Structure–Activity Relationships of C-17 Cyano-Substituted Estratrienes as Anticancer Agents†

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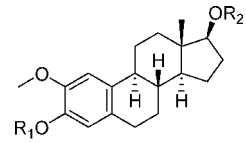
The synthesis, SAR, and preclinical evaluation of 17-cyanated 2-substituted estra-1,3,5(10)-trienes as anticancer agents are discussed. 2-Methoxy-17β-cyanomethylestra-1,3,5(10)-trien-3-ol (**14**), but not the related 2-ethyl derivative **7**, and the related 3-*O*-sulfamates **8** and **15** display potent antiproliferative effects (MCF-7 GI_{50} 300, 60 and 70 nM, respectively) against human cancer cells in vitro. Investigation of the SAR reveals that a sterically unhindered hydrogen bond acceptor attached to C-17 is most likely key to the enhanced activity. Compound **8** displayed significant in vitro antiangiogenic activity, and its ability to act as a microtubule disruptor was confirmed. Inhibitory activity of the sulfamate derivatives against steroid sulfatase and carbonic anhydrase II (hCAII) was also observed, and the interaction between **15** and hCAII was investigated by protein crystallography. The potential of these multimechanism anticancer agents was confirmed in vivo, with promising activity observed for both **14** and **15** in an athymic nude mouse MDA-MB-231 human breast cancer xenograft model.

Introduction

The discovery of angiogenesis inhibitors, which block the formation of new blood vessels required by tumors to support their growth,[1] offered a novel therapeutic approach to the treatment of cancer and stimulated extensive research in the area.[2] Although a number of potent angiogenesis inhibitors have been discovered, their application as single agent cancer therapies has not shown the hoped for clinical benefits. However, use in combination with conventional cytotoxic agents has produced more encouraging results. This approach, illustrated by the use of bevacizumab,[3] a monoclonal antibody against vascular endothelial growth factor (VEGF), in combination with chemotherapy has been shown to improve the survival of patients with colorectal,[4] lung, and breast cancer.[5] The success of this treatment strategy highlights the potential that exists for drugs that can exert an antiangiogenic effect in concert with a second antitumor activity.

In previous studies, we detailed the discovery of a number of sulfamoylated 2-substituted estratriene derivatives[6–9] that, like the endogenous estrogen metabolite 2-methoxyestradiol (**1**) (2-MeOE2), exhibit antiproliferative activity against cancer cells and also display an antiangiogenic effect.[10–13] Although notable mechanistic differences between the 2-substituted estratriene-3-*O*-sulfamates **2** and **3** (Figure 1) and **1** have been reported and are discussed in depth elsewhere,[11,14,15] in essence the antitumor effects of both compound series appear to stem from their ability to disrupt normal microtubule dynamics, which arrests the cell cycle and leads the rapidly dividing cancer cells to undergo apoptosis.[11] The estratriene sulfamate series is, however, differentiated from the corresponding substituted estradiol derivatives by the enhanced antitumor activity and high oral bioavailability,[16] which they display and, additionally, by their ability to inhibit both steroid sulfatase (STS[a], irreversibly)[17,18] and carbonic anhydrase (CA, reversibly).[9,19] STS is a clinical target for the treatment of hormone dependent cancer,[20,21] while the carbonic anhydrase IX isoform, which is highly expressed in a number of tumors, has also been proposed as a target for cancer therapy.[22] It should be noted that the activities of these 2-substituted estratriene derivatives are independent of the estrogen receptor, toward which they exhibit negligible affinity. They are thought to bind to the colchicine binding site of α-tubulin.[9]



1 $R_1 = R_2 = H$
2 $R_1 = SO_2NH_2$, $R_2 = H$
3 $R_1 = R_2 = SO_2NH_2$

Figure 1. Structures of 2-methoxyestradiol **1**, 2-MeOE2MATE **2**, and 2-MeOE2bisMATE **3**.

† The X-ray crystal structure of **15** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 658208. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Rd., Cambridge CB2 1 EZ, U.K. (e-mail: deposit@ccdc.cam.ac.uk). Protein crystal data: accession code 3BET.pdb.

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[a] Abbreviations: EMATE, estrone-3-*O*-sulfamate; E2MATE, estradiol-3-*O*-sulfamate; E2bisMATE, estradiol-3,17-*O*,*O*-bis-sulfamate; STS, Steroid sulfatase; CA, carbonic anhydrase; hCAII, human carbonic anhydrase II; ER, estrogen receptor; E2S, estradiol-3-*O*-sulfate; HDBC, hormone-dependent breast cancer; HIF, hypoxia inducible factor; DMA, *N*,*N*-dimethylacetamide.

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SAR studies showed that both substitution at C-2 of the steroidal A-ring and an unsubstituted sulfamate group at C-3 are requisite for high antiproliferative activity.[6] Optimal activity was conferred by a C-2 ethyl, methylsulfanyl, or methoxy substituent. Although a number of 2-substituted estradiol derivatives displayed antiproliferative and antiangiogenic activity,[23–25] these nonsulfamoylated molecules are typically much less potent, as illustrated by the mean activities of **1** and 2-methoxyestradiol-3-*O*-sulfamate (**2**) (2-MeOE2MATE) across the NCI 60 cell line panel (MGM values of 1.3 μM and 110 nM, respectively).[6] This finding is especially significant in light of the extensive evaluation of **1** as an anticancer agent in numerous academic studies and ongoing phase II clinical trials. It would seem that the advantages conferred by the incorporation of the 3-*O*-sulfamate group are manifold, in that this group affords both enhanced antiproliferative activity and resistance to inactivating conjugation of the C-3 hydroxyl group, to which the corresponding estradiol derivatives are subject. Furthermore, estrogen 3-*O*-sulfamates are also highly active reversible inhibitors of carbonic anhydrase II (CAII), an enzyme that is highly expressed in red blood cells, through a coordination of the monoanionic form of the sulfamate moiety to the zinc atom in the enzyme active site.[26] This interaction is believed to underlie the high oral bioavailability observed for estradiol-3-*O*-sulfamate (E2MATE) (which is atypical of estrogen derivatives), wherein reversible uptake by red blood cells and interaction with carbonic anhydrase II results in avoidance of first pass liver metabolism,[27] and it is plausible to suggest that such a mechanism could well operate advantageously for 2-substituted derivatives of E2MATE.

To date, SAR knowledge of these compounds outside the A-ring is limited to variation of the oxidation level of, and/or substitution at, the C-17-position of the estratriene core. 17-Hydroxy, 17-keto, and 17-oximino derivatives at C-17 display similar potency, though the 17-deoxy- and 17α-benzyl derivatives exhibit greatly reduced antiproliferative activity.[8] An improvement in in vitro antiproliferative activity was achieved by installation of a second *O*-sulfamate group at C-17, and significantly, this enhanced activity was seen to translate into enhanced in vivo antitumor effects. The C-17 sulfamate group of the 2-substituted estradiol-3,17-*O,O*-bis-sulfamates thus not only provides for greater activity, by presumably exploiting electrostatic interactions at the site of action unavailable to the smaller oxygen functions of the first generation compounds but also serves to block inactivating metabolism and conjugation reactions to which they (especially the 17-hydroxyl derivatives) are subject. Compound **1** has been shown to be rapidly metabolized to inactive 2-methoxyestrone (2-MeOE1) by 17β-hydroxysteroid dehydrogenase type II (17β-HSD type II), while the 3,17-*O,O*-bis-sulfamate derivatives are not subject to 17β-HSD type II metabolism.[15] The combination of inactivating metabolism with rapid conjugative inactivation through reaction of the hydroxyl groups, to which **1** is subject, emphasizes the need for molecules with improved pharmacokinetic profiles as well as potency, as amply illustrated by a recent clinical trial where a dose escalation of up to 6 g/day of **1** was used in an attempt to achieve satisfactory plasma concentrations.[28,29] Pharmacokinetic studies in rats showed that 2-methoxyestradiol-3,17-*O,O*-bis-sulfamate (**3**) (2-MeOE2bisMATE), however, displayed 85% bioavailability on oral dosing and was still detectable after 24 h, while identically dosed **1** was undetectable after just 5 min.[16] X-ray crystallography was used to examine the interaction of 2-MeOE2bisMATE with human carbonic anhydrase II (hCAII) and proved, unexpectedly, that the 17-*O*-sulfamate group of this compound could also interact with the catalytic zinc of the enzyme active site in a manner analogous to that observed for the 3-*O*-sulfamate group of E2MATE,[9] although interaction also of the 3-*O*-sulfamate in vivo is not necessarily excluded. Thus, it appears that, in addition to the properties discussed above, the 17-*O*-sulfamate of **3** may also enhance oral bioavailability by allowing a reversible uptake into red blood cells and through interaction with hCAII affords a mechanism for avoiding first pass liver metabolism.

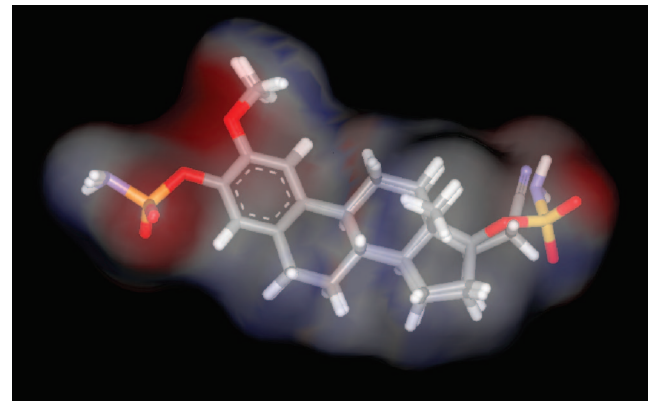


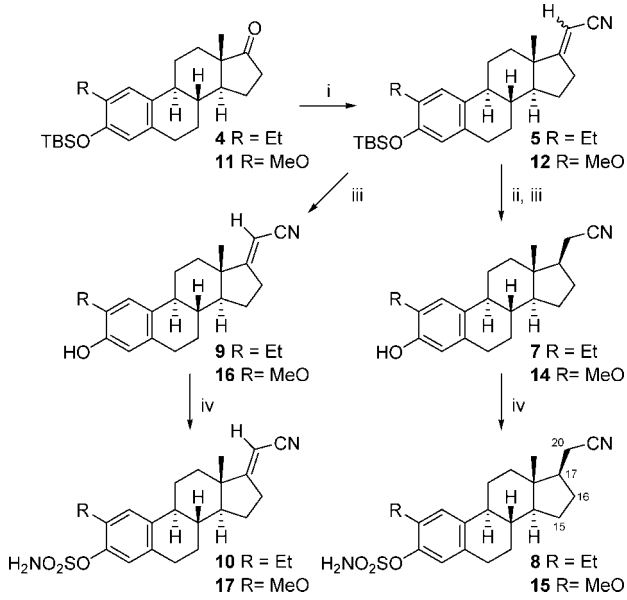
Figure 2. Computational overlay of minimal energy conformations of 17-*O*-sulfamoyl **3** and 17-cyanomethyl **15** functionalized estra-1,3,5[10]-triene derivatives.

Encouraged by the high activity and excellent pharmacokinetic profile displayed by the compounds such as **3**, we explored whether further D-ring modification could afford still greater enhancement in activity. To this end, a series of C-17-carbamate derivatives was synthesized, allowing us to establish that the carbamate function could, in certain cases, successfully function as a bioisostere for the C-17-sulfamate group.[30] We were thus also drawn to examine further C-17 functional groups that could serve as hydrogen bond acceptors in the hope of discovering even more active compounds and herein report the discovery of 17-cyano-substituted estra-1,3,5(10)-trienes as compounds that exhibit potent antiproliferative and antiangiogenic activity, despite only possessing a single sulfamate group.[6]

Chemistry

Earlier SAR studies demonstrated that a 3-*O*-sulfamate group is critical for highly potent activity, but that 2-methoxyestradiol-17-*O*-sulfamate is essentially inactive.[6,9] We were, therefore, drawn to examine whether the 17-*O*-sulfamate group of the 2-substituted estradiol-3,17-*O,O*-bis-sulfamates could be replaced with a small hydrogen bond-accepting group tethered to C-17, reasoning that a sterically smaller functional group might provide enhanced in vitro antiproliferative activity. In order to mimic the distance between the proposed hydrogen bond acceptor and C-17 of the sulfamate group, a two atom linker would be required between C-17 and the H-bond acceptor. We thus decided to install a cyanomethyl group at C-17 since the alkyl linker would afford a reasonable degree of flexibility, and the cyano group should offer sterically unhindered H-bonding interactions through the terminal nitrogen atom. This is clearly illustrated by computational overlay of bis-sulfamate **3** and nitrile compound **15** (vide infra) presented in Figure 2. The close spatial proximity of the nitrile nitrogen and 17-*O*-sulfamate NH_2 would suggest that they could function analogously as H-bond acceptors, albeit with differing electronic and steric properties, which might allow the nitrile to interact more strongly with those residues (Asn349 and Val315) around the colchicine binding site of tubulin identified by docking studies as potential H-bond donors to the 17-*O*-sulfamate group of **3**.[9]

Scheme 1. Synthesis of 17-Cyanomethyl and 17-Cyanomethylene Estra-1,3,5(10)-triene Derivatives[a]

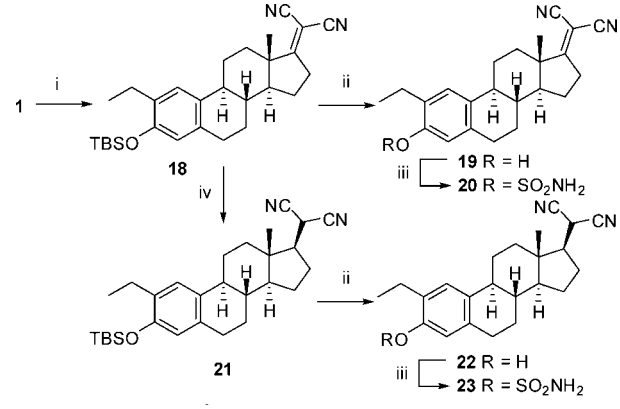


[a] Reagents and conditions: (i) $(EtO)_2POCH_2CN$, NaH, THF; (ii) H_2, Pd/C, THF/EtOH; (iii) TBAF, THF; (iv) H_2NSO_2Cl, DMA.

