European Journal of Medicinal Chemistry 85 (2014) 391–398

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluations of new analogs of 2-methoxyestradiol: Inhibitors of tubulin and angiogenesis

Eirik Johansson Solum [a], Jing-Jy Cheng [b, c], Irene B. Sørvik [d], Ragnhild E. Paulsen [d], Anders Vik [a], Trond Vidar Hansen [a, *]

[a] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, PO Box 1068, Blindern, N-0316 Oslo, Norway
[b] National Research Institute of Chinese Medicine, Taipei, Taiwan
[c] Institute of Biophotonics, National Yang-Ming University, Taipei 112, Taiwan
[d] Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Norway

ARTICLE INFO

Article history:
Received 10 June 2014
Received in revised form
28 July 2014
Accepted 1 August 2014
Available online 2 August 2014

Keywords:
2-Methoxyestradiol
Anti-cancer
Tubulin inhibition
Anti-angiogenesis
Steroids

ABSTRACT

The synthesis, cytotoxicity, inhibition of tubulin polymerization and anti-angiogenic effects of 15 analogs of 2-methoxyestradiol (**1**) are reported. The biological studies revealed that the position of nitrogen atom in the heterocyclic ring is important for inhibition of both tubulin polymerization and angiogenesis. The most potent inhibitors were compounds **11f** and **13e**, with a 6-substituted isoquinoline ring in the 17-position of the steroid skeleton. Moreover, low estrogen activity was observed for the analogs tested at 10 μM concentrations.

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1. Introduction

Several natural products have been reported to be microtubule-binding agents [1]. Such natural products and their derivatives have been widely used in cancer chemotherapy [2]. These anti-cancer agents can either stabilize microtubules and promote polymerization, or destabilize the microtubules and promote depolymerization by inhibition of tubulin [3]. Both types interfere with the mitotic spindle polymerization during cell division that eventually leads to cell death. Studies have shown that most microtubule-binding agents have anti-vascular effects induced by vascular disrupting or from anti-angiogenetic activities, or both [4].

The endogenous steroid 2-methoxyestradiol (2-ME, **1**), see Fig. 1, is a natural product that exhibits anti-vascular effects [5] and anti-angiogenetic activities [6]. 2-Methoxyestradiol (**1**) was for long a period of time believed to be an inactive metabolite, but D'Amoto and co-workers reported in 1994 that **1** was a tubulin polymerization inhibitor [7]. Several studies revealed that the steroid **1** posses a plethora of interesting anti-cancer effects without any undesirable estrogen activity [5–9]. 2-Methoxyestradiol (**1**) has entered several clinical trials [10]. In addition, the steroid **1** has been employed as a lead compound in many structural–activity relationship (SAR) studies [11]. One example that has emerged from these efforts is ENMD-1198 (**2**) that has been the subject of clinical trials and extensive biological evaluations [12].

The cortistatins, exemplified by cortistatins A (**3**), J (**4**) and G (**5**), see Fig. 1, are also examples of natural occurring steroids that have attracted great interest within anti-cancer research [13]. These steroidal alkaloids were first isolated in 2006 from the marine sponge *Corticium simplex* and showed potent anti-angiogenetic effects [14]. It was also reported that cortistatin A (**3**) inhibits the proliferation of human umbilical vein endothelial cells (HUVECs) in the low nano-molar range [14]. The cortistatins have recently attracted a great interest in the synthetic community as targets for total synthesis [15]. In addition, several analogs of **3** with interesting anti-cancer effects have been prepared [16]. These SAR-studies have revealed that the presence of an isoquinoline group is crucial for activity and that the two hydroxyl-groups on the A-ring are removable. Along these lines, one of the most interesting analogs reported so far is the amino steroid **6** reported by Corey and co-workers [16].

* Corresponding author.
E-mail addresses: e.j.solum@farmasi.uio.no (E.J. Solum), verona@nricm.edu.tw (J.-J. Cheng), i.b.sorvik@farmasi.uio.no (I.B. Sørvik), r.e.paulsen@farmasi.uio.no (R.E. Paulsen), anders.vik@farmasi.uio.no (A. Vik), t.v.hansen@farmasi.uio.no (T.V. Hansen).

http://dx.doi.org/10.1016/j.ejmech.2014.08.002
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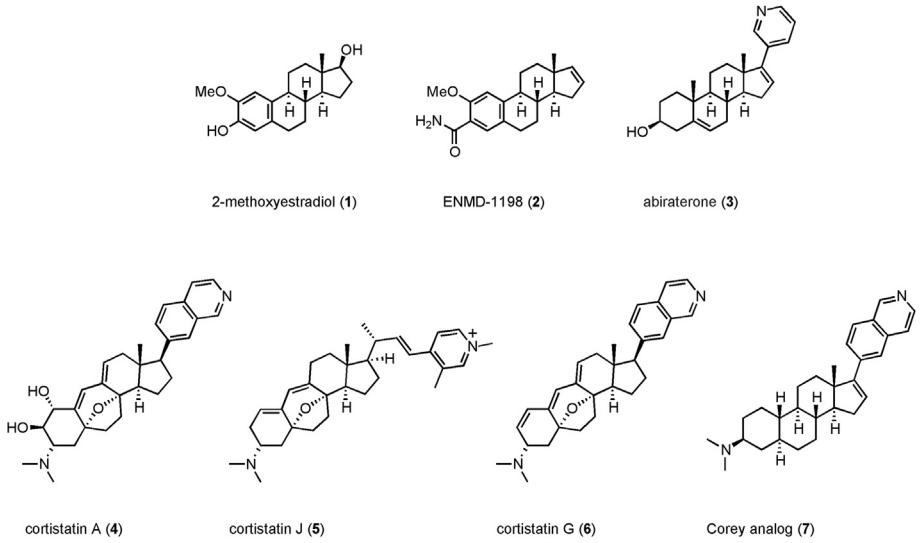


Fig. 1. Examples of steroids exhibiting anti-cancer effects.

Based on the previous SAR-studies, and our interest in analogs of natural products with anti-cancer activities [17], we became interested in preparing analogs with either a pyridine, a quinoline or an isoquinoline ring in the C-17 position of 2-ME (**1**). The synthesis, cytotoxicity, inhibition of tubulin polymerization data and anti-angiogenetic effects of such analogs are presented herein. Some of the new analogs were also tested as agonists for the estrogen receptor α.

2. Chemistry

The new analogs of 2-methoxyestradiol (**1**) were prepared as depicted in Scheme 1. First, **1** was oxidized in an Oppenauer oxidation to ketone **8a** that was converted to the TBS-protected ketone **8b** Then ketone **8b** was converted into the triflate **9** using *N*-phenyl-*bis*(trifluoromethanesulfonimide). This afforded **9** in 69% yield from 2-methoxyestradiol (**1**). Then compound **9** was employed in a Suzuki–Miyaura reaction [18] with different commercially available boronic acids. In the presence of $Pd(PPh_3)_4$ and Cs_2CO_3 in THF/H_2O (1:1), the TBS protected compounds **10a**–**10h** were obtained (Scheme 1). The Stille reaction was also attempted for the synthesis of **10c** and **10d**. However, this proved to be a less efficient method, as demonstrated when the triflate **9** was coupled with either 3- or 4-(tributylstannyl)pyridine in the presence of lithium chloride, copper chloride and $Pd(PPh_3)_4$ in DMSO. The desired analogs **11a**–**11h** were obtained after removal of the TBS-group in **10a**–**10h** under standard conditions [19].

