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Focused Libraries of 16-Substituted Estrone Derivatives and Modified E-Ring Steroids: Inhibitors of 17ß-Hydroxysteroid Dehydrogenase Type 1

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*17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1), an oxidoreductase which has a preferential reductive activity using NADPH as cofactor, converts estrone to estradiol and is expressed in many steroidogenic tissues including breast and in malignant breast cells. As estradiol stimulates the growth and development of hormone-dependent breast cancer, inhibition of the final step of its synthesis is an attractive target for the treatment of this disease. The parallel synthesis of novel focused libraries of 16-substituted estrone derivatives and modified E-ring pyrazole steroids as new potent 17β-HSD1 inhibitors is described. Substituted 3-O-sulfamoylated estrone derivatives were used as templates and were immobilised on 2-chlorotrityl chloride resin to give resin-bound scaffolds with a multi-detachable linker. Novel focused libraries of 16-substituted estrone derivatives and new modified E-ring steroids were assembled from these immobilised templates using solid-phase organic synthesis and solution-phase methodologies. Among the derivatives synthesised, the most potent 17β-HSD1 inhibitors were **25** and **26** with IC_{50} values in T-47D human breast cancer cells of 27 and 165 nM, respectively. Parallel synthesis resulting in a library of C5′-linked amides from the pyrazole E-ring led to the identification of **62** with an IC_{50} value of 700 nM. These potent inhibitors of 17β-HSD1 have a 2-ethyl substituent which will decrease their estrogenic potential. Several novel 17β-HSD1 inhibitors emerged from these libraries and these provide direction for further template exploration in this area. A new efficient diastereoselective synthesis of **25** has also been developed to facilitate supply for in vivo evaluation, and an X-ray crystal structure of this inhibitor is presented.*

Introduction

Breast cancer remains a major cause of death in the Western world and is one of the most common diseases in women, with an estimated global incidence in 2002 of approximately 1150000.[1] The female sex hormones, estrogens, have a central role in the development of breast tumours and the majority are initially hormone-responsive with circulating estrogens playing a vital role in their growth. The highest incidence of breast cancer, approximately 80%, occurs in postmenopausal women when ovarian production of estrogens has stopped and almost all the estrogens are synthesised from inactive precursors in an extra-glandular manner.

Steroidogenic enzyme inhibitors can lower circulating and tissue levels of active estrogens by blocking their biosynthetic pathways and therefore such enzymes are an attractive target for the treatment of hormone-dependent breast cancer (HDBC).[2–6] Aromatase inhibitors, which prevent the conversion of androgens into estrogens, are currently used as an adjuvant therapy to treat HDBC.[5] It has been shown that steroid sulfatase is the main enzyme likely to be responsible for estrone (E1) production in hormone-dependent breast tumours, as estrone production in such tumours is approximately ten times higher per gram of protein for the sulfatase pathway (estrone-3-*O*-sulfate to estrone) than for the aromatase pathway.[7,8]

In postmenopausal women, estrone (E1) is formed almost exclusively from androstenedione, a reaction mediated by the aromatase enzyme complex.[9] Peripheral aromatase activity increases with ageing and is also influenced by body weight.[10,11]

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The 17β-hydroxysteroid dehydrogenase enzymes (17β-HSDs, EC1.1.1.62) catalyze the NAD(P)(H)-dependent oxidoreduction of hydroxy or keto groups at position 17 of androgens and estrogens. In this way they regulate the intracellular availability of steroid hormone substrates to their nuclear receptors. This pathway is pre-receptor controlled, as androgen and estrogen receptors transactivate their target genes by binding the 17β-hydroxy steroids with higher affinity than the 17-keto steroids.[12–15]

Type 1 17β-HSD belongs to a family of HSDs for which 13 mammalian forms have been identified, 11 of which exist in humans. These 17β-HSDs are expressed in a tissue-specific manner and act to interconvert weak androgens and estrogens to their more active counterparts.[16–18] The 17β-HSD1 enzyme, which has a preferential reductive activity using NADPH as cofactor,[19,20] is expressed in many tissues including breast tissue and in malignant breast cells.[21,22] As estradiol (E2) is known to stimulate the growth and development of HDBC,[23] inhibition of the final step in the synthesis of E2 through the design of selective inhibitors of 17β-HSD1 has potential for the treatment of HDBC.

Most human 17β-HSDs belong to the short-chain dehydrogenase/reductase (SDR) superfamily, which comprises a large protein family of oxidoreductases. Present in all forms of life, these SDR enzymes share few distinct sequence motifs and show a low overall homology with other family members of typically 15–30% identity with conserved nucleotide cofactor binding site and catalytic site residues.[24–26]

The 17β-HSD1 enzyme consists of 327 amino acid residues with a subunit mass of ~35 kDa and exists as a homodimer.[27] Numerous protein crystal structures of 17β-HSD1 have been determined, including that for the enzyme in its native form,[28] in complex with estradiol and NADP,[29] with estradiol alone,[30] and with equilin and NADP.[31] This information is valuable in the structure-based drug design of inhibitors of 17β-HSD1.

Although the importance of inhibiting 17β-HSD1 as a potential approach to treating HDBC has been highlighted for many years, the design of selective 17β-HSD1 inhibitors has been a relatively unexplored area until recently. Poirier has reviewed the design of 17β-HSD1 inhibitors.[32] A number of research groups have reported selective inhibitors of 17β-HSD1, of which many share common structural features such as a phenolic ring and hydrophobic scaffolds that interact with the hydrophobic amino acid residues in the active site which recognise the steroid substrate.[32] Poirier and co-workers reported estrone-based steroidal inhibitors of 17β-HSD1 with E2 derivatives bearing a short side chain at positions C17α and C16α of an estrone template.[33] This group also designed hybrid 17β-HSD1 inhibitors, the most potent of which was EM-1745 (IC_{50} = 52 nM), which has an estradiol scaffold linked by an eight-methylene-unit spacer to an adenosine moiety. [34] The crystal structure of 17β-HSD1 and EM-1745 was resolved to 1.6 Å[35] and shows the substrate and cofactor binding sites to be occupied in a similar fashion to that of the ternary complex 17β-HSD1–E_2–$NADP^+$.

EM-1745

Recently, Solvay reported a number of nonsteroidal benzothienopyrimidinones[36] and steroidal compounds as potent and selective 17β-HSD1 inhibitors.[37] Our research group has used either E1 or E2 as a template,[38] and preliminary work on some of the most potent compounds has been published.[39] Further work targeted the modification of estrone at the 6, 16, and 17 positions.[40,41] As indicated,[39] our extended strategy involved the design of 17β-HSD1 inhibitors that bind to the substrate binding site and contain functionality that may interact with the cofactor in the catalytic region. Solid-phase organic synthesis (SPOS) has gained considerable attention in the design of molecules of pharmaceutical interest.[42–44] Library synthesis using the steroid nucleus as a scaffold for SPOS has been relatively unexplored, although a number of novel solid-phase strategies for the synthesis of steroid-derivative libraries have now been designed to expand this relatively unexplored area of combinatorial chemistry.[45–47] The identification of small druglike molecules using steroid templates is undoubtedly a rapidly growing area of research. Estradiol or estrone has been used as a scaffold in a number of drug-discovery programmes.[36,39–41,48] Herein, we highlight a hybrid strategy for the parallel synthesis of novel 2-ethyl-substituted, 16β-substituted estrone compound libraries (Figure 1, **3**).

Recently, we reported the solution-phase synthesis of a lead compound **1** (Figure 1) that shows potent inhibition of 17β-HSD1 with an IC_{50} value of 37 nM; compound **1** is also selective over 17β-HSD2.[39] The pyrazole compound **2** (Figure 1), although less potent than **1** (IC_{50} = 780 nM), is also a highly se-

R^1 = H or SO_2NH_2 R^1 = H or SO_2NH_2

Figure 1. Compounds **1** and **2** are potent 17β-HSD1 inhibitors discovered from solution-phase synthesis; libraries **3** and **4** are 2-ethyl-substituted estrone-derived libraries using SPOS and solution-phase parallel synthesis.

lective inhibitor of 17β-HSD1 over 17β-HSD2.[40] Pyrazoles such as **2** are attractive targets for synthesis owing to the lack of a chiral centre at position 16 and the ease of access to a common pyrazole carboxylic acid intermediate for rapid modification.

Herein, the above SPOS strategy has been extended to new E-ring-modified steroids with the synthesis of a library of functionalised pyrazole derivatives **4** ($R^1 = SO_2NH_2$, Figure 1). Parallel synthesis in solution has also been used to synthesise **4** (R^1 = H). The design and synthesis of novel and potent compounds has the requirement of overcoming the potential estrogenic effects of lead compounds **1** and **2**.

Although several approaches to decrease the estrogenicity of 17β-HSD1 inhibitors with estrone derivatives have been reported,[49] compounds that exhibit druglike properties with potential for development and decreased estrogenic properties have yet to be identified. Several 2-methoxyestrone derivatives have been reported to be less estrogenic than their estrone counterparts,[50,51] and 2-substituted estrone derivatives also show reduced estrogenicity.[52] In silico studies using the crystal structure (PDB code 3ERD)[53] of the ligand-binding domain of the human estrogen receptor alpha (ERα) using Genetic Optimisation of Ligand Docking (GOLD)[54] have shown that compounds substituted at position 2 of the steroid nucleus do not fit well, as the 2-substituent disrupts key hydrophobic interactions in the ERα steroid binding site. This gives an in silico prediction that compounds such as **3** and **4** designed herein would not be strongly estrogenic, as similar docking studies of the binding orientation in ERα have previously proven predictive in the identification of ERα-selective agonists using the same crystal structure.[55] We have shown these in silico predictions to agree with the estrogenicity measured in vitro for compounds similar to **64**.[40]

These initial findings led us to investigate possible combinatorial approaches to explore the estrone template in library design to gain a better understanding of the structure–activity relationships (SAR) for the inhibition of 17β-HSD1. The aim was to discover novel potent compounds that possess 17β-HSD1 inhibitory activities with low estrogenicity. In particular, such an expanded study would enable us to determine the preferred binding modes of **1** and **2** (Figure 1) with 17β-HSD1. The syntheses of 2-ethyl-substituted phenol and sulfamate libraries **3** and **4** (Figure 1) illustrate our efforts to identify novel compounds to investigate the biological significance of 17β-HSD1 inhibition and to evaluate the estrogenic potential of these classes of compounds prior to their pre-clinical evaluation as anticancer drugs.

Chemistry

The synthetic routes to compounds similar to **3** and **4** with a sulfamate linker have been adapted to our strategy by using 2-chlorotrityl chloride resin as shown in Schemes 1 and 2.[47,56] The sulfamate group can be used as an anchoring group or a linker for the solid-phase synthesis of two families of compounds, namely sulfamates and phenols.[47] These routes were used to synthesise small focused libraries (20 and 11 members) of 16-substituted estrone derivatives and modified E-ring steroidal pyrazoles, respectively, to explore the potential design of larger libraries, and to establish SAR. In addition, these smaller libraries were developed to evaluate the scope of the reaction conditions by using various electronically and sterically diverse, commercially available amine building blocks (Tables 1, 2, and 3). By using the hybrid route described (Schemes 1 and 2), we were able to introduce two points of molecular diversity in the target compounds. Further points of diversity may also be introduced on the pyrazole E-ring nitrogen atoms to extend the pyrazole library by, for example, alkylation or acylation to probe hydrogen-bond donor or acceptor, charge-transfer, or dipolar interactions in this region.

Solid-phase synthesis of estrone derivatives functionalised at position 16 containing alkyl amide moieties in the side chain (compounds 3)

The 2-ethylestrone **5** was synthesised in good yield according to a published protocol.[52] Compound **5** was then benzylated to obtain a 3-*O*-benzylestrone intermediate, which was then alkylated at position 16 through enolization by following a modified published procedure on a similar estrone derivative to give the 3-*O*-benzyl-protected ethyl ester **6** in an overall yield of 95 % (Scheme 1).[57] A new chiral centre at position 16 was generated during the formation of **6**, and the resulting diastereomeric mixture was carried through the synthetic route described. The ratio of diastereomers at position 16 was 75:25, as determined by ^{1}H NMR spectroscopy, and the major product was shown to have the 16β configuration. Derivative **6** was sequentially reacted with H_2, Pd/C in methanol/THF to remove the benzyl group, and the product was sulfamoylated using sulfamoyl chloride in DMA to give the sulfamate **8** in an overall yield of 97 %. This library precursor **8** was then loaded onto 2-chlorotrityl chloride resin as a solid support to give the intermediate **9** with high loading (0.78 mmol g^{-1}), under standard conditions (*N,N*-diisopropylethylamine (DIPEA) in CH_2Cl_2).[46] In the loading step it was noted that the resin changed colour from pale yellow to dark yellow. The resin-bound sulfamate ester **9** was then hydrolysed by using NaOH in THF/water to generate the resin-bound acid **10**. At this point, the loading was determined by rinsing the intermediate acid **10** (0.200 g) with CH_2Cl_2/TFA (50:50, 10 mL) for 15 min, after which the sulfamate **11 A** was isolated. Nucleophilic cleavage of the intermediate **10** was also validated with piperazine in THF at reflux using the conditions reported to obtain the phenol **11 B**.[46] The resin-bound sulfamate acid **10** was then coupled with a diverse series of commercially available amines using PyBOP and HOBt to provide resin-bound amides **12**. This series of amides **12** was allowed to react with piperazine to give the target phenol library **13** in moderate to good yields (40–60 %) after chromatography. Treating the resin-bound amides **12** with TFA/CH_2Cl_2 gave the sulfamate library **14** with an average yield of 50 %. The final compounds were synthesised rapidly due to the ease of the work-up procedures in the solid-phase reactions. Libraries **13** and **14** were purified using chromatography over SiO_2 and characterised by ^{1}H NMR, LRMS,

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Scheme 1. Reagents and conditions: a) Benzyl bromide, K_2CO_3, DMF, RT; b) LDA, THF, ethyl bromoacetate, $-78\,^{\circ}C \rightarrow RT$; c) H_2, Pd/C, MeOH/THF (1:1 *v/v*), RT; d) sulfamoyl chloride, $0\,^{\circ}C \rightarrow RT$; e) 2-chlorotrityl chloride resin, DIPEA, CH_2Cl_2, 72 h; f) NaOH, THF/H_2O (1:1 *v/v*), RT; g) EDC, HOBt, PyBOP, DIPEA, CH_2Cl_2, amine, RT, 15 h; h) TFA/CH_2Cl_2 (1:1 *v/v*); i) piperazine, THF, 65–75 °C. Compounds **11 A** and **11 B** present as a 75:25 diastereomeric mixture at position 16 (^1H NMR) with the 16β diastereomer as the major product. Compound **25** (Table 1, a member of the phenol library **13**) was isolated as the 16β diastereomer. DMF = *N*,*N*-dimethylformamide, EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide, LDA = lithium diisopropylamide.

and HRMS. The compounds synthesised are a mixture of diastereomers at position 16 in a ratio of 75:25, as determined by ^1H NMR spectroscopy. The purities of the individual compounds were determined by HPLC analysis (Table 1) to provide the target compounds with average purities ~95%.

