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17β-Arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol as highly potent inhibitors of steroid sulfatase

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ABSTRACT

Steroid sulfatase (STS) catalyzes the desulfation of biologically inactive sulfated steroids to yield biologically active desulfated steroids and is currently being examined as a target for therapeutic intervention for the treatment of breast and other steroid-dependent cancers. Here we report the synthesis of a series of 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol and their evaluation as inhibitors of STS. Some of these compounds are among the most potent reversible STS inhibitors reported to date. Introducing *n*-alkyl groups into the 4′-position of the 17β-benzenesulfonamide derivative resulted in an increase in potency with the *n*-butyl derivative exhibiting the best potency with an IC_{50} of 26 nM. A further increase in carbon units (to *n*-pentyl) resulted in a decrease in potency. Branching of the 4′-*n*-propyl group resulted in a decrease in potency while branching of the 4′-*n*-butyl group (to a *tert*-butyl group) resulted in a slight increase in potency (IC_{50} = 18 nM). Studies with 3′- and 4′-substituted substituted 17β-benzenesulfonamides with small electron donating and electron withdrawing groups revealed the 3′-bromo and 3′-trifluoromethyl derivatives to be excellent inhibitors with IC_{50}'s of 30 and 23 nM, respectively. The 17β-2′-naphthalenesulfonamide was also an excellent inhibitor (IC_{50} = 20 nM) while the 17β-4′-phenylbenzenesulfonamide derivative was the most potent inhibitor of all the compounds studied with an IC_{50} of 9 nM.

1. Introduction

The majority of breast cancers require steroids in their early stages of growth. Hence, compounds capable of interfering with the action of steroids on breast cancer cells or inhibiting the enzymes involved in steroid biosynthesis have been pursued as therapeutics for treating these diseases for several decades now.[1] Aromatase inhibitors are examples of the latter class of compounds and have been used successfully in clinic for treating breast cancer.[1,2] Other enzymes that are being targeted are 17β-hydroxysteroid dehydrogenase (17β-HSD) and steroid sulfatase (STS). STS catalyzes the desulfation of biologically inactive suflated steroids such as estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHEAS) into their desulfated counterparts such as estrone (E1) and dehydroepiandrosterone (DHEA) (Fig. 1). 17β-HSD converts these into estradiol or androstenediol which bind to the estrogen receptor in the nuclear membrane.

Circulating plasma concentrations of E1S and DHEAS are significantly higher than E1 and DHEA.[3] In addition, the half-life of E1S and DHEAS in plasma is about 10–12 h, which is considerably longer than the 30–40 min half-life of E1 and DHEA.[4] For these reasons, the role of sulfated steroids is seen as a storage reservoir that acts as a source of biologically active steroid hormones when activated by STS.

STS expression is detected in 90% of breast tumors,[5,6] which is considerably greater than aromatase expression which is only found in 60–70% of breast tumors.[7,8] Moreover, STS mRNA expression is elevated in breast tumors compared to normal tissue[9] and STS activity in breast tumors is significantly higher than aromatase activity.[10] Clinical studies have now shown that elevated STS mRNA expression may be a predictor of recurrence in breast cancer patients with estrogen receptor positive (ER+) tumors.[11] STS activity is approximately 50 times greater in breast tumors compared with normal breast tissue.[12] Taken together, these findings suggest that STS plays an important role in breast cancer and that STS inhibitors may be effective breast cancer therapeutics.

A vast array of STS inhibitors have been reported.[13] The majority are aryl sulfamates which act as irreversible, suicide inhibitors. 667-Coumate (**1**, Fig. 2) is an example of such a compound (IC_{50} = 350 nM in intact MCF-7 cells[13b]) and has been examined in Phase I clinical trials for treating breast cancer.[14] Reversible STS inhibitors have also been developed.[13] Among these are the 17α-benzylestradiol derivatives of type **2** which are potent inhibitors.[13] It has been proposed that the high affinity of these compounds for STS is a result of the benzyl moiety extending into a hydrophobic channel between two alpha helices located just outside the active site.[13a] As part of our efforts on the development of STS inhibitors,

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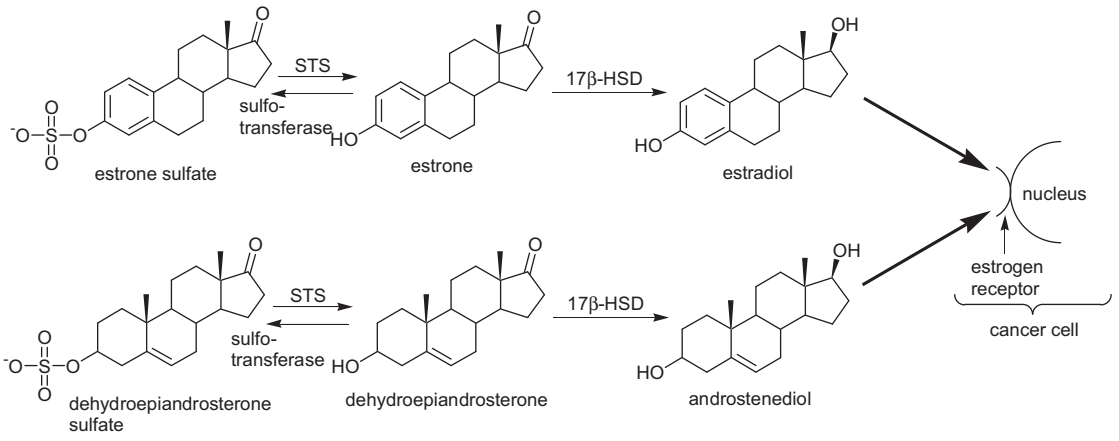


Figure 1. Examples of reactions catalyzed by STS and 17β-HSD.

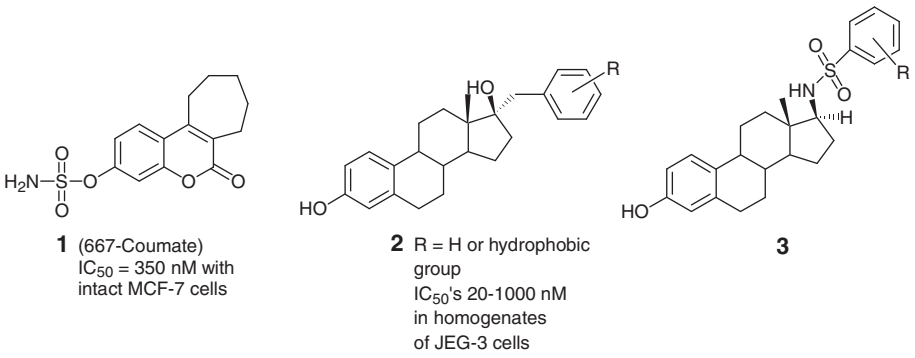


Figure 2. Irreversible and reversible inhibitors of STS.

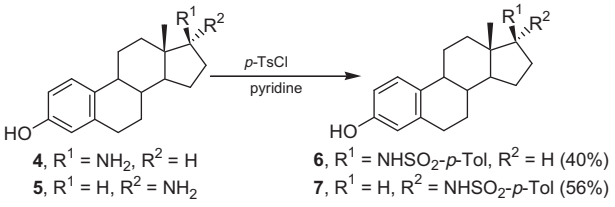
we wished to determine whether other estrogen derivatives bearing other groups at the 17-position could also act as potent STS inhibitors. Here we report that 17β-sulfonamides (**3**, Fig. 2) of 17β-aminoestra-1,3,5(10)-trien-3-ol can be highly potent inhibitors of steroid sulfatase and some of these compounds are among the most potent reversible STS inhibitors reported to date.

2. Results and discussion

2.1. Inhibition studies

We focused upon examining 17-sulfonamides of 17-aminoestra-1,3,5(10)-trien-3-ol as potential STS inhibitors for several reasons. First, we anticipated that their synthesis could be readily accomplished by reacting 17-aminoestra-1,3,5(10)-trien-3-ol with sulfonyl chlorides. Second, dozens of sulfonyl chlorides are commercially available and so a large number of potential inhibitors could be readily prepared. Third, the sulfonamide group is chemically stable and usually exhibits good metabolic stability. It is one of the most important pharmacophores in medicinal chemistry, and numerous bioactive agents bear this functionality.[15]

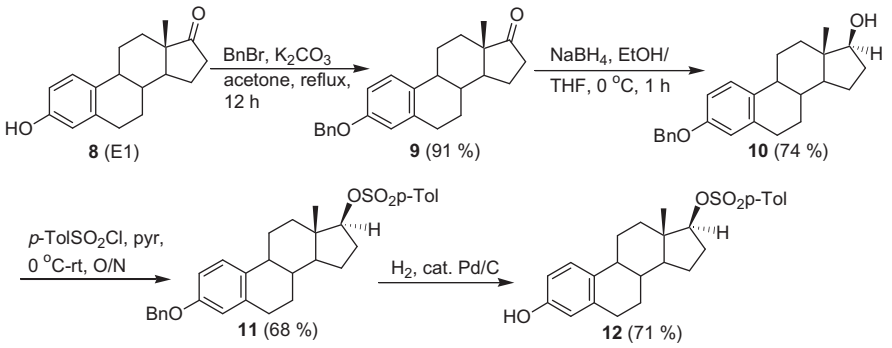
17β- and 17α-*p*-toluenesulfonamides **6** and **7** (Scheme 1) were used as model compounds to determine if this approach had any promise of providing potent STS inhibitors and if the stereochemistry at the 17-position would have an influence on inhibitor potency. 17β-Aminoestra-1,3,5(10)-trien-3-ol (**4**) was synthesized using an efficient two-step sequence we recently reported starting from estrone,[16] and 17α-aminoestra-1,3,5(10)-trien-3-ol (**5**) was easily prepared via a four-step sequence starting from estradiol.[17] Compounds **6** and **7** were prepared by slowly adding a solution of *p*-toluenesulfonyl chloride to a solution of **4** or **5** in pyridine (not optimized).



