# A-Ring-Substituted Estrogen-3-*O*-sulfamates: Potent Multitargeted Anticancer Agents

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*Received January 24, 2005*

Efficient and flexible syntheses of 2-substituted estrone, estradiol and their 3-*O*-sulfamate (EMATE) derivatives have been developed using directed *ortho*-lithiation methodology. 2-Substituted EMATEs display a similar antiproliferative activity profile to the corresponding estradiols against a range of human cancer cell lines. 2-Methoxy (**3**, **4**), 2-methylsulfanyl (**20**, **21**) and 2-ethyl EMATEs (**32**, **33**) proved the most active compounds with 2-ethylestradiol-3-*O*-sulfamate (**33**), displaying a mean activity over the NCI 55 cell line panel 80-fold greater than the established anticancer agent 2-methoxyestradiol (**2**). 2-Ethylestradiol-3-*O*-sulfamate (**33**) was also an effective inhibitor of angiogenesis using three in vitro markers, and various 2-substituted EMATEs also proved to be inhibitors of steroid sulfatase (STS), a therapeutic target for the treatment of hormone-dependent breast cancer. The potential of this novel class of multimechanism anticancer agents was confirmed in vivo with good activity observed in the NCI hollow fiber assay and in a MDA-MB-435 xenograft mouse model.

## Introduction

The discovery that the endogenous estrogen metabolite 2-methoxyestradiol **2** (2-MeOE2) could inhibit cancer cell proliferation and angiogenesis[1] by a mechanism independent of the estrogen receptor has stimulated considerable interest in the mechanism underlying these activities and the potential application of this molecule as a therapeutic agent. The promise displayed by 2-MeOE2 in preclinical studies has led to its evaluation in ongoing Phase I/II clinical trials as a treatment for various cancers although a precise understanding of its mechanism of action has not yet been established.

Metabolism of estradiol (E2) by cytochrome P450 generates 2-hydroxyestradiol which, by catechol *O*-methyl transferase mediated methylation, is then converted to 2-MeOE2.[2] The affinity of 2-MeOE2 for both estradiol receptors $ER_\alpha$ and $ER_\beta$ is approximately one hundredth that of E2.[3] 2-MeOE2 inhibits the proliferation of a range of estrogen-dependent (ER+) and estrogen independent (ER−) cell lines both in vitro[4] and in vivo[1,5] indicating that its underlying mechanism of action is independent of the estradiol receptors. Similarly, when assayed against the proliferation of brain-derived capillary endothelial cells as a measure of antiangiogenic activity, 2-MeOE2 proved 90-times more potent than 2-methoxyestrone (2-MeOE1) and 250-times more potent than estradiol.[1]

A number of potential mechanisms underlying the reversible mitotic arrest[6] and apoptosis[7−9] induced by 2-MeOE2 in various cancer cell lines have been proposed. 2-MeOE2 has been shown to interact with the colchicine binding site primarily on $\beta$-tubulin (although evidence has also been presented for interaction with α-tubulin) and appears to induce metaphase arrest by functioning as an antimicrotubule agent.[10] Several studies have shown that 2-MeOE2 inhibits tubulin assembly in vitro. However, Attalla et al. have shown that in intact cells, 2-MeOE2, like paclitaxel, promotes tubulin polymerization.[11] Given that the concentrations required to achieve biological effects are far lower that those required to affect microtubule structure, it may well be that 2-MeOE2 affects the kinetics of mitotic spindle dynamics in vivo rather than altering microtubule polymerization per se.[12] Cytotoxicity may also arise from the effects of 2-MeOE2 on apoptosis regulators such as p53[8] or BCL-2,[11] or alternatively from interference with DNA synthesis, an effect atypical of microtubule disruptors.[13,14] A recent study has demonstrated that at a concentration of 50 $\mu$M, 2-MeOE2 inhibits HIF-1α at the posttranscriptional level, prevents HIF-1 target gene expression in tumor cells and inhibits HIF-2α in human endothelial cells.[15] These effects occur downstream from the interaction of 2-MeOE2 and microtubules and, inter alia, inhibit the transcriptional activation of the angiogenic promoter vascular endothelial growth factor (VEGF).[15]

Alongside its evaluation as an anticancer agent, 2-MeOE2 has also been proposed as a potential therapy in a wide range of other areas including arthritis,[16] asthma,[14] atherosclerosis,[17] inflammation and cardiovascular disease.[18] The biological activities of 2-MeOE2 have stimulated several groups to investigate novel 2-substituted estradiols as potential therapeutic agents; A-,[13,19−26] B-[27,28] and D-ring[24,29,30] modified analogues of 2-MeOE2 have shown enhanced in vitro activity as

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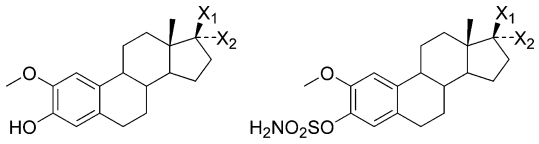
10.1021/jm050066a CCC: $30.25 
Published on Web 07/01/2005




**1** $X_1 = X_2 = O$ **3** $X_1 = X_2 = O$

**2** $X_1 = OH, X_2 = H$ **4** $X_1 = OH, X_2 = H$

**Figure 1.** Chemical structures of 2-MeOE1 **1**, 2-MeOE2 **2**, 2-MeOEMATE **3** and 2-MeOE2MATE **4**.

inhibitors of cancer cell proliferation and tubulin polymerization. To truly improve on the natural product, however, a consideration of the in vivo fate of the molecule must be undertaken, since metabolic oxidation of the 17-hydroxyl group of 2-MeOE2 by 17$\beta$-hydroxysteroid dehydrogenase Type 2 generates inactive 2-MeOE1 **1** (Figure 1). In addition, rapid formation of sulfate conjugates of the 3- and 17-hydroxyl groups of 2-MeOE2 leads to a efficient clearance of 2-MeOE2. Simple 2-MeOE2 analogues are therefore equally likely to suffer from metabolic inactivation and rapid clearance. An appreciation of the importance of these factors can be drawn from a recent clinical trial where a dose escalation up to 6 g/day of 2-MeOE2 was tested in an attempt to achieve a satisfactory plasma concentration.[31]

Our initial focus on 2-MeOE2-like molecules arose from a long standing interest in the development of inhibitors of steroid sulfatase[32−34] which led to the discovery that 2-substituted estrone sulfamates (EMATEs), in addition to being potent nonestrogenic inhibitors of steroid sulfatase,[35] also exhibit antiproliferative effects on human breast cancer cells.[36] Initial work showed that at a concentration of 1 $\mu$M 2-methoxyestrone-3-*O*-sulfamate (2-MeOEMATE) **3**, in contrast to 2-MeOE1 **1**, could cause cell cycle arrest in the $G_2$/M phase; furthermore, this arrest was irreversible while that triggered by 2-MeOE2 was reversible.[36] The effect of 2-MeOEMATE on cancer cells was shown to be independent of the estrogen receptor. A key finding of this study was that 2-MeOEMATE also induced apoptosis in these cell lines; a number of potential pro-apoptotic actions were identified including phosphorylation of BCL-2 and BCL-XL.[37] 2-MeOEMATE also proved to be significantly more active than 2-MeOE1 and 2-MeOE2 in the induction of the tumor suppressor protein p53 and inhibition of the paclitaxel-induced polymerization of tubulin in vitro.[37] Furthermore, initial in vivo work showed that 2-MeOEMATE caused regression of nitrosomethylurea-induced mammary tumors in rats.[36] A noteworthy property of estrogen sulfamates is their high observed oral bioavailability, believed to be due to their uptake by red blood cells and consequent bypassing of first pass liver metabolism.[38] This effect appears to stem from the reversible interaction of these steroidal sulfamates with the catalytic site of carbonic anhydrase II, an enzyme which is highly expressed in red blood cells, through a coordination of the monoanionic form of the sulfamate moiety to the zinc atom.[39] The potential of any such active delivery mechanism acting for 2-substituted estrogen sulfamates would be highly beneficial given the generally poor oral bioavailability of 2-substituted estrogens (vide supra).

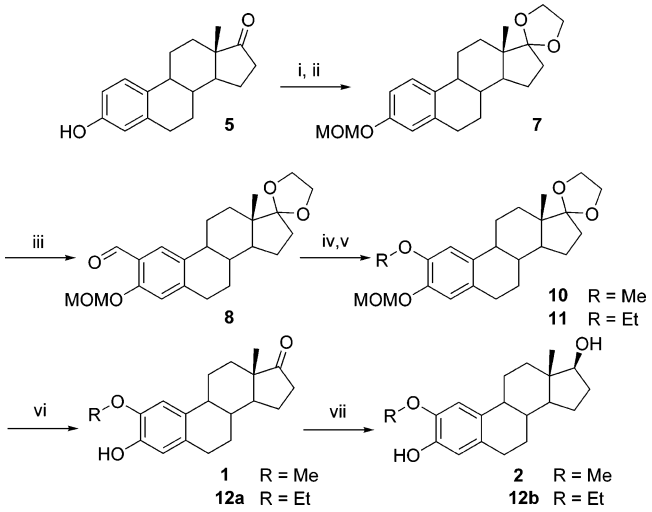
Although, as with 2-MeOE2, a full mechanistic picture of the multiple targeted activity of the 2MeOEMATE remains to be elucidated its spectrum of biological activity encouraged us to explore closely related structures in the search for new antitumor compounds. We thus set out to determine the structural requirements for potency in the 2-substituted estrogen sulfamates, which required an efficient and flexible synthetic approach to 2-substituted estrones and their sulfamate derivatives. Although efficient approaches to 2-substituted estradiols are well-established, we reasoned that direct access to the 2-substituted estrone would afford greater synthetic efficiency by avoidance of the protection, oxidation and deprotection sequence required for transformation of estradiol to estrone. We report here our synthetic approach to such A-ring-substituted estrogen sulfamates with their biological activity in vitro and in vivo and the structure−activity relationship for these compounds. A preliminary account of the work on 2-alkyl sulfanyl derivatives been published.[19]

## Results and Discussion

**Chemistry**. An initial series of A-ring modified 3-*O*-sulfamoylated estrogens was targeted. Maximum flexibility in this route was to be achieved by initial elaboration of the parent estrone **5** (E1), which could then be sulfamoylated prior to adjustment of the oxidation state at C-17. A number of reports had appeared on the synthesis of 2-methoxyestrogens, primarily in the estradiol series, and these could be classified into two distinct strategies: introduction of an oxidizable function by electrophilic means[40] or activation of the 2-position toward nucleophilic substitution.[41,42] The latter approach involves intrinsically fewer steps since no intermediate protection of the 3-hydroxyl function is required. The major drawback of this approach is, however, the lack of selectivity of the reactions used to activate the ring; iodination, for example, gives a mixture of the 2-, 4-, and 2,4-iodinated estrones with literature reports indicating isolated yields of the desired 2-iodo compounds to be around 50%.[41,43] This approach was also hampered by the need to use heavy metal salts as catalysts to obtain optimal yield and selectivity. An elegant approach to 2-MeOE2 involves formation of a $\eta_5$-iridium oxo-complex on the A-ring which can then undergo nucleophilic substitution with methoxide at the 2-position.[44] Unfortunately, this approach is stoichiometric in iridium thus making it prohibitively expensive for scale-up purposes.

