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Two Limonoids from The Seeds of *Chisocheton Macrophyllus* and Their Cytotoxic Activity Against Mcf-7 Breast Cancer Cells

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ABSTRACT. Limonoids (tetranortriterpenoids) are triterpenoid compounds that lose four terminals in their structural framework. These compounds have a wide variety of structures and interesting activities including anti-inflammatory, anti-cancer, anti-tumor and anti-malarial properties. The purpose of this study was to find limonoid compounds from the Indonesian *Chisocheton* plant, and one of which is *Chisocheton macrophyllus*. The dried and powdered seeds of *C. macrophyllus* (4.5 kg) were extracted with methanol and partitioned successively with *n*-hexane, ethyl acetate and *n*-butanol. Evaporation of each extract resulted in the crude extracts of *n*-hexane (346.6 g), ethyl acetate (60.8 g) and *n*-butanol (14.6 g). The *n*-hexane fraction was subjected to a silica gel vacuum-liquid chromatography (VLC) column packed with silica gel 60 using gradient of *n*-hexane, ethyl acetate and methanol (10% stepwise) to afford thirteen fractions (A-M). Fraction F (4.4 g) was subjected to silica gel column chromatography using gradient of *n*-hexane and ethyl acetate (5% stepwise). Subfraction F5 (1.2 g) was chromatographed on a column of silica gel eluted with *n*-hexane: CH_2Cl_2: EtOAc (2:7.5:0.5) to give compound **1** (19.7 mg) and fraction H (1.8 g) was subjected to silica gel column chromatography using gradient of *n*-hexane and ethyl acetate (5% stepwise) as eluting solvent to give **2** (12.0 mg). Chemical structures of **1** and **2** were elucidated by spectroscopic methods and determined as 6α-acetoxyepoxyazadiradione (**1**) and Dysobinin(**2**). Dysobinin (**2**) showed weak cytotoxic activity against MCF-7 breast cancer cells with an IC_{50} value of 228.15 µM

Keywords: 6α-acetoxyepoxyazadiradione, *C. macrophyllus*, dysobinin, limonoid, MCF-7

INTRODUCTION

Limonoids are a class of tetranortriterpenoids that are formed through the loss of four terminal carbons of side chain of euphane (20-*S*) or tirucallane (20-*R*) skeleton that are followed by a cyclization to form a 17β-furan ring (Tan, & Luo, 2011; Shi et al., 2020). Limonoids are classified into ten classes based on the differences on A, B, C, D, and furan ring of the limonoid skeleton and can be identified by their biosynthetic relationships (Fang, Di, & Hao, 2011; Tan & Luo, 2011; Shi *et al*., 2020). Ten classes of limonoid include protolimonoids, apoeuphol skeleton, D-ring *seco*, B, D-ring *seco*, A-ring *seco*, A,B-ring *seco*, C-ring *seco*, A,D-ring *seco* and B-ring *seco* limonoids.

Limonoids occur mainly in the plant order of Rutales and most of them are found in Meliaceae and Rutaceae families (Li, Peng, & Zheng, 2016). Limonoids isolated from species of the family of Meliaceae have been of interest due to their diverse structures and their biological activities, including antifeedant, anticancer, antimicrobial, antimalarial, and antiviral properties (Tan, & Luo, 2011; Wong et al., 2011; Gualdani, Cavalluzzi, Lentini, & Habtemariam, 2016; Shilpi et al., 2016; Chong et al., 2019; Supratman et al., 2020). Nimbolide is a major limonoid isolated from the leaves of *Azadirachta indica* A. Juss or known as neem tree. Nimbolide as a neem limonoid is widely used for anti-malaria, antibacterial activity against *S. aureus* and *S. coagulase*, anti-feedant and insecticidal activity (Kumar & Navaratnam, 2013; Bodduluru, Kasala, Thota, Barua & Sistla, 2014; Wang et al., 2016; Sophia et al., 2018). Nimbolide was presumed to be a more potent anticancer. Nimbolide shows anticancer activity throughout selective modulation of signaling pathways linked to inflammation, survival, growth, invasion, angiogenesis and metastasis. Nimbolide was reported to induce apoptosis by disruption of Mitochondrial Outer Membrane Potential (MOMP) and inhibits tumor cell proliferation through alterations of cyclins, cdks, PCNA and p53 levels. In addition, nimbolide also reducing the nuclear