Scheme 1. Synthesis of sulfonamide **6** and **7**.

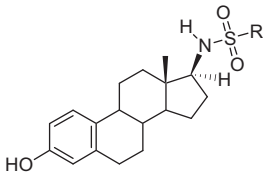
The IC_{50}'s of **6** and **7** and all subsequent IC_{50}'s reported within were obtained using purified STS in Tris buffer at pH 7.0 containing 0.01% Triton X-100, 5% DMSO and using 4-methylumbelliferyl sulfate (4-MUS) as the substrate.[18,19] The β-isomer **6** proved to be a good STS inhibitor with an IC_{50} of 207 nM while the α-isomer **7** was less potent with an IC_{50} of 341 nM. The finding that the β-isomer was the better inhibitor was somewhat surprising as this is the opposite stereochemistry to that of the benzyl groups in the 17α-benzylestradiol inhibitors of type **2**. We also prepared the 17β-sulfonate **12** (Scheme 2). Compound **12** also proved to be a good inhibitor of STS (IC_{50} = 293 nM) but was not as potent as β-sulfonamide analog **6**.

On the basis of the above studies, subsequent efforts focused on preparing a collection of 17β-arylsulfonamides of **6**. These compounds were readily prepared using the same approach as outlined in Scheme 1 for the synthesis of **6**. Our initial efforts focused on phenylsulfonamides substituted at the 4′-position with hydrophobic groups (Table 1) as it has been previously demonstrated that 17α-benzylestradiol derivatives of type **2** bearing certain hydrophobic groups at the 4′-position of the benzyl moiety are potent



Scheme 2. Synthesis of sulfonate **12**.

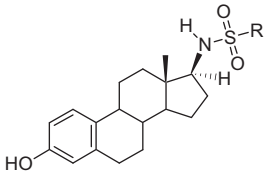
Table 1
Inhibition of steroid sulfatase with 17β-4′-alkylbenzenesulfonamides and arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol



Compound	R	IC_{50}[a] (nM)
13	Benzene	343
14	Benzyl	364
15	4′-Methylbenzyl	261
6	4′-Methylbenzene	207
16	4′-*n*-Propylbenzene	44
17	4′-*n*-Butylbenzene	26
18	4′-*n*-Pentylbenzene	51
19	4′-*iso*Propylbenzene	62
20	4′-*tert*-Butylbenzene	18
21	4′-Phenylbenzene	9
22	4′-Phenoxybenzene	39
23	2′-Naphthalene	20

[a] Errors in IC_{50}'s are within ±5%.

Table 2
Inhibition of Steroid Sulfatase with 17β-benzene sulfonamides **24**–**37**



Compound	R	IC_{50}[a] (nM)
24	2′-Bromobenzene	493
25	3′-Bromobenzene	25
26	4′-Bromobenzene	190
27	3′-Chlorobenzene	67
28	4′-Chlorobenzene	271
29	3′-Nitrobenzene	90
30	3′-Cyanobenzene	137
31	3′-Fluorobenzene	112
32	4′-Fluorobenzene	479
33	3′-Trifluoromethylbenzene	23
34	4′-Trifluoromethylbenzene	73
35	3′-Methylbenzene	65
36	3′-Methoxybenzene	193
37	3′-Trifluoromethoxybenzene	74

[a] Errors in IC_{50}'s are within ±5%.

STS inhibitors.[20a,b] The parent benzenesulfonamide **13** as well as benzyl derivative **14** and 4′-methylbenzyl sulfonamide **15** were poorer inhibitors than tosyl derivative **6** mentioned previously. However, increasing the length of the *n*-alkyl group at the 4′-positon in the benzenesulfonamide moiety from one (methyl, compound **6**) to four carbons (*n*-butyl derivative **17**) resulted in a significant increase in binding affinity with *n*-butyl derivative **17** being a potent inhibitor with an IC_{50} of 26 nM. However, a decrease in affinity occurred with when a fifth carbon unit was present (*n*-pentyl derivative **18**). Branching of the n-propyl group proved to be slightly detrimental to binding affinity (isopropyl derivative **19**) while the *tert*-butyl derivative **20** proved to be more potent than the *n*-butyl derivative with an IC_{50} of 18 nM. The presence of a phenyl group at the 4′-position (biphenyl derivative **21**) yielded a highly potent inhibitor with an IC_{50} of 9 nM. The 4′-phenoxy derivative **22** proved to be over four-fold less potent than the biphenyl derivative. The 2′-naphthalene derivative **23** was also an excellent inhibitor with an IC_{50} of 20 nM.

Further studies were conducted with a series of compounds bearing small electron withdrawing and electron donating substituents at the 3′- and/or 4′-positions (Table 2). It has been reported that the 17β-3′-bromobenzyl estradiol is a potent inhibitor of STS with an IC_{50} of 24 nM.[20a,c] The 3′-bromo derivative **25** also proved to be a potent STS inhibitor with an IC_{50} of 25 nM while the 2′- and 4′-bromo derivatives **24** and **26** were considerably less potent. Further interrogation of the 3′-, and 4′-position with a variety of electron withdrawing and electron donating groups (Table 2) failed to produce a more potent inhibitor than **25** with the exception of the 3′-trifluoromethyl derivative **33** which had an IC_{50} of 23 nM. The 3′-bromo derivative, **25**, was chosen for more detailed kinetics analysis. This compound exhibited mixed inhibition (Fig. 3) with

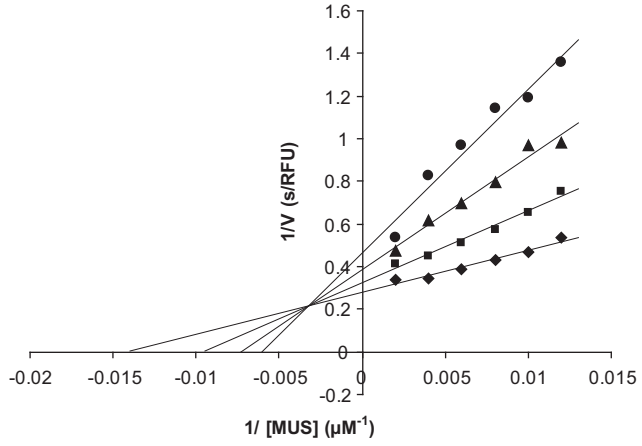
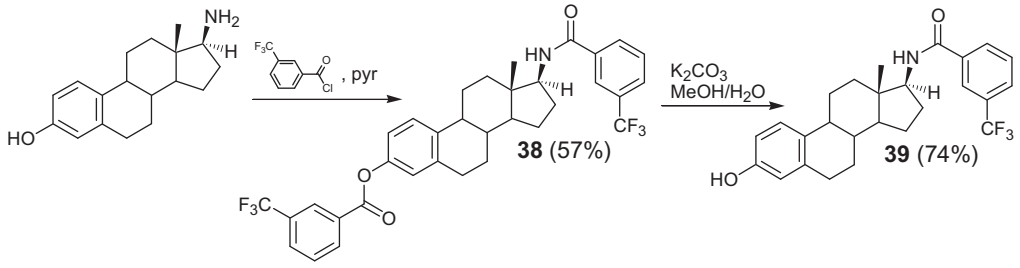


Figure 3. Lineweaver–Burk plot for the inhibition of STS by compound **25**. ♦ (no inhibitor), ■ (25 nM **25**), ▲ (50 nM **25**), ● (75 nM **25**).



Scheme 3. Synthesis of compound **39**.

a K_i of 23 nM and an αK_i of 108 nM. We reported that a compound of type **2** (R = H) also exhibited mixed inhibition.[21]

To determine if a 17β-amide linkage between the aryl moiety and C-17 is as effective as a 17β-sulfonamide linkage we prepared the amide analog of **33** (compound **39**, Scheme 3) and evaluated it as an STS inhibitor. Surprisingly, amide **39** was a much poorer inhibitor with an IC_{50} of 749 nM. The trifluoromethyl group in **33** may be interacting with a site on STS that is not accessible to the trifluoromethyl group in the benzamide. This may be a result of the significant structural differences between amides and sulfonamides.[22]