Solid-phase synthesis of novel E-ring-modified estrone pyrazole derivatives containing alkyl amide moieties in the side chain (compounds 4,$R^1 = SO_2NH_2$)

The synthesis of the novel pyrazole library **4** (Figure 1) with a sulfamate group at position 3 of the scaffold on a solid support was carried out in a similar manner to the libraries prepared in the preceding section. The solid-phase precursor pyrazole ester **38** was synthesised in high yield as shown in Scheme 2. Condensation of 3-*O*-benzyl-protected 2-ethylestrone **35** with diethyl oxalate in the presence of alkoxide has been reported in the literature, and the subsequent formation of pyrazole E-ring systems using hydrazine monohydrate is well known.[58] Thus, **36** was condensed with hydrazine monohydrate in ethanol at room temperature to give a hydrated cyclic product, and addition of *p*-toluenesulfonic acid monohydrate followed by heating for 5 min gave the desired pyrazole **37** in good yield. The remaining deprotection and sulfamoylation reactions were slightly more problematic, as the debenzylation of **37** with Pd/C (5% wt.) in methanol is slow, which is probably a result of steric hindrance by the adjacent ethyl group, but the reaction was high-yielding and complete over a period of 2–5 days to give 85–90% yield of the desired product **38**. Sulfamoylation of the phenol intermediate **38** by using sulfamoyl chloride in DMA gave the desired precursor **39** in 69% yield.

The loading of the precursor **39** onto 2-chlorotrityl chloride resin was performed by the same method as described for compound **9** to give compound **40**. Validation of the saponification reaction that yields **41** was carried out with a small-scale hydrolysis using NaOH followed by TFA-promoted cleavage to give the acid **42**. This showed that saponification was complete after 24 h, and the loading was calculated as 0.8 mmol g^{-1}. Coupling of **41** to form amides was not successful with DMF as the solvent but proceeded in CH_2Cl_2 to give the resin-bound library **43**. Removal of the 2-chlorotrityl group using TFA in CH_2Cl_2 gave the library **44** with an average overall yield after purification of 12%. The yields are lower than those

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Table 1. HPLC purity and biological activity of 16-substituted estrone derivatives.[a]

Compd	R^1	R^2	Purity [%][b]	Inhibition [%][c]
15	SO_2NH_2		95	64 at 10 μM (5.1 μM[d])
16	SO_2NH_2		91	IA[e]
17	SO_2NH_2		99	37
18	SO_2NH_2		91	50
19	SO_2NH_2		98	25
20	SO_2NH_2		90	43
21	SO_2NH_2		89	41
22	SO_2NH_2		95	15
23	SO_2NH_2		92	21
24	SO_2NH_2		97	94
25	OH		99	94 (27 nM[d])
26	OH		88	89 (165 nM[d])
27	OH		95	45 (3.62 μM[d])
28	OH		99	57 (3.31 μM[d])
29	OH		99	35 (1.31 μM[d])
30	OH		95	IA[e]
31	OH		99	IA[e]
32	OH		99	IA[e]
33	OH		90	IA[e]
34	OH		84	IA[e]

[a] The average yield was 50%. [b] Purity after chromatography. [c] At 1 μM unless stated otherwise; data reflect the mean of at least two measurements with SD typically ±5% on a 75:25 (α/β) mixture of diastereomers. [d] IC_{50} value. [e] IA = inactive with <10% inhibition at 1 μM.

Table 2. HPLC purity and biological activity of modified E-ring estrone derivatives.[a]

Compd	R	Purity [%][b]	Inhibition [%][c]
45		99	17
46		97	12
47		95	20
48		98	15
49		99	IA[d]
50		88	IA[d]
51		91	NT[e]
52		93	NT[e]
53		93	NT[e]
54		98	NT[e]
55		98	NT[e]

[a] The average yield was 12%. [b] Purity after chromatography. [c] At 1 μM unless stated otherwise; data reflect the mean of at least two measurements with SD typically ±5%. [d] IA = inactive with <10% inhibition at 1 μM. [e] Not tested.

for compounds obtained as shown in Scheme 1, suggesting that the pyrazole acid, which is conjugated, is much less reactive than the acid in the side chain of position 16 (compound **10**), for which decreased activity due to conjugation is not applicable (Scheme 1). The yields are, however, similar to those for the corresponding solution-phase amide couplings of the phenolic starting material with the use of EDC, DMAP, and NEt_3 in CH_2Cl_2, indicating that the reactivity in these series is decreased.

Solution-phase synthesis of novel E-ring-modified estrone pyrazole derivatives containing alkyl amide moieties in the side chain (compounds 4,R^1 = H)

As there were some problems with the sulfamate pyrazole library **4** using SPOS, it was decided that a small focused pyrazole library be made in solution with a phenol moiety at posi-

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Scheme 2. Reagents and conditions: a) $(CO_2Et)_2$, KO*t*Bu, RT; b) $NH_2NH_2 \cdot H_2O$, EtOH, RT; c) H_2, Pd/C (5% wt), MeOH, RT; (d) H_2NSO_2Cl, DMA; e) 2-chlorotrityl chloride resin, DIPEA, CH_2Cl_2, RT, 3 days; f) NaOH, THF/H_2O, RT, 24 h; g) R_1R_2NH, HOBt, PyBOP, DIPEA, CH_2Cl_2, RT 3 days; h) TFA/CH_2Cl_2 (1:1 *v*/*v*). DMA = *N*,*N*-dimethylacetamide.

tion 3 (Scheme 3). The set of C5′ side-chain substituents chosen was based on side chains from active compounds synthesised using the estrone template (Table 3).

2-Ethylestrone was initially protected as a 3-*tert*-butyldimethylsilyl ether **56** and subsequently functionalised at C16 by reaction with diethyl oxalate in toluene in the presence of potassium *tert*-butoxide to give **57**, as described previously (Scheme 3).[4] Condensation of **57** with hydrazine monohydrate in ethanol gave **58**, which upon treatment with aqueous sodium hydroxide in methanol afforded **59.** The overall yield for compounds **56**–**59** was 51%. Diversity was then introduced by amide coupling to **59** after activation of the carboxylic acid moiety with EDC in CH_2Cl_2, followed by treatment with the required amine in the presence of DMAP. After purification by flash chromatography, the amides **60**–**66** were isolated with the average yield of 44%.

Results and Discussion

As we reported in a preliminary communication, the most potent compound from library **3** was **25**, which showed similar potency to its 2-desethyl analogue **1**.[39] Compounds active in

Scheme 3. Reagents and conditions: a) $(EtOCO)_2$, *t*BuOK, toluene; b) $NH_2NH_2 \cdot H_2O$, EtOH; c) NaOH (aq), MeOH, Δ; d) amine, EDC, DMAP, CH_2Cl_2. DMAP = 4-dimethylaminopyridine.

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Table 3. HPLC purity and biological activity of modified E-ring estrone derivatives.[a]

Compd	R	Purity [%][b]	Inhibition [%][c]
60		97	1.97 μм[d]
61		97	68
62		98	0.7 μм[d]
63		92	13
64		92	32
65		94	19
66		98	27

[a] The average yield was 12%. [b] Purity after chromatography. [c] At 1 μм unless stated otherwise; data reflect the mean of at least two measurements with SD typically ±5%. [d] IC_{50} value.

this series and others were also shown to be selective over 17β-HSD2.[39–41] The X-ray crystal structure of compound **25** confirms the β orientation of the *m*-pyridylmethylamidomethyl side chain at position 16 (Figure 2). Interestingly, compound **15**, the sulfamate analogue of **25**, is ~190-fold less active with an IC_{50} value of 5.1 μм, indicating that favourable interactions of the 2- and 3-substituents on the steroid template with key amino acids in the active site have been diminished. This may not be surprising, as the 2-ethyl and 3-sulfamoyl groups are in close proximity in a region where hydrogen bonding to His 221 is known to be important for activity. Recently, it was shown that the 3-hydroxy group could be replaced with a methoxy moiety to give potent 17β-HSD1 inhibitors, indicating that a hydrogen-bond donor at position 3 is not essential.[37] The rationale for introducing a sulfamate group at position 3 was threefold. First, the tolerance in this region is explored by replacing the hydroxy group with a larger hydrogen-bond donor/acceptor group. Second, as we have previously shown that steroidal sulfamates bind to carbonic anhydrase II (CA II) in red blood cells in a reversible fashion, we reasoned that this may be a mechanism whereby they can be transported to tumours and released in the more acidic environment of the tumour.[59] Hence, a steroidal sulfamate that inhibits 17β-HSD1 may be delivered to the tumour bound to CA II and released at its site of action. Third, even if the sulfamates themselves are only weak 17β-HSD1 inhibitors, it is likely that steroid sulfatase will cleave the sulfamate group in vivo to some degree to release the corresponding phenols, which are potent 17β-HSD1 inhibitors.[7] Generally, the sulfamate analogues in this series show weak to moderate activity and this trend is also observed in **45**, a member of the pyrazole library. This loss of activity may either be due to the sulfamate group being unable to form important hydrogen-bond interactions with His 221 and Glu 282 or to weaker interactions in this region than with the corresponding phenol. In addition, as the sulfamate group is larger than the phenol moiety, some of the hydrophobic interactions of the 2-ethyl group with lipophilic amino acid residues on the protein may be disrupted. An exception to this is compound **24**, which shows 94% inhibition at 10 μм, unlike its corresponding phenol derivative, **33**, which is inactive at 1 μм. Compound **33** has a tertiary amide in the side chain at position 16 of the steroid template and consequently, it may bind differently than **24** and disrupt interactions that are important for activity. Compound **26**, which has an *o*-pyridylmethylamidomethyl side chain at position 16, is a potent 17β-HSD1 inhibitor with an IC_{50} value of 165 nм. The 2-desethyl analogue of **26** has also been shown to be a potent inhibitor of 17β-HSD1 with selectivity over 17β-HSD2.[41] In general terms, the SAR of the phenol library is similar in the 2-ethyl series and the 2-desethyl series.[41] In the phenol series, compounds **25**–**29** containing an aryl or heteroarylmethylamidomethyl side chain at position 16 were potent inhibitors of 17β-HSD1. Compound **27** with a substituted pyrimidine group showed an IC_{50} value of 3.62 μм and a significant drop in inhibitory activity in comparison with compounds **25** and **26**, which contain pyridine groups in the side chain. Compounds **28** and **29**, with defined chirality in the

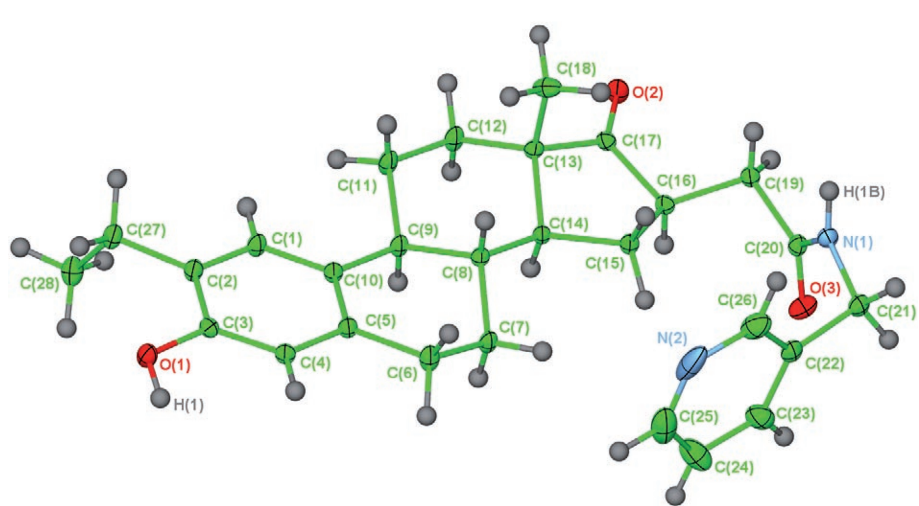

Figure 2. ORTEX[62] plot of the X-ray crystal structure of **25**. Ellipsoids are shown at the 30% probability level.

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α-methylbenzylamidomethyl side chain at position 16, showed similar activities with IC_{50} values of 3.31 μM and 1.31 μM, respectively. The points above show how sensitive this region is to small changes in the structure of the inhibitor and they also indicate the importance of binding interactions between the inhibitors and the protein and/or the cofactor in this region. This is clearly demonstrated by the sixfold difference in activity between compounds **25** and **26** in which the pyridyl nitrogen atom is moved by one position, and the 134-fold difference in activity between compounds **25** and **27** with the introduction of another nitrogen atom and a methyl group into the pyridine ring. Compounds **30**–**34**, which contain an alkyl or dialkylamidomethyl side chain at position 16, are inactive when tested at 1 μM. This indicates that the combination of the 2-ethyl group with these side chains is not additive. In the 2-desethyl series, the analogues of **30**, **31**, **33**, and **34** showed good inhibition of 17β-HSD1 (75–85%) and selectivity over 17β-HSD2 when tested at 10 μM. The added hydrophobic interactions with the 2-ethyl group may result in a change of the inhibitor binding conformation, which results in the loss of more favourable interactions with the 16-position side-chain substituents in the 2-desethyl analogues of **30**–**34** and subsequent loss of activity.

From the sulfamate and phenol libraries, for which estrone was used as the template, compound **25** with the *m*-pyridylmethylamidomethyl side chain at position 16 remains the most potent compound (IC_{50} = 27 nM) and may be the optimal 16β-substituent for this template. The crystal packing in the unit cell is depicted in Figure 3. The six molecules of compound **25** show hydrogen-bond interactions between the C17 keto oxygen atom and the hydrogen atom of the C3 hydroxy moiety. The C16 *m*-pyridylmethylamidomethyl side chain amide group also shows intermolecular hydrogen bonding with the amide carbonyl oxygen atom of one molecule to the amide NH of another. This type of hydrogen bond may be indicative of the interactions of the C16 side-chain amide interactions with key amino acid residues in the active site of 17β-HSD1.

