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Discovery of a Non-Estrogenic Irreversible Inhibitor of 17β-Hydroxysteroid Dehydrogenase Type 1 from 3-Substituted-16β-(*m*-carbamoylbenzyl)-estradiol Derivatives

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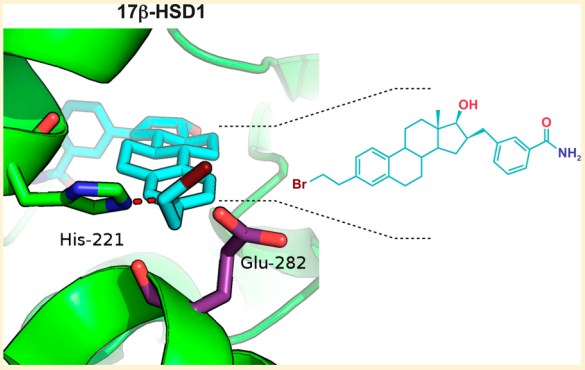
ABSTRACT: 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is thought to play a pivotal role in the progression of estrogen-sensitive breast cancer by transforming estrone (E1) into estradiol (E2). We designed three successive series of E2-derivatives at position C3 of the potent inhibitor 16β-(*m*-carbamoylbenzyl)-E2 to remove its unwanted estrogenic activity. We report the chemical synthesis and characterization of 20 new E2-derivatives, their evaluation as 17β-HSD1 inhibitors, and their proliferative (estrogenic) activity on estrogen-sensitive cells. The structure−activity relationship study provided a new potent and steroidal nonestrogenic inhibitor of 17β-HSD1 named 3-{[(16β,17β)-3-(2-bromoethyl)-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl}benzamide (**23b**). In fact, this compound inhibited the transformation of E1 into E2 by 17β-HSD1 in T-47D cells (IC_{50} = 83 nM), did not inhibit 17β-HSD2, 17β-HSD7, 17β-HSD12, and CYP3A4, and did not stimulate the proliferation of estrogen-sensitive MCF-7 cells. We also discussed the results of kinetic and molecular modeling (docking) experiments, suggesting that compound **23b** is a competitive and irreversible inhibitor of 17β-HSD1.



INTRODUCTION

Breast cancer is the most frequent cancer among American women, with an estimate of 288130 new cases diagnosed and 39520 related deaths in 2011.[1,2] This disease is more frequent in postmenopausal women, representing about three-quarters of total breast cancer cases. Importantly, almost 65% of postmenopausal breast cancer tumors are known to be estrogen-dependent in their progression.[3] 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is suspected to play a pivotal role in the progression of these breast cancers, transforming estrone (E1) into estradiol (E2), the most potent endogenous ligand for estrogen receptors (ERα and ERβ).[4−6] 17β-HSD1 also catalyzes the reduction of dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol (5-diol), a weaker estrogen that becomes more important after menopause and could also stimulate breast cancer cell proliferation.[7] Importantly, several immunohistochemical studies have reported the presence of 17β-HSD1 in breast carcinoma tissue and the enzyme was detected in approximately 50−60% of cancer cells.[8−14] Furthermore, a significant increment of 17β-HSD1 expression following antiaromatase therapy in breast cancer patients has also been observed, suggesting a compensatory response of the enzyme to estrogen depletion.[15] These observations suggest that a 17β-HSD1 inhibitor could be useful in blocking estrogen biosynthesis in a large number of breast cancers and could be advantageous toward a maximal estrogen biosynthesis blockade, considering that aromatase inhibitors are unable to block 5-diol production (Figure 1).

With the emergence of personalized medicine and diagnostic tests, the arrival of a potent 17β-HSD1 inhibitor in a clinical setting is highly expected to give a new option for the treatment of women detected with a high expression of 17β-HSD1 and a low expression of aromatase in breast cancer tumor biopsies. Finally, the use of 17β-HSD1 inhibitors is also a promising approach for the treatment of other estrogen-dependent diseases, such as endometrial cancer[16] and endometriosis,[17] where the enzyme has been shown to be overexpressed.[18−20]

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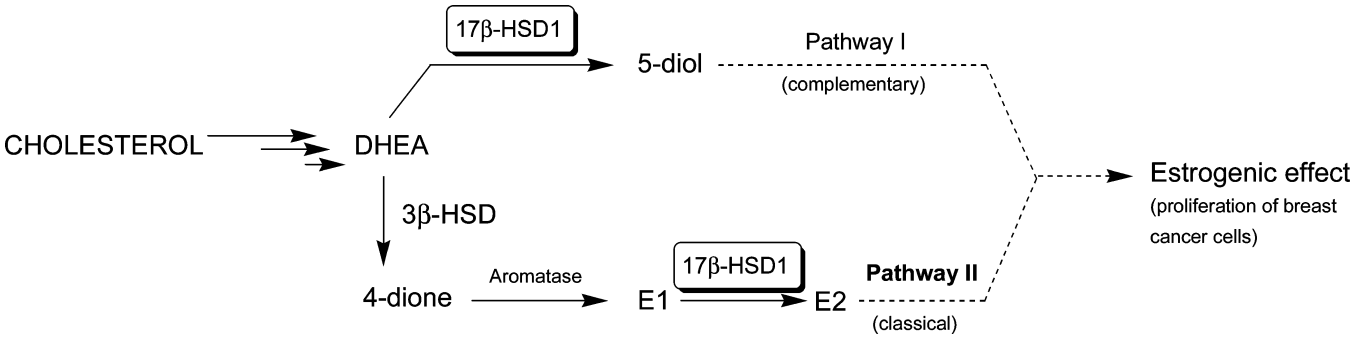
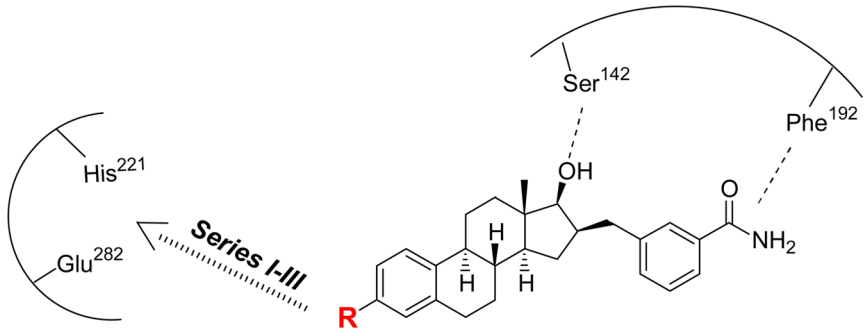


Figure 1. Two pathways involved in the formation of strong estrogen E2 (pathway II) and weak estrogen 5-diol (pathway I) from key steroid DHEA.



R = OH (**1**)

R = $(CH_2)_n$-X (X = alkyl, alcohol, amine, amide or halogen)

(n = 0 (Series I), n = 1 (Series II) or n = 2 (Series III))

Figure 2. Key interactions observed in a ternary complex of 17β-HSD1/inhibitor **1**/cofactor NADP and representation of new E2 derivatives modified at position 3 (series I, II, and III). The scope of this side-chain (R) is dual: (1) reaching a third interaction with an amino acid and (2) removing the undesirable estrogenic activity of the first generation inhibitor **1**.

Over the past 30 years, many efforts were dedicated to designing potent inhibitors of the key steroidogenic enzyme 17β-HSD1, but it is only recently that lead candidates have been reported with very high inhibitory activity.[21−28] The presence of residual estrogenic activity associated with steroidal inhibitors, which are often built around an estrane nucleus, is a major drawback to their development and their use as therapeutic agents. Thus, despite the strong potential of 17β-HSD1 inhibitors for the treatment of estrogen-dependent diseases, validation of this pharmaceutical target remains to be confirmed. New potent and specific inhibitors with a nonestrogenic profile as well as selectivity toward other 17β-HSD isoforms, especially type 2, are thus strongly needed to validate the therapeutic in vivo approach and to engage the first clinical trial with human subjects.

16β-(*m*-Carbamoylbenzyl)-E2 (**1**) has already been reported as a potent inhibitor of 17β-HSD1.[29,30] Despite its good inhibitory potency, this compound was found to stimulate the MCF-7 and T-47D estrogen-sensitive breast cancer cell lines in vitro, thus compromising its therapeutic potential.[29] To remove the undesirable residual estrogenic activity of E2 derivative **1**, we explored the impact on both 17β-HSD1 inhibition and estrogenicity of a series of differently functionalized small chains in the replacement of the hydroxyl (OH) group at position 3. In fact, this OH is well-known to be very important for ER binding affinity and, consequently, to produce an estrogenic effect.[31] Because replacing the 3-OH group by a hydrogen atom did not allow full blockade of the estrogenic activity, as assessed by the proliferation of estrogen-sensitive cells,[29,32] additional modifications should be tested. To reach a new anchoring point with an amino acid in proximity to position 3 of compound **1** (Figure 2),[33] and to potentially remove the undesirable estrogenic activity by disturbing the binding on ER, we selected different functional groups and side chain lengths (0, 1, or 2 carbon spacer) in order to promote hydrogen bonding ($B(OH)_2$, NH_2, F, $CONH_2$, COOH, CH_2OH, $CONR_1R_2$, CH_2NH_2) or hydrophobic interactions (CH_3, CH_2Br, CH_2Cl, CH_2I, CH_2Ph).

The successive synthesis of three series of E2 derivatives, combined with structure−activity relationships (SAR), provided a new potent and nonestrogenic steroidal inhibitor of 17β-HSD1 named 3-{[(16β,17β)-3-(2-bromoethyl)-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl} benzamide (**23b**). After publication of preliminary results,[34] we now report the full details of chemical synthesis and characterization of 20 new E2 derivatives, their evaluation as 17β-HSD1 inhibitors, and their proliferative (estrogenic) activity on estrogen-sensitive cells. We also determine the selectivity of the best inhibitor **23b** for other enzymes and used molecular modeling to obtain insight into its binding mechanism.

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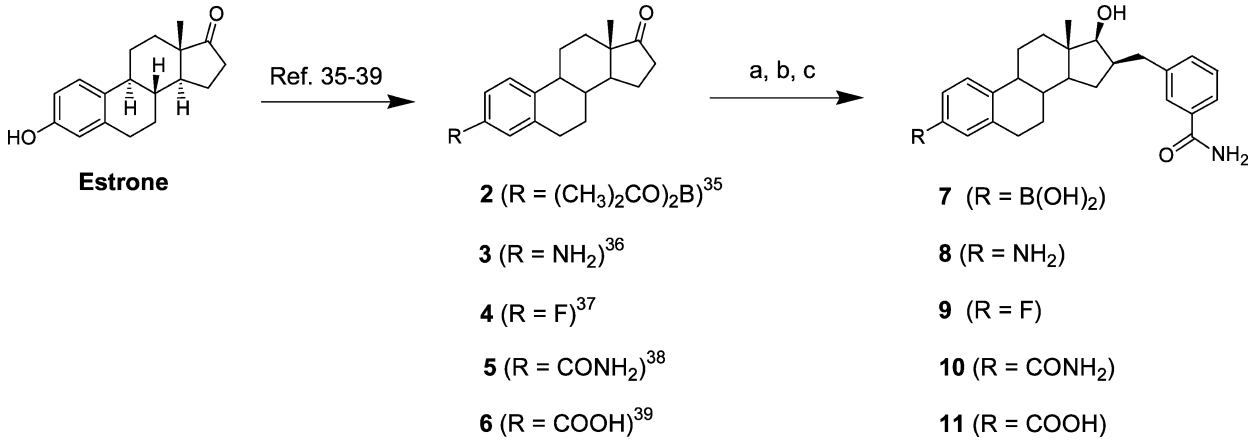
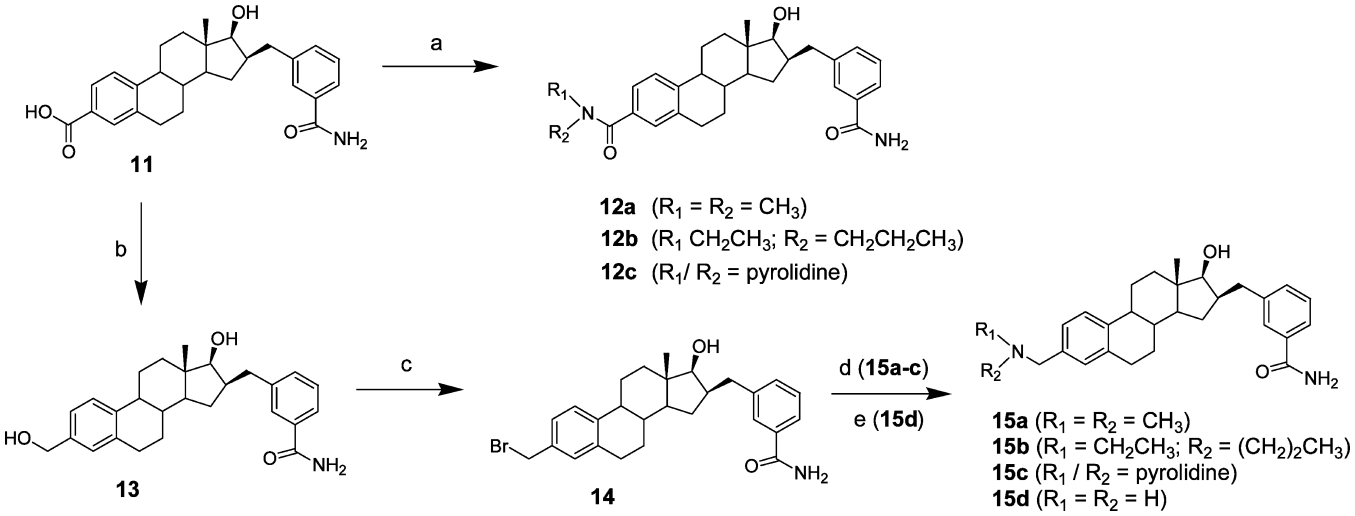
Scheme 1[a]



[a]Reagents and conditions: (a) 3-carboxamide-benzaldehyde, KOH, EtOH, reflux; (b) $NaBH_4$, MeOH, rt; (c) H_2, Pd/C, MeOH, rt.

Scheme 2[a]



[a]Reagents and conditions: (a) R_1R_2NH, BOP, DIPEA, DMF, rt; (b) (i) BOP, DIPEA, THF, rt, (ii) $NaBH_4$, rt; (c) PPh_3, CBr_4, DCM, rt; (d) NHR_1R_2, Et_3N, DCM, rt; (e) (i) NaN_3, DMF, 60 °C, (ii) H_2, Pd/C (10%), MeOH, rt.

RESULTS AND DISCUSSION

Chemistry. Compounds reported in this SAR study were all synthesized from E1 as a common synthetic precursor (Schemes 1−4). The 3-substituted-16β-(*m*-carbamoylbenzyl)-E2 derivatives were regrouped in three different series (I−III) relative to the spacers present between the functional group and the A-ring of steroid core (Figure 2).

Series I Compounds (Schemes 1 and 2). The functional groups ($B(OH)_2$, NH_2, F, $CONH_2$, COOH, CH_2OH, $CONR_1R_2$, CH_2Br, and CH_2NH_2) that constituted the first series of derivatives were selected with the intention of exploring the tolerance of the enzyme for substituents of different natures (H-donor, H-acceptor, hydrophilic, and hydrophobic) and to provide useful SAR data. These functional groups were directly attached to position 3 of the A-ring as illustrated with compounds **7**, **8**, **9**, **10**, **11**, **12a−c**, **13**, **14**, and **15a−d** (Table 1). Our strategy toward obtaining these compounds consisted in functionalizing E1 with the appropriate substituent at C3 and then introducing the *m*-carbamoylbenzyl moiety at C16β. The inverse strategy consisted in introducing the different chains at C3 after the installation of carbamoylbenzyl moiety and was much more uncertain considering the reactivity of the carboxamide functionality. Thus, we first prepared the intermediates **2** (boronate-ester),[35] **3** (amino),[36] **4** (fluoro),[37] **5** (carboxamide),[38] and **6** (carboxy)[39] following previously reported synthetic procedures (Scheme 1). These intermediates were then submitted to a sequence of three chemical steps to give their corresponding 16β-(*m*-carbamoylbenzyl) derivatives **7**, **8**, **9**, **10**, and **11**. These three steps, already reported for the synthesis of **1**,[29,40,41] consisted in an aldolization reaction with the 3-carboxamide-benzaldehyde followed by a reduction with $NaBH_4$ and a catalytic (Pd/C) hydrogenation of the allylic alcohol. This sequence of reactions gave low to modest yields (6−49% for 3 steps) depending of the 3-substituted E1 derivative used as starting material, with the lower yields observed for the boronic acid **7**, aniline **8**, and carboxamide **10**.

The other derivatives of series I, compounds **12a−c**, **13**, **14**, and **15a−d**, were synthesized from 3-carboxy derivative **11** (Scheme 2). An amidation of the mixed anhydride generated from the 3-carboxy group of **11** with appropriate amines using BOP as a coupling agent gave the carboxamide derivatives

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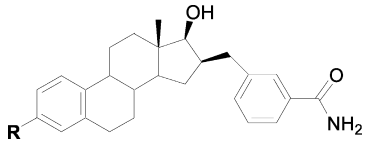
Table 1. Inhibition of 17β-HSD1 (Compounds of Series I)



compd no.	R	inhibition[a] % at 0.1 μM	estrogenicity[b] at 0.1 μM	estrogenicity[b] at 1.0 μM
1	−OH	72 ± 6	+++	+++
7	$-B(OH)_2$	36 ± 4	+++	+++
8	$-NH_2$	31 ± 6	−	+
9	−F	10 ± 5	−	+
10	$-CONH_2$	2 ± 2	−	++
11	−COOH	3 ± 3	+	+++
12a	$-CON(CH_3)_2$	1 ± 4	+	++
12b	−CON(Et)Pr	10 ± 12	−	−
12c	$-CON(CH_2)_4$	3 ± 3	−	−
13	$-CH_2OH$	37 ± 14	−	+
14	$-CH_2Br$	30 ± 12	−	−
15a	$-CH_2N(CH_3)_2$	19 ± 9	−	−
15b	$-CH_2N(Et)Pr$	14 ± 12	−	−
15c	$-CH_2N(CH_2)_4$	10 ± 12	−	+
15d	$-CH_2NH_2$	44 ± 8	+++	+++

[a]Inhibition of the transformation of [^{14}C]-E1 (60 nM) into [^{14}C]-E2 by 17β-HSD1 in T-47D intact cells. Inhibition values are represented as means (±SD) of at least two independent experiments performed in triplicate. [b]Effect of inhibitors on the growth of estrogen-starved estrogen-sensitive MCF-7 cells after 7 days of treatment. Legend for estrogenicity: "−" = no (0−5%), "+" = weak (5−15%), "++" = medium (15−30%), "+++" = strong (>30%) vs control (basal cell proliferation fixed at 0%).

