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Synthesis, Antitubulin, and Antiproliferative SAR of Analogues of 2-Methoxyestradiol-3,17-*O*,*O*-bis-sulfamate

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The synthesis and antiproliferative activity of analogues of estradiol 3,17-*O*,*O*-bis-sulfamates (E2bisMATEs) are discussed. Modifications of the C-17 substituent reveal that an H-bond acceptor is essential for high antiproliferative activity. The local environment in which this H-bond acceptor lies can be varied to an extent. The C-17-oxygen linker can be deleted or substituted with an electronically neutral methylene group, and replacement of the terminal NH_2 with a methyl group is also acceptable. Mesylates **10** and **14** prove equipotent to the E2bisMATEs **2** and **3**, while sulfones **20** and **35** display enhanced in vitro antiproliferative activity. In addition, the SAR of 2-substituted estradiol-3-*O*-sulfamate derivatives as inhibitors of tubulin polymerization has been established for the first time. These agents inhibit the binding of radiolabeled colchicine to tubulin.

Introduction

In previous reports, we and others described the discovery of sulfamoylated derivatives of 2-methoxyestradiol **1** (Figure 1) as promising anticancer agents with dual activity against cancer cell proliferation and angiogenesis.[1−7] The appeal of agents that act against these two targets has been underlined by the positive outcomes of clinical trials combining cytotoxic agents with the anti-VEGF antibody bevacizumab, an inhibitor of angiogenesis.[8−12] 2-Methoxyestradiol-3,17-*O*,*O*-bis-sulfamate **2** and 2-ethylestradiol-3,17-*O*,*O*-bis-sulfamate **3** (E2bisMATEs[a]) differ from **1** because of their enhanced biological activity and superior drug-like properties. The excellent oral bioavailability of **2** (> 85% in rodents) appears to derive from the ability of the sulfamate group to block inactivating metabolism and deactivating conjugation and to interact reversibly with carbonic anhydrase.[2,7,13−15] This latter reversible interaction, which has been characterized by protein crystallography, may minimize first pass liver metabolism through sequestration of the sulfamates in red blood cells. Moreover, as with other aryl sulfamates, **2** and **3** are irreversible inhibitors of steroid sulfatase (STS), itself a target for the treatment of hormone dependent cancer.[7,16] Although a full mechanistic picture for the activity of these compounds continues to emerge, all evidence collected to date suggests that their ability to disrupt the tubulin-microtubule equilibrium in cells is critical for their antitumor activity.[2] It is also important to note, first, that these compounds are not substrates for the P-glycoprotein pump and are thus active against taxane-resistant tumors,[17,18] and second, that their activity is independent of the estrogen receptor even though they are estrogen derivatives.

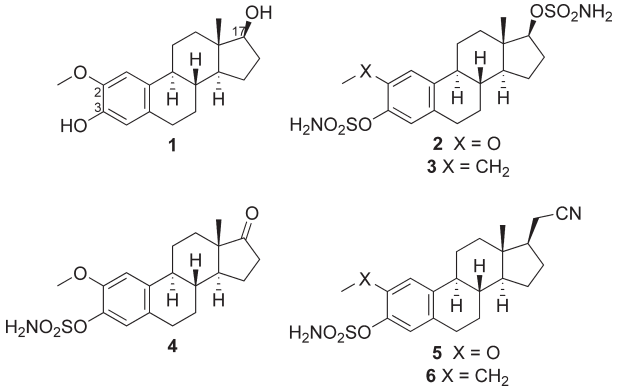

Figure 1. Structures of 2-methoxyestradiol **1**, 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate **2**, 2-ethylestradiol-3,17-*O*,*O*-bis-sulfamate **3**, 2-methoxyestrone-3-*O*-sulfamate **4**, 2-methoxy-3-*O*-sulfamoyl-17*β*-cyanomethyl estra-1,3,5(10)-triene **5**, and 2-ethyl-3-*O*-sulfamoyl-17*β*-cyanomethyl estra-1,3,5(10)-triene **6**.

A-ring modified estrogen-3-*O*-sulfamates were initially synthesized with the goal of obtaining potent nonestrogenic, STS inhibitors.[19] It was subsequently discovered that 2-methoxyestrone-3-*O*-sulfamate **4** also exhibited an antiproliferative effect against a range of estrogen-independent human cancer cells in vitro.[3] SAR studies on the A-ring estrogen-3-*O*-sulfamates demonstrated that optimal antiproliferative activity is obtained with a 2-methoxy, 2-ethyl, or 2-methyl sulfanyl group.[1] Further SAR studies focused on D-ring modifications, principally at the C-17 position of the estratriene

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[a] Abbreviations: E2bisMATEs, estradiol-3,17-*O*,*O*-bis-sulfamates; VEGF, vascular endothelial growth factor; STS, steroid sulfatase; *m*-CPBA, 3-chloroperoxybenzoic acid; DMA, *N*,*N*-dimethylacetamide; TEA, triethylamine; GTP, guanosine triphosphate.

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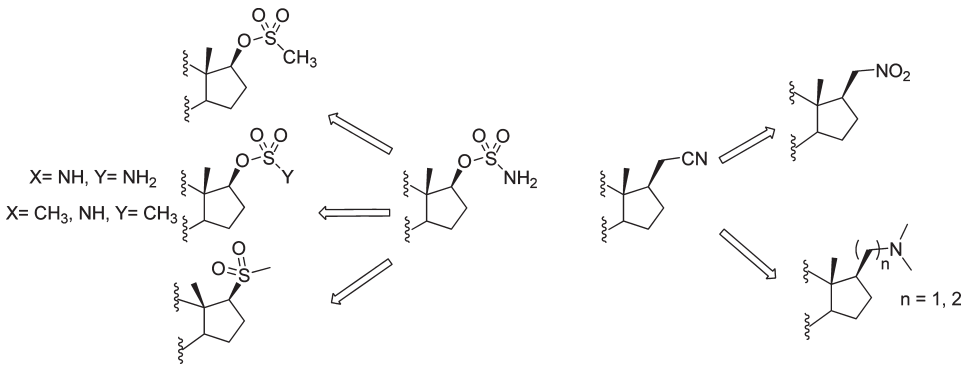


Figure 2. Proposed modifications to E2bisMATEs and 17β-cyanomethyl estradiol-3-*O*-sulfamates at C-17.

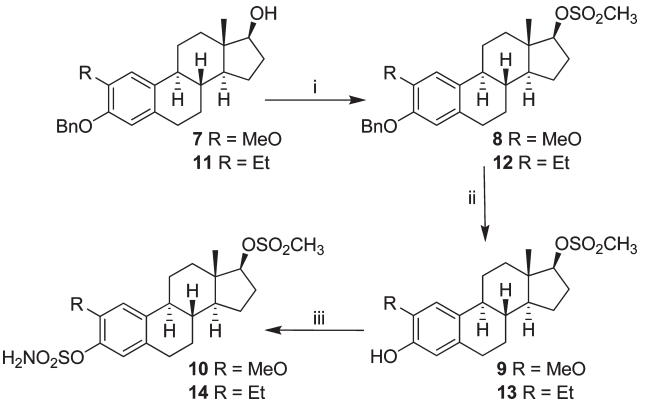
skeleton. A number of modifications were explored, including variation of the oxidation level of C-17 and substitution at this position. While 17β-hydroxy, 17-keto, and 17-oximino derivatives show similar antiproliferative activity, the 17-deoxy and 17α-benzyl analogues were considerably less active.[20] Sulfamoylation of the 17β-hydroxy group gave the E2bisMATEs **2** and **3** that exhibit slightly enhanced in vitro activity and greatly improved in vivo antitumor effects.[2] In addition, a SAR study of C-17 cyanated analogues revealed that 2-methoxy-3-*O*-sulfamoyl-17β-cyanomethyl estra-1,3,5(10)-triene **5** and 2-ethyl-3-*O*-sulfamoyl-17β-cyanomethyl estra-1,3,5(10)-triene **6** offered further enhanced antiproliferative and antiangiogenic activities relative to **2** and **3**.[21] We have also shown that a high level of antiproliferative activity can be retained when a heterocycle possessing a well exposed H-bond acceptor is installed at C-17.[22] It thus appears that optimal activity results from the combined presence of a C-2 XMe group (X = O, CH_2 or S), 3-*O*-sulfamate, and a H-bond acceptor around the C-17 position.

