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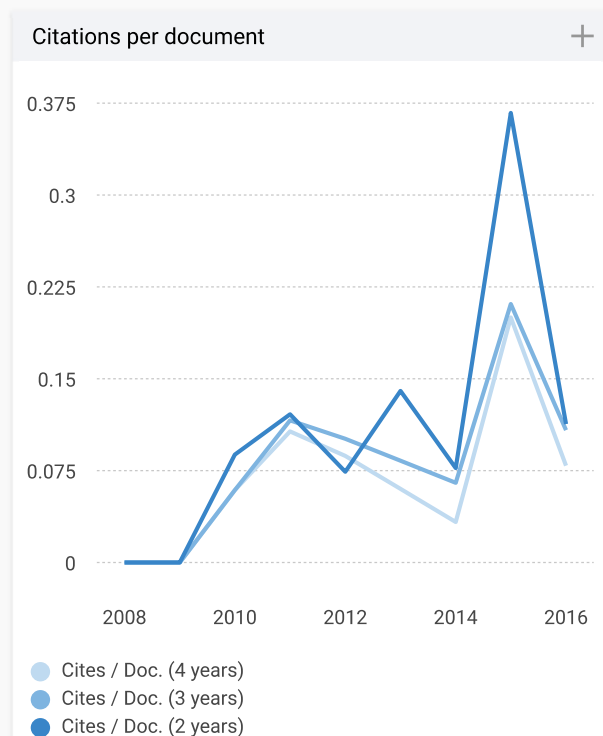
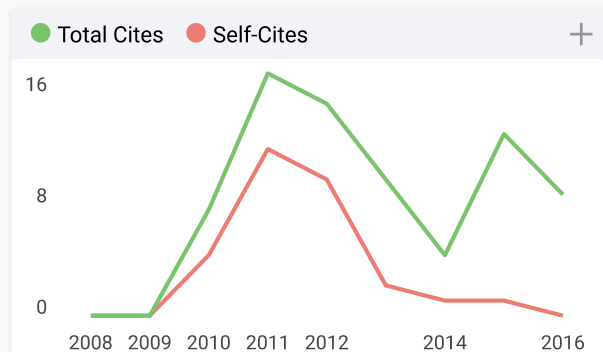
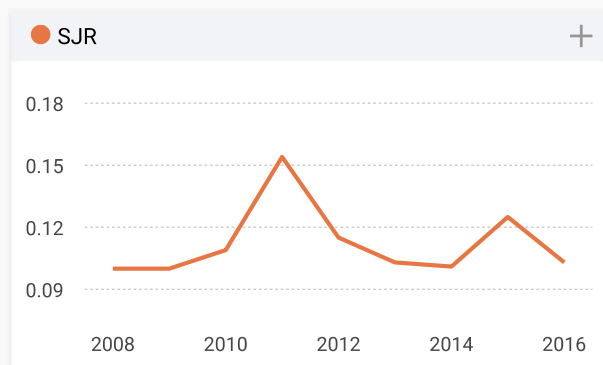
