Modification of Estrone at the 6, 16, and 17 Positions: Novel Potent Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1

Gillian M. Allan,[†] Harshani R. Lawrence,[†] Josephine Cornet,[†] Christian Bubert,[†] Delphine S. Fischer,[†] Nigel Vicker,[†] Andrew Smith,[†] Helena J. Tutill,[‡] Atul Purohit,[‡] Joanna M. Day,[‡] Mary F. Mahon,[§] Michael J. Reed,[‡] and Barry V. L. Potter[†,*]

Medicinal Chemistry, Department of Pharmacy and Pharmacology and Sterix Ltd., University of Bath, Claverton Down, BA2 7AY, UK, Endocrinology and Metabolic Medicine and Sterix Ltd., Faculty of Medicine, Imperial College London, St Mary's Hospital, London, W2 1NY, UK, and Department of Chemistry, University of Bath, BA2 7AY, UK

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The 17β-hydroxysteroid dehydrogenases (17β-HSDs) catalyze the interconversion between the oxidized and reduced forms of androgens and estrogens at the 17 position. The 17β-HSD type 1 enzyme (17β-HSD1) catalyzes the reduction of estrone to estradiol and is expressed in malignant breast cells. Inhibitors of this enzyme thus have potential as treatments for hormone dependent breast cancer. Here we report the syntheses and biological evaluation of novel inhibitors based on the estrone or estradiol template. These have been investigated by modification at the 6, 16 or 17 positions or combinations of these in order to gain information about structure−activity relationships by probing different areas in the enzyme active site. Activity data have been incorporated into a QSAR with predictive power, and the X-ray crystal structures of compounds **15** and **16c** have been determined. Compound **15** has an IC_{50} of 320 nM for 17β-HSD1 and is selective for 17β-HSD1 over 17β-HSD2. Three libraries of amides are also reported that led to the identification of inhibitors **19e** and **20a**, which have IC_{50} values of 510 and 380 nM respectively, and **20h** which, having an IC_{50} value of 37 nM, is the most potent inhibitor of 17β-HSD1 reported to date. These amides are also selective for 17β-HSD1 over 17β-HSD2.

Introduction

Breast cancer is one of the most common cancers in women with an estimated global incidence in 2002 of approximately 1 150 000.[1] Of these breast tumors the majority are initially hormone-responsive with circulating estrogens playing a vital role in their growth. Steroidogenic enzyme inhibitors can reduce circulating and tissue levels of active estrogens by blocking their biosynthetic pathways and thus can represent an effective treatment for hormone dependent breast cancer (HDBC).

Aromatase inhibitors, which prevent the conversion of androgens into estrogens, are currently used as an adjuvant therapy to treat HDBC.[2] It has been proposed, however, that a more important source of estrone (E1) in breast tumors is the body's reservoir of estrone 3-*O*-sulfate (E1S), that can be converted to E1 by the action of estrone sulfatase: E1 production in HDBC via the sulfatase pathway has been found to be approximately 10-fold higher per gram of enzyme than via the aromatase pathway.[3] Estrone sulfatase inhibitors have also been investigated and are now progressing into clinical trials.[4]

Estrone itself, however, is not the most potent human estrogen and, while inhibitors of E1 formation have reached an advanced stage of use and development, another attractive target for the treatment of HDBC is inhibition of 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). This enzyme is responsible for the reduction of the keto group of the weakly active E1 at the 17 position to give the most potent of the human estrogens, 17β estradiol (E2).

This enzyme is one of a class of enzymes known as the 17β-hydroxysteroid dehydrogenases (17β-HSDs) that catalyze the interconversion between the oxidized and reduced forms of androgens and estrogens at the 17 position. Although reversible, their activity is mainly unidirectional and thus they can be classified as oxidative or reductive. Thirteen members of this enzyme family have been identified to date, eleven of which exist in humans where they regulate the bioavailability of active androgens and estrogens.[5] While all require NAD(P)H or NAD(P)$^+$ as cofactor, each type has a selective substrate affinity, directional activity and a particular tissue distribution.

The 17β-HSD1 enzyme, which has a preferentially reductive activity using NADPH as cofactor,[6,7] is expressed in many steroidogenic tissues, including breast tissue, and has been found to be more highly expressed in malignant breast cells.[8,9] E2 is known to stimulate the growth and development of HDBC[10] therefore inhibition of the final step in the synthesis of E2, by the design of selective inhibitors of 17β-HSD1, is an attractive option for the treatment of HDBC. Little progress has yet been made in identifying inhibitors with proven in vivo activity using this concept.

The 17β-HSD1 enzyme consists of 327 amino acid residues, with a subunit mass of 35 kDa, and exists as a homodimer.[11] Much crystallographic information has been determined, including that for the enzyme in its native form,[12] in complex with estradiol and NADP,[13] with estradiol alone,[14] with equilin and NADP[15] and with the inhibitor EM-1745.[16] This structural information is an invaluable aid for identifying potential inhibitors of 17β-HSD1 using molecular modeling.

Inhibitors of 17β-HSD1 have been reported by several groups, and this field has recently been reviewed by Poirier.[17] Common structural features can be identified which aid binding at the active site. These include a phenol, which can undergo bifurcated hydrogen bonding to His221 and Glu282 residues of the protein, and a hydrophobic scaffold which inhabits the hydrophobic area in the active site.

The group of Poirier et al. has reported a number of inhibitors of 17β-HSD1. These include E2 derivatives bearing a short side

* Author to whom correspondence should be addressed. E-mail: B.V.L.Potter@bath.ac.uk. Phone +44 1225 386639; Fax +44 1225 386114.
[†] University of Bath, Department of Pharmacy & Pharmacology.
[‡] Imperial College London.
[§] University of Bath, Department of Chemistry.

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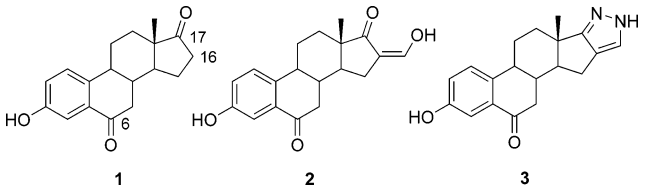


Figure 1. 6-Oxo derivatives of E1.

chain at the C17α and C16α positions on the E2 skeleton[18] and, more recently, novel hybrid inhibitors of 17β-HSD1.[16,19] The most potent of these hybrid inhibitors, EM-1745, has an IC_{50} of 52 nM in an enzymatic assay and consists of an E2 template which is linked via an eight-methylene spacer to an adenosine moiety. The crystal structure of the complex EM-1745/17β-HSD1 was resolved to 1.6 Å and confirms that this inhibitor interacts with both the substrate and the cofactor enzyme-binding domains; it is, however, not drug-like. Two patents from Solvay Pharmaceuticals have also recently been published detailing nonsteroidal thiopyrimidone inhibitors[20] and steroidal inhibitors of 17β-HSD1 based on C15 substituted E1.[21] The most active of these is a 3-*O*-methyl-15β-propyl-*N*-cyclooctyl amide E1 derivative which shows 85% inhibition of 17β-HSD1 at 100 nM in a purified enzyme assay.

As part of our ongoing program to synthesize inhibitors of 17β-HSD1 we used E1 or E2 as a convenient scaffold and investigated substitution at the 6, 16 and 17 positions of these steroid templates, resulting in the filing of a patent by our group.[22] Herein is described the synthesis, molecular modeling studies, and biological activities of some of these novel inhibitors which have been investigated by expansion from the 6, 16 and 17 positions of the E1/E2 scaffold. The information gained about the structure−activity relationships (SAR) for these inhibitors of 17β-HSD1 will also be discussed in detail.

Results and Discussion

Our interest in the 6 position of the E1 template arose as a result of docking studies, using the crystal structure of 17β-HSD1 with E2 and NADP (PDB code 1FDT),[13,23] that suggested that a carbonyl at this position might display favorable interactions in the active site. In particular, these studies indicated that a small hydrogen bond accepting group at the 6 position might capitalize on potentially beneficial interactions with the hydroxyl of Ser222. Compounds **1**−**3** (Figure 1) were therefore synthesized for comparison of the inhibitory activities of 6-oxo compounds with their nonoxidized E1 counterparts. The nonoxidized 16-hydroxymethylene and pyrazole derivatives of E1 have been previously reported by our group to be potent inhibitors of 17β-HSD1.[24] It was hypothesised that the combination of substituents at the 6 and the 16 or the 17 positions might lead to additive effects.

This investigation was expanded by synthesis of oxime derivatives **4**−**8**, Figure 2. Compounds **4** and **5** were prepared to create a pseudo E-ring system as discussed in our recent publication;[24] compounds **6**−**8** were synthesized with the aim of further investigating the SAR around the 6 position in combination with expansion from positions 16 and 17. It would be desirable initially to convert the 6-oxo to another fairly small substituent which would retain similar hydrogen bonding properties. The oxime group was an attractive option for this expansion as it can be easily introduced in one step without 3-*O*-protection of starting material and a large number of hydroxylamines are commercially available for rapid lead identification.

As an extension of this work, the 16 position was further explored in combination with the 17 position in the form of

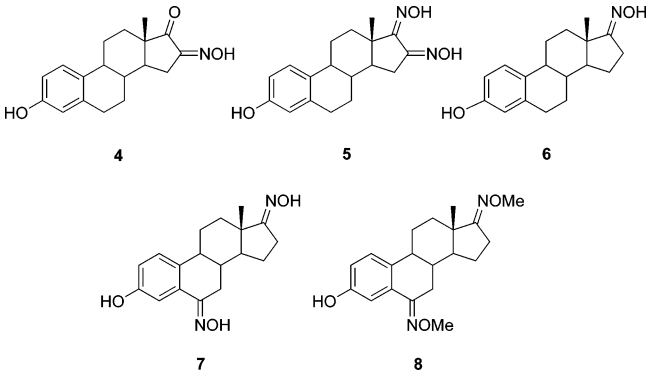


Figure 2. Oxime derivatives of E1 and 6-oxo-E1.

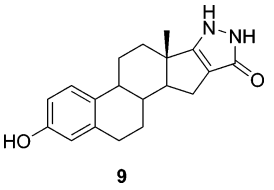


Figure 3. Pyrazolone derivative of E1 (**9**).

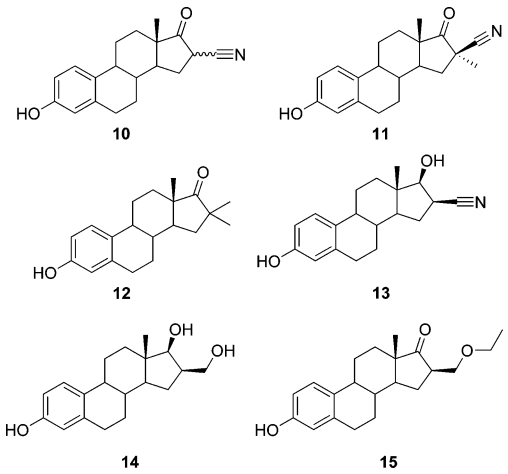


Figure 4. Derivatives of E1 and E2 with small substitutents at the 16 position.

novel E ring pyrazole and pyrazolone steroids. These have the benefit of ease of further elaboration in different areas of space and are achiral at the 16 and 17 positions. Some pyrazole compounds have been reported previously by our group;[24] here we discuss the synthesis and activity of the novel pyrazolone derivative of E1 **9** (Figure 3).

Expansion from the 16 position alone is also an attractive option, as the docking studies using 1FDT also suggested space for significant extension from this position of the E1 scaffold, possibly extending to interactions with the cofactor.

The scope for expansion at the 16 position was investigated in a stepwise manner, starting with incorporation of small substituents initially to give compounds **10**−**14** (Figure 4). Compound **15** was obtained initially as a byproduct, as will be discussed. Compounds **10**−**12** have been reported previously to show promising inhibition of 17β-HSD1 at 10 μM of 77, 85 and 95% respectively.[24]

Further to the high inhibitory activity of **12**, the tolerance of the active site for larger hydrophobic groups at the 16 position was investigated by the incorporation of alkenyl substituents to give a series of compounds **16** as shown in Figure 5. The 16-methylene derivative of E1 has been reported to be an active-site directed alkylating agent of 17β-HSD1 which can be formed

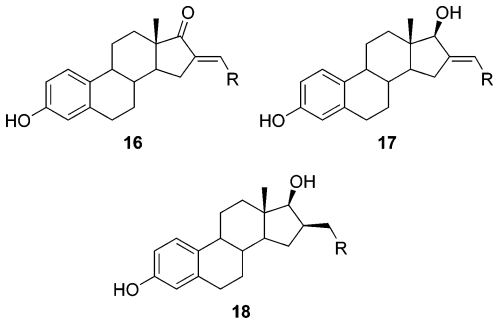


Figure 5. Alkenyl derivatives of E1 (**16**) and E2 (**17**) and 16β-alkyl derivatives of E2 (**18**).

Table 1 [a]

R	Compound number		
	**16a**	**17a**	**18a**
	**16b**	**17b**	**18b**
	**16c**	**17c**	**18c**
	**16d**	**17d**	**18d**
	**16e**	**16e**	ns
	**16f**	**17f**	ns
	**16g**	**17g**	ns
	**16h**	**17h**	ns
	**16i**	**17i**	ns
	**16j**	**17j**	ns
	**16k**	**17k**	ns
	**16l**	ns	ns

[a] ns = not synthesized.

in situ by oxidation of the suicide inhibitor 16-methylene-E2.[25] Therefore 16-methylene-E1 and 16-methylene-E2 were synthesized from 3-*O*-acetyl E1, using the method of Ringold and Rosenkranz,[26] for use as standards for comparison with 16-alkenyl derivatives. Alkyl substituents were investigated by synthesis of isopropyl and isobutyl compounds and aryl substituents containing heteroatoms in different areas in space were also investigated. These were accessed via condensation reactions of various aldehydes with E1 and are listed in Table 1. The effect of reduction to the E2 derivatives **17** was also investigated, and, for selected compounds, hydrogenation

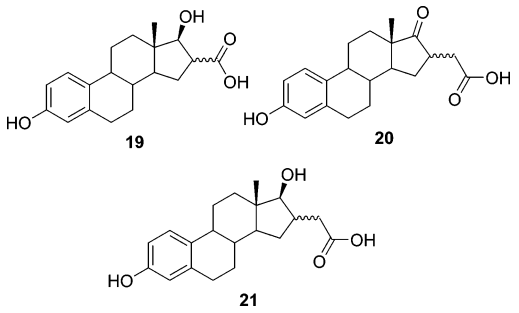
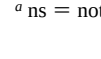
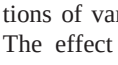
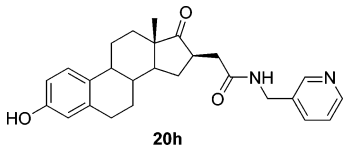


Figure 6. Carboxyl derivative of E2 (**19**) and methyl carboxyl derivatives of E1 (**20**) and E2 (**21**).

Table 2 [a]

R	Compound number		
	19a	**20a**	**21a**
	**19b**	**20b**	**21b**
	**19c**	**20c**	**21c**
	ns	**20d**	**21d**
	ns	**20e**	**21e**
	ns	**20f**	**21f**
	**19d**	**20g**	ns
	**19e**	**20h**	ns
	ns	**20i**	ns
	ns	**20j**	ns
	ns	**20k**	ns
	**19f**	**20l**	ns
	ns	**20m**	ns
	**19g**	ns	ns
	19h	ns	ns
	19i	ns	ns
	19j	ns	ns

[a] ns = not synthesized.



20h

Figure 7. The 16β-diastereomer of compound **20h**: the most potent inhibitor of 17β-HSD1 reported to date.

of the alkene double bond was performed to give 16β-alkyl compounds **18**.

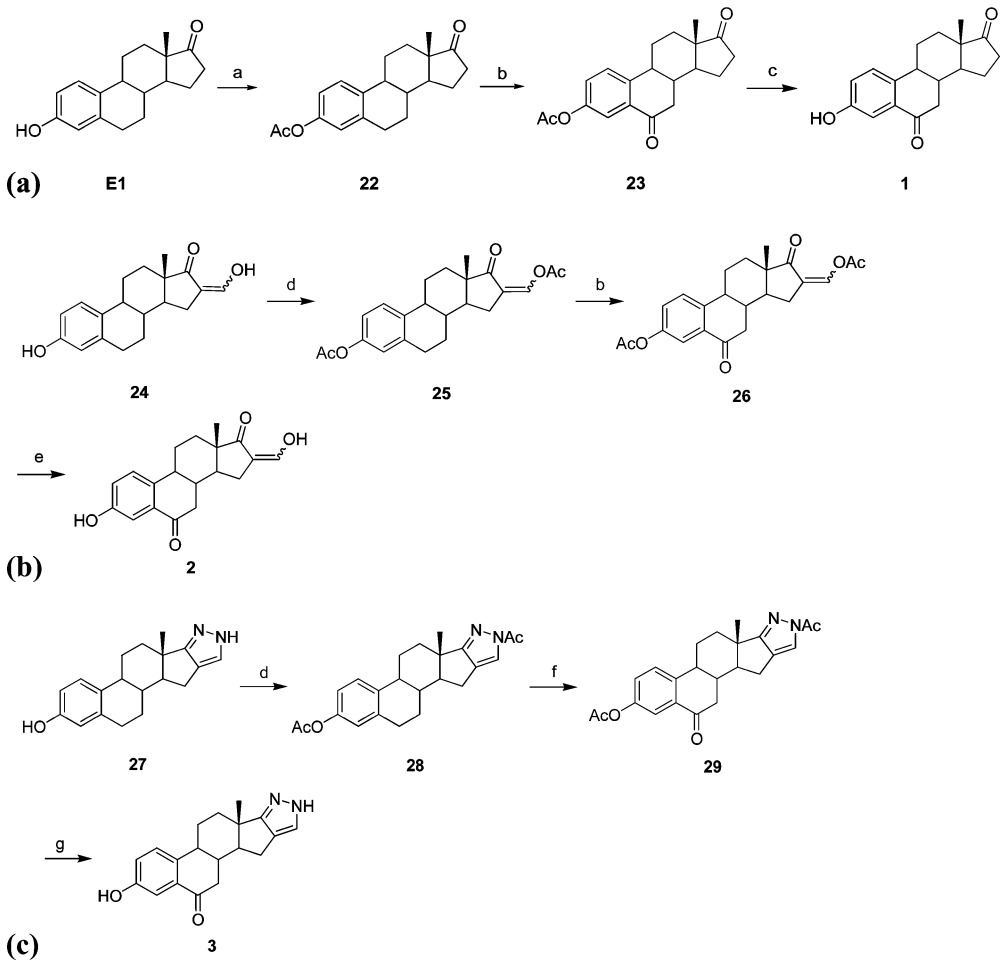
Since the activities of these compounds seemed promising it was decided to incorporate a more flexible, functionalizable substituent at the 16 position from which to probe this area further in the form of syntheses of focused libraries. Two of such linkers were investigated initially: a carboxylic acid in **19** and a methyl carboxylic acid in both **20** and **21**, as shown in Figure 6. These could then be reacted with a diverse set of commercially available amines which was chosen to exhibit a range of electron accepting, electron donating, charge transfer, hydrophobic, hydrophilic and π-stacking properties, with the aim of exploring the SAR in this area of the active site. The amides synthesized for each of these templates are listed in Table 2.