The first synthesis of 2-methoxyestrogens reported by Fishman involves construction of an oxidizable xanthylium salt onto the estrane A-ring.[40] To obtain 2-MeOE1 it proved necessary to first access protected 2-MeOE2 since the 17-keto function did not tolerate the oxidative conditions of the key rearrangement. However, this route only yields small amounts of the desired compound after a convoluted synthesis. Acylation at the 2-position by Friedel−Crafts and Fries rearrangement chemistry was explored by Nambara and co-workers with oxidation of this group by the Baeyer−Villiger reaction giving access to the key 2-hydroxy intermediate.[45] Although successful, this synthesis is lengthened by the protecting group chemistry required to access the target estrone and gives a low overall yield. This strategy has subsequently been refined in a recent synthesis of 2-MeOE2 using a Fries rearrangement to

**Scheme 1**[a]

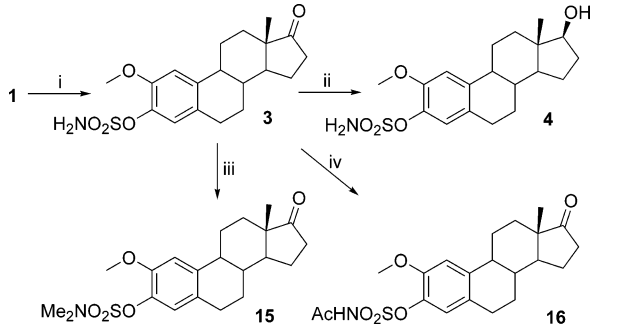



[a] Reagents and conditions: (i) ethylene glycol, *p*-TsOH, Dean−Stark, PhMe, quant; (ii) NaH, MOMCl, DMF, 95%; (iii) *sec*-BuLi, THF, −78 °C, 1 h then DMF −78 °C to room temperature, 86%; (iv) *m*CPBA, $Na_2HPO_4$, DCM then NaOH, MeOH, 84%; (v) $K_2CO_3$, TBAI, DMF and MeI (93%) or EtI (89%); (vi) 6 M HCl /THF (R = Me 90%, R = Et 92%) vii) $NaBH_4$, THF/IPA (R = Me 85%, R = Et 83%).

introduce the 2-acyl group in a selective and high yielding manner.[46] We decided not to use this approach since we wished to adopt a strategy which would allow access to a wider range of 2-substituents. Formylation at the 2-position was used by Cushman et al. in two different syntheses of 2-MeOE2. The earlier approach involved an unselective formylation with hexamethylenetetramine, and this gave only 25% of the desired 2-formylE2 which could then be transformed into 2-MeOE2 in 5% overall yield.[47] A second approach proved far superior,[22] with a 2-formyl group being introduced at the 2-position after the ortho-lithiation method of Pert and Ridley,[48] wherein bis-methoxymethyl-protected estradiol undergoes a sterically directed lithiation at the 2-position. The lithiated intermediate can then be reacted with a range of electrophiles,[49] with DMF quench, affording the 2-formyl derivative in good yield and selectivity.[48] Given this selectivity and the scalable nature of the chemistry involved, along with the opportunity to introduce a range of other functionality, we were attracted to modify this approach to give direct access to 2-substituted estrones and the corresponding estradiols. Conversion of the 17-carbonyl group to the 17$\beta$-hydroxyl is facile and high yielding, in contrast to the reverse process.

A protecting group for the 17-keto group of E1 **5** was required to complement the MOM protection required to direct the ortho-lithiation reaction. A 17-ethylene dioxolone group appeared ideal since it offered a facile introduction and deprotection, the latter being realized in tandem with the acidic MOM cleavage at the 3-*O*-position of the steroid if so desired. Reaction of E1 with ethylene glycol under Dean−Stark conditions delivered the ketal **6** in quantitative yield (Scheme 1). MOM protection was then incorporated under standard conditions by addition of chloromethyl methyl ether to the sodium phenolate of **6** in DMF solution. The desired MOM/ketal **7** was thus available in a rapid manner and high 95% yield over two steps. A directed metalation

**Scheme 2**[a]




[a] Reagents and conditions: (i) $H_2NSO_2Cl$, DBMP, DCM, 0 °C to room temperature, 84%; (ii) $NaBH_4$, THF/IPA, 85%; (iii) NaH, MeI, DMF 58%; (iv) $Ac_2O$, pyridine, 78%.

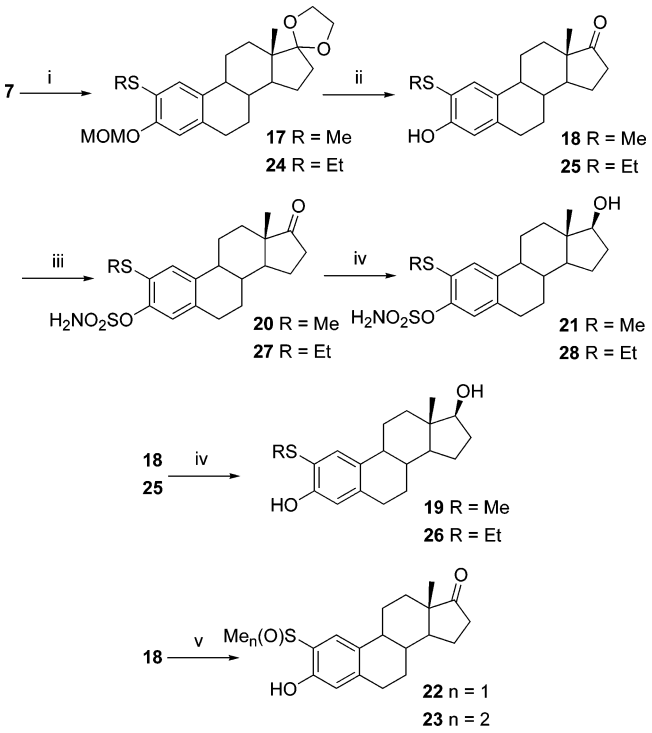
performed on a −78 °C THF solution of the MOM/ketal **7** with *sec*-butyllithium was then followed by a DMF quench to deliver the 2-formyl compound **8** in 86% yield accompanied by a small amount (ca. 3%) of the 4-formyl isomer which was removed by flash chromatography. The *ortho*-lithiation step proved highly dependent on reagent quality, and hence the use of freshly distilled DMF and good quality *sec*-butyllithium solution was key to successful reaction. Completion of the synthesis was achieved in much the same manner as the synthesis of 2-MeOE2.[22] The formyl group was transformed into the formate intermediate by Baeyer−Villiger oxidation accomplished with *m*-chloroperbenzoic acid in buffered dichloromethane and the product then saponified to give the desired 2-hydroxy compound **9**. Alkylation of this with potassium carbonate and the appropriate alkyl iodide in the presence of a catalytic amount of *tetra*-*n*-butylammonium iodide in DMF delivered the protected 2-alkoxy estrones **10** and **11**. Simple acidic deprotection could then be applied to give the desired 2-alkoxy estrones in good overall yield. 2-Methoxyestrone **1** and 2-ethoxyestrone **12a** were thus available for further manipulation.

The transformation of the 2-alkoxy estrones **1** and **12a** into their respective sulfamates could then be achieved with either sulfamoyl chloride[50] in DMA[51] or with 2,6-di-*tert*butyl-4-methylpyridine in dichloromethane[52] to give high yields of the desired 2-substituted estrone-3-*O*-sulfamate **3** or **13** (2-substituted EMATE) respectively as shown in Scheme 2. A selective reduction of the 17-keto function could then be achieved with sodium borohydride in THF/methanol to give the 2-substituted estradiol-3-*O*-sulfamates (2-substituted E2MATEs) **4** and **14**. 2-MeOE2 **2** and 2-EtOE2 **12b** were prepared from the corresponding estrones **1** and **12a** under analogous conditions. A series of 2-methoxy and 2-ethoxy estrones, estradiols and their respective 3-*O*-sulfamate derivatives was thus available for biological evaluation.

The effect of sulfamate *N*-substitution on the activity on 2-MeOEMATE was examined with alkylation (sodium hydride/methyl iodide) and acylation (pyridine/acetic anhydride) of the sulfamate proceeding in a smooth manner (Scheme 2) to afford the desired sulfamate derivatives **15** and **16** in good yield. The *N*-monomethyl sulfamate derivative is not readily accessible by this approach.

We were interested to compare the activities of the 2-alkyl sulfanyl estradiols, their 3-*O*-sulfamate derivatives and the corresponding 2-alkoxy estradiol and

**Scheme 3**[a]

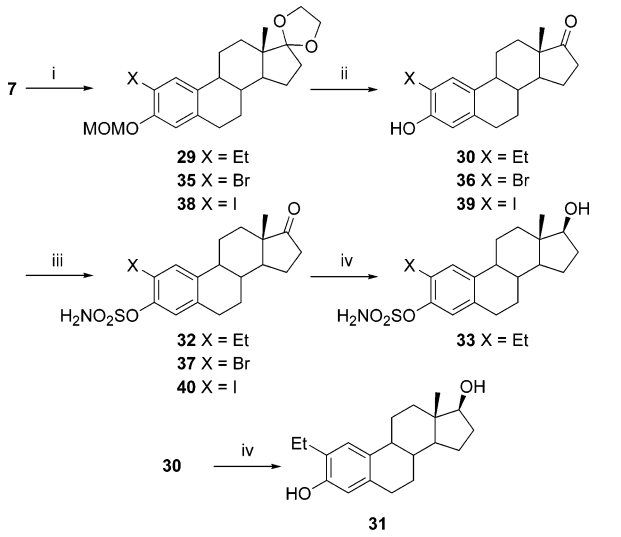



[a] Reagents and conditions: (i) *sec*-BuLi, THF, −78 °C, 1 h then RSSR, −78 °C to room temperature; (ii) 4 M HCl /MeOH; (iii) $H_2NSO_2Cl$, DBMP, DCM, rt iv) $NaBH_4$, THF/IPA; (v) *m*CPBA, DCM, $CHCl_3$.

estradiol-3-*O*-sulfamates. 2-Ethylsulfanyl estradiol, 2-EtSE2, **25**, was previously synthesized by Cushman and co-workers in moderate yield by a nucleophilic substitution reaction between the sodium salt of ethanethiol and protected 2-iodo estradiol. Given the success of our approach to alkoxy estrones, we perceived that the use of disulfides to quench the lithio-anion of MOM/ketal **7** would provide a rapid, flexible entry to the 2-alkylsulfanyl estrogens. As anticipated, disulfides proved to be excellent electrophiles in these reactions with protected 2-methylsulfanyl and 2-ethylsulfanyl estrones, **17** and **24,** being obtained in excellent 91% and 97% yields respectively as shown in Scheme 3. Acidic deprotection at the 3- and 17-positions then afforded the desired 2-alkylsulfanyl estrones **18** and **25** which were then available for transformation into their respective 2-substituted estradiols **19** and **26**, 2-substituted EMATEs **20** and **27** and 2-substituted E2MATEs **21** and **28**, as previously described for the alkoxy series. We were thus able to compare the effect of transposition of the oxygen to sulfur at the 2-position.[19] The oxidation of 2-MeSE1 **18** to the corresponding sulfoxide **22** and sulfone **23** was achieved by use of the appropriate quantity of *m*CPBA in dichloromethane (Scheme 3). The high yields obtained in the synthesis suggest that this approach is the most efficient and flexible entry to 2-alkylsulfanyl estrones presently available.

2-Alkyl estrones were also available from this approach by quenching the lithio-anion of MOM/ketal **7** with the appropriate alkyl iodide. The 2-ethyl MOM/ketal **29** was obtained in high (84%) yield, allowing, after the methods described above, access to 2-ethyl estrone **30**, 2-ethyl estradiol **31**, 2-EtEMATE **32** and 2-EtE2MATE **33** in a direct manner. Evaluation of these

**Scheme 4**[a]




[a] Reagents and conditions: (i) *sec*-BuLi, THF, −78 °C, 1 h then EtI for **29**, $CF_3CH_2Br$ for **35** or $CF_3CH_2I$ for **38**, −78 °C to room temperature; (ii) 4 M HCl /MeOH; (iii) $H_2NSO_2Cl$, DMA, 0 °C to room temperature; (iv) $NaBH_4$, THF/IPA, 73%.