Reduction of the 16,17-double bond in analogs **11b**–**11h** was performed in the presence of one atmosphere of hydrogen and Pd/C with EtOAc as the solvent. The reduction of **11d** was sluggish. Reduction of the benzene ring in **11d** was observed. After five days the 4-substituted 5,6,7,8-tetrahydro-isoquinoline product **12c** was isolated in 36% yield after chromatography. The structure of **13c** was confirmed by 2D-NMR and MS-analyses, see Supporting information. Examples of similar reductions of aromatic compounds have been reported [20]. Reduction of the heterocyclic ring in compound **11h** was also observed; the 5-substituted 1,2,3,4-tetrahydroquinoline compound **13g** was obtained in 33% yield after chromatographic purification. The reduction of the 16,17-double bond in the other analogs occurred smoothly; the TBS-protected compounds **12a**–**b** and **12d**–**12f** were obtained in 53–77% yields after chromatographic purification. Again, deprotection [19] of the TBS-group was achieved with TBAF in THF yielding the analogs **13a**–**13g**. In all cases, only one diastereomer was obtained after chromatography.

3. Biological evaluation

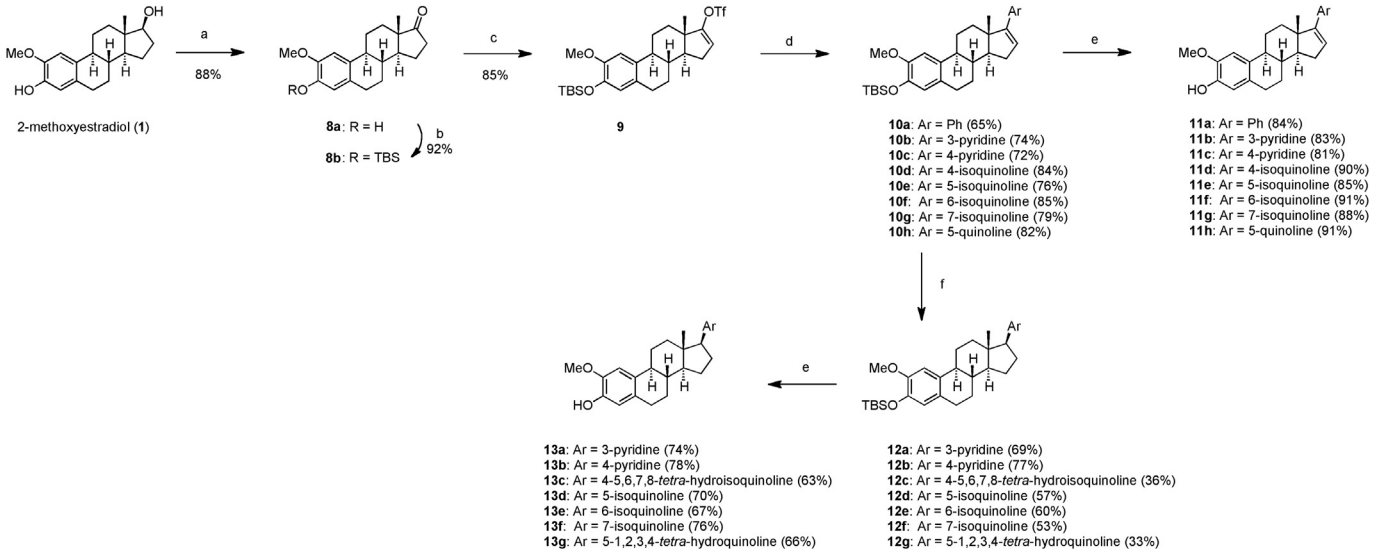
To investigate the anti-cancer effects of the prepared compounds, cell growth inhibition assay against the three different human cancer cell lines K562, OVCAR-3 and WM35 were performed [21]. The most cytotoxic compounds were also subjected to a tubulin polymerization inhibition assay [22]. All new analogs were also tested as inhibitors of angiogenesis [23]. Finally, some of the compounds were also evaluated as agonists against the estrogen receptor β [24,25]. PC-12 cells were co-transfected with ERE-luc and ER. Cells were treated with 10 μM of the analogs **11b**, **11d**, **13a**, **13c**, **13g** and 10 μM of 17β-estradiol and 2-methoxyestradiol for comparison. Luciferase activity was measured 24 h after initiation of the treatments. 2-Methoxyestradiol (**1**) was included for reference in all assays.

4. Results and discussion

All compounds with a 16,17-double bond tested in the K562 cell line, except **11a**, **11b** and **11g**, exhibited cell growth inhibition in the micromolar range, see Table 1.

The most potent analogs of the 16,17-unsaturated compounds in the leukemia cell line K562 were **11c**, **11d**, and **11f** with IC_{50}-values of 0.9, 0.5 and 0.4 μM, respectively. All three analogs were as active or slightly more active than the lead compound 2-methoxyestradiol (**1**), IC_{50} = 0.8 μM, see Table 1. The 5-substituted isoquinoline analog **11e** and the 5-substituted quinoline analog **11h** showed less potent inhibitory effects than **1**. The same trends were more or less observed both in the OVCAR-3 and the WM35 cell assays. Noteworthy, in the ovarian cancer cell line OVCAR-3, the 6-substituted isoquinoline analog **11f** was six-fold more potent than 2-methoxyestradiol (**1**) with IC_{50} = 0.2 μM. This analog was also the most potent compound (IC_{50} = 1.0 μM) subjected to the WM-35 melanoma cancer cell line assay. In both the OVCAR-3 and WM35 cell assays, analog **11d** also displayed cytotoxic effects in the low μM range.

The analogs **13b**–**13h** were then tested for their cytotoxic effects. All analogs, except **13f**, showed activity with IC_{50}-values in



Scheme 1. Synthesis of analogs **11a**–**11h** and analogs **13a**–**13g**. Reagents and conditions: (a) cyclohexanone, Al(O-*i*Pr)$_3$, toluene, Δ, 88%; (b) TBSCl, imidazole, DMF, rt., 92%; (c) KHMDS, PhN(SO$_2$CF$_3$)$_2$, THF, −78 °C, 85%; (d) ArB(OH)$_2$, Cs$_2$CO$_3$, Pd(Ph$_3$P)$_4$, THF:H$_2$O (1:1), 60 °C; (e) Pd/C, EtOAc, rt.; (f) TBAF, THF, rt.

the low micromolar range against the three human cancer lines. The tetrahydroisoquinoline analog **13c** showed cytotoxic activities in the three cell lines similar to the 4-substituted isoquinoline analog **11d**. The 6-substituted isoquinoline compound **13e** also displayed potent cytotoxic effects in all cell lines, comparably to its congener **11d**. The analog **13c** displayed potent activity ($IC_{50} = 0.5$ μM) against the ovarian cancer cell line. The absence of a double bond in the 16,17-position of the 3-substituted pyridine analog **13a** proved beneficial, as the cytotoxicity enhanced in all cell lines compared to compound **11b**.

All analogs except **11a**, **11b** and **11g** were tested in a tubulin inhibition assay. In this assay, the analog **13f** was inactive ($IC_{50} > 10$ μM). The most potent tubulin polymerization inhibitors were **11f** ($IC_{50} = 2.2$ μM), **13c** ($IC_{50} = 2.5$ μM) and **13e** ($IC_{50} = 2.1$ μM). All three compounds were as potent as 2-methoxyestradiol (**1**) ($IC_{50} = 2.2$ μM). The other compounds submitted to the tubulin polymerization inhibition assay were all less active than the lead compound **1**.

