Cytotoxic Constituents from the Stem Bark of *Chisocheton pentandrus*

Rurini Retnowati[1],*, Hermin Sulistyarti[1], Nikmatus Zahro Wahidah[1], Anisa Lailatusy Syarifah[2], Suprianto Salam[3], Nurlelasari[3], Agus Safari[3], Desi Harneti[3], Mulyadi Tanjung[4], Ace Tatang Hidayat[3,5], Rani Maharani[3,5], Unang Supratman[3,5], and Yoshihito Shiono[6]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang 65145, Indonesia*
[2]*Academy of Pharmacy of Putra Indonesia, Malang 65123, Indonesia*
[3]*Department of Chemistry, Faculty of Mathematics and Natural Sciences,*
Universitas Padjadjaran, Jatinangor 45363, Indonesia
[4]*Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60286, Indonesia*
[5]*Central Laboratory, Universitas Padjadjaran, Jatinangor 45363, Indonesia*
[6]*Department of Food, Life, and Environmental Science, Faculty of Agriculture,*
Yamagata University, Tsuruoka, Yamagata 997-8555, Japan.

Abstract – Eight cytotoxic constituents, consisting of six triterpenoids, cabralealactone (**1**), cabraleadiol (**2**), prototiamin A (**3**), 23-desmethyllimocin B (**5**), melianodiol (**7**) and indicalilacol (**8**) along with one limonoid, neemfruitins A (**4**) and one protolimonoid, protoxylocarpin G (**6**), were isolated from the extract of *n*-hexane of the stembark of *Chisocheton pentandrus*. The chemical structures were identified on the basis of spectroscopic evidence and compared to previously reported spectra. These isolated compounds appear for the first time in the plant. Compounds **1 - 8** were evaluated for their cytotoxic effect against MCF-7 breast cancer lines *in vitro*. Among the isolated compounds, melianodiol (**7**) showed the strongest cytotoxic activity with IC$_{50}$ values of 16.8 μM.
Keywords – *Chisocheton pentandrus*, MCF-7 cell lines, Meliaceae, limonoid, triterpenoids

Introduction

The genus *Chisocheton* has approximately 150 species that are widely distributed throughout the tropical regions such as Thailand, Malaysia, and Indonesia.[1,2] Many *Chisocheton* plants have been traditionally used by local people to treat various diseases,[3,4] and previous studies on the chemical constituents of the this genus have led to the isolation and determination of a number of compounds, such as triterpenoids[5-7] and limonoids.[8-12] In addition, biological effects of the constituents have been reported, such as cytotoxic, anti-inflammatory, antifungal, antimalarial and antimycobacterial effects.[2,8,13-15] During the course of our continuing search for anticancer substances from Indonesia *Chisocheton* plants,[1,7,16,17] the methanol extract of the stem bark of *Chisocheton pentandrus* was found to inhibit significant cytotoxic against MCF-7 breast cancer lines.

C. pentandrus is distributed in Sulawesi and Sumatera islands of Indonesia and previous investigation have led to the isolation of cytotoxic triterpenoids and limonoids.[17,18] In the further search for cytotoxic constituent from this plant, we investigated the *n*-hexane extract of the bark of *C. pentandrus* and obtained six triterpenoids and two limonoids (Fig. 1). The cytotoxic effects of these compounds against MCF-7 breast cancer cells were evaluated. Here, we described the structural elucidation of the isolates and their cytotoxic activity.

Experimental

General experimental procedures – The IR spectra were obtained in KBr on a SHIMADZU IR Prestige-2. Mass spectra were measured with a Water QTOF-HR-TOFMS-XEV$^{\text{otm}}$ mass spectrometer. NMR spectra was recorded using a JEOL ECZ-500 and ECZ-600 spectrometer using tetramethyl silane (TMS) as an internal standard. Furthermore, chromatographic separations was carried out on silica gel 60 (70-230 mesh and 230-400 mesh, Merck). The PTLC glass plates were precoated

*Author for correspondence
Rurini Retnowati, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang 65145, Indonesia
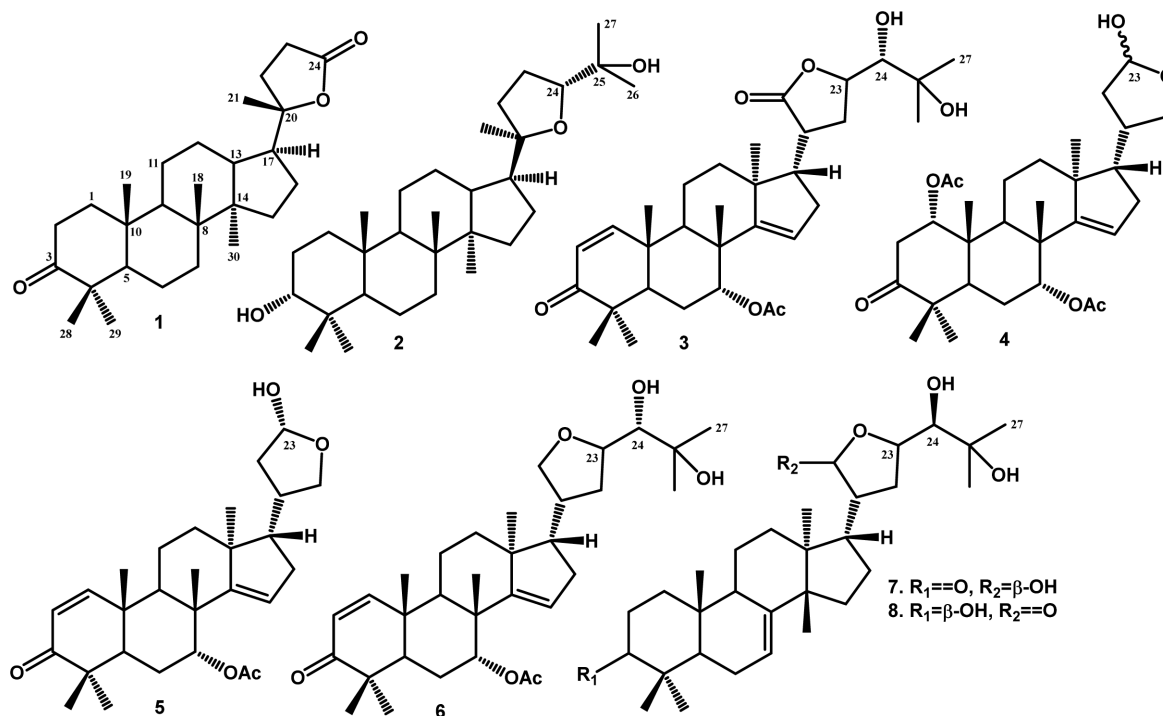Tel: +62-341-361876; E-mail: rretnowati@ub.ac.id;
unang.supratman@unpad.ac.id

Fig. 1. Chemical structures of compounds **1 - 8**.

with silica gel GF$_{254}$ (Merck, 0.25 mm). Similarly, the TLC plates were precoated with silica gel GF$_{254}$ (Merck, 0.25 mm), and the detection was carried out using 10% H$_2$SO$_4$ in ethanol, followed by heating.

Plant materials – The stem bark of *C. pentandrus* was collected at Bogor Botanical Garden, West Java Province, Indonesia in June 2016. The plant was identified by the staff of the Herbarium Bogoriense, Bogor and an exhibition specimen (No. Bo-104) was deposited in the herbarium.

Extraction and isolation – The dried stem bark of *C. pentandrus* (1.8 kg) was grounded to powder and extracted with methanol (4 × 4 L, 4 days each) at room temperature, and concentrated using a rotary evaporator, yielding a concentrated extract (340 g). Furthermore, about 300 g of the methanol extract was suspended in water (600 mL) and successively partitioned with *n*-hexane, ethyl acetate and *n*-butanol. The mixture was then evaporated under reduced pressure, resulting in 10.9, 25.2, and 228.6 g of crude extracts, respectively. In addition, 10.0 g of the *n*-hexane fraction was column chromatographed (CC) using silica gel (70-230 mesh), and eluted with *n*-hexane-ethyl acetate-methanol (10% stepwise), resulting to eight fraction groups (A-H).

