


Cite this: *RSC Adv.*, 2024, **14**, 25042

# The isolation of novel pregnane steroids from *Aglaia pachyphylla* Miq. and the cytotoxicity against breast cancer cell lines (MCF-7)<sup>†</sup>

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Steroid groups isolated from many plants are known to play a significant role in various biological systems. Therefore, this research aimed to analyze two novel pregnane steroids, pachylenone A (**1**) and pachylenone B (**2**), isolated from *Aglaia pachyphylla* Miq. The cytotoxicity of the steroids was evaluated against MCF-7 breast cancer cell lines with other known steroid compounds, namely 5 $\alpha$ -dihydroprogesterone (**3**), GSD-8 (**4**), *trans*-5 $\alpha$ -pregn-17(20)-en-3,16-dion (**5**), 20 $\beta$ -hydroxy-5 $\alpha$ H-pregnan-3-one (**6**), 3 $\beta$ -hydroxy-5 $\alpha$ -pregnan-20-one (**7**), aglaiasterol B (**8**), and 2 $\beta$ ,3 $\beta$ -dihydroxypregnan-16-one (**9**). Meanwhile, structural elucidation was achieved through different spectroscopic methods including one and two-dimensional NMR, as well as mass spectroscopy and quantum chemical calculations (TD-DFT and NMR DP4+ probability). The cytotoxic effects of steroid compounds (**1**–**9**) on MCF-7 lines were also examined. The results showed that compound **8** had the strongest activity with an IC<sub>50</sub> value of 228  $\mu$ M, followed by compound **6** (IC<sub>50</sub> 568.76  $\mu$ M), and pachylenone A (**1**) (IC<sub>50</sub> 768.73  $\mu$ M). As a recommendation for future research, other activities of these compounds should be evaluated.

Received 29th June 2024  
Accepted 5th August 2024

DOI: 10.1039/d4ra04727c

rsc.li/rsc-advances

## Introduction

Pregnane steroids are secondary metabolites with a significant role in various biological systems used by humans.<sup>1</sup> These compounds are typically derived from higher plants and often show a wide range of biological activities due to diverse structures, which have 21 carbons known as the pregnane skeleton (Fig. 1).<sup>2</sup> In addition, the syntheses from cholesterol are carried out through complex biosynthetic pathways and are known for structural complexity, which includes multiple rings as well as functional groups and substituents. Steroids are also described as natural hormones found in the human body.<sup>3</sup>

Over 500 unique pregnane steroids have been isolated, showing the extraordinary chemical diversity found in plants. These structural variations contribute to the diverse biological activities, including anti-inflammatory, antibacterial, antifungal, antiviral, and anticancer properties. Previous research

reported the cytotoxic potential of pregnane steroids against cancer cell lines.<sup>4</sup>

The Meliaceae family is well-known for producing a variety of bioactive compounds, including steroids, sesquiterpenoids, and triterpenoids<sup>5–7</sup> and is used as traditional medicine.<sup>8</sup> Important genera containing biologically active steroids include *Aglaia*, *Trichilia*, and *Dysoxylum*. *Aglaia* is the most extensive genus, comprising 65 of the 150 species found in Indonesia.<sup>9</sup> Several pregnane steroids isolated from the *Aglaia* genus showed significant cytotoxic activity against cancer cells.<sup>10</sup> Previous research reported that pregnane steroids Aglaian A and B were also isolated from *A. lawii* with a significant value of IC<sub>50</sub> against breast cancer cell lines (MCF-7) (50  $\mu$ M).<sup>11</sup>

*Aglaia pachyphylla* Miq., a species within the *Aglaia* genus, is a tropical plant commonly found in Southeast Asia, including in Indonesia, Malaysia, and the Philippines.<sup>8</sup> The cytotoxic potential of pregnane steroids from the species against breast cancer cell lines (MCF-7) has not been extensively analyzed. In the ongoing quest for new bioactive metabolites, a chemical investigation was conducted on *Aglaia pachyphylla* Miq. obtained from the Kutai Kartanegara, East Kalimantan. Using a variety of methods, two novel pregnane compounds, pachylenone A and B (**1** and **2**) as well as seven known steroids (**3**–**9**) were obtained. The final steps comprised the description of molecule separation, structural analysis, and biological assessment.

