RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2015, **5**, 32497

Synthesis, biological evaluation and molecular modeling of new analogs of the anti-cancer agent 2-methoxyestradiol: potent inhibitors of angiogenesis†

Eirik Johansson Solum,[a] Jing-Jy Cheng,*[bc] Ingebrigt Sylte,[d] Anders Vik[a] and Trond Vidar Hansen*[a]

Received 27th February 2015
Accepted 27th March 2015

DOI: 10.1039/c5ra03570h

www.rsc.org/advances

The synthesis, cytotoxicity, inhibition of tubulin polymerization and anti-angiogenic effects of 10 analogs of 2-methoxyestradiol are reported. These efforts revealed that the analog with a 4-pyridine ring in the 17-position, in combination with 2-ethyl- and 3-sulfamate substituents on the steroid A-ring, is the most interesting anti-cancer agent. This compound showed potent inhibitory effects against angiogenesis (IC_{50} = 0.1 ± 0.02 μM) and selective cytotoxic effects towards the CEM, H460 and HT-29 cancer cell lines, with no cytotoxicity observed against the healthy VERO cell line. The most interesting analog also displayed inhibition of tubulin polymerization (IC_{50} = 4.3 μM) almost as potent as 2-methoxyestradiol (IC_{50} = 3.5 μM). Molecular modeling experiments showed that this analog interacts within the colchicine-binding site of β-tubulin *via* multiple bonding with several amino acids. These observations provide support that the cytotoxic and anti-angiogenic effects observed for this novel analog are, at least in part, mediated by binding to tubulin.

Introduction

Several steroids, exemplified by compounds **1–6** in Fig. 1, display anti-cancer effects and some have entered the drug market. The endogenous steroid 2-methoxyestradiol (2-ME, **1**) exhibits anti-vascular effects[1] and anti-angiogenic activities.[2] In 1989, Seegers *et al.* reported that high micro-molar concentrations of **1** affected dividing cancer cells.[3] Five years later, D'Amato *et al.* reported that **1** was a tubulin polymerization inhibitor and a competitive inhibitor of colchicine.[4] A number of biological studies followed which showed that the steroid **1** possesses many interesting anti-cancer effects without any undesirable estrogen activity.[5,6] 2-ME (**1**) has entered several clinical trial development programs,[7] and some structural-activity relationship (SAR) studies have been conducted with **1** as the lead compound.[8] ENMD-1198 (**2**) is one example that emerged from these efforts.[9] Another anti-cancer steroid is abiraterone (**3**) that is used, as its acetate prodrug, in combination with prednisone (**4**) against metastatic castration-resistant prostate cancer. Abiraterone (**3**) is marketed under the trade name Zytiga™,[10] see Fig. 1.

Recently we reported that the two compounds **5** and **6**, see Fig. 1, with a 4- or a 6-substituted isoquinoline ring in the 17-position of the steroid skeleton of 2-ME (**1**), respectively, showed inhibition of tubulin polymerization and anti-angiogenic effects in the low micro-molar range.[11] It has been reported that substituting the methoxy group with an ethyl group in the 2-position of **1** has resulted in new analogs with interesting anti-cancer effects.[12] Based on our previous findings, we wanted to conduct a SAR-study substituting the 2-methoxy group with an ethyl group, as well as introducing an aryl moiety in the 17-position of **1**. Previously, it has also been reported that replacing the phenol in the A-ring of 2-ME (**1**) with a sulfamate group has resulted in enhanced cytotoxicity.[13] Hence, we wanted to include also this substituent in our studies. Overall, this resulted in the design of the novel steroids **7a–7e** and **8a–8e**. The synthetic work, molecular modeling studies and the biological evaluation of these novel 2-ME (**1**) analogs are presented herein.

[a]*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, PO Box 1068 Blindern, N-0316 Oslo, Norway. E-mail: e.j.solum@farmasi.uio.no; anders.vik@farmasi.uio.no; t.v.hansen@farmasi.uio.no; Fax: +47 22855947; Tel: +47 22857450*

[b]*National Research Institute of Chinese Medicine, 155-1 Li-Nung Street, Section 2, Shih-Pai, Taipei, Taiwan. E-mail: verona@nricm.edu.tw; Fax: +886-2-2825-0743; Tel: +886-2-2820-1999-3671*

[c]*Institute of Biophotonics, National Yang-Ming University, Taipei 112, Taiwan*

[d]*Department of Medical Biology, Faculty of Health Sciences, UiT – The Arctic University of Norway, 9037 Tromsø, Norway. E-mail: ingebrigt.sylte@uit.no*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ra03570h

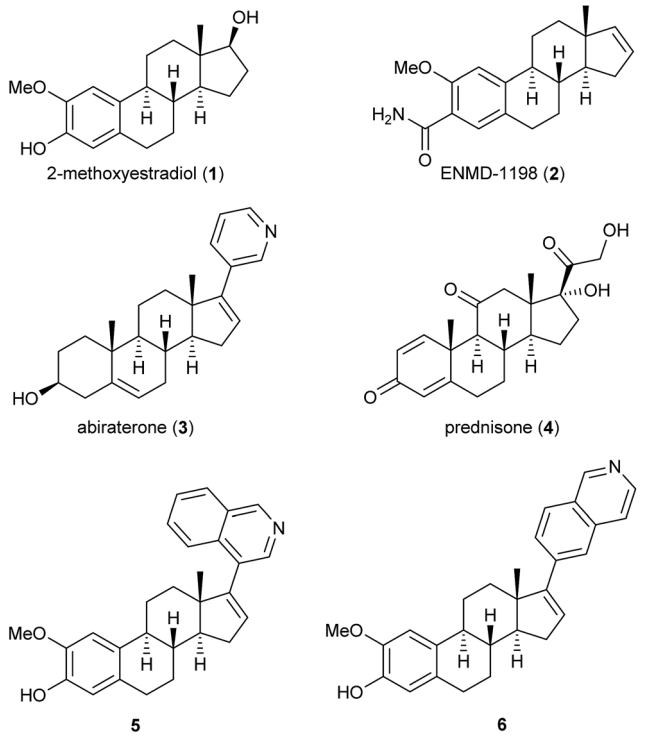


Fig. 1 Examples of steroids with anti-cancer effects.