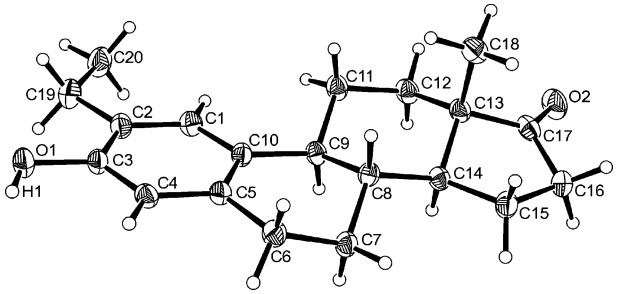



**Figure 2.** X-ray crystal structure showing of one of the molecules in the asymmetric unit of 2-ethylestrone **30**. Ellipsoids are represented at 30% probability level.

compounds would thus determine whether an H-bond acceptor group at the 2-position is necessary for biological activity. 2-Methylestrone-3-*O*-sulfamate **34**, 2-MeEMATE, was also synthesized by this method.[53]

Attempts to introduce a 2,2,2-trifluororoethyl group at the 2-position by quenching the lithiated MOM/ketal with 2-bromo-1,1,1-trifluoroethane and 2-iodo-1,1,1-trifluoroethane proved unsuccessful, with the 2-bromo MOM/ketal **35** and 2-iodo MOM/ketal **38** being the only isolated products of the respective reactions (Scheme 4). The outcome of these reactions is not surprising given the literature precedents for halogen exchange[54] and the strong deactivating effect the trifluoromethyl group exerts on the neighboring carbon.[55] Deprotection to give the 2-halo estrones **36** and **39** was accomplished under acidic conditions in modest overall yield; no attempt was made to optimize this reaction. 2-Bromoestrone-3-*O*-sulfamate **37** (2-BrEMATE) and 2-iodoestrone-3-*O*-sulfamate **40** (2-IEMATE) were also derived for evaluation under the standard conditions described above.

X-ray crystal structures of 2-EtE1 **30** and 2-MeOEMATE **3** were obtained and are presented in Figures 2 and 3, respectively. The two structures confirm that the integrity of the chiral centers at C-8, -9, -13 and -14 of the estrane core is maintained. Adjacent 2-EtE1 units align to allow hydrogen bonding interactions between the C-3-OH group and the C-17-ketone. Interestingly,

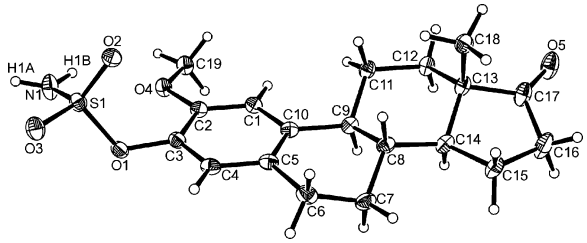
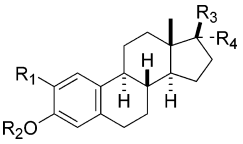



**Figure 3.** X-ray crystal structure showing one of the molecules in the asymmetric unit of 2-methoxyestrone-3-*O*-sulfamate (2-MeOEMATE) **3**. Ellipsoids are represented at 30% probability level.

**Table 1.** Antiproliferative Activities of Estrogen Derivatives against MCF-7 Human Breast Cancer Cells[a]




| compound | $R^1$ | $R^2$ | $R^3$ | $R^4$ | MCF-7 $GI_{50}$ ($\mu$M) |
|---|---|---|---|---|---|
| **1** | MeO | H | O | O | 21.3 |
| **2** | MeO | H | OH | H | 2.35 |
| **3** | MeO | $SO_2NH_2$ | O | O | 0.30 |
| **4** | MeO | $SO_2NH_2$ | OH | H | 0.36 |
| **15** | MeO | $SO_2NMe_2$ | O | O | >10 |
| **16** | MeO | $SO_2NHAc$ | O | O | 168 |
| **12a** | EtO | H | O | O | 9.04 |
| **13** | EtO | $SO_2NH_2$ | O | O | 4.84 |
| **14** | EtO | $SO_2NH_2$ | OH | H | 0.61 |
| **18** | MeS | H | O | O | 33.4 |
| **19** | MeS | H | OH | H | 3.96 |
| **20** | MeS | $SO_2NH_2$ | O | O | 0.40 |
| **21** | MeS | $SO_2NH_2$ | OH | H | 0.43 |
| **22** | MeSO | H | O | O | >10 |
| **23** | $MeSO_2$ | H | O | O | 66 |
| **25** | EtS | H | O | O | 31.9 |
| **26** | EtS | H | OH | H | 23.2 |
| **27** | EtS | $SO_2NH_2$ | O | O | 35.2 |
| **28** | EtS | $SO_2NH_2$ | OH | H | 23.4 |
| **30** | Et | H | O | O | 57.6 |
| **31** | Et | H | OH | H | 10.5 |
| **32** | Et | $SO_2NH_2$ | O | O | 0.34 |
| **33** | Et | $SO_2NH_2$ | OH | H | 0.07 |
| **34** | Me | $SO_2NH_2$ | O | O | 0.26 |
| **36** | Br | H | O | O | 33 |
| **37** | Br | $SO_2NH_2$ | O | O | 6.95 |
| **39** | I | H | O | O | 38 |
| **40** | I | $SO_2NH_2$ | O | O | 1.59 |

[a] $GI_{50}$ figures are the mean values obtained from experiments performed in triplicate, SEM = ±7%.

the crystal structure of 2-MeOEMATE reveals that the $NH_2$ group of the sulfamate is appropriately (but possibly not optimally) rotated about the 01-S1 vector to facilitate a H-bond interaction with the oxygen of the neighboring methoxy group, thus illustrating the H-bonding potential of the sulfamate group and, perhaps, the ability of the neighboring methoxy group to stabilize the conformation of an adjacent sulfamate.

**Biology.** As a first stage predictor of biological activity, the compounds were evaluated for their ability to inhibit the proliferation of MCF-7 cells, a ER+ human breast cancer cell line. Previous experience had shown that results from this assay were predictive of the spectrum of desirable biological properties already obtained with 2-MeOEMATE. The results of this assay are presented in Table 1.

A brief examination of the results obtained for the 2-substituted estrone derivatives shows that all of these molecules display relatively modest growth inhibitory effects, with only 2-EtOE1 **12a** causing more than 50% growth inhibition at 10 $\mu$M concentration. This result correlates with earlier studies in the estradiol series where 2-EtOE2 proved to be the most potent of the 2-alkoxy estradiols.[22] As had been previously reported, the 2-substituted estradiol derivatives display far greater activity than their estrone equivalents, a general trend across the substituents explored here and clearly illustrated by $GI_{50}$ values of 2.35 $\mu$M and 21.3 $\mu$M (Table 1) obtained for 2-MeOE2 **2** and 2-MeOE1 **1** respectively. 2-MeSE2 **19**, which had not previously been synthesized proved to be one of the most active 2-substituted estradiols displaying similar activity to that of 2-MeOE2 **2** (Table 1, 3.96 vs 2.35 $\mu$M respectively). The activity of 2-MeSE2 **19** contrasts with that observed for 2-EtSE2 **26** in both the present and previous studies[22] where the ethylsulfanyl group proved a poor isostere for the ethoxy group. It is not clear if this reduced antiproliferative activity is due to steric factors (the ethylsulfanyl group is larger than the 2-ethoxy group) or the reduced ability of sulfur to act as a hydrogen bond acceptor with respect to oxygen.

A marked increase in growth inhibitory activity was observed in both the estrone and estradiol series upon sulfamoylation of the 3-hydroxyl group. Transformation of the phenolic estrones to their 3-*O*-sulfamoylated (EMATE) derivatives, with the exception of the relatively inactive 2-EtS series, produced an increase in activity ranging from a 2-fold increase from 2-EtOE1 **12** to 2-EtOEMATE **13** to a 170-fold increase in activity from 2-EtE1 **30** and 2-EtEMATE **32** (respective $GI_{50}$s of 57.6 $\mu$M and 0.34 $\mu$M). The EMATE derivatives are also observed to be significantly more active in this assay than the nonsulfamoylated estradiols, the most active of which, 2-MeOE2 **2**, is eight times less active than the corresponding estrone-3-*O*-sulfamate, 2-MeOEMATE **3**, suggesting that the estrogen 3-*O*-sulfamates are unlikely to act simply as prodrugs of the corresponding estrogen. This premise is further supported by the observation that the 2-substituted estrone derivatives display only very modest activity as noted above. Substitution on the nitrogen in the case of the *N,N*-dimethyl **15** and *N*-acyl 2-MeOEMATEs **16** abolished activity discouraging any further investigation into modification of the sulfamate group at this time. While the exact role of the sulfamate moiety in the high inhibitory potency observed for these 2-substituted estrogen sulfamates remains unestablished, the biological activity displayed by **15** and **16** clearly suggested that an unsubstituted sulfamate group (i.e. $H_2NSO_2O$) is crucial for high potency. This is to be contrasted, at least for the *N*-acetyl sulfamates, by the excellent inhibitory activity of *N*-acetyl EMATE against the steroid sulfatase mediated conversion of estradiol sulfate to estradiol.[56,57] The antiproliferative activity of 2-substituted estradiol 3-*O*-sulfamates (E2MATEs) in the MCF-7 cell line proved to be equivalent or, in the case of 2-EtE2MATE **33**, slightly enhanced with respect to their 2-substituted EMATE equivalents.

Inspection of Table 1 reveals that the steric size of the 2-substituent is crucial for high activity, with the larger 2-ethylsulfanyl **27** and 2-ethoxy EMATEs **13** exhibiting significantly lower activity to the homologous

**Table 2.** $GI_{50}$ Values Obtained from the NCI 55 Cell Panel and Mean Graph Midpoint (MGM) Values

| | $GI_{50}$ ($\mu$M) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 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 ($\mu$M) |
| **2** | 0.7 | 0.47 | 0.32 | 0.36 | 0.21 | 0.95 | 1.8 | 0.08 | 1.3 |
| **3** | 0.16 | 0.065 | 0.12 | 0.017 | <0.01 | 0.6 | 0.32 | <0.01 | 0.095 |
| **4** | 0.21 | 0.066 | 0.044 | 0.039 | 0.026 | 0.32 | 0.19 | 0.02 | 0.11 |
| **18** | 27 | 20 | 14 | 12 | 22 | 19 | 85 | 12 | 20.4 |
| **20** | 35 | 0.088 | 0.045 | 0.052 | 0.012 | 0.76 | 22 | <0.01 | 0.229 |
| **26** | 16 | 8.5 | 16 | 11 | 14 | 18 | 24 | 2 | 10 |
| **27** | 6.9 | 3.2 | 1.4 | 5.9 | 2.3 | 11 | 13 | 2.2 | 5.62 |
| **31** | 6.1 | 3.5 | 3.3 | - | 2.9 | 7.3 | 11 | 1.7 | 6.5 |
| **32** | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.014 |
| **33** | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.04 | <0.01 | <0.01 | 0.016 |

[a] Data for compounds **2**, **26** and **31** are taken from ref 22.

2-methylsulfanyl and 2-methoxy EMATEs **20** and **4**. The equivalence in activity of the 2-$CH_3X$ (where X is either O, S, or $CH_2$)-substituted EMATEs **4**, **21** and **32** in this assay in conjunction with the previously demonstrated lack of estrogenicity for 2-MeOEMATE **3**[35] highlights these molecules as being of particular interest and would suggest that groups of this size with similar electronic influence on the aromatic A-ring are optimal on the grounds of antiproliferative activity and absence of uterotropic activity. This is supported by the observed activity of 2-MeEMATE **34**[53] which is equal to that observed for the larger but electronically similar 2-EtEMATE **32**, and far greater than that obtained for the sterically similar 2-IEMATE **40** and 2-BrEMATE **37**. It would seem that electronic-withdrawing 2-substituents diminish the antiproliferative activity in the estrogen-3-*O*-sulfamate series, with the relative antiproliferative activities of 2-IEMATE **40** and 2-BrEMATE **37** reflecting the electron-withdrawing ability of their respective substituents.

