

Anticancer Meroterpenoids from *Centrapalus pauciflorus* leaves: Chromone- and 2,4-Chromadione-Monoterpene Derivatives

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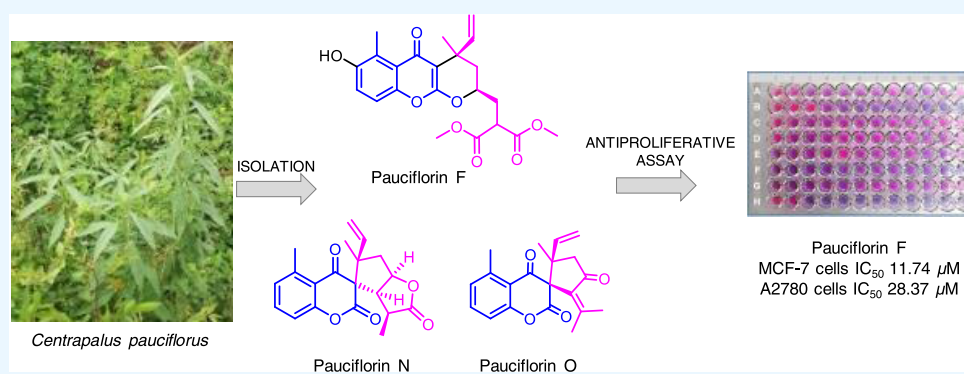
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ABSTRACT: Eight previously undescribed chromones, named pauciflorins F–M and two 5-methyl-2,4-chromadione derivatives named as pauciflorins N and O, were isolated from the methanol extract of the leaves of *Centrapalus pauciflorus* (Willd.) H. Rob. together with the known (+)-spiro-ethuliacoumarin. The structures were determined via extensive spectroscopic analyses, including HRESIMS, 1D NMR (^1H , ^{13}C JMOD), and 2D NMR (HSQC, HMBC, ^1H – ^1H COSY, and NOESY) experiments. Through an MTT assay, seven isolated compounds were tested for their antiproliferative properties against human adherent breast (MCF-7, MDA-MB-231), cervical (HeLa, SiHa), and ovarian (A2780) cancer cell lines. Pauciflorin F was effective against MCF-7 breast cancer cells, its activity (IC_{50} 5.78 μM) was comparable to that of the reference agent cisplatin (IC_{50} 5.78 μM).

INTRODUCTION

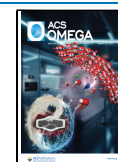
Meroterpenoids are a group of terpenoid-containing hybrid natural products with unique structural architectures and impressive pharmacological properties. Meroterpenoids can be classified based on their nonterpenoid moiety into polyketide-terpenoids, shikimate-terpenoids, and alkaloid-terpenoids. These compounds are synthesized by a wide range of organisms, including bacteria, fungi, algae, plants, animals, and marine organisms.^{1–4} Notably, meroterpenoids derived from chromane/chromene can be condensed with hemi-, mono-, sesqui-, and diterpenoid units; their presence has been observed in various organisms, such as tunicates (*Botryllus*),⁵ brown macroalgae (*Sargassum*, *Cystoseira*),⁶ and *Rhododendron* (Ericaceae),⁷ *Sarcandra* (Chloranthaceae),⁸ and *Mimosa* (Fabaceae)⁹ plant species. In addition, the genera of the Asteraceae family have been discovered to produce uncommon monoterpenoid-coupled chromones, which typically coexist with structurally related monoterpenoid coumarins.^{10–12} These intriguing compounds have been identified in genera such as *Nassauvia*,¹³ *Triptilion*,¹⁴ and *Polyachyrus* from the Nassauvieae tribe, *Gerbera*¹¹ and *Mutisia*¹⁵ from the Mutisieae

tribe, and *Bothriocline* genus¹⁶ from the Vernonieae tribe and reported to accumulate as both coumarin- and chromone-based meroterpenoids. The biosynthesis of these compounds involves the acetate–malonate pathway, where 5-methylcoumarins and 5-methylchromones serve as the main building blocks for chromone-, coumarin-based meroterpenoids, which is catalyzed by polyketide synthase enzymes.¹⁷ Chromane/chromene meroterpenoids have displayed cytotoxic activity against different tumor cell lines, as well as antioxidant and antimicrobial activities. Furthermore, they have been reported to inhibit various enzymes, including protein tyrosine phosphatase 1B (PTP1B), butyrylcholinesterase (BChE), β -

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site amyloid precursor protein cleavage enzyme 1 (BACE1), and protein farnesyl transferase (PFTase).^{1,18}

Continuing our ongoing investigation on the metabolites of *Centrapalus pauciflorus* (Willd.) H. Rob.,^{19,20} this study reports the isolation and structure determination of eight chromone-meroterpenoids named pauciflorins F–M (1–10) and two 5-methyl-2,4-chromadione-meroterpenoids pauciflorins N and O (10–11), extracted from the leaves of *C. pauciflorus*. This plant, belonging to the Asteraceae family, is also known by synonymous names, such as *Centrapalus galamensis* Cass., *Conyza pauciflora* Willd., *Vernonia afromontana* R.E. Fr., and *Vernonia pauciflora* (Willd.) Less., etc. *C. pauciflorus* is native to tropical African countries, spanning from Cape Verde and Senegal in West Africa to Somalia in East Africa and reaching down to Southern Africa, encompassing Zimbabwe and Mozambique.^{21,22} It is a mainly unbranched, annual plant that grows 3–5 m tall. In folklore medicine, its leaves are cooked in porridge or brewed as tea to alleviate chest pain. The plant is also used to relieve stomach pain.²⁰

MATERIALS AND METHODS

General Experimental Procedures. The optical rotations were determined using a JASCO P-2000 polarimeter (JASCO International Co. Ltd., Hachioji, Tokyo, Japan). NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz (¹H) and 125 MHz (¹³C). The two-dimensional (2D) experiments were conducted using standard Bruker software. Gradient-enhanced versions were applied in correlation spectroscopy (¹H–¹H COSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear single quantum coherence spectroscopy (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments. The signals of the deuterated solvent were taken as references. High-resolution electrospray ionization–mass spectroscopy (HRESIMS) was measured using a Thermo Scientific Q-Exactive Plus Orbitrap mass spectrometer in positive ionization mode equipped with an electrospray ionization source. The data were acquired and processed using MassLynx software. Vacuum liquid chromatography (VLC) was made on silica gel (15 μm, Merck) (NP-VLC); LiChroprep RP-18 (40–63 μm, Merck) stationary phase was used for reversed-phase VLC (RP-VLC); open column chromatography (OCC) was conducted on polyamide (MP Biomedicals). Flash column chromatography (FCC) was performed using a CombiFlash Rf+ Lumen instrument with integrated ultraviolet (UV), UV–visible (UV–vis), and electrophoretic light scattering detection using a column (3.5 cm × 14 cm) filled with 40 g of reversed-phase (RP) silica C₁₈. Thin-layer chromatography (TLC) monitored the OCC, VLC, and FCC separations and was carried out on silica gel 60 F₂₅₄ plates (Merck). High-performance liquid chromatography (HPLC) was performed on Agilent, WUFENG, and WATERS HPLC instruments using normal-phased (NP) LiChrospher Si 60 (4 mm × 250 mm, 5 μm) and Luna (R) Silica (2) 100 (250 mm × 21.2 mm, 5 μm), as well as RP Kinetex C₁₈ 100A (4.6 mm × 150 mm, 5 μm) and Agilent ZORBAX ODS C₁₈ 100A (9.4 mm × 250 mm, 5 μm) columns. The TLC plates were detected under a UV light at 254 nm by spraying with concentrated H₂SO₄, followed by heating. All solvents used for chromatography were analytical or HPLC grade (VWR Ltd., Hungary).

Plant Material. The leaves of the plant were gathered in August 2018 in Zaria, Nigeria (11°7′19.758″N 7°43′23.1672″E) and were identified by Umar Shehu Gallah

(National Research Institute for Chemical Technology, NARICT), Zaria, Nigeria. A voucher specimen was deposited in NARICT under the number Narict/Biores/321 and in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary, No. 897.