12a−c in low yields (13−30%). The 3-methylalcohol derivative **13** was obtained by activating the carboxy group with BOP and then reducing the anhydride with $NaBH_4$. The bromide derivative **14** was obtained in good yield (60%) by bromination of **13** using triphenylphosphine and carbon tetrabromide in DCM. From **14**, we generated the corresponding amines **15a−c** by a nucleophilic displacement reaction using the appropriate amines in DCM and TEA as base. The amine **15d** was however synthesized in two steps by using first the sodium azide in DMF for a substitution of the bromide and next by reducing the intermediate azide.

Series II Compounds (Scheme 3). In this second series of compounds represented by **18**, **20**, **22**, **23a−c**, **24**, and **25**, one additional carbon spacer (CH_2) was introduced between the functional group (CH_2Ph, CH_3, CH_2OH, CH_2Cl, CH_2Br, CH_2I, COOH, and $CONHCH_3$) and the A-ring of the steroid core (Scheme 3). The vinyl group was identified as the common functionality for the synthesis of each compound of this series. Thus, the key intermediate **16** was obtained from E1 by a dioxolane protection of the C17-ketone,[42] an activation of the phenol by a triflate formation, and a carbonylative vinylation. The 2-phenylethyl derivative **18** was obtained from a metathesis reaction between the vinyl intermediate **16** and styrene using the Grubb (II) catalyst, followed by deprotection of dioxolane to give **17**, and the addition of the 16β-(*m*-carbamoylbenzyl) moiety using the previously described three-step sequence of reactions. The synthesis of the ethyl derivative **20** was generated by deprotection of **16** and installation of the 16β-side chain. The oxidative hydroboration of **16** using a dimethylsulfide borane complex and hydrogen peroxide gave the corresponding primary alcohol, which was protected as the benzyl ether **21**. This compound was next transformed to **22** using the three-step sequence of reactions. The last step, the catalytic hydrogenation, also allows regeneration of the free alcohol. The hydroxyl group of **22** was substituted using (chloro-phenylthio-methylene)-dimethylammonium chloride (CPMA),[43] CBr_4 and PPh_3, or NaI in acetone to provide the chloride **23a**, bromide **23b**, or iodide **23c**. The alcohol **22** was also transformed into the corresponding carboxylic acid **24** in three steps: a) a Dess−Martin oxidation, b) an in situ oxidation with $NaClO_2$, and c) a $NaBH_4$ reduction of the C17-ketone. Finally, the *N*-methylamide **25** was obtained from **22** by activating the carboxylic acid group the intermediate keto acid with BOP reagent to permit the methylamine nucleophilic displacement.

Series III Compounds (Scheme 4). Compounds **27**, **28**, and **30** contain a long spacer ($(CH_2)_3$ or OCH_2CH_2) at position 3 of the steroid nucleus. This series was designed to see the impact of spacer length on 17β-HSD1 inhibitory activity of compound **23b**, the best inhibitor identified in the series II. The 3-vinyl-17-dioxolane-estra-1(10),2,4-triene (**16**) was first submitted to a metathesis reaction with the allyloxymethyl-benzene to give **26** in a low yield. The usual sequence of reactions was next used to install the 16β-side chain to give the diol **27**. The primary alcohol of **27** was then transformed into bromide **28** using CBr_4 and PPh_3. Finally, compounds **29** and **30**, two analogues of **27** and **28** both bearing an oxygen atom rather than a CH_2 group as point of attachment on the ring A (OCH_2CH_2 instead of $CH_2CH_2CH_2$), were synthesized in three steps. Starting from **1**, an *O*-allylation with allylbromide followed by the ruthenium-catalyzed oxidative cleavage of the terminal olefin gave the corresponding aldehyde,[44] which was then reduced with $NaBH_4$ to give the alcohol **29**. This compound was next transformed to bromide **30**. We were particularly interested by derivative **30** considering the presence of an oxygen atom directly attached to the steroid A-ring. In fact, the CH_2CH_2O side chain of **30** could allow supplemental interaction with the enzyme compared to the $(CH_2)_3$ spacer of **28**.

*Optimization of the Chemical Synthesis of **23b** (Scheme 5).* Small quantities of compounds **23b** and **23c** were initially obtained for the purpose of in vitro assays, but larger quantities were thereafter necessary for in vivo assays. We thus designed a shorter and more efficient chemical route that enabled the preparation of multigram quantities of **23b** or **23c**. Briefly, 3-vinyl-estra-1(10),2,4-triene-17-one (**19**)[45] was oxidized with oxone[46] to give the oxirane **31** in excellent yield. The epoxide group of **31** was then transformed to primary alcohol **32** by performing a regiospecific palladium catalyzed transfer hydrogenation[47] using ammonium formate and palladium on charcoal in refluxing methanol. The usual sequence of three reactions for the introduction of the 16β-carbamoyl-*m*-benzamide side chain[34] was used to provide the diol **22** in a very good yield of 84%. Finally, the bromination of **22** using triphenylphosphine and carbon tetrabromide in DCM gave the bromide **23b**. With only eight steps compared to 10 steps (from estrone), this new strategy to generate **23b** was found advantageous over the first one. Importantly, the second chemical synthesis of **23b** was achieved with a global yield of 17% compared to 7% for the first synthesis. Interestingly, only four chromatographic purifications were required along the chemical synthesis, thus reducing the time and cost needed to obtain multigram of **23b** in very good HPLC purity of 98.5%.

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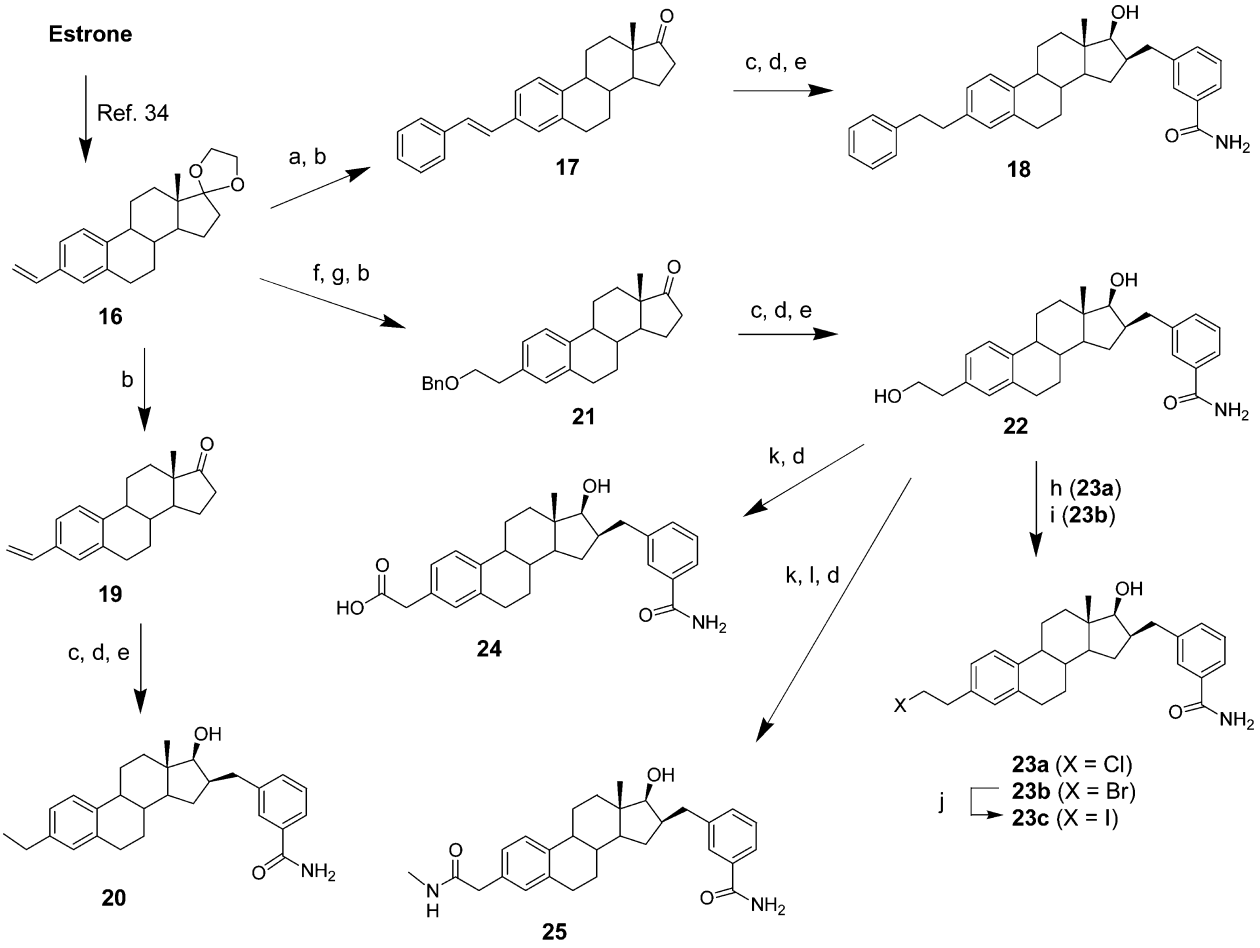
Scheme 3[a]



[a]Reagents and conditions: (a) styrene, Grubb (II) catalyst, dichloroethane, reflux; (b) HCl 10% in MeOH, rt; (c) 3-carboxamide-benzaldehyde, KOH, EtOH, reflux; (d) $NaBH_4$, MeOH, rt; (e) H_2, Pd/C (10%), MeOH, rt; (f) (i) BH_3-DMS, THF, −78 °C, (ii) H_2O_2, $NaHCO_3$; (g) NaH, benzylbromide; (h) CPMA, DCM, rt; (i) PPh_3, CBr_4, DCM, rt; (j) NaI, acetone, rt; (k) (i) Dess−Martin reagent, DCM, rt, (ii) $NaClO_2$, *t*-BuOH, 2-methyl-butene, KH_2PO_4, rt; (l) CH_3NH_2 in THF, BOP, DIPEA, DMF, rt.

Structure−Activity Relationship Study. *Series I Compounds (Inhibition of 17β-HSD1 and Estrogenicity).* The first series of 3-substituted-16β-(*m*-carbamoylbenzyl)-E2 derivatives contains 15 compounds with substituents of different natures that were selected to study the impact on enzyme inhibition of various factors like hydrophobicity, H-bond donor/acceptor capacity, electrostatic charge, and steric hindrance. Compounds were tested for their ability to inhibit the transformation of E1 into E2 by 17β-HSD1 in intact T-47D cells, a cell line that expresses endogenous 17β-HSD1.[30,48] As an initial observation (Table 1), five compounds of this series showed a significant inhibition of 17β-HSD1 (>30% at 0.1 μM), including boronic acid **7**, amines **8** and **15d**, methylalcohol **13**, and bromomethyl **14**, whereas nine compounds gave weak inhibition values (<30% at 0.1 μM), including fluoride **9**, amides **10**, **12a**, **12b**, and **12c**, carboxylic acid **11**, and tertiary amines **15a**, **15b**, and **15c**. When we consider these results more closely, we observe a good inhibition for derivatives with a potential H-bond donor group like primary amines, boronic acid, and alcohol. This could be due to the capacity of these substituents to form H-bond with Glu-282 or His-221 as previously observed for the phenolic derivative **1** (Figure 2). For example, the aromatic amine **8** (31% of inhibition at 0.1 μM) showed a lower inhibition value than the aminomethyl **15d** (44% at 0.1 μM). This could be explained by the fact that the NH_2 of aniline **8** is a poor H-bond acceptor group contrary to the CH_2NH_2 group of **15d**, which has an acceptor/donor capacity close to that of the OH of **1**, a phenol that can form two H-bond interactions with Glu-282 and His-221. A protonation of the CH_2NH_2 group of **15d** could also lead to a stronger interaction with Glu-282 and His-221. This limited H-bond acceptor capacity of **8** could thus explain its lower activity compared to **1** and **15d**. Having a H-bond acceptor/donor capacity, the primary alcohol **13** gave an inhibition close to that of the corresponding primary amine **15d** (37 and 44% at 0.1 μM) and lower than the inhibition of phenol **1** (70%). Also, the interaction between the substituent at position C3 and the enzyme seems to be influenced by the distance between the functionality and the amino acid to favor the formation of H-bond as seen by the difference of activity between the alcohol **13** and phenol **1**.

The very low inhibition at 0.1 μM of the carboxamide **10** (2%) compared to aminomethyl **15d** (44%), H-donor and H-acceptor, and of dimethylcarboxamide **12a** (1%) compared to corresponding amine **15a** (19%), only H-acceptor, reveals that the flexibility and orientation of the substituent to deliver the H-donor group could be an important factor for enzyme inhibition. The presence of a C═O, responsible for the difference of basicity between the amide and amine

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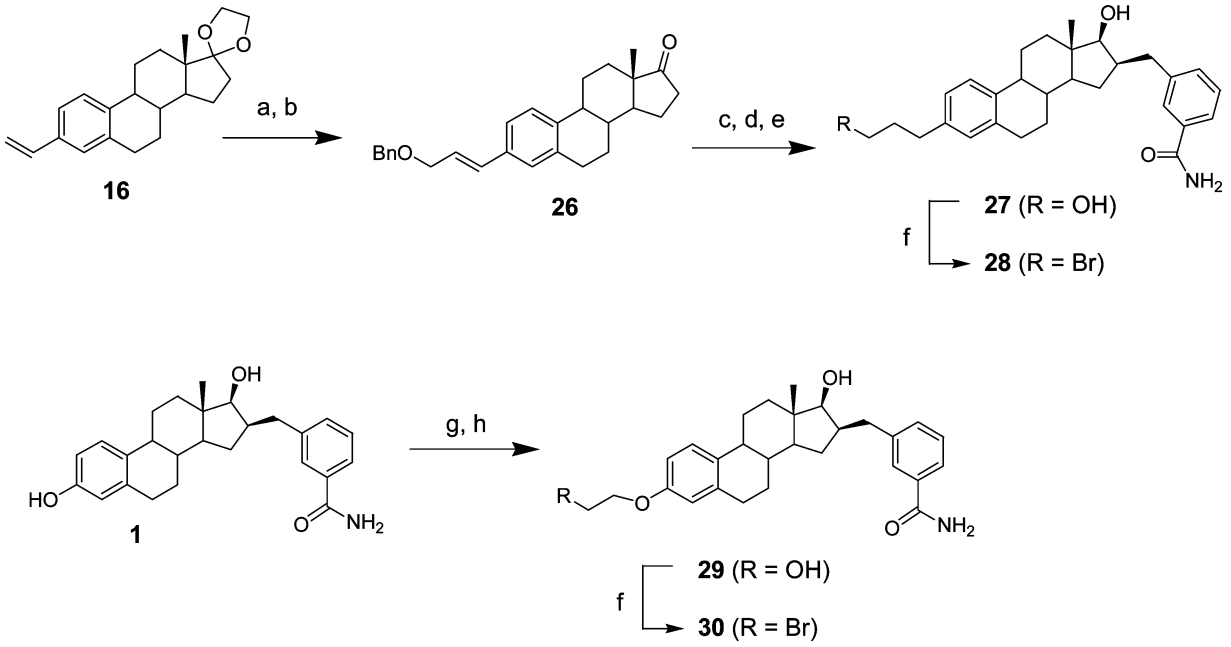
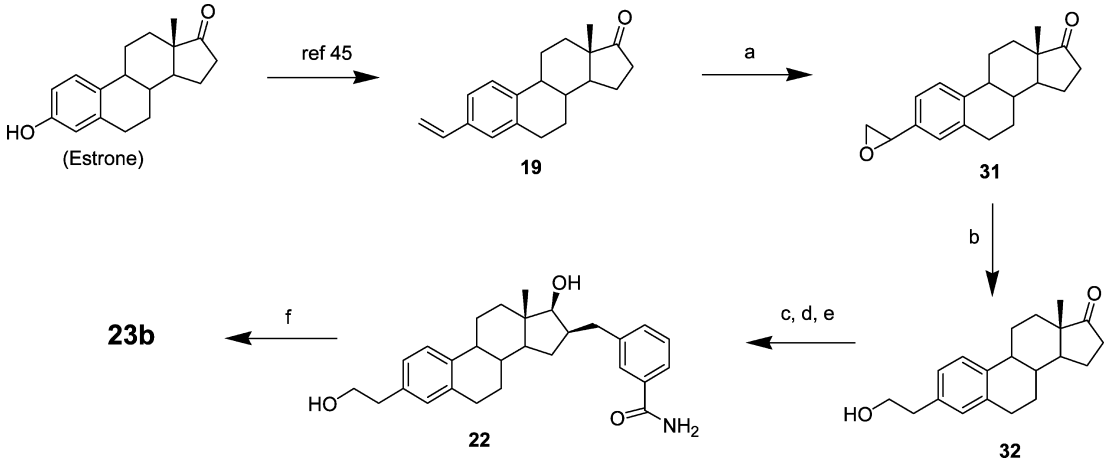
Scheme 4[a]



[a]Reagents and conditions: (a) Grubb II catalyst, allyloxymethyl-benzene; (b) HCl 10% in MeOH, rt; (c) 3-carboxamide-benzaldehyde, KOH, EtOH, reflux; (d) $NaBH_4$, MeOH, rt; (e) H_2, Pd/C (10%), MeOH, rt; (f) PPh_3, CBr_4, DCM, rt; (g) allylbromide, NaOH, acetone, reflux; (h) (i) $NaIO_4$, $RuCl_3$–H_2O, EtOAc/ACN, 0 °C, (ii) $NaBH_4$, THF:H_2O (1:1), rt.

Scheme 5[a]



[a]Reagents and conditions: (a) oxone, $NaHCO_3$, acetone/ACN (1:2), rt; (b) Pd/C (10%), ammonium acetate, MeOH, 70°C; (c) 3-carboxamide-benzaldehyde, KOH, EtOH, reflux; (d) $NaBH_4$, MeOH, rt; (e) H_2, Pd/C (10%), MeOH, rt; (f) PPh_3, CBr_4, DCM, rt.

functionalities or producing a repulsive interaction with Glu-282, could also explain the lower inhibitory activity of amides **10** and **12a**. Furthermore, in the case of a tertiary amine like dimethylamine **15a**, where the amine acted only as H-bond acceptor group via the lone electron pair on nitrogen, the interaction with His-221 seems to be less favored than in the case of the corresponding primary amine **15d** (H-donor and H-acceptor groups). However, the lower inhibition of tertiary amines could also be explained by steric hindrance of the dimethyl group of **15a** (182.5 cm^3/mol), and a loss of inhibitory activity becomes obvious with more bulky chains like diethylamine **15b** (294.5 cm^3/mol) and pyrolidine derivative **15c** (238.5 cm^3/mol). Despite these first interesting SAR observations, the most surprising result of the series was the good inhibition (30% at 0.1 μM) obtained with the hydrophobic bromomethyl substituent (compound **14**), which we had not anticipated.

