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Phenethyisoquinoline alkaloids from the leaves of *Androcymbium palaestinum*

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ABSTRACT

Thirteen compounds were isolated from the methanolic extract of the leaves of *Androcymbium palaestinum* Baker (Colchicaceae). Of these, three were new, two were new natural products, and eight were known. The new isolated compounds were (+)-1-demethylandrocine (**5**), (−)-andropalaestine (**8**), and (+)-2-demethyl-*β*-lumicolchicone (**10**), while the new natural products were (+)-*O*-methylkreysigine-*N*-oxide (**3**) and (+)-*O*,*O*-dimethylautumnaline (**9**). Moreover, two known compounds are reported for the first time from this species, specifically (−)-colchicine (**11**) and (−)-3-demethyldemecolcine (**13**). The structures of the isolated compounds were elucidated using a series of spectroscopic and spectrometric techniques, principally HRESIMS, 1D-NMR (^{1}H and ^{13}C NMR) and 2D-NMR (COSY, edited-HSQC, and HMBC). ECD spectroscopy was used for assigning the absolute configurations of compounds **3**, **5**, and **10**. The cytotoxic activities of the isolated compounds were evaluated using the MDA-MB-435 (melanoma), MDA-MB-231 (breast), and OVCAR3 (ovary) cancer cell lines. Compound **11** was the most potent against all tested cell lines, with IC_{50} values of 12, 95 and 23 nM, respectively.

1. Introduction

Throughout history natural products have contributed immensely to the drug discovery process [1–4]. In the area of cancer, for example, and over the time frame from 1940s to 2014, of the 175 small molecules approved, 131, or 75%, are other than synthetic, with 85, or 49%, actually being either natural products or directly derived therefrom [2]. Moreover, of the 13 natural product-derived drugs that were approved in the U.S. between 2005 and 2007, five were the first members of new classes [1].

Jordan, with its unique position in the heart of the Middle East, acts as a floral bridge between the continents of Asia, Africa, and Europe [5,6]. This geographical position bestows the country with ecologically diverse habitats and a rich variety of wild plants [7,8]. For example, more than 2000 plant species were reported to grow in the wild [6], with less than 5% explored previously for bioactive secondary metabolites.

The Colchicaceae, a family of 16 genera and more than 250 species [9], is represented in Jordan by two genera: *Androcymbium* and *Colchicum* [6]. While *Colchicum* sp. in Jordan have been investigated to some degree [10–15], members of the *Androcymbium* are less well studied. The genus *Androcymbium* is native to Africa, the Mediterranean, and the Middle East, and includes about 56 species [9,16], many of which have been reported in folk medicine for the treatment of a variety of illnesses [17]. Several subclasses of alkaloids, including colchicinoids, dibenzocycloheptylamines, homoaporphines, and l-phenethyltetrahydroisoquinolines, have been reported previously from this genus [18–23].

Androcymbium palaestinum Baker (Colchicaceae), which is found flowering from December to February, is the only species reported to

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grow in Jordan [6]. It is a perennial plant with small underground corms covered with brown scales. It has white showy flowers with brown midribs. The leaves are basal folded in the midrib, wider at base and narrow towards the tip. It is known by local people as "Zanbaq Alghor", which translates to lily of the Jordan Valley. It flourishes in warm sandy soils, such as those found in the Jordan Valley, near the Dead Sea, and in Wadi Araba and Wadi Rum [6]. Over 30 years ago, the corms of *A. palaestinum* were studied, yielding 14 compounds from three different alkaloid classes, specifically, the homoaporphine alkaloids: (+)-*O*-methylkreysigine, (+)-kreysigine, (+)-androcine, (+)-androcimine, (+)-androbine, (+)-nor-*O*-methylkreysigine, (+)-norandrobine, and (+)-szovistamine; the dibenzocycloheptylamine: (−)-androbiphenyline, K-3, and K-4; the colchicinoids: (−)-demecolcine and (−)-3-demethylcolchiceine; and the homomorphinandienone (−)-collutine (Table S1, Supplementary Data) [18,19].

While the flower of *A. palestinum* is beautiful, there are no reports of the use of this plant by local people for traditional medicine; interestingly, it is avoided by grazing animals, such as goats (Personal Communication). Natural products chemistry studies on a methanolic extract of the leaves of this plant resulted in the isolation and identification of thirteen compounds; of which eight were known (**1**, **2**, **4**, **6**, **7**, and **11–13**), two were new natural products: (+)-*O*-methylkreysigine-*N*-oxide (**3**) and (+)-*O*,*O*-dimethylautumnaline (**9**), and three were new compounds: (+)-1-demethylandrocine (**5**), (−)-andropalaestine (**8**), and (+)-2-demethyl-*β*-lumicolchicone (**10**). ECD spectroscopy was used to confirm the absolute configurations of the known compounds and to assign the absolute configurations of the new compounds **3**, **5**, and **10**. A scheme has been added to the supplementary data file to summarize the biogenetic relationships between the various compounds reported in the current manuscript (Scheme S1, Supplementary Data). The isolated compounds (**1**−**13**) were tested for their cytotoxicity using three human cancer cell lines, specifically MDA-MB-435 (melanoma), MDA-MB-231 (breast), and OVCAR3 (ovarian).

2. Experimental

2.1. General experimental procedures

Optical rotations, UV data, and ECD spectra were obtained using a Rudolph Research Autopol III polarimeter (Rudolph Research Analytical), a Varian Cary 100 Bio UV–vis spectrophotometer (Varian Inc.), and an Olis DSM 17 ECD spectrophotometer (Olis, Inc.). NMR data were collected using either a JEOL ECA-500 NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C or a JEOL ECS-400 NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C and equipped with a high sensitivity JEOL Royal probe and a 24-slot autosampler (both from JEOL Ltd.) or an Agilent 700 MHz NMR spectrometer (Agilent Technologies), equipped with a cryoprobe, operating at 700 MHz for ^{1}H and 175 MHz for ^{13}C. Residual solvent signals were utilized for referencing. HRMS data were acquired using a Thermo QExactive Plus mass spectrometer equipped with an electrospray ionization source (Thermo Fisher Scientific). Gemini–NX C_{18} analytical (5 μm; 250 × 4.6 mm) and preparative (5 μm; 250 × 21.2 mm), Luna PFP C_{18} analytical (5 μm; 250 × 4.6 mm), semipreparative (5 μm; 250 × 10.0 mm), and preparative (5 μm; 250 × 19.0 mm) columns (all from Phenomenex) along with Waters Atlantis T3 C_{18} analytical (5 μm; 250 × 4.6 mm), semipreparative (5 μm; 250 × 10.0 mm), and preparative (5 μm; 250 × 19.0 mm) columns (all from Waters Corp.) were used on a Varian Prostar HPLC system equipped with ProStar 210 pumps and a Prostar 335 photodiode array detector (PDA), with data collected and analyzed using Galaxie Chromatography Workstation software (version 1.9.3.2, Varian Inc.). Flash chromatography was performed on a Teledyne ISCO CombiFlash Rf 200 using Silica Gold columns (both from Teledyne ISCO) and monitored by UV and evaporative light-scattering detectors. All other reagents and solvents were obtained from Fisher Scientific and were used without further purification.

