Effects of C-17 heterocyclic substituents on the anticancer activity of 2-ethylestra-1,3,5(10)-triene-3-*O*-sulfamates: synthesis, *in vitro* evaluation and computational modelling

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The potent activity of 2-substituted estra-1,3,5(10)-triene-3-*O*-sulfamates against the proliferation of cancer cells *in vitro* and tumours *in vivo* highlights the therapeutic potential of such compounds. Optimal activity is derived from a combination of a 2-XMe group (where X = CH_2, O or S), a 3-*O*-sulfamate group in the steroidal A-ring and a H-bond acceptor around C-17 of the D-ring. Herein, we describe the synthesis and anti-proliferative activities of a series of novel 2-substituted estra-1,3,5(10)-triene-3-*O*-sulfamates bearing heterocyclic substituents (oxazole, tetrazole, triazole) tethered to C-17. *In vitro* evaluation of these molecules revealed that high anti-proliferative activity in breast and prostate cancer cells lines (GI_{50} of 340–850 nM) could be retained when the heterocyclic substituent possesses H-bond acceptor properties. A good correlation between the calculated electron density of the heterocyclic ring and anti-proliferative activity was observed. Docking of the most active compounds into their putative site of action, the colchicine binding site of tubulin, suggests that they bind through a different mode to the previously described bis-sulfamate derivatives **1** and **2**, which possess similar *in vitro* activity.

Introduction

In previous studies, we explored the structure–activity relationships of 2-substituted estra-1,3,5(10)-triene-3-*O*-sulfamates as multi-mechanism anti-cancer agents.[1–5] 2-Methoxyestradiol-3,17-*O,O*-bis-sulfamate **1** (2-MeOE2bisMATE) and 2-ethylestradiol-3,17-*O,O*-bis-sulfamate **2** (2-EtE2bisMATE) are members of a series of orally bioavailable compounds that inhibit both cancer cell proliferation and angiogenesis (Fig. 1).[6–10] The potential of compounds such as **1**, which combine an anti-angiogenic effect in concert with a second anti-tumour activity, has been underlined by recent studies demonstrating that administration of a pure anti-angiogenic agent such as the anti-VEGF antibody bevacizumab (an intravenously dosed regimen) alongside conventional chemotherapeutic agents provides for improved clinical outcomes in the treatment of colorectal, lung and breast cancers.[11]

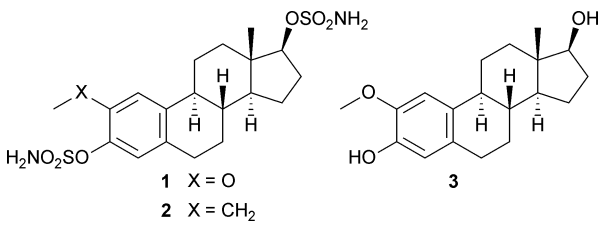

Fig. 1 Structures of 2-methoxyestradiol-3,17-*O,O*-bis-sulfamate **1**, 2-ethylestradiol-3,17-*O,O*-bis-sulfamate **2** and 2-methoxyestradiol **3**.

Although 2-MeOE2bisMATE is an estrogen derivative, its activity is independent of the estrogen receptor (to which it displays negligible affinity) and thus its anti-proliferative effects are expressed against both estrogen receptor positive (ER+) and negative (ER−) human cancer cell lines.[3] The structurally related endogenous estrogen metabolite 2-methoxyestradiol (2-MeOE2),[12,13] a molecule that was until very recently in phase II clinical trials as a treatment for a range of cancers, displays similar anti-proliferative effects, albeit at far higher concentrations (the mean concentrations required to cause 50% growth inhibition across the NCI 60 cell line panel are 1.3 μM for 2-MeOE2 and 87 nM for 2-MeOE2bisMATE).[3] As this result indicates, and as discussed in depth elsewhere,[7,14,15] sulfamoylation of the 3-hydroxyl group delivers greatly enhanced anti-proliferative activity.[10] A number of additional advantages are also conferred by incorporation of the sulfamate group. The sulfamate groups of 2-MeOE2bisMATE block conjugation of the 3- and 17-hydroxyl groups, which leads to inactivation and accelerated systemic clearance in the parent 2-substituted estradiol series. In addition, the 17-*O*-sulfamate group is resistant to metabolism by 17β-hydroxy steroid dehydrogenase (17β-HSD II) which converts 2-MeOE2 into inactive 2-methoxyestrone (2-MeOE1) in the non-sulfamoylated series.[15] Finally, many estrogen sulfamates, including 2-MeOE2bisMATE, reversibly inhibit carbonic anhydrase,[3,16] an enzyme highly expressed in red blood cells. This interaction is believed to underlie the high oral bioavailability observed for estradiol-3-*O*-sulfamates (E2MATEs) *in vivo*, wherein reversible uptake by red blood cells and interaction with carbonic anhydrase

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II (CAII) results in avoidance of first pass liver metabolism.[17] It is also possible that some of the anti-tumour effects observed with sulfamate ester based drugs *in vivo* can be ascribed to inhibition of the hypoxia-induced carbonic anhydrase isozymes CAIX and CAXII.[18] Protein crystallographic studies investigating the interaction of 2-MeOE2bisMATE and related compounds with CAII revealed coordination of a sulfamate NH_2 group to the zinc ion of the enzyme active site.[3] Pharmacokinetic studies in rodents showed the excellent oral bioavailability (>85%) and long half-life of 2-MeOE2bisMATE, which contrasts to the poor bioavailability of p.o. or i.v. dosed 2-MeOE2.[10] Confirmation that the *in vitro* activity could be translated into *in vivo* activity was achieved in NMU-induced tumors in rats and in various xenograft models in athymic nude mice,[3,6] with treatment of nude mice bearing MDA-MB 231 human breast cancer tumour xenografts with 2-MeOE2bisMATE (20 mg kg^{-1}, p.o., qd 28d) causing tumor regression in 8/8 animals and, in 2/8 animals, complete tumor disappearance.[19]

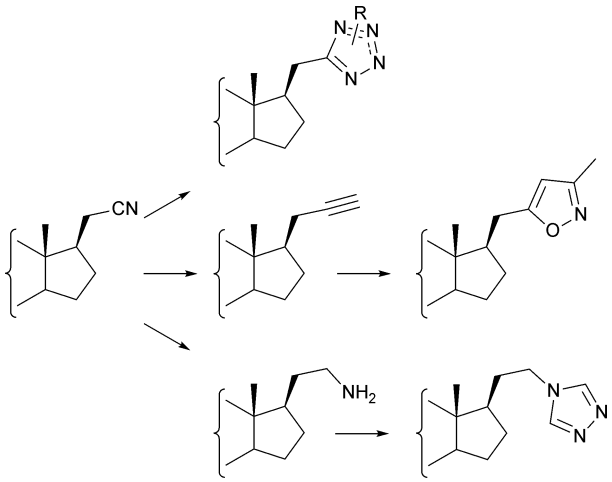
Mechanistic studies have indicated that both the anti-proliferative and anti-angiogenic effects of 2-MeOE2bisMATE are, like 2-MeOE2, principally derived from its ability to disrupt the dynamic polymerisation of tubulin to form the microtubules required for normal cell division.[7] Microtubule disruptor drugs (*e.g.* Taxol and Vinca alkaloids) are widely used in the clinic primarily as intravenous agents but suffer from problems of resistance and toxicity. Recent work from this group has shown that compounds such as 2-MeOE2bisMATE are not only efficacious against multidrug resistant tumours, including those resistant to taxanes,[19] but they are also not a substrate for the P-glycoprotein pump.[20] The development of novel microtubule disruptors, particularly with oral bioavailability, is thus highly appealing and our sulfamate-based approach holds considerable promise to this end.

Having established that optimal activity is derived from a combination of a 2-XMe group (where X = CH_2, O or S) and 3-*O*-sulfamate group in the steroidal A-ring and a H-bond acceptor around C-17 of the D-ring,[2] we were drawn to explore whether 2-substituted estra-1,3,5(10)-trienes bearing a heterocyclic substituent linked to C-17 would exhibit similar, or even enhanced, activity. Herein we report the synthesis of a series of such compounds, their *in vitro* activity and the correlation of this activity to the steric and electronic properties of the putative H-bond acceptor.