A Horner-Wadsworth-Emmons reaction between TBS-protected 2-ethylestrone **4** and diethylcyanomethyl phosphonate was thus carried out in refluxing THF to give the cyanomethylene derivative **5** in an excellent 94% yield as a mixture of its *E*- and *Z*-isomers (Scheme 1). Hydrogenation of the double bond delivered the 17β-alkane **6**, which was then desilylated with TBAF in THF to deliver phenol **7**. Sulfamoylation of **7** was then achieved by reaction with a solution of sulfamoyl chloride in DMA following the method of Okada and co-workers[31] to give sulfamate **8**. Desilylation of alkene **5** was also carried out to deliver the phenol as a mixture of the geometric isomers from which the *E*-alkene **9** could be isolated by chromatography. Sulfamoylation of **9** was then carried out to deliver sulfamate **10**. The analogous series of reactions was performed in the 2-methoxy series with **11** being transformed into the respective phenol (**14** and **16**) and sulfamate (**15** and **17**) derivatives.

An X-ray crystal structure of **15** was obtained and is presented in Figure 3. This structure confirms the β-stereochemistry of

Scheme 2. Synthesis of 17-(1,1-Dicyano)methyl Estra-1,3,5(10)-triene Derivatives[a]



[a] Reagents and conditions: (i) $CH_2(CN)_2$, β-alanine, PhMe; (ii) TBAF, THF; (iii) H_2NSO_2Cl, DMA; (iv) $NaBH_4$, THF/MeOH.

the alkyl substituent C-17 and confirms that the integrity of the chiral centers of the estra-1,3,5(10)-triene core are retained throughout the synthesis. Intermolecular hydrogen bonding between the sulfamate NH_2 group and both the nitrile of crystallographically adjacent **15** and acetone (the crystallization solvent) molecules is observed. The X-ray crystal structure of 2-methoxyestrone-3-*O*-sulfamate showed no such intermolecular interaction, with the sulfamate appearing instead to hydrogen bond to the adjacent methoxy group in an intramolecular fashion.[6] The appearance of intra or intermolecular hydrogen bonding may simply derive from the choice of crystallization solvent.

To assess whether the presence of a second cyano group would be beneficial, the (dicyano)methylene and (dicyano)-methyl derivatives of 2-ethylestrone were synthesized (Scheme 2). Condensation of **4** with malononitrile in the presence β-alanine was thus carried out. Although this reaction proved relatively slow, requiring the addition of extra aliquots of malononitrile after 24 and 48 h, the yield of **18**, however (91%), proved excellent. Sequential desilylation of **18** and sulfamoylation afforded phenol **19** and sulfamate **20**, respectively. Reduction of the Δ-17 double bond of estratetraene **18** was achieved with sodium borohydride in THF/MeOH to give **21**,

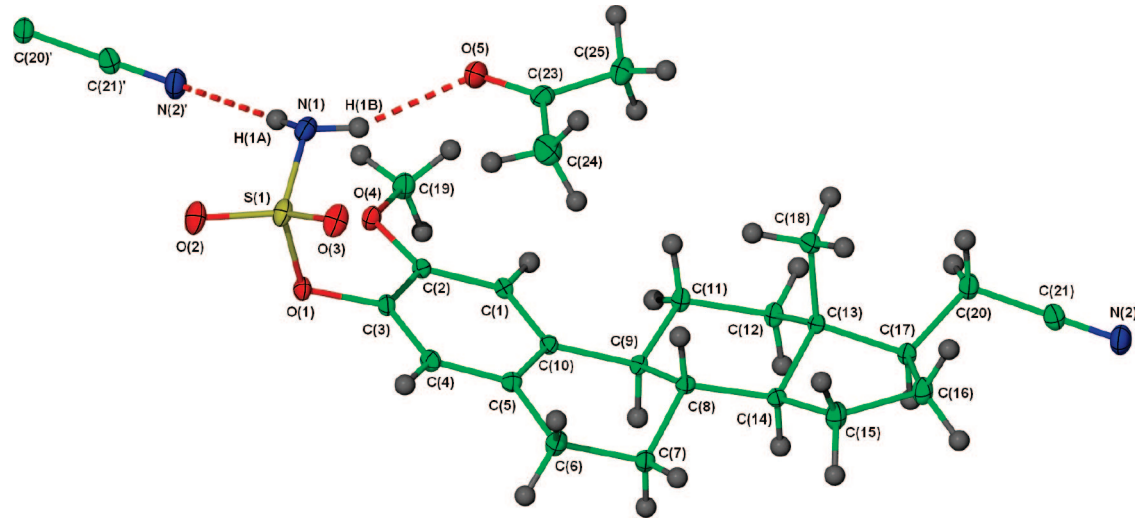
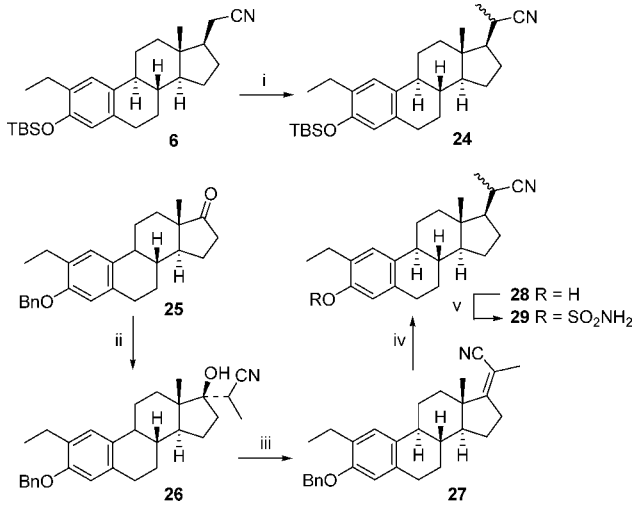


Figure 3. X-ray crystal structure of **15** displaying the intermolecular hydrogen bonding through the sulfamate *NH* to the nitrile of the neighboring **15** and also to the carbonyl oxygen in the solvent of crystallization (acetone). Ellipsoids are represented at 30% probability. Primed labeled atoms were generated via the $-1 + x, y, 1 + z$ symmetry operation.

Scheme 3. Synthesis of 1-(Cyano)ethyl Side Chains at C-17[a]



[a] Reagents and conditions: (i) LDA, THF, MeI, −78 °C to rt; (ii) EtCN, LDA, THF, −78 °C; (iii) MsOH, Et_3N, DCM, or Burgess reagent, DMF; (iv) H_2, Pd/C, THF/EtOH; (v) H_2NSO_2Cl, DMA.

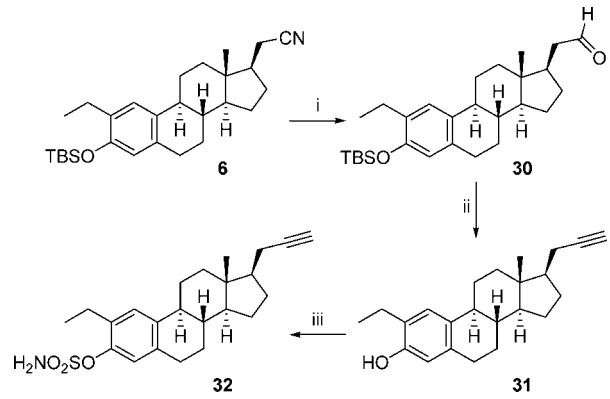
which was then converted to phenol **22** and sulfamate **23** as described above.

In order to study further steric effects around C-17, we were drawn to methylate **6** at C-20. After some investigation, it was determined that deprotonation of the C-20 methylene group could be achieved with 2.2 equivalents of LDA in THF at −78 °C with methyl iodide quench delivering C-20 monomethyl compound **24** in good yield (Scheme 3). Attempts to introduce a *gem*-dimethyl group in a one pot procedure directly from **6** or by lithiation and electrophilic quench of **24**, however, proved abortive, and we thus decided to inspect an alternate approach to compounds with alkyl substituted side chains. Reaction of benzyl protected 2-ethylestrone **25** with the lithio-anion of propionitrile afforded the 17α-alkylated estradiol derivative **26** in reasonable 76% yield, which could then be dehydrated with methanesulfonic acid/triethylamine to give a 95:5 mixture of the Δ-17 and Δ-16 dehydration products with the former, **27**, being isolated by column chromatography as a single isomer (*Z*-stereochemistry as assigned by NOE experiments) in 85% yield. Dehydration of **26** with Burgess reagent[32] delivered spectroscopically identical material. Concurrent debenzylation and double bond hydrogenation delivered 17*β*-(1-cyano)ethyl phenol (**28**), which was then sulfamoylated to give **29**. Reaction of the lithio-anion of isobutyronitrile with benzyl estrone was performed to test the approach to C-20 *gem*-dimethyl target compounds, and though this reaction proved successful, dehydration of the product alcohol proved intractable.

To confirm whether the activities observed for the nitrile series (vide infra) were attributable to their H-bond acceptor capabilities, the isosteric 17*β*-prop-2-ynyl compound **32** was synthesized (Scheme 4). DIBALH reduction of the nitrile group of **6** was thus carried out to give the aldehyde **30** in reasonable 61% yield, which could then be reacted with Ohira's reagent[33] to afford the 3-hydroxy alkyne **31**, as the phenolic TBS group proved unstable to the conditions of the reaction. The desired sulfamate **32** was then formed by reaction with sulfamoyl chloride in DMA.

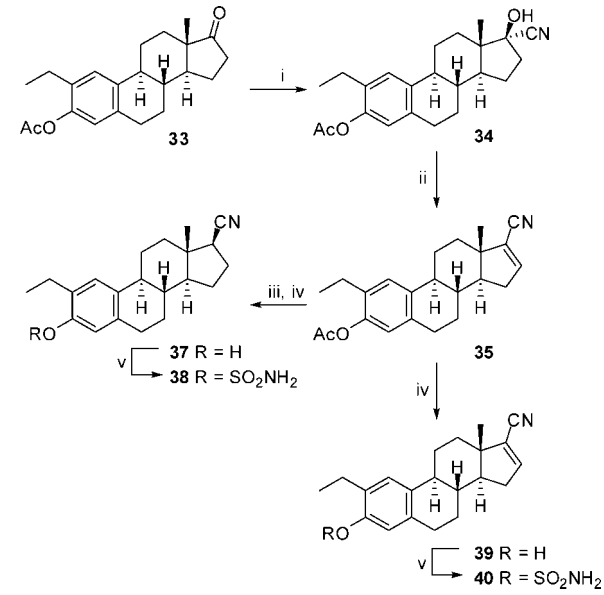
To assess the effects of direct linkage of the nitrile group to C-17 the cyanohydrin **34** was formed by reaction of 2-ethyl-3-*O*-acetyl estrone **33** with potassium cyanide over 5 days at room temperature (Scheme 5). Dehydration of the cyanohydrin with thionyl chloride in refluxing pyridine gave the Δ-16 product

Scheme 4. Synthesis of 2-Ethyl-3-*O*-sulfamoyl-17*β*-prop-2-ynyl Estra-1,3,5(10)-triene[a]



[a] Reagents and conditions: (i) DIBALH, $PhMe/CH_2Cl_2$ 0 °C to rt; (ii) $(MeO)_2POC(N_2)COMe$, MeOH, K_2CO_3; (iii) H_2NSO_2Cl, DMA.

Scheme 5. Synthesis of 17-Cyano Estra-1,3,5(10)-triene Derivatives[a]



[a] Reagents and conditions: (i) KCN, AcOH, MeOH; (ii) $SOCl_2$, pyridine, reflux; (iii) H_2, Pd/C, THF/MeOH; (iv) $NaHCO_3$, MeOH/acetone; (v) H_2NSO_2Cl, DMA.

35, which could then be deprotected and sulfamoylated to give **40** or hydrogenated to give the 17*β*-cyano derivative **36**, which was then converted to phenol **37** and sulfamate **38** derivatives, as previously described.

Results and Discussion

As a first stage predictor of antitumor activity, compounds were assayed for their ability to inhibit the proliferation of a panel of cancer cell lines in vitro. All compounds were screened against DU-145 cells, a human androgen receptor negative (AR−) prostate cancer cell line. The majority of compounds were additionally screened for their ability to inhibit the proliferation of MCF-7 and MDA MB-231 cells (human breast cancer estrogen receptor positive (ER+) and estrogen receptor negative (ER−), respectively). The results obtained from this in vitro screening are presented in Table 1. The first striking result in the table is the activity of the 2-methoxy-17-cyanomethyl estra-1,3,5(10)-trien-3-ol (**14**), which is 2.5-fold more potent against the proliferation of DU-145 cells and ca. 8-fold

Table 1. Antiproliferative Activities of Estratriene Derivatives against Human Cancer Cells in Vitro[a]

No	R_1	R_2	R_3	DU-145 (μM)	MCF-7 (μM)	MDA MB-231 (μM)
1	MeO	H	17β-OH	1.22	2.35	0.94
2	MeO	SO_2NH_2	17β-OH	0.39	0.36	0.170
3	MeO	SO_2NH_2	17β-OSO_2NH_2	0.34	0.25	0.28
41	Et	H	17β-OH	10.3	10.5	8.0
42	Et	SO_2NH_2	17β-OSO_2NH_2	0.21	0.07	0.21
14	MeO	H	17β-CH_2CN	0.485	0.3	0.117
15	MeO	SO_2NH_2	17β-CH_2CN	0.062	0.07	0.071
16	MeO	H	(*E*) =CHCN		28	
17	MeO	SO_2NH_2	(*E*) =CHCN		0.24	
7	Et	H	17β-CH_2CN	2.5	3.24	
8	Et	SO_2NH_2	17β-CH_2CN	0.054	0.06	0.141
9	Et	H	(*E*) =CHCN		>100	
10	Et	SO_2NH_2	(*E*) =CHCN		0.48	
19	Et	H	:$C(CN)_2$	>100	25.9	20.0
20	Et	SO_2NH_2	:$C(CN)_2$	0.267	0.21	0.289
22	Et	H	17β-$CH(CN)_2$	2.99	3.9	2.62
23	Et	SO_2NH_2	17β-$CH(CN)_2$	0.159	0.33	0.249
29	Et	H	17β-CHMe	>100	29.1	7.50
30	Et	SO_2NH_2	17β-CHMe	9.9	0.32	10.3
31	Et	H	17β-CH_2CCH	>100		
32	Et	SO_2NH_2	17β-CH_2CCH	1.69		
37	Et	H	17β-CN	n/a	>100	>100
38	Et	SO_2NH_2	17β-CN	<0.5	0.324	0.342
39	Et	H	Δ-16,17-CN		>100	57.3
40	Et	SO_2NH_2	Δ-16,17-CN		>100	75.4

[a] Data for compounds **1**–**3**, **41**, and **42** are taken from the literature.[9,30] GI_{50} figures are the mean values obtained from experiments performed in triplicate; s.e.m. = ±7%.

more potent against the proliferation of both breast cancer cell lines than **1**. To the best of our knowledge, this is the first time substitution at C-17 alone has provided such an enhancement in antiproliferative activity with respect to the corresponding 17-hydroxy compound, although derivatives of **1** bearing unsaturation in the D-ring, notably the 14-dehydro derivative, have been reported to have significantly enhanced effects on cancer cell proliferation.[34] In comparison, the corresponding 17-*O*-sulfamate derivative of **1** proved less active than **1** with a GI_{50} of >10 μM.[9] Interestingly, the corresponding 2-ethyl substituted phenol **7** though 5−10-fold less active than **14**, was found to be 4-fold more active than 2-ethylestradiol **41** against DU-145 cells, thus mirroring the beneficial effects of the C-17 cyanomethyl group on antiproliferative activity in the phenol series, an effect that could potentially be used to provide for further enhanced activity if used in conjunction with C-2 substituents, which are reported to deliver enhanced activity in the estradiol series. The 17-(*E*)-cyanomethylene functionalized phenols **9** and **16**, in contrast, displayed no significant activity, a result that suggests that rotation around the C-17-C-20 bond is required to allow either optimal electrostatic interaction or minimize disfavorable steric effects.