A selection of these compounds was then screened in the NCI 55 human cancer cell line panel, thus allowing an evaluation of activity across various cancer phenotypes.[58] Data obtained in eight individual cell lines from this assessment are presented in Table 2. The cell lines were selected due to the availability of comparative data for 2-MeOE2 **2**, 2-EtE2 **31** and 2-EtSE2 **26** which are drawn from the literature.[22] The MDA-MB-435 cell line has been highlighted as a cell line especially sensitive to 2-MeOE2 **2**, which generally shows higher activity in the breast cancer cell lines of the panel.[22] The mean graph midpoint (MGM) data represent the mean concentration required to cause 50% growth inhibition in all the cell lines successfully evaluated and thus indicates the relative activity of compounds across the panel. MGM ($\mu$M) values are restricted by definition by the range of concentrations at which the assays are performed, in this case $10^{-4}$ to $10^{-8}$M, thus a numerical value of 0.01 is maximal and would be obtained for a compound which causes 50% growth inhibition at concentrations of $10^{-8}$M or less in all 55 cell lines.

Examination of the data displayed for individual cell lines and the MGM values show that the EMATE derivatives **3**, **4**, **20**, **32** and **33** (with the exception of 2-EtSEMATE **27**) exhibit their antiproliferative effects at much lower concentrations than 2-MeOE2 **2** as, by extension, do their analogous estradiol derivatives. In the 2-MeO series both EMATE **3** and E2MATE **4** derivatives are more active than 2-MeOE2 **2** in all the cell lines for which comparative data are available; the degree of enhancement afforded by introduction of the sulfamate is clearly illustrated by the MGM values which indicate that across the panel the mean activity of the 2-MeO estrogen sulfamates **3** and **4** across the panel is over 10-fold greater than that of 2-MeOE2 **2** (values **3** 0.095 $\mu$M, **4** 0.11 $\mu$M and **2** 1.3 $\mu$M). All data obtained are consistent with those obtained in our preliminary MCF-7 screen with, for example, the influence of the large 2-ethylsulfanyl substituent proving deleterious for antiproliferative activity (vide supra). The 2-$CH_3X$ (where X is either O, S, or $CH_2$)-substituted EMATEs **3**, **20** and **32** and E2MATEs **4** and **33** all proved to be highly active. In particular, 2-EtEMATE **32** and 2-EtE2MATE **33** display 50% growth inhibition at less than 10 nM concentration almost uniformly across the panel as reflected in the 90- and 80-fold greater MGM values than 2-MeOE2 **2**, figures which are intrinsically limited due to the concentration surveyed being greater than that required for 50% growth inhibition for these highly active molecules.

The ability of estrone-3-*O*-sulfamate, EMATE, to act as an irreversible inhibitor of steroid sulfatase (STS), a clinical target for the treatment of hormone-dependent breast cancer, is well documented (vide supra).[32] Previous studies have shown that selected 2-substituted EMATEs, including 2-MeOEMATE, are potent STS inhibitors.[35;53] The activity of 2-MeO-, 2-EtO-, 2-MeS- and 2-Et-substituted EMATE derivatives to inhibit STS activity in placental microsomes was evaluated alongside EMATE ($IC_{50}$ 22 nM). 2-MeOEMATE **3** ($IC_{50}$ 42 nM) and 2-MeOE2MATE **4** ($IC_{50}$ 34 nM) proved to be the most active of the substituted EMATEs assayed, with $IC_{50}$ values slightly higher than EMATE. Unsurprisingly, the *N*,*N*-dimethyl sulfamate **15** showed no inhibition of STS activity at a concentration of 1 $\mu$M. Introduction of the larger 2-EtO substituent caused a significant reduction in STS inhibition (2-EtOEMATE $IC_{50}$ 890 nM), indicating that the ethoxy group is disfavored relative to the methoxy group presumably due to steric constraints at the site of action. 2-MeSEMATE ($IC_{50}$ 120 nM) however proved highly active as an inhibitor of STS; clearly this group is of a similar steric size to the methoxy group and can participate in H-bond acceptor interactions in the binding pocket in an analogous manner to 2-MeOEMATE. It appears reasonable to suggest that the relative activities of 2-MeOEMATE and 2-MeSEMATE are due to the respective H-bond acceptor potential of the ether and thioether groups. The importance of the putative H-bonding interactions is further illustrated by the relative

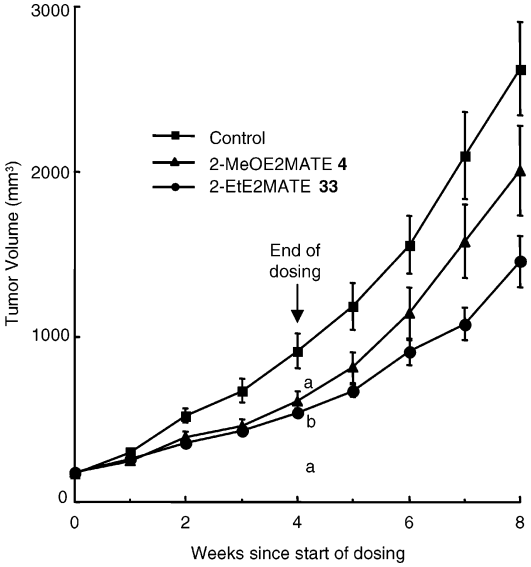



**Figure 4.** In vivo effects of 2-MeOE2MATE or 2-EtE2MATE on the growth of MDA-MB-435 tumor xenografts in nude mice. Vehicle (THF/propylene glycol) or compounds were administered orally (20 mg/kg) daily for 28 days. Tumor volumes were monitored at weekly intervals for the duration of drug administration and continued for a further 4 weeks postdosing. (means ± SEM, $n = 12$) (a, $p < 0.05$ vs controls; b, $p < 0.01$ vs controls, Student's $t$ test).

inactivity of 2-EtEMATE and 2-EtE2MATE with respect to 2-MeOEMATE ($IC_{50}$ values of 1.0 $\mu$M, 1.8 $\mu$M and 42 nM). The inability of the ethyl group to act as an H-bond acceptor results in the greater than 25-fold increase in STS $IC_{50}$ value observed for 2-EtEMATE. These results are in agreement with a previous study in which the excellent STS inhibition ($IC_{50}$ 100 pM) displayed by 2-difluoromethyl EMATE was ascribed to the H-bond interactions between the difluoromethyl group and H-bond donor residues of the active site.[53] Thus, in addition to their direct antiproliferative effect against tumors, these compounds should act against hormone-dependent tumors by inhibiting the STS mediated conversion of estradiol sulfate to estradiol, thereby causing a reduction in the circulatory estrogens required to stimulate their growth. The SAR of the 2-substituted EMATEs as inhibitors of STS clearly contrasts with the SAR for antiproliferative effects discussed above (see Figure 5).

2-EtE2MATE **33** was also evaluated using the NCI antiangiogenesis resource, which assesses HUVEC growth inhibition, and uses cord formation and chemotaxis assays to select compounds with antiangiogenic activity.[59] 2-EtE2MATE **33** gave $IC_{50}$ values of 0.33 $\mu$M, 0.06 $\mu$M and 0.36 $\mu$M in each respective assay, thus confirming the antiangiogenic properties of this molecule in agreement with results previously reported for 2-MeOEMATE.[60]

On the basis of the in vitro activity displayed by 2-EtE2MATE **33**, this compound was selected for evaluation by the NCI in the in vivo hollow fiber assay which entails the intraperitoneal (ip) and subcutaneous (sc) implanting of hollow polyvinylidene fluoride fibers in an animal, each of which contains a culture of one of twelve human tumor cell lines.[61] Each tumor line is thus examined in the ip and sc environment. The vehicle animals are then treated in an ip manner at two dose levels (100 and 150 mg/kg, 25% and 37.5% respectively of the MTD) on a daily basis for 4 days, and after a further day the fibers are collected for analysis and the analyzed compound awarded a score of 2 if a 50% reduction in viable cell mass compared to the vehicle control samples is observed. In this assay 2-EtE2MATE **33** obtained an ip score of 10 and an sc score of 8 (i.e. 50% growth inhibition was observed in 5 of the ip implanted fibers and four of the sc implanted fibers, the maximum possible score is 96), a result which demonstrates the good in vivo activity of this class of molecules and the ability of the compound to be transported from the peritoneum to peripheral tissue.

A further assessment of the ability of 2-MeOE2MATE **4** and 2-EtE2MATE **33** to inhibit tumor growth in vivo was performed using xenografts derived from MDA-MB-435 (ER−) human breast cancer cells transplanted into female mice. The oral administration of either 2-MeOE2MATE or 2-EtE2MATE significantly reduced the growth of tumors derived from MDA-MB-435 cancer cells (Figure 4). A 20 mg/kg dose, administered orally for 28 days, of 2-MeOE2MATE reduced tumor growth by 33% ($p < 0.05$) while 2-EtE2MATE reduced tumor growth by 40% ($p < 0.01$) compared with the growth of tumors in untreated animals. Four weeks after the cessation of dosing the tumor volumes in mice that had received 2-EtE2MATE remained significantly smaller (45% reduction, $p < 0.01$) than those for the control group. The difference in tumor volumes for the 2-MeOE2MATE treated group was not significant at this time point. From this study, therefore, it would appear that the in vivo efficacy of 2-EtE2MATE is greater than that of 2-MeOE2MATE and that both of these molecules are orally bioavailable.

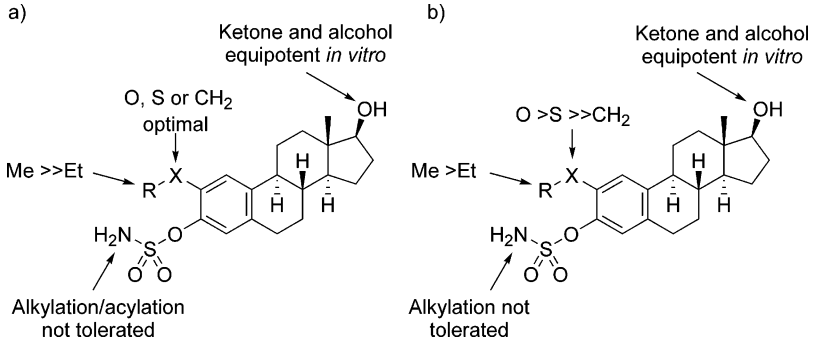



**Figure 5.** In vitro SAR of 2-RX-substituted estradiol-3-*O*-sulfamates as (a) antiproliferative agents and (b) irreversible inhibitors of steroid sulfatase.

Thus, in summary, in the present study a flexible practical approach to 2-substituted estrone derivatives, 2-substituted estrone-3-*O*-sulfamates (EMATEs) and 2-substituted estradiol-3-*O*-sulfamates (E2MATEs) has been developed. Of the molecules synthesized the 2-substituted estrogen-3-*O*-sulfamates exhibited high antiproliferative activity in vitro against a wide range of tumor cells. The structure activity relationship for antiproliferative activity obtained for these molecules is summarized in Figure 5. These 3-*O*-sulfamate derivatives are significantly more active in vitro than the closely related nonsulfamoylated 2-substituted estradiol derivatives and exhibit a contrasting SAR. Substitution at the 2-position for the 3-*O*-sulfamates series is optimally a group of the formula $CH_3X$ (X is either O, S, or $CH_2$) which appear ideal on both steric and electronic grounds. Substitution of the sulfamate moiety leads to a reduction in activity while the oxidation state of C-17, of key importance in the estradiol series, has little effect on in vitro activity. Further assessment of one of these compounds, 2-EtE2MATE **33**, has also demonstrated antiangiogenic activity as well as in vivo antitumor activity. Mechanistic studies have shown that these molecules inhibit the taxol-promoted polymerization of tubulin, and, additionally, they also trigger BCL-2 phosphorylation and upregulation of p53 and induce apoptosis.[36,37] We have also previously shown that 2-MeOEMATE inhibits the growth of NMU-induced tumors in rats.[36] When considered alongside our previous observations that 2-substituted EMATEs are additionally nonestrogenic irreversible inhibitors of steroid sulfatase,[35] itself a clinical target for the treatment of hormone-dependent breast cancer, these molecules are clearly highlighted as a promising class of potential therapeutic agents which have multitargeted antitumor activities. Additionally, the sequestration of such 3-*O*-sulfamate derivatives into red blood cells by binding to CAII provides a route to minimize metabolism and maximize the oral bioavailability of such compounds. We are actively investigating the effects of further modification of the 2-substituted estrogen-3-*O*-sulfamates with a view to producing new classes of potent anticancer agents.