Chemistry

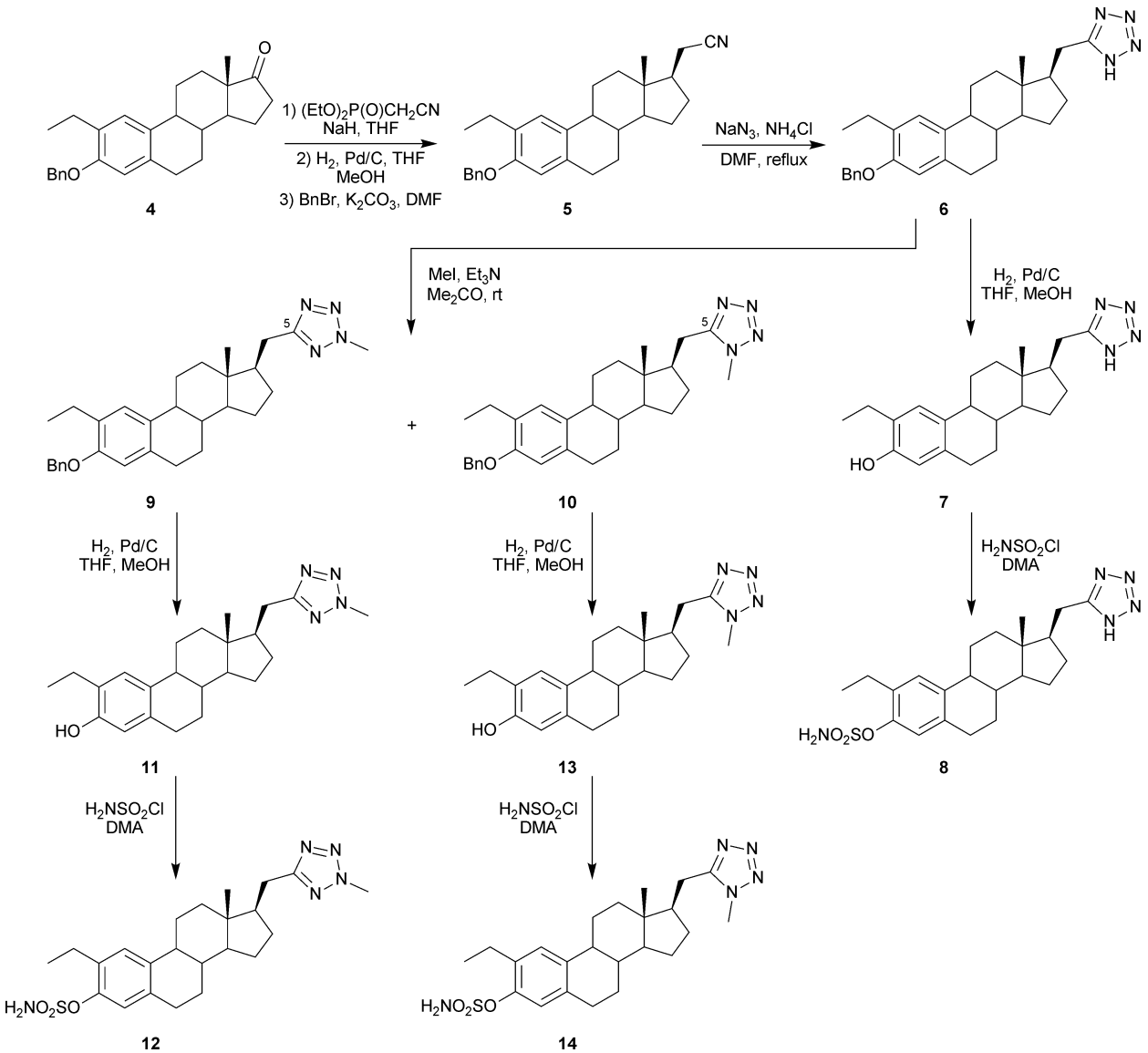
Selection of the heterocyclic substituents and the nature of their attachment to C-17 was based on synthetic accessibility and their potential to exploit those H-bonding interactions around C-17 identified as key to the high anti-proliferative activity of the 2-substituted E2bisMATEs **1** and **2**. We envisaged that a set of heterocycles with diverse electronic properties/H-bonding potential could be synthesised from the relevant nitrile, either by direct dipolar cycloaddition reaction, conversion to the alkyne followed by cycloaddition, or by reduction to the corresponding amine followed by annulation. A set of exploratory tetrazoles, isoxazoles and triazoles was thus targeted (Scheme 1).

The synthesis of the cyanomethyl derivative **5** was achieved in three steps from 2-ethyl-3-*O*-benzylestrone **4** as shown in Scheme 2.[2] Horner–Wadsworth–Emmons olefination with diethylcyanomethyl phosphonate afforded the 17-cyanomethylene derivative. Catalytic hydrogenation over 10% palladium on charcoal effected concomitant debenzylation and double bond reduction. Reprotection of the phenol with benzyl bromide and potassium carbonate allowed access to the nitrile intermediate **5** in high overall yield (86%, 3 steps) from which the tetrazoles, isoxazoles and triazoles would be elaborated. The tetrazole group was installed by dipolar cycloaddition of nitrile **5** and sodium azide to give pentacycle **6**. Hydrogenolysis of **6** over Pd/C gave phenol **7**, which was converted to the corresponding sulfamate **8** by reaction with sulfamoyl chloride in DMA.[21] Alkylation of **6** with methyl iodide in DMF in the presence of potassium carbonate afforded **9** as the sole product, albeit in a modest 28% yield. However, alkylation of **6** with methyl iodide in acetone in the presence of triethylamine delivered the isomeric *N*-methyl tetrazoles **9** and **10**, which were separable by chromatography and were isolated in yields of 41% and 33% respectively. The position of *N*-methylation of the tetrazole ring was determined by NMR spectroscopy using the HMBC ^{1}H-^{13}C long range heterocorrelation experiment, with the spectrum of **10** revealing a correlation between the protons of the methyl group and carbon C5 of the tetrazole. The corresponding HMBC spectrum of **9**, in contrast, showed no correlation between the methyl group and carbon C5 of the tetrazole. Hydrogenolysis of **9** and **10** furnished the corresponding phenols **11** and **13**, which were then sulfamoylated as described above to afford the sulfamoylated tetrazole derivatives **12** and **14**.

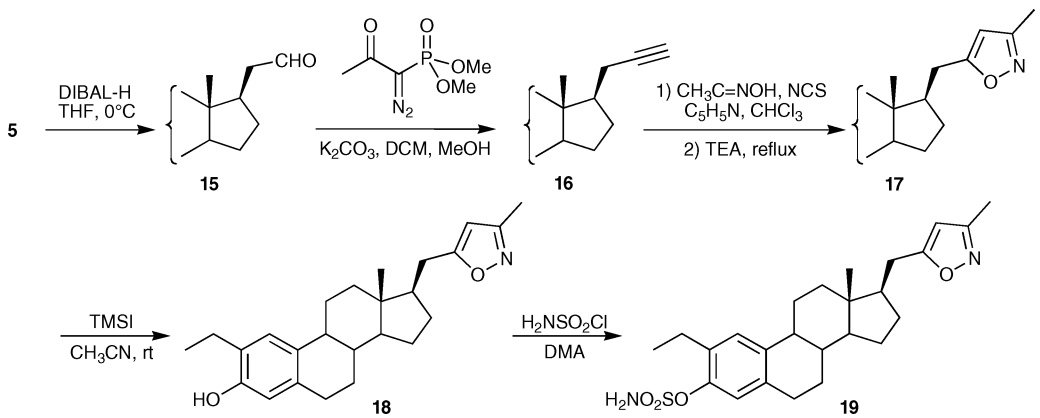


Scheme 1 Proposed elaboration of various heterocycles from the 17β-cyanomethyl group.

Conversion of the common nitrile intermediate **5** into 2-ethyl-17β-(3-methyl-isoxazol-5-ylmethyl)-3-*O*-sulfamoyl-estra-1,3,5(10)-triene **19** required a dipolar cycloaddition of an alkyne with a nitrile oxide. Conversion of the nitrile **5** into the alkyne was achieved in two steps with DIBAL-H reduction of the nitrile **5** delivering the aldehyde **15**, which was then reacted with Ohira's diazophosphonate reagent[22] to deliver the alkyne **16** (Scheme 3) (51% yield, 2 steps). The isoxazole ring was then installed by reaction of **16** in pyridine with acetonitrile oxide (generated *in situ* from the acetaldehyde oxime and *N*-chlorosuccinimide).[23] The 3-*O*-benzyl protecting group of **17** was then removed with trimethylsilyl iodide in acetonitrile to give phenol **18**, which in turn



Scheme 2 The synthesis of C-17 linked tetrazole derivatives.



Scheme 3 Synthesis of C-17 tethered isoxazole derivatives.

was sulfamoylated as described above to give the sulfamoylated oxazole target **19** (43% yield over 2 steps).

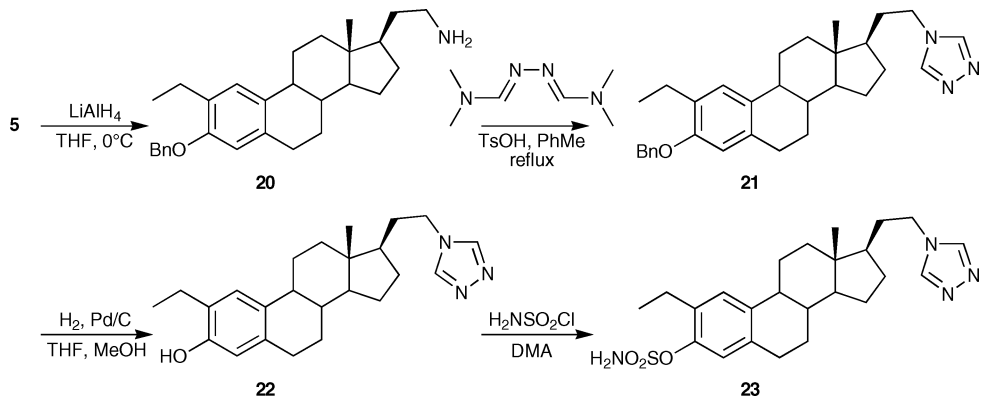
1,2,4-Trisubstituted triazoles are readily accessible by reaction of primary amines and DMF-azine as described by Bartlett *et al.*[24] Thus, 3-*O*-benzyl-2-ethyl-17β-(cyanomethyl)-estra-1,3,5(10)-triene **5** was reduced with lithium aluminium hydride in THF to give the amine **20** in 46% yield (Scheme 4). Acid catalyzed cyclization of **20** with DMF-azine then afforded triazole **21** in 73% yield, which could be transformed into the phenol **22** and sulfamate derivatives **23** as described above.

In addition to the annulation approaches described above, a heterocyclic substituent could also be conveniently introduced by condensation of the C-17 ketone of estrone with an appropriate nucleophile. Thus, acid catalyzed reaction of unprotected 2-methoxyestrone **24** with 4-amino-1,2,4-triazole at 130 °C in ethanol in a sealed tube gave access to the 3-hydroxy-2-methoxy-17-(1,2,4-triazol-4-ylimino)-estra-1,3,5(10)-triene **25** (Scheme 5). Sodium borohydride reduction afforded the phenol **26** whilst sulfamoylation of **25** following the conditions described above led to the sulfamate **27**. Reduction of **27** with sodium borohydride in methanol gave the 2-methoxy-3-*O*-sulfamoyl-17-(1,2,4-triazol-4-ylamino)-estra-1,3,5(10)-triene **28**. Compounds **27** and **28** were of interest to explore any effects of C-17 stereochemistry and rotational freedom on biological activity.

