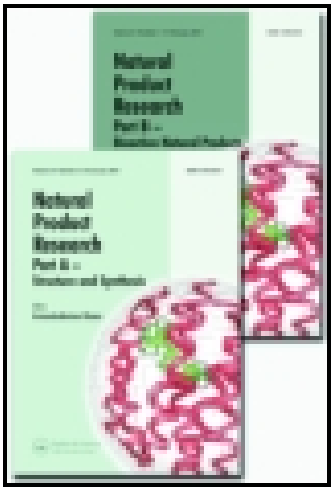

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Urgineaglyceride A: a new monoacylglycerol from the Egyptian Drimia maritima bulbs

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Urgineaglyceride A: a new monoacylglycerol from the Egyptian *Drimia maritima* bulbs

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One new compound, (2*S*)-1-*O*-(*Z*)-tetracos-6-enoate glycerol (**1**) named urgineaglyceride A, along with six known compounds, 3,5,7,3′,5′-pentahydroxydihydroflavonol (**2**), stigmasterol (**3**), (25*S*)-5β-furostane-3β-22α-26-triol (**4**), scillaridin A (**5**), (2*S*)-(+)-2-hydroxynaringenin-4′-*O*-β-D-glucopyranoside (**6**) and quercetin-3′-*O*-β-D-glucopyranoside (**7**), were isolated from the EtOAc fraction of *Drimia maritima* (L.) Stearn bulbs. Their structures were secured based on their IR, UV, 1D and 2D NMR data, in addition to HR-MS data and comparison with the literature data. The isolated compounds were evaluated for their *in vitro* growth inhibitory activity against A549 non-small cell lung cancer (NSCLC), U373 glioblastoma (GBM) and PC-3 prostate cancer cell lines. Compounds **2** and **3** displayed variable activities against the tested cancer cell lines. Compound **2** was a selective inhibitor of the NSCLC cell line with an IC_{50} of 2.3 μM, whereas **3** was selective against GBM with IC_{50} of 0.5 μM and against PC-3 with 2.0 μM.

Keywords: *Drimia maritima*; monoacylglycerol; urgineaglyceride A; *in vitro* growth inhibitory activity

1. Introduction

Drimia maritima (L.) Stearn (syn. *Urginea maritima*) (Asparagaceae) is a native plant found in the Mediterranean area, North Africa and India. It is known in Arabic as Basal Farion, Onsul, Basal el-far, Samm el-far and Ishkil (Boulos 2002). It is used as a cardiotonic diuretic for the treatment of cardiac marasmus and oedema (Iizuka et al. 2001; Metin & Bürün 2010; Bozcuk et al. 2011). It is also used in bronchitis, bronchial asthma, whooping cough and cancer (Duke & Ayensu 1985; Bown 1995). Externally, the bulb is applied for skin problems such as injury, haemorrhoids, warts, dandruff and seborrhoea (Metin & Bürün 2010). It exhibits insecticidal and cytotoxic effects (Iizuka et al. 2001; Boulos 2002; Metin & Bürün 2010; Bozcuk et al. 2011; El-Seedia et al. 2013). Previous phytochemical studies of *D. maritima* bulbs resulted in the isolation of cardiac glycosides (Patel et al. 1986; Kopp et al. 1996; Krenn et al. 2000; Iizuka et al. 2001; Metin & Bürün 2010; El-Seedia et al. 2013), anthocyanins, lignans, flavonoids, fatty acids

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and polysaccharides (Fernandez et al. 1972; Vega et al. 1972; Fernandez et al. 1975; Iizuka et al. 2001). Bioassay-directed fractionation of the EtOAc fraction of *D. maritima* bulbs using different cancer cell lines (A549 non-small cell lung cancer (NSCLC), U373 glioblastoma (GBM) and PC-3 prostate cancer) led to the isolation of one new compound, (2*S*)-1-*O*-(*Z*)-tetracos-6-enoate glycerol (**1**) named urgineaglyceride A, together with six known compounds, 3,5,7,3′,5′-pentahydroxydihydroflavonol (**2**) (Wang et al. 2008), stigmasterol (**3**) (Mohamed & Ibrahim 2007), (25*S*)-5β-furostane-3β-22α-26-triol (**4**) (Yang et al. 2002; Jin et al. 2004), scillaridin A (**5**) (Koorbanally et al. 2004), (2*S*)-(+)-2-hydroxynaringenin-4′-*O*-β-D-glucopyranoside (**6**) (Kim et al. 2000; Ti et al. 2011) and quercetin-3′-*O*-β-D-glucopyranoside (**7**) (Malikov & Yuldashev 2002) (Figure 1). Compounds **4** and **7** are reported here for the first time from *D. maritima* and **2** and **6** for the first time from this family. This study reports the isolation and structure characterisation of the isolated compounds using spectroscopic analyses, especially 1D and 2D NMR studies and HRMS. In addition, the *in vitro* growth inhibitory activity of the isolated compounds was also evaluated.