2.2. Plant material

Leaves of *A. palaestinum* were collected during flowering stage in February/March 2016 from the Jordan Valley, Waqqas city (32°32′34.7712" N; 35°36′18.6624" E, 152.4 m below sea level). The plant material was identified by Dr. Mohammed Gharaibeh, Plant Taxonomist, Faculty of Agriculture, JUST. A voucher specimen (PHS-121) was deposited in the herbarium of the Faculty of Pharmacy, JUST, Irbid, Jordan. The collected plant material was air-dried in the shade away from direct sunlight. This dried plant material was ground into powder using a laboratory mill, stored at room temperature, and protected from light until required for extraction and analysis.

2.3. Extraction and isolation

Extraction and fractionation was carried out as reported by Alali et al (2008) [24]. Briefly, air-dried leaves (600 g) of *A. palaestinum* were extracted via infusion by soaking in MeOH (3 × ~5 L) at rt. for three days followed by filtration to separate the marc. The filtrates were combined and dried under reduced pressure to yield a MeOH extract (111 g).

The MeOH extract was reconstituted in 1000 mL of 5% acetic acid. The acidic solutions were then extracted three times using petroleum ether (3 × 1000 mL) to yield fraction A (3.8 g), and then three times with diethyl ether (3 × 1000 mL) to yield fraction B (5 g). The acidic aqueous residues were then made alkaline (pH = 9) using 10% ammonium hydroxide (NH_4OH), and extracted three times with dichloromethane (3 × 1000 mL) to yield fraction C (979 mg). The pH of the basic aqueous residues were adjusted to pH = 12 using sodium hydroxide and extracted three times with dichloromethane (3 × 1000 mL) to yield fraction D (100 mg). Finally, the fractions were brought to dryness under vacuum.

The dried alkaloid rich fraction (Fraction C) of the leaves (~979 mg) was dissolved in $CHCl_3$ and mixed with Celite 545. Normal-phase flash chromatography was performed using a gradient solvent system of hexanes-$CHCl_3$-MeOH, at a flow rate of 30 mL/min, and 64.4 column volumes over a total run time of 36 min using a 12 g silica RediSep column to yield eight fractions, which were subjected to purifications using HPLC methods, both preparative and semipreprative, leading to the isolation of **1–13**.

Fraction 3 (251.6 mg) of the normal-phase flash chromatography was subjected to preparative HPLC over a Gemini column using a gradient system of 40:60–60:40 of MeOH–H_2O (0.1% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield 16 subfractions. Subfraction 5 (32.4 mg) was subjected preparative HPLC over an Atlantis T3 column using a gradient system of 30:70–40:60 of MeOH–H_2O (0.1% formic acid) over 30 min, no hold, at a flow rate of 17 mL/min to yield 6 subfractions. Subfraction 3 (9.3 mg) was subjected to preparative HPLC over a PFP column using a gradient system of 0:100–50:50 of CH_3CN-H_2O (10% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield compound **12** (5.4 mg). Subfraction 11 that had resulted from fraction 3 of the normal-phase flash chromatography was subjected to semipreparative HPLC over an Atlantis T3 column using a gradient system of 40:60–70:30 of MeOH–H_2O (0.1% formic acid) over 15 min, no hold, at a flow rate of 4.6 mL/min to yield 4 subfractions. Subfraction 3 (8.6 mg) was subjected to preparative HPLC over a PFP column using a gradient solvent system of 0:100–20:80 of CH_3CN-H_2O (10% formic acid) over 5 min, to 40:60 over 15 min at a flow rate of 21.24 mL/min to yield compound **11** (6.4 mg). Moreover, semipreparative HPLC purification of subfraction 1 (1.3 mg) over a PFP column using a gradient solvent system of 0:100–20:80 of CH_3CN-H_2O (10% formic acid) over 5 min to 40:60 over 15 min at a flow rate of 4.62 mL/min yielded compound **10** (0.7 mg).

Subfraction 7 (9.6 mg) that had resulted from fraction 3 of the normal-phase flash chromatography was subjected to semipreparative HPLC over an Atlantis T3 column using a gradient solvent system of 40:60–70:30 of MeOH:H_2O (0.1% formic acid) over 15 min, no hold, at a flow rate of 4.6 mL/min to yield 3 subfractions. Subfraction 1 (3.4 mg) was subjected to semipreparative HPLC over a PFP column using a gradient system of 0:100–10:90 of CH_3CN:H_2O (10% formic acid) over 5 min to 30:70 over 15 min at a flow rate of 4.62 to yield compound **9** (2.2 mg).

Fraction 4 (138 mg) of the normal-phase flash chromatography was subjected to preparative HPLC over a PFP column using a gradient system of 40:60–60:40 of MeOH–H_2O (0.1% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield 12 subfractions. Subfraction 4 (48.8 mg) was subjected to preparative HPLC over a PFP column using a gradient of 7:93 to 13:87 of CH_3CN-H_2O (0.1% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield 3 subfractions. Subfraction 3 (12.9 mg) was subjected to preparative HPLC over a PFP column using a gradient system of 0:100–10:90 over 5 min to 20:80 over 15 min of CH_3CN-H_2O (10% formic acid) at a flow rate of 21.24 mL/min to yield compound **1** (5 mg). Subfraction 7 (31.6 mg) that had resulted from fraction 4 of the normal-phase flash chromatography was subjected to preparative HPLC over an Atlantis T3 column using a gradient system of 30:70–50:50 over 15 min, no hold, of MeOH-H_2O (0.1% formic acid) at a flow rate of 17 mL/min to yield 5 subfractions. Subfraction 1 (10 mg) was subjected to preparative HPLC over a PFP column using a gradient system of 0:100–10:90 over 5 min to 30:70 over 15 min of CH_3CN-H_2O (10% formic acid) at a flow rate of 21.24 mL/min to yield compound **2** (5.4 mg). Subfraction 5 (16.6 mg) that had resulted from fraction 4 was subjected to preparative HPLC over a PFP column using a gradient system of 7:93–13:87 over 13 min of CH_3CN:H_2O (0.1% formic acid) at a flow rate of 21.24 mL/min to yield 5 subfractions. Subfraction 2 (2.1 mg) was subjected to semi-prepartive HPLC over a PFP column using a gradient system of 0:100–10:90 of CH_3CN:H_2O (10% formic acid) over 5 min to 20:80 over 15 min at a flow rate of 4.62 mL/min to yield compound **13** (1.8 mg).