## Experimental Section

**Materials and Methods. Chemistry.** All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, UK), Fluka (Gillingham, UK) or Lancaster Synthesis (Morecambe, UK). Organic solvents of A. R. grade were supplied by Fisher Scientific (Loughborough, UK) and used as supplied. Anhydrous *N,N*-dimethylformamide (DMF) and *N,N*-dimethylacetamide (DMA) were 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 adaption of the method of Appel and Berger[50] and was stored in the refrigerator under positive pressure of $N_2$ as a solution in toluene as described by Woo et al.[62] An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. E1 was purchased from Sequoia Research Products (Oxford, UK). Flash column chromatography was performed on silica gel (MatrexC60).

$^1$H NMR and $^{13}$C NMR spectra were recorded in chloroform solution (unless otherwise indicated) with a JMN-GX 400 NMR spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm, $\delta$) relative to tetramethylsilane (TMS) as an 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 Reichert-Jung Thermo Galen Kofler block and are uncorrected.

The X-ray crystallographic studies of 2-EtE1 and 2-MeOEMATE were carried out on a Nonius Kappa CCD diffractometer with area detector. Crystallographic data for 2-EtE1 and 2-MeOEMATE have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications CCDC 260114 and 260115, respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax(+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

**Crystal Data for 2-EtE1**: $C_{20}$ $H_{26}$ $O_2$, $M$ = 298.41, $\lambda$ = 0.71073 Å, monoclinic, space group $P2_1$, $a$ = 12.0690(3), $b$ = 11.1390(3), $c$ = 12.7150(4)Å, $\beta$ = 90.702(1)°, $U$ = 1709.23(8) Å$^3$, Z = 4, $D_c$ = 1.160 Mg/m$^3$, $\mu$ = 0.073 mm$^{-1}$, $F$(000) = 648, crystal size 0.20 × 0.13 × 0.13 mm, unique reflections = 7656 [$R$(int) = 0.0779], observed $I > 2\sigma(I)$ = 5186, data/restraints/parameters = 7656/1/403, $R1$ = 0.0496 w$R2$ = 0.1026 (obs. data), $R1$ = 0.0897 w$R2$ = 0.1174 (all data), max peak/hole 0.179 and −0.177 eÅ$^{-3}$. Absolute structure parameter = 0.6(11).

**Crystal Data for 2-MeOEMATE**: $C_{19}$ $H_{25}$ N $O_5$ S, $M$ = 379.46, $\lambda$ = 0.71073 Å, orthorhombic, space group $P2_12_12_1$, $a$ = 7.6190(1), $b$ = 15.2780(2), $c$ = 15.4400(2)Å, $U$ = 1797.26(4) Å$^3$, Z = 4, $D_c$ = 1.402 Mg/m$^3$, $\mu$ = 0.211 mm$^{-1}$, $F$(000) = 808, crystal size 0.20 × 0.15 × 0.15 mm, unique reflections = 4104 [$R$(int) = 0.0558], observed $I > 2\sigma(I)$ = 3433, data/restraints/parameters = 4104/2/246, $R1$ = 0.0352 w$R2$ = 0.0798 (obs. data), $R1$ = 0.0491 w$R2$ = 0.0860 (all data), max peak/hole 0.164 and −0.252 eÅ$^{-3}$. Absolute structure parameter = 0.02(6).

**Biology.** Cell proliferation was analyzed using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (Promega, WI). In brief, MCF-7 cells were seeded in a 96-well multiplate at a density of 4000 cells/well (0.05 mL) in MEM with 10% FBS and supplements. Four to five hours after seeding, medium alone or medium containing the test compounds (0.002−20.0 $\mu$M, 0.05 mL) was added. After an additional 96 h of culture, cells were incubated for 2h at 37 °C with MTS (0.02 mL/well, 3-(4,3-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium reagent). The absorbance of the colored formazan product was recorded at 492 nm. Data were expressed as percentage inhibition of MCF-7 proliferation, and the $GI_{50}$ values were obtained from these data. STS inhibition was assayed by the method of Purohit et al.[63] The ability of 2-MeOE2MATE and 2-EtE2MATE to inhibit tumor growth in vivo was examined using xenografts derived from MDA-MB-435 (ER−) human breast cancer cells transplanted into female mice with 12 mice per group. These studies were carried out by AntiCancer Inc. (San Diego, CA). Treatments were initiated when tumor volumes reached 100−200 mm$^3$. Drugs were dissolved in a minimum volume of tetrahydrofuran (THF), diluted with propylene glycol and administered at 20 mg/kg orally, daily for 28 days. The length ($l$) and width ($w$) of tumors was measured at weekly intervals, from which tumor volumes were calculated using the formula ($l \times w^2/2$). Monitoring of tumor volumes continued for a further 28 day period after the end of drug administration.

**17,17-Ethylenedioxy estrone 6.** A suspension of estrone **5** (12.5 g, 46.2 mmol), toluene (150 mL), ethylene glycol (14 mL) and tosic acid (120 mg) was refluxed for 14 h under Dean−Stark conditions. The resultant pale pink solution was poured onto saturated sodium bicarbonate solution (150 mL) and diluted with ethyl acetate (250 mL). The organic layer was separated, the aqueous layer extracted with a further aliquot of ethyl acetate (100 mL) and the combined organics were washed with water (150 mL) and brine (150 mL), dried and evaporated to yield crude dioxolone **6** (15 g, 103%) as an off-white crystalline solid mp 183−184 °C [lit. 180−181 °C[64]

which was used without further purification ($^1$H NMR shows purity >95%).

**3-*O*-Methoxymethyl-17,17-ethylenedioxyestrone.** Sodium hydride (3.48 g, 87 mmol) was added in a portionwise manner to a stirred 0 °C solution of protected estrone **6** (18.2 g, 58 mmol) in dimethylformamide (250 mL). Methyl chloromethyl ether (8.81 mL, 2 equiv) was then introduced in a dropwise manner after evolution of $H_2$ had ceased. On completion of addition, the external cooling was removed and the reaction was left to stir at room temperature for 16 h. Ammonia (100 mL, 2 M) was then added to destroy any residual MOMCl before extraction of the mixture into ethyl acetate (500 mL). The aqueous layer was then separated, and the organic layer was washed with brine (5 × 200 mL), then dried and evaporated. The resultant pale yellow oil was purified by column chromatography (4:1 hexane/ethyl acetate) to give compound **7** as a colorless oil which on standing gave a white solid (19.8 g, 95%): mp 62−63 °C, $R_f$ 0.55 (3:1 hexane/ethyl acetate). $^1$H NMR $\delta$ 7.21 (1H, d, *J* 8.6), 6.82 (1H, dd, *J* 8.6 and 2.7), 6.77 (1H, d, *J* 2.7), 5.13 (2H, s), 3.89−3.98 (4H, m), 3.46 (3H, s), 2.83 (2H, m), 2.27 (2H, m), 1.25−2.05 (11H, m) and 0.88 (3H, s, $CH_3$); *m/z* [EI] 358.2 (100%, $M^+$); HRMS [EI] 358.214410 $C_{22}H_{30}O_4$ requires 358.21441.

**2-Formyl-3-*O*-methoxymethyl-17,17-ethylenedioxyestrone 8.** A well stirred solution of protected estrone **7** (24.3 g, 67.9 mmol) in THF (400 mL) was cooled to −78 °C and then treated with *sec*-butyllithium (156 mL, 203 mmol) in a dropwise manner over 1 h. Cooling was maintained for a further 2 h prior to introduction of dimethylformamide (21 mL, 272 mmol) over 10 min after which time the reaction was allowed to come to 0 °C over 1 h. The reaction was then quenched with aqueous ammonium chloride (20 mL, saturated) and then partitioned between ethyl acetate (500 mL) and water (200 mL). The organic layer was separated, washed with water (3 × 100 mL) and brine (200 mL), dried and evaporated. The desired product **8** was isolated by column chromatography (gradient hexane to 10% ethyl acetate in hexane) as a colorless oil (22.5 g, 86%) which solidified on standing to give a cream solid mp 108−110 °C. $^1$H NMR $\delta$ 10.42 (1H, s), 7.77 (1H, s), 6.91 (1H, s), 5.26 (2H, s), 3.89−3.98 (4H, m), 3.51 (3H, s), 2.80−2.94 (2H, m), 1.30−2.46 (13H, m), and 0.87 (3H, s); *m/z* [EI$^+$] 386.2 (100%, $M^+$); HRMS [EI] 386.20932, calculated 386.20932.

**2-Methylsulfanyl-3-*O*-methoxymethylene-17,17-ethylenedioxyestrone 17.** Protected estrone **7** (20 g, 55.8 mmol) in THF (400 mL) was reacted with *sec*-butyllithium (129 mL, 167 mmol) as described for the synthesis of **8**. The resulting anion was quenched with dimethyl disulfide (20 mL, 223 mmol) over a period of 5 min. After a further 2 h, the reaction was subjected to the standard workup to yield a pale yellow oil which was purified by chromatography (hexane/ethyl acetate gradient 100:0 to 85:15). The desired sulfide **17** (20.5 g, 91%), a clear colorless oil, showed $^1$H NMR $\delta$ 7.16 (1H, s), 6.81 (1H, s), 5.21 (2H, s), 3.86−3.98 (4H, m) 3.51 (3H, s), 2.80−2.88 (2H, m), 2.43 (3H, s), 1.25−2.38 (13H, m) and 0.88 (3H, s); *m/z* [FAB$^+$] 404.1 ($M^+$); HRMS [FAB$^+$] 404.2004, calculated 404.2021.

**2-Ethylsulfanyl-3-*O*-methoxymethylene-17,17-ethylenedioxyestrone 24.** Protected estrone **7** (5 g, 13.9 mmol) in THF (100 mL) was reacted with *sec*-butyllithium (32.2 mL, 41.8 mmol) as described for the synthesis of **8**. The resulting anion was quenched with diethyl disulfide (9.1 mL, 70 mmol) over a period of 5 min. Standard workup yielded a very pale yellow purified by chromatography (hexane/ethyl acetate gradient 100:0 to 85:15) to give the desired sulfide **24** (5.3 g, 12.6 mmol, 97%) as a clear colorless oil which showed $^1$H NMR $\delta$ 7.24 (1H, s), 6.82 (1H, s), 5.21 (2H, s), 3.86−3.98 (4H, m), 3.51 (3H, s), 2.79−2.94 (4H, m) 1.20−2.36 (16H, m [including 1.30 (3H, t)) and 0.88 (3H, s); *m/z* [FAB+] 418.1 ($M^+$); HRMS [FAB$^+$] 418.1275, calculated 418.2178.

**2-Ethyl-3-*O*-methoxymethyl-17,17-ethylenedioxyestrone 29.** Protected estrone **7** (40 g, 112 mmol) in THF (400 mL) was reacted with *sec*-butyllithium (258 mL, 336 mmol) as described for the synthesis of **8**. The resulting anion was quenched with ethyl iodide (23.7 mL, 336 mmol) over 10 min after which time the reaction was allowed to come to 0 °C over 2 h. Ammonium chloride (50 mL, saturated) quench was followed by dilution in ethyl acetate (500 mL). The organic layer was separated, washed with sodium thiosulfate (100 mL, 10% aqueous), water (3 × 100 mL) and brine (200 mL), dried and evaporated. The desired product **29** was isolated by column chromatography (hexane/ethyl acetate gradient 10:0 to 9:1) as a colorless oil (36.5 g, 84%) which showed $^1$H NMR $\delta$ 7.08 (1H, s), 6.78 (1H, s), 5.16 (2H, s), 3.86−3.98 (4H, m), 3.48 (3H, s), 2.78−2.98 (2H, m), 2.61 (2H, q, *J* 7.4), 1.22−2.44 (13H, m), 1.21 (3H, t, *J* 7.4) and 0.88 (3H, s); *m/z* [FAB$^+$] 386.2 (100%, $M^+$); HRMS [FAB$^+$] 386.24571, calculated 386.24571.