The current synthesis of **25** results in a mixture of diastereomers, and careful chromatography is required to isolate **25**. The synthesis of this compound necessitated improvement, as quantities of **25** are required for in vivo evaluation. A much improved diastereoselective synthesis was devised and is outlined in Scheme 4. This initially involves reaction of 2-ethylestrone **5** with ethyl glyoxalate in aqueous methanolic sodium hydroxide to give **67**. A THF and methanolic solution of **67** was then treated with hydrogen by using Pd/C (5%) as a catalyst to give **68** as a single diastereomer, a consequence of forced hydrogen delivery in a *cis* manner from the least hindered face opposite the C18 methyl group. The overall yield of **68** from **5** was 64% when performed on a multi-gram scale. An amide coupling of **68** after activation under our previously described conditions with 3-aminomethylpyridine gave **25**.

Scheme 4. Reagents and conditions: a) EtOCOCHO, NaOH (aq), MeOH; b) H_2, Pd/C, THF/MeOH; c) amine, EDC, DMAP, NEt_3, CH_2Cl_2.

The 2-ethyl-3-sulfamoyl-modified C5′-linked amides **45**–**55** (Table 2) were essentially inactive in tests for the inhibition of 17β-HSD1 at 1 μM. It appears that, even with the variation of the C5′-amide side chains, a combination of these substituents is detrimental. This concurs with findings we described earlier for the 2-ethyl-3-sulfamoyl series with position 16 substituted on the estrone template (compounds **16**–**23**). As already discussed, the close proximity of a 3-sulfamoyl substituent to the 2-ethyl group most likely disrupts essential binding interactions of the inhibitor candidate to important amino acid residues in the active site of the protein. The inhibitory activity against 17β-HSD1 is restored when the 3-sulfamoyl group is removed, resulting in a phenolic group at position 3 (Table 3). The best inhibitors in this series were obtained with compounds containing a pyridyl side chain, namely **60**–**62**. The position of the heteroatom was found not to be crucial for the activity and the 3-pyridyl analogues **60** and **62** are more potent than the respective corresponding 2-pyridyl analogues **61** and **63**. The activities observed in this series are in agreement with those noted previously in the 2-desethyl

Figure 3. Hydrogen-bonded sheet formation in the structure of compound **25**.

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series,[40] and homologation of the side chain of **60** (IC_{50} = 1.97 μM) by one carbon atom gave **62**, the most potent inhibitor of this series with an IC_{50} value of 700 nM. For the library in which 2-ethylestrone is functionalised at position 16, we found that a *m*-pyridylmethylamidomethyl side chain substitution gave the most potent compound **25**, but that the same side chain at the C5′ position of the pyrazole series is not optimal (compound **60**). Overlays of these compounds indicate that homologation of **60** with the *m*-pyridylmethylamidoethyl side chain of **62** provides a better comparison with **25**. This is confirmed by docking **60** and **62** into the active site of human 17β-HSD1; **62** has a higher docking score and shows better interactions with the protein and cofactor, which explains its improved activity over **60.**

Molecular modelling

Compound **62**, with a 2-ethyl group on the steroid template, was identified as the most potent inhibitor of 17β-HSD1 from our pyrazole library, showing 89% inhibition at 1 μM and an IC_{50} value of 700 nM. The crystal structure of 17β-HSD1 in complex with E2 and NADP (PBD code 1FDT) has previously been used by our group in docking studies of potent 17β-HSD1 inhibitors to identify likely binding modes.[39,40] To compare the proposed binding mode of pyrazole **62**, it was docked in a similar manner into 1FDT with E2 removed using the flexible docking programme GOLD.[54] The most highly ranked conformation had a high GOLD score of 78 and this is depicted in Figure 4. The 2-ethyl group of **62** is clearly in a hydrophobic region of the active site and is likely to interact with amino acid residues Met 147, Leu 149, Phe 259, and Leu 262 which are, respectively, 3.3 Å, 3.7 Å, 3.6 Å, and 3.3 Å away from the closest interaction point of the 2-ethyl group in this docked conformation. With no substitution at position 2, these added interactions are absent, and this compound has been shown previously to have an IC_{50} value of 300 nM.[40] The phenol moiety on the steroid template is predicted to form a hydrogen bond with His 221 1.8 Å away and this is also observed with the substrate. The amide at the C5′ position on the pyrazole E-ring appears over the amide of the nicotinamide group and could form π-stacking interactions, as the carbonyl groups of both the inhibitor and the cofactor are ~3 Å apart. The N1′ and N2′ positions of the pyrazole E-ring are 1.9 Å and 2.1 Å from the Pro-*S* hydrogen atom on the nicotinamide ring, and thus, compound **62** acts as an inhibitor of 17β-HSD1. The pyridylethyl group on the side chain from the C5′ position of the pyrazole in **62** extends to the phosphate backbone region of the cofactor. The pyridyl nitrogen atom is ~2.8 Å from the first oxygen link of the phosphate chain from the ribose ring attached to the nicotinamide ring. The pyridyl nucleus positions itself toward the phosphate backbone between the two ribose rings of the cofactor and is thereby able to interact with the cofactor to aid binding.

Compound **62** was docked into the crystal structure (PDB code 3ERD)[60] of the ligand-binding domain in human ERα using GOLD. Poor docking scores indicated that **62** did not fit well in ERα; the maximum score observed was 22. This gives an in silico prediction that **62** would most likely not be strongly estrogenic. Similar docking studies that examine the orientation of binding in ERα have previously proven predictive in identifying ERα-selective agonists using the same crystal structure.[40,61]

Conclusions

The parallel synthesis of novel focused libraries of 16-substituted estrone derivatives and new modified E-ring steroids that contain a pyrazole E-ring has been carried out using SPOS and solution-phase parallel synthesis. The application of this chemistry to the estrone template has resulted in four novel series of diverse 2-ethylestrone derivatives, thus extending the knowledge of the modification of steroid scaffolds in this region. The SPOS of sulfamate and phenol libraries with a multi-detachable linker in these series can now be adapted to larger library design using the estrone scaffold in this ongoing programme. As the inhibition of 17β-HSD1 is currently of significant interest as a therapeutic target for breast cancer, the above strategy was implemented to identify novel inhibitors of 17β-HSD1. Among the 16-substituted estrone derivatives synthesised, the most potent 17β-HSD1 in-

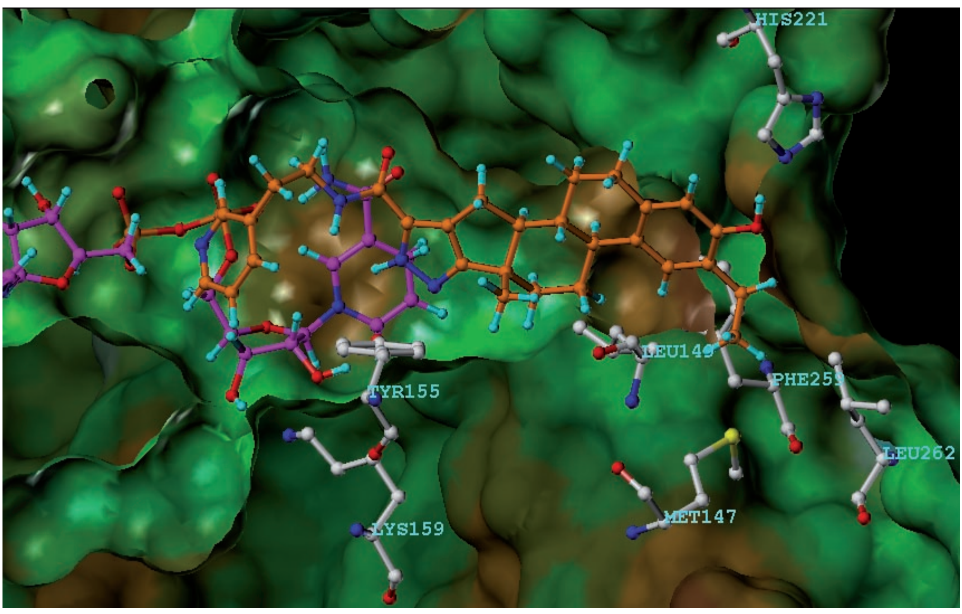

Figure 4. Compound **62** (brown) docked in 17β-HSD1 (PDB code 1FDT), with the NADP cofactor shown in pink and active site amino acids in grey.

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hibitors were **25** and **26** with IC_{50} values of 27 and 165 nм, respectively, in T-47D human breast cancer cells. Parallel synthesis resulting in a library of C5′-linked amides from the pyrazole E-ring of the template led to the identification of **62** with an IC_{50} value of 700 nм. The activities for the 2-ethyl-substituted steroids described herein compare well to their 2-desethyl analogues described earlier. This indicates that a 2-position substituent on the estrone template is tolerated in the active site of 17β-HSD1 and indeed, in the case of the 2-ethyl substituent, may pick up some added hydrophobic interactions. Conversely, a 2-ethyl substituent on the estrone template gives compounds that do not dock to the active site of ERα as described previously in a predictive in silico method for assessing estrogenicity.[39,40] As the 2-ethyl group in these novel series is in close proximity to the 3-phenolic moiety, potential conjugation to this group, a common metabolic issue with phenolic compounds, is likely to be retarded. Several novel 17β-HSD1 inhibitors emerged from these libraries and have provided a clear direction for further novel template exploration in this area. A new efficient diastereoselective synthesis of **25** has been developed for the supply of **25** for in vivo evaluation.

Experimental Section

General: All chemicals were purchased from Aldrich Chemical Co. (Gillingham, UK) or Lancaster Synthesis (Morecambe, UK). All organic solvents of A. R. grade were supplied by Fisher Scientific (Loughborough, UK). E1 was purchased from Sequoia Research Products (Oxford, UK). Reactions using anhydrous solvents were carried out under nitrogen.

Thin layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminium sheets silica gel 60 F_{254}). Product(s) and starting material(s) were detected by either viewing under UV light and/or treating with an ethanolic solution of phosphomolybdic acid followed by heating. Flash column chromatography was performed on silica gel (Sorbsil/Matrex C60) or using Argonaut prepacked columns with a FlashMaster II. ^1H NMR (270 MHz or 400 MHz) spectra were recorded with a Jeol Delta 270 or a Varian Mercury VX 400 NMR spectrometer, and chemical shifts are reported in parts per million (ppm, δ). HPLC analyses were performed on a Waters Millenium 32 instrument equipped with a Waters 996 PDA detector and a Symmetry C18 reversed-phase column (4.6× 150 mm) eluting with $MeCN/H_2O$ at 0.3 mL min^{-1}. FAB low- and high-resolution mass spectra were recorded at the Mass Spectrometry Service Center, University of Bath, with *m*-nitrobenzyl alcohol (NBA) as the matrix. ES and APCI low-resolution mass spectra were obtained on a Waters Micromass ZQ. Accurate mass determinations were also performed at the ESPRC national mass spectrometry service centre at the University of Wales Swansea using Finnigan MAT900 or MAT95 instruments. Melting points were determined with a Reichert-Jung Thermo Galen Kofler block and are uncorrected. X-ray crystallographic studies on compound **25** were carried out on a Kappa CCD diffractometer with area detector.

Molecular modelling: For the docking studies of compound **62**, the starting conformations used for receptor docking were generated from an energy minimisation performed with the MMFF94s force field as implemented in Sybyl 7.0. The resulting lowest-energy conformer was then used for docking studies. Charge calculations were determined by using the MMFF94s method, and GOLD version 2.2 was then used with default parameters to perform the docking studies. The active site was defined as a radius of 12 Å around the C^{α} atom of Ser 142, and 30 attempts were computed and scored using Gold Score.

X-ray crystallography: *crystal data*: $C_{28}H_{34}N_2O_3$, $M=446.57$, $\lambda=0.71073$ Å, monoclinic, space group $P2_1$, $a=11.1300(2)$, $b=9.1770(2)$, $c=11.9820(2)$ Å, $\beta=95.213(1)^{\circ}$, $U=1218.78(4)$ Å^3, $Z=2$, $D_c=1.217$ Mg m^{-3}, $\mu=0.079$ mm^{-1}, $F(000)=480$, crystal size 0.45× 0.40×0.40 mm^3, 7000 unique reflections (R(int)=0.0352), observed $I>2\sigma(I)=6118$, data/restraints/paramaters=7000/4/315, $R1=0.0427$ $wR2=0.1082$ (obs. data), $R1=0.0520$ $wR2=0.1135$ (all data), max peak/hole 0.326 and -0.201 e Å^{-3}; software used: ORTEX,[62] SHELXS,[63] and SHELXL.[64] CCDC 287118 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Biology: Radiolabeled E1 and E2 (^3H and ^{14}C) were purchased from New England Nuclear (Boston, MA, USA) or Amersham Biosciences UK Limited (Amersham, UK).

T-47D and MDA-MB-231 cells have previously been shown to possess predominantly reductive or oxidative 17β-HSD activity, respectively.[65]

Inhibition of 17β-HSD type 1: T-47D human breast cancer cells were incubated with [^{3}H]E1 at a concentration of 2 nм in a 24-well tissue culture plate in the absence or presence of the inhibitor (0.1 nм–10 μм). After incubation of the substrate ±inhibitor (30 min, 37 °C), the products were isolated from the mixture by extraction with Et_2O (4 mL), using [^{14}C]E2 (5000 dpm) to monitor procedural losses. Separation of [^{3}H]E2 from the mixture was achieved using TLC (CH_2Cl_2/EtOAc, 4:1 *v*/*v*) and the mass of [^{3}H]E2 produced was calculated from the ^{3}H counts detected and recovery of [^{14}C]E2.

Inhibition of 17β-HSD type 2: MDA-MB-231 human breast cancer cells were incubated with [^{3}H]E2 at a concentration of 2 nм in T25 flasks in the absence or presence of the inhibitor (0.1 nм–10 μм). After incubation of the substrate ±inhibitor (3 h, 37 °C), the products were isolated from the mixture by extraction with Et_2O (4 mL), using [^{14}C]E1 (5000 dpm) to monitor procedural losses. Separation of [^{3}H]E1 from the mixture was achieved using TLC (CH_2Cl_2/EtOAc, 4:1 *v*/*v*) and the mass of [^{3}H]E1 produced was calculated from the ^{3}H counts detected and recovery of [^{14}C]E1.

