Homoisoflavonoids from the bulbs of Bellevalia longipes and an assessment of their potential cytotoxic activity

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El-Elimat, T., Al-Qiam, R., Burdette, J. E., Al Sharie, A. H., Al-Gharaibeh, M., & Oberlies, N. H. (2022). Homoisoflavonoids from the bulbs of Bellevalia longipes and an assessment of their potential cytotoxic activity. Phytochemistry, 203, 113343.

Made available courtesy of Elsevier: http://dx.doi.org/10.1016/j.phytochem.2022.113343

Abstract:

Seven undescribed homoisoflavonoids were identified from the bulbs of Bellevalia longipes Post (Asparagaceae) as well as thirteen known and one natural homoisoflavonoid that had been reported as a synthetic product previously. A general approach for recognizing homoisoflavonoids via NMR spectroscopy data were presented. The undescribed compounds were: 8-dehydroxy-5-O-demethyl-6-hydroxyscillapersicone, 6-methoxyscillapersicone, 5-O-demethyl-6-methoxyscillapersicone, 8-O-methylscillapersicone, 4′ -O-methylscillapersicone, 4′ ,8-O,O-dimethylscillapersicone, 3′ -O-methylscillapersicone, and 3-hydroxy-desmethylophiopogonanone A. Structures were determined based on analysis of HRMS and NMR data, while absolute configurations were assigned using ECD spectroscopy. Human cancer cell lines were used to assess the cytotoxic activities of the isolated compounds, where 3-dehydroxy-3′ -hydroxyeucomol showed IC50 values of 0.62 μM, 5.36 μM, and 2.52 μM, when tested against MDA-MB-435 (melanoma), MDA-MB-231 (breast), and OVCAR3 (ovarian) cells, respectively.

Keywords: Bellevalia longipes Post | Asparagaceae | Homoisoflavonoids | Cytotoxic activity

Article:

1. Introduction

Homoisoflavonoids are an unusual subclass of flavonoids, which possess an additional carbon between rings B and C (Lin et al., 2014). Biosynthetically, chalcones are thought to be the precursors of homoisoflavonoids, where a carbon atom derived from L-methionine is added onto the C15 chalcone-type skeleton (Dewick, 1975; Lin et al., 2008). The term was first used in 1967 to define new compounds isolated from Eucomis bicolor Bak. (Asparagaceae) (Böhler and Tamm, 1967). As of 2019, Abegaz et al. reported the identification of 295 homoisoflavonoids from various plant families with a range of biological activities, including antimutagenic (Miadoková et al., 2002; Siddaiah et al., 2007), antimicrobial (Sharma et al., 2018), antioxidant (Zhou et al., 2015), immunomodulatory (Chen et al., 2009), antidiabetic (Lin et al., 2014), cytotoxic (Lin et al., 2008), vasorelaxant (He et al., 2009), antiangiogenic (Lin et al., 2008), anti-inflammatory (Li et al., 2012), and many others (Castelli and López, 2017). Moreover,

homoisoflavonoids were reported to inhibit protein tyrosine kinase (Castelli and López, 2017) and acetylcholinesterase activity (Mottaghipisheh and Stuppner, 2021), and exhibit estrogenic and antiestrogenic activities (Masi et al., 2021).

Jordan occupies an enviable position at the heart of the Middle East near a point where Europe, Asia, and Africa intersect. Despite its relatively small size, it has rich biodiversity due to its diverse altitude (−408 m below sea level to 1854 m above sea level), which imparts sharp changes in climate, rainfall, soil, and physical environments (Abd-Al Hadi, 2020; Afifi-Yazar et al., 2011; Al-Eisawi, 1998). Jordan has thirteen different types of vegetation, providing habitat for more than 4000 plant species, including 2622 species of vascular plants belonging to 152 families and 719 genera (Al-Eisawi, 1996; Al-Eisawi, 1998; Ministry of Environment, 2014). As part of a continuing project aimed at identifying cytotoxic compounds from Jordanian plants (Alali et al., 2005, 2006, 2014, 2015; El-Elimat et al., 2018, 2020), the bulbs of Bellevalia longipes Post (Asparagaceae) were investigated. A total of 14 species of Bellevalia can be found in Jordan (Abd-Al Hadi, 2020), and B. longipes is a bulbous perennial herb that flourishes in mountains with red heavy soils (Feinbrun-Dothan, 1986). It is known locally as "Bossyl Al-Ghazal", which can be translated into "Deer Onion" in English, and it blooms on long pedicels in mid-spring, between the months of March and May (Feinbrun-Dothan, 1986), with flowers that are of a brownish lavender color. B. longipes bulbs, which are 2.5–3.0 cm in diameter, can be found across the Irano-Turanian region (i.e., Iran, Turkey, Palestine, Syria, and Jordan) (Al-Eisawi, 1998; Feinbrun-Dothan, 1986). The present study reports the isolation, structural elucidation, absolute configuration determination, and cytotoxic activity evaluation of a suite of compounds from the bulbs of B. longipes.

2. Results and discussion

During the past few years, we have become increasingly interested in homoisoflavonoids, an intriguing and rare class of phytochemicals with diverse biological activities. In this study, phytochemical investigation of the bulbs of B. longipes resulted in the isolation of twenty-one compounds (1–21), most of which were homoisoflavonoids (Fig. 1).

The structures of the isolated compounds (1–21) were determined through in-depth analysis of HRESIMS, 1D (1 H, 13C), and 2D (COSY, HSQC, and HMBC) NMR data. The spectroscopic and spectrometric data of 13 of these compounds compared favorably to the literature, revealing: 8-demethoxy-3,9-dihydropunctatin (1) (Adinolfi et al., 1985a), 3,9-dihydroeucomnalin (2) (Sidwell and Tamm, 1970), 8-O-demethyl-7-O-methyl-3,9-dihydropunctatin (3) (Adinolfi et al., 1984), 7-O-methyl-3,9-dihydropunctatin (4) (Pohl et al., 2001), 5-O-demethylscillapersicone (5) (Adinolfi et al., 1986), 3-dehydroxy-6-hydroxyeucomol (6) (El-Elimat et al., 2018), scillapersicone (8) (Hafez Ghoran et al., 2014), 4′ -O-demethylcremastranone (12) (Adinolfi et al., 1986), 3-dehydroxy-3′ -hydroxyeucomol (13) (Adinolfi et al., 1985b), eucomol (17) (Meksuriyen and Cordell, 1988), punctatin (19) (Corsaro et al., 1992), p-hydroxybenzaldehyde (20), and pinoresinol (21) (Ono et al., 2000) (Supplementary data, Figs. S1-S7, S11, S23, S24, S35, S39-S41).

Having worked with homoisoflavonoids for a number of years (Alali et al., 2015; El-Elimat et al., 2018), we have developed a general strategy that capitalizes upon key chemical shifts in the 1 H NMR data (collected in CDCl3, even for compounds of limited solubility) to rapidly classify these structures (Fig. 2). Specifically, a few key resonances can be examined to assist in the subclassification of homoisoflavonoids into one of three categories (see panel 1 in

Fig. 2). The largest class of homoisoflavonoids is recognized by the presence of a multiplet resonating between ~2.5 and ~3.0 ppm, indicative of a 3-benzylchroman-4-one analogue (i.e., compounds 1 to 16). Alternatively, a singlet olefinic proton (δH ~5.0–6.0 ppm) can be observed in the 3-benzylidenechroman-4-one analogues (i.e., compound 19). Moreover, while less abundant, an aliphatic, geminal AB system (δH ~2.5–3.0 and ~4.0–4.5 ppm) indicates a 3-benzyl-3-hydroxychroman-4-homoisoflavonoid analogue (i.e., compounds 17 and 18). Once classified into one of these categories, the substitution of the A and B rings can also be derived from chemical shift values. If a singlet proton resonates above 11.0 ppm, this indicates a hydroxy substituent (not methylated) at C-5 (i.e., chelated proton).

