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Azadirone-Type Limonoids from the Fruit of *Chisocheton lasiocarpus* and Their Cytotoxic Activity Against MCF-7 Breast Cancer Lines

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ABSTRACT. Limonoid is derivatives of triterpenoid compound that have a wide variety of structures due to various ring-opening, rearrangements, and high-degree of oxidation. Limonoid is known as compounds that have wide-range of biological activities, including anticancer activity. This research was aimed to determine the chemical structure and cytotoxic activity of limonoid in the *n*-hexane extract of *Chisocheton lasiocarpus* fruit. Dried powder of *C. lasiocarpus* fruit was extracted using methanol followed by fractionated using *n*-hexane, ethyl acetate, and *n*-butanol. Five azadirone-type limonoids, 6*α*-(acetoxy)-14*β*,15*β*-epoxyazadirone (**1**), dysobinin (**2**), 7*α*-acetylneotricilenone (**3**), 6*α*-O-acetyl-7-deacetylnimocinol (**4**), and 7*α*-hyroxyneotricilenone (**5**), were isolated from the *n*-hexane extract of *Chisocheton lasiocarpus* fruit. The chemical structure of all compounds was identified by spectroscopic analysis, including 1D, 2D-NMR, IR, UV and HR-TOF MS as well as by comparison with previous reported spectra data. Compounds **1**-**5** were investigated from this plant for first time. The cytotoxic activity of the isolated compounds against MCF-7 breast cancer line were examined and the results showed that 7*α*-hyroxyneotricilenone (**5**) showed the moderate activity with IC_{50} values of 53 µM.

Keywords: cytotoxic activity, limonoid, *Chisocheton lasiocarpus*, Meliaceae, MCF-7

INTRODUCTION

Chisocheton is one genus of the of the Meliaceae Family with more than 53 species and distributed in tropical and subtropical region in Asia such as Nepal, Bhutan, India, Myanmar, South China, South East Asia and Papua New Guinea (Heyne, 1982; Vossen & Umali, 2002; Zhang et al., 2012). Plant from *Chisocheton* genus has been used traditionally as medicine for treatment of stomach illness, kidney illness, backache, fever, rheumatism and malaria (Shilpi et al., 2016; Tasyriq, et al., 2012). Plant extract or isolated compounds from *Chisocheton* plants has been reported to showed many bioactivities, including insecticidal, insect antifeedant, antiobesity, antimalarial, antibacterial, antifungal, antiviral, antimelanin, cytotoxic, and anti-inflamantory (Chie et al., 2016; Fang, Di, & Hao, 2011; Mohamad et al., 2008; Retnowati et al., 2021; Salam et al., 2021; Supratman et al., 2019, 2020; Wong et al., 2012; Yadav, Yadav, & Goyal, 2014; Yadav, Kataky, & Matur, 1999).

One of the compounds that responsible for these activities are limonoid which known to be abundance in *Chisocheton* genus. So far more than 50 limonoids has been isolated from *Chisocheton* plants (Shilpi et al., 2016). Limonoid compounds are highly oxygenated modified triterpenoids with moderate polarity and known as tetranortriterenoids (Salam et al., 2021). These structures varied by the oxidation on the backbone, the side chain moiety, ring transformation and rearrangements which attracted great interest (Chong et al., 2019; Mohamad et al., 2008; Najmuldeen et al., 2012; Roy & Saraf, 2006).

C. lasiocarpus known as Maksang in local name and the leaves are usually used for wrapping food, while the chemical report of this species is still limited that only lasiocarpines and 14β,15β-epoxynimonol has been reported isolated from seed

of C. *lasiocarpus* (Whitmore, Mabberley, Pannell, & Sigh, 1996; Fitriana et al., 2021). Therefore, in our continuous investigation for limonoid compounds from Indonesian *Chisocheton* plants, we have been described limonoids, dysobinol from C. *macrophyllus* seed (Nurlelasari et al., 2017) and pentandricine from C. *pentandrus* stembark (Supriatno et al., 2018). In the further search for limonoid substances from Indonesian *Chisocheton* plants, the *n*-hexane extract of C. *lasiocarpus* fruit was found to have high content of limonoid based on its TLC screening using Erlich reagent. In this contribution, we describe the isolation and structural identification of five limonoids (**Figure 1**), as well as their cytotoxic activity.

EXPERIMENTAL SECTION

General

UV spectra were recorded on TECAN Infinite M200 pro with methanol as solvent. The IR spectra were measured on Nicolet Summit FTIR Spectrometer with DTGS KBr detector. Mass Spectra were measured by Waters QTOF-HRTOFMS-XEVotm mass spectrometer. NMR spectra were measured by JEOL JNM-ECZ500R/S1 and TMS as an internal standard. Chromatographic separation was carried out on silica gel 60 (70-230 mesh and 230-400 mesh, Merck). Thin-layer chromatography (Merck, 0.25 mm) and spots were detected under ultraviolet–visible light and followed by spraying with Ehrlich's reagent (*p*-Dimethylaminobenzaldehyde in 1:1 hydrochloric acid and ethanol).

