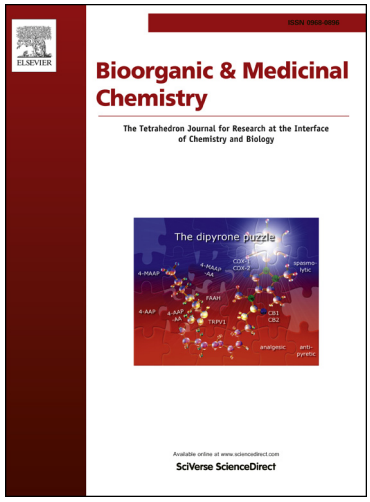

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A-ring Substituted 17β-Arylsulfonamides of 17β-Aminoestra-1,3,5(10)-trien-3-ol as Highly Potent Reversible Inhibitors of Steroid Sulfatase.

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Keywords: steroid sulfatase, hormone therapy, enzyme inhibitors

Abstract

Steroid sulfatase (STS) catalyzes the hydrolysis of the sulfate ester group in biologically inactive sulfated steroids to give biologically active steroids. Inhibitors of STS are considered to be potential therapeutics for treating hormone-dependent cancers such as ER+ breast cancer. A series of 4-substituted 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol were prepared and examined as STS inhibitors. The presence of a NO_2 or Br at the 2-position of the A-ring resulted in a decrease in potency compared to their A-ring-unsubstituted counterparts. However the presence of a nitro group or fluorine atom at the 4-position of the A-ring resulted in an increase in potency and one of these compounds exhibited a K_i^{app} value of 1 nM. Modeling studies provided insight into how these compounds interact with active site residues. The anti-proliferative activity of the 3′-Br, 3′-CF_3, 4-NO_2-3′-Br and 4-NO_2-3′-CF_3 derivatives were examined using the NCI 60-cell-line panel and found to have mean graph midpoint values of 1.9-3.4 μM.

Introduction

It is now well-established that the sex hormones are involved in the development of certain cancers such as breast, prostate and endometrial cancer.[1] Consequently, the development of anticancer drugs that function by blocking the action or limiting the production of these hormones has been the focus of intensive research for many years. This approach to cancer therapy has been used extensively for the treatment of estrogen receptor positive (ER+) breast cancer where drugs such as tamoxifen, which acts as an estrogen receptor antagonist, or letrozole which blocks the production of estrogens by inhibiting the enzyme aromatase, have enjoyed some clinical success.[2] However, many breast cancer patients experience relapse after undergoing these treatments especially those with metastatic ER+ breast cancer.[2] Thus, the pursuit of alternative drugs/therapies for treating ER+ breast cancer has continued unabated.

Steroid sulfatase (STS) catalyzes the hydrolysis of the sulfate ester group in biologically inactive sulfated steroids such as estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHEAS) to give biologically active steroids such as estrone (E1) and dehydroepiandrosterone (DHEA) (Figure 1). Several studies have suggested that STS plays an important role in the progression of ER+ breast cancer. The production of E1 from E1S in breast cancer tissue is approximately 10 times greater than from androstenedione, which is converted to E1 by aromatase.[3] About 90% of Adiol in post-menopausal women originates from DHEAS via desulfation of DHEAS by STS to give DHEA which is converted into Adiol by a dehydrogenase.[4] There is approximately 50-200 times greater STS activity than aromatase activity in malignant breast tissues.[5-7] Sulfatase activity in breast cancer cells is higher than that of normal breast cells.[8] Finally, STS expression in breast tissue is significantly higher than in

normal tissue and STS expression is now used as a prognostic factor in human breast carcinoma.[9-11]

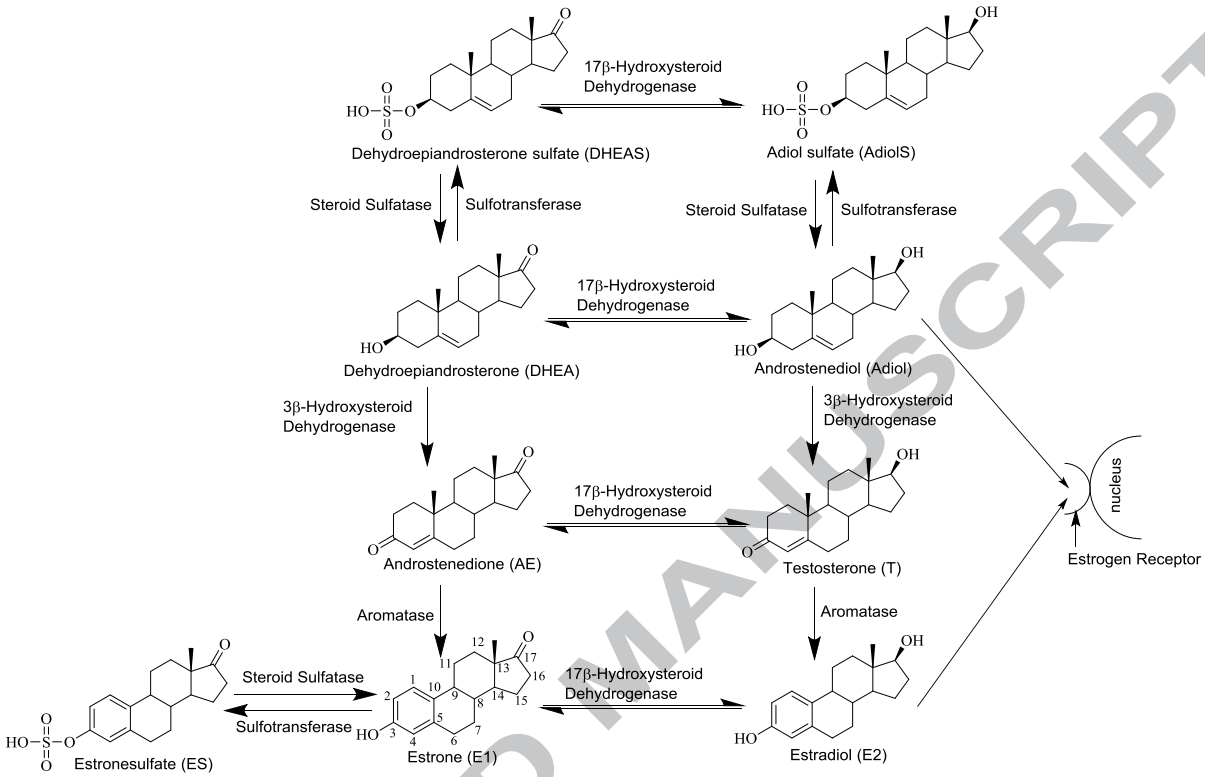


Figure 1. Biosynthesis of estrogenic steroids.

Due the role of STS in promoting ER+ breast cancer and possibly other steroid-dependent cancers such as prostate and endometrial cancer, inhibitors of STS have been pursued as potential anticancer agents for over 20 years.[12a-c] By far the most common class of STS inhibitors are the sulfamates. Since the discovery, in 1994, that estrone sulfamate (EMATE, **1**, Figure 2) is a potent irreversible STS inhibitor,[13] a large number of sulfamates have been prepared and examined as inhibitors of STS.[12a-c,13] However, after over two decades of research, only two sulfamate-based STS inhibitors have entered clinical trials.[14] E2MATE, the estradiol analog of EMATE, is presently in Phase II clinical trials for the treatment of endometriosis.[15] 667-COUMATE (compound **2** in Figure 2), also known as Irusosat,® (**2** in Figure 2) has

undergone clinical trials (Phase I with postmenopausal women having ER+ breast cancer and Phase II with women having advanced endometrial cancer) with mixed results.[16,17] Hence, it is crucial to ascertain if alternative pharmacophores can be developed to broaden the potential for a successful clinical candidate.

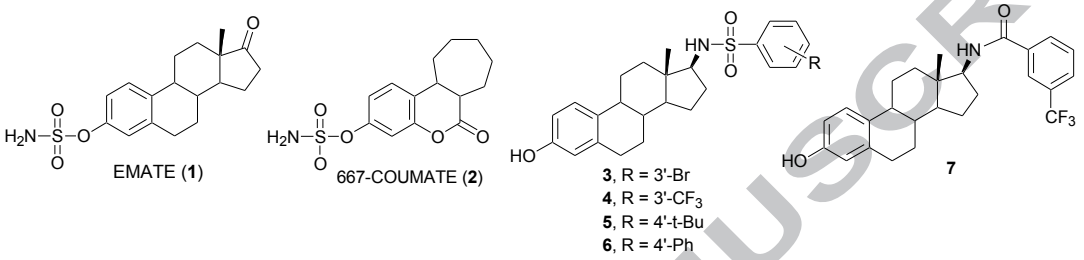


Figure 2. Structures of STS inhibitors **1-7.**

In contrast to irreversible sulfamate-based inhibitors, relatively few reversible, non-sulfamate inhibitors of STS have been reported.[12-14] We previously reported that certain 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol, such as compounds **3-6** (Figure 2), were very good reversible inhibitors of STS (IC_{50} values of **3-6** = 9-25 nM) when assayed with purified STS using 4-methylumbelliferyl sulfate (4-MUS) as the substrate.[18] Replacing the sulfonamide group in **4** with an amide group (compound **7**) yielded a much poorer inhibitor indicating that the sulfonamide group is key to potent inhibition.

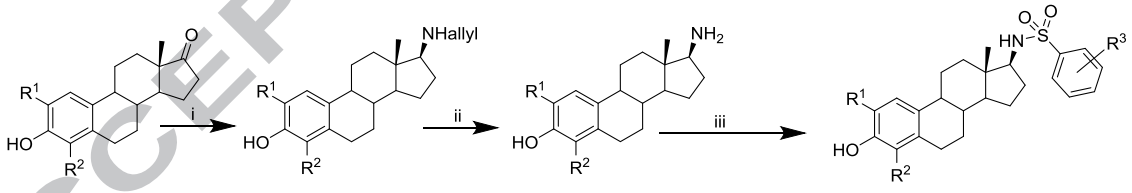
We previously demonstrated that the affinity of E1 or E2 for STS can be increased by introducing a small electron-withdrawing group (EWG) or atom, such as fluorine, bromine, cyano, and nitro, at the 4-position.[19] The reason for this increase in potency is not known though studies showed that it was not due to the decrease in pK_a of the 3-OH group as there was no correlation between the pK_a of the steroid and IC_{50} values. This observation presented to us a potential strategy for further increasing the potency of the 17β-arylsulfonamides. Here we report

the synthesis of a series of 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol bearing a nitro group, or bromine or fluorine atom at the 2- or 4-position and their evaluation as inhibitors of STS. Some of these compounds are highly potent reversible inhibitors of STS with K_i^{app} values as low as 1 nM. Selected inhibitors were evaluated for antiproliferative activity in the NCI 60-cancer cell-line panel. Molecular docking studies into the STS crystal structure were also performed to gain some insight into how they interact with active site residues.