Fraction B (439 mg) was subjected to silica gel CC (50 g, 230-400 mesh) and eluted with *n*-hexane-dichloro-

methane-ethyl acetate (5% stepwise) to give seven sub-fraction groups (B1-B7). Subsequently, part of the group, B3 (230 mg) was purified by crystallization in methanol to yield **1** (86.2 mg), while subfraction B7 (132.3 mg) was chromatographed using silica gel CC (10 g, 230-400 mesh) with gradient elution of *n*-hexane- dichloromethane-ethyl acetate (5% stepwise) to produce seven subfractions (B7A-B7F). Therefore, subfraction B7B (13.2 mg) was separated over silica gel CC (5 g, 230-400 mesh), and eluted with *n*-hexane: dichloromethane (1:1) to yield **2** (2.3 mg). Similarly, subfraction B7C (43.2 mg) was separated over silica gel CC (6 g, 230-400 mesh), and eluted with *n*-hexane-dichloromethane-ethyl acetate (5: 4.5:0.5) to produce **3** (14.2 mg), while Fr. B7D (20.1 mg) was purified by silica gel CC (5 g, 230-400 mesh), and eluted with *n*-hexane: dichloromethane (6:4) to generate **4** (8.1 mg).

Fraction C (700 mg) was separated over silica gel CC (70 g, 70-230 mesh) with a gradient eluent of *n*-hexane-dichloromethane-ethyl acetate (5% stepwise) to produce five subfractions (C1-C5). Therefore, subfraction C3 (300 mg) was further separated over silica gel CC (30 g, 230-400 mesh), and eluted with *n*-hexane: dichloromethane (4:1) to yield four subfractions (C3A-C3D). Subsequently, subfraction C3C (110 mg) was purified by silica gel CC (15 g, 230-400 mesh), and eluted with dichloromethane :

ethyl acetate (7.5:2.5) to generate **5** (14.2 mg), similarly, subfraction C3D (14.5 mg) was separated over silica gel CC (5 g, 230-400 mesh), and eluted with chloroform: ethyl acetate (6:4) for the production of **6** (1.9 mg).

Fraction D (725 mg) was separated on CC silica gel (80 g, 70-230 mesh) using an elution gradient consisting of *n*-hexane-chloroform-ethyl acetate (5% stepwise) to generate seven sub-fractions (D1-D7). Furthermore, sub-fraction D4 (141.5 mg) was separated with CC (15 g, 230-400 mesh), and eluted with *n*-hexane: chloroform : ethyl acetate (7:1.5:1.5), to yield five subfractions (D4A-D4E), while subfraction D4C (41.2 mg) was purified on CC (5 g, 230-400 mesh), and eluted with dichloromethane-chloroform-ethyl acetate (6.0:1.5:2.5) to produce **7** (11.2 mg) and eluted with dichloromethane: ethyl acetate (7.5:2.5) to obtain **8** (2.1 mg).

Cabralealactone (**1**) – colorless crystals; m.p. 138-140 °C; IR (KBr) v_{max} cm^{-1}: 2937, 2870, 1754, 1720, 1464, 1379, 1280, 1056; ^{1}H-NMR (CDCl$_3$, 500 MHz): δ$_H$ 2.52 (1H, d, J = 10 Hz, H-23a), 2.01 (1H, m, H-22b), 1.95 (1H, m, H-5), 1.91 (1H, m, H-12b), 1.90 (1H, m, H-15a), 1.62 (1H, d, J = 10 Hz, H-23b), 1.56 (1H, m, H-7b), 1.53 (1H, m, H-13), 1.52 (1H, m, H-16a), 1.51 (1H, m, H-11b), 1.50 (1H, m, H-1b), 1.49 (1H, m, H-12a), 1.47 (1H, m, H-22a), 1.47 (1H, m, H-2b), 1.41 (1H, dd, J = 2.4, 13.2 Hz, H-9), 1.40 (1H, m, H-2a), 1.39 (1H, m, H-7a), 1.37 (1H, m, H-6b), 1.33 (3H, s, CH$_3$-21), 1.32 (1H, m, H-6a), 1.23 (1H, m, H-17), 1.20 (1H, m, H-11a), 1.17 (1H, m, H-1a), 1.15 (1H, m, H-16b), 1.10 (1H, m, H-15b), 0.92 (3H, s, CH$_3$-18), 0.91 (3H, s, CH$_3$-28), 0.87 (3H, s, CH$_3$-30), 0.82 (3H, s, CH$_3$-19), 0.81 (3H, s, CH$_3$-29); ^{13}C-NMR (CDCl$_3$, 125 MHz), Table 1; HR-TOFMS, *m/z* 415.3311 [M+H]$^+$, (calcd. C$_{27}$H$_{42}$O$_3$ *m/z* 414.3134).

Cabraleadiol (**2**) – colorless needle crystals; m.p. 178-180°C; v_{max} cm^{-1}: 3450, 2942, 1471, 1387, 1075; ^{1}H-NMR (CDCl$_3$, 500 MHz): δ$_H$ 4.33 (1H, ddd, J = 2.4, 6.8, 9.6 Hz, H-3), 3.62 (1H, dd, J = 4.8, 10.2 Hz, H-24), 2.62 (1H, m, H-23b), 2.01 (1H, m, H-22b), 1.90 (1H, m, H-15b), 1.85 (1H, m, H-23a), 1.83 (1H, m, H-17), 1.75 (1H, m, H-12b), 1.62 (1H, m, H-13), 1.55 (1H, m, H-2b), 1.53 (1H, m, H-11b), 1.51 (1H, m, H-16b), 1.50 (1H, m, H-7b), 1.49 (1H, m, H-12a), 1.46 (1H, m, H-16a), 1.44 (1H, dd, J = 2.4, 13.2 Hz, H-9), 1.42 (1H, m, H-1b), 1.39 (1H, m, H-6b), 1.38 (1H, m, H-2a), 1.34 (1H, m, H-7a), 1.32 (1H, m, H-6a), 1.24 (1H, m, H-5), 1.22 (1H, m, H-22a), 1.20 (1H, m, H-11a), 1.17 (1H, m, H-1a), 1.17 (3H, s, CH$_3$-26), 1.13 (3H, s, CH$_3$-21), 1.09 (3H, s, CH$_3$-27), 1.04 (1H, m, H-15a), 0.95 (3H, s, CH$_3$-18), 0.92 (3H, s, CH$_3$-28), 0.87 (3H, s, CH$_3$-30), 0.84 (3H, s, CH$_3$-19), 0.82 (3H, s, CH$_3$-29); ^{13}C-NMR (CDCl$_3$, 125 MHz), Table 1; HR-

TOFMS *m/z* 461.3271 [M+H]$^+$, (calcd. C$_{30}$H$_{52}$O$_3$ *m/z* 460.3916).

Prototiamin A (**3**) – White amorphous powder; IR (KBr) v_{max} cm^{-1}: 3430, 2945, 1720, 1639, 1456, 1387, 1074, 847; ^{1}H-NMR (CDCl$_3$, 600 MHz): δ$_H$ 7.14 (1H, d, J = 10.2 Hz, H-1), 5.86 (1H, d, J = 10.2 Hz, H-2), 5.28 (1H, br.s, H-15a), 5.05 (1H, m, H-7), 4.47 (1H, dd, J = 9.6, 1.8 Hz, H-23), 3.92 (1H, d, J = 9.6 Hz, H-24), 2.71 (1H, m, H-20), 2.53 (1H, m, H-6b), 2.25 (1H, m, H-6a), 2.20 (1H, m, H-5), 2.19 (1H, m, H-17), 2.19 (1H, m, H-9), 2.05 (1H, m, H-22b), 1.94 (3H, s, CH$_3$-2′), 1.82 (1H, m, H-22a), 1.82 (1H, m, H-16b), 1.76 (1H, m, H-12b), 1.63 (1H, m, H-13), 1.57 (1H, m, H-12a), 1.50 (1H, m, H-11b), 1.44 (3H, s, CH$_3$-27), 1.35 (3H, s, CH$_3$-26), 1.22 (1H, m, H-11a), 1.17 (1H, m, H-16a), 1.16 (3H, s, CH$_3$-30), 1.16 (3H, s, CH$_3$-18), 1.07 (3H, s, CH$_3$-19), 1.06 (3H, s, CH$_3$-28), 1.06 (3H, s, CH$_3$-29); ^{13}C-NMR (CDCl$_3$, 150 MHz), Table 1. HR-TOFMS, *m/z* 543.1260 [M+H]$^+$, (calcd. C$_{32}$H$_{46}$O$_7$ *m/z* 542.1244).