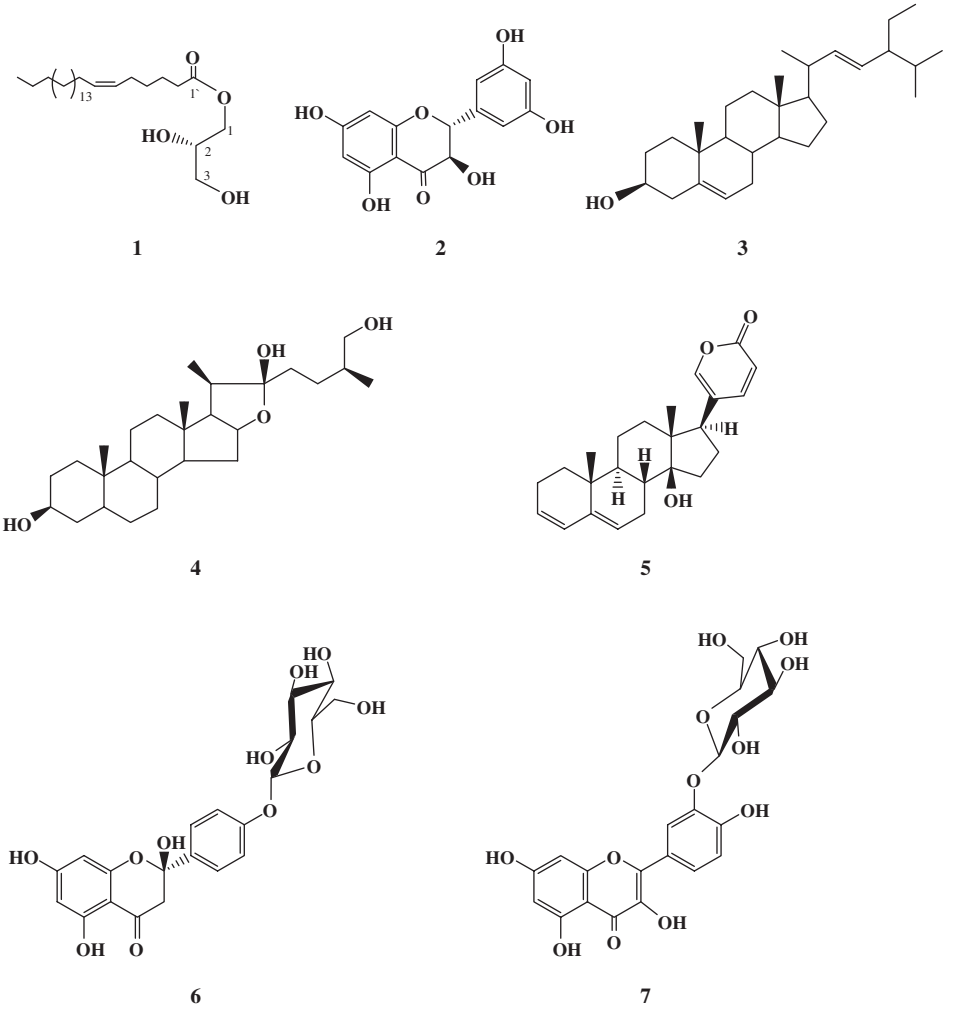

Figure 1. Structures of the isolated compounds **1**–**7**.