Chemistry

2- and 4-Nitro derivatives **20-27** and 2- and 4-bromo derivatives **28** and **29** were prepared via the route outlined in Scheme 1. Reductive amination of 2- or 4-nitroestrone[20] or 2- or 4-bromo estrone[21] with allylamine and sodium triacetoxyborohydride (STAB) gave derivatives **12-15** in reasonable to good yields. Deallylation of **12-15** with dimethylbarbituric acid (DMBA) and $Pd(PPh_3)_4$ gave amines **16-19** in low to good yields. Reaction of these amines with the appropriate sulfonyl chlorides gave target compounds **20-29** in low to moderate yields though these reactions were not optimized.

Scheme 1. Synthesis of 2- and 4-nitro and 2- and 4-bromo derivatives **20-29**[a]



8, R^1= H, R^2= NO_2
9, R^1= NO_2, R^2= H
10, R^1= H, R^2= Br
11, R^1= Br, R^2= H

12, R^1= H, R^2= NO_2 (63%)
13, R^1= NO_2, R^2= H (73%)
14, R^1= H, R^2= Br (78%)
15, R^1= Br, R^2= H (71%)

16, R^1= H, R^2= NO_2 (47%)
17, R^1= NO_2, R^2= H (71%)
18, R^1= H, R^2= Br (63%)
19, R^1= Br, R^2= H (21%)

20, R^1 = H, R^2 = NO_2, R^3 = 3'-Br; **21**, R^1 = H, R^2 = NO_2, R^3 = 3'-CF_3
22, R^1 = H, R^2 = NO_2, R^3 = 4'-t-Bu; **23**, R^1 = H, R^2 = NO_2, R^3 = 4'-Ph
24, R^1 = NO_2, R^2 = H, R^3 = 3'-Br ; **25**, R^1 = NO_2, R^2 = H, R^3 = 3'-CF_3
26, R^1 = NO_2, R^2 = H, R^3 = 4'-t-Bu; **27**, R^1 = NO_2, R^2 = H, R^3 = 4'-Ph
28, R^1 = H, R^2 = Br, R^3 = 3'-CF_3, **29** , R_1 = Br, R^2 = H, R^3 = 3'-CF_3

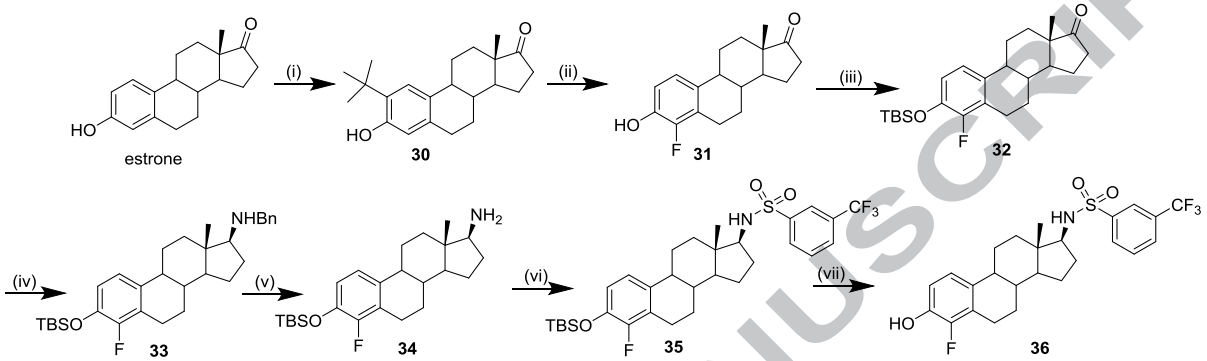
[a]Reagents and conditions: (i) allylamine, STAB, THF, rt, 2 days (63-78%); (ii) cat. $Pd(PPh_3)_4$, DMBA, 35 °C, 1h (21-71%); (iii) $ArSO_2Cl$, pyr CH_2Cl_2, 0 °C then rt O/N (26-67%).

For the evaluation of derivatives bearing a fluorine atom on the A-ring we focussed our efforts on the preparation of the 4-fluoro-3'-CF_3 derivative **36** (Scheme 2). The synthesis of **36**

required the preparation of 4-fluoroestrone (**31**) a compound that has been prepared by several groups by a variety of routes. Compound **31** has been prepared via a multistep procedure in an overall yield of 11% starting from 19-nortestosterone.[22-24] The key step in this procedure was the regiospecific fluorination of the pyrrolidyl enamine of expensive 19-nortestosterone with toxic perchloroylfluoride to give 4β-fluoro-3α-methoxyoestr-5-en-3β, 17β-diol. The 3-*O*-methyl ether of **31** has been prepared by thermal decomposition of estrone 3-*O*-methyl ether 4-diazonium fluoroborate.[23,25] Others have reported that the overall yield of this route is very low (6%).[22] Uemoto and coworkers[26] as well as Page and coworkers[22] have reported the synthesis of **31** and its 2-isomer by subjecting E1 to *N*-fluoropyridinium triflate (NFPT), an electrophilic fluorinating agent. Uemoto obtained the two isomers in a 45% yield in an approximately 1:1 ratio but did not separate them. Page and coworkers reported that their 3-*O*-acetates could be separated by fractional recrystallization from pet. ether-EtOH which gave the 2- and 4-isomers in yields of 53% and 20% respectively. We attempted to obtain **31** using this approach but were unable to separate the two isomers either as their acetates or having a free 3-OH. To avoid formation of the two isomers we first protected the 2-position with a *tert*-butyl group by subjecting estrone to *tert*-butyl alcohol in the presence of cat. boron trifluoride diethyletherate which gave 2-*tert*-butylestrone (**30**) in almost quantitative yield (Scheme 2).[27] Reaction of **30** with NFPT followed by removal of the *tert*-butyl protecting group using $AlCl_3$ in nitromethane gave **31** in a 16% yield (two steps). Although the yield of **31** was low, its synthesis involved only three steps from E1 and no tedious separation of isomers was required. The 3-OH in **31** was protected with a TBS group (84% yield) and the resulting compound, **32**, was subjected to reductive amination with $BnNH_2$ and STAB which gave benzyl protected 17β-amine derivative **33** in an 82% yield. Hydrogenolysis of the benzyl amine moiety in **33** gave 17β-amino derivative **34** in 97% yield.

Reacting **34** with excess of 3'-CF_3-benzenesulfonyl chloride gave sulfonamide **35** in a 79% yield. Removal of the TBS group in **35** gave compound **36** in an 83% yield.

Scheme 2. Synthesis of 4-fluoro-subsituted derivative **36.**[a]



[a]Reagents and conditions: (i) *t*-BuOH, cat. BF_3OEt_2, 12 h (96%); (ii) (a) NFPT, TCE, 135 ^oC, 14 h; (b) $AlCl_3$, $MeNO_2$, CH_2Cl_2, 0 ^oC, 5h (16%, two steps); (iii) TBDMSCl, imidazole, DMF, 2 h, (84%); (iv) $BnNH_2$, STAB, AcOH, DCE, rt, 2 d (83%); (v) H_2, 15 wt % of $Pd(OH)_2$, MeOH/EtOAc (5:1), 12 h (97%); (vi) 3'-$CF_3C_6H_4SO_2Cl$, Et_3N, DMAP, CH_2Cl_2, rt, 48 h (79%); (vii) TBAF, THF, 3h (83%).

The X-ray crystal structure of 2-nitro derivative **25** was obtained and is shown in Figure 3. The structure confirms the β stereochemistry at C-17. The nitro group is coplanar with the aromatic A-ring and forms an intramolecular H-bond with the 3-OH. We were unable to obtain diffraction quality crystals of the corresponding 4-nitro derivative **21**; however, we were able to obtain the crystal structure of 4-nitroestrone (see the supporting information). In 4-nitroestrone, the nitro group is rotated out of the plane of the aromatic ring to avoid steric interactions with the C-6. It is likely that the nitro group in 4-nitro derivative **21** adopts a similar conformation.

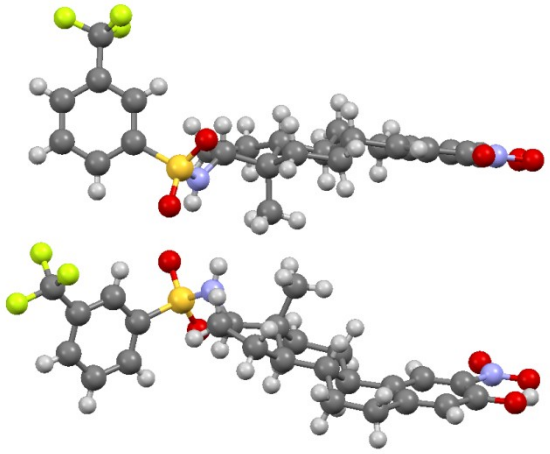
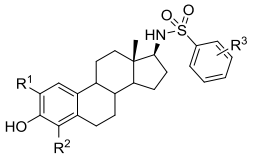


Figure 3. Asymmetric unit in the X-ray crystal structure of compound **25.**

Results and Discussion

To ascertain the effect of C-4 substitution on the STS inhibitory activity of 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ols we initially prepared and evaluated the 2- and 4-nitro derivatives **20-27**. These and subsequent compounds were assayed using purified STS at pH 7.0 in 20 mM Tris-HCl buffer containing 0.1 % triton X-100 and using 4-MUS as the substrate.[28] Their IC_{50} values or apparent K_i values (K_i^{app}) along with their non 4-substituted analogs (**3-6**) are shown in Table 1.

Table 1. STS Inhibitory Activity of Compounds **3**-**6**, **20**-**29** and **36** with purified STS.



Compound	R^1	R^2	R^3	IC_{50} (nM)[a]
3	H	H	3'-Br	25
4	H	H	3'-CF_3	23
5	H	H	4'-*t*-Bu	18
6	H	H	4'-Ph	9
20	H	NO_2	3'-Br	12
21	H	NO_2	3'-CF_3	11
22	H	NO_2	4'-*t*-Bu	8
23	H	NO_2	4'-Ph	1[b]
24	NO_2	H	3'-Br	50
25	NO_2	H	3'-CF_3	67
26	NO_2	H	4'-*t*-Bu	81
27	NO_2	H	4'-Ph	33
28	H	Br	3'-CF_3	49
29	Br	H	3'-CF_3	95
36	H	F	3'-CF_3	2.5[b]

[a]Errors are within ±5%; [b]Apparent K_i

The introduction of a nitro group at the 4-position (R^2) resulted in an increase in potency. This is consistent with our previous finding that 4-NO_2E1 is more potent inhibitor than E1.[19] The effect of the 4-nitro group was most pronounced with the 4'-phenyl derivative **23** which exhibited a K_i^{app} value of 1 nM. The affinity of **23** for STS was such that its IC_{50} depended on the concentration of enzyme (Figure 4) which is expected when the IC_{50} of an inhibitor is lower than

the concentration of enzyme used in the assay.[29,30] The K_i^{app} for **23** was obtained from the Y-intercept the plot of IC_{50} vs. the total enzyme concentration ($[E]_T$) (Figure 4).[29] Introduction of a nitro group at the 2-position resulted in a 2-4.5-fold decrease in potency. This is in contrast to 2-NO_2E1 which was approximately 2-fold more potent than E1.