The 3-*O*-sulfamate derivatives bearing the 17-cyanomethyl group in both the 2-methoxy **15** and 2-ethyl **8** series proved to be exceptionally potent being, in DU-145 cells, 5.5- and 3.5-fold more active than the 2-methoxy- and 2-ethyl-E2bisMATE compounds (**3** and **42**, respectively), which they were designed to mimic. The effects of **15** and **8** against breast cancer cell proliferation were similarly profound with 50% growth inhibition obtained in the low nanomolar range. As observed in the phenol series, the 3-*O*-sulfamoylated 2-methoxy **17** and 2-ethyl **10** compounds bearing a C-17 cyanomethylene group were less potent than the corresponding cyanomethyl compounds, with activities against MCF-7 proliferation reduced by 4- and 8-fold, respectively, presumably reflecting the inability of the rigid 17-substituent of these compounds to attain optimal geometry at the site of action.

The activities of the remaining compounds in the series serve to characterize the spatial and electronic requirements for high activity. Introduction of a second cyano substituent on the C-17 tethered alkyl group resulted in a slight reduction in antiproliferative activity in the case of both phenol **22** and sulfamate **23**, albeit **23** is of a similar potency to the corresponding bis-sulfamate **42**. Introduction of a second H-bond acceptor group thus appears only to hinder the key interaction between the C-17 functionality and the site of action. Introduction of a methyl-substituent on the nitrile substituted carbon **29** caused a reduction in activity similar to that effected by the additional nitrile of **23**, which would suggest that the increased steric bulk around the hydrogen bond acceptor interferes with attainment of the optimal conformation at the site of action. The rigid dicyano-alkene substitution gave a significant decrease in activity in the phenol series **19**, although sulfamate **20** was essentially equipotent with the corresponding alkane **23**. As we had anticipated, alkyne **32** wherein the hydrogen bond acceptor nitrogen of **8** is replaced with with a CH group proved 30-fold less active than the nitrile compound, thus strongly supporting the postulate that the hydrogen bond acceptor group is essential for high antiproliferative activity. By extension, this supports our conclusions on the function of the C-17 sulfamate, principally as a hydrogen bond acceptor, in the bis-sulfamate series. Shortening of the linker group between C-17 and the hydrogen bond acceptor was also found to be deleterious to antiproliferative activity with a 6-fold reduction in activity observed for the 17β-nitrile derivative **38** relative to the corresponding 17-cyanomethyl compound **8**. The Δ-16 unsaturated derivatives proved to be essentially inactive as both phenol **39** and sulfamate **40**.

Thus, it can be seen that C-17 cyanomethyl substitution of 2-substituted estra[1,3,5]-triene-3-*O*-sulfamates affords a series of molecules with enhanced in vitro antiproliferative activity relative to the C-17 sulfamate series. An enhancement in activity is also evident in the nonsulfamoylated series with **7** and **14** found to be more potent than the corresponding estradiol derivatives. The 3-*O*-sulfamates **8** and **15** are the most potent 2-substituted estra[1,3,5]-triene-3-*O*-sulfamates discovered to date, with **15** proving to be up to 33-fold more active than **1** in vitro. Substitution, or deletion, of the CH_2 linker between C-17 and the nitrile group results in reduced activity, while replacement of the hydrogen bond-accepting nitrile group of **8** with an isosteric ethynyl group (compound **32**) results in a 30-fold reduction in activity and a more than 40-fold reduction for the unsulfamoylated pair **7** and **31**. On the evidence of these results, it appears that the C-17 cyanomethyl substituent most likely affords enhanced antiproliferative activity by functioning as a sterically unhindered hydrogen bond acceptor group, even in the simple 2-methoxyestradiol analogue series, which is positioned at the appropriate distance from C-17 in the biological site of action.

A number of the novel 17-cyanomethyl derivatives were screened for antiproliferative activity by the NCI in their 60-cell line assay (Table 2).[35,36] Unfortunately, the most active compounds in the series, **8** and **15**, were not selected by the NCI for evaluation, though the relative activities of compounds **23** and **29** compare favorably with both **1** and **3**, for which comparative data are included. Compounds **23** and **29** caused >50% growth inhibition in the majority of cell lines at concentrations below 10 nM, with mean growth inhibition (MGM) of 30 and 22 nM, respectively (an MGM of 10 nM is

Table 2. Antiproliferative Activity of Selected Compounds in the NCI 60-cell Line Panel[a]

	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12-C	prostate DU-145	breast MDA-MB-435	MGM (μM)
1	0.7	0.47	0.32	0.36	0.21	0.95	1.8	0.08	1.3
3	0.051	0.045	0.036	<0.01	<0.01	0.126	0.083	<0.01	0.087
23		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03
29		<0.01	<0.01	<0.01	NA	<0.01	NA	<0.01	0.022
37	>100	>100	>100	>100	>100	>100	>100	>100	>100
38	0.512	0.724	4.16	0.588	14.7	6.76	2.69	3.16	3.16
40	2.63	4.07	0.524	4.07	3.55	4.47	2.51	2.95	3.63

[a] Results are GI_{50} values in micromolar. Data for **1**[25] and **3**[9] are taken from the literature.

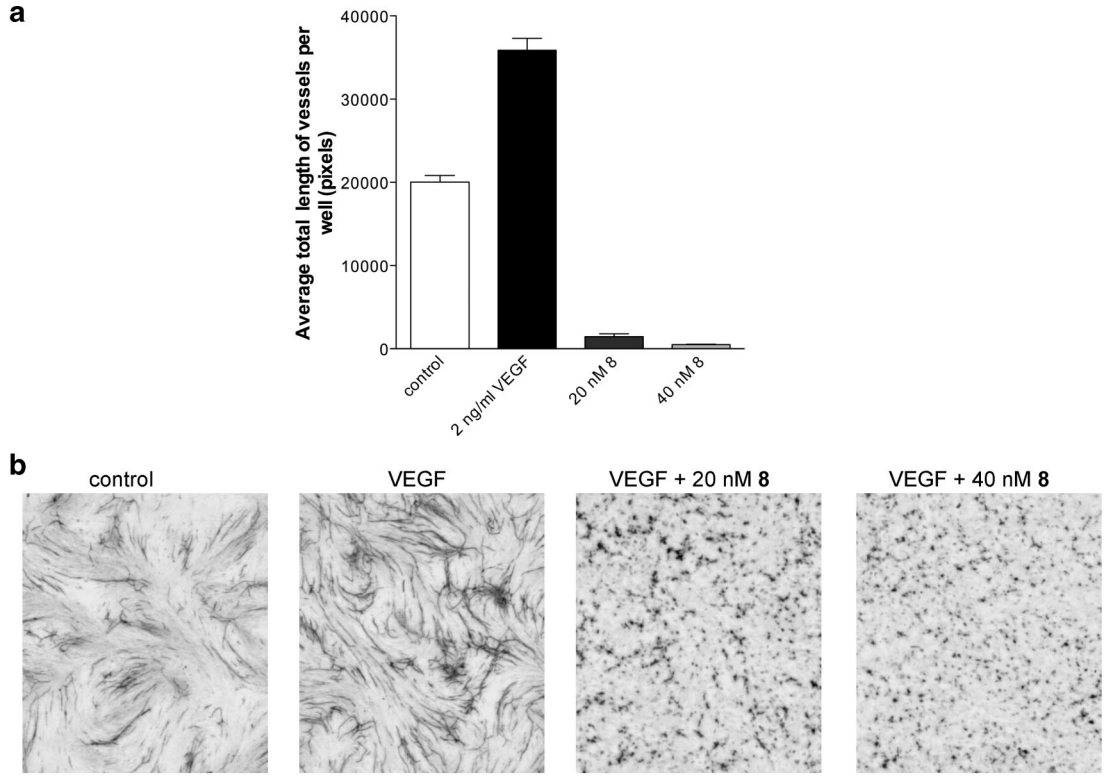


Figure 4. Activity of **8** in an in vitro model of angiogenesis. (a) Plot of quantified tubule length per well in control, VEGF stimulated and **8** treated wells at 20 and 40 nM. Treated wells are stimulated by 2 ng/mL VEGF. (b) Images of the control, stimulated, and treated wells in this assay.

maximal in this case because of the range of concentrations (10 nM to 100 μM) tested and would be achieved only if 50% growth inhibition was observed in all 60 cell lines at 10 nM). The 17-cyanomethyl compounds were thus seen to show slightly greater activity than **3** and are, notably, 40-fold more active than **1** across the NCI panel. In comparison, sulfamates **40** and **38**, whose cyano-groups are directly linked to C-17, showed greatly reduced activity, while **37**, the nonsulfamoylated analogue of **38**, showed no activity. These NCI results confirm the activity observed in our preliminary screens and, furthermore, demonstrate the effects of these compounds against a wide range of cancer phenotypes.

To assess potential antiangiogenic activity, the compounds were assayed for the ability to inhibit the proliferation of human umbilical vein endothelial cells (HUVECs). The IC_{50} values obtained for **1**, **3**, and **15** against HUVEC proliferation were 523, 44, and 30 nM, respectively.[37] It can thus be seen that the sulfamate derivatives express their in vitro antiangiogenic effects at far lower concentrations than **1**. Compound **8** was also assayed for antiangiogenic activity in an in vitro model of angiogenesis, wherein endothelial cells cocultured in a matrix of human dermal fibroblasts are used to assess antiangiogenic potential (Figure 4). When stimulated with VEGF, the endothelial cells proliferate and migrate through the matrix to form tubule-like structures (see Figure 4b) and the extent of tubule formation can be quantified as described elsewhere.[12] As can be seen in Figure 4a, treatment with 20 and 40 nM concentrations of **8** almost completely inhibits the formation of tubule-like structures. Quantification carried out by calculating total pixel length (Figure 4a) reflects the lack of tubule formation, which can be clearly seen in the images of the treated and untreated wells (Figure 4b). We have recently shown that the in vitro antiangiogenic effects of the sulfamoylated 2-substituted estratriene compounds translate well into in vivo activity with compounds including **15** showing impressive activity upon oral administration in mouse Matrigel plug assay for angiogenic activity.[13]

We have previously established that the antiproliferative and antiangiogenic activities of the 2-substituted estratriene-3-*O*-sulfamates are derived from their ability to disrupt normal microtubule dynamics[11] and wished to confirm that the 17-cyanomethyl series was acting, as we anticipated, at this target. We thus inspected the effects of **8** on the taxol stimulated polymerization of tubulin (10 μM Taxol and 20 μM **8**). As can be seen in Figure 5, **8** causes a substantial inhibition of both the rate and extent of microtubule formation stimulated by Taxol, thus supporting the postulate that these compounds, as in previously studied 2-substituted estra-1,3,5(10)-triene-3-*O*-sulfamates and the related estradiol derivatives, act as micro-

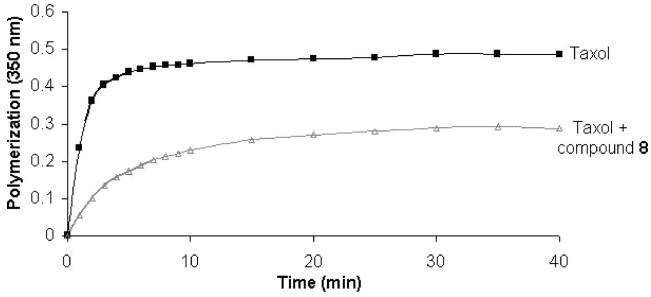


Figure 5. Compound **8** disrupts microtubule dynamics. The ability of compound **8** to inhibit paclitaxel-stimulated tubulin stabilization was assessed. In vitro tubulin assembly was measured by turbidity at 350 nm. Tubulin was preincubated with or without compound **8** (10 μM) for 5 min at 37 °C. Tubulin assembly was then stimulated by adding paclitaxel (10 μM). The change in the absorbance was continuously monitored at 350 nm for 15 min at 37 °C.

tubule disruptors. No further investigation on the interaction of these novel ligands with tubulin has yet been made, although it is notable that these ligands, like the previously studied broadly similar 2-substituted estra-1,3,5(10)-triene-3-*O*-sulfamates, which have been observed to bind tubulin in a competitive manner to radio-labeled colchicine, destabilize the polymerization of tubulin.[11,37]

Having determined that the 17-cyanomethyl compounds act against cancer cell proliferation in vitro, we assayed the compounds for activity against both STS and carbonic anhydrase. It was thought unlikely that the 17β-cyanomethyl substituent would have a profound effect on STS inhibitory activity since the key pharmacophore for inhibition of this enzyme is the phenolic sulfamate group, with activity most strongly influenced by the steric and electronic environment around C-3. Compound **15** was evaluated for its ability to inhibit the STS mediated conversion of tritiated E1S to E1 in placental microsomes.[38] An IC_{50} of 130 nM was obtained, thus confirming that 17-cyanomethyl substitution has little detrimental effect on STS inhibitory activity relative to the analogous 17-hydroxy compound, 2-MeOE2MATE (**2**) (16 nM), or the 17-*O*-sulfamate compound, 2-MeOE2bisMATE (**3**) (39 nM). The 2-ethyl compound **8** was found to be less active as an STS inhibitor with an IC_{50} of 1.4 $\pm$ 0.4 μM, reflecting the lower activity generally observed for 2-ethyl compounds as inhibitors seen in previous studies.[6,9] It is thus evident that in addition to their other anticancer activities, molecules such as **15**, by functioning as inhibitors of STS, could act against hormone dependent cancers by lowering the levels of circulatory estrogen that stimulate their growth.