**2-Bromoestrone 36.** Estrone MOM/ketal 7 (3.58 g, 10 mmol) in THF (70 mL) was reacted with *sec*-BuLi (23 mL, 30 mmol) as described for the synthesis of **8** and then quenched with 1,1,1-trifluoro-2-bromoethane (5 g, 30 mmol). After workup the resulting was partially purified by chromatography (9:1 hexane/ethyl acetate) to give a mixture (2.7 g) of starting material and 2-bromo compound **35** which showed significant resonances at $\delta_H$ ($CDCl_3$) 7.43 (1H, s), 6.86 (1H, s), 5.20 (2H, s, $OCH_2O$), 3.86−3.98 (4H, m), 3.51 (3H, s), 2.76−2.84 (2H, m), and 0.89 (3H, s); *m/z* [FAB+] 437.1 ($M^+$). A sample of this mixture (1 g) was treated with methanolic HCl (made from AcCl and MeOH [5.64 mL and 14.4 mL respectively see above]) for 1 h at room temperature. The white powder obtained from standard workup was purified by column chromatography (3% ethyl acetate in chloroform) to give pure 2-bromoestrone **36** (620 mg, 48% over two steps) mp 211−14 °C [lit 194−195 °C[65]]. $^1$H NMR $\delta$ 7.34 (1H, s), 6.76 (1H, s), 5.37 (1H, s), 2.78−2.86 (2H, m), 1.36−2.56 (13H, m) and 0.91 (3H, s); *m/z* [FAB+] 350.1 ($M^+$ -H, $^{81}$Br, 95%) and 348.1 ($M^+$ -H, $^{79}$Br, 100%); Anal. ($C_{18}H_{21}BrO_2$) C, H.

**2-Iodoestrone 39.** Estrone MOM/ketal **7** (2.86 g, 8 mmol) in THF (50 mL) was reacted with *sec*-BuLi (18 mL, 23.5 mmol) as described for the synthesis of **8** and then quenched with 1,1,1-trifluoro-2-iodoethane (5 g, 23.8 mmol). After standard workup, the crude product was partially purified by chromatography (14:1 hexane/ethyl acetate) to give a mixture (2 g) of starting material **7** and 2-iodinated compound **38** as a pale yellow oil which showed $^1$H NMR $\delta$ 7.65 (1H, s), 6.78 (1H, s), 5.19 (2H, s), 3.88−3.98 (4H, m), 3.50 (3H, s), 2.78−2.86 (2H, m), and 0.87 (3H, s); *m/z* [FAB+] 437.1 ($M^+$). A sample of this mixture (1.95 g) was treated with methanolic HCl (generated from acetyl chloride (11.2 mL) and methanol (28.2 mL)) and then sonicated for 8 min. The resultant orange solution was then stirred for 0.5 h prior to addition of water (50 mL) and neutralization with $NaHCO_3$. The solution was then extracted with ethyl acetate (2 × 100 mL), the organic layers were washed with water (100 mL) and then brine (100 mL), dried and evaporated to give a pale yellow solid. 2-Iodo estrone **39** was isolated by column chromatography (3% ethyl acetate in chloroform) as a white crystalline solid (1.2 g, 38% over two steps) mp 195 °C (dec) [lit. 200−205 °C[43]] which showed $^1$H NMR $\delta$ 7.52 (1H, s, ArH), 6.74 (1H, s, ArH), 5.18 (1H, s, OH), 2.80−2.88 (2H, m, 6-$CH_2$), 1.30−2.56 (13H, m) and 0.91 (3H, s, 18-$CH_3$); *m/z* [APCI$^-$] 395.3 ($M^+$ − H, 100%).

**2-Hydroxy-3-*O*-methoxymethyl-17,17-ethylenedioxyestrone 9.** A room-temperature solution of 2-formyl compound **8** (17.3 g, 44.7 mmol) in dichloromethane (150 mL) and chloroform (150 mL) was treated with sodium hydrogenphosphate (22.2 g, 157 mmol) and then, in a dropwise manner, a solution of *m*CPBA (15.4 g, 80 mmol) in dichloromethane (150 mL). The reaction was stirred for 1 h and then filtered to remove solid residues. The filtrate was then washed with ice−water (400 mL), and the aqueous washings were extracted with dichloromethane (100 mL). The combined organic layers were washed with and saturated sodium bicarbonate (200 mL) then brine (200 mL) prior to evaporation. The resultant white solid was dissolved in degassed methanol (400 mL), treated with sodium hydroxide solution (81 mL, 81 mmol, 1 M) and then stirred for 2 h. The reaction was then carefully neutralized with 2 M HCl, and the methanol was then removed by evaporation. The mixture was then extracted into ethyl acetate (400 mL), the aqueous layer separated and the organic layer

washed with water (200 mL) and brine (200 mL), dried and evaporated. The desired product **9** was isolated by column chromatography (5:1 hexane/ethyl acetate) to give a colorless oil which solidified on standing to give a white crystalline solid (13.8 g, 84%) mp 88−89 °C which showed $^1$H NMR $\delta$ 6.90 (1H, s), 6.78 (1H, s), 5.80 (1H, br), 5.15 (2H, s), 3.88−3.98 (4H, m), 3.51 (3H, s), 2.70−2.82 (2H, m), 1.20−2.28 (13H, m) and 0.87 (3H, s); *m/z* [EI] 374.3 (100%, $M^+$); HRMS [EI] 374.20932, calculated 374.20932; Anal. ($C_{22}H_{30}O_5$) C, H.

**2-Methoxy-3-*O*-methoxymethyl-17,17-ethylenedioxyestrone 10.** A solution of 2-hydroxy compound **9** (14 g, 37.4 mmol) in anhydrous DMF (300 mL) was treated with potassium carbonate (63 g, 455 mmol) and then stirred for 10 min prior to addition of methyl iodide (22.7 mL, 411 mmol) then tetrabutylammonium iodide (100 mg). The reaction was stirred at room temperature for 72 h, then filtered through a sinter to remove the potassium carbonate. The residues were washed with ethyl acetate (400 mL), and then combined filtrate and washings were then poured onto water (200 mL). The organic layer was separated and washed with a further five aliquots of water (100 mL) and then brine (200 mL), dried and evaporated to give **10** as a pale yellow solid (13.5 g, 93%) which was used without further purification. $^1$H NMR $\delta$ 6.86 (1H, s), 6.84 (1H, s), 5.18 (2H, s), 3.88−3.98 (4H, m), 3.51 (3H, s), 2.76−2.83 (2H, m), 1.24−2.36 (13H, m) and 0.89 (3H, s); *m/z* [EI] 388.3 (100%, $M^+$); HRMS [EI] 388.22497, calculated 388.22497.

**2-Ethoxy-3-*O*-methoxymethyl-17,17-ethylenedioxyestrone 11.** A solution of **9** (2.0 g, 5.34 mmol) in DMF (80 mL) was treated with potassium carbonate (7.38 g, 53.4 mmol), and then after 10 minutes, ethyl iodide (4.70 mL, 58.7 mmol) and *tetra-n*-butylammonium iodide (120 mg). After 36 h stirring, the reaction was poured onto brine (150 mL) and extracted with ethyl acetate (200 mL). The organic layer was then separated and washed exhaustively with brine (5 × 100 mL), dried and evaporated to give **11** as a colorless oil (1.91 g, 89%) which was used without further purification. $^1$H NMR $\delta$ 6.88 (1H, s), 6.87 (1H, s), 5.20 (2H, s), 4.11 (2H, q, *J* 7.0), 3.92−4.02 (4H, m), 3.55 (3H, s), 2.80−2.86 (2H, m), 1.30−2.36 (16H, m including 1.46 (3H, t, *J* 7.0)) and 0.93 (3H, s); *m/z* [FAB$^+$] 402.1 (100%, $M^+$); HRMS [FAB$^+$] 402.24062, calculated 402.24062.

**2-Methoxyestrone 1.** A solution of **10** (2.12 g, 5.4 mmol) in THF (50 mL) was treated with hydrochloric acid (20 mL, 6 M). After 1 h stirring, the reaction was poured onto brine (50 mL) and extracted into ethyl acetate (150 mL). The organic layer was separated, washed with water (50 mL), sodium hydrogen carbonate (50 mL), water (50 mL) and brine (50 mL), then dried and evaporated to give an off-white solid. Crystallization from methanol gave 2-methoxyestrone **1** as white crystals (1.45 g, 90%) mp 189−191 °C [lit. 190−192 °C[45]] which showed $^1$H NMR $\delta$ 6.79 (1H, s), 6.65 (1H, s), 5.50 (1H, br), 3.86 (3H, s), 2.76−2.84 (2H, m), 1.35−2.55 (13H, m) and 0.92 (3H, s); *m/z* [FAB$^+$] 300.2 (100%, $M^+$); HRMS [FAB$^+$] 300.17254, calculated 300.17254; Anal. ($C_{19}H_{24}O_3$) C, H.

**2-Ethoxyestrone 12a.** Protected 2-ethoxyestrone **11** (1.91 g, 4.75 mmol) was dissolved in THF (50 mL) and then treated with hydrochloric acid (20 mL, 6 M) as described for the synthesis of **1**. The crude product was purified by column chromatography (4:1 hexane/ethyl acetate) to give **12a** as a white crystalline solid (1.38 g, 92%) mp 145−147 °C [lit. 140−142 °C[24]] which showed $^1$H NMR $\delta$ 6.77 (1H, s), 6.65 (1H, s), 5.55 (1H, br), 4.08 (2H, m), 2.76−2.84 (2H, m), 1.38−2.55 (16H, m including 1.43 (3H, t, *J* 7.0)) and 0.91 (3H, s); *m/z* [FAB$^+$] 314.2 (100%, $M^+$); HRMS [FAB$^+$] 314.18819, calculated 314.18819.

**2-Methylsulfanylestrone 18.** A 4 M solution of methanolic HCl was prepared by cautious addition of acetyl chloride (6.1 mL) to ice cold methanol (15.6 mL). After 5 min of stirring, this solution was poured onto protected estrone **17** (1.5 g, 3.72 mmol). Sonication was applied to speed the dissolution of the protected estrone, a pink color emerged in the reaction mixture after 5 min and, after a further 10 minutes, ethyl acetate (100 mL) and then sufficient sodium hydrogen carbonate to neutralize the reaction mixture was added. The organic layer was then washed with water (2 × 50 mL) and brine (100 mL) before drying ($MgSO_4$) and evaporating. Purification by gradient elution with hexane/ethyl acetate mixtures (100:0 to 80:20) on silica gel gave **18** as a white crystalline solid (1.06 g, 90%) mp 153−5 °C which showed: $^1$H NMR $\delta$ 7.40 (1H, s), 6.73 (1H, s), 6.49 (1H, s), 2.82−2.91 (2H, m), 2.34−2.56 (2H, m), 2.30 (3H, s) 1.36−2.26 (11H, m) and 0.91 (3H, s); *m/z* [FAB$^+$] 316.2 (100%, $M^+$); HRMS [FAB$^+$] 316.149702 calculated 316.14970; Anal. ($C_{19}H_{24}O_2S$) C, H.

**2-Ethylsulfanylestrone 25.** Protected estrone **24** (1.8 g, 4.3 mmol) was reacted with 4 M methanolic HCl as described for the synthesis of **18**. The crude product was crystallized (ethanol) to give white crystals of **25** (1.23 g, 87%) mp 139−141 °C which showed: $^1$H NMR $\delta$ 7.36 (1H, s), 6.73 (1H, s), 6.57 (1H, s), 2.82−91 (2H, m), 2.66 (2H, q, *J* 7.4), 1.36−2.55 (13H, m), 1.21 (3H, t, *J* 7.4) and 0.91 (3H, s); *m/z* [FAB$^+$] 330.2 (100%, $M^+$), HRMS [FAB$^+$] 330.165352, calculated 330.16535; Anal. ($C_{20}H_{26}O_2S$) C, H.