Analogs **11a**–**11h** and **13b**–**13h** were also tested as inhibitors in an angiogenesis growth assay. For the lead compound **1**, an IC_{50}-value of 3.2 μM was obtained. Interestingly, most compounds prepared, except **11a**, **11b** and **11g**, were active as inhibitors in this assay. Compounds **11c** ($IC_{50} = 0.6$ μM), **11d** ($IC_{50} = 0.8$ μM), **11f** ($IC_{50} = 0.7$ μM), **13c** ($IC_{50} = 1.1$ μM), **13d** ($IC_{50} = 1.3$ μM), and **13e** ($IC_{50} = 0.6$ μM) were all more potent than 2-methoxyestradiol (**1**). The other compounds tested showed IC_{50}-values in the 2.4–5.2 μM range. Apparently, the presence of the 6-isoquinoline heterocyclic ring is important for both inhibition of tubulin and angiogenesis, since the 6-isoquinoline substituted compounds **11f** and **13e** displayed strong inhibition in both assays. Moreover, the reduction of the 16,17-double bond proved beneficial for the 3-pyridine substituted analog **13a** since its congener **11b** was inactive in the angiogenesis growth assay.

2-Methoxyestradiol (**1**) has been reported to exhibit very weak estrogenic activity with a 500-fold and 3200-fold lower affinity for the estrogen receptor α and β, respectively, compared to β-estradiol [26]. Hence, the analogs **11b**, **11d**, **13a**, **13c**, and **13g** were tested as potential agonists towards the estrogen receptor. Estradiol was included as positive control and 2-methoxyestradiol (**1**) for comparison. Low agonist activity was observed towards the β-estrogen receptor using a 10 μM concentration of the compounds **11b**, **11d**, **13a**, **13c**, and **13g**, see Fig. 2. Therefore, the compounds reported in this study induce cell death independently of the estrogen receptor β.

5. Conclusions

Corey and co-workers reported that the position of the nitrogen atom in the isoquinoline group is essential for the potent anti-angiogenesis activity of analog **6** [16]. Both the 3-pyridine substituted compound **13a** and the 6-isoquinoline substituted analogs **11f** and **13e** reported herein, displayed higher anti-angiogenetic effects compared to 2-methoxyestradiol (**1**). The position of the nitrogen atom seems to be important for potent inhibition of angiogenesis for the compounds reported herein. The three analogs **11f**, **13a** and **13e** were all also inhibitors of tubulin polymerization. Tubulin polymerization inhibitors leading to decreased angiogenesis in cancer cells are of interest for the development of new remedies against cancer [27]. The most potent compounds presented herein provide new information for further SAR-studies towards the development of new anti-cancer agents.

6. Experimental

6.1. General methods

All reagents and solvents were used as purchased without further purification unless stated otherwise. Melting points are uncorrected. Analytical TLC was performed using silica gel 60 F254 aluminum plates (Merck). Flash column chromatography was performed on silica gel 60 (40–63 μm) produced by Merck. NMR spectra were recorded on a Bruker Avance DPX-300 MHz, DPX-400 MHz or DPX-600 MHz spectrometer for ^{1}H NMR, and 75 MHz, 101 MHz or 151 MHz for ^{13}C NMR. Coupling constants (*J*) are reported in hertz, and chemical shifts are reported in parts per million relative to $CDCl_3$ (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C). Mass spectra were recorded at 70 eV with Fison's VG Pro spectrometer. High-resolution mass spectra were performed with a VG Prospec mass spectrometer and with a Micromass Q-TOF-2™. The HPLC analyses were performed on an Agilent Technologies 1200 Series

Table 1
Biological evaluation of compounds **11a**–**11h** and **13a**–**13g**.

Compound	K562 cell assay IC_{50} (μM)[a]	OVCAR-3 cell assay IC_{50} (μM)[a]	WM35 cell assay IC_{50} (μM)[a]	Tubulin inhibition IC_{50} (μM)[b]	Anti-angiogenesis IC_{50} (μM)[a]
11a	>10	>10	>10	n.d.[c]	>10
11b	>10	>10	>10	n.d.	>10
11c	0.9	1.2	1.0	4.1	0.6
11d	0.5	0.4	0.7	4.8	0.8
11e	3.1	3.9	5.1	7.2	2.4
11f	0.4	0.2	1.0	2.2	0.7
11g	>10	>10	>10	n.d.	>10
11h	1.1	1.2	2.9	3.1	2.0
13a	1.1	0.8	1.9	5.1	5.2
13b	1.4	1.0	1.6	5.8	4.2
13c	0.5	0.4	0.9	2.5	1.1
13d	2.1	3.0	4.1	8.1	1.3
13e	0.7	0.5	0.9	2.1	0.6
13f	>10	>10	>10	>10	3.3
13g	1.3	1.5	1.9	3.7	3.3
2-ME	0.8	1.2	2.1	2.2	3.2

[a] Results of three experiments performed as triplicates.
[b] Results of three experiments performed as duplicates.
[c] n.d. = not determined.

instrument with an Eclipse XDB-C18 (5 mm 4.6 × 150 mm) column. 2-Methoxyestrone (**7**) and 3-*tert*-butyldimethylsiloxy-2-methoxyestrone (**8**) were prepared as previously reported [25]. Protocols for the preparation, physical and spectral data of the intermediates **9** and **10a**–**10h** and **13a**–**13g** are presented in the Supporting information.

6.2. General procedure for the preparation of analogs *11a*–*11h* and *13a*–*13g*

The TBS protected steroids **10a**–**h**, **12a**–**g** (0.3–0.6 mmol, 1 equiv.) were placed in a dry round-bottomed flask under argon atmosphere, and dissolved in dry THF. *Tetra-n*-butylammonium-fluoride (1 M in THF, 1.1 equiv.) was added dropwise. The reaction mixture was stirred at room temperature (16–18 h). Upon completion the reaction the mixture was poured into saturated aqueous $NaHCO_3$ (10 ml), and extracted with ethyl acetate (4 × 5 ml). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The residues were purified by chromatography (silica gel, 5–70% ethyl acetate in hexane) to give the pure products.

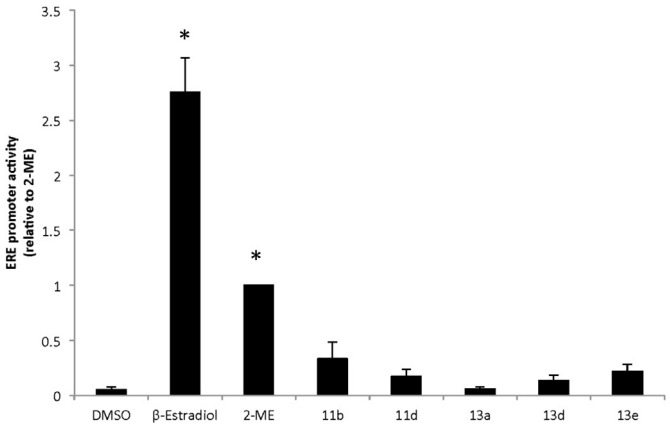


Fig. 2. Analogs show no agonist activity towards the estrogen receptor. Results are corrected for internal standard (*Renilla* luciferase activity) and presented as fraction of 2-methoxyestradiol. Bars show mean ± SE from 2 independent experiments based on $n = 3$–5 replicates. *$p < 0.05$ compared to DMSO control (ANOVA on ranks, Dunn's post hoc test).