Compound 7 (1.36 mg) was obtained as a yellowish powder with a molecular formula of C17H16O7 (index of hydrogen deficiency of 10) as determined by HRESIMS (m/z 333.0962 [M+H]+, calculated 333.0970) (Supplementary Data Fig. S1). Further support for the molecular formula was provided by the 1 H, 13C, and edited HSQC NMR data (Tables 1 and 2, Supplementary Data Figs. S8 and S9). The HRMS data, combined with 1D and 2D NMR, identified 7 as an undescribed 3-benzylchroman- 4-one homoisoflavonoid with structural similarity to 5 (Supplementary Data Table S1). Both compounds (5 and 7) shared the same molecular formula and similar ABX splitting system of ring B (δH 6.67, d, J = 1.8 Hz; 6.70, d, J = 7.8 Hz, and 6.56, dd, J = 7.8, 1.8 Hz, for H-2′ , H-5′ , and H-6′ , respectively for 7). The key differences between 7 and 5 were the singlet aromatic proton position at ring A, which was located at C-6 in 5 vs C-8 in 7. This was verified by HMBC correlations from H-8 (δH 6.15, s) in 7 to C-4 (δC 201.1), C-4a (δC 103.8), and C-6 (δC 129.0) (Fig. 3, Supplementary Data Fig. S10). The methoxy group (δH/δC 3.88/57.0) was located at C-7 based on an HMBC correlation from H3-10 (δH 3.88, s) to C-7 (δC 157.8). Ring B was hypothesized as a dihydroxybenzene based on HMBC correlations of H-2′ and H-6′ to C-4’ (δC 145.5) and from H-5′ to C-3’ (δC 146.8). Moreover, HMBC correlations from H-2 (δH 4.10, dd, J = 11.1, 7.3 Hz; 4.25, dd, J = 11.1, 4.4 Hz) to C-8a (δC 163.6), C-9 (δC 33.5), and C-4 (δC 201.1) and from H-9 (δH 2.60, dd, J = 13.3, 10.3 Hz; 3.04, dd, J = 13.3, 4.5 Hz) to C-3 (δC 50.2), C-2’ (δC 117.4), and C-6’ (δC 121.8) further supported the ring A substitution pattern and established the connectivity of ring C to ring B through position C-9. The trivial name 8-dehydroxy-5-O-demethyl-6-hydroxyscillapersicone was given to compound 7. The absolute configuration of the homoisoflavonoids of the 3-benzylchroman-4-one type is often determined by means of electronic circular dichroism (ECD) spectroscopy (Adinolfi et al., 1988). An ECD curve with a negative Cotton effect at 287–295 nm indicates a 3R configuration (Adinolfi et al., 1988). It was found that compound 7 exhibited a negative Cotton effect at 297 nm (Δε = − 35.3) in the ECD spectrum (Supplementary Data Fig. S43), supporting an R configuration at C-3.

Compound 9 (2.15 mg) was obtained as a yellowish powder with a molecular formula of C19H20O8 (index of hydrogen deficiency of 10) as determined by HRESIMS (m/z 377.1223 [M+H]+, calculated 377.1236) (Supplementary data Fig. S1). The HRMS data along with 1 H, 13C, COSY, HSQC, and HMBC NMR data (Tables 1 and 2, Supplementary data Figs. S12-S15) identified 9 as an undescribed homoisoflavonoid of the 3- benzylchroman-4-one type with structural similarity to 8 (Supplementary Data Table S1). Both 8 and 9 shared the same ABX splitting pattern of ring B (δH 6.67, d, J = 2.8 Hz; 6.70, d, J = 8.0 Hz; 6.55, dd, J = 8.0, 2.8 Hz, for H-2′ , H-5′ , and H-6′ , respectively for 9). A key difference between the two compounds was the absence of aromatic protons in ring A of 9, as opposed to 8, indicating a hexa-substituted ring A in 9. The deshielded C-6 (δC 143.0) in 9 relative to that in 8 (δC 93.3), along with a 30 Da difference in the HRMS data indicated an extra methoxy group at C-6, which was confirmed by

an HMBC correlation from H3-11 (δH/δC 3.82/ 62.5) to C-6. Based on the HMBC correlations from H3-10 (δH/δC 3.97/ 62.7) to C-5 (δC 149.2) and H3-12 (δH/δC 3.78/62.0) to C-7 (δC 147.7), the positions of the remaining two methoxy groups of ring A in 9 were established. COSY data identified two-spin systems as H2-2/H-3/H2-9 and H-5'/H-6' (Fig. 3, Supplementary data Fig. S13). The structure of 9 was established as 6-methoxyscillapersicone. The ECD spectrum of 9 showed a negative Cotton effect (Δε = − 7.6) at 297 nm (Supplementary Data Fig. S43) indicating a 3R configuration.

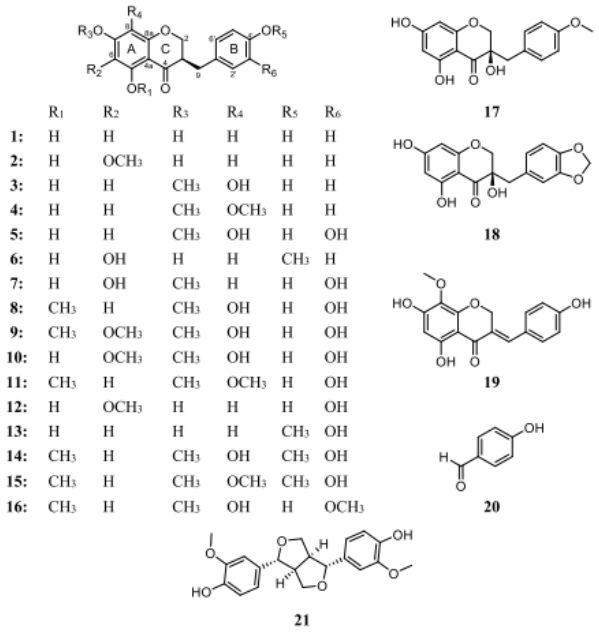

Fig. 1. Structures of the compounds isolated from the bulbs of B. longipes (1–21).

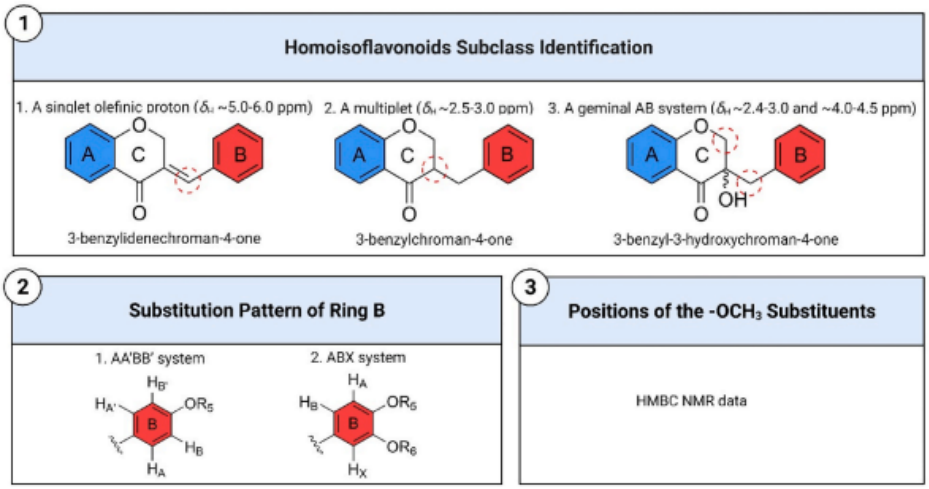

Fig. 2. The NMR methodology used to rapidly classify the structures of the homoisoflavonoids.

Table 1.
^{1}H NMR data for compounds 7, 9, 10, 11 [500 MHz, CD_3OD]

No.	δH, mult (J in Hz)			
	7	**9**	**10**	**11**
2	4.10, dd (11.1, 7.3) 4.25, dd (11.1, 4.4)	4.18, dd (11.3, 7.5) 4.35, dd (11.3, 4.1)	4.12, dd (11.3, 7.5) 4.28, dd (11.3, 4.2)	4.18, dd (11.3, 6.7) 4.30, dd (11.3, 4.0)
3	2.80, m	2.77, m	2.85, m	2.70, m
6	-	-	-	6.33, s
8	6.15, s	-	-	-
9	2.60, dd (13.3, 10.3) 3.04, dd (13.3, 4.5)	2.58, dd (13.9, 10.2) 3.02, dd (13.9, 4.7)	2.58, dd (13.8, 10.3) 3.04, dd (13.8, 4.5)	2.57, dd (13.7, 10.3) 2.98, dd (13.7, 4.6)
10	3.88, s	3.97, s	3.96, s	3.71, s
11	-	3.82, s	3.77, s	3.95, s
12	-	3.78, s	-	3.89, s
2’	6.67, d (1.8)	6.67, d (2.8)	6.65, d (2.4)	6.67, d (2.1)
5′	6.70, d (7.8)	6.70, d (8.0)	6.68, d (7.9)	6.70, d (8.0)
6′	6.56, dd (7.8, 1.8)	6.55, dd (8.0, 2.8)	6.54, dd (7.9, 2.4)	6.55, dd (8.0, 2.1)