Fraction 5 (124.3 mg) of the normal-phase flash chromatography was subjected to preparative HPLC over a PFP column using a gradient mobile phase of 0:100–20:80 of CH_3CN:H_2O (0.1% formic acid) over 15 min, hold for 5 min, then to 50:50 over 15 min at a flow rate of 21.24 mL/min to yield 7 subfractions. Subfraction 4 (14.6 mg) was subjected to semipreparative HPLC over an Atlantis T3 column using a gradient system of 20:80–40:60 of MeOH:H_2O (0.1% formic acid) over 15 min, no hold, at a flow rate of 4.6 mL/min to yield 4 subfractions. Subfraction 2 (4.5 mg) was subjected to preparative HPLC over a PFP column using a gradient system of 0:100–10:90 of CH_3CN:H_2O (10% formic acid) over 5 min to 30:70 over 15 min at a flow rate of 21.24 mL/min to yield compound **5** (4.1 mg). Subfraction 6 (25.9 mg) that had resulted from fraction 5 of the normal-phase flash chromatography was subjected to preparative HPLC over an Atlantis T3 column using a gradient mobile phase of 30:70–40:60 of MeOH:H_2O (0.1% formic acid) over 15 min at a flow rate of 17 mL/min to yield 2 subfractions. Subfraction 2 (10.6 mg) was subjected to preparative HPLC over a PFP column using a gradient mobile phase of 0:100–10:90 of CH_3CN:H_2O (10% formic acid) over 5 min to 20:80 over 15 min at a flow rate of 21.24 mL/min to yield compound **4** (5.9 mg).

Fraction 6 (151.4 mg) of the normal-phase flash chromatography was subjected to preparative HPLC over a PFP column using a gradient system of 0:100–20:80 of CH_3CN:H_2O (0.1% formic acid) over 15 min, hold for 5 min, then to 50:50 over 15 min at a flow rate of 21.24 mL/min to yield 8 subfractions. Subfraction 4 (8.6 mg) was subjected preparative HPLC over a PFP column using a gradient mobile phase system of 10:90–15:85 of CH_3CN:H_2O (0.1% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield 2 subfractions. Subfraction 2 (6 mg) was subjected to preparative HPLC over a PFP column using a gradient system of 0:100–15:85 CH_3CN:H_2O (10% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield compound **7** (1.9 mg). Subfraction 8 (39.6 mg) that had resulted from fraction 6 of the normal-phase flash chromatography was subjected to preparative HPLC over an Atlantis T3 column using a gradient system of 20:80–40:60 of MeOH:H_2O (0.1% formic acid) over 15 min, no hold, at a flow rate of 17 mL/min to yield 3 subfractions. Subfractions 2 (4.4 mg) and 3 (7 mg) were subjected to HPLC over a PFP column using a gradient system of 0:100–10:90 of CH_3CN:H_2O (10% formic acid) over 5 min to 20:80 over 15 min at a flow rate of 21.24 mL/min to yield compounds **6** (1.4 mg). and **8** (3.1 mg), from fractions 2 and 3, respectively.

Fraction 7 (28.8 mg) of the normal-phase flash chromatography was subjected to preparative HPLC over a PFP column using a gradient system of 0:100–20:80 of CH_3CN:H_2O (0.1% formic acid) over 15 min, hold for 5 min, then to 50:50 over 15 min at a flow rate of 21.24 mL/min to yield 9 subfractions. Subfraction 9 (12.8 mg) was subjected to preparative HPLC over an Atlantis T3 column using a system of 40:60–70:30 of MeOH:H_2O (0.1% formic acid) over 15 min, no hold, at a flow rate of 17 mL/min to yield 4 subfractions. Subfraction 4 (2.1 mg) was subjected to semipreparative HPLC over a PFP column using a gradient system of 0:100–10:90 of CH_3CN:H_2O (10% formic acid) over 5 min to 30:70 over 15 min at a flow rate of 4.62 mL/min to yield compound **3** (0.8 mg).

2.3.1. (+)-O-Methylkreysigine-N-oxide (3)

White powder; $[\alpha]_D^{24}$ + 20 (*c* 0.001, MeOH)]; UV (MeOH) λ_{max} (log ε) 260 (3.18), 227 (3.46) nm; ECD (*c* 0.001 M, MeOH) λ ($\Delta\varepsilon$) 223 (+4.10) nm, 259 (−6.50) nm (Fig. 3A); HRESIMS *m/z* 416.2065 $[M + H]^+$ (calcd for $C_{23}H_{30}NO_6$, 416.2068).

2.3.2. (+)-1-Demethylandrocine (5)

Light brown amorphous solid; $[\alpha]_D^{24}$ + 126 (*c* 0.001, MeOH)]; UV (MeOH) λ_{max} (log ε) 298 (2.15), 259 (2.44), 224 (2.63) nm; ECD (*c* 0.3 × 10^{-3} M, MeOH) λ ($\Delta\varepsilon$) 258 (−8.58) nm, 283 (+1.02) nm (Fig. 3B); HRESIMS *m/z* 372.1802 $[M + H]^+$ (calcd for $C_{21}H_{26}NO_5$, 372.1805).

2.3.3. (−)-Andropalaestine (8)

Light brown amorphous solid; $[\alpha]_D^{24}$ − 60 (*c* 0.001, MeOH)]; UV (MeOH) λ_{max} (log ε) 281 (2.88), 236 (3.08) nm; HRESIMS *m/z* 388.2116 $[M + H]^+$ (calcd for $C_{22}H_{30}NO_5$, 388.2118).

2.3.4. (+)-O,O-Dimethylautumnaline (9)

White powder; $[\alpha]_D^{24}$ + 3 (*c* 0.001, MeOH)]; UV (MeOH) λ_{max} (log ε) 279 (2.88), 256 (3.28), 228 (3.35), 216 (3.26) nm; HRESIMS *m/z* 402.2276 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_5$, 402.2275).