Extension from the 16 position of E1 via the methyl carboxylic linker resulted in the discovery of a highly potent inhibitor **20h** (Figure 7) that has an $IC_{50} = 37$ nM and shows selectivity for 17β HSD1 over 17β HSD2, as recently reported by our group in a preliminary report.[27]

Chemistry

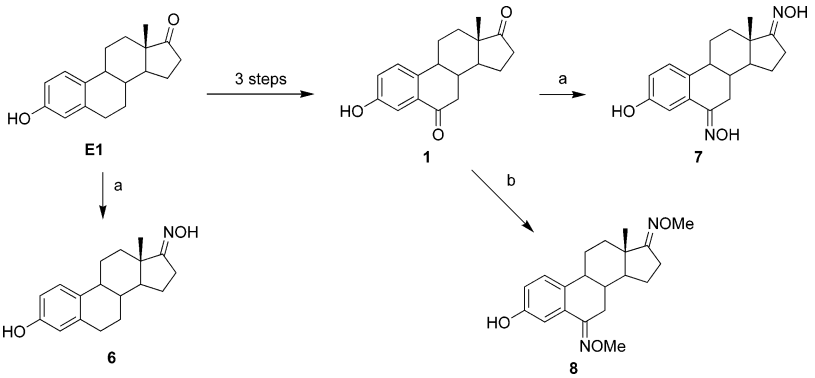
Oxidation at the 6 Position of E1 and Derivatives. The syntheses of compounds **1**−**3** are shown in Scheme 1(**a**−**c**). The oxidation of E1 at the 6 position was performed in the presence of chromic acid by an adaptation of the procedure of Schwenk and Montclair.[28] To prevent oxidation at the 3 position E1 was first protected as the 3-*O*-acetate derivative before reaction with chromium trioxide in AcOH/water at ~15 °C. The yield of **23** after recrystallization was 20% which is in accordance with literature values, as formation of byproducts accounts for the low yield.[29] Removal of the acetate using KOH/MeOH gave compound **1** in 58% yield. Compound **2** was prepared in a similar manner from the hydroxymethylene derivative of E1, **24**, the synthesis of which has been previously described.[30] This was protected as its bis-acetate derivative **25** and in this case the oxidation of **25** progressed in only 10% yield. Removal of the acetate groups using K_2CO_3/MeOH gave the compound **2** in 54% yield. In a similar manner the E-ring pyrazole derivative of E1 was protected as the bis-acetate **28**, at the 3-*O*-position and on the 1′-N or 2′-N of the pyrazole ring, and oxidation was attempted as before. In this case, however, no 6-oxo product was observed (^{1}H NMR showed no C4−H shift associated with deshielding by the new carbonyl group). The use of chromium hexacarbonyl and *tert*-butyl hydroperoxide in acetonitrile at reflux, as described by Pearson and Han,[31] did give the oxidized product **29** in low yield after purification by

Scheme 1[a]



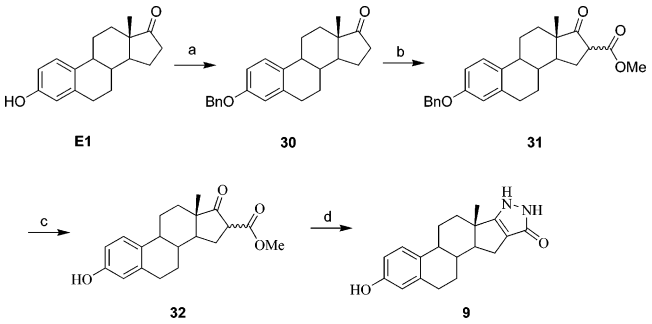
[a] Reagents and conditions: (a) Ac_2O, pyr, 0 °C then Δ; (b) CrO_3, AcOH, 10−15 °C then rt; (c) KOH, MeOH, rt; (d) Ac_2O, pyr, 0 °C then Δ; (e) K_2CO_3 (aq), MeOH, rt; (f) *t*-BuO_2H, $Cr(CO)_6$, MeCN, Δ; (g) KOH(aq), EtOH, rt.

Scheme 2[a]



[a] Reagents and conditions: (a) $NH_2OH \cdot HCl$, NaOAc, $MeOH/H_2O$, rt; (b) $NH_2OMe \cdot HCl$, NaOAc, $MeOH/H_2O$, rt.

Scheme 3[a]



[a] Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt; (b) $CH_3OCO_2CH_3$, NaH, THF, Δ; (c) H_2, Pd−C, THF, rt (d) $NH_2NH_2 \cdot H_2O$, PhMe, Δ.

chromatography. Removal of both acetate groups using KOH/EtOH yielded product **3**.

Syntheses of Oxime Derivatives of E1. The syntheses of compounds **4** and **5** have been previously described.[24,32] Compound **6** (Scheme 2) was prepared in the same manner by adaptation of a literature procedure using hydroxylamine hydrochloride and sodium acetate in ethanol/water.[33] Reaction of **1** with hydroxylamine hydrochloride or methoxylamine hydrochloride under the same conditions gave compounds **7** or **8** respectively. Compound **7** was obtained in 41% yield after purification by flash chromatography; the less polar compound **8** was obtained in a high 92% yield after recrystallization from ethyl acetate/hexane. Compound **6** has been shown previously to exist as the *anti*-isomer,[33] and compounds **4**−**8** were all found by NMR to consist of single geometrical isomers; however, as yet these have not been assigned. It is likely that these are the *anti*-isomers as these are the favored thermodynamic products.

Substitution at the 16/17 Positions. The route to the pyrazolone compound **9** is shown in Scheme 3. Estrone was protected as its 3-*O*-benzyl ether by a literature procedure using benzyl bromide and K_2CO_3 in DMF.[34] The protected compound **30** was then reacted using the procedure of Paquette et al. with sodium hydride and dimethyl carbonate in THF at reflux followed by acidification at 0 °C then recrystallization from MeOH to give **31** in 81% yield.[35] Hydrogenation using 10% Pd−C (catalytic) gave **32** in 93% yield after recrystallization from methanol. The pyrazolone compound **9** was obtained from condensation of **32** with hydrazine monohydrate, although this condensation reaction was found not to be successful using general literature described methods at reflux in EtOH.[36] This is probably due to the fact that the new E-ring formed will be strained due to being attached to the five-membered D-ring and thus the reaction will be more difficult than if attaching the pyrazolone to a six membered ring or an acyclic system. Fortunately, the reaction does proceed under forcing conditions at reflux in toluene in a sealed tube. Purification was problematic, however, as in addition to the desired pyrazolone product the hydrazone derivative of E1 is formed (with loss of the carboxyl ester functionality) and is difficult to separate. The product was precipitated selectively from AcOH/water to give 18% of pure compound **9**. This compound can exist in different tautomeric forms as shown in Figure 8. To investigate which forms may predominate in solution and solid phases, ^{13}C NMR was performed in DMSO-d_6 and IR spectroscopy in solid form (KBr disk). The ^{13}C NMR spectrum showed one major set of peaks with a peak at 171 ppm which is indicative that in this solution a keto-form predominates; the IR spectrum showed a strong absorption at 1600 cm^{-1} which also suggests the presence of a carbonyl functionality in the solid form.

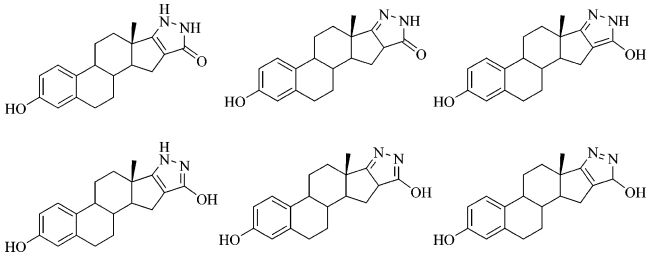
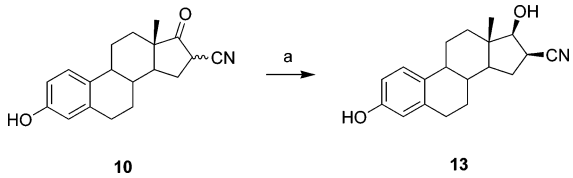


Figure 8. Possible tautomeric forms of compound **9**.

Scheme 4[a]

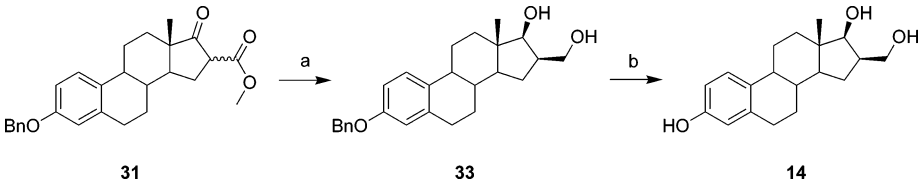


[a] Reagents and conditions: (a) $NaBH_4$, THF/EtOH, rt.

Expansion from the 16 Position of E1. The syntheses of compounds **10**−**12** have been described previously.[24] Compounds **13**−**15** were synthesized as shown in Schemes 4−6. The 16 cyano substituted E2 derivative **13** was obtained by reduction of **10** using sodium borohydride in THF/EtOH in 37% yield after recrystallization from IPA/water. The stereochemistry of this compound at the 16 position was assigned as β by comparison of NMR coupling constants with those reported for the α isomer.[37] ^1H NMR of the crude product prior to recrystallization showed the presence of the 16α isomer as only 5% of the reduced products obtained.

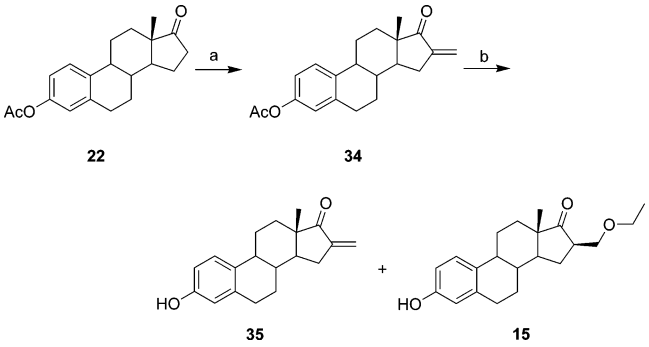
Compound **14** was obtained in two steps from **31**. Reduction of the latter using sodium borohydride at 0 °C in THF/MeOH

Scheme 5[a]



[a] Reagents and conditions: (a) $NaBH_4$, THF/MeOH, 0−20 °C; (b) H_2, Pd−C, THF, rt.

Scheme 6[a]



[a] Reagents and conditions: (a) $(CH_2O)_n$, *i*-AmOH, Δ; (b) KOH(aq), EtOH, 0 °C.

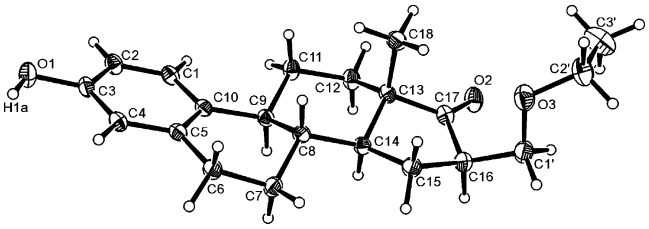


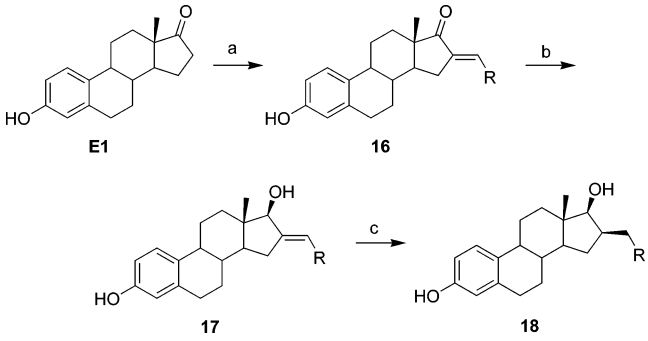
Figure 9. ORTEX[40] plot of the X-ray crystal structure of **15**. Ellipsoids are shown at the 30% probability level.

followed by acidification gave **33** in 13% yield as one of the products isolated by flash chromatography (the other products being the separated diastereomers of 3-*O*-benzyl-16-carboxymethyl-E2 **31**). Subsequent hydrogenation of **33** using catalytic Pd−C (10wt %) in THF gave compound **14** in 93% yield. Compounds **33** and **14** have been described in the literature therefore assignment of the 16-substituent as β was based on comparison with reported NMR data.[38]

The 16-ethoxymethyl derivative **15** was initially isolated as a byproduct, in this case from deprotection of 3-*O*-acetyl-16-methylene E1 using KOH in EtOH as shown in Scheme 6. Compound **15** was obtained from this reaction in 13% yield and was shown by X-ray crystallography to possess the ethyl ether substituent in the β orientation (Figure 9). This compound was subsequently prepared in higher yield in two steps from the 3-*O*-benzyl protected version of the hydroxymethylene compound **24**. This could be alkylated according to literature procedure[39] using potassium carbonate and ethyl iodide before hydrogenation of the alkene double bond. This hydrogenation did yield a mixture of products; however, the desired product **15** was obtained as the major product in 46% yield.

Compounds **16** were formed by condensation of E1 with the appropriate aldehyde (Scheme 7). For **16e**−**f** reactions were started at −78 °C using LDA as base then allowed to warm to ambient temperature to give products in 64 and 21% yield respectively after work up and purification. These were assigned as the more thermodynamically stable *E* isomer based on comparison of NMR with published data.[41] The remaining compounds **16** were obtained in high yields (average 87%) by

Scheme 7[a]



[a] Reagents and conditions: (a) RCHO, LDA, THF, −78 °C or ArCHO, NaOH, EtOH, rt; (b) $NaBH_4$, THF/EtOH, 0 °C then rt; (c) H_2, Pd−C, THF/EtOH, rt.

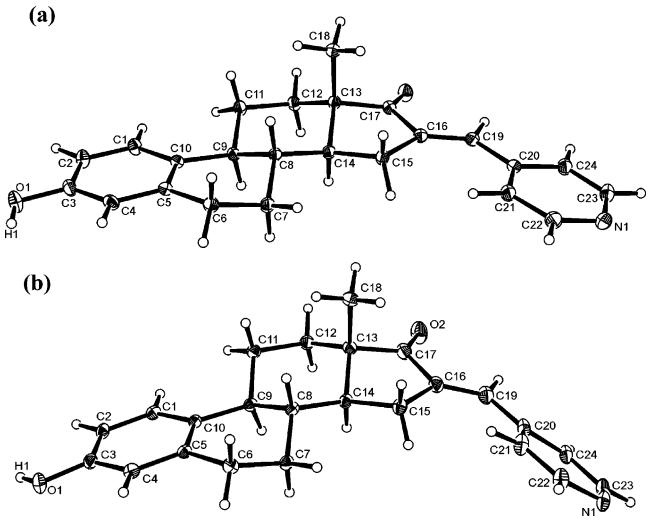
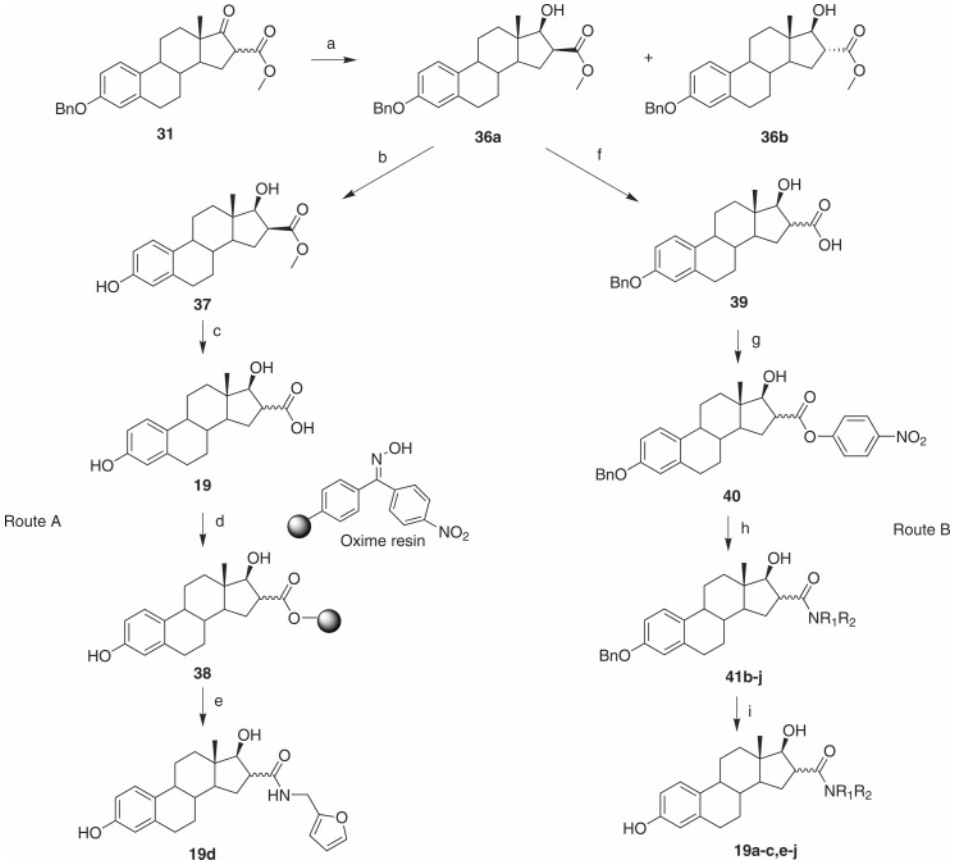


Figure 10. (a) ORTEX[40] plot of the X-ray crystal structure of a polymorph of **16c** in which pyridyl ring is in plane with E1 skeleton (blocklike crystals) and (b) ORTEX[40] plot of the X-ray crystal structure of a polymorph of **16c** in which the pyridyl ring is out of plane with E1 skeleton (needlelike crystals). Ellipsoids are shown at the 30% probability level.

