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Pregnanes with antiproliferative activity from the gorgonian *Eunicella cavolini*

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ABSTRACT

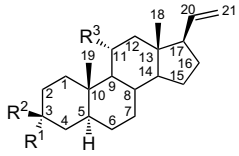
Four new natural products (**1**–**4**), along with three previously reported metabolites (**5**–**7**), all belonging to the pregnane class of steroids were isolated from the organic extract of the gorgonian *Eunicella cavolini*. The structures and relative configurations of the isolated compounds were determined through NMR spectroscopic techniques and chemical interconversions, while their absolute stereochemistry was determined using the modified Mosher's method. Activity evaluation of **1**–**7** towards MCF-7 human breast cancer cells showed partial growth inhibitory effects for all metabolites bearing an 11α-acetoxy moiety.

1. Introduction

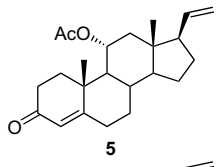
Marine animals, especially those belonging to the phylum Cnidaria, are considered a prolific source of secondary metabolites featuring a wide variety of skeletons and exhibiting a diverse array of pharmacological activities.[1,2] Among them, gorgonians (Anthozoa, Gorgonacea) have been the subject of numerous chemical investigations, which have yielded mainly sesquiterpenes, diterpenes, prostanoids, highly functionalized steroids, acetogenins and alkaloids.[1,2] A number of those metabolites have been proven to possess antibacterial,[3] antiviral,[4] anti-inflammatory,[5,6] antiplasmodial,[7] antifouling,[8] antiproliferative,[9] cytotoxic[10,11] and insecticidal[12] activities. Species of the genus *Eunicella* (Gorgoniidae) have been shown to produce mainly diterpenes.[13–15] Pregnanes and their glucosides[16,17] and tryptamine derivatives[18] have been reported only once so far in the relevant literature.

Eunicella cavolini is one of the most abundant gorgonian species in the Mediterranean Sea.[19] Therefore, in continuation of our research programme for the isolation of bioactive natural products from marine organisms of the Greek Seas,[20–22] we decided to carry out an investigation of its chemical composition. In this report, we describe the isolation and structure elucidation of four new (**1**–**4**) and three previously reported (**5**–**7**) pregnanes, the determination of their absolute stereochemistry, as well as the evaluation of their effect on the growth of MCF-7 human breast adenocarcinoma cells.

2. Results and discussion



1 R^1, R^2=O, R^3=OAc
2 R^1=H, R^2=OH, R^3=OAc
3 R^1=OH, R^2=H, R^3=OAc
4 R^1=R^2=OMe, R^3=OAc
6 R^1=R^3=H, R^2=OH



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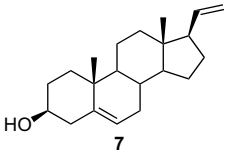

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Colonies of the gorgonian *E. cavolini*, collected from Lichadonissia Isles, Greece, were exhaustively extracted with a mixture of CH_2Cl_2/MeOH and the organic extract was subsequently subjected to a series of chromatographic separations to allow the isolation of compounds **1**–**7** in pure form.

Compound **1** was isolated as yellowish oil. Combination of ^{13}C NMR and HR-FABMS data suggested a molecular formula of $C_{23}H_{34}O_3$ (*m*/*z* 359.2573 for $[M+H]^+$). The ^{13}C NMR spectrum

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Table 1
^{1}H NMR data of compounds **1**–**5**