HRESIMS and NMR data of compound 10 (17.91 mg), which was isolated as a yellowish powder, established the molecular formula as C18H18O8 (index of hydrogen deficiency of 10) (m/z 363.1066 [M+H]+, calculated 363.1074) (Tables 1 and 2, Supplementary data Fig. S1, S16-18). Compound 10 was identified as an undescribed homoisoflavonoid, belonging to the 3-benzylchroman-4-one class bearing structural similarity to 5 (Supplementary Data Table S1). Similar features between 5 and 10 included the ABX splitting pattern of ring B (δH 6.65, d, J = 2.4 Hz; 6.68, d, J = 7.9 H; 6.54, dd, J = 7.9, 2.4 Hz, for H-2′ , H-5′ , and H-6′ , respectively for 10) and the characteristic downfield singlet at 11.55 ppm for the OH group at the C-5 position (shown in the 1 H NMR spectrum that was collected using CDCl3, Supplementary data Fig. S16). However, the aromatic proton H-6 in compound 5 was replaced with a methoxy group (δH/δC 3.96/61.9) in compound 10, consistent with the additional 30 Da in the HRMS data of 10 as opposed to 5. HMBC correlations from H3-10 (δH 3.96) to C-6 (δC 135.2) and from H3-11 (δH 3.77) to C-7 (δC 149.4) established the methoxy positions at C-6 and C-7, respectively (Fig. 3). The OH singlet (δH 11.56) at C-5 (δC 151.4) displayed three HMBC correlations to C-4a (δC 104.8), C-5 (δC 151.4), and C-6 (δC 135.2). The structure of 10 was established as 5-O-demethyl-6- methoxyscillapersicone. A negative Cotton effect (Δε = − 13.5) at 300 nm in the ECD spectrum established the absolute configuration of 10 at C-3 as R (Supplementary Data Fig. S43).

Compound 11 (2.15 mg) was isolated as a white amorphous powder with a molecular formula of C19H20O7 (index of hydrogen deficiency of 10) as determined by HRESIMS (m/z 361.1276 [M+H]+, calculated 361.1282) and 1 H, 13C, and edited HSQC NMR data (Supplementary data Figs. S1, S19-21). Based on spectroscopic and spectrometric analyses, 11 was identified as an undescribed homoisoflavonoid belonging to the 3-benzylchroman-4-one class with structural similarity to 8 (Supplementary Data Table S1). Both compounds shared a similar ABX splitting system of ring B (δH 6.67, d, J = 2.1 Hz; 6.70, d, J = 8.0 Hz; and 6.55, dd, J = 8.0, 2.1 Hz, for H-2′ , H-5′ , and H-6′ , respectively for 11) and a singlet aromatic proton at C-6 (δH/δc 6.33/90.7 for 11). However, the additional 14 Da in 11 vs 8 in the HRMS data indicated methylation of one of the phenolic groups, as confirmed by HMBC correlations from H3-12 (δH 3.89) to C-8 (δC 131.6) (Fig. 3). HMBC data analysis showed that H-6 (δH 6.33) is correlated to C-4a (δC 106.2) and to C-8 (δC 131.6). Furthermore, the positions of the remaining two methoxy groups were determined by HMBC, where 10-OCH3 (δH 3.71) and 11-OCH3 (δH 3.95) were correlated to C-5 (δC 159.9) and C-7 (δC 160.7), respectively (Fig. 3). The trivial name 8-O-methylscillapersicone was assigned. Compound 11 showed a negative Cotton effect on the ECD spectrum (Fig. 3) (Δε = − 10.8) at 295 nm indicating a 3R configuration.

Table 2.
13 C NMR data for compounds 7, 9, 10, 11 [125 MHz, CD3OD], 14 [125 MHz, CDCl3], 15 and 16 [100 MHz, CDCl3].

No.	δC, Type							
	7	9	10	11	14	15	16	18
2	70.8, CH_2	71.1, CH_2	70.6, CH_2	70.2, CH_2	69.9, CH_2	69.4, CH_2	69.9, CH_2	71.4, CH_2
3	50.2, CH	50.8, CH	48.7, CH	50.0, CH	49.2, CH	48.6, CH	49.5, CH	78.6, C
4	201.1, C	195.1, C	201.2, C	194.3, C	191.9, C	191.7, C	192.1, C	192.0, C
4a	103.8, C	112.3, C	104.8, C	106.2, C	105.8, C	104.1, C	105.9, C	105.8, C
5	152.9, C	149.2, C	151.4, C	159.9, C	155.7, C	158.6, C	155.8, C	160.2, C
6	129.0, C	143.0, C	135.2, C	90.7, CH	89.8, CH	89.3, CH	89.8, CH	97.1, CH
7	157.8, C	147.7, C	149.4, C	160.7, C	152.4, C	161.4, C	152.6, C	154.0, C
8	92.4, CH	137.3, C	131.6, C	131.6, C	127.7, C	130.7, C	127.8, C	95.7, CH
8a	163.6, C	149.3, C	146.5, C	157.6, C	149.9, C	158.3, C	149.9, C	156.0, C
9	33.5, CH_2	33.7, CH_2	33.0, CH_2	33.6, CH_2	32.6, CH_2	32.3, CH_2	33.2, CH_2	41.9, CH_2
10	57.0, CH_3	62.7, CH_3	61.6, CH_3	56.4, CH_3	56.4, CH_3	56.2, CH_3	56.4, CH_3	101.1, CH_2
11	-	62.5, CH_3	61.4, CH_3	56.7, CH_3	56.6, CH_3	56.3, CH_3	56.7, CH_3	-
12	-	62.0, CH_3	-	61.4, CH_3	56.7, CH_3	55.9, CH_3	56.8, CH_3	-
13	-	-	-	-	-	61.4, CH_3	-	-

1′	131.2, C	131.6, C	130.7, C	131.1, C	132.0, C	131.8, C	130.7, C	131.2, C
2′	117.4, CH	117.6, CH	117.1, CH	117.1, CH	115.6, CH	115.3, CH	114.8, CH	111.3, CH
3′	146.8, C	147.0, C	146.5, C	146.4, C	146.0, C	145.4, C	147.0, C	148.1, C
4′	145.5, C	145.6, C	145.1, C	145.1, C	145.7, C	145.7, C	144.7, C	146.1, C
5′	116.8, CH	116.9, CH	116.4, CH	116.4, CH	111.1, CH	110.9, CH	111.9, CH	108.5, CH
6′	121.8, CH	122.0, CH	121.5, CH	121.5, CH	121.1, C	120.8, CH	122.4, CH	123.8, CH

[a] The ^{13}C NMR chemical shift values were based on the edited-HSQC and HMBC 2D NMR spectra.

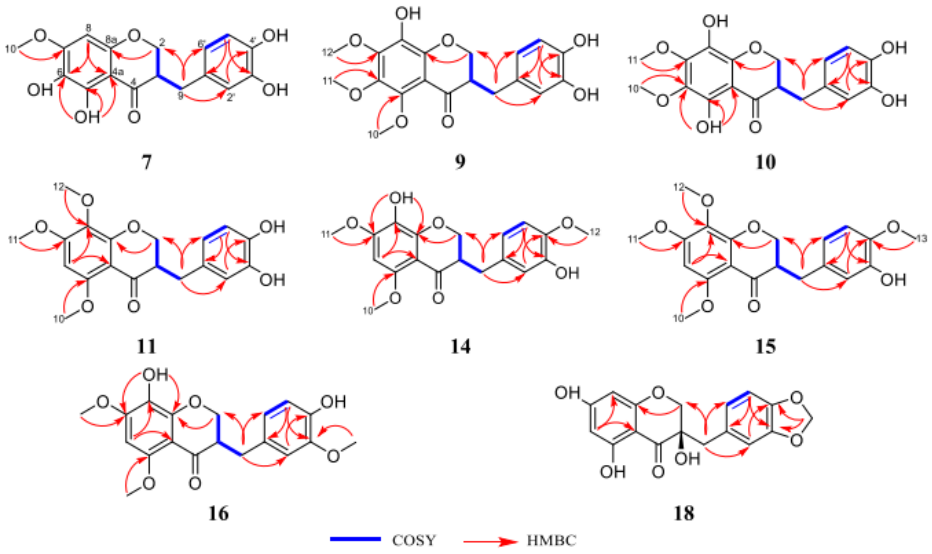

Fig. 3. Key COSY and HMBC correlations of **7, 9–11, 14–16**, and **18.**

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ECD spectrum established the absolute configuration of 10 at C-3 as R (Supplementary Data Fig. S43).