Extraction and Isolation. The air-dried and powdered leaves of the plant (548 g) was extracted using percolation with methanol (45 L) at room temperature until all possible extract was obtained. The methanol extract was concentrated in a vacuum to yield 133 g of extract, representing 24.3% of the plant material. The extract was dissolved in 1 L MeOH–H₂O (1:1) and subjected to solvent–solvent partition with CHCl₃ (3 × 1 L) to yield the lipophilic phase. After concentration, the CHCl₃ phase (65.81 g) was separated using OCC on polyamide (250 g), eluting with methanol–water (1:4, 2:3, 3:2, 4:1, and 5:0) mixtures as eluents. Five fractions were collected according to the eluents. The fraction obtained with MeOH–H₂O (3:2) showed the highest antiproliferative activity against MCF-7, MDA-MB-231, HeLa, and A2780 cell lines with growth inhibition of 70.7%, 85.3%, 63.7%, and 68.2%, respectively at 30 μg/mL,¹⁹ and it was chosen for further chromatographic purification. VLC was conducted on that fraction (14 g) on silica gel using a gradient system of cyclohexane–EtOAc–EtOH (9:1:0, 8:2:0, 7:3:0, 50:20:1.5, 50:20:3, 50:20:6, 50:20:9, 50:20:12, 50:20:15, 5:2:2, 5:2:4, 5:2:6, and 5:2:8), which yielded fractions A–I. Fractions A–C obtained with elution of cyclohexane–EtOAc–EtOH (8:2:0, 50:20:1.5 and 50:20:3) were further chromatographed on NP- and RP-VLC as follows.

NP-VLC was conducted on fraction A using a gradient system of cyclohexane–EtOAc (98:2 to 80:20) as eluent. Two subfractions were obtained A/I and A/II. Subfraction A/I was purified further using RP-HPLC, affording nine fractions A/I/1–9. Further purification of fraction A/I/9 on NP-HPLC with *n*-hexane–EtOAc (95:5) as mobile phase furnished compound 7 (0.9 mg, R_t 7.70 min).

Fraction B was separated via RP-VLC using MeOH–H₂O mixtures (from 4:6 to 9:1) as eluents, affording subfractions B/I–III. Subfraction B/II was subjected to NP-VLC with *n*-hexane–CHCl₃ gradient system (from 9:1 to 3:7), yielding subfractions B/II/1–2. Subfraction B/II/2 was further purified using NP-VLC with cyclohexane–EtOAc (from 98:2 to 80:20) mixtures as eluents, isolating four fractions denoted as B/II/2/a–d. The following RP-HPLC purification of B/II/2/c using MeOH–H₂O (68:32) mixtures as mobile phase led to the isolation of compound 2 (15.2 mg, R_t 21.50 min). Subfraction B/III was subjected to NP-VLC using a cyclohexane–EtOAc gradient solvent system (from 98:2 to 80:20), affording subfractions B/III/1–2. The NP-HPLC separation of fraction B/III/1 with a mobile phase of *n*-hexane–EtOAc–MeOH (90:9:1) yielded subfractions B/III/1/a–c. RP-HPLC further purified subfraction B/III/1/b with MeOH–H₂O (75:25) as mobile phase, isolating pure compound 6 (0.9 mg, R_t 17.87 min). Furthermore, the RP-HPLC of B/III/2 using MeOH–H₂O (78:22) mixtures as mobile phase resulted in the isolation of compounds 5 (10.9 mg, R_t 12.80 min) and 4 (32.1 mg, R_t 15.20 min).

Fraction C was subjected to RP-FCC with MeOH–H₂O (from 30:70 to 75:25) mixtures as eluents, isolating seven subfractions, namely C/I–VII. Subfraction C/III was further purified using NP-VLC with a mobile phase of *n*-hexane–CHCl₃ (from 9:1 to 2:8) resulting subfractions C/III/1–5. Subfraction C/III/1 was subsequently subjected to another

Table 1. ¹H NMR Data of Compounds 1–8 [δ ppm (J = Hz), CDCl₃, 500 MHz]

Position	1	2	3	4	5	6	7	8
6	-	7.06 d (7.9)	7.06 d (7.9)	7.06 d (7.5)	7.06 d (7.7)	7.05 d (8.0)	7.08 d (7.8)	-
7	7.07 d (8.9)	7.39 t (7.9)	7.38 t (7.9)	7.38 t (7.5)	7.39 t (7.7)	7.38 t (8.0)	7.40 t (7.8)	7.06 s
8	7.03 d (8.9)	7.14 d (7.9)	7.15 d (7.9)	7.16 d (7.5)	7.16 d (7.7)	7.17 d (8.0)	7.17 d (7.8)	7.06 s
9	2.74 s	2.82 s	2.83 s	2.83 s	2.83 s	2.83 s	2.83 s	2.76 s
1'a	5.10 d (17.5)	5.12 d (17.1)	5.11 d (17.6)	5.12 dd (17.7, 1.9)	5.12 d (17.7)	5.13 d (17.5)	5.14 t (17.4)	5.10 d (17.4)
1'b	5.07 d (10.7)	5.09 d (10.5)	5.08 d (10.8)	5.09 dd (10.6, 1.9)	5.09 d (11.0)	5.09 d (10.6)	5.11 d (10.5)	5.08 d (10.6)
2'	6.13 dd (17.5, 10.7)	6.16 dd (17.1, 10.5)	6.18 dd (17.6, 10.8)	6.19 ddd (17.7, 10.6, 1.9)	6.17 dd (17.7, 11.0)	6.18 dd (17.5, 10.6)	6.18 dd (17.4, 10.5)	6.14 dd (17.4, 10.6)
4'	1.84 dd (14.0, 11.7)	1.86 dd (14.0, 12.0)	1.84 dd (13.8, 11.7)	1.88 dd (14.0, 12.1)	1.85 dd (14.0, 11.8)	1.95 dd (14.2, 12.0)	2.02 dd (14.2, 12.1)	1.82 dd (14.0, 12.0)
	1.70 dd (14.0, 1.5)	1.72 dd (14.0, 1.7)	1.70 dd (13.8, 1.3)	1.70 m	1.70 dd (14.0, 1.5)	1.66 dd (14.2, 1.7)	1.70 dd (14.2, 1.9)	1.68 dd (14.0, 1.6)
5'	4.40 m	4.42 m	4.41 m	4.47 m	4.40 m	5.07 m	5.19 m	4.39 m
6'	2.34 m (2H)	2.35 m (2H)	2.28 m	2.15 m	2.09 m (2H)	5.35 brd (8.5)	6.83 dd (7.9, 1.4)	2.26 m
			1.75 ddd (14.3, 7.0, 4.5)	1.98 m		-	-	1.74 m
7'	3.82 dd (8.4, 6.1)	3.82 dd (8.6, 5.9)	2.79 m	3.00 m	2.62 m (2H)	-	-	2.80 m
8'	-	-	-	-	-	1.82 s	-	-
9'	-	-	1.28 d (7.0)	3.89 m (2H)	-	1.79 s	1.97 s	1.28 d (7.0)
10'	1.55 s	1.57 s	1.57 s	1.57 s	1.57 s	1.62 s	1.64 s	1.56 s
8'-OCH ₃	3.79 s	3.81 s	3.74 s	3.78 s	3.72 s	-	3.80 s	3.74 s
9'-OCH ₃	3.80 s	3.80 s	-	-	-	-	-	-
6-OH	5.47 s	-	-	-	-	-	-	5.07 s

Table 2. ¹³C NMR Data of Compounds 1-8 (δ ppm, CDCl₃, 125 MHz)