Neemfruitin A (**4**) – Colorless solid; IR (KBr) v_{max} cm^{-1}: 3360, 2939, 1725, 1710, 1458, 1380, 1109; ^{1}H-NMR (CDCl$_3$, 600 MHz): δ$_H$ 5.34 (1H, t, J = 2.4 Hz, H-1), 5.33 (1H, t, J = 2.4 Hz, H-7), 5.28 (1H, dd, J = 1.8, 3.6 Hz, H-15), 4.57 (1H, dd, J = 9.6, 1.8 Hz, H-23), 4.04 (2H, d, J = 9.6 Hz, H-21), 2.70 (1H, m, H-20), 2.53 (1H, m, H-6b), 2.50 (1H, dd, J = 8.0, 10.4 Hz, H-5), 2.25 (1H, m, H-6a), 2.20 (1H, m, H-9), 2.17 (1H, m, H-22a), 2.16 (1H, m, H-22b), 2.04 (1H, m, H-16a), 1.97 (3H, s, CH$_3$-2″), 1.93 (1H, m, H-2a), 1.93 (1H, m, H-11a), 1.91 (3H, s, CH$_3$-2′), 1.73 (1H, m, H-12b), 1.70 (1H, dt, J = 3.0, 10.2 Hz, H-17), 1.61 (1H, m, H-2b), 1.56 (1H, m, H-11b), 1.54 (1H, m, H-12a), 1.45 (1H, m, H-16b), 1.16 (3H, s, CH$_3$-30), 1.16 (3H, s, CH$_3$-19), 1.09 (3H, s, CH$_3$-29), 1.06 (3H, s, CH$_3$-28), 1.00 (3H, s, CH$_3$-18); ^{13}C-NMR (CDCl$_3$, 150 MHz), Table 1. HR-TOFMS, *m/z* 517.4498 [M+H]$^+$, (calcd. C$_{30}$H$_{44}$O$_7$ *m/z* 516.4087).

23-Desmethyllimocin B (**5**) – white solid; IR (KBr) v_{max} cm^{-1}: 3300, 2949, 1705, 1620, 1457, 1380, 1080; ^{1}H-NMR (CDCl$_3$, 600 MHz): δ$_H$ 7.14 (1H, d, J = 10.2 Hz, H-1), 5.86 (1H, d, J = 10.2 Hz, H-2), 5.33 (1H, t, J = 2.4 Hz, H-7), 5.28 (1H, dd, J = 1.8, 3.6 Hz, H-15), 4.57 (1H, dd, J = 9.6, 1.8 Hz, H-23), 4.04 (2H, d, J = 9.6 Hz, H-21), 2.70 (1H, m, H-20), 2.53 (1H, m, H-6b), 2.50 (1H, dd, J = 8.0, 12.4 Hz, H-5), 2.25 (1H, m, H-6a), 2.20 (1H, m, H-10), 2.17 (1H, m, H-22a), 2.16 (3H, s, CH$_3$-2′), 2.16 (1H, m, H-22b), 2.04 (1H, m, H-16a), 1.93 (1H, m, H-11a), 1.92 (1H, m, H-16b), 1.73 (1H, m, H-12b), 1.70 (1H, dt, J = 3.0, 10.2 Hz, H-17), 1.58 (1H, m, H-11b), 1.54 (1H, m, H-12a), 1.16 (3H, s, CH$_3$-19), 1.06 (3H, s, CH$_3$-29), 1.06 (3H, s, CH$_3$-30), 1.00 (3H, s, CH$_3$-18); ^{13}C-

Table 1. ^{13}C-NMR data for compounds **1 - 8**

Position carbon	Compounds							
	1*	**2***	**3***	**4***	**5****	**6****	**7****	**8****
	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)
1	35.2 (t)	33.7 (t)	158.8 (d)	75.4 (d)	158.8 (d)	158.8 (d)	31.2 (t)	31.2 (t)
2	33.7 (t)	25.4 (t)	125.7 (d)	25.6 (t)	125.7 (d)	125.7 (d)	25.6 (t)	25.6 (t)
3	218.2 (s)	73.2 (d)	204.6 (s)	218.3 (s)	204.6 (s)	204.6 (s)	218.4 (s)	72.4 (d)
4	37.3 (s)	37.3 (s)	44.6 (s)	39.9 (s)	44.6 (s)	44.6 (s)	40.3 (s)	40.3 (s)
5	55.4 (d)	49.6 (d)	46.2 (d)	49.8 (d)	49.8 (d)	46.2 (d)	44.6 (d)	44.6 (d)
6	18.3 (t)	18.3 (t)	27.5 (t)	27.5 (t)	27.5 (t)	27.5 (t)	24.8 (t)	24.8 (t)
7	34.7 (t)	35.4 (t)	75.5 (d)	75.6 (d)	74.4 (d)	34.9 (t)	118.8 (d)	118.8 (d)
8	40.6 (s)	40.7 (s)	42.8 (s)	43.0 (s)	43.0 (s)	42.8 (s)	145.3 (s)	145.3 (s)
9	50.4 (d)	50.7 (d)	37.4 (d)	37.4 (d)	37.4 (d)	37.4 (d)	48.9 (d)	48.9 (d)
10	37.7 (s)	37.7 (s)	39.7 (s)	47.2 (s)	47.2 (s)	39.7 (s)	34.6 (s)	34.6 (s)
11	25.4 (t)	21.7 (t)	34.1 (t)	16.2 (t)	16.2 (t)	34.1 (t)	17.4 (t)	17.4 (t)
12	21.3 (t)	27.1 (t)	38.3 (t)	40.0 (t)	40.0 (t)	38.3 (t)	31.2 (t)	31.2 (t)
13	43.2 (d)	42.8 (d)	46.8 (s)	50.2 (s)	50.2 (s)	46.8 (s)	43.8 (s)	43.8 (s)
14	50.3 (s)	50.2 (s)	157.9 (s)	158.9 (s)	158.9 (s)	157.9 (s)	50.7 (s)	50.7 (s)
15	31.2 (t)	31.5 (t)	119.0 (d)	118.0 (d)	118.0 (d)	119.0 (d)	33.9 (t)	33.9 (t)
16	25.1 (t)	25.9 (t)	33.8 (t)	34.6 (t)	34.6 (t)	33.8 (t)	23.9 (t)	23.9 (t)
17	49.5 (d)	49.8 (d)	58.2 (d)	55.3 (d)	55.3 (d)	58.2 (d)	47.2 (d)	47.2 (d)
18	15.6 (q)	16.2 (q)	16.2 (q)	19.7 (q)	19.7 (q)	16.2 (q)	23.5 (q)	23.5 (q)
19	16.1 (q)	16.6 (q)	19.1 (q)	15.3 (q)	15.3 (q)	19.1 (q)	13.1 (q)	13.1 (q)
20	90.3 (s)	86.7 (s)	44.7 (s)	37.6 (d)	37.6 (d)	44.7 (s)	40.7 (d)	40.7 (d)
21	25.4 (q)	27.3 (q)	178.6 (s)	70.3 (d)	72.5 (d)	72.5 (d)	99.3 (d)	178.3 (s)
22	31.3 (t)	35.3 (t)	33.1 (t)	34.2 (t)	34.2 (t)	33.1 (t)	29.6 (t)	29.6 (t)
23	29.3 (t)	26.4 (t)	78.6 (d)	99.8 (d)	99.2 (d)	78.2 (d)	86.4 (d)	86.4 (d)
24	176.9 (s)	86.3 (d)	72.4 (d)	-	-	74.4 (d)	71.3 (d)	71.3 (d)
25	-	70.3 (s)	70.5 (s)	-	-	72.5 (s)	70.3 (s)	70.3 (s)
26	-	27.9 (q)	26.9 (q)	-	-	26.9 (q)	26.5 (q)	26.5 (q)
27	-	24.1 (q)	23.8 (q)	-	-	23.8 (q)	27.0 (q)	27.0 (q)
28	28.4 (q)	28.4 (q)	20.0 (q)	21.4 (q)	-	20.0 (q)	21.6 (q)	21.6 (q)
29	22.2 (q)	22.2 (q)	21.4 (q)	27.1 (q)	21.4 (q)	21.4 (q)	27.4 (q)	27.4 (q)
30	16.4 (q)	15.6 (q)	27.1 (q)	27.5 (q)	27.1 (q)	27.1 (q)	27.8 (q)	27.8 (q)
1′	-	-	170.1 (s)	170.1 (s)	27.5 (q)	170.1 (s)		
2′	-	-	21.2 (q)	21.1 (q)	170.1 (s)	21.2 (q)		
1″	-	-	-	170.0 (s)				
2″	-	-	-	21.0 (q)				