translocation and DNA-binding activity of NF-κB in cancer cells (Kumar & Navaratnam, 2013; Bodduluru, Kasala, Thota, Barua, & Sistla, 2014; Wang et al., 2016; Sophia et al., 2018). Beside nimbolide, other limonoids, such azadirachtin, salannin, nimbin and nimbic acid, have been *isolated* from *A. indica* (Wang et al., 2016). Azadirachtin-A (AzaA) is a prominent limonoid known as strong antifeedant and has been exploited commercially. AzaA is present in seed, leaves and other parts of *A. indica*. Natural pesticide like AzaA is widely used to control the insect. AzaA can keep the insect engaged in defensive while reducing food consumption. *In silico* studies suggests that AzaA accommodated in the hydrophobic pocket of juvenile hormone esterase and interact with active site residues. AzaA generally targets more than one protein and was presumed to be a potent biopesticide (Dawkar et al., 2019). Other limonoids from Meliaceae family also have potential applications in the food and pharmaceutical industries and have been used as food additives and pesticides (Gualdani, Cavalluzzi, Lentini, & Habtemariam, 2016; Shi et al., 2020).

Chisocheton plant is a genus from Meliaceae that consists of more than 50 species. The genus is distributed mainly in India, Thailand, Malaysia, Indonesia and becomes the second largest genus of family Meliaceae (Katja et al., 2016; Supriatno et al., 2018). Previous phytochemical studies on *Chisocheton* have discovered several limonoid compounds, such as malayanines A and B, two novel limonoids, that were isolated from the bark of Malaysian *C. erythrocarpus* Hiern (Chong et al., 2012), chisomicines D and E, two new limonoids, that have been isolated from the bark of Malaysian *C. ceramicus* (Miq.) (Najmuldeen et al., 2012), chisotrijugin, a trijugin-type limonoid, from the bark of *C. cumingianus* (Katja et al., 2016), and pentandricine, a new vilacinine-type limonoid, that was isolated from the stembark of *C. pentandrus,* together with ceramicine B, 6-de(acetyloxy)-23-oxochisocheton, and 6-de(acetyloxy)-23-oxo-7-*O*-deacetylchisocheton that have been (Supriatno et al., 2018). *Chisocheton* genus has also been known as the producers of limonoid compounds with interesting biological activities, for example, ceramicine G and I from *C. ceramicus*, which have cytotoxic activity against MCF-7 breast cancer cells (Wong *et al.,* 2011) and erythrocarpine E from *C. erythrocarpus*, which has anticancer properties against HSC-4 human oral cancer cells (Nagoor et al., 2011).

In order to investigate cytotoxic limonoids from Indonesian *Chisocheton* plants (Katja et al., 2016; Nurlelasari et al., 2017), we continue to carry out a phytochemical investigation on *Chisocheton macrophyllus* seeds. *C. macrophyllus* species are distributed in Nicobar Islands, peninsular Thailand, Peninsular Malaysia, Singapore, Sumatra, Anambas Islands, Java and also Borneo. *C. macrophyllus* is a higher plant with the tree up to 35 m tall. The oil isolated from *C. macrophyllus* seed has been used for lighting in Indonesia. The wood of *C. macrophyllus* are used as timber because it is not durable and splits easily (Vossen, & Umali, 2002; Nurlelasari et al., 2017). Previous investigation on limonoids from *C. macrophyllus* seeds has showed that the plant resulted in bioactive limonoids including dysobinol, 7α-hydroxyneotricilenone, dysobinin and nimonol with cytotoxic activity against P-388 murine leukemia cells (Nurlelasari et al., 2017). In this paper, we describe the isolation, structure elucidation and cytotoxic properties against MCF-7 breast cancer cells of 6α-acetoxyepoxyazadiradione (**1**) and dysobinin (**2**), isolated from *C. macrophyllus* seeds.