Position	1	2	3	4	5	6	7	8
2	162.2	162.3	162.6	162.5	162.6	166.0	162.2	162.5
3	103.3	103.7	103.7	103.5	103.7	103.5	103.7	103.3
4	179.7	179.3	179.3	179.8	179.3	179.4	179.3	179.7
5	124.0	141.2	141.2	141.9	141.2	141.5	141.2	123.9
6	151.0	128.0	127.9	128.0	127.9	127.8	128.0	150.8
7	120.4	131.7	131.7	131.7	131.7	131.6	131.9	120.3
8	115.2	115.1	115.1	115.1	115.1	115.2	115.2	115.3
8a	148.5	154.3	154.3	154.0	154.2	154.3	154.3	148.7
4a	122.1	121.7	121.8	121.5	121.7	121.8	121.7	122.2
9	12.7	22.9	22.8	22.8	22.9	22.9	22.9	12.6
1'	111.8	111.8	111.6	111.7	111.7	111.5	111.8	111.6
2'	145.6	145.5	145.8	145.7	145.8	145.8	145.1	145.8
3'	36.5	36.5	36.6	36.6	36.5	36.6	36.5	36.6
4'	43.4	43.4	43.5	43.8	43.3	43.9	42.5	43.5
5'	74.2	74.3	74.7	75.0	75.7	73.9	73.6	74.6
6'	34.1	34.1	38.7	34.3	30.2	122.2	136.9	38.7
7'	47.9	47.9	36.0	43.6	29.6	140.0	131.3	36.0
8'	169.4	169.3	176.5	174.8	173.4	25.9	167.6	176.7
9'	169.6	169.6	17.3	63.7	-	18.7	13.3	17.4
10'	24.9	24.9	25.0	25.0	24.9	24.8	24.8	24.8
8'-OCH ₃	53.0	53.0	51.9	52.2	52.0	-	52.4	52.0
9'-OCH ₃	53.0	53.0	-	-	-	-	-	-

round of RP-VLC using MeOH–H₂O (from 98:2 to 80:20) as eluent, isolating subfractions C/III/1/a–f. Subfraction C/III/1/f yielded compound **11** (2.0 mg) in NP-HPLC analysis using a mobile phase of *n*-hexane–EtOAc–MeOH (80:19:1). Further purification of subfraction C/III/1/f₄ using RP-HPLC with a mobile phase of MeOH–H₂O (75:25) isolated compound **10** (0.8 g, R_t 17.57 min). Subfraction C/III/5 was subjected to NP-HPLC using a mobile phase of *n*-hexane–EtOAc–MeOH (90:9:1) which generated five fractions denoted as C/III/5/a–e. Compound **8** (4.7 mg, R_t 12.60

min) was isolated in pure form from subfraction C/III/5/b via RP-HPLC using MeOH–H₂O (72:28) as eluent. Furthermore, RP-HPLC analysis of subfraction C/III/5/e applying a solvent system of MeOH–H₂O (72:28) resulted in the isolation of compounds **1** (12.3 mg, R_t 8.20 min) and **3** (0.9 mg, R_t 10.40 min).

Pauciflorin F (1). Colorless oil; [α]_D²⁷ + 89.1 (*c* 0.1, CHCl₃); ¹H and ¹³C NMR data, see Tables 1 and 2; HRESIMS *m/z* 417.1538 [M + H]⁺ (calcd for C₂₂H₂₅O₈⁺ 417.1544).

Pauciflorin G (2). Colorless oil; $[\alpha]_D^{27} - 73.5$ (c 0.1, CHCl_3); ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 401.1593 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{22}\text{H}_{25}\text{O}_7^+$ 401.1595), 423.1413 (calcd for $\text{C}_{22}\text{H}_{24}\text{O}_7\text{Na}$ 423.1414).

Pauciflorin H (3). Colorless oil; $[\alpha]_D^{27} + 124.5$ (c 0.1, CHCl_3); ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 357.1693 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{25}\text{O}_5^+$ 357.1697).

Pauciflorin I (4). Colorless oil; $[\alpha]_D^{27} + 79.3$ (c 0.05, CHCl_3); ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS peak at m/z 373.1640 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{25}\text{O}_6^+$ 373.1646).

Pauciflorin J (5). Colorless oil; $[\alpha]_D^{27} + 124.5$ (c 0.1, CHCl_3); ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS peak at m/z 343.1532 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{23}\text{O}_5^+$ 343.1540).

Pauciflorin K (6). Colorless oil; $[\alpha]_D^{26} + 6.8$ (c 0.05, CHCl_3); ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS peak at m/z 311.1639 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{23}\text{O}_3^+$ 311.1642), 333.1459 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{22}\text{O}_3\text{Na}$ 333.1461).

Pauciflorin L (7). White amorphous powder; $[\alpha]_D^{26} + 53.3$ (c 0.05, CHCl_3); ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS m/z 355.1534 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{23}\text{O}_5^+$ 355.1540).

Pauciflorin M (8). Colorless oil; $[\alpha]_D^{25} + 10.2$ (c 0.1, CHCl_3); ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS peak at m/z 373.1650 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{25}\text{O}_6^+$ 373.1646).

Pauciflorin N (10). White amorphous solid; $[\alpha]_D^{27} - 38.3$ (c 0.05, CHCl_3); ^1H and ^{13}C NMR data, see Table 3; positive-ion HRESIMS peak at m/z 341.1386 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{21}\text{O}_5^+$ 341.1384), 363.1204 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{20}\text{O}_5\text{Na}^+$ 363.1203).

Pauciflorin O (11). White amorphous solid; $[\alpha]_D^{26} + 80.1$ (c 0.1, CHCl_3); ^1H and ^{13}C NMR data, see Table 3; positive-ion HRESIMS peak at m/z 325.1431 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{21}\text{O}_4^+$ 325.1434), 347.1250 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{20}\text{O}_4\text{Na}^+$ 347.1254).

Determination of Antiproliferative Properties. The cell culturing and evaluation of the antiproliferative effects of the isolated compounds against a panel of human cancer cell lines derived from gynecological origin were conducted using a methodology described previously.²⁰

RESULTS AND DISCUSSION

The fraction obtained from the chloroform leaf extract of *C. pauciflorus* with antiproliferative activity was selected for detailed phytochemical investigation and subjected to multi-step chromatographic purification. Through this process, 11 compounds (1–11) (Figure 1) were isolated in pure form, and their structures were determined using spectroscopic analysis, including HRESIMS, 1D [^1H and ^{13}C J -modulated spin-echo (JMOD)] and 2D (^1H – ^1H COSY, HSQC, HMBC, and NOESY) NMR experiments.

Structure elucidation. Pauciflorin F (1) was isolated as a colorless oily substance with an optical rotation of $[\alpha]_D^{27} + 89.1$ (c 0.1, CHCl_3). The molecular formula of compound 1 was shown to be $\text{C}_{22}\text{H}_{24}\text{O}_8$ based on the HRESIMS peak at m/z 417.1538 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{22}\text{H}_{25}\text{O}_8^+$ 417.1544). The ^1H NMR, ^{13}C NMR JMOD, and HSQC spectra of compound 1 revealed the presence of a 1,2,3,4-tetrasubstituted aromatic ring [δ_{H} 7.07 d (8.9 Hz), 7.03 d (8.9 Hz); δ_{C} 122.1, 124.0, 151.0, 120.4, 115.2, and 148.5], two tertiary methyl groups (δ_{H} 1.55 s, and 2.74 s; δ_{C} 24.9 and 12.7), two methoxy groups (δ_{H} 3.79 s, and 3.80 s; δ_{C} 2 \times 53.0), and a vinyl group [δ_{H} 5.10 d (17.5 Hz), 5.07 d (10.7 Hz), 6.13 dd (17.5, 10.7 Hz); δ_{C} 111.8 and 145.6] (Tables 1 and 2). Additionally, three carbonyl functionalities were evident from the carbon resonances at δ_{C} 169.4, 169.6, and 179.7. The ^1H – ^1H COSY spectrum revealed a sequence of correlated protons at δ_{H} 1.70 dd, 1.84 dd, 4.40 m, 2.34 m, and 3.82 dd, indicating a partial structure of $-\text{CH}_2-\text{CH}(\text{OR})-\text{CH}_2-\text{CH}(\text{R})-$ (C-4'–C-7'). This structural unit, along with the quaternary carbons (δ_{C} 179.7, 169.6, 169.4, 162.2, 103.3, and 36.5), aromatic ring, methyl, and vinyl groups were connected using long-range heteronuclear correlations extracted from an HMBC spectrum. The correlations of H-7 (δ_{H} 7.07 d) with C-5 (δ_{C} 124.0) and C-8a (δ_{C} 148.5); H-8 (δ_{H} 7.03 d) with C-4a (δ_{C} 122.1) and C-6 (δ_{C} 151.0); H₃-9 (δ_{H} 2.74 s) with C-4a, C-5, and C-6 provided evidence for the presence of a chromone structural part in pauciflorin F (1). In addition, the attachment of a C₁₀ monoterpene unit to the chromone moiety at positions C-2 and C-3 was supported using the HMBC cross-peaks observed between H-2' (δ_{H} 6.13 dd), H-4' (δ_{H} 1.84 dd and 1.70 dd), H₃-10' (δ_{H} 1.55 s), and C-3 (δ_{C} 103.3) and C-3' (δ_{C} 36.5), between H-4', H-6' (δ_{H} 2.34 m), and C-5' (δ_{C} 74.2), and between H-5' (δ_{H} 4.40 m), H-6', and C-7' (δ_{C} 47.9). Long-range correlations were also observed between H-6' and methoxy groups (δ_{H} 3.79 s, 3.80 s) with C-8' (δ_{C} 169.4) and C-9' (δ_{C} 169.6), confirming the connection of two carboxymethyl group at C-7 (Figure 2).