Chemistry:

Ethyl(2-ethyl-3-benzyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-yl) acetate (6): A solution of LDA (13.9 mmol, 7.7 mL, 1.8 м in heptane, THF, and ethylbenzene) was added to a stirred solution of 3-*O*-benzyl-2-ethylestrone **35** (5.0 g, 12.8 mmol) in anhydrous THF (100 mL) under inert conditions at −10 °C over a period of 20 min. The reaction mixture was cooled to −60 °C and stirred for 15 min before ethyl bromoacetate (1.85 mL, 16.8 mmol) was added dropwise over 10 min. The reaction was allowed to warm to room temperature overnight (approximately 15–18 h) before the crude mixture was diluted with CH_2Cl_2. Saturated NH_4Cl was added, and the products were extracted with CH_2Cl_2. The combined organic phases were dried and concentrated to obtain a yellow solid which was then purified with flash chromatography (50 g column, CH_2Cl_2/MeOH, gradient elution) to obtain the pure product **6** (6.101 g, 99 % yield) as a pale yellow solid: mp=92–95 °C; ^1H NMR (270 MHz, $CDCl_3$): $\delta=0.869$ and 0.978 (2×s, 3 H, 18-CH_3), 1.17–1.23 (t, $J=7.6$ Hz, 3 H, CH_2-CH_3), 1.24–1.29 (t, $J=7.1$ Hz, 3 H, CH_2-CH_3), 1.32–2.59 (broad m, 12 H), 2.62–2.70 (q, $J=7.4$ Hz, 2 H, 2-CH_3-CH_2),

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2.78–3.03 (broad m, 2H, 6-CH_2), 4.11–4.19 (q, J=7.17 Hz, 2H, CH_3-CH_2-O), 5.0 (s, 2H, CH_2-phenyl), 6.63 (s, 4-CH), 7.09 (s, 1-CH), 7.30–7.45 ppm (m, 5H); HPLC: 96% (t_R=7.25 min, 70% MeCN/water); FAB MS [M^+]: 474 m/z; FAB HRMS calcd for $C_{31}H_{38}O_4$: 474.2770, found [M^+]: 474.2694.

Ethyl(2-ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-yl) acetate (7): A solution of benzyl ester **6** (2.80 g, 5.9 mmol) in THF/MeOH (1:1, 40 mL) was degassed by bubbling nitrogen through the mixture) for 20 min. Addition of 5% Pd on C (10% wt, 300 mg) and debenzylation was carried out using a balloon of H_2 for 24 h. The completion of the reaction was monitored by TLC (EtOAc/hexane, 90:10). The crude mixture was filtered using a pad of celite, concentrated to obtain the debenzylated product **7** (2.21 g, 97%) as a colourless solid after purification using flash chromatography (50 g column, EtOAc/hexane gradient elution): mp=97–102°C; ^{1}H NMR (270 MHz, $CDCl_3$): δ=0.85 and 0.96 (2×s, 3H, 18-CH_3), 1.17–1.28 (2×t overlapped, 6H, 2×CH_2-CH_3), 1.49–2.49 (m, 12H), 2.54–2.62 (q, J=7.4 Hz, 2H, 2-CH_3-CH_2), 2.72–3.02 (m, 4H), 4.11–4.16 (q, J=7.1 Hz, 2H, CH_3-CH_2-O), 4.54 (s, 1H), 6.50 (s, 1H, 4-CH), 7.03 ppm (s, 1H, 1-CH); HPLC: >97% (t_R=6.53 min, 70% MeCN/water); (−ve)ES MS [$M-H^+$]: 383 m/z; FAB HRMS calcd for $C_{24}H_{32}O_4$: 384.2301, found [M^+]: 384.2289.

Ethyl(2-ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-yl) acetate (8): A solution of sulfamoyl chloride (57 mL, 0.68 M solution in toluene) was evaporated (below 30°C) using a rotary evaporator. The brown oil obtained was cooled to 0°C under an inert atmosphere, dissolved in anhydrous DMA (30 mL), and treated dropwise with **7** (2.00 g, 5.20 mmol) in anhydrous DMA (30 mL) using a cannula. The reaction mixture was left to stir at 0°C and warmed to room temperature overnight (15 h). Reaction completion was gauged by TLC (CH_2Cl_2/MeOH, 98:2). The crude mixture was diluted with EtOAc and washed with water (5×30 mL) and brine (30 mL). The organic phase was dried and concentrated to obtain a pale yellow solid which was purified using flash chromatography (CH_2Cl_2/MeOH, gradient elution, 50 g column) to obtain the pure product **8** (5.901 g, 98%) as a colourless foamy solid: mp=110–112°C; ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ=0.81 and 0.92 (2×s, 3H each, 18-CH_3), 1.09–1.20 (2×t, J=4.9 and 7.4 Hz, 6H, 2×CH_2-CH_3), 1.33–2.47 (m, 12H), 2.58–2.66 (q, J=7.6 Hz, 2H, 2-CH_2-CH_3), 2.89–2.97 (m, 3H), 4.04–4.09 (q, J=6.9 Hz, 2H, 2-CH_3-CH_2) 7.02 (s, 1H, 4-CH), 7.21 (s, 1H, 1-CH), 7.95 ppm (s, 2H, SO_2NH_2); HPLC: 90% (t_R=5.90 min, 70% MeCN/water); (−ve)ES MS [$M-H^+$]: 462 m/z; FAB HRMS calcd for $C_{24}H_{34}NO_6S$: 464.2062115, found [M^++H^+]: 464.2115.

Synthesis of resin-bound derivative of ethyl(2-ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-yl) acetate (9): The 2-chlorotrityl chloride resin (2.60 g, Novabiochem, loading 1.1 mmol g^{-1}) was allowed to swell in anhydrous CH_2Cl_2 (25 mL) under an inert atmosphere and treated with estrone sulfamate **8** (2.00 g, 4.31 mmol) and DIPEA (7.50 mL, 43.1 mmol). The crude mixture was shaken for approximately 75 h. Acidic mini-cleavage (50% TFA/CH_2Cl_2, 20 min) of a sample from the reaction confirmed the completion of the reaction. The crude mixture was then filtered, washed with DMF, CH_2Cl_2, and MeOH (5 cycles each), and finally with MeOH (3 cycles). This solid-bound intermediate was dried under vacuum to a constant weight to obtain **9** (3.78 g, loading 0.78 mmol g^{-1}, dark yellow) as determined by a mini-cleavage of **9** (50 mg) to give **8** (18 mg, 0.039 mmol). The ^{1}H NMR and other analytical data of the cleaved compound were the same as those reported for compound **8** above. Compound **9** was stored in a refrigerator until taken to the next stage.

Synthesis of resin-bound library precursor 2-ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-yl) acetic acid (10): Resin-bound **9** (6.00 g) was allowed to swell in THF (60 mL), and an aqueous solution of NaOH (4 M, 7 mL) was added slowly. The mixture was shaken at room temperature for approximately 30 h, then filtered and washed with THF/H_2O (1:1, 3×50 mL), THF, CH_2Cl_2, and MeOH (5 cycles each). The resin-bound intermediate was isolated and dried under vacuum to a constant weight to obtain **10** (5.25 g) as yellow beads. Acidic cleavage (50% TFA/CH_2Cl_2, 20 min) of a small sample of **10** confirmed the complete hydrolysis of the ester to the acid. This compound exists as a mixture of diastereomers, and the ^{1}H NMR spectrum of this compound is the same as that reported for compound **11 A** below.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-yl) acetic acid (11 A): Solid-bound **10** (200 mg, 0.154 mmol) was rinsed with a solution of TFA/CH_2Cl_2 (50:50, 10 mL) over a period of 20 min, and the filtrate obtained was evaporated under decreased pressure. The residue was recrystallised from hot methanol to yield **11 A** (50 mg, 74%) as a white solid, a mixture of diastereomers: ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ=0.81 (s, 3H, 18-CH_3), 1.13–1.15 (t, J=7.5 Hz, 2-CH_2CH_3), 1.45–2.50 (m, 13H), 2.57–2.64 (q, 2H, J=7.5 Hz, 2-CH_2CH_3), 2.66–2.83 (m, 2H), 7.22 (s, 1H), 7.03 (s, 1H), 7.96 ppm (s, 2H, NH_2); HPLC: >94% (t_R=1.89 min, 10% water/acetonitrile); (−ve)ES MS: [$M-H^+$], 434 m/z.

2-Ethyl-3-hydroxyoxy-17-oxo-estra-1,3,5(10)-trien-16α/β-yl) acetic acid (11 B): Piperazine (199 mg, 2.31 mmol) was added to a mixture of resin **10** (300 mg, 0.231 mmol) in anhydrous THF (5 mL). The mixture was heated at reflux under N_2 (65°C) for 48 h (without stirring), after which time the mixture was filtered, and the resin was rinsed with MeOH and CH_2Cl_2 (5 cycles each). The combined washings were evaporated under decreased pressure and purified by flash chromatography on silica gel (eluted with 5→15% methanol in CH_2Cl_2; piperazine sticks to baseline) to afford **11 B** (60 mg, 73%) as a white solid: ^{1}H NMR (270 MHz, $CDCl_3$): δ=0.98 and 0.87 (s, 18-CH_3, two diastereomers in approx. 1:3 ratio), 1.21 (t, 3H, J=7.5 Hz, 2-CH_2CH_3), 1.20–1.38 (t, J=7.5 Hz, 3H, 2-CH_2CH_3), 1.45–2.50 (m, 13H), 2.54–2.64 (q, J=7.5 Hz, 2H, 2-CH_2CH_3), 2.79–2.82 (m, 2H), 2.96–2.98 (dd, 1H, J=16.3, 4.2 Hz, 16-H), 6.51 (s, 1H), 7.03 ppm (s, 1H); HPLC: >99% (10% water/acetonitrile); (−ve)ES MS [$M-H^+$]: 356 m/z.

General procedure for the synthesis of the sulfamate library 14: A stock solution of PyBOP [(benzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate] was prepared in 14 mL of CH_2Cl_2, and 2.0 mL of this stock solution was used for each reaction. A stock solution of HOBt (N-hydroxy benzotriazole, 0.185 g per reaction) and DIPEA (diisopropylethyl amine, 0.297 mL per reaction) was prepared in CH_2Cl_2 (9.0 mL), and 1.0 mL of this stock solution was used for each reaction. The resin-bound acid **10** (0.400 g, loading 0.86 mmol g^{-1}) was suspended in CH_2Cl_2 (4.0 mL) under an inert atmosphere in a 25-mL round-bottom flask. PyBOP (0.715 g, 1.37 mmol) was then added followed by the mixture of HOBt and DIPEA, and the requisite amine (4 equiv). The mixture was shaken for approximately 72 h. The crude resins were then filtered and washed with DMF, CH_2Cl_2, and MeOH (5 cycles each) using an Argonaut Flash vacuum manifold and fritted syringes. The washed resins were dried in vacuo, and half of each resin was then transferred to Radleys Green House Synthesiser tubes to generate the phenol library (see below). The remaining half was treated with 50% TFA in CH_2Cl_2 over 20 min and then the TFA and CH_2Cl_2 were removed by evaporation using the Genevac DD4. The products were purified using flash chromatography (Flash Master 2 system, using pre-packed SiO_2 columns (5 or 10 g, CH_2Cl_2/MeOH, gradient

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elution) to generate the sulfamate library **14**. The compounds generated exist as a mixture of diastereomers at position 16 in a ratio of 75:25 as observed by ^{1}H NMR spectroscopy, with the 16β-*R* diastereomer as the major product.

