SHORT REPORT

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A Novel Limonoid from the Seeds of *Chisocheton macrophyllus*

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Abstract: This study aimed to isolate a novel limonoid compound, (6R,7S, 8R, 9R, 10R, 11R, 13S, 17R)-17-(furan-3-yl) -4, 4, 8, 10, 13-pentamethyl-3-oxo-4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 17-dodecahydro-3H-cyclopenta[a]phenanthrene-6,7,11-triyltriacetate (11α-acetoxydysobinin) (**1**), and 3 pre-existing limonoids, namely dysobinin (**2**), dysobinol (**3**), and 7-deacetylepoxyazadiradione (**4**) from the seeds of *Chisocheton macrophyllus* (Meliaceae). In addition, the structure of the isolated compounds was determined using various spectroscopic techniques, including UV, IR, HRTOFMS, 1D, and 2D NMR. The cytotoxic effects of each compound were then evaluated against breast cancer cells of the Michigan Cancer Foundation-7 (MCF-7), but no significant activity was observed.

Keywords: *Chisocheton macrophyllus;* Meliaceae; limonoid; cytotoxic activity; MCF-7.

1. Plant Source

Chisocheton is a genus in the family Meliaceae, consisting of 53 species, which are widely distributed in Thailand, India, Malaysia, and Indonesia [1]. This current study aimed to reports the isolation and structural analysis of a novel limonoid (**1**) and 3 pre-existing limonoids (**2-4**) from *C. macrophyllus* seeds. The cytotoxic effects of the isolated compounds on MCF-7 breast cancer cells were also examined.

2. Previous Studies

The seeds of *Chisocheton macrophyllus* have been shown to contain bioactive limonoids, such as dysobinin, dysobinol, 7α-hydroxyneotricilenone, nimonol and other limonoids [2-6].

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3. Present Study

In this study, *Chisocheton macrophyllus* (Miq.) seeds were gathered in June 2022 from the Bogor Botanical Garden, Bogor, Indonesia (-6.601812, 106.799269). The identification of the plant was confirmed by Bogoriense Herbarium staff member, Mr Harto, and a voucher specimen (No. Bo-1295452) was subsequently lodged in the herbarium.

A total of 3.5 kg of *C. macrophyllus* dried seeds were macerated for 6 days in methanol. The seeds were then subjected to extraction, leading to the production of 414 g of crude extract by evaporating the solvent. Subsequently, the crude extract obtained was fractionated with *n*-hexane, ethyl acetate, and *n*-butanol to produce 197g, 41.7g, and 15g of concentrated extract, respectively. The *n*-hexane extract was subjected to vacuum liquid chromatography on silica gel G60, and the eluent system was a gradient of 10% stepwise *n*-hexane-ethyl acetate-methanol. The resulting process produced 9 fractions (A-I), with fraction D (50 mg) being recrystallized using chloroform-methanol to yield fraction **2** (30 mg). Fraction E (2.04 g) underwent chromatography on silica gel CC (70-230 mesh) with elution using a 2.5% gradient of ethyl acetate in *n*-hexane. This process yielded 7 subfractions (E1-E12), and E9 (289.4 mg) underwent chromatography on a silica gel column, followed by recrystallization with chloroform, leading to the production of **1** (14 mg). In addition, fractions E2 to E5 were pooled (142.5 mg) and purified on a silica gel column to produce **3** (16.1 mg). Fractions E9-E12 (96 mg) were combined and subjected to chromatography on a silica gel column, leading to the isolation of **4** (18 mg).