Compound 11 (2.15 mg) was isolated as a white amorphous powder with a molecular formula of C19H20O7 (index of hydrogen deficiency of 10) as determined by HRESIMS (m/z 361.1276 [M+H]+, calculated 361.1282) and 1 H, 13C, and edited HSQC NMR data (Supplementary data Figs. S1, S19-21). Based on spectroscopic and spectrometric analyses, 11 was identified as an undescribed homoisoflavonoid belonging to the 3-benzylchroman-4-one class with structural similarity to 8 (Supplementary Data Table S1). Both compounds shared a similar ABX splitting system of ring B (δH 6.67, d, J = 2.1 Hz; 6.70, d, J = 8.0 Hz; and 6.55, dd, J = 8.0, 2.1 Hz, for H-2′ , H-5′ , and H-6′ , respectively for 11) and a singlet aromatic proton at C-6 (δH/δc 6.33/90.7 for 11). However, the additional 14 Da in 11 vs 8 in the HRMS data indicated methylation of one of the phenolic groups, as confirmed by HMBC correlations from H3-12 (δH 3.89) to C-8 (δC 131.6) (Fig. 3). HMBC data analysis showed that H-6 (δH 6.33) is correlated to C-4a (δC 106.2) and to C-8 (δC 131.6). Furthermore, the positions of the remaining two methoxy groups were determined by HMBC, where 10-OCH3 (δH 3.71) and 11-OCH3 (δH 3.95) were correlated to C-5 (δC 159.9) and C-7 (δC 160.7), respectively (Fig. 3). The trivial name 8-O-methylscillapersicone was assigned. Compound 11 showed a negative Cotton effect on the ECD spectrum (Fig. 3) (Δε = − 10.8) at 295 nm indicating a 3R configuration.

Compound 14 (2.68 mg) was isolated as a white amorphous powder with a molecular formula of C19H20O7 (index of hydrogen deficiency of 10) as determined by HRESIMS (m/z 361.1273 [M+H]+, calculated 361.1282) and further supported by 1 H, 13C, and edited-HSQC NMR data (Supplementary data Figs. S1, S25-S27). Compound 14 showed NMR signals indicative of an undescribed homoisoflavonoid analogue belonging to the 3-benzylchroman-4-one class, also sharing structural similarities with 8 (Tables 2 and 3, Supplementary Data Table S1). For example, 8 and 14 shared a similar ABX splitting pattern of ring B and possessed an aromatic singlet proton at C-6 (δH/δC 6.16/89.8 for 14). The 1 H and 13C NMR data for 14 suggested that one of the phenolic hydroxy groups had been methylated (δH/δC 3.86/56.4), which was consistent with the 14 Da difference in the HRMS data of 14 relative to 8. HMBC correlations from H3-10 (δH 3.86) to C-4’ (δC 145.7) confirmed its connectivity (Fig. 3, Supplementary data Fig. S28). HMBC correlations from H3-11 (δH 3.90) to C-5 (δC 155.7), H3-12 (δH 3.97) to C-7 (δC 152.4) confirmed the connectivity of the other two methoxy groups. Additionally, the exchangeable proton at 5.59 showed HMBC correlations with C-4′ and C-2’ (δC 115.6), while the exchangeable proton at 5.08 displayed HMBC correlations with C-8a (δC 149.9) and C-7 (δC 152.4), confirming their connectivity to C-3′ (δC 146.0) and C-8 (δC 127.7), respectively. The compound was given the trivial name 4′ -O-methylscillapersicone. The absolute configuration of 14 was established as 3R based on the negative Cotton effect in the ECD spectrum (Δε = − 6.7) at 293 nm.

HRESIMS along with 1 H, 13C, and edited-HSQC NMR data identified the molecular formula of compound 15 (0.52 mg), which was isolated as a white amorphous powder, as C20H22O7 (index of hydrogen deficiency of 10) (m/z 375.1433 [M+H]+, calculated 375.1438) (Tables 2 and 3, Supplementary data Figs. S1, S29, and S31). Spectroscopic and spectrometric analysis including HMBC data revealed that 15 is identical to 3-(3′ -hydroxy-4′ -methoxybenzyl)-5,7,8-trimethoxychroman-4-one, a homoisoflavonoid that was recently reported by Kwon et al. (2020) as an intermediate in the total synthesis of 5,7,8-trioxygenated homoisoflavonoids (Kwon et al., 2020) (Fig. 3, Supplementary data Fig. S32). The trivial name 4′ ,8-O,O-dimethylscillapersicone was ascribed to it. A negative Cotton effect at 294 nm in the

ECD spectrum of compound 15 (Δε = − 3.2) indicated an R configuration at C-3 (Supplementary Data Fig. S43).

Compound 16 (3.34 mg) was isolated as a white amorphous powder. It showed high similarity to compound 14, including a molecular formula of C19H20O7 (HRESIMS m/z 361.1276 [M+H]+, calculated 361.1281) and NMR data, which were indicative of a 3-benzylchroman4-one homoisoflavonoid analogue (Tables 2 and 3, Supplementary data Figs. S1, S33, and S34). At 295 nm in the ECD spectrum of 16, there was a negative Cotton effect (Δε = − 1.7), indicating a 3R configuration, as in 14 (Supplementary Data Fig. S43). NMR data indicated a difference in the position of the 12-OCH3 group. In 14, H3-12 (δH 3.97) showed an HMBC correlation with C-4' (δC 145.7), confirming the methoxy at C-4′ (Fig. 3). Alternatively, in 16, an HMBC correlation from H3-12 (δH 3.91) to C-3' (δC 147.0) confirmed a methoxy at C-3′ (Fig. 3). The HMBC data (Supplementary data Fig. S34) also showed key correlations from H-5' (δH 6.84) to C-3′ , and from H-2' (δH 6.86) and H-6' (δH 6.73) to C-4' (δC 144.7). Additionally, the exchangeable 4′ -OH (δH 5.53) showed HMBC correlations to C-5' (δC 111.9) and C-3′ further verifying the ring B substitution pattern. HMBC correlations from H-6 (δH 6.17) to C-8 (δC 127.8) and C-4a (δC 105.9), from H3-11 (δH 3.91) to C-5 (δC 155.8), and from H3-12 (δH 3.97) to C-7 (δC 152.6) identified this compound as the 3′ -methoxy analogue of 14, which was given the trivial name 3′ -Omethylscillapersicone.

Compound 18 (0.74 mg, molecular formula C17H14O7) was obtained as an amorphous white powder. HRESIMS (m/z 331.0807 [M+H]+, calculated 331.0818) (index of hydrogen deficiency of 11) along with 1D and 2D-NMR data (Tables 2 and 3, Supplementary data Figs. S36- S38) indicated 18 as an undescribed 3-benzyl-3-hydroxychroman-4-one homoisoflavonoid analogue. The compound showed high structural similarity to 17. For instance, both shared a similar ring A substitution pattern with two meta-coupled aromatic protons (δH 6.04, d, J = 2.2 Hz and δH 5.99, d, J = 2.2 Hz for H-6, and H-8, respectively for 18). Both compounds also showed an aliphatic geminal AB system of H2-2 (δH 4.05, d, J = 11.1 Hz and 4.21, d, J = 11.1 Hz for 18) and H2-9 (δH 2.88, d, J = 14.0 Hz and 2.93, d, J = 14.0 Hz for 18). The major differences between the NMR data of 17 vs 18 were in the ring B splitting patterns. Compound 17 had an aromatic A2B2 system, while 18 displayed an ABX system. Moreover, compound 18 had a characteristic methylenedioxy ring attached to C-3′ and C-4′ , which was confirmed based on an extra unit of unsaturation in 18 vs 17 and upon characteristic HMBC correlations from H2-10 (δH 5.94 and 5.95, δC 101.1, CH2) to C-3' (δC 148.1) and C-4' (δC 146.1) (Fig. 3). The trivial name 3-hydroxy-desmethylophiopogonanone A was ascribed to 18. Based on the positive Cotton effect at the ECD spectrum (Δε = +19.5) at 295 nm, the absolute configuration of 18 was determined as 3R (Supplementary Data Fig. S43).

The cytotoxicity of the isolated compounds (1–21) was tested in vitro against MDA-MB-435 (melanoma), MDA-MB-231 (breast), and OVCAR3 (ovary) cancer cell lines. Compounds 9, 11, 13, 14, 17, and 19 were active against at least one cancer cell line (Table 4). Compound 13 was the most potent with IC50 values of 0.62 μM, 5.36 μM, and 2.52 μM, when tested against those three cancer cell lines, respectively. Compound 13 was previously reported to be active against colon cancer (HT29 cell line, ED50 = 2.78 μM) and breast cancer (MDA-MB-435 cell line, ED50 = 1.33 μM) (Abegaz, 2002). The rest of the compounds were inactive (IC50 values > 25 μM).

Table 3

^{1}H NMR data for compounds **14, 18** [500 MHz, CDCl3], **15** and **16** [400 MHz, CDCl3].