General procedure for the synthesis of the phenol library 13: Resin-bound intermediates **12** (0.200 g, 0.86 mmol g^{-1}) were suspended in anhydrous THF (4 mL) under an inert atmosphere (a Radleys Green House Parallel Synthesiser was used to carry out these reactions), treated with piperazine (0.75 g, 8.7 mmol), and heated at reflux for approximately 70 h. The resins were filtered, and the filtrates were collected into tubes and concentrated using the Genevac DD4 solvent evaporator. These compounds were preabsorbed onto silica gel and purified using flash chromatography (FlashMaster II system, using SiO_2 columns (5 or 10 g), ethyl acetate/hexane, gradient elution) to give the sulfamate library **13**. The compounds generated exist as a mixture of diastereomers at position 16 in a ratio of 75:25 as observed by ^{1}H NMR spectroscopy, with the 16β-*R* diastereomer as the major product.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-(pyridine-3-ylmethyl) amide (15): (TFA salt): ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.45 and 0.56 (2×s, 3H each, 18-CH$_3$), 0.75–0.79 (t, J = 7.3 Hz, 3H, CH$_2$-C*H$_3$*), 1.33–2.47 (m, 12H), 2.26–2.29 (q, J = 7.3 Hz, 2H, C*H$_2$*-CH$_3$), 2.42–2.59 (m, 3H), 3.98–4.00 (m, 2H, 2-CH$_3$-C*H$_2$*), 6.65 (s, 1H, 4-CH), 6.84 (s, 1H, 1-CH), 7.20 (broad s, 1H) 7.52 (broad m, 1H), 7.59 (s, 2H, NH$_2$, disappeared on D$_2$O shake), 8.21–8.17 ppm (m, 3H, aromatic); HPLC: >95% (t_R = 12.13 min, 5→95% MeCN/water); (+ve)ES MS [M+H$^+$]: 526 m/z; FAB HRMS m/z calcd for $C_{28}H_{35}N_3O_5S$: 526.2375, found [M+H$^+$]: 526.2379.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16-methylcarboxylic acid-(pyridine-2-ylmethyl) amide (16): (TFA salt): ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.81 (s, 3H, 18-CH$_3$), 1.09–1.15 (t, J = 7.1 Hz, 3H, CH$_2$-C*H$_3$*), 1.33–2.47 (m, 12H), 2.61–2.64 (q, J = 7.3 Hz, 2H, C*H$_2$*-CH$_3$), 2.82–2.90 (m, 3H), 4.36–4.38 (m, 2H, 2-CH$_3$-C*H$_2$*), 7.02 (s, 1H, 4-CH), 7.21 (s, 1H, 1-CH), 7.28–7.30 (m, 2H, aromatic) 7.52 (broad m, 1H), 7.96 (s, 2H, NH$_2$), 8.48–8.51 ppm (m, 2H, aromatic); HPLC: >91% (t_R = 2.12 min, 70% MeCN/water); (+ve)ES MS [M+H$^+$]: 526 m/z; FAB HRMS m/z calcd for $C_{28}H_{35}N_3O_5S$: 526.2375, found [M+H$^+$]: 526.2374.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-(5-methylpyrimidinyl-2-ylmethyl) amide (17): (TFA salt): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.805 (s, 3H, 18-CH$_3$), 1.10–1.13 (t, J = 7.4 Hz, 3H, CH$_2$-C*H$_3$*), 1.37–2.45 (m, 12H), 2.58–2.62 (q, J = 7.8 Hz, 2H, C*H$_2$*-CH$_3$), 2.80–2.87 (m, 3H), 4.34–4.36 (m, 2H, CH$_3$-C*H$_2$*), 6.99 (s, 1H, 4-CH), 7.18 (s, 1H, 1-CH), 7.92 (s, 2H, NH$_2$), 8.43 (2×s, 2H, aromatic) 8.51–8.84 ppm (t, J = 5.85 Hz, 1H, NH); HPLC: >99% (t_R = 2.0 min, 80% MeCN/water); (+ve)ES MS [M+H$^+$]: 541, [M+H-18$^+$]: 523 m/z; FAB HRMS m/z calcd for $C_{28}H_{36}N_4O_5S$: 541.2484, found [M+H$^+$]: 541.2478.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16-methylcarboxylic acid-*S*-α-methylbenzyl amide (18): (TFA salt): ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.80 and 0.83 (s, 3H, 18-CH$_3$), 1.09–1.14 (t, J = 7.4 Hz, 3H, CH$_2$-C*H$_3$*), 1.32–1.37 (d, J = 6.8 Hz, 3H, CH-C*H$_3$*), 1.39–2.45 (m, 12H), 2.60–2.63 (q, J = 7.6 Hz, 2H, C*H$_2$*-CH$_3$), 2.80–2.81 (m, 3H), 4.95–4.99 (m, 1H, CH$_3$-C*H*), 7.01 (s, 1H, CH), 7.20–7.22 (m, 2H, aromatic), 7.30–7.32 (m, 4H, aromatic), 7.95 (broad m, 2H, aromatic), 8.35–8.39 ppm (broad t, 1H, NH); HPLC: >91% (t_R = 2.0 min, 70% MeCN/water); (+ve)ES MS [M+H$^+$]: 539 m/z; FAB HRMS m/z calcd for $C_{30}H_{38}N_2O_5S$: 539.2579, found [M+H$^+$]: 539.2579.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-(*R*)-α-methylbenzyl amide (19): (TFA salt): ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.80 and 0.83 (2×s, 3H, 18-CH$_3$), 1.09–1.15 (t, J = 7.1 Hz, 3H, CH$_2$-C*H$_3$*), 1.32–1.35 (d, J = 6.8 Hz, 3H, CH-C*H$_3$*), 1.39–2.45 (m, 12H), 2.57–2.66 (q, J = 7.9 Hz, 2H, C*H$_2$*-CH$_3$), 2.80–2.84 (m, 3H), 4.91–4.94 (m, 1H, CH$_3$-C*H*), 7.02 (s, 1H, CH), 7.21–7.23 (m, 2H, aromatic), 7.30–7.32 (m, 4H, aromatic), 7.94 (broad m, 2H, aromatic), 8.31–8.35 ppm (broad t, J = 3.57 Hz, 1H, NH); HPLC: >98% (t_R = 2.69 min, 70% MeCN/water); (+ve)ES MS [M+H$^+$]: 539 m/z; FAB HRMS m/z calcd for $C_{30}H_{38}N_2O_5S$: 535.2579, found [M+H$^+$]: 539.2574.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-(tetrahydrofuran-2-ylmethyl) amide (20): (TFA salt): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 0.81 and 0.89 (2×s, 3H, 18-CH$_3$), 1.09–1.13 (t, J = 7.1 Hz, 3H, CH$_2$-C*H$_3$*), 1.23–2.45 (m, 16H), 2.58–2.63 (q, J = 7.4 Hz, 2H, C*H$_2$*-CH$_3$), 2.79–2.81 (m, 3H), 3.10–3.12 (m, 2H), 3.62–3.80 (m, 3H), 6.99 (s, 1H, CH), 7.18 (s, 1H, aromatic), 7.92 ppm (broad m, 2H, aromatic); HPLC: >90% (t_R = 2.42 min, 70% MeCN/water); (+ve)ES MS [M+H$^+$]: 519 m/z; FAB HRMS m/z calcd for $C_{28}H_{38}N_2O_6S$: 519.2528, found [M+H$^+$]: 519.2539.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N*-ethyl amide (21): (TFA salt): ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.80 and 0.89 (2×s, 3H, 18-CH$_3$), 0.98–1.03 (t, J = 7.1 Hz, 3H, CH$_2$-C*H$_3$*), 1.09–1.15 (t, J = 7.4 Hz, 3H, CH$_2$-C*H$_3$*), 1.23–2.45 (m, 12H), 2.57–2.66 (q, J = 7.4 Hz, 2H, C*H$_2$*-CH$_3$), 2.79–2.82 (m, 3H), 3.02–3.08 (q, J = 7.4 Hz, C*H$_2$*-CH$_3$), 7.01 (s, 1H, CH), 7.21 (s, 1H, aromatic), 7.88 (broad t, 1H, NH), 7.95 ppm (s, 2H, NH$_2$); HPLC: >89% (t_R = 3.51 min, 5→95% MeCN/water); (+ve)ES MS [M+H$^+$]: 463 m/z; FAB HRMS m/z calcd for $C_{24}H_{34}N_2O_5S$: 463.2266, found [M+H$^+$]: 463.2282.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N*-methyl-*N*-ethyl amide (22): (TFA salt): ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.82 and 0.91 (2×s, 3H, 18-CH$_3$), 0.96–1.02 (t, J = 7.1 Hz, 3H, CH$_2$-C*H$_3$*), 1.09–1.15 (t, J = 7.4 Hz, 3H, CH$_2$-C*H$_3$*), 1.23–2.45 (m, 12H), 2.58–2.66 (q, J = 7.4 Hz, 2H, C*H$_2$*-CH$_3$), 2.79–2.82 (m, 4H), 2.92 (s, N-CH$_3$), 7.01 (s, 1H, CH), 7.21 (s, 1H, aromatic), 7.95 ppm (s, 2H, NH$_2$); HPLC: >95% (t_R = 2.44 min, 70% MeCN/water); (+ve)ES MS [M+H$^+$]: 477 m/z; FAB HRMS m/z calcd for $C_{25}H_{36}N_2O_5S$: 477.2423, found [M+H$^+$]: 477.2414.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N*-butyl amide (23): (TFA salt): diagnostic signals ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.81 (s, 3H, 18-CH$_3$), 0.83–0.90 (m, 6H) 1.09–1.15 (t, J = 7.1 Hz, 3H, CH$_2$-C*H$_3$*), 1.23–2.45 (m, 12H), 2.59–2.66 (q, J = 7.4 Hz, 2H, C*H$_2$*-CH$_3$), 2.79–2.82 (m, 3H), 3.00–3.06 (m, 2H) 7.02 (s, 1H, CH), 7.21 (s, 1H, aromatic), 7.82–7.87 (m, 1H, NH), 7.96 ppm (s, 2H, NH$_2$); HPLC: >92% (t_R = 2.45 min, 70% MeCN/water); (+ve)ES MS [M+H$^+$]: 491 m/z; FAB HRMS m/z calcd for $C_{26}H_{38}N_2O_5S$: 491.2579, found [M+H$^+$]: 491.2581.

2-Ethyl-3-sulfamoyloxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N,N*-diethyl amide (24): (TFA salt): ^{1}H NMR (270 MHz, [D$_6$]DMSO): δ = 0.82 and 0.91 (2×s, 3H, 18-CH$_3$), 0.96–1.03 (t, J = 6.8 Hz, 3H, CH$_2$-C*H$_3$*), 1.05–1.15 (m, 6H, 2×CH$_2$-C*H$_3$*), 1.23–2.45 (m, 12H), 2.58–2.63 (q, J = 7.6 Hz, 2H, C*H$_2$*-CH$_3$), 2.79–2.82 (m, 3H), 3.25–3.10 (q, J = 7.1 Hz, C*H$_2$*-CH$_3$) 7.01 (s, 1H, CH), 7.21 (s, 1H, aromatic), 7.94 ppm (s, 2H, NH$_2$); HPLC: >97% (t_R = 2.65 min, 70% MeCN/water); (+ve)ES MS [M+H$^+$]: 491 m/z; FAB HRMS m/z calcd for $C_{26}H_{38}N_2O_5S$: 491.2575, found [M+H$^+$]: 491.2568.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16β-methylcarboxylic acid-(pyridine-3-ylmethyl) amide (25): ^{1}H NMR (270 MHz, CDCl$_3$): δ = 0.79 (s, 3H, 18-CH$_3$), 1.17–1.22 (t, J = 7.6 Hz, 3H, CH$_2$-

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CH_3), 1.32–2.40 (m, 13 H), 2.54–2.63 (q, $J=7.6$ Hz, 2 H, CH_2-CH_3), 2.67–2.82 (m, 3 H), 4.43–4.46 (m, 2 H, NH-CH_2), 6.12 (broad s, 1 H, OH), 6.41 (broad t, 1 H, NH), 6.51 (s, 1 H, aromatic), 6.39–6.41 (apparent t, $J=5.9$ Hz, 1 H, NH), 6.70–6.72 (apparent t, $J=5.9$ Hz, 1 H, NH), 7.01 (s, 1 H, CH), 7.28–7.30 (m, 1 H, aromatic) 7.64–7.67 (apparent t, $J=7.6$ Hz, 1 H, aromatic), 8.50–8.53 ppm (m, 2 H, aromatic); HPLC: >99% ($t_R=5.20$ min, 5→99% MeCN/water); (−ve)ES MS [$M-H^+$]: 445 m/z; FAB HRMS m/z calcd for $C_{28}H_{35}N_2O_3$: 447.2647, found [$M+H^+$]: 447.2654.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-(pyridine-2-ylmethyl) amide (26): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.46$ (s, 3 H, 18-CH_3), 0.81–0.88 (m, 2 H), 1.14–1.24 (m, 4 H), 1.32–2.85 (m, 15 H), 4.39–4.90 (m, 2 H, NH-CH_2), 6.48 (s, 1 H, aromatic), 6.90–6.95 (broad t, 1 H, NH), 6.97 (s, 1 H, aromatic), 7.01–7.06 (s, 1 H, aromatic), 7.27–7.39 (m, 1 H, aromatic), 7.67–7.74 (m, 1 H, aromatic), 8.52–8.54 ppm (m, 1 H, aromatic); HPLC: >99% ($t_R=2.25$ and 2.76 min, 75% MeCN/water); (−ve)ES MS [$M+H^+$]: 445 m/z; FAB HRMS m/z calcd for $C_{28}H_{34}N_2O_3$: 447.2647, found [$M+H^+$]: 447.2655.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-(5-methylpyrimidinyl-2-ylmethyl) amide (27): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.81$ and 0.84 (2×s, 3 H, 18-CH_3), 1.17–1.22 (m, 3 H, 2×CH_2-CH_3), 1.32–2.40 (m, 13 H), 2.54–2.63 (q, $J=7.6$ Hz, 2 H, CH_2-CH_3), 2.67–2.82 (m, 3 H), 4.55–4.57 and 4.80–4.82 (m, 2 H, NH-CH_2), 6.19 (broad s, 1 H, OH), 6.49 (s, 1 H, CH), 7.02 (s, 1 H, CH), 8.24, 8.37, 8.47, 8.60, 8.61 ppm (4×s, 2 H, CH); HPLC: >95% ($t_R=2.16$ and 2.38 min, 80% MeCN/water); (−ve)ES MS [$M+H^+$]: 445 m/z; FAB HRMS m/z calcd for $C_{28}H_{35}N_3O_3$: 462.4756, found [$M+H^+$]: 462.2744.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*S*-α-methylbenzyl amide (28): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.74$ and 0.94 (2×s, 3 H, 18-CH_3), 1.16–1.22 (t, $J=7.6$ Hz, 3 H, CH_2-CH_3), 1.46–1.49 (d, $J=6.8$ Hz, 3 H, CH-CH_3), 2.65–2.72 (dd, $J=5.4$ and 5.8 Hz, 1 H), 1.32–2.40 (m, 12 H), 2.53–2.61 (q, $J=7.6$ Hz, 2 H, CH_2-CH_3), 2.67–2.82 (m, 3 H), 4.9 and 5.0 (2×s, 1 H, OH), 5.08–5.13 (m, CH-CH_3), 6.06–6.03 (d, $J=8.2$ Hz, 1 H, NH), 6.30–6.33 (d, $J=7.9$ Hz, 1 H, NH), 6.50 (s, 1 H, CH), 7.02 (s, 1 H, CH), 7.32–7.40 ppm (m, 5 H, aromatic); HPLC: >99% ($t_R=3.36$ min, 80% MeCN/water); (+ve)ES MS [$M+H^+$]: 460 m/z; FAB HRMS m/z calcd for $C_{30}H_{37}NO_3$: 460.2851, found [$M+H^+$]: 460.2841.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-(*R*)-α-methylbenzyl amide (29): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.84$ and 0.94 (2×s, 3 H, 18-CH_3), 1.17–1.22 (t, $J=7.4$ Hz, 3 H, CH_2-CH_3), 1.46–1.49 (d, $J=6.8$ Hz, 3 H, CH-CH_3), 1.32–2.40 (m, 12 H), 2.53–2.61 (q, $J=7.6$ Hz, 2 H, CH_2-CH_3), 2.65–2.72 (dd, $J=5.4$ and 5.8 Hz, 1 H) 2.79–2.82 (m, 3 H), 4.71 and 4.75 (2×s, 1 H, OH), 5.06–5.11 (m, CH-CH_3), 5.59–6.03 (d, $J=8.2$ Hz, 1 H, NH), 6.30–6.33 (d, $J=7.9$ Hz, 1 H, NH), 6.50 (s, 1 H, CH), 7.02 (s, 1 H, CH), 7.31–7.40 ppm (m, 5 H, aromatic); HPLC: >99% ($t_R=3.36$ min, 70% MeCN/water); (+ve)ES MS [$M+H^+$]: 460 m/z; FAB HRMS m/z calcd for $C_{30}H_{37}NO_3$: 460.2851, found [$M+H^+$]: 460.2842.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N*-ethyl amide (30): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.81$ and 0.95 (2×s, 3 H, 18-CH_3), 1.10–1.16 (t, $J=7.4$ Hz, 3 H, CH_2-CH_3), 1.16–1.12 (t, $J=7.4$ Hz, 3 H, CH_2-CH_3), 1.42–2.43 (m, 13 H), 2.55–2.62 (q, $J=7.9$ Hz, 2 H, CH_2-CH_3), 2.65–2.82 (m, 3 H), 3.22–3.32 (m, 2 H, N-CH_2-CH_3), 4.94 and 5.03 (2×s, 1 H, OH), 5.72 and 5.93 (2×broad t, 1 H, NH), 6.51 (s, 1 H, CH), 7.01 ppm (s, 1 H, CH); HPLC: >95% ($t_R=5.20$ min, 70% MeCN/water); (+ve)ES MS [$M+H^+$]: 384 m/z; FAB HRMS m/z calcd for $C_{24}H_{33}NO_3$: 385.2538, found [$M+H^+$]: 384.2532.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N*-methyl-*N*-ethyl amide (31): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.82$ and 0.94 (2×s, 3 H, 18-CH_3), 1.06–1.12 (t, $J=7.1$ Hz, 3 H, CH_2-CH_3), 1.16–1.22 (t, $J=7.6$ Hz, 3 H, CH_2-CH_3), 1.92–2.43 (m, 13 H), 2.53–2.61 (q, $J=7.6$ Hz, 2 H, CH_2-CH_3), 2.78–2.82 (m, 3 H), 2.91–2.95 (2×s, 3 H, N-CH_3), 3.31–3.38 (m, 1 H), 4.78–4.82 (broad s, 1 H, OH), 6.51 (s, 1 H, CH), 7.02 ppm (s, 1 H, CH); HPLC: >99% ($t_R=5.20$ min, 70% MeCN/water); (+ve)ES MS [$M+H^+$]: 398 m/z; FAB HRMS m/z calcd for $C_{25}H_{35}NO_3$: 398.2695, found [$M+H^+$]: 398.2695.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N*-butyl amide (32): Diagnostic signals ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.82$ (s, 3 H, 18-CH_3), 0.91–0.94 (m, 3 H), 1.17–1.22 (t, $J=7.6$ Hz, 3 H, CH_2-CH_3), 1.29–2.43 (m, 15 H), 2.53–2.62 (q, $J=7.9$ Hz, 2 H, CH_2-CH_3), 2.78–2.82 (m, 3 H), 3.20–3.25 (m, 1 H), 4.82 and 4.89 (2×broad s, 1 H, OH), 5.72 and 5.96 (2×broad t, 1 H, NH), 6.51 (s, 1 H, CH), 7.02 ppm (s, 1 H, CH); HPLC: >99% ($t_R=2.96$ min, 70% MeCN/water); (+ve)ES MS [$M+H^+$]: 412 m/z; FAB HRMS m/z calcd for $C_{26}H_{37}NO_3$: 412.2851, found [$M+H^+$]: 412.2857.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N,N*-diethyl amide (33): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.82$ and 0.94 (2×s, 3 H, 18-CH_3), 1.08–1.22 (2×t, $J=7.1$ and 7.4 Hz, 3 H each, 2×CH_2-CH_3), 1.92–2.43 (m, 13 H), 2.53–2.62 (q, $J=7.6$ Hz, 2 H, CH_2-CH_3), 2.78–2.81 (m, 3 H), 3.25–3.42 (m, 4 H, N-CH_2-CH_3), 4.77 and 4.81 (broad s, 1 H, OH), 6.51 (s, 1 H, CH), 7.02 ppm (s, 1 H, CH); HPLC: >96% ($t_R=13.41$ min, 5→95% MeCN/water); (+ve)ES MS [$M+H^+$]: 412 m/z; FAB HRMS m/z calcd for $C_{26}H_{37}NO_3$: 412.2851, found [$M+H^+$]: 412.2854.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16α/β-methylcarboxylic acid-*N*-isopropyl amide (34): ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.79$ and 0.93 (2×s, 3 H, 18-CH_3), 1.12–1.16 (2×d, $J=6.3$ Hz each, 6 H, 2×CH_3), 1.19–1.21 (t, $J=7.1$ Hz, 3 H, CH_2-CH_3), 1.92–2.43 (m, 13 H), 2.53–2.62 (m, 2 H, CH_2-CH_3), 2.78–2.85 (m, 3 H), 4.04–4.08 (m, 4 H, NH-CH-$(CH_3)_2$), 5.28 and 5.42 (2×s, 1 H, 2×OH), 5.57–5.59 and 5.83–5.86 (2×d, $J=7.6$ Hz each, 1 H, NH), 6.52 (s, 1 H, CH), 7.01 ppm (s, 1 H, CH); HPLC: >84% ($t_R=3.90$ min, 5→95% MeCN/water); (+ve)ES MS [$M+H^+$]: 398 m/z; FAB HRMS m/z calcd for $C_{25}H_{35}NO_3$: 398.2695, found [$M+H^+$]: 398.2692.