EXPERIMENTAL SECTION

Material and instrumentation

Seeds of *C. macrophyllus* were collected from Bogor Botanical Garden, Bogor, West Java Province, Indonesia with voucher specimen (No. Bo-1295453).

IR spectra and mass spectra were recorded on an One PerkinElmer spectrum-100 FT-IR in KBr and Waters Xevo QTOF MS respectively. NMR spectra were obtained with JEOL JNM-ECZ500R/S1 at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Chromatographic separations were carried out on the silica gel 60 (70–230 and 230–400 mesh, Merck). TLC analysis was carried out on 60 GF_{254} (Merck, 0.25 mm) using various solvent systems, and detection by irradiating under ultraviolet-visible light Vilber Lourmat (λ 254 nm dan λ 365 nm) followed by heating of silica gel plates, sprayed with 10% H_2SO_4 in ethanol and Ehrlich's reagent (*p*-dimethylaminobenzaldehyde).

Extraction and isolation

The dried and powdered seeds of *C. macrophyllus* (4.5 kg) was extracted with methanol at room temperature for 3 x 4 L x 24 hours. After the solvent removal under vacuum, a total 560 g of methanol extract was obtained and partitioned with *n*-hexane, ethyl acetate and *n*-butanol. Evaporation on each extract resulted the crude extracts of *n*-hexane (346.6 g), ethyl acetate (60.8 g) and *n*-butanol (14.6 g).

The *n*-hexane soluble fraction was subjected to a silica gel vacuum-liquid chromatography (VLC) column packed with silica gel 60 using gradient of *n*-hexane, ethyl acetate and methanol (10% stepwise) to afford thirteen fractions (A-M). Fraction F (4.4 g) was subjected to silica gel column chromatography using gradient of *n*-hexane and ethyl acetate (5% stepwise) as eluting solvent to afford twelve subfractions (F1-F12). Subfraction F5 (1.2 g) was chromatographed on a column of silica gel eluted with *n*-hexane: CH_2Cl_2: EtOAc (2:7.5:0.5) to give compound **1** (19.7 mg). Fraction H (1.8 g) was subjected to silica gel column chromatography using gradient of *n*-hexane and ethyl acetate (5% stepwise) as eluting solvent to give **2** (12.0 mg).

6α-acetoxyepoxyazadiradione (1)

Colorless crystals 220-221°C; IR (KBr) ν_{max} 2922, 1741, 1668, 1503, 1365 and 1244 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) see **Table 1**; HR-TOFMS *m/z* 547.2302 $[M+Na]^+$, (calcd. $C_{30}H_{36}O_8Na$ *m/z* 547.2308).

Dysobinin *(2)*

Colorless crystals 196-197°C ; IR (KBr) ν_{max} 2919, 1740, 1667, 1502, 1382; 1362, 1234 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) see **Table 1**; HR-TOFMS *m/z* 495.2741 $[M+H]^+$, (calcd. $C_{30}H_{39}O_6$ *m/z* 495.2747).

Bioassays for cytotoxic activity

The cytotoxicity assay was conducted according to the method previously described by Examinati, Wulandari, Harneti, & Poniah (2018) and Supriatno *et al*., (2018). MCF-7 cells plated in 96 multiwell culture plates at a density of 1.7×10^4 cells/well. After twenty-four hours, medium was discarded and fresh medium containing sample with different concentrations 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500.00, 1000.00 μ g/mL and control was added. After incubation with sample for 24h, prestoBlue® reagent (resazurin dye) was added. The PrestoBlue® assay results were read using multimode reader at 570 nm. The IC_{50} values were determined by linier regression method using Microsoft Excel software. The IC_{50} value corresponds to the concentration of compounds that decreases 50% number of viable cells and the absorbance in control corresponds to 100% viability.