No.	1	2	3	4	5
1	1.84 m, 1.56 m	1.49 m, 1.30 m	1.60 m, 1.24 m	1.39 m	1.96 m
2	2.36 m, 2.22 m	1.74 m, 1.32 m	1.63 m	1.81 m, 1.40 m	2.31 m
3		3.55 tt (10.4, 5.1)	3.99 br s		
4	2.26 m, 2.08 m	1.54 m, 1.22 m	1.47 m, 1.36 m	1.61 m, 1.27 m	5.74 s
5	1.60 m	1.21 m	1.64 m	1.39 m	
6	1.35 m, 1.26 m	1.29 m	1.22 m	1.29 m, 1.22 m	2.39 m
7	1.73 m, 1.00 m	1.72 m, 0.99 m	1.69 m, 1.01 m	1.70 m, 1.01 m	1.89 m, 1.13 m
8	1.50 m	1.44 m	1.45 m	1.46 m	1.64 m
9	1.18 m	1.09 m	1.20 m	1.18 m	1.42 t (10.5)
11	5.17 td (10.5, 5.4)	5.11 td (10.5, 5.4)	5.11 td (10.5, 5.5)	5.11 td (10.5, 5.4)	5.22 td (10.5, 5.1)
12	2.02 m, 1.04 m	2.02 m, 1.01 m	2.01 m, 1.02 m	2.01 m, 1.02 m	2.05 m, 1.09 m
14	1.16 m	1.14 m	1.13 m	1.13 m	1.18 m
15	1.67 m, 1.14 m	1.68 m, 1.11 m	1.66 m, 1.14 m	1.66 m, 1.15 m	1.70 m, 1.22 m
16	1.76 m, 1.55 m	1.78 m, 1.56 m	1.78 m, 1.53 m	1.80 m, 1.54 m	1.84 m, 1.60 m
17	1.98 m	1.97 m	1.96 m	1.98 m	2.01 m
18	0.65 s	0.63 s	0.62 s	0.61 s	0.70 s
19	1.09 s	0.90 s	0.87 s	0.87 s	1.25 s
20	5.68 ddd (17.1, 10.4, 7.8)	5.68 ddd (17.8, 10.4, 7.8)	5.68 ddd (17.4, 10.1, 7.7)	5.67 ddd (17.8, 10.2, 8.0)	5.69 ddd (17.5, 9.8, 7.3)
21	4.96 m, 4.93 m	4.95 m, 4.93 m	4.95 m, 4.92 m	4.95 m, 4.92 m	4.98 m, 4.95 m
OAc	1.96 s	1.96 s	1.95 s	1.95 s	1.99 s
OMe				3.15 s	
OMe				3.12 s	

Recorded at 400 MHz in $CDCl_3$. Chemical shifts are expressed in parts per million and *J* values in parentheses are reported in hertz.

revealed 23 carbon signals, which as determined from DEPT experiments corresponded to four quaternary, seven methine, nine methylene and three methyl carbon atoms. The structural elements displayed in the ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) included one ketone functionality (δ_C 211.3), one ester carbonyl (δ_C 170.2), one oxygenated methine ($\delta_{H/C}$ 5.17/71.3), one monosubstituted double bond ($\delta_{H/C}$ 5.68/138.8; 4.93, 4.96/115.3), two tertiary methyl groups ($\delta_{H/C}$ 0.65/13.5; 1.09/12.0) and one acetate methyl group ($\delta_{H/C}$ 1.96/22.0). Since the carbon–carbon double bond and the two carbonyls accounted for three of the seven degrees of unsaturation, the structure of **1** was determined as tetracyclic. These data were consistent with the reported values for a pregn-20-ene skeleton.[23]

Table 2
^{13}C NMR data of compounds **1**–**5**

No.	1		2		3		4		5	
1	39.1	t	37.5	t	32.8	t	35.6	t	36.5	t
2	38.3	t	31.9	t	29.0	t	28.9	t	34.1	t
3	211.3	s	70.7	d	66.2	d	99.7	s	199.3	s
4	45.0	t	38.6	t	36.3	t	35.7	t	124.7	d
5	47.0	d	44.8	d	38.7	d	42.2	d	145.4	s
6	29.4	t	29.1	t	29.2	t	28.8	t	33.5	t
7	31.8	t	32.2	t	32.3	t	32.1	t	32.0	t
8	35.1	d	35.1	d	35.1	d	35.1	d	35.5	d
9	56.3	d	56.6	d	56.6	d	56.4	d	55.8	d
10	37.3	s	37.1	s	37.9	s	37.4	s	39.9	s
11	71.3	d	71.4	d	71.4	d	71.4	d	71.0	d
12	44.2	t	44.2	t	44.3	t	44.2	t	44.2	t
13	43.6	s	43.8	s	43.7	s	43.7	s	43.7	s
14	54.1	d	54.2	d	54.3	d	54.2	d	54.0	d
15	24.8	t	24.8	t	24.7	t	24.8	t	24.7	t
16	27.3	t	27.3	t	27.3	t	27.3	t	27.3	t
17	55.1	d	55.2	d	55.2	d	55.1	d	55.0	d
18	13.5	q	13.5	q	13.5	q	13.5	q	13.5	q
19	12.0	q	12.8	q	11.7	q	12.1	q	18.4	q
20	138.8	d	139.0	d	139.0	d	139.0	d	138.6	d
21	115.3	t	115.1	t	115.1	t	115.1	t	115.5	t
OAc	170.2	s	170.3	s	170.4	s	170.4	s	169.9	s
OAc	22.0	q	22.0	q	22.0	q	22.0	q	21.9	q
OMe							47.4	q		
OMe							47.5	q		