After investigating the inhibitory potency on 17β-HSD1, we evaluated the proliferative (estrogenic) activity of synthesized compounds on MCF-7 estrogen-sensitive cell line. In fact, for a potential use in breast cancer, an enzyme inhibitor should be devoid of estrogenic activity. Despite the promising 17β-HSD1 inhibitory results obtained with boronic acid **7**, aniline **8**, methylalcohol **13**, and methylamine **15d**, these compounds were all estrogenic at 1 μM and, consequently, they were disqualified for their use in the context of breast cancer treatment. However, we were pleased to see that bromomethyl derivative **14** did not stimulate the cell proliferation at 0.1 and 1

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μM. Other compounds, such as **12b**, **12c**, **15a**, and **15b**, were nonestrogenic on MCF-7 cells, but they inhibit weakly 17β-HSD1. Finally, compounds **9**, **10**, **11**, and **12a** were found to be estrogenic and weak inhibitors. Thus, on the basis of the 17β-HSD1 inhibition and estrogenicity results of series I compounds, we planned the synthesis of new compounds (series II) in order to investigate the effect of an additional carbon spacer to move away the functionality from A-ring on both enzyme inhibition and estrogenic activity.

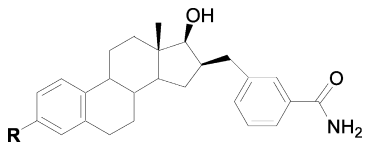
Series II Compounds (Inhibition of 17β-HSD1 and Estrogenicity). To extend our SAR studies, we added a methylene (CH_2) spacer between some of the most active functionalities of the first series of compounds and the steroid A-ring. The tolerance of this enzyme to different types of hydrophobic substituents (Ph, H, Cl, Br, and I) was also investigated. The results obtained in this second series were very interesting (Table 2) because the bromoethyl derivative **23b** and the iodoethyl derivative **23c** gave good 17β-HSD1 inhibition values (51 and 54% at 0.1 μM, respectively). An improvement in inhibitory activity was thus observed with **23b** ($2 \times CH_2$) over **14** ($1 \times CH_2$) when tested at 0.1 μM (51 and 30%, respectively). However, replacement of the bromide of **23b** by a lipophilic and nonelectronegative substituent like phenyl or methyl produced very weak inhibitions at 0.1 μM (17 and 16% for **18** and **20**, respectively). As seen with tertiary amide **25**, the size of the phenyl ring substituent is also detrimental to inhibition. In the same manner, the chain extension was ineffective for carboxylic acid **11** and alcohol **13** because the homologue compounds **24** and **22** had a weak inhibitory effect on 17β-HSD1.

Despite the results pointing toward the importance of electronegativity of the substituent, this factor does not seem to be the most important as observed with the poor inhibition value (12% at 0.1 μM) obtained with chloroethyl derivative **23a**, the more electronegative substituent of the series. The leaving group capability could represent a more coherent explanation for the stronger inhibition observed with bromide and iodide atoms compared to lower leaving capability of the chloride atom. This hypothesis suggests an irreversible inhibition of the enzyme by a nucleophilic attack of the bromide or iodide atom by an amino acid residue. In fact, it was previously reported that a bromopropyl chain added at position 16 of E2 provided an irreversible inhibition of 17β-HSD1.[49,50]

The estrogenicity of series II compounds was assessed by evaluating the proliferation of estrogen-sensitive cells. The presence of OH group (compound **22**) is sufficient to induce a cell proliferation that is higher than the level observed with alcohol **13**. A weak estrogenicity was also obtained for the carboxylic acid derivative **24**. None of the other compounds stimulated the proliferation of cells at 0.1 and 1 μM, including the two most active inhibitors of the series II (compounds **23b** and **23c**). These two compounds have a hydrophobic group at position 3 of steroid core that, contrary to **22** and **24**, cannot generate a hydrogen bonding with ER. In fact, the C3-OH of E2 is well-known to be involved in binding with ER.[51,52]

Series III Compounds (Inhibition of 17β-HSD1 and Estrogenicity). A short series of compounds was designed to study the tolerance of the enzyme for a longer side chain at position 3 (Table 3). We first added a CH_2 group to the bromoethyl side chain of compound **23b**, the most potent inhibitor and nonestrogenic compound of the first two series, to obtain the bromopropyl derivative **28**. We also tested the intermediate alcohol **27** to validate the tendency observed in series I and II with alcohol derivatives **13** and **22**. Finally, we tested compound **30**, a derivative that integrated an oxygen atom in the bromopropyl side chain to reach additional interactions with the enzyme. The inhibition assay revealed that the longer $(CH_2)_3Br$ side chain did not increase the inhibition compared to the shorter $(CH_2)_2Br$ side chain (41 and 51% at 0.1 μM for **28** and **23b**, respectively). For the different alcohol derivatives synthesized in the three series, the proximity of the

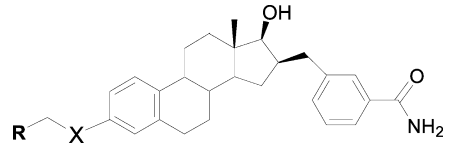
Table 2. Inhibition of 17β-HSD1 (Compounds of Series II)



compd no.	R	inhibition[a] % at 0.1 μM	estrogenicity[b] at 0.1 μM	estrogenicity[b] at 1.0 μM
11 (series I)	$-COOH$	3 ± 3	+	+++
13 (series I)	$-CH_2OH$	37 ± 14	−	+
14 (series I)	$-CH_2Br$	30 ± 12	−	−
18	$-CH_2CH_2Ph$	17 ± 5	−[c]	−[c]
20	$-CH_2CH_3$	6 ± 8	−	−
22	$-CH_2CH_2OH$	18 ± 1	+	+
23a	$-CH_2CH_2Cl$	12 ± 7	−[c]	−[c]
23b	$-CH_2CH_2Br$	51 ± 2	−	−
23c	$-CH_2CH_2I$	54 ± 2	−[c]	−[c]
24	$-CH_2COOH$	7 ± 7	−	+
25	$-CH_2CONHCH_3$	11 ± 8	−	−

[a]Inhibition of the transformation of [^{14}C]-E1 (60 nM) into [^{14}C]-E2 by 17β-HSD1 in T-47D intact cells. Inhibition values are represented as means (±SD) of at least two independent experiments performed in triplicate. [b]Effect of inhibitors on the growth of estrogen-starved estrogen-sensitive MCF-7 cells after 7 days of treatment. [c]Effect of inhibitors on the growth of estrogen-starved estrogen-sensitive T-47D cells after 7 days of treatment. Legend for estrogenicity: "−" = no (0−5%), "+" = weak (5−15%), "++" = medium (15−30%), "+++" = strong (>30%) vs control (basal cell proliferation fixed at 0%).

Table 3. Inhibition of 17β-HSD1 (Compounds of Series III)



compd no.	R	X	inhibition[a] % at 0.1 μM	estrogenicity[b] at 0.1 μM	estrogenicity[b] at 1.0 μM
23b (series II)	$-Br$	CH_2	51 ± 2	−	−
27	$-CH_2OH$	CH_2	20 ± 5	−	+
28	$-CH_2Br$	CH_2	41 ± 5	−	−
30	$-CH_2Br$	O	37 ± 3	−	−

[a]Inhibition of the transformation of [^{14}C]-E1 (60 nM) into [^{14}C]-E2 by 17β-HSD1 in T-47D intact cells. Inhibition values are represented as means (±SD) of at least two independent experiments performed in triplicate. [b]Effect of inhibitors on the growth of estrogen-starved estrogen-sensitive T-47D cells after 7 days of treatment at different concentrations. Legend for estrogenicity: "−" = no (0−5%), "+" = weak (5−15%), "++" = medium (15−30%), "+++" = strong (>30%) vs control (basal cell proliferation fixed at 0%).

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hydroxyl group to the steroid A-ring was an important factor and inhibition values decreased with the spacer length (72, 37, 18, and 20% of inhibition at 0.1 μM for n = 0−3 or compounds **1**, **13**, **22**, and **27**, respectively). As a second relevant result of series III, the presence of an oxygen atom in the side chain of compound **28** (compound **30**) did not increase the inhibition (41 and 37% for **28** and **30**, respectively). Regarding the ability of series III compounds to stimulate estrogen-sensitive cell proliferation, the alcohol derivative **27** was found to be estrogenic but interestingly not the bromide derivatives **28** and **30**. This result once again illustrates the importance of a hydrogen bonding between the C-3 OH and an amino acid known to be involved in the binding of E2 to ER.[51,52]

Determination of IC_{50} Values (17β-HSD1 Inhibition). The IC_{50} values of bromide derivatives **14**, **23b**, **28**, and **30**, the most promising compounds for inhibitory as well as nonestrogenic activity, were determined to compare their potency to inhibit 17β-HSD1 in T-47D cells (Figure 3). The

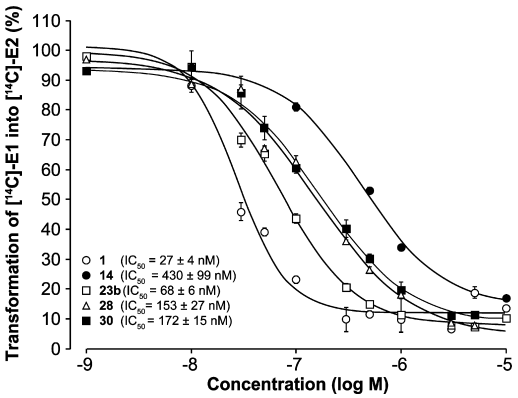

Figure 3. 17β-HSD1 inhibitory potency of compounds **1**, **14**, **23b**, **28**, and **30** in T-47D intact cells. Breast cancer cells expressing 17β-HSD1 were incubated with various concentrations of inhibitor for 24 h in presence of labeled [^{14}C]-E1 (60 nM). IC_{50} represents the concentration that inhibited the transformation of E1 into E2 by 50%. Results are representative of two experiments performed in triplicate except for compound **14** (tested one time).

bromomethyl derivative **14** with a shorter spacer (1 × CH_2) was found to be six times less potent than the bromoethyl derivative **23b** (2 × CH_2) (IC_{50} = 430 nM and 68 nM, respectively), and the bromopropyl derivative **28** with a longer spacer (3 × CH_2) was found to be half as potent (IC_{50} = 153 nM) than **23b**. The presence of an oxygen atom instead of a CH_2 group in the side chain of **30** did not increase the inhibition value of **28** (IC_{50} = 172 and 153 nM, respectively). Thus, the bromoethyl derivative **23b** was the most potent 17β-HSD1 inhibitor of the bromide series with IC_{50} value of 68 nM in this assay (Figure 3) and 97 nM in another assay both performed in T-47D cells (mean value = 83 nM). On the basis of these screening results, **23b** was selected for subsequent biological in vivo assays.[53]

Selectivity of 23b over Other Enzymes. *Inhibition of 17β-HSD2, 17β-HSD7, and 17β-HSD12.* The selectivity of the17β-HSD1 inhibitor **23b** over 17β-HSD2 was evaluated to ensure that it does not deactivate the oxidation of E2 into E1 by inhibiting 17β-HSD2 (Table 4). The assay was performed with stably transfected 17β-HSD2 in intact HEK-293 cells using [^{14}C]-E2 as substrate.[54] As a result, compound **23b** did show a very low inhibition of 17β-HSD2 (only 7% at the higher concentration of 10 μM) for the conversion of E2 to E1. We were also interested in the selectivity of compound **23b** for other 17β-HSDs, such as type 7 and type 12,[55,56] which have been reported to convert E1 to E2 in breast cancer cells.[30] Similarly to 17β-HSD2, the assays were done with stably transfected 17β-HSD7 or 17β-HSD12 in HEK-293 cells but using [^{14}C]-E1 as substrate instead of [^{14}C]-E2. The compound **23b** was found to be highly selective over 17β-HSD7 (9% of inhibition at 10 μM) and less selective over 17β-HSD12 (34% of inhibition at 10 μM).

Inhibition of CYP3A4. We were concerned about the selectivity of action of compound **23b** toward CYP3A4 (Table 4), one of the most important liver enzymes involved in drug metabolism. We used the P450 Inhibition Kit CYP3A4/DBF as suggested by the manufacturer with the exception that **23b** was dissolved in a mixture of DMSO/ACN (5:95) instead of only acetonitrile. Ketoconazole was used as a positive reference inhibitor giving an IC_{50} of 0.024 μM, which is in agreement with reported values.[57] When tested for CYP3A4 inhibition, the bromoethyl derivative **23b** and the phenolic derivative **1**, we obtained IC_{50} values of 3.9 ± 0.5 μM for **23b** and 1.5 ± 0.4 μM for **1**, thus indicating a very low risk of drug−drug interactions for both compounds but especially for **23b**.[57]

Competitive Nature and Irreversibility of Inhibitor 23b. The leaving group ability of halogen atoms (Cl, Br, and I) and the potency of corresponding compounds to inhibit the transformation of [^{14}C]-E1 to [^{14}C]-E2 by 17β-HSD1 suggests the formation of a covalent bond. To verify this hypothesis and to provide some information about the mechanism of action, we performed an inactivation assay with a representative inhibitor and purified 17β-HSD1. Figure 4 shows the inhibition curves for the bromide derivative **23b** (0, 0.1, and 0.5 μM) according to preincubation time. As expected, a slight decrease of the enzyme activity was observed in the absence of inhibitor, but a progressive inhibition of 17β-HSD1 was observed with

Table 4. Selectivity of Compounds 1 and 23b on Four Enzymes (17β-HSD2, 17β-HSD7, 17β-HSD12, and CYP3A4)[a]

compd no.	inhibition of 17β-HSD1[b] IC_{50} (μM)	inhibition of 17β-HSD2% at 10 μM	inhibition of 17β-HSD7% at 10 μM	inhibition of 17β-HSD12% at 10 μM	inhibition of CYP3A4 IC_{50} (μM)
1	0.036 ± 0.012	0 ± 12	0 ± 1	6 ± 7	1.5 ± 0.4
23b	0.083 ± 0.021	7 ± 16	9 ± 5	34 ± 10	3.9 ± 0.5
INH-2[c]		48 ± 13			
INH-7[d]			81 ± 3		
INH-12[e]				39 ± 3	
ketoconazole					0.024 ± 0.004

[a]Inhibition values are represented as means (±SD) of at least two experiments performed in triplicate. [b]17β-HSD1 in T-47D cells. [c]Potent inhibitor of 17β-HSD2 (see compound **1** in ref 54). [d]Potent inhibitor of 17β-HSD7 (see compound **81** in ref 55). [e]Weak inhibitor of 17β-HSD12 (see compound **55** in ref 56).

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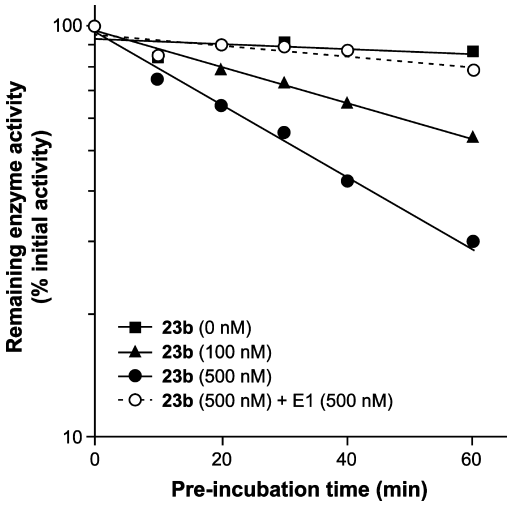

Figure 4. Time-dependent inactivation of 17β-HSD1 by compound **23b**. The transformation of [^{14}C]-E1 to [^{14}C]-E2 by purified enzyme was assessed after preincubation with compound **23b** (0, 100, or 500 nM), with or without the natural substrate E1 (500 nM), and expressed as the percentage of initial enzyme activity. See the Experimental Section for the conditions of the enzymatic assay.

the time at both concentrations for **23b**. The rate of this time-dependent inhibition was higher at 0.5 μM than at 0.1 μM of inhibitor. The very fact that the preincubation time affects the enzyme activity indicates an irreversible effect and strongly suggests the inactivation of the enzyme caused by a covalent binding of the inhibitor considering its nature. As previously reported,[58] a reversible inhibitor would generate an inhibition curve closely similar to that of the control because the same enzymatic activity would be recovered after washing out the inhibitor independently of the preincubation time. In the inactivation assay discussed above, we also addressed the competitiveness of inhibitor **23b**. Moreover, the rate of 17β-HSD1 inactivation ([^{14}C]-E1 to [^{14}C]-E2) is slowed by the presence of unlabeled E1 (0.5 μM), which thus appear to compete with **23b** (0.5 μM) for the substrate binding site of the enzyme.

Molecular Modeling. Since we demonstrated the competitive nature of the inhibitor **23b** for the substrate binding site (Figure 4), we were interested in studying the potential 17β-HSD1/**23b** interactions by performing some docking experiments. For discussion purposes, we also studied the competitive reversible inhibitor **1**, an E2 derivative that was crystallized with 17β-HSD1.[33] The hydroxyl group at position C3 of compound **1** produces H-bonds with side chains of residues Glu-282 and His-221 (Figure 5). Compound **23b** differs from compound **1** as it does not have an H-bond donor group at position C3. Its inhibition potency was however shown to be very good, suggesting a different mode of action.