A comparison of the IC_{50}'s of the compounds described here to the 17α-benzylestradiol inhibitors reported by Poirier and co-workers is of interest as this analysis reveals some similarities as well as some significant differences. Poirier and co-workers have suggested that the enhanced affinity of the 17α-benzylestradiol inhibitors compared to estradiol is a result of the benzyl group projecting into a channel between the two hydrophobic alpha helices that insert STS into the membrane of the endoplasmic reticulum.[13a] It is likely that the aryl sulfonamide moiety of the compounds reported here also projects into this tunnel. The finding that the IC_{50}'s of the benzyl (**14**), 4′-*n*-butyl (**17**) and 4′-*tert*-butyl (**20**) inhibitors are very similar to the analogous 17α-benzylestradiol inhibitors[20a] is probably because the alkyl group in both series of compounds form non-specific hydrophobic interactions with the hydrophobic residues located in the channel. The finding that 3′-bromo derivatives of both series are potent inhibitors with similar IC_{50}'s would further support the suggestion that both series of compounds interact with STS in a similar manner in spite of the fact that the 17β-sulfonamide link between the aryl moieties and carbon-17 in the inhibitors described here is structurally, electronically and spatially (β vs α) very different from the 17α-CH_2 unit in the 17α-benzylestradiol inhibitors and lacks a 17-OH group. However, some differences in IC_{50}'s between the two sets of compounds suggest that this may not always be the case. For examples, the 4′-biphenyl derivative **21** (IC_{50} = 9 nM) and naphthyl derivative **23** (IC_{50} = 20 nM) are 4-fold and 6-fold more potent than 17α-4′-phenylbenzylestradiol (IC_{50} of 35 nM) and the 17α-naphth-2′-ylmethylestradiol (IC_{50} = 120 nM).[20b,c] The 3′-trifluoromethylbenzenesulfonamide derivative **33** (IC_{50} = 23 nM) is almost six-fold more potent than 17β-3′-trifluoromethylbenzylestradiol (IC_{50} = 126 nM).[20b,c] In any case, it should be pointed out that the two classes of compounds were assayed under different conditions (the IC_{50}'s of 17α-benzyl estradiol inhibitors were determined using homogenates of JEG-3 cells in Tris-acetate buffer, 10% glycerol, pH 7.0 and [^{3}H]E1S as substrate[20b] while our work was done with pure STS in Tris buffer, pH 7.0, Triton, and 5% DMSO using 4-MUS as substrate) suggesting that caution should be applied when comparing their IC_{50}'s.

3. Conclusions

We have shown that 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol can be highly potent inhibitors of STS. Some of these compounds, such as the 4′-*tert*-butyl (**20**), 4′-biphenyl (**21**), 2′-naphthalene (**23**), 3′-bromo (**25**), and 3′-trifluoromethyl (**33**) derivatives are among the most potent reversible STS inhibitors reported to date. Studies on the antiproliferative activity of these compounds with various breast cancer cell lines are in progress and will be reported in due course.

4. Experimental

4.1. General

All starting materials and reagents were obtained from Sigma Aldrich Canada Ltd. THF was distilled from sodium-benzophenone. CH_2Cl_2 was distilled from calcium hydride under nitrogen. Silica gel chromatography was performed using silica gel (60 Å, 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 trichlorofluoromethane (δ 0.0 ppm) external standard. Low-resolution (LRMS) and high-resolution (HRMS) electron impact (EI) mass spectra were obtained on a JEOL HX110 double focusing mass spectrometer. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Steroid sulfatase from human placenta was purified as previously described.[19] Fluorimetry was performed using a Spectramax GeminiXS plate reader (Molecular Devices, CA) at 25 °C.

4.2. Syntheses

4.2.1. 3-Benzyloxyestrone (9)

Potassium carbonate (1.38 mmol) was added to a stirred solution of estrone (**E1**, 0.9 mmol) in anhydrous acetone (10 mL), and the resulting suspension was stirred for 1 h. Benzyl bromide (1.1 mmol) was added and the mixture was heated to reflux for 4 h. The mixture was poured into ice/water then extracted with ethyl acetate. The combined extracts were washed with water then dried (Na_2SO_4), filtered, and concentrated. The resulting pale yellow crude solid was recrystallized from ethanol to yield compound **10** as white crystals (91%). Mp 132–133 °C (lit 132–134 °C)[23]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42–7.29 (m, 5H, C_6H_5), 7.18 (d, J = 8.6 Hz, 1H, H-1), 6.77 (d, J = 8.5 Hz, 1H, H-2), 6.72 (br s, 1H, H-4), 5.02 (s, 2H, $C_6H_5CH_2$), 2.88–2.86 (m, 2H), 2.53–2.35 (m, 2H), 2.24–1.88 (m, 4H), 1.63–1.41 (m, 7H), 0.89 (s, 3H, CH_3, H-18).

4.2.2. 3-Benzyloxyestra-1,3,5(10)-trien-17β-ol (10)

To a solution of compound **9** (0.5 mmol) in ethanol/THF (6 mL, 5:1) at 0 °C was added $NaBH_4$ (1 mmol). The resulting mixture was stirred for 1 h at 0 °C then the reaction was quenched with 1 M HCl. After extraction with ethyl acetate, the combined extracts were washed with water and satd brine then dried (Na_2SO_4),

filtered, and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 1:4) gave compound **10** as a white solid (74%). Mp 62–63 °C (lit 61–63 °C)[24]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44–7.31 (m 5H, C_6H_5), 7.21 (d, J = 8.6 Hz, 1H, H-1), 6.78 (d, J = 8.5 Hz, 1H, H-2), 6.73 (br s, 1H, H-4), 5.03 (s, 2H, $C_6H_5CH_2$), 3.72 (t, J = 7.8 Hz, 1H, H-17), 2.85–2.83 (m, 2H), 2.33–2.28 (m, 1H), 2.23–2.08 (m, 2H), 1.97–1.86 (m, 2H), 1.71–1.68 (m, 1H), 1.51–1.14 (m, 7H), 0.78 (s, 3H, CH_3, H-18).

4.2.3. 3-Benzyloxy-17β-toluenesulfonyl-estra-1,3,5(10)-trien-17β-ol (11)

To a stirred solution of compound **10** (0.69 mmol) in anhydrous pyridine (2 mL) at 0 °C was added *p*-toluenesuflonylchloride (1.03 mmol) portion-wise. The solution was stirred at room temperature for 16 h. The pyridine was removed under vacuum, the residue was dissolved in ethyl acetate, then washed with 2 N HCl, water and brine then dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (ethyl acetate/hexane, 7:3), to give **11** as a white solid (68%). Mp 115–117 °C (lit 115–117 °C)[25]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (d, J = 7.8 Hz, 2H, 2ArH-a), 7.42–7.32 (m, 7H, ArH's), 7.15 (d, J = 8.5 Hz, 1H, H-1), 6.76 (d, J = 8.4 Hz, 1H, H-2), 6.70 (br s, 1H, H-4), 5.01 (s, 2H, C_6H_5–CH_2), 4.35 (t, J = 8.1 Hz, 1H, H-17), 2.81 (m, 2H), 2.45 (s, 3H, $OSO_2C_6H_4$–CH_3), 2.24–2.10 (m, 2H), 1.99–1.92 (m, 1H), 1.85–1.61 (m, 4H), 1.43–1.26 (m, 4H), 1.16–1.08 (m, 2H), 0.83 (s, 3H, CH_3, H-18).

4.2.4. 17β-Toluenesulfonyloxy-estra-1,3,5(10)-trien-3-ol (12)

To a solution of compound **11** (0.14 mmol) in a methanol/ethyl acetate (1:1, 5 mL) and a catalytic amount of acetic acid (100 μL) was added $Pd(OH)_2$ (0.8 equiv). The mixture was stirred under H_2 gas for 16 h then filtered and concentrated. Purification of the residue by flash chromatography (ethyl acetate/hexane, 8:2) gave compound **12** as a white solid (71%). Mp 185–186 °C (lit 186–187 °C)[26]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (dd, J = 6.6 and 1.7 Hz, 2H, ArH-a), 7.33 (d, J = 6.6 Hz, 2H, ArH-b), 7.08 (d, J = 8.5 Hz, 1H, H-1), 6.59 (dd, J = 8.4 and 2.7 Hz, 1H, H-2), 6.52 (d, J = 2.7 Hz, 1H, H-4), 4.59 (s, 1H, Ar-O*H*), 4.33 (q, J = 7.7 Hz, 1H, H-17), 2.78–2.75 (m, 2H), 2.43 (s, 3H, $OSO_2C_6H_4$–CH_3), 2.09–1.95 (m, 3H), 1.78–1.67 (m, 4H), 1.41–1.33 (m, 4H), 1.14–1.09 (m, 2H), 0.81 (s, 3H, CH_3, H-18).

4.2.5. General procedure for synthesis of sulfonamides of 17-amino-1,3,5(10)-estratrien-3-ol

To a stirred solution of 17-amino-1,3,5(10)-estratrien-3-ol (0.38 mmol) in dry pyridine (3 mL) at 0 °C was added a solution of the sulfonyl chlorides (0.40 mmol) in dichloromethane (1 mL) via a syringe pump over 30 min. After addition, the reaction was stirred for 16 h at room temperature. The pyridine was removed under vacuum, the residue was dissolved in ethyl acetate, washed with water and brine then dried (Na_2SO_4,), filtered, and concentrated and the residue purified by column chromatography.

4.2.6. 17β-(4′-Methylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (6)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **6** as a white solid (33%). Mp 179–180 °C (lit. 184–185 °C)[27]; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.75 (d, J = 8.2 Hz, 2H, ArH-a), 7.28 (d, J = 8.0 Hz, 2H, ArH-b), 7.08 (d, J = 8.4 Hz, 1H, H-1), 6.58 (dd, J = 8.3 and 2.7 Hz, 1H, H-2), 6.52 (d, J = 2.6 Hz, 1H, H-4), 4.73 (br s, 1H, Ar-O*H*), 4. 50 (d, J = 9.2 Hz, 1H, NH), 3.13 (q, J = 8.9 Hz, 1H, H-17), 2.76 (m, 2H), 2.41 (s, 3H, C_6H_4–CH_3), 2.23–2.10 (m, 2H), 1.83–1.71 (m, 3H), 1.62–1.60 (m, 1H), 1.39–1.08 (m, 7H), 0.68 (s, 3H, CH_3, H-18).