RESULTS AND DISCUSSION

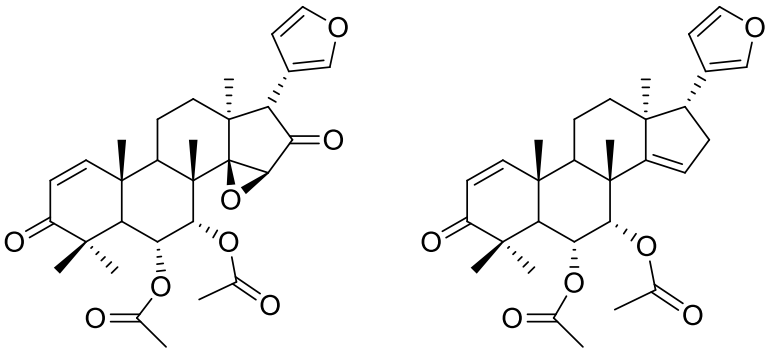
The *n*-hexane extract from the seeds of *C. macrophyllus* was subjected to a silica gel vacuum-liquid chromatography (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to normal phase column chromatography on silica gel to yield compounds **1** and **2** (**Figure 1**).

Compound **1** was isolated as a colorless crystals with 220-221°C. The HR-TOFMS showed the presence of $[M+Na]^+$ peak at m/z 547.2302 (calcd *m/z* for $C_{30}H_{36}O_8Na$, 547.2308), indicating the molecular formula of $C_{30}H_{36}O_8$. UV spectrum in MeOH showed λ_{max} 220 nm and IR absorptions suggested the presence of aliphatic (ν_{max} 2922 cm^{-1}), carbonyl ester (ν_{max} 1741 cm^{-1}), α,β-unsaturated carbonyl (ν_{max} 1668 cm^{-1}), olefinic (ν_{max} 1503 cm^{-1}), *gem* dimethyl (ν_{max} 1365 cm^{-1}), and ether groups (ν_{max} 1244 cm^{-1}). The ^{1}H-NMR spectrum showed five tertiary methyls at δ_H 1.01 (3H, s, Me-29), 1.14 (3H, s, Me-18), 1.19 (3H, s, Me-30), 1.23 (3H, s, Me-19) and 1.30 (3H, s, Me-28). Two acetoxyl groups δ_H 2.06 (3H, s, H-1') and 2.00 (3H, s, H-1''). Three oxygenated protons at δ_H 5.34 (1H, dd, *J* = 2.6, 12.5 Hz, H-6), 5.01 (1H, d, *J* = 2.6 Hz, H-7) and 3.40 (1H, s, H-15), a β-furan moiety at δ_H 6.21 (1H, d, *J* = 1.45 Hz, H-22), 7.38 (1H, d, *J* = 1.45 Hz, H-23) and 7.54 (1H, s, H-21) and two olefinic protons at δ_H 5.93 (1H, d, *J* = 10.5 Hz, H-2), and 7.11 (1H, d, *J* = 10.5 Hz, H-1). The ^{13}C-NMR and DEPT spectra showed thirty carbons consisting of an α,β-unsaturated carbonyl at δ_C 204.5 (C-3), carbonyl ketone at δ_C 208.1 (C-16), two acetoxyl groups at δ_C 21.2 (C-1'), 170.0 (C-2'), 21.3 (C-1'') and 170.1 (C-2''), five methyls at δ_C 19.1 (Me-28), 20.3 (Me-18), 21.7 (Me-30), 24.8 (Me-29) and 31.7 (Me-19). The spectra also showed two methylene carbons at δ_C 16.2 (C-11) and 28.7 (C-12), three sp^3 methine carbons at δ_C 38.5 (C-9), 48.5 (C-5) and 50.9 (C-17), three sp^2 methine carbons at δ_C 111.0 (C-22), 126.6 (C-2) and 156.9 (C-1), three oxygenated sp^3 methine carbons at δ_C 57.1 (C-15), 69.9 (C-6) and 73.1 (C-7), two oxygenated sp^2 methine carbons at δ_C 141.7 (C-21) and 142.6 (C-23), four sp^3 quaternary carbons at δ_C 40.5 (C-10), 42.5 (C-8), 43.3 (C-4), and 45.3 (C-13), an oxygenated sp^3 quaternary carbons at δ_C 72.4 (C-14), and one sp^2 quaternary carbons at δ_C 116.5 (C-20). These functionalities accounted for seven out of the total thirteen degrees of unsaturation ($C_{30}H_{36}O_8$), while the remaining six degrees of unsaturation corresponded to the pentacyclic limonoid structure (Wong et al., 2011; Najmuldeen et al., 