(6R, 7S, 8R, 9R, 10R, 11R, 13S, 17R)-17-(furan-3-yl)-4, 4, 8, 10, 13-pentamethyl-3-oxo-4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 17-dodecahydro-3H-cyclopenta[a]phenanthrene-6, 7, 11-triyl triacetate (11 α-acetoxydysobinin) ***(1)***: Colorless crystals; mp: 203–205°C; $[\alpha]_D^{26.0}$= -1.4° (c 0.05, $CHCl_3$); UV (CH_3OH): λ_{max} (log ε): 212 (1.42); IR (KBr) ν_{max} 2979, 1737, 1666, 1502, 1366, 1377, 1244 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H (ppm) = 0.87 (3H, s, H-18), 1.17 (3H, s, H-28), 1.23 (3H, s, H-19), 1.24 (3H, s, H-29), 1.34 (3H, s, H-30), 2.01 (3H, s, H-1′′), 2.03 (3H, s, H-1′), 2.06 (3H, s, H-1′′′), 2.47 (1H, m, H-16), 2.56 (1H, d, *J* = 12.4 Hz, H-5), 2.57 (1H, m, H-9), 2.63 (2H, d, *J* = 12.4 Hz, H-12), 2.81 (1H, dd, *J* = 11.0, 7.3 Hz, H-17), 5.40 (1H, d, *J*=7.7 Hz, H-15), 5.43 (1H, d, J= 2.5 Hz, H-7), 5.45 (1H; d, *J*=7.7 Hz), 5.46 (1H, m, H-6), 5.86 (1H, d, *J* = 10.3 Hz, H-2), 6.23 (1H, dd, *J*= 1.8, 0.9 Hz, H-22), 7.23 (1H, s, H-21), 7.36 (1H, d, *J*= 10.3 Hz, H-1), 7.43 (d, *J* = 10.3 Hz, H-23); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C (ppm) = 20.1 (CH_3-1′′′), 20.5 (CH_3-28), 20.9 (CH_3-18), 21.1 (CH_3-1′′), 21.4 (CH_3-1′), 22.0 (CH_3-29), 28.4 (CH_3-30), 31.7 (CH_3-19), 34.5 (CH_2, C-16), 41.5 (C, C-10), 42.0 (CH, C-9), 42.6 (CH_2, C-12), 42.8 (C, C-8), 45.3 (CH, C-5), 46.4 (C, C-13), 47.3 (C, C-4), 51.4 (CH, C-17), 69.5 (CH, C-6), 70.6 (CH, C-11), 73.9 (CH, C-7), 111.0 (CH, C-22), 120.0 (CH, C-15), 123.9 (C, C-20), 125.4 (CH_2, C-2), 139.9 (CH, C-21), 142.9 (CH, C-23), 157.1 (C, C-14), 158.3 (CH, C-1), 170.1 (C, C-2′), 170.1 (C, C-2′′) 170.3 (C, C-2′′′), 204.1 (C, C-3); HRTOFMS (positive ion mode): *m/z* 553.2816 $[M+H]^+$ (calcd. for $C_{32}H_{41}O_8$; 553.2801).

Compound **1** appeared as colorless crystals in chloroform, with a molecular formula of $C_{32}H_{40}O_8$, which was determined by HRTOFMS investigation of the positive ion peak at *m/z* 553.2801 $[M+H]^+$, necessitating 12 degrees of unsaturation. The IR spectrum revealed absorption spectra, suggesting the presence of aliphatic (2979 cm^{-1}), 2 carbonyl for ester and *α,β*-unsaturated ketone (1737 cm^{-1} and 1666 cm^{-1}), alkene (1502 cm^{-1}), and *gem*-dimethyl groups (1366 and 1377 cm^{-1}). The ^{1}H-NMR spectrum exhibited the presence of 8 tertiary methyls, with 5 in upfield chemical shift (δ_H 0.87, 1.17, 1.23, 1.24 and 1.34), and 3 in downfield chemical shift at (δ_H 2.01, 2.03, and 2.06, each 3H). In addition, the results showed the presence of oxymethine protons for H-6, H-7, and H-11 at δ_H 5.46 (1H, m), 5.43 (1H, d, *J*=2.6 Hz) and 5.45 (1H, m, *J*=7.7 Hz), respectively. Furan skeleton was also observed for H-21, H-22, and H-23 at δ_H 6.23 (1H, d, *J*=1.45 Hz), 7.23 (1H, s), and 7.36 (1H, d, *J*=1.45 Hz), respectively. A total of 4 olefinic methine group resonating at δ_H 5.40 (1H, d, *J*=7.7 Hz), 5.86 (1H, d, *J*=10.3 Hz), 6.23 (1H, dd, *J*= 1.8, 0.9 Hz) and 7.36 (1H, d, *J*=10.3 Hz) each for H-15, H-2, H-1 and H-22 were displayed in the ^{1}H-NMR spectrum.