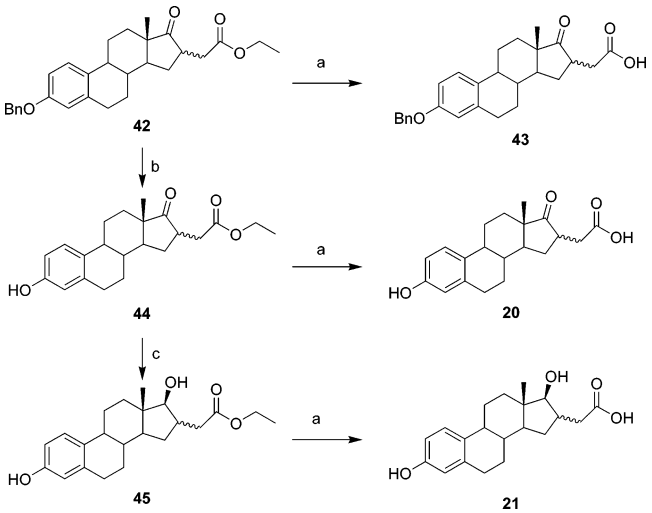
condensation of aromatic aldehydes with E1 using NaOH in EtOH at room temperature. The syntheses of some of these compounds at high temperature have been previously reported to yield the *Z*-isomers.[42] However compound **16c** was crystallized from EtOH and shown by X-ray crystallography to possess the alkene double bond in the *E*-orientation. Two polymorphs were obtained from this recrystallization: colorless blocks and needle-like crystals (Figures 10a and 10b, respectively). These exhibited differing orientations of the phenolic OH and of the pyridyl ring, and this impacted heavily on the hydrogen bonds that direct the crystal packing. The remaining aromatic analogues of the series were assigned the geometry *E* by analogy since they were prepared in the same manner and showed similar chemical shifts for their alkene protons. Subsequent reduction using $NaBH_4$ gave compounds **17** in high yield (average 85%)

Scheme 8[a]



[a] Reagents and conditions: (a) $NaBH_4$, THF/MeOH, 0 °C; (b) H_2, Pd−C, THF, rt; (c) NaOH(aq), MeOH, rt; (d) Oxime resin, DIC, HOBt, DMF, rt; (e) furfurylamine, DCM, 40 °C; (f) NaOH (aq), THF, rt; (g) $(p\text{-}NO_2C_6H_4O)_2CO$, NEt_3, DMF, rt; (h) R_1R_2NH, MeCN, rt; (i) H_2, Pd−C, THF, rt.

Scheme 9[a]

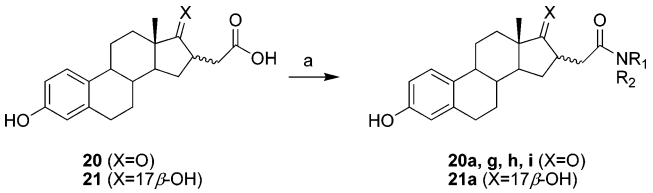


[a] Reagents and conditions: (a) NaOH (aq), THF/MeOH, Δ; (b) H_2, Pd−C, EtOH, rt; (c) $NaBH_4$, MeOH, 0 °C to room temperature.

and then hydrogenation using Pd−C catalyst yielded compounds **18** (average yield 71% after purification by chromatography). Compounds **18** were assigned as having the 16-substituent in the expected β orientation based on comparison of NMR spectra with those of known structures.[43]

A similar approach was used to attach the carboxymethyl linker using 3-*O*-benzyl-E1 with NaH and dimethyl carbonate to give **31** (Scheme 3) which was obtained in a 1:1.9 ratio of diastereomers after recrystallization from methanol. The initial intention was then to saponify the ester and form a library of

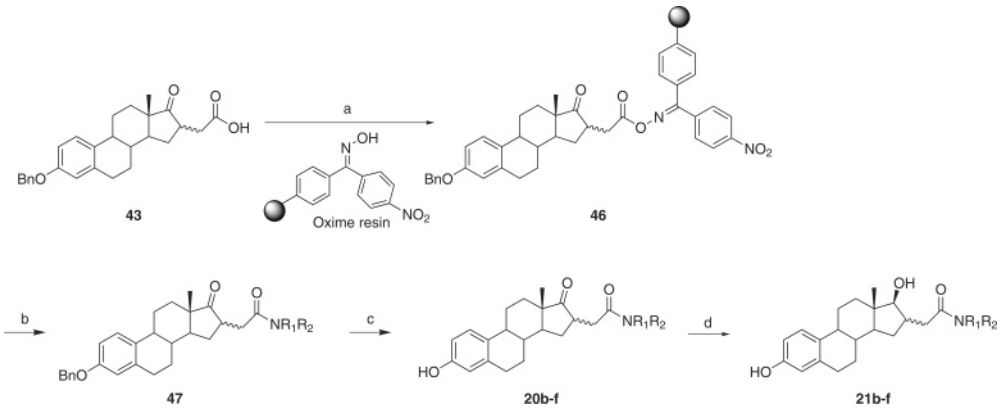
Scheme 10[a]



[a] Reagents and conditions: (a) R_1R_2NH, EDC, DMAP, DCM, rt.

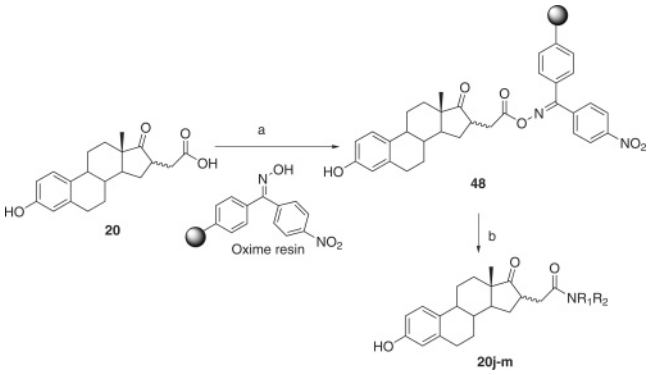
amides; however, decarboxylation of the β-keto acid proved to be a significant problem with this approach. The carboxymethyl amides were therefore investigated using the reduced (E2) scaffold which was obtained by reduction with sodium borohydride at 0 °C to give **36a** and **36b** as shown in Scheme 8. This reduction was kept at 0 °C for 4 h since allowing the reaction to warm to 20 °C over a longer time (18 h) resulted in further reduction to form **33** (Scheme 5). In this case the diastereomers were separated by chromatography in 48% and 18% yields and the major product **36a** was used for further reaction. This was assigned as the 16β-carboxyl ester by comparison of ^{1}H NMR coupling constants for the two isomers, and comparison of the ^{1}H NMR of **37** with published NMR data for the 16α diastereomer.[39] The carbon center at the 16 position was however found to epimerize during subsequent saponification. Initially, saponification followed by amide coupling was attempted; however, amide coupling reactions were found to be problematic using standard coupling conditions. Two routes were thus investigated for amide synthesis, both involving activation of the starting carboxylic acid: (Route A) removal the benzyl protecting group first to give **37**, followed by saponification and loading of **19** onto Oxime resin for solid-

Scheme 11[a]



[a] Reagents and conditions: (a) Oxime resin, DIC, HOBt, DMF, rt; (b) R_1R_2NH, DCM, 40 °C; (c) H_2, Pd−C, MeOH, rt; (d) $NaBH_4$, THF/EtOH, 0 °C to room temperature.

Scheme 12[a]



[a] Reagents and conditions: (a) Oxime resin, DIC, HOBt, DMF, rt; (b) R_1R_2NH, DCM, 40 °C.

phase synthesis, or (Route B) saponification first to give **39** followed by formation of the active ester **40** for amide coupling before deprotection. Route A was used to synthesize the furfuryl amide **19d** but in only 4% yield over the final two steps (loading on resin followed by amide coupling). Route B was found to be superior since the active ester **40** was obtained in 52% yield, and the average yields for amide coupling and debenzylation were 80% and 36% respectively. Amides **19a**−**c** and **19e**−**j** were thus synthesized using Route B.

The methylcarboxyl linker was attached to 3-*O*-benzyl-E1 in 70% yield using LDA and bromoethyl acetate to give **42** as previously described (Scheme 9).[39] In this case decarboxylation of the resulting γ-keto acid was no longer a problem, enabling the synthesis of libraries of both E1 and E2 derivatives. The syntheses of methyl carboxylic acid intermediates are also shown in Scheme 9. Compound **42** could be debenzylated first to give **44**, which was then saponified to give **20**, or could be reduced to the E2 derivative **45** followed by saponification to give **21**. Acids **20** and **21** were then used for solution phase amide couplings using standard conditions (EDC, DMAP, DCM) (Scheme 10). Other amides were synthesized via solid-phase reactions using Oxime resin (Schemes 11 and 12). Scheme 11 shows the solid-phase route using the 3-*O*-benzyl intermediate **43**; Scheme 12 shows this route using acid **20**. All of these amide couplings progressed in moderate yields. In the case of the E1 derivatives, amides **20a**, **20g**, **20h** and **20i** were prepared by solution phase chemistry as shown in Scheme 10 with yields of 40, 33, 50, and 40% respectively. Amides **20b**−**f** were prepared by solid-phase amide formation followed by debenzylation as illustrated in Scheme 11. The average overall yield for these compounds was 40% based on a loading of 1.06 mmol/g on Oxime resin. Amides **20j**−**m** were also formed via a solid-phase route but in these cases the unprotected precursor **20** was loaded onto Oxime resin to give intermediate **48** (Scheme 12). The amides were therefore obtained directly by reaction of **48** with the specific amines at 40 °C. Once again, an average yield of 40% was obtained.

Of inhibitors **21a**−**f**, amide **21a** was obtained by solution phase amide coupling of **21** with a yield of 33%; **21b**−**f** were obtained by sodium borohydride reduction of **20b**−**f**. This reduction progressed cleanly in quantitative yield.

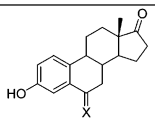
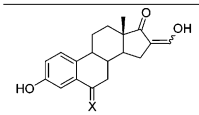
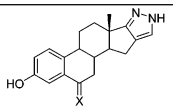
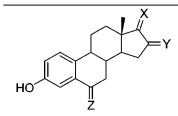
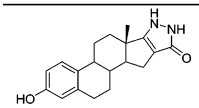
Biological Activity: Inhibition of 17β-HSD Type 1 and Type 2

The compounds synthesized were tested for their ability to inhibit 17β-HSD1 activity in T47-D cells. As a measure of selectivity, their ability to inhibit 17β-HSD2 activity was also measured in MDA-MB-231 cells. Since 17β-HSD2 catalyses the oxidative (inactivating) process, ability to inhibit this enzyme is not a desirable property for potential treatments for HDBC. These evaluations of inhibition were performed by measuring the amount of labeled E1 or E2 formed from the labeled natural substrate in the presence of the required cofactor. The percentage of inhibition is then calculated by comparison of conversion in the absence and presence of inhibitor. The percentages of inhibition achieved for a 10 μM concentration of the inhibitors, as well as the IC_{50} values for some of the most potent compounds, are shown in Tables 3−8. Compounds which gave less than 10% inhibition at 10 μM in the 17β-HSD2 assay were considered to be inactive against this enzyme.

Oxo, Oxime, and Pyrazolone Derivatives of E1. 6-Oxo Derivatives of E1. The inhibition data for the 6-oxo derivatives of E1 are shown in Table 3. The activities of the corresponding nonoxidized E1 derivatives are also shown for comparison. From these results it can be concluded that the presence of the 6-oxo substituent does not significantly enhance activity. The 6-oxo derivative of E1, **1**, has comparable activity to the natural substrate itself which indicates that although the keto group is well tolerated it does not enhance binding in the active site. For compounds **2** and **3** a slightly detrimental effect was observed which suggests that these combinations of substitution at the 6 and 17 positions are not favored.

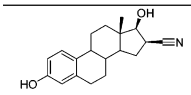
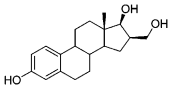
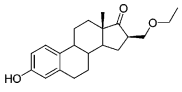
Oxime Derivatives of E1. By comparing the activities of compounds **4**−**8** information can be gained about the combination of effects of the oxime functionality at different positions on the E1 skeleton. These results are shown in Table 3. Comparison of **4** with E1 shows that oxime substitution at the

Table 3. Inhibition of 17β-HSD Type 1 and Type 2 by 6-oxo, 6-, 16- and 17-Oxime and Pyrazolone Derivatives of E1[a,b]

Structure	Compound	Inhibition of 17β-HSD (% at 10 μM) Type 1	Type 2	IC_{50} (μM) Type1
	**1** (X=O)	98	<10	0.34
	E1 (X=H,H)	nd	nd	0.33
	**2** (X=O)	97	<10	0.70
	24 (X=H,H)	97	15	0.11
	**3** (X=O)	96	<10	0.46
	27 (X=H,H)	97	32	0.18
	**4** (X=O;Y=NOH; Z=H,H)	96	<10	1.10
	5 (X=NOH; Y=NOH; Z=H,H)	55	<10	nd
	6 (X=NOH; Y=H,H; Z=H,H)	20	nd	nd
	7 (X=NOH; Y=H,H; Z=NOH)	83	27	1.90
	8 (X=NOMe; Y=H,H; Z=NOMe)	31	15	nd
	**9**	86	<10	nd

[a] Mean of at least two measurements with typically a SD or spread of ±5%. [b] nd = not determined.

Table 4. Inhibition of 17β-HSD Type 1 and Type 2 by Derivatives of E1 and E2 Possessing a Small Substituent at the 16 Position[a,b]

Structure	Compound	Inhibition of 17β-HSD (% at 10 μM) Type 1	Type 2	IC_{50} (μM) Type1
	**13**	56	<10	nd
	**14**	86	17	nd
	**15**	92	19	0.32

[a] Mean of at least two measurements with typically a SD or spread of ±5%. [b] nd = not determined.

16 position is slightly detrimental to potency although **4** does still show very high inhibition of 17β-HSD1 at 10 μM and low inhibition of 17β-HSD2 at the same concentration. Comparison of **6** with E1 shows that replacing the 17-carbonyl with an oxime moiety is not tolerated. It is interesting therefore that a slightly better inhibition is exhibited by compound **5** in which there is an oxime moiety at the both 16 and 17 positions. Compound **7** also shows improved inhibition compared with **6**, indicating that having the oxime moiety at the 6 position must have a beneficial effect on binding in the active site, sufficient to substantially compensate for the detrimental effect of the 17-oxime. This illustrates how a combination of substituents may lead to a different more favored orientation. The methyl-oxime compound **8** does not show good inhibition of 17β-HSD1, which could be due to steric effects of the methyl group clashing with residues in the active site or perhaps due to removal of hydrogen bonding interactions by replacement of the oxime hydrogen with a methyl group. It can be concluded from these results that substitution at the 6 position in combination with the 17 position could in some cases lead to improved synergistic effects. One can also deduce that disfavored interactions can to some degree be alleviated by substitution at a different position on the E1 skeleton.

Pyrazolone Derivative of E1. The pyrazolone compound **9** (Table 3) showed 86% inhibition of 17β-HSD1 at 10 μM and

Table 5. Inhibition of 17β-HSD Type 1 and Type 2 by 16 Alkenyl Derivatives of E1 and E2 and 16 β-alkyl Derivatives of E2[a−c]

R	Compounds **16**		Compounds **17**			Compounds **18**	
	Inhibition of 17β-HSD (% at 10 μM)		Inhibition of 17β-HSD (% at 10 μM)		$1C_{50}$ (μM)	Inhibition of 17β-HSD (% at 10 μM)	
	Type1	Type2	Type1	Type2	Type1	Type1	Type2
H	70	10	64	20	nd	ns	ns
(a)	44	10	52	43	nd	87	11
(b)	23	16	55	17	nd	88	11
(c)	34	25	77	<10	4.1	84	37
(d)	72	13	72	81	nd	41	25
(e)	71	18	68	<10	nd	ns	ns
(f)	82	31	84	27	nd	ns	ns
(g)	17	<10	26	<10	nd	ns	ns
(h)	15	15	33	38	nd	ns	ns
(i)	39	18	57	31	nd	ns	ns
(j)	24	<10	48	<10	nd	ns	ns
(k)	67	14	41	25	nd	ns	ns
(l)	26	17	ns	ns	ns	ns	ns

[a] Mean of at least two measurements with typically a SD or spread of ±5%. [b] nd = not determined. [c] ns = not synthesized.

was selective for Type 1 over Type 2 (inhibition of 17β-HSD2 was 7% at 10 μM). However, these results were less promising than for the corresponding E-ring pyrazole **27** that showed 97% inhibition of 17β-HSD1 at 10 μM. Compound **9** can exist as the different tautomeric forms shown in Figure 8, four of which can be thought of as a hydroxyl-substituted pyrazole. As mentioned previously, however, ^{13}C NMR of **9** in deuterated DMSO suggests that in polar solution a keto form predominates. The inferior activity of **9** compared to **27** is probably due to differences in the electronic properties of the E-ring system.