2012; Nurlelasari et al., 2017; Supriatno et al., 2018) with an additional cyclic. The NMR spectra data of **1** were resembled to those of previously reported dysobinin (Nurlelasari et al., 2017), except for the absence of olefinic signals at C-14/C-15 and instead the appearance of oxygenated signals [δ_H 3.40 (1H, s), δ_C 57.1 and δ_C 72.4] and carbonyl at δ_C 208.1, thus suggesting the appearance epoxide between C-14 and C-15 and carbonyl at C-16 in structure **1**. Position of these carbonyl at C-16 and epoxide between C-14 and C-15 was determined through the ^{1}H-^{1}H COSY and HMBC experiments (**Figure 2**). ^{1}H-^{1}H COSY spectrum of **1** in $CDCl_3$ (**Figure 2**), showed correlation in H_1-H_2, H_5-H_6-H_7, H_{11}-H_{12} and H_{22}-H_{23}. Four partial structures **a** (from C-1 to C-2), **b** (from C-5 to C-7), **c** (C-11 to C-12), and **d** (from C-22 to C-23) were deduced from this ^{1}H-^{1}H COSY data and supporting the presence of a havanensin-type of limonoid structure in **1**. Data from HMBC spectrum showed ^3J correlations between sp^2 methine proton signal δ_H 7.11 (H-1) and δ_C 48.5 (C-5) and carbonyl at δ_C 204.5 (C-3) and a correlation between δ_H 5.93 (H-2) and δ_C 40.5 (C-10). Furthermore, olefinic protons at δ_H 7.11 (1H, d, *J* = 10.5 Hz, H-1) and δ_H 5.93 (1H, d, *J* = 10.5 Hz, H-2) are coupled each other indicating that an α,β-unsaturated carbonyl was located at C-1, C-2 and C-3. Correlations from oxygenated sp^3 methine protons at δ_H 5.34 (H-6) to δ_C 48.5 (C-5), δ_C 73.1 (C-7) and δ_C 170.0 (C-2'), δ_H 5.01 (H-7) to δ_C 48.5 (C-5), δ_C 69.9 (C-6), δ_C 42.5 (C-8), δ_C 38.5 (C-9) and δ_C 170.1 (C-2''), δ_H 2.06 (H-1') to δ_C 170.0 (C-2') and δ_H 2.00 (H-1'') to δ_C 170.1 (C-2'') indicating that acetyl group was attached at C-6 and C-7.

Correlations from oxygenated sp^3 methine protons at δ_H 3.40 (H-15) to δ_C 208.1 (C-16) and δ_C 50.9 (C-17) and correlations from δ_H 3.86 (H-17) to δ_C 28.7 (C-12), δ_C 45.3 (C-13), δ_C 208.1 (C-16), δ_C 116.5 (C-20), δ_C 141.7 (C-21) and δ_C 111.0 (C-22) were used to assign position of an epoxide between C-14 and C-15, a carbonyl located on C-16 and a furan ring attached at C-17. Chemical structure of **1** was presumed to be the same as 6 α -acetoxyepoxyazadiradione due to the high similarity of the NMR chemical shifts that was previously reported (**Table 1**.) (Pereira et al., 2014). The indicating the relative stereochemistry of epoxide between C-14/C-15 of **1** are β-oriented and acetyl group at C-6 and C-7 is α -oriented. Therefore, compound **1** was identified as a 6 α -acetoxyepoxyazadiradione and showed in this plant for the first time.