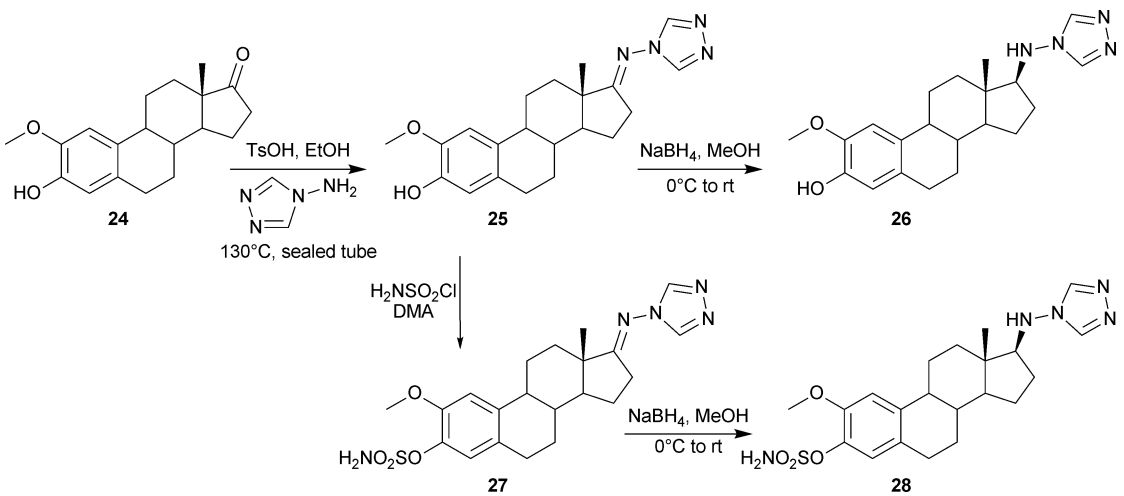
Results and discussion

To assess their potential as anti-cancer agents, members of the series of novel C-17 heterocycle substituted 2-ethyl estratriene derivatives were evaluated for their ability to inhibit the proliferation of DU-145 (prostate) and MDA-MB 231 (ER–) breast cancer cells *in vitro*. In addition, compounds **12**, **14**, **19** and **23** were also evaluated for their ability to inhibit the proliferation of MCF-7 (ER+ breast) cancer cells. The results of these assays and comparator data for 2-MeOE2, 2-MeOE2bisMATE and 2-EtE2bisMATE are presented in Table 1.

As can be seen from the data presented in Table 1, the phenolic compounds displayed little or no activity in the assay (GI_{50} > 10 μM or 100 μM, depending on cell lines), although tetrazole **11** exhibited modest activity against the proliferation of MDA MB-231 cells. Clearly, in the 2-substituted estradiol series, substitution of the 17β-hydroxyl group with the heterocyclic groups selected in this study does not yield more active compounds, albeit the parent 2-ethyl estradiol only displays modest activity compared to 2-MeOE2. In order to assess the possibility that these results might be linked to a decrease in cellular permeability of the heterocyclic compounds, their ClogP values were calculated by using ChemDraw software version 11.0. The ClogP of the phenols and sulfamates, although higher than those of the bis-sulfamates **1**



Scheme 4 Synthesis of 1,2,4-triazole derivatives.



Scheme 5 Synthesis of C-17 4-amino-(1,2,4)-triazole derivatives.

Table 1 Anti-proliferative activities of substituted estradiol derivatives against DU-145 human prostate cancer cells, MDA-MB-231 and MCF-7 human breast cancer cells

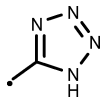
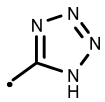
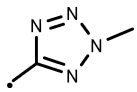
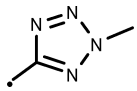
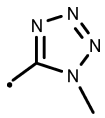
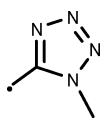
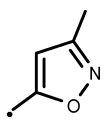
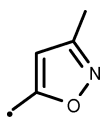
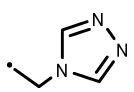
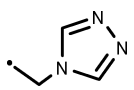
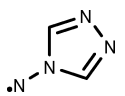
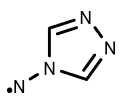
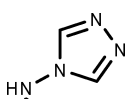
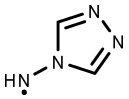
					$GI_{50}/\mu M$		
Compound	R_1	R_2	R_3	Clog*P*	DU-145	MDA-MB-231	MCF-7
7	Et	H		2.33	>100	>100	ND
8	Et	SO_2NH_2		1.47	>100	>100	ND
11	Et	H		2.37	47.0	19.2	ND
12	Et	SO_2NH_2		1.52	1.64	1.71	0.8
13	Et	H		2.28	>100	>100	ND
14	Et	SO_2NH_2		1.42	0.39	0.55	0.34
18	Et	H		3.18	>100	ND	ND
19	Et	SO_2NH_2		2.32	1.83	ND	1.62
22	Et	H		1.97	>100	>100	ND
23	Et	SO_2NH_2		1.11	0.85	0.61	0.34
25	MeO	H		–0.56	ND	ND	>10
27	MeO	SO_2NH_2		–1.40	ND	ND	10
26	MeO	H		1.77	ND	ND	>10

Table 1 *(Contd.)*

Compound	R_1	R_2	R_3	Clog*P*	GI_{50}/μM		
					DU-145	MDA-MB-231	MCF-7
28	MeO	SO_2NH_2		0.93	ND	ND	>10
3	MeO	H	OH	0.60	1.22	0.94	2.35
	Et	H	OH	1.73	10.3	8.0	10.5
1	MeO	SO_2NH_2	OSO_2NH_2	–0.69	0.34	0.28	0.25
2	Et	SO_2NH_2	OSO_2NH_2	0.42	0.21	0.21	0.07

and **2** (Table 1), were found to be within reasonable values for good absorption and permeation. The benefits of the 3-*O*-sulfamate substituent on anti-proliferative activity in this series of C-17 heterocycle substituted compounds are, however, evident in Table 1 and in agreement with those results obtained in previous studies.[1–4] A number of the novel, sulfamoylated estratriene derivatives displayed anti-proliferative activity in the micromolar (tetrazole **12**, oxazole **19** and triazole **27**) to sub-micromolar (tetrazole **14** and triazole **23**) range (Table 1).

In the tetrazole series, the 1-methyl derivative **14** proved to be 4-fold more active than the isomeric 2-methyl compound **12** and greater than 250-fold more active than the non-methylated tetrazole **8**, which can exist in both the 1-*H* and 2-*H* tautomeric forms. One potential explanation for this difference in activity is the relative potential of **14**, **12** and **8** to exploit lipophilic interactions at the site of interaction with tubulin with, presumably, the 1-methyl derivative **14** possessing optimal substitution. Support for this postulate can be drawn from the bisMATE series, where steric factors are seen to be key determinants of activity and *N*-methylation causes a radical reduction in activity.[6] However, since the C-17 sulfamate appears to act as a H-bond acceptor[3] and alternate H-bond acceptor groups at C-17 also confer high anti-proliferative activity,[2,5] it is also necessary to consider the electronic character of the tetrazole series and, indeed, that of the other heterocycles appended to C-17. In order to determine the electron densities around the heterocyclic ring and thus the potential of the respective heterocycles and their substituted derivatives to participate in electrostatic interactions with the residues of tubulin proximal to the site of interaction, a series of calculations using Mulliken population analysis was performed. Compounds **2**, **12**, **14**, **19** and **23** were initially built and energy minimized in the Sybyl 7.0 molecular modelling suite using the MMFFs94 force field and charges.[25] The calculations were then carried out at the end of a full geometry optimisation using the PM3 level of calculation as implemented within the ArgusLab 4.0.1 molecular modelling package.[26] The calculated electron densities are displayed in Fig. 2. Importantly, we only considered compound **2** in its neutral form. Indeed, although the pK_as of aromatic sulfamates (typically around 7–9) allow for partial ionization at physiological pH, aliphatic sulfamates are slightly less acidic (pK_a around 8–10), as shown, for instance, by a comparative study of the pK_as of imidazolylphenyl sulfamates and (imidazolylphenoxy)alkyl sulfamates[27] and it is likely that the D-ring sulfamate is mostly unionized. As can be seen from Fig. 2, and unsurprisingly, the D-ring sulfamate of compound **2** can clearly act as a strong H-bond acceptor with its two electron rich oxygen atoms. Likewise, Fig. 2 shows that both the *N*-5 nitrogen of **14** and the carbon of the *N*-Me group of *N*-1 are electron rich and are thus able to undergo electrostatic interaction with electron deficient residues of the active site. The *N*-5 and

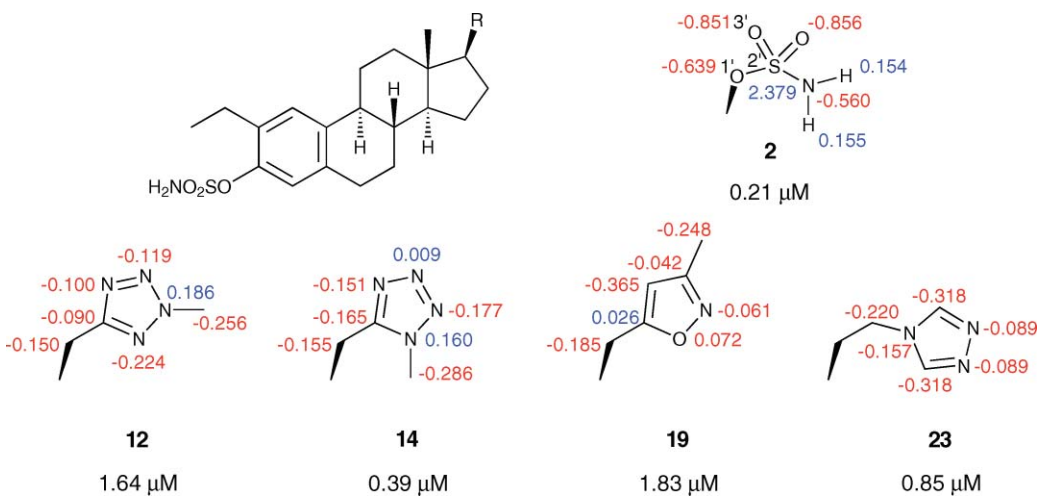
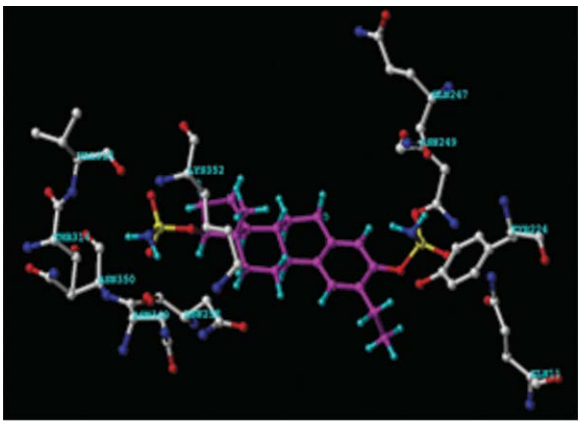


Fig. 2 Mulliken charges as calculated following the Mulliken population analysis for compounds **2**, **12**, **14**, **19** and **23**. GI_{50} values against the proliferation of DU-145 human prostate cancer cells are also presented.

