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C6-(*N*,*N*-butyl-methyl-heptanamide) derivatives of estrone and estradiol as inhibitors of type 1 17β-hydroxysteroid dehydrogenase: Chemical synthesis and biological evaluation

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Abstract—A series of estrone and estradiol derivatives having an *N*-butyl,methyl heptanamide side chain at C6-position were synthesized, tested as inhibitors of type 1 17β-HSD and assessed for their possible estrogenic activity. A better type 1 17β-HSD inhibition was obtained for the 6β-side chain orientation over 6α; the C17-alcohols are more potent inhibitors than the corresponding ketones; introducing a 2-methoxy group decreased the inhibitory potency; and the replacement of a C–S bond by a C–C bond in the C6β-side chain is not detrimental to inhibition. Interestingly, the new inhibitors were also found less estrogenic than the lead compound in two breast cancer cell lines, T-47D and MCF-7.

1. Introduction

Breast cancer is the most frequent cancer in women and the second cause of cancer death. In fact, one in eight North American women suffers from breast cancer during her lifetime, and 570,280 women were expected to die of this disease in 2005.[1]

The growth of most cancerous breast tissue is stimulated by estrogens. It is thus of interest that, in adult women, a large proportion of active estrogens are synthesized in peripheral target tissues such as breast from inactive precursor steroids.[2] Among enzymes involved in steroidogenesis,[3] type 1 17β-hydroxysteroid dehydrogenase (17β-HSD) is responsible for the conversion of estrone (E_1) into estradiol (E_2), the most potent estrogen, and dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol (Δ5-diol) (Fig. 1).[4,5] Types 7 and 12 of 17β-HSD are the two other isoforms which convert the weak E_1 into the potent E_2.[6] The importance of 17β-HSD activity in breast tumour development and growth is indicated by higher intratumoral levels of E_2.[7–10] Moreover, although the conversion of E_1 into E_2 has been observed both in normal human breast and malignant breast tumours, the reductive activity is stronger in tumours than in normal breast tissue.[11–15] Thus, in the perspective of designing drugs to control E_2 formation, we are interested in synthesizing potent inhibitors of type 1 17β-HSD.[16]

Our group has recently reported the synthesis and the inhibitory potency on type 1 17β-HSD of a number of E_2 derivatives functionalised at position 6.[17,18] Compound **1**, an E_2 derivative bearing a 6β-thiaheptanamide side chain (Fig. 2), was found to be a good inhibitor of the synthesis of E_2 from E_1, whereas the corresponding 6α-epimer is inactive. Unfortunately, compound **1** also exerts a proliferative (estrogenic) activity as illustrated by its ability to stimulate growth of estrogen-sensitive cells in culture,[17] thus reducing its possible therapeutic interest. Three strategies to modify the biological profile (estrogenicity and inhibitory potency) of lead compound **1** were then tested by synthesizing compounds **2**–**4**. Thus, the replacement of the 3-OH by a hydrogen atom as well as that of the amide group by a methyl was clearly unfavourable for the inhibition activity. Changing the thioether for an ether bond decreased 10-fold the estrogenic profile of lead compound **1** while the inhibitory potency was only decreased 5-fold.[18]

As another strategy for reducing the estrogenicity of **1**, we tried adding a short alkylether (MeO or EtO) at

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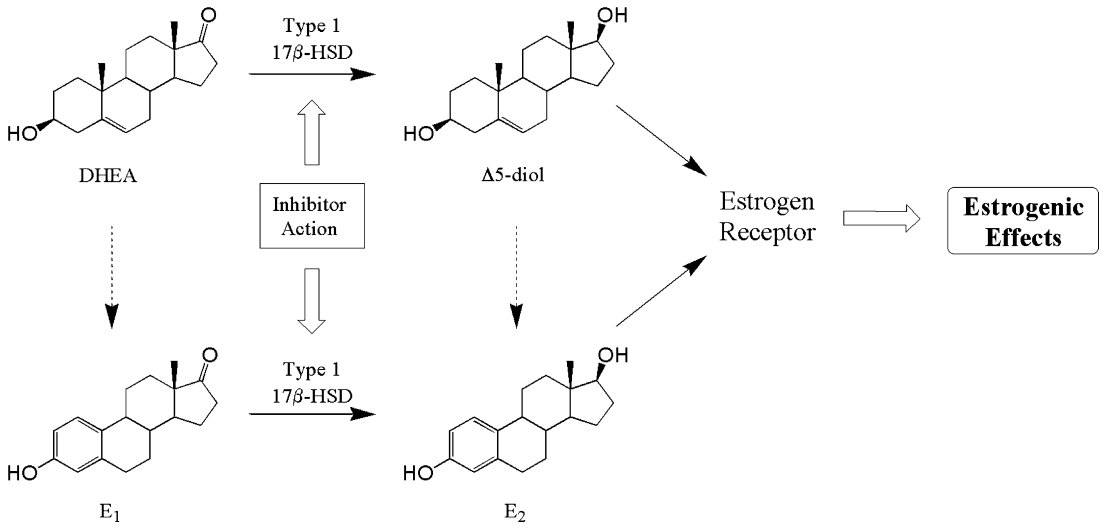


Figure 1. Role of type 1 17β-HSD inhibitors.