Recorded at 50.3 MHz in $CDCl_3$. Chemical shifts are expressed in parts per million.

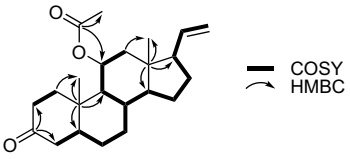

Figure 1. Key COSY and HMBC correlations for compound **1**.

Analysis of the 2D NMR spectra (HSQC, HMBC and COSY) of **1** verified the pregn-20-ene skeleton and suggested the positions of the ketone functionality and the acetoxy group (Fig. 1). More specifically, C-3 displayed HMBC correlations with H_2-2 and H_2-4, whereas these showed cross peaks in the COSY spectrum with H_2-1 and H-5, respectively. The correlations of C-1 with H_3-19 and C-10 with H-5, H-9 and H_3-19 observed in the HMBC spectrum concluded the assignment of ring A. The COSY correlations of H-5/H_2-6, H_2-6/H_2-7, H_2-7/H-8 and H-8/H-9 identified ring B. The HMBC correlations of C-12 with H_3-18 and C-13 with H-14, H-17 and H_3-18, in conjunction with the cross peaks of H-8/H-14, H-9/H-11 and H-11/H_2-12 confirmed the assignment of ring C. The methine proton H-11 displayed in the HMBC spectrum a correlation with the ester carbonyl, thus fixing the position of the acetoxy group at C-11. The cross peaks of H-14/H_2-15, H_2-15/H_2-16, H_2-16/H-17, H-17/H-20 and H20/H_2-21 provided evidence for ring D and the exocyclic Δ^{20} double bond.

The relative configuration of **1** was established by analysis of the key correlations displayed in the NOESY spectrum (Fig. 2). NOE enhancements observed for H-8/H-11, H-8/H_3-18, H-8/H_3-19, H-11/H_3-18, H-11/H_3-19 and H_3-18/H-20, as well as for H-5/H-9 and H-14/H-17 provided evidence that H-8, H-11, H_3-18 and H_3-19 were

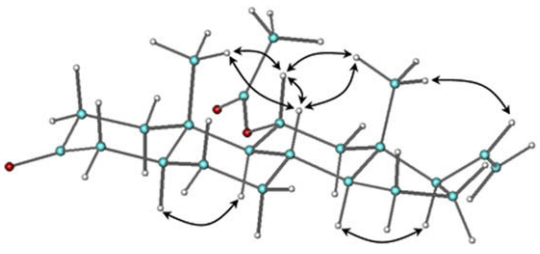

Figure 2. Key NOESY correlations for compound **1**.

cofacial on one side of the molecule, while H-5, H-9, H-14 and H-17 were on the opposite side.

Compound **2**, obtained as colourless oil, displayed spectroscopic characteristics (Tables 1 and 2) quite similar to those of metabolite **1**. However, the absence of the carbon signal at δ 211.3, as well as the presence of a second oxygenated methine ($\delta_{H/C}$ 3.55/70.7) indicated that the ketone functionality at C-3 had been replaced by a hydroxy group. The peak at m/z 359.2594 for $[M-H]^+$ observed in the HR-FABMS confirmed the molecular formula of **2** as $C_{23}H_{36}O_3$. A triplet of triplets at δ 3.55, which was assigned to the H-3, indicated the configuration of the hydroxy group at C-3 as β.