*Measured in 500 MHz for ^{1}H and 125 MHz for [13]C
** Measured in 600 MHz for ^{1}H and 150 MHz for [13]C

NMR (CD₃OD, 150 MHz), Table 1. HR-TOFMS, *m/z* 455.0712 [M+H] [+], (calcd. $C_{28}H_{40}O_5$ *m/z* 456.2876).

Protoxylocarpin G (**6**) – White solid; IR (KBr) ν_{max} cm^{-1}: 3420, 2949, 1715, 1645, 1457, 1380, 1080; ^{1}H-NMR (CD₃OD, 600 MHz): δ_H 7.14 (1H, d, *J* = 10.2 Hz, H-1), 5.86 (1H, d, *J* = 10.2 Hz, H-2), 5.28 (1H, br.s, H-15), 4.16 (2H, d, *J* = 9.6 Hz, H-23), 3.92 (1H, d, *J* = 9.6 Hz, H-24), 2.71 (H, m, H-20), 2.53 (1H, m, H-6b), 2.25 (1H, m, H-6a), 2.20 (1H, m, H-5), 2.19 (1H, m, H-17), 2.19 (1H, m, H-9), 1.94 (3H, s, CH₃-2′), 1.87 (1H, m, H-16a), 1.78 (1H, m, H-12a), 1.69 (1H, m, H-7b), 1.65 (m, H-7a), 1.57 (1H, m, H-12b), 1.52 (1H, m, H-11a), 1.50 (1H, m, H-11b), 1.44 (3H, s, CH₃-27),1.35 (3H, s, CH₃-26), 1.17 (1H, m, H-16b), 1.16 (3H, s, CH₃-30), 1.16 (3H, s, CH₃-18), 1.01 (3H, s, CH₃-19), 1.06 (3H, s, CH₃-28), 1.06 (3H, s, CH₃-29); ^{13}C-NMR (CD₃OD, 150 MHz)

Table 1. HR-TOFMS, *m/z* 527.2984 [M+H] $^+$, (calcd. $C_{32}H_{48}O_6$ *m/z* 528.2951).

Melianodiol (7) – Colorless needle crystal; m.p. 216-218ºC; IR (KBr) v_{max} cm^{-1} : 3350, 2935, 1725, 1645, 1457, 1380, 1055; ^{1}H-NMR (CDCl$_3$, 600 MHz): δ_H 5.26 (1H, dd, *J* = 2.6, 5.8 Hz, H-7), 4.63 (1H, d, *J* = 5.6 Hz, H-21), 4.05 (1H, br.s, H-24), 3.64 (1H, ddd, *J* = 1.3, 6.2, 8.7 Hz, H-23), 2.67 (1H, ddd, *J* = 5.7, 8.4, 11.7 Hz, H-20), 2.38 (1H, m, H-22a), 2.34 (1H, m, H-17), 2.24 (1H, m, H-9), 2.20 (1H, ddd, *J* = 6.2, 8.4, 11.7 Hz, H-22b), 2.09 (1H, d, *J* = 14.7, H-6a), 1.93 (1H, m, H-2a), 1.78 (1H, m, H-5), 1.76 (1H, m, H-12a), 1.73 (1H, m, H-16b), 1.63 (1H, m, H-11a), 1.61 (1H, m, H-2b), 1.61 (1H, m, H-12b), 1.57 (1H, m, H-16a), 1.53 (1H, m, H-11b), 1.52 (1H, m, H-15b), 1.49 (1H, m, H-1a), 1.41 (1H, m, H-6b), 1.39 (3H, s, CH$_3$-27), 1.38 (1H, dt, *J* = 3.2, 12.2 Hz, H-1b), 1.32 (3H, s, CH$_3$-26), 1.06 (1H, m, H-15a), 1.01 (3H, s, CH$_3$-30), 0.93 (3H, s, CH$_3$-18), 0.93 (3H, s, CH$_3$-28), 0.92 (3H, s, CH$_3$-29), 0.78 (3H, s, CH$_3$-19); ^{13}C-NMR (CDCl$_3$, 150 MHz) Table 1. HR-TOFMS, *m/z* 487.4535 [M+H] $^+$, (calcd. $C_{32}H_{50}O_7$ *m/z* 488.4502).

Indicalilacol B (8) – White solid; IR (KBr) v_{max} cm^{-1}: 3430, 2930, 1760, 1655, 1420, 1378, 1210, 1110; ^{1}H-NMR (CDCl$_3$, 600 MHz): δ_H 5.26 (1H, dd, *J* = 2.6, 5.8 Hz, H-7), 4.63 (1H, d, *J* = 5.6 Hz, H-21), 4.32 (1H, d, *J* = 5.4 Hz, H-3), 4.05 (1H, br.s, H-24), 3.64 (1H, ddd, *J* = 1.3, 6.2, 8.7 Hz, H-23), 2.67 (1H, ddd, *J* = 5.7, 8.4, 11.7 Hz, H-20), 2.38 (1H, m, H-22a), 2.34 (1H, m, H-17), 2.24 (1H, m, H-9), 2.20 (1H, ddd, *J* = 6.2, 8.4, 11.7 Hz, H-22b), 2.09 (1H, d, *J* = 14.7, H-6a), 1.93 (1H, m, H-2a), 1.78 (1H, m, H-5), 1.76 (1H, m, H-12a), 1.73 (1H, m, H-16b), 1.63 (1H, m, H-11a), 1.61 (1H, m, H-12b), 1.61 (1H, m, H-2b), 1.57 (1H, m, H-16a), 1.53 (1H, m, H-11b), 1.52 (1H, m, H-15b), 1.49 (1H, m, H-1a), 1.41 (1H, m, H-6b), 1.39 (3H, s, CH$_3$-27), 1.38 (1H, dt, *J* = 3.2, 12.2 Hz, H-1b), 1.32 (3H, s, CH$_3$-26), 1.01 (3H, s, CH$_3$-30), 0.93 (3H, s, CH$_3$-28), 0.92 (3H, s, CH$_3$-29), 0.84 (3H, s, CH$_3$-18), 0.78 (3H, s, CH$_3$-19); ^{13}C-NMR (CDCl$_3$, 160 MHz) see Table 1. HR-TOFMS, *m/z* 487.4535 [M+H] $^+$, (calcd. $C_{32}H_{50}O_7$ *m/z* 488.4502).