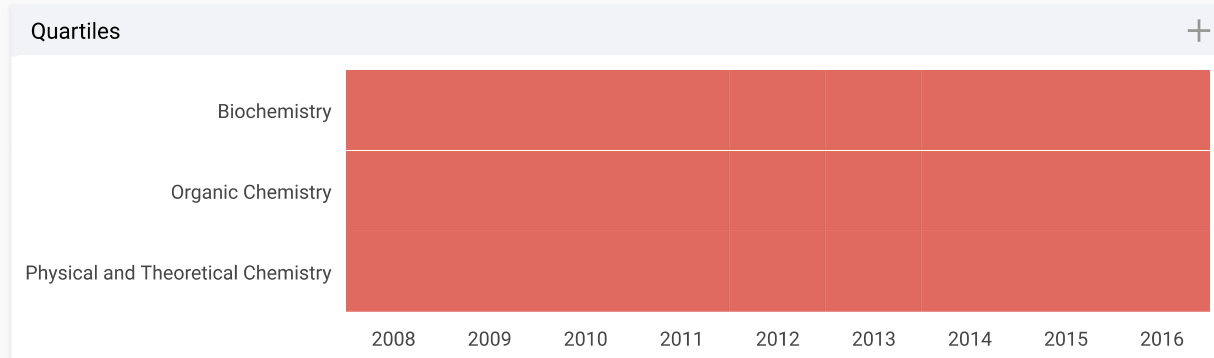
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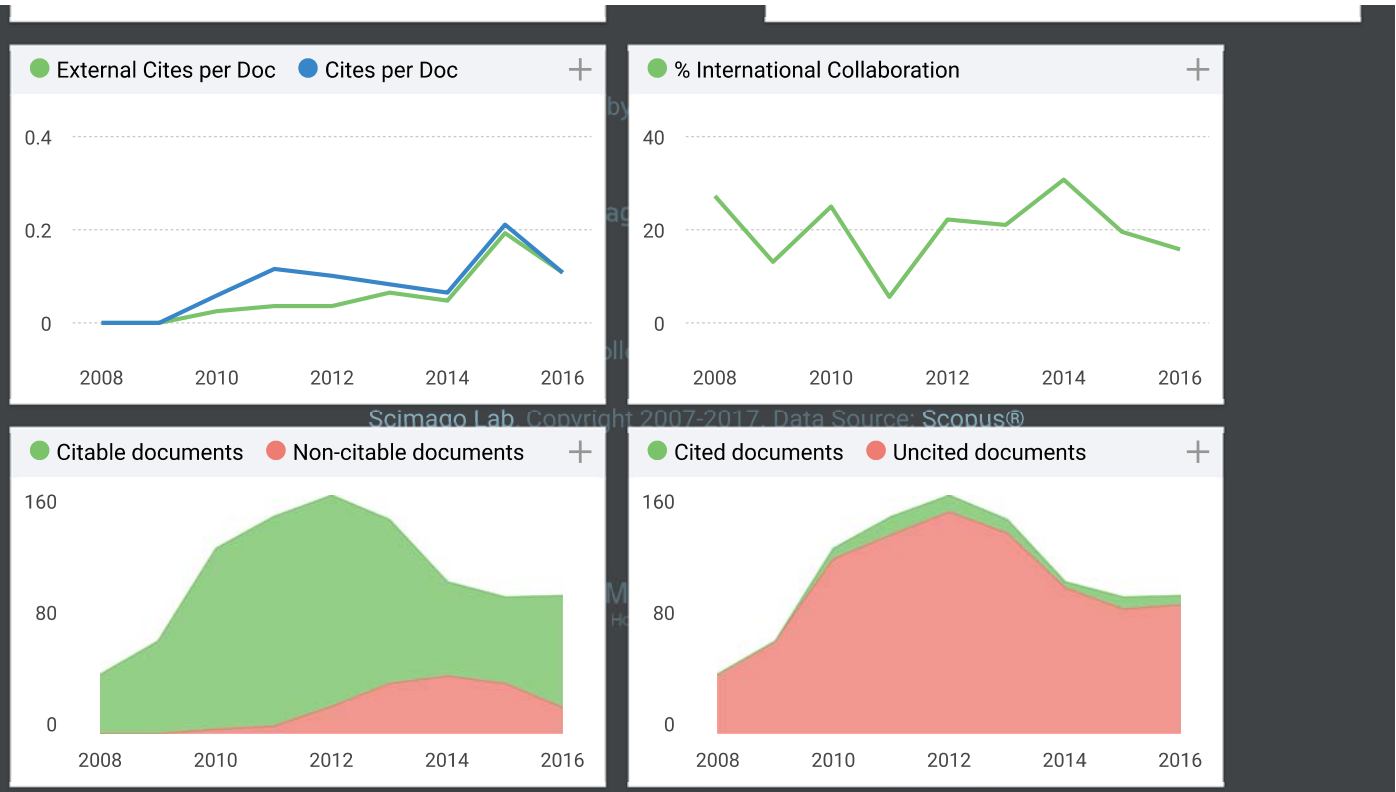
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3