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<sup>†</sup> Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d4ra04727c>



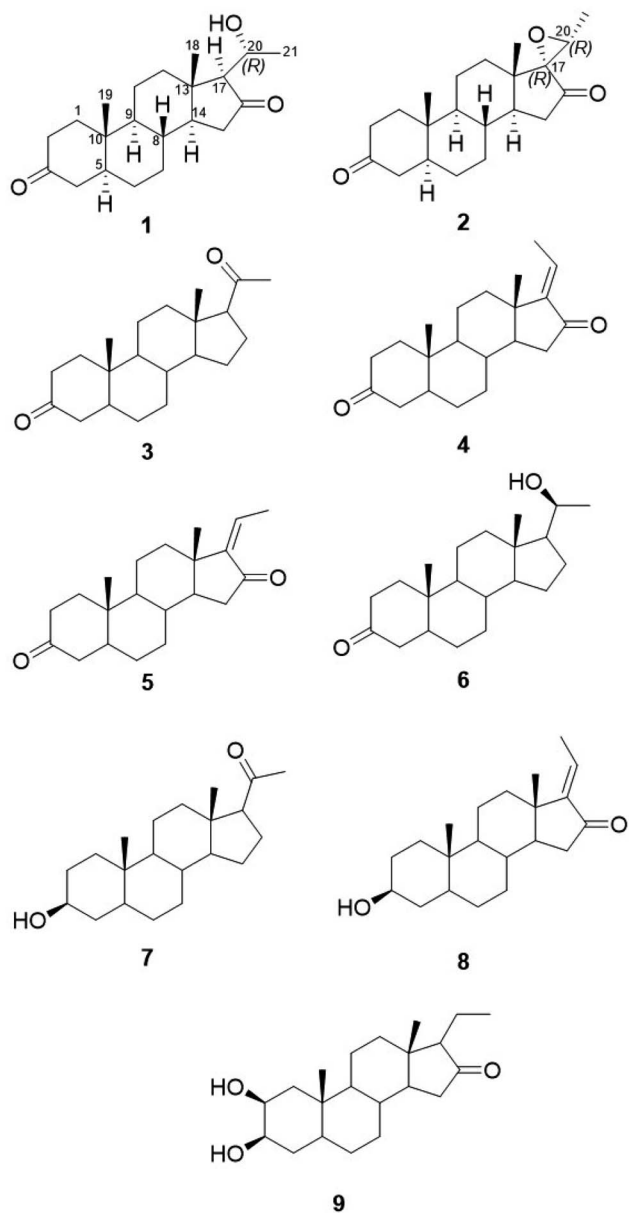


Fig. 1 Chemical structures of compounds 1–9.

## Result and discussion

The concentrated ethanolic extract of stem bark of *A. pachyphylla* Miq. was dissolved in water and extracted respectively with *n*-hexane, ethyl acetate, and *n*-butanol. The *n*-hexane extract was separated using vacuum liquid chromatography (VLC), followed by a combination of column chromatography on normal and reverse phase columns (silica gel G<sub>60</sub> and ODS) to gain nine pregnane steroids including two novel pregnane steroids compounds 1 and 2.

Compound 1 was obtained as white amorphous powder, and the molecular formula was established as C<sub>21</sub>H<sub>32</sub>O<sub>3</sub> based on HRTOF-MS ( $M + H$ )<sup>+</sup> at  $m/z$  333.2426 (calcd 333.2430), indicating six double bond equivalents (DBEs). The IR spectrum showed absorption bands of hydroxy (3391 cm<sup>−1</sup>), C–H

(2938 cm<sup>−1</sup>), and two carbonyl stretches (1732 and 1717 cm<sup>−1</sup>). Moreover, the <sup>1</sup>H NMR spectrum reported two methyl singlets ( $\delta_H$  0.95 and 1.05), one methyl doublet ( $\delta_H$  1.42, d, 7 Hz), and one hydroxy ( $\delta_H$  4.06, m). The <sup>13</sup>C NMR and DEPT spectra also contained 21 carbon resonances, including three methyls at  $\delta_C$  11.6, 13.9, and 23.3, eight methylenes at  $\delta_C$  21.0, 28.8, 31.9, 38.2, 38.5, 39.2, 39.5, and 44.7 ppm, five methines at  $\delta_C$  34.4, 44.7, 50.3, 53.8, and 69.7 ppm, two quaternary carbons at  $\delta_C$  35.9 and 43.1 ppm, one oxymethine at  $\delta_C$  66.5 ppm, as well as two ketones at  $\delta_C$  211.7 and 217.5 ppm.