Cytotoxicity assay – The MCF-7 Cells were plated at a density of 5 × 10^3 cells/well in 96-well plate, incubated at 37ºC overnight and treated in triplicate with decreasing concentrations isolated compound from *C. pentandrus* extract. Furthermore, all the extracted were dissolved in DMSO and *Cis*-platin (Sigma) was used as a positive control. After 48 hours of treatment, cell viability was evaluated using the MTT reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was added according to the manufactured protocols, followed by a 4-hour

incubation. The results were expressed as relative viable cells compared to the controls (untreated cells). The 50% inhibitory concentration (IC$_{50}$) values of antiproliferative activity were calculated and converted into the IC$_{50}$ Dose.

Results and Discussion

The methanol extract of the of *C. pentandrus* stem bark was partitioned successfully with *n*-hexane, ethyl acetate and *n*-butanol. Column chromatography was repeated using silica gel of the soluble fraction in *n*-hexane led to the isolation of eight isolated compounds (Fig. 1). The structures of the isolated compounds were identified by spectroscopic methods including 1D, 2D NMR and HR-TOFMS. To the best our knowledge, compounds **1 - 8**, were isolated from *C. pentandrus* for the first time.

Compound **1** was obtained as colorless crystals (MeOH). The molecular composition was determined was $C_{27}H_{42}O_3$ based on HR-TOFMS ([M+H]$^+$ *m/z* 415.3311, calcd. for $C_{27}H_{42}O_3$ *m/z* 414.3334) and NMR analysis (Table 1), and seven degrees of unsaturation is required. Furthermore, IR spectra showed absorption peaks at 2937 and 2870 cm^{-1} (C-H sp^3), 1754 cm^{-1} (C=O), 1720 (C=O, lactone) 1464 cm^{-1} (C=C), 1379-1280 cm^{-1} (*gem*-dimethyl), and 1056 cm^{-1} (C-O groups). The ^{1}H-NMR spectrum showed the presence of six tertiary methyl groups, resonating at δ_H 1.33 (CH$_3$-21), 0.92 (CH$_3$-18), 0.91 (CH$_3$-28), 0.87 (CH$_3$-30), 0.82 (CH$_3$-19) and 0.81 (CH$_3$-29). The aliphatic protons in the upfield region were observed also in the ^{1}H-NMR spectra. The ^{13}C-NMR, HMQC and DEPT 135° spectra of **1** showed the presence of six methyl groups, exhibiting the characteristics of dammarane-type triterpenoid, one oxygenated quaternary carbon at δ_C 90.3 (C-20), one carbonyl lactone at δ_C 176.9 (C-24) and one carbonyl ketone at δ_C 218.2 (C-3). These functions represented two of the total of seven degrees of unsaturation, and the remaining five degrees of unsaturation matched the dammarane-type triterpenoid framework with an addition of a five-membered lactone ring.[19,20] The ^{1}H-^{1}H correlations (Fig. 2) from H-1/H-2, H-5/H-6/H-7, H-9/H-11/H-12/H-13, H-15/H-16/H-17 and H-22/H-23 were aided by the presence of the dammarane-type in compound **1**. The HMBC cross peaks (Fig. 2) from H-1 (δ_H 1.17 and 1.50) and H-2 (δ_H 1.40 and 1.47) to the carbonyl ketone at C-3 (δ_C 218.2) indicated the presence of a ketone group at C-3. Protons signal H-17 (δ_H 1.23) and CH$_3$-21 (δ_H 1.33) were correlated to C-20 (δ_C 90.3), whereas methylene protons at C-23 (δ_H 2.52 and 2.62) was correlated to C-24 (δ_C 176.9) indicated the presence of five-membered lactone ring at C-17. The relative
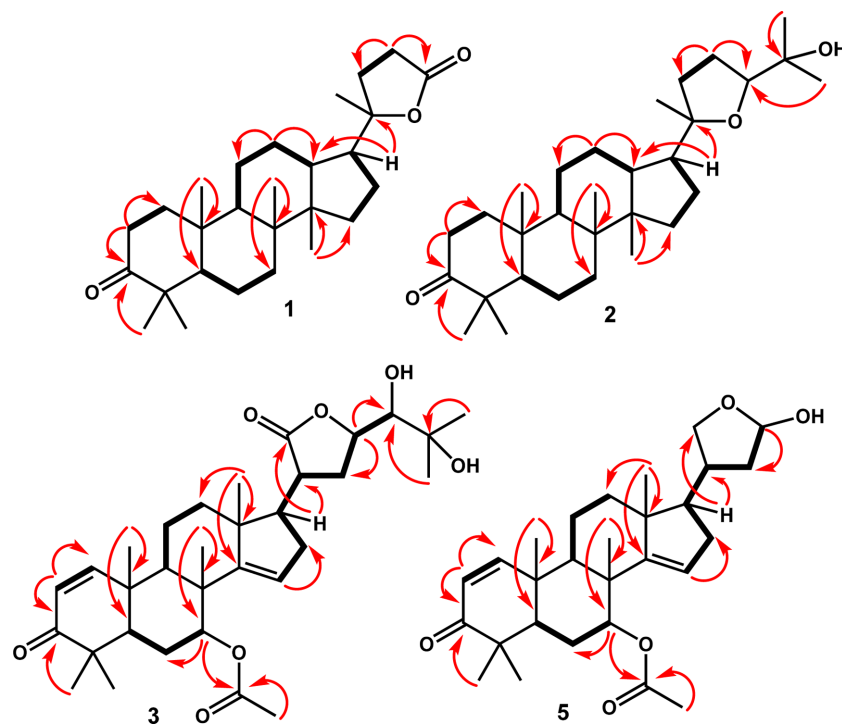
Fig. 2. Selected ¹H-¹H COSY and HMBC correlations of compounds **1**, **2**, **3** and **5**.
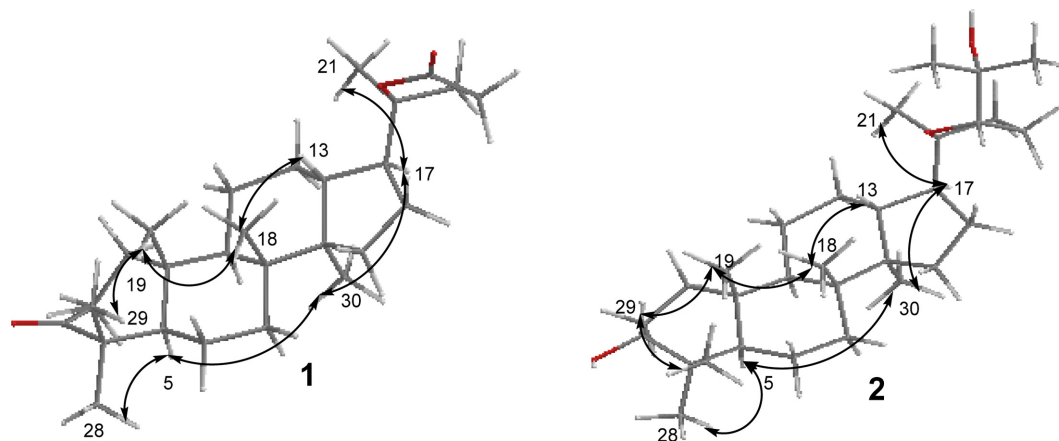

Fig. 3. Selected NOESY correlations for compounds **1** and **2**.

configuration of **1** was determined by NOESY experiments, which is supported by the presence of dammarane-type triterpenoids in *Chisocheton* species.[19,20] The NOESY correlations (Fig. 3) from H-17/CH$_3$-30 identified the γ-lactone ring at C-17 as β-oriented, while the cross peak observed between CH$_3$-30/H-17/CH$_3$-21, indicates the CH$_3$-21 as α-oriented. A comparison of the NMR data of **1** with cabralealactone obtained from the whole plant of *Cleome africana*,[19] revealed that the structures of the two compounds were very similar, consequently, compound **1** was identified as cabralealactone.