No.	δH, mult (J in Hz)			
	14	15	16	18
2	4.20, dd (11.2, 6.9) 4.36, dd (11.2, 4.0)	4.18, dd (11.2, 7.5) 4.34, dd (11.2, 4.1)	4.22, dd (11.2, 6.6) 4.36, dd (11.2, 3.9)	4.05, d (11.1) 4.21, d (11.1)
3	2.78, m	2.78, m	2.77, m	-
6	6.16, s	6.12, s	6.17, s	6.04, d (2.2)
8	-	-	-	5.99, d (2.2)
9 2.88, d (14.0) 2.93, d (14.0)	2.60, dd (13.8, 10.9) 3.17, dd (13.8, 4.3)	2.57, dd (14.0, 10.8) 3.18, dd (14.0, 4.4)	2.63, dd (13.9, 10.9) 3.16, dd (13.9, 4.2)	2.88, d (14.0) 2.93, d (14.0)
10	3.86, s	3.87, s	3.88, s	5.94, d (1.5) 5.95, d (1.5)
11	3.90, s	3.94, s	3.91, s	
12	3.97, s	3.92, s	3.97, s	-
13	-	3.79, s	-	-
2′	6.73, d (2.1)	6.78, d (2.1)	6.86, d (2.6)	6.72, d (1.7)
5′	6.80, d (7.8)	6.81, d (8.3)	6.84, d (8.9)	6.75, d (7.8)
6′	6.79, dd (7.8, 2.1)	6.71, dd (8.3, 2.1)	6.73, dd (8.9, 2.6)	6.64, dd (7.8, 1.7)
5-OH	-	-	-	11.24
8-OH	5.08	-	-	-
3′-OH	5.59	5.58	-	-
4′-OH	-	-	5.53	-

3. Conclusions

This is the first study to explore the chemical composition and cytotoxic activities of the chemical constituents of the bulbs of B. longipes. A number of Bellevalia species have been chemically investigated throughout the world. It was found that homoisoflavonoids and triterpenoids were two of the most important chemical classes of compounds identified (Adinolfi et al., 1989, 1990; Alali et al., 2015; El-Elimat et al., 2018). A total of twenty-one compounds, mostly belonging to the homoisoflavonoid family of secondary metabolites (1–21), were identified in the present study. Of these, seven (7, 9–11, 14, 16, and 18) were never reported before, while one had been reported as a synthetic intermediate (15). When tested against three human cancer cell lines: MDA-MB-435 (melanoma), MDA-MB-231 (breast), and OVCAR3 (ovarian), compounds 9, 11, 13, 14, 17, and 19 showed cytotoxic activity with IC50 values in the range of 0.62–24.99 μM, with compound 13 as the most potent. The homoisoflavonoids isolated in this study, as well as those described in our two previous publications (Alali et al., 2006; Corsaro et al., 1992), have provided us with deep insights into the structure-activity relationship of this very promising and intriguing class of natural compounds. Briefly, there is a correlation between ring B substitution patterns and cytotoxicity. Reduced polarity of ring B substituents improves activity. Furthermore, adding an OH group at C-3 significantly increases activity.

Table 4
Cytotoxic activities of compounds 9, 11, 13, 14, 17, and 19 against three human tumor cell lines.

Compound[a]	IC_{50} (μM)[b]		
	MDA-MB-435	MDA-MB-231	OVCAR-3
13	0.62	5.36	2.52
14	9.81	>25	>25
9	19.39	>25	>25
17	19.69	>25	>25
19	21.32	>25	22.58
11	24.99	>25	>25
Taxol[c]	0.52×10^{-3}	5.26×10^{-3}	4.23×10^{-3}

a The remaining compounds were inactive (IC50 values > 25 μM).
b IC50 is the concentration inhibiting 50% of growth with a 72-h incubation.
c Positive control.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained using a Rudolph Research Autopol III polarimeter (Rudolph Research Analytical). UV data, and ECD spectra were collected via a Varian Cary 100 Bio UV–Vis spectrophotometer (Varian Inc.) and an Olis DSM 17 ECD spectrophotometer (Olis, Inc.), respectively. A JOEL EC5 500 NMR spectrometer, operating at 500 MHz for 1 H and 125 MHz for 13C, and a JOEL EC5 400 NMR spectrometer, operating at 400 MHz for 1 H and 100 MHz for 13C (both from JEOL Ltd.), were used to obtain the nuclear magnetic resonance data. The JOEL EC5 400 NMR spectrometer was equipped with a JEOL Royal probe and a 24- slot autosampler for automated data collection. Signals from residual solvents were used for referencing. The high-resolution mass spectrometry (HRMS) data were collected using a Thermo Q Exactive Plus Orbitrap mass spectrometer equipped with an electrospray ionization source (Thermo Fisher Scientific San Jose, CA, USA). Flash chromatography was carried out using a Teledyne CombiFlash Rf 200 and Silica Gold columns (both from Teledyne ISCO). Monitoring of the eluted peaks was performed by means of ultraviolet (UV) and evaporative light scattering detectors. HPLC was performed using a Varian Prostar system with ProStar 210 pumps and a Prostar 335 photodiode array detector (PDA). A Galaxie Chromatography Workstation (version 1.9.3.2, Varian Inc.) was used to collect and analyze chromatographic data. A set of columns was used for the purification of the compounds, including Gemini-NX C18 analytical (5 μm; 250 × 4.6 mm), preparative (5 μm; 250 × 21.2 mm), and semipreparative (5 μm; 250 × 10.0 mm) (all from Phenomenex, Torrance, CA, USA). Luna PFP C18 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 C18 analytical (5 μm; 250 × 4.6 mm), semipreparative (5 μm; 250 × 10.0 mm), and preparative (5 μm; 250 × 21.2 mm) columns.

4.2. Plant collection and identification

Around 50 bulbs of Bellevalia longipes Post (Asparagaceae) were collected at Al-Mugair in Irbid, Jordan (latitude 32.6141505, longitude 35.9272285, elevation 498 m), during the flowering stage between March and May of 2017. Dr. Mohammed Gharaibeh, a Plant Taxonomist at the Jordan University of Science and Technology (JUST), identified and supervised the collected plant materials, and a voucher specimen (PHS-123) was deposited in the herbarium of the Faculty of Pharmacy, JUST. The collected plant materials were cleaned of mud, sliced into small pieces, and then air dried in a shaded, well-ventilated area away from direct sunlight. The dried bulb pieces were ground using a laboratory mill to a fine powder and then stored at room temperature, protected from light, until used for extraction and analysis.

4.3. Extraction and isolation

The extraction and fractionation procedures were performed as described previously (2015) (Alali et al., 2015). Briefly, powdered B. longipes bulbs (530 g) were extracted in CHCl3 (~2.5 L) by means of a Soxhlet apparatus. The solvent was evaporated under reduced pressure to produce approximately 9.0 g of dried extract. About 5 g of this extract was then reconstituted in 400 mL CHCl3, 100 mL CH_3OH, and 500 mL of water. At room temperature, the mixture was stirred for approximately 30 min, transferred to a separatory funnel (2 L), shaken vigorously, and allowed to separate into two layers. The bottom layer was drawn off into a round-bottom flask and evaporated to dryness under reduced pressure using a rotatory evaporator. This dried organic layer was then reconstituted in 400 mL hexanes and 400 mL of 1:1 CH_3OH/CH_3CN and shaken vigorously in a separatory funnel. The CH_3OH/CH_3CN layer was drawn off and evaporated to dryness under vacuum. The dried CH_3OH/CH_3CN fraction (2.1 g) was dissolved in $CHCl_3$ and mixed with Celite 545. Normal-phase flash chromatography was performed using a gradient solvent system of $CHCl_3$ in hexanes (0%–100%) followed by CH_3OH in $CHCl_3$ (0%–100%) at a flow rate of 40 mL/min, and 40 column volumes over a total run time of 43.9 min to generate twelve fractions.

Fraction five (37.18 mg) was subjected to preparative HPLC over a Gemini column using a gradient solvent system of 30–80% of CH3CN: H2O (0.1% formic acid) over 40 min at a flow rate of 21.24 mL/min to yield 17 subfractions. From them, subfractions 2, 4, 7, 11, 12, and 13 were identified as compounds **20** (0.53 mg, tR = 6.5 min), **21** (1.54 mg, tR = 13.5 min), **15** (0.52 mg, tR = 16.3 min), **18** (0.74 mg, tR = 23.0 min), **17** (0.53 mg, tR = min 23.5), and **4** (1.92 mg, tR = 26.0 min), respectively.