Detailed NMR data analysis suggested that compound 1 was similar to pregnane steroid with 21 carbons.<sup>12</sup> The HMBC correlation (Fig. 2) of H-1 ( $\delta_H$  2.37), H-2 ( $\delta_H$  2.04), and H-4 ( $\delta_H$  2.28) to C-3 ( $\delta_C$  211.7) also reported the presence of a ketone group at C-3. Meanwhile, the position of ketone at C-16 was shown by the correlation of H-15 ( $\delta_H$  2.25), and H-17 ( $\delta_H$  1.91) to C-16 ( $\delta_C$  217.5). Correlation between CH<sub>3</sub>-21 ( $\delta_H$  1.42) to C-20 ( $\delta_C$  66.5), H-20 ( $\delta_H$  4.06) to C-21 ( $\delta_C$  23.3), and C-17 ( $\delta_C$  69.7) indicated the presence of oxymethine and methyl doublet at C-20 and C-21, respectively. In addition, <sup>1</sup>H–<sup>1</sup>H COSY (H-1/H-2, H-5/H-6, H-8/H-9, H-11/H-12, and H-8/H-14/H-15) and H-17/H-20/H-21 correlations confirmed four rings of pregnane skeleton and the position of oxymethine at C-20, respectively. The relative configuration of compound 1 was determined by the NOESY experiment. The correlations (Fig. 3) of H-5 ( $\delta_H$  1.58) and H-14 ( $\delta_H$  1.46) with H-9 ( $\delta_H$  0.93) and H-17 ( $\delta_H$  1.91) showed that H-5, H-9, H-14 and H-7 were in the  $\alpha$  orientation. The correlations of CH<sub>3</sub>-19 ( $\delta_H$  1.05) and CH<sub>3</sub>-18 ( $\delta_H$  0.95) with H-8 ( $\delta_H$  1.60) and H-20 ( $\delta_H$  4.06) reported that CH<sub>3</sub>-18, CH<sub>3</sub>-19, H-8 and H-20 were  $\beta$ -orientation. In addition, hydroxy at C-20 had  $\alpha$ -orientation and the configuration was confirmed by ECD experiment (solvent = MeOH) and NMR calculation (DP4+ analysis). In this context, density functional theory (DFT) was stimulated to calculate the <sup>13</sup>C NMR chemical shift of two probability C-20 isomers, denoted as (20*R*)-1a and (20*S*)-1b. The strong correlation ( $R^2 = 0.9994$ ) was reported by (20*R*)-1a between the

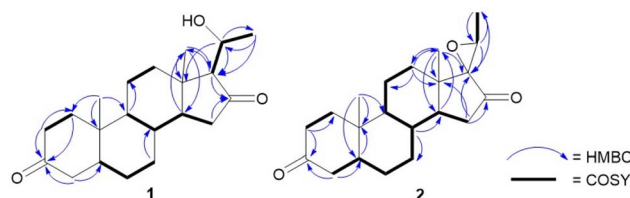


Fig. 2 Key HMBC and <sup>1</sup>H–<sup>1</sup>H COSY correlations of pachylenone A and B (1 and 2).

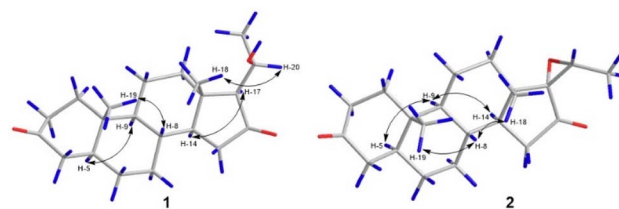


Fig. 3 Key NOESY correlations of pachylenone A and B (1 and 2).