The presence of hydroxyl group (δ_{H} 5.47 s) at position C-6 was deduced from the downfield shift of the aromatic carbon C-6 (δ_{C} 151.0). The chemical shift assignments of the ring system of compound 1 agreed with the published data for *nassauvia* chromones.¹³ Additionally, the relative configuration

Table 3. NMR Data of Compounds 10, 11 [δ ppm ($J = \text{Hz}$), CDCl_3 , 500 MHz (^1H), and 125 MHz (^{13}C)]

Position	^1H NMR		^{13}C NMR	
	10	11	10	11
2	-	-	167.8	167.1
3	-	-	69.3	72.6
4	-	-	193.1	191.6
4a	-	-	118.7	119.2
5	-	-	142.8	142.3
6	7.04, d (7.8)	7.11, d (7.8)	128.6	128.4
7	7.46, t (7.8)	7.52, t (7.8)	135.8	135.6
8	7.00, d (7.8)	7.10, d (7.8)	115.0	115.3
8a	-	-	155.9	155.8
9	2.57, s	2.60, s	22.8	21.8
1'a	4.94, d (17.3)	4.93, d (17.2)	115.8	114.9
1'b	4.71, d (10.7)	4.90, d (10.7)	-	-
2'	5.31, dd (17.3, 10.7)	5.56, dd (17.2, 10.7)	138.6	139.9
3'	-	-	56.9	48.0
4' α	2.20, dd (14.0, 8.0)	2.45, d (16.3)	42.9	50.1
4' β	2.50, dd (14.0, 5.6)	2.70, d (16.3)	-	-
5'	5.13, td (8.0, 5.6)	-	78.6	201.1
6'	4.33, dd (10.7, 8.0)	-	48.9	130.9
7'	2.88, dq (10.7, 7.1)	-	36.3	155.7
8'	-	2.44, s	177.5	21.7
9'	0.98, d (7.1)	1.60, s	11.2	27.6
10'	1.22, s	1.27, s	25.0	23.1

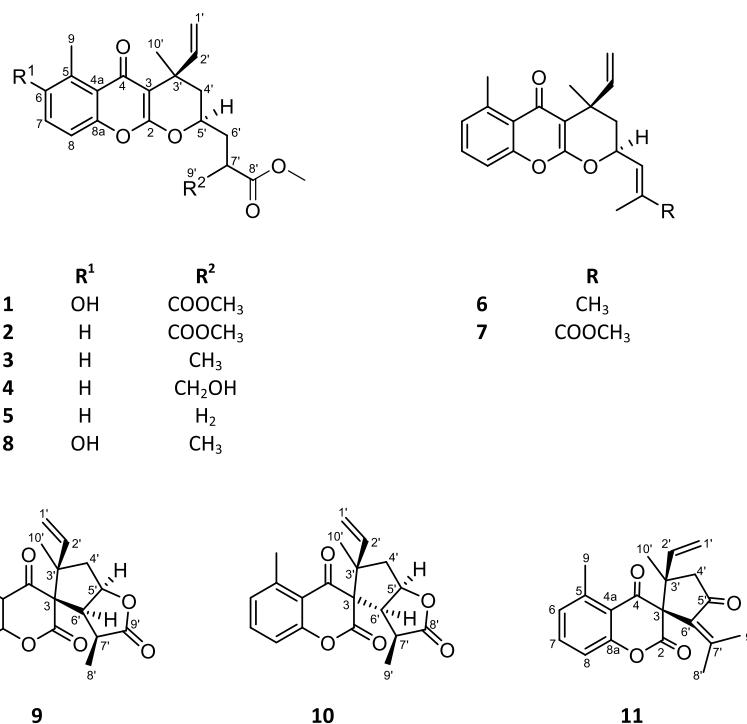


Figure 1. Structures of the Compounds Isolated from *C. pauciflorus*.

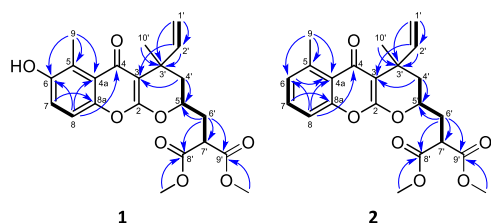


Figure 2. Key HMBC Correlations of Compounds 1 and 2.

of compound 1 was determined using the NOESY experiment. The strong Overhauser effect observed between H₃-10' (δ_{H} 1.55 s) and H-5' (δ_{H} 4.40 m) indicated their same α -orientation (Figure 3), thereby confirming the complete structure of pauciflorin F (1), as depicted on Figure 1.

Pauciflorin G (2) was isolated as a colorless oily substance with an optical rotation of $[\alpha]_{\text{D}}^{27} - 73.5$ (c 0.1, CHCl₃). The molecular formula of compound 2 was deduced from the observed peak at m/z 401.1593 $[\text{M} + \text{H}]^+$ (calcd for C₂₂H₂₅O₇⁺ 401.1595) in the positive-ion HRESIMS spectrum. The ¹H and ¹³C NMR data of compound 2 revealed a chromone-coupled monoterpene structure with the same monoterpene moiety as compound 1 but with a different chromone moiety. Three *ortho*-coupled aromatic protons were detected at δ_{H} 7.06 d ($J = 7.9$ Hz), 7.39 t ($J = 7.9$ Hz), and 7.14 d ($J = 7.9$ Hz), corresponding to H-6, H-7, and H-8, respectively. The chemical shifts of C-5, C-6, and C-7 at δ_{C} 141.2, 128.0, and 131.7, respectively, further supported the characterization of pauciflorin G (2) as the 6-deoxy derivative of pauciflorin F (1). The HMBC (Figure 2) and NOESY

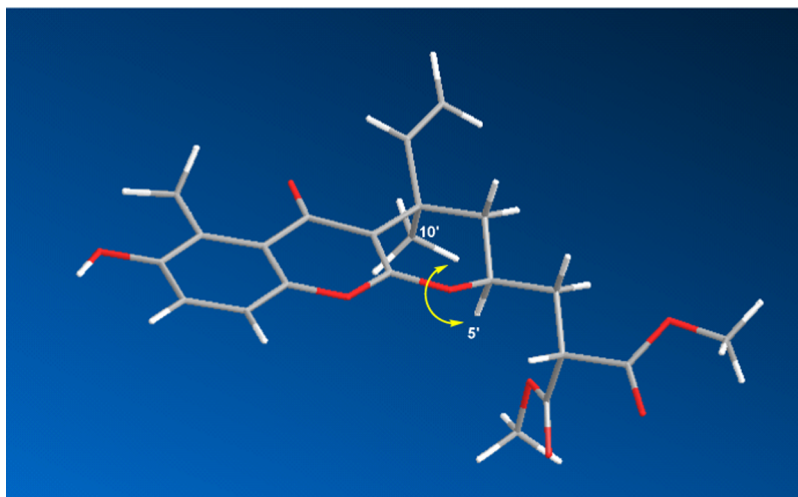


Figure 3. NOESY Correlation (yellow \leftrightarrow) of Pauciflorin F (1).

correlations agreed with the proposed structure of compound 2.