Table 6. Inhibition of 17β-HSD Type 1 and Type 2 by Carboxyl Derivatives of E2[a,b]

R	Compound	Inhibition of 17β-HSD (% at 10 μM)		IC_{50} (μM)
		Type 1	Type 2	Type1
	37	63	<10	nd
	19	39	<10	nd
	19a	82	11	nd
	19b	79	<10	nd
	19c	70	<10	nd
	19d	87	<10	nd
	19e	91	<10	0.51
	19f	90	12	0.81
	19g	87	<10	nd
	19h	85	<10	nd
	19i	86	<10	nd
	19j	74	<10	nd

[a] Mean of at least two measurements with typically a SD or spread of ±5%. [b] nd = not determined.

16-Substituted Derivatives of E1 and E2. Small Substituents. The results for compounds **13**−**15** were encouraging (Table 4), as all inhibited 17β-HSD1 to a moderate or higher degree and all showed reasonable selectivity for Type 1 over Type 2. Compound **15**, in particular, showed a promising inhibition of 17β-HSD1 of 92% at 10 μM with an IC_{50} value of 0.32 μM. This emphasizes that there is potential for expansion at the 16 position, with the caveat of substituent optimization, and also suggests that β-substitution is well tolerated. This has significance for compounds **18** since, due to steric factors, the β-substituted products would be those expected from the hydrogenation of compounds **17**.

Alkenyl Substituents. Compounds **16** showed a wide range of inhibitory activity as shown in Table 5. The methylene derivative of E1,[25] **35** (**16**, R = H, Table 5) showed 70% inhibition of 17β-HSD1 at 10 μM, and 10% inhibition of Type 2 in our assay. The isopropyl analogue **16e** showed similar inhibition and the isobutyl version **16f** gave an inhibition of 82% for Type 1 but was less selective, with 31% inhibition of Type 2. Of the aryl substituents the only promising compounds were **16d** and **16k** which showed 72 and 67% inhibition of Type 1 at 10 μM, respectively. Reduction of the 17-carbonyl to give the E2 derivatives **17** yielded interesting results. The methylene-E2 derivative **17a**[25] and the two other alkyl derivatives (**17e**

Table 7. Inhibition of 17β-HSD Type 1 and Type 2 by Methyl Carboxyl Derivatives of E1[a,b]

R	Compound	Inhibition of 17β-HSD (% at 10 μM) Type 1	Type 2	IC_{50} (μM) Type1
	44	97	18	0.30
	20	13	<10	nd
	**20a**	91	21	0.38
	**20b**	79	<10	nd
	**20c**	75	<10	nd
	**20d**	90	<10	1.5
	**20e**	82	<10	nd
	**20f**	84	<10	nd
	**20g**	85	<10	nd
	**20h**	95	26	0.037
	**20i**	82	<10	nd
	**20j**	36	13	nd
	**20k**	53	<10	nd
	**20l**	83	10	nd
	**20m**	58	14	nd

[a] Mean of at least two measurements with typically a SD or spread of ±5%. [b] nd = not determined.

and **17f**) did not show much difference in activity from their E1 analogues. For the aryl compounds, activities were improved in all cases except for the two most active compounds from series **16**: compound **17d** showed no improvement in inhibition of Type 1 compared with **16d**, but interestingly the selectivity for Type 1 over Type 2 was lost; in fact **17d** showed a high 81% inhibition of Type 2 at 10 μM, and compound **17k** showed decreased inhibition when compared with **16k** and also showed less selectivity. Other examples (**17a**, **17h** and **17i**) also exhibited some drop in selectivity compared with their **16** series counterparts. Generally, however, inhibition of Type 1 was improved by reduction of the E1 derivatives **16** to the E2 derivatives **17**. As yet the irreversibility of the inhibition shown by compounds **16a**−**l** and **17a**−**k** has not been fully investigated.

16β-Alkyl Substituents. Compounds **18** were synthesized to further investigate the potential of β-substitution at the 16 position. Here the results were more promising, with inhibitions of 84% and over for compounds **18a**−**c** at 10 μM. These findings suggest that some flexibility of the substituent may be important for binding in the active site. The results also show that bulky aryl substituents are tolerated although optimization of these systems would be needed to obtain a potent inhibitor.

Table 8. Inhibition of 17β-HSD Type 1 and Type 2 by Methyl Carboxyl Derivatives of E2[a]

R	Compound	Inhibition of 17β-HSD (% at 10 μM) Type 1	Type 2
	45	67	18
	21	58	<10
	**21a**	63	25
	**21b**	78	16
	**21c**	67	11
	**21d**	63	11
	**21e**	79	14
	**21f**	76	13

[a] Mean of at least two measurements with typically a SD or spread of ±5%.

Carboxyl Derivatives of E2. The inhibitory activities of the carboxylic acid derivatives of E2 are shown in Table 6. These were tested as mixtures of diastereomers in ratios which in most cases were undetermined as for the majority of the amides only one set of peaks was observed by ^{1}H NMR. Apart from the ester and acid intermediates **37** and **19** which showed 63 and 39% inhibition of 17β-HSD1 at 10 μM respectively, the carboxyl derivatives of E2 show good inhibition of 17β-HSD1 (70−91%). It can also be seen from the results that aromatic amide moieties show better inhibition than the alkyl amides. This indicates the potential for enhancing activity through beneficial π−π interactions. The best compound in the series proved to be the *m*-pyridyl amide **19e** which has an IC_{50} of 510 nM. All of the compounds in this series showed good selectivity for Type 1 over Type 2.

Methyl Carboxyl Derivatives of E1. The results for the methyl carboxylic acid derivatives of E1, shown in Table 7, were the most promising of all the series investigated to date. For this series the ester intermediate **44** itself, tested as a 3:1 mixture of diastereomers, showed 97% inhibition of 17β-HSD1 at 10 μM, with an IC_{50} value of 300 nM. This activity was lost upon hydrolysis to the acid **20**, which was also tested as an approximately 3:1 ratio of diastereomers. The majority of the amides **20a**−**m**, however, showed excellent inhibition of 17β-HSD1 and, importantly, selectivity for Type 1 over Type 2. It can be deduced from the comparison of results for **20a** (which shows good inhibition) with **20j** and **20k** that very flexible

moieties are less well tolerated in the active site. Also, from comparison of the inhibition by **20m** with **20i**, one can deduce that there is an optimum distance at which the pyridyl moiety interacts (**20m** appears to have exceeded this). By far the best result was for compound **20h** which, as mentioned previously, is the most potent inhibitor of 17β-HSD1 reported to date.[27] This compound was initially tested as a mixture of diastereomers, along with the other members of the library, before the major diastereomer (the 16β-substituted compound) was separated and its biological activity evaluated. This single 16β-substituted diastereomer was found to have the same potency as the 2:1 mixture; as yet the 16α-substituted compound has not been isolated for testing. The diastereomers of the other less active amides were not separated.

Methyl Carboxyl Derivatives of E2. The percentage inhibition of 17β-HSD1 and 17β-HSD2 at 10 μM for the methyl carboxylic acid derivatives of E2, compounds **21**, are listed in Table 8. These analogues showed reduced inhibitory activities compared with compounds **20** and also showed less activity than the carboxyl derivatives **19**. The selectivity for inhibition of 17β-HSD1 over 17β-HSD2 was however conserved.

Comparison of results for series **19** and **21** may suggest that the carboxyl linker in series **19** might be preferable to the methyl carboxyl, since overall the activities of compounds **19** are higher than those of **21**. However, this conclusion can only be tentative since most of series **21** contain only simple alkyl amides (ideally a more comprehensive library **21** including greater variety of amides might confirm the preferential binding of one series over another). It can be concluded, however, that the linker/amide combination is more successful for **19a** than for **21a** and that compounds **19** appear to be more selective for 17β-HSD1 over 17β-HSD2. Comparison of activities for series **20** and **21** suggest that E1 is preferable as a template for this particular methyl carboxyl system than E2.

However, since inhibitor **20h**, *N*-(pyridin-3-ylmethyl)estrone-16-methylcarboxamide, showed vastly superior potency over the other compounds it seems reasonable to pursue further investigation into compounds which to a large degree mimic the attributes of this compound in similar areas of space.

Molecular Modeling. To investigate the binding of our inhibitors in the active site of 17β-HSD1, the crystal structure of 17β-HSD1 in complex with E2 and NADP (PDB code 1FDT) was used.[13,23] Compounds were docked into 1FDT with E2 removed using the docking program GOLD version 2.2.[44] For all compounds docked, the steroid backbone overlaid closely with that of E2 as it is positioned in the crystal structure 1FDT. When E2 is docked back into the active site after being removed it docks back into the same position as it is in the crystal structure. Shown in Figure 11 is inhibitor **1** docked into 1FDT with a docking score of 50; the docked E2 is left out for clarity as the steroid backbones of E2 and **1** overlay. The distance between the 6-oxo carbonyl oxygen of **1** and the hydroxyl on serine 222 is 2.21 Å; from the carbonyl oxygen to the tyrosine phenolic oxygen is 2.86 Å. These distances suggest that hydrogen bonding in this area is possible for 6-oxo compounds, and it was hoped that the presence of a 6-oxo substituent might enhance the binding of inhibitors. SAR results for the examples synthesized, however, showed no improvement in inhibitory activity of the 6-oxo over nonoxidized compounds.

Also shown (Figure 12) is the potent inhibitor **20h** docked into 1FDT. This docking gives an indication of what interactions in the active site might confer superiority to **20h** over the other compounds synthesized. For example, in addition to the common hydrophobic interactions of the steroid backbone with residues

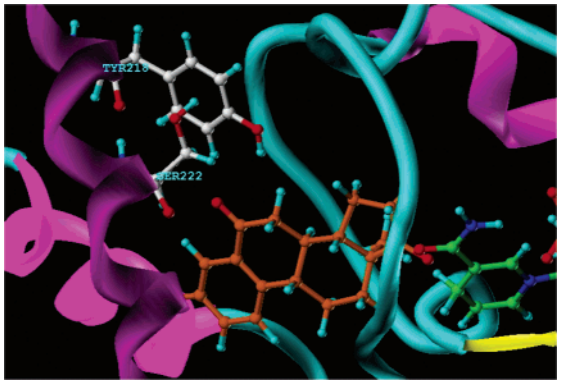


Figure 11. Compound **1** (orange) docked into 1FDT in the presence of NADP with substrate removed. E2 docked (not shown) overlays with the steroid template of **1**.

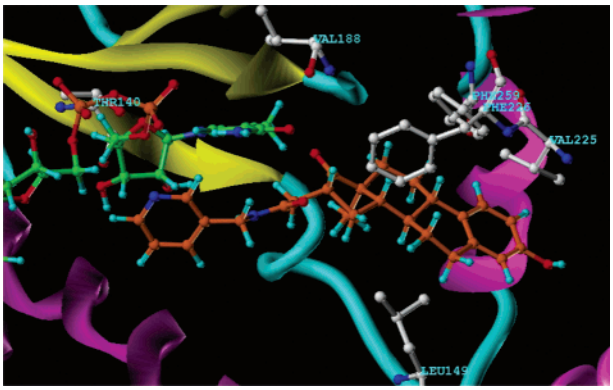


Figure 12. Compound **20h** (orange) docked into 1FDT in the presence of NADP with substrate removed.

Leu149, Val255, Phe226, and Phe259, the pyridyl nitrogen is only 3.36 Å from the nearest phosphate oxygen of the cofactor. In addition, the hydrogen atoms in the methyl linker of the side chain are 1.71 and 3.33 Å from the Pro(*S*) hydrogen of the cofactor, which could interfere with the transfer of the Pro(*S*) hydrogen to the 17-carbonyl of **20h**.

The novel 17β-HSD1 inhibitors described herein, along with compounds previously published by our group, for which an IC_{50} had been determined and which possess only defined stereocenters were used to develop a quantitative structure−activity relationship (QSAR). This was achieved using the three-dimensional method of comparative molecular similarity indices analysis (CoMSIA), part of the SYBYL suite, where a set of aligned molecules is used in QSAR generation.[45] These molecules include the highly potent compound **20h** (the separated diastereomer which is β-substituted at the 16 position), the equipotent 2-ethyl analogue,[27] which has also been confirmed by X-ray crystallography as β-substituted at the 16 position, and a variety of pyrazole derivatives. The set of molecules was aligned using FlexS[46,47] and CoMSIA was then used to assess steric, electrostatic, hydrophobic, and hydrogen bond donor/acceptor fields. The two parameters which were given the highest relative contributions by CoMSIA were electrostatic and hydrogen bond acceptor fields: the aligned molecules with these fields are shown in Figure 13. Areas of favored and disfavored hydrogen bond acceptor interactions are shown by green and yellow contours, respectively. The electrostatic contours can be interpreted as the blue contour defining a region where increased positive charge will result in increased activity (or a more negative charge will result in decreased activity); the red contour defines a region of space where electron density is favorable.

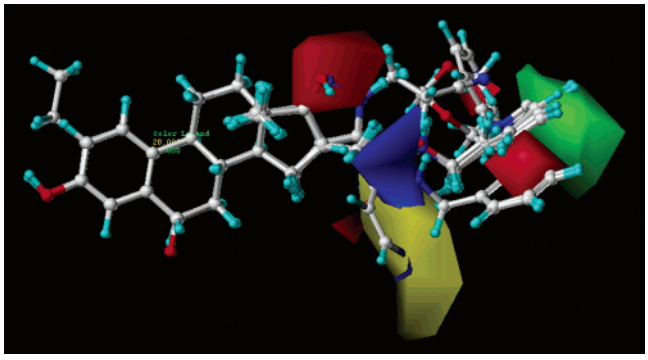


Figure 13. CoMSIA alignment showing hydrogen bond acceptor and electrostatic fields: regions where a hydrogen bond acceptor is favorable or unfavorable are shown in green and yellow respectively; blue contours indicate regions where increased positive charge is favorable and red contours show regions where electron density is favorable.

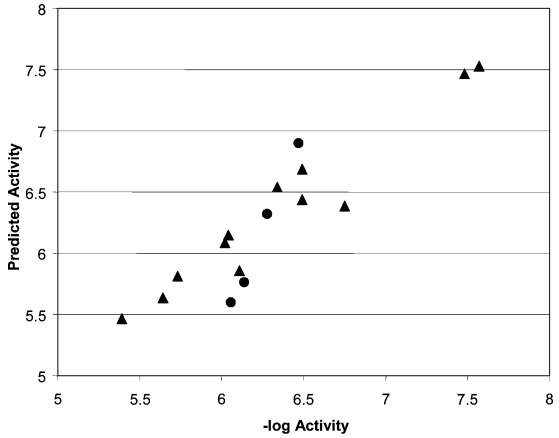


Figure 14. QSAR showing activity against predicted activity (units are −log IC_{50} values). Triangles are the training set; circles are the validation set.

Using the IC_{50} values as continuous response data, the statistical tool in QSAR for CoMSIA utilizes partial least squares (PLS) for regression analysis.[48] The results of the QSAR are shown in Figure 14, where the −log of the observed IC_{50} is plotted against the −log of the predicted IC_{50}. Compound **1** was included in the validation set; compounds **3**, **15**, **17c**, **20h**, and **27** were included in the training set to predict activity. The full list of compounds used with their IC_{50} values can be found in Supporting Information. The graph in Figure 14 shows good correlation of observed versus predicted activity with an r^2 value of 0.94 and q^2 value of 0.86, indicating that the QSAR can be used in a predictive fashion to calculate activities in silico. This QSAR will be further refined as more data become available from other series.

Conclusions

Novel potent inhibitors of 17β-HSD1 have been identified by the modification of E1 at the 6, 16 and 17 positions. This work has provided valuable information about the SAR around the substituted D ring of the E1/E2 template for this emerging and topical area of research. A number of conclusions can be drawn from these results about the effects of substitution at positions 6, 16 and 17 and combinations of these. Docking studies indicate there is limited space for expansion at the 6 and 17 positions and results for the oxime compounds agree with this hypothesis (the methyl-oxime compound **8** being a lot less active than compound **7**). This diminished activity could also be a consequence of disrupted hydrogen bonding interactions in an important substrate binding region. Although the presence of a 6-oxo functionality does not significantly enhance inhibitory activity, and in some cases can be detrimental, the presence of a carbonyl group at C6 can block a known[49] point for metabolism of the substrate, and these compounds are novel and interesting inhibitors in their own right. A combination of substitutions at different positions on the E1 scaffold can lead to improved activity, as exemplified by the activity of compounds **6** and **7** where an oxime moiety at the 6 position can alleviate the highly detrimental effect of 17-oxime group. Comparison of the inhibitory activities of compounds **5** and **6** show that the combination of an oxime moiety at the 16 and 17 positions is also more favorable than having the oxime at the 17 position alone. Our group further investigated this 16/17 combined substitution in the form of E-ring pyrazole steroids, which have been reported previously, and has extended the SAR in this area.[24] Herein is reported the novel pyrazolone compound **9** which has been found not to show such high inhibition as the E-ring pyrazole compound. This highlights the importance of electronic effects in this area on binding in the active site.

In addition, a diverse array of new E1 and E2 derivatives with substitution at the 16 position has been synthesized. From their biological activities it can be concluded that compounds containing a flexible linker to the 16 position give better inhibition than those with a rigid alkene linker and, in the majority of cases, substitution at the 16 position of E2 confers selectivity for 17β-HSD1 over 17β-HSD2. This work has led to the discovery of novel potent inhibitors of 17β-HSD1 including compounds **15** and **20a** which show IC_{50} values of 320 and 380 nM, respectively. Also discovered was compound **20h** which, with an IC_{50} of 37 nM, is the most potent inhibitor of 17β-HSD1 reported to date.[27] In addition a predictive QSAR has been developed which will be modified to identify novel nonsteroidal 17β-HSD1 inhibitors by in silico screening.

Experimental Section

Chemistry. All chemicals were purchased from Aldrich Chemical Co. (Gillingham, UK) or Lancaster Synthesis (Morecambe, U.K.). All organic solvents of A. R. grade were supplied by Fisher Scientific (Loughborough, U.K.). 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 aluminum 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. IR spectra were recorded on a Perkin-Elmer Spectrum RXI FT-IR as KBr disks and peak positions are expressed in cm^{-1}. ^1H NMR (270 or 400 MHz) and DEPT-edited ^{13}C NMR (100.4 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 using either a Waters Radialpack C_{18} reversed phase column (8 × 100 mm) eluting with MeOH/H_2O at 2 mL/min, or a Symmetry C18 reverse phase column (4.6 × 150 mm) eluting with MeCN/H_2O at 0.3 mL/min. FAB low and high-resolution mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath, using *m*-nitrobenzyl alcohol (NBA) as the matrix. ES and APCI low resolution mass spectra were obtained on a Waters Micromass ZQ. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block and are uncorrected. X-ray crystallographic studies of compounds **15** and **16c** were carried out on a kappa CCD diffractometer with area detector.