**2-Ethylestrone 30.** A solution of methanolic HCl was generated by cautious addition of acetyl chloride (122 mL) to ice cold methanol (324 mL) and then poured onto **29** (21 g, 54.3 mmol). Sonication was applied for 5 min to aid solution and after 10 min (400 mL) water was added to the reaction. The mixture was cooled in an ice bath causing precipitation of the product as a white powder which was collected by filtration and then crystallized from methanol to give **30** white crystals (14.7 g over three crops, 91%) mp 208−210 °C. $^1$H NMR $\delta$ 7.03 (1H, s), 6.51 (1H, s), 4.92 (1H, s), 2.78−2.86 (2H, m), 2.59 (2H, q, *J* 7.4), 1.30−2.54 (13H, m), 1.21 (3H, t, *J* 7.4) and 0.91 (3H, s); *m/z* [ES$^-$] 297.1 (100%, $M^+ - H$); Anal. ($C_{20}H_{30}O_2$.EtOAc) C, H.

**2-Methylsulfoxyestrone 22.** A solution of 2-methylsulfanylestrone **18** (632 mg, 2 mmol) in dichloromethane (40 mL) at 10 °C was treated with *m*CPBA (652 mg, 3.4 mmol) in a portionwise manner over 2 min. After 15 min, the reaction was diluted with dichloromethane (50 mL) and then washed with brine, sodium hydrogen carbonate solution, water (50 mL each) and brine (2 × 50 mL), dried and evaporated to give an off white solid which was crystallized from ethanol to give **22** as a white crystalline solid (420 mg, 63%) mp 250 °C (dec); $^1$H NMR $\delta$ 10.0 (1H, s), 6.95 (1H,s), 6.70 (1H, s), 2.80−2.96 (2H, m), 2.95 (3H, s), 1.40−2.56 (13H, m) and 0.91 (3H, s,); *m/z* [ES$^-$] 331.1 ($M^+ - H$, 35%) and 316.5 ($M^+ - OH$, 100%); Anal. ($C_{19}H_{24}O_3S$) C, H.

**2-Methylsulfonylestrone 23.** A solution of 2-methylsulfanylestrone **18** (632 mg, 2 mmol) in dichloromethane (40 mL) was treated with *m*CPBA (1.34 g, 7 mmol) at room temperature. After 1 h, TLC analysis showed no sign of starting material or intermediate sulfoxide. The reaction was filtered, washed with saturated sodium hydrogen carbonate solution (2 × 50 mL), water (50 mL) then brine (50 mL), dried and evaporated to give a white solid. The product **23** was isolated by crystallization from ethanol as colorless prisms (553 mg, 78%) mp 265−266 °C which showed $^1$H NMR $\delta$ 8.59 (1H, s), 7.54 (1H, s), 6.78 (1H, s), 3.10 (3H, s), 2.84−3.00 (2H, m), 1.40−2.56 (13H, m) and 0.92 (3H, s); *m/z* [ES-] 347.2 ($M^+ - H$); Anal. ($C_{19}H_{24}O_4S$) C, H.

**2-Methoxyestradiol 2.** A room-temperature solution of 2-methoxyestrone **1** (900 mg, 3 mmol) in THF (5 mL) and isopropyl alcohol (25 mL) was treated with sodium borohydride (111 mg, 3 mmol) in a portionwise manner. The reaction was stirred for 16 h then treated with saturated ammonium chloride solution (10 mL) and then partitioned between ethyl acetate (50 mL) and water (30 mL). The organic layer was separated, washed with water (2 × 25 mL) and brine (50 mL), dried and evaporated to give a white solid. Recrystallization from chloroform gave the pure estradiol **2** as white crystals (770 mg, 85%) mp 189−190 °C [lit. 188−190 °C[40]] which showed $^1$H NMR $\delta$ ($d_6$-DMSO) 8.57 (1H, s), 6.76 (1H, s), 6.43 (1H, s), 4.48 (1H, d, *J* 4.9) 3.70 (3H, s), 3.45−3.55 (1H, m), 2.60−2.68 (2H, m), 1.05−2.35 (13H, m) and 0.67 (3H, s); Anal. ($C_{19}H_{26}O_3$) C, H.

**2-Ethoxyestradiol 12b.** A solution of 2-ethoxyestrone **12a** (237 mg, 0.75 mmol) in THF (6 mL) and isopropyl alcohol (6

mL) was cooled to 0 °C then treated with sodium borohydride (114 mg, 3.02 mmol). The reaction was stirred for 14 h then brought to pH 6 by addition of glacial acetic acid. Solvents were then removed by evaporation and the desired product **12b** isolated by column chromatography (3:1 hexane/ethyl acetate) as a white crystalline solid (197 mg, 83%) which showed mp 153−155 °C [lit. 154−155 °C[22]] $^1$H NMR $\delta$ 6.78 (1H, s), 6.64 (1H, s), 5.58 (1H, s), 4.10 (2H, m), 3.72 (1H, dd, *J* 8.6 and 8.2), 2.72−2.80 (2H, m), 1.16−2.30 (16H, m including 1.42 (3H, t, *J* 7.0)), and 0.78 (3H, s); *m/z* [FAB$^+$] 316.2 (100%, M$^+$); HRMS [FAB$^+$] 316.20385, calculated 316.20385.

**2-Methylsulfanylestradiol 19.** Sodium borohydride (37 mg) was added to a solution of 2-methylsulfanylestrone **18** (316 mg, 1 mmol) in THF/IPA (8 mL each) at 0 °C. After 14 h, stirring saturated ammonium chloride (5 mL) was added to quench the reaction and the mixture was then extracted with ethyl acetate (50 mL). The organic layers were washed with water (3 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$) and evaporated to give a white solid which was crystallized from ethanol to give **19** white crystals (296 mg, 83%) mp 163−164 °C which showed: $^1$H NMR $\delta$ 7.40 (1H, s), 6.71 (1H, s), 6.47 (1H, s), 3.73 (1H, dd, *J* 8.4 and 8.4), 2.80−2.86 (2H, m), 1.14−2.36 (16H, m including 2.29 (3H, s)) and 0.91 (3H, s); *m/z* [FAB$^+$] 318.2 (100%, M$^+$); HRMS [FAB$^+$] 318.16535 calculated 316.16535; Anal. ($C_{19}H_{26}O_2S$) C, H.

**2-Ethylsulfanylestradiol 26.** Sodium borohydride (37 mg) was added to a room-temperature solution of 2-ethylsulfanylestrone **25** (330 mg, 1 mmol) in THF/isopropyl alcohol (8 mL each). After 14 h stirring, saturated ammonium chloride (5 mL) was added to quench the reaction and the mixture was then extracted with ethyl acetate (50 mL). The organic layers were washed with water (3 × 20 mL) and brine (2 × 20 mL), dried ($MgSO_4$) and evaporated to give a white solid which was crystallized from ethanol/hexane to give 2-ethylsulfanylestradiol **26** as white crystals (305 mg, 92%) mp 165−166 °C [lit. 166−167 °C[22]] which showed $^1$H NMR $\delta$ 7.37 (1H, s), 6.72 (1H, s), 6.56 (1H, s), 3.73 (1H, m), 2.80−86 (2H, m), 2.69 (2H, qd, *J* 7.4 and 1.5), 1.20−2.46 (16H, m including 1.23 (3H, td, *J* 7.4 and 1.5) and 0.79 (3H, s); *m/z* [FAB$^+$] 332.1 (100%, M$^+$), HRMS [FAB$^+$] 332.18100, calculated 332.18100; Anal. ($C_{20}H_{28}O_2S$) C, H.

**2-Ethylestradiol 31.** 2-Ethylestrone **30** (1.19 g, 4 mmol) was dissolved in THF (5 mL) and isopropyl alcohol (20 mL), cooled to 0 °C and treated with sodium borohydride (151 mg, 4 mmol) in a portionwise manner. Monitoring of the reaction by TLC showed no residual starting material remained after 2 h, at which stage ammonium chloride (10 mL) was added to quench excess borohydride. The reaction was then extracted with ethyl acetate (50 mL), and the organic layers were washed with water and brine, dried and evaporated to give the product as a white powder which was recrystallized from ethyl acetate/hexane to give **31** as a white crystalline solid (1.03 g, 86%) mp 168−169 °C [lit. 165−166 °C[22]] which showed $^1$H NMR (DMSO) $\delta$ 8.80 (1H, s), 6.90 (1H, s), 6.40 (1H, s), 4.47 (1H, d, *J* 4.7), 3.47−3.54 (1H, m), 2.58−2.72 (2H, m), 2.45 (2H, q, *J* 7.4), 1.00−2.28 (16H, m including 1.05 (3H, t, *J* 7.4)), and 0.66 (3H, s); Anal. ($C_{20}H_{28}O_2$) C, H.

**2-Methoxyestrone-3-*O*-sulfamate 3.** To a solution of 2-methoxyestrone **1** (300 mg, 1 mmol) and 2,6-di-*tert*-butyl-4-methyl pyridine (616 mg, 3 mmol) in dichloromethane (16 mL) was added sulfamoyl chloride (4.2 mL, 0.7M, 2.94 mmol). The reaction was left to stir overnight, the mixture then poured onto water (25 mL) and the organic layer was then separated and washed with water (3 × 10 mL), dried and evaporated. The desired product **3** was isolated by column chromatography (3:2 hexane/ethyl acetate) as a white crystalline solid mp 183−185 °C (321 mg, 84%) [lit. 183−186 °C[52]] which showed $^1$H NMR $\delta$ 7.04 (1H, s), 6.91 (1H, s), 5.03 (2H, br), 3.87 (3H, s), 2.82−2.88 (2H, m, 6-$CH_2$), 1.36−2.55 (13H, m), and 0.92 (3H, s, $CH_3$); *m/z* [FAB+] 379.2 (100%, M+); HRMS [FAB+] 379.14534, calculated 379.14534; Anal. ($C_{19}H_{25}NO_5S$) C, H, N.

**2-Ethoxyestrone-3-*O*-sulfamate 13.** A solution of 2-ethoxyestrone **12** (395 mg, 1.26 mmol), 2,6-di-*tert*-butyl-4-methyl pyridine (820 mg, 4 mmol) in dichloromethane (20 mL) was reacted with sulfamoyl chloride (5.4 mL, 0.7 M, 3.77 mmol) as described for the synthesis of **3**. The desired product **13** was isolated by column chromatography (3:2 hexane/ethyl acetate) as a white solid (282 mg, 57%), a sample was crystallized from ethyl acetate/hexane to give as white needles mp 179−181 °C which showed $^1$H NMR $\delta$ 7.30 (1H, s), 6.92 (1H, s), 5.20 (2H, s), 4.11 (2H, m), 2.78−2.86 (2H, m), 1.12−2.55 (16H, m including 1.43 (3H, t, *J* 7.0)), and 0.91 (3H, s); *m/z* [FAB$^+$] 395.2 (100%, M$^+$); HRMS [FAB$^+$] 393.16099, calculated 393.16099; Anal. ($C_{20}H_{27}NO_5S$) C, H, N.

**2-Methylsulfanylestrone-3-*O*-sulfamate 20.** A stirred solution of 2-methylsulfanylestrone **19** (400 mg, 1.26 mmol) and 2,6-di-*tert*-butyl-4-methyl pyridine (776 mg, 3.78 mmol) in dichloromethane (20 mL) was reacted with sulfamoyl chloride (5.4 mL, 3.78 mmol, 0.7 M in toluene) as described for the synthesis of **3**. The desired sulfamate **20** was purified by column chromatography (chloroform/ethyl acetate 9:1) to give a white crystalline solid (358 mg, 72%) mp 184−185 °C which showed $^1$H NMR ($CD_3OD$) $\delta$ 7.23 (1H,s), 7.12 (1H, s), 4.88 (2H, br s), 2.82−2.90 (2H, m), 1.38−2.54 (16H, m including 2.41 (3H, s)) and 0.91 (3H, s); *m/z* [FAB$^+$] 395.2 (M$^+$, 55%), 255.2 (17%); HRMS [FAB$^-$] 394.1 (100%); HRMS [FAB$^+$] 395.12250, calculated 395.12250; Anal. ($C_{19}H_{25}NS_2O_4 \cdot H_2O$) C, H, N.