The ability of estrogen-3-*O*-sulfamates to reversibly inhibit carbonic anhydrase is well established, with the key interaction being the coordination of the monoanionic form of the sulfamate to the zinc ion of the active site. This reversible interaction of phenolic sulfamates with carbonic anhydrase II could potentially allow an estratriene sulfamate derivative the advantage of sequestration by red blood cells in vivo and consequent avoidance of first pass liver metabolism.[27] Compound **15** was thus assayed for inhibitory activity against CAII and was found to have an IC_{50} value of 1.5 μM against this enzyme. The activity of **15** is thus approximately 4-fold lower than the activity of a number of 2-substituted estratriene-3-*O*-sulfamate derivatives (e.g., 2-methoxyestrone-3-*O*-sulfamate (2-MeOEMATE), IC_{50} of 376 nM and **3**, IC_{50} of 379 nM) and also 3-fold less active than 2-methoxyestradiol-17-*O*-sulfamate (2-MeOE2–17MATE, IC_{50} of 526 nM), which possesses a less acidic alkyl sulfamate group. The detrimental effects of 2-substitution on CA inhibitory effects are known, as estrone-3-*O*-sulfamate (IC_{50} 42nM) is 8-fold more active than 2-MeOEMATE,[19] and *o*-bromination of even a simple aryl monosulfamate reduces CAII binding from 27 to 137 nM.[39] This reflects, presumably, simple steric constraints close to the sulfamate binding region of the CAII active site. However, we had not previously observed that a 17-substituent, which we would expect to occupy the large lipophilic pocket above the enzyme active site, could have such an effect on inhibitory activity. We were thus drawn to further explore the interaction of **15** with hCAII to see if we could elucidate the origins of the effect of the C-17 substituent.

As can been seen in Figure 6b, the sulfamate *NH* of **15** coordinates to the catalytic zinc ion ($N-Zn^{2+} = 2.08$ Å) of the enzyme active site displacing the hydroxide ion present in the active site of the uninhibited enzyme. The zinc ion is also coordinated to three histidine residues (His94, His96, and His119) with an overall tetrahedral geometry. Two hydrogen bonds between the sulfamate and Thr 199 through their respective *NH* and *O* groups (see Figure 6b) are also apparent, with the steroid nucleus lying in the hydrophobic part of the active site pocket. The presence of the 2-substituent has previously proved to be detrimental to CAII inhibitory activity, and it would thus appear that groups at the 2-position render the interaction between the sulfamate group and the zinc atom less favorable through steric hindrance with the proximal residues of the active site. It is not, however, readily apparent from this structure why **15** should be 4-fold less active than either 2-MeOEMATE or **3** as an inhibitor of CAII. The electron density around C-17 is ill defined in this structure, presumably reflecting the rotational freedom of the cyanomethyl group and in turn the absence of substantial energetically beneficial or detrimental interactions with the protein at this site. The structure of **3**/hCAII did contrast to that of **15** in two respects, namely, the unexpected coordination of the C-17 sulfamate to the catalytic zinc and the presence of a second binding site for the ligand, not observed in the **15**/hCAII structure. Given the small difference in inhibitory activity of the respective 3- and 17-*O*-sulfamate derivatives of **1** (376 and 526 nM), we believe that the bis-sulfamate derivative can bind through both of its sulfamate groups and that the crystal structure only reflected one of these modes. The presence of a second binding site (ca. 60% occupancy) may, however, be more relevant since it could be possible that the nitrile substituted compound **15** has lower, or negligible affinity, for this second site, thus resulting in a lower overall affinity between the ligand and enzyme and consequently a reduced inhibitory activity. Given the large size of the pocket above the enzyme active site, it may be possible that occupation of both of the ligand binding sites identified in the **3**/hCAII structure is required for high activity. Even with a protein crystal structure, it is not possible to draw a definitive conclusion on the origin of the reduced inhibitory activity of **15** against hCAII relative to other 2-substituted estratriene sulfamate derivatives.

In order to evaluate this class of compounds for in vivo antitumor activity, it was decided to test 2-methoxy-17-cyanomethyl estratriene-3-*O*-sulfamate (**15**) and the corresponding 3-hydroxy compound **14** for their ability to inhibit the growth of MDA-MB-231 xenografts in female nude athymic mice ($n = 6$). The compounds were dosed orally once daily over a period of 28 days as a solution in THF/PG (10%/90%) at doses of 40 and 80 mg/kg (**15**) and 120 mg/kg (**14**). As can be seen in Figure 7, promising inhibition of tumor growth was achieved with both compounds. The higher 80 mg/kg dose of sulfamate **15** proved

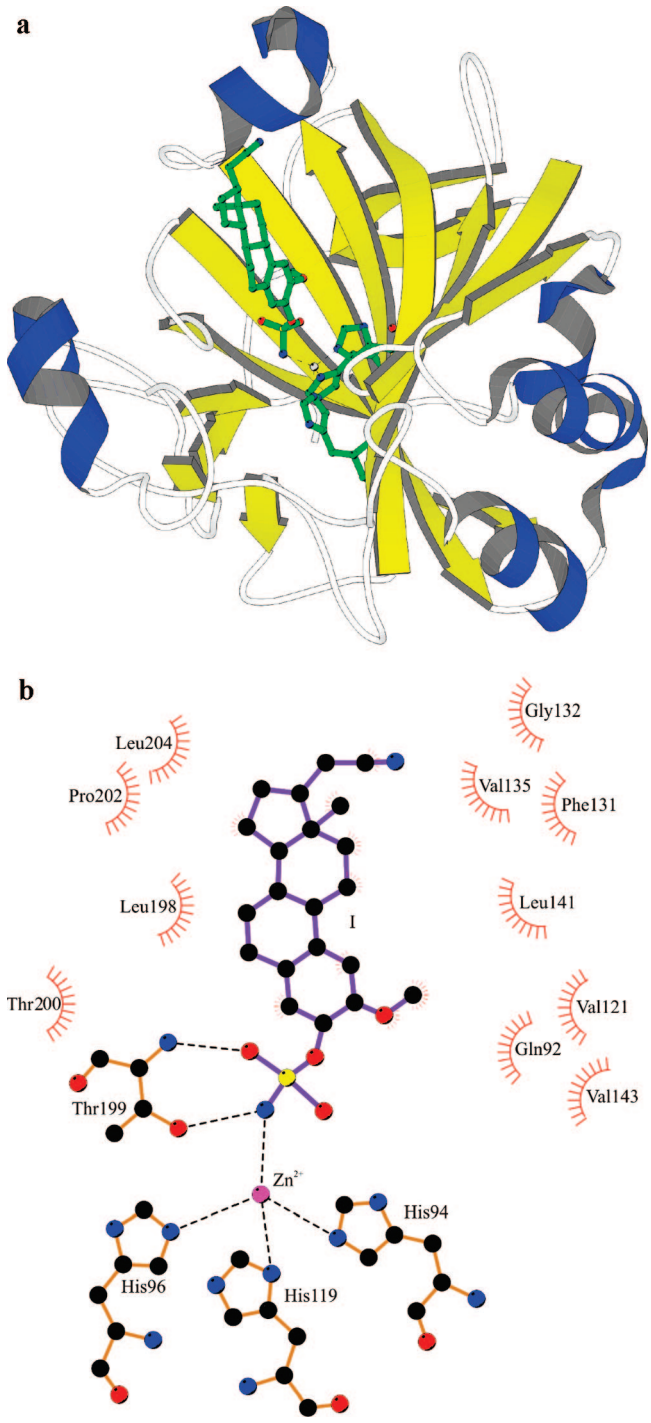


Figure 6. (a) Ribbon diagram of the **15**−hCAII complex obtained by X-ray crystallography. The inhibitor molecule, metal coordinating residues His94, His96, and His119, and the zinc ion are represented in ball and stick format. (b) Detailed schematic representation of **15** within the hCAII active site.

more effective than the 40 mg/kg dose, with growth inhibitions of 66% and 34% being achieved, respectively. Dosing with the phenol **14,** albeit at the higher 120 mg/kg dose, caused a 44% inhibition of tumor growth. No toxic effects were observed at these dose levels (data not shown). A dose dependent inhibition of tumor growth was thus observed with **15** treatment, while significant activity was observed with **14** treatment. The relative in vivo activities were found to correlate with their relative in vitro activities. It is notable that **15** proved less active than the corresponding bis-sulfamate, 2-MeOE2bisMATE **3** which, in

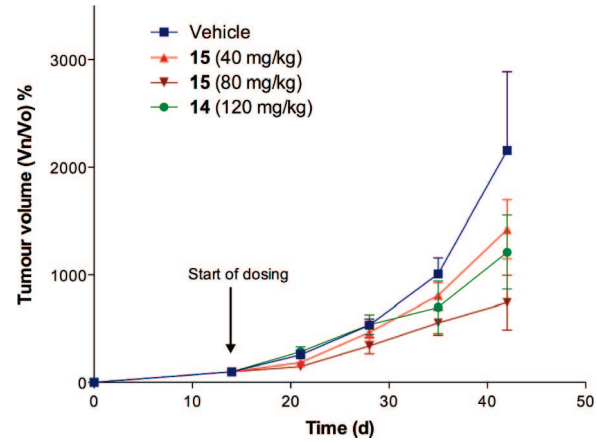


Figure 7. Effects of daily oral administration of **14** and **15** on the growth of MDA-MB-231 xenografts in athymic female nude mice.

the same model, caused tumor regression, although it is 4-fold less potent than **15** in vitro. The difference in in vitro and in vivo activities observed for **15** and **3** may derive from a number of factors. The formulation used for this study has not been rigorously investigated, and we have no information on the oral bioavailability achieved for **15**. It may also be that the nitrile group of **15** is subject to rapid metabolism, a factor that could lead to inactivation or accelerated clearance. In contrast, pharmacokinetic studies have shown that **3** displays high oral bioavailability in rodents (>85%) and does not undergo significant metabolism. As noted above, the relative inhibitory activity of **15** for carbonic anhydrase is 4-fold less than that observed for **3**, and this could possibly result in increased exposure to metabolism for **15** relative to the bis-sulfamate. It is thus clear that, although promising in vivo activity has been observed for **15**, pharmacokinetic studies would be highly advantageous to establish the in vivo fate of the compound and could direct the development of an enhanced formulation or further structural modification to block potential metabolism. Nevertheless, the structural modification intrinsic to **15** is clearly worthy of further investigation, particularly that for **14**, given the problems already noted with 2-MeOE2.

Conclusions

In conclusion, we have discovered that a number of 17-cyanomethyl functionalized 2-substituted estra-1,3,5(10)-triene derivatives display high activity against the proliferation of cancer cells in vitro. Enhanced activity is observed in both the 3-hydroxy and 3-*O*-sulfamoyl series of compounds, although as observed in previous studies, the sulfamoylated compounds display the most potent antiproliferative effects. SAR studies defined that the C-17 cyanomethyl group most likely functions as a hydrogen bond acceptor and that substitution of the methylene linker between the nitrile and C-17, presumably due to steric factors, is deleterious to activity. Direct linkage of the nitrile group to C-17 and replacement of the nitrile group or replacement an isosteric ethynyl group caused a great reduction in activity. Antiproliferative activity against a wide range of hormone dependent and independent cancer cells was observed, and it appears that this activity, as with previously studied 2-substituted estra-1,3,5(10)-triene-3-*O*-sulfamates, stems from their ability to interfere with the dynamics of tubulin polymerization. The sulfamate compounds showed activity against the proliferation of HUVECs and in an in vitro model of angiogenesis. Furthermore, the antiangiogenic profiles of these compounds in vivo has recently been established.[13] In addition,

activity of the sulfamoylated molecules against two tumor relevant enzymes, namely, steroid sulfatase (a clinical target for the treatment of HDBC) and carbonic anhydrase was observed. The interaction of **15** with carbonic anhydrase was studied by protein crystallography revealing, as expected, coordination of the 3-*O*-sulfamate group to the catalytic zinc of the hCAII active site. Confirmation of the potential of these molecules for development as anticancer agents was demonstrated by the significant growth inhibition achieved on treatment of human breast cancer xenografts in mice with two of the novel compounds being orally bioavailable. Further development of antitumor agents based on structural modification of 2-MeOE2 is presently underway.

Experimental Procedures

Materials and Methods. Chemistry. All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, U.K.), Fluka (Gillingham, U.K.) or Lancaster Synthesis (Morecambe, U.K.). Organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, U.K.) and used as supplied. Anhydrous *N,N*-dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMA) were purchased from Aldrich and stored under positive pressure of N_2 after use. THF was distilled from sodium with benzophenone indicator. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[40] and was stored in the refrigerator under positive pressure of N_2 as a solution in toluene as described by Woo et al.[41] An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. Estrone was purchased from Sequoia Research Products (Oxford, U.K.). Reactions were carried out at room temperature (rt) unless otherwise stated. Flash column chromatography was performed on silica gel (MatrexC60).

^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury VX400 NMR spectrometer at 400 and 100 MHz, respectively, and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded at the Mass Spectrometry Service Center, University of Bath, U.K. or at the EPSRC National Mass Spectrometry service, Swansea. FAB-MS was carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses were performed by Microanalysis Service, University of Bath. Melting points were determined using a Reichert−Jung Thermo Galen Kofler block and are uncorrected.

Biology. The effects of compounds on MCF-7, DU-145 and MDA MB-231 cell growth were determined using a microtiter plate assay as described previously.[6,30] STS inhibition was assayed by the method of Purohit et al.,[38] and carbonic anhydrase inhibition was carried out as described elsewhere.[19] The effects of compound **8** on in vitro polymerization of purified bovine brain tubulin (Cytoskeleton, Denver, CO) were measured by turbidometry using a previously validated assay.[11,37] Tubulin (1 mg/mL) in MES buffer (0.1 M MES at pH 6.5, 0.5 mM $MgCl_2$, 1 M monosodium glutamate, and 1 mM GTP) was preincubated with or without test compounds (10 μM, 1% v/v ethanol) for 5 min at 37 °C. Tubulin assembly was then stimulated by adding paclitaxel (10 μM). The change in the absorbance was continuously monitored at 350 nm for 15 min at 37 °C. Because the amount of tubulin polymerized is directly proportional to the area under the curve (AUC), it was used to determine the extent of tubulin polymerization by different test compounds. The AUC of paclitaxel alone, with a maximal extent of polymerization, was set to 100% polymerization.

In Vivo Efficacy Studies. Female MF-1 nu/nu mice from Harlan (Bicester, Oxon, U.K.) were injected subcutaneously (s.c.) in the flank with 2×10^6 MDA-MB-231 cells, which resulted in a single tumor per animal. All experiments were carried out under conditions that complied with institutional guidelines. Daily oral administration of **15** (40 and 80 mg/kg) and **14** (120 mg/kg) was initiated when the tumors reached approximately 100–150 mm^3 in volume. Dosing was performed for 28 days. Animal weights and tumor measurements were taken weekly using electronic callipers. Tumor volume (V), in mm^3, was determined using the following equation: length $\times$ width^2/2 ($l \times w^2$/2).