2.3.5. (+)-2-Demethyl-β-lumicolchicone (10)

Light yellow powder; $[\alpha]_D^{24}$ + 66 (*c* 0.0009, MeOH)]; UV (MeOH) λ_{max} (log ε) 376 (2.99), 353 (3.01), 327 (2.94), 259 (2.93) nm; ECD (*c* 0.3 × 10^{-3} M, MeOH) λ ($\Delta\varepsilon$) 205 (+6.99) nm, 226 (−3.82) nm, 253 (+7.83) nm, 266 (+5.82), 278 (+7.75) nm, 317 (−9.83) nm, 368 (+4.32) nm (Fig. 3C); HRESIMS *m/z* 343.1173 $[M + H]^+$ (calcd for $C_{19}H_{19}O_6$, 343.1176).

2.4. Cytotoxicity assay

Compounds **1–13** were tested for cytotoxicity against human melanoma cancer cells MDA-MB-435 [25], human breast cancer cells MDA-MB-231, and human ovarian cancer cells OVCAR3 as described previously [26,27]. Briefly, the cell lines were propagated at 37 °C in 5% CO_2 in RPMI 1640 medium, supplemented with fetal bovine serum (FBS) (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL). Cells in log phase growth were harvested by trypsinization followed by two washings to remove all traces of trypsin. A total of 5000 cells were seeded per well of a 96-well clear, flat-bottom plate (Microtest 96, Falcon) and incubated overnight (37 °C in 5% CO_2). Samples dissolved in DMSO were then diluted and added to the appropriate wells. The

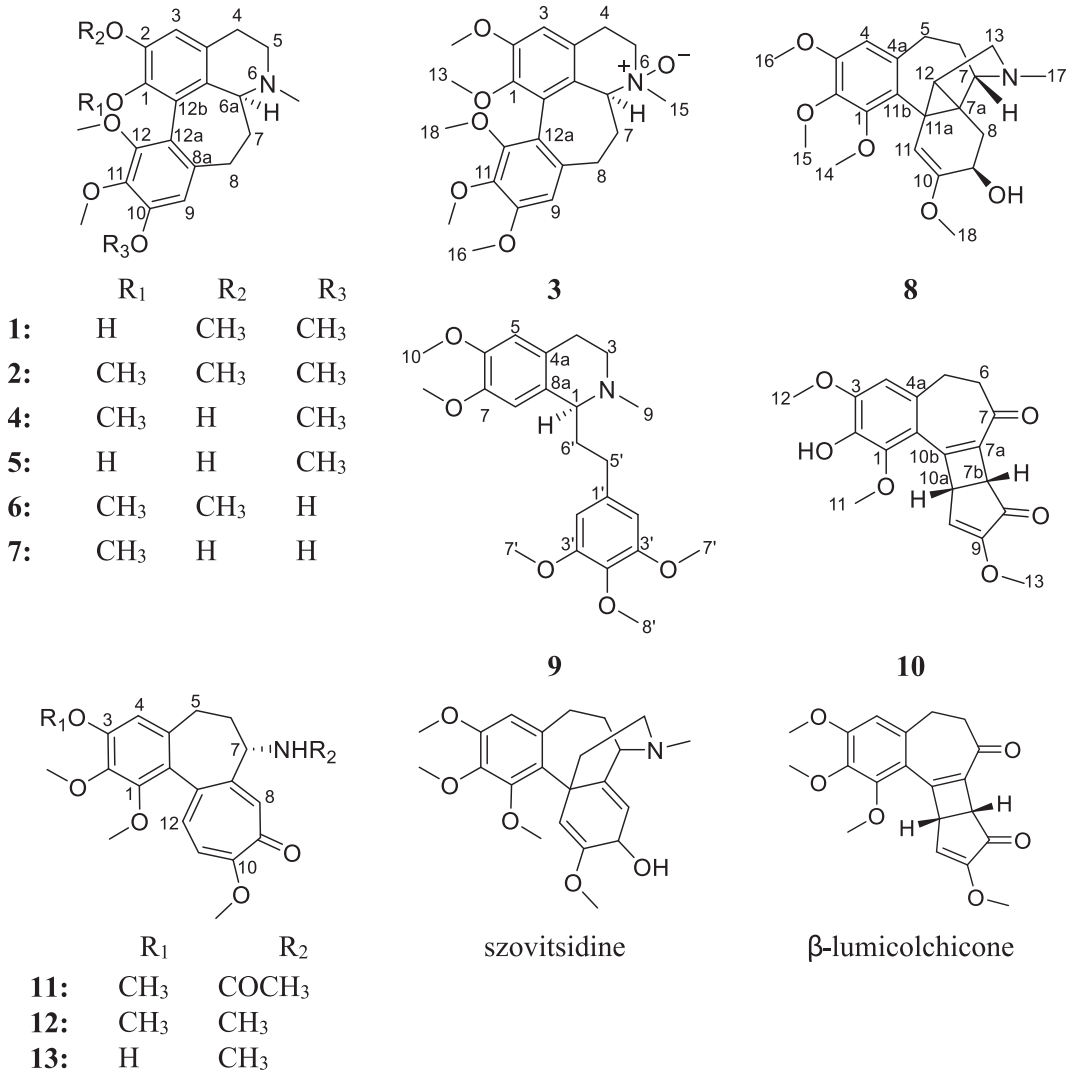

Fig. 1. Structures of compounds **1–13**, szovitsidine, and *β*-lumicolchicone.

cells were incubated in the presence of test substance for 72 h at 37 °C and evaluated for viability with a commercial absorbance assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega Corp, Madison, WI) that measured viable cells. IC_{50} values are expressed in μM relative to the solvent (DMSO) control. Taxol (paclitaxel) was used as a positive control.

3. Results and discussion

The dried leaves of *A. palaestinum* were extracted with MeOH, and the resulting crude extract was reconstituted in 5% acetic acid and defatted using, in order, petroleum ether and diethyl ether. The defatted acidic solution was then made alkaline and partitioned with dichloromethane. The resulting dried alkaloid-rich dichloromethane extract (i.e. fraction C) was fractionated using normal-phase flash chromatography. Extensive purifications of the resulting fractions using reversed-phase HPLC methods, both preparative and semipreparative, resulted in the isolation of thirteen compounds (**1–13**), most of which were alkaloids (Fig. 1).

Compounds (**1**, **2**, **4**, **6**, **7**, and **11–13**) were identified as the known homoaporhine/colchicinoid alkaloids. Their structures were established by comparison of NMR (1D/2D), HRMS, specific rotation, and ECD data with literature values and were identified as: (+)-kreysigine (**1**) [22], (+)-*O*-methylkreysigine (**2**) [22], (+)-androcine (**4**) [22], (+)-androcimine (**6**) [22], (+)-androbine (**7**) [22], (−)-colchicine (**11**) [28,29], (−)-demecolcine (**12**) [28,30], and (−)-3-demethyldemecolcine (**13**) [28,30] (Figs. S1-S4, S6, S8, S9, S13-S15, Table S2, Supplementary Data).