The first docking attempt for compound **23b** led to a root-mean-square deviation (RMSD) value of 1.49 Å when compared to the compound **1** position obtained from the crystallographic structure. As shown in Figure 6A, the Glu-282 side chain is oriented toward the binding site to make an H-bond with compound **1**, leaving no space for the bromoethyl side chain of compound **23b**. However, Glu-282 is a solvent exposed flexible residue as indicated by its high *B*-factor value of 45. To account for this flexibility in the docking calculations, we generated a binding site conformation for which the Glu-282 side chain is exposed to the solvent (see Experimental Section). As shown in Figure 6B, this latter conformation accommodates

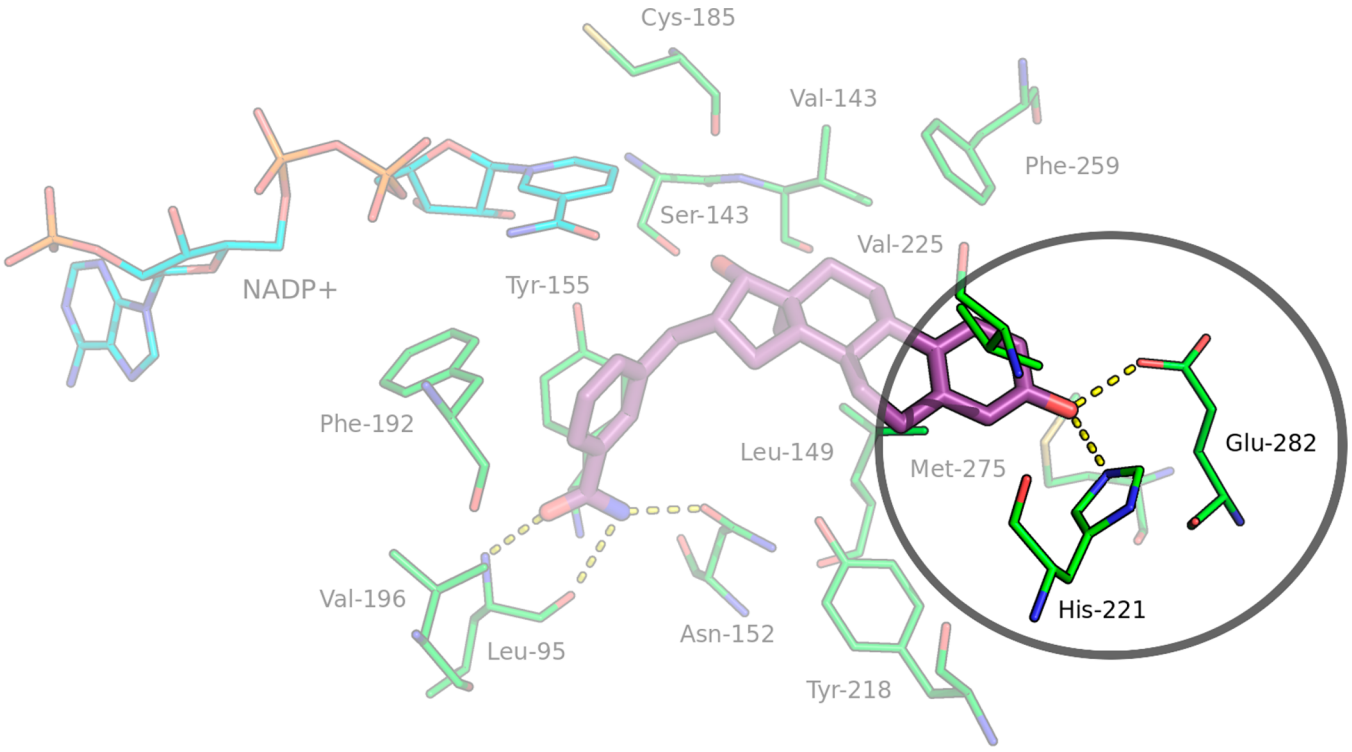

Figure 5. Representation of compound **1** in the active site of 17β-HSD1. Coordinates are from PDB 3HB5.[33] Compound **1** is represented by the thick purple sticks, NADP$^+$ by the small cyan sticks, and the amino acids are represented by the small green sticks. H-bonds between compound **1** hydroxyl at position C3 and Glu-282/His-221 are highlighted.

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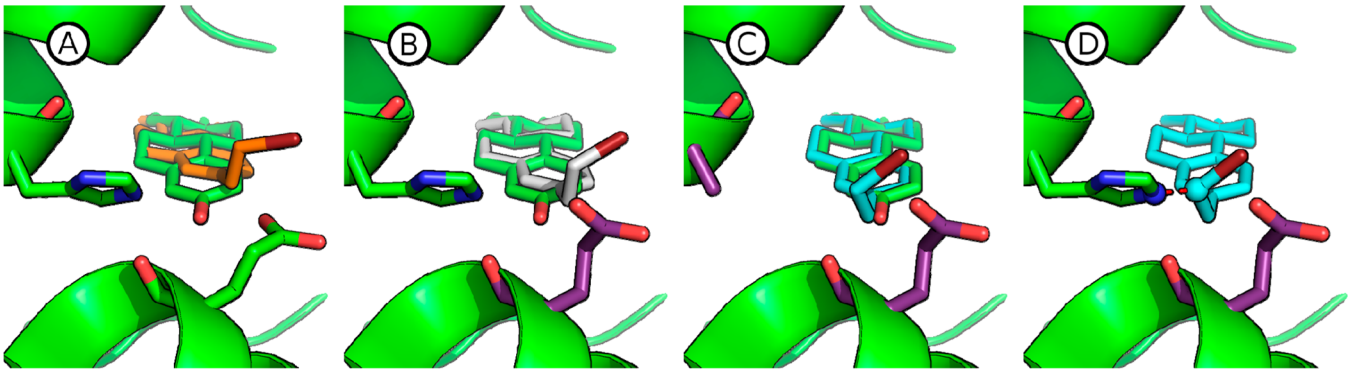

Figure 6. Results from the docking of compound **23b** at the binding site of 17β-HSD1: (A) using the crystallographic binding site conformation, (B) with Glu-282 side chain solvent-oriented, (C) with Glu-282 side chain solvent-oriented and His-221 mutated into Ala, and (D) superposition of receptor from (B) with the docked compound **23b** from (C). The structure of compound **1** is shown for purposes of comparison with the docked compound **23b** and is not included in the docking calculations. Atoms from PDB 3HB5 are represented in green (protein in cartoon and compound **1** in sticks), the modified residues are represented by the purple sticks, and docked compound **23b** by the orange (A), white (B), and cyan (C,D) sticks.

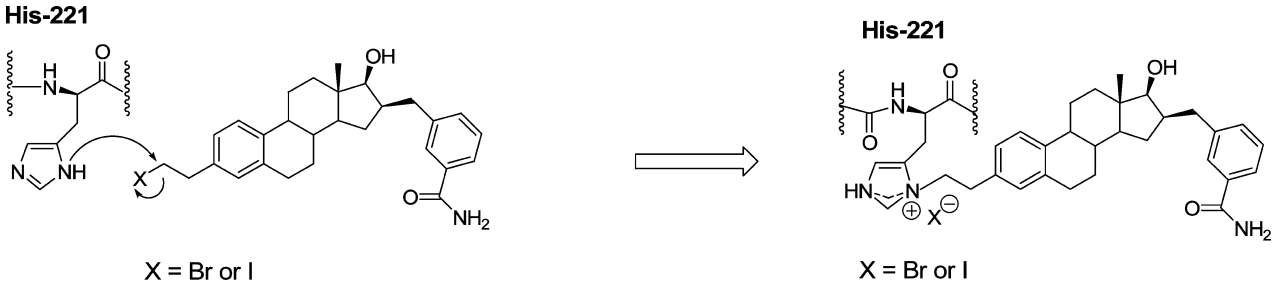

Figure 7. Proposed irreversible mechanism of compound **23b** (X = Br) and **23c** (X = I) on 17β-HSD1.

the docking of compound **23b** with a much better RMSD of 0.76 Å. Nonetheless, the distance between His-221 side chain and the bromide of compound **23b** is 3.8 Å, too long to account for an irreversible binding as hypothesized earlier. To get around the force field limitations that do not allow for covalent reactions between the bromoethyl moiety and His-221 side chain, this latter residue was mutated into an Ala, which has a smaller side chain. The docking results using this binding site conformation are presented in Figure 6C,D. The RMSD of the resulting compound **23b** structure is 0.59 Å, and the distance between the CH_2 of the bromoethyl side chain and the NH of reconstituted His-221 side chain is now 1.7 Å, indicating the possibility of a covalent reaction (Figure 6D). This doubly mutated docking was made to demonstrate that without the conformational limitations of His-221 and Glu-282, the core with the bromoethyl moiety is very well placed in the enzyme pocket to generate a covalent bond.

On the basis of the results of kinetics and molecular modeling with bromide derivative **23b**, and the similar reactivity of both bromide and iodide, we are confident that the iodide of **23c** will be released in the enzyme active site after the formation of a covalent bond with an amino acid. Therefore, it could be caught by this amino acid to form the corresponding iodoimidium salt[59] and to be retained within the active site of 17β-HSD1 (Figure 7). In the event that the iodide would be expelled from the enzyme active site and then to the cell, it could be caught by the NIS symporter,[60] a specialized protein known to fix iodide and to be selectively expressed in thyroid and breast cancer cells.[61] Interestingly, the use of an irreversible inhibitor of 17β-HSD1 could open the door to molecular imaging and radiotherapeutics by adding the appropriate iodo-radioisotope (^{123}I and ^{131}I, respectively) on parent inhibitor **23c**. Advantageously, the iodo-radioisotope derivatives could be readily accessible from a simple substitution reaction between compound **23b** and Na*I.

CONCLUSION

Three successive series (I–III) of 3-substituted-16β-(*m*-carbamoylbenzyl)-E2 derivatives has provided important SAR data regarding enzyme tolerance for substituents of different natures (hydrophilic, hydrophobic, H-bond donor, H-bond acceptor, basic, acidic, etc.) and different chain lengths (0–3 atom spacer). In the first series of synthesized compounds, different types of substituents like alcohol and amine gave acceptable inhibition levels but their significant estrogenic activity disqualified them for treatment of estrogen-related diseases. The most promising substituent of the first series was undoubtedly the bromomethyl derivative **14**, which showed an acceptable level of inhibition and a nonestrogenic profile. The second series of compounds thus focused on the carbon chain extension of bromomethyl derivative **14** as well as other promising compounds of the first series. These new series of compounds converged toward the identification of 3-(2-bromoethyl)-16β-(*m*-carbamoylbenzyl)-estra-1(10),2,4-trien-17β-ol (**23b**) as potent inhibitor of 17β-HSD1 in T-47D cells (IC_{50} = 68 and 97 nM) without any estrogenic activity detected on estrogen-sensitive cells. This bromo derivative was found to be a competitive and irreversible inhibitor of 17β-HSD1. Molecular modeling with **23b** docked in 17β-HSD1 showed the potential key interactions with His-221, highlighting the possibility of a nucleophilic attack of the His-221 on the bromoethyl group. Otherwise, this novel inhibitor represents an

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important evolution relative to lead compound **1**, which was a potent inhibitor of 17β-HSD1 but with an unwanted estrogenic activity. Also, compound **23b** had a selectivity of action over 17β-HSD2, 17β-HSD7, 17β-HSD12, and liver enzyme CYP3A4, thus showing a promising profile toward in vivo assays. Importantly, the evaluation of bromo derivative **23b** in vivo reveals the efficiency of this inhibitor to completely block the tumor growth of estrone stimulated cancer cells (T-47D) expressing the 17β-HSD1 enzyme.[53] Finally, as interesting perspective, the iodide derivative **23c** could provide an opportunity for molecular imaging of tissues expressing 17β-HSD1 as well as selective radiotherapeutic treatment. These new 17β-HSD1 inhibitors **23b** and **23c**, developed through a SAR study, thus represent promising candidates toward clinical studies for the treatment and diagnosis of estrogen-dependent diseases like breast cancer and endometriosis.

EXPERIMENTAL SECTION

General. Chemical reagents were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). The usual solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and were used as received. Anhydrous dichloromethane (DCM), dimethylformamide (DMF), and tetrahydrofuran (THF) were obtained from Sigma-Aldrich. Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.20- mm silica gel 60 F254 plates (E. Merck; Darmstadt, Germany) and with 230−400 mesh ASTM silica gel 60 (Silicyle, Québec, QC, Canada), respectively. Infrared spectra (IR) were recorded on a Horizon MB 3000 ABB FTIR spectrometer (Québec, QC, Canada), and only the significant bands reported in cm^{-1}. Samples were prepared as KBr pellet. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^1H and 100.6 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer (Billerica, MA, USA). The chemical shifts (δ) were expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm), acetone (2.06 and 29.1 ppm), or methanol (3.33 and 49.0 ppm) for ^1H and ^{13}C NMR, respectively. The numbering reported in Figure 8 was used for the assignment of ^1H and ^{13}C NMR signals. Low-resolution mass spectra (LRMS) were recorded on a PE Sciex API-150ex apparatus (Foster City, CA, USA) equipped with a turbo ion-spray source. The HPLC purity of the final compounds to be tested was determined with a Shimadzu apparatus using a Shimadzu SPD-M20A photodiode array detector, an Altima HPC18 reversed-phase column (250 mm × 4.6 mm, 5 μM), and a solvent gradient of MeOH:H_2O. The wavelength of the UV detector was selected between 190 and 205 nm. All final compounds shown a purity ≥95% (95.0−99.9%) except for compounds **13**, **15b**, **15c**, **18**, **20**, **24**, **25**, and **27** (90.2−93.8%). The IUPAC nomenclature was used in the experimental part and the names of steroid derivatives were generated using ACD/Laboratories (Chemist'version) software (Toronto, ON, Canada).

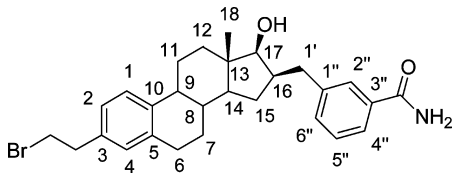

Figure 8. Carbon numbering used for the assignment of representative ^1H NMR signals.

Synthesis of 7, 8, 9, 10, and 11. *General Procedure for the Introduction of 16β-Carbamoyl-m-benzamide Side Chain.* (a) Aldolization reaction: To a solution of the appropriate steroidal ketone **2**, **3**, **4**, **5**, or **6** (0.3 mmol) in EtOH (0.04 M) was added 3-formyl-benzamide (0.6 mmol) and an aqueous KOH (10%) solution (15% v/v). The solution was heated at reflux for 30 min. The resulting solution was diluted with water, neutralized with aqueous HCl (10%), and extracted with EtOAc. The organic layers were combined, washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by flash chromatography to give the corresponding enone. (b) Reduction of enone: To a solution of enone (0.25 mmol) in MeOH (0.03 M) was added $NaBH_4$ (0.38 mmol), and the mixture was stirred at room temperature. After 1 h, the resulting solution was concentrated under vacuo, diluted with EtOAc, washed with water and brine, and dried with $MgSO_4$. After the organic phase was evaporated under reduced pressure, the crude compound was purified by flash chromatography to give the corresponding allylic alcohol. (c) Catalytic hydrogenation of allylic alcohol: To a solution of allylic alcohol (0.2 mmol) in EtOH (0.03 M) under argon atmosphere at room temperature was added palladium on charcoal (10% w/w). The reaction vessel was flushed three times with hydrogen and stirred for 24 h at room temperature. The resulting solution was filtered on Celite and then evaporated under reduced pressure. The crude compound was purified by flash chromatography using DCM/MeOH (95:5) as eluent to give the desired 16β-carbamoyl-benzamide derivative **7**, **8**, **9**, **10**, or **11**.

*[(16β,17β)-16-(3-Carbamoylbenzyl)-17-hydroxyestra-1(10),2,4-trien-3-yl]boronic Acid (**7**).* Yield: 13 mg, 8% (for 3 steps). IR (KBr): 1659 (C═O, amide), 3380 (OH and NH_2). ^1H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.13 (m, 14α-CH and 15β-CH), 1.20−1.55 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.69 (t, J = 6.0 Hz, 15α-CH), 1.84 (broad d, J = 11.8 Hz, 7β-CH), 2.05 (d, J = 12.2 Hz, 12β-CH), 2.26 (m, 9α-CH), 2.35 (m, 11α-CH), 2.47 (q, J = 12.5 Hz, 1H of 1′-CH_2), 2.52 (m, 16α-CH), 2.83 (m, 6-CH_2), 3.17 (d, J = 12.1 Hz, 1H of 1′-CH_2), 3.84 (d, J = 9.4 Hz, 17α-CH), 7.22−7.50 (m, 4-CH, 2-CH, 1-CH, 5″-CH, and 6″-CH), 7.70 (dd, J_1 = 1.2 Hz, J_2 = 7.4 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.2 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.6 (C8), 43.4 (C16), 45.4 (C13), 46.0 (C9), 50.1 (C14), 83.0 (C17), 125.4 (C1), 126.0 (C4″), 129.1 (C2″), 129.4 (C5″), 131.9 (C2), 133.5 (C6″), 134.8 (C3 and C3″), 135.4 (C4), 136.5 (C5), 136.7 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{27}H_{37}BNO_5$ [M + CH_3OH + H]$^+$ = 466.3. HPLC purity of 97.9% (retention time = 10.6 min).

*3-[(16β,17β)-3-Amino-17-hydroxyestra-1(10),2,4-trien-16-yl]-methyl}benzamide (**8**).* Yield: 5 mg, 8% yield (for 3 steps). IR (KBr): 1663 (C═O, amide), 3364 (OH and NH_2). ^1H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.14 (m, 14α-CH and 15β-CH), 1.20−1.50 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.66 (t, J = 7.0 Hz, 15α-CH), 1.84 (m, J = 11.8 Hz, 7β-CH), 2.02 (d, J = 12.4 Hz, 12β-CH), 2.15 (m, 9α-CH), 2.30 (m, 11α-CH), 2.47 (q, J = 12.3, 1H of 1′-CH_2), 2.50 (m, 16α-CH), 2.72 (m, 6-CH_2), 3.17 (dd, J_1 = 2.2 Hz, J_2 = 12.0 Hz, 1H of 1′-CH_2), 3.83 (d, J = 9.4 Hz, 17α-CH), 6.46 (d, J = 2.1 Hz, 4-CH), 6.54 (dd, J_1 = 2.3 Hz, J_2 = 8.2 Hz, 2-CH), 7.04 (d, J = 8.3 Hz, 1-CH), 7.40 (m, 5″-CH and 6″-CH), 7.70 (d, J = 7.4 Hz, 4″-CH), 7.75 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.5 (C11), 28.7 (C7), 30.7 (C6), 33.0 (C15), 38.9 (C12), 39.0 (C1′), 40.0 (C8), 43.4 (C16), 45.1 (C13), 45.5 (C9), 49.9 (C14), 83.1 (C17), 114.9 (C2), 117.2 (C4), 126.0 (C4″), 126.8 (C1), 129.2 (C2″), 129.5 (C5″), 132.0 (C10), 133.5 (C6″), 134.8 (C3"), 138.2 (C5), 144.4 (C1″), 145.5 (C3), 172.7 ($CONH_2$). LRMS for $C_{27}H_{37}N_2O_3$ [M + CH_3OH + H]$^+$ = 437.3. HPLC purity of 96.9% (retention time = 5.3 min).