3-*O*-Benzyl-2-ethylestrone (35): Potassium carbonate (14.0 g, 100 mmol) was added to a stirred solution of 2-ethylestrone **5** (10.00 g, 33.5 mmol) in anhydrous DMF (100 mL) under an inert atmosphere, followed by benzyl bromide (4.78 mL, 40 mmol). The reaction was stirred at room temperature for 2 days, and the solid obtained was collected by filtration, dissolved in CH_2Cl_2, filtered to remove any inorganics, and concentrated to obtain a white solid. The white solid obtained was recrystallised from CH_2Cl_2/MeOH (90:10) to obtain the pure benzyl ether estrone derivative, the precursor of **6** (12.00 g, 92%), as a white solid: mp = 156–160 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta=0.90$ (s, 3 H, 18-CH_3), 1.17–1.23 (t, $J=7.4$ Hz, CH_2-CH_3), 1.51–2.29 (m, 14 H), 2.45–2.55 (m, 4 H, 16-CH_2), 2.62–2.70 (q, $J=7.6$ Hz, 2 H, CH_3-CH_2), 2.84–2.90 (m, 2 H, 6-CH_2), 5.04 (s, 2 H, CH_2-phenyl) 6.64 (s, 4-H), 7.10 (s, 1-CH), 7.28–7.45 ppm (m, 5 H); HPLC: 98% ($t_R=3.24$ min, 80% MeCN/water); FAB HRMS m/z calcd for $C_{27}H_{32}O_2$: 388.2357, found [M^+]: 388.2389.

2-Ethyl-3-*O*-benzyl-16-(1′-hydroxy-2′-carboxylic acid ethyl ester-ethylidene) estrone 36: Diethyl oxalate (1.40 mL, 10.20 mmol) was added to a stirred solution of 3-*O*-benzyl-2-ethylestrone **35** (2.00 g, 5.15 mmol) in toluene (20 mL) followed by potassium *tert*-butoxide (0.693 g, 6.18 mmol), and the reaction was stirred at room temperature for 16 h. The solution was acidified to pH 5 with glacial acetic acid before being concentrated in vacuo. EtOAc (30 mL) was

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added to the residue, and the solution was washed with water (30 mL) and brine (30 mL). The organic layer was concentrated in vacuo to give a white powder, which was washed with ethanol/water and collected by filtration to give the product **36** (2.44 g, 97%) as a white solid: mp = 135–138°C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 0.99$ (s, 3H, 18-CH_3), 1.20 (t, $J = 7.5$ Hz, 3H, $ArCH_2CH_3$), 1.38 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.41–1.69 (6H), 2.00–2.05 (m, 2H), 2.30–2.50 (m, 3H), 2.66 (q, $J = 7.5$ Hz, 2H, $ArCH_2CH_3$), 2.88–2.91 (m, 2H), 3.04–3.10 (m, 1H), 4.35 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 5.04 (s, 2H, $ArCH_2O$), 6.64 (s, 1H, 4-H), 7.09 (s, 1H, 1-H), 7.26–7.45 ppm (m, 5H); HPLC: > 97% ($t_R = 2.86$ min, 98% MeCN in H_2O); (−ve)ES MS [$M-H^+$]: 487 *m/z*; FAB HRMS *m/z* calcd for $C_{31}H_{36}O_5$: 488.2563, found [M^+]: 488.2569.

3-Benzyloxy-2-ethyl-1,3,5(10)-triene-[17,16-c]-[5′-(carboxylic acid ethyl ester)] pyrazole 37: Hydrazine monohydrate (0.3 mL, 6 mmol) was added to a stirred suspension of ester **36** (2.44 g, 5 mmol) in ethanol (50 mL), at which time the starting material dissolved. Stirring at room temperature was continued overnight before the white precipitate was collected by filtration and washed with water. The filtrate was acidified to pH 5 with glacial acetic acid; more precipitate formed and was collected. The precipitates were combined and suspended in ethanol before *p*-toluene sulfonic acid monohydrate (~0.1 g) was added. The mixture was heated for 5 min to aromatise the pyrazole E-ring and then stirred at room temperature for 1 h. The pale-yellow solution was concentrated in vacuo until a precipitate began to form, after which water was added. The precipitate was collected by filtration, washed with water and air-dried to give the pyrazole product **37** (2.33 g, 96%) as a white solid: mp = 103–107°C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta =$ 1.03 (s, 3H, 18-CH_3), 1.21 (t, $J = 7.5$ Hz, 3H, $ArCH_2CH_3$), 1.37 (t, $J =$ 7.2 Hz, 3H, OCH_2CH_3), 1.45–2.20 (6H), 2.32–2.50 (m, 4H), 2.67 (q, $J = 7.5$ Hz, 2H, $ArCH_2CH_3$), 2.82–2.96 (m, 3H), 4.35 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 5.05 (s, 2H, $ArCH_2O$), 6.65 (s, 1H, 4-H), 7.12 (s, 1H, 1-H), 7.28–7.46 ppm (m, 5H); HPLC: > 92% ($t_R = 5.17$ min, 94% MeCN in H_2O); (−ve)ES MS [$M-H^+$]: 483 *m/z*; FAB HRMS *m/z* calcd for $C_{31}H_{36}N_2O_3$: 484.2726, found [M^+]: 484.2730.

2-Ethyl-3-hydroxy-1,3,5(10)-triene-[17,16-c]-[5′-(carboxylic acid ethyl ester)] pyrazole 38: A solution of pyrazole **37** (3.40 g, 7 mmol) in MeOH (70 mL) was degassed with bubbling nitrogen for 30 min. Pd/C (5% wt., catalytic) was added to this, and degassing was continued for a further 10 min before hydrogen gas was passed over the reaction; stirring under hydrogen was continued for a further 3 days. The mixture was filtered through a pad of celite, rinsed with ethyl acetate and methanol, and the filtrate was concentrated in vacuo. The product obtained was purified by flash chromatography (hexane/ethyl acetate, gradient elution, 50 g column) to give the deprotected pyrazole **38** (2.47 g, 89%) as a white solid: mp = 155–159°C; ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.03$ (s, 3H, 18-CH_3), 1.15 (t, $J = 7.5$ Hz, 3H, $ArCH_2CH_3$), 1.36 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.39–2.20 (6H), 2.26–2.44 (m, 4H), 2.53 (q, $J = 7.3$ Hz, 2H, $ArCH_2CH_3$), 2.79–2.86 (m, 3H), 4.34 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 6.46 (s, 1H, 4-H), 6.97 ppm (s, 1H, 1-H); (+ve)ES MS [MH^+]: 395 *m/z*; FAB HRMS *m/z* calcd for $C_{24}H_{30}N_2O_3$ 395.2335, found [$M+H^+$]: 395.2312.

2-Ethyl-3-sulfamoyloxy-1,3,5(10)-triene-[17,16-c]-[5′-(carboxylic acid ethyl ester)] pyrazole 39: A solution of sulfamoyl chloride (30 mL of a 0.68 M solution in toluene, 20.4 mmol) was concentrated in vacuo without heating and cooled on ice under nitrogen. DMA (15 mL) was added to this, and the mixture was added to a cooled (ice/acetone bath) solution of pyrazole **38** (2.42 g, 6.13 mmol) in DMA (10 mL). The stirred solution was allowed to warm to room temperature overnight before being diluted with water (60 mL), and the product was extracted with ethyl acetate (100 mL) to give an oily solution in DMA. This was re-dissolved in ethyl acetate and washed again with water before concentrating to give a cream-coloured crystalline solid. Recrystallisation from CH_2Cl_2/hexane gave the required sulfamate **39** (2 g, 69%) as a white solid: mp > 200°C (dec.); ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.04$ (s, 3H, 18-CH_3), 1.20 (t, $J = 7.5$ Hz, 3H, $ArCH_2CH_3$), 1.36 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.39–2.20 (6H), 2.29–2.50 (m, 4H), 2.71 (q, $J = 7.5$ Hz, 2H, $ArCH_2CH_3$), 2.81–2.92 (m, 3H), 4.33 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.09 (s, 1H, 4-H), 7.22 ppm (s, 1H, 1-H); HPLC: > 97% ($t_R =$ 2.40 min, 80% MeCN in H_2O); FAB LRMS [$M+H^+$]: 474 *m/z*; FAB HRMS *m/z* calcd for $C_{24}H_{32}N_3O_5S$: 474.2063, found [$M+H^+$]: 474.2067.

Synthesis of resin-bound derivative of 2-ethyl-3-sulfamoyloxy-1,3,5(10)-triene-[17,16-c]-[5′-(carboxylic acid ethyl ester)] pyrazole 40: Sulfamate **39** (2.00 g, 4.22 mmol) was added as a solid in portions to a suspension of 2-chlorotrityl chloride resin (2.27 g, 1.1 mmol g^{-1} theoretical loading) swollen in anhydrous CH_2Cl_2 (12 mL) and DIPEA (5.34 mL, 29.5 mmol) under nitrogen, followed by a further volume of CH_2Cl_2 (5 mL). The reaction was shaken at room temperature for 96 h before the resin was collected by filtration and washed three times with CH_2Cl_2, methanol, and finally with CH_2Cl_2 (5 cycles) to give the resin-bound intermediate **40** (3.74 g).