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[Website \(http://nphs.kmu.edu.tw/index.php/en-GB/faculty\)](http://nphs.kmu.edu.tw/index.php/en-GB/faculty) | [E-Mail \(\)](#)

Interests: natural products chemistry; medicinal chemistry; transgenic plant (arabidopsis) reporter assay; epigenetic modulation for microbial secondary metabolites; functional food; ethnopharmacology

Prof. Dr. Ping-Jyun Sung

1. National Museum of Marine Biology and Aquarium, Pingtung 944, Taiwan
2. Graduate Institute of Marine Biology, National Dong Hwa University, Pingtung 944, Taiwan
3. Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan
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Oceanography Section, Science Research Center, Kochi University, 200 Otsu, Monobe, Nankoku, Kochi 783-8502, Japan

[Website \(http://www.cc.kochi-u.ac.jp/~htanaka/\)](http://www.cc.kochi-u.ac.jp/~htanaka/) | [E-Mail \(\)](#)

Interests: Organic chemistry; Carbohydrate chemistry; Natural products

Dr. Bernd Schneider

Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, Beutenberg Campus, 07745 Jena, Germany

[Website \(www.ice.mpg.de\)](http://www.ice.mpg.de) | [E-Mail \(\)](#)

Interests: natural products chemistry; chemical ecology; plant natural products; NMR of small molecules

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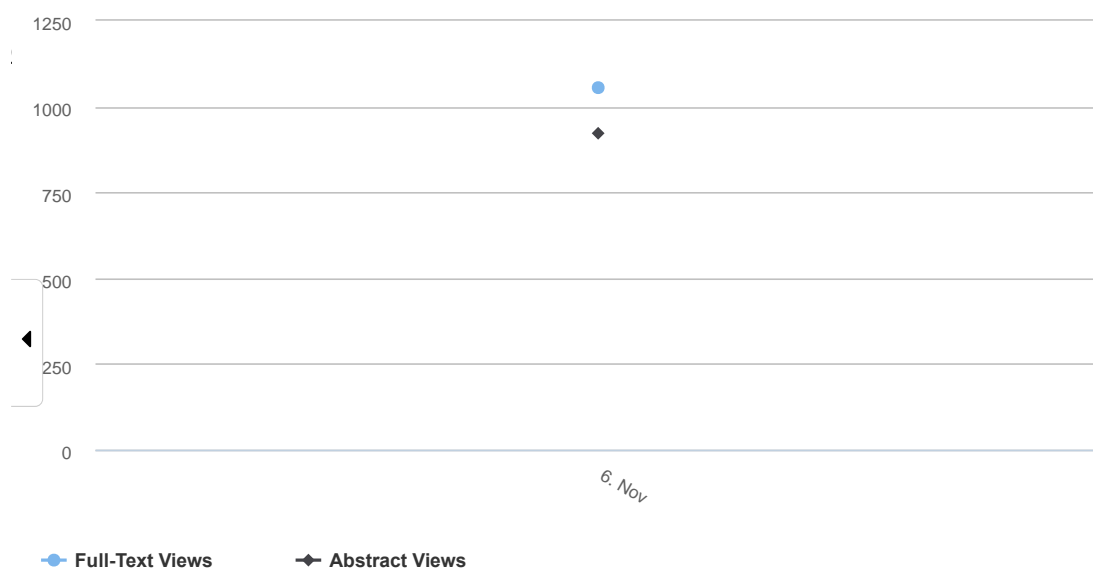
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Supplementary File 2:

INCHI-Document (/1422-8599/2017/2/M939/s2) (INCHI, 710 B)

Supplementary File 3:

MOL-Document (/1422-8599/2017/2/M939/s3) (MOL, 2 KB) [Display in MOL Viewer \(/1422-8599/2017/2/M939/s3\)](#)

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Short Note

4-Methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one from *Melicope Moluccana* T.G. Hartley

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Abstract: 4-Methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one (**1**) was isolated from the leaves of *Melicope moluccana* T.G. Hartley. The chemical structure of **1** was elucidated using mainly UV, IR, HRESIMS, 1D and 2D-NMR spectroscopy.

Keywords: 4-Methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one; 2-quinolone alkaloid; *Melicope moluccana*

1. Introduction

Melicope is one of the genus of the family Rutaceae, consisting of about 320 species growing in the world [1]. Phytochemical studies have shown that the species produce a variety of alkaloids [2,3], flavonoids [4,5], coumarins [6], acetophenones [7], and lignans [8], which exhibit various biological activities, including antioxidant [8,9], anticancer [10,11], and antiinflammatory [12]. This study is part of our research on the chemical constituents of *Melicope* species found in Indonesia. In continuation of our research for alkaloid compounds in this medicinal plant, we report the isolation of 4-methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one (**1**) from the methanol extract of the leaves of *Melicope moluccana* T.G. Hartley. The chemical structure of compound **1** were established by UV, IR, HRESIMS, 1D and 2D-NMR, and by comparison with those related compounds previously reported. Cytotoxic and antiplasmodial activities of isolated compound from this species are also briefly described.