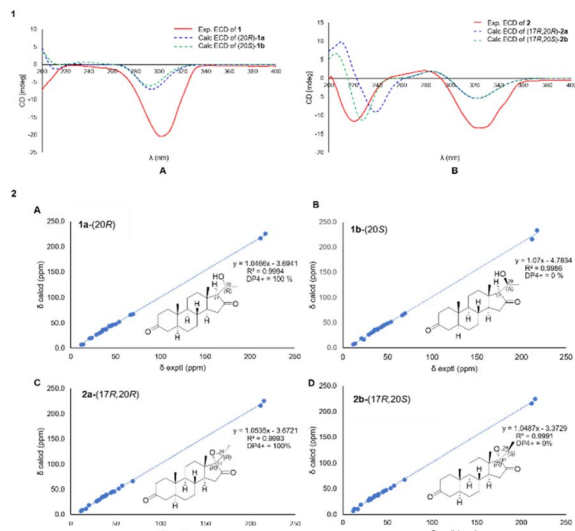


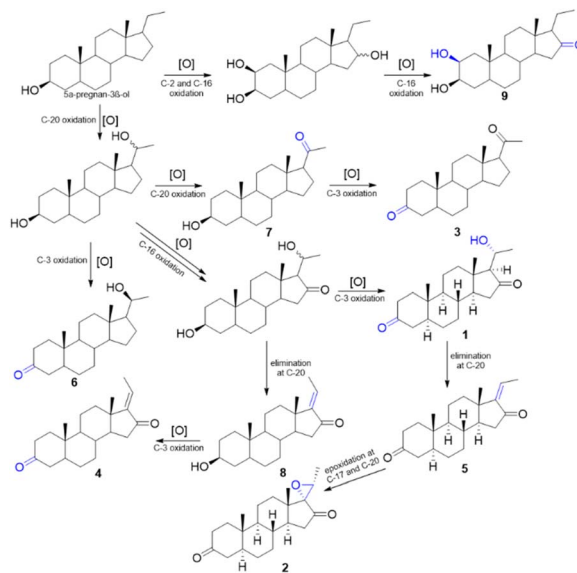
Fig. 4 [1] (A) Calculated and experimental ECD spectra of compound 1, (B) calculated and experimental ECD spectra of compound 2. [2] (A) (1a-20R) and (B) (1b-20S) NMR calculation of compound 1, (C) (2a-17R, 20R) and (D) (2b-17R, 20S) NMR calculation of compound 2.

experimental and calculated data based on TD-DFT calculation (Fig. 4). The absolute configuration of compound 1 was shown as 5*S*, 8*R*, 9*S*, 10*S*, 13*S*, 14*S*, 17*S*, and 20*R*. Therefore, compound 1 was determined as a novel pregnane steroid and named pachylenone A. The 2D NMR data served as the primary building block for the architecture, as reported in Fig. 1.

Compound 2, colorless needles, had a molecular formula of  $C_{21}H_{30}O_3$  based on HRTOF-MS ( $M + H$ )<sup>+</sup> at  $m/z$  331.2266 (calcd 331.2273), indicating seven DBEs. The IR spectrum showed absorption bands C–H stretch ( $2924\text{ cm}^{-1}$ ), and two carbonyl stretches ( $1740$  and  $1710\text{ cm}^{-1}$ ). Additionally, the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data were almost similar to compound 1, except for the presence of epoxide at C-17 and C-20. The HMBC correlations (Fig. 2) of H-15 ( $\delta_{\text{H}}$  2.37), H-18 ( $\delta_{\text{H}}$  1.02), H-20 ( $\delta_{\text{H}}$  3.15) and H-21 ( $\delta_{\text{H}}$  1.58) with C-17 ( $\delta_{\text{C}}$  69.0) reported a quarternary carbon at C-17. The correlation between H-21 ( $\delta_{\text{H}}$  1.58) and C-20 ( $\delta_{\text{C}}$  57.2) was confirmed as a methine epoxide at C-20, and the presence was supported by the addition of a double bond equivalent (DBE). The pregnane skeleton and one epoxide ring were shown by the  $^1\text{H}$ – $^1\text{H}$  COSY correlation between H-1/H-2, H-4/H-5/H-6/H-7/H-8/H-9/H-11/H-12 and H-8/H-14/H-15 for four rings pregnane and the correlation between H-20 and H-21 for epoxide ring.

