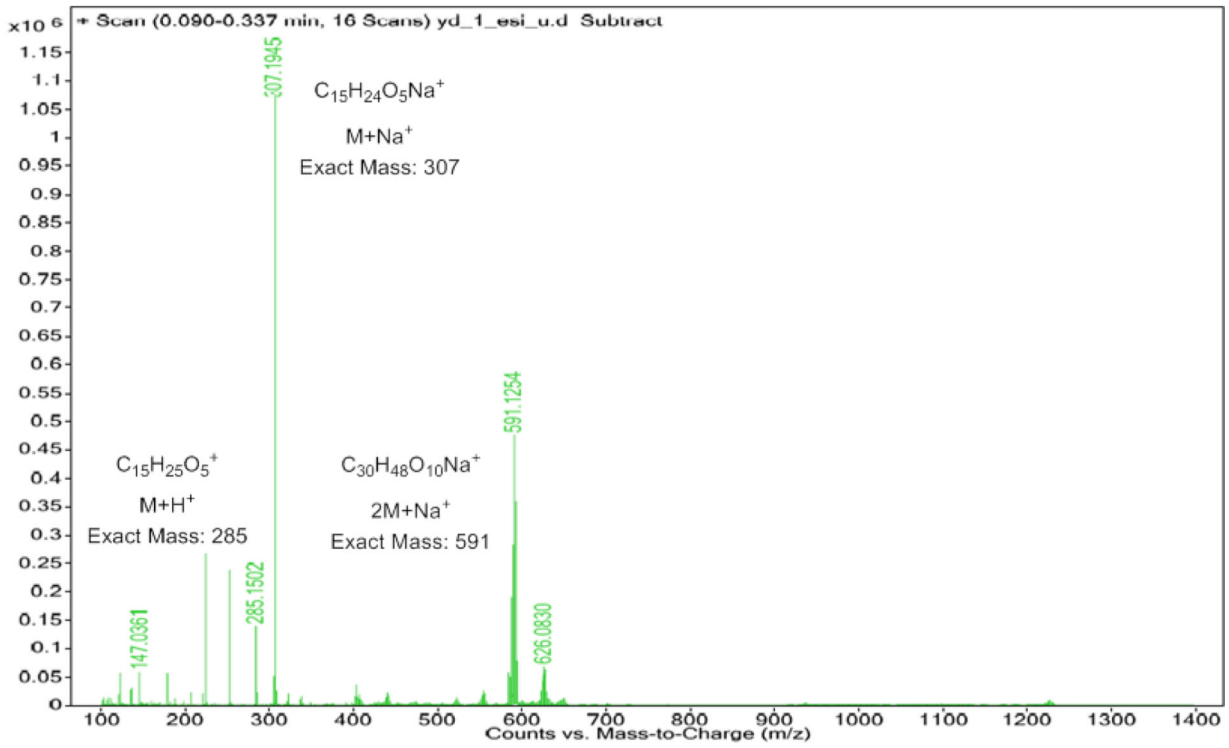


Figure 3.