Compound **3** (0.8 mg) was obtained as a white powder with a molecular formula of $C_{23}H_{29}NO_6$ as determined by HRESIMS (*m/z* 416.2065 $[M + H]^+$, calcd 416.2068) and NMR data (Table 1, Figs. S1 and S5, Supplementary Data), establishing an index of hydrogen deficiency of 10. Analysis of the NMR data indicated a new homoaporphine alkaloid with structural similarity to **2** (Fig. 1). A key difference was an *N*-oxide moiety, consistent with a 16 amu difference in the HRMS data of compound **3** relative to **2**, indicating oxidation of the tertiary nitrogen into *N*-oxide to give (+)-*O*-methylkreysigine-*N*-oxide, which was reported previously as a semisynthetic product [31]. Inspection of the NMR data of **3** showed signals characteristic of two singlet aromatic protons (δ_H/δ_C 6.74/111.4, H-3 and 6.59/107.3, H-9), nine aliphatic protons (δ_H 3.17, H_2–4; 3.84 and 3.55, H_2–5; 3.86, H-6a; 2.06 and 2.97, H_2–7 and 2.22 and 2.58, H_2–8), one *N*-methyl group (δ_H/δ_C 3.24/56.1), and five methoxy functionalities (δ_H/δ_C 3.56/60.6; 3.75/61.1; 3.88/61.1; 3.90/56.2 and 3.91/56.2) (Table 1, Fig. S5, Supplementary Data). HMBC correlations from the 1-OCH_3, 2-OCH_3, 10-OCH_3, 11-OCH_3 and 12-OCH_3 protons to C-1 (δ_C 146.1), C-2 (δ_C 152.9), C-10 (δ_C 153.9), C-11 (δ_C 141.0), and C-12 (δ_C 151.5), respectively, confirmed their connectives (Fig. 2). HMBC correlations from H-3 to C-1 (δ_C 146.1), C-4 (δ_C

Table 1
NMR data for compound **3** (700 MHz for ^{1}H and 175 MHz for ^{13}C, $CDCl_3$) and compound **5** (400 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$).

Position	3		5	
	δc, type	δ_H, mult (*J* in Hz)	δc, type	δ_H, mult (*J* in Hz)
1	146.1, C	–	146.9, C	–
2	152.9, C	–	139.2, C	–
3	111.4, CH	6.74, s	109.5, CH	6.68, s
3a	123.0, C	–	123.4	–
3b	123.6, C	–	122.2	–
4	27.3, CH_2	3.17, m	23.3, CH_2	2.82, dd (15.9, 5.2) 3.12, m
5	58.7, CH_2	3.84, m 3.55, m	43.7, CH_2	3.11, m 3.40, m
6a	72.8, CH	3.86, m	58.7, CH	3.63, dd (11.2, 6.2)
7	34.2, CH_2	2.06, m 2.97, m	34.6, CH_2	2.16, m 2.52, m
8	30.1, CH_2	2.22, m 2.58, m	30.4, CH_2	2.33, m 2.53, m
8a	134.1, C	–	135.3, C	–
9	107.3, CH	6.59, s	114.1, CH	6.79, s
10	153.9, C	–	153.7, C	–
11	141.0, C	–	141.2, C	–
12	151.5, C	–	149.5, C	–
12a	119.0, C	–	119.7, C	–
12b	128.9, C	–	128.9, C	–
13	60.6, CH_3	3.56, s	39.9, CH_3	2.58, s
14	56.2, CH_3	3.90, s	56.3, CH_3	3.93, s
15	56.1, CH_3	3.24, s	61.6, CH_3	3.94, s
16	56.2, CH_3	3.91, s	62.6, CH_3	3.66, s
17	61.1, CH_3	3.88, s		
18	61.1, CH_3	3.75, s		

27.3), and C-3b (δ_C 123.6); H-9 to C-8 (δ_C 30.1), C-11 (δ_C 141.0), and C-12a (δ_C 119.0); H-6a to C-5 (δ_C 58.7), *N*-methyl, C-7 (δ_C 34.2) and C-12b (δ_C 128.9) were also observed confirming the structure of **3**. COSY data identified two-spin systems as H-4/H-5 and H-6a/H-7/H-8 (Fig. 2).

The absolute configurations of homoaporphine alkaloids are assigned using specific rotation and electronic circular dichroism (ECD) spectroscopy [18,22,32], in which a clockwise rotation of plane polrized light (dextrorotatory) along with a negative Cotton effect in the 254–258 nm region of the ECD spectra are indicative of a C-6a*S* configuration [18,22]. A specific rotation of $[\alpha]_D^{24} = +20$ (*c* 0.001, MeOH) for **3**, along with a negative Cotton effect at 259 nm ($\Delta\varepsilon = -6.5$) in the ECD spectrum, supported an *S*-configuration at C-6a (Fig. 3A).

Compound **5** (4.1 mg) was obtained as a light brown amorphous solid with a molecular formula of $C_{21}H_{25}NO_5$ as determined by HRESIMS (*m/z* 372.1802 $[M + H]^+$, calcd 372.1805) and NMR data (Table 1, Figs. S1 and S7, Supplementary Data), establishing an index of hydrogen deficiency of 10. Analysis of the NMR data indicated a new homoaporphine alkaloid with structural similarity to **4** (Fig. 1). Compound **5** lacked a methoxy group, which was replaced by an exchangeable proton, consistent with a 14 amu difference in the HRMS data of **5** relative to **4**. Inspection of the NMR data showed signals characteristic of two singlet aromatic protons (δ_H/δ_C 6.68/109.5; H-3 and 6.79/114.1; H-9), nine aliphatic protons (δ_H 2.82 and 3.12, H_2–4; 3.11 and 3.40, H_2–5; 3.63, H-6a; 2.16 and 2.52, H_2–7 and 2.33 and 2.53, H_2–8), one *N*-methyl group (δ_H/δ_C 2.58/39.9) and three methoxy functionalities (δ_H/δ_C 3.66/62.6; 3.93/56.3, and 3.94/61.6) (Table 1, Fig. S7, Supplementary Data). HMBC correlations from the 10-OCH_3, 11-OCH_3, and 12-OCH_3 protons to C-10 (δ_C 153.7), C-11 (δ_C 141.2), and C-12 (δ_C 149.5), respectively, confirmed their connectives (Fig. 2). HMBC correlations from H-3 to C-1 (δ_C 146.9), C-2 (δ_C 139.2), C-3b (δ_C 122.2), and C-4 (δ_C 23.3); H-9 to C-8 (δ_C 30.4), C-10 (δ_C 153.7), C-11 (δ_C 141.2), and C-12a (δ_C 119.7) and H-6a to C-3a (δ_C 123.4), C-3b and C-5 (δ_C 43.7) were also observed (Fig. 2). COSY data showed two-spin systems as H-4/H-5 and H-6a/H-7/H-8 (Fig. 2). The trivial name 1-demethylandrocine was assigned to compound **5**. A negative Cotton effect at 258 nm ($\Delta\varepsilon = -8.58$) in the ECD spectrum along with a specific rotation value of $[\alpha]_D^{24} = +126$ (*c* 0.001, MeOH), supported an *S*-configuration at C-6a (Fig. 3B).