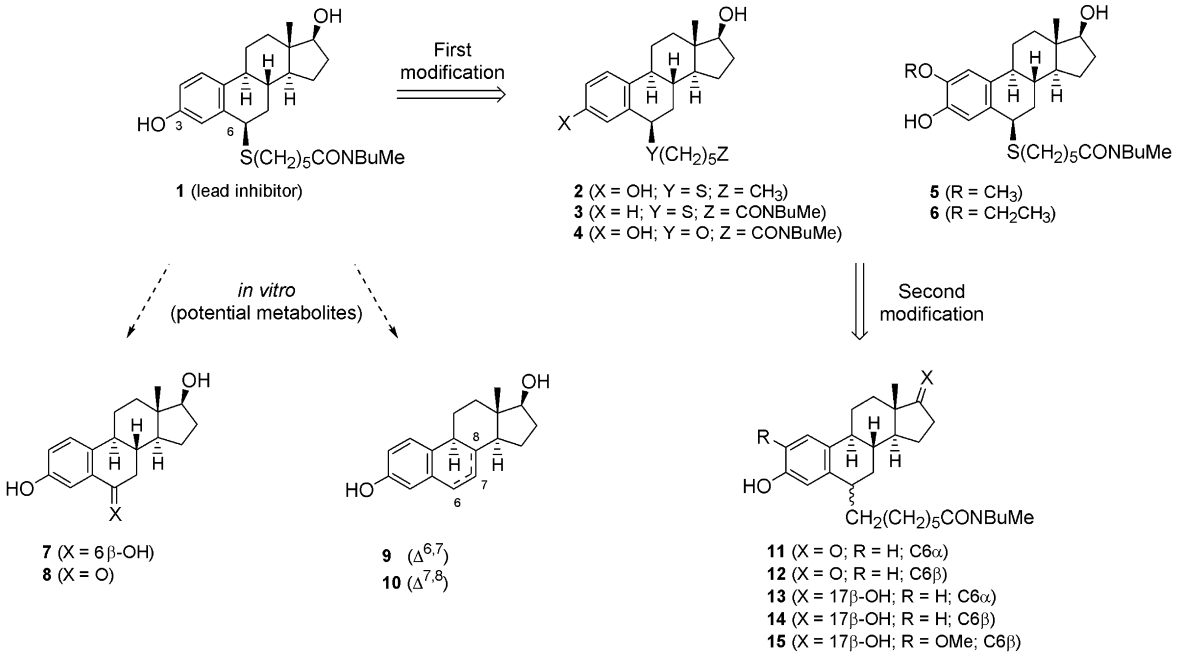


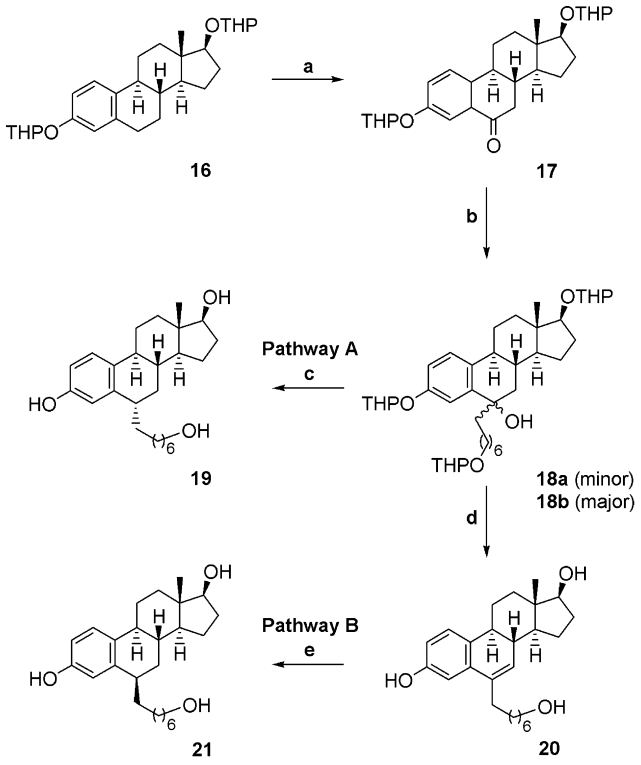
Figure 2. Chemical structures of type 1 17β-HSD inhibitors **1**–**6**, of potential metabolites **7**–**10** and of new inhibitors **11**–**15**.

the C2-position, but stability problems prevented the synthesis and limited the usefulness of **5** and **6**.[19] The instability of the thioether bond was hypothesized to cause the problems in the chemical synthesis of compounds **5** and **6** as well as the estrogenicity of **1**. To confirm or infirm this last hypothesis, potential estrogenic metabolites **7**–**10** resulting from the cleavage of the thioether bond were tested. Furthermore, considering the chemical and biological results obtained with compounds **1**–**10**, it was concluded that the thioether (C–S) or ether (C–O) bond must be replaced by a carbon-carbon (C–C) bond, which should be much less likely to be modified by metabolizing enzymes. We also investigated the role of the 17β-OH versus the 17-ketone and the presence of a 2-methoxy group to reduce the estrogenic properties of the best inhibitors. The chemical synthesis of **11**–**15**, inhibitory potency on types 1, 7 and 12 of 17β-HSD as well as proliferative activity on estrogen sensitive (ER+) breast cancer cell lines T-47D and MCF-7 are reported in this paper.

2. Results and discussion

2.1. Chemistry

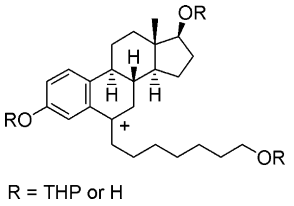
As reported in Scheme 1, compounds **11**–**14** were synthesized from key intermediates **19** and **21**, differing only in their stereochemistry in C6-position. They both were synthesized from the same precursor **18**, which was obtained from diTHP-E_2 (**16**) by an oxidation at C6 giving **17**[20,21] and the addition of a Grignard reagent.



Scheme 1. Reagents and conditions: (a) i—BuLi, diisopropylamine, *t*-BuOK, steroid **16**, −78 °C, 3 h; ii—$B(OMe)_3$, 0 °C, 2 h; iii—H_2O_2, 1 h, rt; iv—PCC, NaOAc, CH_2Cl_2, 0 °C, 15 min, then 1 h, rt (78%); (b) i—Mg, $Br(CH_2)_7OTHP$, Et_2O, THF, rt, 1.5 h; ii—$CeCl_3$, THF, −78 °C, 1.5 h, then steroid **17**, −78 °C, 5 min; iii—−40 °C, 4 h (99%); (c) Et_3SiH, BF_3–Et_2O, CH_2Cl_2, rt, 1 h (56%); (d) 5% HCl, EtOH, THF, 90 °C, 1 h (72%); (e) 10% Pd/C (15% w/w), H_2, EtOAc, rt, 4.5 h (95%).

An excess of in situ generated tetrahydropyranyloxyheptyl magnesium bromide[22] and cerium chloride was used for the Grignard reaction[23,24] that afforded a 5:2 ratio of diastereomers **18a** and **18b** in a nearly quantitative yield.

Compound **19**, 6α-(7-hydroxyheptyl)-E_2, was obtained in 56% yield by a treatment of **18** with triethylsilane and borontrifluoride etherate (Pathway A in Scheme 1).[25,26] A secondary minor product of unknown structure was also isolated. In the conditions of deoxygenation, the tetrahydropyranyl (THP) groups were cleaved to give the free hydroxyl groups. Only one isomer was obtained because the intermediate carbocation was attacked by the in situ generated hydride using the less hindered β-face. Indeed the 7α-H and 9α-H direct the hydride attack to the β-face (Fig. 3). For the synthesis of 6β-(7-hydroxyheptyl)-E_2 (**21**), the deoxygenation step was replaced by two steps:[27] a dehydration of **18** with 5% HCl in ethanol at reflux to give the olefin **20**, followed by a catalytic hydrogenation with palladium/carbon in ethylacetate (Pathway B) to produce a 95:5 mixture of the desired 6β-isomer **21** and 6α-isomer **19** in excellent yield. The stereoselectivity of the double bond reduction is explained by the palladium complexation, which was achieved on the less hindered α-face of olefin **20**.



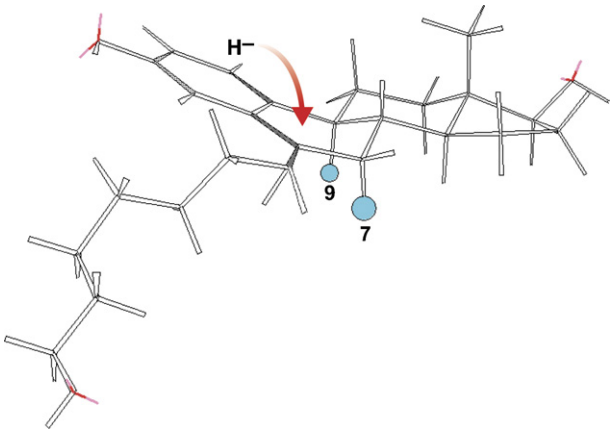


Figure 3. Attack of in situ generated hydride on the β face of the intermediate carbocation. The 3D structure was generated with CSChem 3D std 5.0 (Cambridge Soft Corporation, Cambridge, USA).

The C6-stereochemistry of **19** and **21** was established by NMR analysis. Using a combination of NMR experiments (COSY, APT, HSQC, HMBC and NOESY),[28,29] all proton and carbon signals were fully identified for **19**. Using the signal at 2.88 ppm (6β-CH), the NOESY spectra allowed identifying four signals of different intensity with 4-CH (medium), 7β-CH (strong), 1′-CH_2 (medium) and 8β-CH (strong). Furthermore, no NOE was observed between the C-6 hydrogen and the 9α-CH. Taken together, these data clearly established the 6β-CH (or 6α-side chain) stereochemistry of **19** (Fig. 4).