Molecular Modeling. For the docking studies of compound **1** in 1FDT the starting conformations used for receptor docking were generated from an energy minimization performed using the MMFF94s force field, with MMFF94 charges applied, as implemented in Sybyl 7.0. The resulting lowest energy conformer was then used for docking studies using Gold version 2.2 with default parameters. The active site was defined as a 12 Å radius around the C alpha atom of Serine 142 and 30 attempts were computed and scored using Gold Score.

To develop a QSAR using CoMSIA, the ligands were initially minimized using the MMFF94s force field as implemented within the Sybyl 7.0 package. The molecules were aligned using FlexS, with a common core elucidated by DISTILL which corresponded to the steroid backbone. Gastegier−Hückel charges were used for the charge descriptors in FlexS and CoMSIA was performed using the aligned compounds and the standard Sybyl 7.0 CoMSIA fields.

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

T47-D and MDA-MB-231 cells have previously been shown to possess predominantly reductive or oxidative 17B-HSD activity, respectively.[50]

Measurement of Inhibition of 17β-HSD Type 1. T47-D human breast cancer cells were incubated with ^{3}H-E1 at a concentration of 2 nM per well, in a 24-well tissue culture plate, in the absence or presence of the inhibitor (0.1 nM to 10 μM). After incubation of the substrate ± inhibitor for 30 min at 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 (DCM/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.

Measurement of Inhibition of 17β-HSD Type 2. MDA-MB-231 human breast cancer cells were incubated with ^{3}H-E2 at a concentration of 2 nM per T25 flask, in the absence or presence of the inhibitor (0.1 nM to 10 μM). After incubation of the substrate ± inhibitor for 3 h at 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 (DCM/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.

Compounds **4**,[32] **5**,[24] **6**,[33,51] **10**,[24] **21**,[39] **22**,[52] **24**,[30] **27**,[53] **30**,[34] **42**,[39] and **45**[39] have been described in the literature. Experimental details and data for compounds **23** and **1** are reported in Supporting Information as are analytical and spectroscopic data for compounds **16b−d**, **16f−l**, **17b−d**, **17h**, **17i**, **18b−d**, **19b**, **19c**, **19e−j**, **20b−m**, and **21b−f**.

3-*O*-Acetyl-16-acetoxymethylene-estrone (25). To a stirred solution of 16-formyl-estrone **24** (230 mg, 0.77 mmol) in anhydrous pyridine (15 mL) at 0 °C was added dropwise over 10 min acetic anhydride (8.34 mL, 88.4 mmol). The resulting yellow mixture was then heated to reflux for 1 h. The final brown solution was cooled then poured into ice/water (50 mL) and acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 50 mL), washed with H_2O (30 mL), Na_2CO_3 10% (30 mL), then brine (30 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to give orange foam (250 mg). This was purified by flash chromatography using $CHCl_3$/EtOAc (95:5) as eluent to give the title compound as a pale yellow oil that crystallized on standing (140 mg, 47%): mp 115−117 °C; TLC ($CHCl_3$/EtOAc, 9:1) R_f 0.70 cf. R_f 0.30; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (s, 3H, H-18), 1.42−2.84 (m, 11H), 2.24 (s, 3H, OAc), 2.29 (s, 3H, OAc), 2.89−2.96 (m, 2H, H-6), 6.81 (d, J = 2.6 Hz, 1H, H-4), 6.86 (dd, J = 8.3, 2.6 Hz, 1H, H-2), 7.29 (d, J = 8.3 Hz, 1H, H-1), 8.15 (dd, J = 2.9, 1.7 Hz, 1H, =CH); LRMS (FAB+) m/z 383.0 [100, $(M + H)^+$]; HRMS (FAB+) m/z calcd. for $C_{23}H_{27}O_5$ $(M + H)^+$ 383.1858, found 383.1852.

6-Oxo-3-*O*-acetyl-16-acetoxymethylene-estrone (26). To a stirred solution of 3-*O*-acetyl-16-acetoxymethylene-estrone **25** (120 mg, 0.31 mmol) in AcOH at 10−15 °C in an ice/water bath was added dropwise over 30 min a solution of CrO_3 (132 mg, 1.32 mmol) in aq. AcOH (0.75 mL, 10%). The resulting dark brown solution was stirred for 40 h at room temperature. The solvent was then removed under reduced pressure and ice/water (50 mL) was added. The organics were extracted with EtOAc (2 × 50 mL), washed with H_2O (2 × 30 mL), then brine (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a brown crude product. This was purified by flash chromatography with $CHCl_3$/EtOAc (8:2) as eluent to give the title compound as a pale yellow solid (13 mg, 10%): mp 96−101 °C; TLC ($CHCl_3$/EtOAc, 4:1) R_f 0.40 cf. R_f 0.63; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (s, 3H, H-18), 1.24−2.92 (m, 11H), 2.26 (s, 3H, OAc), 2.32 (s, 3H, OAc), 7.29 (dd, J = 8.4, 2.6 Hz, 1H, H-2), 7.46 (d, J = 8.4 Hz, 1H, H-1), 7.77 (d, J = 2.6 Hz, 1H, H-4), 8.17 (dd, J = 2.9, 1.7 Hz, 1H, =CH); LRMS (FAB+) m/z 663.5 [48], 397.2 [38, $(M + H)^+$], 73.0 [100]; HRMS (FAB+) m/z calcd. for $C_{23}H_{25}O_6$ $(M + H)^+$ 397.1651, found 397.1670.

6-Oxo-16-formyl-estrone (2). To a stirred solution of 6-oxo-3-*O*-acetyl-16-acetoxymethylene-estrone **26** (30 mg, 0.08 mmol) in MeOH (10 mL) was added dropwise aq. K_2CO_3 (63 mg, 0.46 mmol in 1 mL). The resulting pale yellow solution was stirred at room temperature for 45 min before the final pale brown mixture was acidified with 3 drops of 5 M HCl and concentrated in vacuo. H_2O was added (30 mL) and the organics were extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with H_2O (2 × 30 mL) then brine (2 × 30 mL), dried (Na_2SO_4), and concentrated in vacuo. The resulting beige crude product was recrystallized from EtOAc/hexane to give the title compound as white powder (13 mg, 54%): mp 224−227 °C; ^{1}H NMR (400 MHz, DMSO-d_6) 0.80 (s, 3H, H-18), 1.41−2.65 (m, 11H), 7.01 (dd, J = 8.4, 2.7 Hz, 1H, H-2), 7.28 (d, J = 2.7 Hz, 1H, H-4), 7.31(d, J = 8.4 Hz, 1H, H-1), 7.41 (1H, s, 1H, C-1′-H), 9.64 (1H, s, exchanged with D_2O, C-3-OH) and 10.77 (1H, br s, exchanged with D_2O, C-1′-OH); LRMS (FAB-) m/z 311.2 [100, $(M - H)^-$]; HRMS (FAB+) m/z calcd. for $C_{19}H_{21}O_4$ $(M + H)^+$ 313.1440, found 313.1450.

Acetic Acid 8-Acetyl-6a-methyl-4b,5,6,6a,8,10,10a,10b,11,12-decahydro-7,8-diaza-pentaleno[2,1-*a*]phenanthene-2-yl Ester (28). To a solution of **27** (0.598 g, 2.0 mmol) in dry pyridine (8 mL) was added acetic anhydride (2 mL), and the mixture was refluxed under nitrogen for 16 h. The mixture was then cooled and poured onto ice, and the resulting cream colored precipitate was collected by filtration and purified by column chromatography using EtOAc/hexane (1/1) to give the title compound as white powder (0.303 g, 40%): TLC (EtOAc/hexane, 1/1) R_f 0.48; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.07 (s, 3H, H-18), 1.40−1.60 (m, 1H), 1.62−2.10 (m, 5H), 2.28 (s, 3H, $OCOCH_3$), 2.28−2.48 (m, 4H), 2.64 (s, 3H, $NCOCH_3$), 2.64−2.72 (m, 1H), 2.87−2.96 (m, 2H), 6.82 (d, J = 2.2 Hz, 1H, H-4), 6.86 (dd, J = 8.4, 2.5 Hz, 1H, H-2), 7.30 (d, J = 8.4 Hz, 1H, H-1), 7.86 (s, 1H); LCMS (ES+) 380.23 $(M + 2H)^+$.

Acetic Acid 8-Acetyl-6a-methyl-12-oxo-4b,5,6,6a,8,10,10a,-10b,11,12-decahydro-7,8-diaza-pentaleno-[2,1-*a*]phenanthren-2-yl Ester (29). To a solution of **28** (0.121 g, 0.32 mmol) in MeCN (2.5 mL) was added *tert*-butyl hydroperoxide (0.14 mL of a 70wt % solution in water, 1 mmol) and chromium hexacarbonyl (0.021 g, 0.09 mmol). The solution was heated to reflux for 24 h before being cooled to room temperature. Water (10 mL) was added, and the products were extracted with diethyl ether (3 × 10 mL). The ether extracts were combined, washed with H_2O, saturated aqueous $NaHCO_3$ and brine before being dried ($MgSO_4$) and concentrated in vacuo. The title compound was isolated by flash column chromatography using ethyl acetate/hexane (1/1) as eluent (29 mg, 23%): TLC (EtOAc/hexane 1/1) R_f = 0.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 3H, 18-H), 1.8−2.0 (m, 2H), 2.2−2.5 (m, 4H), 2.33 (s, 3H), 2.5−2.9 (m, 5H), 2.65 (s, 3H), 7.34 (dd, J = 8.4, 2.5 Hz, 1H, H-2), 7.65 (d, J = 8.6 Hz, 1H, H-1), 7.79 (d, J = 2.3 Hz, 1H, H-4), 7.88 (s, 1H).

2-Hydroxy-6a-methyl-5,6,6a,8,10,10a,10b,11-octahydro-4b*H*-7,8-diaza-pentaleno[2,1-*a*]phenanthren-12-one (3). To a solution of **29** (0.014 g, 0.04 mmol) in EtOH (1 mL) was added a solution of KOH (0.004 g) in ethanol/water (2 mL of 1/1), and the mixture was shaken then left at room temperature for 30 min. TLC at this

stage showed no starting material remaining; therefore, the mixture was acidified with glacial AcOH, concentrated in vacuo and H_2O was added. The solution was left standing until a pale orange precipitate formed. This powder was collected by filtration and washed with water to yield the title compound (6 mg, 46%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91 (s, 3H, 18-H), 1.60−1.82 (m, 2H), 2.1−2.35 (m, 5H), 2.4−2.7 (m, 5H), 7.02 (dd, J = 8.6, 2.7 Hz, 1H, H-2), 7.29 (d, J = 2.7 Hz, 1H, H-4), 7.34 (d, J = 8.6 Hz, 1H, H-1), 9.65 (s, 1H), 12.04 (bs, 1H); LCMS (ES+) 309.99 (M + 2H)$^+$; HRMS (FAB+) calcd. for $C_{19}H_{21}N_2O_2$ (M + H)$^+$ 309.16030, found 309.16132.

3-Hydroxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-cyclopenta[*a*]phenanthren-17-one Oxime (6). To a suspension of E1 (500 mg, 1.85 mmol) in a mixture of MeOH/H_2O (5:1, 90 mL) was added NaOAc (1.50 g, 18.80 mmol) followed by hydroxylamine hydrochloride (1.40 g, 20.72 mmol). The resulting suspension was stirred at room temperature overnight. The solvent was then removed under reduced pressure and H_2O (100 mL) added. The organics were extracted with EtOAc (100 mL + 50 mL), washed with H_2O (50 mL), then brine (50 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to give a white crystalline crude product (627 mg). This was recrystallized from MeOH to give the title compound as white crystals (426 mg, 81%): mp 251−253 °C [lit. 248−250 °C];[51] TLC ($CHCl_3$/EtOAc, 4:1) R_f 0.13 cf. R_f 0.55 (E1); IR (KBr) 3415 (NOH), 3270 (OH), 2930 (aliph CH), 1620 (C═N or arom C═C), 1585−1460 (arom C═C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (s, 3H, H-18), 1.32−2.41 (m, 13H), 2.65−2.80 (m, 2H, H-6), 6.44 (d, J = 2.6 Hz, 1H, H-4), 6.50 (dd, J = 8.5, 2.6 Hz, 1H, H-2), 7.05 (d, J = 8.5 Hz, 1H, H-1), 9.01 (s, exchanged with D_2O, 1H, OH) and 10.10 (s, exchanged with D_2O, 1H, NOH); LRMS (FAB+) m/z 286.1 (100, [M + H]$^+$); HRMS (FAB+) m/z calcd. for $C_{18}H_{24}NO_2$ (M + H)$^+$ 286.1807, found 286.1809.

3-Hydroxy-13-methyl-8,9,11,12,13,14,15,16-octahydro-7*H*-cyclopenta[*a*]phenanthrene-6,17-dione Dioxime (7). To a solution of **1** (100 mg, 0.35 mmol) in a mixture of MeOH/H_2O (5:1, 18 mL) was added NaOAc (293 mg, 3.58 mmol) followed by hydroxylamine hydrochloride (274 mg, 3.94 mmol). The resulting solution was stirred at room temperature overnight before the solvent was removed under reduced pressure and H_2O (50 mL) was added. The organics were extracted with EtOAc (50 mL + 20 mL) and the combined organic layers washed with H_2O (2 × 20 mL), then brine (2 × 20 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to give a light brown crude product (120 mg). This was purified by flash chromatography using a gradient of $CHCl_3$/EtOAc (7:3 to 1:1) then $CHCl_3$/EtOAc/acetone (2:2:1 to 1:1:2) as eluent to give the title compound as cream colored powder (46 mg, 41%): mp 341−344 °C; TLC ($CHCl_3$/EtOAc, 7:3) R_f 0.11 cf. R_f 0.44; IR (KBr) 3410, 3265−3050 (br, NOH, OH), 2930−2850 (aliph CH), 1705 (C═N), 1580−1495 (arom C═C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.83 (s, 3H, H-18), 1.14−2.42 (m, 12H), 3.06 (m, 1H, H-7), 6.73 (dd, J = 8.3, 2.5 Hz, 1H, H-2), 7.14 (d, J = 8.3 Hz, 1H, H-1), 7.29 (d, J = 2.8 Hz, 1H, H-4) and 9.28, 10.15, 11.08 (each 1H, s, 2×NOH, OH); LRMS (FAB+) m/z 315.1 [82, (M + H)$^+$], 73.0 [100]; HRMS (FAB+) m/z calcd. for $C_{18}H_{23}N_2O_3$ (M + H)$^+$ 315.1709, found 315.1715.

3-Hydroxy-13-methyl-8,9,11,12,13,14,15,16-octahydro-7*H*-cyclopenta[*a*]phenanthrene-6,17-dione Bis-(*O*-methyl-oxime) (8). To a solution of **1** (50 mg, 0.18 mmol) in a mixture of MeOH/H_2O (5:1, 9 mL) was added NaOAc (146 mg, 1.79 mmol) followed by *O*-methyl-hydroxylamine hydrochloride (164 mg, 1.97 mmol). The resulting solution was stirred at room temperature overnight before the solvent was removed under reduced pressure and H_2O (30 mL) was added. The organics were extracted with EtOAc (20 mL + 10 mL), and the combined organic layers were washed with H_2O (2 × 10 mL) then brine (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo. The white crystalline crude product was recrystallized from EtOAc/hexane to give the title compound as white crystals (55 mg, 92%): mp 206−208 °C; TLC ($CHCl_3$/EtOAc, 8:2) R_f 0.70 cf. R_f 0.28; IR (KBr) 3134 (br, OH), 2995 (arom CH), 2935−2890 (aliph CH), 1570−1490 (arom C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, 3H, H-18), 1.24−2.58 (m, 12H), 3.12 (dd, J = 18.1, 4.5 Hz, 1H, H-7), 3.84 (s, 3H, OMe), 3.99 (s, 3H, OMe), 4.85 (br s, exchanged with D_2O, 1H, OH), 6.84 (dd, J = 8.6, 2.7 Hz, 1H, H-2), 7.20 (d, J = 8.6 Hz, 1H, H-1), 7.29 (d, J = 2.7 Hz, 1H, H-4); LRMS (FAB+) m/z 343.2 [100, (M + H)$^+$]; HRMS (FAB+) m/z calcd. for $C_{20}H_{27}N_2O_3$ (M + H)$^+$ 343.2022, found 343.2033. Anal. ($C_{20}H_{26}N_2O_3$) C, H, N.

3-*O*-Benzyl-estrone-16-carboxylic Acid Methyl Ester (31). To a stirred suspension of NaH (3.48 g of a 60% dispersion, 87 mmol) in anhydrous THF (50 mL) was added dimethyl carbonate (6.11 mL, 72 mmol), and the mixture was heated to reflux. To this was added dropwise a solution of 3-*O*-benzylestrone **30** (10.45 g, 29 mmol) in THF (50 mL), and the reaction was refluxed for 8 h before being cooled to 0 °C. The mixture was acidified with 3 M AcOH (50 mL) before being poured into brine. The product was extracted with $CHCl_3$ and the solution dried over Na_2SO_4 before concentration in vacuo. Recrystallization of the solid residue from MeOH gave the title compound as beige solid (9.8 g, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (s, 1.05H, CH_3), 0.99 (s, 1.95H, CH_3), 1.34−2.50 (m, 11H), 2.91−2.93 (m, 2H, H-6), 3.22 (dd, J = 10.1, 8.6 Hz, 0.65H, H-16), 3.58 (dd, J = 9.4, 1.7 Hz, 0.35H, H-16), 3.77 (s, 3H, OCH_3), 5.03 (s, 2H, CH_2Ph), 6.73 (d, J = 2.5 Hz, 1H, H-4), 6.80 (dd, J = 8.6, 2.5 Hz, 1H, H-2), 7.20 (d, 1H, J = 8.6 Hz, 1H, H-1), 7.30−7.50 (m, 5H); LRMS (FAB+) m/z 418.20 (M$^+$).

