Cytotoxic Triterpenoids from the Bark of *Chisocheton patens* Blume (Meliaceae)

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ABSTRACT

Four new triterpenoids, namely, chisopaten A-D (1-4), were isolated from the *n*-hexane extract of the bark of *Chisocheton patens* Blume. The chemical structures of new compounds were elucidated on the basis of spectroscopic data interpretation. All isolated compounds were evaluated for their cytotoxic activity against MCF-7 breast cancer lines. Chisopaten A and C, showed strongest cytotoxicity activity with IC_{50} values of 4.01 ± 0.008 and 4.33 ± 0.009 μM, respectively.

1. Introductions

The *Chisocheton* genus, belong to Meliaceae family, comprises more than 53 species and widely distributed mainly in Malaysia, Indonesia and Australia (Stevens, 1975; Yang et al., 2009; Shilpi et al., 2016). Previous phytochemical investigations of this genus resulted in the isolation of mainly limonoids (Yadav et al., 1999; Awang et al., 2007; Laphookhieo et al., 2008; Mohamad et al., 2008; Maneerat et al., 2008; Najmuldeen et al., 2012; Chong et al., 2012; Katja et al., 2016a, 2016b; Nurlelasari Katja et al., 2017; Supriatno et al., 2018), apotirucallane-type triterpenoids (Yadav et al., 1999; Xie Bojun et al., 2009; Yang et al., 2011; Zhang et al., 2012), lanostane-type triterpenoids (Nurlelasari Katja et al., 2017) and dammarane-type triterpenoids (Chan et al., 2012). Reported bioactivities of the isolated compounds from *Chisocheton* genus include cytotoxic (Awang et al., 2007; Mohamad et al., 2008; Maneerat et al., 2008; Phongmaykin et al., 2008; Yang et al., 2009; Wong et al., 2011; Nagoor et al., 2011; Huang et al., 2016; Katja et al., 2016a, 2016b; Nurlelasari Katja et al., 2017), anti-inflammatory (Najmuldeen et al., 2011; Yang et al., 2011; Chan et al., 2012), antifungal (Bordoloi et al., 1993), antimalarial (Maneerat et al., 2008), antimycobacterial (Maneerat et al., 2008; Phongmaykin et al., 2008), anti-melanin (Iijima et al., 2016) and antiplasmodial (Mohamad et al., 2008). As part of our continuing search for anticancer candidate compounds against MCF-7 breast cancer cells from indonesian *Chisocheton* plants, we isolated and described a new limonoid, pentandricine from the bark of *C. pentandrus* (Supriatno et al., 2018). In the further screening for cytotoxic compounds from Indonesia *Chisocheton* plants, we found that the *n*-hexane extract of the bark of *Chisocheton patens* Blume, exhibited strong cytotoxic activity against MCF-7 breast cancer cells with an IC_{50} value of 2.01 μg/mL. We described herein the isolation and structural elucidation of new triterpenoid compounds, chisopaten A-D (**1-4**), along with their cytotoxic activity against MCF-7 breast cancer cells.

2. Results and discussion

The dried bark of *C. patens* Blume was extracted with MeOH at room temperature. The crude MeOH extract was partitioned between *n*-hexane and H_2O to give the concentrated *n*-hexane extract (67.20 g) and aqueous layer. The *n*-hexane extract exhibited significant cytotoxic activity against MCF-7 breast cancer cells. By using a cytotoxic assay to guide separations, the *n*-hexane extract was chromatographed over a vacuum-liquid chromatographed (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to normal column chromatography and preparative TLC on silica gel GF254 to afford four new cytotoxic triterpenoids **1- 4** (Fig. 1).

Compound **1** was isolated as a white crystal with $[\alpha]_D^{23}$ +15.6 (*c* 0.26, ethanol). Its molecular formula was determined to be $C_{30}H_{48}O_3$ with seven degrees of unsaturation from its hydrogen molecular ion peak $[M-H]^-$ at *m/z* 455.3571 (calcd. for $C_{30}H_{48}O_3$, 456.3525) in the HR-TOFMS. The IR spectrum showed absorption peaks due to hydroxyl from carboxyl (3435 cm^{-1}), carbonyl (1698 cm^{-1}) and olefinic groups