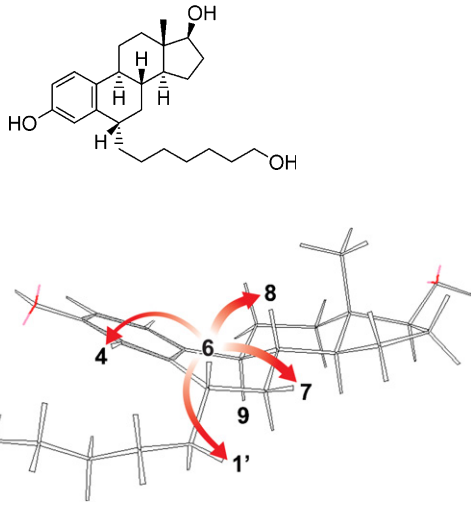
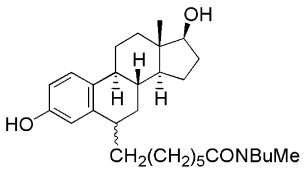
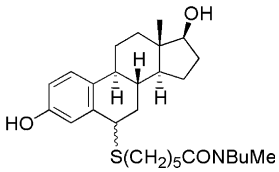


Figure 4. 2D and 3D representations of **19**. The important NOE results are represented by four arrows. The 3D structure was generated with CSChem 3D std 5.0 (Cambridge Soft Corporation, Cambridge, USA).

Table 1. Characteristic ^{1}H NMR signals (ppm) observed for compounds **19** and **21** in $CDCl_3$ and compound **1** and its epimer in acetone-d_6





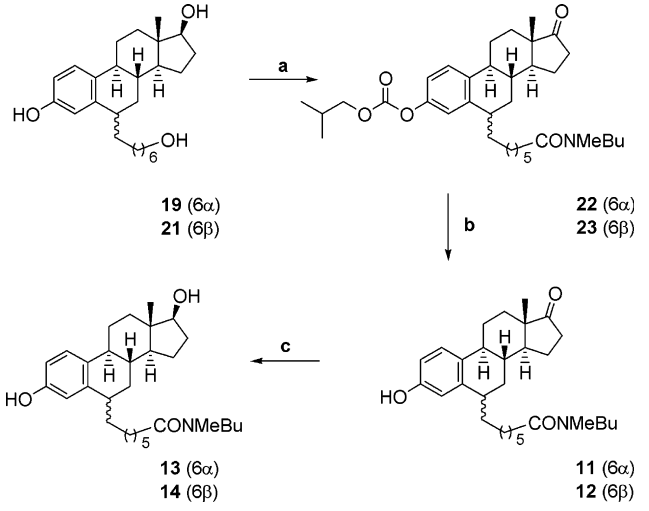
Signal	**19** (6α-side chain)	**21** (6β-side chain)	Epimer of **1**[a] (6α-side chain)	**1**[a] (6β-side chain)
4-CH	6.75	6.54	7.22	6.88
18-CH_3	0.74	0.78	0.76	0.81

[a] Data from Ref. 17.

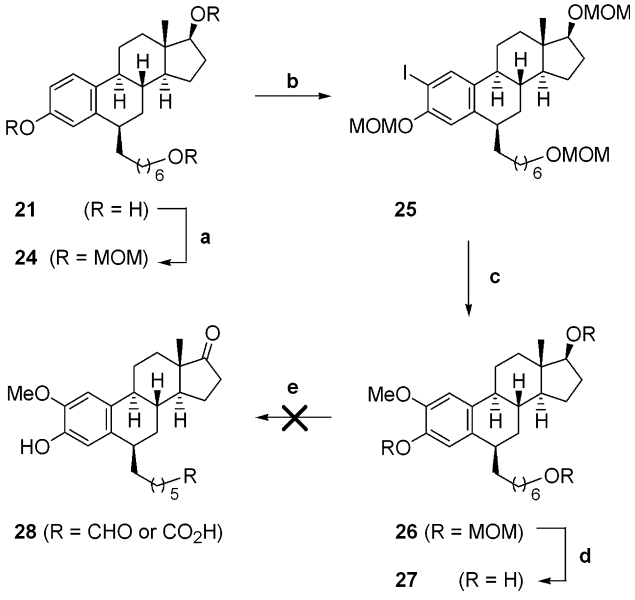
The combination of NMR experiments reported above was also performed for **21**, but the 6α-CH (or 6β-side chain) stereochemistry was not clearly established. The chemical shift of two protons (4-CH and 18-CH_3) of lead compound **1** and its epimer was next compared to the corresponding signals of **19** and **21** (Table 1). For these signals, the chemical displacement tendencies were the same for the two α isomers (**19** and epimer of **1**) as well as for the two β isomers (**21** and **1**).

In the next synthesis step, triols **19** and **21** were oxidized with Jones' reagent to give the corresponding carboxylic acid which, upon treatment with tributylamine, isobutylchloroformate and *N*-methyl-*N*-butylamine,[30] yielded 50% and 59% of amides **22** and **23**, respectively (two steps, Scheme 2). In the results of ^{1}H NMR spectroscopy, the methyl of the amide group ($CONCH_3$) appeared as two singlets at 2.91/2.96 and 2.91/2.97 ppm for **22** and **23**, respectively, while the methylene of $CONCH_2$ exhibited two triplets at 3.25/3.35 and 3.26/3.36 ppm. Moreover, the CH_3 of the *N*-butyl group as well as protons and carbons surrounding the amide function were also duplicated. The duplication of these NMR signals can be explained by the two conformations of the amide bond and is typical of such alkylamide compounds.[31,32] In the process, the 17β-hydroxyl group was oxidized to a ketone and the phenolate group was protected as an isobutylcarbonate group. The deprotection was easily performed using a solution of aqueous potassium carbonate in methanol to give **11** and **12** in 94% and 96% yield, respectively. These compounds were next treated with sodium borohydride to stereoselectively reduce the C17-carbonyl into alcohols **13** and **14** in excellent yields.

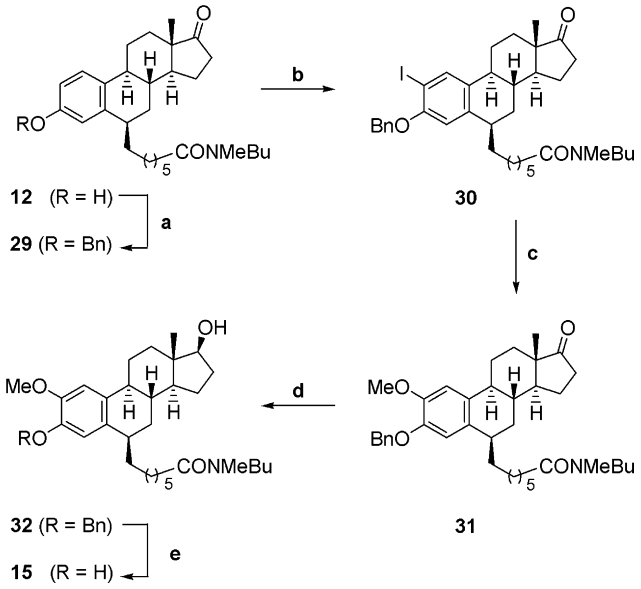
The synthesis of the 2-methoxy analogue of **12**, compound **15**, is reported in Schemes 3 and 4. In the first strategy (Scheme 3), triol **21** was protected by three methoxymethylether (MOM) groups to obtain **24** which was treated with sodium carbonate, iodine and an excess of silver trifluoroacetate[33] to give in quantitative yield a mixture of 2-iodo and 4-iodo compounds with a 95/5 regioselectivity. The major product **25** was then transformed into C2-methoxy derivative **26** in 74% yield by a treatment with sodium methylate, 15-crown-5 ether



Scheme 2. Reagents and conditions: (a) i—Jones' reagent, acetone, rt, 20 min; ii—Bu_3N, $(CH_3)_2CHCH_2OCOCl$, CH_2Cl_2, −10 °C, 40 min, then BuMeNH, −10 °C, 5 min; iii—3 h, rt (50% for **22** and 59% for **23**); (b) 1% K_2CO_3, H_2O, MeOH, rt, 3 h (94% for **11** and 96% for **12**); (c) $NaBH_4$, MeOH, 0 °C, 0.5 h (99% for **13** and 91% for **14**). 6α and 6β correspond to the isomer with a 6α- and a 6β-side chain, respectively.