2. Results and discussion

Compound **1** was obtained as colourless oil. The HR-ESI-MS spectrum showed a molecular ion peak at *m/z* 441.3852 $[M + H]^+$, which is consistent with a formula of $C_{27}H_{52}O_4$. This requires two double bond equivalents. The IR spectrum revealed absorption bands at 3435 (OH) and 1742 (C═O) cm^{-1}. The ^1H and ^{13}C NMR spectra for **1** revealed the presence of a fatty acid moiety as reflected by the signals at δ_C 174.3 (s, C1′), δ_H 2.35 (2H, t, $J = 7.6$ Hz, H-2′)/δ_C 34.1 (t, C-2′), δ_H 1.30–1.25 (m, $(CH_2)_n$)/δ_C 29.7–29.1 (t, $(CH_2)_n$), δ_H 0.88 (3H, t, $J = 7.2$ Hz, H-24)/δ_C 14.1 (q, C-24′) and confirmed by the observed $^1H-^1H$ COSY and HMBC correlations (Supplementary Figure S7). The proton signals at δ_H 5.34 (m, H-6′) and 5.33 (m, H-7′) correlated with the carbon signals at δ_C 129.7 (C-6′) and 130.0 (C-7′) in the HSQC spectrum, respectively, indicating the presence of an olefinic double bond in **1**. The position of the double bond was established by the HMBC correlations from H-5′ to C-6′ and C-7′ and from H-6′ and H-7′ to C-5′ and confirmed by the fragment ion peaks at *m/z* 350 $[M\text{-}C_3H_7O_3]^+$ and 265 $[M\text{-}C_8H_{15}O_4]^+$ in the ESI-MS spectrum (Supplementary Figure S8). The geometry of the double bond between C-6′ and C-7′ was deduced to be *Z* from the $^1H-^1H$ coupling constant of 7.6 Hz between H-6′ and H-7′ (Bankeu et al. 2010). The signals at δ_C 65.2 (C-1)/δ_H 4.19 (1H, dd, $J = 11.6$ and 4.8 Hz, H-1a) and 4.15 (1H, dd, $J = 11.6$ and 4.8 Hz, H-1b), δ_C 63.3/δ_H 3.69 (1H, dd, $J = 12.0$ and 6.4 Hz, H-3a) and 3.60 (1H, dd, $J = 12.0$ and 6.4 Hz, H-3b) and δ_C 70.3/δ_H 3.93 (1H, m, H-2) indicated the presence of an asymmetrical substituted glycerol moiety (Vlahov 1999). This was supported by the fragment ion peaks at *m/z* 350 $[M\text{-glycerol}]^+$, 423 $[M\text{-}H_2O]^+$ and 406 $[M\text{-}2H_2O]$ in the ESI-MS spectrum, also was confirmed by the HMBC correlations of H-1 and H-3 with C-2 and H-2 with C-1 and C-3. The attachment of the fatty acid moiety at C-1 of glycerol was confirmed by the HMBC correlation of H-1 with C-1′. According to the ESI-MS spectrum, the fatty acid moiety should be (*Z*)-tetracos-6-enoic acid. This was established by alkaline hydrolysis of **1** using NaOMe–MeOH (Kim et al. 2005; Al-Musayeib et al. 2013). The resulting fatty acid methyl ester (FAME) was extracted with *n*-hexane and subjected to GC–MS analysis, which afforded molecular ion peak at *m/z* 380 $[M]^+$ and was identified as (*Z*)-tetracos-6-enoic acid methyl ester ($C_{25}H_{48}O_2$). The stereochemistry at C-2 was assigned as 2*S* based on REOESY experiment as well as comparison of ^1H and ^{13}C NMR chemical shifts and optical rotation with the reported data for glyceride analogue (Dharma et al. 1985; Ibrahim et al. 2010; Ibrahim 2014) (Supplementary Figures S1–S6). Thus, the structure of **1** was elucidated as (2*S*)-1-*O*-(*Z*)-tetracos-6-enoate glycerol and a trivial name urgineaglyceride A was given.

The known compounds were identified by analysing the spectroscopic data (1D, 2D NMR and MS) and comparing their data with those in the literature as 3,5,7,3′,5′-pentahydroxydihydroflavonol (**2**) (Wang et al. 2008), stigmasterol (**3**) (Mohamed & Ibrahim 2007), (25*S*)- 5β-furostane-3β-22α-26-triol (**4**) (Yang et al. 2002; Jin et al. 2004), scillaridin A (**5**) (Koorbanally et al. 2004), (2*S*)-(+)-2-hydroxynaringenin-4′-*O*-β-D-glucopyranoside (**6**) (Kim et al. 2000; Ti et al. 2011) and quercetin-3′-*O*-β-D-glucopyranoside (**7**) (Malikov & Yuldashev 2002). Compounds **4** and **7** are reported here for the first time from the plant, and **2** and **6** for the first time from the family.