Results and discussions

Chemistry

The synthesis of compounds **7a–7e** and **8a–8e** commenced with the *ortho*-formylation reaction[14] with estradiol **9** as the substrate, in the presence of a mixture of para-formaldehyde, $MgCl_2$ and Et_3N in refluxing THF. As previously reported,[15] the regioisomeric ratio was observed to be 13 : 1 in favor of the desired salicylaldehyde **10**. Regioisomeric pure product was obtained in 81% yield after chromatography. Then a Wittig-reaction between **10** and the yield of methyl-triphenylphosphonium bromide, the latter obtained after reaction with sodium *tert*-butoxide, afforded the styrene **11**. Reduction of the double bond in **11** with hydrogen in the presence of palladium on carbon gave the desired 2-ethyl substituted estradiol **12** in 61% yield over the three steps (Scheme 1). Further modification of the 17-position was achieved in a three-step protocol. Oxidation of **12**, followed by TBS-protection of the phenol in **13**, yielded the ketone **14**. Compound **14** was converted, as previously reported,[16] to the enol triflate **15**. The triflate **15** was reacted in a Suzuki–Miyaura cross-coupling reaction with the enumerated boronic acids (Scheme 1), affording the desired products **16a–16e** in 73–84% yields. Finally, deprotection of the TBS-group using an excess of tetra-*n*-butyl ammonium fluoride followed by purification by column chromatography yielded the desired 2-ethyl estrone analogs **7a–7e** as stereoisomeric pure products. The introduction of the sulfamate in the 3-position of **1** was achieved by reacting the phenol with sulfamoyl chloride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) in dichloromethane as solvent. The products **8a–8e** were obtained in 69–76% yields.

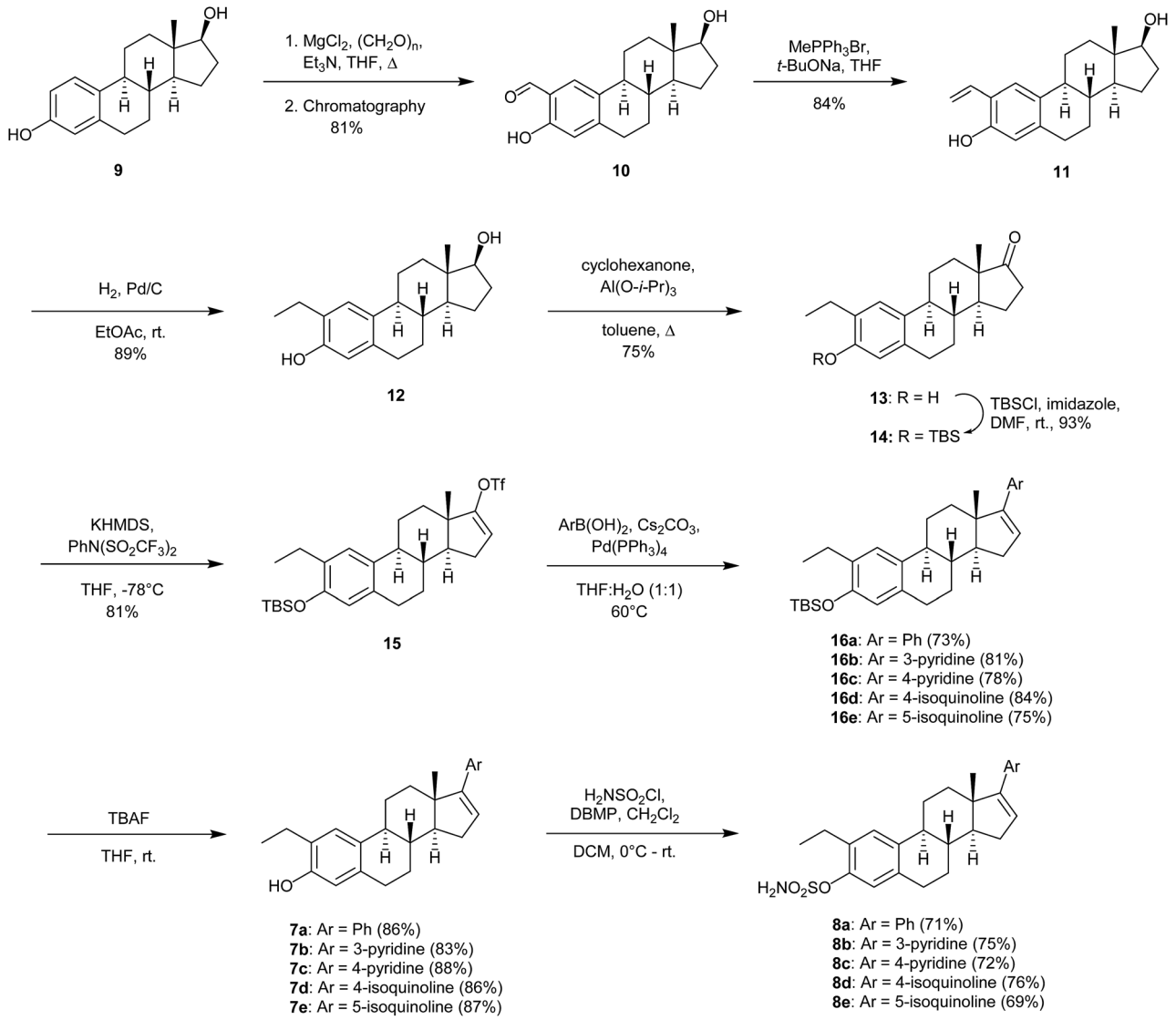
Biological evaluations

Cytotoxicity. The products **7a–7e** and **8a–8e** were evaluated, together with 2-ME (**1**), for their cytotoxic effects[17] in three different cancer cell lines, and also against the non-cancer VERO cell line. Cytotoxicity of each compound was determined using the SRB assay and the O.D. of each compound for the SRB assay was obtained. The IC_{50}-value was calculated from the curve between OD and concentrations. The results are compiled in Table 1.