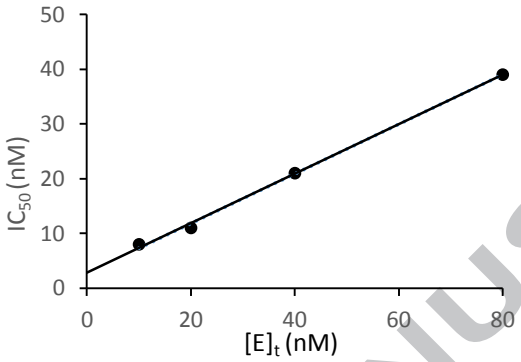


Figure 4. Plot of IC_{50} values for compound **23** as a function of $[E]_T$.

Interestingly, nitrated EMATE derivatives exhibit trends, in terms of their ability to inhibit STS, that are similar to those reported here for the 17β-arylsulfonamide-based inhibitors in that 4-nitroEMATE (**37**, Figure 5) is a 5-fold more potent inhibitor than EMATE while the 2-nitro analog of EMATE is 18-fold less potent than EMATE when assayed with crude STS from placental microsomes.[31] The reason for these differences is not known though it was suggested that intramolecular hydrogen bonding between the 2-nitro group and the sulfamoyl moiety in EMATE may occur which could potentially affect its ability to interact with STS and that this type of intramolecular interaction does not occur with the 4-nitro derivative.[31] The enhanced affinity of 4-nitroEMATE compared to EMATE was attributed to favorable interactions between the nitro group and residues in the STS active site. The similar trends in inhibitory potency between the nitrated 17β-arylsulfonamide-based inhibitors presented here and the nitrated EMATES suggest that 17β-arylsulfonamide-based inhibitors in Table 1 might initially interact

with STS in a manner that is similar to EMATE and its derivatives even though their mechanisms of action are completely different.

For studies on compounds bearing a Br or F at the 2- or 4-positions we focussed on preparing and evaluating the 3'-CF_3 derivatives (**28**, **29** and **36**). Compound **28** which contained a Br at the 4-position was 2-fold less potent than its A-ring unsubstituted analog, compound **3** (Table 1). This is in contrast to our results with E1 in that 2-BrE1 is a 10-fold more potent inhibitor than E1.[19] The 2-Br analog **29** was 4-fold less potent than **3**. Introduction of a fluorine to the 4-position, compound **36**, resulted in a 9-fold increase in potency with a K_i^{app} of 2.5 nM (obtained from the Y-intercept of a plot of IC_{50} vs. $[E]_T$ - see the supporting information).

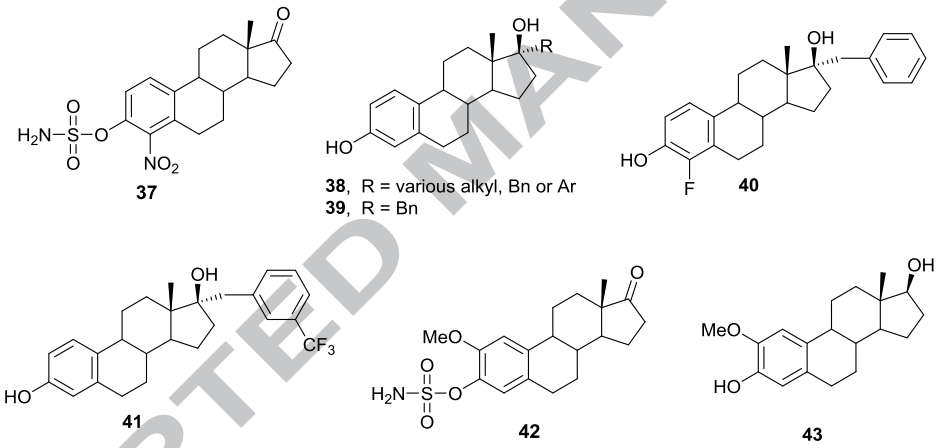


Figure 5. Structures of STS inhibitors **37-42** and 2-MeOE2 (**43**).

The development of future STS inhibitors based on the scaffold described here would be greatly assisted by knowing how these inhibitors interact with STS. Poirier and coworkers have reported that 17α-benzylE2 inhibitors of type **38** are potent reversible inhibitors of STS (IC_{50} values as low as 21 nM when assayed using crude STS obtained from homogenates of HEK or JEG-3 cells at pH 7.0 and [^{3}H]E1S as substrate).[32-36] They suggested that the benzyl group extends into the hydrophobic tunnel between the two alpha helices of STS that insert into the

membrane of the ER and this results in an increase in potency compared to E2. We reported previously that compound **40**, which has a fluorine at the 4-position is a 15-fold more potent inhibitor (IC_{50} = 40 nM) than **39** under our assay conditions.[19] Moreover, under our assay conditions, we have shown that 17α-benzylE2 inhibitors and 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol exhibit mixed inhibition suggesting that these compounds are capable of binding at both the active site and at a secondary site outside the active site.[18,19,37]

To study possible active site binding interactions the 3'-CF_3 series of inhibitors were docked into the crystal structure of STS (PDB ID: 1P49), after conversion of the sulfated formylglycine75 (FGly75) hydrate to an FGly75 hydrate, using the build tool in Discovery Studio (DS) – Structure-Based Design (SBD) software. The LibDock docking algorithm in DS was used to investigate active site binding modes of the STS inhibitors. Figures 6A-C show the docking poses obtained for compounds **4** (Figure 6A), **21** (Figure 6B) and the corresponding 3'-trifluoromethyl-17α-benzylE2 inhibitor **41** (Figure 6C) which has been reported by Poirier and coworkers to have an IC_{50} of 126 nM when assayed using crude STS obtained homogenates of JEG-3 cells at pH 7.0 and [^{3}H]E1S as substrate.[35] We also show the docking pose for compound **7** (Figure 6D) which is the amide analog of **4** which we had previously shown to have an IC_{50} value 31-times greater than **4**.

The A-, B-, C- and D-rings of compounds **4**, **21** and **41** share common hydrophobic interactions with a variety of hydrophobic amino acids such as Val101, Leu74, Val486, Phe488 and Val177. These hydrophobic interactions are shown for compound **21** (Figure 6B) but are not shown for compounds **4** and **41** (Figures 6A and 6C) so that other interactions can be visualized more clearly. When bound in the active site in this fashion the aryl sulfonamide groups in **4** and

21 and the benzyl portion in **41** do not extend to any significant extent down the hydrophobic tunnel.

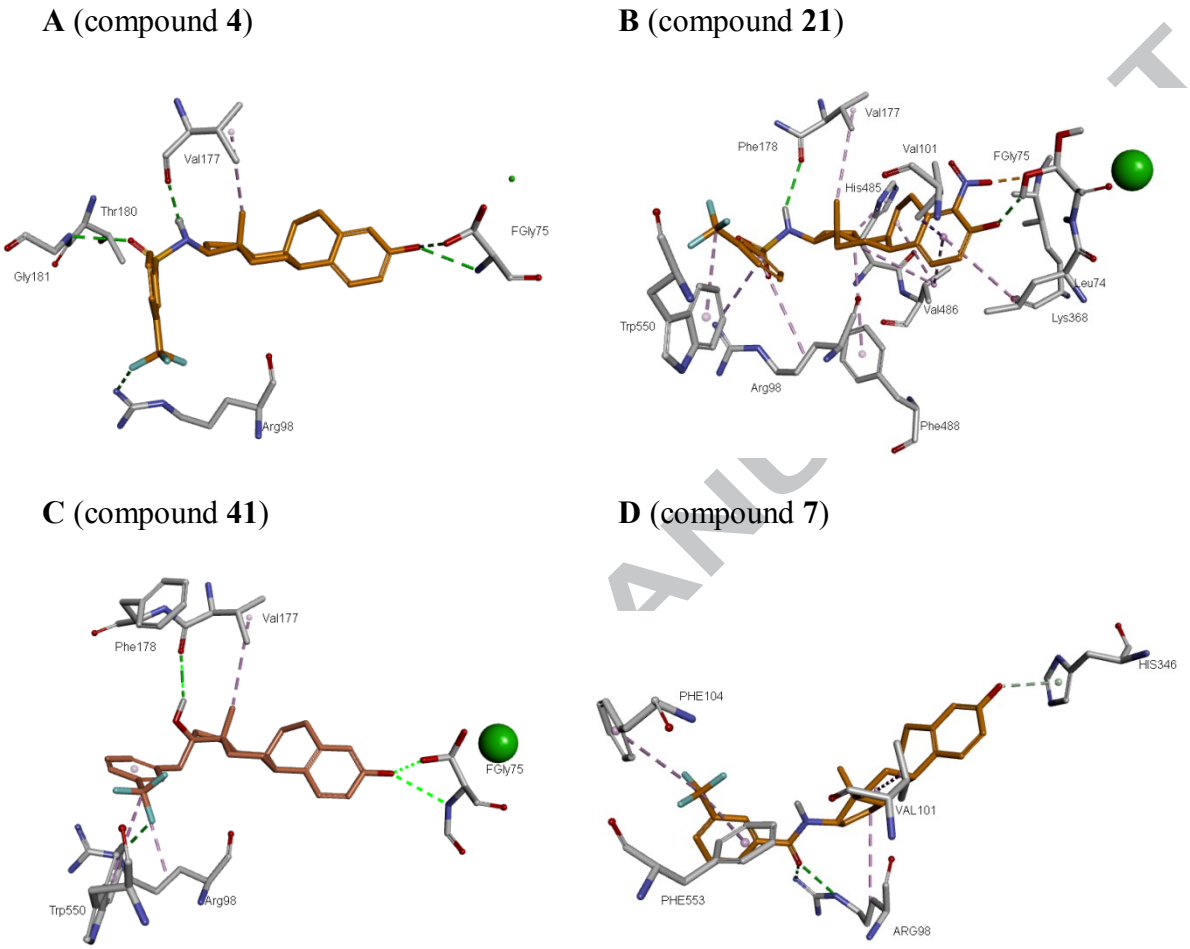


Figure 6. The binding poses of compounds **4** (**A**), **21** (**B**), **41** (**C**) and **7** (**D**). All hydrogens, with the exception of the hydrogens bonded to the nitrogen of the sulfonamide groups in **4** and **21**, the amide group in **7** and the 17-*O* of compound **41**, have been removed for reasons of clarity. H-bonds are shown as dashed green lines and are 2.4-3.5 Å. Hydrophobic interactions (pi-alkyl, alkyl-alkyl) are indicated by dashed purple lines and are 3.9-5.5 Å. The pi-cation interaction involving Trp550 and the benzene sulfonamide moiety in **21** shown in panel **B** is indicated by a dashed dark purple line (3.95 Å). The pi-donor H-bond interaction between His346 and the 3-OH of 7 shown in panel **D** is indicated by a dashed light green line (4.05 Å). The electrostatic interaction between the nitro group in **21** and Lys368 shown in panel **B** is indicated by a dashed orange line (3.81 Å). The Ca^{+2} ion is shown as a green sphere.