Having shown that there is a reasonable degree in flexibility in the nature of the C-17 substituent and that the in vivo profile of **2** and **3** remained the most promising of the structural class, we were drawn to modify the 17-*O*-sulfamate group in the hope of discovering additional antitumor compounds with improved activity. We thus set out to synthetically replace the constituent atoms of the sulfamate group with isosteric groups as outlined in Figure 2. In addition, amino and nitro derivatives were elaborated to compare their activities with those of the cyanomethyl compounds **5** and **6**. We describe here our synthetic approaches to these compounds together with their in vitro biological activity. We also report herein the structure−activity relationships of this compound class as inhibitors of tubulin polymerization and colchicine binding.

Results and Discussion

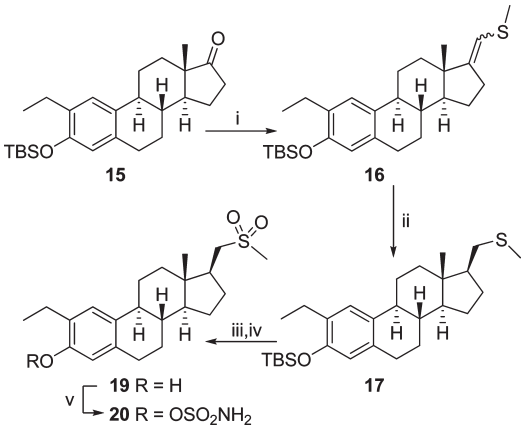
Chemistry. Our previous SAR studies on C-17 modified estradiol-3-*O*-sulfamates showed that E2bisMATEs **2** and **3** possess an excellent in vivo profile and are the most promising of their class of compounds.[2] To assess the contribution of the sulfamate NH_2 group to activity, the 17-β-*O*-methanesulfonyl analogues of **10** and **14** were prepared (Scheme 1). 2-Methoxy-3-*O*-benzyl estradiol **7** was reacted with methanesulfonyl chloride to afford the 2-methoxy-3-*O*-benzyl-17-*O*-methanesulfanylestra-1,3,5(10)-triene **8** in excellent yield. Hydrogenolysis of **8** furnished the corresponding phenol **9**, which was then treated with sulfamoyl chloride to afford the corresponding sulfamate derivative **10**. 2-Ethyl-3-*O*-benzyl estradiol **11** was transformed to give compounds **12**−**14** under analogous conditions.

Scheme 1[a]



[a] Reagents and conditions: (i) CH_3SO_2Cl, pyridine; (ii) H_2, Pd/C, THF, MeOH; (iii) H_2NSO_2Cl, DMA.

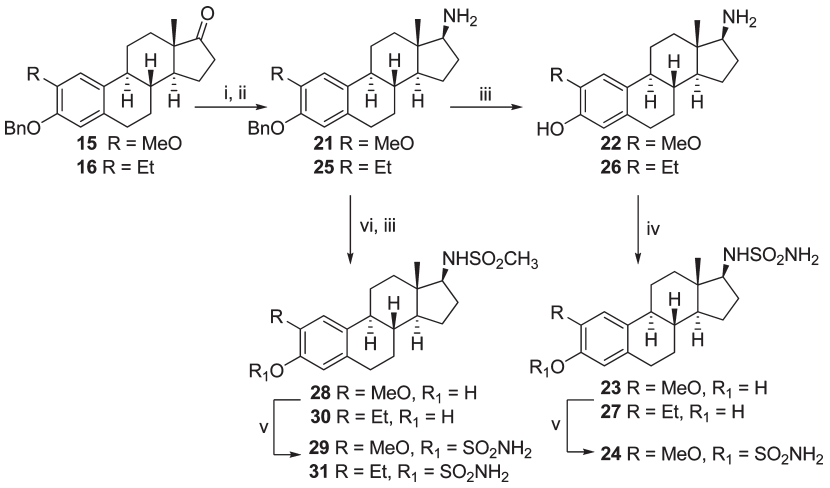
Scheme 2[a]



[a] Reagents and conditions: (i) NaH, $CH_3SCH_2PPh_3Cl$, THF; (ii) H_2, Pd/C, THF, MeOH; (iii) *m*-CPBA, DCM; (iv) TBAF, THF; (v) H_2NSO_2Cl, DMA.

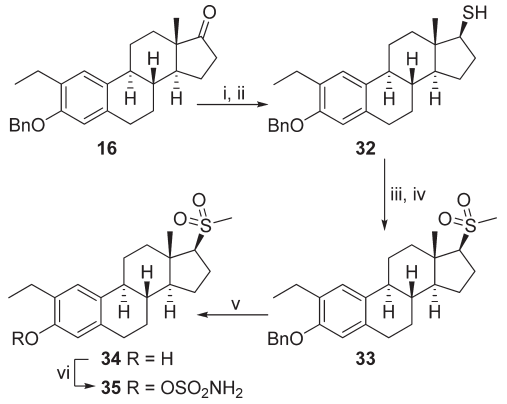
Having substituted the sulfamate NH_2 group for a methyl, we proceeded to additionally substitute the 17β-oxygen atom with a methylene group and thus prepared sulfone **20**. Wittig reaction of (methylthiomethyl)triphenylphosphonium chloride with 2-ethyl-3-*O*-*tert*-butyldimethylsilylestrone **15** gave methyl vinyl sulfide **16** as a 3:2 mixture of *E*- and *Z*-isomers (Scheme 2). Hydrogenation delivered **17** which, upon *m*-CPBA oxidation and silyl deprotection, gave sulfone **19**.

Scheme 3[a]



[a]Reagents and conditions: (i) hydroxylamine hydrochloride, pyridine, reflux; (ii) $NaBH_4$, MoO_3, THF, MeOH, 0 °C; (iii) H_2, Pd/C, THF, MeOH; (iv) sulfamide, 1,4-dioxane, reflux; (v) H_2NSO_2Cl, DMA; (vi) CH_3SO_2Cl, pyridine, 0 °C to rt.

Scheme 4[a]



[a] Reagents and conditions: (i) Lawesson's reagent, THF, 150 °C, microwave; (ii) $NaBH_4$, THF, 0 °C, 1 h; (iii) MeONa, MeI, THF, MeOH, rt, 16 h; (iv) *m*-CPBA, DCM, 0 °C, 2 h; (v) H_2, Pd/C, THF, MeOH; (vi) H_2NSO_2Cl, DMA.

Subsequent sulfamoylation under standard conditions afforded sulfamate **20**.

We had earlier established that the C-17 oxygen is not essential for activity[21] and were thus drawn to explore what effect its replacement with a nitrogen would have while otherwise retaining all other elements of the C-17 sulfamate. Amination of C-17 was thus required to access the C-17 sulfamides. This was effected by synthesizing the corresponding oxime, followed by diasteroselective reduction with sodium borohydride and molybdenum trioxide (Scheme 3), as described by Gonschior et al.,[23] to afford 17β-amine **21**. Deprotection of the benzyl ether by hydrogenolysis gave phenol **22**. Attempts to directly install the two sulfamate groups with sulfamoyl chloride in DMA[24] or 2,6-di-*tert*-butyl-4-methylpyridine in DCM proved unsuccessful, and we thus adopted a two-step approach toward the sulfamate/sulfamide derivatives. First, **22** was *N*-sulfamoylated with sulfamide in refluxing 1,4-dioxane to afford 2-methoxy-17β-aminosulfonamide-3-hydroxyestra-1,3,5(10)-triene **23** in 61% yield. Next, compound **23** was sulfamoylated with sulfamoyl chloride in DMA to give **24** in 23% yield. The same approach was used in the ethyl series, but the target compound could not be isolated in satisfactory purity.

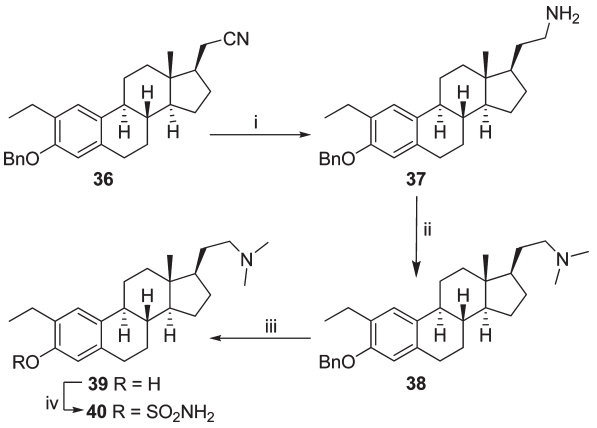
17β-*N*-Methylsulfonyl analogues of bis-sulfamates **2** and **3** were synthesized from intermediates **21** and **25** (Scheme 3) by reaction with methanesulfonyl choride. Deprotection and sulfamoylation yielded phenols (**28** and **30**) and sulfamate derivatives (**29** and **31**) in succession.