The two compounds **7a** and **8a** exhibited potent cytotoxic effects against all of the cancer cell lines. Unfortunately, these compounds also inhibited the growth of the VERO cell line. The phenyl ring attached at the 17-position of the steroid skeleton is apparently detrimental for any selective inhibition towards cancer cell growth. Among the other analogs tested, several of the compounds proved to be active, especially towards the human CEM leukemia cell line. Noteworthy, all of the compounds except 2-ME (**1**) exhibited lower activity towards the lung cancer cell line H460 and the colon cancer cell line HT-29. Compound **8c** showed good activity towards the CEM leukemia cell line ($IC_{50} = 8.0 \pm 1.4$ μM). To our delight, this compound did not exhibit any activity towards the VERO cell line. Analogs **8a–8e** exhibited better cytotoxic properties than **7a–7e**. Among these analogs, the only active analogs were compounds **7b** and **7e** that showed cytotoxic effects in the CEM cell line. The introduction of the 2-ethyl and the sulfamate substituents gave better selectivity as well as cytotoxicity against the human CEM leukemia cell line.

Inhibition of tubulin polymerization. All compounds were submitted to the tubulin polymerization assay[18] at 10 μM with colchicine and paclitaxel as positive and negative controls, respectively (Table 1). The inhibition rate was calculated as described in the ESI.† The IC_{50}-value against tubulin polymerization inhibition was determined for each of the compounds **8a–8d**. Among these compounds, **8c** displayed the most potent inhibition of tubulin polymerization ($IC_{50} = 4.3$ μM). For the lead compound, 2-ME (**1**), the IC_{50}-value was determined as 3.5 μM. The IC_{50}-values for **8a**, **8b** and **8d** were determined to be 8.1 μM, 6.1 μM and 7.7 μM, respectively.

Anti-angiogenic activity. The anti-angiogenetic activity of the prepared analogs was tested using an endothelial cell tube formation assay[11,17] Among the prepared analogs, seven proved more active than **1**. Interestingly, the three compounds **8b**, **8c** and **8d** were considerably more potent than 2-ME (**1**), with IC_{50}-values of 0.2 ± 0.03, 0.1 ± 0.02 and 0.7 ± 0.04 μM, respectively. In the anti-angiogenetic assay, the IC_{50}-value for **1** was determined to be 3.2 ± 0.22 μM. The rest of the compounds did not show any anti-angiogenetic activity ($IC_{50} > 10$ μM). The substitution pattern with an ethyl and a sulfamate group in the 2- and the 3-position, respectively, on the A-ring, gave the most potent anti-angiogenetic compounds. However, removing the sulfamate group in the 2-ethyl analogs, as in compounds **7a–7e**, reduced the activity in the endothelial cell tube formation assay.



Scheme 1 Synthesis of estrogen analogs **7a–7e** and **8a–8e**.

The most potent compounds in this assay were compounds **8b** and **8c** that revealed anti-angiogenetic effects in the nanomolar range, with IC_{50}-values of 0.2 ± 0.03 and 0.1 ± 0.02 μM, respectively.

Molecular modeling. The Internal Coordinate Mechanics (ICM) program[19] was used for docking of compounds **8a–8d** and 2-Me (**1**) into the β-subunit of tubulin using the 1SA0 X-ray structure.[20] The docking showed that the orientations of the lead compound 2-ME (**1**) and the compounds **8a–8d** are similar in the colchicine binding pocket of tubulin (Fig. 2). It has previously been reported that 2-ME (**1**), in the micromolar range, is a competitive inhibitor of colchicine.[4] Several studies have confirmed this observation.[21,22] Our molecular modeling studies revealed that the methoxy-group of 2-ME (**1**) interacts with the side chain of Thr314, the backbone amino acids of Val315 and Asn350, while the phenol group in **1** interacts with the side chain of Lys352 and the backbone amino group of Asn349. The docking indicates that a hydrogen bond between the phenol group in the A ring of 2-ME (**1**) and the side chain of Lys352 is very likely. The hydroxyl group at the C-17 position in **1** interacts with the side chain of Cys241 (Fig. 2) also by hydrogen bonding.

The molecular modeling studies of the novel analogs showed that the sulfamate group present in compounds **8a–8d** interacts within a pocket consisting Asn349 (backbone oxygen), Asn258 (side chain oxygen) and Lys352 (terminal side chain hydrogens). Hydrogen bonding interactions were formed between the side chain of Lys352 and the sulfamate group (Fig. 2) and all compounds **8a–8d**. The 2-ethyl group of **8a–8d** form hydrophobic interactions with Thr314, Val315, Asn350 and the side chain of Lys352. The phenyl group of **8a**, the 3-pyridine group of **8b**, the 4-pyridine group of **8c**, and the 4-isoquinoline group of **8d** all interacted in a hydrophobic pocket consisting of Val238, Cys241, Leu248, Ala250, Leu255, Ala316, Val318, Ala354, and Ile378.

Table 1 Biological evaluation of compounds **7a–7e** and **8a–8e**

Compound	CEM cell assay IC_{50}[a] (μM)	H460 cell assay IC_{50}[a] (μM)	HT-29 cell assay IC_{50}[a] (μM)	VERO cell assay IC_{50}[a] (μM)	Anti-angiogenesis IC_{50}[a] (μM)	Tubulin polymerization inhibition[b] (%)	Tubulin polymerization inhibition IC_{50}[c] (μM)
Colchicine	n.d.	n.d.	n.d.	n.d.	n.d.	100	n.d.[d]
Paclitaxel	n.d.	n.d.	n.d.	n.d.	n.d.	0	n.d.
7a	12.9 ± 2.1	16.9 ± 3.1	19.3 ± 2.3	29.8 ± 2.6	>10	41	n.d.
7b	66.9 ± 6.1	>256	>256	>256	>10	51	n.d.
7c	>256	>256	>256	>256	>10	38	n.d.
7d	>256	>256	>256	>256	>10	54	n.d.
7e	160.9 ± 23.5	>256	>256	>256	>10	58	n.d.
8a	5.4 ± 0.7	9.3 ± 2.3	7.9 ± 1.1	9.3 ± 1.7	>10	109	8.1
8b	86.5 ± 9.8	>256	49.7 ± 5.9	>256	0.2 ± 0.03	158	6.1
8c	8.0 ± 1.4	110.3 ± 13.6	28.3 ± 3.7	>256	0.1 ± 0.02	109	4.3
8d	46.2 ± 5.4	>256	>256	>256	0.7 ± 0.04	36	7.7
8e	67.0 ± 9.5	>256	160.9 ± 18.5	>256	>10	56	n.d.
2-ME (**1**)	84.9 ± 9.7	63.3 ± 7.1	>256	>256	3.2 ± 0.22	138	3.5

[a] Results of three experiments performed as triplicates. [b] Determined at 10 μM. [c] Results of two experiments performed as triplicates. [d] n.d = not determined.