2. Result and Discussion

The dried and powdered leaves of *M. moluccana* (2.0 kg) were extracted with methanol. The residue was partitioned with *n*-hexane. The methanol extract was then adjusted to pH 3–4 with 3% sulfuric acid and partitioned with ethyl acetate to separate the non-alkaloid compound. Acid extracts were basified with ammonia solution (pH 8–9) and partitioned with ethyl acetate to give the crude alkaloid.

The crude alkaloid (5 g) was fractionated by column chromatography on silica gel eluted with mixtures of *n*-hexane-ethyl acetate (9:1 to 1:1) to give four major fractions A–D. Fraction A (325 mg) was further separated by radial chromatography to yield two sub-fractions (A₁, A₂). Sub-fraction A₁ was purified using radial chromatography eluted with *n* hexane-chloroform (from 1:1 to 3:7) to give compound **1** (Figure 1).

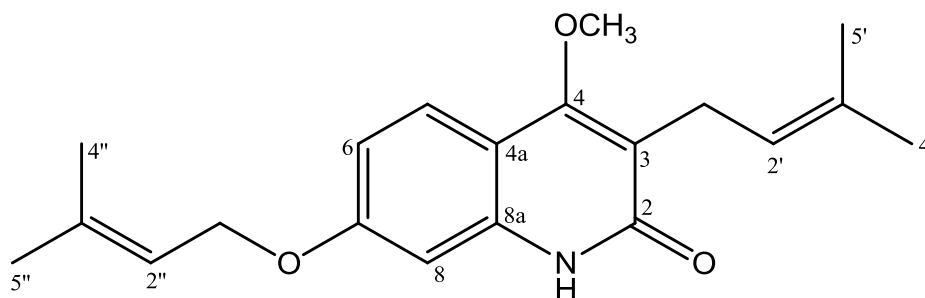


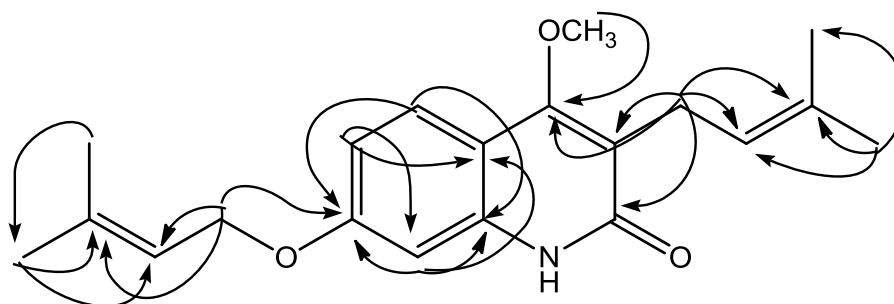
Figure 1. Structures of 4-methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one.