About 166.46 mg of fraction six was subjected to preparative HPLC over a Gemini column using a gradient system of 30–80% of $CH_3CN:H_2O$ (0.1% formic acid) over 15 min at a flow rate of 21.24 mL/min to yield 5 subfractions. Subfraction 1 was identified as compound 2 (70.73 mg, tR = 14.5 min). Subfraction 2 (2.34 mg, tR = 15.2 min) was further purified using semipreparative HPLC equipped with an Atlantis T3 column, and a gradient system of 40–60% of $CH_3CN:H_2O$ (0.1% formic acid) over 20 min at a flow rate of 4.6 mL/min to yield 1.21 mg of compound 13 (tR = 17.1 min).

Fraction seven (140.53 mg) was subjected to preparative HPLC over a Gemini column using a gradient system of 30–80% of $CH_3CN:H_2O$ (0.1% formic acid) over 30 min at a flow rate of 21.24 mL/min to yield 9 subfractions. Subfractions 1, 5, and 7 were identified as compounds 16 (0.91 mg, tR = 8.3 min), 6 (0.94 mg, tR = 13.0 min), and 2 (40.94 mg, tR = 14.5 min), respectively.

Fraction eight (143.75 mg) was subjected to preparative HPLC over a Gemini column using a gradient system of 20–100% CH3CN:H2O (0.1% formic acid) over 55 min at a flow rate of 21.24 mL/min to yield 17 subfractions. Subfractions 1, 2, 4, and 5 were identified as compounds 16 (2.43 mg, tR = 13.1 min), 14 (2.68 mg, tR = 14.3 min), 6 (0.87 mg, tR = 21.6 min), and 3 (1.82 mg, tR = 22.8 min), respectively. Sub-fraction 11 (1.22 mg, tR = 28.3 min) was subjected to a semipreparative HPLC purification step over a PFP column using a gradient system of 60–80% CH3OH:H2O (0.1% formic acid) over 15 min at a flow rate of 4.62 mL/ min to yield compound 19 (0.55 mg, tR = 23.8 min).
Fraction nine (931.54 mg) was subjected to preparative HPLC over an Atlantis T3 column using an isocratic mobile phase of 30:70 CH3CN: H2O (0.1% formic acid) over 40 min at a flow rate of 17 mL/min to yield 9 subfractions, from which, subfractions 1 and 6 were identified as compounds 8 (62.33 mg, tR = 7.0 min) and 5 (243.74 mg, tR = 22.1 min), respectively. Subfraction 3 (4.61 mg, tR = 15.9 min) was subjected to semipreparative HPLC purification over a Gemini column using a gradient system of 15–30% of CH3CN:H2O (0.1% formic acid) over 30 min at a flow rate of 4.6 mL/min to yield 4 subfractions. Subfractions 2 and 3 from the semipreparative purification step were identified as compounds 1 (0.52 mg, tR = 15.5 min) and 9 (2.15 mg, tR = 23.5 min), respectively. Subfraction 4 (4.52 mg, tR = 16.5 min) from the preparative HPLC purification was subjected to semipreparative HPLC purification over a Gemini column using a gradient system of 15–30% of CH_3CN:H_2O (0.1% formic acid) over 30 min at a flow rate of 4.6 mL/min to yield compound 11 (1.73 mg, tR = 24.5 min). Subfraction 5 (3.05 mg, tR = 18.3 min) from the preparative HPLC purification was subjected to semipreparative HPLC over a Gemini column using a gradient system of 15–30% of CH_3CN:H_2O (0.1% formic acid) over 30 min at a flow rate of 4.6 mL/min to yield compound 7 (1.36 mg, tR = 25.2 min). Subfraction 7 (73.64 mg, tR = 27.5 min) from the preparative HPLC purification was subjected to a second cycle of preparative HPLC purification over an Atlantis T3 column using a gradient system of 20–30% of CH_3CN:H_2O (0.1% formic acid) over 44 min at a flow rate of 17 mL/min to yield additional 3 subfractions, of which subfraction 1 (5.92 mg, tR = 19.8 min) was purified over a semipreparative Atlantis T3 column using a gradient system of 30–40% of CH3CN:H2O (0.1% formic acid) over 15 min at a flow rate of 4.6 mL/min to yield 1.38 mg of compound 10 (tR = 17.5 min). Subfraction 3 (47.83, tR = 38.3 min) from the second cycle was further purified by semipreparative HPLC over an Atlantis T3 column using a gradient system of 40–70% of CH_3CN:H_2O (0.1% formic acid) over 30 min at a flow rate of 4.6 mL/min to yield 3 subfractions. From them, subfraction 3 (tR = 27.3 min) was further purified over preparative PFP column using an isocratic system of 22:78 of CH_3OH:H_2O (0.1% formic acid) for 76 min at a flow rate of 17 mL/min to yield 2 subfractions, which were identified as compound 10 (4.39 mg, tR = 64.1 min) and 12 (2.39 mg, tR = 69.5 min), for subfractions 1 and 2, respectively.
8-Dehydroxy-5-O-demethyl-6-hydroxyscillapersicone (7): Yellowish powder; $[\alpha]_D^{24}$ = − 20 (c = 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 350 (3.05), 290 (3.54), 237 (3.47) nm; ECD (c = 3.0 mM, CH_3OH) λ (Δε) 280 (+2.4) nm, 297 (− 35.3) nm, 320 (+1.8) nm; 1 H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) (see Tables 1 and 2); HRESIMS m/z 333.0962 [M+H]+ (calcd. for $C_{17}H_{17}O_7$, 333.0970).
6-Methoxyscillapersicone (9): Yellowish powder; $[\alpha]_D^{24}$ = − 18 (c = 0.1, CH_3OH)]; UV (CH_3OH) λmax (log ε) 350 (3.26), 282 (3.66), 233 (3.62) nm; ECD (c = 2.6 mM, CH_3OH) λ (Δε) 270 (+7.8) nm, 297 (− 7.6) nm, 330 (+1.6) nm; 1 H NMR (CD_3OD, 500 MHz) and ^{13}C NMR

(CD_3OD, 125 MHz) (see Tables 1 and 2); HRESIMS m/z 377.1223 [M+H]+ (calcd. for $C_{19}H_{21}O_8$, 377.1236).

5-O-Demethyl-6-methoxyscillapersicone (10): Yellowish powder; $[\alpha]_D^{24}$ = − 18 (c = 0.1, CH_3OH)]; UV (CH_3OH) λ_{max} (log ε) 375 (3.02), 288 (3.61), 230 (3.52) nm; ECD (c = 2.7 mM, CH_3OH) λ (Δε) 270 (+6.3), 300 (− 13.5), 350 (− 0.5) nm; 1 H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) (see Tables 1 and 2); HRESIMS m/z 363.1066 [M+H]+ (calcd. for $C_{18}H_{19}O_8$, 363.1074).

8-O-Methylscillapersicone (11): White amorphous powder; $[\alpha]_D^{24}$ = − 28 (c = 0.1, CH_3OH)]; UV (CH_3OH) λ_{max} (log ε) 326 (3.34), 284 (3.66), 236 (3.60) nm; ECD (c = 2.7 mM, CH_3OH) λ (Δε) 270 (+12.7), 295 (− 10.8), 350 (− 5.1) nm; 1 H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) (see Tables 1 and 2); HRESIMS m/z 361.1274 [M+H]+ (calcd. for $C_{19}H_{21}O_7$, 361.1282).

4′ -O-Methylscillapersicone (14): White amorphous powder; $[\alpha]_D^{28}$ = − 18 (c = 0.1, CH_3OH)]; UV (CH_3OH) λ_{max} (log ε) 349 (3.18), 287 (3.43), 243 (3.39) nm; ECD (c = 2.7 mM, CH_3OH) λ (Δε) 270 (+3.3), 293 (− 6.7), 350 (− 1.1) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Tables 2 and 3); HRESIMS m/z 361.1273 [M+H]+ (calcd. for $C_{19}H_{21}O_7$ 361.1282).

4′ ,8-O,O-Dimethylscillapersicone (15): White amorphous powder; $[\alpha]_D^{24}$ = − 10 (c = 0.05, CH_3OH)]; UV (CH_3OH) λ_{max} (log ε) 284 (3.07), 233 (2.99) nm; ECD (c = 2.6 mM, CH_3OH) λ (Δε) 270 (+1.3), 294 (− 3.2), 350 (− 0.8) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) (see Tables 2 and 3); HRESIMS m/z 375.1433 [M+H]+ (calcd. for $C_{20}H_{23}O_7$, 375.1438).