Plant Material

Fruit of C. *lasiocarpus* (Miq.) Valeton was found from Bogor Botanical Garden, Bogor, West Java, Indonesia in August 2019. The identification of plant was carried out by Mr. Harto, the staff of Bogoriense Herbarium, Bogor, Indonesia and voucher specimen (VII. G. 168) was deposited at the herbarium.

Extraction and Isolation

The dried fruit of C. *lasiocarpus* (1.2 kg) was extracted with methanol for 6 days (6 x 3L) and evaporated the solvent to produce dark brown residue (270 g). The residue was fractionated successively with *n*-hexane, ethyl acetate and *n*-butanol followed with solvent evaporation in vacuum to afford concentrated extract of *n*-hexane (36.5 g), ethyl acetate (28.1 g) and *n*-butanol (23.6 g). All of the extracts were examined for their cytotoxic activity against MCF-7 breast cancer cell line and showed activity with IC_{50} values of 168; 389 and 404 μg/mL, respectively. The *n*-hexane showed the strongest cytotoxic activity and contents of limonoid compounds based on the TLC analysis under UV light and positive result on Ehrlich's reagent sprayer. Therefore, the further isolation was focused on the *n*-hexane extract.

The *n*-hexane extract was separated by vacuum liquid chromatography (VLC) on silica gel 60 using gradient system eluent of *n*-hexane, ethyl acetate, and methanol to afford nine fractions (A-I). Fraction C (16.7 g) was subjected to VLC on silica gel with *n*-hexane and ethyl acetate as a gradient eluent to give seven subfractions (C1-C7). Subfraction C3 (2.1 g) was subjected to silica gel column chromatography using gradient solvent of *n*-hexane and ethyl acetate to afford five subfractions (C3A-C3E). Furthermore, subfraction C3B was column chromatographed on a silica gel (230-400 mesh) eluted by *n*-hexane : dichloromethane : ethyl acetate (6:3:1) to give **1** (33.2 mg) and **2** (30.1 mg). Fraction C4 (1.1 g) was column chromatographed on a silica gel (230-400 mesh), eluted with *n*-hexane : ethyl acetate (8:2) to give five subfractions (C4A-C4E). In addition, subfraction C4D (280 mg) was separated by column chromatographed on ODS eluted with methanol : water (8:2) to give compound **3** (9.6 mg) and **4** (6.5 mg). Finally, subfraction C4E (59.3 mg) was separated by column chromatography on silica gel (230-400 mesh), eluted by *n*-hexane : chloroform : Ethyl acetate (6:2:2) to afford compound **5** (11.5 mg).

6α-(acetoxy)-14β,15β-epoxyazadirone (1). Colorless needle crystal; mp: 210-213 °C; UV (MeOH) λ_{max} (log ε): 230 nm (2.63); IR (KBr plate) ν_{max} cm^{-1}: 2922, 1739, 1675, 1030; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 7.36 (1H, t, *J*= 1.5 Hz, H-23), 7.14 (1H, d, *J*= 10.5 Hz, H-1), 7.09 (1H, s, H-21), 6.15 (1H, d, *J* = 1.5 Hz, H-22), 5.92 (1H, d, *J*= 10.5 Hz, H-2), 5.35 (1H, dd, *J*= 2.5, 12.5 Hz, H-6), 5.03 (1H, d, *J*= 2.5 Hz, H-7), 3.43 (1H, m, H-15), 2.62 (1H, td, *J*= 4.5, 11.0 Hz, H-17), 2.50 (1H, d, *J*= 12.5 Hz, H-5), 2.13 (1H, d, *J* = 4.5 Hz, H-16b), 2.11 (1H, s, CH_3-1'), 2.01 (1H, s, CH_3-1''), 1.89 (2H, m, H-12), 1.81 (1H, m, H-11), 1.59 (1H, dd, *J*= 11.0, 13.5 Hz, H-16a), 1.24 (1H, s, CH_3-30), 1.22 (1H, s, CH_3-29), 1.17 (1H, s, CH_3-28), 1.15 (1H, s, CH_3-19), 0.95 (1H, s, CH_3-18); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOF MS *m/z* 511.2693 $[M+H]^+$ (calcd. *m/z* 511.2696 for $C_{30}H_{39}O_7$).