The determination of the *in vitro* growth inhibitory activity using the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay for *n*-BuOH, EtOAc and EtOAc subfractions, as well as the isolated pure compounds, revealed that the subfractions UM-2 to UM-7 showed significant activity against A549 NSCLC, PC-3 prostate cancer and U373 GBM cell lines, with IC_{50} values from 0.02 to 3.7 μg/mL (Table 1). Concerning the pure compounds, **2** and **3** displayed *in vitro* growth inhibitory activity in the three cancer cell lines under study, with magnitude of effects that ranged between those of cisplatin (IC_{50} from 0.4 to 4.0 μM, Table 1). Compound **2** has selectivity against A549 NSCLC

Table 1. *In vitro* growth inhibitory activity of the tested fractions and compounds **1**–**7**.

Sample	IC_{50} (μM)			
	A549 NSCLC	U373 GBM	PC-3 prostate cancer	Mean ± SEM
EtOAc[a]	0.009	0.04	0.06	0.04 ± 0.01
n-BuOH[a]	0.03	0.1	0.2	0.11 ± 0.05
UM-1[a]	>100	>100	>100	>100
UM-2[a]	0.006	0.03	0.2	0.08 ± 0.06
UM-3[a]	0.005	0.03	0.01	0.02 ± 0.01
UM-4[a]	0.006	0.04	0.08	0.04 ± 0.02
UM-5[a]	0.01	0.3	0.2	0.17 ± 0.08
UM-6[a]	0.2	4	1	1.7 ± 1.2
UM-7[a]	1	6	4	3.7 ± 1.4
UM-8[a]	3	30	11	15 ± 8
UM-9[a]	2	22	11	12 ± 6
1	41	>100	>100	>100
2	2.3	>100	>100	>100
3	10	0.5	2	4.4 ± 0.6
4	38.6	>100	>100	88.6
5	>100	>100	>100	>100
6	NT	NT	NT	–
7	>100	53.8	>100	>100
Cisplatin[b]	4	0.4	NT	–

Note: NT, not tested.
[a] The growth inhibitory effects of the tested fractions are expressed as μg/mL.
[b] The cisplatin-related data have already been published (Ibrahim et al. 2012; Ibrahim et al. 2014).

at a concentration 2.3 μM. However, **1** and **4** displayed moderate activity against A549 NSCLC model (Table 1), while **7** displayed moderate *in vitro* growth inhibitory activity against U373 GBM cell line. In addition, **5** had no activity towards the different cell lines.

3. Experimental

3.1. *General experimental procedures*

Melting points were obtained in an Electrothermal 9100 Digital Melting Point (Electrothermal Engineering, Southend-on-Sea, Essex, UK). The UV spectra were carried out in MeOH (Merck, Darmstadt, Germany) using a Perkin-Elmer Lambda 25 UV/VIS spectrophotometer (Perkin-Elmer, Waltham, MA, USA). IR was measured with a Shimadzu Infrared-400 spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotation was recorded on a Perkin-Elmer Model 341 LC Polarimeter (Perkin-Elmer). HR-ESI-MS was determined with a Micromass Qtof 2 mass spectrometer (Micromass, Manchester, UK). EI-MS spectra were recorded on JEOL JMS-SX/SX 102A mass spectrometer (ThermoFinnigan, Bremen, Germany). ^{1}H and ^{13}C NMR spectra were measured on a Bruker DRX 400 spectrometer (Bruker, Rheinstetten, Germany). 1D NMR spectroscopic data were measured at a temperature of 24.2°C and 2D NMR (^{1}H–^{1}H COSY, ROESY, HSQC and HMBC) spectroscopic data were measured at a temperature of 27°C. A GC–MS was performed on Clarus 500 GC–MS (Perkin-Elmer, Shelton, CT, USA). The software controller/integrator was Turbo Mass, version 4.5.0.007 (Perkin-Elmer). An Elite 5MS GC capillary column (30 mm × 0.25 mm × 0.5 μM, Perkin-Elmer) was used. The carrier gas was helium (purity 99.9999%) at a flow rate of 2 mL/min (32 psi, flow initial 55.8 cm/s, split; 1:40). Temperature conditions were as follows: inlet line temperature, 200°C; source temperature, 150°C; trap emission, 100°C and