4.2.7. 17α-Toluenesulfonamide-1,3,5(10)-estratrien-3-ol (7)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **7** as a white solid (47%). Mp 131–132 °C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.72 (d, J = 8.2 Hz, 2H, ArH-a), 7.28 (d, J = 8.0 Hz, 2H, ArH-b), 7.08 (d, J = 8.4 Hz, 1H, H-1), 6.64 (dd, J = 8.4 and 2.7 Hz, 1H, H-2), 6.53 (d, J = 2.5 Hz, 1H, H-4), 5.03 (br s, 1H, Ar-O*H*), 4. 50 (d, J = 9.1 Hz, 1H, NH), 3.29 (t, J = 8.3 Hz, 1H, H-17), 2.75 (m, 2H), 2.41 (s, 3H, C_6H_4–CH_3), 2.25–2.21 (m, 2H), 2.08–2.03 (m, 1H), 1.79–1.58 (m, 3H), 1.52–1.51 (m, 1H), 1.41–1.40 (m, 1H), 1.37–1.05 (m, 6H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 143.1 (*C*–CH_3), 138.3 (*C*–SO_2NH–), 138.0 (C-5), 132.4 (C-6), 129.0 (2ArCH-b), 127.1 (2ArCH-a), 126.5 (C-1), 115.2 (C-4), 112.8 (C-2), 62.5 (C-17), 49.6 (C-14), 44.89 (C-13), 43.2 (CH), 38.9 (CH), 32.7 (CH_2), 31.2 (CH_2), 29.6 (CH_2), 27.8 (CH_2), 26.0 (CH_2), 24.0 (CH_2), 21.5 (C_6H_4–CH_3), 18.2 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 426 (M+H, 20), 255 (100); HRMS (ESI$^+$) calcd for $C_{25}H_{32}NO_3S$ $(M+H)^+$ 426.2103; found 426.2104.

4.2.8. 17β-Benzenesulfonamide-1,3,5(10)-estratrien-3-ol (13)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:4) which provided **13** as a white solid (67%). Mp 234–235 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88 (d, J = 8.1 Hz, 2H, ArH-a & a′), 7.58–7.47 (m, 3H, ArH-b, b′ & c), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.59 (d, J = 8.4 Hz, 1H, H-2), 6.52 (s, 1H, H-4), 4.60 (s, 1H, Ar-O*H*), 4.48 (d, J = 9.2 Hz, 1H, NH), 3.15 (q, J = 8.9 Hz, 1H, H-17), 2.75 (m, 2H), 2.21–2.16 (m, 2H), 1.88–1.60 (m, 4H), 1.42–1.07 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 141.1 (*C*–SO_2NH–), 138.1 (C-5), 132.5 (ArCH), 132.4 (C-6), 129.0 (2ArCH), 127.1 (2ArCH), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 412 (M+H, 100), 255 (18); HRMS (ESI$^+$) calcd for $C_{24}H_{30}NO_3S$ $(M+H)^+$ 412.1946; found 412.1950.

4.2.9. 17β-Benzylsulfonamide-1,3,5(10)-estratrien-3-ol (14)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **14** as a white solid (26%). Mp 202–203 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.35 (m, 5H, ArH), 7.11 (d, J = 8.4 Hz, 1H, H-1), 6.60 (dd, J = 8.4 and 2.6 Hz, 1H, H-2), 6.53 (d, J = 2.5 Hz, 1H, H-4), 4.79 (br s, 1H, Ar-O*H*), 4.23 (AB system, 2H, J = 13.9 and 13.9 Hz, 2H, Ar-CH_2), 4.09 (d, J = 9.2 Hz, 1H, NH), 3.23 (q, J = 8.4 Hz, 1H, H-17), 2.77 (m, 2H), 2.28–2.09 (m, 3H), 1.94–1.68 (m, 3H), 1.42–1.18 (m, 7H), 0.65 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 138.1 (C-5), 132.4 (C-6), 130.7 (2ArCH), 129.4, 128.7 (2ArCH), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.8 (C-17), 59.7 (ArCH_2SO_2NH), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.9 (CH), 36.5 (CH_2), 29.9 (CH_2), 29.5 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) m/z (%) 425 (M^+, 100), 270 (35), 213, 91; HRMS (EI) calcd for $C_{25}H_{31}NO_3S$ 425.2025; found 425.2018.

4.2.10. 17β-(4′-Methylbenzylsulfonamide-1,3,5(10)-estratrien-3-ol (15)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **15** as a white solid (38%). Mp 140–141 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27 (d, J = 7.9 Hz, 2H, ArH-a), 7.17 (d, J = 7.9 Hz, 2H, ArH-b), 7.11 (d, J = 8.4 Hz, 1H, H-1), 6.60 (dd, J = 8.4 and 2.5 Hz, 1H, H-2), 6.53 (d, J = 2.2 Hz, 1H, H-4), 4.66 (br s, 1H, Ar-O*H*), 4.18 (AB system, 2H, J = 13.9 and 13.9 Hz, 2H, Ar-CH_2), 3.99 (d, J = 9.2 Hz, 1H, NH), 3.27 (q, J = 8.9 Hz, 1H, H-17), 2.78 (m, 2H), 2.34 (s, 3H, Ar-CH_3), 2.30–2.25 (m, 1H), 2.15–2.10 (m, 2H), 1.95–1.69 (m, 3H), 1.43–1.13 (m, 7H), 0.65 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ

153.4 (C-3), 138.6 (*C*–CH_2SO_2NH), 138.1 (C-5), 132.4 (C-6), 130.6 (2ArCH), 129.4 (2ArCH), 126.5 (C-1), 126.4 (ArCH), 115.2 (C-4), 112.7 (C-2), 63.8 (C-17), 59.3 (*C*H_2SO_2NH), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.9 (CH), 36.5 (CH_2), 30.0 (CH_2), 29.5 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.1 (CH_2), 21.2 (C_6H_4–*C*H_3), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m*/*z* (%) 440 (M+H, 31), 377 (28), 376 (100), 270 (28); HRMS (ESI$^+$) calcd for $C_{26}H_{34}NO_3S$ $(M+H)^+$ 440.2259; found 440.2265.

4.2.11. 17β-(4′-*n*-Propylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (16)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **16** as a white solid (27%). Mp 217–218 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, *J* = 8.1 Hz, 2H, ArH-a), 7.27 (d, *J* = 8.1 Hz, 2H, ArH-b), 7.08 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (d, *J* = 8.4 Hz, 1H, H-2), 6.52 (br s, 1H, H-4), 4.59 (br s, 1H, Ar-O*H*), 4.39 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.64 (t, *J* = 7.6 Hz, 2H, C*H*$_2$–CH_2–CH_3), 2.20–2.07 (m, 2H), 1.89–1.61 (m, 6H), 1.42–1.06 (m, 7H), 0.92 (t, *J* = 7.3 Hz, 3H, CH_2–CH_2–*C*H_3), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 147.9 (*C*-propyl), 138.3 (*C*–SO_2NH–), 138.0 (C-5), 132.4 (C-6), 129.0 (2ArCH-b), 127.1 (2ArCH-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 37.8 (*C*H$_2$–CH_2–CH_3), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 24.2 (CH_2–*C*H_2–CH_3), 23.1 (CH_2), 13.6 (CH_2–CH_2–*C*H_3), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m*/*z* (%) 454 (M+H, 72), 256 (20), 255 (100); HRMS (ESI$^+$) calcd for $C_{27}H_{36}NO_3S$ $(M+H)^+$ 454.2416; found 454.2410.

4.2.12. 17β-(4′-*n*-Butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (17)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **17** as a white solid (56%). Mp 221–222 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, *J* = 8.3 Hz, 2H, ArH-a), 7.28 (d, *J* = 8.3 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.3 and 2.6 Hz, 1H, H-2), 6.52 (d, *J* = 2.6 Hz, 1H, H-4), 4.50 (br s, 1H, Ar-O*H*), 4.32 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.9 Hz, 1H, H-17), 2.76 (m, 2H), 2.67 (t, *J* = 7.5 Hz, 2H, C*H*$_2$–CH_2–CH_2–CH_3), 2.20–2.11 (m, 2H), 1.83–1.58 (m, 6H), 1.37–1.10 (m, 9H), 0.92 (t, *J* = 7.3 Hz, 3H, CH_2–CH_2–CH_2–*C*H_3), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 148.1 (*C*-Butyl), 138.3 (*C*–SO_2NH–), 138.1 (C-5), 132.5 (C-6), 129.0 (2ArCH-b), 127.1 (2ArCH-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 35.5 (*C*H$_2$–CH_2–CH_2–CH_3), 33.2 (CH_2–*C*H$_2$–CH_2–CH_3), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 22.2 (CH_2–CH_2–*C*H$_2$–CH_3), 13.9 (CH_2–CH_2–CH_2–*C*H_3), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m*/*z* (%) 468 (M+H, 100), 255 (74), 219 (29), 152 (31); HRMS (ESI$^+$) calcd for $C_{28}H_{38}NO_3S$ $(M+H)^+$ 468.2572; found 468.2559.