6.3. (8S,9S,13S,14S)-2-methoxy-13-methyl-17-phenyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (*11a*)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.41 (m, 2H), 7.35 (t, $J = 7.5$ Hz, 2H), 7.29 (d, $J = 7.6$ Hz, 1H), 6.83 (s, 1H), 6.71 (s, 1H), 5.98 (m, 1H), 5.48 (s, 1H), 3.90 (s, 3H), 2.97–2.75 (m, 2H), 2.35 (m, 3H), 2.24 (m, 1H), 2.16 (m, 1H), 2.03–1.92 (m, 1H), 1.83 (m, 1H), 1.78–1.65 (m, 3H), 1.50 (m, 1H), 1.11 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.0, 144.7, 143.6, 137.5, 132.2, 129.7, 128.3, 127.3, 126.9, 126.8, 114.8, 108.0, 57.0, 56.2, 47.7, 44.5, 37.5, 35.7, 31.5, 29.1, 28.0, 27.0, 16.9. Eluent 20% EtOAc in hexane, Rf = 0.57, yield 91 mg, 84%, product: colorless solid, mp 135–137 °C. HRMS calcd. for $C_{25}H_{28}O_2$ $[M]^+$: 360.2089. Found 360.2078.

6.4. (8S,9S,13S,14S)-2-methoxy-13-methyl-17-(pyridin-3-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (*11b*)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (d, $J = 2.4$ Hz, 1H), 8.51 (dd, $J = 4.6$, 1.7 Hz, 1H), 7.72 (dt, $J = 7.9$, 1.9 Hz, 1H), 7.30–7.27 (m, 1H), 6.82 (s, 1H), 6.70 (s, 1H), 6.06 (s, 1H), 5.98 (s, 1H), 3.89 (s, 3H), 2.97–2.66 (m, 2H), 2.44–2.28 (m, 2H), 2.25–2.11 (m, 2H), 2.02–1.92 (m, 1H), 1.90–1.78 (m, 1H), 1.78–1.62 (m, 3H), 1.55–1.37 (m, 1H), 1.08 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 151.9, 147.8, 144.9, 143.8, 143.8, 134.0, 133.2, 131.8, 129.6, 129.4, 123.3, 115.1, 115.0, 108.1, 108.1, 56.9, 56.2, 47.8, 44.5, 37.4, 35.5, 31.6, 29.0, 27.9, 26.9, 16.9. Eluent 70% EtOAc in hexane, Rf = 0.50, yield 90 mg, 83%, product colorless solid, mp 203–208 °C decomp. HRMS calcd. for $C_{24}H_{27}NO_2$ $[M]^+$: 361.2033. Found 361.2035.

6.5. (8S,9S,13S,14S)-2-methoxy-13-methyl-17-(pyridin-4-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (*11c*)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (s, 2H), 7.31 (d, $J = 4.7$ Hz, 2H), 6.79 (s, 1H), 6.67 (s, 1H), 6.22 (s, 1H), 3.87 (s, 3H), 2.97–2.67 (m, 2H), 2.42–2.26 (m, 3H), 2.23 (d, $J = 8.4$ Hz, 1H), 2.21–2.10 (m, 1H), 1.98–1.89 (m, 1H), 1.86–1.75 (m, 1H), 1.73–1.60 (m, 3H), 1.53–1.39 (m, 1H), 1.26 (s, 1H), 1.09 (s, 3H) ^{13}C NMR (101 MHz, $CDCl_3$): δ 152.7, 149.5, 145.0, 144.9, 143.8, 131.9, 131.8, 129.6, 121.4, 114.9, 108.0, 56.9, 56.3, 47.7, 44.5, 37.4, 35.4, 31.7, 29.0, 27.9, 26.9, 16.9. Eluent 70% EtOAc in hexane, Rf = 0.47, yield 89 mg, 81% product light yellow

solid, mp 212–217 °C decomp. HRMS calcd. for $C_{24}H_{27}NO_2$ $[M]^+$: 361.2042. Found 361.2038.

*6.6. (8S,9S,13S,14S)-17-(isoquinolin-4-yl)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (**11d**)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19 (s, 1H), 8.39 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 8.01–7.97 (m, 1H), 7.77–7.65 (m, 1H), 7.67–7.57 (m, 1H), 6.76 (s, 1H), 6.69 (s, 1H), 5.91 (dd, J = 3.0, 1.6 Hz, 1H), 5.81 (s, 1H), 3.84 (s, 3H), 2.96–2.75 (m, 2H), 2.55–2.44 (m, 1H), 2.43–2.21 (m, 3H), 2.09–1.93 (m, 2H), 1.79–1.67 (m, 2H), 1.64–1.47 (m, 3H), 1.03 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 151.2, 149.7, 144.8, 143.8, 141.5, 135.8, 132.0, 132.0, 130.3, 129.6, 127.9, 127.2, 125.7, 114.9, 108.1, 77.5, 77.4, 77.2, 76.8, 56.8, 56.2, 50.1, 44.7, 37.9, 35.3, 32.3, 29.1, 28.2, 26.9, 16.6. Eluent 70% EtOAc in hexane, Rf = 0.58, yield 74 mg, 90%, product light yellow solid, mp 123–126 °C. HRMS calcd. for $C_{28}H_{29}NO_2$ $[M]^+$: 411.2203. Found 411.2205.

*6.7. (8S,9S,13S,14S)-17-(isoquinolin-5-yl)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (**11e**)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.26 (s, 1H), 8.51 (d, J = 5.9 Hz, 1H), 7.94–7.85 (m, 2H), 7.64–7.47 (m, 2H), 6.76 (s, 1H), 6.69 (s, 1H), 5.85 (dd, J = 3.1, 1.6 Hz, 1H), 3.84 (s, 3H), 2.99–2.76 (m, 2H), 2.61–2.46 (m, 1H), 2.42–2.22 (m, 3H), 2.11–1.90 (m, 2H), 1.80–1.66 (m, 2H), 1.66–1.46 (m, 3H), 1.02 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 152.6, 151.3, 144.9, 143.8, 142.8, 135.6, 135.2, 131.9, 131.0, 129.8, 129.6, 126.7, 126.5, 119.5, 115.0, 108.1, 56.8, 56.2, 50.0, 44.7, 37.8, 35.3, 32.2, 29.1, 28.1, 26.9, 16.7. Eluent 70% EtOAc in hexane, Rf = 0.55, yield 105 mg, 85%, product light yellow solid, mp 212–217 °C decomp. HRMS calcd. for $C_{28}H_{29}NO_2$ $[M]^+$: 411.2203. Found 411.2203.

*6.8. (8S,9S,13S,14S)-17-(isoquinolin-6-yl)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (**11f**)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.25 (s, 1H), 8.50 (d, J = 5.8 Hz, 1H), 7.97 (d, J = 8.6 Hz, 1H), 7.84 (s, 1H), 7.81–7.64 (m, 2H), 6.80 (s, 1H), 6.68 (s, 1H), 6.25 (s, 1H), 5.72 (s, 1H), 3.87 (s, 3H), 2.96–2.74 (m, 2H), 2.48–2.27 (m, 4H), 2.29–2.14 (m, 1H), 2.01–1.93 (m, 1H), 1.91–1.80 (m, 1H), 1.77–1.63 (m, 3H), 1.55–1.38 (m, 1H), 1.17 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.1, 150.5, 144.8, 143.8, 140.8, 140.7, 136.8, 131.8, 131.6, 129.6, 128.0, 128.0, 127.4, 123.0, 121.6, 114.9, 108.0, 57.0, 56.3, 48.0, 44.5, 37.4, 35.7, 31.8, 29.0, 27.9, 27.0, 17.0. Eluent 70% EtOAc in hexane, Rf = 0.56, yield 56 mg, 91%, product light yellow solid, mp 248–255 °C decomp. HRMS calcd. for $C_{28}H_{29}NO_2$ $[M]^+$: 411.2190. Found 411.2190.