Synthesis of resin-bound derivative of 2-ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-(5′-carboxylic acid) pyrazole 41: Solid-bound sulfamate **40** (3.74 g) was allowed to swell in THF (25 mL) before aqueous sodium hydroxide (1 M, 30 mL) was added slowly, and the mixture was shaken at room temperature for 24 h. The resin was collected by filtration, washed with THF/water (1:1) and washed with MeOH and CH_2Cl_2 before being dried in vacuo to obtain solid-bound acid **41** as yellow beads (3.53 g, loading 0.8 mmol g^{-1} as calculated from cleavage to give **42**).

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-(5′-carboxylic acid) pyrazole 42: Solid-bound **41** (0.028 g) was placed in a fritted syringe and rinsed with TFA/CH_2Cl_2 (20 mL of a 1:1 mixture, added portion-wise with 5 min between washings) followed by CH_2Cl_2. The washings were combined and concentrated in vacuo. Purification of the crude product **42** by flash chromatography (gradient elution of CH_2Cl_2 to 10% MeOH in CH_2Cl_2) gave the pure product **42** (0.010 g) as a white solid: mp > 250°C (dec.); ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.04$ (s, 3H, 18-CH_3), 1.20 (t, $J = 7.5$ Hz, 3H, $ArCH_2CH_3$), 1.46–1.90 (5H), 1.99–2.07 (m, 1H), 2.12–2.23 (m, 1H), 2.31–2.51 (4H), 2.71 (q, $J = 7.5$ Hz, 2H, $ArCH_2CH_3$), 2.82–2.92 (3H), 7.09 (s, 1H, 4-H), 7.22 ppm (s, 1H, 1-H); HPLC: > 93% ($t_R =$ 1.45 min, 70% MeCN in H_2O); (+ve)ES MS [$M+H^+$]: 446 *m/z*; FAB HRMS *m/z* calcd for $C_{22}H_{28}N_3O_5S$: 446.1750, found [$M+H^+$]: 446.1713.

General procedure for the synthesis of the sulfamate library 45–55: Solid-bound acid **41** (0.200 g) was allowed to swell in dry CH_2Cl_2 (3 mL) under N_2. Bromotripyrrolidinophosphonium hexafluorophosphate (PyBOP, 0.260 g, 0.5 mmol) was added to this, followed by *N*-hydroxybenzotriazole (HOBt, 0.070 g, 0.5 mmol) and DIPEA (0.18 mL, 1.0 mmol). The mixture was shaken for 10 min before addition of the amine (0.06 mL) and shaking under N_2 at room temperature was continued for 72 h to give the resin-bound library **43**. The resin was collected in a fritted syringe and washed with DMF, CH_2Cl_2, and MeOH (3 cycles) and again with CH_2Cl_2 (3 cycles) before cleavage of the product using TFA/CH_2Cl_2 (1:1). Purification by flash chromatography (gradient elution, CH_2Cl_2 to 10%

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MeOH in CH_2Cl_2) gave the required sulfamates **45–55** in up to 53% yield and with an average purity of 90%.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-3′′-ylmethyl)carbamoyl] pyrazole (45): ^{1}H NMR (400 MHz, CD_3OD): δ = 1.06 (s, 3H, 18-CH_3), 1.21 (t, J = 7.4 Hz, 3H, $ArCH_2CH_3$), 1.47–1.57 (m, 1H), 1.65–1.90 (3H), 2.04–2.07 (m, 1H), 2.14–2.33 (2H), 2.39–2.53 (3H), 2.72 (q, J = 7.4 Hz, 2H, $ArCH_2CH_3$), 2.90–2.94 (3H), 4.58 (s, 2H), 7.09 (s, 1H, 4-H), 7.21 (s, 1H, 1-H), 7.40–7.43 (m, 1H), 7.85 (d, J = 7.8 Hz, 1H), 8.42 (d, J = 4.7 Hz, 1H), 8.54 ppm (d, J = 2.0 Hz, 1H); HPLC: >99% (t_R = 1.91 min, 80% MeCN in H_2O); (+ve)ES MS [M+H$^+$]: 536.31 m/z; FAB HRMS m/z calcd for $C_{28}H_{34}N_5O_4S$: 536.2332, found [M+H$^+$]: 536.2330.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-2′′-ylmethyl)carbamoyl] pyrazole (46): ^{1}H NMR (270 MHz, CD_3OD): δ = 1.04 (s, 3H, 18-CH_3), 1.19 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.39–1.55 (m, 1H), 1.59–1.88 (m, 3H), 1.98–2.04 (m, 1H), 2.14–2.58 (5H), 2.70 (q, J = 7.5 Hz, 2H, $ArCH_2CH_3$), 2.78–2.98 (3H), 4.64 (s, 2H), 7.08 (s, 1H, 4-H), 7.20 (s, 1H, 1-H), 7.28–7.33 (m, 1H), 7.42 (d, J = 7.9 Hz, 1H), 7.80 (dt, J = 7.8, 1.7 Hz, 1H), 8.49 ppm (d, J = 4.5 Hz, 1H); HPLC: >97% (t_R = 2.11 min, 70% MeCN in H_2O); (−ve)ES MS [M−H$^+$]: 534 m/z; FAB HRMS m/z calcd for $C_{28}H_{34}N_5O_4S$: 536.2332, found [M+H$^+$]: 536.2330.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-3′′-ylethyl)carbamoyl] pyrazole (47): ^{1}H NMR (270 MHz, CD_3OD): δ = 1.02 (s, 3H, 18-CH_3), 1.20 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.46–1.89 (4H), 1.98–2.04 (m, 1H), 2.15–2.52 (5H), 2.65–2.98 (7H), 3.60 (t, J = 6.7 Hz, 2H, –CH_2CH_2–), 7.04 (s, 1H, 4-H), 7.21 (s, 1H, 1-H), 7.36–7.41 (m, 1H), 7.78 (dt, J = 6.2, 1.7 Hz, 1H), 8.39 (dd, J = 4.8, 1.7 Hz, 1H), 8.44 ppm (d, J = 1.7 Hz, 1H); LC: purity > 95%; (−ve)ES MS [M−H$^+$]: 548 m/z; FAB HRMS m/z calcd for $C_{29}H_{36}N_5O_4S$: 550.2488, found [M+H$^+$]: 550.2499 m/z.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-2′′-ylethyl)carbamoyl] pyrazole (48): ^{1}H NMR (270 MHz, CD_3OD): δ = 1.02 (s, 3H, 18-CH_3), 1.20 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.46–1.88 (4H), 1.98–2.05 (m, 1H), 2.12–2.52 (5H), 2.71 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.77–2.92 (3H), 3.07 (t, J = 6.9 Hz, 2H, –CH_2CH_2–), 3.71 (dt, J = 7.2, 1.8 Hz, 2H, –CH_2CH_2–), 7.09 (s, 1H, 4-H), 7.20 (s, 1H, 1-H), 7.26–7.29 (m, 1H), 7.36 (d, J = 7.9 Hz, 1H), 7.77 (dt, J = 7.7, 1.9 Hz, 1H), 8.47–8.50 ppm (m, 1H); HPLC: >98% (t_R = 1.96 min, 90% MeCN in H_2O); (−ve)ES MS [M−H$^+$]: 548 m/z; FAB HRMS m/z calcd for $C_{29}H_{36}N_5O_4S$: 550.2488, found [M+H$^+$]: 550.2483.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(5′′-methylpyrazin-2′′-ylmethyl)carbamoyl] pyrazole (49): ^{1}H NMR (400 MHz, CD_3OD): δ = 1.06 (s, 3H, 18-CH_3), 1.22 (t, J = 7.4 Hz, 3H, $ArCH_2CH_3$), 1.47–1.57 (m, 1H), 1.65–1.90 (3H), 2.03–2.07 (m, 1H), 2.14–2.55 (5H), 2.72 (q, J = 7.4 Hz, 2H, $ArCH_2CH_3$), 2.90–2.94 (3H), 4.66 (s, 2H), 7.09 (s, 1H, 4-H), 7.21 (s, 1H, 1-H), 8.48 (s, 1H), 8.49 ppm (s, 1H); HPLC: >99% (t_R = 1.93 min, 80% MeCN in H_2O); (+ve)ES MS [M+H$^+$]: 551.18 m/z; FAB HRMS m/z calcd for $C_{28}H_{35}N_6O_4S$: 551.2441, found [M+H$^+$]: 551.2447.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-methoxyethylcarbamoyl] pyrazole (50): ^{1}H NMR (400 MHz, CD_3OD): δ = 1.06 (s, 3H, 18-CH_3), 1.22 (t, J = 7.4 Hz, 3H, $ArCH_2CH_3$), 1.47–1.57 (m, 1H), 1.65–1.90 (3H), 2.03–2.09 (m, 1H), 2.18–2.55 (5H), 2.72 (q, J = 7.4 Hz, 2H, $ArCH_2CH_3$), 2.84–2.95 (3H), 3.39 (s, H), 3.55 (s, H), 7.09 (s, 1H, 4-H), 7.22 ppm (s, 1H, 1-H); HPLC: >88% (t_R = 3.28 min, 5→95% MeCN in H_2O over 10 min); (+ve)ES MS [M+H$^+$]: 503.24 m/z; FAB HRMS m/z calcd for $C_{25}H_{35}N_4O_5S$: 503.2328, found [M+H$^+$]: 503.2325.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(furan-2′′-ylmethyl)carbamoyl] pyrazole (51): ^{1}H NMR (270 MHz, CD_3OD): δ = 1.02 (s, 3H, 18-CH_3), 1.20 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.46–1.89 (4H), 1.95–2.06 (m, 1H), 2.12–2.52 (5H), 2.71 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.81–2.91 (3H), 4.52 (s, 2H), 6.28–6.36 (m, 2H), 7.09 (s, 1H, 4-H), 7.21 (s, 1H, 1-H), 7.43 ppm (s, 1H); HPLC: >91% (t_R = 2.17 min, 70% MeCN in H_2O); (−ve)ES MS [M−H$^+$]: 523.27 m/z; FAB HRMS m/z calcd for $C_{27}H_{33}N_4O_5S$: 525.2172, found [M+H$^+$]: 525.2176.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-phenylethylcarbamoyl] pyrazole (52): ^{1}H NMR (270 MHz, CD_3OD): δ = 1.03 (s, 3H, 18-CH_3), 1.20 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.46–1.90 (4H), 1.98–2.05 (m, 1H), 2.12–2.52 (5H), 2.71 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.77–2.99 (5H), 3.55–3.61 (2H, –CH_2CH_2–), 7.10 (s, 1H, 4-H), 7.20–7.30 ppm (6H); HPLC: >93% (t_R = 2.83 min, 70% MeCN in H_2O); (−ve)ES MS [M−H$^+$]: 547.27 m/z; FAB HRMS m/z calcd for $C_{30}H_{37}N_4O_4S$: 549.2536, found [M+H$^+$]: 549.2532.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(benzo[1′′,3′′]dioxol-5′′-ylmethyl)carbamoyl] pyrazole (53): ^{1}H NMR (270 MHz, CD_3OD): δ = 1.06 (s, 3H, 18-CH_3), 1.20 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.46–2.54 (10H), 2.71 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.81–3 (3H), 4.45 (s, 2H), 5.91 (s, 2H), 6.74–6.85 (2H), 7.09 (s, 1H, 4-H), 7.21 ppm (s, 1H, 1-H); HPLC: >93% (t_R = 5.39 min, 5→95% MeCN in H_2O over 10 min); (−ve)ES MS [M−H$^+$]: 577.26 m/z; FAB HRMS m/z calcd for $C_{30}H_{35}N_4O_6S$: 579.2277, found [M+H$^+$]: 579.2257.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(1*H*-indol-3′′-ylethyl)carbamoyl] pyrazole (54): ^{1}H NMR (270 MHz, CD_3OD): δ = 1.00 (s, 3H, 18-CH_3), 1.20 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.46–2.52 (11H), 2.71 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.90–2.92 (2H), 3.03–3.08 (2H, –CH_2CH_2–), 3.63–3.67 (2H, –CH_2CH_2–), 6.95–7.12 (4H), 7.21 (s, 1H, 1-H), 7.34 (d, J = 8.0 Hz, 1H), 7.56–7.59 ppm (1H); HPLC: >98% (t_R = 2.22 min, 70% MeCN in H_2O); (−ve)ES MS [M−H$^+$]: 586.33 m/z; FAB HRMS m/z calcd for $C_{32}H_{38}N_5O_4S$: 588.2645, found [M+H$^+$]: 588.2659.

2-Ethyl-3-sulfamoyloxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-*N*-methyl-*N*-carbamoyl] pyrazole (55): ^{1}H NMR (400 MHz, CD_3OD): δ = 1.08 (s, 3H, 18-CH_3), 1.20–1.25 (6H), 1.30–1.6 (1H), 1.65–1.90 (3H), 2.01–2.09 (m, 1H), 2.16–2.50 (5H), 2.72 (q, J = 7.4 Hz, 2H, $ArCH_2CH_3$), 2.84–2.93 (3H), 3.06–3.26 (3H), 3.46–3.64 (m, 2H), 7.09 (s, 1H, 4-H), 7.22 ppm (s, 1H, 1-H); HPLC: >98% (t_R = 2.43 min, 5→95% MeCN in H_2O over 10 min); (+ve)ES MS [M+H$^+$]: 487.24 m/z; FAB HRMS m/z calcd for $C_{25}H_{35}N_4O_4S$: 487.2379, found [M+H$^+$]: 487.2396.

2-Ethyl-3-*O*-*tert*-butyl-dimethylsilyl-16-(1′-hydroxy-2′-carboxylic acid ethyl ester-ethylidene) estrone (57): Diethyl oxalate (0.84 mL, 6.2 mmol) and potassium *tert*-butoxide (0.45 g, 4 mmol) were added to a stirred solution of 3-*O*-TBDMS-2-ethylestrone **56** (1.275 g, 3.1 mmol) in toluene (30 mL),and the reaction was stirred at room temperature overnight. The reaction was acidified with glacial acetic acid and concentrated in vacuo before ethyl acetate (30 mL) was added. The solution was washed with water (30 mL) and brine (30 mL) before being concentrated in vacuo to give a white powder. This powder was washed with ethanol/water and collected by filtration to give the title compound in quantitative yield: mp = 114–116 °C; ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.21 (s, 6H, $(CH_3)_2Si$), 0.98 (s, 3H, 18-CH_3), 0.99 (s, 9H, $(CH_3)_3C$), 1.15 (t, J = 7.5 Hz, 3H, $ArCH_2CH_3$), 1.38 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.54–1.69 (m, 6H), 2.00–2.05 (m, 2H), 2.27–2.47 (m, 3H), 2.55 (q, J = 7.5 Hz, 2H, $ArCH_2CH_3$), 2.80–2.86 (m, 2H), 3.03–3.11 (m, 1H), 4.35 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 6.48 (s, 1H, 4-H), 7.03 ppm (s, 1H, 1-H);

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FAB LRMS [MH^+]: 513.3 *m/z*; FAB HRMS *m/z* calcd for $C_{30}H_{44}O_2Si$: 512.2958, found [M^+]: 512.2965.