electron energy, 70 eV. The column temperature program was as follows: 50 °C for 5 min, increased to 220°C (rate, 20°C/min) and held for 5 min. The injector temperature was 220°C. MS scan was from 50 to 650 *m/z*. Vacuum liquid chromatography (VLC) was performed using silica gel 60 (0.04–0.063 mm; 500 g; Merck). Column chromatographic separations were performed on silica gel 60 (0.04–0.063 mm; Merck) and Sephadex LH-20 (0.25–0.1 mm, Merck). TLC analyses were conducted on pre-coated silica gel F_{254} aluminium sheets (Merck). Compounds were detected by spraying the sheets with an anisaldehyde/H_2SO_4 reagent followed by heating at 110°C for 1–2 min. The solvent systems used for TLC analyses were $CHCl_3$–MeOH (95:5, Figure S1), $CHCl_3$–MeOH (90:10; Figure S2) and *n*-BuOH–acetone–formic acid–H_2O (60:17:8:15, Figure S3).

3.2. *Plant materials*

The fresh bulbs of *D. maritima* (red squill) were collected in July 2009 from the desert of Matrouh, Egypt. The plant material was kindly identified by Prof. Dr A. Fayed (Professor of Plant Taxonomy, Faculty of Science, Assiut University, Egypt). A voucher specimen has been deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt (Registration code DY-UM-2009).

3.3. *Extraction and isolation*

The fresh bulbs were cleaned from sandy soil, cut into small slices (3 kg) and extracted with MeOH (5 L × 4) at r.t. The combined extracts were concentrated under reduced pressure to afford a dark reddish residue (140.0 g). Eighty grams of the latter was suspended in distilled water (400 mL) and then successively partitioned between EtOAc (800 mL × 4) and *n*-BuOH (800 mL × 3). Each fraction was concentrated under reduced pressure to give EtOAc (16.5 g) and *n*-BuOH (48.0 g). The EtOAc fraction was subjected to silica gel VLC using *n*-hexane–EtOAc gradients, nine subfractions were collected as follows: UM-1 (0.9 g, *n*-hexane–EtOAc; 90:10), UM-2 (1.3 g, *n*-hexane–EtOAc; 80:20), UM-3 (1.1 g, *n*-hexane–EtOAc; 70:30), UM-4 (1.9 g, *n*-hexane–EtOAc; 60:40), UM-5 (0.95 g, *n*-hexane–EtOAc; 50:50), UM-6 (1.8 g, *n*-hexane–EtOAc; 40:60), UM-7 (2.1 g, *n*-hexane–EtOAc; 30:70), UM-8 (1.9 g, *n*-hexane–EtOAc; 10:90) and UM-9 (1.9 g, EtOAc; 100%) (1 L for each fraction was collected and evaporated to afford corresponding weight). The *in vitro* growth inhibitory activity of EtOAc subfractions (Table 1) were subjected to different chromatographic techniques to isolate compounds **1**–**7**. Fraction UM-2 (1.3 g) was subjected to silica gel column chromatography (80 g × 50 cm × 2 cm) using *n*-hexane–EtOAc (98:2 to 90:10) to afford compounds **3** (70 mg, colourless needles) and **4** (20 mg, colourless needles). Fraction UM-3 (1.1 g) was chromatographed over silica gel column (70 g × 50 cm × 2 cm) using *n*-hexane–EtOAc in order of increasing polarity to afford compound **5** (18 mg, colourless needles). Compounds **3**–**5** were re-crystallised from MeOH–Me_2CO (1:1) to furnish colourless needles. Similarly, fraction UM-4 (1.9 g) was subjected to silica gel column chromatography (100 g × 50 cm × 2 cm) using *n*-hexane–EtOAc gradients to afford compound **1** (28 mg, colourless oil). Repeated silica gel column chromatography of fraction UM-5 (0.95 g) using $CHCl_3$–MeOH (96:4 to 90:10) gave compound **2** (15 mg, yellow crystals). Fraction UM-6 (1.8 g) was subjected to silica gel column chromatography (90 g × 50 cm × 2 cm) using $CHCl_3$–MeOH (95:5 to 85:15) to afford compound **6** (2.9 mg, yellow crystals). Fraction UM-7 (2.1 g) was chromatographed over Sephadex LH-20 column (0.25–0.1 mm; 100 g × 50 cm × 3 cm) using MeOH as an eluent to give impure **7**, which was further purified on a silica gel column (30 g × 50 cm × 2 cm) using $CHCl_3$–MeOH (90:10 to 80:20) to give **7** (28 mg, yellow amorphous powder).