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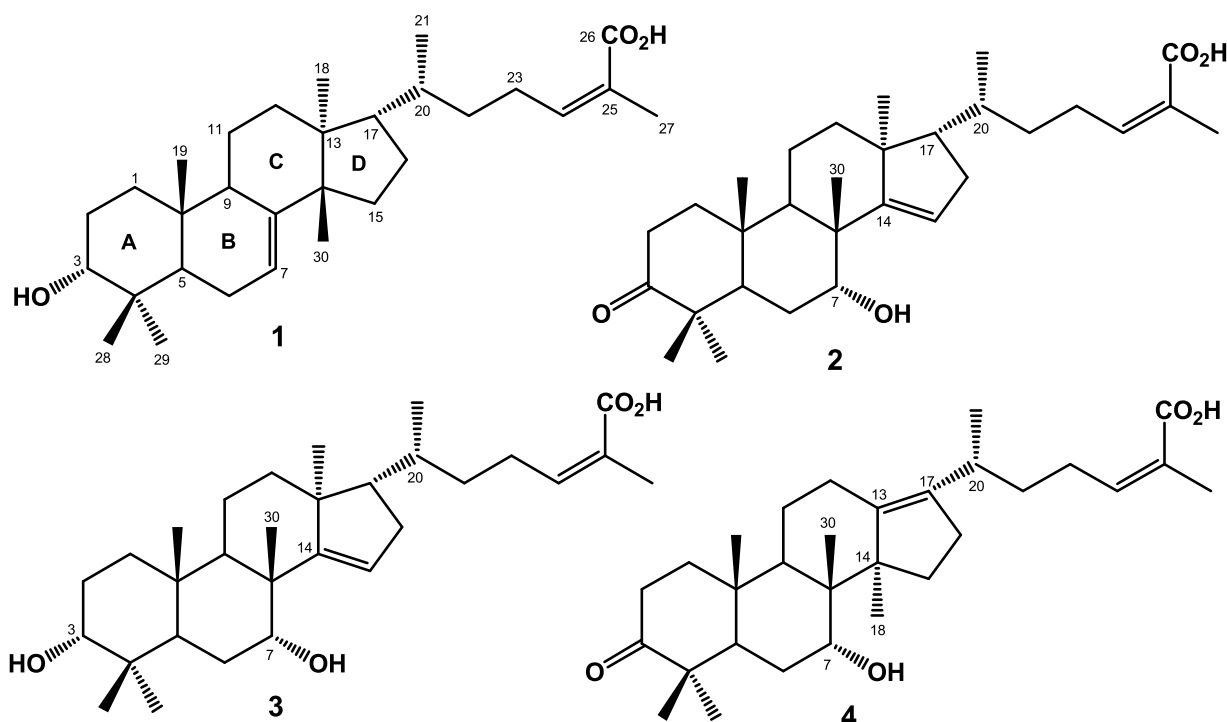
Fig. 1. Structures of Compounds **1**-**4**.
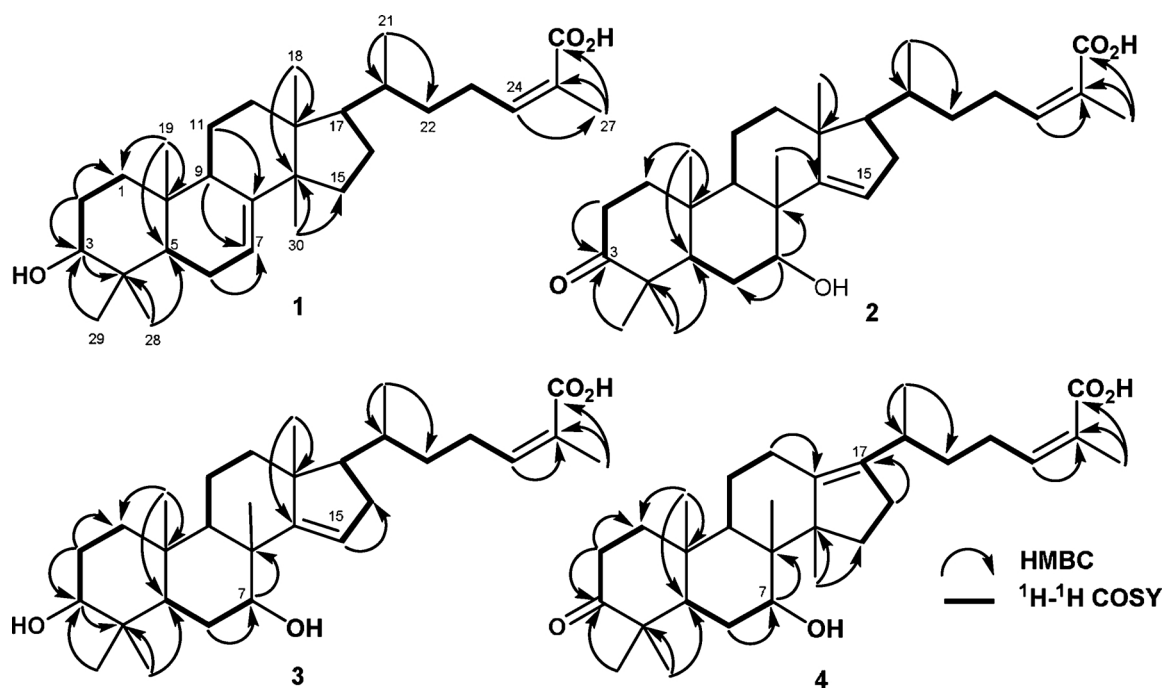
Fig. 2. Selected HMBC and ¹H-¹H COSY correlations for **1**-**4**.