The NOESY correlations (Fig. 3) of H-5 ( $\delta_{\text{H}}$  1.58) and H-9 ( $\delta_{\text{H}}$  0.93) with H-9 ( $\delta_{\text{H}}$  0.93) and H-14 ( $\delta_{\text{H}}$  1.71) reported the protons in the  $\alpha$ -orientation. Meanwhile, the correlations of H-8 ( $\delta_{\text{H}}$  1.67) with  $\text{CH}_3$ -18 ( $\delta_{\text{H}}$  1.02) and  $\text{CH}_3$ -19 ( $\delta_{\text{H}}$  1.05) showed  $\text{CH}_3$ -18 and  $\text{CH}_3$ -19 in the  $\beta$ -orientation.

The unambiguous configuration for epoxide ring was characterized by the ECD experiment (solvent = MeOH) and calculation of the spectra using TD-DFT method and NMR (DP4+ analysis) (Fig. 4). The closely fitting ECD curve showed that the absolute configuration of compound 2 was determined as 5*S*, 8*R*, 9*S*, 10*S*, 13*S*, 14*S*, 17*R*, 20*R*. This was supported by the



Scheme 1 A plausible biosynthetic pathway of compounds 1–9.

excellent correlation ( $R^2 = 0.9993$ ) observed between the experimental and calculated data. Therefore, compound 2 had been determined as a novel pregnane-type of steroid and was named pachylenone B.

The biogenesis pathway for 1 and 2 was described based on Scheme 1 from 5 $\alpha$ -pregnan-3 $\beta$ -ol. This was initiated by oxidation in C-20 and C-16 to suggest a hydroxy group and ketone, respectively. In addition, the oxidation process at C-3 converted the hydroxy group to ketone, producing compound 1. The elimination process at C-20, which converted hydroxy to an olefinic group, was suggested for compound 5. Subsequently, further epoxidation of the carbon atoms at C-17 and C-20 produced compound 2.

A total of seven known pregnane compounds were obtained from this species including 5 $\alpha$ -dihydroprogesterone (3),<sup>13</sup> GSD-8 (4),<sup>14</sup> *trans*-5 $\alpha$ -pregn-17(20)-en-3,16-dione (5),<sup>15</sup> 20 $\beta$ -hydroxy-5 $\alpha$ H-pregnan-3-one (6),<sup>16</sup> 3 $\beta$ -hydroxy-5 $\alpha$ -pregnan-20-one (7),<sup>17</sup> aglaiasterol B (8),<sup>18</sup> and 2 $\beta$ ,3 $\beta$ -dihydroxypregnan-16-one (9).<sup>19</sup>

According to Table 2,  $\text{IC}_{50}$  values were obtained by assessing the cytotoxic activity of 1–9 against breast cancer cell lines (MCF-7) using the Resazurin (PrestoBlue) assay. Aglaiasterol B (8) possessed the highest activity among pregnane steroids, followed by 20 $\beta$ -hydroxy-5 $\alpha$ H-pregnan-3-one (6) ( $\text{IC}_{50} = 568.76\text{ }\mu\text{M}$ ) and pachylenone A (1) ( $\text{IC}_{50} = 768.73\text{ }\mu\text{M}$ ) with an  $\text{IC}_{50}$  value of  $228\text{ }\mu\text{M}$ . Meanwhile, compounds 1–9 of the pregnane steroids were categorized as inactive against breast cancer cell lines (MCF-7).

A brief structure–activity relationship (SAR) analysis showed that the cytotoxic activity of aglaiasterol B (8) against MCF-7 cancer cells could be increased directly due to the presence of hydroxyl, carbonyl, and double bond groups at C-3, C-16, and C-17/C-20, respectively. In addition, the presence of the carbonyl substituent (C-16) and the *R* configuration of the hydroxyl group (C-20) in compound 1 decreased the value of the cytotoxic activity.