Scheme 3. Reagents and conditions: (a) MOMCl, diisopropylethylamine, CH_2Cl_2, THF, rt, 18 h (59%); (b) $NaHCO_3$, CF_3COOAg, I_2, CH_2Cl_2, −40 °C, 4 h (100%); (c) MeONa, 15-crown-5, CuI, MeOH, DMF, 120 °C, 12 h (74%); (d) 5% HCl, MeOH, 90 °C, 2.5 h (91%); (e) Jones' reagent, rt or TPAP, NMO, rt or Dess–Martin, rt or IBX polystyrene, 0 °C.



Scheme 4. Reagents and conditions: (a) Cs_2CO_3, benzyl bromide, CH_3CN, 100 °C, 2.5 h (100%); (b) $NaHCO_3$, CF_3COOAg, I_2, CH_2Cl_2, −40 °C, 4 h (85%); (c) MeONa, CuI, MeOH, DMF, 120 °C, 12 h (66%); (d) $NaBH_4$, MeOH, 0 °C, 0.5 h (99%); (e) $Pd(OH)_2$ (10% w/w), H_2, EtOAc, rt, 12 h (98%).

and a catalytic amount of copper iodide warmed at 120 °C overnight.[34] After removal of MOM groups with 5% HCl in methanol, triol **27** was submitted to oxidation using a variety of reagents. Unfortunately, all the methods we tried (Jones' reagent, TPAP/NMO, Dess–Martin or IBX-polystyrene) failed to provide **28** as the corresponding carboxylic acid or aldehyde. Contrary to phenols **19** and **21**, which were oxidized with Jones' reagent, the 2-methoxy phenol nucleus of **27** is too sensitive to oxidants and undergoes degradation. Although a selective protection of the phenol over the two remaining alcohols would also be a valuable alternative strategy, we decided to use instead the second strategy reported in Scheme 4.

In the second approach, we started by a C3-benzylation of **12**, yielding compound **29** (Scheme 4). The iodation at the C2-position of **29** gave the 2-iodo (85%) and 4-iodo (3%) compounds after chromatography. Nucleophilic substitution of the iodide of compound **30** with sodium methylate and a stoichiometric amount of copper iodide afforded **31**, bearing the C2-methoxy group, in 66% yield. It was not possible to increase this yield when we tried milder conditions using a catalytic amount of copper iodide (0.3 or 0.7 equiv). Under such conditions, the nucleophilic substitution was obtained in 50% yield, but the mixture of starting material **30** and methoxylated product **31** was not separable by chromatography. Finally, the reduction of 17-ketosteroid **31** gave **32**, which after removal of benzyl ether protection afforded **15**.

2.2. Biological activity

The four possible metabolites of **1,** compounds **7**–**10**, were tested as inhibitors in intact cells. None of them however inhibited the type 1 17β-HSD overexpressed in HEK-293 cells (data not shown). In addition of confirming the importance of the C6-side chain for enzyme inhibition, this result also indicated that the residual estrogenicity observed for **1**[17] was not the result of metabolites such as **7**–**10**, which are known to be estrogenic compounds.[35] Although we concluded that a metabolite of **1** was not responsible for its estrogenicity, the presence of a thioether at the C6 position of E_2 prevented the synthesis of methoxy derivative **5**. The thioether (C–S) bond was then replaced by a more stable C–C bond allowing the chemical synthesis of **11**–**15**.

2.2.1. Inhibitory potency in homogenated HEK-293 cells. We used homogenated HEK-293 cells overexpressing type 1 17β-HSD to evaluate the ability of compounds **11**–**14** to inhibit the transformation of E_1 into E_2 (Table 2). We first compared the inhibitory potency of 17-ketones **11** and **12** and that of 17β-alcohols **13** and **14**. For compounds **12** and **14** (β-oriented side chain at position C6), it is clear that the alcohol **14** was a better inhibitor (46% of inhibition, as compared to 23%) at 1 μM. Compounds **11** and **13** with an α-oriented C6 side chain produced equivalent and very low inhibition values (16% and 17%). We next determined the effect of the α and β orientation of the C6 side chain (**13** vs **14** and **11** vs **12**). Results previously obtained clearly demonstrated that the β orientation was crucial to the inhibitory activity of type 1 17β-HSD.[17] As expected, the new results indicated that compound **14** with a β-oriented side chain is a better inhibitor at 1 μM than compound **13** with an α orientation. At 0.1 μM, the difference of inhibitory activity was however not significant. For less potent 17-ketone inhibitors **11** and **12**, the differences were not significant at both concentrations, but the inhibitory values are too low to allow a good comparison. Moreover, we compared **1** (C–S bond at position 6) with its C–C analogue **14** and this modification decreased the inhibition from 74% to 46% at 1 μM.

2.2.2. Inhibitory potency in intact HEK-293 cells. Purified enzymes, as well as homogenated cells overexpressing a functional enzyme, are certainly useful models for development of inhibitors. However, they differ much from actual intact breast cancer cells exerting a 17β-HSD activity, an experimental model that constitutes a better approximation of physiological conditions. So we also performed the inhibition assay in intact HEK-293 cells overexpressing type 1 17β-HSD, and in a tumoral cell line (next section). All inhibition values obtained with **11**–**14** are weaker in intact cells than in homogenated cells. The significant drop in inhibitory potency could be explained by the fact that compounds must cross the cell membrane and resist to metabolization. Nonetheless, inhibition values obtained with **11**–**14** showed the same pattern of inhibition as in homogenated cells (Table 2). Inhibitor **1** with a C–S bond has again a better inhibitory potency (63% and 45%) than **14** (49% and 21%) at 10 and 1 μM, respectively. The β orientation of the side chain is clearly important for the inhibitory activity as well as the presence of a 17β-hydroxyl group. Since the steroidal nucleus may confer estrogenic activities to inhibitors **11**–**14**, we synthesized an additional

Table 2. Percentage of inhibition of type 1 17β-HSD using several inhibition assays

Compound	C17 group	C6 side chain	HEK-293 homogenated[a] (%)		HEK-293 intact cells[b] (%)			T-47D intact cells[c] (%)		
			1 μM	0.1 μM	10 μM	1 μM	0.1 μM	10 μM	1 μM	0.1 μM
1	17β-OH	β	74	33	63	45	11	95	82	31
11	C=O	α	16	3	14	5	3	71	19	7
12	C=O	β	23	12	31	20	8	92	57	19
13	17β-OH	α	17	9	15	11	3	74	21	9
14	17β-OH	β	46	15	49	21	11	95	82	29
15	17β-OH	β	N/D	N/D	28	15	13	81	32	11
E_1	C=O	—	59	19	83	38	8	93	85	36

N/D, not determined.
[a] SD < ±5%. One run in triplicate.
[b] SD < ±8%. Mean of five experiments in quadruplicate.
[c] SD < ±5% . Mean of two experiments in triplicate.

compound, the 2-MeO analogue of **14**, compound **15**. The 2-MeO group decreased the inhibitory activity of **14** from 49% to 28% at 10 μM.