(1645 cm⁻¹). The ¹H NMR spectrum displayed six tertiary methyl singlets [δ_H 0.77, 0.83, 0.89 (2×), 0.98 and 1.84 (each 3H, s)], a secondary methyl doublet [δ_H 0.89 (3H, d, J = 6.2, CH₃-21)], an oxymethine proton [δ_H 3.37 (1H, J = 3.4, 1.8, H-3)] and two olefinic protons [δ_H 5.24 (1H, d, J = 3.0, H-7) and 5.94 (1H, t, J = 7.8, H-24)]. The ¹³C NMR and DEPT spectra exhibited the presence of seven methyls [δ_C 12.3 (C-19), 17.5 (C-21), 19.8 (C-27), 21.0 (C-29), 21.1(C-18), 27.3 (C-28) and 26.6 (C-30)], nine methylenes [δ_C 17.8 (C-11), 23.7 (C-6), 25.2 (C-23), 26.3 (C-2), 27.9 (C-16), 31.2 (C-1), 33.8 (C-12 and C-15) and 35.5 (C-22)], seven methines [δ_C 36.0 (C-20), 44.4 (C-5), 48.6 (C-

9), 52.8 (C-17), 75.6 (C-3), 118.0 (C-7), 142.9 (C-24)] and seven quartenary carbon signals [δ_C 34.5 (C-10), 37.0 (C-4), 43.3 (C-13), 51.1 (C-14), 127.1 (C-25), 145.9 (C-8) and 170.3 (C-26)]. The ¹H and ¹³C NMR data suggested that **1** had a triterpenoid tetracyclic skeleton similar to masticadienolic acid with an lanostane derivative skeleton (Camacho et al., 2000; Wang et al., 2003; Isaka et al., 2017). The structure of the tetracyclic system (A, B, C and D) was determined by analysis of COSY and HMBC spectra (Fig. 2). Key HMBC spectra were the ²J correlations from the seven methyl groups (CH₃-18, CH₃-19, CH₃-21, CH₃-27, CH₃-28, CH₃-29 and CH₃-30) to their attached carbons C-
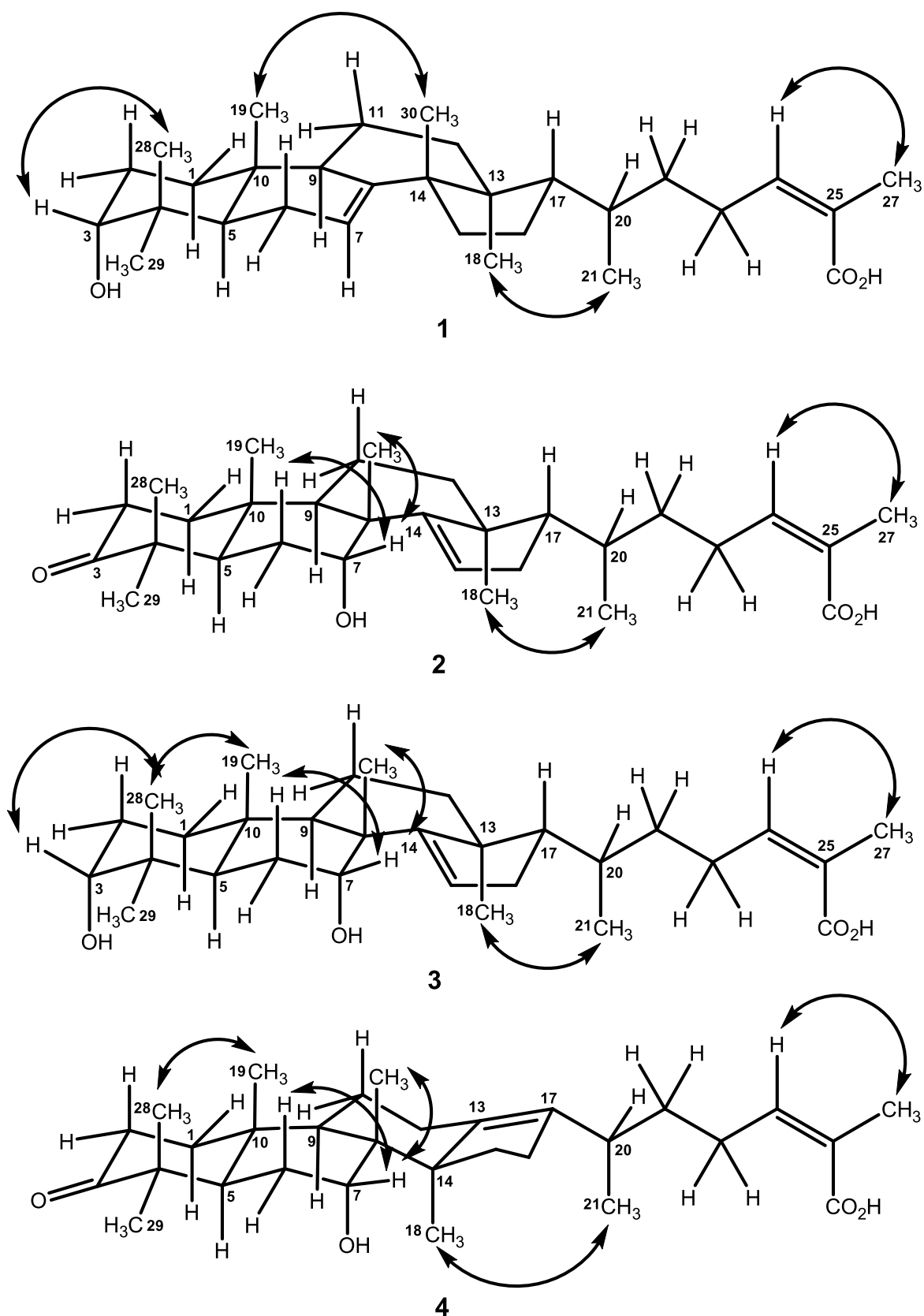
Fig. 3. Key NOESY correlations of compounds **1**-**4**.