Compound **3** was obtained as colourless oil. The ^{13}C NMR spectrum and the ion peak at m/z 359.2597 ($[M-H]^+$) observed in the HR-FABMS suggested the same molecular formula as **2**. Moreover, both ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) closely resembled those of compound **2**. The main difference between the two metabolites was that H-3 appeared in **3** as a broad singlet at δ 3.99, thus indicating an α configuration for the hydroxy group at C-3.

Compound **4**, isolated as colourless oil, had a molecular formula of $C_{25}H_{40}O_4$ (m/z 403.2854 for $[M-H]^+$), as deduced from the HR-FABMS and ^{13}C NMR spectrum. In the ^1H NMR spectrum (Table 1), evident was the presence of two methoxy groups at δ 3.16 and 3.12 and the simultaneous absence of the second oxygenated methine attributable to H-3. In the ^{13}C NMR spectrum (Table 2), besides the presence of two more carbons signals at δ 47.5 and 47.4 accounting for the carbon atoms of the two methoxy groups, obvious was the replacement of C-3 with a quaternary carbon resonating at δ 99.7. Analysis of the 2D NMR spectra (HSQC, HMBC and COSY) confirmed the position of the two methoxy groups at C-3. Since MeOH was one of the solvents used for the extraction of the organism and the fractionation of the extract thereafter, the possibility of **4** being an artefact cannot be excluded.

Reduction of **1** with $NaBH_4$ yielded both compounds **2** and **3**, while ketalization of **1** afforded compound **4**. This verified that the hydroxy groups in **2** and **3**, as well as both methoxy groups in **4** were positioned at C-3, replacing in all cases the ketone functionality present in **1**. Moreover, this confirmed that the relative configurations of **2**–**4** were the same with that of **1**.

The absolute stereochemistry of **3** was determined by application of the modified Mosher's method.[24] When **3** was treated with (*R*)- and (*S*)-MTPA chloride, the secondary hydroxy group at C-3 reacted to give the (*S*)- and (*R*)-MTPA derivatives, respectively. The ^1H NMR chemical shifts of the MTPA derivatives of **3** were assigned by analysis of ^1H, HSQC and COSY NMR spectra. The calculation of the $\Delta\delta_{S-R}$ values, shown in Figure 3, defined the absolute stereochemistry of C-3 as *R* and subsequently, on the basis of its relative configuration, established the absolute stereochemistry of **3** as depicted. Since compounds **1**–**4** were clearly correlated through the chemical interconversions described above, the absolute stereochemistry of **1**, **2** and **4** is as shown.

Compounds **5**–**7** were identified by comparison of their spectroscopic and physical characteristics with those reported in the literature as 11α-acetoxy-pregna-4,20-dien-3-one (**5**),[16] 5α-pregn-20-en-3β-ol (**6**)[25,26] and pregna-5,20-dien-3β-ol (**7**).[27,28] Analysis of the ^{13}C and 2D (HSQC, HMBC and COSY) NMR spectra allowed the assignment of the ^1H and ^{13}C NMR chemical shifts for **5** (Tables 1 and 2), for which only a few characteristic ^1H NMR resonances had been reported.[16]

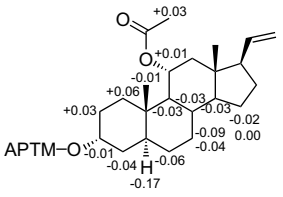

Figure 3. $\Delta\delta_{S-R}$ values (ppm) for the MTPA derivatives of **3** in $CDCl_3$.

Progesterone analogues and derivatives can inhibit estrogen-dependent, estrogen receptor (ER)-mediated proliferation of MCF-7 human breast cancer cells in culture[29] and have been extensively used in the treatment of breast cancer.[30] We sought to compare the effect of **1**–**7** on the growth of MCF-7 cells to that of ICI 182,780 (Faslodex®), an ER-specific antagonist of MCF-7 cell growth that is also used to treat advanced breast cancer. To this end, we exposed MCF-7 cells, exponentially growing in the presence of 17β-estradiol (1 nM), to **1**–**7** (10 μM), ICI 182,780 (1 μM) or to vehicle alone (0.1% DMSO). The partial growth inhibitory effects of **1**–**5** (49±3, 41±3, 41±4, 40±4 and 48±3%, respectively, of the effect of ICI 182,780 set equal to 100) were significant ($p<0.05$; ANOVA), whereas those of **6** (13%) and **7** (5%) were non-significant, suggesting that the 11α-acetoxy moiety is instrumental in inhibiting the estrogen-dependent growth of these cells. It is possible that **1**–**5** behave like the 16α-substituted analogues of the antiprogestin RU486 in inducing progesterone receptor partial antagonism.[31] In addition, **1**–**5** might interfere with pregnane X receptor, a highly promiscuous nuclear receptor involved in the detoxification of xenobiotics (e.g., CYP3),[32] which is reportedly capable of affecting the growth of MCF-7 cells.[33]