Compound **8** (3.1 mg) was obtained as a light brown amorphous solid with a molecular formula of $C_{22}H_{29}NO_5$ as determined by HRESIMS (*m/z* 388.2116 $[M + H]^+$, calcd 388.2118) and analysis of NMR data (Table 2, Figs. S1 and S10, Supplementary Data), establishing an index of hydrogen deficiency of 9. Inspection of the NMR data showed signals characteristic of one singlet aromatic proton (δ_H/δ_C 6.34/107.2), one singlet olefinic proton (δ_H/δ_C 4.98/103.9), eight aliphatic protons displayed between 1.76 and 2.83 ppm, one *N*-methyl

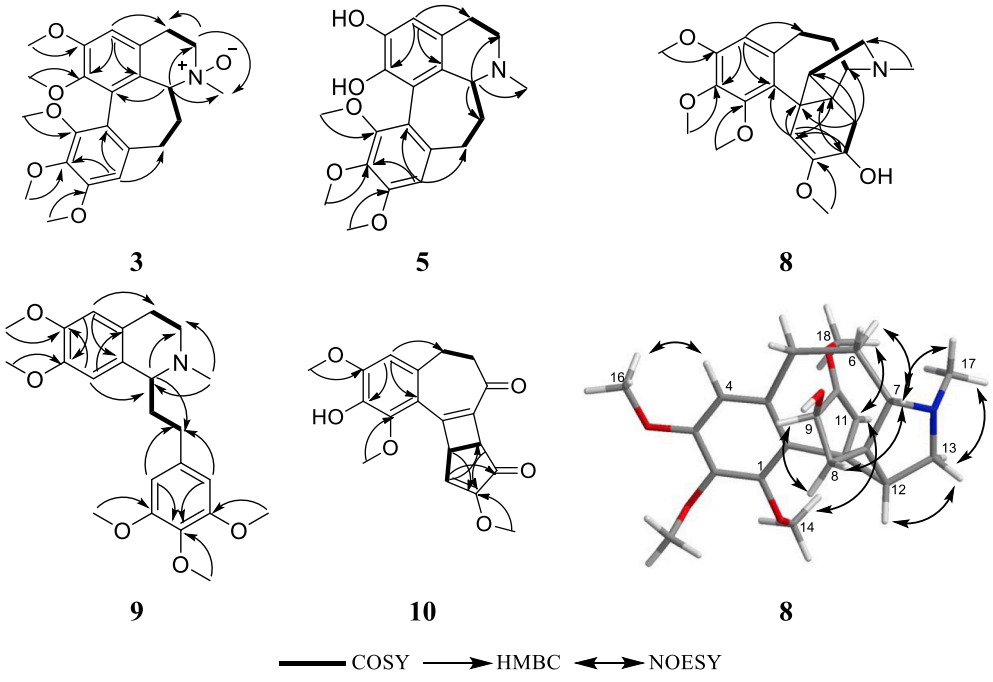

Fig. 2. Key COSY and HMBC correlations of **3**, **5**, and **8–10**, and NOESY correlations of **8**.

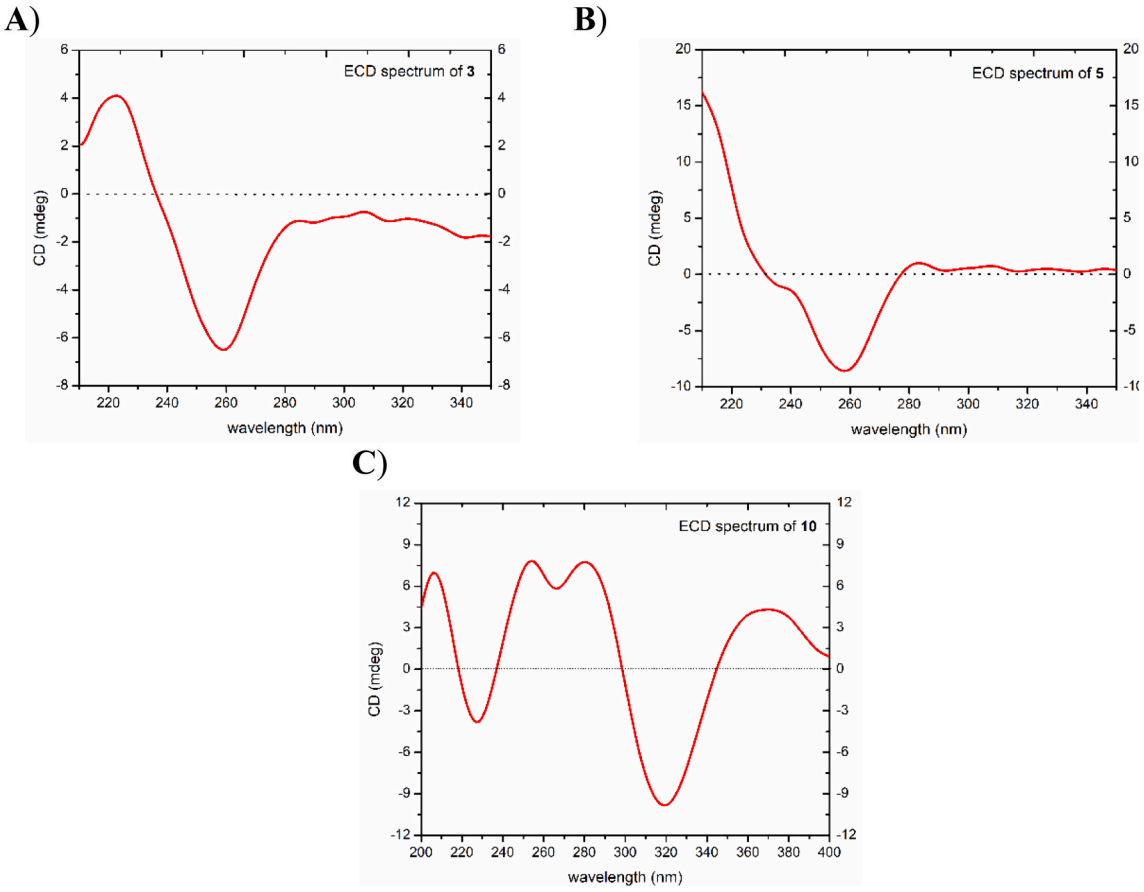

Fig. 3. ECD spectra for compounds A) **3** (1 mM), B) **5** (0.3 mM), and C) **10** (0.3 mM) [MeOH, cell length 2 cm].