Protein Crystallography. The hCA II−**15** complex was obtained by adding a 5-molar excess of the inhibitor to a 10 mg/mL protein solution in 100 mM Tris-HCl at pH 8.5. Crystals of the complex were obtained by the hanging drop vapor diffusion technique at 20 °C. The drops were prepared by mixing 2 μL of the complex solution and 2 μL of the precipitant solution containing 2.8 M $(NH_4)_2SO_4$, 300 mM sodium chloride in 100 mM Tris-HCl (pH 8.4), and 1 mM dithiothreitol to improve the crystal quality. A complete data set was collected at 1.85 Å resolution from a single crystal of about 0.2 mm $\times$ 0.3 mm $\times$ 0.3 mm. Data collection was carried out at Synchrotron source Elettra in Trieste, using a Mar CCD detector. The crystal belonged to the $P2_1$ space group with unit cell parameters $a = 42.17$ Å, $b = 41.49$ Å, $c = 71.58$ Å, and $\beta = 104.07°$. Diffracted intensities were processed using the HKL crystallographic data reduction package (Denzo/Scalepack).[42] A total of 59499 reflections was measured and reduced to 20038 unique reflections. Crystal parameters and data processing statistics are summarized in tabular form in the Supporting Information. The structure of the complex was analyzed by difference Fourier techniques, using the PDB file 1CA2[43] as a starting model for refinement. Water molecules were removed from the starting model prior to structure factor and phase calculations. The inspection of the Fourier maps, calculated with 3Fo − 2Fc and Fo − Fc coefficients, revealed prominent electron density features in the active site region. After an initial refinement limited to the enzyme structure, a model for the inhibitor was easily built and introduced into the atomic coordinates set for further refinement, which proceeded to convergence with continuous map inspections and model updates. The refinement was carried out with the program CNS,[44] and model building and map inspections were performed using the O program.[45] The final model contains 2068 protein atoms, 28 inhibitor atoms, and 292 water molecules, and has *R*factor and *R*free values of 17.0 and 20.3%, respectively. The correctness of stereochemistry was finally checked using PROCHECK.[46] The statistics for refinement are summarized in the Supporting Information. Coordinates and structure factors have been deposited in the Brookhaven Protein Data Bank (accession code 3BET.pdb).

Crystal Data for 15. Empirical formula: $C_{24}H_{31}N_2O_5S$, $M = 459.57$, $T = 150(2)$ K, $\lambda = 0.71073$ Å, monoclinic, space group $P2_1$, $a = 8.2860(2)$ Å, $b = 13.6050(4)$ Å, $c = 11.2540(1)$ Å, $\beta = 107.430(1)°$, $U = 1210.42(6)$ Å^3, $Z = 2$, $D_c = 1.261$ gcm^{-3}, $\mu = 0.170$ mm^{-1}; $F(000) = 490$, crystal size 0.35 $\times$ 0.35 $\times$ 0.30 mm, unique reflections = 7011 [R(int) = 0.0759], observed $I > 2\sigma(I) = 5870$, data/restraints/parameters: 7011/3/303, $R1 = 0.0470$ $wR2 = 0.1085$ (obs. Data), $R1 = 0.0636$, $wR2$ - 0.1162 (all data), max. peak/hole: 0.493 and −0.293 eÅ^{-3}, absolute structure parameter = 0.00(6). Data were collected on a Nonius kappaCCD diffractometer.

2-Ethyl-3-*O*-(*t*-butyldimethylsilyl)estrone (4). A solution of 2-ethylestrone[6] (2.0 g, 6.7 mmol) and imidazole (1.14 g, 16.8 mmol) in DMF (20 mL) was treated with *t*-butyl-dimethylsilyl chloride (1.11 g, 7.38 mmol) and stirred for 14 h. Ethyl acetate (50 mL) and water (100 mL) were then introduced and the organic layer separated. After washing with water (3 $\times$ 50 mL), and brine (50 mL), the organic layer was dried and evaporated to give a colorless oil, which solidified on standing. Crystallization from ethyl acetate/hexane gave a white powder (1.98 g, 72%) with mp 95–97 °C, which showed δ_H 0.22 (6H, s), 0.91 (3H, s), 1.01 (9H, s), 1.16 (3H, t, *J* 7.4), 1.32–2.50 (13H, m), 2.56 (2H, q, *J* 7.4), 2.78–2.88 (2H, m), 6.49 (1H, s) and 7.05 (1H, s); HRMS [ES$^+$] *m/z* found 413.2851; $C_{26}H_{41}O_2Si$ ($M^+ + H$) requires 413.2870.

2-Ethyl-3-*O*-(*t*-butyldimethylsilyl)-17-cyanomethylene Estra-1,3,5(10)-triene (5). Sodium hydride (302 mg, 7.6 mmol) was washed with three aliquots of hexane (1 mL each), dried under nitrogen, then treated with THF (10 mL), and then diethyl (cyanomethyl)phosphonate (1.17 mL, 7.3 mmol), at which point vigorous gas evolution was observed. The resultant solution was then treated with a solution of **4** (1.82 g, 4.4 mmol) in THF (20 mL) and brought to reflux for 18 h. The reaction mixture was then

cooled to rt, diluted with ethyl acetate (25 mL), and poured onto water (10 mL); the aqueous layer was separated and the organic layer washed with water (3 × 25 mL) and brine (25 mL), dried, and evaporated. The crude product was purified by column chromatography (3% ethyl acetate/hexane) to give the desired product **5** as a mixture of isomers. White foam (1.81 g, 4.16 mmol, 94%). δ_H (selected) 0.21 (6H, s), 0.87 and 0.98 (3H, 2 × s), 0.99 (9H, s), 1.15 (3H, t, *J* 7.4), 2.55 (2H, q, *J* 7.4),:CH at 5.12 (app t, *J* 2.0) and 5.04 (app t, *J* 2.4), 6.47 (1H, s) and 7.03, 7.04 (1H, 2 × s); MS [FAB$^+$] *m/z* 435.3 (M$^+$, 100%); HRMS [FAB$^+$] *m/z* found 435.2961, $C_{28}H_{41}ONSi$ requires 435.2957.

2-Ethyl-3-*O*-(*t*-butyldimethylsilyl)-17*β*-cyanomethyl Estra-1,3,5(10)-triene (6). Compound **5** (1.80 g, 4.13 mmol) was dissolved in THF (5 mL) and ethanol (50 mL) then treated with Pd/C (250 mg, 10%). The degassed solution was then stirred under a hydrogen atmosphere for 16 h before filtering through a pad of celite. Evaporation gave the desired product **6** (1.58 g, 87%) as a white foam. δ_H 0.21 (6H, s), 0.66 (3H, s), 0.99 (9H, s), 1.16 (3H, t, *J* 7.4), 1.22–2.42 (16H, m), 2.55 (2H, q, *J* 7.4), 2.76–2.84 (2H, m), 6.47 (1H, s) and 7.03 (1H, s); MS [FAB$^+$] *m/z* 437.4 (M$^+$, 100%); HRMS [FAB$^+$] *m/z* found 437.3121; $C_{28}H_{43}OSiN$ requires 437.3114. Anal. ($C_{28}H_{43}NO_2Si$) C, H, N.

2-Ethyl-17*β*-cyanomethyl Estra-1,3,5(10)-trien-3-ol (7). A solution of **6** (1.18 g, 2.70 mmol) in THF (12 mL) was treated with TBAF (4 mL, 1 M in THF). After 1 h, the reaction was diluted with ethyl acetate (25 mL) and then washed with water (3 × 30 mL), brine (30 mL), dried, and evaporated to give a yellowy solid. The crude product was suspended in hot hexane, and the desired product **7**, a white solid with mp 180–182 °C, was collected by filtration (770 mg, 88%). δ_H 0.66 (3H, s), 1.15–2.45 (19H, m including 1.21 (3H, t, *J* 7.6)), 2.58 (2H, q, *J* 7.6), 2.75–2.85 (2H, m), 4.46–4.56 (1H, br), 6.49 (1H, s) and 7.03 (1H, s); MS [FAB$^+$] *m/z* 323.2 (M$^+$, 100%); HRMS [FAB$^+$] *m/z* found 323.2252; $C_{22}H_{29}ON$ requires 323.2249.

2-Ethyl-3-*O*-sulfamoyl-17*β*-cyanomethyl Estra-1,3,5(10)-triene (8). An ice cold solution of sulfamoyl chloride (4.76 mmol) in DMA (5 mL) was added to **7** (770 mg, 2.38 mmol). After 14 h of stirring, ethyl acetate (25 mL) was added, and the solution was washed with water (3 × 20 mL) and brine (20 mL), dried (Na_2SO_4), and evaporated. The crude product was purified by column chromatography (10% acetone in chloroform) to give **8** as a white crystalline solid with mp 175–177 °C (700 mg, 73%). δ_H 0.67 (3H, s), 1.21 (3H, t, *J* 7.4), 1.25–2.42 (16H, m) 2.68 (2H, q, *J* 7.4), 2.80–2.88 (2H, m), 4.91 (2H, br), 7.07 (1H, s) and 7.17 (1H, s); MS [FAB$^+$] *m/z* 402.0 (M$^+$, 100%); HRMS [FAB$^+$] *m/z* found 402.1975; $C_{22}H_{30}O_3N_2S$ requires 402.1977. Anal. ($C_{22}H_{30}N_2O_3S$).

***E*-2-Ethyl-17-cyanomethylene Estra-1,3,5(10)-trien-3-ol (9).** A solution of **5** (540 mg, 1.24 mmol) in THF (10 mL) was reacted with TBAF (3 mL, 3 mmol) as described for the synthesis of **7**. Column chromatography (20% ethyl acetate/hexane) afforded the desired phenol **9** as a pale yellow oil (284 mg, 0.882 mmol, 71%). Crystallization from ethyl acetate/hexane gave white crystals with mp 168–170 °C. δ_H 0.88 (3 H), 1.22 (3 H, t, *J* 7.4), 1.25–2.45 (11 H, m), 2.59 (2 H, q, *J* 7.4), 2.63–2.84 (4 H, m), 4.53 (1 H, s), 5.04 (1 H, t, *J* 2.3), 6.49 (1 H, s) and 7.02 (1 H, s); MS [FAB$^+$] *m/z* 321.3 (M$^+$, 100%); HRMS [FAB$^+$] *m/z* found 321.2088; $C_{22}H_{27}ON$ requires 321.2093. Anal. ($C_{21}H_{25}NO_2$) C, H, N.

***E*-2-Ethyl-3-*O*-sulfamoyl-17-cyanomethylene Estra-1,3,5(10)-triene (10).** Compound **9** (110 mg, 0.34 mmol) was reacted with sulfamoyl chloride (2 equiv) in DMA (1.5 mL) as described for the synthesis of **8**. Column chromatography (10% acetone in chloroform) gave the desired product as a white foam (86 mg, 63%). δ_H 0.87 (3H, s), 1.21 (3H, t, *J* 7.4), 1.23–1.63 (6H, m), 1.90–1.99 (3H, m), 2.20–2.29 (1H, m), 2.39–2.46 (1H, m), 2.58–2.82 (4H, m, including 2.69 (2H, q, *J* 7.4)), 2.83–2.89 (2H, m), 5.00 (2H, br), 5.05 (1H, t, *J* 2.6), 7.09 (1H, s) and 7.18 (1H, s); MS [ES$^-$] *m/z* 399.5 (M$^+$ − H, 100%). Anal. ($C_{21}H_{28}N_2O_4S$) C, H, N.

2-Methoxy-3-*O*-(*t*-butyldimethylsilyl)estrone (11). 2-Methoxyestrone[6] (1.5 g, 5 mmol) and imidazole (408 mg, 6 mmol) in DMF (20 mL) was reacted with *t*-butyl-dimethylsilyl chloride (829 mg) as described for the synthesis of **4**. The resultant crude oil was purified by chromatography (9:1 hexane/EtOAc) to give a white powder (1.5 g, 73%) with mp 162–64 °C, which showed δ_H 0.15 (6H, s), 0.92 (3H, s), 0.99 (9H, s), 1.38–2.54 (13H, m), 2.75–2.83 (2H, m), 3.77 (3H, s), 6.56 (1H, s), 6.76 (1H, s); MS [ES$^-$] *m/z* 413.3 (70%, M$^+$ − H); HRMS [ES$^+$] *m/z* found 415.2660; $C_{25}H_{39}O_3Si$ (M$^+$ + H) requires 415.2663.

2-Methoxy-3-*O*-(t-butyldimethylsilyl)-17-cyanomethylene Estra-1,3,5(10)-triene (12). Sodium hydride (204 mg, 5.1 mmol) in THF (10 mL) was reacted with diethyl (cyanomethyl)phosphonate (776 *μ*L, 4.8 mmol) and 2-methoxy-3-*O*-*t*-butyldimethylsilyl estrone (1.82 g, 4.4 mmol) in THF (10 mL) as described for the synthesis of **5**. The crude product was purified by column chromatography (4% ethyl acetate/hexane) to give the desired product **12** (a 3:2 mixture of *E*- and *Z*-isomers) as a white solid (1.04 g, 79%). δ_H (selected) 0.13 (6H, s), 0.98 (9H, s), 0.99, 0.88 (3H, 2 × s), 3.77 (3H, s), 5.12, 5.04 (1H, 2 × m, :CH, both isomers), 6.54 (1H, s) and 6.75, 6.74 (1H, 2 × s); MS [FAB$^+$] *m/z* 438.1 [(M + H)$^+$, 50%] and 380.1 (100%); HRMS [FAB$^+$] *m/z* found 437.2731; $C_{27}H_{39}NO_2Si$ requires 437.2750.

2-Methoxy-3-*O*-(t-butyldimethylsilyl)-17*β*-cyanomethyl Estra-1,3,5(10)-triene (13). A solution of **12** (0.98 g, 2.24 mmol) in THF (15 mL) and ethanol (100 mL) was reacted with Pd/C (200 mg, 5%) and hydrogen over 48 h as described for the synthesis of **6**. The desired product **13** was isolated as a white solid (0.92 g, 94%) with mp 149–151 °C. δ_H 0.14 (6H, s), 0.68 (3H, s), 0.98 (9H, s), 1.24–2.42 (16H, m), 2.70–2.76 (2H, m), 3.76 (3H, s), 6.53 (1H, s) and 6.75 (1H, s). Anal. ($C_{27}H_{41}NO_2Si$) C, H, N.