We also wished to evaluate the impact of deletion of the 17β-oxygen linker to the sulfamate on antiproliferative activity. We thus prepared 17β-methylsulfonyl-3-*O*-sulfamoylestra-1,3,5(10)-triene **34**, following the route outlined in Scheme 4. Treatment of 2-ethyl-3-*O*-benzylestrone **16** with Lawesson's reagent in refluxing toluene/xylene, followed by reduction with sodium borohydride in THF, afforded thiol **32**. *S*-Methylation with methyl iodide in the presence of sodium methoxide and subsequent oxidation with *m*-CPBA in DCM produced sulfone **33**. Conversion to the corresponding phenol **34** and sulfamate **35** was as described for the other analogues in the series.

As noted above, screening of various C-17 modified 2-substituted estrogen-3-*O*-sulfamates for antiproliferative activity indicated that hydrogen bonding interactions around C-17 are key to a high level of activity. We therefore investigated whether this positive interaction could be accessed by a *N*,*N*-dimethylamino group linked to C-17 by an alkyl linker. Nitrile **36**[22] was thus converted to 17β-(2-(*N*,*N*-dimethylamino)ethyl)-2-ethyl-3-*O*-sulfamoylestra-1,3,5(10)-triene **36**, as shown in Scheme 5. Reduction of **37** with lithium aluminum hydride in THF afforded **37**, which was doubly methylated in a refluxing mixture of formic acid and aqueous formaldehyde. Hydrogenolysis of the resultant 17β-(2-(*N*,*N*-dimethylamino)ethyl)-2-ethyl-3-*O*-benzylestra-1,3,5(10)-triene **37** gave the phenol **38**, which was converted to the sulfamate **39** as described above.

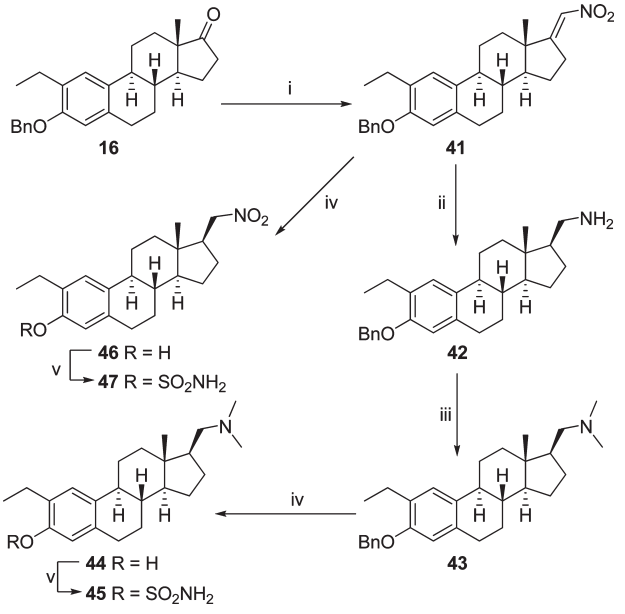
To access 17β-(*N*,*N*-dimethylaminomethyl)-2-ethyl-3-*O*-sulfamoylestra-1,3,5(10)-triene **45**, a Henry-type reaction under conditions modified from Tamura et al.[25] was used to give **41**. This compound was then reduced with lithium aluminum hydride to give 17β-aminomethyl-2-ethyl-3-*O*-benzylestra-1,3,5(10)-triene **42** in 40% overall yield. Reductive alkylation of **42** was carried out under the conditions described for the synthesis of **38**. Deprotection of **43** and sulfamoylation delivered successively phenol **44** and sulfamate **45**. Hydrogenation of **41** over Pd/C was also carried out to afford 17β-nitromethyl derivative **46**,

Scheme 5[a]



[a] Reagents and conditions: (i) $LiAlH_4$, THF, rt; (ii) 37% aq formaldehyde/HCOOH/reflux; (iii) H_2, Pd/C, THF, MeOH; (iv) H_2NSO_2Cl, DMA.

Scheme 6[a]



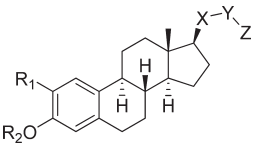
[a] Reagents and conditions: (i) CH_3NO_2, cat. $(CH_3)_2N(CH_2)_2NH_2$, reflux; (ii) $LiAlH_4$, THF, rt; (iii) 37% aq formaldehyde, HCOOH, reflux; (iv) H_2, Pd/C, THF, MeOH; (v) H_2NSO_2Cl, DMA.

which was subsequently converted into sulfamate **47** (Scheme 6).

Biology. To assess their potential as anticancer agents, we evaluated the series of novel 2-ethyl- and 2-methoxyestradiol derivatives for their ability to inhibit the proliferation of DU-145 (androgen receptor negative) prostate cancer cells and MDA-MB-231 (estrogen receptor negative) breast cancer cells in vitro. The results of these assays and comparative data for compounds **1**−**6** are presented in Table 1.

We reported earlier on the promising multitargeted antitumor agents **2** and **3** (Figure 1), both of which exhibit excellent activities against cancer cell proliferation and angiogenesis and possess excellent oral bioavailability.[2,6,15] These studies showed that *N*,*N*-bismethylation of the 17β-sulfamate causes a marked decreased in antiproliferative activity, the detrimental effect being attributed to steric factors, resulting from the size of the *N*,*N*-dimethylsulfamate

Table 1. Antiproliferative Activities of 2-Ethyl- and 2-Methoxyestradiol Derivatives against DU-145 Human Prostate Cancer Cells and MDA-MB-231 Human Breast Cancer Cells in Vitro[a]



						GI_{50} (μM)	
compd	R_1	R_2	X	Y	Z	DU-145	MDA MB-231
1	MeO	H	OH	na	na	1.22	0.94
2	MeO	SO_2NH_2	O	SO_2	NH_2	0.34	0.28
3	Et	SO_2NH_2	O	SO_2	NH_2	0.21	0.21
4	MeO	SO_2NH_2	CH_2	CN	na	0.062	0.071
5	Et	SO_2NH_2	CH_2	CN	na	0.054	0.141
6	MeO	SO_2NH_2	=O	na	na	0.46	0.12
9	MeO	H	O	SO_2	CH_3	4.2	2.37
10	MeO	SO_2NH_2	O	SO_2	CH_3	0.52	0.23
13	Et	H	O	SO_2	CH_3	5.58	nd
14	Et	SO_2NH_2	O	SO_2	CH_3	0.57	0.20
20	Et	SO_2NH_2	CH_2	SO_2	CH_3	0.19	0.23
22	MeO	H	NH_2	na	na	2.64	3.56
23	MeO	H	NH	SO_2	NH_2	58.6	nd
24	MeO	SO_2NH_2	NH	SO_2	NH_2	6.13	5.6
26	Et	H	NH_2	na	na	60.8	na
27	Et	H	NH	SO_2	NH_2	>100	nd
28	MeO	H	NH	SO_2	CH_3	>100	nd
29	MeO	SO_2NH_2	NH	SO_2	CH_3	9.9	5.2
30	Et	H	NH	SO_2	CH_3	>100	nd
31	Et	SO_2NH_2	NH	SO_2	CH_3	0.84	3.52
34	Et	H	na	SO_2	CH_3	>100	>100
35	Et	SO_2NH_2	na	SO_2	CH_3	0.11	0.23
39	Et	H	$(CH_2)_2$	NMe_2	na	7.96	4.74
40	Et	SO_2NH_2	$(CH_2)_2$	NMe_2	na	3.54	2.96
44	Et	H	CH_2	NMe_2	na	10.1	9.5
45	Et	SO_2NH_2	CH_2	NMe_2	na	3.05	4.03
46	Et	H	CH_2	NO_2	na	12.2	10.4
47	Et	SO_2NH_2	CH_2	NO_2	na	0.14	0.17
48	MeO	H	CH_2	CN	na	0.49	0.12