Table 2
NMR data for compound **8** (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$).

Position	δc, type	δ_H, mult (*J* in Hz)	Position	δc, type	δ_H, mult (*J* in Hz)
1	152.0, CH	–	10	153.1, C	–
2	139.9, C	–	11	103.9, CH	4.98, s
3	152.5, C	–	11a	26.9, C	–
4	107.2, C	6.34, s	11b	126.8, C	–
4a	136.5, C	–	12	38.4, CH	1.76, m
5	30.7, CH_2	2.30, ddd (13.3, 4.8, 2.1) 2.83, m	13	56.9, CH_2	2.49, dd (11.1, 5.7) 2.77, m
6	25.4, CH_2	1.76, m	14	61.2, CH_3	4.02, s
7	68.4, CH	2.75, m	15	60.6, CH_3	3.85, s
7a	33.6, C	–	16	55.9, CH_3	3.83, s
8	31.3, CH_2	2.20, m	17	40.4, CH_3	1.97, s
9	65.9, CH	4.29, dd (4.0, 3.0)	18	54.5, CH_3	3.49, s

group (δ_H/δ_C 1.97/40.4), and four methoxy functionalities (δ_H 3.49; 3.83; 3.85, and 4.02) (δ_C 54.5; 55.9; 60.6, and 61.2) (Table 2, Fig. S10, Supplementary Data). HMBC correlations from the 1-OCH_3, 2-OCH_3, 3-OCH_3, and 10-OCH_3 protons to C-1 (δ_C 152.0), C-2 (δ_C 139.9), C-3 (δ_C 152.5), and C-10 (δ_C 153.1), respectively, confirmed their connectives. HMBC correlations from H-4 to C-2 (δ_C 139.9), C-3 (δ_C 152.5), C-5 (δ_C 30.7), and C-11b (δ_C 126.8); H-11 to C-9 (δ_C 65.9), C-11b, C-11a (δ_C 26.9), and C-7a (δ_C 33.6); H-9 to C-11 (δ_C 103.9), C-10 and C-7a; H-8 to C-10, C-7 (δ_C 68.4), C-11a and C-12 (δ_C 38.4) and H-12 to C-11, C-8 (δ_C 31.3) and C-13 (δ_C 56.9) were also observed (Fig. 2). COSY data showed three spin systems as H_2–8/H-9, H_2–5/H_2–6/H-7, and H-12/H_2–13 (Fig. 2). These data suggested that **8** was related to the androcymbines (homomorphinans/homomorphinandienone) class of alkaloids, showing structural similarity to szovitsidine, which was isolated from *Colchicum szovitsii* (Fig. 1) [33]. Compound **8** and szovitsidine showed identical molecular weight, molecular formula, and unsaturation number. However, the NMR data of **8** indicated the lack of the olefinic C7a-C8 bond in **8** relative to szovitsidine, which was replaced by a quaternary aliphatic carbon (δ_C 33.6 for C-7a) and a methylene group (δ_H/δ_C 2.20/31.3 for H_2–8/C-8). Moreover, the methylene group at C-12 in szovitsidine was replaced by a methine (δ_H/δ_C 1.76/38.4 for H-12/C-12) in **8**. These data, along with HMBC correlations from H_2–8 to C-7 (δ_C 68.4), C-12, and C-11a (δ_C 26.9), indicated the formation of a cyclopropane ring (C-7a, C-11a, and C-12), which account for the unsaturation unit that was lost due to the saturation of the C7a—C—8 double bond in **8** relative to szovitsidine. An attempt to assign the absolute configuration of **8** using Mosher's esters methodology [34] was unsuccessful. The relative configuration of **8** was assigned by NOESY NMR data (Fig. 2).

Compound **9** (2.2 mg) was obtained as a white powder with a molecular formula of $C_{23}H_{31}NO_5$ as determined by HRESIMS (*m/z* 402.2276 $[M + H]^+$, calcd 402.2275) and NMR data (Table 3, Figs. S1 and S11, Supplementary Data), establishing an index of hydrogen deficiency of 9. Inspection of NMR data indicated **9** as a phenethylisoquinoline alkaloid with structural similarity to the known alkaloids (+)-autumnaline [35] and (+)-homolaudanosine [36]. However, compound **9** has an additional two methyl groups consistent with a 28 amu difference in the HRMS data of **9** relative to (+)-autumnaline and an extra methoxy group consistent with a 30 amu difference in the HRMS data of **9** relative to (+)-homolaudanosine (Table 3, Fig. S11, Supplementary Data). HMBC correlations from the 6-OCH_3, 7-OCH_3, 2 × 3′-OCH_3, and 4′-OCH_3 protons to C-6 (δ_C 147.51), C-7 (δ_C 147.47), 2 × C-3′ (δ_C 153.2), and C-4′ (δ_C 136.1), respectively, confirmed their connectives and established the structure of **9** (Fig. 1). Other HMBC correlations observed were from H-1 to C-3 (δ_C 48.1), C-4a (δ_C 126.8),

Table 3
NMR data for compound **9** (400 MHz for ^{1}H and 100 MHz for ^{13}C, $CDCl_3$).

Position	δc, type	δ_H, mult (*J* in Hz)	Position	δc, type	δ_H, mult (*J* in Hz)
1	62.8, CH	3.46, dd (5.44, 4.24)	10	56.2, CH_3	3.83, s
3	48.1, CH_2	2.70, m 3.14, m	11	56.0, CH_3	3.86, s
4	25.4, CH_2	2.70, m	1′	138.8, C	–
4a	126.8, C	–	2′, 2′	105.5, CH	6.40, s
5	111.5, CH	6.58, s	3′, 3′	153.2, C	–
6	147.51, C	–	4′	136.1, C	–
7	147.47, C	–	5′	32.2, CH_2	2.05, m
8	110.3, CH	6.54, s	6′	37.1, CH_2	2.50, m 2.70, m
8a	129.7, C	–	7′, 7′	56.2, CH_3	3.83, s
9	42.8, CH_3	2.50, s	8′	61.0, CH_3	3.81, s

Table 4
NMR data for compound **10** (700 MHz for ^{1}H and 175 MHz for ^{13}C, $CDCl_3$).