*3-{[(16β,17β)-3-Fluoro-17-hydroxyestra-1(10),2,4-trien-16-yl]-methyl}benzamide (**9**).* Yield: 59 mg, 26% (3 steps). IR (KBr): 1639 (C═O, amide), 3356 (OH and NH_2). ^1H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.15 (m, 14α-CH and 15β-CH), 1.22−1.56 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.67 (t, J = 7.0 Hz, 15α-CH), 1.82 (m, 7β-CH), 2.04 (d, J = 12.2 Hz, 12β-CH), 2.21 (m, 9α-CH), 2.35 (m, 11α-CH), 2.47 (q, J = 12.5, 1H of 1′-CH_2), 2.52 (m, broad, 16α-CH), 2.81 (m, 6-CH_2), 3.17 (dd, J_1 = 3.2 Hz, J_2 = 12.6 Hz, 1H of 1′-CH_2), 3.84 (d, J = 9.4 Hz, 17α-CH), 6.75 (dd, J_1 = 2.6. Hz, J_2 = 9.8 Hz, 4-CH), 6.81 (dt, J_1 = 2.8 Hz, J_2 = 8.6 Hz, 2-CH), 7.27 (dd, J_1 = 6.0 Hz, J_2 = 8.4 Hz, 1-CH), 7.40 (5″-CH and 6″-CH), 7.71 (td, J_1 = 1.5 Hz, J_2 = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.4 (C11), 28.3 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C1′ and C12), 39.4 (C8), 43.3 (C16), 45.2 (C9), 45.3 (C13), 49.8 (C14), 82.9 (C17), 113.1 (d, $J_{CC\text{-}F}$ = 21.0 Hz, C2), 115.7 (d, $J_{CC\text{-}F}$ = 20.2 Hz, C4), 125.9 (C4″), 127.9 (d, $J_{CC\text{-}F}$ = 8.0 Hz, C1), 129.1 (C2″), 129.4 (C5″),

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133.5 (C6″), 134.7 (C3″), 137.3 (C10), 140.1 (d, J_{C-F} = 6.8 Hz, C5), 144.3 (C1″), 162.2 (d, J_{C-F} = 242.0 Hz, C3), 172.6. LRMS for $C_{27}H_{35}FNO_3$ [M + CH_3OH + H]$^+$ = 440.3. HPLC purity of 98.1% (retention time = 17.5 min).

*(16β,17β)-16-(3-Carbamoylbenzyl)-17-hydroxyestra-1(10),2,4-triene-3-carboxamide (**10**).* Yield: 21 mg, 6% (for 3 steps). IR (KBr): 1647 (C═O, amide), 3213, 3329, 3483, and 3533 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.93 (s, 18-CH_3), 1.18 (m, 14α-CH and 15β-CH), 1.27−1.60 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.70 (m, 15α-CH), 1.88 (m, 7β-CH), 2.07 (d, J = 12.6 Hz, 12β-CH), 2.32 (m, 9α-CH), 2.40 (m, 11α-CH), 2.48 (q, J = 12.4, 1H of 1′-CH_2), 2.52 (m, broad, 16α-CH), 2.89 (m, 6-CH_2), 3.17 (m, 1H of 1′-CH_2), 3.86 (d, J = 9.4 Hz, 17α-CH), 7.35−7.45 (m, 1-CH, 5″-CH and 6″-CH), 7.58 (s, 4-CH), 7.62 (dd, J_1 = 1.8 Hz, J_2 = 8.2 Hz, 2-CH), 7.70 (dd, J_1 = 1.4 Hz, J_2 = 7.0 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.2 (C11), 28.4 (C7), 30.5 (C6), 33.1 (C15), 38.9 (C12 and C1″), 39.3 (C8), 43.4 (C16), 45.4 (C13), 45.9 (C9), 50.0 (C14), 82.9 (C17), 126.0 (C1 and C2), 126.5 (C4″), 129.2 (C2″), 129.3 (C5″), 129.5 (C4), 132.0 (C3), 133.6 (C6″), 134.8 (C3″), 138.2 (C5), 144.4 (C1″), 146.0 (C10), 172.6 (2 × $CONH_2$). LRMS for $C_{28}H_{37}N_2O_4$ [M + CH_3OH + H]$^+$ = 465.3. HPLC purity of 96.4% (retention time = 6.4 min).

*(16β,17β)-16-(3-Carbamoylbenzyl)-17-hydroxyestra-1(10),2,4-triene-3-carboxylic Acid (**11**).* Yield: 320 mg, 49% (for 3 steps). IR (KBr): 1666 (C═O, amide, and acid), 3198, 3283, 3383, and 3553 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.17 (m, 14α-CH and 15β-CH), 1.26−1.58 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.70 (t, J = 7.0 Hz, 15α-CH), 1.87 (m, 7β-CH), 2.06 (d, J = 12.4 Hz, 12β-CH), 2.31 (m, 9α-CH), 2.42 (m, 11α-CH), 2.48 (q, J = 12.6, 1H of 1′-CH_2), 2.53 (m, 16α-CH), 2.88 (m, 6-CH_2), 3.18 (dd, J_1 = 3.2 Hz, J_2 = 12.6 Hz, 1H of 1′-CH_2), 3.86 (d, J = 9.4 Hz, 17α-CH), 7.36−7.45 (m, 1-CH, 5″-CH and 6″-CH), 7.69 (d, J = 7.3 Hz, 2-CH), 7.71(s, 4-CH), 7.75 (d, J = 7.4 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.2 (C11), 28.3 (C7), 30.4 (C6), 33.0 (C15), 38.8 (C12), 38.9 (C1′), 39.3 (C8), 43.3 (C16), 45.4 (C13), 46.1 (C9), 50.0 (C14), 82.9 (C17), 126.0 (C1), 126.4 (C4″), 128.0 (C2), 129.1 (C2″), 129.4 (C5″), 131.3 (C4), 133.5 (C6″), 134.8 (C3″), 138.0 (C3 and C5), 144.3 (C1″), 146.8 (C10), 171.0 (COOH), 172.7 ($CONH_2$). LRMS for $C_{28}H_{36}NO_5$ [M + CH_3OH + H]$^+$ = 466.3. HPLC purity of 95.1% (retention time = 13.8 min).

Synthesis of 12a−c. *(General Procedure for N-Acylation of **11**).* To a solution of acid **11** (0.12 mmol) in DMF (3 mL) was added BOP (0.14 mmol), the appropriate amine (0.36 mmol), and DIPEA (28 μL, 0.17 mmol). The solution was stirred at room temperature for 2 h. The mixture was poured into water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude *N*-acylated derivative **12a**, **12b**, or **12c** was purified by flash chromatography (typically DCM/MeOH, 95:5 to 9:1).

*(16β,17β)-16-(3-Carbamoylbenzyl)-17-hydroxy-N,N-dimethylestra-1(10),2,4-triene-3-carboxamide (**12a**).* Yield: 7 mg, 13%. IR (KBr): 1666 (C═O, amide), 3379 and 3456 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.92 (s, 18-CH_3), 1.17 (m, 14α-CH and 15β-CH), 1.22−1.60 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.70 (t, J = 7.0 Hz, 15α-CH), 1.87 (m, 7β-CH), 2.08 (dt, J_1 = 2.9 Hz, J_2 = 12.3 Hz, 12β-CH), 2.30 (m, 9α-CH), 2.35−2.70 (m, 11α-CH, 1H of 1′-CH_2 and 16α-CH), 2.86 (m, 6-CH_2), 3.02 (s, NCH_3), 3.10 (s, NCH_3), 3.17 (dd, J_1 = 3.2 Hz, J_2 = 12.5 Hz, 1H of 1′-CH_2), 3.87 (d, J = 9.4 Hz, 17α-CH), 7.11 (s, 4-CH), 7.17 (d, J = 8.0 Hz), 7.38−7.44 (m, 1-CH, 5″-CH and 6″-CH), 7.70 (d, J = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.2 (C11), 28.3 (C7), 30.4 (C6), 33.0 (C15), 38.8 (C12), 38.9 (C1′), 39.4 (C8), 39.7 and 40.1 ($N(CH_3)_2$), 43.3 (C16), 45.4 (C13), 45.8 (C9), 50.0 (C14), 83.0 (C17), 125.3 (C1), 126.0 (C2), 126.5 (C4″), 128.5 (C4), 129.2 (C2″), 129.4 (C5″), 133.5 (C6″), 134.3 (C3), 134.8 (C3″), 138.3 (C5), 143.6 (C10), 144.3 (C1″), 172.6 ($CONH_2$), 174.2 ($CON(CH_3)_2$). LRMS for $C_{29}H_{37}N_2O_3$ [M + H]$^+$ = 461.3. HPLC purity of 97.6% (retention time = 10.2 min).

*(16β,17β)-16-(3-Carbamoylbenzyl)-N-ethyl-17-hydroxy-N-propylestra-1(10),2,4-triene-3-carboxamide (**12b**).* Yield: 7 mg, 30%. IR (KBr): 1666 (C═O, amide), 3209 and 3406 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.78 and 1.00 (2t, J = 7.1 Hz, $CH_3CH_2CH_2N$), 0.92 (s, 18-CH_3), 1.12 and 1.26 (2t, J = 6.4 Hz, CH_3CH_2N), 1.17 (m, 14α-CH and 15β-CH), 1.20−1.75 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH, 15α-CH and $CH_3CH_2CH_2N$), 1.85 (m, 7β-CH), 2.05 (d, J = 12.2 Hz, 12β-CH), 2.30 (m, 9α-CH), 2.48 (m, 11α-CH, 1H of 1′-CH_2 and 16α-CH), 2.85 (m, 6-CH_2), 3.10−3.60 (m, 1H of 1′-CH_2, $CH_3CH_2CH_2N$ and CH_3CH_2N), 3.85 (d, J = 9.4 Hz, 17α-CH), 7.11 (s, 4-CH), 7.04 (d, J = 8.0 Hz, 2-CH), 7.35−7.44 (m, 1-CH, 5″-CH, and 6″-CH), 7.70 (d, J = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 11.7 (CH_3CH_2N), 13.3 (C18), 14.3 ($CH_3CH_2CH_2N$), 23.0 ($CH_3CH_2CH_2N$), 27.2 (C11), 28.3 (C7), 30.4 (C6), 33.0 (C15), 38.8 (C12), 38.9 (C1′), 39.4 (C8), 43.3 (C16), 45.4 (C13), 45.8 (C9), 47.5 (CH_3CH_2N), 50.0 (C14), 51.9 ($CH_3CH_2CH_2N$), 83.0 (C17), 124.6 (C2), 126.1 (C1), 126.5 (C4″), 127.8 (C4), 129.2 (C2″), 129.4 (C5″), 133.5 (C6″), 134.3 (C3), 134.8 (C3″), 138.3 (C5), 143.5 (C10), 144.3 (C1″), 172.6 ($CONH_2$), 174.2 (CON). LRMS for $C_{32}H_{43}N_2O_3$ [M + H]$^+$ = 503.3. HPLC purity of 98.8% (retention time = 15.4 min).

*3-{[(16β,17β)-17-Hydroxy-3-(pyrrolidin-1-ylcarbonyl)estra-1-(10),2,4-trien-16-yl]methyl}benzamide (**12c**).* Yield: 17 mg, 30%. IR (KBr): 1666 (C═O, amide), 3402 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.15 (m, 14α-CH and 15β-CH), 1.25−1.60 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.69 (t, J = 7.0 Hz, 15α-CH), 1.89−1.99 (2t, J = 6.5 Hz, CH_2CH_2 of pyrolidine and 7β-CH), 2.03 (m, 12β-CH), 2.29 (m, 9α-CH), 2.39 (m, 11α-CH), 2.47 (q, J = 12.5, 1H of 1′-CH_2), 2.52 (m, 16α-CH), 2.85 (m, 6-CH_2), 3.17 (dd, J_1 = 3.0 Hz, J_2 = 12.6 Hz, 1H of 1′-CH_2), 3.47 and 3.58 (2t, J = 7.0 Hz, 2 × CH_2N of pyrolidine), 3.85 (d, J = 9.4 Hz, 17α-CH), 7.21 (s, 4-CH), 7.26 (d, J = 8.2 Hz, 2-CH), 7.34−7.44 (m, 1-CH, 5″-CH and 6″-CH), 7.70 (d, J = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 25.3 (CH_2 of pyrolidine), 27.2 (C11 and CH_2 of pyrolidine), 28.3 (C7), 30.4 (C6), 33.0 (C15), 38.8 (C12), 38.9 (C1′), 39.3 (C8), 43.3 (C16), 45.4 (C13), 45.8 (C9), 47.4 and 50.0 (2 × CH_2N of pyrolidine), 50.0 (C14), 82.9 (C17), 125.3 (C2), 126.0 (C1), 126.4 (C4″), 128.6 (C4), 129.1 (C2″), 129.2 (C5″), 133.5 (C6″), 135.1 (C3″), 134.9 (C3), 138.2 (C5), 144.1 (C10), 144.3 (C1″), 172.1 (CON), 172.6 ($CONH_2$). LRMS for $C_{31}H_{39}N_2O_3$ [M + H]$^+$ = 487.3. HPLC purity of 95.1% (retention time = 13.3 min).

Synthesis of 13. To a solution of acid **11** (300 mg, 0.70 mmol) in anhydrous THF (20 mL) was successively added BOP (338 mg, 0.76 mmol) and DIPEA (145 μL, 0.84 mmol) under an argon atmosphere at room temperature. The solution was stirred for 10 min and $NaBH_4$ (30 mg, 0.79 mmol) was added in one portion and stirred again for 1 h. The resulting solution was poured into water, extracted with EtOAc, washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by two successive flash chromatography procedures, first using DCM/MeOH (95:5) and second acetone/hexanes (1:1) to give 88 mg (30%) of **13** (3-{[(16β,17β)-17-Hydroxy-3-(hydroxymethyl)estra-1(10),2,4-trien-16-yl]methyl}benzamide). IR (KBr): 1663 (C═O, amide), 3356 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.92 (s, 18-CH_3), 1.16 (m, 14α-CH and 15β-CH), 1.24−1.54 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.69 (t, J = 7.0 Hz, 15α-CH), 1.85 (m, 7β-CH), 2.05 (m, J = 12.2 Hz, 12β-CH), 2.26 (m, 9α-CH), 2.37 (m, 11α-CH), 2.47 (q, J = 12.4, 1H of 1′-CH_2), 2.53 (m, broad, 16α-CH), 2.83 (m, 6-CH_2), 3.17 (dd, J_1 = 3.6 Hz, J_2 = 13.0 Hz, 1H of 1′-CH_2), 3.85 (d, J = 9.5 Hz, 17α-CH), 7.03 (s, 4-CH), 7.09 (d, J = 8.0 Hz, 2-CH), 7.27 (d, J = 8.0 Hz, 1-CH), 7.38−7.45 (m, 5″-CH and 6″-CH), 7.70 (d, J = 6.0 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.4 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.7 (C8), 43.4 (C16), 45.4 (C13), 45.8 (C9), 50.0 (C14), 65.1 (CH_2OH), 83.0 (C17), 125.5 (C1), 126.0 (C2), 126.3 (C4″), 128.6 (C4), 129.1 (C2″), 129.4 (C5″), 133.5 (C6″), 134.8 (C3″), 137.6 (C3), 139.6 (C5), 140.6 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{28}H_{38}NO_4$ [M + CH_3OH + H]$^+$ = 452.3. HPLC purity of 91.8% (retention time = 9.7 min).

Synthesis of 14. To a solution of alcohol **13** (65 mg, 0.15 mmol) in DCM (7 mL) was added at 0 °C triphenylphosphine (61 mg, 0.23 mmol), DIPEA (58 mg, 80 μL, 0.45 mmol), and carbon tetrabromide (77 mg, 0.23 mmol). The solution was stirred at room temperature for

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15 h. The resulting solution was poured into water, extracted with DCM, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by flash chromatography (DCM/MeOH, 97:3) to give 45 mg (60%) of **14** (3-{[(16β,17β)-3-(bromomethyl)-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl}-benzamide). ^{1}H NMR (CD_3COCD_3): 0.92 (s, 18-CH_3), 1.16 (m, 14α-CH and 15β-CH), 1.20−1.56 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.69 (t, J = 6.2 Hz, 15α-CH), 1.84 (m, 7β-CH), 2.02 (m, under solvent peak, 12β-CH), 2.24 (m, 9α-CH), 2.35 (m, 11α-CH), 2.47 (q, J = 12.2 Hz, 1H of 1′-CH_2), 2.52 (m, broad, 16α-CH), 2.81 (m, 6-CH_2), 3.22 (dd, J_1 = 2.9 Hz, J_2 = 12.4 Hz, 1H of 1′-CH_2), 3.87 (m, 17α-CH and 17β−OH), 4.58 (s, CH_2Br), 6.5 (broad s, 1H of $CONH_2$), 7.12 (s, 4-CH), 7.19 (d, J = 8.1 Hz, 2-CH), 7.29 (d, J = 8.0 Hz, 1-CH), 7.35 (t, J = 7.6 Hz, 5″-CH). 7.41 (d, J = 7.6 Hz, 6″-CH), 7.42 (broad s, 1H of $CONH_2$), 7.75(d, J = 7.5 Hz, 4″-CH), 7.83 (s, 2″-CH). ^{13}C NMR (CD_3COCD_3): 12.4 (C18), 26.1 (C11), 27.3 (C7), 29.2 (C6), 32.1 (C15), 34.0 (CH_2Br), 37.8 (C1′ and C12), 38.2 (C8), 42.3 (C16), 44.4 (C13), 44.6 (C9), 48.9 (C14), 81.3 (C17), 124.8 (C4″), 125.8 (C1), 126.6 (C2), 128.0 (C2″), 128.2 (C5″), 129.7 (C4) 131.9 (C6″), 134.5 (C3″), 135.4 (C3), 137.1 (C5), 141.0 (C10), 143.3 (C1″), 168.4 ($CONH_2$). LRMS for $C_{28}H_{37}BrNO_3$ [M + CH_3OH + H]$^+$ = 514.3 and 516.3. HPLC purity of 97.8% (retention time = 18.3 min).

Synthesis of 15a−c. *General Procedure for N-Akylation of Bromide **14**.* To a solution of **14** (25 mg, 0.06 mmol) in DCM (3 mL) was added triethylamine (43 μL, 3.0 mmol) and the appropriate amine (3.0 mmol). The solution was stirred at room temperature for 3 h. The resulting solution was poured into water, extracted with DCM, dried over a phase separator device (Biotage), and evaporated under reduced pressure to give the desired *N*-alkylated derivative after purification by flash chromatography (typically DCM/MeOH, 95:5 to 9:1).