Discussions

The introduction of an ethyl group in the 2-position of 2-ME (**1**) has previously been reported to afford potent inhibitors against angiogenesis.[12,23] This knowledge, together with the introduction of new analogs substituted in the 17-position, gave several potent cytotoxic agents that also inhibited tubulin depolymerization. Moreover, introducing the sulfamate group in the 3-position enhanced both the cytotoxicity and the tubulin inhibition, but also resulted in better inhibition of angiogenesis, as seen for compounds **8a–8d**. Overall, compound **7a** displayed the most potent cytotoxic effects. However, towards the development of new anti-cancer agents, the most promising candidate is compound **8c**. This compound showed cytotoxic effects in all three cancer cell lines, but no such effects against the VERO cells. In addition, this compound also exhibited very potent anti-angiogenic activities in the nanomolar range. Corey and co-workers have reported that the position of the nitrogen atom in the heterocyclic substituent at the C-17 position in some steroids is essential for potent anti-angiogenesis activity.[24] Moreover, It has been reported that sterols interacts with several proteins and biological targets.[25] The compounds reported herein may exhibit their mode of actions by interacting with multiple biological targets. The anti-angiogenic activities displayed by the new analogs reported herein are also dependent on the substitution pattern. Compound **8c** also displayed inhibition of tubulin polymerization (IC_{50} = 4.3 μM) in the same range as for 2-ME (**1**), IC_{50} = 3.5 μM. Compounds **8a**, **8b** and **8d** showed slightly less effects towards inhibition of tubulin than both **1** and **8c**.

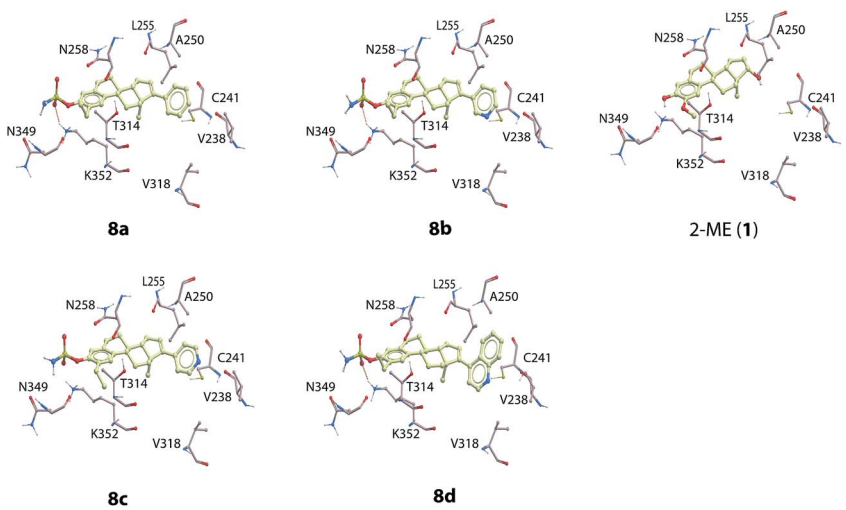


Fig. 2 The docked compounds (**8a–8d** and 2-ME (**1**)) in the colchicine binding site of β-tubulin. The most important amino acids for ligand binding are included in the figure. Color coding of atoms in the compounds and the amino acids: red; oxygen, blue; nitrogen, yellow: carbon, dark-yellow: sulfur. Ligand hydrogen atoms are not displayed.

The molecular docking showed that the virtual ligand screening (VLS) scoring function values for of compounds **8a–8d** with tubulin were all in the same range as the scoring of 2-ME. The new analogs **8a–8d** exhibited similar binding mode in the colchicine binding pocket as the lead compound 2-ME (**1**) (Fig. 2). These observations were also reflected in the comparable IC_{50}-values obtained from the tubulin inhibition studies, see Table 1. However, compound **8a** had a slightly better scoring in tubulin than **8a–8d** indicating that changing the phenyl group of compound **8a** into a 3- or a 4-substituted pyridine ring, as for **8b** and **8c**, respectively, or into a 4-isoquinoline group as for **8d**, decreased the binding affinity to β-tubulin compared to **8a**. These parts of the compounds interact in a hydrophobic region of β-tubulin and the phenyl group of **8a** seems to obtain more favorable interactions than the nitrogen containing ring systems (Fig. 2).

Conclusion

In total, 10 new analogs of 2-ME (**1**) have been prepared using the *ortho*-formylation and the Suzuki–Miyaura reactions. All analogs were evaluated for their cytotoxic effects, as well as their anti-angiogenic activity and inhibition of tubulin polymerization. Compound **8c** exhibited more potent cytotoxic and anti-angiogenic effects than **1**. This compound has a sulfamate group in the 3-position of ring A in the steroid skeleton. Such sulfamates of estrogens have been reported to be multi-targeted anti-cancer agents,[22] and the biological effects of the new analogs reported herein may also arise *via* such mechanisms. Such types of anti-cancer agents are of current interest towards the potential development of remedies against cancer, including leukemia.[26] The results presented herein provide new information on such new lead compounds.