[a] Data for compounds **1**−**6** are taken from the literature.[2,21,26] Abbreviations: na, not applicable; nd, not determined.

moiety.[2] In an attempt to find a bioisosteric replacement of the 17-sulfamate, we also showed that 17β-carbamoyloxy-2-ethyl estradiol-3-*O*-sulfamate essentially retained the antiproliferative activity of **2** and **3**. In contrast, introduction of a 17β-*O*-acetate led to greatly reduced inhibition of cancer cell proliferation.[26]

In an attempt to better understand the role played by each constituent (O, SO_2, NH_2) of the 17β-*O*-sulfamate group in the antitumor activity of compounds **2** and **3**, and to possibly achieve enhanced activity, we designed a number of analogues. First, we replaced the NH_2 group with a methyl group. Hence, phenols (**9**, **13**) and sulfamates (**10**, **14**) were prepared and screened against DU-145 and MDA-MB-231 cancer cells. As can be seen from the data presented in Table 1, phenols **9** and **13** proved 3−4 fold less active than 2-methoxyestradiol **1**. With the notable exception of compound **48**,[21] which is the only C-17 substituted estratriene-3-ol derivative that possesses significantly enhanced antiproliferative activity relative to **1**, these results are in agreement with those of previous studies wherein simple modification of position C-17 alone does not yield greatly improved activities relative to the parent estradiol.[1,2,20,21,26,27] Sulfamates **10** and **14**,

Table 2. GI_{50} (μM) and MGM (μM) Values Obtained from the NCI Screening Panel[a]

compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12-C	MGM
1	0.7	0.47	0.32	0.36	0.21	0.95	1.3
2	0.051	0.045	0.036	< 0.01	< 0.01	0.126	0.087
3	< 0.01	nd	< 0.01	< 0.01	< 0.01	0.028	0.018
8	6.9	5.9	4.6	14.4	2.9	19.9	5.9
10	1.17	0.78	0.34	0.65	0.54	3.1	1.58
14	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.028
20	< 0.01	< 0.01	< 0.01	10.7	< 0.01	< 0.01	0.030
28	> 100	60.3	> 100	> 100	> 100	> 100	89.1
29	9.6	5.6	3.2	7.1	3.5	23.4	7.1
31	4.17	4.37	0.47	5.89	0.71	6.92	2.69
44	21.4	11.5	16.2	18.6	39.8	43.7	20.9
45	13.5	9.1	12.6	11.7	12.6	18.2	10.2
47	0.031	0.041	0.021	0.063	0.025	0.066	0.071

[a] Results are micromolar GI_{50} values. Data for **1**, **2**, and **3** are taken from the literature.[2,30] The MGM represents the mean concentration that caused 50% growth inhibition in all 60 cell lines. Abbreviation: nd, not determined.

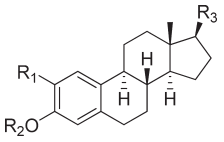
on the other hand, displayed excellent antiproliferative activities, being slightly less potent than **2** and **3** in DU-145 cells but equipotent in MDA-MB-231 cells. Thus, the C-17 terminal NH_2 can be replaced by a small alkyl group and the amino group's potential H-bond donating properties are not required for a compound to maintain good antiproliferative activity. Compounds **10** and **14**, together with a selection of other phenols and sulfamates, were also tested for antiproliferative activity in the NCI 60-cell line assay.[28,29] Data obtained for six individual cell lines are presented in Table 2. Compound **14** caused > 50% growth inhibition at concentrations below 10 nM in the majority of the cell lines, with MGM value of 28 nM, which correlates well with our DU-145 and MDA-MB-231 assay results. Compound **10** was much less active, with a MGM value of 1.58 μM.

The next change we made in the 17-*O*-sulfamate moiety was replacement of the oxygen linker between C-17 and the SO_2 group with a methylene group (sulfamate **20**) or a NH group (sulfamates **24**, **29**, **31**). Compound **20** was equipotent with derivatives **2** and **3** in both the DU-145 and MDA-MB-231 cells. It was also highly active in the NCI cell lines, yielding a MGM value of 30 nM. Conversely, the 17-β-aminosulfonamide **24** (X = NH) was 20- to 30-fold less active than E2bisMATEs **2** and **3**. When both the oxygen linker and terminal NH_2 were replaced, as in 17-β-aminomethanesulfonyl derivatives **29** and **31** (X = NH, Z = CH_3), the two resulting compounds had different activities. Compound **29** was dramatically less potent than **2** and **3** in the DU-145 and MDA-MB-231 cells and in the NCI cell lines. Compound **30** was more active, with a submicromolar GI_{50} in the DU-145 cells. In summary, while an H-bond acceptor group (X = O) and an electronically neutral group (X = CH_2) were tolerated, an H-bond donating group (X = NH) led to a marked decrease in antiproliferative activity. Interestingly, sulfamate **35**, in which the methylsulfonyl group is directly linked to C-17, was slightly more active than **2** and **3** in DU-145 cells and equipotent with them in MDA-MB-231 cells. Thus, the oxygen linker to the sulfamate at C-17 is not required for antiproliferative activity. These results also illustrate the importance of the SO_2 hydrogen-bond accepting group because replacement of both the oxygen linker and final NH_2 group (compound **20**) or deletion of the oxygen linker (compound 35), can still lead to compounds displaying remarkable antiproliferative activities.

In light of the above results, we sought to evaluate the impact of the H-bond accepting group Y (see Figure 2) on the antiproliferative activity of the resulting sulfamates by replacing the SO_2 group with a *N*,*N*-dimethylamino group (sulfamates **39** and **44**) and a nitro group (sulfamate **46**). As can be seen from Table 1, compounds **39** and **44** proved 10- to 15-fold less active than the E2bisMATEs **2** and **3** and between 20- and 60-fold less active than the cyanomethyl derivatives **4** and **5**, indicating that the *N*,*N*-dimethylamino group is not a suitable replacement for the SO_2 and the cyano groups, regardless of the length of the alkyl linker (CH_2 or $(CH_2)_2$). This might be due to the size of the NMe_2 group, with steric hindrance around the nitrogen atom reducing its accessibility and in turn its ability to form the requisite H-bond. This postulate is supported by the excellent antiproliferative activities in DU-145 and MDA-MB-231 cells shown by the 17β-nitromethyl derivative **46**, which had a MGM value of 71 nM in the NCI panel.

The antiproliferative effect of 2-methoxyestradiol **1** has been shown to stem from its ability to disrupt tubulin polymerization by interacting at the colchicine site of tubulin.[31] Likewise, mechanistic studies carried out on 2-methoxyestrone-3-*O*-sulfamate **4** and its 2-ethyl analogue have shown that they induce mitotic arrest and apoptosis, properties that are most likely attributable to their antimicrotubule activity.[4] To explore their likely mechanism of action and construct an on target SAR for this class of compounds, the E2bisMATEs **2** and **3** and a number of the most active 2-substituted estradiol-3-*O*-sulfamate derivatives were evaluated for their inhibitory effects on tubulin polymerization and on the binding of [^{3}H]colchicine to tubulin (Table 3). With the exception of compounds **50**, **53**, and **54**, which had little effect on tubulin assembly (and indeed are only very modest inhibitors of cell proliferation), all the tested derivatives were strong inhibitors of tubulin polymerization, with compounds **3**, **5**, **14**, **47**, **49**, and **52** essentially equipotent to combretastatin A-4 in this assay and 2- to 5-fold more active than 2-methoxyestradiol **1**. A clear correlation between the ability to inhibit tubulin assembly and cell proliferation is evident. Indeed, all of the compounds with submicromolar GI_{50}'s against DU-145 and MDA-MB-231 proliferation were good inhibitors of tubulin assembly (IC_{50} values between 1.3 and 3.6 μM), while the compounds with weak antiproliferative activity (**50**, **53**, and **54**) had negligible effects on tubulin assembly. Importantly, sulfamates **2** and **51** are markedly more active than their