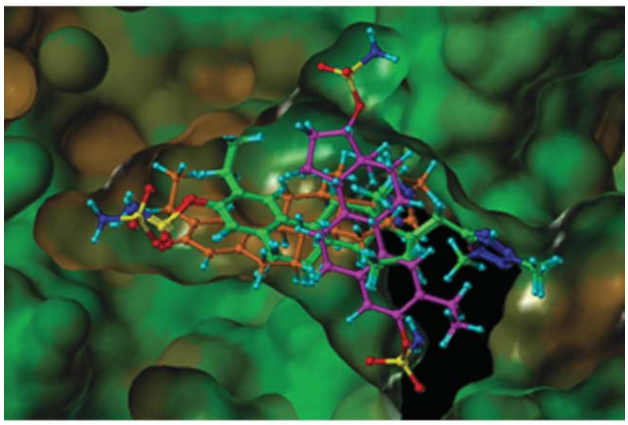
N-1 groups of **12** are also electron rich and would thus offer similar potential for electrostatic interaction as those available to **14**, although methylation of *N*-2 may sterically impede the ligand–tubulin interaction and thus result in the 4-fold difference in anti-proliferative activity between **14** and **12**. Interestingly, isoxazole **19**, which displays a similar electron density profile across the heterocycle to tetrazole **12**, shows comparable anti-proliferative activity. In contrast, the non-methylated tetrazole **8** is clearly less able to participate in electrostatic interaction as the tautomeric forms interconvert rapidly in solution and thus offer no stable dipole with which to interact with the proximal residues of the site of action. Additionally, since the pK_a of compound **8** is probably in the same range as the one of tetrazole (pK_a = 4.89), the predominant form of **8** in physiological solution is very likely to be its aromatic tetrazole anion species, which offers little H-bonding potential. It would therefore appear that there is a good correlation between the anti-proliferative activity across the oxazole/tetrazole series and the ability of the respective oxazole and tetrazole derivatives to undergo electrostatic interaction (**14** > **12** and **19** >> **8**) and furthermore that steric effects also play a significant role in differentiating between the activity of **12** and **14**. The electron density calculated for triazole **23** indicates the presence of different electron rich atoms (*N*-4, *C*-3 and *C*-5), which offer potential for electrostatic interactions with electron deficient residues of the active site and shows two unfavourable electron deficient nitrogen atoms.

In order to investigate the potential mode of binding of compounds **12**, **14**, **19** and **23**, these compounds, together with 2EtE2bisMATE **2** were energy minimized using the MMFFs94 force field as implemented within Sybyl 7.0 then docked into the 1SA0 crystal structure of tubulin[28] using the GOLD package. The colchicine binding site was defined as a 8.5 Å radius around the terminal carbon of Leu255 and each ligand was docked a total of 30 times and scored with the GOLD Score scoring function. Docking results obtained for 2EtE2bisMATE **2** are presented in Fig. 3. Optimal docking shows the H-bonding interactions of the 3-*O*-sulfamate group with the amide of the side chain of Asn249 and the OH group of Tyr224. The C-17 sulfamate group of **2** occupies a small, essentially lipophilic pocket (Fig. 3a) where potential H-bonding interactions are available with the backbones of Asn 258, Val315, Asn349, Asn350 and Lys352 and the side chain hydroxy group of Thr314.

Due to size constraints, the binding pocket around the C-17 *O*-sulfamate of 2-EtE2bisMATE **2** is unable to accommodate the different heterocycles of compounds **12**, **14**, **19** and **23**. As a result, the highest scoring binding modes obtained for these compounds differ noticeably from that of **2** (Fig. 3b). A consistent binding mode was observed for compounds **12**, **14** and **19** in which the tetrazole and isoxazole rings lie in a lipophilic pocket formed by Tyr224, Ser178 and Thr179 (Fig. 4). These residues provide several potential H-bonding interactions with the heterocycles. In particular, Ser178 and the OH group of Tyr224 and NH of Thr179 are likely to interact through H-bonding with the heterocycles. The difference in activity between the most active 1-methyltetrazole compound **14** and its *N*-2 isomer **12** and the isoxazole **19** appears to be due to the position of the methyl group which, in the case of **14**, better favours H-bonding interactions. Indeed, the methyl group of compounds **12** and **19** looks likely to partly shield the tetrazole and isoxazole respectively and prevent them from interacting with Tyr224.



(a)



(b)

Fig. 3 (a) Docking of 2-EtE2bisMATE **2** into the 1SA0 crystal structure of tubulin and (b) docking of 2-EtE2bisMATE **2** (magenta), compounds **12**, **14**, **19** (green) and **23** (orange). The surface of the protein was generated to show a colour gradient from electron donors as red, to lipophilic areas in green.

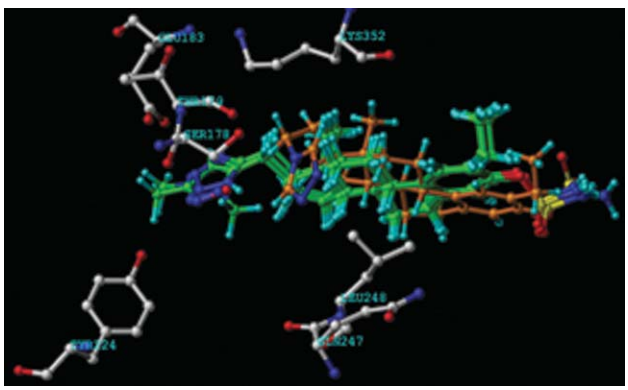


Fig. 4 Docking of compounds **12**, **14**, **19** (green) and **23** (orange) in the colchicine binding site of tubulin.

An alternate binding mode was obtained for compound **23** (Fig. 4). Whilst the steroid skeleton and 3-*O*-sulfamate group of **12**, **14**, **19** and **23** occupy the same area of space, the triazole group at position C-17 of **23** projects into a distinct area of space with respect to the tetrazole and isoxazole rings of **12**, **14** and **19**. Thus, for **23**, the highest scored dock suggests that triazole

can potentially form a favorable cation–π interaction with Lys352 in addition to lipophilic interactions (in particular with Leu248). These interactions offer a possible explanation as to why **23** retains a good level of anti-proliferative activity even though it differs significantly from other active members of the screening set since, although the nitrogen atom at position 3′ of compound **23** displays a similar electron density to the corresponding nitrogen atom in compound **14** (Fig. 2), it is unlikely to act as a H-bond acceptor, its lone pair being involved in the aromaticity of the triazole ring.

2-Methoxy-3-*O*-sulfamoyl-17-(4*H*-1,2,4-triazol-4-ylimino)-estra-1,3,5(10)-triene **27** exhibited modest activity (GI_{50} = 10 μM in MCF-7 cell line) indicating that C-17β stereochemistry is not absolutely essential for anti-proliferative activity as previously observed in earlier studies on *O*-substituted C-17-oximo-derivatives of 2-methoxy-3-*O*-sulfamoylestrone.[5] The related amine **28** was, however, inactive, suggesting that the activity of **27** may derive from the imino group and not the tethered heterocycle.

Conclusion

Our study has shown that high anti-proliferative activity can be retained in 2-substituted estratriene-3-*O*-sulfamates by substitution with an appropriate heterocycle (**14**, **23**) at C-17. As demonstrated by the range of activities obtained for the tetrazoles **8**, **12** and **14**, the presence of both a hydrogen bond acceptor and its steric environment are likely key to high anti-proliferative activity. Docking studies of these compounds and isoxazole **19** in the colchicine binding site of tubulin suggest that they have a similar binding mode. A number of potential H-bonding interactions between the C-17 heterocyclic substituent and the postulated site of interaction were identified and used to explain some of these requirements. Based on the computational analysis, the distinct binding mode observed for compound **23** suggests that cation–π and lipophilic interactions around C-17 could explain the anti-proliferative activity of this compound more than those of H-bonding. The results obtained in this SAR study, particularly those for compounds **23** and **32**, are currently being used to design further candidate microtubule disruptor compounds as potential therapeutic agents for the treatment of cancer.

Experimental section

Materials and methods

Chemistry. All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, UK), Fluka (Gillingham, UK) or Lancaster Synthesis (Morecambe, UK). Organic solvents of A.R. grade were supplied by Fisher Scientific (Loughborough, UK) and used as supplied. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[29] and was stored in the refrigerator under positive pressure of N_2 as a solution in toluene as described by Woo *et al.*[30] An appropriate volume of this solution was freshly concentrated *in vacuo* immediately before use. Reactions were carried out at room temperature (rt) unless otherwise stated. Flash column chromatography was performed on silica gel (MatrexC60). ^{1}H NMR and ^{13}C NMR spectra were recorded with either a JMN-GX270 at 270 and 67.5 MHz respectively or a Varian Mercury VX400 spectrometer at 400 and 100.6 MHz. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS) as an internal standard and coupling constants in hertz (J). Mass spectra were recorded at the Mass Spectrometry Service Center, University of Bath, UK. FAB-MS were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Stuart SMP3 melting point apparatus and are uncorrected.

Biology. The effects of compounds on MCF-7, DU-145 and MDA MB-231 cell growth were determined using a microtiter plate assay as described previously.[4,31]

Molecular modeling. Compounds were built in the SYBYL 7.0 molecular modelling program.[25] To obtain low energy conformations of these models, energy minimization was performed to convergence using the MMFF94s forcefield with MMFF94 charges. The calculation of Mulliken populations was then performed at the end of a full geometry optimisation using the PM3 level of calculation as implemented within ArgusLab 4.0.1 molecular modelling package.[26] Compounds were then docked into the 1SA0 crystal structures[28] using the GOLD package. The active site was defined as an 8.5 angstrom radius around the terminal carbon of Leu255. Each ligand was docked a total of 30 times and scored with the GOLD Score fitness function.