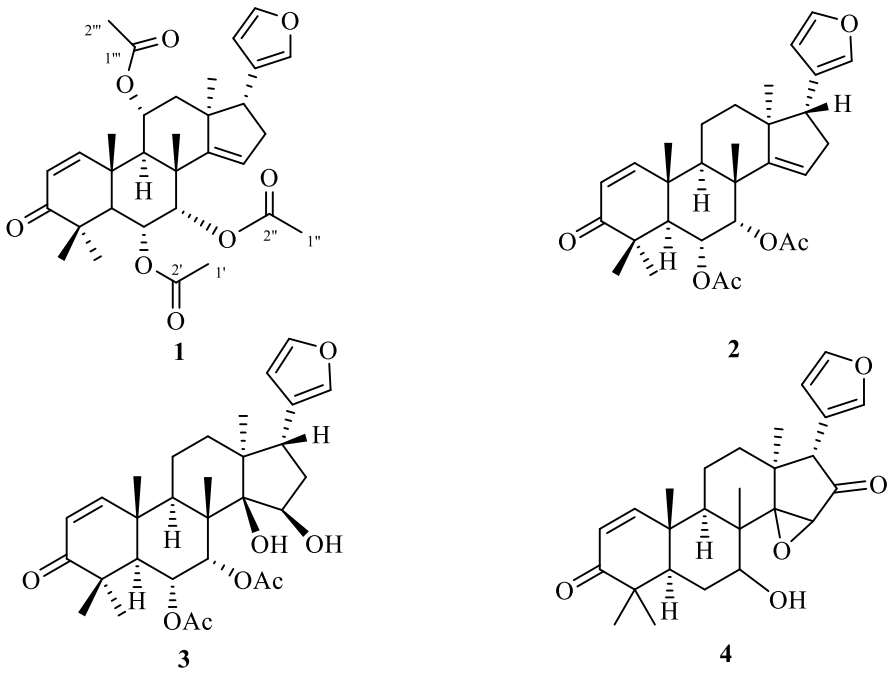

Figure 1. Structures of Compounds **1-4**

The ^{13}C-NMR, DEPT135, and (HSQC) spectra showed the presence of 32 carbons containing carbonyl for ketone and ester group (δ_C 204.1, 170.1, 170.1, 170.3) as well as 8 methyl tertiary (δ_C 21.4, 21.1, 20.1, 20.5, 20.9, 22.0, 28.4, and 31.7). The ^{13}C NMR spectra also displayed 2 methylenes (δ_C 42.6 and 34.5), along with 3 methines aliphatic (δ_C 42.0, 45.3, and 51.4). In addition, there were 4 methines olefinic (δ_C 111.0, 120.0, 125.4, and 158.3), 3 oxymethines (δ_C 69.5, 70.6, and 73.9), 2 oxygenated sp^2 methine carbons (δ_C 139.9, and 142.9), and 6 quartenary carbons including sp^3 and sp^2 quartenary carbon (δ_C 41.5, 42.8, 47.3, 46.4, 123.9, and 157.1). In this study, 7 out of 12 degrees of unsaturation could be attributed to the functionalities, and the pentacylic limonoid structure accounted for the remaining 5 degrees [3,4].

The NMR data of compound **1** were similar to those reported for dysobinin [5], except for the presence of acetoxy signals [δ_H 5.45 (1H, d, J=7.7 Hz), δ_C 70.6], indicating that **1** was an acetoxy analog of dysobinin. In addition, 3 acetoxy groups were located at C-6 (δ_C 69.5), C-7 (δ_C 73.9), and C-11 (δ_C 70.6), respectively elucidated through (^{1}H-^{1}H COSY) and (HMBC) experiments (Figure 2). HMBC correlations were observed between H-6 (δ_H 5.46, m), H-7 (δ_H 5.43, d, J=2.5 Hz), and H-11 (δ_H 5.45, d, J=7.7 Hz) and each corresponding acetyl carbons, namely δ_C 170.1 (C-2′), 170.1 (C-2″), 170.3 (C-2‴). The positions of 3 acetoxy were also revealed by ^{1}H-^{1}H COSY from H_5/H_6/H_7 and H_9/H_{11}/H_{12}. The results showed that the position of an *α,β*-unsaturated ketone was assigned to be in C-1/C-2/C-3 from the correlations of H-1(δ_H 7.36, t, 10.3 Hz) to carbonyl ketone δ_C 204.1 (C-3) with ^{1}H-^{1}H COSY correlation H_1/H_2.