Compound (**2**) was isolated as a colorless crystals with 196-197°C. The $[M+H]^+$ peak at *m/z* 495.2741 (calcd for $C_{30}H_{39}O_6$, 495.2747), HR-TOFMS indicated the molecular formula $C_{30}H_{38}O_6$, and NMR data (**Table 2**), thus requiring twelve degrees of unsaturation. IR absorptions suggested the presence of aliphatic (ν_{max} 2919 cm^{-1}), carbonyl ester (ν_{max} 1740 cm^{-1}), α,β -unsaturated carbonyl (ν_{max} 1667 cm^{-1}), olefinic (ν_{max} 1502 cm^{-1}), *gem* dimethyl (ν_{max} 1382; 1362 cm^{-1}), and ether groups (ν_{max} 1234 cm^{-1}). The ^{1}H-NMR spectrum showed five tertiary methyls at δ_H 0.78 (3H, s, Me-28), 1.17 (3H, s, Me-19), 1.17 (3H, s, Me-30), 1.23 (3H, s, Me-29) and 1.31 (3H, s, Me-18). Two acetoxyl groups δ_H 1.99 (3H, s, H-1') and 2.03 (3H, s, H-1''). Two oxygenated protons at δ_H 5.36 (1H, dd, J = 4.5, 3.5 Hz, H-6) and 5.41 (1H, d, J = 2.6 Hz, H-7), a β-furan moiety at δ_H 6.25 (1H, d, J = 1.45 Hz, H-22), 7.36 (1H, d, J = 1.45 Hz, H-23) and 7.22 (1H, s, H-21) and two olefinic protons at δ_H 5.90 (1H, d, J = 10.5 Hz, H-2), and 7.12 (1H, d, J = 10.5 Hz, H-1). The ^{13}C NMR and DEPT spectra showed thirty carbons consisting of an α,β-unsaturated carbonyl at δ_C 204.8 (C-3), two acetoxyl groups at δ_C 21.0 (C-1'), 170.2 (C-2'), 21.4 (C-1'') and 170.3 (C-2''), five methyls at δ_C 20.5 (Me-28), 20.8 (Me-29), 22.7 (Me-30), 26.8 (Me-18) and 31.7 (Me-19). The spectra also showed three methylene carbons at δ_C 16.5 (C-11), 34.4 (C-12) and 32.7 (C-16), three sp^3 methine carbons at δ_C 37.3 (C-9), 47.9 (C-5) and 51.6 (C-17), four sp^2 methine carbons at δ_C 111.0 (C-22), 119.8 (C-15), 126.2 (C-2) and 157.4 (C-1), two oxygenated sp^3 methine carbons at δ_C 70.0 (C-6) and 74.6 (C-7), two oxygenated sp^2 methine carbons at δ_C 139.8 (C-21) and 142.7 (C-23), four sp^3 quaternary carbons at δ_C 40.8 (C-10), 43.0 (C-8), 45.0 (C-4), and 47.1 (C-13) and two sp^2 quaternary carbons at δ_C 124.5 (C-20) and 158.2 (C-14). These functionalities accounted for seven out of the total twelve degrees of unsaturation, while the remaining five degrees of unsaturation corresponded to the pentacyclic limonoid structure (Wong et al., 2011; Najmuldeen et al., 2012; Nurlelasari et al., 2017; Supriatno et al., 2018). Structure of **2** was presumed to be the same as dysobinin because of the high similarity of the NMR chemical shifts of the backbone skeleton (Nurlelasari et al., 2017). Therefore, the structure of **2** was elucidated as havanensin-type of limonoid and namely as dysobinin.

Compounds **1** and **2** were evaluated for their cytotoxic activity against MCF-7 breast cancer cell and cisplatin as a positive control according to a method previously described (Examinati, Wulandari, Harneti, & Poniah, 2018; Supriatno et al., 2018). 6 α -Acetoxyepoxyazadiradione (**1**) was found to be inactive and dysobinin (**2**) demonstrated weak cytotoxic activity against MCF-7 breast cancer cell line with IC_{50} values of 228.15 μM whereas cisplatin as the positive control has IC_{50} value of 11.42 μM. The bioassay result, suggested that the carbonyl at C-16 and epoxide at C-14/C-15 in 6 α -acetoxyepoxyazadiradione (**1**) decreased the cytotoxic activity.