Table 1 NMR data (700 MHz for  $^1\text{H}$  and 175 MHz for  $^{13}\text{C}$  in  $\text{CDCl}_3$ ) for **1** and **2**

Position	<b>1</b>		<b>2</b>	
	$^{13}\text{C}$ NMR	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR	$^1\text{H}$ NMR
	$\delta_{\text{C}}$ (mult.)	$\delta_{\text{H}}$ (integral, mult, $J$ Hz)	$\delta_{\text{C}}$ (mult.)	$\delta_{\text{H}}$ (integral, mult, $J$ Hz)
1	38.2 (t)	2.04 (1H, m) 1.37 (1H, m)	38.1 (t)	2.03 (1H, m) 1.46 (1H, m)
2	38.5 (t)	2.37 (1H, m) 2.33 (1H, m)	38.0 (t)	2.35 (1H, m) 2.03 (1H, m)
3	211.7 (s)		211.4 (s)	
4	44.7 (t)	2.11 (1H, m) 2.28 (1H, m)	44.5 (t)	2.27 (1H, m) 2.11 (1H, m)
5	46.7 (d)	1.58 (1H, m)	46.5 (d)	1.58 (1H, m, overlap)
6	28.8 (t)	1.38 (2H, m)	28.6 (t)	1.37 (2H, m)
7	31.9 (t)	1.66 (1H, m) 1.01 (1H, dd, 14; 7)	31.4 (t)	1.70 (1H, m) 1.03 (1H, m)
8	34.4 (d)	1.60 (1H, m)	34.6 (d)	1.67 (1H, m)
9	53.8 (d)	0.93 (1H, m)	53.4 (d)	0.93 (1H, m)
10	35.9 (s)		35.9 (s)	
11	21.0 (t)	1.65 (1H, m) 1.49 (1H, m)	20.1 (t)	1.71 (1H, m) 1.46 (1H, m)
12	39.2 (t)	1.86 (2H, m)	30.2 (t)	1.46 (1H, m) 1.28 (1H, dd, 14; 7)
13	43.1 (s)		39.8 (s)	
14	50.3 (d)	1.46 (1H, m)	48.5 (d)	1.71 (1H, m, overlap)
15	39.5 (t)	2.25 (1H, m) 1.48 (1H, m)	39.7 (t)	2.37 (1H, d, 7) 2.15 (1H, d, 7)
16	217.5 (s)		215.2 (s)	
17	69.7 (d)	1.91 (1H, d, 7)	69.0 (s)	
18	13.9 (q)	0.95 (3H, s)	16.8 (q)	1.02 (3H, s)
19	11.6 (q)	1.05 (3H, s)	11.5 (q)	1.05 (3H, s)
20	66.5 (d)	4.06 (1H, m)	57.2 (d)	3.15 (1H, q, 7)
21	23.3 (q)	1.42 (3H, d, 7)	12.7 (q)	1.58 (3H, d, 7)

Table 2 Cytotoxic activities of pregnane steroids **1–9** against breast cancer MCF-7 cell lines

No.	Compound	$\text{IC}_{50}$ ( $\mu\text{M}$ )
1	Pachylenone A ( <b>1</b> )	768.73
2	Pachylenone B ( <b>2</b> )	>1000
3	5 $\alpha$ -Dihydroprogesterone ( <b>3</b> )	>1000
4	GSD-8 ( <b>4</b> )	>1000
5	<i>trans</i> -5 $\alpha$ -Pregn-17(20)-en-3,16-dione ( <b>5</b> )	>1000
6	20 $\beta$ -Hydroxy-5 $\alpha$ H-pregnan-3-one ( <b>6</b> )	568.76
7	3 $\beta$ -Hydroxy-5 $\alpha$ -pregnan-20-one ( <b>7</b> )	>1000
8	Aglaiasterol B ( <b>8</b> )	228
9	2 $\beta$ ,3 $\beta$ -Dihydroxypregnan-16-one ( <b>9</b> )	>1000
Positive control	(Cisplatin)	43.18

## Experimental section

### General experimental procedures

IR spectra were obtained using a PerkinElmer spectrum-100 FT-IR in the plate of KBR (Waltham, Massachusetts, USA) and Nicolet Summit (Thermo scientific). Meanwhile, optical rotations were measured using an ATAGO AP-300 automatic polarimeter (Saitama, Japan). Electronic circular dichroism spectra were also recorded on a JASCO J-1500 with the detector PM-539. High-resolution mass spectra (HRTOF-MS) were gained on