2-Methoxy-17*β*-cyanomethyl Estra-1,3,5(10)-trien-3-ol (14). A solution of **13** (0.92 g, 2.09 mmol) in THF (20 mL) was reacted with TBAF (3 mL, 1 M in THF) as described for the synthesis of **7**. The desired phenol **14** was crystallized from ethyl acetate/hexane to give colorless crystals (600 mg,88%) with mp 172–174 °C. δ_H 0.68 (3H, s), 1.24–2.42 (16H, m), 2.72–2.80 (2H, m), 3.85 (3H, s), 5.42 (1H, s), 6.63 (1H, s) and 6.77 (1H, s); MS [ES$^-$] *m/z* 309 ((M$^+$ −H)$^-$, 100%). Anal. ($C_{21}H_{27}NO_2$) C, H, N.

2-Methoxy-3-*O*-sulfamoyl-17*β*-cyanomethyl Estra-1,3,5(10)-triene (15). Compound **14** (570 mg, 1.75 mmol) was reacted with sulfamoyl chloride and DMA as described for the synthesis of **8**. Column chromatography (chloroform/aceone 9:1) followed by recrystallization from acetone/hexane afforded the sulfamate as a white crystalline solid (**15**) (590 mg, 83%) with mp 183–185 °C. δ_H 0.69 (3H, s), 1.20–2.50 (16H, m, alkyl H), 2.74–2.84 (2H, m), 3.87 (3H, s), 5.06 (2H, s), 6.92 (1H, s) and 7.04 (1H, s); MS [FAB$^+$] *m/z* 404.1 (M$^+$, 100%); HRMS [FAB$^+$] *m/z* found 404.1767; $C_{21}H_{28}O_4N_2S$ requires 404.1770. Anal. ($C_{21}H_{28}O_4N_2S$) C, H, N.

***E*-2-Methoxy-17-cyanomethylene Estra-1,3,5(10)-trien-3-ol (16).** A solution of **12** (400 mg, 0.95 mmol) in THF (10 mL) was reacted with TBAF (3 mL) as described for the synthesis of **7**. Column chromatography (10% ethyl acetate in hexane) afforded two fractions, the first being a mixture of the two isomers as a white powder (91 mg, 30%) and the second *E*-isomer **16** as a white solid with mp 183–185 °C (115 mg, 0.36 mmol, 37%). δ_H ($CDCl_3$) 0.89 (3H, s), 1.25–2.39 (11H, m), 2.56–2.82 (4H, m), 3.86 (3H, s), 5.05 (1 H, t, *J* 2.7), 5.43 (1H, s), 6.65 (1H, s), 6.77 (1H, s); MS (FAB$^+$) *m/z* 323.1 (M$^+$, 100%); HRMS [ES$^+$] *m/z* found 324.1961; $C_{21}H_{26}O_2N$ (M$^+$+H) requires 324.1958. Anal. ($C_{22}H_{27}NO$) C, H, N.

2-Methoxy-3-*O*-sulfamoyl-17-cyanomethylene Estra-1,3,5(10)-triene (17). Compound **16** (40 mg, 0.124 mmol) was treated with sulfamoyl chloride (1.5 mmol) in DMA (1.5 mL) as described for the synthesis of **8**. Flash column chromatography (hexane/ethyl acetate 4:1) afforded the desired sulfamate **17** as white needles with mp 202–204 °C (36 mg, 72%). δ_H (d_6-acetone) 0.95 (3H, s), 1.25–2.66 (12H, m), 2.74–2.84 (3H, m), 3.84 (3H, s), 5.27 (1H, t, *J* 2.3), 6.90 (2H, s), 7.02 (1H, s), 7.04 (1H, s); MS [FAB$^+$] *m/z* 402.0 (M$^+$, 100%); HRMS [FAB$^+$] *m/z* found 402.1611; $C_{21}H_{26}O_4N_2S$ requires 402.1613. Anal. ($C_{22}H_{28}N_2O_3S$) C, H, N.

2-Ethyl-3-*O*-(*t*-butyldimethylsilyl)-17-(1,1-dicyano)methylene Estra-1,3,5(10)-triene (18). A solution of **4** (830 mg, 2 mmol), malononitrile (0.38 mL, 10 mmol), and *β*-alanine (535 mg, 6 mmol) in toluene (150 mL) and acetic acid (30 mL) was refluxed for 3 days with extra aliquots of malononitrile (0.13 mL, 2 mmol)

added after 24 and 48 h. The solution was then cooled to rt, the solvents evaporated, and the residual solid stirred with water (50 mL). The mixture was then extracted with ethyl acetate (2 × 100 mL), and the resultant organic layer was washed with water and brine, then dried, and evaporated. Column chromatography (hexane/ethyl acetate 5:1) gave the desired alkene **18** as a white powder with mp 168–169 °C (840 mg, 91%). δ_H 0.21 (6H, s), 1.00 (9H, s), 1.06 (3H, s), 1.15 (3H, t, *J* 7.4), 1.40–2.26 (9H, m), 2.45–3.05 (m, 7H), 6.40 (1H, s) and 7.02 (1H, s); MS [ES$^-$] *m*/*z* 459.4 ($M^+ - H)^-$, 100%); HRMS [ES$^+$] *m*/*z* found 461.2985; $C_{29}H_{41}N_2OSi$ (M^++H) requires 461.2983.

2-Ethyl-3-*O*-hydroxy-17-(1,1-dicyano)methylene Estra-1,3,5(10)-triene (19). A solution of **18** (230 mg, 0.5 mmol) in THF (50 mL) was cooled to 0 °C and treated with TBAF (0.6 mmol, 0.6 mL) in a dropwise manner. After 2 h at 0 °C, the reaction was allowed to come to rt and then treated with water (10 mL) and ethyl acetate (80 mL). The resultant organic layer was separated, washed with water and brine, then dried, and evaporated. The residual solid was purified by column chromatography (hexane/ethyl acetate 5:1 to 2:1) to give phenol **19** as a white solid with mp 269–270 °C (125 mg, 72%). δ_H 1.06 (3H, s), 1.21 (3H, t, *J* 7.5), 1.39–2.52 (10H, m), 2.61 (2H, q, *J* 7.5), 2.63–3.02 (5H, m), 4.52 (1H, s), 6.50 (1H, s) and 7.02 (1H, s); HPLC: 98.9%, MS [APCI$^-$] *m*/*z* 345.4 (($M^+ - H)^-$, 100%).

2-Ethyl-3-*O*-sulfamoyl-17-(1,1-dicyano)methylene Estra-1,3,5(10)-triene (20). Compound **19** (69 mg, 0.2 mmol) was reacted with sulfamoyl chloride (0.6 mmol) in DMA (2 mL) as described for the synthesis of **8**. Column chromatography (hexane/ethyl acetate 5:1 to 3:1) gave the desired sulfamate **20** as a white powder with mp 227–228 °C (62 mg, 73%). δ_H 1.06 (s, 3H), 1.20 (3H, t, *J* 7.4), 1.44–2.55 (10H, m), 2.69 (2H, q, *J* 7.4), 2.75–3.04 (5H, m), 5.03 (2H, s), 7.09 (1H, s) and 7.16 (1H, s); HPLC 100%. Anal. ($C_{23}H_{27}N_3O_3S$) C, H, N.

2-Ethyl-3-*O*-*t*-butyldimethylsilyl-17*β*-(1,1-dicyano)methyl Estra-1,3,5(10)-triene (21). A solution of **18** (460 mg, 1 mmol) in methanol (50 mL) and THF (5 mL) was cooled to −10 °C, then treated with $NaBH_4$ (76 mg, 2 mmol) in a portionwise manner. After 4 h of stirring, water (50 mL) was added, and then sufficient ammonium chloride was added to acidify the solution. The mixture was then extracted with ethyl acetate (2 × 60 mL) and the combined organics washed with water and brine, dried, and evaporated. Column chromatography (hexane/ethyl acetate 5:1) gave the desired product **21** as a white powder with mp 161–162 °C (380 mg, 83%). δ_H 0.22 (6H, s). 0.80 (3H, s), 1.00 (9H, s), 1.16 (3H, t, *J* 7.5), 1.33–2.40 (14H, m), 2.55 (2H, q, *J* 7.5), 2.78 (2H, m), 3.56 (1H, d, *J* 9.9), 6.47 (1H, s) and 7.03 (1H, s); MS [ES$^-$] *m*/*z* 461.3 (($M^+ - H)^-$, 100%); HRMS [ES$^+$] *m*/*z* found 463.3152; $C_{29}H_{43}N_2OSi$ (M^++H) requires 463.3139.

2-Ethyl-17*β*-(1,1-dicyano)methyl Estra-1,3,5(10)-trien-3-ol (22). A solution of **21** (230 mg, 0.5 mmol) in THF (50 mL) was reacted with TBAF (0.6 mmol, 0.6 mL) as described for the synthesis of **6**. Column chromatography (hexane/ethyl acetate 5:1 to 2:1) gave **22** as a white solid with mp 247–248 °C (124 mg, 71%). δ_H 0.80 (3H, s), 1.21 (3H, t, *J* 7.4), 1.30–2.41(14H, m), 2.59 (2H, q, *J* 7.4), 2.81 (2H, m), 3.56 (1H, d, *J* 9.9), 4.51 (1H, br), 6.49 (1H, s) and 7.02 (1H, s); MS [APCI$^-$] *m*/*z* 347.4 ($M^+ - H)^-$, HRMS [ES$^+$] *m*/*z* found 349.2264; $C_{23}H_{29}N_2O$ ($M^+ + H$) requires 349.2274. Anal. ($C_{23}H_{28}N_2O$) C, H, N.

2-Ethyl-3-*O*-sulfamoyl-17*β*-dicyanomethyl Estra-1,3,5(10) -triene (23). A solution of **22** (0.2 mmol) was reacted with sulfamoyl chloride (0.6 mmol) in DMA (2 mL) as described for the synthesis of **8**. Column chromatography (hexane/ethyl acetate 5:1 to 3:1) gave the desired sulfamate **23** (170 mg, 80%) as a white powder with mp 169–170 °C. δ_H 0.80 (3H, s), 1.21 (3H, t, *J* 7.4), 1.32–2.38 (14H, m), 2.68 (2H, q, *J* 7.4), 2.83 (2H, m), 3.57 (1H, d, *J* 9.9), 4.91 (2H, br), 7.08 (1H, s) and 7.16 (1H, s); MS [FAB$^-$] *m*/*z* 347.4 (($M^+ - H)^-$, 100%). Anal. ($C_{23}H_{29}N_3O_3S$) C, H, N.

2-Ethyl-3-*O*-(*t*-butyldimethylsilyl)-17*β*-(1-cyanoethyl) Estra-1,3,5(10)-triene (24). A stirred −78 °C solution of **6** (220 mg, 0.5 mmol) in THF (50 mL) was treated with LDA (1.2 mL, 1.2 mmol, 1 M in THF) in a dropwise manner, and then after a further 0.5 h, methyl iodide (212 mg, 1.5mmol) was added. The mixture was stirred for 4 h at −78 °C and 12 h at rt before the addition of ethyl acetate (100 mL) and water (30 mL). The organic layer was then separated, washed with water and brine, dried, and evaporated. The crude yellow oil was purified by column chromatography (hexane/ethyl acetate 20:1) to give the desired product **24** as a colorless oil (176 mg, 78%) as a 1:1 mixture of diastereoisomers. δ_H (selected) 0.23 (6H, s), 0.76 and 0.78 (3H, 2 × s), 1.01 (9H, s), 1.17 (2H, t, *J* 7.4), 1.25–1.96 (13H, m), 2.05–2.50 (3H, m), 2.57 (2H, q, *J* 7.4), 2.66–2.88 (4H, m), 6.48 and 6.50 (1H, 2 × s), 7.03 and 7.06 (1H, 2 × s); HRMS [ES$^+$] *m*/*z* found 452.3349; $C_{29}H_{46}OSi$ ($M^+ + H$) requires 452.3347.

2-Ethyl-3-*O*-benzyl Estrone (25). A mixture of 2-ethyl estrone[6] (5.6 g, 18.8 mmol) and potassium carbonate (5.19 g, 37.5 mmol) in DMF (100 mL) was treated with benzyl chloride (2.81 mL) and then heated to 120 °C for 4 h. The reaction was then cooled to rt prior to the addition of sufficient water to dissolve the potassium carbonate then precipitate the product. The resultant beige powder was removed by filtration then resuspended in hot methanol to remove colored impurities. The product was then collected by filtration to give **25** as a white powder (6.1 g, 84%) with mp 169–171 °C, which showed 0.90 (3H, s), 1.20 (3H, t, *J* 7.4), 1.34–2.56 (13H, m), 2.66 2H, q, *J* 7.4), 2.83–2.92 (2H, m), 5.04 (2H, s), 6.64 (1H, s), 7.10 (1H, s) and 7.28–7.45 (5H, m); HRMS [ES$^+$] *m*/*z* found 389.2466; $C_{27}H_{33}O_2$ ($M^+ + H$) requires 389.2475. Anal. ($C_{27}H_{32}O_2$) C, H.

2-Ethyl-3-*O*-benzyl-17α-hydroxy-17*β*-(1-cyanoethyl) Estra-1,3,5(10)-triene (26). A −78 °C solution of propionitrile (50 mmol) in dry THF (50 mL) was treated with LDA (50 mmol) in a dropwise manner, and then stirred for 1 h prior to the addition of **25** (8 mmol) in THF (20 mL) over 3 h. The reaction was then stirred for an additional 3 h prior to ammonium chloride quench. The mixture was then extracted into ethyl acetate and the organic layer washed with water and brine, dried, and evaporated. The crude oil was purified by column chromatography (hexane/ethyl acetate gradient 10:1 to 4:1) to give the desired alcohol **26** (2.70 g, 76%) as a white powder with mp 75–77 °C, which showed δ_H 0.97 (3H, s), 1.20 (3H, t, *J* 7.6), 1.25–2.49 (16H, m), 2.66 (2H, q, *J* 7.6), 2.77–2.91 (3H, m), 5.04 (2H, s), 6.62 (1H, s), 7.09 (1H, s) and 7.28–7.46 (5H, m). MS [ES$^+$] *m*/*z* 466.36 ($M^+ + Na)^+$, 100%).