Experimental

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 instrument with an Eclipse XDB-C18 (5 mm 4.6 × 150 mm) column. Optical rotations were measured using a 1 ml cell with 1.0 dm path length on Perkin-Elmer 341 polarimeter in dedicated solvent. Protocols for the preparation, physical and spectral data of the intermediates **10–16** and products **7a–7e** and **8a–8e** are presented in the ESI.†

Cancer cell growth inhibition

To assess cell viability, the AlamarBlue® (AB) assay (dye purchased from Biosource International, Nivelles, Belgium) was used as previously described.[11,17] This involved aspirating medium at the end of each treatment period and adding 100 μl of fresh medium containing 10% v/v AB to control and treated wells. Plates were incubated at 37 °C for six hours prior to measuring the absorbance at 540 nm and at 595 nm wavelengths using a spectrophotometric plate reader (DYNEX Technologies, USA). Experimental data were normalized to control values.

Inhibition of tubulin polymerization

The method applied was that described by Lawrence and coworkers.[18] The assay was performed using a commercial kit (Cytoskeleton Inc., Denver, USA). Briefly, samples were prepared directly in a 96-well microtiter 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^{-1} in Mes buffer) and the test compound (20 μl in DMSO). The tubulin and samples of test compounds were immediately placed in a 96-well plate reader, alongside the blank samples containing Mes buffer (198 μl) and the analogs (10 μl, same concentration). The absorbance (λ 340 nm) was recorded at 25 °C temperature for a period of 60 min. The polymerization curve was made as OD of each sample (*Y* axis) *vs.* recording time (*X* axis). The AUC (area under curve) between zero to 30 minutes was obtained to present the polymerization degree using Sigmaplot software. After AUC was obtained, the average AUC was calculated (see ESI†) to get the inhibition percentage. Colchicine was set as 100% inhibition and paclitaxel as 0%. The IC_{50}-value was calculated after obtaining the curve equation of inhibition % (*Y* axis) and concentration (*X* axis) using Excel.

Inhibition of angiogenesis

The method applied was essential that described previously.[17] Endothelial cell tube formation assay was modified from a method previously described.[11] Matrigel (12.5 mg ml^{-1}) was thawed at 4 °C, and 50 μl was 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 endothelial cell (30 000 cells per 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 tube formation areas were obtained using KURABO Angiogenesis Image Analysis Software. The length of the capillary network was quantified with a map scale calculator (KURABO Angiogenesis Image Analysis Software). Inhibition curve was obtained between areas and concentrations to get the IC_{50}-value.

Molecular modelling

The Internal Coordinate Mechanics (ICM) program[19] was used for docking of compounds **8a–8d** and 2-ME (**1**) into the β-subunit of tubulin. The docking studies were performed with the tubulin structure from the X-ray complex of tubulin with colchicine (PDB id: 1SA0).[20] Crystallographic water molecules, ions and co-crystallized inhibitors were removed from the X-ray complexes and hydrogen atoms were added and optimized using the ECEPP/3 force field of ICM. The compounds were built using ICM and optimized before docking. A grid map that included the amino acids within 5 Å of the co-crystallized inhibitors was calculated, and semi-flexible docking with flexible ligands was performed. Each docking was run in three parallels. The docking poses were scored using the Virtual ligand scoring (VLS) module of the ICM program.