In conclusion, four new pregnanes (**1**–**4**) were isolated from the organic extract of the gorgonian *E. cavolini*, along with three previously reported metabolites of the same class (**5**–**7**). Their structures and relative configurations were established through analysis of their NMR spectroscopic characteristics and chemical interconversions, whereas their absolute stereochemistry was determined using the modified Mosher's method. Compounds **1**–**5**, but not **6** or **7**, inhibited MCF-7 cell growth, indicating that the 11α-acetoxy moiety may relate to the antiproliferative activity of these pregnanes.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer model 341 polarimeter with a 1 dm cell. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer. IR spectra were obtained on a Paragon 500 Perkin–Elmer spectrometer. NMR spectra were recorded on Bruker AC 200 and Bruker DRX 400 spectrometers. Chemical shifts are given on the δ (ppm) scale using TMS as internal standard. The 2D experiments (HSQC, HMBC, COSY, NOESY) were performed using standard Bruker microprograms. High resolution mass spectra were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana, USA. Low resolution CI mass spectra were measured in positive mode on a Thermo Electron Corporation DSQ mass spectrometer using a Direct-Exposure Probe and methane as the CI reagent gas. Column chromatography separations were performed using Kieselgel 60 (Merck). HPLC separations were conducted using a CECIL 1100 Series liquid chromatography pump equipped with a GBC LC-1240 refractive index detector, using a Kromasil 100 C-18 (MZ-Analysentechnik, 25 cm×8 mm) column. TLC were performed using Kieselgel 60 F_{254} (Merck aluminium support plates) and spots were detected after spraying with 15% H_2SO_4 in MeOH reagent and heating at 100 °C for 1 min.

3.2. Animal material

E. cavolini was collected from Lichadonissia Isles in Maliakos Gulf, Greece, at a depth of 15–20 m in October 2004. A voucher specimen of the soft coral is kept at the Herbarium of the Department of Pharmacognosy and Chemistry of Natural Products, University of Athens (ATPH/MO/164).

3.3. Extraction and isolation

Colonies of the gorgonian (1.7 kg) were exhaustively extracted with CH_2Cl_2/MeOH (3:1) at room temperature. Evaporation of the solvent in vacuo afforded a dark orange oily residue (48 g) that was subjected to gravity column chromatography on silica gel, using *c*-hexane with increasing amounts of EtOAc, followed by EtOAc with increasing amounts of MeOH as the mobile phase, to afford 18 fractions (I–XVIII). Fraction V (25% EtOAc, 3.6 g) was further fractionated by gravity column chromatography on silica gel, using *c*-hexane/EtOAc (75:25) as the mobile phase, to yield 16 fractions (V1–V16). Fraction V3 (1.1 g) was further fractionated by gravity column chromatography on silica gel, using *c*-hexane with increasing amounts of EtOAc as the mobile phase, to yield 19 fractions (V3a–V3s). Fractions V3g (15% EtOAc, 40.0 mg), V3h (15% EtOAc, 48.0 mg), V3i (15% EtOAc, 49.0 mg), V3j (15% EtOAc, 31.0 mg) and V3k (15% EtOAc, 42.0 mg) were subjected repeatedly to reversed phase HPLC, using MeOH/H_2O (92:8) as eluent, to yield compounds **1** (44.0 mg), **4** (20.1 mg), **6** (24.0 mg) and **7** (7.0 mg) in pure form. Fraction V8 (130.7 mg) was subjected repeatedly to reversed phase HPLC, using MeOH/H_2O (90:10) as eluent, to yield compounds **2** (5.3 mg), **3** (6.0 mg) and **5** (3.2 mg) in pure form.