Table 3. Inhibition of Tubulin Polymerization and Colchicine Binding by 2-Ethyl and 2-Methoxyestradiol Derivatives and Combretastatin A-4 (CA-4)



compd	R_1	R_2	R_3	GI_{50}(μM) DU-145	inhibition of tubulin assembly [c] IC_{50} (μM) ± SD	colchicine binding,[d] % inhibition ± SD
CA-4	na	na	na		1.2 ± 0.2	99 ± 1
1	MeO	H	OH	1.22	7.0 ± 0.8	17 ± 4
51	MeO	SO_2NH_2	OH	0.19[a]	3.0 ± 0.4	29 ± 6
52	Et	SO_2NH_2	OH	< 0.01[a]	1.3 ± 0.04	57 ± 0.8
4	MeO	SO_2NH_2	=O	0.32[a]	3.3 ± 0.2	22 ± 5
53	MeO	SO_2NMe_2	=O	> 10[b]	> 40	nd
54	MeO	H	OSO_2NH_2	18.3[a]	> 40	nd
2	MeO	SO_2NH_2	OSO_2NH_2	0.34	2.2 ± 0.3	28 ± 3
3	Et	SO_2NH_2	OSO_2NH_2	0.21	1.3 ± 0.01	45 ± 4
49	Me	SO_2NH_2	OSO_2NH_2	0.38	1.3 ± 0.2	46 ± 4
50	^{n}Pr	SO_2NH_2	OSO_2NH_2	3.4	20 ± 1	nd
14	Et	SO_2NH_2	OSO_2CH_3	0.57	1.6 ± 0.2	49 ± 0.2
20	Et	SO_2NH_2	$CH_2SO_2CH_3$	0.19	2.1 ± 0.2	37 ± 5
35	Et	SO_2NH_2	SO_2CH_3	0.11	3.6 ± 0.2	28 ± 5
5	MeO	SO_2NH_2	CH_2CN	0.062	1.3 ± 0.08	78 ± 0.9
47	Et	SO_2NH_2	CH_2NO_2	0.14	1.7 ± 0.3	74 ± 2

[a] Data taken from the literature.[1,2] [b] GI_{50} in MCF-7 cells.[1] [c] Tubulin was at 10 μM, and compounds were evaluated at various concentrations. Inhibition of the extent of assembly at 20 min was determined. [d] Inhibition of [^{3}H] colchicine binding: tubulin was at 1.0 μM, colchicine was at 5.0 μM, and tested compounds were at 5.0 μM. na: not applicable, nd: not determined.

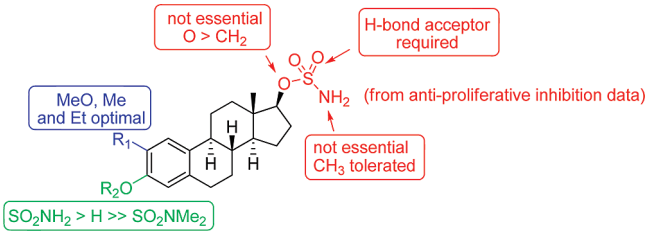


Figure 3. In vitro SAR of 2-substituted estradiol-3-*O*-sulfamate derivatives as antiproliferative agents and tubulin polymerization inhibitors.

corresponding phenols **1** and **54**, a result that also correlates with our previous observation that sulfamoylation at the phenolic position is a key factor for obtaining a good level of antiproliferative activity.[1−3,20−22,26,27] Similarly, in the bis-sulfamate series, optimal activity was obtained for the C-2 methyl, ethyl, and methoxy derivatives (compounds **49**, **2** and **3**), while the bulkiest group in the series (*n*-propyl, compound **49**) was detrimental to activity, with a sharp, > 10-fold decrease in the GI_{50} obtained as well as sharply reduced activity as an inhibitor of tubulin assembly. *N*,*N*-Dimethylation of sulfamate **52** (IC_{50} = 3.3 μM) to yield compound **53** led to a sharp decrease in tubulin assembly activity, in line with the deleterious effect of this substituent on antiproliferative activity.[1]

Study of the effects of this series of compounds on inhibition of colchicine binding was performed with the agents that inhibited tubulin assembly with IC_{50}'s in the low micromolar range, with a comparison to the known weak effect of **1** and the known potent effect of combretastatin A-4 (CA-4). All the sulfamates examined were more potent than **1** but substantially less active than CA-4. When present in equimolar concentration with [^{3}H]colchicine (5.0 μM each), there was a reasonable correlation between potency as an inhibitor of tubulin assembly and potency as an inhibitor of colchicine binding. Among the most active inhibitors of assembly, the greatest inhibition of colchicine binding was observed with compound **5** (78%) and the least with compound **3** (45%).

Conclusions

This study was aimed at identifying the minimum requirements to maintain or enhance the high level of antiproliferative activity of E2bisMATEs **2** and **3** by modification of the C-17β-*O*-sulfamate. A summary of the in vitro SAR of the 2-substituted estradiol-3-*O*-sulfamate compounds as antiproliferative agents and tubulin polymerization inhibitors is presented in Figure 3.

We first showed that the hydrogen bonding potential of the terminal NH_2 group of the sulfamate is not essential and that high antiproliferative activity is retained when it is replaced by a CH_3, as illustrated by compounds **10**, **14**, and **20**. We also established that, while the 17β-aminosulfamate **24** and 17β-aminomesylates **29** and **31** show much reduced antiproliferative activities, substitution of the oxygen linker remains possible. This was shown by the substantial antiproliferative activity obtained with sulfone **20**. We also demonstrated that the oxygen linker of the 17β-sulfamate can be deleted, as demonstrated by the antiproliferative potency of compound **35**. The high antiproliferative activity shown by 2-ethyl-17β-nitromethyl-3-*O*-sulfamoylestra-1,3,5(10)-triene **46** contrasts with the 20−25-fold lower antiproliferative activity observed with the *N*,*N*-dimethylaminomethyl derivative **45** and the *N*,*N*-dimethylaminoethyl derivative **40**. This result is in concert with the excellent antiproliferative activity obtained with compounds **10**, **14**, **20**, and **35**, all of which possess a SO_2 group able to function as a H-bond acceptor moiety, which thus far is required at position C-17. Finally, evaluation of a number of 2-substituted estradiol-3-*O*-sulfamate derivatives that possess high antiproliferative activity demonstrated that this activity probably derives from their ability to inhibit tubulin assembly. Over the course of a number of studies,

we have found that the sulfamate group yields strong interactions with enzymes such as sulfatase and carbonic anhydrase and, in the present case, proteins such as tubulin. The results presented here serve to define which components of the sulfamate group are key to its interaction with tubulin and should thus facilitate the design of further microtubule disruptors and optimization of sulfamate bearing lead compounds in the future.

Experimental Section

Biology. In Vitro Studies: Cell Lines. DU-145 (brain metastasis carcinoma of the prostate) and MDA-MB-231 (metastatic pleural effusion of breast adenocarcinoma) established human cell lines were obtained from the ATCC Global Bioresource Center. Cells were maintained in a 5% CO_2 humidified atmosphere at 37 °C in RPMI-1640 medium, supplemented with 10% fetal bovine serum, penicillin, and streptomycin.

Antiproliferative Assays. DU-145 and MDA-MB-231 cells were seeded into 96-well microtiter plates (5000 cells/well) and treated with $10^{-9}-10^{-4}$ M compounds or with vehicle control. At 96 h post-treatment, live cell counts were determined by the WST-1 cell proliferation assay (Roche, Penzberg, Germany) as per the manufacturer's instructions. Viability results were expressed as a percentage of mean control values resulting in the calculation of the 50% growth inhibition (GI_{50}). All experiments were performed in triplicate.

Tubulin Assays. Bovine brain tubulin, prepared as described previously,[32] was used in the studies presented here. Assembly IC_{50}'s were determined as described in detail elsewhere.[33] Briefly, 1.0 mg/mL (10 μM) tubulin was preincubated without GTP with varying compound concentrations for 15 min at 30 °C. The reaction mixtures were placed on ice, and GTP (final concentration, 0.4 mM) was added. The reaction mixtures were transferred to cuvettes, held at 0 °C in a recording spectrophotometer. Baselines were established at 0 °C, and increase in turbidity was followed for 20 min following a rapid (< 30 s) jump to 30 °C. Compound concentrations required to reduce the turbidity increase by 50% were determined. The method for measuring inhibition of the binding of [^{3}H]colchicine to tubulin was described in detail previously.[34] Reaction mixtures contained 0.1 mg/mL (1.0 μM) tubulin, 5.0 μM [^{3}H]colchicine, and potential inhibitor at 5.0 μM. Compounds were compared to CA-4, a particularly potent inhibitor of the binding of colchicine to tubulin.[35] Reaction mixtures were incubated 10 min at 37 °C, a time point at which the binding of colchicine in control reaction mixtures is generally 40−60% complete.