General procedure for the preparation of compounds 7a–e

The TBS protected steroids **16a–e** (0.3–0.4 mmol, 1.0 equiv.) were placed in a dry round-bottomed flask under argon atmosphere, and dissolved in dry THF. *Tert*-butylamoniumfluoride (1 M in THF, 1.1 equiv.) was added drop vise. 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, 20–50% ethyl acetate in hexane) to give the pure products.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-13-methyl-17-phenyl-7,8,9,11,12,13,-14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-ol (7a). $[\alpha]_D^{20}$ = 85 (c = 0.04, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46–7.40 (m, 2H), 7.38–7.30 (m, 2H), 7.27–7.23 (m, 1H), 7.06 (s, 1H), 6.52 (s, 1H), 5.95 (dd, J = 3.2, 1.8 Hz, 1H), 4.51 (s, 1H), 3.00–2.76 (m, 2H), 2.62 (q, J = 7.5 Hz, 2H), 2.46–2.25 (m, 3H), 2.25–2.04 (m, 2H), 2.02–1.89 (m, 1H), 1.86–1.75 (m, 1H), 1.72–1.57 (m, 3H), 1.56–1.37 (m, 1H), 1.24 (t, J = 7.6 Hz, 3H), 1.08 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2, 151.3, 137.5, 135.7, 133.1, 128.3, 127.2, 126.9, 126.9, 126.2, 115.4, 57.0, 47.8, 44.3, 37.6, 35.7, 31.5, 29.3, 27.9, 26.9, 23.2, 16.9, 14.6. Eluent 20% EtOAc in hexane R_f = 0.59 yield 113 mg, 86%, product white solid. HRMS calcd for $C_{26}H_{30}O$ $[M]^{\bullet+}$ 358.2297. Found 358.2295.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-13-methyl-17-(pyridin-3-yl)-7,8,9,11,-12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-ol (7b). $[\alpha]_D^{20}$ = 25 (c = 0.06, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.50 (d, J = 4.1 Hz, 1H), 7.76 (dt, J = 8.0, 1.8 Hz, 1H), 7.31 (dd, J = 7.8, 4.9 Hz, 1H), 7.07 (s, 1H), 6.60 (s, 1H), 6.06 (dd, J = 3.1, 1.7 Hz, 1H), 2.96–2.75 (m, 2H), 2.66 (q, J = 7.5 Hz, 2H), 2.48–2.25 (m, 3H), 2.23–2.07 (m, 2H), 2.01–1.89 (m, 1H), 1.89–1.77 (m, 1H), 1.76–1.58 (m, 3H), 1.56–1.38 (m, 1H), 1.26 (t, J = 7.5 Hz, 3H), 1.05 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.3, 151.6, 147.1, 147.0, 135.2, 134.7, 133.6, 131.9, 129.9, 127.9, 126.1, 123.6, 115.5, 56.9, 47.9, 44.2, 37.5, 35.5, 31.7, 29.3, 27.9, 26.7, 23.3, 16.9, 14.7. Eluent 50% EtOAc in hexane R_f = 0.27, yield 121 mg, 83%, product white solid, mp. °C decomp. HRMS calcd for $C_{25}H_{29}NO$ $[M]^{\bullet+}$ 359.2249. Found 359.2238.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-13-methyl-17-(pyridin-4-yl)-7,8,9,11,-12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-ol (7c). $[\alpha]_D^{20}$ = 12 (c = 0.05, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (s, 2H), 7.31 (d, J = 5.2 Hz, 2H), 7.05 (s, 1H), 6.54 (s, 1H), 6.22 (dd, J = 3.2, 1.8 Hz, 1H), 3.03–2.74 (m, 2H), 2.62 (q, J = 7.5 Hz, 2H), 2.50–2.06 (m, 6H), 1.99–1.89 (m, 1H), 1.86–1.76 (m, 1H), 1.73–1.53 (m, 3H), 1.60–1.37 (m, 1H), 1.24 (t, J = 7.5 Hz, 3H), 1.08 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.5, 151.4, 149.2, 144.7, 135.1, 132.1, 131.5, 127.3, 125.8, 121.2, 115.2, 56.6, 47.4, 44.0, 37.1, 35.1, 31.4, 29.0, 27.6, 26.4, 22.9, 16.6, 14.3. Eluent 50% EtOAc in hexane R_f = 0.25, yield 123 mg, 88%, product white solid. HRMS calcd for $C_{25}H_{29}NO$ $[M]^{\bullet+}$ 359.2249. Found 359.2255.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-17-(isoquinolin-4-yl)-13-methyl-7,8,-9,11,12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-ol (7d). $[\alpha]_D^{20}$ = 15 (c = 0.03, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.83 (s, 1H), 8.30 (s, 1H), 8.14 (d, J = 8.1 Hz, 1H), 8.03 (d, J = 8.4 Hz, 1H), 7.79 (t, J = 7.6 Hz, 1H), 7.69 (t, J = 7.5 Hz, 1H), 6.88 (s, 1H), 6.47 (s, 1H), 5.88 (s, 1H), 2.93–2.56 (m, 2H), 2.45 (q, J = 7.5 Hz, 2H), 2.34–2.15 (m, 3H), 2.05–1.80 (m, 2H), 1.75–1.64 (m, 1H), 1.64–1.52 (m, 1H), 1.51–1.34 (m, 3H), 1.06 (t, J = 7.5 Hz, 3H), 0.95 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.6, 151.3, 149.2, 141.2, 134.7, 134.0, 131.4, 130.5, 130.2, 128.4, 128.0, 127.7, 127.3, 127.0, 125.4, 125.0, 114.7, 56.0, 49.4, 43.7, 37.5, 34.6, 31.7, 28.7, 26.2, 22.8, 16.2, 14.6. Eluent 50% EtOAc in hexane R_f = 0.23, yield 148 mg, 86%, product white solid. HRMS calcd for $C_{29}H_{31}NO$ $[M]^{\bullet+}$ 409.2406. Found 409.2395.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-17-(isoquinolin-5-yl)-13-methyl-7,8,-9,11,12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-ol (7e). $[\alpha]_D^{20}$ = 16 (c = 0.06, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.51 (d, J = 5.9 Hz, 1H), 7.92 (d, J = 7.1 Hz, 2H), 7.69–7.50 (m, 2H), 7.02 (s, 1H), 6.57 (s, 1H), 5.84 (dd, J = 3.0, 1.5 Hz, 1H), 5.76 (s, 1H), 3.02–2.82 (m, 2H), 2.62 (q, J = 7.5 Hz, 2H), 2.56–2.46 (m, 1H), 2.42–2.24 (m, 3H), 2.08–1.93 (m, 2H), 1.80–1.66 (m, 2H), 1.64–1.47 (m, 3H), 1.22 (t, J = 7.5 Hz, 3H), 1.02 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.6, 151.8, 151.4, 142.6, 135.7, 135.5, 135.3, 132.6, 131.0, 129.9, 129.2, 127.6, 126.8, 126.6, 126.2, 119.6, 115.5, 56.8, 50.1, 44.5, 38.0, 35.3, 32.2, 29.4, 28.1, 26.7, 23.27, 16.7, 14.6. Eluent 50% EtOAc in hexane R_f = 0.24, yield 134 mg, 87%, product white solid. HRMS calcd for $C_{29}H_{31}NO$ $[M]^{\bullet+}$ 409.2406. Found 409.2408.

General procedure for the synthesis of 8a–e

The estrogen (1 equiv.) and 2,6-di-*tert*-butyl-4-methylpyridine (3.0 equiv.) were added to a round bottomed flask and dissolved in dichloromethane under argon atmosphere. The mixture was cooled to 0 °C before sulfamoyl chloride (2.95 equiv.) was added. The reaction mixture was stirred at 0 °C for additional 30 min, and then at room temperature for 16–18 h. Sodium bicarbonate (saturated) was added and the mixture was extracted with EtOAc, dried over $MgSO_4$ and evaporated. Flash