*3.3.1. 11α-Acetoxy-5α-pregn-20-en-3-one (**1**)*

Yellowish oil; $[\alpha]_D^{20}$ −23.0 (*c* 0.100, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 244.0 (2.36) nm; IR (thin film) ν_{max} 2937, 1738, 1713, 1637, 1249, 983 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; PCIMS (CH_4) *m*/*z* (rel int. %) 359 (100), 299 (99), 283 (31), 231 (20), 105 (19); HR-FABMS *m*/*z* 359.2573 $[M+H]^+$ (calcd for $C_{23}H_{35}O_3$, 359.2586).

*3.3.2. 11α-Acetoxy-5α-pregn-20-en-3β-ol (**2**)*

Colourless oil; $[\alpha]_D^{20}$ −40.0 (*c* 0.025, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243.0 (2.53) nm; IR (thin film) ν_{max} 3467, 2942, 1746, 1632, 1237, 991 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; PCIMS (CH_4) *m*/*z* (rel int. %) 343 (2), 301 (3), 283 (100), 267 (39), 215 (21); HR-FABMS *m*/*z* 359.2594 $[M-H]^+$ (calcd for $C_{23}H_{35}O_3$, 359.2586).

*3.3.3. 11α-Acetoxy-5α-pregn-20-en-3α-ol (**3**)*

Colourless oil; $[\alpha]_D^{20}$ −22.0 (*c* 0.050, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243.5 (2.62) nm; IR (thin film) ν_{max} 3452, 2953, 1733, 1627, 1246, 997 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; PCIMS (CH_4) *m*/*z* (rel int. %) 343 (4), 301 (25), 283 (100), 267 (9), 215 (8); HR-FABMS *m*/*z* 359.2597 $[M-H]^+$ (calcd for $C_{23}H_{35}O_3$, 359.2586).

*3.3.4. 11α-Acetoxy-3,3-dimethoxy-5α-pregn-20-ene (**4**)*

Colourless oil; $[\alpha]_D^{20}$ −24.0 (*c* 0.100, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245.0 (2.51) nm; IR (thin film) ν_{max} 2953, 1719, 1631, 1247, 989 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; PCIMS (CH_4) *m*/*z* (rel int. %) 391 (24), 387 (19), 373 (12), 359 (100), 313 (26), 299 (57), 279 (34); HR-FABMS *m*/*z* 403.2854 $[M-H]^+$ (calcd for $C_{25}H_{39}O_4$, 403.2848).

3.4. Reduction of 1

Compound **1** (9.8 mg) was treated with $NaBH_4$ (10 mg) in MeOH (2 mL) and left under constant stirring at room temperature for 30 min. The reaction was quenched by the addition of H_2O (2 mL) and the mixture was evaporated in vacuo. The residue was purified by reversed phase HPLC, using MeOH/H_2O (92:8) as eluent, to obtain **2** (4.0 mg) and **3** (1.5 mg).

3.5. Ketalization of 1

Aminopropylated silica gel hydrochloride (APSG·HCl) was prepared as previously described.[34] Compound **1** (10.2 mg) was treated with APSG·HCl (1 mg) in anhydrous MeOH (1 mL) and left under constant stirring at room temperature for 14 h. The reaction mixture was filtered through a sintered funnel and subsequently concentrated in vacuo to afford **4** (10.7 mg) in 98% yield.

3.6. Preparation of MTPA derivatives of 3

Compound **3** (2.5 mg) was treated with (*R*)-MTPA chloride (5 μL) in freshly distilled dry pyridine (1 mL) and left under constant stirring at room temperature for 18 h. The reaction was quenched by the addition of H_2O (1 mL) and CH_2Cl_2 (5 mL), and the mixture was partitioned between the aqueous and the organic layer. After evaporation of the organic layer in vacuo, the residue was purified by reversed phase HPLC, using MeOH/H_2O (90:10) as eluent, to obtain the (*S*)-MTPA derivative (1.3 mg). The (*R*)-MTPA derivative (1.5 mg) was prepared with (*S*)-MTPA chloride and purified in the same manner.