Dysobinin (2). White crystal; mp: 190-194 °C; UV (MeOH) λ_{max} (log ε): 229 nm (2.45); IR (KBr) Vmax cm^{-1}: 2938, 1721, 1664, 1503, 1022; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 7.37 (1H, t, *J*= 1.5 Hz, H-23), 7.23 (1H, s, H-21), 7.14 (1H, d, *J*= 10.0 Hz, H-1), 6,27 (1H, d, *J*= 1.5 Hz, H-22), 5.91 (1H, d, *J*= 10.0 Hz, H-2), 5.45 (1H, d, *J* = 3.0 Hz H-15), 5.42 (1H, d, *J*= 3.0 Hz, H-7), 5.37 (1H, m, H-6), 2.81 (1H, dd, *J* = 7.5, 10.5 Hz, H-17), 2.50 (1H, d, *J*= 12.0 Hz, H-5), 2.39-2.42 (1H, m, H-12b), 2.34-2.36 (1H, m, H-12a), 2.25 (1H, dd, *J* = 7.5, 10.5 Hz, H-16b), 2.04 (3H, s, CH_3-1'), 2.02 (1H, d, *J* = 3.0 Hz, H-16a), 2.00 (3H, s, CH_3-1''), 1.93 (1H, m, H-11b), 1.73 (1H, m, H-11a), 1.65 (1H, m, H-9), 1.32 (3H, s, CH_3-30), 1.25 (3H, s, CH_3-29), 1.18 (3H, s, CH_3-19), 1.18 (3H, s, CH_3-28), 0.79 (3H, s, CH_3-18); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOFMS *m/z* 495.2743 $[M+H]^+$ (calcd. *m/z* 495.2747 for $C_{30}H_{39}O_6$).

7α-acetylneotricilenone (3). White crystal; m.p 207-210 °C; UV (MeOH) λ_{max} (log ε): 230 nm (2.68); IR (KBr) Vmax cm^{-1}: 2956, 1722, 1662, 1026; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 7.39 (1H, t, *J* = 1.5 Hz, H-23), 7.28 (1H, s, H-21), 7.15 (1H, d, *J* = 10.0 Hz, H-1), 6,29 (1H, t, *J* = 1.5 Hz, H-22), 5.88 (1H, d, *J* = 10.0 Hz, H-2), 4.96 (1H, t, *J* = 2.5 Hz, H-7), 3.48 (1H, t, *J* = 10.0 Hz, H-17), 2.50 (1H, t, *J* = 10.0 Hz, H-16), 2.43 (1H, s, H-14), 2.08 (1H, s, CH_3-1'), 2.07 (1H, dd, *J* = 5.0, 10.5 Hz, H-5), 2.03 (1H, td, *J* = 3.0, 14 Hz, H-12b), 1.87-1.90 (2H, m, H-6), 1.69-179 (2H, m, H-11), 1.38 (1H, dd, *J* = 2.5, 11.5 Hz, H-9), 1.25-1.32 (1H, m, H-12a), 1.15 (3H, s, CH_3-30), 1.13 (3H, s, CH_3-19), 1.06 (3H, s, CH_3-29), 1.05 (3H, s, CH_3-28), 0.78 (3H, s, CH_3-18); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOFMS *m/z* 475.2462 $[M+Na]^+$, (calcd. *m/z* 475.2460 for $C_{28}H_{36}O_5Na$).

6α-O-acetyl-7-deacetyl nimocinol (4). Colorless crystal; m.p 189-191 °C; UV (MeOH) λ_{max} (log ε): 229 nm (2.64); IR (KBr) Vmax ν_{max} cm^{-1}: 3384, 2943, 1739, 1658, 1019. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 7.38 (1H, t, *J* = 1.5 Hz, H-23), 7.27 (1H, s, H-21), 7.11 (1H, d, *J* = 10.0 Hz, H-1), 6,28 (1H, d, *J* = 1.5 Hz, H-22), 5.88 (1H, d, *J* = 10.0 Hz, H-2), 5.57 (1H, d, *J* = 3.5 Hz, H-15), 5.46 (1H, dd, *J* = 2.0, 12.0 Hz, H-6), 4.07 (1H, d, *J* = 2.0 Hz, H-7), 2.86 (1H, dd, *J* = 7.5, 11.0 Hz, H-17), 2.74 (1H, d, H-16b), 2.42 (1H, ddd, *J* = 3.5, 7.5, 15.0 Hz, *J* = 12.0 Hz, H-5), 2.57 (1H, dd, *J* = 11.0, 15.0 Hz, H-16a), 2.29 (1H, dd, *J* = 12.0, 6.5 Hz, H-9), 2.18 (3H, s, CH_3-1'), 1.93-1.96 (1H, m, H-11b), 1.88-1.92 (1H, m, H-12b), 1.70-1.77 (1H, m, H-11a), 1.63-1.66 (1H, m, H-12a), 1.32 (3H, s, CH_3-29), 1.28 (3H, s, CH_3-30), 1.19 (3H, s, CH_3-28), 1.17 (3H, s, CH_3-19), 0.83 (3H, s, CH_3-18); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOFMS *m/z* 475.2462 $[M+H]^+$, (calcd. *m/z* 475.2460 for $C_{28}H_{36}O_5Na$).