Pauciflorin H (3), a colorless oil with an optical rotation value of $[\alpha]_D^{27} + 124.5$ (c 0.1, CHCl_3), had a molecular composition of $\text{C}_{21}\text{H}_{25}\text{O}_5$ based on the observed peak at m/z 357.1693 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{25}\text{O}_5^+$ 357.1697) in the positive-ion HRESIMS. The ^1H and ^{13}C NMR JMOD assignments of compound 3, obtained via the analysis of the ^1H – ^1H COSY, HSQC, and HMBC spectra, showed that chemical shifts of compound 3 were very similar to those of compound 2 with differences observed only in the resonances of the C-7'–C-9' region of the molecule (Tables 1 and 2). The 9'-methylcarboxylate group of 2 was replaced by a methyl group [δ_{H} 1.28 d ($J = 7.0$ Hz), δ_{C} 17.3] in compound 3, as confirmed using the HMBC correlation observed between H₃-9' and C-6' (δ_{C} 38.7), C-7' (δ_{C} 36.0), and C-8' (δ_{C} 176.5). The key NOESY correlations were observed between H₃-9 (δ_{H} 2.83 s) and H-6 (δ_{H} 7.06 d), as well as between H₃-10' (δ_{H} 1.57 s) and H-5' (δ_{H} 4.41 m), revealing the stereostructure of compound 3, as shown in Figure 1. However, the configuration of C-7' could not be determined based on NMR studies.

Pauciflorin I (4) was isolated as a colorless oily substance with an optical rotation of $[\alpha]_D^{27} + 79.3$ (c 0.05, CHCl_3). The molecular formula of compound 4 was found to be $\text{C}_{21}\text{H}_{20}\text{O}_5$ based on the protonated molecular ion at m/z 373.1640 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{20}\text{O}_5^+$ 373.1646) detected in the HRESIMS spectrum. The ^1H and ^{13}C NMR JMOD data (Tables 1 and 2) of 4 showed a similar structural pattern as that of compound 3, with only a difference in the functionality at C-9' (δ_{C} 63.7 for 4, and 17.3 for 3) and a less extent in the neighboring carbons. The 9'-methyl group (δ_{H} 1.28 s) of compound 3 was replaced by a hydroxymethyl group [δ_{H} 3.89 m (2H)] in 4. The HMBC correlation between the 9'-methylene protons and C-8' (δ_{C} 174.8) further supported the presence of a hydroxymethyl group at C-7'.

Pauciflorin J (5), a colorless oily substance with an optical rotation value of $[\alpha]_D^{27} + 124.5$ (c 0.1, CHCl_3), was determined to have the molecular formula of $\text{C}_{20}\text{H}_{22}\text{O}_5$ based on the HRESIMS by the presence of a protonated molecular ion peak at m/z 343.1532 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{22}\text{O}_5^+$ 343.1540). Analysis of the ^1H NMR and ^{13}C NMR JMOD spectra of 5 revealed the presence of the same dihydropyranochromone ring system, which was substituted with two methyl groups (C-9 and C-10') and a vinyl group (C-1'–C-2'), as observed in compounds 1–4. However, compound 5 exhibited the presence of a C₉ monoterpene unit, while the C-9' position was missing. This observation was supported by the sequence of correlated protons in the ^1H – ^1H COSY spectrum, which indicated the structural unit of $-\text{CH}_2-\text{CH}(\text{OR})-\text{CH}_2-\text{CH}_2-$ [δ_{H} 1.85 dd, 1.70 dd, 4.40 m, 2.09 m (2H), and 2.62 m (2H)] (C-4'–C-7'). Furthermore, the HMBC correlation of H₂-7' (δ_{H} 2.62 m) and methoxy group (δ_{H} 3.72 s) with C-8' (δ_{C} 173.4) confirmed the presence of a carboxymethyl group at C-7'. The NOESY cross-peaks between H-5' (δ_{H} 4.40 m) and H-10' (δ_{H} s) showed the same stereochemistry of 5 as that of compounds 1–4. These findings were consistent with the proposed structure of pauciflorin J (5), as depicted in Figure 1.

Pauciflorin K (6) was isolated as a colorless oily substance with an optical rotation of $[\alpha]_D^{26} + 6.8$ (c 0.05, CHCl_3). The molecular formula of compound 6 was determined to be $\text{C}_{22}\text{H}_{22}\text{O}_3$ based on the HRESIMS peak observed at m/z 311.1639 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{23}\text{O}_3^+$ 311.1642). The ^1H

NMR and ^{13}C NMR JMOD spectra of compound 6 exhibited similar chemical shifts of protons and carbons as those observed in compound 5, except for C-2 (δ_{C} 5: 162.6 vs 6: 166.0), C-5' (δ_{C} 5: 75.7 vs 6: 73.9), H-5' (δ_{H} 5: 4.40 m vs 6: 5.07 m), and the C-6'–C-9' side chain at C-5' (Tables 1 and 2). This side chain was identified as an isobutenyl group, as evidenced by the HMBC correlations of H-5' (δ_{H} 5.07 m) with C-7' (δ_{C} 140.0), as well as H₃-8' (δ_{C} 1.82 s) and H₃-9' (δ_{C} 1.79 s) with C-6' (δ_{C} 122.2) and C-7'. The NOESY spectrum of compound 6 revealed the presence of the characteristic Overhauser effect between the α -oriented 10'-methyl and H-5'.

Pauciflorin L (7) was isolated as a white amorphous powder with an optical rotation of $[\alpha]_D^{26} + 53.3$ (c 0.05, CHCl_3). Its molecular formula was deduced from the peak observed at m/z 355.1534 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{23}\text{O}_5^+$ 355.1540) in the positive-ion HRESIMS spectrum. The 1D and 2D NMR spectra demonstrated that compound 7 shares a similar structure to compound 6, but the C-6'–C-9' structural part of 7 differs (Tables 1 and 2). The side chain connected at C-5' consists of two quaternary carbons (δ_{C} 131.3, 167.6), a methine (δ_{C} 136.9, δ_{H} 6.83 dd), a methoxy (δ_{C} 52.4, δ_{H} 3.80 s), and a methyl group (δ_{C} 13.3, δ_{H} 1.97 s). The HMBC correlations of H-5' (δ_{H} 5.19 m) with C-6' (δ_{C} 136.9), H₃-9' (δ_{H} 1.97 s) with C-7' (δ_{C} 131.3), 8-OCH₃ (δ_{H} 3.80 s), H₃-9', and H-6' (δ_{H} 6.83 dd) with C-8' (δ_{C} 167.6) confirmed the $-\text{CH}=\text{C}(\text{CH}_3)-\text{COOCH}_3$ side chain at C-5' in compound 7. The relative configuration was elucidated based on the NOESY correlations observed between H-2'/H-4' β , H-4' β /H-6', H-5'/H-4' α , H-5'/H₃-9', and H-5'/H₃-10', providing evidence for the α position of H-5' and H₃-10', as well as *trans* geometry of the C-6'/C-7' olefin group.

Pauciflorin M (8), a colorless oil with an optical rotation of $[\alpha]_D^{25} + 10.2$ (c 0.1, CHCl_3), displayed the molecular composition $\text{C}_{21}\text{H}_{24}\text{O}_6$ according to the peak observed at m/z 373.1650 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{24}\text{O}_6^+$ 373.1646) in the positive-ion HRESIMS. The NMR characteristics of compound 8 revealed similarities to 1 regarding the aromatic part of the molecule and compound 3 regarding the monoterpene moiety. The ^1H and ^{13}C NMR chemical shift assignments, performed using the HSQC, HMBC, and ^1H – ^1H COSY spectra, confirmed the presence of a 5-methyl-6-hydroxychromone and a monoterpene carboxylic acid methyl ester adduct structure in compound 8 (Tables 1 and 2).