**2-Ethylsulfanylestrone-3-*O*-sulfamate 27.** A stirred solution of 2-ethylsulfanylestrone **25** (300 mg, 0.91 mmol) and 2,6-di-*tert*-butyl-4-methyl pyridine (615 mg, 3 mmol) in dichloromethane (20 mL) was treated with sulfamoyl chloride (4.28 mL, 3 mmol, 0.7 M in toluene) as described for the synthesis of **3**. Column chromatography (chloroform: ethyl acetate 9:1) gave the desired sulfamate **27** (300 mg, 81%) as a white solid, a sample of this material was crystallized from ethyl acetate/hexane to give white crystals mp 179−181 °C, which showed $^1$H NMR $\delta$ 7.34 (1H, s), 7.16 (1H, s), 5.13 (2H, br s), 2.86−2.98 (4H, m), 1.40−2.57 (13H, m), 1.29 (3H, t, *J* 7.2) and 0.92 (3H, s); *m/z* [FAB$^+$] 409.2 (100%, M$^+$), HRMS [FAB$^+$] found 409.13815, calculated 409.13815; Anal. ($C_{20}H_{27}NO_4S_2 \cdot H_2O$) C, H, N.

**2-Ethylestrone-3-*O*-sulfamate 32.** 2-Ethylestrone **30** (600 mg, 2 mmol) was added to a 0 °C solution of sulfamoyl chloride (3 mmol) in DMA (1.5 mL). After 16 h stirring, ethyl acetate (25 mL) and then water (25 mL) were added, and the organic layer was separated and then washed with water (4 × 25 mL) and brine (25 mL), dried and evaporated to give a white solid. Column chromatography (chloroform to 10% acetone in chloroform) gave the desired sulfamate **32** (660 mg, 87%) as a white crystalline solid mp 161−163 °C which showed $^1$H NMR $\delta$ 7.18 (1H, s), 7.10 (1H, s), 5.23 (2H, s), 2.85−2.90 (2H, m,), 2.70 (2H, q, *J* 7.6), 1.32−2.56 (H, m), 1.21 (3H, t, J 7.6) and 0.93 (3H, s); *m/z* [APCI$^-$] 376.2 (100%, M$^+$ − H); Anal. ($C_{20}H_{27}NO_4S$) C, H, N.

**2-Bromoestrone-3-*O*-sulfamate 37.** An ice cold solution of sulfamoyl chloride (1 mmol) in dimethylacetamide (1.5 mL) was reacted with 2-bromoestrone **36** (150 mg, 0.43 mmol) as described for the synthesis of **32**. The product **37**, a white powder (168 mg, 91%), was recrystallized from acetone/hexane to give colorless needles mp 225−6 °C which showed $^1$H NMR $\delta$ 7.52 (1H, s), 7.24 (1H, s), 4.99 (2H, s), 2.84−2.94 (2H, m), 1.36−2.58 (13H, m) and 0.92 (3H, s); *m/z* (AP-) 428.2 (M$^+$ − H, $^{81}$Br, 30%) and 426.2 (M$^+$ -H, $^{79}$Br, 40%); Anal. ($C_{18}H_{22}BrSO_4N$) C, H, N.

**2-Iodoestrone-3-*O*-sulfamate 40.** An ice cold solution of sulfamoyl chloride (0.5 mmol) in dimethylacetamide (1.5 mL) was reacted with 2-iodoestrone **39** (120 mg, 0.30 mmol) as described for the synthesis of **32**. The desired product **40**, a white powder (123 mg, 85%), was recrystallized from acetone/hexane to give white needles mp 225−226 °C which showed $^1$H NMR $\delta$ 7.72 (1H, s), 7.23 (1H, s), 5.01 (2H, s), 2.86−2.96 (2H, m), 1.36−2.58 (13H, m) and 0.91 (3H, s); *m/z* (AP-) 474.2 (M$^+$ − H, 70%) and 395.2 (100%); Anal. ($C_{18}H_{22}ISO_4N$) C, H, N.

**2-Methoxyestrone-3-*O*-(*N,N*-dimethyl)sulfamate 15.** To a solution of **3** (136 mg, 0.36 mmol) in DMF (5 mL) was added sodium hydride (32 mg, 0.79 mmol) and then, after 15 min,

methyl iodide (67 μL, 1.08 mmol). The reaction was stirred at room temperature for 16 h then poured onto brine (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was then washed with brine (4 × 15 mL), dried and evaporated. The product was isolated by column chromatography (3:2 hexane/ethyl acetate) as a white solid (86 mg, 59%), a sample of which was recrystallized from ethyl acetate/hexane to give **15** as fine white needles mp 218−220 °C. $^1$H NMR δ 7.06 (1H, s), 6.88 (1H, s), 3.85 (3H, s), 2.98 (6H, s), 2.78−2.86 (2H, m), 1.23−2.60 (15H, m), and 0.92 (3H, s); *m/z* [FAB$^+$] 407.2 (100%, M$^+$); HRMS [FAB$^+$] 407.17664, calculated 407.17664; Anal. ($C_{21}H_{29}NO_5S$) C, H, N.

**2-Methoxyestrone-3-*O*-(*N*-acetyl)sulfamate 16.** A solution of **3** (379 mg, 1 mmol) in pyridine (10 mL) at room temperature was treated with acetic anhydride (10 mL). After 2 h, the reaction was treated with ice (20 g) then chloroform (20 mL), causing precipitation of the product in the aqueous layer. The product **16**, a white powder mp 175−177 °C (329 mg, 78%), was collected by filtration. $^1$H NMR δ 7.15 (1H, s), 6.84 (1H, s), 3.81 (3H, s), 3.10 (1H, br), 2.78−2.86 (2H, m), 1.32−2.64 (16H, m including 1.96 (3H, s)), and 0.91 (3H, s); *m/z* [FAB$^+$] 421.1 (100%, M$^+$); HRMS [FAB$^+$] 421.15591, calculated 421.15591.

**2-Methoxyestradiol-3-*O*-sulfamate 4.** A solution of **3** (1.2 g, 3,16 mmol) in THF (8 mL) and isopropyl alcohol (16 mL) was added sodium borohydride (239 mg, 6.32 mmol) in a portionwise manner. The reaction was stirred at room temperature for 3 h then quenched with ammonium chloride solution. The mixture was then extracted with ethyl acetate (2 × 50 mL), and the organic layers were washed with water (50 mL) and brine (50 mL), dried and evaporated. The desired product was isolated by chromatography (chloroform/ethyl acetate gradient 8:1 to 4:1) to give the desired product as an off-white solid. Crystallization from ethyl acetate/hexane gave **4** as colorless crystals (1.02 g, 85%) mp 187−188 °C. $^1$H NMR δ 7.80 (2H, s), 6.96 (1H, s), 6.95 (1H, s), 4.52 (1H, s), 3.75 (3H, s), 3.48−3.56 (1H, m), 2.68−2.76 (2H, m), 1.08−2.40 (13H, m), and 0.67 (3H, s); *m/z* [ES-] 380.2 (M+ − H, 100%); Anal. ($C_{19}H_{27}NO_5S$) C, H, N.

**2-Ethoxyestradiol-3-*O*-sulfamate 14.** 2-Ethoxyestrone-3-*O*-sulfamate **13** (100 mg, 0.25 mmol) was dissolved in THF (3 mL) and methanol (3 mL), and the solution was cooled to 0 °C and then treated with sodium borohydride (32 mg, 3.3 eq) in the same manner as described for the synthesis of **4**. Column chromatography (3:2 hexane/ethyl acetate) gave the desired product **14** as a white foam (78 mg, 78%). A sample of this material was recrystallized from chloroform/hexane to give colorless prisms mp 103−105 °C which showed $^1$H NMR δ 7.01 (1H, s), 6.92 (1H, s), 5.36 (2H, s), 4.10 (2H, m), 3.70 (1H, dd, *J* 8.6 and 8.6), 2.73−2.83 (2H, m), 1.12−2.30 (16H, m including 1.43 (3H, t, *J* 7.0)), and 0.75 (3H, s); *m/z* [FAB$^+$] 395.2 (100%, M$^+$); HRMS [FAB$^+$] 395.17665, calculated 393.17665. Anal. ($C_{20}H_{29}NO_5S\cdot H_2O$) C, H, N.

**2-Methylsulfanyl-3-*O*-sulfamoylestradiol 21.** 2-Methylsulfanylestrone-3-*O*-sulfamate **20** (150 mg, 0.38 mmol) was dissolved in THF (4 mL) and methanol (4 mL), and the solution was cooled to 0 °C and then treated with sodium borohydride (20 mg, 0.53 mmol). After 15 min, no starting material was present by TLC, and the reaction was brought to pH 6 by dropwise addition of glacial acetic acid. Solvent was then removed by evaporation, and the desired product **21** was crystallized from ethanol to give colorless crystals mp 179−180 °C (113 mg, 75%) which showed $^1$H NMR ($CDCl_3$ with $CD_3$-OD) δ 7.21 (1H,s), 7.10 (1H, s), 6.05 (2H, br), 3.67 (1H, dd, *J* 8.5,8.4), 2.76−2.84 (2H, m), 2.42 (3H, s), 1.10−2.32 (13H, m) and 0.74 (3H, s); *m/z* [APCI$^+$] 398.4 (80%, M$^+$ + H); Anal. ($C_{19}H_{27}NS_2O_4$) C, H, N.

**2-Ethylsulfanylestradiol-3-*O*-sulfamate 28.** A room-temperature solution of **27** (150 mg, 0.37 mmol) in THF (2 mL) and methanol (5 mL) was treated with sodium borohydride (38 mg, 1 mmol) as described for the synthesis of **4**. The crude product, a white solid was purified by crystallization from ethyl acetate/hexane to give **28** as colorless needles (124 mg, 82%) mp 110−111 °C which showed $^1$H NMR δ 7.33 (1H, s), 7.13 (1H, s), 5.16 (2H, br s), 3.71 (1H, dd, *J* 8.6 and 7.7), 2.92 (2H, q, *J* 7.4), 2.78−2.88 (2H, m), 1.10−2.36 (17H, m including 1.26 (3H, t, *J* 7.4)) and 0.76 (3H, s); *m/z* [ES-] 410.2 (M$^+$ − H, 50%) 316.5 (100%); Anal. ($C_{20}H_{29}NO_4S_2\cdot H_2O$) C, H, N.

**2-Ethylestradiol-3-*O*-sulfamate 33.** A solution of **32** (300 mg, 0.8 mmol) in THF (5 mL) and isopropyl alcohol (20 mL) was treated with sodium borohydride (60 mg, 1.58 mmol) as described for the synthesis of **4**. The crude product, a white solid, was recrystallized from ethyl acetate/hexane to give **33** as a fine white powder (220 mg, 73%): mp 193−195 °C which showed $^1$H NMR (DMSO) δ 7.94 (2H, s), 7.20 (1H, s), 6.99 (1H, s), 4.52 (1H, d, *J* 5.0), 3.45−3.55 (1H, m), 2.72−2.82 (2H, m), 2.63 (2H, q, *J* 7.4), 1.05−2.40 (H, m including 1.12 (3H, t, *J* 7.4)) and 0.67 (3H, s); *m/z* [APCI$^-$] 379.2 (100%, M$^+$ − H); Anal. ($C_{20}H_{29}NO_4S$) C, H, N.

**Acknowledgment.** This work was supported by Sterix Ltd. as a member of the IPSEN group. We thank Dr. G. Sohal and D. Bennetto for technical support and Drs. L.W.L. Woo and B. LeBlond for useful discussions during the course of this work.

**Supporting Information Available:** Microanalysis and $^{13}$C NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050066A