2.2.3. Inhibitory potency in breast cancer cell line T-47D. After we performed inhibition assays of compounds **11**–**15** with HEK-293 cells overexpressing type 1 17β-HSD in homogenated and intact cells, we repeated the assays with the ER+ breast cancer cell line T-47D (Table 2). This cell line, which is known for its expression of type 1 17β-HSD,[36] yet constitutes a more physiological model than transfected laboratory HEK-293 cells. All inhibitors have a better inhibitory activity in T-47D cells than in homogenated or intact HEK-293 cells. The better inhibitory effect on T-47D intact cells (specific activity = 10 pmol/h/10^6 cells) than intact HEK-293 cells (specific activity = 12.5 nmol/h/10^6 cells) could probably be due to higher amount of enzymes found in transfected HEK-293 cells (1250-fold). Unlike results obtained above with **1** and **14** either in homogenates or in intact HEK-293 cells, in T-47D cells these compounds have the same inhibitory potency at 10, 1 and 0.1 μM. Changing from a C–S bond to a C–C bond did not influence the inhibitory activity. Results also indicated that inhibitors **11** and **13**, with the α-oriented side chain, showed weaker inhibition values with about 70% and 20% at 10 and 1 μM, respectively. Among the C-6β series of inhibitors, the best inhibitory activity at 1 μM (82%) was obtained for compound **14** with the hydroxyl group at position 17. The keto analogue **12** inhibited 57% of the transformation of E_1 into E_2. As in the HEK-293 model, alcohol **13** and ketone **11,** with an α-orientation of the side chain, gave similar low percentages of inhibition. Again, compound **15** (2-MeO) has a lower inhibitory activity than **14** (2-H) with 82% and 32% of inhibition at 1 μM, respectively.

2.2.4. Selectivity towards other reductive isoforms of 17β-HSD (types 7 and 12). We studied the selectivity of **11**–**15** over other reductive isoforms of 17β-HSDs, types 7 and 12. All enzymatic assays were performed with intact HEK-293 cells overexpressing type 7 or 12, and we tested the ability of compounds to inhibit the transformation of E_1 into E_2 (Table 3). Compounds **11**–**15** weakly inhibited type 7 17β-HSD with inhibition values of 0% to 11% at 1 μM and 17% to 31% at the higher concentration of 10 μM. Type 12 17β-HSD was not inhibited by any compound at 1 μM but inhibition values of 22% to 44% were obtained at 10 μM. Enzymatic assays performed in our laboratory with other compounds having an alkylamide chain have however demonstrated that this kind of inhibitor was less selective at higher concentrations.

Table 3. Percentage of inhibition of other 17β-HSD isoforms using intact cells HEK-293 overexpressing type 7 or 12 17β-HSD

Compound	Type 7[a] (%)		Type 12[b] (%)	
	10 μM	1 μM	10 μM	1 μM
1	25	9	22	0
11	29	5	44	3
12	31	7	40	0
13	24	11	30	1
14	27	0	30	1
15	17	0	22	0
E_1	0	0	0	0

[a] SD < ±5%. One run in triplicate.
[b] SD < ±5%. Mean of two experiments in triplicate.

2.3. Proliferative activity

In order to determine the possible undesirable estrogenic activity of our inhibitors, we selected compounds **12**, **14** and **15** as well as lead compound **1** for performing proliferative assays with two breast cancer cell lines, T-47D and MCF-7, which are known to express the estrogen receptor (ER). For comparison purposes, cell growth in absence of E_2 was assigned to be 100% and in presence of E_2 or other compounds was expressed as percentage of control (Fig. 5). Our data showed that a treatment of estrogen-starved T-47D cells with 10 nM of E_2 for 10 days increased the cell growth 3-fold over basal level. Only compound **1** at 1 μM produced a similarly strong proliferative effect. At lower concentrations, 0.1 and 0.01 μM, compound **1** augmented 2.3-fold the cell proliferation. Switching from a C–S bond (compound **1**) to a C–C bond (compound **14**) decreased significantly the proliferative activity. Although we were expecting a much weaker proliferative effect with **12** because of the lower affinity of a ketone (like E_1 derivative) for the ER, the ketone analogue **12** stimulated cell growth similarly as **14**. Results showed a

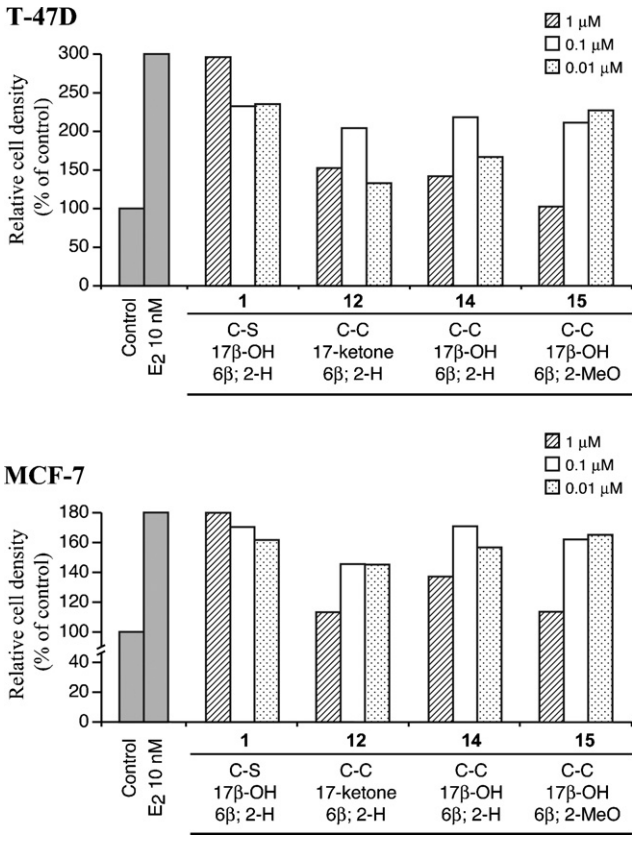


Figure 5. Effects of selected inhibitors on the growth of estrogen-starved T-47D and MCF-7 cells after 10 days of treatment. Control is fixed as 100%. SEM $\leqslant$ 5%.

slight decrease of the proliferative activity at 0.1 and 0.01 μM for **12** when compared with **14**. This result is supported by the stronger binding affinity on ER for E_2 than for E_1.[37,38] The 2-MeO derivative **15** has no proliferative activity on T-47D cells at 1 μM, but we observed a higher cell growth at lower concentrations that reached 2.3-fold over basal level at 0.01 μM. This increasing proliferative activity with decreasing concentration is supported by the fact that 2-MeO-E_2 decreases cell growth of many cancer cell lines at high concentrations but exerts some affinity for the ER at lower concentrations.[39–41]

To improve the evaluation of proliferative activity of our compounds, we used another ER+ breast cancer cell line, MCF-7 (Fig. 5; lower panel). The results indicated that a 10-day treatment of estrogen-starved MCF-7 cells with 10 nM E_2 increased the cell growth 1.8-fold over control level. As observed in T-47D cells, compound **1** was the only one to stimulate cell growth to this level. At a lower concentration of 0.01 μM, data showed that the proliferative effect decreased to 1.6-fold over control. Comparison between **1** and **14** indicated a decrease of proliferative activity to 1.3-fold at 1 μM only by switching the C–S bond to a C–C bond. As expected, the ketone (C=O) group at C17-position of **12** decreased the proliferative activity at all three concentrations when compared to **14** (17β-OH). Results for the 2-MeO compound **15** indicated an increasing proliferative activity from 1.1-fold at 1 μM to 1.7-fold at 0.01 μM over basal level. These results showed the same tendencies as proliferative activities on T-47D.