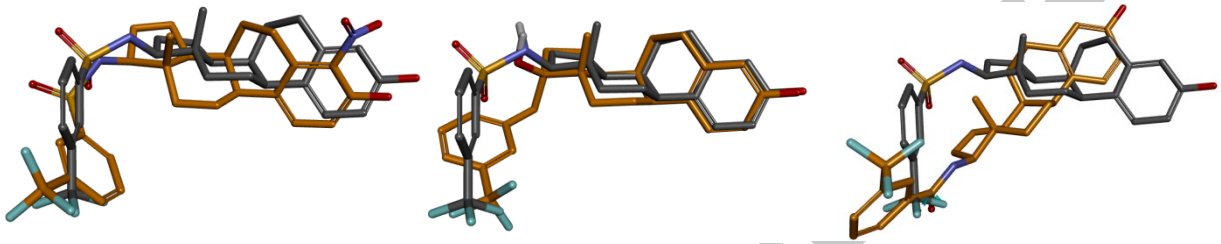
The 3-OH in compounds **4**, **21** and **41** forms H-bonds with the OH and/or C=O of the FGly hydrate. It would be expected that this interaction would be affected by the presence of an EWG attached to the A-ring. However, there does not appear to be a relationship between inhibitor potency (i.e. the potency of 4-nitro inhibitor **21**, 4-bromo inhibitor **28** and 4-fluoro inhibitor **36**) and the pK_a's of the corresponding ortho-substituted phenols.[38] This result suggests that the effect of these EWG's on the potency of the inhibitors may also be attributed to the EWG's being involved in specific interactions (H-bonding, electrostatic interaction etc.) with active site residues. The docking pose for **21** shows the 4-NO_2 group rotated out of the plane of the aromatic A-ring, similar to the nitro group in 4-nitroestrone (see the supporting information), and is involved in electrostatic interactions with the side chain of Lys 368. This results in a slight rotation and shifting of the steroid backbone in **21** in relation to the steroid backbone in compounds **4** and **41** whose A-D rings almost completely overlap as seen in Figures 7A and 7B.

The sulfonamide N-H in compounds **4** and **21** as well as the 17-OH in compound **41** form an H-bond with the carbonyl of Val177. One of the S=O oxygens in compound **4** H-bonds with the N-H of Gly181.

Although the phenyl portion of the aryl sulfonamide group of **4** is at almost a 90^o angle to the phenyl group of the benzyl moiety in **41** (see Figure 7B), a fluorine atom in both **4** and **41** forms an H-bond with the side chain of Arg98. In contrast, the CF_3 group in **21** is rotated slightly away from Arg98 and is involved in a hydrophobic interaction with side chain of Trp550 and the side chain of Arg98 is involved in a pi-cation interaction with the phenyl group of the sulfonamide moiety.

Amide **7**, which is a much poorer inhibitor than its sulfonamide analog **4**, exhibits a docking pose that is quite different from compounds **4**, **21** or **41** (Figures 6D and 7C). In

compound **7**, no H-bond is formed between the 3-OH and FGly hydrate as the entire inhibitor is shifted away from the FGly hydrate towards the entrance of the active site. There is a weak pi-donor H-bond interaction between the 3-OH and the imidazole ring of His346. The carbonyl of the amide is involved in two H-bonds with the side chain of Arg98. The CF_3 group is involved in weak hydrophobic interactions with Phe104 and Phe553.



A **B** **C**

Figure 7. Overlay of the binding poses of (**A**); **4** (grey) and **21** (gold), (**B**); **4** (grey) and **41** (gold) and, (**C**); **4** (grey) and **7** (gold).

These differences in binding modes may, at least in part, explain the differences in potency between these compounds; however, as 17α-benzylE2 inhibitors and 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol exhibit mixed inhibition,[19] which indicates that these inhibitors bind at both the active site and a site outside the active site, then these modeling studies do not provide a complete picture of all inhibitor-protein interactions. In the absence of an x-ray structure of an STS-inhibitor complex, the location of the additional binding site would be entirely speculative.

Many sulfamate-based STS inhibitors have been examined for antiproliferative activity in cell-based assays. Previous reports have demonstrated that the antiproliferative activity displayed in in vitro assays using MCF-7 cells were predictive of the spectrum of desirable biological properties obtained with certain sulfamate-based STS inhibitors such as 2-MeOEMATE (**42**, Figure 5).[39-41] It is believed that the antitumor activity of compounds such as

42 is due to their ability to disrupt the tubulin-microtubule equilibrium in cells.[40] In contrast, very few reversible STS inhibitors have been evaluated for their antiproliferative activity in cell-based assays. Potter and coworkers reported 51% inhibition of growth of MCF-7 cells with 10 μM compound **39** which had been previously shown by Poirier and coworkers to be a moderately good STS inhibitor (IC_{50} = 310 nM) when assayed using STS obtained from homogenates of JEG-3 cells and [^{3}H]-E1S as substrate.[39]

Compounds **3**, **4**, **20**, and **21** were screened for their antiproliferative activity in the NCI human 60-cancer cell line panel which included two ER^+ breast cancer cell lines (MCF-7 and T-47D) and four ER^- breast cancer cell lines (HS578T, BT-549, MDA-MB-468, MDA-MB-231). The GI_{50} values with these breast cancer cell lines are shown in Table 2. The GI_{50}'s of these compounds with these breast cancer cell lines ranged from for 1.8-5.0 μM and there was little difference in cytotoxicity between the ER+ and ER^- lines. There was no correlation between STS inhibitory activity and GI_{50} values though this was not unexpected. In comparison, 2-methoxyestradiol (MeOE2, **43**, Figure 5) an experimental drug candidate (under trade name of Panzem®) undergoing clinical trials for treating breast cancer and ovarian cancer, exhibits a GI_{50} value of 2.4 μM with MCF-7 cells.[39-41] The GI_{50} values of 4-substituted EMATE derivatives such as 4-nitroEMATE with MCF-7 cells have not been reported. In contrast, many 2-substituted EMATE derivatives have been examined for their ability to inhibit the growth of MCF-7 cells.[39-41] The GI_{50} values of these compounds depended greatly on the appended substituents and the presence of a sulfamate group at the 3-position was found to be important for antiproliferative activity. Some of these compounds exhibited GI_{50} values with MCF-7 and other cancer cell lines in the low nanomolar range. The GI_{50} values for compounds **3**, **4**, **20**, and **21** with the other 54 cell lines evaluated in the screen were between 1-10 μM. The mean graph

midpoint (MGM) data in Table 2 which represent the average of the GI_{50} values with all 60 cell lines, were between 1.9-3.4 μM for these four compounds. In comparison, MeOE2 exhibited an MGM value of 1.3 μM in this screen.[39-41]

Table 2. GI_{50} values (μM) and MGM (μM) values of compounds **3**, **4**, **20**, **21** obtained from the NCI screening.

Compound	MCF-7	T-47D	HS578T	BT-549	MDA-MB-468	MDA-MB-231	MGM
3	3.7	5.0	4.0	3.1	2.4	3.9	3.3
4	2.7	3.7	3.2	2.7	4.1	2.4	3.4
20	1.9	2.5	2.1	1.6	2.2	1.5	1.9
21	1.8	1.8	2.2	1.9	2.7	1.9	2.2

Conclusions

In this study we set out to further refine STS inhibitory activity of 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol by examining the effect of small EWGs at the 2- and or 4- position of the A-ring. The presence of a NO_2, F or Br at the 2-position of the A-ring resulted in a decrease in potency compared to their A-ring-unsubstituted counterparts. In contrast, the presence of a nitro group or fluorine atom at the 4-position of the A-ring resulted in an increase in potency. Compounds **23** and **36** were found to be tight-binding inhibitors with K_i^{app} values of 1 and 2.5 nM respectively. Modeling studies performed on the 3′-CF_3 series revealed possible reasons for differences in binding affinity amongst this series of compounds. The anti-proliferative activity of the 3′-Br, 3′-CF_3, 4-NO_2-3′-Br and 4-NO_2-3′-CF_3 derivatives were examined using the NCI 60 cell-line panel. The mean graph midpoint (MGM) values of these compounds, 1.9-3.4 μM, were similar to that previously reported for 2-methoxyestradiol (1.3 μM) an experimental drug candidate undergoing clinical trials for treating breast cancer and ovarian cancer. Further studies to determine the estrogenic properties of these compounds and if

these compounds interact with STS at more than one site are in progress.

Experimental Section

General Procedures. All buffers, starting materials and reagents were obtained from commercial suppliers. THF was distilled from sodium-benzophenone, pyridine was distilled from KOH pellets, 1,2-DCE and 1,1,2-TCE were dried by standing over activated type 4A molecular sieves, CH_2Cl_2 was distilled from calcium hydride under nitrogen. Benzylamine and allylamine were dried by distillation from KOH pellets and stored in the dark over type 4 Å molecular sieves. Dioxane was distilled from Na and stored over type 4 Å molecular sieves. Silica gel chromatography was performed using silica gel (60Å, 230-400 mesh) obtained from Silicycle (Laval, Quebec, Canada). ^{1}H-, ^{13}C-, and ^{19}F-NMR spectra were recorded on a Bruker Avance 300 spectrometer. For NMR spectra obtained using $CDCl_3$ as the solvent, chemical shifts (δ) for ^{1}H NMR spectra are reported relative to internal Me_4Si (δ 0.0 ppm), chemical shifts for ^{13}C spectra are relative to the residual solvent peak (δ 77.0 ppm, central peak), and chemical shifts for ^{19}F-NMR are relative to a $CFCl_3$ (δ 0.0 ppm) external standard. High-resolution (HRMS) electrospray ionization (ESI) mass spectra were obtained on a Waters/Micromass QTOF Ultima Global mass spectrometer or a Thermo Q-Exactive Orbitrap mass spectrometer. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Cellular studies were done using human tumor cell lines of the cancer screening panel (NCI60) at the U.S. National Cancer Institute (Bethesda, USA). STS was purified as previously described.[42] All fluorescent measurements were carried out on a SpectraMax GeminiXS® fluorimeter (Molecular Devices, Sunnyvale, CA, USA) at 24 °C in black microtiter plates from Corning (Corning, MA, USA). All determinations were carried out in triplicate and errors reported as ± 5% of obtained results.

2- and 4-Nitroestrone (8 and 9). To a stirred solution of E1 (1.7 g, 6.4 mmol) in glacial acetic acid (90 mL) at 70-75 ^{o}C was added a mixture of concentrated nitric acid (0.4 mL) and glacial acetic acid (10 mL). Stirring was continued at room temperature for 18 h during which a precipitate formed. The mixture was filtered and washed with hot glacial acetic acid to afford (200 mg, 20%) of **8** as a pale yellow powder. The filtrate was concentrated, the residue was dissolved in benzene (30 mL), aq. $NaHCO_3$ (2%, 20 mL) was added and the mixture stirred for 6 h. The layers were separated and the aqueous layer was acidified with conc. HCl (10 mL). The mixture was filtered and the filtrate was concentrated and recrystallized from ethanol (95%, 10 mL) to afford **9** as bright yellow crystals (250 mg, 23%). Characterization data for **8**: Mp: 277-280^{o}C (lit[20] 273-276^{o}C); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.40 (brs, 1H, ArOH), 7.44 (d, J = 8.8 Hz, 1H), 6.94 (d, J = 8.8 Hz, 1H), 3.20-3.14 (m, 1H), 3.02-2.94 (m, 1H), 2.55-2.46 (m, 1H), 2.35-1.94 (m, 6H), 1.67-1.23 (m, 8H), 0.90 (s, 3H,). Characterization data for **9**: Mp: 181-183 ^{o}C (lit:[20] 183-184^{o}C); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 10.32 (brs, 1H, ArOH), 7.90 (s, 1H), 6.80 (s, 1H), 2.95-2.92 (m, 2H), 2.54-2.39 (m, 2H), 2.17-1.96 (m, 5H), 1.61-1.41 (m, 7H), 0.89 (s, 3H, H-18).