Compound **2** was obtained as a colorless needle crystal. Its molecular composition C$_{30}$H$_{52}$O$_3$, was determined from HR-TOFMS spectra (*m/z* 461.3271 [M+H] $^+$, calculated, C$_{30}$H$_{52}$O$_3$ *m/z* 460.3274) and NMR data (Table 1). The IR spectra showed absorption peaks at 3450 cm^{-1} (OH), 2942 cm^{-1} (C-H sp^3), 1471 and 1387 cm^{-1} (*gem*-dimethyl), and 1075 cm^{-1} (C-O) groups. The ¹H-NMR spectrum showed the presence of eight tertiary methyl groups at δ$_H$ 1.17 (CH$_3$-26), 1.13 (CH$_3$-21), 1.09 (CH$_3$-27), 0.95 (CH$_3$-18), 0.92 (CH$_3$-28), 0.87 (CH$_3$-30), 0.84 (CH$_3$-19) and 0.82 (CH$_3$-29) resonances. Two oxygenated

methines resonances at δ_H 4.33 (1H, ddd, $J = 2.4, 6.8, 9.6$ Hz, H-3) and δ_H 3.62 (1H, dd, $J = 4.8, 10.2$ Hz, H-24) were also observed in the ^{1}H-NMR spectra. The ^{13}C-NMR spectra showed 30 carbons and were classified by DEPT 135° and HMQC experiments as eight methyl groups, exhibiting the characteristics of dammarane-type triterpenoid,[19,20] an oxymethine group at δ_C δ_C 86.3 (C-24) and 73.2 (C-3) as well as an oxygenated quaternary carbon at δ_C 70.3 (C-25). The ^{1}H-^{1}H correlations (Fig. 2) from H-1/H-2/H-3, H-5/H-6/H-7, H-9/H-11/H-12/H-13, H-15/H-16/H-17 and H-22/H-23 were aided by the presence of the dammarane-type in compound **2**[19,20]. The HMBC cross peaks (Fig. 2) from CH$_3$-28 (δ_H 0.92), CH$_3$-29 (δ_H 0.82), and the methylene protons at H-2 (δ_H 1.55 and 1.38) to the oxymethine carbon at C-3 (δ_C 73.2) indicated the presence of a hydroxy group at C-3. The correlation of H-22 (1.22 and 2.01) and H-23 (2.52 and 2.62) to C-24 (δ_C 86.3) and C-20 (δ_C 86.7) indicates that the position of furan ring at C-20/C-24. Furthermore, methyl protons at δ_H 1.17 (CH$_3$-26) and 1.09 (CH$_3$-27) as well as the oxygenated proton at δ_H 3.62 correlated with oxygenated quaternary carbon at δ_C 86.3 (C-24), indicating that an isopropyl alcohol moiety was located at C-24. In addition, the key NOESY correlation (Fig. 3) between CH$_3$-21 and CH$_3$-26, CH$_3$-27 specifies the isopropyl group at C-24 as a α-orientation, which is supported by the presence of dammarane-type triterpenoids in *Chisocheton* species.[19,20] The NMR spectra of **2** were in good agreement with that reported cabraleadiol isolated from *Chisocheton penduliflorus*,[20] therefore, compound **2** was identified as a cabraleadiol.

Compound **3** was isolated as a white amorphous powder. Its molecular composition was assigned as C$_{32}$H$_{46}$O$_7$ on the basis of the HRTOFMS (*m/z* 543.1260 [M+H]$^+$, (calcd. C$_{32}$H$_{46}$O$_7$ *m/z* 542.1244 NMR data (Table 1), therefore required ten double-bond equivalents. IR spectra showed absorption peaks at 3430 cm^{-1} (OH), 2945 cm^{-1} (C-H sp^3), 1720 (C=O), 1639 cm^{-1} (C=C), 1456 and 1378 cm^{-1} (*gem*-dimethyl) and 1076 cm^{-1} (C-O) groups. The ^{1}H-NMR spectrum showed the presence of seven tertiary methyl groups, resonance at δ_H 1.44 (CH$_3$-27), 1.35 (CH$_3$-26), 1.16 (CH$_3$-30), 1.16 (CH$_3$-18), 1.07 (CH$_3$-19), 1.06 (CH$_3$-28) and 1.06 (CH$_3$-29). The signal of one acetyl at δ_H 1.94 (CH$_3$-2′), two oxygenated methines at δ_H 4.47 (1H, dd, $J = 9.6, 1.8$ Hz, H-23), 3.92 (1H, d, $J = 9.6$ Hz, H-24), three methine sp^2 at δ_H 7.14 (1H, d, $J = 10.2$ Hz, H-1), 5.86 (1H, d, $J = 10.2$ Hz, H-2) and 5.28 (1H, br.s, H-15) were observed also in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum (Table 1) showed the presence 32 carbons, which were assigned to

one ketone (δ_C 204.6), one ester (δ_C 170.1), one lactone (δ_C 178.6), four sp^2 carbons (δ_C 158.8, 157.9, 125.7 and 119.0), eight methyls (δ_C 27.1, 26.9, 23.8, 21.4, 21.2, 20.0, 19.1 and 16.2), six methylenes and seven methines. These properties represented a total of five or ten degrees of unsaturation, and the remaining five degrees of unsaturation corresponded to the pentacyclic framework of the apotirucallan-type triterpenoid.[21] The proton pairing was also confirmed with the ^{1}H-^{1}H COSY spectrum (Fig. 2) and supported the presence of apotirucallan-type triterpenoid in **3**. The HMBC crosspeaks (Fig. 2) from H-2 (δ_H 5.86) to C-1 (δ_C 158.8) and carbonyl at C-3 (δ_C 204.6), indicated the presence of α,β-unsaturated ketone at C-1, C-2 and C-3, respectively. Correlation which was arising from H-7 (δ_H 5.33) to C-1' (δ_C 170.1) and C-6 (δ_C 27.5) as well as from CH$_3$-2″ (δ_H 1.94) to to C-1' (δ_C 170.1), indicate that acetyl group was attached at C-7. The correlation from H-15 (δ_H 5.28) to C-16 (δ_C 33.8) and C-14 (δ_C 157.9), indicated that another double-bond was located at C-14 and C-15, respectively. An oxygenated proton at H-23 (δ_H 4.47) and methine proton at H-17 (δ_H 2.19) was correlated to carbonyl at C-21 (δ_C 178.6), indicate the lactone ring was attached at C-17. Furthermore, the correlation from CH$_3$-26 (δ_H 1.35) to C-24 (δ_C 72.4) and C-25 (δ_C 70.5), suggesting that 1,2-diol side chain was attached at C-23. The detailed examination of the NMR data and comparison with those reported for prototiamin A, an apotirucallane-type triterpenoid isolated from the bark of *Entandrophragma congoense*,[21] showed that the structures of the two compounds were very similar. Therefore, compound **3** was identified as proto-tiamin A.

Compound **4** was isolated as colorless solid, with the molecular formula determined as C$_{30}$H$_{44}$O$_7$ based on HRTOFMS (*m/z* 517.4498 [M+H]$^+$, (calcd. C$_{30}$H$_{44}$O$_7$ *m/z* 516.4087) and NMR spectra (Table 1). In addition, ^{13}C NMR analysis show that nine degrees of unsaturation is required, while, the IR absorption bands at 3560, 2939, 1725 and 1109 cm^{-1} imply the presence of hydroxyl, aliphatic, carbonyl ester and ether functionalities. The ^{1}H-NMR spectrum showed the presence of five methyl tertiary signals at δ_H 1.16 (CH$_3$-19), 1.16 (CH$_3$-30), 1.06 (CH$_3$-28), 1.06 (CH$_3$-29), 1.00 (CH$_3$-18) and two acetyl signals at δ_H 1.91 and 1.97. One olefinic proton at δ_H 5.28 (1H, dd, $J = 1.8, 3.6$ Hz, H-15) and three oxygenated methane signals at δ_H [5.39 (1H, m, H-1), 5.33 (1H, m, H-7) and 4.57 (1H, dd, $J = 9.6, 1.8$ Hz, H-23)] were also observed in the ^{1}H-NMR spectrum. Moreover, the ^{13}C NMR spectrum (Table 1) recognized 30 nonequivalent carbon signals, which are characterized by one carbonyl

ketone (δ_C 218.2), two acetyls (δ_C 170.1 and 170.0), two sp^2 carbon (δ_C 158.9 and 118.0), seven methyl signals at (δ_C 27.5, 27.1, 21.4, 21.1, 21.0, 19.7 and 15.3), seven sp^3 methylenes (including one oxygenated methylene type at δ_C 70.3), seven sp^3 methynes (including two oxygenated types at δ_C 75.4, 75.6 and a hemiacetal at δ_C 99.8) and four quaternary carbons. These properties represented a total of four or nine degrees of unsaturation, and the remaining five degrees of unsaturation corresponded to limonoid framework.[16,17,22] Comprehensive analyses of the above mentioned ^{1}H and ^{13}C NMR data exhibited that the structure of **4** was similar to that of neemfruitin,[22] a limonoid isolated from *Azadirachta indica*. Consequently, compound **4** was identified as neemfruitin A.