Compound 9 was isolated as colorless oil and identified based on its 1D and 2D NMR spectroscopic data as (+)-spiroethuliacoumarin (9). This compound was previously isolated from *Ethulia conyzoides*.²³ The complete structure and the relative stereochemistry of compound 9 were established by Mahmoud et al. through X-ray crystallography.

Pauciflorin N (10) was obtained as a white amorphous powder with an optical rotation of $[\alpha]_D^{27} - 38.3$ (c 0.05, CHCl_3). The molecular formula of compound 10 was determined to be $\text{C}_{20}\text{H}_{20}\text{O}_5$ based on the positive-ion HRESIMS peak observed at m/z 341.1386 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{21}\text{O}_5^+$ 341.1384). The ^1H and ^{13}C JMOD NMR spectra of compound 10 showed a structural pattern similar to that of compound 9 (Table 3).

In the ^1H – ^1H COSY spectrum, identical spin systems were identified for both compounds, concluding that compounds 9 and 10 are stereoisomers. Notable differences in chemical shifts were observed in their ^1H and ^{13}C NMR spectra in the carbon resonances around the spiro C-3 stereocenter (C-3, C-

Table 4. Antiproliferative Properties of the Isolated Compounds 1–3, 5, 8, 9, and 11

Compound	Conc. (μM)	Inhibition (%) \pm SEM and calculated IC ₅₀ (μM)				
		MCF-7	MDA-MB-231	HeLa	SiHa	A2780
1	10	40.47 \pm 2.18	22.79 \pm 0.80	– ^a	–	–
	30	84.14 \pm 0.45	47.32 \pm 1.44	–	36.75 \pm 1.63	66.23 \pm 0.66
	IC ₅₀	11.74	– ^b	– ^b	– ^b	28.37
2	10	28.02 \pm 1.87	–	–	26.76 \pm 1.05	–
	30	38.50 \pm 2.18	27.37 \pm 1.92	30.53 \pm 1.13	41.53 \pm 0.73	24.34 \pm 3.72
3	10	–	–	–	–	–
	30	25.36 \pm 3.73	–	40.61 \pm 2.33	27.70 \pm 1.41	–
5	10	34.65 \pm 1.47	22.87 \pm 2.64	39.32 \pm 3.34	32.71 \pm 2.21	22.52 \pm 1.31
	30	44.90 \pm 2.17	23.67 \pm 2.71	41.26 \pm 3.72	46.57 \pm 0.32	23.49 \pm 2.06
8	10	–	22.26 \pm 1.10	31.60 \pm 3.01	–	20.38 \pm 1.73
	30	22.88 \pm 2.40	24.87 \pm 0.56	44.62 \pm 1.22	21.51 \pm 1.96	41.00 \pm 2.75
9	10	–	–	–	–	–
	30	–	–	23.71 \pm 0.58	–	–
11	10	22.43 \pm 0.81	–	–	20.62 \pm 1.61	–
	30	34.92 \pm 0.85	–	22.51 \pm 1.42	30.77 \pm 2.35	21.80 \pm 1.11
cisplatin ^c	10	66.91 \pm 1.81	42.72 \pm 2.68	42.61 \pm 2.33	60.98 \pm 0.92	83.57 \pm 2.21
	30	96.80 \pm 0.35	86.44 \pm 0.42	99.93 \pm 0.26	88.95 \pm 0.53	95.02 \pm 0.28
	IC ₅₀	5.78	10.17	12.43	4.29	1.30

^aInhibition values lower than 20% are considered negligible and not given numerically. ^bNot determined. ^cResults from ref 20.

4, C-1', C-4', C-6', and C-10') and the corresponding proton resonances (H-1' *cis*, H-2', H-4' β , H-6', and H₃-10'). The NOESY correlations of **10** between H₃-10' and H-5', H-6', H-4' α , between H-6' and H-7', and between H-2' and H-4' β agreed those published for compound **9**.²¹ These correlations confirmed the α -position of H₃-10', H-5', and H-6', as well as β -position of vinyl and 9'-methyl groups. The only possible difference in compounds **9** and **10** is likely the opposite stereochemistry of C-3. This is supported by the considerable difference in the chemical shifts of the 10'-methyl group (δ_{H} **9**: 1.01 s vs **10**: 1.22 s) and the key NOESY correlation between H₃-9 and H₃-10'. Such correlation is only possible when the α -oriented H₃-10' methyl group is connected to the spiro-structure opposite to that of compound **9**. In the 3D model of **10**, the distance between H₃-9 and H₃-10' protons was 2.9 Å (Figure S73). Consequently, the structure of pauciflorin N was elucidated, as presented in the structural formula of compound **10**.

Pauciflorin O (**11**) was isolated as a white amorphous solid material with an optical rotation value of $[\alpha]_{\text{D}}^{26} + 80.1$ (*c* 0.1, CHCl₃). Its molecular formula was found to be C₂₀H₂₀O₄ from the protonated molecular ion observed at *m/z* 325.1431 [*M* + H]⁺ (calcd for C₂₀H₂₁O₄⁺ 325.1434) detected in the MS spectrum. HRESIMS, ¹H and ¹³C NMR JMOD data indicated that the molecule has 11 degrees of unsaturation. In the ¹³C NMR spectrum, the presence of two keto groups (δ_{C} 191.6 and 201.1) and one ester functionality (δ_{C} 167.1) were detected beside an aromatic nucleus and two other double bonds—one monosubstituted (δ_{C} 114.9, 139.9) and the other tetrasubstituted (δ_{C} 130.9, 155.7). These structural elements contribute to nine unsaturations, which indicates the presence of two additional rings in the molecule: the 2,4-chromandione part and another ring in the terpene segment. The ¹H–¹H COSY spectrum showed two spin systems, namely CH₂=CH– (δ_{H} 4.90, 4.93, and 5.56 dd) (C-1'–C-2") and –CH=CH– (δ_{H} 7.11, 7.52, and 7.10 d) (C-6–C-8). The planar structure of compound **11** was constructed using the HMBC correlations. Noteworthy correlations were observed between C-3/H₃-10', C-3/H₂-4', C-4/H-6, C-4/H₃-9, C-8a/

H-7, C-8a/H-8, C-6'/H₃-8', C-6'/H₃-9', C-6'/H₂-4', C-5'/H₂-4', and C-5'/H₃-9'. However, the characteristic NOESY correlation between the H₃-9 and H₃-10' characteristic to the spiro-structure of **10** was absent in the case of **11**. Additionally, a comparison of ¹³C NMR chemical shifts of C-2–C-4 and C-10' of compounds **9**, **10**, and **11** was also conducted to determine the relative configuration. The chemical shifts of C-2 (δ_{C} **9**: 167.4, **10**: 167.8, **11**: 167.1), C-3 (δ_{C} **9**: 70.8, **10**: 69.3, **11**: 72.6), C-4 (δ_{C} **9**: 191.0, **10**: 193.1, **11**: 191.6), and C-10' (δ_{C} **9**: 23.1, **10**: 25.0, **11**: 23.1) of **11** were more similar to those of compound **9**, proving the same stereochemistry of C-3 in pauciflorin O (**11**).

Assay for Antiproliferative Activity. Seven of the isolated compounds, namely pauciflorins F, G, H, J, M, and O (**1–3**, **5**, **8**, **11**) and (+)-spiro-ethuliacoumarin (**9**), were investigated for their potential antiproliferative activity against a panel of adherent human malignant cell lines comprising breast (MCF-7, MDA-MB-231), cervical (HeLa, SiHa), and ovarian (A2780) cancer cells. In the first MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test, two final concentrations (10 and 30 μM) were tested after 72 h of incubation. Subsequently, a broad range of concentrations (0.1–30 μM) was employed when at least 50% cell growth inhibition was detected at 30 μM . Among the compounds tested, only pauciflorin F (**1**) elicited >50% inhibition of proliferation against MCF-7 and A2780 cells. Notably, its activity against MCF-7 was comparable to that of the reference agent cisplatin, as evidenced by the IC₅₀ values (Table 4). However, pauciflorin F (**1**) was ineffective against HeLa cells and exhibited modest activity on MDA-MB-231 and SiHa cells. Pauciflorins G, H, J, and M (**2**, **3**, **5**, and **8**) showed weak antiproliferative effects, with maximal inhibition of 40%–50% on some cell lines. Conversely, (+)-spiro-ethuliacoumarin (**9**) and pauciflorin O (**11**) demonstrated limited efficacy, eliciting <35% growth inhibition against the tested cell lines.