7α-hyroxyneotricilenone (5). White needle crystal; m.p 215-217 °C; UV (MeOH) λ_{max} (log ε): 230 nm (2.63); IR (KBr) Vmax cm^{-1}: 3435, 2939, 1715, 1666, 1636, 1025; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 7.40 (1H, t, *J* = 1.5 Hz, H-23), 7.29 (1H, s, H-21), 7.13 (1H, d, *J* = 10.0 Hz, H-1), 6,30 (1H, d, *J* = 1.5 Hz, H-22), 5.86 (1H, d, *J* = 10.0 Hz, H-2), 3.89 (1H, br.s, H-7), 3.49 (1H, s, H-17), 2.72 (1H, s, H-14), 2.52 (2H, d, *J* = 10.5 Hz, H-16), 2.29 (1H, dd, *J* = 2.5, 13.0 Hz, H-12b), 2.27 (1H, dd, *J* =2.5, 13, H-5), 2.01 (1H, dt, *J* = 3.5, 14.5 Hz, H-12a), 1.94 (1H, td, *J* = 2.5, 13.0, 14.5 Hz, H-6a), 1.72 (1H, m, H-6b), 1.37 (1H, dd, *J* = 2.5, 11.0 Hz, H-9), 1.15 (3H, s, CH_3-29), 1.10 (3H, s, CH_3-19), 1.09 (3H, s, CH_3-28), 1.09 (3H, s, CH_3-30), 0.79 (3H, s, CH_3-18); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOFMS *m/z* 433.2353 $[M+H]^+$, (calcd. *m/z* 433.2355 for $C_{26}H_{34}O_4$ Na).

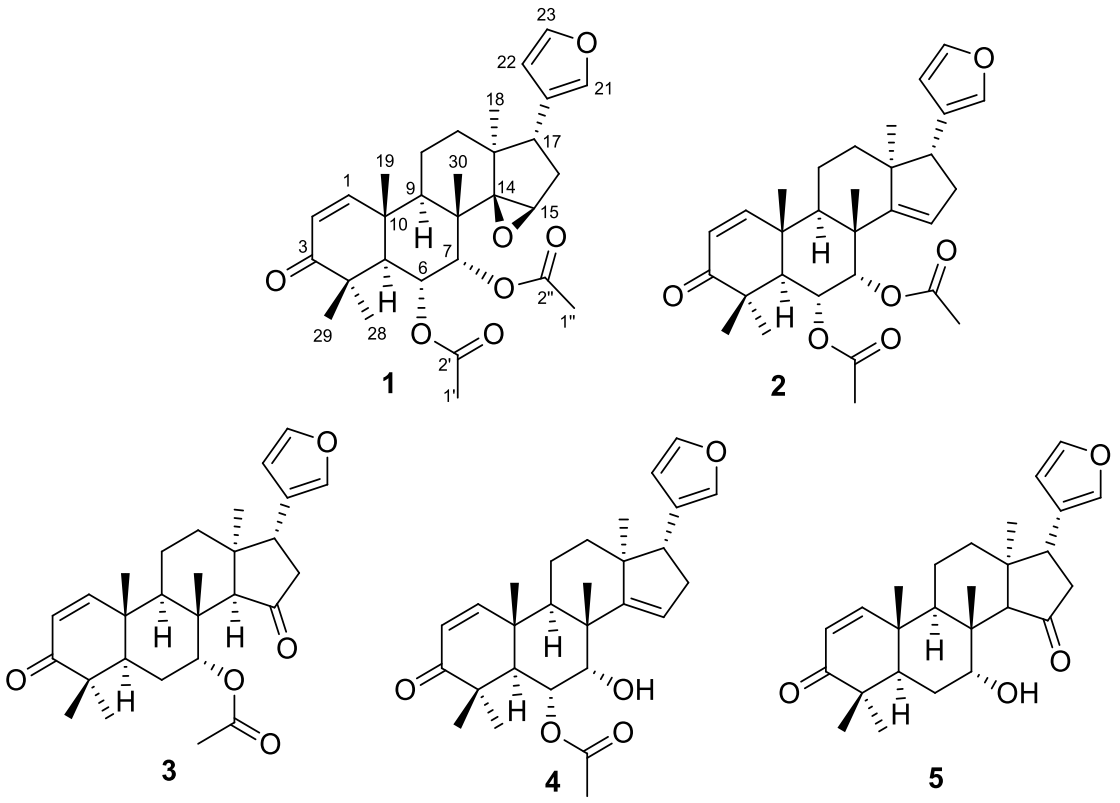

Figure 1. Structures of Compounds **1-5**

Determination of Cytotoxic Activity

The cytotoxicity of compounds **1-5** was measured with a cell viability test by PrestoBlue® assay. The cells were maintained in a Roswell Park Memorial Institute (RPMI) medium with 10% (v/v) Fetal Bovine Serum (FBS) and 1 µL/1 mL antibiotics (1% Penicillin-Streptomycin). Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2. MCF-7 cells plated in 96 multi-well culture plates at a density of 1.7×10^4 cells/well. After 24 h, the 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 µg/mL and control was added. After incubation with the sample for 48h, PrestoBlue® reagent (resazurin dye) was added. The PrestoBlue® assay results were read using a 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 by 50% the number of viable cells and the absorbance in control corresponds to 100% viability (Nurlelasari et al., 2021).