column chromatography (50% EtOAc in hexane) afforded the products as colorless solids.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-13-methyl-17-phenyl-7,8,9,11,12,13,-14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl sulfamate (8a). $[\alpha]_D^{20} = 18$ ($c = 0.04$, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.37 (m, 2H), 7.35–7.29 (m, 2H), 7.26–7.24 (m, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 5.95 (dd, $J = 3.1$, 1.7 Hz, 1H), 4.94 (s, 2H), 3.00–2.83 (m, 2H), 2.71 (q, $J = 7.5$ Hz, 2H), 2.45–2.37 (m, 1H), 2.38–2.28 (m, 2H), 2.25–2.19 (m, 1H), 2.19–2.07 (m, 1H), 2.04–1.92 (m, 1H), 1.86–1.75 (m, 1H), 1.75–1.62 (m, 3H), 1.54–1.40 (m, 1H), 1.37–1.29 (m, 1H), 1.23 (t, $J = 7.6$ Hz, 3H), 1.07 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.0, 146.3, 139.9, 137.4, 136.2, 133.7, 128.3, 127.2, 127.0, 126.9, 126.8, 121.5, 57.0, 47.7, 44.5, 37.1, 35.6, 32.0, 31.5, 29.3, 27.7, 26.6, 23.2, 16.9, 14.8. Eluent 50% EtOAc in hexane $R_f = 0.44$, yield 98 mg, 71%, product white solid. HRMS calcd for $C_{26}H_{31}NO_3S$ $[M]^{\bullet+}$ 437.2025. Found 437.2001.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-13-methyl-17-(pyridin-3-yl)-7,8,9,11,-12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl sulfamate (8b). $[\alpha]_D^{20} = 15$ ($c = 0.06$, MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (d, $J = 1.6$ Hz, 1H), 8.46 (dd, $J = 4.7$, 1.3 Hz, 1H), 7.95 (s, 2H), 7.80 (dt, $J = 8.0$, 1.8 Hz, 1H), 7.37 (dd, $J = 7.4$, 4.8 Hz, 1H), 7.20 (s, 1H), 7.03 (s, 1H), 6.16 (s, 1H), 2.93–2.78 (m, 2H), 2.63 (q, $J = 7.4$ Hz, 2H), 2.48–2.41 (m, 1H), 2.38–2.24 (m, 2H), 2.21–2.04 (m, 2H), 1.98–1.87 (m, 1H), 1.83–1.69 (m, 1H), 1.70–1.54 (m, 3H), 1.51–1.34 (m, 1H), 1.13 (t, $J = 7.5$ Hz, 3H), 1.03 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 151.2, 147.8, 147.1, 146.1, 138.1, 135.0, 134.0, 133.3, 132.1, 128.9, 126.2, 123.4, 121.6, 56.2, 47.0, 44.0, 36.5, 34.7, 31.0, 28.5, 27.0, 26.0, 22.4, 16.3, 14.7. Eluent 50% EtOAc in hexane $R_f = 0.29$, yield 111 mg, 75%, product white solid. HRMS calcd for $C_{25}H_{30}N_2O_3S$ $[M]^{\bullet+}$ 438.1977. Found 438.1990.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-13-methyl-17-(pyridin-4-yl)-7,8,9,11,-12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl sulfamate (8c). $[\alpha]_D^{20} = 49$ ($c = 0.07$, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (s, 2H), 7.94 (s, 2H), 7.40 (d, $J = 5.7$ Hz, 2H), 7.20 (s, 1H), 7.03 (s, 1H), 6.36 (s, 1H), 2.90–2.80 (m, 2H), 2.68–2.59 (m, 2H), 2.47–2.41 (m, 1H), 2.38–2.23 (m, 3H), 2.07–1.96 (m, 1H), 1.96–1.86 (m, 1H), 1.80–1.69 (m, 1H), 1.68–1.53 (m, 3H), 1.50–1.38 (m, 1H), 1.13 (t, $J = 7.5$ Hz, 3H), 1.05 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 151.8, 149.7, 146.2, 143.4, 138.1, 135.0, 133.5, 131.6, 126.2, 121.6, 120.7, 56.2, 46.8, 43.7, 36.4, 34.6, 31.0, 28.5, 27.0, 26.0, 22.4, 16.3, 14.7. Eluent 50% EtOAc in hexane $R_f = 0.26$, yield 108 mg, 72%, product white solid. HRMS calcd for $C_{25}H_{30}N_2O_3S$ $[M]^{\bullet+}$ 438.1977. Found 438.1950.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-17-(isoquinolin-4-yl)-13-methyl-7,8,-9,11,12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl sulfamate (8d). $[\alpha]_D^{20} = 14$ ($c = 0.04$, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.32 (s, 1H), 8.20–7.90 (m, 2H), 7.78–7.68 (m, 1H), 7.67–7.59 (m, 1H), 7.14 (s, 3H), 5.91 (d, $J = 1.5$ Hz, 1H), 5.44 (s, 2H), 2.98–2.82 (m, 2H), 2.69 (q, $J = 7.5$ Hz, 2H), 2.59–2.46 (m, 1H), 2.44–2.23 (m, 3H), 2.03–1.91 (m, 1H), 1.82–1.65 (m, 2H), 1.65–1.51 (m, 3H), 1.19 (t, $J = 7.6$ Hz, 3H), 0.99 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.0, 149.5, 146.5, 140.9, 139.6, 136.0, 135.9, 133.8, 132.1, 130.7, 129.6, 128.6, 128.1, 127.4, 126.8, 125.7, 121.6, 56.8, 50.0, 44.7, 37.5, 35.2, 32.3, 29.3, 27.9, 26.5, 23.3, 16.6, 14.8. Eluent 50% EtOAc in hexane $R_f = 0.22$, yield 134 mg, 76%, product white solid. HRMS calcd for $C_{29}H_{32}N_2O_3S$ $[M]^{\bullet+}$ 488.2134. Found 488.2130.

(8*S*,9*S*,13*S*,14*S*)-2-Ethyl-17-(isoquinolin-4-yl)-13-methyl-7,8,-9,11,12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl sulfamate (8e). $[\alpha]_D^{20} = 18$ ($c = 0.06$, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.35 (d, $J = 5.9$ Hz, 1H), 7.91 (d, $J = 8.0$ Hz, 1H), 7.86 (d, $J = 6.0$ Hz, 1H), 7.60 (t, $J = 7.6$ Hz, 1H), 7.54 (d, $J = 6.3$ Hz, 1H), 7.15 (s, 1H), 7.13 (s, 1H), 5.93–5.75 (m, 1H), 5.57 (s, 2H), 3.01–2.80 (m, 2H), 2.69 (q, $J = 7.5$ Hz, 2H), 2.59–2.45 (m, 1H), 2.43–2.24 (m, 3H), 2.12–1.92 (m, 2H), 1.84–1.65 (m, 2H), 1.65–1.42 (m, 3H), 1.19 (t, $J = 7.6$ Hz, 3H), 1.01 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.3, 150.1, 145.3, 141.4, 138.4, 134.8, 134.5, 133.9, 132.7, 129.8, 128.7, 128.0, 125.7, 125.6, 125.5, 120.6, 118.3, 55.7, 48.8, 43.5, 36.3, 34.1, 31.0, 28.1, 26.7, 25.3, 22.1, 15.5, 13.6. Eluent 50% EtOAc in hexane $R_f = 0.21$, yield 110 mg, 69%, product white solid. HRMS calcd for $C_{29}H_{32}N_2O_3S$ $[M]^{\bullet+}$ 488.2134. Found 488.2133.