*3-{[(16β,17β)-3-[(Dimethylamino)methyl]-17-hydroxyestra-1-(10),2,4-trien-16-yl]methyl}benzamide (**15a**).* Yield: 10 mg, 43%. IR (KBr): 1663 (C═O, amide), 3364 and 3429 (OH and NH_2). ^{1}H NMR ($CDCl_3$): 0.91 (s, 18-CH_3), 1.16 (m, 14α-CH and 15β-CH), 1.25−1.57 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.69 (t, J = 7.0 Hz, 15α-CH), 1.84 (m, 7β-CH), 2.05 (d, J = 12.2 Hz, 12β-CH), 2.24 (s, 2 × NCH_3), 2.26 (m, 9α-CH), 2.37 (m, 11α-CH), 2.47 (q, J = 12.3 Hz, 1H of 1′-CH_2), 2.52 (m, 16α-CH), 2.83 (m, 6-CH_2), 3.17 (dd, J_1 = 2.7 Hz, J_2 = 12.5 Hz, 1H of 1′-CH_2), 3.41 (s, C$\underline{H_2}$N(CH_3)$_2$), 3.85 (d, J = 9.4 Hz, 17α-CH), 7.00 (s, 4-CH), 7.05 (d, J = 8.0 Hz, 2-CH), 7.27 (d, J = 8.0 Hz, 1-CH), 7.35−7.45 (m, 5″-CH and 6″-CH), 7.70 (d, J = 6.0 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.3 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C12), 39.0 (C1′), 39.6 (C8), 43.4 (C16), 45.1 (N(CH_3)$_2$), 45.4 (C13), 45.8 (C9), 50.0 (C14), 64.6 (CH_2N), 83.0 (C17), 126.0 (C4″), 126.3 (C1), 128.0 (C2), 129.2 (C2″), 129.4 (C5″), 131.3 (C4), 133.5 (C6″), 134.8 (C3″), 135.4 (C3), 137.7 (C5), 140.9 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{29}H_{39}N_2O_2$ [M + H]$^+$ = 447.2. HPLC purity of 97.4% (retention time = 4.1 min).

*3-{[(16β,17β)-3-[Ethyl(propyl)amino)methyl]-17-hydroxyestra-1-(10),2,4-trien-16-yl]methyl}benzamide (**15b**).* Yield: 5 mg, 21%. IR (KBr): 1663 (C═O, amide), 3398 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.90 (t, J = 7.2 Hz, C$\underline{H_3}$$CH_2CH_2$N), 0.92 (s, 18-$CH_3$), 1.10 (t, J = 7.2 Hz, C$\underline{H_3}$$CH_2$N), 1.16 (m, 14$\alpha$-CH and 15$\beta$-CH), 1.25−1.60 (m, 7$\alpha$-CH, 12$\alpha$-CH, 8$\beta$-CH, 11$\beta$-CH, and CH_2C$\underline{H_2}$$CH_3$), 1.69 (t, J = 7.1 Hz, 15α-CH), 1.85 (m, 7β-CH), 2.05 (d, J = 12.8 Hz, 12β-CH), 2.26 (m, 9α-CH), 2.33−2.60 (11α-CH, 1H of 1′-CH_2, 16α-CH, NC$\underline{H_2}$$CH_3$, and NC$\underline{H_2}$$CH_2CH_3$), 2.83 (m, 6-$CH_2$), 3.17 (dd, J_1 = 3.1 Hz, J_2 = 12.6 Hz, 1H of 1′-CH_2), 3.58 (s, ArC$\underline{H_2}$N), 3.85 (d, J = 9.4 Hz, 17α-CH), 7.01 (s, 4-CH), 7.07 (d, J = 8.1 Hz, 2-CH), 7.26 (d, J = 8.0 Hz, 1-CH), 7.36−7.45 (m, 5″-CH and 6″-CH), 7.70 (d, J = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 11.1 (CH_3), 12.1 (CH_3), 13.3 (C18), 20.1 (CH_3C$\underline{H_2}$$CH_2$N), 27.3 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C12), 39.0 (C1′), 39.6 (C8), 43.4 (C16), 45.4 (C13), 45.8 (C9), 48.1 (CH_3CH_2C$\underline{H_2}$N), 50.0 (C14), 55.8 (CH_3C$\underline{H_2}$N), 58.4 (ArC$\underline{H_2}$N), 83.0 (C17), 126.0 (C4″), 126.3 (C1), 128.1 (C2), 129.1 (C2″), 129.4 (C5″), 131.3 (C4), 133.5 (C6″), 134.8 (C3 and C3″), 137.7 (C5), 140.8 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{32}H_{45}N_2O_2$ [M + H] = 489.4. HPLC purity of 91.5% (retention time = 2.1 min).

*3-{[(16β,17β)-17-Hydroxy-3-(pyrrolidin-1-ylmethyl)estra-1-(10),2,4-trien-16-yl]methyl}benzamide (**15c**).* Yield: 7 mg, 28%. IR (KBr): 1663 (C═O, amide), 3205 and 3383 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.16 (m, 14α-CH and 15β-CH), 1.25−1.57 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.68 (t, J = 7.0 Hz, 15α-CH), 1.82 (broad s, 7β-CH and CH_2CH_2 of pyrolidine), 2.04 (d, J = 12.3 Hz, 12β-CH), 2.25 (m, 9α-CH), 2.37 (m, 11α-CH), 2.47 (q, J = 12.3 Hz, 1H of 1′-CH_2), 2.55 (broad s, 2 × CH_2N of pyrolidine and 16α-CH), 2.82 (m, 6-CH_2), 3.17 (dd, J_1 = 3.0 Hz, J_2 = 12.6 Hz, 1H of 1′-CH_2), 3.57 (s, C$\underline{H_2}$NAr), 3.85 (d, J = 9.4 Hz, 17α-CH), 7.02 (s, 4-CH), 7.08 (d, J = 8.1 Hz, 2-CH), 7.26 (d, J = 8.0 Hz, 1-CH), 7.36−7.44 (m, 5″-CH and 6″-CH), 7.70 (d, J = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 24.0 (CH_2CH_2 of pyrolidine), 27.3 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C12), 39.0 (C1′), 39.6 (C8), 43.4 (C16), 45.4 (C13), 45.8 (C9), 50.0 (C14), 54.8 (2(CH_2N), 61.1 (Ar$\underline{C}$$H_2$N), 83.0 (C17), 126.0 (C4″), 126.3 (C1), 128.2 (C2), 129.1 (C2″), 129.4 (C5″), 131.0 (C4), 133.5 (C6″), 134.9 (C3″), 136.0 (C3), 137.7 (C5), 140.8 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{31}H_{41}N_2O_2$ [M + H]$^+$ = 473.3. HPLC purity of 93.8% (retention time = 2.0 min).

Synthesis of 15d. To a solution of bromide **14** (30 mg, 0.06 mmol) in anhydrous DMF (3 mL) was added sodium azide (12 mg, 0.18 mmol). The solution was stirred at 60 °C for 3 h under an argon atmosphere. The resulting solution was poured into water, extracted with EtOAc, washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound (25 mg) was dissolved in ethanol (3 mL), and palladium on charcoal (10%) was added (10 mg). The reaction vessel was then flushed three times with hydrogen and the solution stirred for 24 h under an argon atmosphere at room temperature. The resulting solution was filtered on Celite and evaporated under reduced pressure. The crude compound was purified by flash chromatography using DCM/MeOH (95:5) as eluent to give **15d** (3-{[(16β,17β)-3-(aminomethyl)-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl}benzamide). Yield: 15 mg, 58%. IR (KBr): 1663 (C═O, amide), 3418 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.16 (m, 14α-CH and 15β-CH), 1.22−1.56 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.69 (t, J = 7.0 Hz, 15α-CH), 1.84 (m, 7β-CH), 2.04 (m, 12β-CH), 2.24 (m, 9α-CH), 2.37 (m, 11α-CH), 2.47 (q, J = 12.4 Hz, 1H of 1′-CH_2), 2.52 (m, broad, 16α-CH), 2.82 (m, 6-CH_2), 3.17 (dd, J_1 = 2.9 Hz, J_2 = 12.4 Hz, 1H of 1′-CH_2), 3.84 (d, J = 9.4 Hz, 17α-CH), 6.94 (s, 4-CH), 7.00 (d, J = 9.2 Hz, 2-CH), 7.25 (d, J = 8.0 Hz, 1-CH), 7.35−7.46 (m, 5″-CH and 6″-CH), 7.70 (d, J = 6.0 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.4 (C11), 28.5 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C12), 39.0 (C1′), 39.7 (C8), 43.4 (C16), 45.4 (CH_2NH_2), 45.7 (C9), 45.9 (C13), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.2 (C1), 126.7 (C2), 129.1 (C4), 129.2 (C2″), 129.4 (C5″), 133.5 (C6″), 134.8 (C3″), 138.0 (C5), 138.8 (C3), 140.7 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{27}H_{35}N_2O_2$ [M + H]$^+$ = 419.3. HPLC purity of 95.5% (retention time = 2.0 min).

Synthesis of 17. To a solution of compound **16**[34] (350 mg, 1.07 mmol) in DCM (75 mL) under argon atmosphere was added styrene (257 μL, 233 mg, 2.24 mmol). The solution was purged by argon bubbling for 5 min, and Grubb (II) catalyst (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene)-(tricyclohexylphosphine)ruthenium) (48 mg, 0.056 mmol) was added. The solution was heated at reflux for 24 h under argon atmosphere. The solution was poured into water, extracted two times with DCM, filtered under separator phase device (Biotage), and evaporated under reduced pressure. The crude compound was purified by flash chromatography (EtOAc/hexanes: 95:5) to give 50 mg (11%) of metathesis product. This later compound (42 mg, 0.105 mmol) was then dissolved in methanol (3 mL), and an aqueous solution of HCl 10% (1 mL) was added. The reaction mixture was stirred at room temperature for 2 h. The solution was poured into a saturated $NaHCO_3$ solution (50 mL), extracted with EtOAc, washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure to give compound **17** (3-[(*E*)-2-phenylethenyl]estra-1(10),2,4-trien-17-one).

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Yield: 40 mg, 78%. IR (KBr): 1736 (C═O). ^{1}H NMR ($CDCl_3$): 0.92 (s, 18-CH_3), 1.40−2.58 (residual CH and CH_2), 2.95 (m, 6-CH_2), 7.08 (s, C<u>H</u>═C<u>H</u>), 7.23−7.38 (m, 1-CH, 2-CH, 4-CH, and 3-CH of styrene), 7.51 (d, J = 7.2 Hz, 2CH of styrene). ^{13}C NMR ($CDCl_3$): 13.8 (C18), 21.6 (C15), 25.7 (C11), 26.5 (C7), 29.4 (C6), 31.6 (C12), 35.9 (C16), 38.2 (C8), 44.5 (C14), 48.0 (C13), 50.5 (C9), 124.0 (C4), 125.7 (C2), 126.4 (CH of Ph), 127.1 (C1), 127.4 (CH of Ph), 128.0 (CH of Ph), 128.4 (CH═CH), 128.6 (CH of Ph), 134.9 (C10), 136.7 (C5), 137.5 (C of Ph), 139.4 (C3), ∼225 (too weak not recorded). LRMS for $C_{26}H_{29}O$ $[M + H]^+$ = 357.3.

Synthesis of 18. Compound **18** (6 mg, 11% yield) was prepared from **17** using the general three-step procedure for introducing the 16β-carbamoyl-*m*-benzamide side chain. 3-{[(16β,17β)-17-hydroxy-3-(2-phenylethyl)estra-1(10),2,4-trien-16-yl]methyl}benzamide (**18**). IR (KBr): 1643 (C═O, amide), 3379 (OH and NH_2). ^{1}H NMR ($CDCl_3$): 0.88 (s, 18-CH_3), 1.15 (m, 14α-CH and 15β-CH), 1.26−1.60 (m, 7α-CH, 12α-CH, 8β-CH and 11β-CH), 1.75 (t, J = 7.2 Hz, 15α-CH), 1.83 (m, 7β-CH), 2.00 (m, 12β-CH), 2.25 (m, 9α-CH), 2.35 (m, 11α-CH), 2.48 (q, J = 12.8, 1H of 1′-CH_2), 2.55 (m, 16α-CH), 2.80 (m, 6-CH_2 and PhC<u>H</u>$_2$C<u>H</u>$_2$Ph), 3.17 (dd, J_1 = 3.8 Hz, J_2 = 12.9 Hz, 1H of 1′-CH_2), 3.88 (m, 17α-CH), 5.65 and 6.10 (2 broad s, $CONH_2$), 6.94 (s, 4-CH), 7.01 (d, J = 8.0 Hz, 2-CH), 7.18−7.42 (m, 1-CH, 5″-CH, 6″-CH and <u>Ph</u>CH_2), 7.60 (d, J = 7.3 Hz, 4″-CH), 7.72 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.4 (C11), 28.7 (C7), 30.5 (C6), 33.0 (C15), 38.7 (Ph<u>C</u>H_2), 38.8 (C12), 39.0 (C1′), 39.2 (Ph<u>C</u>H_2), 39.7 (C8), 43.4 (C16), 45.4 (C13), 45.7 (C9), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.1 (C2), 126.8 (C1), 129.1 (<u>Ph</u>CH_2CH_2), 129.2 (2 × <u>Ph</u>CH_2CH_2), 129.4 (<u>Ph</u>CH_2CH_2), 129.5 (2 × <u>Ph</u>CH_2CH_2), 129.9 (C4), 133.5 (C6″), 134.8 (C3″), 137.4 (C3), 139.0 (C5), 140.1 (C10), 143.3 (<u>Ph</u>CH_2CH_2), 144.4 (C1″), 167.6 ($CONH_2$). LRMS for $C_{34}H_{40}NO_2$ $[M + H]^+$ = 494.3. HPLC purity of 93.5% (retention time = 21.6 min).

Synthesis of 19. To a solution of compound **16**[34] (180 mg, 0.64 mmol) in acetone (18 mL) was added an aqueous solution of HCl 10% (2 mL), and the mixture was stirred at room temperature for 2 h. The solution was then poured into a saturated solution of $NaHCO_3$ (50 mL), extracted with EtOAc, washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure to give 140 mg of deprotected compound **19** (3-ethenylestra-1(10),2,4-trien-17-one).[42] ^{1}H NMR (CD_3COCD_3): 0.90 (s, 18-CH_3), 1.37−2.48 (residual CH and CH_2), 2.88 (m, 6-CH_2), 5.16 (d, J = 10.9 Hz, 1H of CH═C<u>H</u>$_2$), 5.74 (d, J = 17.6 Hz, 1H of PhCH═C<u>H</u>$_2$), 6.69 (dd, J_1 = 10.9 Hz, J_2 = 17.6 Hz, C<u>H</u>═CH_2), 7.16 (s, 4-CH), 7.26 (m, 1-CH and 2-CH). ^{13}C NMR (CD_3COCD_3): 13.4 (C18), 21.4 (C15), 25.8 (C11), 26.5 (C7), 29.3 (C6, under solvent peaks), 31.9 (C12), 35.3 (C16), 38.3 (C8), 44.6 (C14), 47.7 (C13), 50.5 (C9), 112.4 (CH═<u>C</u>H_2), 123.6 (C4), 125.7 (C2), 126.9 (C1), 135.2 (C10), 136.7 (C5), 137.1 (<u>C</u>H═CH_2), 139.9 (C3), 218.6 (C17). LRMS for $C_{20}H_{25}O$ $[M + H]^+$ = 281.2.

Synthesis of 20. Compound **20** (40 mg, 54% yield) was prepared from **19** using the general procedure for introduction of the 16β-carbamoyl-*m*-benzamide side chain. 3-{[(16β,17β)-3-ethyl-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl}benzamide (**20**). IR (KBr): 1666 (C═O, amide), 3186 and 3367 (OH and NH_2). ^{1}H NMR ($CDCl_3$): 0.87 (s, 18-CH_3), 1.12 (m, 14α-CH and 15β-CH), 1.22 (t, J = 7.6 Hz, C<u>H</u>$_3$$CH_2$), 1.20.-1.65 (m, 7$\alpha$-CH, 12$\alpha$-CH, 8$\beta$-CH, and 11$\beta$-CH), 1.74 (t, J = 7.0 Hz, 15α-CH), 1.83 (m, 7β-CH), 2.00 (m, 12β-CH), 2.24 (m, 9α-CH), 2.34 (m, 11α-CH), 2.48 (q, J = 12.3, 1H of 1′-CH_2), 2.53 (m, 16α-CH), 2.58 (q, J = 7.6 Hz, CH_3C<u>H</u>$_2$Ph), 2.82 (m, 6-CH_2), 3.16 (dd, J_1 = 4.3 Hz, J_2 = 12.8 Hz, 1H of 1′-CH_2), 3.87 (m, 17α-CH), 5.75 and 6.12 (broad s, $CONH_2$), 6.92 (s, 4-CH), 6.99 (d, J = 8.1 Hz, 2-CH), 7.22 (d, J = 8.0 Hz, 1-CH), 7.34−7.42 (m, 5″-CH and 6″-CH), 7.60 (d, J = 8.8 Hz, 4″-CH), 7.72 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 16.3 (<u>C</u>H_3CH_2), 27.4 (C11), 28.7 (C7), 29.4 (CH_3<u>C</u>H_2), 30.5 (C6), 33.0 (C15), 38.9 (C1′), 39.0 (C12), 39.7 (C8), 43.3 (C16), 45.4 (C13), 45.6 (C9), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.1 (C2), 126.2 (C1), 129.1 (C4), 129.2 (C2″), 129.4 (C5″), 133.5 (C6″), 134.8 (C3″), 137.4 (C3), 138.6 (C5), 142.4 (C10), 144.3 (C1″), 172.7 ($CONH_2$). LRMS for $C_{29}H_{40}NO_3$ $[M + CH_3OH + H]^+$ = 450.3. HPLC purity of 93.6% (retention time = 19.8 min).