*6.9. (8S,9S,13S,14S)-17-(isoquinolin-7-yl)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (**11g**)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.27 (s, 1H), 8.48 (d, J = 5.6 Hz, 1H), 7.99 (s, 1H), 7.82 (q, J = 8.6 Hz, 2H), 7.69 (d, J = 5.7 Hz, 1H), 6.81 (s, 1H), 6.68 (s, 1H), 6.18 (s, 1H), 5.85 (s, 1H), 3.87 (s, 3H), 2.96–2.71 (m, 2H), 2.49–2.27 (m, 4H), 2.27–2.12 (m, 1H), 2.02–1.92 (m, 1H), 1.92–1.80 (m, 1H), 1.79–1.65 (m, 3H), 1.55–1.37 (m, 1H), 1.17 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.0, 151.7, 144.9, 143.8, 141.1, 136.9, 135.3, 131.8, 131.2, 129.9, 129.6, 128.8, 126.5, 124.3, 120.9, 114.9, 108.1, 57.0, 56.3, 47.9, 44.5, 37.4, 35.8, 31.7, 29.0, 27.9, 27.0, 17.0. Eluent 70% EtOAc in hexane, Rf = 0.55, yield 54 mg, 88%, product light yellow solid, mp 253–261 °C decomp. HRMS calcd. for $C_{28}H_{29}NO_2$ $[M]^+$: 411.2203. Found 411.2203.

*6.10. (8S,9S,13S,14S)-2-methoxy-13-methyl-17-(quinolin-5-yl)-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-ol (**11h**)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (d, J = 3.9 Hz, 1H), 8.48 (d, J = 8.5 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.46–7.29 (m, 2H), 6.65 (s, 1H), 6.58 (s, 1H), 5.73 (s, 1H), 5.53 (s, 1H), 3.74 (s, 3H), 2.88–2.65 (m, 2H), 2.49–2.35 (m, 1H), 2.30–2.12 (m, 3H), 1.96–1.79 (m, 2H), 1.70–1.30 (m, 5H), 0.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 151.3, 148.4, 144.8, 143.8, 137.3, 136.7, 131.9, 131.5, 129.9, 129.6, 128.3, 126.8, 120.7, 114.8, 108.0, 56.9, 56.2, 50.1, 44.7, 37.8, 35.3, 32.2, 29.1, 28.1, 26.9, 16.7. Eluent 70% EtOAc in hexane, Rf = 0.54, yield 56 mg, 91%, product light yellow solid, mp 207–213 °C decomp. HRMS calcd. for $C_{28}H_{29}NO_2$ $[M]^+$: 411.2198. Found 411.2201.

*6.11. (8S,9S,13S,14S,17S)-2-methoxy-13-methyl-17-(pyridin-3-yl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-ol (**13a**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (d, J = 5.9 Hz, 2H), 7.61 (d, J = 7.6 Hz, 1H), 7.29 (s, 1H), 6.77 (s, 1H), 6.65 (s, 1H), 5.68 (s, 1H), 3.85 (s, 3H), 2.79 (s, 3H), 2.46–1.82 (m, 6H), 1.68 (d, J = 8.4 Hz, 1H), 1.47 (d, J = 10.9 Hz, 6H), 0.53 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.1, 147.2, 145.1, 144.0, 137.2, 136.7, 132.0, 129.9, 123.4, 115.1, 108.5, 56.5, 55.7, 54.9, 45.2, 44.6, 39.6, 37.9, 29.5, 28.3, 27.0, 26.4, 24.6, 13.2. Eluent 70% EtOAc in hexane Rf = 0.43, yield 55 mg, 74%, product colorless solid, mp 197–201 °C decomp. HRMS calcd. for $C_{24}H_{29}NO_2$ $[M]^+$: 363.2198. Found 363.2192.

*6.12. (8S,9S,13S,14S,17S)-2-methoxy-13-methyl-17-(pyridin-4-yl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-ol (**13b**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 4.2 Hz, 2H), 7.21 (d, J = 5.2 Hz, 2H), 6.79 (s, 1H), 6.67 (s, 1H), 3.87 (s, 4H), 2.79 (t, J = 8.1 Hz, 3H), 2.36–1.83 (m, 6H), 1.73 (d, J = 9.4 Hz, 1H), 1.60–1.35 (m, 6H), 0.53 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.6, 148.5, 144.8, 143.7, 131.7, 129.6, 124.4, 114.8, 108.2, 56.6, 56.2, 55.6, 45.2, 44.3, 39.3, 37.8, 29.2, 28.0, 26.7, 25.7, 24.3, 13.0. Eluent 70% EtOAc in hexane Rf = 0.39, yield 65 mg, 78%, product colorless solid, mp 203–206 °C decomp. HRMS calcd. for $C_{24}H_{29}NO_2$ $[M]^+$: 363.2198. Found 363.2207.

*6.13. (8S,9S,13S,14S,17S)-2-methoxy-13-methyl-17-(5,6,7,8-tetrahydroisoquinolin-4-yl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-ol (**13c**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.07 (s, 1H), 6.69 (s, 1H), 6.58 (s, 1H), 3.76 (s, 3H), 3.03 (t, J = 9.8 Hz, 1H), 2.85–2.47 (m, 6H), 2.30–2.09 (m, 2H), 2.06–1.96 (m, 2H), 1.95–1.59 (m, 6H), 1.59–1.22 (m, 7H), 0.64 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.1, 146.4, 146.3, 144.9, 143.8, 134.9, 132.7, 131.7, 129.6, 114.9, 108.3, 56.2, 55.8, 48.9, 46.1, 44.4, 39.3, 38.6, 29.2, 28.8, 28.0, 27.2, 26.8, 26.7, 24.6, 23.0, 22.1, 13.5. Eluent 70% EtOAc in hexane Rf = 0.42, yield 639 mg, 63%, product colorless solid, mp 127–130 °C. HRMS calcd. for $C_{28}H_{35}NO_2$ $[M]^+$: 417.2668. Found 417.2662.

*6.14. (8S,9S,13S,14S,17S)-17-(isoquinolin-5-yl)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-ol (**13d**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.34 (d, J = 6.2 Hz, 1H), 7.89 (d, J = 6.3 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 7.0 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 6.57 (s, 1H), 6.49 (s, 1H), 3.63 (s, 3H), 3.56 (t,

J = 9.6 Hz, 1H), 2.77–2.51 (m, 2H), 2.20–1.90 (m, 4H), 1.90–1.68 (m, 2H), 1.60–1.10 (m, 8H), 0.44 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.0, 144.9, 143.8, 141.9, 137.0, 136.3, 131.7, 129.9, 129.5, 129.2, 126.6, 126.4, 117.6, 115.0, 108.3, 56.2, 56.0, 49.9, 45.9, 44.4, 39.5, 38.7, 29.2, 28.4, 28.0, 26.9, 24.6, 13.4. Eluent 70% EtOAc in hexane Rf = 0.47, yield 49 mg 70%, product colorless solid. mp 207–210 °C decomp. HRMS calcd. for $C_{28}H_{31}NO_2$ $[M]^+$: 413.2355. Found 413.2361.