13, C-10, C-20, C-25, C-4, C-4, and C-14, respectively, enabled the assignment of the six singlet methyls and one secondary methyl. An olefin was assigned to C-7/C-8 by the HMBC correlations from H-5, H-6 and H-9 to C-7 (δ_C 118.0) and from H-6, H-9 and H-11 to C-8 (δ_C 145.9). The HMBC correlations from H-24 to C-25 (δ_C 127.1), C-27 (δ_C 19.8) and H-27 to C-24 (δ_C 142.9), C-25 (δ_C 127.1), C-26 (δ_C 170.3), indicated

the presence of the α,β-unsaturated carboxylic acid. Correlations from H-1, H-2 and CH₃-29 to C-3 (δ_C 75.6), suggested that a hydroxy group was located at C-3. The side chain of **1** was assigned by a continuous sequence from C-15 to C-24 as deduced from ¹H-¹H COSY and HMBC spectra. Moreover, the HMBC cross-peak of CH₃-21 to C-20 (δ_C 36.0), requires that the side chain is connected to C-20. In the NOESY spectrum (Fig. 3), the are correlations between H-3 with CH₃-28, as well as from its coupling constant values (δ_H 3.37, *J* = 3.4, 1.8 Hz), indicated that the hydroxy group at C-3 is α-oriented. A detailed comparison of NMR data of **1** to those of 3α-hydroxytirucalla-7,24-*Z*-dien-26-oic acid (Camacho et al., 2000; Wang et al., 2003; Isaka et al., 2017) was very similar. The main difference is orientation of methyl group at C-20. The NOE correlation between CH₃-21 and CH₃-18, indicated that methyl group at C-20 is α-oriented, consequently **1** as an euphane type-triterpenoid. This configuration was very important to distinguish between tirucallane-type (H-20α) with euphane-type (H-20β) triterpenoids (Wang et al., 2003). In addition, in the ¹H-NMR spectrum of CH₃-21 (δ_H 0.89 (3H, d, *J* = 6.2 Hz) and the optical rotation (+15.6), indicated that **1** belong to the euphane-type triterpenoid rather than the tirrucallane-type. Therefore, compound **1** was determined as a new euphane-type triterpenoid, 3α-hidroxyeupha-7,24-*Z*-diene-26-oic acid, and namely chisopaten A.

Compound **2** was obtained as a colorless solid with [α]_D²³ +14.5 (*c*, 0.28, ethanol) and gave a molecular formula of C₃₀H₄₆O₄ by HR-

TOFMS ([M-H]⁻ *m/z* 469.3308, calcd. 469.3318) and NMR data (Table 1), thus requiring eight degrees of unsaturation. The IR spectrum of **2** showed absorption peaks due to the presence of a hydroxyl (3449 cm⁻¹), carbonyl (1702 cm⁻¹), an isolated double bond (1645 cm⁻¹) and an ether groups (1230 cm⁻¹). The ¹H-NMR spectrum displayed a total of seven methyl signals, including one secondary methyl (δ_H 0.93, d, *J* = 6.6 Hz, CH₃-21), and six tertiary methyls (δ_H 0.89, 0.97, 0.98, 1.01, 1.08 and 1.84), and two olefinic methines (δ_H 5.42, d, *J* = 3.8 Hz and 5.97, t, *J* = 7.2 Hz), one oxygenated methine proton (δ_H 3.91, d, *J* = 2.1 Hz). The ¹³C NMR and DEPT spectra revealed 30 carbon signals, consisting of 7 methyls, 8 methylenes, 7 methines (including two olefinic carbons), and 8 quaternary carbons (including two olefinic, one carbonyl group and one carboxylic group). Comparing the NMR spectra of **2** to those of **1**, showed the same skeleton. The significant differences were the position of double bond and hydroxyl group as well as the presence of newly carbonyl group. The location of double bond was confirmed by HMBC correlation from H-15, CH₃-18 and CH₃-30 to C-14 (δ_C 161.2), suggested that position of newly double bond at C-14/C-15. The corelation of H-2 and CH₃-29 to a carbonyl carbon at δ_C 215.0, revealed the position of carbonyl is located at C-3. The correlation from an oxygenated methine at δ_H 3.91 to C-8 (δ_C 43.7) and C-6 (δ_C 25.0), suggested that a hydroxyl group was located at C-7. In addition, compound **2** was contain of α,β-unsaturated carboxylic acid at C-24−C-27, which was confirmed by HMBC

Table 1

NMR Data (600 MHz for ¹H and 150 MHz for ¹³C) for **1**- **4**.