Cross-metathesis reaction of **1** and the mass spectrum of the crude product

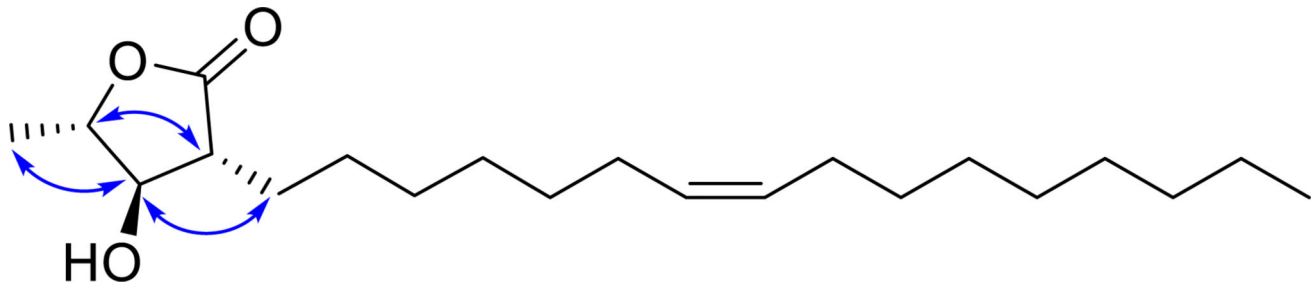


Figure 4.
NOESY correlations for malleastrumolide A

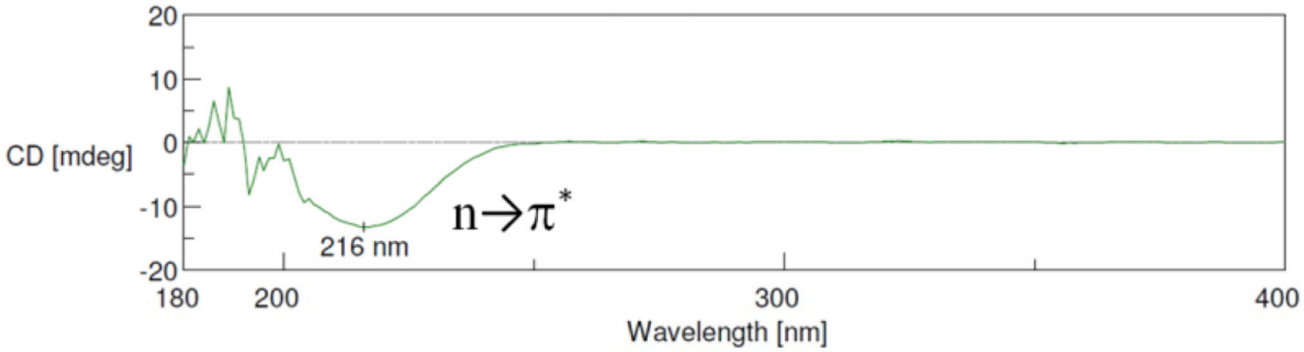


Figure 5.
The ECD spectra of malleastrumolide A

Table 1

^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) data for compound 1 in $CDCl_3$

Position	δ_H (*J* in Hz)	δ_C, type
1		175.8, C
2	2.55 td, 8.1, 5.7	48.6, CH
3	3.84 dd, 8.1, 6.4	79.1, CH
4	4.20 dq, 6.4, 6.4	79.8, CH
5	1.46 d, 6.4	18.2, CH_3
1'	1.82 – 1.91, m	28.4, CH_2
	1.55 – 1.64 m	
2'	1.47 – 1.54 m	26.8, CH_2
	1.29 – 1.35 m	
3'	1.24 – 1.29, m	29.4, CH_2
4'	1.24 – 1.29, m	29.4, CH_2
5'	1.31 – 1.35, m	29.5, CH_2
6'	1.98 – 2.05, m	27.2, CH_2
7'	5.30 – 5.39, m	129.9, CH
8'	5.30 – 5.39, m	130.0, CH
9'	1.98 – 2.05, m	27.2, CH_2
10'	1.31 – 1.35, m	29.5, CH_2
11'	1.31 – 1.35, m	29.5, CH_2
12'	1.24 – 1.29, m	29.4, CH_2
13'	1.24 – 1.29, m	29.4, CH_2
14'	1.24 – 1.29, m	31.9, CH_2
15'	1.24 – 1.29, m	22.6, CH_2
16'	0.88 t, 6.9	14.1, CH_3

Table 2

Bioactivities of malleastrumolide A

	Malleastrumolide A	Artemisinin	Paclitaxel
P. falciparum Dd2 (μM)	2.74 ± 0.27	0.007 ± 0.002	NT
A2780 (μM)	17.4 ± 2.1	NT	0.028 ± 0.002