6.15. (8S,9S,13S,14S,17S)-17-(isoquinolin-6-yl)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a] phenanthren-3-ol (**13e**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.49 (d, J = 5.7 Hz, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.78–7.63 (m, 2H), 7.56 (d, J = 8.4 Hz, 1H), 6.78 (s, 1H), 6.66 (s, 1H), 3.84 (s, 3H), 3.05–2.96 (m, 1H), 2.90–2.73 (m, 2H), 2.37–2.19 (m, 3H), 2.17–2.05 (m, 1H), 2.05–1.92 (m, 2H), 1.81–1.71 (m, 1H), 1.66–1.20 (m, 7H), 0.56 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.3, 145.3, 144.8, 143.7, 141.6, 136.2, 131.8, 129.9, 129.7, 127.6, 127.1, 125.3, 121.0, 114.8, 108.3, 57.7, 56.2, 55.6, 45.5, 44.4, 39.4, 38.0, 29.2, 28.0, 26.8, 26.4, 24.4, 13.2. Eluent 70% EtOAc in hexane Rf = 0.49, yield 50 mg, 67%, product colorless solid, mp 235–237 °C. HRMS calcd. for $C_{28}H_{31}NO_2$ $[M]^+$: 413.2355. Found 413.2347.

6.16. (8S,9S,13S,14S,17S)-17-(isoquinolin-7-yl)-2-methoxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a] phenanthren-3-ol (**13f**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.31 (d, J = 5.7 Hz, 1H), 7.71 (s, 1H), 7.66 (d, J = 8.5 Hz, 1H), 7.61–7.47 (m, 2H), 6.61 (s, 1H), 6.49 (s, 1H), 3.68 (s, 3H), 2.84 (t, J = 9.7 Hz, 1H), 2.72–2.55 (m, 2H), 2.25–2.02 (m, 3H), 2.02–1.90 (m, 1H), 1.89–1.70 (m, 2H), 1.65–1.50 (m, 1H), 1.47–1.08 (m, 7H), 0.39 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.8, 144.8, 143.7, 142.0, 139.7, 135.4, 133.9, 131.8, 129.7, 128.5, 126.8, 125.9, 121.3, 114.8, 108.3, 57.4, 56.3, 55.5, 45.3, 44.4, 39.4, 38.0, 29.2, 28.0, 26.8, 26.5, 24.4, 13.1. Eluent 70% EtOAc in hexane Rf = 0.50, yield 35 mg 76%, product colorless solid, mp 241–248 °C decomp. HRMS calcd. for $C_{28}H_{31}NO_2$ $[M]^+$: 413.2355. Found 413.2362.

6.17. (8S,9S,13S,14S,17S)-2-methoxy-13-methyl-17-(1,2,3,4-tetrahydroquinolin-5-yl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-ol (**13g**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (t, J = 7.8 Hz, 1H), 6.78 (s, 1H), 6.74 (d, J = 7.5 Hz, 1H), 6.65 (s, 1H), 6.42 (d, J = 7.5 Hz, 1H), 3.85 (s, 3H), 3.34–3.21 (m, 2H), 3.13 (t, J = 9.7 Hz, 1H), 2.95–2.71 (m, 4H), 2.31–2.15 (m, 2H), 2.05–1.81 (m, 6H), 1.73–1.62 (m, 1H), 1.58–1.20 (m, 7H), 0.69 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.6, 144.3, 143.5, 140.4, 132.1, 129.7, 125.4, 121.7, 118.7, 114.7, 113.0, 108.2, 56.2, 55.8, 50.5, 45.9, 44.5, 41.7, 39.3, 38.5, 29.2, 29.0, 28.1, 26.9, 24.8, 24.6, 22.8, 13.6. Eluent 70% EtOAc in hexane Rf = 0.46, yield 18 mg 66%, product colorless solid, mp.131–133 °C. HRMS calcd. for $C_{28}H_{31}NO_2$ $[M]^+$: 417.2668. Found 417.2685.

6.18. Cancer cell growth inhibition (cytotoxicity)

The method applied was that described by Edmondson and coworkers [21] and is exemplified for the K562 cell line. K562 human chronic myelogeneous leukemia cells were cultivated in RMPI medium, free of antibiotics and containing 2-mercaptoethanol (2 μM) and L-glutamine (2 mM), supplemented with fetal calf serum (FCS) (10% v/v). The cells were adjusted to a concentration depending on their observed doubling time (ca. 40,000 cells/ml), in RPMI medium supplemented with FCS (10% v/v). The test compound was dissolved in DMSO. A solution of 100 μl in medium was added to 100 μl of cell solution (40,000 cells/ml) in a 96-well microtitre testplate (4 μl of the compound solution diluted in medium in order to reach decreasing concentrations). This series of dilutions was continued to afford samples at different concentrations leaving one cell solution free of drug acting as a control. The plates were incubated at 37 °C (5% CO_2 in air) for 5 days. The plate was then removed from the incubator and 50 μl of a solution of MTT (3 mg/ml in PBS) was added to each well. After incubation (37 °C, 5% CO_2 in air, 3 h) the medium was carefully removed from each well by suction and the resulting formazan precipitate re-dissolved in 200 μl DMSO. The optical density of each well was read at two wavelengths (λ 540 and 690 nm) using a Titretek Multiscan MCC/340 plate reader. After processing and analysis through the application of an 'in-house' software package, the results enabled the calculation of the drug dose required to inhibit cell growth by 50% (IC_{50} value), determined by graphical means as percentage of the control growth.

6.19. Inhibition of tubulin polymerization

The method applied was that described by Lawrence and coworkers [22]. Tubulin was isolated from porcine brain and stored at −78 °C. Samples were prepared directly in a 96-well microtitre plate that was pre-incubated at 4 °C in the fridge for 30 min and contained Mes buffer [128 μl (0.1 M Mes, 1 mM EGTA, 0.5 mM $MgCl_2$, distilled water, pH 6.6)], GTP (20 μl, 5 mM in Mes buffer), tubulin (50 μl, 11 mg/ml in Mes buffer) and the candidate drug (20 μl, with appropriate concentration of sample in DMSO). The tubulin/drug samples were immediately placed in a 96-well plate reader, alongside blank samples containing Mes buffer (198 μl) and the analogs (10 μl, same concentration). The absorbance (λ 350 nm) was recorded at 25 °C temperature for a period of 60 min, and the results were compared to untreated controls to evaluate the relative degree of change in optical density.

6.20. Inhibition of angiogenesis

Endothelial cell tube formation assay was modified from a method previously described [23]. Matrigel (12.5 mg/ml) was thawed at 4 °C, and 50 μl were quickly added to each well of a 96-well plate and allowed to solidify for 10 min at 37 °C. Once solid, the wells were incubated for 30 min with BAECs (30,000 cells/well). After adhesion of the cells, the medium was removed and replaced by fresh medium supplemented with compounds with five different concentrations ranging from 10 μM to 0.001 μM and incubated at 37 °C for 18 h. The tubes of growth were visualized with an inverted ZEISS microscope at a magnification of 10. The length of the capillary network was quantified with a map scale calculator (KURABO Angiogenesis Image Analysis Software).