1 **2**

Figure 1. Chemical Structures of Compound **1** and **2**.

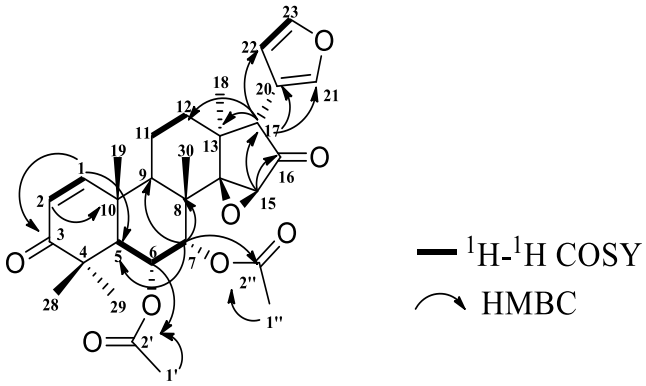


Figure 2. Selected ^{1}H-^{1}H COSY and HMBC Correlations for **1**.

Tabel 1. NMR data for compounds **1** dan 6α-acetoxyepoxyazadiradione (Pereira *et al*., 2014) ($CDCl_3$, 500 MHz for ^{1}H and 125 for ^{13}C)

Posisi C	*F5		**6α-acetoxyepoxyazadiradione	
	^{1}H -NMR δ_H ppm (ΣH; mult; *J*=Hz)	^{13}C-NMR δ_C ppm	^{1}H -NMR δ_H ppm (ΣH; mult; *J*=Hz)	^{13}C-NMR δ_C ppm
1	7.11 (1H; d; 10.5)	156.9	7.16 (1H; d; 10.1)	156.7
2	5.93 (1H; d; 10.5)	126.6	5.99 (1H; d; 10.1)	126.5
3	-	204.5	-	204.4
4	-	43.3	-	45.2
5	2.48 (1H; d; 12.5)	48.5	2.54 (1H; d; 12.5)	48.5
6	5.34 (1H; dd; 2.6; 12.5)	69.9	5.40 (1H; dd; 2,6; 12.5)	69.8
7	5.01 (1H; d; 2.6)	73.1	5.06 (1H; d; 2.6)	73.0
8	-	42.5	-	43.2
9	2.64 (1H; dd; 2.6; 12.5)	38.5	2.71 (1H; dd; 4.0; 12.5)	38.4
10	-	40.5	-	40.5
11	1.90 (2H; m)	16.2	2.19 (1H; m); 1.88 (1H; m)	16.1
12	2.65 (2H; dd; 4.0; 12.5)	28.7	2.03 (1H; m); 1.95 (1H; m)	28.6
13	-	45.3	-	42.5
14	-	72.4	-	72.2
15	3.40 (1H; s)	57.1	3.46 (1H; s)	57.0
16	-	208.1	-	207.9
17	3.86 (1H; s)	50.9	3.92 (1H; s)	50.8
18	1.14 (3H; s)	20.3	1.07 (3H; s)	24.7
19	1.23 (3H; s)	31.7	1.24 (3H; s)	21.5
20	-	116.5	-	116.4
21	7.54 (1H; s)	141.7	7.43 (1H; t; 1.6)	142.5
22	6.21 (1H; d; 1.45)	111.0	6.26 (1H; d; 1.6)	110.9
23	7.38 (1H; d; 1.45)	142.6	7.41 (1H; s)	141.6
28	1.30 (3H; s)	19.1	1.29 (3H; s)	31.6
29	1.01 (3H; s)	24.8	1.20 (3H; s)	20.2
30	1.19 (3H; s)	21.7	1.36 (3H; s)	19.0
1'	2.06 (3H; s)	21.2	2.11 (3H; s)	21.2
2'	-	170.0	-	169.9
1"	2.00 (3H; s)	21.3	2.05 (3H; s)	21.1
2"	-	170.1	-	169.8

* ($CDCl_3$; ^{1}H-NMR 500 MHz; ^{13}C-NMR 125 MHz)

**($CDCl_3$; ^{1}H-NMR 500 MHz; ^{13}C-NMR 125 MHz)

Tabel 2. NMR data for compounds **2** and dysobinin (Nurlelasari *et al.*, 2016).