Position	δc, type	δ_H, mult (*J* in Hz)	Position	δc, type	δ_H, mult (*J* in Hz)
1	146.6, C	–	8	197.3, C	–
2	137.2, C	–	9	158.3, C	–
3	149.3, C	–	10	125.0, CH	6.56, d (3.4)
4	108.2, CH	6.60, s	10a	43.8, CH	4.24, dd (3.4, 2.8)
4a	135.9, C	–	10b	158.3, C	–
5	30.8, CH_2	2.85, m	10c	118.7, C	–
6	40.8, CH_2	2.64, m	11	61.2, CH_3	4.04, s
7	194.8, C	–	12	56.5, CH_3	3.95, s
7a	133.6, C	–	13	57.0, CH_3	3.68, s
7b	48.6, CH	3.90, d (2.8)	2-OH		5.52, s

C-8 (δ_C 110.3), and C-5′ (δ_C 32.2); H-5 to C-4 (δ_C 25.4), C-7 (δ_C 147.47) and C-8a (δ_C 129.7); H-8 to C-1 (δ_C 62.8), C-4a (δ_C 126.8), and C-6 (δ_C 147.51). H-6′ has HMBC correlations with C-5′, C-4′ (δ_C 136.1), C-3′ (δ_C 153.2), and C-2′ (δ_C 105.5), and H-2′ to C-5′, C-2′ (δ_C 105.5), C-3′ (δ_C 153.2) and C-4′ (Fig. 2). COSY data showed two spin systems as H-1/H-6′/H-5′ and H-3/H-4 (Fig. 2). Interestingly, compound **9** was reportsed previously by synthesis [32,37,38]. However, this is the first report of **9** from nature, to which the trivial name *O*,*O*-dimethylautumnaline was assigned. The specific rotation of **9** was determined as $[\alpha]_D^{24} = +3$ (*c* 0.001, MeOH) supporting an *S*-configuration at C-1, consistent with reported values in literature [32,37,38].

Compound **10** (0.70 mg) was obtained as a light yellow powder with a molecular formula of $C_{19}H_{18}O_6$ as determined by HRESIMS (*m/z* 343.1173 $[M + H]^+$, calcd 343.1176) and NMR data (Table 4, Figs. S1 and S12, Supplementary Data), establishing an index of hydrogen deficiency of 11. Analysis of the NMR data suggested **10** as a new colchicone derivative with a structural similarity to the known alkaloid *β*-lumicolchicone (Fig. 1), which was isolated from the tubers of *Gloriosa superba* [39]. Compound **10** lacked a methoxy group at C-2, which was replaced by an exchangeable proton (Table 4, Fig. S12, Supplementary Data), consistent with a 14 amu difference in the HRMS data of **10** relative to *β*-lumicolchicone. Inspection of the NMR data showed signals characteristic of one singlet aromatic proton (δ_H/δ_C 6.60/108.2), one singlet olefinic proton (δ_H/δ_C 6.56/125.0), six aliphatic protons that are displayed between 2.64 and 4.24, and three methoxy functionalities (δ_H 3.68; 3.95 and 4.04) (δ_C 57.0, 56.5, and 61.2) (Table 4, Fig. S12, Supplementary Data). HMBC correlations from the 1-OCH_3, 3-OCH_3 and 9-OCH_3 protons to C-1 (δ_C 146.6), C-3 (δ_C 149.3), and C-9 (δ_C 158.3), respectively, confirmed their connectives (Fig. 2). HMBC correlations from H-4 to C-2 (δ_C 137.2), C-10c (δ_C 118.7), and C-5 (δ_C 30.8); H-7b to C-7a (δ_C 133.6), C-8 (δ_C 197.3), C-9 (δ_C 158.3) and C-10a (δ_C 43.8); H-10 to C-7b (δ_C 48.6), C-8 and C-10a; H-10a to C-9 and C-10 (δ_C 125.0) were also observed (Fig. 2). COSY data showed two spin systems as H-5/H-6 and H-10/H-10a/H-7b (Fig. 2). The trivial name 2-demethyl-*β*-lumicolchicone was assigned to **10**, in deference to the known compound *β*-lumicolchicone. A NOESY correlation from H-7b to H-10a along with coupling constant value of 2.8 ppm confirmed the *syn*-fusion at C-7b/C-10a. The absolute configuration was established using CD spectra of structurally related compounds [40]. The CD spectrum of **9** was similar to that reported for *β*-lumicolchicine but opposite to that of γ-lumicolchicine establishing the configuration as 7b*R*,10a*S* (Fig. 3C) [40].

Table 5
Cytotoxic activities of compounds **11–13**.

Compound[a]	IC_{50} values in nM[b]		
	MDA-MB-435	Ovcar3	MDA-MB-231
11	12	23	95
12	21	77	113
13	800	2230	9940
taxol[c]	0.1	1.45	171

[a] Compounds **1–10** were inactive, IC_{50} values > 25 μM.
[b] IC_{50} is the concentration to inhibit 50% of growth with a 72 h incubation.
[c] Positive control.

The cytotoxicities of **1–13** were tested against MDA-MB-435 (melanoma), MDA-MB-231 (breast), and OVCAR3 (ovary) cancer cell lines. Colchicine (**11**) was the most potent, with IC_{50} values in the range of 10 to 100 nM, depending on the cell line (Table 5); these data were consistent with previous studies on this well-known alkaloid [41–43]. The cytotoxicity data facilitated conclusions on the structure-activity relationships (i.e. SARs). For instance, compounds **1–10**, all of which lacked the tropolone ring, were inactive, demonstrating the importance of the tropolone moiety for cytotoxic activity. Replacing the *N*-acetyl group in **11** by an *N*-methyl group in **12** reduced the activity against MDA-MB-435 and OVCAR3 by factors of 2, and 3, respectively, although the activity vs MDA-MB-231 cells remained intact. Moreover, demethylation of the 3-OCH_3 group, as noted in compounds **13** vs **12**, diminished the cytotoxicity in MDA-MB-435, MDA-MB-231, and OVCAR3 cells by a factor of ~40, ~90, and ~ 30, respectively.

Declaration of Competing Interest

All the authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2020.104706.

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