Position Carbons	1		2		3		4	
	¹³C NMR δ_C (mult)	¹H NMR δ_H (Integral, mult, *J* = Hz)	¹³C NMR δ_C (mult)	¹H NMR δ_H (Integral, mult, *J* = Hz)	¹³C NMR δ_C (mult)	¹H NMR δ_H (Integral, mult, *J* = Hz)	¹³C NMR δ_C (mult)	¹H NMR δ_H (Integral, mult, *J* = Hz)
1	31.2 (t)	1.34 (1H, m) 1.55 (1H, m)	38.7 (t)	1.47 (1H, m) 1.85 (1H, m)	32.5 (t)	1.29 (1H, m) 1.38 (1H, m)	39.6 (t)	1.55 (1H, m) 1.88 (1H, m)
2	26.3 (t)	2.37 (1H, m) 2.50 (1H, m)	33.6 (t)	1.60 (1H, m) 1.35 (1H, m)	24.0 (t)	1.66 (1H, m) 1.74 (1H, m)	34.6 (t)	2.40 (2H, m)
3	75.6 (d)	3.37 (1H, 3.4, 1.8)	215.0 (s)	–	75.6 (d)	3.88 (1H, 3.6, 1.4)	218.0 (s)	–
4	37.0 (s)	–	46.4 (s)	–	36.8 (s)	–	46.7 (s)	–
5	44.4 (d)	1.79 (1H, dd, 9.6, 3.6)	46.6 (d)	2.08 (1H, d 2.4)	41.2 (d)	2.04 (2.02 (1H, dd 11.4, 4.8)	45.7 (d)	1.84 (1H, d, 2.5)
6	23.7 (t)	1.94 (2H, m)	25.0 (t)	1.60 (1H, m) 2.05 (1H, m)	24.8 (t)	1.50 (1H, m) 1.93 (1H, m)	28.7 (t)	1.38 (1H, m) 1.80 (1H, m)
7	118.0 (d)	5.24 (1H, d, 3,0)	71.9 (d)	3.91 (1H, d, 2.1)	72.8 (d)	3.31 (3.85 (1H, d, 1.7)	74.6 (d)	3.84 (1H, d, 1.9)
8	145.9 (s)	–	43.7 (s)	–	43.8 (s)	–	44.5 (s)	–
9	48.6 (d)	2.36 (1H, m)	41.2 (d)	2.06 (1H, m)	40.2 (d)	1.98 (1H, m)	45.3 (d)	2.01 (1H, m)
10	34.5 (s)	–	37.1 (s)	–	37.4 (s)	–	37.0 (s)	–
11	17.8 (t)	1.54 (1H, m) 1.80 (1H, m)	16.5 (t)	1.54 (1H, m) 1.70 (1H, m)	16.4 (t)	2.00 (1H, m) 2.24 (1H, m)	22.3 (t)	1.17 (1H, m) 1.42 (1H, m)
12	33.8 (t)	1.62 (1H, m) 1.81 (1H, m)	34.8 (t)	1.97 (1H, m) 2.23 (1H, m)	34.7 (t)	1.43 (1H, m) 1.96 (1H, m)	22.1 (t)	1.90 (1H, m) 2.01 (1H, m)
13	43.3 (s)	–	46.7 (s)	–	46.7 (s)	–	141.2 (s)	–
14	51.1 (s)	–	161.2 (s)	–	161.9 (s)	–	56.7 (s)	–
15	33.8 (t)	1.43 (1H, m) 1.64 (1H, m)	119.4 (d)	5.42 (1H, d, 3.8)	119.3 (t)	5.39 (1H, d, 1.8)	30.4 (t)	1.81 (1H, m) 1.95 (1H, m)
16	27.9 (t)	1.24 (1H, m) 1.94 (1H, m)	34.8 (t)	1.80 (1H, m) 1.96 (1H, m)	34.5 (t)	2.00 (1H, m) 2.26 (1H, m)	29.4 (t)	2.23 (1H, m) 2.40 (1H, m)
17	52.8 (s)	1.50 (1H, m)	60.6 (s)	1.44 (1H, m)	60.5 (d)	1.41 (1H, m)	132.3 (d)	–
18	21.1 (q)	0.83 (3H, s)	18.2 (q)	1,01 (3H, s)	17.5 (q)	1.01 (3H, s)	27.7 (q)	1.30 (3H, s)
19	12.3 (q)	0.77 (3H, s)	14.5 (q)	0.89 (3H, s)	14.6 (q)	0.89 (3H, s)	16.3 (q)	0.85 (3H, s)
20	36.0 (d)	1.39 (1H, m)	33.6 (d)	2.54 (2H, m)	33.6 (d)	1.95 (1H, m)	31.6 (d)	2.40 (2H, m)
21	17.5 (q)	0.89 (3H, d, 6.2)	18.2 (q)	0.93 (3H, d, 6.6)	17.8 (q)	0.92 (0.92 (3H, d, 6.0)	20.0 (q)	0.90 (1H, d, 6.0)
22	35.5 (t)	1.13 (2H, m)	34.5 (t)	1.49 (2H, m)	35.2 (t)	1.46 (2H, m)	35.1 (t)	1,.33 (2H, m)
23	25.2 (t)	1.54 (2H, m)	26.0 (t)	2.32 (1H, m) 2.53 (1H, m)	25.9 (t)	2.37 (1H, m)	28.2 (t)	2.40 (2H, m)
24	142.9 (d)	5.94 (1H, t, 7.8)	143.1 (d)	5.97 (1H, t, 7.2)	142.7 (d)	5.93 (5.96 (1H, t, 7.2)	146.6 (d)	5.98 (1H, t, 7.2)
25	127.1 (s)	–	127.0 (s)	–	127.2 (s)	–	125.7 (s)	–
26	170.3 (s)	–	168.3 (s)	–	170.3 (s)	–	170.0 (s)	–
27	19.8 (q)	1.84 (3H, s)	20.3 (q)	1.84 (3H, s)	19.7 (q)	1.84 (3H, s)	20.5 (q)	1.83 (3H, s)
28	27.3 (q)	0.98 (3H, s)	26.8 (q)	1.08 (3H, s)	27.2 (q)	1.05 (3H, s)	27.4 (q)	0.70 (3H, s)
29	21.0 (q)	0.89 (3H, s)	20.6 (q)	0.98 (3H, s)	21.4 (q)	0.81 (3H, s)	21.1 (q)	1.01 (3H, s)
30	26.6 (q)	0.89 (3H, s)	25.6 (q)	0.97 (3H, s)	27.4 (q)	0.88 (3H, s)	26.9 (q)	0.96 (3H, s)