2-Ethyl-3-O-benzyl-17β-(cyanomethyl)-estra-1,3,5(10)-triene ***5***. To a suspension of NaH (10 mmol, 400 mg of a 60% dispersion in mineral oil) in dry THF (50 mL) stirred at 0 °C under nitrogen was added dropwise diethylcyanomethylphosphonate (1.77 g, 10 mmol). After completion, the mixture was stirred for an additional 30 minutes before **4** (1.94 g, 5 mmol) in THF (10 mL) was added dropwise. The solution was stirred at room temperature for 18 h, cooled to 0 °C and quenched with water (30 mL). The mixture was then extracted with ethyl acetate, the combined organic layers washed with water, brine and dried and evaporated. Chromatography (hexane–ethyl acetate 25 : 1 to 20 : 1) gave the olefin as a mixture of *Z* and *E* isomers. The olefin was then dissolved in THF (10 mL) and methanol (30 mL) and treated with 10% Pd/C (100 mg) prior to stirring under an atmosphere of hydrogen for 18 h. The resultant suspension was filtered through celite, washed with ethyl acetate and then evaporated. The resultant solid was stirred with K_2CO_3 (1.38 g, 10 mmol) and benzyl bromide (0.59 mL, 5 mmol) in DMF (20 mL) for 24 h at room temperature. After addition of water (50 mL), the organics were extracted with ethyl acetate (2 × 80 mL), the combined organic layers washed with water, brine, dried and evaporated. Flash chromatography (hexane–ethyl acetate 20 : 1) gave **5** (1.7 g, 86% over 3 steps) as a white solid. Mp 158–159 °C; δ_H (270 MHz, $CDCl_3$): 0.67 (3H, s), 1.21 (3H, t, *J* 7.4), 1.26–1.56 (6H, m), 1.60–1.66 (1H, m), 1.72–2.01 (5H, m), 2.12–2.34 (2H, m), 2.62 (2H, q, *J* 7.4), 2.76–2.81 (2H, m), 2.84 (1H, dd, *J* 14.9 and 5.4), 3.10 (1H, dd, *J* 14.9 and 5.7), 5.07 (2H, s), 6.70 (1H, s), 7.06 (1H, s) and 7.28–7.50 (5H, m); δ_C (67.5 MHz, $CDCl_3$) 12.3, 14.6, 17.7, 23.5, 24.0, 26.3, 27.8, 28.3, 29.7, 37.4, 38.9, 42.7, 43.9, 54.4, 69.8, 111.9, 119.8, 126.2, 127.0, 127.6, 128.4, 130.3, 132.0, 134.9, 137.7, 145.5 and 154.5; *m/z* (ESI+) 414.1 ([M + H]$^+$, 100%).

2-Ethyl-3-O-benzyl-17β-(1H-tetrazol-5-ylmethyl)-estra-1,3,5(10)-triene ***6***. A mixture of **5** (0.83 g, 2 mmol), sodium azide (0.26 g, 4 mmol) and ammonium chloride (214 mg, 4 mmol) in DMF (10 mL) was refluxed for 24 h. Four further aliquots of sodium

azide (0.13 g, 1 mmol) were added after 24, 48, 72 and 96 h prior to cessation of reflux after 120 h and cooling to room temperature. Water (50 mL) and ethyl acetate (100 mL) were then added and the separated organic layer washed successively with water and brine, dried and evaporated. Purification (chromatography hexane–ethyl acetate 5 : 1 to 3 : 2) gave a white solid (0.78 g, 85%). Mp 214–215 °C; δ_H (270 MHz, CD_3COCD_3) 0.79 (3H, s), 1.15 (2H, t, *J* 7.4), 1.22–1.48 (6H, m), 1.60–1.66 (1H, m), 1.72–2.01 (5H, m), 2.12–2.34 (2H, m), 2.62 (2H, q, *J* 7.4), 2.78 (2H, m), 2.84 (1H, dd, *J* 14.9 and 5.4), 3.10 (1H, dd, *J* 14.9 and 5.7), 5.07 (2H, s), 6.70 (1H, s), 7.06 (1H, s) and 7.28–7.50 (5H, m); δ_C (100 MHz, CD_3COCD_3) 11.8, 14.3, 23.3, 23.9, 24.0, 26.3, 27.8, 28.0, 29.5, 37.4, 39.0, 42.5, 44.1, 49.4, 54.5, 69.4, 111.9, 126.1, 127.2, 127.6, 128.4, 129.6, 132.1, 134.8, 138.1, 154.4 and 164.7; *m/z* (FAB–) 455.23 ([M – H]$^-$, 100%).

2-Ethyl-3-hydroxy-17β-(1H-tetrazol-5-ylmethyl)-estra-1,3,5(10)-triene 7. A solution of **6** (228 mg, 0.5 mmol) in THF (5 mL) and methanol (15 mL) was treated with 10% Pd/C (50 mg) then stirred under an atmosphere of hydrogen for 48 h. The resultant suspension was then filtered through celite, washed with ethyl acetate and evaporated under reduced pressure. The crude solid was purified by flash chromatography (hexane–ethyl acetate 4 : 1 to 1 : 1) and recrystallised from ethyl acetate to give a white powder (155 mg, 85%). Mp 248–249 °C; δ_H (400 MHz, CD_3COCD_3) 0.81 (3H, s), 1.15 (3H, t, *J* 7.4), 1.20–1.47 (6H, m), 1.60–1.66 (1H, m), 1.72–2.01 (5H, m), 2.12–2.34 (2H, m), 2.60 (2H, q, *J* 7.4), 2.78 (2H, m), 2.94 (1H, dd, *J* 14.5 and 5.5), 3.10 (1H, dd, *J* 14.9 and 5.8), 6.54 (1H, s) and 7.02 (1H, s); δ_C (100 MHz, CD_3COCD_3) 11.2, 13.6, 22.5, 23.3, 25.4, 25.8, 27.2, 27.6, 29.0, 37.0, 38.5, 41.8, 43.5, 49.2, 53.9, 114.1, 125.4, 126.8, 130.3, 133.9, 151.9 and 164.9; *m/z* (FAB+): 367.2488 [M + H]$^+$. $C_{22}H_{31}N_4O$ requires 367.2498.

*2-Ethyl-3-O-sulfamoyl-17β-(1H-tetrazol-5-ylmethyl)-estra-1,3,5(10)-triene **8**.* Phenol **7** (80 mg, 0.22 mmol) was added to an ice cold solution of sulfamoyl chloride (0.87 mmol) in DMA (1 mL). The resulting mixture was stirred for 16 hours at room temperature then diluted with ethyl acetate (50 mL), and washed with water (3 × 20 mL), brine (20 mL), dried and evaporated. The resulting solid was purified by flash chromatography (hexane–ethyl acetate 1 : 1) to give a white powder (50 mg, 52%). (Found C, 59.00; H, 7.05; N, 15.50 requires $C_{22}H_{31}N_5O_3S$ C, 59.30; H, 7.01; N, 15.72%); mp 223–224 °C. δ_H (400 MHz, CD_3COCD_3) 0.68 (3H, s), 1.04 (3H, t, *J* 7.3), 1.08–1.40 (7H, m), 1.49–1.55 (1H, m), 1.62–1.80 (3H, m), 1.82–1.94 (2H, m), 2.07–2.14 (1H, m), 2.18–2.24 (1H, m), 2.56 (2H, q, *J* 7.3), 2.66–2.76 (3H, m), 2.98 (1H, dd, *J* 14.8 and 5.9), 6.95 (1H, s) and 7.01 (1H, s); δ_C (100 MHz, CD_3COCD_3) 11.8, 14.2, 22.8, 23.9, 26.1, 27.5, 28.0, 28.9, 29.2, 37.3, 38.6, 42.5, 44.2, 49.4, 54.4, 121.8, 126.7, 133.9, 135.4, 138.6, 146.6 and 164.9; *m/z* (FAB+) 446.4 ([M + H]$^+$, 100%).

*2-Ethyl-17β-(2-methyl-2H-tetrazol-5-ylmethyl)-3-O-benzyl estra-1,3,5(10)-triene **9** and 2-ethyl-17β-(1-methyl-2H-tetrazol-5-ylmethyl)-3-O-benzyl estra-1,3,5(10)-triene **10***. **Method A**: A mixture of **6** (225 mg, 0.49 mmol), methyl iodide (0.1 mL, 1.6 mmol) and potassium carbonate (207 mg, 1.5 mmol) in DMF (2 mL) was stirred at room temperature for 24 h. The mixture was stirred for an additional 72 h during which time additional aliquots of methyl iodide (0.5 mL, 8 mmol) were added every 24 hours. After addition of water (20 mL), the organics were extracted with ethyl acetate (2 × 50 mL) and the combined organic layers were washed with water, brine, dried and evaporated. The residual solid was purified by flash chromatography (hexane–ethyl acetate 10 : 1 to 1 : 1) to give **9** (65 mg, 28%) and **6** (30 mg, 13%) as white powders.

Method B: A solution of **6** (456 mg, 1 mmol), methyl iodide (0.12 mL, 2 mmol) and triethylamine (0.28 mL, 2 mmol) in acetone (10 mL) was stirred at room temperature for 5 h. After addition of water (20 mL), the mixture was extracted with ethyl acetate (2 × 50 mL) and the combined organic layers were washed with water, brine, dried and evaporated. The residual solid was purified by flash chromatography (hexane–ethyl acetate 10 : 1 to 3 : 1) to give **9** (155 mg, 33%) and **10** (195 mg, 42%) as white powders.