Waters Q-TOF-HRTOFMS-XEVOtm mass spectrometer (Milford, MA, USA). Subsequently, NMR spectra were conducted on Bruker ASCEND 700 MHz for  $^1\text{H}$ ,  $^{13}\text{C}$ , and 2D-NMR using TMS as the internal standard. Silica gel  $\text{G}_{60}$  (Merck, Darmstadt, Germany, 70–230 and 230–400 mesh) and octadecyl silane (ODS, Fuji Sylisia Chemical LTD, Chromatorex®  $\text{C}_{18}$  DM1020 M, 100–200 mesh) were also adopted for normal and reverse phase column chromatography (CC). For TLC, Merck pre-coated silica gel 60  $\text{F}_{254}$  plates were performed and spots were detected by spraying with 10%  $\text{H}_2\text{SO}_4$  in ethanol before heating.

### Plant material

The stem bark of *A. pachyphylla* Miq. was collected from KHDTK, Kutai Kartanegara, East Kalimantan, Indonesia in December 2020, and was determined at Herbarium Wanariset (WAN), Balikpapan. In addition, a voucher specimen (no. FF11.20) was deposited in the Faculty of Forestry, Mulawarman University.

### Extraction and isolation

A total of 4.8 kg of stem bark powder of *A. pachyphylla* Miq. was macerated in a macerator using ethanol 96% (20 L)  $8 \times 24$  hours at room temperature to obtain the ethanol extract (685 g, 14.2% yield) after the removal of solvent. Furthermore, the





concentrated extract was sequentially extracted using *n*-hexane, EtOAc, and *n*-BuOH at room temperature. After evaporating with a rotary evaporator, *n*-hexane (26 g), EtOAc (221 g), and *n*-BuOH (72 g) extracts were obtained. The *n*-hexane extract (26 g) was subjected to column chromatography using silica gel, eluted with *n*-hexane/EtOAc (10:0–0:10, 10%) and EtOAc/MeOH (10:0–8:2, 10%) to obtain nine fractions (Fr. A – Fr. I).

Fr. C (4.2 g) was chromatographed using silica gel by a gradient system of *n*-hexane/EtOAc (10:0–8:2, 1%) to obtain ten subfractions (Fr. C1–Fr. C10). In addition, Fr C.4 (119 mg) was divided using reverse phase column chromatography (MeOH/water, 9:1) to afford compounds 3 (4 mg) and 4 (7.5 mg). Recrystallization process with MeOH was also used to purify Fr C6 and C7 in obtaining compounds 5 (14 mg) and 6 (55 mg). Fr C8 was separated on RP-18 silica gel CC (MeOH/water, 9:1) to provide compound 7 (7 mg), while Fr. C9 (150 mg) obtained 1 (7 mg) and 2 (8.5 mg). A total of five subfractions (Fr E1–E5) was reported by subjecting Fr E (3.3 g) to silica gel CC eluted with *n*-hexane/EtOAc (8:2–6:4, 2%). An isocratic system *n*-hexane/DCM/EtOAc (6:2:2) was used to purify Fr E3 (803 mg) in obtaining compound 8 (8.3 mg). Fr F (2.6 g) was separated using a gradient system of normal phase column chromatography, eluted with *n*-hexane/EtOAc (7:3–1:1, 2%) to obtain seven subfractions (Fr F1–F7). Subsequently, Fr F4 (469 mg) was treated to obtain four subfractions (Fr F4a–F4d). Compound 9 (5.2 mg) was reported by purifying Fr F4b on RP-18 silica gel CC (MeOH/water, 7:3).

Pachylenone A (1): white amorphous powder,  $[\alpha]_D^{26}$  -95.2 (c 0.4, CHCl<sub>3</sub>), IR (KBr)  $\nu_{\max}$  3391, 2938, 2854, 1732, 1717 cm<sup>-1</sup>, HRTOF-MS  $m/z$  333.2426 [M+H]<sup>+</sup> (calc. for C<sub>21</sub>H<sub>33</sub>O<sub>3</sub>, 333.2430), <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 700 MHz) and <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 175 MHz), as shown in Table 1.