4.2.13. 17β-(4′-*n*-Pentylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (18)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:4), which provided **18** as a white solid (21%). Mp 168–169 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, *J* = 7.8 Hz, 2H, ArH-a), 7.28 (d, *J* = 8.0 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.2 and 2.2 Hz, 1H, H-2), 6.52 (br s, 1H, H-4), 4.50 (br s, 1H, Ar-O*H*), 4.35 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.66 (t, *J* = 7.4 Hz, 2H, C*H*$_2$–CH_2–CH_2–CH_2–CH_3), 2.21–2.11 (m, 2H), 1.85–1.80 (m, 2H), 1.77–1.59 (m, 4H), 1.38–1.09 (m, 11H), 0.87 (t, *J* = 6.3 Hz, 3H, CH_2–CH_2–CH_2–CH_2–*C*H_3), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 148.2 (*C*-pentyl), 138.3 (*C*–SO_2NH–), 138.1 (C-5), 132.5 (C-6), 129.0 (2ArCH-b), 127.1 (2ArCH-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 35.8 (*C*H$_2$–CH_2–CH_2–CH_2–CH_3), 31.3 (CH_2–*C*H$_2$–CH_2–CH_2–CH_3), 30.7 (CH_2–CH_2–*C*H$_2$–CH_2–CH_3), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 22.4 (CH_2–CH_2–CH_2–*C*H$_2$–CH_3), 13.9 (CH_2–CH_2–CH_2–CH_2–*C*H_3), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m*/*z* (%) 482 (M+H, 100), 255 (80); HRMS (ESI$^+$) calcd for $C_{29}H_{40}NO_3S$ $(M+H)^+$ 482.2729; found 482.2721.

4.2.14. 17β-(4′-*iso*-Propylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (19)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **19** as a white solid (43%). Mp 206–207 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, *J* = 8.4 Hz, 2H, ArH-a), 7.33 (d, *J* = 8.3 Hz, 2H, ArH-b), 7.08 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.4 and 2.7 Hz, 1H, H-2), 6.52 (d, *J* = 2.6 Hz, 1H, H-4), 4.61 (br s, 1H, Ar-O*H*), 4.42 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.8 Hz, 1H, H-17), 3.01–2.92 (m, 1H), 2.76 (m, 2H), 2.20–2.11 (m, 2H), 1.82–1.61 (m, 4H), 1.38–1.10 (m overlapping d of –CH(C*H*$_3$)$_2$ with *J* = 7.0 Hz, 13H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.0 (*C*-*i*-propyl), 153.3 (C-3), 138.4 (*C*–SO_2NH–), 138.1 (C-5), 132.4 (C-6), 127.1 (4ArCH-b & a overlapping), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 34.1 (*C*H(CH$_3$)$_2$), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.7 ($2CH_3$, CH(*C*H$_3$)$_2$), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 453 (M^+, 100), 270 (90), 253 (30); HRMS (ESI$^+$) calcd for $C_{27}H_{36}NO_3S$ $(M+H)^+$ 454.2416; found 454.2403.

4.2.15. 17β-(4′-*t*-Butylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (20)

Purification was achieved by flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **20** as a white solid (53%). Mp 218–219 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, *J* = 8.4 Hz, 2H, ArH-a), 7.48 (d, *J* = 8.5 Hz, 2H, ArH-b), 7.09 (d, *J* = 8.4 Hz, 1H, H-1), 6.58 (dd, *J* = 8.3 and 2.5 Hz, 1H, H-2), 6.52 (d, *J* = 2.5 Hz, 1H, H-4), 4.56 (br s, 1H, Ar-O*H*), 4.32 (d, *J* = 9.2 Hz, 1H, NH), 3.15 (q, *J* = 8.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.15–2.03 (m, 2H), 1.86–1.61 (m, 4H), 1.39–1.11 (m, 16H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.3 (*C*-*t*-butyl), 153.3 (C-3), 138.1 (*C*–SO_2NH–), 138.0 (C-5), 132.5 (C-6), 126.9 (2ArCH-b), 126.5 (C-1), 125.9 (2ArCH-a), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 35.1 (*C*(CH$_3$)$_3$), 31.1 (C(*C*H$_3$)$_3$), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 467 (M^+, 91), 270 (100), 253 (32); HRMS (ESI$^+$) calcd for $C_{28}H_{38}NO_3S$ $(M+H)^+$ 468.2572; found 468.2566.

4.2.16. 17β-(4′-Phenylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (21)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 1:9) which provided **21** as a white solid (31%). Mp 223–224 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.92 (d, *J* = 8.4 Hz, 2H, ArH-a), 7.70 (d, *J* = 8.3 Hz, 2H, ArH-b), 7.62 (dd overlapping, *J* = 8.4 and 1.5 Hz, 2H, ArH-a′), 7.50–7.38 (m, 3H, ArH-b′ and c′), 7.09 (d, *J* = 8.3 Hz, 1H, H-1), 6.58 (dd, *J* = 8.4 and 2.4 Hz, 1H, H-2), 6.52 (br s, 1H, H-4), 4.45 (br s, 1H, Ar-O*H*), 4.40 (d, *J* = 9.3 Hz, 1H, NH), 3.20 (q, *J* = 8.5 Hz, 1H, H-17), 2.76 (m, 2H), 2.23–2.12 (m, 2H), 1.91–1.63 (m, 2H), 1.40–1.12 (m, 7H), 0.70 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 145.4 (*C*-Phenyl), 139.7 (*C*–SO_2NH–), 139.3 (C-$C_{1'}$-Phenyl), 138.1 (C-5), 132.5 (C-6), 129.0 (2ArCH), 128.5 (ArCH), 127.6 (4ArCH overlapping), 127.3 (2ArCH), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) *m*/*z* (%) 488 (M+H, 17), 391 (47), 323 (40), 219 (100), 173 (48); HRMS (ESI$^+$) calcd for $C_{30}H_{34}NO_3S$ $(M+H)^+$ 488.2259; found 488.2262.

4.2.17. 17β-(4′-Phenoxybenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (22)

Purification was achieved using flash chromatography (100% chloroform then methanol/chloroform, 2:3) which provided **22** as a white solid (38%). Mp 184–185 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81 (d, J = 8.5 Hz, 2H, ArH-a), 7.39 (dd overlapping, J = 7.7 and 7.7 Hz, 2H, ArH-b′), 7.18 (d, J = 7.1 Hz, 1H, ArH-c′), 7.06 (d overlapping dd, J = 8.4, 8.2 and 8.6 Hz respectively, 5H, H-1 and 4ArH-a′ & b), 6.58 (d, J = 8.5 Hz, 1H, H-2), 6.52 (br s, 1H, H-4), 4.49 (br s, 1H, Ar-O*H*), 4.35 (d, J = 9.3 Hz, 1H, NH), 3.15 (q, J = 8.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.23–2.09 (m, 2H), 1.90–1.61 (m, 2H), 1.40–1.09 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.3 (C-Phenyl), 155.3 (O-*C*-Phenyl), 153.3 (C-3), 138.1 (C-5), 134.8 (*C*–SO_2NH–), 132.4 (C-6), 130.1 (2ArCH-b′), 129.3 (2ArCH-a), 126.5 (C-1), 124.8 (ArCH-c′), 120.1 (2ArCH-b), 117.7 (2Ar-CH-a′), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.8 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26.1 (CH_2), 23.2 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 504 (M+H, 100), 256 (15), 255 (75); HRMS (ESI$^+$) calcd for $C_{30}H_{34}NO_4S$ $(M+H)^+$ 504.2209; found 504.2207.

4.2.18. 17β-2′-Naphthylsulfonamide-1,3,5(10)-estratrien-3-ol (23)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:3) which provided **23** as a white solid (25%). Mp 136–137 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (br s, 1H, ArH), 7.96–7.82 (m, 4H, ArH), 7.66–7.57 (m, 2H, ArH), 7.06 (d, J = 8.3 Hz, 1H, H-1), 6.58 (d, J = 8.4 Hz, 1H, H-2), 6.51 (br s, 1H, H-4), 4.54 (s overlapping d, J = 8.8 Hz, 2H, Ar-O*H* and NH), 3.20 (q, J = 8.6 Hz, 1H, H-17), 2.74 (m, 2H), 2.18–2.02 (m, 2H), 1.83–1.73 (m, 2H), 1.60–1.55 (m, 1H), 1.41–1.12 (m, 7H), 0.70 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 138.1 (C-5), 137.8, 134.7, 132.5 (C-6), 132.1, 129.4, 129.2, 128.7, 128.3, 127.9, 127.5, 126.5 (C-1), 122.4, 115.1 (C-4), 112.6 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 462 (M+H, 100), 256 (20), 255 (97); HRMS (ESI$^+$) calcd for $C_{28}H_{32}NO_3S$ $(M+H)^+$ 462.2103; found 462.2102.

4.2.19. 17β-(2′-Bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (24)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:4) which provided **24** as a white solid (67%): Mp 225–226 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (dd, J = 8.9 and 1.3 Hz 1H, ArH-a), 7.72 (d, J = 7.4 Hz, 1H, ArH-d), 7.42 (m, 2H, ArH-c & b), 7.07 (d, J = 8.4 Hz, 1H, H-1), 6.58 (d, J = 8.3 Hz, 1H, H-2), 6.52 (br s, 1H, H-4), 5.06 (d, J = 8.9 Hz, 1H, NH), 4.64 (s, 1H, Ar-O*H*), 3.12 (q, J = 8.8 Hz, 1H, H-17), 2.75 (m, 2H), 2.20–2.05 (m, 2H), 1.80–1.58 (m, 6H), 1.39–0.82 (m, 6H), 0.74 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 139.9 (C–SO_2NH–), 138.0 (C-5), 134.9 (ArCH-c), 133.5 (ArCH-a), 132.3 (C-6), 131.4 (ArCH-d), 127.8 (ArCH-b), 126.5 (C-1), 119.9 (*C*-Br), 115.2 (C-4), 112.7 (C-2), 63.7 (C-17), 51 (C-14), 43.7 (CH), 43.0 (C-13), 38.7 (CH), 36.2 (CH_2), 29.4 (CH_2), 28.7 (CH_2), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.9 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 492 (M+H+2, 28), 491 (M+2, 9), 490 (M+H, 28), 256 (20), 255 (100); HRMS (ESI$^+$) calcd for $C_{24}H_{29}BrNO_3S$ $(M+H)^+$ 490.1052; found 490.1053.