Chemistry. All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, UK) or Alfa Aesar (Heysham, UK). Organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK) and used as supplied. DMA was purchased from Aldrich and stored under a 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[36] and was stored in the refrigerator under positive pressure of N_2 as a solution in toluene as described by Woo et al.[37] An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. Reactions were carried out at rt unless stated otherwise. Flash column chromatography was performed on silica gel (MatrexC60).

^{1}H NMR and ^{13}C NMR spectra were recorded with either a JMN-GX 270 at 270 and 67.5 MHz, respectively, or a Varian Mercury VX 400 NMR spectrometer at 400 and 100 MHz, respectively, and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS) as internal standard. Mass spectra were recorded at the Mass Spectrometry Service Center, University of Bath, UK. FAB-MS were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Stuart SMP3 melting point apparatus and are uncorrected. HPLC analyses were performed on a Waters Millenium 32 instrument equipped with a Waters 996 PDA detector. A Waters Sunfire C18 reversed-phase column (8 mm × 100 mm) was eluted with a methanol/water gradient at 2 mL/min. The purity of final compounds was greater than 95% unless specified otherwise.

2-Methoxy-3-*O*-benzyl-17β-*O*-methanesulfonylestra-1,3,5(10)-triene 8. Methanesulfonyl chloride (0.09 mL, 1.2 mmol) was added to an ice-cooled solution of 2-methoxy-3-*O*-benzyl-estradiol **7** (390 mg, 1 mmol) in dry pyridine (5 mL) under nitrogen. The solution was stirred at 0 °C for 2 h, and the reaction mixture was quenched with water (20 mL). The organics were extracted with ethyl acetate (2 × 60 mL), and the organic layer was washed successively with water and brine and then dried over $MgSO_4$. After evaporation of the solvents under vacuum, the resultant crude solid was purified by flash chromatography (hexane/ethyl acetate 5:1) to give **8** as a white powder (0.44 g, 93%), mp 163−164 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.86 (3H, s), 1.19−1.52 (6H, m), 1.70−1.91 (3H, m), 2.05 (1H, m), 2.17−2.32 (3H, m), 2.72 (2H, m), 3.00 (3H, s), 3.85 (3H, s), 4.56 (1H, dd, *J* 8.6 and 8.1 Hz), 5.09 (2H, s), 6.60 (1H, s), 6.82 (1H, s), 7.28−7.46 (5H, m). HRMS (ES+) *m*/*z* found 471.2188; $C_{27}H_{35}O_5S$ ($M^+ + H$) requires 471.2200.

2-Methoxy-3-hydroxy-17β-*O*-methanesulfonylestra-1,3,5(10)-triene 9. A solution of **8** (375 mg, 0.8 mmol) in THF (10 mL) and methanol (30 mL) was treated with 10% Pd/C (40 mg) and then stirred under an atmosphere of hydrogen for 24 h. The resultant suspension was then filtered through celite, washed with ethyl acetate, and evaporated under reduced pressure. The resultant crude solid was purified by flash chromatography (hexane/ethyl acetate 2:1) to give a white powder (260 mg, 86%), mp 167−168 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s), 1.33−1.60 (6H, m), 1.76−1.94 (3H, m), 2.05−2.09 (1H, m), 2.18−2.34 (3H, m), 2.80 (2H, m), 3.06 (3H, s), 3.90 (3H, s), 4.61 (1H, dd, *J* 9.1 and 7.7 Hz), 5.52 (1H, s), 6.68 (1H, s), 6.81 (1H, s). HRMS (ES+) *m*/*z* found 381.1743; $C_{20}H_{29}O_5S$ ($M^+ + H$) requires 381.1730. Anal. ($C_{20}H_{28}O_5S$) C, H, N.

2-Methoxy-3-*O*-sulfamoyl-17β-*O*-methanesulfonylestra-1,3,5(10)-triene 10. Compound **9** (115 mg, 0.3 mmol) was added to an ice cold solution of sulfamoyl chloride (0.6 mmol) in DMA (1.0 mL). The resulting mixture was stirred for 16 h at rt and then diluted with ethyl acetate (50 mL) and washed with water (4 × 20 mL), brine (20 mL), dried, and evaporated. Flash chromatography (hexane/ethyl acetate 5:1 to 2:1) gave a white powder (120 mg, 86%), mp 152−153 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 0.76 (3H, s), 1.18−1.25 (2H, m), 1.31−1.44 (4H, m), 1.62−1.81 (3H, m), 1.84−1.96 (1H, m), 2.08−2.19 (2H, m), 2.22−2.34 (1H, m), 2.65 (2H, m), 2.96 (3H, s), 3.71 (3H, s), 4.44 (1H, dd, *J* 8.9 and 7.7 Hz), 6.78 (2H, s), 6.88 (1H, s), 6.90 (1H, s). HRMS (ES+) *m*/*z* found 459.1372; $C_{20}H_{29}NO_7S_2$ (M^+) requires 459.1385. Anal. ($C_{20}H_{29}NO_7S_2$) C, H, N.

2-Ethyl-3-hydroxy-17β-*O*-methanesulfonylestra-1,3,5(10)-triene 13. A solution of **12** (330 mg, 0.7 mmol) in THF (10 mL) and methanol (30 mL) was treated with 10% Pd/C (40 mg) and then stirred under an atmosphere of hydrogen for 24 h, as described for the synthesis of **9**. The resultant crude solid was purified by flash chromatography (hexane/ethyl acetate 2:1) to give a white powder (190 mg, 77%), mp 195−196 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.86 (3H, s), 1.21 (3H, t, *J* 7.7 Hz), 1.25−1.60 (6H, m), 1.71−1.91 (3H, m), 2.03 (1H, m), 2.13−2.38 (3H, m), 2.58 (2H, q, *J* 7.7 Hz), 2.79 (2H, m), 3.01 (3H, s), 4.53 (1H, s), 4.56 (1H, dd, *J* 9.1 and 7.9 Hz), 6.49 (1H, s), 7.03 (1H, s). HRMS (ES+) *m*/*z* found. 379.1946; $C_{21}H_{31}O_4S$ ($M^+ + H$) requires 379.1938. Anal. ($C_{21}H_{30}O_4S$) C, H, N.

2-Ethyl-3-*O*-sulfamoyl-17β-*O*-methanesulfonylestra-1,3,5(10)-triene 14. Compound **13** (115 mg, 0.3 mmol) was reacted with sulfamoyl chloride (0.6 mmol) in DMA (1 mL), as described for the synthesis of **10**. Flash chromatography (hexane/ethyl acetate

5:1 to 2:1) gave a white powder (105 mg, 77%), mp 179−180 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.85 (3H, s), 1.20 (3H, t, J 7.4 Hz), 1.30−1.55 (6H, m), 1.73−1.87 (3H, m), 2.04 (1H, m), 2.16−2.36 (3H, m), 2.68 (2H, q, J 7.4 Hz), 2.82 (2H, m), 3.01 (3H, s), 4.57 (1H, dd, J 8.7 and 8.1 Hz), 5.08 (2H, s), 6.49 (1H, s), 7.03 (1H, s). HRMS (ES+) m/z found 458.1672; $C_{21}H_{32}NO_6S_2$ ($M^+ + H$) requires 458.1666. Anal. ($C_{21}H_{31}NO_6S_2$) C, H, N.

(*E*- and *Z*-)-2-Ethyl-3-*O*-*tert*-butyldimethylsilyl-17-methylsulfanylmethylene Estrone 16. A rt suspension of hexane-washed sodium hydride (12 mmol) in THF (20 mL) was treated with diethyl (methylthiomethyl)phosphonate (2.14 mL, 12 mmol) and then brought to reflux. On cessation of gas evolution, the clear colorless solution was treated with a solution of 2-ethyl-3-*O*-tert-butyldimethylsilyl estrone (1.68 g, 4 mmol) in THF (20 mL). After 18 h at reflux, the reaction was cooled, diluted with ethyl acetate (60 mL), poured onto water (40 mL), and the aqueous layer separated. The organic layer was then washed with water (3 × 35 mL) and brine (50 mL) and then dried and evaporated. Column chromatography (5% ethyl acetate/hexane) yielded the desired TBS-protected alkene **16** (1.13 mg, 62%), a colorless oil, as a mixture of isomers, as well as an amount of the corresponding desilylated product (300 mg, 22%). ^{1}H NMR ($CDCl_3$) δ 0.21 (6H, s), 0.92, 0.81 (3H, 2*s*), 1.00 (9H, s), 1.19 (3H, t, J 7.4), 2.26, 2.21 (3H, 2*s*), 2.56 (2H, q, J 7.4), 2.75 (2H, m), 5.48−5.55 (1H, 2*m*, both isomers), 6.54, 6.47 (1H, s, both isomers), 7.05 (1H, s). m/z [FAB+] found 456.2882, $C_{28}H_{44}SOSi$ requires 456.2882.