Posisi C	*H		**Dysobinin	
	^{1}H -NMR δ_H ppm (ΣH; mult; *J*=Hz)	^{13}C-NMR δ_C ppm	^{1}H -NMR δ_H ppm (ΣH; mult; *J*=Hz)	^{13}C-NMR δ_C ppm
1	7.12 (1H; d; 10.5)	157.4	7.3 (1H; d; 10.3)	158.2
2	5.90 (1H; d; 10.5)	126.2	5.84 (1H; d; 10.3)	126.6
3	-	204.8	-	204.1
4	-	45.0	-	45.6
5	2.48 (1H; d; 14.0)	47.9	2.5 (1H; m)	48.9
6	5.36 (1H; dd; 4.5; 3.5)	70.0	5.40 (1H; m)	70.0
7	5.41 (1H; d; 2.6)	74.6	5.40 (1H; m)	75.1
8	-	43.0	-	43.9
9	2.41 (1H; m)	37.3	1.28 (1H; m)	38.3
10	-	40.8	-	41.6
11	1.62 (2H; m)	16.5	1.80 (2H; m)	17.0
12	2.32 (2H; m)	34.4	2.3 (1H; m); 2.5 (1H; m)	35.3
13	-	47.1	-	47.9
14	-	158.2	-	159.7
15	2.24 (1H; m)	119.8	2.26 (1H; m)	120.2
16	1.73 (1H; m); 1.91 (1H; m)	32.7	1.73 (1H; m); 1.93 (1H; m)	33.6
17	2.79 (1H; dd; 11.0; 18.5)	51.6	2.84 (1H; dd; 7.4; 11.3)	52.7
18	1.31 (3H; s)	26.8	1.35 (3H; s)	27.1
19	1.17 (3H; s)	31.7	1.22 (3H; s)	32.1
20	-	124.5	-	125.5
21	7.22 (1H; s)	139.8	7.40 (1H; s)	140.9
22	6.25 (1H; d; 1.45)	111.0	6.40 (1H; s)	112.0
23	7.36 (1H; d; 1.45)	142.7	7.50 (1H; s)	143.7
28	0.78 (3H; s)	20.5	1.15 (3H; s)	20.7
29	1.23 (3H; s)	20.8	1.22 (3H; s)	20.9
30	1.17 (3H; s)	22.7	1.22 (3H; s)	21.2
1'	1.99 (3H; s)	21.0	2.00 (3H; s)	21.3
2'	-	170.2	-	170.6
1"	2.03 (3H; s)	21.4	2.00 (3H; s)	22.4
2"	-	170.3	-	170.6

* ($CDCl_3$; ^{1}H-NMR 500 MHz; ^{13}C-NMR 125 MHz)

**($CDCl_3$; ^{1}H-NMR 500 MHz; ^{13}C-NMR 125 MHz)

CONCLUSIONS

6α-Acetoxyepoxyazadiradione (**1**) and dysobinin (**2**) have been isolated from the seeds of *C. macrophyllus*. The discovery of **1** and **2** supported the occurrence of limonoid in the *Chisocheton* genus. Compound **1** and **2** were evaluated for their cytotoxic activity against MCF-7 breast cancer cell line. Compound **1** was inactive and compound **2** demonstrated weak cytotoxic activity (228.15 μM) against MCF-7 breast cancer cell line. The bioassay data suggested that the carbonyl at C-16 and epoxide at C-14/C-15 in 6α-acetoxyepoxyazadiradione (**1**) decreased the cytotoxic properties.

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