2-Ethyl-17β-(2-methyl-2*H*-tetrazol-5-ylmethyl)-3-*O*-benzyl estra-1,3,5(10)-triene **9**; mp 144–145 °C. δ_H (270 MHz, $CDCl_3$) 0.74 (3H, s), 1.19 (3H, t, *J* 7.4), 1.21–1.51 (7H, m), 1.60–2.03 (6H, m), 2.15–2.34 (2H, m), 2.62 (2H, q, *J* 7.4), 2.66 (1H, dd, *J* 14.4 and 9.7), 2.81 (2H, m), 2.99 (1H, dd, *J* 14.9 and 5.0), 4.23 (3H, s), 5.03 (2H, s), 6.61 (1H, s), 7.09 (1H, s) and 7.26–7.45 (5H, m); δ_C (100 MHz, $CDCl_3$) 12.5, 14.7, 23.5, 24.2, 26.2, 26.5, 27.9, 28.4, 29.8, 37.6, 39.0, 39.2, 42.7, 44.1, 49.6, 54.5, 69.9, 111.9, 126.2, 127.1, 127.6, 128.5, 130.2, 132.5, 135.1, 137.8, 154.5 and 167.0; *m/z* (ESI+) 471.3107 [M + H]$^+$. $C_{30}H_{39}N_4O$ requires 471.3118.

2-Ethyl-17β-(1-methyl-2*H*-tetrazol-5-ylmethyl)-3-*O*-benzyl estra-1,3,5(10)-triene **10**; mp 125–126 °C. δ_H (270 MHz, $CDCl_3$) 0.69 (3H, s), 1.13 (3H, t, *J* 7.3), 1.15–1.52 (7H, m), 1.59–1.97 (5H, m), 2.09–2.27 (2H, m), 2.58 (2H, q, *J* 7.3), 2.60 (1H, m), 2.74 (2H, m), 2.87 (1H, dd, *J* 14.9 and 4.6), 3.91 (3H, s), 4.95 (2H, s), 6.55 (1H, s), 7.00 (1H, s) and 7.26–7.40 (5H, m); δ_C (100 MHz, $CDCl_3$) 12.6, 14.7, 23.5, 23.9, 24.2, 26.4, 27.8, 28.4, 29.7, 33.4, 37.4, 38.9, 42.8, 44.0, 48.6, 54.3, 69.9, 111.9, 126.2, 127.1, 127.7, 128.5, 130.3, 132.2, 135.0, 137.8 and 154.5, 155.1; *m/z* (ESI+) 471.3101 [M + H]$^+$. $C_{30}H_{39}N_4O$ requires 471.3118.

*2-Ethyl-3-hydroxy-17β-(2-methyl-2H-tetrazol-5-ylmethyl)estra-1,3,5(10)-triene **11**.* A solution of **9** (165 mg, 0.35 mmol) in THF (5 mL) and methanol (15 mL) was treated with 10% Pd/C (40 mg) then stirred under an atmosphere of H_2 for 24 h as described for the synthesis of **7**. The resultant crude solid was purified by flash chromatography (hexane–ethyl acetate 8 : 1) and recrystallised (hexane–diethyl ether 2 : 1) to give a white powder (85 mg, 64%). (Found C, 72.50; H, 8.50; N, 15.16 requires $C_{23}H_{32}N_4O$ C, 72.60; H, 8.48; N, 14.72%); mp 117–118 °C; δ_H (270 MHz, CD_3COCD_3) 0.73 (3H, s), 1.19 (3H, t, *J* 7.3), 1.21–1.53 (7H, m), 1.58–2.00 (5H, m), 2.11–2.29 (2H, m), 2.58 (2H, q, *J* 7.3), 2.71 (1H, dd, *J* 14.6 and 9.9), 2.76 (2H, m), 2.97 (1H, dd, *J* 14.6 and 5.0), 4.29 (3H, s), 4.73 (1H, s), 6.48 (1H, s) and 7.02 (1H, s); δ_C (100 MHz, CD_3COCD_3) 12.5, 14.4, 23.1, 24.2, 26.2, 26.5, 27.8, 28.3, 29.3, 37.6, 38.9, 39.3, 42.7, 44.1, 49.6, 54.5, 115.2, 126.3, 127.2, 132.7, 135.5, 151.2 and 167.0; *m/z* (FAB+): 381.2648 [M + H]$^+$. $C_{23}H_{32}N_4O$ requires 381.2654.

*2-Ethyl-3-O-sulfamoyl-17β-(2-methyl-2H-tetrazol-5-ylmethyl)-estra-1,3,5(10)-triene **12**.* Phenol **11** (65 mg, 0.17 mmol) was reacted with sulfamoyl chloride (0.34 mmol) in DMA (1 mL) as described for the synthesis of **8**. Flash chromatography (hexane–ethyl acetate 5 : 1 to 2 : 1) gave a white powder which was recrystallized (hexane–ethyl acetate 3 : 1) (70 mg, 90%). (Found C, 59.80; H, 7.33; N, 14.90 requires $C_{23}H_{33}N_5O_3S$ C, 60.11; H, 7.24; N,

15.24%); mp 86–87 °C; δ_H (270 MHz, $CDCl_3$) 0.67 (3H, s), 1.13 (3H, t, *J* 7.3), 1.19–1.44 (6H, m), 1.60–1.96 (6H, m), 2.09–2.24 (2H, m), 2.61 (2H, q, *J* 7.3), 2.65 (1H, dd, *J* 14.6 and 9.7), 2.75 (2H, m), 2.92 (1H, dd, *J* 14.6 and 5.0), 4.23 (3H, s), 5.01 (2H, br s), 7.00 (1H, s) and 7.10 (1H, s); δ_C (100 MHz, $CDCl_3$) 12.5, 14.6, 23.5, 24.2, 26.2, 26.3, 27.6, 28.3, 29.2, 37.5, 38.4, 39.3, 42.6, 44.3, 49.5, 54.5, 121.4, 127.0, 133.6, 136.0, 139.7, 146.1 and 166.9; *m/z* (FAB+): 460.2381 $[M + H]^+$. $C_{23}H_{34}N_5O_3S$ requires 460.2382.

2-Ethyl-3-hydroxy-17β-(1-methyl-2H-tetrazol-5-ylmethyl)estra-1,3,5(10)-triene ***13***. A solution of **10** (110 mg, 0.23 mmol) in THF (5 mL) and methanol (20 mL) was treated with 10% Pd/C (30 mg) then stirred under an atmosphere of hydrogen for 24 h as described for the synthesis of **7**. The resultant crude solid was recrystallised (hexane–ethyl acetate 1 : 5) to give pale yellow needles (80 mg, 90%). (Found C, 72.60; H, 8.51; N, 14.30 requires $C_{23}H_{32}N_4O$ C, 72.60; H, 8.48; N, 14.72%); mp 206–207 °C; δ_H (270 MHz, $CDCl_3$) 0.76 (3H, s), 1.22 (3H, t, *J* 7.3), 1.24–1.55 (7H, m), 1.65–2.03 (5H, m), 2.14–2.32 (2H, m), 2.60 (2H, q, *J* 7.3), 2.67 (1H, dd, *J* 14.8 and 5.2), 2.73–2.82 (2H, m), 2.96 (1H, dd, *J* 14.8 and 4.5), 4.02 (3H, s), 5.18 (1H, s), 6.53 (1H, s) and 7.03 (1H, s); δ_C (100 MHz, $CDCl_3$) 12.5, 14.4, 23.0, 23.8, 24.1, 26.3, 27.7, 28.3, 29.2, 33.4, 37.3, 38.8, 42.7, 43.9, 48.5, 54.2, 115.2, 126.2, 127.3, 132.2, 135.3, 151.3 and 155.0; *m/z* (FAB+): 381.2645 $[M + H]^+$. $C_{23}H_{32}N_4O$ requires 381.2654.

2-Ethyl-3-O-sulfamoyl-17β-(1-methyl-2H-tetrazol-5-ylmethyl)-estra-1,3,5(10)-triene ***14***. Phenol **13** (50 mg, 0.13 mmol) was reacted with sulfamoyl chloride (0.26 mmol) in DMA (1 mL) as described for the synthesis of **8**. Flash chromatography (hexane–ethyl acetate 1 : 1 to 1 : 3) gave a white powder (30 mg, 52%). (Found C, 59.80; H, 7.22; N, 15.20 requires $C_{23}H_{33}N_5O_3S$ C, 60.11; H, 7.24; N, 15.24%); mp 218–219 °C; δ_H (270 MHz, $CDCl_3$) 0.76 (3H, s), 1.19 (3H, t, *J* 7.3), 1.20–1.51 (7H, m), 1.54–2.01 (8H, m), 2.12–2.30 (2H, m), 2.67 (2H, q, *J* 7.3), 2.70 (1H, dd, *J* 14.9 and 5.3), 2.78–2.83 (2H, m), 2.95 (1H, dd, *J* 14.9 and 4.6), 4.00 (3H, s), 5.05 (2H, s), 7.06 (1H, s) and 7.15 (1H, s); δ_C (100 MHz, $CDCl_3$) 12.5, 14.7, 23.1, 23.9, 24.2, 26.2, 27.5, 28.4, 29.2, 33.5, 37.3, 38.4, 42.7, 44.2, 48.6, 54.3, 121.5, 126.9, 133.7, 136.0, 139.3, 146.2 and 155.0; *m/z* (FAB+): 460.2376 $[M + H]^+$. $C_{23}H_{34}N_5O_3S$ requires 460.2382.

2-Ethyl-3-O-benzyl-estra-1,3,5(10)-triene 17β-acetaldehyde ***15***. A THF solution of DIBAL (3.6 mL, 1.5 M, 5.4 mmol) was added in a dropwise manner to a 0 °C solution of **5** (0.75 g, 1.80 mmol). The reaction was then allowed to warm to rt and stirred for a further 3 h before acidifying with HCl (2 M aqueous). After 0.5 h, the mixture was extracted with ethyl acetate (3 × 30 mL), the combined organics were washed with water, then brine, dried and evaporated to give a white powder (480 mg, 64%); mp 135–136 °C; δ_H (270 MHz, $CDCl_3$) 0.69 (3H, s), 1.26 (3H, t, *J* 7.3), 1.29–1.59 (7H, m), 1.81–1.87 (2H, m), 1.91–2.09 (3H, m), 2.24–2.41 (3H, m), 2.58 (1H, ddd, *J* 15.7, 4.3 and 2.2), 2.71 (2H, q, *J* 7.3), 2.86–2.90 (2H, m), 5.09 (2H, s), 6.68 (1H, s), 7.15 (1H, s), 7.33–7.50 (5H, m) and 9.84 (1H, dd, *J* 2.5 and 2.2); δ_C (100 MHz, $CDCl_3$) 12.2, 14.6, 19.1, 23.4, 24.0, 26.4, 27.9, 28.4, 29.7, 37.9, 38.9, 42.5, 44.1, 45.6, 49.6, 54.6, 68.1, 69.8, 84.5, 111.8, 126.2, 127.0, 127.6, 128.4, 130.2, 132.5, 135.0, 137.7, 154.5 and 203.1; *m/z* (FAB+) 417.3 ($[M + H]^+$, 100%); *m/z* (ESI+): 417.2773 $[M + H]^+$. $C_{29}H_{37}O_2$ requires 417.2788.