3.4. *Alkaline hydrolysis of compound 1*

A solution of **1** (10 mg) in 3% NaOMe–MeOH (2 mL) was stirred at 40°C for 2 h. The reaction mixture was neutralised with 2 N HCl in MeOH and partitioned between MeOH and *n*-hexane. The *n*-hexane layer was concentrated under reduced pressure to yield FAME, which was analysed as (*Z*)-tetracos-6-enoic acid methyl ester by GC–MS from the molecular ion peaks at *m/z* 380 $[M]^+$ (Kim et al. 2005; Al-Musayeib et al. 2013).

3.5. *Acid hydrolysis*

Solutions of the isolated glycosides (**6** and **7**) (5 mg in 10 mL MeOH) were treated with 3% H_2SO_4 (1.5 mL) and heated at 100°C for 1 h. The aglycones were extracted with EtOAc, concentrated under reduced pressure, purified on Sephadex LH-20 column using MeOH. The sugars in the aqueous layer were identified by co-PC (paper chromatography) with authentic materials using solvent system S3 (El-Shanawany et al. 2012).

3.6. *Spectral data*

(2*S*)-1-*O*-(*Z*)-Tetracos-6-enoate glycerol (**1**): Colourless oil; R_f 0.57, silica gel 60 F_{254} (Figure S1); $[\alpha]_D + 14.8°$ ($c = 0.5$, $CHCl_3$); IR (KBr) γ_{max}: 3435, 2966, 1742, 1472, 1072, 952 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ_H 4.19 (1H, dd, $J = 11.6$ and 4.8 Hz, H-1A), 4.15 (1H, dd, $J = 11.6$ and 4.8 Hz, H-1B), 3.93 (1H, m, H-2), 3.69 (1H, dd, $J = 12.0$ and 6.4 Hz, H-3A), 3.60 (1H, dd, $J = 12.0$ and 6.4 Hz, H-3B), 2.35 (2H, t, $J = 7.6$ Hz, H-2′), 1.64 (2H, m, H-3′), 2.01 (2H, m, H-5′), 5.34 (1H, dt, $J = 7.6$ and 6.8 Hz, H-6′), 5.33 (1H, dt, $J = 7.6$ and 6.8 Hz, H-7′), 1.30–1.25 (2H × 14, m, H-4′, H-8′-H-21′), 0.88 (3H, t, $J = 7.2$ Hz, H-24′); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 65.2 (C-1), 70.3 (C-2), 63.3 (C-3), 174.3 (C-1′), 34.1 (C-2′), 24.9 (C-3′), 27.2 (C-5′), 129.7 (C-6′), 130.0 (C-7′), 29.7–29.1 (CH_2 × 15, C-4′, C-8′-C-21′), 31.9 (C-22′), 22.7 (C-23′), 14.1 (C-24′); HR-ESI-MS *m/z* 441.3852 (calcd for $C_{27}H_{53}O_4$ $[M + H]^+$, 441.3866).

3.7. *Determination of in vitro growth inhibitory activity (cytotoxicity)*

The *in vitro* growth inhibitory activity of the *n*-BuOH and EtOAc fractions, as well as EtOAC subfractions and isolated compounds was determined using the A549 NSCLC (Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ); code ACC107), PC-3 prostate cancer (DSMZ; code ACC465) and U373 GBM (European Collection of Cell Culture; code 89081403) cell lines. The cells were cultured in RPMI (Lonza, Braine-l'Alleud, Belgium) media supplemented with 10% heat-inactivated foetal bovine serum (Lonza), 4 mM glutamine, 100 μg/mL gentamicin, 200 U/mL penicillin and 200 μg/mL streptomycin (Lonza). The overall growth of the human cancer cell lines was determined using a colorimetric MTT assay (Sigma, Diegem, Belgium) as previously detailed (Mijatovic et al. 2006; Ibrahim et al. 2012; Ibrahim et al. 2014). Each experimental condition was performed in six replicates. Cisplatin (Sigma-Aldrich, Bornem, Belgium) was used as a positive control. The results are presented in Table 1.

4. Conclusion

Investigation of the *in vitro* growth inhibitory activity of the EtOAc subfractions of *D. maritima* bulbs afforded the isolation of one new and six known compounds. Their structures were established by different spectroscopic analyses. The *in vitro* growth inhibitory activity of the *n*-BuOH, EtOAc and EtOAc subfractions, as well as the isolated compounds was evaluated against

three different human cancer cell lines. Compounds **4** and **7** are reported here for the first time from the plant and **2** and **6** for the first time from this family.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S8.

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