4-Methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one (**1**) was isolated as yellow solid, reacted positively with Dragendorff reagent to give reddish brown. The HRESIMS displayed a positive molecular ion peak at m/z 328.1928, indicating a molecular formula of $C_{20}H_{26}NO_3$ (see Figure S6, supporting material). The UV spectrum exhibited absorption maxima λ_{max} (nm) ($\log \epsilon$): 227 (4.57), 269 (3.86), 282 (3.85), 322 (4.09), and 336 (3.98) typical for a 2-quinolone skeleton [11]. The IR spectrum (see Figure S7, supporting information) indicated absorptions for hydroxyl (3421 cm^{-1}), conjugated carbonyl (1645 cm^{-1}), aromatic ($1562, 1512, 1461\text{ cm}^{-1}$) and ether (1093 cm^{-1}) groups [13]. The ^1H NMR spectrum of **1** (Table 1, Figures S1 and S2, supporting material) showed the presence of ABX coupling aromatic systems at δ_{H} 7.65 (d, $J = 8.8\text{ Hz}$; H-5), 7.65 (dd, $J = 8.8; 2.4\text{ Hz}$; H-6), and 7.05 (d, $J = 2.4\text{ Hz}$; H-8) characteristic for 2-quinolone with three substituents. Furthermore, the ^1H NMR spectrum also showed an isoprenyl (3-methyl-2-butenyl) group [δ_{H} 1.83 (3H, s, H-4'), 1.79 (3H, s, H-5'), 3.39 (2H, d, $J = 6.8\text{ Hz}$, H-1'), 5.29 (1H, t, $J = 6.8\text{ Hz}$, H-2')], an oxyisoprenyl (3-methylbut-2-en-1-yl)oxy group [δ_{H} 1.83 (3H, s, H-4''), 1.70 (3H, s, H-5''), 4.59 (2H, d, $J = 6.8\text{ Hz}$, H-1''), 5.52 (1H, t, $J = 6.8\text{ Hz}$, H-2'')], and a singlet proton of methoxy group at δ_{H} 3.93. The ^{13}C -NMR spectrum (Table 1) of **1** showed 20 carbon signals. The assignment of ^{13}C -NMR spectrum was confirmed by HMQC and HMBC spectra. The placement of isoprenyl, oxyisoprenyl, and methoxy groups in 2-quinolone skeleton was established by HMQC and HMBC spectra. Long-range correlation was observed in the HMBC spectrum of **1** between the proton signal of a methoxy group at δ_{H} 3.93 with an oxyaryl atom (δ_{C} 162.5). The methylene proton signal of an isoprenyl group at δ_{H} 3.39 (H-1') showed long-range correlations with a carbonyl carbon (δ_{C} 166.0; C-2), a oxyaryl carbon (δ_{C} 162.5), two quaternary carbons (δ_{C} 132.2, 119.3), and a methine carbon signal (δ_{C} 121.8), showing a methoxy group attached at C-4. The proton signal of an aromatic region at δ_{H} 7.65 (H-5) showed long-range correlations with an oxyaryl carbon signals (δ_{C} 160.6) and a quaternary carbon (δ_{C} 139.1). The result revealed δ_{C} 160.6 has position at C-7 and δ_{C} 139.1 at C-8a. Furthermore, the methylene proton signal at 4.59 (H-1'') showed long-range correlations with an oxyaryl carbon (δ_{C} 160.6), a quaternary carbon (δ_{C} 139.0), and a methine carbon signal (δ_{C} 118.9), showing that the oxyisoprenyl group attached at C-7. See also Figure S3-S5, supporting information).

On the basis of above spectral evidence, the structure of **1** was elucidated as 4-methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one. Other HMBC correlations consistent with structure **1** are shown in Table 1 and Figure 2. The structure of **1** has not been previously reported, and it is a novel compound. On cytotoxic evaluation against P-388 cells, compound **1** exhibited IC_{50} values of $0.63\text{ }\mu\text{g/mL}$. That cytotoxic data suggested that compound **1** has high activity. The antiplasmodial activity of **1** showed IC_{50} values of $4.28\text{ }\mu\text{g/mL}$, which categorized as moderate activity. The data on cytotoxic and antiplasmodial activity are presented in Table 2.

Table 1. NMR spectroscopic data of 4-methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one in CDCl₃.

No. C	δ_H (mult, J Hz)	δ_C	HMBC
2	-	166.0	-
3	-	119.3	-
4	-	162.5	-
4a	-	111.0	-
5	7.65 (d, 8.8)	124.2	C-7, C-8a
6	6.83 (dd, 8.8; 2.4)	112.4	C-4a, C-8
7	-	160.6	-
8	6.79 (d, 2.4)	98.8	C-4a, C-7, C-8a
8a	-	139.1	-
1'	3.39 (d, 6.8)	23.2	C-2, C-3, C-4, C-2', C-3'
2'	5.29 (t, 6.8)	121.8	C-4', C-5'
3'	-	132.2	-
4'	1.83 (s)	18.0	C-2', C-3', C-5'
5'	1.79 (s)	25.7	C-2', C-3', C-4'
1''	4.59 (d, 6.8)	65.1	C-7, C-2'', C-3''
2''	5.52 (t, 6.8)	118.9	C-4'', C-5''
3''	-	139.0	-
4''	1.83 (s)	18.3	C-2'', C-3'', C-5''
5''	1.70 (s)	25.9	C-2'', C-3'', C-4''
4-OCH ₃	3.93 (s)	61.8	C-4
NH	11.29 (s)	-	-

**Figure 2.** Selected HMBC correlations for 1.**Table 2.** Cytotoxic analysis against P-388 cells and antiplasmodial activity of compound 1.