4-Bromoestrone (10). To a stirred solution of **E1** (500 mg, 1.8 mmol) in ethanol (50 mL), was added NBA (255 mg, 1.8 mmol). The mixture was stirred for 24 h during which a precipitate formed. The mixture was filtered and washed with cold ethanol. Recrystalization from ethanol afforded **10** as white solid (575 mg, 89%). Mp: 264-265 ^{o}C (lit:[22] 263-265 ^{o}C); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 9.80 (s, 1H, ArOH), 7.07 (d, J = 8.8 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 2.87-2.79 (m, 1H), 2.63-2.51 (m, 1H), 2.42-1.92 (m, 6H), 1.72-1.65 (m, 1H), 1.55-1.27 (m, 5H), 0.77 (s, 3H).

2-Bromoestrone (11). To a stirred solution of estratrien-1,3,5(10)-3-acetate[22] (1 g, 3.2 mmol) in TFA (20 mL) at 0 ^{o}C (ice bath) was added thallium trifluoroacetate (3.5 g, 6.4 mmol), and the mixture stirred for 24 h. The TFA was removed by rotary evaporation, the residue was washed with DCE (2 x 10 mL) then dried under high vacuum for 6 h. The residue was dissolved in 1,4-dioxane (50 mL), $CuBr_2$ (1.14 g, 7.9 mmol) was added and mixture was refluxed for 3 h. The solvent was removed, the residue was dissolved in dichloromethane (25 mL), washed with water (2 x 20 mL), brine (2 x 20 mL), dried (Na_2SO_4) and concentrated. Purification by flash chromatography (ethyl acetate/hexane, 4:1) afforded 2-bromo-estratrien-1,3,5(10)-3-acetate as white solid (0.9 g, 73%). Mp: 166-167 ^{o}C (lit:[22]146-147 ^{o}C); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.46 (s, 1H), 6.82 (s, 1H), 2.85-2.82 (m, 2H), 2.53-2.44 (m, 1H), 2.31-1.93 (m, 9H), 1.59-1.39 (m, 6H), 0.88 (s, 3H). To a stirred solution of 2-bromo-estratrien-1,3,5(10)-3-acetate (0.50 g, 1.3 mmol) in methanol (20 mL) was added potassium carbonate (0.8 g, 6.3 mmol). The mixture was stirred under reflux for 3 h. The solvent was then removed and water (20 mL) and dichloromethane (20 mL) added to the residue. The layers were separated and the aq. layer extracted with dichloromethane (20 mL). The combined organics were washed with brine (20 mL), dried (Na_2SO_4) and then concentrated. Purification by flash chromatography (ethyl acetate/hexane, 3:2) afforded **11** as white solid (0.45 g, 91%). Mp: 194-196 ^{o}C (lit:[22] 194-195^{o}C); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.32 (s, 1H), 6.74 (s, 1H), 5.26 (s, 1H, ArOH), 2.83-2.80 (m, 2H), 2.53-2.44 (m, 1H), 2.32-1.93 (m, 6H), 1.59-1.39 (m, 7H), 0.88 (s, 3H).

General procedure for the reductive amination of 8-11. To a stirred solution of **8-11** in THF (for **8** and **9**, 0.050 g/mL THF) or DCE (for **10** and **11**, 0.020 g/mL DCE) was added 4 equiv allylamine, 4 equiv glacial acetic and 2.5 equiv sodium triacetoxyborohydride (STAB). The mixture was stirred for 1-3 d, after which aq. saturated $NaHCO_3$ (20 mL) was added and the

mixture stirred was for additional 10 min. The mixture was extracted with dichloromethane, the combined organics washed with aq. saturated $NaHCO_3$, water and brine, then dried (Na_2SO_4) and concentrated. Purification by flash chromatography gave compounds **12-15**.

17β-Allylamino-4-nitro-estratrien-1,3,5(10)-3-ol (12). Purification by flash chromatography (methanol/chloroform, 1:9) afforded **12** as a yellow solid (350 mg, 63%); Mp 150-151°C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.20 (d, J = 8.9 Hz, 1H), 6.77 (d, J = 8.9 Hz, 1H), 5.90-5.70 (m, 1H), 5.15 (d, J = 17 Hz, 1H), 5.00 (d, J = 10 Hz, 1H), 3.18 (m, 1H, overlaps with H_2O peak), 3.15 (m, 1H), 2.25-2.00 (m, 2H), 2.00-1.70 (m, 3H), 1.49-1.02 (m, 7H), 0.64 (s, 3H,); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 147.7, 141.0, 138.2, 131.2, 128.3, 128.1, 115.7, 115.1, 68.2, 51.8, 51.2, 43.7, 43.1, 38.1, 37.8, 29.2, 26.5, 26.3, 24.1, 23.4, 12.2; HRMS (ESI$^+$) calcd. for $C_{21}H_{29}N_2O_3$ $(M+H)^+$ 357.2178; found 357.2186.

17β-Allylamino-2-nitro-estratrien-1,3,5(10)-3-ol (13). Purification by flash chromatography (ethyl acetate/hexane, 3:7) afforded **13** as a yellow solid (415 mg, 73%); Mp 163-164 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 1H), 6.81 (s, 1H), 5.90 (dddd, J = 6.0, 10.2, 11.9, 16.3 Hz, 1H), 5.51 (brs, 1H), 5.09 (ddd, J = 1.7, 17.2, 18.8 Hz, 2H), 3.27 (dd, J = 1.3 and 6.0 Hz, 2H), 2.92-2.82 (m, 2H), 2.67-2.61 (m, 1H), 2.30-2.26 (m, 1H), 2.15-1.98 (m, 3H), 1.89 (m, 1H), 1.71-1.20 (m, 8H), 0.73 (s, 3H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.8, 149.2, 137.4, 133.9, 131.7, 121.4, 118.8, 115.5, 68.3, 52.2, , 51.1, 43.5, 42.9, 38.1, 37.6, 29.7, 26.7, 26.2, 23.4, 11.8; HRMS (ESI$^+$) calcd. for $C_{21}H_{29}N_2O_3$ $(M+H)^+$ 357.2178; found 357.2176.

4-Bromo-17β-allylamino-estratrien-1,3,5(10)-3-ol (14). Purification by flash chromatography (ethyl acetate/hexane, 1:4) afforded **14** as white solid (160 mg, 71%). Mp: 145-147 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.14 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 5.96-

5.83 (m, 1H), 5.18-5.04 (m, *2*H), 3.46-3.27 (brs overlapped by an AB system, 3H), 2.93-2.85 (m, 1H), 2.72-2.62 (m, 2H), 2.28-1.91 (m, 5H), 1.72-1.70 (m, 1H), 1.47-1.23 (m, 7H), 0.72 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 150.1, 137.4, 136.5, 134.4, 125.4 , 115.6, 113.7, 112.6, 68.3, 52.1, 51.4, 44.1, 42.9, 38.0, 37.9, 31.1, 29.7, 27.5, 26.7, 23.4, 11.8; HRMS (ESI^+) calcd. for $C_{21}H_{29}NOBr$ $(M+H)^+$ 390.1433; found 390.1443.

2-Bromo-17β-allylamino-estratrien-1,3,5(10)-3-ol (15). Purification by flash chromatography (ethyl acetate/hexane, 1:4) afforded **15** as white solid (120 mg, 71%). Mp: 173-175^{o}C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.30 (s, 1H), 6.65 (s, 1H), 5.94-5.87 (m, 1H), 5.2-5.05 (m, 2H), 4.10 (brs, 2H), 3.86 (t, J = 5.7 Hz, 1H), 3.30-3.28 (m, 2H), 2.75-2.63 (m, 3H), 2.20-1.97 (m, 5H), 1.84-1.81 (m, 1H), 1.70-1.67 (m, 1H), 1.43-1.23 (m, 7H), 0.72 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 150.4, 137.7, 136.8, 134.0, 128.9, 116.1, 116.0, 107.4, 68.2, 52.1, 51.2, 43.7, 43.0, 38.4, 37.8, 29.2, 27.1, 26.4, 23.4, 11.7; HRMS (ESI^-) calcd. for $C_{21}H_{27}NOBr$ $(M-H)^-$ 388.1276; found 388.1272.

General method for deallylation of 12-15. To a stirred solution of compounds **12-15** in dry DCM (0.020 g/mL) was added a solution of *N,N*-dimethylbarbituric acid (DMBA, 3 equiv) and 15 mol% $Pd(Ph_3)_4$ in dry dichloromethane (0.17 g DMBA/mL dichloromethane). The mixture was stirred for 3 h at 35^{o}C under an argon atmosphere and then diluted with dichloromethane. The mixture was washed with sat. $NaHCO_3$, water, brine then dried (Na_2SO_4) and concentrated under vacuum. Pure **16-19** was obtained using flash chromatography.

17β-Amino-4-nitro-estratrien-1,3,5(10)-3-ol (16). Purification by flash chromatography (methanol/chloroform/NH_4OH, 9:90:1) afforded **16** as a yellow solid (150 mg, 47%). Mp 167-169 ^{o}C; This compound exhibited very limited solubility, with or without heating, in all organic solvents tested including $CHCl_3$, MeOH, H_2O and DMSO. Prolonged heating in DMSO results

in some decomposition. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.44 (d, J = 8.8 Hz, 1H), 6.92 (d, J = 9.0 Hz, 1H), 3.20 (m, 2H), 2.96-2.78 (m, 3H), 2.23-2.15 (m, 4H), 1.97-1.92 (m, 2H), 1.70 (m, 1H), 1.46-1.17 (m, 8H), 0.72 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 151.9, 134.2, 134.1, 132.8, 116.4, 115.8, 68.2, 52.0, 51.4, 44.5, 43.0, 38.0, 37.5, 29.6, 27.8, 26.83, 26.80, 23.3, 11.9; HRMS (ESI^+) calcd. for $C_{18}H_{25}N_2O_3$ $(M+H)^+$ 317.18597; found 317.18601.

17β-Amino-2-nitro-estratrien-1,3,5(10)-3-ol (17). Purification by flash chromatography (methanol/chloroform/NH_4OH, 9:90:1) afforded **17** as an orange solid (220 mg, 71%). Mp 133-135 ^{o}C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 1H), 6.81 (s, 1H), 3.77 (brs, 2H), 2.92-2.70 (m, 3H), 2.33-2.28 (m, 1H), 2.14-2.00 (m, 2H), 1.87 (m, 2H), 1.70-1.64 (m, 1H), 1.53-1.48 (m, 1H), 1.38-1.15 (m, 6H), 0.65 (s, 3H). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.8, 149.2, 133.8, 131.7, 121.4, 118.8, 62.7, 51.9, 43.4, 42.8, 38.4, 36.3 , 30.9, 29.8, 26.7, 26.1, 23.3, 11.1; HRMS (ESI^+) calcd for $C_{18}H_{25}N_2O_3$ $(M+H)^+$ 317.1865; found 317.1853.