Estrone-16-carboxylic Acid Methyl Ester (32). To a solution of **31** (2.5 g, 5.97 mmol) in THF (30 mL) was added Pd/C (10wt %, catalytic), and the reaction mixture was stirred at room temperature under H_2 (balloon) for 8 h. The mixture was filtered through Celite, and the filtrate was evaporated in vacuo. Recrystallization of the solid residue from MeOH gave the title compound as white solid (1.82 g, 93%): mp 215−217 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (s, 1.95H, CH_3), 0.91 (s, 1.05H, CH_3), 1.20−2.40 (m, 11H), 2.73−2.76 (m, 2H, H-6), 3.39 (dd, J = 9.8, 8.6 Hz, 0.65H, H-16), 3.65 (s, 1.05H, OCH_3), 3.65 (s, 1.95H, OCH_3), 3.80 (dd, J = 9.8, 8.6 Hz, 0.35H, H-16), 6.45 (d, J = 2.3 Hz, 1H, H-4), 6.05 (dd, J = 8.6, 2.7, 1H, H-2), 7.04 (d, J = 8.2 Hz, 1H, H-1), 9.04 (s, 1H, OH); HRMS (FAB+) m/z calcd. for $C_{20}H_{24}O_4$ (M$^+$) 328.1675, found 328.1684; HPLC > 97% (t_R = 2.75, 10:90 H_2O:MeOH). Anal. ($C_{20}H_{24}O_4$) C, H.

2-Hydroxy-6a-methyl-5,6,6a,7,8,10,10a,10b,11,12-decahydro-4b*H*-7,8-diaza-pentaleno[2,1-*a*]phenanthren-9-one (9). To a suspension of **32** (0.657 g, 2 mmol) in toluene (10 mL) was added hydrazine monohydrate (0.11 mL, 2.2 mmol), and the mixture was heated at 150 °C in a sealed tube for 4 h. During this time the starting material dissolved then another white precipitate formed. The reaction was then cooled to room temperature and acidified with glacial AcOH, and H_2O was added to ensure that all product precipitated. The white powder was collected by filtration and washed with H_2O, EtOH (minimum), diethyl ether, and hexane. LCMS showed two compounds with masses corresponding to the desired product and the hydrazone derivative of E1. This mixture was suspended in EtOH, and aq. NaOH was added slowly until the solid dissolved. The solution was then acidified to pH 3 with glacial AcOH, and a small amount of H_2O was added until a fine white powder precipitated (0.112 g, 18%): mp > 275 °C (dec); IR (KBr) 3164 (OH), 2910 (NH), 1599 (C═O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (s, 3H, H-18), 1.30−1.70 (m, 4H), 1.80−1.87 (m, 1H), 1.93−2.11 (m, 3H), 2.17−2.40 (m, 3H), 2.71−2.78 (m, 2H), 6.43 (d, J = 2.2 Hz, 1H), 6.50 (dd, J = 8.1, 2.6 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 9.01 (s, 1H), 10.3 (bs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.2 (CH_3), 23.3 (CH_2), 25.9 (CH_2), 27.0-(CH_2), 29.0(CH_2), 33.7(CH_2), 37.2(CH), 41.1(C), 43.9(CH), 61.0-(CH), 102.4(C), 112.8(CH), 115.0(CH), 125.9(CH), 130.4(C), 137.1(C), 155.0(C), 158.7(C), 170.8(C); LCMS (APCI+) m/z 311.29 (M + H)$^+$; HRMS (FAB+) m/z calcd. for $C_{19}H_{23}N_2O_2$ (M + H)$^+$ 311.1759, found 311.1761.

16β-Cyano-estradiol (13). To a stirred solution of 16-cyano-estrone **10** (150 mg, 0.51 mmol) in a mixture of THF/EtOH (3:2, 5 mL) at room temperature was added portionwise $NaBH_4$ (50 mg, 1.32 mmol). The resulting pale yellow solution was stirred for 30 min before the solvent was removed under reduced pressure and

H_2O (20 mL) was added. The organics were extracted with EtOAc (20 mL + 10 mL), and the combined organic layers washed with H_2O (20 mL) then brine (20 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to give a light yellow crude product (159 mg). This was recrystallized from isopropyl alcohol/H_2O to give white crystals (16 mg) and a further crop of the product (40 mg) was obtained from the residue of the mother liquor upon recrystallization from EtOH (overall yield 37%): mp 252−254 °C; IR (KBr) 3430−3235 (OH), 2925−2850 (aliph CH), 2255 (CN), 1610−1500 (arom C═C) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.79 (s, 3H, H-18), 1.10−2.27 (m, 11H), 2.66−2.76 (m, 2H, H-6), ∼3.33 (m, ∼1H, H-16, under solvent peaks), 3.68 (dd, J = 9.4, 4.7 Hz, 1H, H-17), 5.50 (d, J = 4.7 Hz, 1H, C-17-OH), 6.43 (d, J = 2.5 Hz, 1H, H-4), 6.50 (dd, J = 8.5, 2.5 Hz, 1H, H-2), 7.03 (d, J = 8.5 Hz, 1H, H-1) and 9.01 (s, exchanged with D_2O, 1H, C-3-OH); LRMS (FAB+) m/z 297.1 [100, (M^+)]; HRMS (FAB+) m/z calcd. for $C_{19}H_{23}NO_2$ (M^+) 297.1729, found 297.1724.

3-*O*-Benzyl-16β-hydroxymethyl-estradiol (33). To a cold (0 °C), stirred solution of **31** (5 g, 12 mmol) in THF/MeOH (90:10) was added $NaBH_4$ (452 mg, 12 mmol), and the reaction mixture was stirred for 18 h at 0−20 °C, acidified with 2 N HCl, and extracted with ether (2×). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The solid was purified by column chromatography using $CHCl_3$/EtOAc (90:10) as eluent to separate **36** (2.50 g, 57%) and the title compound **33**, 0.63 g, 13%: ^1H NMR (270 MHz, DMSO-d_6) δ 0.68 (s, 3H, H-18), 0.95−1.45 (m, 4H), 1.70−1.90 (m, 4H), 2.00−2.35 (m, 4H), 2.74−2.76 (m, 2H, H-6), 3.29 (m, 1H), 3.67 (m, 2H), 4.13 (dd, J = 5.9, 4.4 Hz, 1H, H-17), 4.60 (d, J = 4.4 Hz, 1H, OH), 5.03 (s, 2H, CH_2Ph), 6.69 (d, J = 2.7 Hz, 1H, H-4), 6.74 (dd, J = 8.4, 2.7 Hz, 1H, H-2), 7.14 (d, J = 8.6 Hz, 1H, H-1), 7.25−7.46 (m, 5H, Ph); HRMS (FAB+) m/z calcd. for $C_{26}H_{32}0_3$ (M^+) 392.2351, found 392.2342.

16β-Hydroxymethyl-estradiol (14). Prepared by the same method as **32**. Yield = 78%, white solid: mp > 275 °C (dec); ^1H NMR (400 MHz, DMSO-d_6) δ 0.72 (s, 3H, H-18), 1.02−1.13 (m, 2H), 1.19−1.42 (m, 4H), 1.78−1.89 (m, 3H), 2.10 (td, J = 10.0, 3.3 Hz, 1H), 2.23−2.27 (m, 2H), 2.70−2.77 (m, 2H, H-6), 3.30−3.36 (m, 1H), 3.65−3.72 (m, 2H), 4.18 (dd, J = 6.2, 4.8 Hz, 1H, H-17), 4.64 (d, J = 4.8 Hz, 1H, OH), 6.45 (d, J = 2.6 Hz, 1H, H-4), 6.52 (dd, J = 8.4, 2.6 Hz, 1H, H-2), 7.06 (d, J = 8.4 Hz, 1H, H-1), 9.02 (s, 1H, phenol); LRMS (FAB+) m/z 302.1 [100, (M^+)]; HPLC > 99% (t_R = 1.74, 100% MeOH); Anal. ($C_{19}H_{26}O_3$) C, H.

3-*O*-Acetyl-16-methylene-estrone (34). To a stirred solution of **22** (1.00 g, 3.21 mmol) in anhydrous isoamyl alcohol (8 mL) were added paraformaldehyde (480 mg, 16.0 mmol for one unit) and dimethylamine hydrochloride (1.6 g, 19.6 mmol). The resulting mixture was heated to reflux for 24 h. The resulting light yellow solution was poured into H_2O (30 mL) and acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 50 mL), and the combined organic layers washed with saturated aqueous $NaHCO_3$ (20 mL), H_2O (20 mL), then brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo to give a light yellow oil. The isoamyl alcohol was distilled off and the oily residue crystallized overnight. Purification by flash chromatography using $CHCl_3$/EtOAc (95:5) as eluent gave the title compound as white solid (527 mg, 51%). For analysis some crystals were obtained by adding hexane to the oily crude residue and allowing to stand for 1 h: ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (s, 3H, H-18), 1.41−2.72 (m, 11H), 2.29 (s, 3H, OAc), 2.90−2.95 (m, 2H, H-6), 5.41−5.43 (m, 1H, H-1′trans), 6.09−6.12 (m, 1H, H-1′cis), 6.81 (d, J = 2.5 Hz, 1H, H-4), 6.86 (dd, J = 8.3, 2.5 Hz, 1H, H-2), 7.29 (d, J = 8.3 Hz, 1H, H-1); LRMS (FAB+) m/z 325.2 [100, (M + H)$^+$]; HRMS (FAB+) m/z calcd. for $C_{21}H_{25}O_3$ (M + H)$^+$ 325.1804, found 325.1822.

16β-Ethoxymethyl-estrone (15). To a stirred solution of 3-*O*-acetyl-16-methylene-estrone **34** (200 mg, 0.62 mmol) in EtOH (20 mL) at 0 °C was added dropwise aq. KOH (41 mg, 0.74 mmol in 2 mL). The resulting light yellow solution was stirred for 30 min at 0 °C. The solvent was then removed under reduced pressure, and H_2O was added (40 mL), followed by a few drops of 5 M HCl. The resulting white precipitate was collected by filtration and dried under high vacuum. This was purified by flash chromatography with $CHCl_3$/EtOAc (95:5) as eluent to give the title compound as pale yellow powder (26 mg, 13%). This was recrystallized from EtOH/H_2O to give pale yellow crystals (13 mg, 6%): mp 208−210 °C; TLC ($CHCl_3$/EtOAc, 8:2) R_f 0.40 cf. R_f 0.86; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, s, H-18), 1.16 (t, J = 7.0 Hz, 3H, H-3′), 1.36−2.42 (m, 12H), 2.81−2.85 (m, 2H, H-6), 3.42−3.50 (m, 2H, H-2′), 3.59 (m, 2H, H-1′), 4.66 (s, 1H, OH), 6.58 (d, J = 2.8 Hz, 1H, H-4), 6.64 (dd, J = 8.1, 2.8 Hz, 1H, H-2) and 7.15 (1H, d, J = 8.1 Hz, H-1); LRMS (FAB+) m/z 329.1 [100, (M + H)$^+$]; HRMS (FAB+) m/z calcd. for $C_{21}H_{29}O_3$ (M + H)$^+$ 329.2117, found 329.2124. Anal. ($C_{21}H_{28}O_3$) C, H.

16-(Pyridin-3-yl)methylene-estrone (16a). To a stirred suspension of E1 (1.35 g, 5.0 mmol) and pyridine-3-carbaldehyde (595 mg, 5.0 mmol) in EtOH (40 mL) at room temperature was added NaOH (1.0 g, 25 mmol). The resulting dark orange solution was stirred at room temperature for 4 h before glacial AcOH (ca. 10 mL) was added with stirring. The color changed to light yellow, and a light yellow solid precipitated. The solid was collected by filtration and washed with H_2O (50 mL), EtOH (20 mL), diethyl ether (50 mL), and hexane (50 mL) and dried under high vacuum to give the title compound as yellow powder (1.63 g, 90%): mp > 280 °C (dec); ^1H NMR (400 MHz, DMSO-d_6) δ 0.90 (s, 3H, H-18), 1.26−1.66 (m, 5H), 1.82−1.90 (m, 1H), 1.95−2.02 (m, 1H), 2.14−2.22 (m, 1H), 2.30−2.38 (m, 1H), 2.57−2.66 (m, 1H), 2.70−2.81 (m, 2H), 2.83−2.92 (m, 1H), 6.45 (d, J = 2.3 Hz, 1H, H-4), 6.51 (dd, J = 8.2, 2.3 Hz, 1H, H-2), 7.04 (d, J = 8.2 Hz, 1H, H-1), 7.34 (s, 1H), 7.47 (dd, J = 8.2, 5.1 Hz, 1H), 8.06 (d, J = 8.2 Hz, 1H), 8.56 (dd, J = 4.7, 1.2 Hz, 1H), 8.82 (d, J = 1.6 Hz, 1H), 9.03 (s, 1H, −OH); LRMS (FAB+) m/z 360.2 [100, (M + H)$^+$]; LRMS (FAB-) m/z 276.1 [100], 358.2 [90, (M − H)$^-$]; HRMS (FAB+) m/z calcd. for $C_{24}H_{26}NO_2$ (M + H)$^+$ 360.1963, found 360.1964.

16-Isobutylidene-estrone (16e). A solution of E1 (420 mg, 1.55 mmol) in dry THF (5 mL) was added dropwise to a stirred solution of LDA (2.47 mL of a 1.8 M solution in heptane/THF/ethyl benzene, 4.44 mmol) in dry THF (2 mL) at −78 °C, under an atmosphere of N_2. After stirring for 2 h at −78 °C, isobutyraldehyde (185 μL, 2.03 mmol, freshly distilled from Na_2SO_4) was added. The resulting mixture was allowed to warm to room temperature with stirring overnight. The solvent was then removed under reduced pressure and H_2O (50 mL) added. The organics were extracted with EtOAc (50 mL + 20 mL), and the combined organic layers washed with H_2O (20 mL) then brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo to give white foam. This was purified by flash chromatography using $CHCl_3$/EtOAc (9:1) as eluent to give the title compound as white foam (401 mg, 79%). This was recrystallized from EtOAc/hexane to give white crystals (325 mg, 64%): mp 188−190 °C; TLC (chloroform/EtOAc, 8:2) R_f 0.65 cf. R_f 0.74 (E1); IR (KBr) 3370 (OH), 2930−2890 (aliph CH), 1710 (C═O), 1645−1445 (arom C═C and exocyclic C═C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, 3H, H-18), 1.06 (d, J = 6.6 Hz, 3H, H-3′), 1.04 (d, J = 6.6 Hz, 3H, H-4′), 1.40−2.70 (m, 12H), 2.84−2.90 (m, 2H, H-6), 4.67 (s, exchanged with D_2O, 1H, OH), 6.46 (dd, J = 9.8, 1.9 Hz, 1H, H-1′), 6.59 (d, J = 2.7 Hz, 1H, H-4), 6.64 (dd, J = 8.3, 2.7 Hz, 1H, H-2), 7.16 (d, J = 8.3 Hz, 1H, H-1); ^{13}C NMR (100 MHz, $CDCl_3$) 14.99 (C-18), 22.34 (2×CH_3), 26.42 (CH_2), 26.44 (CH_2), 27.18 (CH_2), 29.69 (CH), 29.94 (CH_2), 31.98 (CH_2), 38.32 (CH), 44.38 (CH), 48.34 (CH), 48.79 (C-13), 113.12 (CH), 115.54 (CH), 126.66 (CH), 132.20 (C), 134.73 (C), 138.12 (C), 144.25 (CH, C-1′), 153.78 (C-3), 210.04 (C═O); LRMS (FAB+) m/z 325.1 [100, (M + H)$^+$]; HRMS (FAB+) m/z calcd. for $C_{22}H_{29}O_2$ (M + H)$^+$ 325.2167, found 325.2166. Anal. ($C_{22}H_{28}O_2$) C, H.

16-(Pyridin-3-yl)methylene-estradiol (17a). To a solution of 16-(pyridin-3-yl)methylene-estrone **16a** (360 mg, 1.0 mmol) in EtOH (20 mL) and THF (20 mL), cooled to 0 °C (ice bath), was added $NaBH_4$ (0.100 g, 2.6 mmol). The reaction mixture was allowed to warm to room temperature with stirring overnight. The clear and colorless solution was concentrated in vacuo to ca. 20 mL volume, and H_2O (50 mL) was added to precipitate the product. This was collected by filtration and washed with H_2O (50 mL),

MeOH (20 mL), and diethyl ether (50 mL) and dried under high vacuum to give the title compound as white powder (333 mg, 92%): mp > 150 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.58 (s, 3H, H-18), 1.15−1.43 (m, 5H), 1.83−1.96 (m, 2H), 2.12−2.31 (m, 3H), 2.62−2.80 (m, 3H), 3.99 (bs, 1H, H-17), 5.25 (bs, 1H, −OH), 6.41 (d, J = 2.0 Hz, 1H), 6.43 (d, J = 2.3 Hz, 1H, H-4), 6.49 (dd, J = 8.6, 2.3 Hz, 1H, H-2), 7.04 (d, J = 8.6 Hz, 1H, H-1), 7.34 (dd, J = 8.2, 5.1 Hz, 1H), 7.80 (d, J = 8.2 Hz, 1H), 8.36 (dd, J = 4.7, 1.2 Hz, 1H), 8.56 (d, J = 2.0 Hz, 1H), 9.00 (bs, 1H, −OH); LRMS (FAB+) *m/z* 362.2 [100, (M + H)$^+$]; LRMS (FAB-) *m/z* 360.2 [100, (M − H)$^-$]; HRMS (FAB+) *m/z* calcd. for $C_{24}H_{28}NO_2$ (M + H)$^+$ 362.2120, found 362.2117.