RESULTS AND DISCUSSIONS

Dried and grinded fruit of *C. lasiocarpus* (1.2 kg) was extracted with methanol and further evaporated to afford dark brown residue (270 g) and fractionated successively with *n*-hexane, EtOAc and *n*-butanol. The *n*-hexane extract (36.5 g) was subjected to several chromatography procedures including vacuum-liquid chromatography (VLC), column chromatography and crystallization, the separation process was observed by TLC using silica gel GF_{254} to afford five limonoid compound **1-5** (**Figure 1**).

Compound **1** was obtained as a colorless needle crystal (MeOH), having the molecular formula of $C_{30}H_{38}O_7$ based on analysis of HR-TOFMS spectra with peak ion molecule $[M+H]^+$ *m/z* 511.2693 (calcd. for $C_{30}H_{39}O_7$, *m/z* 511.2696), with twelve degrees of unsaturation. The UV spectrum showed maximum absorption at 230 nm (log ε 2.63), suggesting the presence of an enone group. The IR spectrum showed the presence of aliphatic (2922 cm^{-1}), carbonyl ester (1739 cm^{-1}), carbonyl (1775 cm^{-1}) and ether group (1030 cm^{-1}). The ^{1}H-NMR spectrum displayed seven tertiary methyl singlets, consisting of five from limonoid skeleton and two from acetoxy group δ_H 0.95 (3H, s, CH_3-18), 1.15 (3H, s, CH_3-19), 1.17 (3H, s, CH_3-28), 1.22 (3H, s, CH_3-29), 1.24 (3H, s, CH_3-30), 2.11 (3H, s, CH_3-1') and 2.01 (3H, s, CH_3-1"), five olefinic protons δ_H 5.92 (1H, d, *J*= 10.5, H-2), 6.15 (1H, d, *J*=1.5 Hz, H-22), 7.14 (1H, d, *J*=10.5 Hz, H-1), 7.09 (1H, s, H-21), and 7.36 (1H, t, *J*=1.5 Hz, H-23), three oxymethines δ_H 5.35 (1H, dd, *J*= 2.5, 12.5 Hz, H-6), 5.03 (1H, d, *J*= 2.5 Hz, H-7) and 3.43 (1H, m, H-15). The ^{13}C NMR and DEPT 135° spectra revealed 30 carbon signals, exhibited the presence of five quaternary methyl groups that is a characteristic of intact limonoid at δ_C 21.5 (C-18), 21.8 (C-19), 31.6 (C-28), 20.1 (C-29), 18.8 (C-30), and two additional quaternary methyl groups came from two acetoxy group at δ_C 21.2 (C-1') and 21.1 (C-1"). The ^{13}C-NMR spectrum also showed signals at δ_C 157.6 (C-1), 126.1 (C-2) and 204.6 (C-3), a characteristic of an α,β-unsaturated ketone at the ring A. In addition, signals at 123.6 (C-20), 139.5 (C-21), 110.8 (C-22) and 142.9 (C-23) is a typical peak for a furan side chain. Four oxygenated carbon signals at δ_C 70.1 (C-6), 73.5 (C-7), 72.7 (C-14) and 57.1 (C-15), two oxygenated carbons are the carbon that bond to the acetoxy group and the signal at chemical shift region of C-14 (δ_C 72.7) and C-15 (δ_C 57.1) inferred a pair of carbon signal for epoxide ring. The functionalities accounted for seven out of the twelve degrees of unsaturation, and five degrees of unsaturation belongs to pentacyclic of limonoid skeleton (Nurlelasari et al., 2021; Wong et al., 2011; Yang, Wang, Luo, Wang, & Kong, 2012). The NMR data of compound **1** resembled a similar result with reported for compound 6*α*-(acetoxy)-14β,15β-epoxyazadirone that isolated from seeds of *Toona ciliata* (Neto *et al*., 1995). Therefore, the structure of compound **1** was identified as 6*α*-(acetoxy)-14β,15β-epoxyazadirone.

To determine the stereochemistry of the substituents at C-6 and C-7 besides from NOESY experiment, it also can be defined based on its vicinal coupling constant, the vicinal coupling constant for proton at C-5, C-6 and C-7 is (J_{HH} =12.3 Hz), (J_{HH} =12.3 and 2.5 Hz), and (J_{HH} = 2.5 Hz) respectively. The orientation of H-5 based on biosynthesis approach is β-oriented with axial position, the large vicinal coupling constant (J_{HH} =12.3 Hz) indicate axial-axial correlation (vicinal coupling constant correlation value J_{aa}= 7-12 Hz; J_{ee}= J_{ae} = 2-5 Hz), inferred the proton on C-6 at the axial position which is β-orientation and the acetoxy group at the opposite plane at α-orientation. The vicinal coupling constant between H-6 and H-7 have small value (J_{HH} =2.5 Hz) that indicate axial-equatorial correlation and it inferred H-7 at the equatorial position (β-orientation) and the acetoxy group at axial position at α-orientation.