2-Ethyl-3-*O-tert*-butyl-dimethylsilyl-1,3,5(10)-triene-[17,16-c]-[5′-(carboxylic acid ethyl ester)] pyrazole (58): Hydrazine monohydrate (0.17 mL, 3.4 mmol) was added to a stirred suspension of **57** (1.53 g, 3 mmol) in EtOH (25 mL), at which time the starting material dissolved. Stirring at room temperature was continued overnight, during which time the solution became cloudy. *p*-Toluene sulfonic acid (~0.1 g) was added, and the mixture was heated for 5 min to aromatise the pyrazole E-ring. The solution was concentrated in vacuo until precipitate began to form. Water was then added, and the white powder was collected by filtration, washed with water, and air-dried to give quantitative yield: mp = 123–127 °C; ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 0.22$ (s, 6H, $(CH_3)_2Si$), 0.98 (s, 3H, 18-CH_3), 0.99 (s, 9H, $(CH_3)_3C$), 1.16 (t, $J = 7.5$ Hz, 3H, $ArCH_2CH_3$), 1.37 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.40–2.18 (6H), 2.30–2.50 (4H), 2.56 (q, $J = 7.5$ Hz, 2H, $ArCH_2CH_3$), 2.81–2.96 (m, 3H), 4.35 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 6.49 (s, 1H, 4-H), 7.06 ppm (s, 1H, 1-H); HPLC: >96% ($t_R = 6.69$ min, 100% MeCN); FAB LRMS [MH^+]: 509.2 *m/z*; FAB HRMS *m/z* calcd for $C_{34}H_{41}N_2O_3$: 509.3168, found [MH^+]: 509.3178.

2-Ethyl-3-hydroxy-1,3,5(10)-triene-[17,16-c]-(5′-carboxylic acid) pyrazole (59): NaOH (aq, 0.211 g in 16 mL) was added to a stirred suspension of **58** (1.27 g, 2.64 mmol) in MeOH (30 mL), and the mixture was heated at reflux overnight (the starting material dissolved upon heating). The reaction was cooled, acidified with glacial acetic acid, and concentrated in vacuo until precipitate began to form (~half volume). Water (20 mL) was added, and the resulting precipitate was collected by filtration. This was dissolved in MeOH, concentrated, and dried in vacuo: yield: 57%; mp > 210 °C (dec.); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.02$ (s, 3H, 18-CH_3), 1.16 (t, $J = 7.4$ Hz, 3H, $ArCH_2CH_3$), 1.36–1.47 (m, 1H), 1.56–1.83 (3H), 1.93–1.99 (m, 1H), 2.02–2.15 (m, 1H), 2.22–2.28 (m, 2H), 2.36–2.43 (m, 2H), 2.55 (q, $J = 7.4$ Hz, 2H, $ArCH_2CH_3$), 2.72–2.90 (m, 3H), 6.44 (s, 1H, 4-H), 6.95 ppm (s, 1H, 1-H); HPLC: >92% ($t_R = 1.49$ min, 70% MeCN in H_2O); (−)ES MS [$M-H^+$]: 365.16 *m/z*; FAB HRMS *m/z* calcd for $C_{22}H_{27}N_2O_3$: 367.2022, found [MH^+]: 367.2010.

General procedure for the solution-phase synthesis of library 4 from 2-ethyl-3-hydroxy-1,3,5(10)-triene-[17,16-c]-(5′-carboxylic acid) pyrazole (59): DMAP (catalytic), EDC (0.071 g, 0.37 mmol), and NEt_3 (20 μL) were added to a stirred suspension of **59** (0.045 g, 0.123 mmol) in dry CH_2Cl_2 (8 mL) under N_2, and the solution was stirred at room temperature for 30 min. Amine (20 μL) was then added to this, and stirring was continued for 4 days. The solution was washed with saturated bicarbonate, the organic layer was separated, and the aqueous layer was washed with CH_2Cl_2. The organic layers were combined and concentrated in vacuo, and the product was purified by flash chromatography (FlashMaster II) using an elution gradient of CH_2Cl_2 to 10% MeOH in CH_2Cl_2.

2-Ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-3′′-ylmethyl)carbamoyl] pyrazole (60): Yield: 39%; ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.03$ (s, 3H, 18-CH_3), 1.15 (t, $J = 7.4$ Hz, 3H, $ArCH_2CH_3$), 1.39–1.52 (m, 1H), 1.59–1.87 (m, 3H), 1.94–2.00 (m, 1H), 2.14–2.50 (5H), 2.55 (q, $J = 7.4$ Hz, 2H, $ArCH_2CH_3$), 2.78–2.90 (3H), 4.58 (s, 2H), 6.45 (s, 1H, 4-H), 6.97 (s, 1H, 1-H), 7.38–7.43 (m, 1H), 7.85 (d, $J = 7.7$ Hz, 1H), 8.43 (d, $J = 3.7$ Hz, 1H), 8.56 ppm (s, 1H); HPLC: >97% ($t_R = 2.11$ min, 70% MeCN in H_2O); (+)ES MS [MH^+]: 457.38 *m/z*; FAB HRMS *m/z* calcd for $C_{28}H_{33}N_4O_2$: 457.2603, found [MH^+]: 457.2589.

2-Ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-2′′-ylethyl)carbamoyl] pyrazole (61): Yield: 46%; ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.00$ (s, 3H, 18-CH_3), 1.14 (t, $J = 7.5$ Hz, 3H, $ArCH_2CH_3$), 1.33–1.49 (m, 1H), 1.54–1.83 (3H), 1.93–2.02 (m, 1H), 2.04–2.43 (5H), 2.54 (q, $J = 7.5$ Hz, 2H, CH_2CH_3), 2.71–2.90 (3H), 3.07 (t, $J = 7.1$ Hz, 2H, $-CH_2CH_2-$), 3.70 (dt, $J = 6.9$, 1.7 Hz, 2H, $-CH_2CH_2-$), 6.45 (s, 1H, 4-H), 6.96 (s, 1H, 1-H), 7.26–7.31 (m, 1H), 7.36 (d, $J = 7.7$ Hz, 1H), 7.77 (dt, $J = 7.7$, 1.7 Hz, 1H), 8.47–8.50 ppm (m, 1H); HPLC: >97% ($t_R = 3.41$, 70% MeCN in H_2O); (−)ES MS [$M-H^+$]: 469.55 *m/z*; FAB HRMS *m/z* calcd for $C_{29}H_{35}N_4O_2$: 471.2760, found [MH^+]: 471.2747.

2-Ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-3′′-ylethyl)carbamoyl] pyrazole (62): Yield: 51%; ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.02$ (s, 3H, 18-CH_3), 1.15 (t, $J = 7.4$ Hz, 3H, $ArCH_2CH_3$), 1.30–1.49 (m, 1H), 1.59–1.86 (m, 3H), 1.93–2.01 (m, 1H), 2.10–2.45 (5H), 2.55 (q, $J = 7.4$ Hz, 2H, $-CH_2CH_3$), 2.72–2.85 (3H), 2.95 (t, $J = 7.1$ Hz, 2H, $-CH_2CH_2-$), 3.61 (t, $J = 7.3$ Hz, 2H, $-CH_2CH_2-$), 6.46 (s, 1H, 4-H), 6.97 (s, 1H, 1-H), 7.37–7.42 (m, 1H), 7.79 (d, $J = 7.7$ Hz, 1H), 8.40 (d, $J = 4.2$ Hz, 1H), 8.45 ppm (s, 1H); HPLC: >98%; ($t_R = 3.09$ min, 70% MeCN in H_2O); (−)ES MS [$M-H^+$]: 469.49 *m/z*; FAB HRMS *m/z* calcd for $C_{29}H_{35}N_4O_2$: 471.2760, found [MH^+]: 471.2756.

2-Ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(pyridin-2′′-ylmethyl)carbamoyl] pyrazole (63): Yield: 41%; ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.01$ (s, 3H, 18-CH_3), 1.14 (t, $J = 7.4$ Hz, 3H, $ArCH_2CH_3$), 1.31–1.47 (m, 1H), 1.57–1.83 (m, 3H), 1.93–2.00 (m, 1H), 2.14–2.50 (5H), 2.54 (q, $J = 7.4$ Hz, 2H, $ArCH_2CH_3$), 2.71–2.86 (3H), 4.64 (s, 2H), 6.44 (s, 1H, 4-H), 6.95 (s, 1H, 1-H), 7.27–7.31 (m, 1H), 7.45 (d, $J = 8.0$ Hz, 1H), 7.78 (dt, $J = 7.7$, 1.6 Hz, 1H), 8.48 ppm (d, $J = 4.7$ Hz, 1H); HPLC: >92% ($t_R = 2.15$ min, 80% MeCN in H_2O); (−)ES MS [$M-H^+$]: 455.36 *m/z*; FAB HRMS *m/z* calcd for $C_{28}H_{33}N_4O_2$: 457.2603, found [MH^+]: 457.2607.

2-Ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-methoxy-ethylcarbamoyl] pyrazole (64): Yield: 36%; ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.02$ (s, 3H, 18-CH_3), 1.15 (t, $J = 7.4$ Hz, 3H, $ArCH_2CH_3$), 1.34–1.50 (m, 1H), 1.53–1.85 (m, 3H), 1.93–2.00 (m, 1H), 2.17–2.47 (5H), 2.54 (q, $J = 7.4$ Hz, 2H, $-CH_2CH_3$), 2.73–2.87 (3H), 3.3–3.4 (4H, $-CH_2CH_2-$), 3.53 (s, 3H, O–CH_3), 6.45 (s, 1H, 4-H), 6.96 ppm (s, 1H, 1-H); HPLC: >96% ($t_R = 2.11$ min, 80% MeCN in H_2O); (−)ES MS [$M-H^+$]: 422.35 *m/z*; FAB HRMS *m/z* calcd for $C_{25}H_{34}N_3O_3$: 424.2600, found [MH^+]: 424.2606.

2-Ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(5′′-methyl-pyrazin-2′′-ylmethyl)carbamoyl] pyrazole (65): Yield: 42%; ^{1}H NMR (270 MHz, CD_3OD): $\delta = 1.01$ (s, 3H, 18-CH_3), 1.14 (t, $J = 7.4$ Hz, 3H, $ArCH_2CH_3$), 1.31–1.47 (m, 1H), 1.52–1.83 (m, 3H), 1.92–2.00 (m, 1H), 2.15–2.58 (7H), 2.52 (s, 3H, $ArCH_3$), 2.69–2.86 (3H), 4.64 (s, 2H), 6.44 (s, 1H, 4-H), 6.95 (s, 1H, 1-H), 7.27–7.31 (m, 1H), 8.46 (d, $J = 1.1$ Hz, 1H), 8.49 ppm (s, 1H); HPLC: >94% ($t_R = 2.08$ min, 80% MeCN in H_2O); (−)ES MS [$M-H^+$]: 470.35 *m/z*; FAB HRMS *m/z* calcd for $C_{28}H_{34}N_5O_2$: 472.2713, found [MH^+]: 472.2718.

2-Ethyl-3-hydroxy-estra-1,3,5(10)-triene-[17,16-c]-[5′-(benzo-[1′′,3′′]dioxol-5′′-ylmethyl) carbamoyl] pyrazole (66): Yield: 56%; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 1.08$ (s, 3H, 18-CH_3), 1.19 (t, $J = 7.4$ Hz, 3H, $ArCH_2CH_3$), 1.48–2.52 (10H), 2.59 (q, $J = 7.8$ Hz, 2H), 2.80–2.95 (m, 3H), 4.48 (s, 2H), 5.95 (s, 2H), 6.49 (s, 1H), 6.79–6.89 (m, 3H), 7.01 ppm (s, 1H); HPLC: >98% ($t_R = 7.29$ min, 90% MeCN in H_2O); APCI [$M-H^+$]: 498.32 *m/z*; FAB HRMS *m/z* calcd for $C_{30}H_{34}N_3O_4$: 500.2549, found [MH^+]: 500.2548.

2-Ethyl-3-hydroxy-17-oxo-estra-1,3,5(10)-trien-16β-yl) acetic acid (68): NaOH (aq, 3.19 g, 32 mL) was added to a stirred suspension of 2-ethylestrone (5.50 g, 19 mmol) in MeOH (30 mL), and the reac-

tion was stirred for 10 min. Ethyl glyoxylate (7 g of a ~50% solution in toluene; ~34 mmol) was then added, and the reaction was stirred at room temperature for 26 h. The mixture was acidified to pH 5 with glacial acetic acid, water (50 mL) was added, and the resulting white precipitate was collected by filtration and washed with water. This was used without further purification for the reduction step. A solution of **67** (~19 mmol) in THF (50 mL) and MeOH (20 mL) was degassed with bubbling N_2 for 40 min. Pd/C (5% wt., catalytic) was added, and degassing was continued for a further 10 min before H_2 gas (balloon) was passed over the reaction; stirring under a hydrogen blanket was continued until the reaction reached apparent completion (72 h), as determined by TLC. The mixture was then filtered through celite, and the filtrate was concentrated in vacuo. Purification by flash chromatography using a gradient elution of CH_2Cl_2 to 10% MeOH in CH_2Cl_2 gave the title compound **68** as a white powder with a yield of 4.36 g (64%) over 2 steps. LCMS (AP-): 355.42; ^{1}H NMR (270 MHz, [D$_6$]DMSO): $\delta=0.76$ (3H, s), 1.04 (t, 3H, $J=7.4$ Hz), 1.25–1.49 (m, 7H), 1.70–1.89 (m, 2H), 2.13–2.33 (m, 3H), 2.43 (q, 2H, $J=7.4$ Hz), 2.57 (dd, 1H, $J=15.5$, 4.1 Hz), 2.62–2.66 (m, 2H), 6.42 (s, 1H), 6.90 (s, 1H), 8.85 ppm (s, 1H).

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