4.2.20. 17β-(3′-Bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (25)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **25** as a white solid (82%). Mp 182–183 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (dd overlapping, J = 1.7 and 1.6 Hz, 1H, ArH-d), 7.80 (d, J = 7.9 Hz, 1H, ArH-a), 7.67 (dd, J = 7.7 and 6.9 Hz, 1H, ArH-c), 7.37 (dd, J = 7.9 and 7.9 Hz, 1H, ArH-b), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.60 (dd, J = 8.3 and 2.6 Hz, 1H, H-2), 6.52 (d, J = 2.5 Hz, 1H, H-4), 4.76 (s, 1H, Ar-O*H*), 4.63 (d, J = 9.4 Hz, 1H, NH), 3.17 (q, J = 8.8 Hz, 1H, H-17), 2.76 (m, 2H), 2.23–2.12 (m, 2H), 1.84–1.65 (m, 4H), 1.39–1.13 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 143.0 (*C*–SO_2NH–), 138.0 (C-5), 135.5 (ArCH-c), 132.3 (C-6), 130.6 (ArCH-b), 130.0 (ArCH-d), 126.5 (C-1), 125.5 (ArCH-a), 122.9 (*C*-Br), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.0 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^-$) m/z (%) 491 (M+2, 27), 490 (M−H+2, 98), 489 (M^+, 28), 488 (M−H, 97), 255 (100), HRMS (ESI$^-$) calcd for $C_{24}H_{27}BrNO_3S$ $(M-H)^-$ 488.0895; found 488.0908.

4.2.21. 17β-(4′-Bromobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (26)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **26** as a white solid (78%). Mp 115–116 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, J = 8.7 Hz, 2H, ArH-a), 7.64 (d, J = 8.7 Hz, 2H, ArH-b), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.58 (dd, J = 8.5 and 2.5 Hz, 1H, H-2), 6.52 (d, J = 2.5 Hz, 1H, H-4), 4.54 (br s, 1H, Ar-O*H*), 4.42 (d, J = 9.4 Hz, 1H, NH), 3.15 (q, J = 8.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.24–2.08 (m, 2H), 1.88–1.60 (m, 4H), 1.40–1.12 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 140.2 (*C*–SO_2NH–), 138.1 (C-5), 132.3 (C-6), 132.2 (2ArCH), 128.6 (2ArCH), 127.4 (*C*-Br), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 492 (M+H+2, 75), 490 (M+H, 72), 256 (20), 255 (100); HRMS (ESI$^+$) calcd for $C_{24}H_{29}BrNO_3S$ $(M+H)^+$ 490.1052; found 490.1046.

4.2.22. 17β-(3′-Chlorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (27)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **27** as a white solid (73%). Mp 186–187 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87 (dd overlapping, J = 1.8 and 1.7 Hz, 1H, ArH-d), 7.75 (ddd, J = 7.7, 1.6, and 1.2 Hz, 1H, ArH-a), 7.53 (dddd, J = 8.02, 2.0, 1.9, and 1.2 Hz, 1H, ArH-c), 7.43 (dd overlapping, J = 7.9 and 7.9 Hz, 1H, ArH-b), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.59 (dd, J = 8.4 and 2.7 Hz, 1H, H-2), 6.52 (d, J = 2.6 Hz, 1H, H-4), 4.53 (s, 1H, Ar-O*H*), 4.50 (d,, J = 9.5 Hz, 1H, NH), 3.18 (q, J = 8.9 Hz, 1H, H-17), 2.76 (m, 2H), 2.24–2.12 (m, 2H), 1.85–1.69 (m, 4H), 1.40–1.13 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 142.9 (*C*–SO_2NH–), 138.0 (C-5), 135.1 (C-6), 132.6 (ArCH-c), 132.3 (*C*–Cl), 130.3 (ArCH-b), 127.2 (ArCH-d), 126.5 (C-1), 125.1 (ArCH-a), 115.2 (C-4), 112.6 (C-2), 63.5 (C-17), 51 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) m/z (%) 447 (M+2, 40), 445 (M^+, 100), 270 (43), 253 (25), 213 (30), HRMS (EI) calcd for $C_{24}H_{28}ClNO_3S$ 445.1478; found 445.1474.

4.2.23. 17β-(4′-Chlorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (28)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **28** as a white solid (69%). Mp 124–125 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (d, J = 8.4 Hz, 2H, ArH-a), 7.45 (d, J = 8.4 Hz, 2H, ArH-b), 7.07 (d, J = 8.5 Hz, 1H, H-1), 6.60 (d, J = 8.4 Hz, 1H, H-2), 6.53 (br s, 1H, H-4), 5.29 (br s, 1H, Ar-O*H*), 4.98 (d, J = 9.2 Hz, 1H, NH), 3.13 (q, J = 8.6 Hz, 1H, H-17), 2.74 (m, 2H), 2.21–2.05 (m, 2H), 1.87–1.60 (m, 4H), 1.42–1.07 (m, 7H), 0.67 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 139.7 (*C*–SO_2NH–), 138.9 (*C*–Cl), 138.0 (C-5), 132.3 (C-6), 129.3 (2ArCH-b), 128.5 (2ArCH-a), 126.5 (C-1), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (ESI$^+$) m/z (%) 448 (M+H+2, 38), 447

(M+2, 30), 446 (M+H, 100), 255 (18), 239 (38); HRMS (ESI^+) calcd for $C_{24}H_{29}ClNO_3S$ $(M+H)^+$ 446.1557; found 446.1542.

4.2.24. 17β-(3′-Nitrobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (29)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:2) which provided **29** as a yellow solid (49%). Mp 200–201 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.72 (br s, 1H, ArH-d), 8.41 (m, 1H, ArH-c), 8.20 (m, 1H, ArH-a), 7.72 (m, 1H, ArH-b), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.58 (dd, J = 8.3 and 2.4 Hz, 1H, H-2), 6.52 (br s, 1H, H-4), 4.53 (d,, J = 9.5 Hz, 1H, NH), 4.45 (s, 1H, Ar-O*H*), 3.25 (q, J = 8.6 Hz, 1H, H-17), 2.76 (m, 2H), 2.24–2.13 (m, 2H), 1.92–1.66 (m, 4H), 1.40–1.14 (m, 7H), 0.71 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 148.2 (*C*–NO_2), 143.5 (*C*–SO_2NH–), 138 (C-5), 132.5 (ArCH), 132.2 (C-6), 130.4 (ArCH), 127 (ArCH), 126.5 (C-1), 122.3 (ArCH), 115.2 (C-4), 112.7 (C-2), 63.6 (C-17), 51 (C-14), 43.7 (CH), 43 (C-13), 38.7 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.9 (CH_3, C-18); LRMS (EI) m/z (%) 456 (M^+, 100), 270 (18), 213 (20); HRMS (ESI^+) calcd for $C_{24}H_{29}N_2O_5S$ $(M+H)^+$ 457.1797; found 457.1807.

4.2.25. 17β-(3′-Cyanobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (30)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:3) which provided **30** as a white solid (53%). Mp 127–128 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.16 (s, 1H, ArH-a), 8.10 (d, J = 7.9 Hz, 1H, ArH-d), 7.84 (d, J = 7.8 Hz, 1H, ArH-c), 7.64 (dd, J = 7.7 and 7.9 Hz, 1H, ArH-b), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.59 (dd, J = 8.4 and 2.5 Hz, 1H, H-2), 6.53 (br s, 1H, H-4), 4.48–4.46 (s and d overlapping, 2H, Ar-O*H* and NH), 3.20 (q, J = 8.9 Hz, 1H, H-17), 2.77 (m, 2H), 2.25–2.13 (m, 2H), 1.89–1.79 (m, 2H), 1.70–1.66 (m, 2H), 1.40–1.09 (m, 7H), 0.70 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.3 (C-3), 143.0 (*C*–SO_2NH–), 138.0 (C-5), 135.6 (ArCH-c), 132.2 (C-6), 130.9 (ArCH-a), 130.6 (ArCH-d), 130.1 (ArCH-b), 126.4 (C-1), 117.1 (*C*N), 115.2 (C-4), 113.6 (*C*–CN), 112.7 (C-2), 63.5 (C-17), 51.0 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 27.0 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) m/z (%) 436 (M^+, 100), 253 (15), 213 (22); HRMS (EI) calcd for $C_{25}H_{28}N_2O_3S$ 436.1821; found 436.1811.