Compound **2** was obtained as a white crystal (MeOH) with melting point, 210-213 °C. The molecular formula was identified to be $C_{30}H_{38}O_6$ with twelve degrees of unsaturation based on analysis of HR-TOFMS spectra with peak ion molecule $[M+H]^+$ *m/z* 495.2743 (calcd. *m/z* 495.2747 for $C_{30}H_{39}O_6$). The IR and UV spectra of compound **2** exhibited similar spectral data to compound **1**, and the ^{1}H-NMR spectrum displayed seven tertiary methyl singlets δ_H 0.79 (3H, s, CH_3-18), 1.18 (3H, s, CH_3-19), 1.18 (3H, s, CH_3-28), 1.25 (3H, s, CH_3-29), 1.32 (3H, s, CH_3-30), 2.04 (3H, s, CH_3-1') and

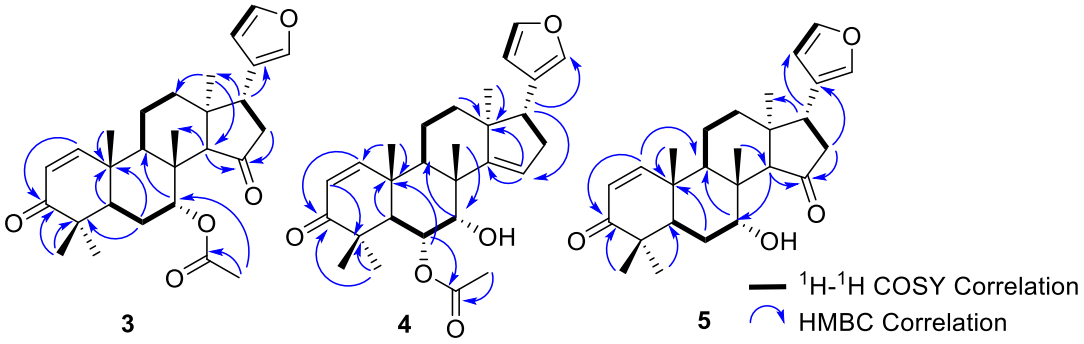

Figure. 2 Selected HMBC and ^{1}H-1H COSY correlation of compound **3**-**5**.

2.00 (3H, s, CH_3-1''), but different from compound **1**, compound **2** have six olefinic protons at δ_H 7.14 (1H, d, *J*=10.0 Hz, H-1), 5.91 (1H, d, *J*= 10.0, H-2), 7.23 (1H, s, H-21), 6.27 (1H, d, *J*=1.5 Hz, H-22), 7.37 (1H, t, *J*=1.5 Hz, H-23), 5.42 (1H, d, *J*= 3.0 Hz, H-15), and only two oxygenated methine proton δ_H 5.42 (1H, d, *J*= 3.0 Hz, H-7) and 5.37 (1H, m, H-6), indicating one oxygenated group was changed into double bond group. The ^{13}C-NMR and DEPT 135° spectra revealed 30 carbon signals, including one ketone δ_C 204.6 (C-3), two ester δ_C 170.1 (C-2') and 170.2 (C-2''), unlike compound **1**, compound **2** have eight *sp*^{2} carbons δ_C 158.1 (C-14), 157.2 (C-1), 142.6 (C-23), 139.7 (C-21), 126.1 (C-2), 124.4 (C-20), 119.7 (C-15), and 110.9 (C-22) while the four of them represent furan group, seven methyl groups δ_C 26.7 (C-18), 31.6 (C-19), 20.4 (C-28), 20.7 (C-29), 20.8 (C-30), 21.4 (CH_3-1') and 21.0 (CH_3-1''), two oxygenated methines δ_C 74.5 (C-7) and 69.9 (C-6), three methines, four quaternary carbons, and three methylenes. A comparison of the NMR data of **2** with dysobinin isolated from fruit of *Dysoxylum binectariferum* (Singh, Garg & Khanna, 1976) inferred that the two compounds were similar, compound **2** was identified as dysobinin.