16-Isobutylidene-estradiol (17e). To a stirred solution of 16-isobutylidene-estrone **16e** (100 mg, 0.31 mmol) in a mixture of MeOH/THF (3:1, 9 mL) at 0 °C was added dropwise a solution of $NaBH_4$ (57 mg, 1.51 mmol) in H_2O (3 mL). The resulting solution was stirred at 0 °C for 20 min before glacial AcOH (5 drops) was added, followed by aq. NaCl (10% solution, 20 mL), and the white precipitate that formed was collected by filtration and dried under high vacuum (102 mg). This was recrystallized from acetone/hexane to give the title compounds as white crystals (60 mg, 60%): TLC ($CHCl_3$/EtOAc, 8:2) R_f 0.72 cf. R_f 0.82; mp 143−145 °C; IR (KBr) 3435−3325 (OH), 2955−2865 (aliph CH), 1695−1500 (arom C═C and exocyclic C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.67 (s, 3H, H-18), 0.96 (d, J = 6.6 Hz, 3H, H-3′), 0.99 (d, J = 6.6 Hz, 3H, H-4′), 1.22−2.02 (m,13H), 2.81−2.87 (m, 2H, H-6), 3.91−3.98 (m, 1H, H-17), 4.66 (s, exchanged with D_2O, 1H, OH), 5.32 (dd, J = 9.4, 2.3 Hz, 1H, H-1′), 6.56 (d, J = 2.7 Hz, 1H, H-4), 6.63 (dd, J = 8.6, 2.7 Hz, 1H, H-2), 7.16 (d, J = 8.6 Hz, 1H, H-1); LRMS (FAB+) *m/z* 326.2 [95, (M$^+$)], 309.2 [100, (M − OH)$^+$]; HRMS (FAB+) *m/z* calcd. for $C_{22}H_{30}O_2$ (M$^+$) 326.2246, found 326.2252.

16-(2′,2′-Dimethyl)-propylidene-estradiol (17f). To a stirred solution of 16-(2′,2′-dimethylpropylidene)-estrone **16f** (100 mg, 0.31 mmol) in a mixture of MeOH/THF (2:1, 15 mL) at 0 °C was added dropwise a solution of $NaBH_4$ (44 mg, 1.16 mmol) in H_2O (2.5 mL). The resulting solution was stirred at 0 °C for 1 h before glacial AcOH (4 drops) was added, followed by aq. NaCl (10% solution, 15 mL), and the white precipitate that formed was collected by filtration and dried (79 mg). This was recrystallized from EtOAc/hexane (1:8) to give the title compound as white crystals (63 mg, 79%): mp 219−222 °C; IR (KBr) 3550, 3140 (OH), 2960−28765 (aliph CH), 1610, 1505 (aliph C═C and arom C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.65 (s, 3H, H-18), 1.10 (s, 9H, $C(CH_3)_3$), 1.18−2.59 (m, 12H), 2.82−2.88 (m, 2H, H-6), 3.85−3.90 (m, 1H, H-17), 4.60 (s, exchanged with D_2O, 1H, OH), 5.46 (dd, J = 4.9, 2.5 Hz, 1H, H-1′), 6.55 (d, J = 2.8 Hz, 1H, H-4), 6.61 (dd, J = 8.5, 2.8 Hz, 1H, H-2), 7.14 (d, J = 8.5 Hz, 1H, H-1); LRMS (FAB+) *m/z* 340.2 [49, (M$^+$)], 323.2 [100, (M − OH)$^+$], 283.2 [92, (M − $C(CH_3)_3$)$^+$]; HRMS (FAB+) *m/z* calcd. for $C_{23}H_{32}O_2$ (M$^+$) 340.2402, found 340.2390.

16-Benzylidene-estradiol (17g). To a solution of 16-benzylidene-estrone **16g** (179 mg, 0.50 mmol) in EtOH (10 mL) and THF (10 mL), cooled to 0 °C (ice bath), was added $NaBH_4$ (100 mg, 2.64 mmol). The reaction was stirred overnight at room temperature before being concentrated in vacuo and the residue dissolved in EtOAc (40 mL). The solution was washed with H_2O (2 × 25 mL) and brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was dissolved in a small amount of EtOAc and precipitated by addition of hexane. The white solid was collected by filtration and dried under high vacuum to give the title compound (175 mg, 97%): mp 213−216 °C; ^{1}H NMR (270 MHz, DMSO-d_6) δ 0.61 (s, 3H, H-18), 1.20−1.50 (m, 5H), 1.82−1.98 (m, 2H), 2.20−2.35 (m, 3H), 2.58−2.82 (m, 3H), 3.95−4.05 (m, 1H, H-17), 5.15 (d, J = 6.2 Hz, 1H), 6.43 (s, 1H), 6.46 (d, J = 2.3 Hz, 1H, H-4), 6.52 (dd, J = 8.2, 2.3 Hz, 1H, H-2), 7.06 (d, J = 8.2 Hz, 1H, H-1), 7.15−7.20 (m, 1H), 7.31−7.41 (m, 4H); LRMS (FAB+) *m/z* 360.2 [100, (M$^+$)]; LRMS (FAB-) *m/z* 359.3 [100, (M − H)$^-$]; HRMS (FAB+) *m/z* calcd. for $C_{25}H_{28}O_2$ (M$^+$) 360.2089, found 360.2097.

16-(3,4,5-Trimethoxy-benzylidene)-estradiol (17j). To a solution of 16-(3,4,5-trimethoxy-benzylidene)-estrone **16j** (448 mg, 1.0 mmol) in THF/MeOH (1/1, 40 mL) cooled to 0 °C (ice bath) was added $NaBH_4$ (100 mg, 2.64 mmol). The reaction was stirred for 2 h at this temperature, while the color changed from slightly yellow to colorless. The solution was diluted with EtOAc (100 mL) and washed with water (100 mL + 50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo to give the title compound as white solid (445 mg, 99%): mp 135−140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 3H, H-18), 1.20−1.60 (m, 6H), 1.90−1.98 (m, 1H), 2.01−2.08 (m, 1H), 2.21−2.41 (m, 3H), 2.74−2.90 (m, 3H), 3.86 (s, 3H), 3.89 (s, 6H), 4.15 (d, J = 9.4 Hz, 1H, H-17), 4.62 (s, 1H, −OH), 6.49−6.51 (m, 1H), 6.59 (d, J = 2.3 Hz, 1H, H-4), 6.63−6.68 (m, 3H), 7.18 (d, J = 8.2 Hz, 1H); LRMS (FAB+) *m/z* 451.3 [100, (M + H)$^+$].

16-(Thiophen-2-yl)methylene-estradiol (17k). To a stirred solution of 16-(thiophenen-2-yl)methylene-estrone **16k** (365 mg, 1.0 mmol) in THF (20 mL) and EtOH (40 mL) at 0 °C was added $NaBH_4$ (100 mg, 2.64 mmol), and the reaction was allowed to warm to room temperature overnight before being concentrated in vacuo. The residue was dissolved in EtOAc (40 mL) and the solution washed with water (40 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The white crude product was crystallized from EtOAc/hexane to give the title compound as colorless crystals (208 mg, 57%): mp > 180 °C (dec); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 (s, 3H, H-18), 1.38−1.58 (m, 6H), 1.98−2.06 (m, 2H), 2.12−2.22 (m, 1H), 2.26−2.40 (m, 2H), 2.68 (dd, J = 16.9, 6.6 Hz, 1H), 2.82−2.92 (m, 2H), 4.17 (d, J = 9.4 Hz, 1H, H-17), 4.56 (s, 1H, −OH), 6.59 (d, J = 2.3 Hz, 1H, H-4), 6.64 (dd, J = 8.6, 2.3 Hz, 1H, H-2), 6.81 (d, J = 2.3 Hz, 1H), 7.00−7.06 (m, 2H), 7.18 (d, J = 8.6 Hz, 1H, H-1), 7.27 (d, J = 4.7 Hz, 1H); LRMS (FAB+) *m/z* 366.2 [100, (M$^+$)].

General Procedure for Hydrogenation: 18a−d. The starting 16-methylene estradiol was dissolved in a volume of THF and the same volume of EtOH added to the solution. The solution was then degassed by bubbling nitrogen through for 40 min before Pd/C (5wt %, catalytic) was added and hydrogen gas (balloon) was passed over the reaction. The reaction was stirred under hydrogen at room-temperature overnight before being filtered through Celite and purified by flash chromatography.

Representative example:

16-(Pyridin-3-yl)methyl-estradiol (18a). Purification by column chromatography using an elution gradient of 100% hexane to 100% EtOAc gave the title compound in 88% yield: mp 224−227 °C; TLC (EtOAc/hexane, 1:3) R_f 0.1; ^{1}H NMR (270 MHz, DMSO-d_6) δ 0.76 (s, 3H, H-18), 1.00−1.04 (m, 2H), 1.20−1.35 (m, 5H), 1.54−1.56 (m, 1H), 1.73−1.90 (m, 2H), 2.13−2.32 (m, 3H), 2.64 (m, 2H), 3.01 (d, J = 9.4 Hz, 1H), 4.77 (d, J = 4.5 Hz, 1H), 6.41 (d, J = 2.5 Hz, 1H, H-4), 6.49 (dd, J = 8.4, 2.5 Hz, 1H, H-2), 7.03 (d, J = 8.4 Hz, 1H, H-1), 7.25−7.31 (m, 1H), 7.60−7.64 (m, 1H), 8.34−8.39 (m, 1H), 8.41 (d, J = 1.7 Hz, 1H), 9.02 (s, 1H); LRMS (FAB+) *m/z* 364.1 [100, (M + H)$^+$]; HRMS (FAB+) *m/z* calcd. for $C_{24}H_{30}NO_2$ (M + H)$^+$ 364.2276, found 364.2287.

3-*O*-Benzyl-estradiol-16-carboxylic Acid Methyl Ester (36). To a cooled (0 °C), stirred solution of **31** (5 g, 12 mmol) in THF/MeOH (90:10) was added $NaBH_4$ (456 mg, 12 mmol), the reaction mixture was stirred for 4 h at 0 °C, acidified with 2 N HCl, and the products were extracted with ether (2×). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The solid was purified by column chromatography using $CHCl_3$/EtOAc (90:10) as eluent to give 2.48 g of the 16β-carboxyl diastereomer **36a** (48%) and 0.91 g of the 16α **36b** (18%) as white solids.

36a: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 3H, H-18), 1.19 (ddd, J = 12.9, 10.9, 6.2 Hz, 1H), 1.29−1.43 (m, 2H), 1.46−1.56 (m, 2H), 1.76 (dt, J = 12.9, 8.6 Hz, 1H), 1.87−1.93 (m, 1H), 2.00−2.13 (m, 2H), 2.20−2.27 (m, 1H), 2.30−2.35 (m, 1H), 2.83−2.89 (m, 2H, H-6), 3.15 (q, J = 9.8 Hz, 1H, H-16α), 3.34 (d, J = 8.2 Hz, 1H, OH), 3.72 (s, 3H, OCH_3), 3.89 (dd, J = 9.8, 8.2 Hz, 1H, H-17α), 5.04 (s, 2H, CH_2Ph), 6.72 (d, J = 2.7 Hz, 1H, H-4), 6.79 (dd, J = 8.4, 2.7 Hz, 1H, H-2), 7.21 (d, J = 8.6 Hz, 1H, H-1),

7.30−7.46 (m, 5H, Ph); LRMS (FAB+) *m/z* 91.0 [100], 420.1 [42, (M^+)]; HRMS (FAB+) *m/z* calcd. for $C_{27}H_{32}O_4$ (M^+) 420.2301, found 420.2298. Anal. ($C_{27}H_{32}O_4$) C, H.

36b: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H, H-18), 1.20−1.60 (m, 5H), 1.72 (app. q, J = 12.4 Hz, 1H), 1.85−1.88 (m, 1H), 1.97 (dt, J = 12.1, 3.3 Hz, 1H), 2.02−2.10 (m, 2H), 2.21−2.26 (m, 1H), 2.31−2.35 (m, 1H), 2.77 (ddd, J = 12.1, 8.4, 4.0 Hz, 1H, H-16β), 2.86 (m, 2H, H-6), 3.75 (s, 3H, OCH_3), 3.89 (dd, J = 8.1, 4.4 Hz, 1H, H-17α), 5.04 (s, 2H, CH_2Ph), 6.73 (d, J = 2.9 Hz, 1H, H-4), 6.79 (dd, J = 8.4, 2.6 Hz, 1H, H-2), 7.21 (d, J = 8.1 Hz, 1H, H-1), 7.30−7.46 (m, 5H, Ph); ^{1}H NMR (400 MHz, $CDCl_3$, D_2O) δ 3.80 (d, J = 7.8 Hz, H-17α); LRMS (FAB+) *m/z* 91.0 [100], 420.1 [37, (M^+)]; HRMS (FAB+) *m/z* calcd. for $C_{27}H_{32}O_4$ (M^+) 420.2301, found 420.2301; HPLC > 99% (t_R = 3.03, 4:96 H_2O:MeOH). Anal. ($C_{27}H_{32}O_4$) C, H.

Methyl Estradiol-16β-carboxylate (37). Synthesized from **36a**, procedure as for **32**. Recrystallization of the solid residue from MeOH/H_2O gave the title compound as white solid (92%): mp 222−224 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75 (s, 3H, H-18), 1.04−1.46 (m, 5H), 1.64−1.86 (m, 4H), 2.08 (td, J = 10.5, 3.3 Hz, 1H), 2.21−2.25 (m, 1H), 2.66−2.79 (m, 2H, H-6), 3.05 (q, J = 9.0 Hz, 1H), 3.56 (s, 3H, OCH_3), 3.83 (dd, J = 10.5, 5.5 Hz, 1H, H-17α), 5.02 (d, J = 5.0 Hz, 1H, OH), 6.42 (d, J = 2.7 Hz, 1H, H-4), 6.48 (dd, J = 8.2, 2.7 Hz, 1H, H-2), 7.03 (d, J = 8.6 Hz, 1H, H-1), 8.99 (s, 1H, OH); LRMS (FAB+) *m/z* 331.1 [100, (M + H)$^+$]; HRMS (FAB+) calcd. for $C_{20}H_{26}O_4$ (M^+) 330.1831, found 330.1831; HPLC > 95% (t_R = 2.35, 8:92 H_2O:MeOH). Anal. ($C_{20}H_{26}O_4$) C, H.

Estradiol-16-carboxylic Acid (19). To a suspension of **37** (1.1 g, 3.20 mmol) in MeOH (10 mL) was added aq. NaOH (0.58 g, 14 mmol in 3 mL). The solution was stirred at room temperature for 5 h before solvents were removed in vacuo and 6 N HCl was added to the residue. The products were extracted with EtOAc (5×), and the combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Recrystallization of the solid residue from MeCN gave the title compound as white solid (470 mg, 46%): mp > 235 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.69 (s, 1.86H, H-18), 0.73 (s, 1.14H, H-18), 1.20−1.40 (m, 5H), 1.45−1.85 (m, 4H), 2.08−2.12 (m, 1H), 2.22−2.33 (m, 1H), 2.45 (m, 0.62H, H-16), 2.70−2.75 (m, 2H, H-6), 2.95 (q, J = 9.0 Hz, 0.38H, H-16), 3.69 (d, J = 8.0 Hz, 0.62H, H-17), 3.60 (d, J = 10.8 Hz, 0.38H, H-17), 4.92 (bs, 1H, OH), 6.42 (d, J = 2.7 Hz, 1H, H-4), 6.48 (dd, J = 8.2, 2.7 Hz, 1H, H-2), 7.03 (d, J = 8.2 Hz, 1H, H-1), 8.99 (s, 1H); LRMS (FAB+) *m/z* 316.1 [100, (M^+)]; HRMS (FAB+) calcd. for $C_{19}H_{24}O_4$ (M^+) 316.1675, found 316.1677.

Loading of 19 onto Oxime Resin (38). Oxime resin (0.500 g, loading of 1.06 mmol/g resin) was swollen in dry DMF (7 mL) for 15 min after which **19** (0.251 g, 0.79 mmol) was added, followed by DIC (41 μL) and HOBt (0.358 g, 2.61 mmol). The mixture was shaken for 48 h at room temperature under inert atmosphere, and the resin was filtered, washed with DCM, DMF, MeOH (three cycles), and MeOH (5 times) and dried in vacuo. IR (KBr) 1750 (C═O, ester), 1600 (C═N, oxime) cm^{-1}.

***N*-(Furan-2-ylmethyl)estradiol-16-carboxamide (19d).** To a suspension of loaded resin **38** (200 mg, maximum loading of 1.06 mmol/g resin) swollen in dry DCM (4 mL) was added furfurylamine (47 μL, 0.53 mmol). The mixture was shaken at 40 °C under nitrogen atmosphere for 24 h. After filtration, the resin was washed with DCM (five times), and the filtrated was evaporated in vacuo. The resulting brown oil was purified by column chromatography using EtOAc/hexane (50:50) as eluent to give the title compound as a yellow solid (3 mg, 3.6% over two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 2.49H, H-18), 0.84 (s, 0.51H, H-18), 1.08−1.60 (m, 6H), 1.84−1.89 (m, 2H), 2.16−2.24 (m, 2H), 2.28−2.36 (m, 1H), 2.56−2.62 (m, 1H, H-16), 2.80−2.83 (m, 2H, H-6), 3.74 (d, J = 8.4 Hz, 0.83H, H-17), 3.80 (d, J = 8.9 Hz, 0.17H, H-17), 4.41 (d, J = 5.7 Hz, 2H, CH_2N), 6.15 (dd, J = 3.2, 1.7 Hz, 1H, furan), 6.25 (m, 1H, furan), 6.50 (d, J = 2.7 Hz, 1H, H-4), 6.55 (dd, J = 8.4, 2.7 Hz, 1H, H-2), 7.04 (d, J = 8.1 Hz, 1H, H-1), 7.28 (dd, J = 2.0; 1.0 Hz, 1H, furan); LCMS (APCI+) *m/z* 396.18 (M + H)$^+$.