4.2.26. 17β-(3′-Fluorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (31)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **31** as a white solid (65%). Mp 182–183 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.67 (d, J = 7.8 Hz, 1H, ArH-a), 7.58 (d, J = 8.2 Hz, 1H, ArH-d), 7.48 (m, 1H, ArH-b), 7.26 (m, overlapped with $CDCl_3$, 1H, ArH-c), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.59 (dd, J = 8.4 and 2.5 Hz, 1H, H-2), 6.52 (d, J = 2.3 Hz, 1H, H-4), 4.60 (s, 1H, Ar-O*H*), 4.56 (d,, J = 9.3 Hz, 1H, NH), 3.18 (q, J = 8.8 Hz, 1H, H-17), 2.76 (m, 2H), 2.22–2.12 (m, 2H), 1.88–1.63 (m, 4H), 1.39–1.12 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.3 (d, J = 250 Hz, *C*–F), 153.3 (C-3), 143.2 (d, J = 6.6 Hz, *C*–SO_2NH–), 138 (C-5), 132.3 (C-6), 130.8 (d, J = 7.6 Hz, ArCH-b), 126.5 (C-1), 122.7 (d, J = 3.3 Hz, ArCH-a), 119.6 (d, J = 21.0 Hz, ArCH-c), 115.2 (C-4), 114.4 (d, J = 24.1 Hz, ArCH-d), 112.7 (C-2), 63.5 (C-17), 51.0 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -109; LRMS (EI) m/z (%) 429 (M^+, 100), 270 (25), 213 (22); HRMS (EI) calcd for $C_{24}H_{28}FNO_3S$ 429.1774; found 429.1782.

4.2.27. 17β-(4′-Fluorobenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (32)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **32** as a white solid (61%). Mp 140–141 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.89 (m, 2H, ArH-a), 7.16 (dd overlapping, J = 8.5 and 8.5 Hz, 2H, ArH-b), 7.08 (d, J = 8.4 Hz, 1H, H-1), 6.59 (dd, J = 8.4 and 2.5 Hz, 1H, H-2), 6.53 (d, J = 2.5 Hz, 1H, H-4), 4.89 (br s, 1H, Ar-O*H*), 4.71 (d, J = 9.2 Hz, 1H, NH), 3.13 (q, J = 8.8 Hz, 1H, H-17), 2.75 (m, 2H), 2.22–2.10 (m, 2H), 1.85–1.61 (m, 4H), 1.38–1.09 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.9 (d, J = 253.0 Hz, *C*–F), 153.4 (C-3), 138.0 (C-5), 137.2 (d, J = 3.2 Hz, *C*–SO_2NH–), 132.3 (C-6), 129.7 (d, J = 9.9 Hz, 2ArCH-a), 126.5 (C-1), 116.1 (d, J = 22.4 Hz, 2ArCH-b), 115.2 (C-4), 112.7 (C-2), 63.4 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -105; LRMS (ESI^+) m/z (%) 430 (M+H, 100), 255 (13), 223 (15); HRMS (ESI^+) calcd for $C_{24}H_{29}FNO_3S$ $(M+H)^+$ 430.1852; found 430.1847.

4.2.28. 17β-(3′-Trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (33)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:4) which provided **33** as a white solid (48%). Mp 196–197 °C; ^1H NMR ($CDCl_3$, 300 MHz), δ 8.14 (br s, 1H, ArH-d), 8.07 (d, J = 7.9 Hz, 1H, ArH-a), 7.82 (d, J = 7.8 Hz, 1H, ArH-c), 7.65 (dd overlapping, J = 7.8 and 7.8 Hz, 1H, ArH-b), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.59 (dd, J = 8.3 and 2.5 Hz, 1H, H-2), 6.52 (d, J = 2.5 Hz, 1H, H-4), 4.47 (s overlapping d, J = 8.8 Hz, 2H, Ar-O*H* and N*H*), 3.21 (q, J = 8.8 Hz, 1H, H-17), 2.76 (m, 2H), 2.22–2.12 (m, 2H), 1.86–1.65 (m, 4H), 1.39–1.12 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 142.5 (*C*–SO_2NH–), 138.0 (C-5), 132.3 (C-6), 131.7 (q, J = 33.2 Hz, *C*–CF_3), 130.2 (d, J = 1.0 Hz, ArCH), 129.8 (ArCH), 129.1 (q, J = 3.7 Hz,, ArCH), 126.5 (C-1), 124.1 (q, J = 3.9 Hz, ArCH), 123.2 (q, J = 271.3 Hz, C–*C*F_3), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.0 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz), δ -62.8; LRMS (EI) m/z (%) 479 (M^+, 100), 270 (21), 213 (22); HRMS (EI) calcd for $C_{25}H_{28}F_3NO_3S$ 479.1742; found 479.1732.

4.2.29. 17β-(4′-Trifluoromethylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (34)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:1) which provided **34** as a white solid (34%). Mp 138–139 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, J = 8.1 Hz, 2H, ArH-a), 7.76 (d, J = 8.0 Hz, 2H, ArH-b), 7.09 (d, J = 8.4 Hz, 1H, H-1), 6.58 (d, J = 8.8 Hz, 1H, H-2), 6.53 (br s, 1H, H-4), 4.51 (d overlapping s, 2H, Ar-O*H* and N*H*), 3.20 (q, J = 8.6 Hz, 1H, H-17), 2.77 (m, 2H), 2.23–2.09 (m, 2H), 1.92–1.61 (m, 4H), 1.44–1.10 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 144.8 (d, J = 1.2 Hz, *C*–SO_2NH–), 138.0 (C-5), 134.2 (d, J = 33.0 Hz, *C*–CF_3), 132.3 (C-6), 127.5 (2ArCH-a), 126.5 (C-1), 126.2 (q, J = 3.7 Hz, 2ArCH-b), 123.0 (q, J = 271.2 Hz, C–*C*F_3), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.1 (C-14), 43.7 (CH), 43.0 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −62.5; LRMS (ESI^+) m/z (%) 480 (M+H, 43), 256 (19), 255 (100); HRMS (ESI^+) calcd for $C_{25}H_{29}F_3NO_3S$ $(M+H)^+$ 480.1820; found 480.1813.

4.2.30. 17β-(3′-Methylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (35)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **35** as a white solid (43%). Mp 202–203 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.67 (m, 2H, ArH-d & a), 7.36 (m, 2H, ArH-c & b), 7.09 (d, J = 8.5 Hz, 1H, H-1), 6.59 (dd, J = 8.4 and 2.7 Hz, 1H, H-2), 6.51 (d, J = 2.5 Hz, 1H, H-4), 4.47 (s, 1H, Ar-O*H*), 4.34 (d, J = 9.3 Hz, 1H, NH), 3.16 (q, J = 8.7 Hz, 1H, H-17), 2.76 (m, 2H), 2.4 (s, 3H, Ar-CH_3), 2.23–2.11 (m, 2H), 1.82–1.72 (m, 4H), 1.35–1.12 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$,

75 MHz) δ 153.3 (C-3), 140.9 (*C*–SO_2NH–), 139.1 (*C*–CH_3), 138 (C-5), 133.2 (ArCH), 132.4 (C-6), 128.8 (ArCH), 127.4 (ArCH), 126.5 (C-1), 124.1 (ArCH), 115.2 (C-4), 112.7 (C-2), 63.3 (C-17), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 21.3 (Ar-CH_3), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 425 (M^+, 100), 270 (60), 253 (25), 213 (20); HRMS (EI) calcd for $C_{25}H_{31}NO_3S$ 425.2025; found 425.2035.

4.2.31. 17β-(3′-Methoxybenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (36)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 2:3) which provided **36** as a white solid (70%). Mp 198–199 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 (m, 3H, ArH-a,b,d), 7.09 (m, 2H, ArH-c overlapping H-1), 6.58 (d, *J* = 8.3 Hz, 1H, H-2), 6.52 (s, 1H, H-4), 4.49 (s, 1H, Ar-O*H*), 4.39 (d, *J* = 9.1 Hz, 1H, NH), 3.85 (s, 3H, $ArOCH_3$), 3.16 (pseudo t, 1H, H-17), 2.76 (m, 2H), 2.23–2.11 (m, 2H), 1.91–1.62 (m, 4H), 1.43–1.14 (m, 7H), 0.69 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.8 (*C*–OCH_3), 153.3 (C-3), 142.3 (*C*–SO_2NH–), 138.1 (C-5), 132.4 (C-6), 130 (ArCH), 126.5 (C-1), 119.2 (ArCH), 118.8 (ArCH), 115.2 (C-4), 112.7 (C-2), 111.8 (C-d), 63.4 (C-17), 55.6 (OCH_3), 51.1 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.5 ($2CH_2$), 27.1 (CH_2), 26 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); LRMS (EI) *m*/*z* (%) 441 (M^+, 100), 270 (70), 253 (26), 213 (20); HRMS (EI) calcd for $C_{25}H_{31}NO_4S$ 441.1974; found 441.1979.

4.2.32. 17β-(3′-Trifluoromethoxybenzene)sulfonamide-1,3,5(10)-estratrien-3-ol (37)

Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:1) which provided **37** as a white solid (31%). Mp 152–153 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.84 (d, *J* = 7.8 Hz, 1H, ArH-a), 7.75 (br s, 1H, ArH-d), 7.55 (dd overlapped, *J* = 8.0 and 7.9 Hz, 1H, ArH-b), 7.41 (d, *J* = 8.1 Hz, 1H, ArH-c), 7.07 (d, *J* = 8.4 Hz, 1H, H-1), 6.59 (d, *J* = 8.4 Hz, 1H, H-2), 6.53 (br s, 1H, H-4), 4.98 (s, 1H, Ar-O*H*), 4.92 (d, *J* = 9.3 Hz, 1H, NH), 3.18 (q, *J* = 8.9 Hz, 1H, H-17), 2.75 (m, 2H), 2.20–2.06 (m, 2H), 1.89–1.69 (m, 2H), 1.68–1.59 (m, 2H), 1.42–1.07 (m, 7H), 0.68 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.4 (C-3), 149.3 (q, *J* = 2.0 Hz, *C*–OCF_3), 143.3 (*C*–SO_2NH–), 138.0 (C-5), 132.3 (C-6), 130.7 (ArCH), 126.5 (C-1), 120.3 (q, *J* = 275.2 Hz, OCF_3), 125.3 (ArCH), 124.9 (ArCH), 119.7 (d, *J* = 0.8 Hz, ArCH), 115.2 (C-4), 112.7 (C-2), 63.5 (C-17), 51.0 (C-14), 43.7 (CH), 42.9 (C-13), 38.8 (CH), 36.3 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 27.1 (CH_2), 26.0 (CH_2), 23.1 (CH_2), 11.8 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −57.6; LRMS (ESI^+) *m*/*z* (%) 496 (M+H, 32), 255 (41); HRMS (ESI^+) calcd for $C_{25}H_{29}F_3NO_4S$ $(M+H)^+$ 496.1769; found 496.1758.