Synthesis of 21 and 22. These compounds were prepared from **16** as previously published in our preliminary report.[34]

Synthesis of 23a. To a solution of alcohol **22**[34] (20 mg, 0.05 mmol) in DCM (1.0 mL) was added chlorodimethyl(phenylthio)-chloride methanaminium (CPMA) (45 mg, 0.19 mmol) at 0 °C under an argon atmosphere. The solution was then allowed to return at room temperature and stirred for 3 h. The crude compound was purified by flash chromatography (DCM/MeOH, 97:3) to give 12 mg (57%) of chloride **23a** (3-{[(16β,17β)-3-(2-chloroethyl)-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl}benzamide). IR (KBr): 1639 (C═O, amide) and 3364 (OH and NH_2). ^{1}H NMR (CD_3OD) 0.91 (s, 18-CH_3), 1.15 (m, 14α-CH and 15β-CH), 1.25−1.56 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.68 (t, J = 7.0 Hz, 15α-CH), 1.83 (m, 7β-CH), 2.04 (m, 12β-CH), 2.23 (m, 9α-CH), 2.36 (m, 11α-CH), 2.47 (q, J = 12.3, 1H of 1′-CH), 2.53 (m, 16α-CH), 2.81 (m, 6-CH_2), 2.96 (t, J = 7.3 Hz, C<u>H</u>$_2$$CH_2$Cl), 3.17 (dd, J_1 = 2.9 Hz, J_2 = 12.4 Hz, 1H of 1′-CH_2), 3.70 (t, J = 7.4 Hz, CH_2C<u>H</u>$_2$Cl), 3.84 (d, J = 9.4 Hz, 17α-CH), 6.92 (s, 4-CH), 6.98 (d, J = 8.0 Hz, 2-CH), 7.22 (d, J = 8.0 Hz, 1-CH), 7.36−7.44 (m, 5″-CH and 6″-CH), 7.70 (d, J = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.3 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.6 (C8), 39.8 (<u>C</u>H_2CH_2Cl), 43.3 (C16), 45.4 (C13), 45.7 (C9), 46.0 (CH_2<u>C</u>H_2Cl), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.4 (C1), 127.1 (C2), 129.1 (C2″), 129.4 (C5″), 130.3 (C4), 133.5 (C6″), 134.8 (C3″), 136.7 (C3), 137.8 (C5), 140.0 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{29}H_{39}ClNO_3$ $[M + CH_3OH + H]^+$ = 484.2. HPLC purity of 98.7% (retention time = 19.2 min).

Synthesis of 23b. This compound was prepared from **22** as previously published in our preliminary report.[34]

Synthesis of 23c. To a solution of bromide **23b** (35 mg, 0.07 mmol) in acetone (5 mL) was added sodium iodide (15 mg, 0.1 mmol), and the solution was stirred at room temperature under argon atmosphere for 24 h. Another portion of sodium iodide (52 mg, mmol) was added and the solution stirred for an additional 24 h. The resulting solution was poured into water (100 mL) and extracted with EtOAc. The combined organic layer was washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by flash chromatography (DCM/MeOH, 95:5) to give 18 mg (47%) of iodure **23c** (3-{[(16β,17β)-17-hydroxy-3-(2-iodoethyl)estra-1(10),2,4-trien-16-yl]methyl} benzamide). IR (KBr): 1636 (C═O, amide), 3379 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.14 (m, 14α-CH and 15β-CH), 1.22−1.55 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.69 (t, J = 7.1 Hz, 15α-CH), 1.82 (m, 7β-CH), 2.04 (m, 12β-CH), 2.22 (m, 9α-CH), 2.36 (m, 11α-CH), 2.47 (q, J = 12.3 Hz, 1H of 1′-CH), 2.52 (m, 16α-CH), 2.82 (m, 6-CH_2), 3.07 (t, J = 7.6 Hz, C<u>H</u>$_2$$CH_2$I), 3.17 (dd, J_1 = 3.0 Hz, J_2 = 12.5 Hz, 1H of 1′-CH_2), 3.36 (t, J = 7.6 Hz, CH_2C<u>H</u>$_2$I), 3.84 (d, J = 9.4 Hz, 17α-CH), 6.89 (s, 4-CH), 6.95 (d, J = 8.0 Hz, 2-CH), 7.22 (d, J = 8.0 Hz, 1-CH), 7.36−7.44 (m, 5″-CH and 6″-CH), 7.69 (dd, J_1 = 1.4 Hz, J_2 = 6.0 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 6.3 (CH_2I), 13.3 (C18), 27.3 (C11), 28.4 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.6 (C8), 41.1 (<u>C</u>H_2CH_2I), 43.4 (C16), 45.4 (C13), 45.7 (C9), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.5 (C1), 126.6 (C2), 129.1 (C4), 129.4 (C2″), 129.8 (C5″), 133.5 (C6″), 134.8 (C3″), 137.9 (C5), 139.2 (C3), 140.0 (C10), 144.4 (C1″), 172.7 ($CONH_2$). LRMS for $C_{29}H_{39}INO_3$ $[M + CH_3OH + H]^+$ = 576.2. HPLC purity of 95.0% (retention time = 16.6 min).

Synthesis of 24. Dess−Martin periodinane (67 mg, 0.16 mmol) was added in one portion to a solution of alcohol **22** (50 mg, 0.12 mmol) in DCM (4 mL) at room temperature. After 1 h, the reaction mixture was treated with saturated $NaHSO_3$ (0.25 mL), followed by a saturated solution of $NaHCO_3$ (5 mL) and the aqueous layer was extracted with EtOAc. The combined organic phase was dried over $MgSO_4$, filtered, and evaporated to give a crude compound (49 mg) that was taken up in *t*-BuOH (2.2 mL) and water (0.2 mL). 2-Methyl-2-butene (64 μL, 0.76 mmol), $NaClO_2$ (13 mg, 0.14 mmol), and KH_2PO_4 (19 mg, 0.14 mmol) were then sequentially added, and the mixture was stirred for 12 h at room temperature. The organic solvent was evaporated under vacuum, and the resulting aqueous solution was acidified using 1 N HCl (1 mL) and extracted with EtOAc. The

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organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The crude compound was purified by trituration from MeOH to give 30 mg of an aldehyde. To a solution of the later compound (30 mg, 0.067 mmol) in MeOH (5 mL) was added $NaBH_4$ (7 mg, 0.18 mmol). The solution was stirred at room temperature for 2 h, and two other portions of $NaBH_4$ were sequentially added (7 mg, 0.18 mg) over a period of 2 h. The resulting solution was concentrated under vacuo, diluted with DCM (25 mL), washed with water, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by flash chromatography (DCM/MeOH, 9:1) to give 15 mg (50%) of compound **24** ([(16β,17β)-16-(3-carbamoylbenzyl)-17-hydroxyestra-1(10),2,4-trien-3-yl]acetic acid). IR (KBr): 1659 (C═O, amide), 1705 (C═O, acid), 2300−3600 (OH, acid), 3388 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.15 (m, 14α-CH and 15β-CH), 1.24 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.68 (t, J = 7.1 Hz, 15α-CH), 1.82 (m, 7β-CH), 2.04 (m, 12β-CH), 2.23 (m, 9α-CH), 2.36 (m, 11α-CH), 2.47 (q, J = 12.3 Hz, 1H of 1′-CH_2), 2.53 (m, 16α-CH), 2.81 (m, 6-CH_2), 3.17 (dd, J_1 = 3.1 Hz, J_2 = 12.7 Hz, 1H of 1′-CH_2), 3.51 (s, C$\underline{H}_2$COOH), 3.84 (d, J = 9.4 Hz, 17α-CH), 6.96 (s, 4-CH), 7.01 (d, J = 8.0 Hz, 2-CH), 7.23 (d, J = 8.0 Hz, 1-CH), 7.36−7.46 (m, 5″-CH and 6″-CH), 7.70 (td, J_1 = 1.5 Hz, J_2 = 7.5 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.3 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.6 (C8), 43.4 (C16), 45.4 (C13), 45.7 (C9), 49.2 (under solvent peaks (CH_2COOH)), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.4 (C1), 127.5 (C2), 129.1 (C2″), 129.4 (C5″), 130.8 (C4), 133.3 (C3), 133.5 (C6″), 134.8 (C3″), 137.8 (C5), 140.1 (C10), 144.4 (C1″), 163.0 (COOH), 172.7 ($CONH_2$). LRMS for $C_{29}H_{38}NO_5$ [M + CH_3OH + H]$^+$ = 480.3. HPLC purity of 91.2% (retention time = 5.7 min).

Synthesis of 25. To the C17-ketonic form of **24** (37 mg, 0.08 mmol), obtained from Dess−Martin oxidation of **22**, dissolved in anhydrous DMF (3 mL) and under argon atmosphere was added BOP (40 mg, 0.09 mmol), methyl amine (115 μL, 0.03 mmol, 2.0 M in THF), and DIPEA (18 μL, 0.11 mmol). The solution was stirred at room temperature. After 3 h, the mixture was poured into water, extracted with EtOAc, washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure to give 41 mg of a crude amide. This compound was taken up into a mixture of MeOH/DCM (9:1), then treated with $NaBH_4$ (15 mg, 0.40 mmol) and stirred for 30 min at room temperature. The resulting solution was poured into water, extracted with EtOAc, washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure. The crude amide compound was purified by flash chromatography (DCM/MeOH, 95:5) to give 6 mg (15%) of **25** (3-{[(16β,17β)-17-hydroxy-3-[2-(methylamino)-2-oxoethyl]estra-1(10),2,4-trien-16-yl]methyl}benzamide). IR (KBr): 1655 (C═O, amide), 3360 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.15 (m, 14α-CH and 15β-CH), 1.25−1.50 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.68 (t, J = 7.0 Hz, 15α-CH), 1.83 (m, 7β-CH), 2.04 (m, 12β-CH), 2.24 (m, 9α-CH), 2.36 (m, 11α-CH), 2.47 (q, J = 12.3, 1H of 1′-CH_2), 2.52 (m, 16α-CH), 2.71 (s, C$\underline{H}_3$NHCO), 2.86 (m, 6-CH_2), 3.17 (dd, J_1 = 2.5 Hz, J_2 = 12.3 Hz, 1H of 1′-CH_2), 3.41 (s, Ar$\underline{C}H_2$CO), 3.84 (d, J = 9.4 Hz, 17α-CH), 6.96 (s, 4-CH), 7.01 (d, J = 8.0 Hz, 2-CH), 7.23 (d, J = 8.0 Hz, 1-CH), 7.36−7.44 (m, 5″-CH and 6″-CH), 7.70 (d, J = 7.4 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 26.5 (C$\underline{H}_3$NHCO), 27.3 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.7 (C8), 43.3 (C16), 43.4 ($\underline{C}H_2$CONH), 45.4 (C13), 45.7 (C9), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.5 (C1), 127.3 (C2), 129.1 (C2″), 129.4 (C5″), 130.5 (C4), 133.5 (C6″), 133.8 (C3), 134.8 (C3″), 138.0 (C5), 140.2 (C10), 144.4 (C1″), 167.7 ($\underline{C}$ONHCH$_3$), 175.1 ($CONH_2$). LRMS for $C_{29}H_{37}N_2O_3$ [M + H]$^+$ = 461.2. HPLC purity of 90.2% (retention time = 4.1 min).

Synthesis of 26. To a solution of compound **16**[34] (1.5 g, 4.82 mmol) in DCM (400 mL) was added allyloxymethyl-benzene (1.3 g, 9.55 mmol), and the mixture was stirred under an argon atmosphere for 5 min. Grubb's catalyst (204 mg, 0.24 mmol) was then added, and the solution was heated at 60 °C under argon atmosphere for 48 h. The solution was evaporated and the residue purified by flash chromatography using EtOAc/hexanes (5:95) to give 130 mg (6%) of metathesis product. To a solution of this compound (120 mg, 0.28 mmol) in acetone (3 mL) was added aqueous 10% HCl (3 mL), and the solution was stirred at room temperature for 6 h. The resulting solution was diluted with water (60 mL), neutralized with a saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic layers were combined and washed with brine, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by flash chromatography (EtOAc/hexanes, 5:95) to give 80 mg (69%) of compound **26** (3-[(1*E*)-3-(benzyloxy)prop-1-en-1-yl]estra-1(10),2,4-trien-17-one). ^{1}H NMR ($CDCl_3$): 0.91 (s, 18-CH_3), 1.40−2.45 (residual CH and CH_2), 2.52 (dd, J_1= 8.6 Hz, J_2 = 18.7 Hz, 16β-CH), 2.92 (m, 2H, 6-CH_2), 4.19 (dd, J_1 = 1.1 Hz, J_2 = 6.1 Hz, OC$\underline{H}_2$CH═CH), 4.57 (s, OC$\underline{H}_2$Ph), 6.30 (m, CH═C$\underline{H}$CH$_2$O), 6.58 (d, J = 16.0 Hz, C$\underline{H}$═CHCH$_2$O), 7.14 (s, 4-CH), 7.18−7.39 (m, OCH$_2$Ph, 1-CH, and 2-CH). ^{13}C NMR ($CDCl_3$): 13.8 (C18), 21.6 (C15), 25.7 (C11), 26.5 (C7), 29.4 (C6), 31.6 (C12), 35.8 (C16), 38.1 C8), 44.4 (C14), 48.0 (C13), 50.5 (C9), 70.8 (O$\underline{C}$H$_2$Ph), 72.0 (PhO$\underline{C}$H$_2$═CH), 123.9 (C4), 125.4 (C2), 125.6 (CH of Ph), 127.1 (C1), 127.6 (CH of Ph), 127.8 (CH of Ph), 128.4 (CH of Ph), 132.4 (Ph-$\underline{C}$H═CH$_2$), 134.3 (C10), 136.6 (C5), 138.3 (C of Ph), 139.4 (C3), 217.0 (C17). LRMS for $C_{28}H_{33}O_2$ [M + H]$^+$ = 401.3.

Synthesis of 27. Compound **27** (38 mg, 55%) was obtained from **26** using the general three-step procedure used for introducing the 16β-carbamoyl-*m*-benzamide side chain. 3-{[(16β,17β)-17-hydroxy-3-(3-hydroxypropyl)estra-1(10),2,4-trien-16-yl]methyl}benzamide (**27**). IR (KBr): 1663 (C═O, amide), 3383 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.15 (m, 14α-CH and 15β-CH), 1.20−1.58 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.68 (t, J = 7.0 Hz, 15α-CH), 1.80 (m, 7β-CH and CH$_2$C$\underline{H}_2$CH$_2$OH), 2.04 (m, 12β-CH), 2.21 (m, 9α-CH), 2.35 (m, 11α-CH), 2.47 (q, J = 12.2, 1H of 1′-CH), 2.52 (m, 16α-CH), 2.59 (t, J = 7.2 Hz, ArC$\underline{H}_2$CH$_2$), 2.79 (m, 6-CH_2), 3.17 (d, J = 12.5 Hz, 1H of 1′-CH_2), 3.56 (t, J = 6.5 Hz, CH$_2$C$\underline{H}_2$OH), 3.84 (d, J = 9.3 Hz, 17α-CH), 6.87 (s, 4-CH), 6.93 (d, J = 8.0 Hz, 2-CH), 7.18 (d, J = 8.0 Hz, 1-CH), 7.36−7.44 (m, 5″-CH and 6″-CH), 7.70 (d, J = 6.0 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.4 (C11), 28.7 (C7), 30.5 (C6), 32.6 (CH$_2$$\underline{C}H_2CH_2$), 33.0 (C15), 35.5 (Ar$\underline{C}H_2CH_2$), 38.9 (C1′), 39.0 (C12), 39.7 (C8), 43.4 (C16), 45.4 (C13), 45.7 (C9), 50.0 (C14), 62.3 (CH$_2$OH), 83.0 (C17), 126.0 (C4″), 126.2 (C1), 127.2 (C2), 129.1 (C2″), 129.4 (C5″), 129.9 (C4), 133.5 (C6″), 134.8 (C3″), 137.5 (C5), 138.9 (C3), 140.3 (C10), 144.3 (C1″), 172.7 ($CONH_2$). LRMS for $C_{30}H_{42}NO_4$ [M + CH_3OH + H] = 480.3. HPLC purity of 90.6% (retention time = 13.5 min).

Synthesis of 28. To a solution of compound **27** (28 mg, 0.063 mmol) in DCM (3 mL) was added at 0 °C triphenylphosphine (33 mg, 0.13 mmol) and carbon tetrabromide (42 mg, 0.13 mmol). The solution was stirred at 0 °C for 40 min, and second portions of triphenylphosphine (13 mg, 0.05 mmol) and carbon tetrabromide (17 mg, 0.05 mmol) were added. After 1 h at 0 °C, the resulting solution was poured into water (50 mL), extracted with DCM, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by flash chromatography (DCM/MeOH: 97:3) to give 8 mg (25%) of **28** (3-{[(16β,17β)-3-(3-bromopropyl)-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl} benzamide). IR (KBr): 1663 (C═O, amide), 3383 (OH and NH_2). ^{1}H NMR ($CDCl_3$): 0.88 (s, 18-CH_3), 1.12 (m, 14α-CH and 15β-CH), 1.20−1.58 (m, 7α-CH, 12α-CH, 8β-CH, and 11β-CH), 1.75 (t, J = 7.1 Hz, 15α-CH), 1.83 (m, 7β-CH), 2.00 (m, 12β-CH), 2.15 (m, C$\underline{H}_2$CH$_2$Br), 2.23 (m, 9α-CH), 2.33 (m, 11α-CH), 2.48 (q, J = 12.3, 1H of 1′-CH), 2.53 (m, 16α-CH), 2.70 (t, J = 7.2 Hz, ArC$\underline{H}_2$CH$_2$), 2.82 (m, 6-CH_2), 3.17 (dd, J_1 = 4.3 Hz, J_2 = 12.9 Hz, 1H of 1′-CH_2), 3.41 (t, J = 6.6 Hz, CH$_2$C$\underline{H}_2$Br), 3.86 (m, 17α-CH), 5.60 and 6.10 (broad s of CON$\underline{H}_2$), 6.91 (s, 4-CH), 6.98 (d, J = 7.9 Hz, 2-CH), 7.22 (d, J = 8.0 Hz, 1-CH), 7.34−7.42 (m, 5″-CH and 6″-CH), 7.60 (d, J = 7.3 Hz, 4″-CH), 7.72 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.4 (C11), 28.6 (C7), 30.5 (C6), 33.0 (C15), 33.7 (CH$_2$$\underline{C}H_2CH_2$Br), 34.4 (Ar$\underline{C}H_2CH_2$), 35.7 (CH$_2$Br), 38.9 (C1′), 39.0 (C12), 39.7 (C8), 43.3 (C16), 45.4 (C13), 45.7 (C9), 50.0 (C14), 83.0 (C17), 126.0 (C4″), 126.4 (C1), 126.8 (C2), 129.1 (C2″), 129.4 (C5″), 130.0 (C4), 133.5 (C6″), 134.8 (C3″), 137.7 (C5), 139.0 (C3), 139.3 (C10), 144.3 (C1″), 172.7

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($CONH_2$). LRMS for $C_{30}H_{41}BrNO_3$ $[M + CH_3OH + H]^+$ = 542.2 and 544.3. HPLC purity of 97.5% (retention time = 16.6 min).