Compound	(IC ₅₀ , $\mu\text{g/mL}$)	
	Cytotoxic Activity (P-388 Cells)	Antiplasmodial
4-methoxy-3-(3-methylbut-2-en-1-yl)-7-[(3-methylbut-2-en-1-yl)oxy]quinolin-2(1H)-one (1)	0.63	4.28
Artonin E	1.33	
Chloroquine		1.03

3. Experimental Section

3.1. General

The UV and IR spectra were measured with a Shimadzu 1800 spectrophotometer (Kyoto, Japan) and Perkin Elmer Spectrum One FTIR spectrophotometer (Waltham, MA, USA). NMR spectra were recorded on a JEOL ECA 400 spectrometer (Tokyo, Japan) in CDCl₃ at 400 (¹H) and 100 (¹³C) MHz using TMS as the internal standard. The mass spectra were recorded using a Waters LCT Premier XE

(Santa Clara, CA, USA). Column chromatography and radial chromatography were carried out using Si gel 60 G 1.07734.1000 and Si gel 60 PF₂₅₄ 1.07749.1000 (Merck, Darmstadt, Germany).

3.2. Plant Material

The leaves of *M. moluccana* T.G. Hartley were collected in July 2015 from the conserved forest of Weda, Central Halmahera, North Maluku, Indonesia. The plant was identified at the Herbarium Bogoriense, and a voucher specimen number 46889 was deposited in the Bogor Botanical Garden, Bogor, Indonesia.

3.3. Extraction and Isolation

The dried and powdered leaves of *M. moluccana* T.G. Hartley (2.0 kg) were macerated in methanol at room temperature for 24 h two times, and the methanol extract was evaporated under reduced pressure to give methanol extract with dark brown residue (108 g). The methanol extract was partitioned with *n*-hexane (1:1 *v/v*) and then evaporated by rotavapor to give *n*-hexane extract (15 g). To the methanol extract was then added 3% sulfuric acid and adjusted to pH 3–4. Furthermore, the acid extracts were partitioned with ethyl acetate and then evaporated by rotavapor, yielding non-alkaloid (phenolic compound) extract (8 g). The acid extracts were basified with ammonia solution and adjusted to pH 8–9 and then partitioned with ethyl acetate to give crude alkaloid. The crude alkaloid (5 g) of *M. moluccana* was separated by column chromatography on silica gel. Elution with *n*-hexane-ethyl acetate mixture by increasing amount of ethyl acetate (9:1 to 1:1) to give four major fractions A–D. On thin layer chromatographic (TLC) analysis, fraction A (325 mg) showed two major spots. Separation of fraction A using planar radial chromatography and eluted with *n*-hexane-acetone mixture (from 9:1 to 7:3) to give two subfractions (A₁, A₂). Purification of subfraction A₁ by planar radial chromatography with eluent *n*-hexane-chloroform (from 1:1 to 3:7) yielded compound **1** (14 mg).

3.4. Cytotoxic Assay

Preliminary cytotoxic evaluation of compound **1** was carried out against murine leukemia P-388 cells according to the MTT assay, as previously described [14,15].

3.5. Antiplasmodial Analysis

The antiplasmodial activity of compound **1** was expressed by the 50% inhibitory concentrations (IC₅₀), representing the concentration of chloroquine that induced a 50% parasitaemia decrease compared to the positive control culture referred as 100% parasitaemia [16,17].

Supplementary Materials: ¹H-NMR, ¹³C-NMR, HMQC, HMBC, and HRESIMS spectra are reported in the supplementary materials as Figure S1–S7 and structure refinement parameters as Table S1.

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Author Contributions: M.T. and T.S.T. designed the whole experiment. R.D.S. and R.A.W. executed the experiment. All authors interpreted data and prepare the manuscript in the same contribution.

Conflicts of Interest: The authors declare no conflict of interest.

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