4-Bromo-17β-amino-estratrien-1,3,5(10)-3-ol (18). Purification by flash chromatography (methanol/chloroform/NH_4OH, 9:90:1) afforded **18** as a white solid (58 mg, 63%). This compound exhibited very limited solubility, with or without heating, in all organic solvents tested including $CHCl_3$, MeOH, H_2O and DMSO. Prolonged heating in DMSO results in some decomposition. Mp: 163-165 ^{o}C. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.11 (broad d, J = 7.90 Hz, 1H), 6.83 (broad d, J = 7.0 Hz, 1H), 3.4 (bs, 1H), 2.81 (broad d, J = 17.5 Hz, 1H), 2.25 (m, 1H), 2.11 (broad s, 1H), 1.95-1.70 (m, 3H), 1.69-1.55 (m, 1H), 1.37-1.03 (m, 7H), 0.58 (s, 3H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 151.7, 136.3, 132.6, 124.8, 113.1, 112.4, 51.3, 43.6, 40.3, 37.9, 36.2, 30.8, 27.1, 26.1, 22.9, 10.9; HRMS (ESI^+) calcd. for $C_{18}H_{25}NOBr$ $(M+H)^+$ 350.1120; found 350.1127.

2-Bromo-17β-amino-estratrien-1,3,5(10)-3-ol (19). Purification by flash chromatography (methanol/chloroform/NH_4OH, 9:90:1) afforded **4.26** as a white solid (20 mg, 21%). Mp: 190-191 ^oC; ^1H-NMR ($CDCl_3$, 300 MHz) δ 7.33 (s, 1H), 6.69 (s, 1H), 3.47 (s, 1H), 2.73-2.78 (m, 3H), 2.62 (br s, 2H), 2.29-2.04 (m, 3H), 1.90-1.81 (m, 2H), 1.75-1.64 (m, 1H), 1.52-1.13 (m, 7H), 0.67 (s, 3H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 152.0, 136.6, 132.1, 129.0, 116.2, 106.6, 62.3, 51.3, 43.1, 42.4, 38.4, 36.1, 30.6, 28.5, 26.8, 25.8, 22.9, 10.9; HRMS (ESI^+) calcd. for $C_{18}H_{25}NOBr$ $(M+H)^+$ 350.1120; found 350.1111.

General procedure for synthesis of sulfonamides 20-28. To a stirred solution of **16-19** in dry pyridine (0.16 mmol/mL) at 0 ^oC was added a solution of the appropriate sulfonyl chloride (1.2 equiv) in dichloromethane (1 mL) drop-wise via a syringe pump over 10 min. The reaction was stirred for 16 h at room temperature. The mixture was concentrated by rotary evaporation pyridine and the residue dissolved in chloroform or ethyl acetate , washed with water and brine, then dried with anhydrous Na_2SO_4 and concentrated. Purification was achieved using flash chromatography.

4-Nitro-17β-(3'-bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (20). Purification by flash chromatography (ethyl acetate/hexane, 3:7) afforded **20** as a yellow solid (41 mg, 48%). Mp 230-231 ^oC; ^1H-NMR ($CDCl_3$, 300 MHz) δ 9.40 (s, 1H), 8.02 (s, 1H), 7.80 (d, J = 6.9 Hz, 1H), 7.68 (d, J = 7.4 Hz, 1H), 7.44-7.35 (m, 2H), 6.92 (d, J = 8.7 Hz, 1H), 4.55 (d, J = 9.2 Hz, 1H), 3.21-3.09 (m, 2H), 2.94-2.88 (m, 1H), 2.23-2.15 (m, 2H), 1.89-1.65 (m, 4H), 1.44-1.19 (m, 8H), 0.72 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.1, 143.1, 136.1, 135.6, 134.0, 133.6, 132.8, 130.6, 130.0, 125.5, 122.9, 121.5, 116.5, 63.3, 50.8, 44.2, 42.9, 37.6, 36.3, 29.5, 27.8, 26.5, 22.9, 11.8; HRMS (ESI^+) calcd. for $C_{24}H_{28}N_2O_5SBr$ $(M+H)^+$ 535.0902; found 535.0909.

4-Nitro-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (21). Purification by flash chromatography (ethyl acetate/hexane, 1:4) afforded **21** as a yellow solid (44 mg, 53%). Mp 263-264°C; ^{1}H-NMR (CD_3OD, 300 MHz) δ 8.15 (s, 1H), 8.11 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.73 (overlapping dd, *J* = 8.0 Hz), 7.18 (d, *J* = 8.9 Hz, 1H), 6.74 (d, *J* = 8.9 Hz, 1H), 3.20-3.08 (m, 2H), 2.73-2.51 (m, 1H), 2.24-2.12 (m, 1H), 2.08-1.94 (m, 1H), 1.85-1.50 (m, 4H), 1.41-1.02 (m, 8H), 0.69 (s, 3H); ^{13}C-NMR (CD_3OD, 75 MHz) δ 147.9, 144.9, 142.1, 133.4, 132.4 (q, *J* = 33.5 Hz), 131.7, 131.4, 129.9 (q, *J* = 3.2 Hz), 129.7, 129.0, 126.7, 124.9 (q, *J* = 271.6 Hz), 124.84 (q, *J* = 3.2 Hz), 115.4; 64.8, 52.0, 44.9, 44.1, 39.5, 37.7, 29.1, 27.3, 25.2, 23.9, 12.4 ^{19}F-NMR ($CDCl_3$, 282 MHz), δ -63.1; HRMS (ESI^+) calcd for $C_{25}H_{28}N_2O_5F_3S$ $(M+H)^+$ 525.1671; found 525.1667.

4-Nitro-17β-(4'-*t*-butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (22). Purification by flash chromatography (ethyl acetate/hexane, 2.5:7.5) afforded **22** as a yellow solid (32 mg, 40%). Mp 228-229 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.40 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 1H), 4.35 (d, *J* = 9.3 Hz, 1H), 3.22-3.06 (m, 2H), 2.95-2.87 (m, 1H), 2.20-2.11 (m, 2H), 1.90-1.77 (m, 3H), 1.67-1.59 (m, 1H), 1.43-1.12 (m, 17 H), 0.71 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 156.4, 152.2, 138.0, 136.1, 134.1, 133.7, 132.9, 126.9, 125.9, 116.5, 63.1, 50.9, 44.3, 42.8, 37.6, 36.2 , 35.1, 31.1, 29.5, 27.8, 26.5, 22.9, 11.8; HRMS (ESI^+) calcd. for $C_{28}H_{37}N_2O_5S$ $(M+H)^+$ 513.2423; found 513.2432.

4-Nitro-17β-4'-biphenylsulfonamide-1,3,5(10)-estratrien-3-ol (23). Purification by flash chromatography (methanol/chloroform, 1:8) afforded **23** as a yellow solid (23 mg, 27%). Mp 270-271 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.40 (s, 1H), 7.93 (d, *J* = 8.2 Hz, 2H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 6.8 Hz, 2H), 7.49-7.38 (m, 4H), 6.80 (d, *J* = 8.6 Hz, 1H), 4.51 (d, *J* =

8.9 Hz, 1H), 3.47-3.17 (m, *2*H), 3.12-3.06 (m, 1H), 2.22-2.15 (m, 3H), 1.91-1.80 (m, 3H), 1.66-1.60 (m, 2H), 1.44-1.15 (m, 8H), 0.73 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.1, 145.4, 139.7, 139.2, 136.1, 134.0, 133.7, 132.7, 129.0, 128.5, 127.6, 127.3, 116.5, 63.3, 51.1, 44.3, 42.8, 37.6, 36.3, 29.5, 27.8, 26.5, 23.0, 11.8; HRMS (ESI^+) calcd. for $C_{30}H_{33}N_2O_5S$ $(M+H)^+$ 533.2110; found 533.2098.

2-Nitro-17β-(3'-bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (24). Purification by flash chromatography (ethyl acetate/hexane, 3:7) afforded **24** as a yellow solid (56 mg, 66%). Mp 242-243 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H), 8.03 (s, 1H), 7.91 (s, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.38 (dd, *J* = 7.9 and 7.9 Hz, 1H), 6.80 (s, 1H), 4.68 (d, *J* = 9.3 Hz, 1H), 3.19 (q, *J* = 8.7 Hz, 1H), 2.86-2.80 (m, 2H), 2.27-2.23 (m, 1H), 2.11 (m, 1H), 1.91-1.65 (m, 4H), 1.46-1.14 (m, 7H), 0.70 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.8, 148.9, 143.0, 135.6, 133.3 (C-5), 131.7, 130.6, 130.0, 125.5, 122.9, 121.5, 118.8, 63.3, 51.0, 43.2, 42.8, 38.2, 35.9, 29.5, 26.5, 25.8, 23.1, 11.8; HRMS (ESI^+) calcd. for $C_{24}H_{28}N_2O_5SBr$ $(M+H)^+$ 535.0902; found 535.0902.

2-Nitro-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (25). Purification by flash chromatography (methanol/chloroform, 0.5:9.5) afforded **25** as a yellow solid (55 mg, 67%). Mp 278-279 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H), 8.15 (s, 1H), 8.07 (d, *J* = 8.07 Hz, 1H), 7.91 (s, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.66 (dd, *J* = 7.4 and 7.9 Hz, 1H), 6.80 (s, 1H), 4.59 (d, *J* = 9.6 Hz, 1H), 3.22 (q, *J* = 8.5 Hz, 1H), 2.89 (m, 2H), 2.26-2.22 (m, 1H), 2.11 (m, 1H), 1.91-1.85 (m, 2H), 1.73-1.68 (m, 2H), 1.49-1.10 (m, 7H), 0.70 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.8, 148.9, 142.5, 133.3, 131.7 (q, *J* = 33.3 Hz), 131.7, 130.2, 129.8, 129.1 (q, *J* = 3.6 Hz, Ar-CH), 124.1 (q, *J* = 3.8 Hz, Ar-CH), 123.2 (q, *J* = 271.3 Hz, C-$\underline{C}F_3$),

121.5, 118.8, 63.4, 51.0, 43.2, 42.8, 38.2, 35.9, 29.5, 26.5, 25.8, 23.0, 11.8; ^{19}F-NMR ($CDCl_3$, 282 MHz), δ -63.1; HRMS (ESI^+) calcd. for $C_{25}H_{28}N_2O_5F_3S$ $(M+H)^+$ 525.1671; found 525.1656.

2-Nitro-17β-(4'-*t*-butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (26). Purification by flash chromatography (ethyl acetate/hexane, 1:4) afforded **26** as a yellow solid (21 mg, 26%). Mp 212-213 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 10.37 (app. d, 1H), 7.91 (s, 1H), 7.80-7.77 (2 overlapping d, *J* = 8.4 Hz, 2H), 7.50-7.47 (2 overlapping d, *J* = 8.4 Hz, 2H), 6.80 (s, 1H), 4.57 (d, J = 9.2 Hz, 1H), 3.15 (apparent t, *J* = 8.6 Hz, 1H), 2.85 (m, 2H), 2.24-2.20 (m, 1H), 2.09-2.01 (m, 1H), 1.84-1.75 (m, 3H), 1.65-1.62 (m, 1H), 1.49-1.14 (m, 17 H), 0.69 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 156.4,152.8, 148.9, 137.9, 133.5, 131.7, 126.9, 125.9, 121.5, 118.8, 63.1, 51.1, 43.2, 42.8, 38.2, 35.9, 35.1, 31.1, 29.5, 26.5, 25.8, 23.1, 11.7; HRMS (ESI^+) calcd. for $C_{28}H_{37}N_2O_5S$ $(M+H)^+$ 513.2423; found 513.2424.