Compound **5** was obtained as a white solid with a proposed molecular formula of $C_{28}H_{40}O_5$ based on HRTOFMS (*m/z* 455.0712 [M+H][+], (calcd. $C_{28}H_{40}O_5$ *m/z* 456.2876) and NMR data (Table 1), therefore nine indices of hydrogen deficiency were required. Furthermore, the IR absorption bands at 3300, 1705 and 1080 cm^{−1} show the presence of hydroxyl, carbonyl and ether properties, while the NMR data was highly similar to **4**. However, differences were observed in the disappearance of one of acetyl group at [δ_H 1.91 (3H, s), δ_C 21.7, 1701.1], oxygenated methyne at [δ_H 5.39 (1H, m), δ_C 75.4] and the appearance of olefinic signals at [δ_H 7.14 (1H, d, $J = 10.2$ Hz), 5.86 (1H, d, $J = 10.2$ Hz); δ_C 158.8, 125.7), suggesting **5** as a 1-deacetyl derivative of **4**. The proton pairing was also confirmed with the ^{1}H-^{1}H COSY spectrum (Fig. 2) and supported the presence of limonoid structure in **5**.[22,23] The HMBC crosspeaks (Fig. 2) from H-2 (δ_H 5.86) to C-1 (δ_C 158.8) and carbonyl at C-3 (δ_C 204.6), indicated the presence of α,β-unsaturated ketone at C-1, C-2 and C-3, respectively. Correlation which was arising from H-7 (δ_H 5.33) to C-1' (δ_C 170.1) and C-6 (δ_C 27.5) as well as from CH$_3$-2" (δ_H 1.94) to to C-1' (δ_C 170.1), indicate that acetyl group was attached at C-7. The above-mentioned NMR data suggested that compound **5** is a limonoid, for which NMR data (Table 1) showed many similarities to those of 23-desmethyllimocin-B, a limonoid isolated from the seed of *Azadirachta indica*.[23] Therefore, compound **5** was identified as 23-desmethyllimocin-B.

Compound **6** was obtained as a white solid with a molecular composition of $C_{32}H_{48}O_6$, based on HRTOFMS (*m/z* 527.2984 [M+H][+], (calcd. $C_{32}H_{48}O_6$ *m/z* 528.2951) and NMR analysis, therefore nine degrees of unsaturation are required. Furthermore, the IR spectrum showed the absorption bands for hydroxyl (3420 cm^{−1}), aliphatic (2949 and 2860 cm^{−1}), carbonyl (1715 cm^{−1}) and ether (1080 cm^{−1}) moieties, while the NMR data observed in

Table 2. IC$_{50}$ inhibition values of compounds **1 - 8** against MCF-7 breast cancer cell line

Compounds	IC$_{50}$ (µM)
Cabrealeolactone (**1**)	61.18
Cabreadiol (**2**)	33.12
Prototiamin A (**3**)	76.08
Neemfruitins A (**4**)	181.12
Desmethyllimocin B (**5**)	98.18
Protoxylocarpin G (**6**)	90.24
Melianodiol (**7**)	16.84
Indicalilacol B (**8**)	20.23
Cisplatin*	13.20

*positive control

Table 1 was highly similar to **5**. However, the difference was identified in the absence of a hemiacetal group at [δ_H 4.57 (1H, dd, $J = 9.6$, 1.8 Hz), δ_C 99.2], and also the appearance of a newly oxygenated methynes at [δ_H 4.47 (1H, dd, $J = 9.6$, 1.8 Hz), 3.92 (1H, d, $J = 9.6$ Hz), δ_C 78.2, 74.4], two methyls at [δ_H 1.35 (3H, s), 1.44 (3H, s), δ_C 26.9, 23.8] and one oxygenated quaternary carbon at δ_C 72.5. These observations suggest that **6** is a 2-methylbutane-2,3-diol derivative of **5** and is attached to C-23. The aforementioned data as well as biogenetic considerations suggested that compound **6** is protolimonoid, with a structure similar to that of protoxylocarpin G, a protolimonoid isolated from *Xylocarpus granatum*.[24] Consequently, compound **6** was identified as protoxylocarpin G.

Compound **7** was obtained as a colorless crystal. Its molecular composition $C_{30}H_{48}O_5$, was determined from HRTOFMS (*m/z* 487.4535 [M+H][+], (calcd. $C_{32}H_{50}O_7$ *m/z* 488.4502) and NMR data (Table 1), therefore seven unsaturated degrees were required. The IR spectra showed absorption peaks at 3350 cm^{−1} (OH), 2935 cm^{−1} (C-H sp^3), 1725 (C=O), 1645 (C=C), 1457 and 1380 cm^{−1} (*gem*-dimethyl groups), and 1055 cm^{−1} (C-O) groups. The ^{1}H-NMR spectrum showed the presence of seven tertiary methyl groups at δ_H 1.39 (CH$_3$-27), 1.32 (CH$_3$-26), 1.01 (CH$_3$-30), 0.93 (CH$_3$-28), 0.92 (CH$_3$-29), 0.84 (CH$_3$-18) and 0.78 (CH$_3$-19). One olefinic proton at δ_H 5.26 (1H, dd, $J = 2.6$, 5.8 Hz, H-7), two oxygenated methyne signals at δ_H 3.64 (1H, ddd, $J = 1.3$, 6.2, 8.7 Hz, H-23), 4.06 (1H, br.s, H-24) and one hemiacetal signal at δ_H 4.63 (1H, d, $J = 5.6$ Hz) were also observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of **7** showed 30 carbons and were classified as seven methyl groups at δ_C 27.8, 27.4, 27.0, 26.5, 23.5, 21.6 and 13.1, one carbonyl ketone at δ_C 218.4 (C-3), two oxymethine signals at δ_C 86.4 (C-23) and 71.3 (C-24), one oxygenated quaternary

carbon at δ_C 70.3 (C-24), hemiacetal signal at δ_C 99.3 (C-21) and two sp^2 carbon at δ_C 118.8 (C-7) and 145.3 (C-8), respectively. These properties represented two of seven total degrees of unsaturation, and the remaining five degrees of unsaturation were consistent with the limonoid structure.[24,25] The above data showed general features similar to those of melianodiol, a limonoid isolated from the leaves of *Aglaia andamanica*.[25] Therefore, compound **7** was identified as melianodiol.

Compound **8** was obtained as a white solid having a molecular composition of $C_{30}H_{48}O_5$ based on HRTOFMS (*m/z* 487.4535 [M+H]$^+$, (calcd. $C_{32}H_{50}O_7$ *m/z* 488.4502) along with NMR analysis, therefore seven degrees of unsaturation are required. Furthermore, the IR spectrum showed the absorption bands for hydroxyl (3430 cm^{-1}), aliphatic (2930), carbonyl (1760 cm^{-1}), *gem*-dimethyl (1420 and 1378 cm^{-1}) and ether (1080 cm^{-1}) moieties, while the NMR data observed in Table 1 was highly similar to **7**. However, the difference was identified in the absence of a hemiacetal signal at [δ_H 4.63 (1H, d, *J* = 5.6 Hz), δ_C 99.3] and ketone signal at δ_C 218.4, and appearance of a newly carbonyl lactone at δ_C 178.3 (C-21) and hydroxyl signal at [δ_H 4.32 (1H, d, *J* = 5.4 Hz), δ_C 72.4]. These observations indicate that **8** is 3-hydroxy and lactone derivative of **7**. The above data revealed that **8** is a tirucallane-type triterpenoid similar to indicalilacol B, isolated from *Azadiracta indica*.[26] Consequently, compound **8** was identified as indicalilacol B.