correlation of H-24 to C-27 (δ_C 20.3) and CH$_3$-27 to C-24 (δ_C 143.1), C-25 (δ_C 127.0) and C-26 (δ_C 168.3). The equatorial, β-orientation of H-7 (3.91, d, *J* = 2.1 Hz) was displayed by its small coupling constants and NOESY correlation between H-7/CH$_3$-19 and H-7/CH$_3$-30, indicated that the hydroxy group at C-7 is α-oriented. The correlation of H-7 to CH$_3$-30 and H-17 showed that methyl (C-30) and proton (C-17) are both β-oriented. A sequence correlation from CH$_3$-30 to H-17 and H-17 to H-20 displayed that H-20 is β-oriented, suggested that **2** is *apo*-euphane-type triterpenoid. In addition, the C-24/C-25 double bond was assigned an *Z*-configuration based on the NOESY correlation of H-24 with CH$_3$-27 and by comparing the spectral data previously reported (Shilpi et al., 2016), especially of the chemical shifts of C-27 between *Z* or *E*, 21 ppm in *Z* configuration and 12 ppm in *E* configuration (Camacho et al., 2000; Shilpi et al., 2016). Therefore, compound **2** was determined as new *apo*-euphane-type triterpenoid, 3-oxo-*apo*-eupha-7α-ol,14,24-*Z*-diene-26-oic acid and named chisopaten B.

Compound **3** was obtained as a white crystals with $[\alpha]_D^{23}$ +17.5 (*c*, 0.26, ethanol). Its molecular formula was established as C$_{30}$H$_{48}$O$_4$ by HR-TOFMS ([M-H]$^-$ *m/z* 471.3471, calcd. 471.3474) and NMR data (Table 1), suggesting the presence of seven degrees of unsaturation. The IR spectrum showed the presence of a hydroxyl (3423 cm^{-1}), a carbonyl (1692 cm^{-1}), an olefinic (1647 cm^{-1}) and ether groups (1170 cm^{-1}). The ^{13}C NMR and DEPT spectra of **3** displayed 30 carbon resonances, consisting of 7 methyls, 8 methylenes, 8 methines (including two olefinic carbon), and 7 quaternary carbons (including an olefinic and carbonyl group). The NMR spectra of **3** is similar to that of **2**, except the absence of carbonyl group and the presence of additional hydroxyl group. This suggestion was clearly supported by HMBC correlations of H-1, H-2, CH$_3$-29 to an oxygenated carbon at δ_H 75.6, revealed that a newly hydroxyl group located at C-3. The ^{1}H-^{1}H COSY correlations of H-1/H-2, H-5/H-6, H-9/H-11/H-12 and H-15 to H-24 confirmed the tetracyclic skeleton and side chains of **3** almost identical with **2**. The NOESY cross-peaks of H-7 with CH$_3$-19 and CH$_3$-30, H-3 with CH$_3$-28 as well as their small coupling constants, suggested that H-3, H-7 and CH$_3$-30 are β-oriented and therefore hydroxyl group at C-3 and C-7 are both α-oriented. The relative configurations of H-17 and H-20 are both β-oriented that was determined by NOESY correlation between CH$_3$-18 and CH$_3$-21, therefore a methyl group at C-20 is α-oriented. In addition, the *Z*-geometry of the C-24/C-25 double bond of **3** was from the NOESY correlation between H-24 and CH$_3$-27. Consequently, compound **3** was established as a new *apo*-euphane triterpenoid, 3α,7α-diol-*apo*-eupha-14,24-*Z*-diene-26-oic acid and namely chisopaten C.