2-Nitro-17β-4'-biphenylsulfonamide-1,3,5(10)-estratrien-3-ol (27). Purification by flash chromatography (ethyl acetate/hexane, 1:9) afforded **27** as a yellow solid (36 mg, 43%). Mp 265-266 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 10.40 (s, 1H), 7.96-7.91 (m, 3H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 7.7 Hz, 2H), 7.49-7.38 (m, 3H), 6.80 (s, 1H), 4.67 (d, *J* = 9.2 Hz, 1H), 3.21 (q, *J* = 8.7 Hz, 1H), 2.90-2.77 (m, 2H), 2.26-2.21 (m, 2H), 1.90-1.63 (m, 4H), 1.40-1.12 (m, 8H), 0.71 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.8, 149.2, 145.5, 139.7, 139.2, 133.8 (C-5), 131.7, 129.1, 128.5, 127.6, 127.3, 121.5, 118.9, 63.3, 51.1, 43.3, 42.8, 38.1, 36.0, 29.5, 26.5, 25.8, 23.1, 11.8; HRMS (ESI^+) calcd. for $C_{30}H_{33}N_2O_5S$ $(M+H)^+$ 533.2110; found 533.2103.

4-Bromo-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (28). Purification by flash chromatography (ethyl acetate/hexane, 1:9) afforded **28** as a white solid (15 mg, 48%). Mp 215-217 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.15 (s, 1H), 8.07 (d, *J* = 7.9 Hz, 1H),

7.82 (d, *J* = 7.9 Hz, 1H), 7.65 (dd, *J* = 7.8 and 7.8 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 5.49 (brs, 1H), 4.72 (d, *J* = 9.4 Hz, 1H), 3.20 (q , *J* = 8.6 Hz, 1H), 2.91-2.83 (m, 1H), 2.69-2.60 (m, 1H), 2.24-2.15 (m, 2H), 1.92-1.83 (m, 2H), 1.72-1.62 (m, 3H), 1.37-1.08 (m, 8H), 0.69 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 150.1, 142.5, 136.2, 133.9, 131.5 (q, *J* = 33.2 Hz) 130.2, 129.8, 129.1 (q, *J* = 3.4 Hz), 125.5, 124.1 (q, *J* = 3.7 Hz), 122.1 (q, *J* = 306.7 Hz), 113.6, 112.7, 63.5, 50.9, 43.8, 42.9, 38.0, 36.3, 30.9, 29.5, 27.2, 26.2, 11.8; ^{19}F-NMR ($CDCl_3$, 282 MHz), δ -63.1; HRMS (ESI^-) calcd. for $C_{25}H_{26}NO_3F_3SBr$ $(M-H)^-$ 556.0769; found 556.0756.

2-Bromo-17β-(3'-trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (29). Purification by flash chromatography (ethyl acetate/hexane, 0.5:9.5) afforded **29** as a white solid (10 mg, 33%). Mp 233-234 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.14 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.65 (dd, *J* = 7.7 and 8.0 Hz, 1H), 7.27 (s, 1H), 6.70 (s, 1H, ArH), 5.23 (brs, 1H), 4.45 (d, *J* = 9.2 Hz, 1H), 3.20 (app. t , *J* = 9.0 Hz, 1H), 2.74 (m, 2H), 2.21-2.12 (m, 2H), 1.90-1.79 (m, 2H), 1.70-1.62 (m, 2H), 1.40-1.12 (m, 8H), 0.69 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 149.9, 142.5, 138.0, 134.1, 131.8 (q, *J* = 33.9 Hz), 130.3, 129.9, 129.2 (q, *J* = 3.7 Hz), 128.7, 124.2 (q, *J* = 3.7 Hz), 115.8, 107.4, 63.5, 51.0, 43.5, 42.9, 38.5, 36.2 , 29.6, 29.0, 26.9, 26.0, 23.1, 11.9; ^{19}F-NMR ($CDCl_3$, 282 MHz), δ -63.0; HRMS (ESI^+) calcd. for $C_{25}H_{28}NO_3F_3SBr$ $(M+H)^+$ 558.0925; found 558.0927.

4-Fluoroestrone (31). A stirred mixture of **30**[27] (0.652 g, 2.00 mmol) and *N*-fluoropyridinium triflate (NFPT, 0.980 g, 4.00 mmol) in dry 1,1,2-trichloroethane (TCE, 24 mL) in a glass pressure tube (CAUTION!: This reaction should be performed in a fume hood behind a blast shield) under argon atmosphere was heated at 135 °C for 15 h. The TCE was then removed *in vacuo*, water (100 mL) was added and reaction mixture was extracted with dichloromethane (4

× 50 mL). The combined organics were washed with water, brine, dried (Na_2SO_4), then concentrated. The residue was subjected to flash chromatography (ethyl acetate/hexane, 1:5) which afforded 2-*t*-butyl-4-fluoroestra-1,3,5(10)-triene-17-one as a pale brown solid (147 mg) along with a small amount of an unidentified impurity which we could not remove. This material was used without further purification for the next step. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.96 (s, 1H), 5.29 (d, *J* = 7.5 Hz, 1H), 2.97 (m, 1H), 2.91-2.89 (m, 1H), 2.49-2.46 (m, 2H), 2.21-1.95 (m, 5H), 1.62-1.34 (m, 16H), 0.90 (s, 3H); ^{19}F-NMR ($CDCl_3$, 282 MHz) δ -147; HRMS (ESI^+) calcd. for $C_{22}H_{30}O_2F$ $(M+H)^+$ 345.22243; found 345.22235. To a cooled (ice bath) mixture of a stirred solution of 2-*tert*-butyl-4-fluoroestra-1,3,5(10)-triene-17-one (0.147g, 0.427 mmol) in dichloromethane (7 mL) was added nitromethane (3 mL) followed by the addition of $AlCl_3$ (0.455 g, 3.41 mmol) and the mixture stirred at 0 ^{o}C for 5 h. The reaction was quenched by the addition of ice then an aq. solution of HCl (1 M, 10 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organics were washed with water, brine then dried (Na_2SO_4) and concentrated under vacuum. Purification by flash chromatography (ethyl acetate/hexane, 1:5) afforded **31** an amorphous white solid (92.4 mg, 16% over two steps). Mp: 221-222 ^{o}C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.93 (d, *J* = 8.6 Hz, 1H), 6.79 (2 overlapping d, *J* = 8.8 Hz, 1H), 5.32 (s, 1H), 2.98 (dd, *J* = 6.0, 17.8 Hz, 1H), 2.77-2.63 (m, 1H), 2.5 (dd, *J* = 8.5, 19.1 Hz, 1H), 2.36-2.33 (m, 1H), 2.22-1.91 (m, 5H), 1.61-1.24 (m, 7H), 0.90 (s, 3H); ^{19}F-NMR ($CDCl_3$, 282 MHz) δ -145.5; ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 149.1 (d, *J* = 235 Hz), 141.1 (d, *J* = 14.6 Hz), 132.9, 124.3 (d, *J* = 14.1 Hz), 120.8 (d, J = 3.2 Hz), 114.0, 104.9, 50.23, 48.0, 43.8, 37.7, 35.9, 31.4, 25.9, 25.5, 22.2, 21.6, 13.8; HRMS (ESI^+) calcd. for $C_{18}H_{22}O_2F$ $(M+H)^+$ 289.1598; found 289.1597.

3-*O-tert*-Butyldimethylsilyl-4-Fluoro-estra-1,3,5(10)-triene (32). To a solution of compound **31** (92.4 mg, 0.319 mmol) and imidazole (95.6 mg, 1.40 mmol) in dry DMF (4 mL) was added TBDMSCl (106 mg, 0.702 mmol). The mixture was stirred for 2 h then diluted with Et_2O (10 mL) and washed with water (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried (Na_2SO_4) and concentrated. Purification by FC (EtOAc/Hex, 1:10) gave pure **32** as an amorphous white solid (107.6 mg, 84%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.88 (d, *J* = 8.8 Hz, 1H), 6.69 (app. t, *J* = 8.7 Hz, 1H), 2.95 (dd, *J* = 6.1, 17.9 Hz, 1H), 2.74-2.62 (m, 1H), 2.50 (dd, *J* = 9.0, 18.8 Hz, 1H), 2.40-1.92 (m, 6H), 1.69-1.31 (m, 6H), 0.99 (s, 9H), 0.90 (s, 3 H), 0.17 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 151.9 (d, *J* = 241.5 Hz), 140.6 (d, *J* = 13.1 Hz), 133.9 (d, *J* = 3.0 Hz), 124.8 (d, *J* = 15.1 Hz), 120.2, 118.9, 50.3, 47.9, 43.9, 37.7, 35.8, 31.5, 25.6, 21.6, 18.3, 13.9, 13.8, 4.62; ^{19}F-NMR ($CDCl_3$, 282 MHz) δ -136.6. HRMS (ESI^+) calcd. for $C_{24}H_{36}O_2FSi$ 403.2463 $(M+H)^+$; found 403.2463

3-*O-tert*-Butyldimethylsilyl-4-Fluoro-17β-benzylamino-estra-1,3,5(10)-triene (33). To a stirred solution of **32** (107 mg, 0.266 mmol) in dry 1,2-dichloroethane (DCE) (3 mL) was added benzylamine (114 mg, 1.06 mmol), glacial acetic acid (0.045 mL, 0.798 mmol), and sodium triacetoxyborohydride (STAB, 112 mg, 0.532 mmol). Stirring was continued for 30 h and then sat. $NaHCO_3$ (10 mL) was added. The mixture was stirred for 30 minutes then extracted with EtOAc (2 x 10 mL). The combined organics were washed with sat. $NaHCO_3$ (2 x 10 mL), water (2 x 10 mL) and brine (1 x 10 mL), then dried (Na_2SO_4) and concentrated. Purification by flash chromatography (methanol/CH_2Cl_2/ammonium hydroxide, 97.3:2.5:0.2) afforded **33** as an amorphous white solid (107.2 mg, 82%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.33-7.20 (m, 5H), 6.91 (d, *J* = 8.6 Hz, 1H), 6.70 (overlapping dd, *J* = 8.6 Hz, 1H), 3.86 (s, 2H), 2.92 (dd, *J* = 6.1, 17.9 Hz, 1H), 2.68-2.60 (m, 2H), 2.35-1.84 (m, 6H), 1.74-1.68 (m, 1H), 1.62-1.20