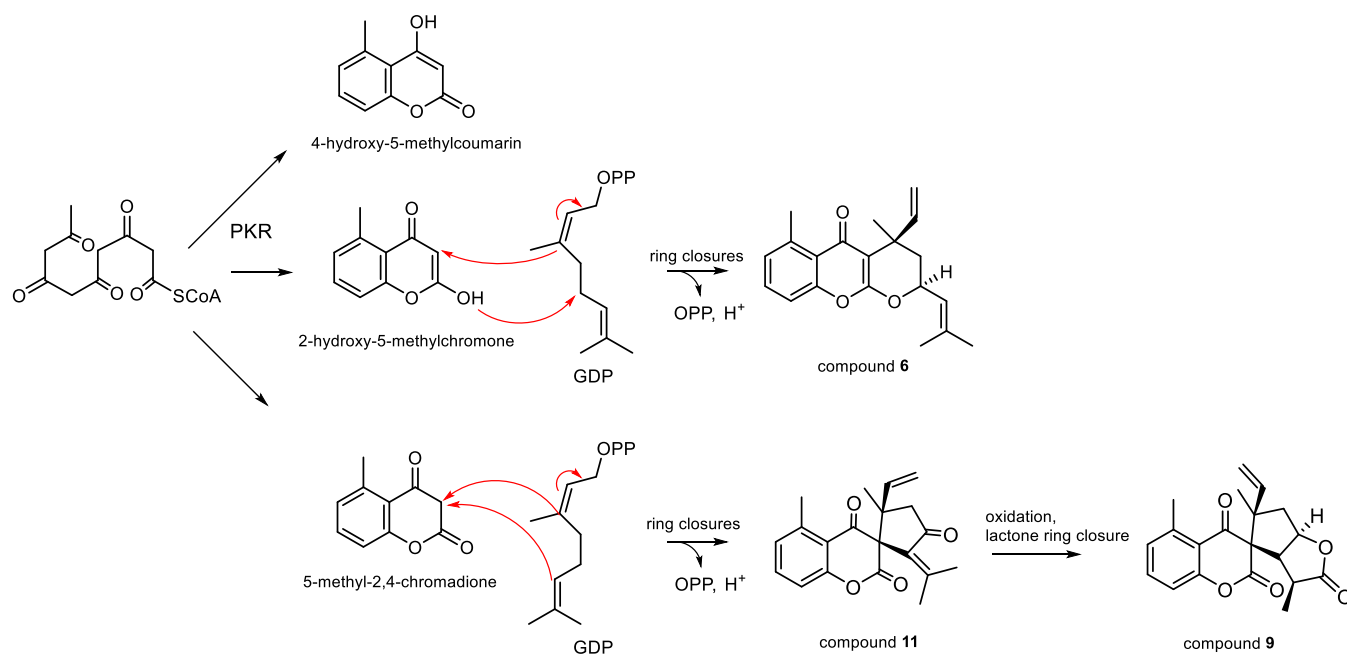


Figure 4. Putative Biogenetic Pathway Proposed for the Main Structural Types Represented by Compounds 6, 9 and 11. PKR: polyketide reductase; GDP: geranyl-diphosphate

CONCLUSION

The chloroform extract of the methanol extract prepared from *C. pauciflorus* leaves was subjected to an activity-guided isolation process to identify compounds with potential antiproliferative activity. The chloroform extract and its fractions were tested against the human breast (MCF-7 and MDA-MB-231), cervical (HeLa and SiHa), and ovarian (A2780) cancer cell lines and fractions with high activity were further purified using multistep chromatographic separations. Ten previously undescribed meroterpenoids (1–8, 10, 11), named pauciflorins F–O, and the known compound (+)-spiro-ethuliacoumarin were isolated, and their structures determined using MS and NMR measurements. These compounds represent three structural types: 5-methylchromone–monoterpene (1–8), tricyclic 5-methyl-2,4-chromadione–monoterpene (11), and tetracyclic 5-methyl-2,4-chromadione–monoterpene derivatives (9, 10). The 2,4-chromadione-based meroterpenoids have a spiro-structure. A common structural feature among these compounds is the presence of a 5-methyl group at C-5, as well as methyl and vinyl groups at C-3'. The structural variations originate from the monoterpene part, which can have lactone, carboxymethyl, hydroxymethyl, methyl, or olefin functionalities. Pauciflorin J (5) is the only compound featuring a C₉ nor-monoterpene moiety.

The occurrence of chromone-monoterpene-based meroterpenoids in plants is sparsely reported in the literature. Gerdelavin B was isolated from the Chinese Asteraceae species *Gerbera delavayi*,¹¹ and additional 5-methyl-chromone–monoterpene adducts were obtained from *Gerbera piloselloides*.¹⁰ Ptaerobliquol, belonging to the same structural type, was found in *Ptaeroxylon obliquum* (Rutaceae).²⁴ Nassauvia chromones, similar to pauciflorins F–M (1–8), were obtained from *Triptilion spinosum*, featuring a tricyclic, 5-methylchromone-containing ring system with methyl and vinyl groups in position C-3', but with a long aliphatic chain attached at C-5'.¹⁴

5-Methyl-2,4-chromadione–monoterpenes are rare in nature. Previously, only the isolation of (+)-spiro-ethuliacoumarin (9) had been reported from the Egyptian plant *Ethulia conyzoides*.²³ In addition, a compound encoded as ZINC31161132 with a 5-methyl-2,4-chromadione–monoterpene structure, was virtually screened for antituberculosis activity using a pharmacophor model.²⁵

As regards, the biosynthesis of the compounds, a common biosynthetic origin can be supposed for the co-occurring 4-hydroxy-5-methylcoumarin, 2-hydroxy-5-methylchromone, and 5-methyl-2,4-chromadione derivatives (Figure 4). The aromatic parts are derived through the acetate-malonate pathway,¹⁷ while the monoterpene parts form from geranyl-diphosphate (GDP). Claisen cyclization of the polyketide precursor affords the aromatic rings by enzymatic route catalyzed by polyketide reductase (PKR), and *O*-heterocyclic rings are formed in the next steps (enolization, Michael-type nucleophilic attack, etc.). The connection of the aromatic parts with GDP includes C-alkylation, oxidative cleavage, cyclization and lactonization.²⁶ Figure 4 shows the putative biogenetic pathway proposed for the main structural types of the isolated compounds represented by 6, 9 and 11.

Seven isolated compounds were assayed for antiproliferative action against human adherent cancer cell lines of gynecological origin using the MTT method. Pauciflorin F (1) exhibited considerable activity against MCF-7 breast cancer cells, with an IC₅₀ value comparable to that of the clinically used drug cisplatin. However, its activity was less pronounced against ovarian (A2780) and triple-negative breast (MDA-MB-231), while no relevant effect was detected on cervical cancer cell lines (HeLa and SiHa). Despite sharing structural similarities, the remaining investigated compounds, namely pauciflorins G, H, J, M, and O (2, 3, 5, 8, and 11) and (+)-spiro-ethuliacoumarin (9), did not exhibit substantial activity against the tested cancer cell lines.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.3c03884>.