*3.6.1. (R)-MTPA derivative of **3***

^1H NMR (400 MHz, $CDCl_3$) δ 7.54–7.50 (m, 2H, Ar–H), 7.42–7.38 (m, 3H, Ar–H), 5.67 (ddd, 17.0, 10.4, 7.9 Hz, 1H, H-20), 5.25–5.21 (m, 1H, H-3), 5.08 (dt, 10.5, 5.4 Hz, 1H, H-11), 4.97–4.93 (m, 1H, H-21a), 4.91–4.89 (m, 1H, H-21b), 3.53 (s, 3H, OMe), 2.00–1.94 (m, 1H, H-12a), 1.97–1.93 (m, 1H, H-17), 1.91 (s, 3H, OAc), 1.82–1.74 (m, 1H, H-16a), 1.70–1.66 (m, 2H, H-2), 1.71–1.65 (m, 1H, H-7a), 1.68–1.62 (m, 1H, H-15a), 1.57–1.51 (m, 2H, H-4), 1.55–1.51 (m, 1H, H-16b), 1.47–1.39 (m, 1H, H-5), 1.45–1.39 (m, 1H, H-8), 1.31–1.27 (m, 2H, H-1), 1.21–1.15 (m, 2H, H-6), 1.13–1.09 (m, 1H, H-15b), 1.13–1.07 (m, 1H, H-14), 1.09–1.03 (m, 1H, H-9), 1.04–0.96 (m, 1H, H-12b), 0.99–0.93 (m, 1H, H-7b), 0.88 (s, 3H, H-19), 0.61 (s, 3H, H-18).

*3.6.2. (S)-MTPA derivative of **3***

^1H NMR (400 MHz, $CDCl_3$) δ 7.55–7.51 (m, 2H, Ar–H), 7.42–7.38 (m, 3H, Ar–H), 5.67 (ddd, 17.0, 10.4, 7.9 Hz, 1H, H-20), 5.24–5.20 (m, 1H, H-3), 5.09 (dt, 10.5, 5.3 Hz, 1H, H-11), 4.97–4.93 (m, 1H, H-21a), 4.91–4.89 (m, 1H, H-21b), 3.56 (s, 3H, OMe), 2.00–1.94 (m, 1H, H-12a), 1.97–1.93 (m, 1H, H-17), 1.94 (s, 3H, OAc), 1.82–1.74 (m, 1H, H-16a), 1.73–1.69 (m, 2H, H-2), 1.68–1.62 (m, 1H, H-15a), 1.67–1.61 (m, 1H, H-7a), 1.55–1.51 (m, 1H, H-16b), 1.53–1.47 (m, 2H, H-4), 1.41–1.37 (m, 1H, H-8), 1.37–1.33 (m, 2H, H-1), 1.29–1.23 (m, 1H, H-5), 1.15–1.09 (m, 2H, H-6), 1.11–1.07 (m, 1H, H-15b), 1.10–1.04 (m, 1H, H-14), 1.07–0.99 (m, 1H, H-9), 1.04–0.96 (m, 1H, H-12b), 0.87 (s, 3H, H-19), 0.89–0.85 (m, 1H, H-7b), 0.61 (s, 3H, H-18).

3.7. Assessment of antiproliferative activity

Pregnane effects on the 17β-estradiol-dependent growth of MCF-7 cells were assessed in 96-well flat-bottomed microculture plates using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], as previously reported.[35] Cell numbers in the presence of **1**–**7** (10 μM), ICI 182,780 (1 μM), or vehicle (0.1% DMSO), as assessed by MTT conversion to coloured formazan monitored by measurement of the optical density (OD) at 550 nm, were expressed relative to the OD of the vehicle alone, set equal to 100. As expected, cell growth was fully inhibited by ICI 182,780 (1 μM).[36] Growth inhibition by the pregnanes was then expressed as % of that of ICI 182,780 by [(OD vehicle−OD pregnane)×100]/(OD vehicle−OD ICI 182,780). Statistically significant growth inhibitory effects (mean±S.E.M; n=4) were classified as full, partial, weak or marginal depending on whether they were >75–100, >25–75, >10–25 and ≤10% of ICI 182,780, respectively.

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