Compound **4** was isolated as a colorless solid with $[\alpha]_D^{23}$ +12.9 (*c*, 0.26, ethanol) and the molecular formula was assigned to be C$_{30}$H$_{46}$O$_4$ through its HR-TOFMS (*m/z* 469.3330, calcd. 469.3318, [M-H]$^-$) and NMR data (Table 1), thus requiring eight degrees of unsaturation. The NMR spectra displayed similarity to those of **2** regarding to the six-membered A and B rings. These assignments were confirmed by the ^2J and ^3J HMBC correlations of methyls (CH$_3$-29) to C-3 (δ_C 218.0), C-4 (δ_C 46.7) and C-5 (δ_C 45.7), methyl (CH$_3$-19) to C-1 (δ_C 39.6), C-5 (δ_C 45.7) C-9 (δ_C 45.3) and C-10 (δ_C 37.0), methyl (CH$_3$-30) to C-7 (δ_C 74.6), C-8 (δ_C 44.5), C-9 (δ_C 45.3) and C-14 (δ_C 56.7). The main differences are the position of methyl group and double bond. The HMBC correlations from H-16 (δ_H 2.23 and 2.40) and H-20 (δ_H 2.40) to C-17 (δ_C 132.3) and from H-12 (δ_H 1.90 and 2.01) to C-14 (δ_C 56.7), suggested that the position of a double bond at C-13/C-17. The ^{1}H-^{1}H COSY cross-peaks of H-1/H-2, H-5/H-6/H-7 and CH$_3$-21 to H-24 confirmed that A, B and C rings as well as side chains of **4**. Furthermore, correlation from methyl signal at δ_H 0.96 to C-14 (δ_C 56.7) and C-15 (δ_C 30.4), indicated that a methyl tertier attached at C-14. The equatorial, β-orientation of H-7 (3.84, d, *J* = 1.9 Hz) was displayed by its small coupling constants and NOESY correlation between H-7/CH$_3$-19 and H-7/CH$_3$-30, indicated that the hydroxy group at C-7 is α-oriented. The NOE correlation between CH$_3$-18 and CH$_3$-21, displayed that the methyl (C-21) is α-oriented. A comparison with dysotrifolin C isolated form *Dysoxylum densiflorum* (Nugroho et al., 2014), consequently,

Table 2

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

Compounds	IC$_{50}$ (μM)
Chisopaten A (**1**)	4.01 ± 0.008
Chisopaten B (**2**)	6.98 ± 0.008
Chisopaten C (**3**)	4.34 ± 0.009
Chisopaten D (**4**)	9.23 ± 0.008

compound **4** is an euphane type-triterpenoid (Camacho et al., 2000; Wang et al., 2003; Isaka et al., 2017). A β-orientation of H-7 (δ_H 3.84, d, *J* = 1.9 Hz) was indicated by its small coupling constants and NOESY correlations from this proton to CH$_3$-19 and H-7. Therefore, the structure of **4** was elucidated as a new euphane type-triterpenoid, 3-oxo-eupha-7α-ol,13,24-*Z*-diene-26-oic acid and namely chisopaten D.

All compounds were assessed for their cytotoxicity against MCF-7 breast cancer cell line according to a method described (Supriatno et al., 2018; Skehan et al., 1990), using Cisplatin as positive control (Hadisaputri et al., 2012) and the results are shown in Table 2. Chisopaten A and C showed the strongest activity against MCF-7, suggested that the position of hydroxyl, olefinic, carbonyl and methyl group are important role for the cytotoxic activity of compounds. The carbonyl group decreased the activity of compounds, was determined by comparing **2** (a carbonyl at C-3) with **3** (a hydroxyl at C-3). Furthermore, location of methyl (C-18) at C-13 and olifenic group at C-14/C-15 assigned that the cytotoxic activity of **2** was stronger than **4** against MCF-7 breast cancer cell line (Fig. 3).

3. Experimental procedures

3.1. General

Optical rotations were measured with an ATAGO AP-300 automatic polarimeter. Melting points were measured on a melting point M-565 apparatus. The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR in KBr. The mass spectra were determined with a Waters Xevo QTOF MS. NMR data were recorded on JEOL ECZ-600 spectrometer at 600 MHz for ^{1}H and 150 MHz using TMS as an internal standard. Column chromatography was performed on silica gel 60 (70–230 and 230–400 mesh). TLC was carried out on silica gel 60 F 254 (Merck, 0.25 mm) using various solvent systems, and spots were detected by irradiating under ultraviolet-visible light (257 and 364 nm) and heating the silica gel plates sprayed with 75% vanillin sulfat in EtOH (v/v = 75:25).