2-Ethyl-3-O-benzyl-17β-prop-2-ynyl-estra-1,3,5(10)-triene ***16***. A mixture of (1-diazo-2-oxo-propyl)-phosphonic acid dimethyl ester (290 mg, 1.5 mmol) and K_2CO_3 (210 mg, 1.5 mmol) in dry methanol (2.5 mL) was stirred under nitrogen and cooled to 0 °C before dropwise introduction of **15** (208 mg, 0.5 mmol) in DCM (2 mL). After 24 h at room temperature, water (10 mL) and DCM (50 mL) were added to the solution, the organic layer was separated and washed with water then brine, dried and evaporated. The resultant oil was purified by flash chromatography (hexane–ethyl acetate 25 : 1) to give a white powder (165 mg, 80%). Mp 78–79 °C; δ_H (270 MHz, $CDCl_3$) 0.67 (3H, s), 1.22 (3H, t *J* 7.3), 1.24–1.59 (8H, m), 1.66–1.80 (2H, m), 1.87–2.14 (4H, m), 2.15–2.38 (3H, m), 2.70 (2H, q, *J* 7.3), 2.84–2.89 (2H, m), 5.01 (2H, s), 6.66 (1H, s), 7.14 (1H, s) and 7.30–7.49 (5H, m); δ_C (67.5 MHz, $CDCl_3$) 12.2, 14.6, 19.1, 23.4, 24.0, 26.4, 27.9, 28.4, 29.7, 37.9, 38.9, 42.5, 44.1, 45.6, 49.6, 54.6, 68.1, 69.8, 84.5, 111.8, 126.2, 127.0, 127.6, 128.4, 130.2, 132.5, 135.0, 137.7 and 154.5; *m/z* (ESI+): 413.2842 $[M + H]^+$. $C_{30}H_{37}O$ requires 413.2844.

2-Ethyl-3-O-benzyl-17β-(3-methyl-isoxazol-5-ylmethyl)-estra-1,3,5(10)-triene ***17***. A mixture of NCS (800 mg, 6 mmol) and pyridine (80 μL, 1 mmol) in $CHCl_3$ (10 mL) was stirred at rt under nitrogen then treated with acetaldoxime (354 mg, 6 mmol) in portion wise manner. After 0.25 h, a solution of **16** (825 mg, 2 mmol) in $CHCl_3$ (2 mL) was added drop wise before addition of Et_3N (0.9 mL, 8 mmol). The mixture was then refluxed for 24 h. The solvents were evaporated and the residual oil dissolved in ethyl acetate (100 mL). The organic solution was washed with water and brine then dried and evaporated. The residual oil was purified by flash chromatography (hexane–ethyl acetate 50 : 1 to 35 : 1) to give **16** (380 mg, 46%) and **17** (350 mg, 37%) as a white powder. Mp 128–129 °C; i_H (270 MHz, $CDCl_3$) 0.70 (3H, s), 1.21 (3H, t, *J* 7.3), 1.22–1.97 (12H, m), 2.18–2.33 (5H, m), 2.54 (1H, dd, *J* 15.1 and 9.4), 2.67 (2H, q, *J* 7.3), 2.78–2.86 (3H, m), 5.04 (2H, s), 5.81 (1H, s), 6.63 (1H, s), 7.10 (1H, s) and 7.28–7.46 (5H, m); δ_C (67.5 MHz, $CDCl_3$) 11.5, 12.5, 14.7, 23.5, 24.3, 26.5, 27.7, 27.9, 28.6, 29.8, 37.6, 38.9, 42.7, 44.1, 49.1, 54.5, 69.9, 101.8, 111.9, 126.3, 127.1, 127.7, 128.5, 130.2, 132.4, 135.0, 137.7, 154.5, 159.7 and 173.3; *m/z* (FAB+) 470.4 ($[M + H]^+$, 100%); *m/z* (ESI+) 470.3034 $[M + H]^+$. $C_{30}H_{40}N_2O_2$ requires 470.3054

2-Ethyl-3-hydroxy-17β-(3-methyl-isoxazol-5-ylmethyl)-estra-1,3,5(10)-triene ***18***. A mixture of TMSCl (0.1 mL, 1.1 mmol) and sodium iodide (165 mg, 1.1 mmol) in dry acetonitrile (5 mL) was stirred for 0.5 h at room temperature under nitrogen. Benzyl ether **17** (200 mg, 0.42 mmol) in dry acetonitrile (2 mL) was then added and the mixture stirred at 50 °C for 2 h. Ethyl acetate (80 mL) was added and the organic layer was washed successively with 1 M sodium thiosulfate, water then brine, dried and evaporated. The resulting oil was purified by flash chromatography (hexane–ethyl acetate 10 : 1 to 8 : 1) to give a white solid (110 mg, 70%). Mp 204–205 °C; δ_H (270 MHz, $CDCl_3$) 0.70 (3H, s), 1.21 (3H, t, *J* 7.3), 1.24–1.55 (7H, m), 1.70–1.77 (2H, m), 1.81–1.97 (3H, m), 2.14–2.32 (5H, m), 2.55 (1H, dd, *J* 14.9 and 9.8), 2.59 (2H, q, *J* 7.3), 2.78 (2H, m), 2.82 (1H, dd, *J* 14.9 and 4.5), 4.67 (1H, s), 5.81 (1H, s), 6.50 (1H, s) and 7.04 (1H, s); δ_C (67.5 MHz, $CDCl_3$) 11.5, 12.4, 14.5, 23.1, 24.2, 26.5, 27.7, 27.8, 28.5, 29.3, 37.5, 38.9, 42.6, 44.1, 49.1, 54.5, 101.8, 115.2, 126.3, 127.2, 132.6, 135.5, 151.2, 159.7 and 173.3; *m/z* (FAB+): 379.2503 $[M]^+$. $C_{25}H_{33}NO_2$ requires 379.2511.

2-Ethyl-17β-(3-methyl-isoxazol-5-ylmethyl)-3-O-sulfamoyl-estra-1,3,5(10)-triene ***19***. Phenol **18** (60 mg, 0.16 mmol) was reacted with sulfamoyl chloride (0.32 mmol) in DMA (1 mL) as described for the synthesis of **8**. Flash chromatography (hexane–ethyl acetate 6 : 1 to 3 : 1) gave a white powder (45 mg, 63%). Mp 103–104 °C; δ_H (270 MHz, $CDCl_3$) 0.68 (3H, s), 1.19 (3H, t, *J* 7.3), 1.22–1.55 (7H, m), 1.70–1.74 (2H, m), 1.80–1.95 (3H, m), 2.17–2.29 (5H, m), 2.53 (1H, dd, *J* 14.9 and 9.4), 2.65 (2H, q, *J* 7.3), 2.76–2.84 (3H, m), 5.01 (2H, s), 5.79 (1H, s), 7.05 (1H, s), 7.16 (1H, s); δ_C (67.5 MHz, $CDCl_3$) 11.4, 12.4, 14.6, 23.0, 24.2, 26.2, 27.5, 27.6, 28.5, 29.2, 37.4, 38.4, 42.5, 44.2, 49.0, 54.5, 101.8, 121.4, 126.9, 133.6, 136.0, 139.6, 146.1, 159.7 and 173.1; *m/z* (FAB+): 458.2240 $[M]^+$. $C_{25}H_{34}N_2O_4S$ requires 458.2239.

2-Ethyl-17β-(2-aminoethyl)-3-O-benzyl estra-1,3,5(10)-triene ***20***. A solution of **5** (1.35 g, 3.3 mmol) in dry THF (50 mL) was cooled to 0 °C under nitrogen then treated with $LiAlH_4$ (495 mg, 13 mmol) in a portion wise manner. The reaction mixture was stirred at room temperature under nitrogen for 8 h. Saturated aqueous NH_4Cl (10 mL) was then added cautiously and the mixture was extracted with ethyl acetate. The organic layer was washed with water, brine, dried and evaporated. The crude solid was purified by flash chromatography (ethyl acetate–methanol–40% aq. NH_4OH 10 : 1 : 0 to 10 : 1 : 0.5) to give a white powder (550 mg, 46%). Mp 58–60 °C; ν_{max} (KBr) 3395 and 3314 cm^{-1} (NH_2); δ_H (400 MHz, $CDCl_3$) 0.62 (3H, s), 1.18–1.98 (19H, m), 2.12–2.35 (2H, m), 2.62–2.93 (6H, m), 5.03 (2H, s), 6.63 (1H, s), 7.11 (1H, s) and 7.28–7.46 (5H, m); δ_C (100 MHz, $CDCl_3$) 12.7, 14.7, 22.0, 23.5, 24.5, 26.6, 28.0, 28.5, 29.8, 34.8, 37.9, 38.9, 41.7, 44.3, 48.7, 54.8, 69.8, 111.9, 126.3, 127.1, 127.7, 128.5, 130.2, 132.7, 135.1, 137.8 and 154.4; *m/z* (FAB+) 418.3 ($[M + H]^+$, 100%).