The double bond in ring D was confirmed using HMBC correlations from H-15 (δ_H 5.40, d, J=7.7 Hz) to similar configuration with dysobinin. The optical rotation of compound **1**, $[\alpha]^{26.0}_D$ -1.4° (c 0.05, $CHCl_3$) was similar to that of dysobinin, as previously reported ($[\alpha]^{20.0}_D$+150°) [7]. Consequently, the structure of compound **1** had been determined as a novel azadirone type of limonoid derivative and was named *11α*-acetoxydysobinin.C-13, C-16, and C-17 (δ_C 46.4, 34.5, 51.4) combined with ^{1}H-^{1}H COSY correlation H_{15}/H_{16}/H_{17}. The furan skeleton was identified by HMBC correlations, from H-22 (δ_H 6.23, dd, 1.8, 0.9 Hz) correlated to oxygenated methine sp^2 C-23 (δ_C 142.9) and from H-21 (δ_H 7.23, s) to C-20, C-22, and C-23 (δ_C 123.9, 111.0, and 142.9), along with ^{1}H-^{1}H COSY correlation H_{22}/H_{23}. The relative configuration of the acetoxy group (C-11) in compound **1** was determined by the NOESY experiment (Figure 3), as well as by comparing the NMR data with

dysobinin. The key correlation observed at H-11/CH_3-19 indicated that the acetoxy group at C-11 was α oriented and 2 acetoxy at C-6 and C-7 had had been determined as a novel azaridone-type of limonoid derivative and was named *11α*-acetoxydysobinin.

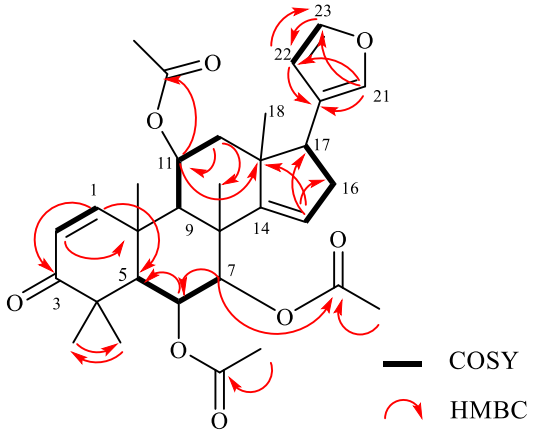

Figure 2. Selected HMBC and ^{1}H-^{1}H COSY correlations for **1**

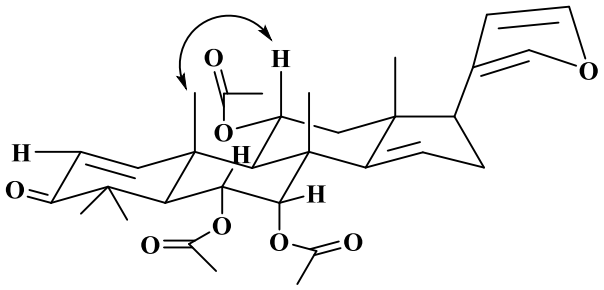

Figure 3. Selected ^{1}H-^{1}H NOESY correlations of **1**

Based on the established procedures [4], all compounds were evaluated for their cytotoxicity against the MCF-7 breast cancer cell line, using cisplatin as a positive control. The results showed that compound **1** did not show any significant activity towards MCF-7 breast cancer cells, with IC_{50} (inhibitory concentration, 50%) values >250 µg/mL, as shown in Table 1. The findings indicated that the presence of acetoxy at C-11 could reduce cytotoxic activity. This was evidenced by compound **2**, which had better activity compared to compound **1** due to the absence of acetoxy group at C-11.

Table 1. Cytotoxicity of compounds **1**–**4** against MCF-7 breast cancer cell.

Compound	**IC_{50} (µg/mL)**
11 α-acetoxydysobinin (**1**)	>250.00
Dysobinin (**2**)	68.15
Dysobinol (**3**)	243.70
7-deacetylepoxyazadiradione (**4**)	94.62
Cisplatin (positive control)	38.06

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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