(m, 9H), 1.02 (s, 9H), 0.80 (s, 3H), 0.20 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.9 (d, *J* = 241.3 Hz), 141.1, 140.5 (d, *J* = 13.8 Hz), 134.8, (d, *J* = 2.2 Hz), 128.3, 128.0, 126.8, 125.1 (d, *J* = 15.2 Hz), 120.1 (d, *J* = 3.2 Hz), 118.7 (d, *J* = 2.2 Hz), 68.4, 52.8, 52.2, 44.0, 43.1, 38.2, 38.1, 29.7, 25.7, 22.4, 18.3, 12.90, -4.63, -4.66; ^{19}F-NMR ($CDCl_3$, 282 MHz) δ -136.9; HRMS (ESI^+) calcd for $C_{31}H_{45}NOFSi$ 494.3249 $(M+H)^+$; found 494.3249

3-*O-tert*-Butyldimethylsilyl-4-Fluoro-17β-amino-estra-1,3,5(10)-triene (34). A flask containing **33** (107 mg, 0.219 mmol) and $Pd(OH)_2$ (15 mg) in MeOH/EtOAc (10 mL, 5:1) was fitted with a balloon filled with H_2 and stirred for 24 h. The mixture was filtered through Celite and concentrated to give pure **34** as an amorphous white solid (85 mg, 97%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.86 (d, J = 8.5 Hz, 1H), 6.66 (overlapping dd, J = 8.5 Hz, 1H), 2.88 (m, 1H), 2.80-2.57 (m, 2H), 2.31-1.10 (m, 16 H), 0.97 (s, 9H), 0/66 (s, 3H), 0.16 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 152.1 (d, *J* = 241.1 Hz), 140.4 (*J* = 13.1 Hz), 134.7, 125.1 (d, *J* = 15.2 Hz), 120.1 (d, *J* = 3.2 Hz), 118.6 (d, *J* = 1.1 Hz), 62.9, 52.0, 44.0, 38.4, 36.7, 31.3, 26.6, 26.3, 25.6, 23.4, 22.47, 22.42, 18.3, 11.1, -4.7; ^{19}F-NMR ($CDCl_3$, 282 MHz) δ -136.9; HRMS (ESI^+) calcd for C24H39NOFSi 404.2780 $(M+H)^+$; found 404.2780

3-*O-tert*-Butyldimethylsilyl-4-Fluoro-17β-(3'-trifluoromethylbenzene)sulfonamide-estra-1,3,5(10)-trien-3-ol (35). To a solution of **34** (85 mg, 0.213 mmol) in dry CH_2Cl_2 (3 mL) was added DMAP (52 mg, 0.426 mmol), Et_3N (0.059 mL, 0.426 mmol) and 3-trifluoromethylbenzenesulfonylchloride (0.070 mL, 0.426 mmol). The mixture was stirred for 48 h. EtOAc (20 mL) was added and the mixture was washed with sat. $NaHCO_3$ (2 x 10 mL), H_2O (1 x 10 mL) and sat. brine (1 x 10 mL) then dried (Na_2SO_4) and concentrated. Flash chromatography of the crude material (EtOAc/hexane, 1:10) afforded **35** as an amorphous white solid (102.2 mg, 79%). TLC of the chromatographed material revealed that it contained a small

amount of impurity which we were unable to remove. It was used without further purification for the next step. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.2 (s, 1H), 8.1 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.67 (overlapping dd, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 8.6 Hz, 1H), 6.67 (overlapping dd, *J* = 8.0 Hz, 1H), 4.98 (d, *J* = 9.3 Hz, 1H), 3.21 (app q, *J* = 8.8 Hz, 1H), 2.87 (dd, *J* = 5.4, 18.1 Hz, 1H), 2.70-2.53 (m, 1H), 2.28-2.06 (m, 2H), 1.93-1.78 (m, 2H), 1.77-1.60 (m, 2H), 1.41-1.10 (m, 7H), 0.98 (s, 9H), 0.72 (s, 3H), 0.16 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.1 (d, *J* = 242 Hz), 142.6, 140.6 (d, *J* = 13.0 Hz), 134.2 (d, *J* = 3.2 Hz), 131.7 (q, *J* = 33.5 Hz), 130.2, 129.8, 129.1 (d, *J* = 3.2 Hz), 124.9 (d, *J* = 15.1 Hz), 124.1 (q, *J* = 3.2 Hz), 123.3 (q, *J* = 272.7 Hz), 120.1 (d, J = 3.2 Hz), 118.8 (d, *J* = 1.6 Hz), 63.5, 51.0, 43.7, 42.9, 38.2, 36.3, 29.3, 26.3, 25.9, 25.6, 23.1, 22.4, 22.4, 22.3, 18.3, 11.8, -4.7; ^{19}F-NMR ($CDCl_3$, 282 MHz) δ -63.1, -136.8.

4-Fluoro-17β-(3'-trifluoromethylbenzene)sulfonamide-estra-1,3,5(10)-trien-3-ol (36). To a solution of **35** (82 mg, 0.135 mmol) in THF (3 mL) was added tetrabutylammonium fluoride (TBAF, 1M in THF, 0.203 mL, 0.203 mmol). The mixture was stirred for 5 h. EtOAc (10 mL) was added and the mixture was washed with 0.1 N HCl (1 x 10 mL), water (1 x 10 mL) and sat. brine (1 x 10 mL) then dried (Na_2SO_4) and concentrated. Purification by flash chromatography (EtOAc/hexane, 1:5) afforded **36** as an amorphous white solid (55.4 mg, 83%). ^{1}H-NMR (CD_3OD, 300 MHz) δ 8.14 (s, 1H), 8.10 (d, *J* = 7.4 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.74 (2 overlapping dd, *J* = 7.7 Hz, 1H, ArH), 6.79 (d, *J* = 8.2 Hz, 1H), 6.62 (overlapping dd, *J* = 8.5 Hz, 1H), 3.11 (overlapping dd, *J* = 7.9 Hz, 1H), 2.81 dd, *J* = 5.3, 18.1 Hz, 1H), 2.56-2.49 (m, 1H), 2.18-2.12 (m, 1H), 2.10-1.93 (m, 1H), 1.84-1.75 (m, 1H), 1.72-1.51 (m, 3H), 1.40-0.98 (m, 8H), 0.67 (s, 3H); ^{13}C-NMR (CD_3OD, 75 MHz) δ 150.7 (d, *J* = 240.0 Hz), 144.9, 143.2 (d, J = 13.0 Hz), 133.7 (*J* = 2.2 Hz), 132.5 (q, *J* = 32.5 Hz), 131.4, 129.9 (q, *J* = 3.2 Hz), 125.5 (d, *J* = 14.1 Hz), 124.9 (q, *J* = 3.2 Hz), 124.5 (q, *J* = 271.6 Hz), 121.6 (d, *J* = 4.3 Hz), 115.5 (d, *J* = 3.2

Hz), 64.9, 52.1, 44.9, 44.1, 39.8, 37.8, 29.1, 27.5, 27.3, 24.0, 23.33, 23.26, 12.4;^{19}F-NMR ($CDCl_3$, 282 MHz) δ -144.8, -64.5; HRMS (ESI^+) calcd for $C_{25}H_{28}NO_3F_4S$ $(M+H)^+$ 498.1726; found 498.1723.

Determination of IC_{50}'s. 20 μL of inhibitor stock solution in DMSO/0.1 M Tris-HCl, pH 7.0 (1:1), were added to the wells of a 96-well microtiter plate containing 140 μL of 0.1 M Tris, pH 7.0. After that, 20 μL of a 2 mM MUS stock solution in 0.1 M Tris–HCl, pH 7.0, was added. The assay was initiated by adding 20 μL STS (100 nM stock solution in 20 mM Tris–HCl, pH 7.4, 0.1% Triton X-100). The final concentration of inhibitor ranged from 5 nM to 5 μM. The final concentration of 4-MUS was 200 μM, and 10 nM for STS. The reactions was followed were followed by detection of fluorescent product, 4-methylumbelliferone (excitation 360 nm, emission, 460 nm), over 10 min at 24°C. Each reaction was performed in quadruplicate. Additional controls were performed in an identical manner but did not contain STS. Eleven concentrations of inhibitor bracketing the IC_{50} value were used for each compound. The initial rates of enzyme activity in relative fluorescence units per second (RFU/s) were used to determine the IC_{50}. The ratio of the initial rate in the presence of inhibitor (V_i) to that in the absence of inhibitor (V_o) was calculated and plotted as a semi-log curve in Grafit (Erithacus Software, Surrey, U.K.), from which the IC_{50} value was calculated based on the following equation: $V_i = V_o/[1 + ([I]/IC_{50})S] + B$, where: V_i is the initial rate of reaction at an inhibitor concentration of [I]; V_o is the velocity in the absence of inhibitor; B is background and s is the slope factor.

Determination of K_i^{app}'s for compounds 23 and 36. The IC_{50}'s for these compounds were determined as described above at four of different enzyme concentrations (10, 20, 40, and 80 nM). For tight binding inhibitors, $IC_{50} = K_i^{app} + ½[E]_t$.[29] Plots of IC_{50} as a function of $[E]_T$

were constructed (see Figure 4 and Figure S2 in the supplementary material). The y-intercept of these plots provided the K_i^{app}.

Molecular Modeling Experiments. Docking experiments were performed using Discovery Studio (DS) Client v2.5.0.9164 (2005-09), BIOVIA/Accelrys Software Inc. running on a HP xw4600 workstation. The coordinates for the X-ray crystal structure of human steroid sulfatase enzyme was obtained from RCSB Protein Data Bank (PDB file: 1P49). The ligand molecules were constructed using the Build Fragment tool and energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The steroid sulfatase enzyme was prepared for docking experiments first by deleting water molecules and then by using the prepare protein tool in DS. The formylglycine (FGly75), present in the catalytic site was modified to the *gem*-diol using the build fragment tools. Subsequently, the enzyme was energy minimized by steepest descent method for 10,000 steps reaching a convergence of 0.1 kcal/mol Å and further by conjugate gradient method for 10,000 steps reaching a convergence of 0.01 kcal/mol Å. The binding site of the enzyme was defined by generating a 10 Å radius sphere, after selecting using the amino acid Thr99. The STS inhibitors were docked in the active site of steroid sulfatase enzyme using the LibDock command under the receptor-ligand protocol in DS using CHARMm force field. The quality of ligand-enzyme complex obtained was evaluated using LibDock scoring function (kcal/mol) and by considering various intermolecular polar and nonpolar interactions between the ligand and the enzyme.

Associated Content

Supporting Information. Experimental procedures for the preparation of compounds **8-29** and **31-36**. ^{1}H-NMR spectra for compounds **8-11**. ^{1}H-, ^{13}C- and ^{19}F- (when applicable) NMR spectra

for compounds **12-29** and **31-36**. X-ray crystallographic data for compound **25** and 4-nitroestrone. Plot of IC_{50} as a function of $[E]_T$ for compound **36.** This material is available free of charge via the Internet at http://pubs.acs.org.

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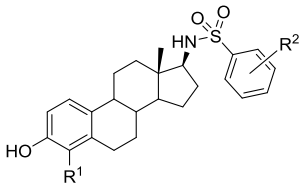
Graphical Abstract



$R^1 = NO_2$ or F; R^2 = 3'-Br, 3'-CF_3, 4'-t-Bu, 4'-Ph

Potent steroid sulfatase inhibition: IC_{50}s = 1-12 nM