3. Conclusion

Five new inhibitors of type 1 17β-HSD, compounds **11**–**15**, were efficiently prepared from E_2, purified by chromatography and fully characterized by spectroscopic analysis (IR, NMR and MS). For the inhibitory activity on type 1 17β-HSD, a better enzyme inhibition was obtained for compounds **12** and **14** having a 6β-orientation of the alkylamide side chain (compared to 6α-analogues **11** and **13**). A slightly better enzyme inhibition was obtained for compounds **13** and **14** having a 17β-hydroxy, when compared to 17-ketone analogues **11** and **12**. The presence of a 2-methoxy group on compound **15** reduced the inhibitory activity compared to that of compound **14**. Compounds **14** and **1**, with a C–C and a C–S bond, respectively, gave the same inhibitory potency for type 1 17β-HSD in T-47D cells, but the C–C bond is more stable. Selectivity of compounds **11**–**15** over types 7 and 12 17β-HSDs was also investigated, and they did not inhibit these isoforms at 1 μM.

For the proliferative activity on ER+ cell lines, the three C–C inhibitors tested (compounds **12**, **14** and **15**) were clearly less estrogenic than the C–S lead compound **1**. The results were less impressive in MCF-7 cells but followed the same pattern observed in T-47D. The use of a more stable C–C alkylamide side chain is not detrimental for enzyme inhibition in T-47D cells and allowed the synthesis of a 2-MeO analogue (which was not possible for the C–S lead compound **1**). Even if the 2-MeO analogue **15** exerted a weak inhibition of type 1 17β-HSD at 1 μM, we succeeded in eliminating all the remaining estrogenic activity in both ER+ cell models. Furthermore, at 1 μM, compound **14** inhibited over 80% of type 1 17β-HSD activity and it is 2.1-fold less estrogenic than compound **1** in T-47D breast cancer cells. Compound **14** arises from this study as the most active inhibitor of type 1 17β-HSD.

4. Experimental

4.1. Chemistry

Compounds **8**–**10** and starting material (estradiol) for the synthesis of **11**–**15** were purchased from Steraloids Inc. (Newport, RI), whereas compound **7** was available in our laboratory. Anhydrous reactions were performed in oven-dried glassware under positive argon pressure using commercially available anhydrous solvents (Gibbstown, NJ, USA), except THF which was distilled from sodium/benzophenone ketyl under argon. Chemical reagents were purchased from Aldrich Chemical Co (Milwaukee, WI, USA). Flash chromatography was performed on Silicycle 60 230–400-mesh silica gel (Québec, Qc, Canada). Thin-layer chromatography (TLC) was performed on 0.25-mm silica gel 60 F_{254} plates (Nepean, Ontario, Canada) and compounds were visualized by exposure to UV light (254 nm), a solution of

ammonium molybdate/sulfuric acid/water and/or a solution of para-anisaldehyde/sulfuric acid/acetic acid/ethanol (plus heating). Infrared (IR) spectra (Perkin-Elmer 1600, Norwalk, CT, USA) were obtained from a thin film of the solubilized compound on NaCl pellets (usually in CH_2Cl_2) or in KBr pellets containing the solid compound. Only significant bands are reported (in cm^{-1}). ^1H and ^{13}C spectra were recorded at 300 (^1H) and 75.5 (^{13}C) MHz or at 400 (^1H) and 100 (^{13}C) MHz using a Bruker AC/F 300 or a Brucker AVANCE 400 spectrometer (Billerica, MA, USA). The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm) or methanol (3.31 and 49.0 ppm) for ^1H and ^{13}C, respectively. Carbon assignments were reported only for key intermediates **19** and **21** and for final compounds **11**–**15**. Low-resolution mass spectra (LRMS) were recorded with an LCQ Finnigan apparatus (San Jose, CA, USA) equipped with an atmospheric pressure chemical ionisation (APCI) source on positive mode. High-resolution mass spectra (HRMS) were provided by the Regional Laboratory for Instrumental Analysis (Université de Montréal, Montréal, Canada). High-performance liquid chromatography (HPLC) analyses were carried out using a Waters Associates System (Milford, MA, USA), a Nova-Pack C18 column (150 × 3.9 mm, 4 μm, 60 Å) and a solution of MeOH containing 20 mM $AcONH_4$ as eluent (1 mL/min flow rate).

4.1.1. Synthesis of 3,17β-(ditetrahydro-2″-*H*-pyran-2″-yloxy)-6-[7′-(tetrahydro-2″-*H*-pyran-2″-yloxy)-heptyl]-estra-1,3,5(10)-trien-6-ol (18). To a stirred solution of magnesium (528 mg, 22.3 mmol) in dry Et_2O (7.5 mL) was added 7-(tetrahydro-2′-*H*-pyran-2′-yloxy)-heptyl bromide[22] (3.07 g, 11.0 mmol) in Et_2O (7.5 mL) and THF (2.5 mL) over a period of 0.5 h. The reaction mixture was stirred under nitrogen for 1 h at rt then THF (1 mL) was added. A mixture of anhydrous $CeCl_3$ (2.71 g, 11.0 mmol) in dry THF (150 mL) was stirred under nitrogen for 18 h at rt, then cooled at −78 °C, and the Grignard reagent solution was added slowly to this reaction mixture. After 1.5 h at −78 °C, ketone **17**[18–21] (1.07 g, 2.35 mmol) in THF (80 mL) was added at −78 °C. After being stirred at −40 °C for 4 h, the reaction mixture was quenched with saturated aq NH_4Cl (100 mL) and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The crude alcohols **18** were a 5:2 mixture of two diastereomers. Purification of this mixture by flash chromatography (hexanes/EtOAc, 90:10 then 85:15) afforded three fractions containing 0.41 g of **18a**, 0.08 g of a mixture of **18a** and **18 b** and 1.03 g of **18 b** (1.52 g, 99% yield). Minor diastereomer **18a**: $R_f = 0.56$ (hexanes/EtOAc, 70:30); Colorless oil; IR (film) ν 3363 (OH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.79 and 0.81 (2s, 18-CH_3), 1.00–2.35 (m, 43H, CH and CH_2 of steroid skeleton, aliphatic chain and THP groups), 3.36 (m, 1H), 3.49 (m, 2H), 3.60 (m, 1H), 3.71 (m, 2H), 3.89 (m, 3H) (9H of 17α-CH and 4× OCH_2 of aliphatic chain and THP groups), 4.56 (m, O–CH–O), 4.66 (m, O–CH–O), 5.36 (m, ArO–CH–O), 6.96 (dd, $J_2 = 8.5$ Hz, $J_1 = 2.5$ Hz, 2-CH), 7.18 (d, $J = 2.5$ Hz, 4-CH), 7.22 (2d, $J = 8.4$ Hz, 1-CH); LRMS calcd for $C_{40}H_{63}O_7$–H_2O $[M+H–H_2O]^+$ 637.4. Major diastereomer **18b**: $R_f = 0.44$ (hexanes/EtOAc, 70:30); Colorless oil; IR (film) ν 3358 (OH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 and 0.82 (2s, 18-CH_3), 1.15–2.35 (m, 43H, CH and CH_2 of steroid skeleton, aliphatic chain and THP groups), 3.38 (m, 1H), 3.50 (m, 2H), 3.64 (m, 1H), 3.72 (m, 2H), 3.90 (m, 3H) (9H of 17α-CH and 4× OCH_2 of aliphatic chain and THP groups), 4.57 (m, O–CH–O), 4.66 (m, O–CH–O), 5.42 (m, ArO–CH–O), 6.92 (dd, $J_2 = 8.5$ Hz, $J_1 = 2.5$ Hz, 2-CH), 7.17 (2d, $J = 8.6$ Hz, 1-CH), 7.23 (t_{app}, $J = 2.7$ Hz, 4-CH); LRMS calcd for $C_{40}H_{62}O_7Na$ $[M+Na]^+$ 677.4.