Synthesis of 29. To a solution of compound **1**[29] (150 mg, 0.37 mmol) in acetone (3 mL) were added NaOH (50 mg, 1.25 mmol) and allyl bromide (40 μL, 0.46 mmol). After the mixture was stirred at 60 °C for 5 h, the resulting solution was diluted with EtOAc, and the solution was washed with a saturated aqueous solution of ammonium chloride, brine, dried with $MgSO_4$, and evaporated under reduced pressure to give 165 mg of the corresponding 3-*O*-allyl compound. The crude compound was found sufficiently pure to pursue to the next step without further purification. Sodium periodate (108 mg, 0.50 mmol) was added to water (0.5 mL) and the solution stirred at 0 °C for 5 min followed by the addition of $RuCl_3$−H_2O (4 mg, 0.02 mmol), EtOAc (1 mL), and acetonitrile (1 mL). The 3-*O*-allyl compound (150 mg, 0.33 mmol) was added to the previous solution and stirred for 90 s. The reaction was quenched by the addition of a saturated aqueous solution of $Na_2S_2O_3$ (2 mL). The aqueous layer was extracted with EtOAc, and the combined organic phase was dried with Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in a mixture of THF (1 mL) and water (1 mL), and $NaBH_4$ (13 mg, 0.34 mmol) was added. The solution was stirred at room temperature for 20 min, water was then added (10 mL), and the mixture was extracted with DCM. The organic phase was washed with a saturated $NaHCO_3$ solution, dried with Na_2SO_4, and evaporated under reduced pressure. The residue was dissolved in THF (1 mL) and water (1 mL) at 0 °C, sodium periodate (144 mg, 0.67 mmol) was added in small portions, and the solution was stirred for 20 min at room temperature. Ethylene glycol (50 μL) was added, the reaction mixture was diluted with water (3 mL), and the mixture was extracted with EtOAc. The combined organic phase was dried with Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by flash chromatography (EtOAc/hexanes, 9:1) to give 25 mg (15%) of compound **29** (3-{[(16*β*,17*β*)-17-hydroxy-3-(2-hydroxyethoxy)estra-1(10),2,4-trien-16-yl]methyl} benzamide). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.14 (m, 14*α*-CH and 15*β*-CH), 1.20−1.50 (m, 7*α*-CH, 12*α*-CH, 8*β*-CH, and 11*β*-CH), 1.67 (m, 15*α*-CH), 1.81 (m, 7*β*-CH), 2.02 (m 12*β*-CH), 2.17 (m, 9*α*-CH), 2.33 (m, 11*α*-CH), 2.47 (q, J = 12.3, 1H of 1′-CH_2), 2.52 (m, 16*α*-CH), 2.78 (m, 6-CH_2), 3.17 (dd, J_1 = 3.0 Hz, J_2 = 12.5 Hz, 1H of 1′-CH_2), 3.85 (m, 17*α*-CH and C$\underline{H}_2$OH), 3.99 (m, OC$\underline{H}_2CH_2$OH), 6.62 (d, J = 2.7 Hz, 4-CH), 6.70 (dd, J_1 = 2.7 Hz, J_2 = 8.6 Hz, 2-CH), 7.17 (d, J = 8.6 Hz, 1-CH), 7.36−7.44 (m, 5″-CH and 6″-CH), 7.70 (dd, J_1 = 1.5 Hz, J_2 = 6.0 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.5 (C11), 28.6 (C7), 30.8 (C6), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.9 (C8), 43.4 (C16), 45.4 (C9), 45.5 (C13), 49.9 (C14), 61.8 (CH_2OH), 70.4 (O$\underline{C}H_2CH_2$), 83.0 (C17), 112.2 (C2), 115.4 (C4), 126.0 (C4″), 127.2 (C1), 129.1 (C2″), 129.4 (C5″), 133.5 (C6″), 134.0 (C5), 134.8 (C3″), 138.9 (C10), 144.4 (C1″), 158.2 (C3), 172.7 ($CONH_2$). LRMS for $C_{29}H_{40}NO_5$ $[M + CH_3OH + H]^+$ = 450.3.

Synthesis of 30. To a solution of compound **29** (20 mg, 0.46 mmol) in DCM (2 mL) and anhydrous THF (1 mL) was added at 0 °C triphenylphosphine (23 mg, 0.87 mmol) and carbon tetrabromide (29 mg, 0.87 mmol). The solution was stirred at 0 °C for 40 min, and second portions of triphenylphosphine (20 mg, 0.46 mmol) and carbon tetrabromide (23 mg, 0.87 mmol) were added. The solution was stirred at 0 °C for 40 min, and then third portions of triphenylphosphine (20 mg, 0.46 mmol) and carbon tetrabromide (23 mg, 0.87 mmol) were added. After 1 h at 0 °C, the resulting solution was poured into water (100 mL), extracted with DCM, dried with $MgSO_4$, and evaporated under reduced pressure. The crude compound was purified by flash chromatography (DCM/diethyl ether/MeOH, 75:20:5) to give 12 mg (52%) of **30** (3-{[(16*β*,17*β*)-3-(2-bromoethoxy)-17-hydroxyestra-1(10),2,4-trien-16-yl]methyl}-benzamide). IR (KBr): 1663 (C═O, amide), 3367 (OH and NH_2). ^{1}H NMR (CD_3OD): 0.91 (s, 18-CH_3), 1.14 (m, 14*α*-CH and 15*β*-CH), 1.24−1.52 (m, 7*α*-CH, 12*α*-CH, 8*β*-CH, and 11*β*-CH), 1.67 (t, J = 6.8 Hz, 15*α*-CH), 1.82 (m, 7*β*-CH), 2.03 (m, 12*β*-CH), 2.18 (m, 9*α*-CH), 2.33 (m, 11*α*-CH), 2.47 (q, J = 12.3 Hz, 1H of 1′-CH_2), 2.52 (m, 16*α*-CH), 2.80 (m, 6-CH_2), 3.17 (d, J = 10.4 Hz, 1H of 1′-CH_2), 3.67 (t, J = 5.6 Hz, $OCH_2C\underline{H}_2Br$), 3.83 (d, J = 9.2 Hz, 17*α*-CH), 4.25 (t, J = 5.6 Hz, $OC\underline{H}_2CH_2Br$), 6.62 (s, 4-CH), 6.70 (d, J_1 = 8.7 Hz, 2-CH), 7.19 (d, J = 8.6 Hz, 1-CH), 7.36−7.44 (m, 5″-CH and 6″-CH), 7.70 (d, J = 7.4 Hz, 4″-CH), 7.76 (s, 2″-CH). ^{13}C NMR (CD_3OD): 13.3 (C18), 27.5 (C11), 28.6 (C7), 30.7 (C6 and $C\underline{H}_2Br$), 33.0 (C15), 38.8 (C1′), 39.0 (C12), 39.8 (C8), 43.4 (C16), 45.4 (C9), 45.4 (C13), 49.9 (C14), 69.2 (O$\underline{C}H_2CH_2Br$), 83.0 (C17), 113.3 (C2), 115.6 (C4), 126.0 (C4″), 127.4 (C1), 129.1 (C2″), 129.4 (C5″), 133.5 (C6″), 134.5 (C5), 134.8 (C3″), 139.1 (C10), 144.4 (C1″), 157.5 (C3), 172.7 ($CONH_2$). LRMS for $C_{29}H_{39}BrNO_4$ $[M + CH_3OH + H]^+$ = 544.2 and 546.3. HPLC purity of 98.0% (retention time = 18.6 min).

Multigram Synthesis of ***23b****. Synthesis of* ***19****.* 3-Vinyl-estra-1(10),2,4-trien-17-one (**19**) was synthesized in two steps (6.4 g, 72%) from estrone (8.6 g, 31.7 mmol) using a published procedure.[45]

Synthesis of ***31*** *from* ***19****.* To a solution of alkene **19** (6.1 g, 21.7 mmol) in a mixture of acetone and acetonitrile (1:2) 450 mL was added a saturated aqueous solution of $NaHCO_3$ (300 mL) and oxone (20.0 g, 65.1 mmol). The solution was stirred at room temperature for 4 h, then poured into water and extracted with EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, and evaporated under reduce pressure. Purification by flash chromatography with hexanes/EtOAc (8:2) yielded 4.9 g (76%) of 3-(oxiran-2-yl)estra-1(10),2,4-trien-17-one (**31**). IR (film): 1736 (C═O, ketone). ^{1}H NMR (CD_3COCD_3): 0.91 (s, 18-CH_3), 1.40−2.50 (residual CH and CH_2), 2.76 and 3.06 (2m, C$\underline{H}_2$OCH), 2.89 (m, 6-CH_2), 3.79 (dd, J_1 = 4.0 Hz, J_2 = 2.6 Hz, C$\underline{H}$OCH_2), 7.01 (s, 4-CH), 7.06 (d, J = 8.1 Hz, 2-CH), 7.29 (d, J = 8.1 Hz, 1-CH). ^{13}C NMR (CD_3COCD_3): 13.4 (C18), 21.4 (C15), 25.8 (C11), 26.5 (C7), 29.3 (C6) (under solvent peaks), 31.9 (C12), 35.3 (C16), 38.3 (C8), 44.6 (C14), 47.7 (C13), 50.2 (C$\underline{H}_2$O of epoxide), 50.5 (C9), 51.6 (C$\underline{H}$O of epoxide), 123.0 (C4), 125.6 (C2), 126.2 (C1), 135.6 (C10), 136.8 (C5), 140.0 (C3), 227.0 (C17). LRMS for $C_{20}H_{25}O_2$ $[M + H]^+$ = 297.2. HPLC purity of 99.9% (retention time = 13.2 min).

Synthesis of ***32*** *from* ***31****.* To a solution of oxirane **31** (4.9 g, 16.5 mmol) in MeOH in a Schlenck reactor were added ammonium formate (10.4 g, 165 mmol) and 10% palladium on charcoal (2.50 g) under an argon atmosphere at room temperature. The solution was heated at 70 °C for 2 h. The suspension was filtered over Celite and the filtrate evaporated under reduced pressure to dryness. Purification by flash chromatography with hexanes/EtOAc (7:3) yielded 3.6 g (73%) of 3-(2-hydroxyethyl)estra-1(10),2,4-trien-17-one (**32**). IR (film): 1728 (C═O, ketone), 3464 (OH). ^{1}H NMR ($CDCl_3$): 0.91 (s, 18-CH_3), 1.38−2.44 (residual CH and CH_2), 2.51 (dd, J_1 = 8.6 Hz, J_2 = 18.8 Hz, 16*β*-CH), 2.82 (t, J = 6.5 Hz, C$\underline{H}_2CH_2$OH), 2.91 (m, 6-CH_2), 3.86 (t, J = 6.5 Hz, $CH_2C\underline{H}_2$OH), 6.98 (s, 4-CH), 7.03 (d, J = 8.0 Hz, 2-CH), 7.25 (d, J = 9.0 Hz, 1-CH). ^{13}C NMR ($CDCl_3$): 13.8 (C18), 21.6 (C15), 25.7 (C11), 26.5 (C7), 29.4 (C6), 31.6 (C12), 35.9 (C16), 38.2 (C8), 38.7 (C$\underline{H}_2CH_2$OH), 44.3 (C14), 48.0 (C13), 50.5 (C9), 63.6 (CH_2OH), 125.6 (C1), 126.4 (C2), 129.7 (C4), 135.8 (C3), 136.7 (C5), 137.9 (C10), 221.5 (C17). LRMS for $C_{20}H_{27}O_2$ $[M + H]^+$ = 299.2. HPLC purity of 99.3% (retention time = 9.7 min).

Transformation of ***32*** *to* ***22*** *and Then to* ***23b****.* The compound **22**[34] was synthesized in a yield of 84% (4.4 g) from compound **32** (3.6 g, 12.1 mmol) using the three-step general procedure for the introduction of 16*β*-carbamoyl-*m*-benzamide side chain described above. The bromination of **22** (4.4 g, 10.1 mmol) was performed using the same procedure described above and provided **23b** (2.5 g, 50%) in excellent HPLC purity (98.5%).

17*β*-HSD1 Inhibition Assay. Breast cancer T-47D cells were seeded in a 24-well plate (3000 cells/well) in RPMI medium supplemented with insulin (50 ng/mL) and 5% dextran-coated charcoal-treated fetal bovine serum (FBS), which was used rather than untreated 10% FBS, to remove the remaining steroid hormones. Stock solution of each compound to be tested was previously prepared in EtOH and diluted with culture medium to achieve appropriate concentrations prior to use. After 24 h of incubation, a diluted solution was added to the cells to obtain the appropriate final concentration (0.1 or 1 μM for screening and ranging from 1 nM to 10 μM for IC_{50} value determination). The final concentration of EtOH in the well was adjusted to 0.1%. Additionally, a solution of [^{14}C]-E1 (American Radiolabeled Chemicals, Inc., St. Louis, MO, USA) was added to

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obtain a final concentration of 60 nM. Cells were incubated for 24 h, and each inhibitor was assessed in triplicate. After incubation, the culture medium was removed and labeled steroids (E1 and E2) were extracted with diethyl ether. The organic phase was evaporated to dryness with nitrogen. Residues were dissolved in DCM, dropped on silica gel thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA), and eluted with toluene/acetone (4:1) as solvent system. Substrate [^{14}C]-E1 and metabolite [^{14}C]-E2 were identified by comparison with reference steroids (E1 and E2) and quantified using the Storm 860 system (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation and the percentage of inhibition were calculated as follow: % transformation = 100[^{14}C]-E2/([^{14}C]-E1 + [^{14}C]-E2) and % of inhibition = 100(% transformation without inhibitor − % transformation with inhibitor)/% transformation without inhibitor.

17β-HSD1 Inactivation Assay. Purified 17β-HSD1 kindly provided by Dr. Sheng-Xiang Lin (CHU de Québec—Research Center)[33] was used for inactivation/competition assays. An enzyme solution was diluted in physiological buffer (100 mM Tris, 20% glycerol, 0.2 mg/mL BSA, 2 mM EDTA) containing 1 mM of NADPH as cofactor and was treated in triplicate with an ethanolic solution of compound **23b** to reach the accurate concentration, with or without unlabeled E1 (500 nM). The mixture was then preincubated at 37 °C with shaking before simultaneous dilution 1:20 in physiological buffer and addition of radiolabeled substrate to a final concentration of 60 nM [^{14}C]-E1. Transformation of substrate was stopped after 45 min of incubation at 37 °C with shaking by cooling down on ice the enzyme medium and adding equal volume of diethyl ether for further extractions, separation by TLC, and quantification of radiolabeled steroids as described above.

Estrogen-Sensitive Cell Proliferation Assays (Estrogenic Activity). Quantification of cell growth was determined by using CellTiter 96 aqueous solution cell proliferation assay (Promega, Nepean, ON, Canada) following the manufacturer's instructions. MCF-7 or T-47D cells were resuspended in their medium (DMEM-F12 or RPMI, respectively) supplemented with insulin (50 ng/mL) and 5% dextran-coated charcoal treated FBS to remove remaining estrogenic hormones present in the serum and medium. Aliquots (100 μL) of the cell suspension were seeded in 96-well plates (3000 cells/well). After 48 h, the medium was changed for a new one containing an appropriate concentration of the steroid derivative to be tested. The medium was replaced every two days. Cells were left to grow for seven days, either in presence or absence of the compound to be tested.

Inhibition Assays for 17β-HSD2, 17β-HSD7, and 17β-HSD12. Selectivity of compounds **1** and **23b** were assessed as previously described[30] in stably transfected HEK-293 cells kindly provided by Dr. Van Luu-The (CHU de Québec—Research Center). Cells were seeded in 24-well plates in protocol medium (MEM medium supplemented with 5% dextran-coated charcoal stripped, G418 (700 μg/mL), penicillin (100 IU/mL), streptomycin (100 μg/mL), insulin (50 ng/mL), glutamine (2 mM), nonessential amino acids (0.1 mM), and pyruvate (1 mM)). After 48 h incubation, treatment with DMSO solution (<0.5% final) of compound **1** or **23b** in protocol medium was conducted. Radiolabeled substrates ([^{14}C]-E2 for 17β-HSD2 and [^{14}C]-E1 for 17β-HSD7 and 17β-HSD12), obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA), were added to obtain a final concentration of 60 nM. Once a substrate transformation of 12−25% was reached, radiolabeled steroids (E1 and E2) were extracted from the supernatant with diethyl ether. Separation and quantification of substrates and metabolites were conducted as previously described in the section on 17β-HSD1 inhibition assays.

Inhibition of CYP3A4. We used the commercially available P450 Inhibition Kit CYP3A4/DBF (BD Gentest) of BD Biosciences (Mississauga, ON, Canada) according to manufacturer's instructions, with the exception that **23b** was dissolved in a mixture of dimethylsulfoxide/acetonitrile (5:95) instead of only acetonitrile. The enzyme activity was measured by the fluorescence caused by the enzymatic transformation of dibenzylfluorescein (DBF). Inhibitory potencies were determined as IC_{50} values, which were calculated with GraphPad Prism 5 software to express inhibition potency.

Molecular Modeling. Docking simulations were performed using MOE 2012.10.[62] The crystal structure coordinates of 17β-HSD1, including inhibitor **1** and cofactor NADP, were taken from PDB ID 3HB5 (PMID: 19929851). Hydrogen atoms were added using the Protonate 3D tool included in MOE. The protein complex was prepared using the LigX tool, included in MOE, to adjust H, rotamers, and to minimize the system's energy as previously described (PMID: 22566074). Docking simulations were performed using the rigid receptor protocol and default parameters. Validation of the docking protocol was carried out by a self-docking of compound **1**, leading to an RMSD of 0.37 Å between the docked and the crystallographic structures. Because compound **23b** shares its core structure with compound **1**, no further optimization of the docking protocol was considered.

Compound **23b** was built in MOE based on compound **1**. Hydrogens were readjusted and molecules were energy-minimized prior to docking using the same protocol as for compound **1**. Three docking calculations were done for compound **23b**, each using a different binding site conformation: (A) using the crystallographic conformation, (B) with Glu-282 side chain removed from the binding site and exposed to the solvent, and (C) in addition to point 2, the mutation of His-221 to Ala. The Glu-282 conformation was modified using the Rotamer Explorer tool in MOE, and the lowest energy conformer not pointing toward the binding site was selected. The mutation of His-221 to Ala was done using the Sequence tool in MOE, and no further energy minimization was required.

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Notes

The authors declare the following competing financial interest(s): R. Maltais and D. Poirier have ownership interest in a patent application. No potential conflicts of interest were disclosed by the other authors.

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ABBREVIATIONS USED

ACN, acetonitrile; BOP, (benzotriazol-1-yloxy)tris-(dimethylamino) phosphonium hexafluorophosphate; DCM, dichloromethane; DHEA, dehydroepiandrosteone; 5-diol, 5-androsten-3β,17β-diol; 4-dione, 4-androstene-3,17-dione; DIPEA, diisopropylethylamine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; E1, estrone; E2, estradiol; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; EtOAc, ethyl acetate; EtOH, ethanol; FBS, fetal bovine serum; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; MeOH, methanol; *p*-TSA, *para*-toluene sulfonic acid; SAR, structure−activity relationship; TEA, triethylamine; THF, tetrahydrofuran

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