3-*O*-Benzyl-estradiol-16-carboxylic Acid (39). To a solution of **36** (2.00 g, 4.75 mmol) in THF (20 mL) was added aq. NaOH (285 mg, 7.13 mmol in 5 mL), and the reaction was stirred at room temperature for 24 h. The solvents were removed in vacuo, and 6N HCl was added to the residue. The organics were extracted with diethyl ether (5×), and the product was extracted into aq. K_2CO_3 (10%, 5×). This basic solution was acidified with 6 N HCl and the product extracted into EtOAc (5×). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The white solid was suspended in hexane and collected by filtration to give the title compound (720 mg, 37%): ^{1}H NMR (270 MHz, DMSO-d_6) δ 0.68 (s, 1.05H, H-18), 0.72 (s, 1.95H, H-18), 1.00−1.50 (m, 6H), 1.60−1.90 (m, 3H), 2.08−2.15 (m, 1H), 2.23−2.29 (m, 1H), 2.72−2.76 (m, 2H, H-6), 2.92 (q, J = 8.9 Hz, 0.65H, H-16), 3.68 (dd, J = 7.2, 5.3 Hz, 0.35H, H-16), 3.80 (d, J = 10.5 Hz, 0.65H, H-17), 4.91 (d, J = 5.3, 0.35H, H-17), 5.03 (s, 2H, CH_2Ph), 6.68 (s, 1H, H-4), 6.74 (dd, J = 8.6, 2.6 Hz, 1H, H-2), 7.14 (d, J = 8.6 Hz, 1H, H-1), 7.25−7.50 (m, 5H, Ph); LRMS (FAB+) *m/z* 91.0 [100], 406.1 [40, (M^+)].

(4′-Nitro-phenyl) 3-*O*-Benzyl-estradiol-16-carboxylate (40). To a stirred solution of **39** (0.400 g, 0.98 mmol) in dry DMF (4 mL) was added NEt_3 (0.14 mL, 0.98 mmol) followed by bis(4-nitrophenyl) carbonate (299 mg, 0.98 mmol). The solution was stirred at room temperature for 2 h under N_2 atmosphere before being cooled to 0 °C and acidified with 2 N HCl. The mixture was extracted with DCM (3×), and the combined organic layers were washed with sat. $NaHCO_3$ (5×) and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography using an elution gradient of hexane to EtOAc to give the title compound as yellow solid (270 mg, 52%). ^{1}H NMR (270 MHz $CDCl_3$,) δ 0.87 (s, 3H, H-18), 1.27−1.50 (m, 4H), 1.80−2.05 (m, 4H), 2.10−2.40 (m, 3H), 2.82−2.88 (m, 2H, H-6), 3.05 (ddd, J = 11.7, 7.7, 3.7 Hz, 1H, H-16), 4.06 (dd, J = 8.1, 5.1 Hz, 1H, H-17), 5.01 (s, 2H, CH_2Ph), 6.70 (d, J = 2.6 Hz, 1H, H-4), 6.76 (dd, J = 8.8, 2.6 Hz, 1H, H-2), 7.19 (d, J = 8.8 Hz, 1H, H-1), 7.25−7.45 (m, 7H), 8.27 (d, J = 12.1 Hz, 2H); LRMS (FAB+) *m/z* 527.4 [20, (M^+)]; 389.4 [6, (M − *p*NO_2PhO)$^+$]; 91.1 [100, ($PhCH_2^+$)].

General Procedure for Amide Coupling: 41a−c and 41e−j. To a solution of the active ester **40** (0.090 g, 0.17 mmol) in dry MeCN (2 mL) was added amine (2 equiv), and the solution was stirred overnight. The solvents were removed in vacuo, and the residue was semipurified by flash column chromatography using an elution gradient of hexane to EtOAc to give 3-*O*-benzyl protected E1-16-carboxyl amides with an average yield of 80%. These were then debenzylated without further purification.

General Procedure for Debenzylation: 19a−c and 19e−j. To a solution of 3-*O*-benzyl amide **41** in THF (3 mL) was added Pd/C (5wt %, catalytic), and the reaction mixture was stirred under H_2 (balloon) for 24 h. The Pd/C was removed by filtration and the filtrate was concentrated in vacuo. The final product was precipitated in a mixture of EtOAc/hexane. Compounds **19a−c and 19e−j** were obtained with an average yield of 36%.

Representative example:

***N*-(Tetrahydro-furan-2-ylmethyl)estradiol-16-carboxamide (19a).** Yield = 12%. ^{1}H NMR (270 MHz, MeOH-d_3) δ 0.81 (s, 3H, H-18), 1.26−1.45 (m, 6H),1.50−1.70 (m, 3H), 1.80−2.00 (m, 6H), 2.10−2.18 (m, 1H), 2.27−2.32 (m, 1H), 2.60 (ddd, J = 11.4, 8.1, 3.7 Hz, 1H, H-16), 2.74−2.79 (m, 2H, H-6), 3.70−3.95 (m, 3H), 3.93−4.00 (m, 1H), 6.45 (d, J = 2.6 Hz, 1H, H-4), 6.52 (dd, J = 8.4, 2.6 Hz, 1H, H-2), 7.06 (d, J = 8.4 Hz, 1H, H-1); LCMS (APCI+) *m/z* 401.34 (M + 2H)$^+$.

Ethyl Estrone-16-methylcarboxylate (44). Procedure as for **18a−d** using EtOH as solvent. The progress of the reaction was monitored by TLC (EtOAc/hexane, 3:7) and was found to be complete after 48 h. The crude product was purified by column chromatography (5−20% EtOAc in hexane) to obtain the debenzylated product **44** (0.828 g, 70%, mixture of two diastereomers 3:1) as a white solid: mp 140−143 °C; ^{1}H NMR (400 MHz, $CDCl_3$)

[chemical shifts of major to minor isomer ratio approximately 3:1 was determined using Me signals at 13-position], δ 0.916 and 0.988 (major isomer) [2 × s, 3H, H-18], 1.28 (t, J = 7.0 Hz, 3H, CH_3), 1.40−1.60 (m, 5H), 1.75−1.80 (m, 1H), 1.92−2.54 (m, 6H), 2.74−2.79 (dd, J = 3.9, 4.2 Hz, 1H), 2.85−2.88 (m, 2H), 2.99−3.02 (m, 1H), 4.15−4.17 (q, 2H, CH_2), 4.63 (1H, OH), 6.57−6.58 (appd, J = 2.7 Hz, 1H), 6.62−6.65 (dd, J = 8.5, 2.7 Hz, 1H), 7.13−7.16 (dd, J = 8.5, 3.9 Hz, 1H); LRMS (FAB+) m/z 357 [100, $(M + H)^+$]; HRMS (FAB+) m/z calcd. for $C_{22}H_{29}O_4$ $(M + H)^+$ 357.2065, found 357.2054.

Estrone-16-methylcarboxylic Acid (20). To a solution of E1-16-methyl carboxylic acid ethyl ester **44** (1.00 g, 2.8 mmol) in THF:MeOH:H_2O (1:1:0.5, 10 mL) was added NaOH (0.56 g, 1.4 mmol), and the mixture was heated to 80 °C in a sealed tube overnight. The reaction was monitored by TLC (EtOAc: hexane, 1:1). The crude mixture was acidified to pH = 3−4, and the organics were extracted with EtOAc and DCM. The combined organic layers were dried ($MgSO_4$) and concentrated to obtain white solid. Recrystallization from MeOH/hexane gave the title compound as a white solid (0.920 g, quantitative yield): mp > 250 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) [chemical shifts of major to minor isomer ratio approximately 3:1 was determined using Me signals at 13-position] δ 0.786 [major isomer] and 0.896 (2 × s, 3H, H-18), 1.31−1.52 (m, 6H), 1.74−1.77 (m, 1H), 1.88−1.91 (m, 1H), 2.16−2.33 (m, 4H), 2.40−2.45 (m, 1H), 2.55−2.62 (m, 1H), 2.72−2.74 (m, 2H), 6.43−6.44 (appd, J = 2.5 Hz, 1H), 6.48−6.51 (d, J = 8.5, 2.4 Hz, 1H, H-2), 7.02−7.04 (d, J = 8.5 Hz, 1H, H-1), 9.0 (bs, 1H); LRMS (FAB+) m/z 329 [100, $(M + H)^+$]; HRMS (FAB+) m/z calcd. for $C_{20}H_{26}O_4$ $(M + 2H)^+$ 330.1831, found 330.1751.

3-*O*-Benzyl-estrone-16-methylcarboxylic Acid (43). The reaction wascarried out using the procedure outlined for compound **20** with 3-*O*-benzyl-estrone-16-methylcarboxylic acid ethyl ester **42** (1.714 g, 3.8 mmol), NaOH (1.537 g, 38.4 mmol) and THF:MeOH:H_2O (1:1:0.5, 10 mL). The title compound (1.114 g, 90%) was isolated as white solid after recrystallization from EtOAc/hexane; ^{1}H NMR (400 MHz,DMSO-d_6) δ 0.81, 0.84 (2 × s, 3H, H-18), 1.36−2.64 (m, 14H), 2.81−2.83 (m, 2H), 5.06 (s, 2H, CH_2Ph), 6.73 (appd, J = 2.3 Hz, 1H), 6.77 (dd, J = 8.5 and 2.5 Hz, 1H), 7.18 (d, J = 8.5 Hz, 1H), 7.31−7.44 (5H); LRMS (FAB+) m/z 90.9 [100], 418.0 [21, (M^+)]; HRMS (FAB+) m/z calcd. for $C_{27}H_{30}O_4$ (M^+) 418.2099, found 418.2148; HPLC > 99% (t_R = 1.89, 100% MeOH).

General Method for Solution Phase Parallel Synthesis of E1-16-Methylcarboxyl-amides. The carboxylic acid **20** (0.050 g, 0.15 mmol) or **21** (0.050 g, 0.15 mmol) was suspended in DCM (1.0 mL) in a glass tube in a Radleys GreenHouse Parallel Synthesizer. To this was added a solution of EDC (0.043 g, 0.227 mmol), DMAP (catalytic), and NEt_3 (0.100 mL) in DCM (1.0 mL). The reaction mixture was stirred for 30 min before the corresponding amine (5 molar equivalents) in DCM (1.0 mL) was added and stirring was continued overnight. The completions of the reactions were monitored by TLC (EtOAc:hexane, 2:7). The crude mixtures were concentrated under reduced pressure and purified using flash chromatography with DCM:MeOH (gradient elution, starting with 2% MeOH in DCM). Average yield 39%.

Synthesis of Resin Bound Intermediate (46). To a suspension of Oxime resin (2.0 g, loading 1.06 mmol/g) swollen in DMF (7 mL) was added **43** (1.428 g, 3.18 mmol) followed by DIC (1.33 g, 10.6 mmol) and HOBt (1.431 g, 10.6 mmol). The reaction mixture was shaken using a flask shaker for approximately 72 h under N_2. The yellow reaction mixture obtained was filtered and the resin washed with DCM, DMF, MeOH (three cycles with each solvent), then MeOH (5×) and dried in vacuo to obtain the resin bound intermediate (2.391 g) as yellow beads: IR (KBr) 3502 (OH), 1739 (CO−O−N═), 1662 (C═O) cm^{-1}.

Parallel Synthesis of E1-Methylcarboxyl-amides Followed by Debenzylation. To a suspension of resin bound intermediate **46** (0.100 g, 0.106 mmol) in anhydrous DCM (3.0 mL) under N_2 in a glass tube in a Radleys GreenHouse Synthesizer was added the corresponding amine (5 molar equivalents), and the reactions were heated at 40 °C for 72 h. The reaction mixtures were filtered and the filtrates concentrated under reduced pressure to obtain pale yellow solids. The crude compounds were purified by flash chromatography using DCM/MeOH (gradient elution, starting with 2% MeOH in DCM) to obtain pure benzylated intermediates (average recovery 15 mg/100 mg of resin bound intermediate, analyzed by ^{1}H NMR). These intermediates were then debenzylated under H_2 in the GreenHouse Synthesizer to obtain final compounds in average 90% purity and with an average overall yield of 40%.

Synthesis of Resin Bound Intermediate (48). To a suspension of Oxime resin (0.500 g, loading 1.06 mmol/g) swollen in DMF (7 mL) was added **20** (0.521 g, 5.3 mmol) followed by DIC (0.667 g, 5.3 mmol) and HOBt (0.715 g, 5.3 mmol). The reaction mixture was shaken using a flask shaker for approximately 72 h under N_2. The yellow reaction mixture obtained was filtered and the resin washed with DCM, DMF, MeOH (three cycles with each solvent), then MeOH (5×) and dried under high vacuum to obtain the resin bound intermediate as yellow beads: IR (cm^{-1}) 1662 (C═O), 1739 (CO−O−N═), 3502 (OH).

Parallel Synthesis of E1-Methylcarboxyl-amides. To a suspension of resin bound **48** (0.100 g, 0.106 mmol) in anhydrous DCM (3.0 mL) under N_2 in a glass tube in Radleys GreenHouse Synthesizer was added the corresponding amine (5 molar equivalents), and the reaction was heated at 40 °C under N_2 for 72 h. The reaction mixtures were filtered, and the filtrates were evaporated using Genevac to obtain yellow solids. The crude compounds were purified by flash chromatography using DCM/MeOH (gradient elution, starting with 2% MeOH in DCM) to obtain compounds **20j**−**m** with an average recovery of 14 mg/100 mg of resin bound intermediate.

Representative example:

***N*-(Tetrahydrofuran-2-ylmethyl)estrone-16-methylcarboxamide (20a).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.69, 0.73, 0.83, 0.87 (4 × s, 3H, H-18), 1.2−3.1 (m, 21H), 3.50−3.95 (m, 4H), 6.0 (bs) and 6.15−6.17 (m, 1H), 6.50 (d, J = 2.3 Hz, 0.8H, H-4, major), 6.55−6.58 (m, 0.8H, H-2, major), 6.76−6.81 (m, H-4 and H-2 minor, 0.4H) 7.05 (d, J = 8.6 Hz, H-1 major + minor underneath, 1H); LCMS (ES-) m/z 410.2 $(M - H)^-$; HRMS (FAB+) m/z calcd. for $C_{25}H_{34}NO_4$ $(M + H)^+$ 412.2487, found 408.2491.

Representative example:

***N*-(Tetrahydro-furan-2-ylmethyl)estradiol-16-methylcarboxamide (21a).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78, 0.79, (2 × s, 3H, H-18), 0.86−0.88 (m, 1H), 1.19−1.55 (m, 16H), 1.67−1.97 (m, 2H), 2.12−2.30 (m, 3H), 2.74−2.85 (m, 3H), 3.73−3.77 (m, 1H), 4.48 (d, J = 9.8 Hz, 1H), 4.63 (bs, 1H), 6.57 (s, 1H, H-4), 6.64 (dd, J = 8.6, 2.8 Hz, 1H, H-2), 7.16 (d, J = 8.5 Hz, 1H, H-1); LCMS (ES-) m/z 311 $(M - C_5H_9NO)^-$.

X-ray Crystallography. Crystal Data for **15**, $C_{21}H_{28}O_3$, M = 328.43, λ = 0.71073 Å, orthorhombic, space group $P2_12_12_1$, a = 7.1090(1), b = 11.9830(2), c = 21.4370(4) Å, U = 1826.16(5) $Å^3$, Z = 4, D_c =1.195 mg/m^3, μ = 0.078 mm^{-1}, $F(000)$ = 712, crystal size 0.40 × 0.13 × 0.04 mm, unique reflections = 4176 [R(int) = 0.0742], observed $I > 2\sigma(I)$ = 3169, data/restraints/paramaters = 4176/0/221, $R1$ = 0.0412 w$R2$ = 0.0857 (obsd data), $R1$ = 0.0663 w$R2$ = 0.0952 (all data), max peak/hole 0.259 and −0.283 $eÅ^{-3}$, software used, SHELXS,[54] SHELXL,[55] and ORTEX.[40]

Crystal Data for **16c** (block crystals), $C_{24}H_{24}NO_2$, M = 358.44, λ = 0.71073 Å, tetragonal, space group $P4_3$, a = 6.8940(1), b = 6.8940(1), c = 38.7530(6)Å, U = 1841.82(5) $Å^3$, Z = 4, D_c = 1.293 mg/m^3, μ = 0.082 mm^{-1}, $F(000)$ = 764, crystal size 0.25 × 0.25 × 0.12 mm, unique reflections = 4064 [R(int) = 0.0383], observed $I > 2\sigma(I)$ = 3861, data/restraints/paramaters = 4064/1/247, $R1$ = 0.0338 w$R2$ = 0.0813 (obs. data), $R1$ = 0.0376 w$R2$ = 0.0842 (all data), max peak/hole 0.201 and −0.228 $eÅ^{-3}$, software used, SHELXS,[54] SHELXL,[55] and ORTEX.[40]

Crystal Data for **16c** (needle crystals), $C_{24}H_{25}NO_2$, M = 359.45, λ = 0.71073 Å, monoclinic, space group $C2$, a = 31.4610(8), b = 6.4880(2), c = 9.2690(3) Å, β = 90.923(1)° U = 1891.73(10) $Å^3$, Z = 4, D_c = 1.262 Mg/m^3, μ = 0.080 mm^{-1}, $F(000)$ = 768, crystal size 0.38 × 0.13 × 0.05 mm, unique reflections = 4183 [R(int) =

0.0627], observed $I > 2\sigma(I) = 3680$, data/restraints/paramaters = 4183/61/293, $R1 = 0.0602$ w$R2 = 0.1044$ (obsd data), $R1 = 0.0741$ w$R2 = 0.1083$ (all data), max peak/hole 0.193 and −0.272 eÅ^{-3}, software used, SHELXS,[54] SHELXL,[55] and ORTEX.[40]

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Supporting Information Available: Experimental details and data for compounds **23** and **1** and analytical and spectroscopic data for compounds **16b**−**d**, **16f**−**l**, **17b**−**d**, **17h**, **17i**, **18b**−**d**, **19b**, **19c**, **19e**−**j**, **20b**−**m**, and **21b**−**f**. Also available are microanalysis data on selected compounds, HPLC data for biologically tested compounds for which microanalysis data are not available, structures and IC_{50} data for compounds used to develop the QSAR, figure showing the crystal packing of polymorphs of **16c**, and crystallographic data for compounds **15** and the two polymorphs of **16c**. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as CCDC 278452, 278453, and 278454, respectively. Copies of these data can be obtained free of charge on application to CCDC, 12 Union Rd., Cambridge CB2 1EZ, UK [fax (+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk]. This material is available free of charge via the Internet at http://pubs.acs.org.

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