4.1.2. Synthesis of 7-(3′,17β′-dihydroxy-estra-1′,3′,5′(10′)-trien-6′α-yl)-heptanol (19). To a stirred solution of **18b** (334 mg, 0.518 mmol) in CH_2Cl_2 (11 mL) were added triethylsilane (2.5 mL) and $BF_3{\cdot}Et_2O$ (5.3 mL) at 0 °C. The reaction mixture was stirred under nitrogen for 1 h at rt, poured into ice-cold aq 10% K_2CO_3 (5 mL) and extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography (hexanes/EtOAc, 40:60 then 30:70) to afford **19** (113 mg, 56% yield) as colourless oil. When this reaction was performed with **18a**, compound **19** was also obtained. IR (film) ν 3315 (OH); ^1H NMR (400 MHz, $CDCl_3$) δ 0.75 (s, 18′-CH_3), 0.90–2.35 (m, 25H, CH and CH_2 of steroid skeleton and aliphatic chain), 2.88 (m, 6′β-CH), 3.66 (m, CH_2OH), 3.72 (t, $J = 8.5$ Hz, 17′α-CH), 6.62 (dd, $J_2 = 8.4$ Hz, $J_1 = 2.5$ Hz, 2′-CH), 6.75 (d, $J = 2.2$ Hz, 4′-CH), 7.14 (d, $J = 8.4$ Hz, 1′-CH);^{13}C NMR (75 MHz, CD_3OD) δ 11.6 (C18′), 24.1 (C15′), 27.0 (C3), 27.1, 30.6 and 31.1 (C4–C6), 27.7 (C11′), 30.7 (C16′), 33.6 (C2), 35.5 (C7′), 37.9 (C12′), 39.0 (C7), 39.2 (C6′), 40.1 (C8′), 44.1 (C13′), 45.0 (C9′), 51.4 (C14′), 63.0 (C1), 82.5 (C17′), 113.5 (C2′), 115.0 (C4′), 126.9 (C1′), 133.4 (C10′), 142.8 (C5′), 156.0 (C3′); LRMS calcd for $C_{25}H_{39}O_3$ $[M+H]^+$ 387.2.

4.1.3. Synthesis of 7-(3′,17β′-dihydroxy-estra-1′,3′,5′(10′),6′(7′)-tetraen-6-yl)-heptanol (20). A solution of **18a** and **18b** (150 mg, 0.233 mmol) in ethanol (2.6 mL) was treated with concentrated HCl (0.13 mL). The resulting mixture was stirred at reflux for 1 h. The reaction mixture was neutralized with saturated aq $NaHCO_3$, extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated. The crude product was purified by chromatography (hexanes/EtOAc, 70:30 then 55:45) to afford **20** (65 mg, 72% yield) as a white solid. IR (film) ν 3352 (OH); ^1H NMR (400 MHz, CD_3OD) δ 0.75 (s, 18′-CH_3), 0.80–2.65 (m, 23H, CH and CH_2 of steroid skeleton and aliphatic chain), 3.53 (m, CH_2OH), 3.67 (t, $J = 8.5$ Hz, 17′α-CH), 5.74 (s, 7′-CH), 6.60 (dd, $J_2 = 8.2$ Hz, $J_1 = 2.5$ Hz, 2′-CH), 6.73 (d, $J = 2.4$ Hz, 4′-CH), 7.07 (d, $J = 8.3$ Hz, 1′-CH); ^{13}C NMR (75 MHz, CD_3OD) δ 11.5, 24.0, 25.6, 26.9, 29.7, 30.4, 30.5, 30.6, 33.6, 33.9, 37.5, 40.0, 43.7, 44.1, 44.8, 63.0, 82.3, 111.3, 113.9, 125.2, 129.6, 132.7, 137.3, 137.9, 156.6; LRMS calcd for $C_{25}H_{37}O_3$ $[M+H]^+$ 385.3.

4.1.4. Synthesis of 7-(3′,17β′-dihydroxy-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanol (21). A mixture of **20** (135 mg, 0.35 mmol) and 10% palladium on charcoal (20 mg, 15% in weight) in EtOAc (3.5 mL) under hydrogen

atmosphere was stirred 4.5 h at rt. Then the palladium catalysis was removed by filtration on Celite, washed with EtOAc and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (hexanes/EtOAc, 50:50 then 40:60) to afford a 5:95 mixture of diastereomers **19** and **21** (128 mg, 95%) as colourless oil. IR (film) ν 3333 (OH); ^{1}H NMR (400 MHz, CD_3OD) δ 0.78 (s, 18′-CH_3), 1.10–2.35 (m, 25 H, CH and CH_2 of steroid skeleton and aliphatic chain), 2.65 (m, 6′α-CH), 3.55 (t, J = 6.6 Hz, CH_2OH), 3.65 (t, J = 8.6 Hz, 17′α-CH), 6.54 (m sharp, 2′-CH and 4′-CH), 7.06 (d, J = 9.1 Hz, 1′-CH); ^{13}C NMR (75 MHz, CD_3OD) δ 11.8 (C18′), 24.1 (C15′), 26.9 (C3), 27.4 (C11′), 29.0, 30.5 and 30.8 (C4–C6), 30.7 (C16′), 31.6 (C7′), 33.7 (C2), 35.2 (C8′), 38.0 (C12′), 39.1 (C6′), 39.4 (C7), 44.6 (C13′), 45.9 (C9′), 51.0 (C14′), 63.0 (C1), 82.4 (C17′), 113.8 (C2′), 116.3 (C4′), 127.0 (C1′), 132.2 (C10′), 144.0 (C5′), 155.9 (C3′); LRMS calcd for $C_{25}H_{39}O_3$–H_2O [M+H-H_2O]$^+$ 369.2.

4.1.5. Procedure for amidation of 19 and 21 (synthesis of 22 and 23). A solution of **19** or **21** (110 mg, 0.284 mmol) and Jones' reagent (0.22 mL, 0.594 mmol) in acetone (36 mL) was stirred for 20 min at rt. The reaction was quenched by addition of isopropanol (2 mL) and acetone was evaporated under reduced pressure at rt. Water (15 mL) was added to the slurry and the aq phase was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and evaporated. To a solution of the crude acid in dry CH_2Cl_2 (28 mL) and tributylamine (0.13 mL, 0.54 mmol) was added isobutylchloroformate (0.065 mL, 0.675 mmol) at −10 °C and the mixture was stirred for 40 min at −10 °C. *N*-Methylbutylamine (0.615 mL, 5.175 mmol) was then added slowly at −10 °C and the mixture was stirred for 3 h at rt. The mixture was poured into an ice-cold aq 1 M HCl, the aq phase was extracted with CH_2Cl_2, the organic phase was washed with saturated aq $NaHCO_3$, dried over $MgSO_4$ and evaporated. The crude compound was next purified by flash chromatography (hexanes/EtOAc, 70:30) as eluent to give the desired compound **22** or **23** (59% and 50% yield, respectively).