6.21. Estrogen agonist assay

Rat pheochromocytoma PC-12 cells were grown in DMEM supplemented with 10% fetal calf serum, 5% horse serum, sodium pyruvate (1 mM), penicillin (100 IE/ml), and streptomycin (100 μg/ml). Cells were kept at 37 °C in a humidified atmosphere with 5% CO_2 and plated with a density of 7×10^4 cells/ml in plastic dishes. Two days after plating cells were exposed to medium containing 1 pM, 10 pM, 50 pM, 0.1 nM, 1 nM and 10 μM of 17-β-estradiol. For 2-methoxyestradiol and the other analogs concentrations of 1 nM, 100 nM, 500 nM, 1 μM, 2.5 μM 5 μM and 10 μM were used. 0.1% DMSO served as solvent control. Cells were harvested for measurement of luciferase after 24 h.

6.22. Transfection and promoter activity measurements

ERE-luc and estrogen receptor α (ERα) (0.5 μg each; kind gift from J. Milbrandt, Washington University, ST-Louis, MO) were transfected into the cells together with pRL-CMV (0.1 μg; purchased from Promega, Madison, WI) to a total of 1.1 μg DNA using 2 μl of Metafectene® Pro one day after plating. DMEM without serum was used for formation of the liposome/DNA-complexes. Medium was replaced with medium containing serum after 3 h. Cells were grown overnight and then treated with the compounds **11a**–**11h** and **13a**–**13g**. Luciferase was measured as previously described [25,26] after 24 h with 1 mM luciferin added by a dispenser in the luminometer (EG&G Berthold Lumat LB9507). *Renilla* luciferase was measured in the luminometer according to the manufacturer's protocol using Dual-Luciferase® Reporter Assay System (Promega, Madison, WI).

Acknowledgment

The School of Pharmacy and the Faculty of Mathematics and Natural Sciences, University of Oslo, is gratefully acknowledged for Ph.D.-scholarships to E. J. S. and I. B. S, respectively.

Appendix A. Supplementary data

Supplementary data related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2014.08.002.

References

[1] R.A. Stanton, K.M. Gernert, J.H. Nettles, R. Aneja, Drugs that target dynamic microtubules: a new molecular perspective, Med. Res. Rev. 31 (2011) 443–481.
[2] G.M. Cragg, P.G. Grothaus, D.J. Newman, Impact of natural products on developing new anti-cancer agents, Chem. Rev. 109 (2009) 3012–3043.
[3] M.A. Jordan, L. Wilson, Microtubule dynamics: mechanisms and regulation by microtubule-associated proteins and drugs in vitro and in cells, in: T. Fojo (Ed.), The Role of Microtubules in Cell Biology, Neurobiology, and Oncology, Humana Press, Totowa, 2008, pp. 47–81.
[4] J.A. Hadfield, S. Ducki, N. Hirst, A.T. McGown, Tubulin and microtubules as targets for anticancer drug, in: L. Meijer, A. Jézéquel, M. Roberge (Eds.), Progress in Cell Cycle Research, Cell Cycle Regulators as Therapeutic Targets, vol. 5, Life in Progress Editions, Roscoff, 2003, pp. 309–325.
[5] S. Bu, A. Blaukat, X. Fu, N.E. Heldin, M. Landstrøm, Mechanisms for 2-methoxyestradiol-induced apoptosis of prostate cancer cells, FEBS Lett. 531 (2002) 141–151.
[6] S. Verenich, P.M. Gerk, Therapeutic promises of 2-methoxyestradiol and its drug disposition challenges, Mol. Pharm. 7 (2010) 2030–2039.
[7] R.J. D'Amato, C.M. Lin, E. Flynn, J. Folkman, E. Hamel, 2-Methoxyestradiol, an endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 3964–3968.
[8] T. Fotsis, Y. Zhang, M.S. Pepper, H. Adlercreutz, R. Montesano, P.P. Nawroth, L. Schweigerer, The endogenous estrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumour growth, Nature 268 (1994) 237–239.
[9] M. Sattler, L.R. Quinnan, Y.B. Pride, J.L. Gramlich, S.C. Chu, G.C. Even, S.K. Kraeft, L.B. Chen, R. Salgia, 2-Methoxyestradiol alters cell motility, migration, and adhesion, Blood 102 (2003) 289–296.
[10] (a) C. Sweeney, G. Liu, C. Yiannoutsos, J. Kolesar, D. Horvath, M.J. Staab, K. Fife, V. Armstrong, A. Treston, C. Sidor, G. Wilding, A phase II multicenter, randomized, double-blind, safety trial assessing the pharmacokinetics, pharmacodynamics, and efficacy of oral 2-methoxyestradiol capsules in hormone-refractory prostate cancer, Clin. Cancer Res. 11 (2005) 6625–6633;
(b) W.L. Dahut, N.J. Lakhani, J.L. Gulley, P.M. Arlen, E.C. Kohn, H. Kotz, D. McNally, A. Parr, D. Nguyen, S.X. Yang, S.M. Steinberg, J. Venitz, A. Sparreboom, W.D. Figg, Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors, Cancer Biol. Ther. 5 (2006) 22–27;
(c) J. James, D.J. Murry, A.M. Treston, A.M. Storniolo, G.W. Sledge, C. Sidor, K.D. Miller, Phase I safety, pharmacokinetic and pharmacodynamic studies of 2-methoxyestradiol alone or in combination with docetaxel in patients with locally recurrent or metastatic breast cancer, Investig. New Drugs 25 (2006) 41–48;
(d) S.V. Rajkumar, P.G. Richardson, M.Q. Lacy, A. Dispenzieri, P.R. Greipp, T.E. Witzig, R. Schlossman, C.F. Sidor, K.C. Anderson, M.A. Gertz, Novel therapy with 2-methoxyestradiol for the treatment of relapsed and plateau phase multiple myeloma, Clin. Cancer Res. 13 (2007) 6162–6167.
[11] J.F. Peyrat, J.D. Brion, M. Alami, Synthetic 2-methoxyestradiol derivatives: structure-activity relationships, Curr. Med. Chem. 19 (2012) 4142–4156.
[12] (a) C. Moser, S.A. Lang, A. Mori, C. Hellerbrand, H.J. Schlitt, E.K. Geissler, W.E. Fogler, O. Stoeltzing, ENMD-1198, a novel tubulin-binding agent reduces HIF-1alpha and STAT3 activity in human hepatocellular carcinoma (HCC) cells, and inhibits growth and vascularization *in vivo*, BMC Cancer 8 (2008) 206–216;
(b) E. Pasquier, S. Sinnappan, M.A. Munoz, M. Kavallaris, ENMD-1198, a new analogue of 2-methoxyestradiol, displays both antiangiogenic and vascular-disrupting properties, Mol. Cancer Ther. 9 (2010) 1408–1418.
[13] (a) S. Aoki, Y. Watanabe, D. Tanabe, M. Arai, H. Suna, K. Miyamoto, H. Tsujibo, K. Tsujikawa, H. Yamamoto, M. Kobayashi, Structure–activity relationship and biological property of cortistatins, anti-angiogenic spongean steroidal alkaloids, Bioorg. Med. Chem. 15 (2007) 6758–6762;
(b) Y. Sato, H. Kamiyama, T. Usui, T. Saito, H. Osada, S. Kuwahara, H. Kiyota, Synthesis and anti-angiogenic activity of cortistatin analogs, Biosci. Biotechnol. Biochem. 72 (2008) 2992–2997;
(c) J. Shi, H. Shigehisa, C.A. Guerrero, R.A. Shenvi, C.C. Li, P.S. Baran, Stereodivergent synthesis of 17-α and 17-β-aryl steroids: application and biological evaluation of D-ring cortistatin analogues, Angew. Chem. Int. Ed. 48 (2009) 4328–4331.
[14] (a) S. Aoki, Y. Watanabe, M. Sanagawa, A. Setiawan, N. Kotoku, M. Kobayashi, Cortistatin A, B, C and D, anti-angiogenic steroidal alkaloids, from the marine sponge *Corticium simplex*, J. Am. Chem. Soc. 128 (2006) 3148–3149;
(b) Y. Watanabe, S. Aoki, D. Tanabe, A. Setiawan, M. Kobayashi, Cortistatins E, F, G and H, four novel steroidal alkaloids from marine sponge *Corticium simplex*, Tetrahedron 63 (2007) 4074–4079;
(c) A. Aoki, Y. Watanabe, D. Tanabe, A. Setiawan, M. Arai, M. Kobyashi, Cortistatins J, K, L, novel abeo-9(10-19)-androstane-type steroidal alkaloids with isoquinoline unit, from marine sponge *Corticium simplex*, Tetrahedron Lett. 48 (2007) 4485–4488.
[15] R.A. Shenvi, C.A. Guerrero, J. Shi, C.C. Li, P.S. Baran, Synthesis of (+)-cortistatin A, J. Am. Chem. Soc. 130 (2008) 7241–7243;
(b) K.C. Nicolaou, Y.P. Sun, X.S. Peng, D. Polet, D.Y.K. Chen, Total synthesis of (+)-cortistatin A, Angew. Chem. Int. Ed. 47 (2008) 7310–7313;
(c) H.M. Lee, C. Nieto-Oberhuber, M.D. Shair, Enantioselective synthesis of (+)-cortistatin A, a potent and selective inhibitor of endothelial cell proliferation, J. Am. Chem. Soc. 130 (2008) 16864–16866;
(d) K.C. Nicolaou, X.S. Peng, Y.P. Sun, D. Polet, B. Zou, C.S. Lim, D.Y. Chen, Total synthesis and biological evaluation of cortistatins A and J and analogues thereof, J. Am. Chem. Soc. 131 (2009) 10587–10597;
(e) A.N. Flyer, C. Si, A.G. Myers, Synthesis of cortistatins A, J, K and L, Nat. Chem. 2 (2010) 886–892;
(f) E.M. Simmons, A.R. Hardin-Narayan, X. Guo, R. Sarpong, Formal total synthesis of (+)-cortistatin A, Tetrahedron 66 (2010) 4696–4700;
(g) M.G. Nilson, R.L. Funk, Total synthesis of (±)-cortistatin J from furan, J. Am. Chem. Soc. 133 (2011) 12451–12453;
(h) J. Shi, G. Manolikakes, C.H. Yeh, C.A. Guerrero, R.A. Shenvi, H. Shigehisa, P.S. Baran, Scalable synthesis of cortistatin A and related structures, J. Am. Chem. Soc. 133 (2011) 8014–8027;
(i) S. Yamashita, K. Iso, K. Kitajima, M. Himuro, M. Hirama, Total synthesis of cortistatins A and J, J. Org. Chem. 76 (2011) 2408–2425.
[16] B. Czako, L. Kurti, A. Mammoto, D.E. Ingber, E.J. Corey, Discovery of potent and practical antiangiogenic agents inspired by cortistatin A, J. Am. Chem. Soc. 131 (2009) 9014–9019.
[17] (a) L. Evensen, K. Odlo, D.R. Micklem, Littlewood-Evans, J. Wood, C. Kuzniewski, K.A. Altmann, T.V. Hansen, J.B. Lorens, Contextual compound screening for improved therapeutic discovery, Chem. Bio. Chem. 14 (2013) 2512–2518;
(b) Ø.W. Akselsen, K. Odlo, J.J. Cheng, G. Maccari, M. Botta, T.V. Hansen, Synthesis, biological evaluation and molecular modeling of 1,2,3-triazole analogs of combretastatin A-1, Bioorg. Med. Chem. 20 (2012) 234–242;
(c) Ø.W. Akselsen, T.V. Hansen, *ortho*-Formylation of estrogens. Synthesis of the anticancer agent 2-methoxyestradiol, Tetrahedron 67 (2011) 7738–7742;
(d) K. Odlo, J. Fournier dit Chabert, S. Ducki, O.A. Gani, I. Sylte, T.V. Hansen, 1,2,3-Triazole analogues of combretastatin A-4 as potential microtubule-binding agents, Bioorg. Med. Chem. 18 (2010) 6874–6885;
(e) K. Odlo, J. Hentzen, J. Fournier dit Chabert, S. Ducki, O.A. Gani, I. Sylte, M. Skrede, V.A. Flørenes, T.V. Hansen, 1,5-Disubstituted 1,2,3-triazoles as cis-restricted analogues of combretastatin A-4: synthesis, molecular modeling and evaluation as cytotoxic agents and inhibitors of tubulin, Bioorg. Med. Chem. 16 (2008) 4829–4838.
[18] N. Miyaura, A. Suzuki, Palladium-catalyzed cross-coupling reactions of organoboron compounds, Chem. Rev. 95 (1995) 2457–2483.
[19] E.J. Corey, A. Venkateswarlu, Protection of hydroxyl groups as *tert*-butyldimethylsilyl derivatives, J. Am. Chem. Soc. 94 (1972) 6190–6191.
[20] (a) J.J. Ginos, Precursors to apomorphine and morphinan analogs. Catalytic reduction of quinoline and isoquinoline, J. Org. Chem. 40 (1975) 1191–1195;
(b) F.W. Vierhapper, E.L. Eliel, Selective hydrogenation of quinoline and its homologs, isoquinoline, and phenyl-substituted pyridines in the benzene ring, J. Org. Chem. 40 (1975) 2729–2734;
(c) F.W. Vierhapper, E.L. Eliel, Selective reduction of the benzene ring in quinolines and isoquinolines, J. Am. Chem. Soc. 96 (1974) 2256–2257.