Compound **3** was obtained as a white crystal (MeOH). The molecular formula was identified as $C_{28}H_{36}O_5$ with eleven degrees of unsaturation based on analysis of HR-TOFMS spectra with peak ion molecule $[M+H]^+$ *m/z* 475.2462 (calcd. *m/z* 475.2460 for $C_{28}H_{36}O_5Na$). The UV spectrum showed maximum absorption at 230 nm (log ε 2.68), indicating the presence of an enone group. The IR spectrum showed the presence of carbonyl ester (1722 cm^{-1}) and an α,β-unsaturated carbonyl (1662 cm^{-1}) functionalities. The ^{1}H-NMR spectrum displayed six tertiary methyl singlets, five from intact limonoid and one from acetoxy group δ_H 0.78 (3H, s, CH_3-18), 1.13 (3H, s, CH_3-19), 1.05 (3H, s, CH_3-28), 1.15 (3H, s, CH_3-30), 1.06 (3H, s, CH_3-29), and 2.08 (3H, s, CH_3-1'), five olefinic protons δ_H 7.15 (1H, d, *J*= 10.0 Hz, H-1) and 5.88 (1H, d, *J*= 10.0 Hz, H-2), a typical proton for ring A 1-en-3 one, 7.28 (1H, s, H-21), 6.29 (1H, t, *J*=1.5 Hz, H-22), and 7.39 (1H, t, *J*= 1.5 Hz, H-23), the three proton signals that belong to furan group and one oxygenated methine proton δ_H 4.96 (1H, d, *J*= 2.5 Hz, H-7). The ^{13}C NMR and DEPT 135° spectra revealed 28 carbon signals (**Table. 1**). In the HMBC spectrum of compound **3**, cross peak from H-1 (δ_H 7.15) to C-3 (δ_C 204.6) and C-10 (δ_C 39.5) and from H-28 (δ_H 1.05) and H-29 (δ_H 1.06) to C-3 (δ_C 204.6) support the the presence of α,β-unsaturated carbonyl in ring-A. The carbonyl at position C-15 was indicated by the correlation on the COSY spectra according for position of ring D that only showed between H-17 (δ_H 3.48) and H-16 (δ_H 2.50), and cross peak shown from the HMBC spectra of H-14 (δ_H 2.43) to C-15 (δ_C 218.8), H-16 (δ_H 2.50) to C-15 (δ_C 218.8) and H-17 (δ_H 3.48) to C-21 (δ_C 140.2). Additionally, one acetoxy group located at C-7 was revealed by the COSY correlation between H-5 (δ_H 2.07), H-6 (δ_H 1.89) and H-7 (δ_H 4.96) and cross peak from proton CH_3-1' (δ_H 2.08) to C-2' (δ_C 169.4) and C-7 (δ_C 73.2) (**Figure 2**). The NMR spectra of **3** were similar with that reported 7*α*-acetylneotricilenone that was isolated previously from seeds of *Azadirachta indica* (Kraus et al., 1981), therefore, compound **3** was identified as a 7*α*-acetylneotricilenone.

Compound **4** was isolated as a colorless crystal (MeOH) and the molecular formula of $C_{28}H_{36}O_5$ was identified based on analysis of HR-TOFMS spectra with peak ion molecule $[M+H]^+$ *m/z* 475.2462 (calcd. *m/z* 475.2460 for $C_{28}H_{36}O_5Na$) with eleven degrees of unsaturation. The spectra of compound **2** exhibited similar spectral data to compound **4**, therefore, it is possible to assign the ^{1}H and ^{13}C-NMR signal of rings A - D and the signal of furan, methyl and C-6 acetoxy by comparison of the spectra. The presence of the acetoxy at C-6 was indicated from the proton signal at δ_H 5.46 (1H, dd, *J*= 2.0, 12.0 Hz, H-6) that from the COSY spectra showed correlation with proton at δ_H 2.74 (1H, d, *J*= 12.0 Hz, H-5) and 4.07 (1H, d, *J*= 2.0 Hz, H-7), and in the HMBC spectrum showed cross peak from H-6 (δ_H 5.46) to C-5 (δ_C 46.3), C-10 (δ_C 41.0) and C-7 (δ_C 170.4). Furthermore, the hydroxyl at C-7 was

inferred by the cross peak from H-7 (δ_H 4.07) to C-9 (δ_C 35.6) and C-30 (δ_C 26.5) (**Figure 2**). The stereochemistry of the hydroxyl and acetoxy group was determined by NOESY experiment, the spectrum showed correlation between H-6/H-7 and CH_3-19/CH_3-30, therefore H-6/H-7 was at the same plane as CH_3-19/CH_3-30 at β-oriented. Thus, the hydroxyl and acetoxy group was *α*-oriented. The NMR data of compound **4** compared with 6*α*-O-acetyl-7-deacetylnimocinol that was isolated from leaves of *Azadirachta indica* (Siddiqui et al., 2000) showed similar data. Therefore, the structure of compound **4** was inferred as 6*α*-O-acetyl-7-deacetylnimocinol.

Compound **5** was obtained as a white needle crystal (MeOH) and the molecular formula of $C_{26}H_{34}O_5$ was identified based on analysis of HR-TOFMS spectra with peak ion molecule $[M+H]^+$ *m/z* 433.2353 (calcd. *m/z* 433.2355 for $C_{28}H_{36}O_5Na$), therefore ten degrees of unsaturation are required. The spectra of compound **5** exhibited similar spectral data to compound **3**. The ^{1}H-NMR spectrum showed the difference of the absence of singlet signal worth three proton a typical methyl for acetyl group signal at δ_H 2.10 ppm. It was supported by the peak ion molecule of **5** [*m/z* 433.2355; $C_{26}H_{34}O_4Na$] and **3** [*m/z* 475.2460; $C_{28}H_{36}O_5Na$] that inferred the lost of acetyl group for compound **5**. Furthermore, the hydroxyl group bond to C-7 (δ_C 69.9) was inferred by its correlation based on COSY experiment that showed cross peak between H-7 (δ_H 3.89) to H-6 (δ_H 1.94 and 1.72) and H-6 to H-5 (δ_H 2.27). From these revealed data indicated that compound **5** is a neotrichilenone derivative limonoid. Therefore, based on the comparison with NMR data of 7*α*-hyroxyneotricilenone from *Trichilia havanensis* (Chan, Gibbs, & Taylor, 1967), the structure of compound **5** was define as 7*α*-hyroxyneotricilenone.