2-Ethyl-3-*O*-benzyl-17-(1-cyano)ethylene Estra-1,3,5(10)-triene (27). Methane sulfonic acid (0.1 mL, 1.4 mmol) was added dropwise to a solution of **26** (500 mg, 1.1 mmol) in triethylamine (5 mL) and DCM (50 mL), and then stirred at 0 °C for 4 h. The reaction mixture was poured onto ice/water (20 mL), and the organics were extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with water then brine, dried, and evaporated to give a crude oil containing a mixture of the two possible dehydration products (Δ17:Δ16 ratio ca. 95:5 as measured by NMR). Column chromatography (hexane/ethyl acetate 10:1) gave the desired Δ-17 alkene **27** (400 mg, 85%) as a white powder with mp 63–64 °C, which showed δ_H 0.95 (3H, s), 1.21 (3H, t, *J* 7.3), 1.30–1.71 (6H, m), 1.84 (3H, s), 1.85–2.51 (6H, m), 2.64 (2H, q, *J* 7.3), 2.75–2.90 (3H, m), 5.04 (2H, s), 6.63 (1H, s), 7.10 (1H, s) and 7.28–7.48 (5H, m); MS [ES$^-$] *m*/*z* 424.35 (($M^+ - H)^-$, 100%); HRMS [ES$^+$] *m*/*z* found 426.2788, $C_{30}H_{37}NO$ (M^++H) requires 426.2791.

Alternatively, a solution of **26** (220 mg, 0.5 mmol) and Burgess reagent (358 mg, 1.5 mmol) in DMF (5 mL) was stirred at rt for 1 day. After the addition of water, the organics were extracted with ethyl acetate, the organic layer washed with water and brine, dried, and evaporated. The resultant yellow oil (230 mg) was purified by chromatography (hexane/EtOAc 20:1 to 15:1) to give **27** as a white powder (165 mg, 78%), which was spectroscopically identical to that described above.

2-Ethyl-3-hydroxy-17*β*-(1-cyano)ethyl Estra-1,3,5(10)-triene (28). A solution of **27** (430 mg, 1mmol) in methanol (45 mL) and THF (5 mL) was treated with 5% Pd/C (50 mg) and then was stirred under an atmosphere of H_2. After standard workup column chromatography (hexane/ethyl acetate 10:1 to 1:1), recrystallization (hexane/ethyl acetate 5:1) gave the desired phenol **28** as a white powder with mp 235–236 °C (225 mg, 67%). δ_H 0.76 (3H, s), 1.20

(3H, t, *J* 7.4), 1.24–1.69 (13H, m), 1.71–2.48 (4H, m), 2.57 (2H, q, *J* 7.3), 2.71 (1H, m), 2.78 (2H, m), 4.47 (1H, br), 6.49 (1H, s) and 7.03 (1H, s); MS [ES$^-$] *m*/*z* 336.3 ((M$^+$ − H)$^-$, 100%). Anal. ($C_{23}H_{31}NO$) C, H, N.

2-Ethyl-3-*O*-sulfamoyl-17*β*-(1-cyano)ethyl Estra-1,3,5(10)-triene (29). Compound **28** (100 mg, 0.3mmol) was reacted with a solution of sulfamoyl chloride (0.8 mmol) in DMA (2 mL) as described for the synthesis of **8**. Column chromatography (hexane/ethyl acetate 5:1 to 2:1), then recrystallization (hexane/ethyl acetate 5:1) gave the desired sulfamate **29** as a white powder with mp 171–172 °C (86 mg, 69%). δ_H 0.76 (3H, s), 1.20 (3H, t, *J* 7.4), 1.22–1.64 (11H, m), 1.75–2.35 (6H, m), 2.66 (2H, q, *J* 7.3), 2.68 (1H, m), 2.84 (2H, m), 4.92 (2H, br), 7.07 (1H, s) and 7.16 (1H,s); MS [ES$^-$] *m*/*z* 415.4 ((M$^+$ − H)$^-$, 100%). Anal. ($C_{23}H_{32}N_2O_3S$) C, H, N.

2-Ethyl-3-*O*-*t*-butyldimethylsilyl-17*β*-(2′-oxoethyl) Estra-1,3,5(10)-triene (30). A 0 °C solution of **6** (550 mg, 1.26 mmol) in dichloromethane (7 mL) and toluene (15 mL) was treated with DIBALH (1.26 mL, 1.26 mmol, THF solution) and then allowed to warm to room temperature. Further aliquots of DIBALH (1 and 0.5 mL) were added (after 1 h and 1.5 h) until TLC analysis showed that no starting material remained. A solution of Rochelle's salt (20 mL, sat.) was then added, and the reaction was stirred for a further 0.5 h before extracting with ethyl acetate (3 × 30 mL). The combined organic layers were then washed with water and brine, then dried, and evaporated. The resulting oil was purified by column chromatography (hexane/ ethyl acetate to 19:1) to afford the desired aldehyde **30** as a white powder with mp 78–80 °C (340 mg, 61%). δ_H 0.25 (6H, s), 0.66 (3H, s), 1.02 (9H, s), 1.19 (3H, t, *J* 7.4), 1.23–2.56 (16H, m), 2.58 (2H, q, *J* 7.4), 2.74–2.88 (2H, m), 6.50 (1H, s), 7.07 (1H, s) and 9.81 (1H, t, *J* 2.2). MS [APCI$^+$] *m*/*z* 441.4 (100%, (M$^+$ + H)).

2-Ethyl-17*β*-prop-2-ynyl Estra-1,3,5(10)-trien-3-ol (31). A mixture of (1-diazo-2-oxo-propyl)-phosphonic acid dimethyl ester[33] (0.19 g, 1.0 mmol) and dry K_2CO_3 (138 mg, 1.0 mmol) in anhydrous methanol (2.5 mL) was stirred under nitrogen and cooled to 0 °C, then treated with a solution of **30** (130 mg, 0.3mmol) in DCM (1 mL). The mixture was stirred for 24 h at room temperature after which water (10 mL) and DCM (50 mL) were added to the solution. The organic layer was washed with water and brine, dried, and evaporated. The resulting oil was purified by flash chromatography (gradient hexane/ethyl acetate 40:1 to 10:1) to give the desired alkyne **31** as a colorless oil (60 mg, 62%), which showed δ_H 0.63 (3H, s), 1.21 (3H, t, *J* 7.3), 1.20–2.31 (17H, m), 2.57 (2H, q, *J* 7.3), 2.78 (2H, m), 4.58 (1H, s), 6.45 (1H, s) and 7.04 (1H, s); HRMS [FAB$^+$] *m*/*z* found 322.2288; $C_{23}H_{30}O$ requires 322.2296. NB: A 7% yield of the TBDMS protected alkyne product was also isolated.

2-Ethyl-3-*O*-sulfamoyl-17*β*-prop-2-ynyl Estra-1,3,5(10)-triene (32). Compound **31** (50 mg, 0.16 mmol) was reacted with sulfamoyl chloride (0.3 mmol) in DMA (1 mL) as described for the synthesis of **8**. Column chromatography (hexane/ethyl acetate 10:1 to 7:1) gave the desired sulfamate **32** as a colorless oil (40 mg, 62%), which showed δ_H 0.61 (3H, s), 1.18 (3H, t, *J* 7.3), 1.20–2.30 (17H, m), 2.66 (2H, q, *J* 7.3), 2.79 (2H, m), 4.97 (2H, br), 7.03 (1H, s) and 7.15 (1H, s); HRMS [FAB$^-$] *m*/*z* found 400.1937; $C_{23}H_{30}NO_3S$ requires 400.1946.

2-Ethyl-3-*O*-acyl Estrone (33). A solution of 2-ethyl estrone[6] (0.89 g, 3 mmol) in DCM (20 mL) and TEA (1 mL, 7.2 mmol) was stirred and cooled to 0 °C. Acetyl chloride (0.29 mL, 4 mmol) was added in a dropwise manner, and the mixture was stirred at 0 °C for an hour. After the addition of DCM (20 mL) and water (20 mL), the organic layer was separated, washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. The crude white powder was purified by flash chromatography (hexane/EtOAc 20/1 to 10:1) to afford the desired acetate **33** (880 mg, 88%) as a white powder with mp 138–139 °C. δ_H ^{1}H NMR ($CDCl_3$, 270 MHz): 0.89 (3H, s, CH_3), 1.16 (3H, t, *J* 7.4 Hz), 1.38–1.68 (6H, m), 1.92–2.25 (5H, m), 2.30 (3H, s), 2.38–2.54 (4H, m), 2.83–2.88 (2H, m), 6.72 (1H, s) and 7.15 (1H, s); HRMS [FAB$^+$] *m*/*z* 341.2098; $C_{22}H_{29}O_3$ requires 341.2111. Anal. ($C_{22}H_{28}O_2$) C, H.

2-Ethyl 3-*O*-acyl-17-hydroxy-17-cyano Estra-1,3,5(10)-triene (34). A solution of **33** (1.7 g, 5 mmol) and KCN (3.26 g, 50mmol) in methanol (20 mL) and acetic acid (5 mL) was stirred at room temperature for 5 days. Ice–water (50 mL) was then added to the mixture, and the resulting precipitate was separated by filtration. The residue was washed with copious amounts of water and then dissolved in ethyl acetate (100 mL). The resultant solution was then washed with water and brine, dried, and evaporated to give **34** as a white solid with mp 147–148 °C (1.6 g, 87%). δ_H 0.85 (3H, s), 1.16 (3H, t, *J* 7.4), 1.33–2.06 (11H, m), 2.30 (3H, s), 2.25–2.58 (4H,m), 2.70 (2H, q, *J* 7.4), 2.82 (2H, m), 6.71 (1H, s) and 7.15 (1H, s); HRMS [FAB$^+$] *m*/*z* found 368.2229; $C_{23}H_{30}NO_3$ (M$^+$ + H) requires 368.2220. Anal. ($C_{23}H_{29}NO_3$) C, H, N.

2-Ethyl-3-*O*-acyl-17-cyano Estra-1,3,5(10),16-tetraene (35). A solution of **34** (1.47 g, 4 mmol) in dry pyridine (10 mL) was treated with $SOCl_2$ (1.46 mL, 20 mmol) in a dropwise manner. The solution was brought to reflux for 1 h, then cooled to 0 °C before bringing to pH 1 with HCl (5 M aqueous). After extraction into ethyl acetate (3 × 30 mL), the combined organic layers were washed with water and brine, then dried and evaporated to give a dark brown oil. Column chromatography (hexane/ethyl acetate 8:1 to 6:1) gave **35** (500 mg, 36%) as a white powder with mp 176–177 °C, which showed δ_H 0.94 (3H, s), 1.16 (3H, t, *J* 7.4), 1.36–2.26 (9H, m), 2.30 (3H, s), 2.39–2.52 (4H, m), 2.86 (2H, m), 6.65 (1H, dd, *J* 3.5 and 2.0), 6.72 (1H, s) and 7.14 (1H, s); HRMS [FAB$^+$] *m*/*z* found 350.2115; $C_{23}H_{28}NO_2$ requires 350.2115. Anal. ($C_{23}H_{28}NO_2$. $0.25H_2O$) C, H, N.

2-Ethyl-3-*O*-acyl-17*β*-cyano Estra-1,3,5(10)-triene (36). A solution of **35** (350 mg, 1 mmol) in THF (5 mL) and methanol (30 mL) was reacted with 5% Pd/C (50 mg) under an atmosphere of hydrogen for 24 h as described for the synthesis of **6**. Column chromatography (hexane/ethyl acetate 8:1 to 3:1) gave **36** as a white powder with mp 195–196 °C (330 mg, 94%). δ_H 0.99 (3H, s), 1.21 (3H, t, *J* 7.4), 1.22–2.32 (12H, m), 2.35 (3H, s), 2.38–2.47 (2H, m), 2.53 (2H, q, *J* 7.4), 2.88 (2H, m), 6.76 (1H, s) and 7.19 (1H, s); MS [APCI$^-$] *m*/*z* 350.11 (M$^+$ − H)$^-$.

2-Ethyl-17*β*-cyano Estra-1,3,5(10)-trien-3-ol (37). A solution of **36** (280 mg, 0.8 mmol) in acetone (10 mL) and methanol (10 mL) was treated with $NaHCO_3$ (0.34 g, 4 mmol) then stirred for 24 h. Solids were then removed by filtration and the filtrate evaporated. The resulting solid was treated with 2 M aqueous HCl to pH 1, and the solution was extracted with ethyl acetate (3 × 25 mL). The combined organics were then washed with water and brine, then dried, and evaporated. The resulting solid was purified by chromatography (hexane/ethyl acetate 8:1 to 6:1) and then recrystallized from hexane/ethyl acetate (4:1) to give **37** as a white powder with mp 284–285 °C (210 mg, 85%). δ_H 0.95 (3H, s), 1.21 (3H, t, *J* 7.4), 1.30–2.42 (14H, m, 2.58 (2H, *J* 7.4), 2.78 (2H, m), 4.50 (1H, s), 6.49 (1H, s) and 7.03 (1H, s); MS [APCI$^-$] *m*/*z* 308.29 (M$^+$ − H)$^-$. Anal. ($C_{21}H_{27}NO$) C, H, N.

2-Ethyl-3-*O*-sulfamoyl-17*β*-cyano Estra-1,3,5(10)-triene (38). Compound **37** (125 mg, 0.4 mmol) was reacted with sulfamoyl chloride (0.8 mmol) in DMA (2 mL) as described for the synthesis of **8**. Column chromatography (hexane/ethyl acetate 5:1) followed by crystallization (hexane/ethyl acetate 5:1) gave the desired sulfamate **38** as a white powder (135 mg, 86%) with mp 193–194 °C, which showed δ_H 1.00 (3H, s), 1.25 (3H, t, *J* 7.4), 1.28–2.42 (14H, m), 2.73 (2H, q, *J* 7.4), 2.88 (2H, m), 4.99 (2H, s), 7.13 (1H, s) and 7.23 (1H, s); MS [APCI$^-$] *m*/*z* 387.31 (M$^+$ − H)$^-$. Anal. ($C_{21}H_{28}N_2O_3S$) C, H, N.

2-Ethyl-17-cyano Estra-1,3,5(10),16-tetraen-3-ol (39). A solution of **35** (210 mg, 0.6 mmol) in acetone (10 mL) and methanol (10 mL) was reacted with $NaHCO_3$ (150 mg, 1.8 mmol) as described for the synthesis of **37**. Column chromatography (hexane/ethylacetate 8:1 to 6:1) followed by recrystallization (hexane/ethyl acetate 6:1) gave the desired phenol **39** as a white powder (165 mg, 90%) with mp 213–214 °C, which showed δ_H 0.88 (3H, s), 1.16 (3H, t, *J* 7.5), 1.31–2.41 (11H, m), 2.53 (2H, q, *J* 7.5), 2.70–2.84 (2H, m), 4.48 (1H, s), 6.44 (1H, s), 6.60 (1H, dd, *J* 3.3 and 1.8) and 6.97 (1H, s); MS [APCI$^-$] *m*/*z* 306.21 (M$^+$ − H)$^-$. Anal. ($C_{21}H_{25}NO$) C, H, N.