3.2. Plant material

The bark of *C. patens* Blume was collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in June 2015. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No.B-3664) was deposited at the herbarium.

3.3. Extraction and isolation

The dried bark of *C. patens* Blume (1.50 kg) was extracted with MeOH at room temperature to give a crude MeOH extract (250 g) after removal of solvent. The crude MeOH extract was partitioned between *n*-hexane and H$_2$O to give the *n*-hexane extract (67.20 g) and after evaporation and aqueous layer. The *n*-hexane soluble fraction was separated by vacum liquid chromatography on silica gel 60 using a gradient of *n*-hexane and EtOAc to give eight fractions (A–H). Fraction F (8.81 g) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane-Me$_2$CO (10:0–8:2), to give seven subfractions (F01-F07).

Subfraction F05 (0.40 g) was recrytallized in methanol to give **1** (300.6 mg). Subfraction F06 (0.41 g) was chromatographed on a column of silica gel, eluted with *n*-hexane-CH₂Cl₂-EtOAc (6:3:1) to give five subfractions (F06A-F06E). Subfraction F06D (74.60 mg) was separated on a column of silica gel, eluted withn *n*-hexane: Me₂CO (8:2) to give **2** (30.9 mg). Fraction G (30.13 g) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane-Me₂CO (10:0-7:3) to give thirteen subfractions (G01-G13). Subfraction G09 (4.57 g) was separated on a column of silica gel, eluted with a gradient of *n*-hexane: EtOAc (6:4), to give seven subfractions (G09A-G09 G). Subfraction G09E (320 mg) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane: EtOAc (10:0-7:3) to afford eight subfractions (G09E1-G09E8). Subfraction G09E6 (110 mg) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane: EtOAc (7:3) with the additon of acetic acid (0.5%), to give **3** and **4** (49.00 mg and 3.10 mg).

3.3.1. Chisopatens A (1)

White crystal; m.p 160.2–174.7 °C; $[\alpha]_D^{23}$ +15.6 (*c* 0.26, ethanol); IR (KBr) v_{max} 3435, 1698 1645 and 1120 cm⁻¹; ¹H-NMR (CD₃OD, 600 MHz), see Table 1; ¹³C-NMR (CD₃OD, 150 MHz), see Table 1; HR-TOFMS (negative ion mode) *m/z* 455.3571 [M-H]-, (calcd. for C₃₀H₄₈O₃, *m/z* 456.3525).

3.3.2. Chisopatens B (2)

Colorless solid; m.p. 79.2–82.3 °C; $[\alpha]_D^{23}$ +14.5 (*c*, 0.28, ethanol); IR (KBr) v_{max} 3449, 2952, 1702, 1457, 1368, 1215, 1068, and 1049 cm⁻¹; ¹H-NMR (acetone-d_6, 600 MHz) see Table 1; ¹³C-NMR (aceton-d_6, 150 MHz), see Table 1; HR-TOFMS (negative ion mode) *m/z* 469.3308 [M-H]- (calcd. for C₃₀H₄₆O₄, *m/z* 470.3318).

3.3.3. Chisopatens C (3)

White crystal; m.p. 165.1–166.3 °C; $[\alpha]_D^{23}$ +17.5 (*c*, 0.26, ethanol); IR (KBr) v_{max} 3423, 2935, 2606, 1692, 1647, 1456, 1387, 1231 and 1038 cm⁻¹; ¹H-NMR (CD₃OD, 600 MHz), ¹³C-NMR (CD₃OD, 150 MHz), see Table 1; HR-TOFMS (negative ion mode) *m/z* 471.3471 [M − H]- (calcd. for C₃₀H₄₈O₄, *m/z* 472.3437).

3.3.4. Chisopatens D (4)

Colorless solid; m.p 93.3–95.2 °C; $[\alpha]_D^{23}$ +12.9 (*c*, 0.26, ethanol); IR (KBr) v_{max} 3443, 2953, 1698, 1457, 1385 and 1242 cm⁻¹; ¹H-NMR (DMSO-d_6, 500 MHz), see Table 1; ¹³C-NMR (DMSO-d_6, 125 MHz), see Table 1; HR-TOFMS (negative ion mode) *m/z* 469.3330 [M − H]- (calcd. for C₃₀H₄₆O₄, *m/z* 470.3318).

3.4. Determination of cytotoxic activity

Cell viability was determined by a MTT assay. Briefly, the MCF-7 cells were seeded to each well of a 96-well plate at optimum cell density of approximately 3×10^4 cells cm⁻³. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The samples were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH 7.30–7.65). Control wells received only DMSO. The assay was terminated after a 48 h incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h incubation was conducted. Optical density was read using a micro plate reader at 550 nm. IC₅₀ values were taken from the plotted graph of percentage living cells compared to control (%), receiving only PBS and DMSO, vs the tested concentration of compounds (μM). The IC₅₀ value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

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