The cytotoxic activity against MCF-7 breast cancer cell line of these isolated compounds (**1**-**5**) were evaluated and cisplatin was used as a positive control based on the method previously reported (Skehan et al., 1990; Supratman *et al*., 2020; Supriatno et al., 2018) and the results shown in **Table 2**.

Table 1. ^{13}C-NMR Data for compound **1**-**5**

Carbon Position	Compounds				
	1	**2**	**3**	**4**	**5**
	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)
1	157.6 (d)	157.2 (d)	157.9 (d)	157.2 (d)	158.2 (d)
2	126.1 (d)	126.1 (d)	125.8 (d)	126.0 (d)	125.8 (d)
3	204.6 (s)	204.6 (s)	204.6 (s)	204.9 (s)	205.1 (s)
4	42.8 (s)	44.9 (s)	44.0 (s)	44.9 (s)	44.2 (s)
5	48.4 (d)	47.8 (d)	45.0 (d)	46.3 (s)	43.7 (d)
6	70.1 (d)	69.9 (d)	22.2 (t)	71.4 (d)	25.3 (t)
7	73.5 (d)	74.5 (d)	73.2 (d)	73.5 (d)	69.9 (d)
8	41.8 (s)	42.9 (s)	40.9 (s)	44.8 (s)	42.4 (s)
9	39.3 (d)	37.2 (d)	46.9 (d)	35.6 (d)	45.9 (d)
10	40.5 (s)	40.7 (s)	39.5 (s)	41.0 (s)	39.4 (s)
11	16.3 (t)	16.4 (t)	17.6 (t)	16.2 (t)	17.7 (t)
12	29.1 (t)	34.5 (t)	34.2 (t)	32.2 (t)	34.1 (t)
13	45.1 (s)	47.1 (s)	42.1 (s)	47.2 (s)	42.1 (s)
14	72.7 (s)	158.1 (s)	61.1 (d)	160.0 (s)	62.0 (d)
15	57.1 (d)	119.7 (d)	218.9 (s)	120.5 (d)	221.4 (s)
16	32.0 (t)	32.6 (t)	43.1 (t)	34.3 (t)	43.2 (t)
17	38.9 (d)	51.5 (s)	37.8 (d)	51.6 (t)	37.9 (d)
18	21.5 (q)	26.7 (q)	27.8 (q)	20.5 (q)	27.7 (q)
19	21.8 (q)	31.6 (q)	19.3 (q)	20.6 (q)	19.1 (q)
20	123.6 (s)	124.4 (s)	122.7 (s)	124.1 (s)	122.8 (s)
21	139.5 (d)	139.7 (d)	140.7 (d)	139.7 (d)	140.1 (d)
22	110.8 (d)	110.9 (d)	110.7 (d)	110.9 (d)	110.7 (d)
23	142.9 (d)	142.6 (d)	142.9 (d)	142.7 (d)	142.9 (d)
28	31.6 (q)	20.4 (q)	27.3 (q)	20.6 (q)	21.3 (q)
29	20.1 (q)	20.7 (q)	21.1 (q)	31.6 (q)	27.4 (q)
30	18.8 (q)	20.8 (q)	18.1 (q)	26.5 (q)	18.6 (q)
2′	169.9 (s)	170.1 (s)	169.4 (s)	170.4 (s)	
1′	21.2 (q)	21.4 (q)	21.2 (q)	21.7 (q)	
2″	170.0 (s)	170.2 (s)			
1″	21.1 (q)	21.0 (q)			

($CDCl_3$, ^{13}C-NMR 125 MHz)

Table 2. Cytotoxic activity of compounds **1**-**5** against MCF-7 breast cancer line

Compounds	IC_{50} (μM)
1	3201.8
2	119.5
3	3609.6
4	7283.5
5	53.4
Cisplatin*	15.9

*positive control

Compound **5** showed the best cytotoxic activity against MCF-7 breast cancer cell with IC_{50} value of 53.4 µM, classified as weak activity. The result indicating that the presence of hydroxyl group at C-7 of compound **5** can increase the cytotoxic activity. Furthermore, compound **1**-**4** based on the IC_{50} value classified as no active compounds against MCF-7 breast cancer cells, and suggesting the presence of epoxy ring and ketone group can decrease activity of each compound.

CONCLUSIONS

Five azadirone-type limonoids, 6*α*-(acetoxy)-14*β*,15*β*-epoxyazadirone (**1**), dysobinin (**2**), 7*α*-acetylneotricilenone (**3**), 6*α*-O-acetyl-7-deacetylnimocinol (**4**), and 7*α*-hyroxyneotricilenone (**5**), were isolated from the *n*-hexane of the fruit of *Chisocheton lasiocarpus*. Compound **5** (7*α*-hyroxyneotricilenone) showed strongest cytotoxic activity with IC_{50} values of 53.4 μM suggesting that hydroxyl group at C-7 can increase the cytotoxic activity, but the presence of epoxy ring and ketone group can decrease activity in azadirone-type limonoid.

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