3′ -O-Methylscillapersicone (16): White amorphous powder; $[\alpha]_D^{24}$ = − 10 (c = 0.09, CH_3OH)]; UV (CH_3OH) λ_{max} (log ε) 350 (2.51), 287 (2.95), 238 (2.86), 216 (2.85) nm; ECD (c = 2.7 mM, CH_3OH) λ (Δε) 270 (+1.1), 295 (− 1.7), 350 (− 0.1) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$,100 MHz) (see Tables 2 and 3); HRESIMS m/z 361.1274 [M + H]+ (calcd. for $C_{19}H_{21}O_7$, 361.1281).

3-Hydroxy-desmethylophiopogonanone A (18): White amorphous powder; $[\alpha]_D^{24}$ = + 42 (c = 0.07, CH_3OH)]; UV (CH_3OH) λ_{max} (log ε) 292 (3.22), 224 (3.13) nm; ECD (c = 3.0 mM, CH_3OH) λ (Δε) 270–4.1), 295 (+19.5), 350 (+3.8) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Tables 2 and 3); HRESIMS m/z 331.0807 [M + H]+ (calcd. for $C_{17}H_{15}O_7$, 331.0818).

4.4. Cytotoxic activity

Compounds (1–21) were evaluated for their cytotoxic activities against three human cancer cell lines: melanoma (MDA-MB-435) (Rae et al., 2007), breast (MDA-MB-231), and ovarian (OVCAR3) as described previously (El-Elimat et al., 2014, 2015). Briefly, the human cancer cell lines were purchased from the American Type Culture Collection (Manassas, VA). The cell lines were propagated at 37 ◦C in 5% CO_2 in RPMI 1640 medium, supplemented with fetal bovine serum (10%), penicillin (100 units/mL), and streptomycin (100 μg/mL). Cells in the log phase of growth were harvested by trypsinization and then washed twice to remove enzyme traces. In a 96-well clear, flat-bottomed plate (Microtest 96, Falcon), 5000 cells were seeded in each well, and the plates were incubated overnight at 37 ◦C in 5% CO_2.

DMSO-dissolved samples were then diluted and added to the appropriate wells. After 72 h of incubation at 37 ◦C, the cells were evaluated for viability using a commercial absorbance assay

(CellTiterBlue Cell Viability Assay, Promega Corp, Madison, WI). IC_{50} values were calculated relative to the solvent (DMSO) as a control. The positive control used was taxol (paclitaxel).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This research was supported, in part, by the Deanship of Research, Jordan University of Science and Technology, Irbid, Jordan (Grant No. 91/2019) and via the National Cancer Institute/National Institutes of Health, Bethesda, MD, USA (Grant No. P01 CA125066).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2022.113343.

References

Abd-Al Hadi, F. (Ed.), 2020. The National Biodiversity Strategy and Action Plan. Ministry of Environment, Amman, Jordan. https://www.cbd.int/nbsap/.

Abegaz, B.M., 2002. Novel phenylanthraquinones, isofuranonaphthoquinones, homoisoflavonoids, and biflavonoids from African plants in the genera Bulbine, Scilla, Ledebouria, and Rhus. Phytochemistry Rev. 1 (3), 299–310. https://doi.org/10.1023/A:1026066626537.

Adinolfi, M., Aquila, T., Barone, G., Lanzetta, R., Parrilli, M., 1989. Homoisoflavanones from Bellevalia romana. Phytochemistry 28 (11), 3244–3246. http://www.sciencedirect.com/science/article/pii/0031942289803206.

Adinolfi, M., Barone, G., Belardini, M., Lanzetta, R., Laonigro, G., Parrilli, M., 1984. 3- Benzyl-4-chromanones from Muscari comosum. Phytochemistry 23 (9), 2091–2093. http://www.sciencedirect.com/science/article/pii/S0031942200849894.

Adinolfi, M., Barone, G., Belardini, M., Lanzetta, R., Laonigro, G., Parrilli, M., 1985a. Homoisoflavanones from Muscari comosum bulbs. Phytochemistry 24 (10), 2423–2426. http://www.sciencedirect.com/science/article/pii/S00319422 00830551.

Adinolfi, M., Barone, G., Corsaro, M.M., Mangoni, L., Lanzetta, R., Parrilli, M., 1988. Absolute configuration of homoisoflavanones from muscari species. Tetrahedron 44 (15), 4981–4988. http://www.sciencedirect.com/science/article/pii/S0040402001862025.

Adinolfi, M., Barone, G., Greca, M., Mangoni, L., Lanzetta, R., Parrilli, M., 1990. Glycosides from Bellevalia romana. Isolation and structure of bellevaliosides A-D. Gazz. Chim. Ital. 120 (7), 427–433. https://www.iris.unina.it/handle/11588/140680?mode=full.1#record.

Adinolfi, M., Barone, G., Lanzetta, R., Laonigro, G., Mangoni, L., Parrilli, M., 1985b. Three 3-benzyl-4-chromanones from Muscari comosum. Phytochemistry 24 (3), 624–626. http://www.sciencedirect.com/science/article/pii/S0031942200807906.

Adinolfi, M., Corsaro, M.M., Lanzetta, R., Laonigro, G., Mangoni, L., Parrilli, M., 1986. Ten homoisoflavanones from two Muscari species. Phytochemistry 26 (1), 285–290. http://www.sciencedirect.com/science/article/pii/S0031942200815290.

Afifi-Yazar, F.U., Kasabri, V., Abu-Dahab, R., 2011. Medicinal plants from Jordan in the treatment of cancer: traditional uses vs. in vitro and in vivo evaluations, part 1. Planta Med. 77 (11), 1203–1209. https://doi.org/10.1055/s-0030-1270832.

Al-Eisawi, D., 1996. Vegetation of Jordan. UNESCO, Regional Office for Science and Technology for the Arab States. https://books.google.jo/books?id=qoPQ0wIMtM0C.

Al-Eisawi, D.M., 1998. Field Guide to Wild Flowers of Jordan and Neighbouring Countries. Jordan Press Foundation, Amman, Jordan. https://books.google.jo/books?id=PvnaAAAAMAAJ.

Alali, F., El-Elimat, T., Albataineh, H., Al-Balas, Q., Al-Gharaibeh, M., Falkinham, J.O., Chen, W.-L., Swanson, S.M., Oberlies, N.H., 2015. Cytotoxic homoisoflavones from the bulbs of Bellevalia eigii. J. Nat. Prod. 78 (7), 1708–1715. https://doi.org/10.1021/acs.jnatprod.5b00357.

Alali, F.Q., Amrine, C.S.M., El-Elimat, T., Alkofahi, A., Tawaha, K., Gharaibah, M., Swanson, S.M., Falkinham, J.O., Cabeza, M., Sanchez, ´ A., Figueroa, M., Oberlies, N. H., 2014. Bioactive withanolides from Withania obtusifolia. Phytochem. Lett. 9, 96–101. https://www.sciencedirect.com/science/article/pii/S1874390014000858.

Alali, F.Q., El-Elimat, T., Li, C., Qandil, A., Alkofahi, A., Tawaha, K., Burgess, J.P., Nakanishi, Y., Kroll, D.J., Navarro, H.A., Falkinham, J.O., Wani, M.C., Oberlies, N. H., 2005. New colchicinoids from a native Jordanian meadow saffron, Colchicum brachyphyllum: isolation of the first naturally occurring dextrorotatory colchicinoid. J. Nat. Prod. 68 (2), 173–178. https://doi.org/10.1021/np0496587.

Alali, F.Q., Tawaha, K., El-Elimat, T., Qasaymeh, R., Li, C., Burgess, J., Nakanishi, Y., Kroll, D.J., Wani, M.C., Oberlies, N.H., 2006. Phytochemical studies and cytotoxicity evaluations of Colchicum tunicatum Feinbr and Colchicum hierosolymitanum Feinbr (Colchicaceae): two native Jordanian meadow saffrons. Nat. Prod. Res. 20 (6), 558–566. https://doi.org/10.1080/14786410500183381.

Bohler, ¨ P., Tamm, C., 1967. The homoisoflavones, a new class of natural product. Isolation and structure of eucomin and eucomol. Tetrahedron Lett. 8 (36), 3479–3483. http://www.sciencedirect.com/science/article/pii/S0040403901898269.

Castelli, M.V., Lopez, ´ S.N., 2017. Chapter 9 - homoisoflavonoids: occurrence, biosynthesis, and biological activity. In: Atta ur, R. (Ed.), Stud. Nat. Prod. Chem., vol. 54 Elsevier, pp. 315–354. http://www.sciencedirect.com/science/article/pii/B9780444639295000097.

Chen, P.Y., Kuo, Y.C., Chen, C.H., Kuo, Y.H., Lee, C.K., 2009. Isolation and immunomodulatory effect of homoisoflavones and flavones from Agave sisalana. Perrine Mol. 14 (5), 1789–1795. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6254440/.