2-Ethyl-3-*O*-*tert*-butyldimethylsilyl-17β-methylsulfanylmethyl Estrone 17. A solution of **16** (1 g, 2.2 mmol) in THF (5 mL) and ethanol (50 mL) was treated with 10% Pd/C (1 g) at 80 psi for 18 h. The material was filtered through celite and then evaporated to give the desired product **17** as a clear colorless oil (570 mg, 56%). ^{1}H NMR ($CDCl_3$) δ 0.21 (6H, s), 0.65 (3H, s), 0.99 (9H, s), 1.15 (3H, t, J 7.4), 2.10 (3H s), 1.20−2.40 (15H, m), 2.56 (2H, q, J 7.4), 2.65−2.85 (3H, m), 6.47 (1H, s) and 7.04 (1H, s). HRMS [FAB+], $C_{28}H_{46}OSSi$ requires 458.3039.

2-Ethyl-3-*O*-*tert*-butyldimethylsilyl-17β-methanesulfonylmethyl Estrone 18. A rt solution of **17** (500 mg, 1.09 mmol) in dichloromethane (25 mL) was treated with *m*-CPBA (764 mg, 4 mmol). The reaction was stirred for 16 h and then washed with aqueous sodium hydroxide (40 mL, 1 M), water (40 mL), and brine (40 mL), dried, and evaporated. The crude product, a yellow oil, was purified by column chromatography (4:1 to 3:1 hexane/ethyl acetate) to give the desired sulfone **18** (170 mg, 35%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.21 (6H, s), 0.64 (3H, s), 0.99 (9H, s), 1.15 (3H, t, J 7.4), 1.20−2.40 (14H, m), 2.55 (2H, q, J 7.4), 2.74−2.94 (3H, m), 2.92 (3H, s), 3.10−3.20 (1H, m), 6.47 (1H, s), 7.03 (1H, s). HRMS [FAB+], $C_{28}H_{46}O_3SSi$ requires 490.2937.

2-Ethyl-17-methanesulfonylmethyl Estrone 19. A solution of **18** (135 mg, 0.29 mmol) in THF (5 mL) was treated with a solution of tetra-butyl ammonium fluoride in THF (0.5 mL. 0.5 mmol) and maintained at rt for 16 h. The reaction was then diluted with ethyl acetate (25 mL), washed with water (20 mL) and brine (25 mL), and then dried and evaporated. The product was crystallized from ether/hexane to give the desired sulfone **19** as a white solid (85 mg over 3 crops, 77%). ^{1}H NMR ($CDCl_3$) δ 0.65 (3H, s), 1.21 (3H, t, J 7.4), 1.26−2.38 (14H, m), 2.58 (2H, q, J 7.4), 2.76−2.84 (2H, m), 2.85−2.92 (1H, m), 2.93 (3H, s), 3.13 (1H, dd, J 13.3 and 2.3), 4.60 (1H, s), 6.48 (1H, s), 7.02 (1H, s). m/z [ES−] 375.3 ($M^+ - H$, 100%). HRMS [FAB+] 376.2072, $C_{22}H_{32}SO_3$ requires 376.2072.

2-Ethyl-3-*O*-sulfamoyl-17-methanesulfonylmethyl Estrone 20. Sulfamoyl chloride (150 mg, 1.3 mmol) was cooled to 0 °C, dissolved in DMA (2 mL) and then after 5 min treated with **19** (60 mg, 0.16 mmol). External cooling was removed after 15 min, and the reaction was left to stir at rt for 3 h. The reaction was then diluted in ethyl acetate (15 mL), poured onto brine (15 mL), and the organic layer was separated. The organic extract was washed with water (3 × 10 mL), brine (10 mL), dried, and evaporated to give a yellow powder. Crystallization from ethyl acetate/hexane afforded the desired product **20** as white crystals (42 mg, 58%), mp 208−210 °C. ^{1}H NMR ($CDCl_3$ + 2 drops CD_3OD) δ 7.17 (1H, s), 7.07 (1H, s), 4.95 (2H, s), 3.10−3.18 (1H, m), 2.76−2.95 (6H, m, including 2.92 (3H, s)), 2.66 (2H, q, J 7.4), 1.16−2.40 (17H, m including 1.20 (3H, t, J 7.4), 0.64 (3H, s). m/z [APCI−] 454.29 ($M^+ - H$, 100%). HRMS [FAB+] 455.1769, $C_{22}H_{33}NO_5S$ requires 455.1800.

2-Ethyl-3-benzyloxyestra-1,3,5(10)-triene-17β-thiol 32. 3-Benzyl-2-ethylestrone (3.1 g, 8.0 mmol), Lawesson's reagent (1.6 g, 4.0 mmol), and a magnetic stirring bar were placed in an oven-dried 10 mL microwave vessel. Anhydrous THF (4.0 mL) was added and the mixture irradiated in the microwave at 150 °C for 30 min. After cooling to rt, additional Lawesson's reagent (1.6 g, 4.0 mmol) was added and the mixture irradiated once more in the microwave at 150 °C for 30 min. To gain enough material, the whole procedure was carried out altogether four times. The resulting thick slurry was taken up in DCM and filtered through a silica gel pad. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography (SiO_2, hexane/DCM 9:1 to 4:1 to 1:1) to give 2-ethyl-3-benzyloxyestra-1,3,5(10)-triene-17-thione as a salmon-red solid (914 mg, 28%), mp 125−129 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.93 (3H, s), 1.21 (3H, t, J 7.5), 1.34−1.92 (6H, m), 1.96−2.32 (4H, m), 2.39−2.54 (1H, m), 2.59−3.08 (4H, m), 2.68 (2H, q, J 7.5 Hz), 5.05 (2H, s), 6.65 (1H, s), 7.12 (1H, s), 7.27−7.46 (5H, m). HRMS (ES+) m/z found 405.2243; $C_{27}H_{33}OS$ ($M^+ + H$) requires 405.2247. Starting material was recovered as beige solid (1.868 g, 60%). The thione (485 mg, 1.2 mmol) was dissolved in anhydrous THF (6.0 mL) and cooled to 0 °C. Sodium borohydride (138 mg, 3.6 mmol) was added portionwise, and the reaction mixture was stirred for 1 h at 0 °C. Hydrochloric acid (1 M, 30 mL) was slowly added at 0 °C, and the mixture was extracted with ethyl acetate (4 × 30 mL), washed with water and brine, dried ($MgSO_4$), filtered, and concentrated to give the title compound as a light-yellow solid (479 mg, 98%); mp 127−132 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.74 (3H, s), 1.10−1.65 (7H, m), 1.21 (3H, t, J 7.5 Hz), 1.70−1.89 (3H, m), 1.93 (1H, dt, J 12.4, 3.0 Hz), 2.12−2.29 (2H, m), 2.37 (1H, dq, J 13.5, 3.0 Hz), 2.67 (2H, q, J 7.5 Hz), 2.68−2.95 (3H, m), 5.04 (2H, s), 6.63 (1H, s), 7.11 (1H, s), 7.27−7.47 (5H, m). HRMS (ES+) m/z found 407.2397; $C_{27}H_{35}OS$ ($M^+ + H$) requires 407.2403.