2-Ethyl-3-*O*-sulfamoyl-17-cyano Estra-1,3,5(10),16-tetraene (40). Compound **39** (75 mg, 0.25 mmol) was reacted with sulfamoyl chloride (0.5 mmol) in DMA (1 mL) as described for the synthesis of **8**. Column chromatography (hexane/ethyl acetate 5:1) followed by crystallization (hexane/ethyl acetate 5:1) gave the desired sulfamate **40** as a white powder (70 mg, 73%) with mp 212–213 °C, which showed δ_H 0.99 (3H,s), 1.26 (3H, t, *J* 7.5), 1.41–2.54 (11H, m), 2.74 (2H, q, *J* 7.5), 2.92 (2H, m), 5.03 (2H, s), 6.71 (1H, dd, *J* 3.3 and 1.9), 7.13 (1H, s) and 7.22 (1H, s); MS [APCI$^-$] *m/z* 385.23 (M − H)$^-$; HRMS [FAB$^+$] *m/z* found 386.1653; $C_{21}H_{23}O_3N_2S$ requires 386.1664. Anal. ($C_{21}H_{26}N_2O_3S$) C, H, N.

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Supporting Information Available: Table of elemental analyses, 13C NMR data and crystallographic data for the hCA II/**15** complex. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Folkman, J.; Bach, M.; Rowe, J. W.; Davidoff, F.; Lambert, P.; Hirsch, C.; Goldberg, A.; Hiatt, H. H.; Glass, J.; Henshaw, E. Tumor angiogenesis: therapeutic implications. *New Engl. J. Med.* **1971**, *285*, 1182–1186.

(2) Ferrara, N.; Kerbel, R. S. Angiogenesis as a therapeutic target. *Nature* **2005**, *438*, 967–974.

(3) Ferrara, N.; Hillan, K. J.; Gerber, H. P.; Novotny, W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat. Rev. Drug Discovery* **2004**, *3*, 391–400.

(4) Hurwitz, H.; Fehrenbacher, L.; Novotny, W.; Cartwright, T.; Hainsworth, J.; Heim, W.; Berlin, J.; Baron, A.; Griffing, S.; Holmgren, E.; Ferrara, N.; Fyfe, G.; Rogers, B.; Ross, R.; Kabbinavar, F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *New Engl. J. Med.* **2004**, *350*, 2335–2342.

(5) Jain, R. K.; Duda, D. G.; Clark, J. W.; Loeffler, J. S. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin. Pract. Oncol.* **2006**, *3*, 24–40.

(6) Leese, M. P.; Hejaz, H. A. M.; Mahon, M. F.; Newman, S. P.; Purohit, A.; Reed, M.; Potter, B. V. L. A-ring substituted estrogen sulfamates: Potent multi-targeted anti-cancer agents. *J. Med. Chem.* **2005**, *48*, 5243–5256.

(7) Leese, M. P.; Newman, S. P.; Purohit, A.; Reed, M. J.; Potter, B. V. L. 2-Alkylsulfanyl estrogen derivatives: synthesis of a novel class of multi-targeted anti-tumor agents. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3135–3138.

(8) Leese, M. P.; Leblond, B.; Newman, S. P.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Anti-cancer activities of novel D-ring modified 2-substituted estrogen-3-*O*-sulfamates. *J. Steroid Biochem. Mol. Biol.* **2005**, *94*, 239–251.

(9) Leese, M. P.; Leblond, B.; Smith, A.; Newman, S. P.; Di Fiore, A.; de Simone, G.; Supuran, C. T.; Purohit, A.; Reed, M. J.; Potter, B. V. L. 2-substituted estradiol bis-sulfamates, multitargeted antitumor agents: Synthesis, in vitro SAR, protein crystallography, and in vivo activity. *J. Med. Chem.* **2006**, *49*, 7683–7696.

(10) Purohit, A.; Hejaz, H. A. M.; Walden, L.; MacCarthy-Morrogh, L.; Packham, G.; Potter, B. V. L.; Reed, M. J. The effect of 2-methoxyoestrone-3-*O*-sulphamate on the growth of breast cancer cells and induced mammary tumours. *Int. J. Cancer* **2000**, *85*, 584–589.

(11) MacCarthy-Morrogh, L.; Townsend, P. A.; Purohit, A.; Hejaz, H. A. M.; Potter, B. V. L.; Reed, M. J.; Packham, G. Differential effects of estrone and estrone-3-*O*-sulfamate derivatives on mitotic arrest, apoptosis, and microtubule assembly in human breast cancer cells. *Cancer Res.* **2000**, *60*, 5441–5450.

(12) Newman, S. P.; Leese, M. P.; Purohit, A.; James, D. R. C.; Rennie, C. E.; Potter, B. V. L.; Reed, M. J. Inhibition of *in vitro* angiogenesis by 2-methoxy- and 2-ethyl-estrogen sulfamates. *Int. J. Cancer* **2004**, *109*, 533–540.

(13) Chander, S. K.; Foster, P. A.; Leese, M. P.; Newman, S. P.; Potter, B. V. L.; Purohit, A.; Reed, M. J. In vivo inhibition of angiogenesis by sulphamoylated derivatives of 2-methoxyoestradiol. *Br. J. Cancer* **2007**, *96*, 1368–1376.

(14) Wood, L.; Leese, M. P.; Mouzakiti, A.; Purohit, A.; Potter, B. V. L.; Reed, M. J.; Packham, G. 2-MeOE2bisMATE induces caspase-dependent apoptosis in CAL51 breast cancer cells and overcomes resistance to TRAIL via cooperative activation of caspases. *Apoptosis* **2004**, *9*, 323–332.

(15) Newman, S. P.; Ireson, C. R.; Tutill, H. J.; Day, J. M.; Parsons, M. F. C.; Leese, M. P.; Potter, B. V. L.; Reed, M. J.; Purohit, A. The role of 17β-hydroxysteroid dehydrogenases in modulating the activity of 2-methoxyestradiol in breast cancer. *Cancer Res.* **2006**, *66*, 324–330.

(16) Ireson, C. R.; Chander, S. K.; Purohit, A.; Perera, S.; Newman, S. P.; Parish, D.; Leese, M. P.; Smith, A. C.; Potter, B. V. L.; Reed, M. J. Pharmacokinetics and efficacy of 2-methoxyoestradiol and 2-methoxyoestradiol-*bis*-sulphamate *in vivo* in rodents. *Br. J. Cancer* **2004**, *90*, 932–937.

(17) Reed, M. J.; Purohit, A.; Newman, S. P.; Potter, B. V. L. Steroid sulfatase: Molecular biology, regulation and inhibition. *Endocr. Rev.* **2005**, *26*, 171–202.

(18) Reed, M. J.; Purohit, A.; Woo, L. W. L.; Potter, B. V. L. The development of steroid sulphatase inhibitors. *Endocr.-Relat. Cancer* **1996**, *3*, 9–23.

(19) Ho, Y. T.; Purohit, A.; Vicker, N.; Newman, S. P.; Robinson, J. J.; Leese, M. P.; Ganeshapillai, D.; Woo, L. W. L.; Potter, B. V. L.; Reed, M. J. Inhibition of carbonic anhydrase II by steroidal and non-steroidal sulphamates. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 909–914.

(20) Stanway, S. J.; Purohit, A.; Woo, L. W. L.; Sufi, S.; Vigushin, D.; Ward, R.; Wilson, R.; Stanczyk, F. Z.; Dobbs, N.; Kulinkaya, E.; Elliott, M.; Potter, B. V. L.; Reed, M. J.; Coombs, R. C. Phase I study of STX64 (667 Coumate) in breast cancer patients: The first study of a steroid sulfatase inhibitor. *Clin. Cancer Res.* **2006**, *12*, 1585–1592.

(21) Stanway, S. J.; Delavault, P.; Purohit, A.; Woo, L. W. L.; Thurieau, C.; Potter, B. V. L.; Reed, M. J. Steroid sulfatase: a new target for the endocrine therapy of breast cancer. *Oncologist* **2007**, *12*, 370–374.

(22) Hussain, S. A.; Ganesan, R.; Reynolds, G.; Gross, L.; Stevens, A.; Pastorek, J.; Murray, P. G.; Perunovic, B.; Anwar, M. S.; Billingham, L.; James, N. D.; Spooner, D.; Poole, C. J.; Rea, D. W.; Palmer, D. H. Hypoxia-regulated carbonic anhydrase IX expression is associated with poor survival in patients with invasive breast cancer. *Br. J. Cancer* **2007**, *96*, 104–109.

(23) Cushman, M.; He, H. M.; Katzenellenbogen, J. A.; Varma, R. K.; Hamel, E.; Lin, C. M.; Ram, S.; Sachdeva, Y. P. Synthesis of analogs of 2-methoxyestradiol with enhanced inhibitory effects on tubulin polymerization and cancer cell growth. *J. Med. Chem.* **1997**, *40*, 2323–2334.

(24) Klauber, N.; Parangi, S.; Flynn, E.; Hamel, E.; D'Amato, R. J. Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and taxol. *Cancer Res.* **1997**, *57*, 81–86.

(25) Cushman, M.; He, H. M.; Katzenellenbogen, J. A.; Lin, C. M.; Hamel, E. Synthesis, antitubulin and antimitotic activity, and cytotoxicity of analogs of 2-methoxyestradiol, an endogenous mammalian metabolite of estradiol that inhibits tubulin polymerization by binding to the colchicine binding site. *J. Med. Chem.* **1995**, *38*, 2041–2049.

(26) Abbate, F.; Winum, J. Y.; Potter, B. V. L.; Casini, A.; Montero, J. L.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: X-ray crystallographic structure of the adduct of human isozyme II with EMATE, a dual inhibitor of carbonic anhydrases and steroid sulfatase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 231–234.

(27) Elger, W.; Schwarz, S.; Hedden, A. M.; Reddersen, G.; Schneider, B. Sulfamates of various estrogens are prodrugs with increased systemic and reduced hepatic estrogenicity at oral application. *J. Steroid Biochem. Mol. Biol.* **1995**, *55*, 395–403.

(28) Dahut, W. L.; Lakhani, N. J.; Gulley, J. L.; Arlen, P. M.; Kohn, E. C.; Kotz, H.; McNally, D.; Parr, A.; Nguyen, D.; Yang, S. X.; Steinberg, S. M.; Venitz, J.; Sparreboom, A.; Figg, W. D. Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. *Cancer Biol. Ther.* **2006**, *5*, 22–27.

(29) Sutherland, T. E.; Anderson, R. L.; Hughes, R. A.; Altmann, E.; Schuliga, M.; Ziogas, J.; Stewart, A. G. 2-Methoxyestradiol: a unique blend of activities generating a new class of anti-tumour/anti-inflammatory agents. *Drug Discovery Today* **2007**, *12*, 577–584.

(30) Bubert, C.; Leese, M. P.; Mahon, M. F.; Ferrandis, E.; Regis-Lydi, S.; Kasprzyk, P. G.; Newman, S. P.; Ho, Y. T.; Purohit, A.; Reed, M. J.; Potter, B. V. L. 3,17-Disubstituted 2-alkylestra-1,3,5(10)-trien-3-ol derivatives: synthesis, in vitro and in vivo anti-cancer activity. *J. Med. Chem.* **2007**, *50*, 4443.

(31) Okada, M.; Iwashita, S.; Koizumi, N. Efficient general method for sulfamoylation of a hydroxyl group. *Tetrahedron Lett.* **2000**, *41*, 7047–7051.

(32) Burgess, E. M.; Penton, H. R.; Taylor, E. A. Synthetic applications of N-carboalkoxysulfamate esters. *J. Am. Chem. Soc.* **1970**, *92*, 5224–5226.

(33) Ohira, S. Methanolysis of dimethyl(1-diazo-2-oxopropyl)phosphonate. Generation of (diazomethyl) phosphonate and reaction with carbonyl compounds. *Synth. Commun.* **1989**, *19*, 561–564.

(34) Tinley, T. L.; Leal, R. M.; Randall-Hlubek, D. A.; Cessac, J. W.; Wilkens, L. R.; Rao, P. N.; Mooberry, S. L. Novel 2-methoxyestradiol analogues with antitumor activity. *Cancer Res.* **2003**, *63*, 1538–1549.

(35) Boyd, M. R.; Paull, K. D. Some practical consideration and applications of the National Cancer Institute *in vitro* anticancer drug discovery screen. *Drug Dev. Res.* **1995**, *34*, 91–109.

(36) Shoemaker, R. H. The NCI 60 human tumor cell line screen: An information-rich screen informing on mechanisms of toxicity. *In Vitro Cell. Dev. Biol.: Anim.* **2006**, *42*, 5A.

(37) Newman, S. P.; Foster, P. A.; Ho, Y. T.; Day, J. M.; Raobaikady, B.; Kasprzyk, P. G.; Leese, M. P.; Potter, B. V. L.; Reed, M. J.; Purohit, A. The therapeutic potential of a series of orally bioavailable anti-angiogenic microtubule disruptors as therapy for hormone-independent prostate and breast cancers. *Br. J. Cancer* **2007**, *97*, 1673–1682.

(38) Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. Inactivation of steroid sulfatase by an active site directed inhibitor, estrone-3-O-sulfamate. *Biochemistry* **1995**, *34*, 11508–11514.

(39) Lloyd, M. D.; Thiyagarajan, N.; Ho, Y. T.; Woo, L. W. L.; Sutcliffe, O. B.; Purohit, A.; Reed, M. J.; Acharya, K. R.; Potter, B. V. L. First crystal structures of human carbonic anhydrase II in complex with dual aromatase-steroid sulfatase inhibitors. *Biochemistry* **2005**, *44*, 6858–6866.

(40) Appel, R.; Berger, G. Uber das hydrazidosulfamid (On hydrazidosulfamide). *Chem. Ber.* **1958**, *91*, 1339–1341.

(41) Woo, L. W. L.; Lightowler, M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Heteroatom-substituted analogues of the active-site directed inhibitor estra-1,3,5(10)-trien-17-one-3-sulphamate inhibit estrone sulphatase by a different mechanism. *J. Steroid Biochem. Mol. Biol.* **1996**, *57*, 79–88.

(42) Otwinowski, Z.; Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Method Enzymol.* **1997**, *276*, 307–326.

(43) Eriksson, A. E.; Jones, T. A.; Liljas, A. Refined structure of human carbonic anhydrase II at 2.0Å resolution. *Proteins* **1988**, *4*, 274–282.

(44) Brunger, A. T.; Adams, P. D.; Clore, G. M.; Delano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J. S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr., Sect. D* **1998**, *54*, 905–921.

(45) Jones, T. A.; Zou, J. Y.; Cowan, S. W.; Kjeldgaard, M. Improved methods for building protein models in electron-density maps and the location of errors in these models. *Acta Crystallogr., Sect. A* **1991**, *47*, 110–119.

(46) Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton, J. M. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* **1993**, *26*, 283–291.

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