Corsaro, M.M., Lanzetta, R., Mancino, A., Parrilli, M., 1992. Homoisoflavanones from Chionodoxa luciliae. Phytochemistry 31 (4), 1395–1397. http://www.sciencedirect.com/science/article/pii/003194229280299T.

Dewick, P.M., 1975. Biosynthesis of the 3-benzylchroman-4-one eucomin in Eucomis bicolor. Phytochemistry 14 (4), 983–988. https://www.sciencedirect.com/science/article/pii/0031942275851727.

El-Elimat, T., Alhawarri, M.B., Rivera-Ch´ avez, J., Burdette, J.E., Czarnecki, A., AlGharaibeh, M., Al Sharie, A.H., Alhusban, A., Alali, F.Q., Oberlies, N.H., 2020. Phenethyisoquinoline alkaloids from the leaves of Androcymbium palaestinum. Fitoterapia 146, 104706. https://www.sciencedirect.com/science/article/pii/S0367326X20302884.

El-Elimat, T., Raja, H.A., Day, C.S., Chen, W.-L., Swanson, S.M., Oberlies, N.H., 2014. Greensporones: resorcylic acid lactones from an aquatic Halenospora sp. J. Nat. Prod. 77 (9), 2088–2098. https://doi.org/10.1021/np500497r.

El-Elimat, T., Raja, H.A., Figueroa, M., Swanson, S.M., Falkinham Iii, J.O., Lucas, D.M., Grever, M.R., Wani, M.C., Pearce, C.J., Oberlies, N.H., 2015. Sorbicillinoid analogs with cytotoxic and selective anti-Aspergillus activities from Scytalidium album. J. Antibiot. (Tokyo) 68 (3), 191–196. https://doi.org/10.1038/ja.2014.125.

El-Elimat, T., Rivera-Chavez, J., Burdette, J.E., Czarnecki, A., Alhawarri, M.B., AlGharaibeh, M., Alali, F., Oberlies, N.H., 2018. Cytotoxic homoisoflavonoids from the bulbs of Bellevalia flexuosa. Fitoterapia 127, 201–206. https://www.sciencedirect.com/science/article/pii/S0367326X17318889.

Feinbrun-Dothan, N., 1986. Flora Palaestina: Alismataceae to Orchidaceae. Israel Academy of Sciences and Humanities. https://books.google.jo/books?id=yjAtAQAAIAAJ.

Hafez Ghoran, S., Saeidnia, S., Babaei, E., Kiuchi, F., Dusek, M., Eigner, V., Dehno Khalaji, A., Soltani, A., Ebrahimi, P., Mighani, H., 2014. Biochemical and biophysical properties of a novel homoisoflavonoid extracted from Scilla persica Hausskn. Bioorg. Chem. 57, 51–56. http://www.sciencedirect.com/science/article/pii/S0045206814000662.

He, W., Fang, T., Zhang, K., Tu, P., 2009. Vasorelaxation effects of homoisoflavonoids from Caesalpinia sappan in rat thoracic aortic rings. China J. Chin. Mater. Med. 34 (6), 731–734.

Kwon, S., Lee, S., Heo, M., Lee, B., Fei, X., Corson, T.W., Seo, S.-Y., 2020. Total synthesis of naturally occurring 5,7,8-trioxygenated homoisoflavonoids. ACS Omega 5 (19), 11043–11057. https://doi.org/10.1021/acsomega.0c00932.

Li, N., Zhang, J.-Y., Zeng, K.-W., Zhang, L., Che, Y.-Y., Tu, P.-F., 2012. Anti-inflammatory homoisoflavonoids from the tuberous roots of Ophiopogon japonicus. Fitoterapia 83 (6), 1042–1045. http://www.sciencedirect.com/science/article/pii/S0367326X12001608.

Lin, L.-G., Xie, H., Li, H.-L., Tong, L.-J., Tang, C.-P., Ke, C.-Q., Liu, Q.-F., Lin, L.-P., Geng, M.-Y., Jiang, H., Zhao, W.-M., Ding, J., Ye, Y., 2008. Naturally occurring homoisoflavonoids function as potent protein tyrosine kinase inhibitors by c-Srcbased high-throughput screening. J. Med. Chem. 51 (15), 4419–4429. https://doi.org/10.1021/jm701501x.

Lin, L.G., Liu, Q.Y., Ye, Y., 2014. Naturally occurring homoisoflavonoids and their pharmacological activities. Planta Med. 80 (13), 1053–1066. https://doi.org/ 10.1055/s-0034-1383026.

Masi, M., Koirala, M., Delicato, A., Di Lecce, R., Merindol, N., Ka, S., Seck, M., Tuzi, A., Desgagne-Penix, I., Calabro, ` V., Evidente, A., 2021. Isolation and biological characterization of homoisoflavanoids and the alkylamide N-p-coumaroyltyramine from

Crinum biflorum Rottb., an Amaryllidaceae species collected in Senegal. Biomolecules 11 (9), 1298. https://doi.org/10.3390/biom11091298.
Meksuriyen, D., Cordell, G.A., 1988. Traditional medicinal plants of Thailand xiii: flavonoid derivatives from Dracaena loureiri (agavaceae). J. Sci. Soc. Thail. 14 (1), 3–24. https://doi.org/10.2306/scienceasia1513-1874.1988.14.003.
Miadokov´ a, E., Ma˘sterova, ´ I., Vl˘ckova, ´ V., Dúhova, ´ V., Toth, ´ J., 2002. Antimutagenic potential of homoisoflavonoids from Muscari racemosum. J. Ethnopharmacol. 81 (3), 381–386. http://www.sciencedirect.com/science/article/pii/S0378874102001356.
Ministry of Environment, J., 2014. 2014: the Fifth National Report on the Implementation of the Convention on Biological Diversity Jordanian Ministry of Environment (MoEnv), in Cooperation with the Global Environmental Facility andThe World Bank Clearing House Mechanism of Biodiversity in Jordan under the Convention on Biological Diversity. http://jo.chm-cbd.net/implementation/nationa l-reports/fifth-national-biodiversity-report.
Mottaghipisheh, J., Stuppner, H., 2021. A comprehensive review on chemotaxonomic and phytochemical aspects of homoisoflavonoids, as rare flavonoid derivatives. Int. J. Mol. Sci. 22 (5), 2735. https://doi.org/10.3390/ijms22052735.
Ono, M., Masuoka, C., Odake, Y., Ikegashira, S., Ito, Y., Nohara, T., 2000. Antioxidative constituents from Tessaria integrifolia. Food Sci. Technol. Res. 6 (2), 106–114. https://doi.org/10.3136/fstr.6.106.
Pohl, T., Koorbanally, C., Crouch, N.R., Mulholland, D.A., 2001. Secondary metabolites of Scilla plumbea, Ledebouria cooperi and Ledebouria ovatifolia (hyacinthaceae). Biochem. Systemat. Ecol. 29 (8), 857–860. https://doi.org/10.1016/S0305-1978(01)00027-8.
Rae, J.M., Creighton, C.J., Meck, J.M., Haddad, B.R., Johnson, M.D., 2007. MDA-MB-435 cells are derived from M14 Melanoma cells—a loss for breast cancer, but a boon for melanoma research. Breast Cancer Res. Treat. 104 (1), 13–19. https://doi.org/10.1007/s10549-006-9392-8.
Sharma, S., Patial, V., Singh, D., Sharma, U., Kumar, D., 2018. Antimicrobial homoisoflavonoids from the rhizomes of Polygonatum verticillatum. Chem. Biodivers. 15 (12), 43–62. https://doi.org/10.1002/cbdv.201800430.
Siddaiah, V., Maheswara, M., Venkata Rao, C., Venkateswarlu, S., Subbaraju, G.V., 2007. Synthesis, structural revision, and antioxidant activities of antimutagenic homoisoflavonoids from Hoffmanosseggia intricata. Bioorg. Med. Chem. Lett. 17 (5), 1288–1290. http://www.sciencedirect.com/science/article/pii/S0960894X06013904.
Sidwell, W.T.L., Tamm, C., 1970. The homoisoflavones II. Isolation and structure of 4′ - Omethyl-punctatin, autumnalin and 3,9-dihydro-autumnalin. Tetrahedron Lett. 11 (7), 475–478. http://www.sciencedirect.com/science/article/pii/0040403970890037.
Zhou, X., Yuping, Z., Zhao, H., Liang, J., Zhang, Y., Shi, S., 2015. Antioxidant homoisoflavonoids from Polygonatum odoratum. Food Chem. 186, 63–68. http://www.sciencedirect.com/science/article/pii/S0308814615002381.