4.2.33. 17β-(3′-Trifluoromethyl)benzamido-estra-1,3,5(10)-trien-3-yl-(3′-trifluoro-methyl)benzoate (38)

To a stirred solution of 17-amino-1,3,5(10)-estratrien-3-ol (150 mg, 0.55 mmol) in dry pyridine (3 mL) at 0 °C was added the *m*-trifluoromethylbenzoyl chloride (230 mg, 1.10 mmol). The reaction was stirred overnight at room temperature. The pyridine was removed under vacuum, the residue was dissolved in chloroform, washed with water, dil. HCl, water, $NaHCO_3$, water, and finally brine then dried (Na_2SO_4,), filtered, and concentrated. Purification was achieved using flash chromatography (ethyl acetate/hexane, 3:7) which provided **38** as a white solid (57%). Mp 118–119 °C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 8.43 (s, 1H, ArH), 8.35 (d, *J* = 8.1 Hz, 1H, ArH), 8.00 (s, 1H, ArH), 7.93 (d, *J* = 7.7 Hz, 1H, ArH), 7.87 (d, *J* = 7.4 Hz, 1H, ArH), 7.74 (d, *J* = 7.6 Hz, 1H, ArH), 7.66–7.54 (m, 2H, ArH), 7.33 (d, *J* = 8.7 Hz, 1H, H-1), 6.94 (d overlapping br s, *J* = 8.9 Hz, 2H, H-2 and H-4, respectively), 5.97 (d, *J* = 8.7 Hz, 1H, NH), 4.22 (q, *J* = 8.9 Hz, 1H, H-17), 2.89 (m, 2H), 2.33–2.26 (m, 3H), 1.94–1.82 (m, 3H), 1.63–1.40 (m, 7H), 0.83 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.1 (*C*(O)NH), 164.2 (*C*(O)O), 148.4 (C-3), 138.3 (2C, C-5 and C-6), 135.8 (*C*–C(O)NH–),133.3 (ArCH), 131.6 (q, *J* = 33 Hz, 2C, *C*–CF_3), 130.6 (C–*C*(O)O–), 130.1 (ArCH), 130.0 (d, *J* = 3.5 Hz, ArCH), 129.2 (d, *J* = 3.4 Hz, 2ArCH), 127.9 (d, *J* = 3.5 Hz, ArCH), 126.9 (d, *J* = 3.7 Hz, ArCH), 126.6 (C-1), 123.8 (q, *J* = 3.7 Hz, ArCH), 123.6 (q, *J* = 270.9 Hz, 2C, C–*C*F_3), 121.4 (C-4), 118.5 (C-2), 59.5 (C-17), 51.7 (C-14), 44.0 (C-13), 43.8 (CH), 38.6 (CH), 37.0 (CH_2), 29.5 (CH_2), 28.7 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 23.4 (CH_2), 12.2 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ -62.5 ($2CF_3$ overlapping); LRMS (ESI^+) *m*/*z* (%) 616 (M+H, 100); HRMS (ESI^+) calcd for $C_{34}H_{32}F_6NO_3$ $(M+H)^+$ 616.2286; found 616.2296.

4.2.34. 17β-(3′-Trifluoromethyl)benzamido-estra-1,3,5(10)-trien-3-ol (39)

To a stirred solution of compound **38** (120 mg, 0.27 mmol) in methanol (15 mL), was added a solution of K_2CO_3 (35 mg, 0.25 mmol) in H_2O (120 μL). The resultant solution was stirred for 3 h, then diluted with water, neutralized with dil. HCl, and extracted with chloroform. The extract was then washed with water, brine, filtered, dried with Na_2SO_4, and concentred. Purification was achieved using flash chromatography (ethyl acetate/hexane, 1:9) which provided **39** as a white solid (74%). Mp 135–136 °C; ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.99 (s, 1H, ArH-d), 7.92 (d, *J* = 7.7 Hz, 1H, ArH-a), 7.74 (d, *J* = 7.8 Hz, 1H, ArH-c), 7.56 (dd overlapping, *J* = 7.7 and 7.8 Hz, 1H, ArH-b), 7.12 (d, *J* = 8.3 Hz, 1H, H-1), 6.61 (d, *J* = 8.3 Hz, 1H, H-2), 6.56 (br s, 1H, H-4), 6.01 (d, *J* = 8.7 Hz, 1H, NH), 5.07 (br s, 1H, O*H*), 4.19 (q, *J* = 9.0 Hz, 1H, H-17), 2.80 (m, 2H), 2.29–2.21 (m, 3H), 1.89–1.85 (m, 3H), 1.58–1.39 (m, 7H), 0.81 (s, 3H, CH_3, H-18); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.1 (*C*(O)NH), 153.3 (C-3), 138.1 (C-5), 135.7 (*C*–C(O)NH–), 132.4 (C-6), 131.0 (d, *J* = 32.5 Hz, *C*–CF_3), 130.0 (d, *J* = 1.3 Hz, ArCH), 129.2 (d, *J* = 2.4 Hz, ArCH), 127.9 (q, *J* = 3.7 Hz, ArCH), 126.5 (C-1), 123.8 (q, *J* = 3.8 Hz, ArCH), 123.7 (q, *J* = 271.0 Hz, C–*C*F_3), 115.3 (C-4), 112.7 (C-2), 59.5 (C-17), 51.6 (C-14), 43.8 (C-13), 43.7 (CH), 38.9 (CH), 37.0 (CH_2), 29.6 (CH_2), 28.8 (CH_2), 27.3 (CH_2), 26.2 (CH_2), 23.4 (CH_2), 12.3 (CH_3, C-18); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −62.9; LRMS (ESI^+) *m*/*z* (%) 444 (M+H, 100); HRMS (ESI^+) calcd for $C_{26}H_{29}F_3NO_2$ $(M+H)^+$ 444.2150; found 444.2135.

4.3. Inhibition studies

4.3.1. IC_{50} determinations

Inhibitor solutions (20 μL) of various concentrations in DMSO/0.1 M Tris–HCl pH 7.0 (1:1) were added to the wells of a 96-well microtiter plate containing 20 μL of a 2 mM 4-MUS solution in 0.1 M Tris–HCl, pH 7.0 and 140 μL of 0.1 M Tris–HCl pH 7.0. The reaction is initiated by the addition of 20 μL of 100 nM STS in 20 mM Tris–HCl, pH 7.4, 0.1% Triton X-100 into to the well, yielding a final concentration of 10 nM STS, 200 μM 4-MUS (K_m concentration) in 0.1 M Tris–HCl, pH 7.0, 0.01% Triton X-100, and 5% DMSO. All reactions were performed in triplicate at 25 °C, and STS activity was measured by monitoring for 10 min the production of 4-MU using a spectrofluorimeter plate reader with excitation and emission at 360 and 460 nM, respectively. The activity of STS in the presence of inhibitor (V_i) was compared to the activity of STS in the absence of inhibitor (V_o), and a percent activity was calculated. IC_{50} was determined by plotting the percent activity on a semi log graph against the log concentration of the inhibitor, and fitted in Grafit from Erithacus Software (Surrey, UK) using the equation: $V_i = V_o/[1+([I]/IC_{50})S] + B$ where V_i = initial rate of reaction at inhibitor concentration [*I*]; V_o = velocity in the absence of inhibitor; B = background activity; *s* = slope factor equal $V_o - B$.

4.3.2. K_i determination

A 2 mM stock solution of compound **25** was made in DMSO (100%), from which dilutions 0.75, 0.50, and 0.25 μM were

prepared in DMSO/0.1 M Tris–HCl pH 7.0 (1:1). Each fixed concentration of inhibitor (20 μL) was added to the wells of a black 96-well microtiter containing 20 μL of varying concentrations of 4-MUS substrate ranging from 833 μM to 5 mM prepared in 0.1 M Tris–HCl pH 7.0 and 140 μL of 0.1 M Tris–HCl pH 7.0. A control was made in which inhibitor storage buffer was added instead. The reaction was initiated by the addition of 20 μL of 100 nM STS in buffer containing 20 mM Tris–HCl 0.1% Triton X-100 pH 7.0. STS activity was monitored as described previously in Section 4.3.1. A control was done in a similar manner, in which enzyme storage buffer was added instead. Therefore, the final concentration of inhibitor in the assay was 75, 50, and 25 nM for each 4-MUS concentration ranging between 83.3 and 500 μM, also, the final concentration of enzyme in the assay was 10 nM. All reactions were performed in triplicate, and the initial rates of the reaction obtained as relative fluorescent units over time (RFU/s) for each 4-MUS concentration are plotted as a Lineweaver–Burk graph using Microsoft Excel. The slopes and intercepts of the Lineweaver–Burk plots were replotted based on the equations for mixed-type inhibition to obtain the desired K_i and αK_i values. All reactions were performed in triplicate.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.036.

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