Acknowledgements

The School of Pharmacy, University of Oslo, is gratefully acknowledged for a Ph.D. scholarship to E. J. S.

References

1 S. Bu, A. Blaukat, X. Fu, N. E. Heldin and M. Landstrom, *FEBS Lett.*, 2002, **531**, 141–151.
2 S. Verenich and P. M. Gerk, *Mol. Pharmaceutics*, 2010, **7**, 2030–2039.
3 J. C. Seegers, M.-L. Aveling, C. H. van Aswegen, M. Cross, F. Koch and W. S. Joubert, *J. Steroid Biochem.*, 1989, **32**, 797–809.
4 R. J. D'Amato, C. M. Lin, E. Flynn, J. Folkman and E. Hamel, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 3964–3968.
5 T. Fotsis, Y. Zhang, M. S. Pepper, H. Adlercreutz, R. Montesano, P. P. Nawroth and L. Schweigerer, *Nature*, 1994, **268**, 237–239.
6 M. Sattler, L. R. Quinnan, Y. B. Pride, J. L. Gramlich, S. C. Chu, G. C Even, S. K. Kraeft, L. B. Chen and R. Salgia, *Blood*, 2003, **102**, 289–296.
7 (*a*) C. Sweeney, G. Liu, C. Yiannoutsos, J. Kolesar, D. Horvath, M. J. Staab, K. Fife, V. Armstrong, A. Treston, C. Sidor and G. Wilding, *Clin. Cancer Res.*, 2005, **11**, 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 and W. D. Figg, *Cancer Biol. Ther.*, 2006, **5**, 22–27; (*c*) J. James, D. J. Murry, A. M. Treston, A. M. Storniolo, G. W. Sledge, C. Sidor and K. D. Miller, *Invest. New Drugs*, 2006, **25**, 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 and M. A. Gertz, *Clin. Cancer Res.*, 2007, **13**, 6162–6167.
8 J. F. Peyrat, J. D. Brion and M. Alami, *Curr. Med. Chem.*, 2012, **19**, 4142–4156.
9 (*a*) C. Moser, S. A Lang, A. Mori, C. Hellerbrand, H. J. Schlitt, E. K. Geissler, W. E. Fogler and O. Stoeltzing, *BMC Cancer*,

2008, **8**, 206–216; (*b*) E. Pasquier, S. Sinnappan, M. A. Munoz and M. Kavallaris, *Mol. Cancer Ther.*, 2010, **9**, 1408–1418.

10 C. J. Ryan, M. R. Smith, J. S. de Bono, A. Molina, C. J. Logothetis, P. de Souza, K. Fizazi, P. Mainwaring, J. M Piulats, S. Ng, J. Carles, P. F. A. Mulders, E. Basch, E. J. Small, F. Saad, D. Schrijvers, H. Van Poppel, S. D. Mukherjee, H. Suttmann, W. R. Gerritsen, T. W. Flaig, D. J. George, E. Y. Yu, E. Efstathiou, A. Pantuck, E. Winquist, C. S. Higano, M.-E. Taplin, Y. Park, T. Kheoh, T. Griffin, H. I. Scher and D. E. Rathkopf, *N. Engl. J. Med.*, 2013, **368**, 138–148.

11 E. J. Solum, J.-J. Cheng, I. B. Sørvik, R. E. Paulsen, A. Vik and T. V. Hansen, *Eur. J. Med. Chem.*, 2014, **85**, 391–398.

12 S. P. Newman, M. P. Leese, A. Purohit, D. R. C. James, C. E. Rennie, B. V. L. Potter and M. J. Reed, *Int. J. Cancer*, 2004, **109**, 533–540.

13 A. B. Edsall, G. E. Agoston, A. M. Treston, S. M. Plum, R. H. McClanahan, T.-S. Lu, W. Song and M. Cushman, *J. Med. Chem.*, 2007, **50**, 6700–6705.

14 (*a*) N. U. Hofsløkken and L. Skattebøl, *Acta Chem. Scand.*, 1999, **53**, 258–262; (*b*) T. V. Hansen and L. Skattebøl, *Org. Synth.*, 2005, **82**, 64–68.

15 Ø. W. Akselsen and T. V. Hansen, *Tetrahedron*, 2011, **67**, 7738–7742.

16 E. J. Solum, A. Vik and T. V. Hansen, *Steroids*, 2014, **87**, 46–53.

17 Ø. W. Akselsen, K. Odlo, J. J. Cheng, G. Maccari, M. Botta and T. V. Hansen, *Bioorg. Med. Chem.*, 2012, **20**, 234–242.

18 N. J. Lawrence, A. T. McGown, S. Ducki and J. A. Hadfield, *Anti-Cancer Drug Des.*, 2000, **15**, 135–141.

19 R. Abagyan, M. Totrov and D. N. Kuznetsov, *J. Comput. Chem.*, 1994, **15**, 488–506.

20 R. B. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel and M. Knossow, *Nature*, 2004, **428**, 198–202.

21 Y. Gökmen-Polar, D. Escuin, C. D. Walls, S. E. Soule, Y. Wang, K. L. Sanders, T. M. LaVallee, M. Wang, B. D. Guenther, P. Giannakou and G. W. Sledge Jr, *Cancer Res.*, 2005, **65**, 9406–9414.

22 J.-F. Peyrat, J.-D. Brion and M. Alami, *Curr. Med. Chem.*, 2012, **19**, 4142–4156.

23 M. P. Leese, H. A. M. Hejaz, M. F. Mahon, S. P. Newman, A. Purohit, M. J. Reed and B. V. L. Potter, *J. Med. Chem.*, 2005, **48**, 5243–5256.

24 B. Czako, L. Kurti, A. Mammoto, D. E. Ingber and E. J. Corey, *J. Am. Chem. Soc.*, 2009, **131**, 9014–9019.

25 U. Westphal, *J. Steroid Biochem.*, 1983, **19**, 1–15.

26 Z. Chen, L. Han, M. Xu, Y. Xu and X. Qian, *Curr. Med. Chem.*, 2013, **20**, 1694–1714.