2-Ethyl-3-*O*-benzyl-17β-methanesulfonylestra-1,3,5(10)-triene 33. Compound **32** (406 mg, 1.0 mmol) was dissolved in anhydrous THF (15 mL) and methanol (15 mL) and cooled to 0 °C. Sodium methoxide (51.4 mg, 0.95 mmol) was added portionwise, and the reaction mixture was stirred for 15 min. After warming to rt, iodomethane (131 mg, 0.92 mmol) was added, and the reaction mixture was stirred for 16 h at rt. After removal of the solvents under reduced pressure, water (100 mL) and glacial acetic acid (5 mL) were added, the organics were extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography (SiO_2, hexane/ethyl acetate 95:5 to 90:10) to give 2-ethyl-3-*O*-benzyl-17β-methanesulfanylestra-1,3,5(10)-triene as a white solid (142 mg, 35%); mp 186−188 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.81 (3H, s), 1.23 (3H, t, J 7.5 Hz), 1.18−1.72 (7H, m), 1.74−1.93 (2H, m), 2.05 (1H, dt, J 12.1, 3.2 Hz), 2.12−2.29 (2H, m), 2.16 (3H, s), 2.31−2.42 (1H, m), 2.62 (1H, t, J 9.1 Hz), 2.69 (2H, q, J 7.4 Hz), 2.75−2.96 (2H, m), 5.05 (2H, s), 6.64 (1H, s), 7.12 (1H, s), 7.28−7.49 (5H, m). HRMS (ES+) m/z found 421.2566; $C_{28}H_{37}OS$ ($M^+ + H$) requires 421.2560. The intermediate sulfide (84 mg, 0.2 mmol) was dissolved in anhydrous DCM and cooled to 0 °C. 3-Chloroperbenzoic acid (70 wt %; 149 mg, 0.6 mmol) was added portionwise. The reaction mixture was stirred for 2 h at 0 °C and then diluted with DCM (30 mL) and washed with a 1 M aqueous sodium hydroxide solution (2 × 15 mL). The organic layer was dried ($MgSO_4$), filtered, and

concentrated to give the title compound as a white solid (91 mg, 99%); mp 168−174 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.08 (3H, s), 1.19 (3H, t, *J* 7.4 Hz), 1.23−1.62 (6H, m), 1.78−1.99 (2H, m), 2.02−2.41 (4H, m), 2.46 (1H, dd, *J* 8.9, 2.5 Hz), 2.65 (2H, q, *J* 7.6 Hz), 2.75−2.91 (2H, m), 2.82 (3H, s), 5.02 (2H, s), 6.61 (1H, s), 7.07 (1H, s), 7.26−7.47 (5H, m). HRMS (ES+) *m*/*z* found 453.2464; $C_{28}H_{37}O_3S$ ($M^+ + H$) requires 453.2458.

2-Ethyl-3-hydroxy-17β-methanesulfonylestra-1,3,5(10)-triene 34. A solution of **33** (90 mg, 0.2 mmol) in THF (5 mL) and methanol (20 mL) was treated with 10% Pd/C (19 mg) as described for the synthesis of **9**. The resultant crude solid was purified by flash column chromatography (SiO_2: hexane/ethyl acetate 9:1 to 4:1) to give the title compound as a light-yellow solid (69 mg, 97%); mp 194−197 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.93 (3H, s), 1.05 (3H, t, *J* 7.5 Hz), 1.11−1.43 (6H, m), 1.64−1.84 (1H, m), 1.92−2.23 (4H, m), 2.26−2.33 (1H, m), 2.45 (2H, q, *J* 7.5 Hz), 2.59−2.72 (2H, m), 2.71 (3H, s), 2.88 (2H, t, *J* 9.4 Hz), 6.40 (1H, s), 6.85 (1H, s), 7.77 (1H, s, br). HRMS (ES−) *m*/*z* found 361.1848; $C_{21}H_{29}O_3S$ (M^- − H) requires 361.1843.

2-Ethyl-3-*O*-sulfamoyl-17β-methanesulfonylestra-1,3,5(10)-triene 35. Compound **34** (43 mg, 0.12 mmol) was reacted with sulfamoyl chloride (0.6 mmol) in DMA (1 mL), as described for the synthesis of **10**. Flash chromatography (hexane/ethyl acetate 4:1) afforded the title compound as a white solid (45 mg, 85%); mp 181−187 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.87 (3H, s), 1.00 (3H, t, *J* 7.5 Hz), 1.06−1.43 (6H, m), 1.64−1.79 (2H, m), 1.84−2.21 (4H, m), 2.26 (1H, dd, *J* 9.0, 2.3 Hz), 2.51 (2H, q, *J* 7.5 Hz), 2.59−2.71 (2H, m), 2.65 (3H, s), 2.84 (2H, t, *J* 9.5 Hz), 6.90 (1H, s), 6.95 (1H, s). HRMS (ES−) *m*/*z* found 440.1573; $C_{21}H_{30}NO_5S_2$ (M^- − H) requires 440.1571.

2-Ethyl-3-*O*-benzyl-17-nitromethyleneestra-1,3,5(10)-triene 41. A solution of 2-ethyl-3-*O*-benzylestrone (1.94 g, 5 mmol) in nitromethane (60 mL) was refluxed in a round-bottom flask equipped with a Dean−Stark trap and a condenser until 10 mL of nitromethane was distilled, and then *N,N*-dimethylethylenediamine (0.1 mL, 0.9 mmol) was added and the solution refluxed for 24 h. After cooling the reaction mixture to rt, the solvent was evaporated under vacuum and the residual solid was purified by column chromatography (hexane/ethyl acetate 10:1 to 8:1) and recrystallized in hexane/ethyl acetate 20:1 to afford a white powder (1.6 g, 74%), mp 78−79 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.96 (3H, s), 1.21 (3H, t, *J* 7.4 Hz), 1.35−1.62 (6H, m), 1.99 (3H, m), 2.27 (1H, m), 2.47 (1H, m), 2.66 (2H, q, *J* 7.4 Hz), 2.85 (2H, m), 3.09 (2H, m), 5.04 (2H, s), 6.64 (1H, s), 6.92 (1H, dd, *J* 2.5 and 2.2 Hz), 7.10 (1H, s), 7.29−7.46 (5H, m). HRMS (ES+) *m*/*z* found 432.2530; $C_{28}H_{34}NO_3$ ($M^+ + H$) requires 432.2534.

2-Ethyl-3-hydroxy-17β-nitromethylestra-1,3,5(10)-triene 46. A solution of **41** (1.29 g, 3 mmol) in THF (10 mL) and methanol (60 mL) was treated with 10% Pd/C (40 mg), as described for the synthesis of **9**. The resultant crude solid was purified by flash column chromatography (hexane/ethyl acetate 10:1 to 5:1) to give a white powder (0.65 g, 65%), mp 132−133 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.70 (3H, s), 1.21 (3H, t, *J* 7.4 Hz), 1.30−1.56 (7H, m), 1.75−2.05 (4H, m), 2.16−2.35 (3H, m), 2.58 (2H, q, *J* 7.4 Hz), 2.79 (2H, m), 4.25 (1H, dd, *J* 11.6 and 9.2 Hz), 4.48 (1H, dd, *J* 11.6 and 5.9 Hz), 4.49 (1H, s), 6.49 (1H, s), 7.02 (1H, s). HRMS (ES+) *m*/*z* found 344.2222; $C_{21}H_{30}NO_3$ ($M^+ + H$) requires 344.2220. Anal. ($C_{21}H_{29}NO_3$) C, H, N.

2-Ethyl-17β-nitromethyl-3-*O*-sulfamoylestra-1,3,5(10)-triene 47. Compound **46** (0.34 g, 1 mmol) was reacted with sulfamoyl chloride (3 mmol) in DMA (1 mL), as described for the synthesis of **10**. Flash column chromatography (hexane/ethyl acetate 5:1 to 2:1) and subsequent recrystallization (hexane/ethyl acetate 6:1) gave a white powder (0.31 g, 76%), mp 203−204 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.70 (3H, s), 1.20 (3H, t, *J* 7.4 Hz), 1.25−1.53 (7H, m), 1.78−2.05 (4H, m), 2.22−2.34 (3H, m), 2.68 (2H, q, *J* 7.4 Hz), 2.84 (2H, m), 4.25 (1H, dd, *J* 11.8 and 9.1 Hz), 4.48 (1H, dd, *J* 11.8 and 6.0 Hz), 4.93 (2H, s), 7.07 (1H, s), 7.16 (1H, s). HRMS (ES+) *m*/*z* found 423.1939; $C_{21}H_{31}N_2O_5S$ ($M^+ + H$) requires 423.1948. Anal. ($C_{21}H_{30}N_2O_5S$) C, H, N.

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Supporting Information Available: Table of elemental analysis, ^{13}C NMR data, and the experimental details for compounds **12**, **21**−**31**, **37**−**40** and **42**−**45** are presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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