2-Ethyl-3-O-benzyl-17β-(2-[1,2,4]triazol-4-yl-ethyl) estra-1,3,5(10)-triene ***21***. A solution of **20** (417 mg, 1 mmol), DMF azine (285 mg, 2 mmol) and *p*-TsOH·H_2O (19 mg, 0.1 mmol) in toluene (30 mL) was refluxed for 24 h. After evaporation of the solvent, the residual solid was dissolved in ethyl acetate and the organic layer washed with water and brine then dried and evaporated. The desired product was isolated by chromatography (ethyl acetate–methanol 1 : 0 to 15 : 1) to give a white solid (340 mg, 73%). Mp 203–204 °C; δ_H (270 MHz, $CDCl_3$) 0.63 (3H, s), 1.20 (3H, t, *J* 7.3), 1.24–1.70 (9H, m), 1.75–1.99 (5H, m), 2.15–2.35 (2H, m), 2.60 (2H, q, *J* 7.3), 2.78–2.83 (2H, m), 3.90–4.10 (2H, m), 5.02 (2H, s), 6.62 (1H, s), 7.08 (1H, s), 7.26–7.45 (5H, s) and 8.16 (2H, s); δ_C (100 MHz, $CDCl_3$) 12.7, 14.7, 23.5, 24.4, 26.4, 27.9, 28.1, 29.8, 31.8, 37.7, 38.8, 42.7, 44.1, 44.8, 47.8, 54.5, 69.9, 111.9, 126.2, 127.1, 127.6, 128.5, 130.3, 132.2, 135.0, 137.8, 142.7 and 154.5; *m/z* (ESI+) 470.3154 $[M + H]^+$. $C_{31}H_{40}N_3O$ requires 471.3166.

2-Ethyl-3-hydroxy-17β-(2-[1,2,4]triazol-4-yl-ethyl) estra-1,3,5(10)-triene ***22***. A solution of **21** (234 mg, 0.50 mmol) in THF (5 mL) and methanol (20 mL) was treated with 10% Pd/C (30 mg) then stirred under an atmosphere of hydrogen for 24 h. The resultant suspension was filtered through celite, the celite washed with ethyl acetate and the combined filtrates evaporated. The residual solid was recrystallized from ethanol–water 5 : 1 to give a white powder (150 mg, 79%). (Found C, 75.70; H, 8.79; N, 10.70 requires $C_{24}H_{33}N_3O$ C, 75.95; H, 8.76; N, 11.07%); mp 257–258 °C; δ_H (270 MHz, CD_3OD) 0.67 (3H, s), 1.13 (3H, t, *J* 7.3), 1.15–1.49 (8H, m), 1.60–2.16 (7H, m), 2.25–2.32 (1H, m), 2.53 (2H, q, *J* 7.3), 2.71 (2H, m), 4.03–4.22 (2H, m), 6.41 (1H, s), 6.93 (1H, s) and 8.57 (2H, s); δ_C (100 MHz, CD_3OD) 11.6, 13.7, 22.9, 24.0, 26.3, 27.6, 27.8, 29.0, 31.4, 37.5, 39.1, 42.4, 44.1, 44.7, 47.8, 54.5, 114.4, 125.6, 127.5, 131.0, 134.4, 137.8 and 152.2; *m/z* (FAB+) 380.5 ($[M + H]^+$, 100%); *m/z* (ESI+) 380.2693 $[M + H]^+$. $C_{24}H_{34}N_3O$ requires 380.2696.

2-Ethyl-3-O-sulfamoyl-17β-(2-[1,2,4]triazol-4-yl-ethyl) estra-1,3,5(10)-triene ***23***. **22** (80 mg, 0.21 mmol) was reacted with sulfamoyl chloride (0.42 mmol) in DMA (1 mL) as described for the synthesis of **8**. Flash chromatography (ethyl acetate–methanol 10 : 1) gave a white powder (65 mg, 68%). (Found C, 62.70; H, 7.63; N, 11.90 requires $C_{24}H_{34}N_4O_3S$ C, 62.85; H, 7.47; N, 12.22%); mp 245–246 °C; δ_H (270 MHz, CD_3OD–DMSO-d_6 10 : 1) 0.70 (3H, s), 1.18 (3H, t, *J* 7.3), 1.20–1.58 (8H, m), 1.65–2.10 (6H, m), 2.21–2.32 (1H, m), 2.32–2.43 (1H, m), 2.72 (2H, q, *J* 7.3), 2.84 (2H, m), 4.09–4.23 (2H, m), 7.06 (1H, s), 7.21 (1H, s) and 8.61 (2H, s); δ_C (100 MHz, CD_3OD–DMSO-d_6 10 : 1) 11.7, 14.0, 22.7, 24.1, 26.1, 27.5, 27.6, 29.0, 31.5, 37.4, 39.1, 42.3, 44.3, 44.6, 47.9, 54.5, 110.1, 121.6, 126.4, 133.9, 135.4, 138.7 and 146.6; *m/z* (FAB+) 458.2347 $[M]^+$. $C_{24}H_{34}N_4O_3S$ requires 458.2352

2-Methoxy-3-hydroxy-17-(4H-1,2,4-triazol-4-ylimino)-estra-1,3,5(10)-triene ***25***. A solution of 2-methoxyestrone **24** (1.00 g, 3.33 mmol), 4-amino-4*H*-1,2,4-triazole (560 mg, 6.66 mmol) and *p*TsOH hydrate (50 mg) in EtOH (5 mL) was heated in an ACE-pressure tube to 100 °C for 20 h. After cooling to rt, a white crystalline solid was filtered off, washed with a small amount of cold EtOH (*ca.* 5 mL) and dried under high vacuum to give **25** (877 mg, 72%) as fine colorless needles. (Found C, 68.7; H, 7.09; N, 15.0 requires $C_{21}H_{26}N_4O_2$ C, 68.83; H, 7.15; N, 15.29%); mp >230 °C (dec.); δ_H (400 MHz, DMSO-d_6) 1.03 (3H, s), 1.24–1.73 (6H, m), 1.84–1.96 (2H, m), 2.02–2.08 (1H, m), 2.19–2.27 (1H, m), 2.36–2.48 (2H, m), 2.62–2.78 (2H, m), 2.86–2.94 (1H, m), 3.74 (3H, s), 6.48 (1H, s), 6.81 (1H, s), 8.67 (1H, s) and 8.76 (2H, s); δ_C (100 MHz, DMSO-d_6) 16.5, 22.7, 25.9, 26.8, 28.3, 28.7, 33.6, 37.8, 43.7, 46.4, 50.7, 55.8, 109.6, 115.6, 128.2, 129.8, 140.1, 144.5, 145.7 and 188.5; *m/z* (FAB+) 367.2144 $[M + H]^+$. $C_{24}H_{34}N_4O_3S$ requires 367.2134.

2-Methoxy-3-hydroxy-17β-(4H-1,2,4-triazol-4-ylamino)-estra-1,3,5(10)-triene ***26***. To a solution of **25** (110 mg, 0.30 mmol) in MeOH (10 mL) was added $NaBH_4$ (38 mg, 1.00 mmol) at 0 °C. The clear solution was stirred for 2 h at this temperature, then water (50 mL) and EtOAc (50 mL) were added. The organic layer was separated, washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4 and evaporated. The residue was dissolved in EtOAc and precipitated by addition of Et_2O to give **26** (69 mg, 62%) as a white solid: mp >190 °C (dec.); δ_H (270 MHz, DMSO-d_6) 0.78 (3H, s, H-18), 1.00–1.54 (7H, m), 1.60–1.92 (4H, m), 1.98–2.22 (2H, m), 2.54–2.78 (2H, m), 3.12–3.24 (1H, m, H-17), 3.68 (3H, s, -OCH_3), 6.42 (1H, s), 6.64–6.74 (2H, s), 8.58 (1H, s) and 8.61 (2H, s); *m/z* (FAB+) 369.2291 $[M + H]^+$. $C_{21}H_{29}N_4O_2$ requires 369.2290.

2-Methoxy-3-O-sulfamoyl-17-(4H-1,2,4-triazol-4-ylimino)-estra-1,3,5(10)-triene ***27***. To a solution of sulfamoyl chloride (566 mg, 4.9 mmol) in DMA (5 mL) was added **25** (550 mg, 1.5 mmol) at 0 °C. The mixture was stirred for 18 h at rt, then ethyl acetate (70 mL) and water (50 mL) were added. The organic layer was separated, washed with water (2 × 30 mL) and brine (20 mL), dried over Na_2SO_4 and evaporated. The residue was dissolved in

a small amount of acetone and Et_2O was added. The resulting precipitate was filtered off and dried under high vacuum to give **27** (568 mg, 85%) as a white solid. Mp 180–184 °C; δ_H (400 MHz, DMSO-d_6) 1.06 (3H, s), 1.24–1.78 (7H, m), 1.88–1.98 (1H, m), 2.04–2.14 (1H, m), 2.27–2.50 (2H, m), 2.76–2.84 (2H, m), 2.88–2.98 (1H, m), 3.80 (3H, s), 7.03 (1H, s), 7.04 (1H, s), 7.85 (2H, s) and 8.79 (2H, s); δ_C (100 MHz, $CDCl_3$) 16.9, 23.2, 26.1, 26.9, 28.6, 29.2, 31.4, 34.0, 37.9, 44.4, 46.8, 51.1, 56.4, 111.0, 123.5, 128.7, 137.4, 138.9, 140.5, 150.0 and 189.0; *m/z* (FAB+) 446.1884 $[M + H]^+$. $C_{21}H_{28}N_5O_4S$ requires 446.1862.

2-Methoxy-3-O-sulfamoyl-17β-(4H-1,2,4-triazol-4-ylamino)-estra-1,3,5(10)-triene ***28***. To a solution of **27** (143 mg, 0.32 mmol) in MeOH (10 mL) was added $NaBH_4$ (38 mg, 1 mmol) at 0 °C. The clear solution was stirred for 2 h at this temperature, then water (50 mL) and EtOAc (50 mL) were added. The organic layer was separated, washed with water (20 mL) and brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in EtOAc and precipitated by addition of Et_2O to give **28** (81 mg, 57%) as a white solid. Mp >145 °C (dec.); δ_H (270 MHz, DMSO-d_6) 0.79 (3H, s, H-18), 1.02–1.54 (7H, m), 1.60–1.92 (4H, m), 2.04–2.22 (2H, m), 2.66–2.78 (2H, m), 3.14–3.24 (1H, m, H-17), 3.73 (3H, s, -OCH_3), 6.71 (1H, d, J = 1.7), 6.93 (1H, s), 6.95 (1H, s), 7.80 (2H, s, -NH_2), 8.61 (2H, s); *m/z* (FAB+) 448.2019 $[M + H]^+$. $C_{21}H_{30}N_5O_4S$ requires 448.2016.

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