The isolated compounds, **1 - 8**, were evaluated for their cytotoxic against MCF-7 breast cancer lines using a previously described method,[17,18,27] using *cis*-Platin as a positive control.[28,29] Among the compounds tested, melianodiol (**7**), exhibited the strongest cytotoxicity with an IC$_{50}$ value of 16.84 μM. Interestingly, Compounds **7** and **8** share the same structure and only differed at the functional of the cyclopentane ring, the cytotoxic activities of compounds **7** and **8** differed greatly, suggested the presence of hemiacetal group increase the cytotoxic activity. These results were supported from previously studies that the presence of a hemiacetal group in triterpenoids structure can increase cytotoxic activity.[17,24,25] In addition, despite close structural similarities to **7**, a low activity was observed in **6**, which indicates the possible contribution of an acetyl group to cytotoxicity.[25] This results was supported from previously studies that the presence of a hemiacetal group in triterpenoids structure can increase cytotoxic activity.[25] Therefore, the structural comparison of compound **7** with the other compounds indicates that carbonyl, hydroxyl and acetyl groups present contribute to the cytotoxic activity.

References

(1) Katja, D. G.; Farabi, K.; Nurlelasari.; Harneti, D.; Mayanti, T.; Supratman, U.; Awang, K.; Hayashi, H. *J. Asian Nat. Prod. Res*. **2017**, *19*, 194-200.

(2) Shilpi, J. A.; Saha, S.; Chong, S. L.; Nahar, L.; Sarker, S. D.; Awang, K. *Chem. Biodivers*. **2016**, *13*, 483-503.

(3) Awang, K.; Lim, C. S.; Mohamad, K.; Hiroshi, M.; Hirasawa, Y.; Takeya, K.; Thoison, O.; Hadi, A. H. A. *Bioorg. Med. Chem*. **2007**, *15*, 5997-6002.

(4) Tan, Q. G.; Luo, X. D. *Chem. Rev*. **2011**, *111*, 7437-7522.

(5) Yang, M. H.; Wang, J. S.; Luo, J. G.; Wang, X. B.; Kong, L. Y. *J. Nat. Prod*. **2009**, *72*, 2014-2018.

(6) Mohamad, K.; Hirasawa, Y.; Litaudon, M.; Awang, K.; Hadi, A. H. A.; Takeya, K.; Ekasari, W.; Widyawaruyanti, A.; Zaini, N. C.; Morita, H. *Bioorg. Med. Chem*. **2009**, *17*, 727-730.

(7) Supratman, U.; Naibaho, W.; Salam, S.; Maharani, R.; Hidayat, A. T.; Harneti, D.; Nurlelasari.; Shiono, Y. *Phytochem. Lett*. **2019**, *30*, 81-87.

(8) Chong, S. L.; Hematpoor, A.; Hazni, H.; Sofian-Azirun, M.; Litaudon, M.; Supratman, U.; Murata, M.; Awang, K. *Phytochem. Lett*. **2019**, *30*, 69-73.

(9) Najmuldeen, I. A.; Hadi, A. H. A.; Mohamad, K.; Awang, K.; Ketuly, K. A.; Mukhtar, M. R.; Taha, H.; Nordin, N.; Litaudon, M.; Guérritte, F.; Nugroho, A. E.; Morita, H. *Heterocycles* **2012**, *84*, 1265-1270.

(10) Fang, X.; Di, Y. T.; Hao, X. J. *Current Organic Chemistry* **2011**, *15*, 1363-1391.

(11) Haldar, S.; Kolet, S. P.; Thulasiram, H. V. *Green Chemistry* **2013**, *15*, 1311-1317.

(12) Nagoor, N. H.; Muttiah, N. S. J.; Lim, C. S.; In, L. L. A.; Mohamad, K.; Awang, K. *PLoS One*. **2011**, *6*, e23661.

(13) Wong, C. P.; Shimada, M.; Nagakura, Y.; Nugroho, A. E.; Hirasawa, Y.; Kaneda, T.; Awang, K.; Hadi, A. H. A.; Mohamad, K.; Shiro, M.; Morita, H. *Chem. Pharm. Bull*. **2011**, *59*, 407-411.

(14) Yang, M. H.; Wang, J. S.; Luo, J. G.; Wang, X. B.; Kong, L. Y. *Bioorg. Med. Chem*. **2011**, *19*, 1409-1417.

(15) Bordoloi, M.; Saikia, B.; Mathur, R. K.; Goswami, B. N. *Phytochemistry* **1993**, *34*, 583-584.

(16) Nurlelasari.; Katja, D. G.; Harneti, D.; Wardayo, M. M.; Supratman, U.; Awang, K. *Chem. Nat. Compd*. **2017**, *53*, 83-87.

(17) Supriatno.; Nurlelasari.; Herlina, T.; Harneti, D.; Maharani, R.; Hidayat, A. T.; Mayanti, T.; Supratman, U.; Azmi, M. N.; Shiono, Y. *Nat. Prod. Res*. **2018**, *25*, 1-7.

(18) Supratman, U.; Salam, S.; Naibaho, W.; Fajar, M.; Nurlelasari.; Katja, D. G.; Harneti, D.; Maharani, R.; Hidayat, A. T.; Lesmana, R.; Nafiah, M. A.; Shiono, Y. *Phytochem. Lett*. **2020**, *35*, 63-67.

(19) Nagaya, H.; Tobita, Y.; Nagae, T.; Itokawa, H.; Takeya, K.; Halim, A. F.; Abdel-Halim, O. B. *Phytochemistry* **1997**, *44*, 1115-1119.

(20) Phongmaykin, J.; Kumamoto, T.; Ishikawa, T.; Suttisri, R.; Saifah, E. *Arch. Pharm. Res*. **2008**, *31*, 21-27.

(21) Happi, G. M.; Kouam, S. F.; Talontsi, F. M.; Lamshöft, M.; Zühlke,

S.; Bauer, J. O.; Strohmann, C.; Spiteller, M. *J. Nat. Prod.* **2015**, *78*, 604-614.

(22) Chianese, G.; Yerbanga, S. R.; Lucantoni, L.; Habluetzel, A.; Basilico, N.; Taramelli, D.; Fattorusso, E.; Taglialatela-Scafati, O. *J. Nat. Prod.* **2010**, *73*, 1448-1452.

(23) Kumar, C. S. S. R.; Srinivas, M.; Yakkundi, S. *Phytochemistry* **1996**, *43*, 451-455.

(24) Pudhom, K.; Sommit, D.; Nuclear, P.; Ngamrojanavanich, N.; Petsom, A. *J. Nat. Prod.* **2009**, *72*, 2188-2192.

(25) Puripattanavong, J.; Weber, S.; Brecht, V.; Frahm, A. W. *Planta Med.* **2000**, *66*, 740-745.

(26) Kurimoto, S.; Takaishi, Y.; Ahmed, F. A.; Kashiwada, Y. *Fitoterapia* **2014**, *92*, 200-205.

(27) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.

(28) Hadisaputri, Y. E.; Miyazaki, T.; Suzuki, S.; Yokobori, T.; Kobayashi, T.; Tanaka, N.; Inose, T.; Sohda, M.; Kuwano, H. *Ann. Surg. Oncol.* **2012**, *19*, S589-S596.

(29) Chavoshi, H.; Vahedian, V.; Saghaei, S.; Pirouzpanah, M. B.; Raeisi, M.; Samadi, N. *Asian Pac. J. Cancer Prev.* **2017**, *18*, 2243-2247.