HRESIMS, NMR spectra of **1–11** and 3D structure of compound **10** (PDF)

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Author Contributions

[#]G. Krstić and M. B. Saidu contributed equally to this study. J.H. and I.Z. performed conceptualization; M.B.S., A.B., R.B., U.S.G., and H.A. conducted experiments; D.R., G.K., R.B. and I.Z. performed the methodologies; M.B.S., G.K., and H.A. wrote the original draft; J.H., D.R., and I.Z. reviewed and edited the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Nazir, M.; Saleem, M.; Tousif, M. I.; Anwar, M. A.; Surup, F.; Ali, I.; Wang, D.; Mamadalieva, N. Z.; Alshammari, E.; Ashour, M. L.; Ashour, A. M.; Ahmed, I.; Elizbit, Green, I. R.; Hussain, H. Meroterpenoids: A comprehensive update insight on structural diversity and biology. *Biomolecules* **2021**, *11*, 957.
- (2) Liu, X.; Fu, Y.; Zhou, Q.; Wang, S.; Gao, L.; Lei, J.; Ke, A.; Li, Y.; Zhang, X.; Huo, C.; Lu, X. Aspergichromones A–E, five chromone derivatives with complicated polycyclic architecture from *Aspergillus deflectus*. *Org. Lett.* **2022**, *24*, 1610–1615.
- (3) Jiang, M.; Wu, Z.; Liu, L.; Chen, S. The chemistry and biology of fungal meroterpenoids (2009–2019). *Org. Biomol. Chem.* **2021**, *19*, 1644.
- (4) Li, C.; Shao, Y.; Li, W.; Yin, T.; Li, H.; Yan, H.; Guo, X.; Liu, B.; He, B. Hybrid diterpenic meroterpenoids from an endophytic *Penicillium* sp. induced by chemical epigenetic manipulation. *J. Nat. Prod.* **2022**, *85*, 1486–1494.
- (5) Menna, M.; Imperatore, C.; D’Aniello, F.; Aiello, A. Meroterpenes from marine invertebrates: structures, occurrence, and ecological implications. *Mar. Drugs* **2013**, *11*, 1602–1643.
- (6) Liu, L.; Heinrich, M.; Myers, S.; Dworjanyn, S. A. Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in Traditional Chinese Medicine: A phytochemical and pharmacological review. *J. Ethnopharmacol.* **2012**, *142*, 591–619.
- (7) Huang, G.-H.; Hu, Z.; Lei, C.; Wang, P.-P.; Yang, J.; Li, J.-Y.; Li, J.; Hou, A.-J. Enantiomeric pairs of meroterpenoids with diverse heterocyclic systems from *Rhododendron nyingchiense*. *J. Nat. Prod.* **2018**, *81*, 1810–1818.
- (8) Bai, M.; Liu, Y.-Y.; Li, Y.-L.; Shi, W.-Y.; Li, K.-X.; Lu, L.-W.; Le Zhou; Bin Lin; Huang, X.-X.; Song, S.-J. Shao-Jiang Song. Meroterpenoids and sesquiterpene dimers from *Sarcandra glabra* with anti-neuroinflammatory activity. *Industrial Crops and Products* **2022**, *183*, 114983.
- (9) Chiou, C.-T.; Shen, C.-C.; Tsai, T.-H.; Chen, Y.-J.; Lin, L.-C. Meroterpenoids and chalcone-lignoids from the roots of *Mimosa diplotricha*. *J. Nat. Prod.* **2016**, *79*, 2439–2445.
- (10) Zhao, C.; Gao, H.; Li, J.; Yu, M.; Wu, J.; Zhang, H.; Zhang, T.; Zou, Z. Bioactive constituents from *Gerbera piloselloides* with anti-inflammatory and antiproliferative activities. *Fitoterapia* **2022**, *161*, 105258.
- (11) Liu, S. Z.; Feng, J. Q.; Jian Wu, J.; Zhao, W. M. A new monoterpene–coumarin and a new monoterpene–chromone from *Gerbera delavayi*. *Helv. Chim. Acta* **2010**, *93*, 2026–2029.
- (12) Li, T.; Ma, X.; Fedotov, D.; Kjaerulff, L.; Frydenvang, K.; Coriani, S.; Hansen, P. R.; Kongstad, K. T.; Staerk, D. Structure elucidation of prenyl- and geranyl-substituted coumarins in *Gerbera piloselloides* by NMR Spectroscopy, electronic circular dichroism calculations, and single crystal X-ray crystallography. *Molecules* **2020**, *25*, 1706.
- (13) Zdero, C.; Bohimann, F.; King, R. M.; Robinson, H. α -Isocedrene derivatives, 5-methyl coumarins and other constituents from the subtribe Nassauviinae of the Compositae. *Phytochemistry* **1986**, *25*, 2873–2882.
- (14) Bittner, M.; Jakupovic, J.; Bohlmann, F.; Grenz, M.; Silva, M. 5-Methyl coumarins and chromones from *Triptilion* species. *Phytochemistry* **1988**, *27*, 3263–3266.
- (15) Viturro, C. I.; de la Fuente, J. R.; Maier, M. S. Antifungal methylphenone derivatives and 5-methylcoumarins from *Mutisia friesiana*. *Z. Naturforsch. C* **2003**, *58*, 533–540.
- (16) Jakupovic, J.; Boeker, R.; Schuster, A.; Bohlmann, F.; Jones, S. B. Further guaianolides and 5-alkylcoumarins from *Gutenbergia* and *Bothriocline* species. *Phytochemistry* **1987**, *26*, 1069–1075.
- (17) Pietiäinen, M.; Kontturi, J.; Paasela, T.; Deng, X.; Ainasoja, M.; Nyberg, P.; Hotti, H.; Teeri, T. H. Two polyketide synthases are necessary for 4-hydroxy-5-methylcoumarin biosynthesis in *Gerbera hybrida*. *Plant J.* **2016**, *87*, 548–558.

(18) Fuloria, N. K.; Raheja, R. K.; Shah, K. H.; Oza, M. J.; Kulkarni, Y. A.; Subramaniyan, V.; Sekar, M.; Fuloria, S. Biological activities of meroterpenoids isolated from different sources. *Front. Pharmacol.* **2022**, *13*, 830103.

(19) Krstić, G.; Saidu, M. B.; Bombicz, P.; De, S.; Ali, H.; Zupkó, I.; Berkecz, R.; Gallah, U. S.; Rédei, D.; Hohmann, J. Pauciflorins A–E, unexpected chromone–monoterpene-derived meroterpenoids from *Centrapalus pauciflorus*. *J. Nat. Prod.* **2023**, *86*, 891–896.

(20) Saidu, M. B.; Krstić, G.; Todorović, N.; Berkecz, R.; Ali, H.; Zupkó, I.; Hohmann, J.; Rédei, D. Monoterpenoid 5-methylcoumarins from *Centrapalus pauciflorus* with antiproliferative activity. *Arab. J. Chem.* **2023**, *16*, 104777.

(21) IPNI 2022. *International Plant Names Index*. Published on the Internet <http://www.ipni.org>, The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Botanic Gardens. [Retrieved 08 September 2022].

(22) Tropical Plants Database, Ken Fern. tropical.theferns.info. 2022–04–16. < [tropical.theferns.info/viewtropical.php?id = Centrapalus+pauciflorus](http://tropical.theferns.info/viewtropical.php?id=Centrapalus+pauciflorus)> PROTA (Plant Resource of Tropical Africa). <http://www.prota.org>.

(23) Mahmoud, A. A.; Ahmed, A. A.; Inuma, M.; Tanaka, T.; Muraoka, O. Structure of a novel spiro-monoterpene-coumarin in *Ethulia conyzoides*. *Tetrahedron Lett.* **1994**, *35*, 6517–6520.

(24) Agostinho, Đ.; Boudesocque, L.; Thery-Kone, I.; Debierre-Grockiego, F.; Gueiffier, A.; Enguehard-Gueiffier, C.; Allouchi, H. A new meroterpenoid isolated from roots of *Ptaeroxylon obliquum* Radlk. *Phytochem. Lett.* **2013**, *6*, 560–566.

(25) Pinto, V. S.; Araújo, J. S. C.; Silva, R. C.; da Costa, G. V.; Cruz, J. N.; Neto, M. F. A.; Campos, J. M.; Santos, C. B. R.; Leite, F. H. A.; Junior, M. C. S. In silico study to identify new antituberculosis molecules from natural sources by hierarchical virtual screening and molecular dynamics simulations. *Pharmaceuticals* **2019**, *12*, 36.

(26) Amen, Y.; Elsbay, M.; Othman, A.; Sallam, M.; Shimizu, K. Naturally occurring chromone glycosides: Sources, bioactivities, and spectroscopic Features. *Molecules* **2021**, *26*, 7646.