Pachylenone B (2): colorless needles,  $[\alpha]_D^{26}$  -55.3 (c 0.4, CHCl<sub>3</sub>), IR  $\nu_{\max}$  2924, 1740, 1710 cm<sup>-1</sup>, HRTOF-MS  $m/z$  331.2266 [M + H]<sup>+</sup> (calc. for C<sub>21</sub>H<sub>31</sub>O<sub>3</sub>, 331.2273); <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 700 MHz) and <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 175 MHz), as shown in Table 1.

### Methods for ECD and NMR calculation

Conformational distribution searches were performed using Spartan 14.0 (Wavefunction Inc., Irvine, CA, USA) software with a Boltzman distribution of 2% using the Merck molecular force field (MMFF). The optimization of conformers was performed using DFT method at B3LYP/6-31g(d) level on the Gaussian 09 program. In addition, the optimized conformer was subjected to TD-DFT calculation at B3LYP/6-311g(d,p) level, nstates = 20, and a polarizable continuum model (IEFPCM, solvent: MeOH). The spectra were generated to SpecDis 1.71 with sigma = 0.25 eV and the Gauge-Independent Atomic Orbital (GIAO) method at MPW1PW91/6-311+g(d,p) (IEFPCM, solvent: chloroform) was used for NMR calculation.<sup>20</sup>

### Cytotoxic bioassay of compounds 1–9

The PrestoBlue assay, as documented by Mulyani and Nurlelari (2024)<sup>21,22</sup> was used to evaluate the cytotoxic potential. Compounds 1–9 were evaluated against breast cancer cell lines (MCF-7) (ATCC HTB-22) cultivated in RPMI medium. These cells

were supplemented with 10% Fetal Bovine Serum (FBS) and 1  $\mu$ L mL<sup>-1</sup> penicillin-type antibiotic (Sigma Aldrich P4333). Additionally, incubation was carried out at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub>. Cell seeding was performed in 96-well plates at a density of  $1.7 \times 10^4$  and the introduction of compounds 1–9 of varying concentrations (2.34, 4.69, 9.38, 18.75, 37.50, 75.00, 150, 300  $\mu$ g mL<sup>-1</sup>) into the wells occurred separately. The stock drug solutions were made with cisplatin (positive control) as much as 1 mg was dissolved in 1 mL phosphate buffered saline (PBS, Gibco 18912-014). After 48 hours, an assessment of cell viability was performed by quantifying the conversion of resazurin to resorufin, emitting a pink fluorescent signal denoting viable cells. Meanwhile, the analysis of results unfolded through a multimode reader at 570 nm, and the IC<sub>50</sub> values were deduced from graphs contrasting the percentage of viable cells to DMSO 2% in media 1 mL as a negative control. The values showed the concentration requisite to impede cell growth by 50%. Preliminary SAR was demonstrated by the substituents at C-3, 5, 6, 7, 15, 16, 17, and 21, which was affecting cytotoxic activity.<sup>23</sup>

## Conclusions

In conclusion, this research was conducted to successfully examine two novel pregnane steroids, pachylenone A (1) and pachylenone B (2), with seven compounds isolated from *Aglaia pachyphylla* Miq. The chemical activities of the steroids were enhanced and the interest in chemical synthesis was promoted to recreate the potency and selectivity of natural compounds. Moreover, other bioactivities and mechanisms of action for compounds (1–9) in killing cancer cells were evaluated.

## Data availability

Data supporting this study are included within the article and ESI.†

## Author contributions

All authors contributed to the writing of the manuscript and have approved the final version.

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

The authors are grateful to the Indonesian Ministry of Research, Technology, and Higher Education for Grant of Pendidikan Magister menuju Doktor untuk Sarjana Unggul (PMDSU) (3166/UN6.3.1/PT.00/2024; 6 Mei 2024) Indonesia, Academic Leadership Grant (ALG) (1433/UN6.3.1/PT.00/2024; 18 Maret 2024) by Desi Harneti, and the Directorate of Research and Community Engagement Universitas Padjadjaran, for providing support during the research. In addition, the authors are thankful to Universitas Padjadjaran for providing the required facilities.



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