[21] J.M. Edmondson, L.S. Armstrong, A.O. Martinez, A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures, Tissue Cult. Methods 11 (1988) 15–17.
[22] N.J. Lawrence, A.T. McGown, S. Ducki, J.A. Hadfield, The interaction of chalcones with tubulin, Anticancer Drug Des. 15 (2000) 135–141.
[23] M.K. Jones, H. Wang, B.M. Peskar, E. Levin, R.M. Itani, I.J. Sarfeh, A.S. Tarnawski, Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing, Nat. Med. 5 (1999) 1418–1423.
[24] R.E. Paulsen, C.A. Weaver, T.J. Fahrner, J. Milbrandt, Domains regulating transcriptional activity of the inducible orphan receptor NGFI-B, J. Biol. Chem. 267 (1992) 16491–16496.
[25] B.O. Strøm, P. Aden, G.H. Mathisen, J. Lomo, S. Davanger, R.E. Paulsen, Transfection of chicken cerebellar granule neurons used to study glucocorticoid receptor regulation by nuclear receptor 4A (NR4A), J. Neurosci. Methods 193 (2010) 39–46.
[26] T.M. LaVallee, X.H. Zhan, C.J. Herbstritt, E.C. Kough, S.J. Green, V.S. Pribluda, 2-Methoxyestradiol inhibits proliferation and induces apoptosis independently of estrogen receptors α and β, Cancer Res. 62 (2002) 3691–3697.
[27] G.R. Cragg, D.G.I. Kingston, D.J. Newman, Development and future trends in anticancer natural products drug discovery, in: G.R. Cragg, D.G.I. Kingston, D.J. Newman (Eds.), Anticancer Agents from Natural Products, CRC Press/Taylor & Francis, Boca Raton, FL, 2012, pp. 699–727.