4.1.5.1. *N*-Butyl-*N*-methyl-7-(3′-isobutyloxycarbonyloxy-17′-oxo-estra-1′,3′,5′(10′)-trien-6′α-yl)-heptanamide (22). Yellow oil (59%). IR (film) ν 1760 and 1739 (C=O, carbonate and ketone), 1620 (C=O, amide); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 18′-CH_3), 0.93 (q_{app}, J = 7.4 Hz, CH_2CH_3), 1.00 (d, J = 6.7 Hz, $CH(CH_3)_2$), 1.00–2.40 (m, 29H, CH and CH_2 of steroid skeleton, chain and isopropylcarbonate), 2.51 (dd, J_2 = 18.3 Hz, J_1 = 8.6 Hz, 16′β-CH), 2.94 (m, 6′β-CH), 2.91 and 2.96 (2s, NCH_3), 3.25 and 3.35 (2m, NCH_2), 4.03 (d, J = 6.7 Hz, OCH_2CH), 6.95 (dd, J_2 = 8.5 Hz, J_1 = 2.3 Hz, 2′-CH), 7.06 (d, J = 2.1 Hz, 4′-CH), 7.27 (d, J = 6.7 Hz, 1′-CH); LRMS calcd for $C_{35}H_{54}NO_5$ [M+H]$^+$ 568.3.

4.1.5.2. *N*-Butyl-*N*-methyl-7-(3′-isobutyloxycarbonyloxy-17′-oxo-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (23). Yellow oil (50%). IR (film) ν 1761 and 1740 (C=O, carbonate and ketone), 1624 (C=O, amide); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (s, 18′-CH_3), 0.94 (m, CH_2CH_3), 1.00 (d, J = 6.7 Hz, $CH(CH_3)_2$), 1.20–2.45 (m, 29 H, CH and CH_2 of steroid skeleton, chain and isopropylcarbonate), 2.51 (dd, J_2 = 18.8 Hz, J_1 = 8.3 Hz, 16′β-CH), 2.79 (m, 6′α-CH), 2.91 and 2.97 (2s, NCH_3), 3.26 and 3.36 (2m, NCH_2), 4.03 (d, J = 6.6 Hz, OCH_2CH), 6.95 (m sharp, 2′-CH and 4′-CH), 7.27 (d, J = 7.7 Hz, 1′-CH); LRMS calcd for $C_{35}H_{54}NO_5$ [M+H]$^+$ 568.2.

4.1.6. Procedure for deprotection of 22 and 23 (synthesis of 11 and 12). Carbonate **22** or **23** (58 mg, 0.102 mmol) was dissolved in MeOH (2.75 mL) and a solution of K_2CO_3 (1%, w:v) in MeOH/H_2O [(25:75, v:v), 2.75 mL] was added. The resulting mixture was stirred at rt for 3 h. Then, the mixture was acidified with aq 1 M HCl; MeOH was evaporated under reduced pressure and the aq phase was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and evaporated. The crude residue was purified by chromatography (hexanes/EtOAc, 60:40) to afford phenol **11** or **12** (96% and 94% yield, respectively).

4.1.6.1. *N*-Butyl-*N*-methyl-7-(3′-hydroxy-17′-oxo-estra-1′,3′,5′(10′)-trien-6′α-yl)-heptanamide (11). White amorphous solid (96%). IR (film) ν 3276 (OH), 1738 (C=O, ketone), 1621 (C=O, amide); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 18′-CH_3), 0.92 and 0.95 (2t, J = 7.2 Hz, CH_2CH_3), 1.20–2.40 (m, 28H, CH and CH_2 of steroid skeleton and chain), 2.50 (dd, J_2 = 18.7 Hz, J_1 = 8.6 Hz, 16′β-CH), 2.91 (m, 6′β-CH), 2.95 and 2.99 (2s, NCH_3), 3.27 and 3.40 (2m, NCH_2), 6.67 (dd, J_2 = 8.4 Hz, J_1 = 2.4 Hz, 2′-CH), 6.93 (dd, J_2 = 7.8 Hz, J_1 = 2.2 Hz, 4′-CH), 7.12 (d, J = 8.5 Hz, 1′-CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8 (C18′), 13.9 (C4″), 19.9 and 20.0 (C3″), 21.6 (C15′), 24.4 and 24.7 (C3), 26.0 (C11′), 27.9, 28.0 and 28.5 (C4–C6), 29.4 and 30.6 (C2″), 31.5 (C12′), 32.7 and 33.3 (C2, C8′), 33.7 and 35.9 (NCH_3), 35.3 (C7′), 35.5 (C16′), 37.8 (C7), 38.2 (C6′), 44.0 (C9′), 47.7 (C1″), 47.8 (C13′), 47.8 and 50.0 (C1″), 50.5 (C14′), 112.9 (C2′), 114.3 (C4′), 126.1 (C1′), 131.7 (C10′), 141.3 (C5′), 154.8 (C3′), 173.4 (C1), 221.2 (C17′); LRMS calcd for $C_{30}H_{46}NO_3$ [M+H]$^+$ 468.3; HRMS calcd for $C_{30}H_{46}NO_3$ [M+H]$^+$ 468.34722, found 468.34686; HPLC purity of 89% (t_R = 31.45 min).

4.1.6.2. *N*-Butyl-*N*-methyl-7-(3′-hydroxy-17′-oxo-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (12). White amorphous solid (96%). IR (film) ν 3265 (OH), 1734 (C=O, ketone), 1621 (C=O, amide); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (s, 18′-CH_3), 0.93 and 0.95 (2t, J = 7.2 Hz, CH_2CH_3), 1.20–2.40 (m, 28 H, CH and CH_2 of steroid skeleton and chain), 2.50 (dd, J_2 = 18.8 Hz, J_1 = 8.2 Hz, 16′β-CH), 2.76 (m, 6′α-CH), 2.94 and 2.99 (2s, NCH_3), 3.28 and 3.37 (2m, NCH_2), 6.67 (dd, J_2 = 8.4 Hz, J_1 = 2.5 Hz, 2′-CH), 6.81 (s, 4′-CH), 7.12 (d, J = 8.4 Hz, 1′-CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9 (C18′ and C4″), 19.9 and 20.0 (C3″), 21.6 (C15′), 24.8 and 25.0 (C3), 25.6 (C11′), 26.9 and 28.4 (2C) (C4–C6), 29.4 and 30.6 (C2″), 30.8 (C7′), 31.5 (C12′), 32.5 and 33.2 (C2), 33.8 and 35.8 (NCH_3),

33.9 (C8′), 35.5 (C16′), 36.6 (C7), 37.2 (C6′), 44.1 (C9′), 47.8 and 49.9 (C1″), 48.2 (C13′), 50.3 (C14′), 112.9 (C2′), 115.5 (C4′), 125.8 (C1′), 131.0 (C10′), 142.7 (C5′), 154.6 (C3′), 173.2 (C1), 221.2 (C17′); LRMS calcd for $C_{30}H_{46}NO_3$ $[M+H]^+$ 468.4; HRMS calcd for $C_{30}H_{46}NO_3$ $[M+H]^+$ 468.34722, found 468.34685; HPLC purity of 77% for **12** (t_R = 31.24 min).

4.1.7. Procedure for reduction of 11 and 12 (synthesis of 13 and 14). $NaBH_4$ (4 mg, 0.105 mmol) was added to a cooled (0 °C) solution of **11** or **12** (34 mg, 0.073 mmol) in MeOH (2 mL). After the mixture was stirred for 0.5 h at 0 °C, the reaction was quenched by addition of water and extraction was performed with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and evaporated to dryness. The crude residue was purified by chromatography (hexanes/EtOAc, 50:50) to afford **13** or **14** (91% and 99% yield, respectively).

4.1.7.1. *N*-Butyl-*N*-methyl-7-(3′,17β′-dihydroxy-estra-1′,3′,5′(10′)-trien-6′α-yl)-heptanamide (13). White amorphous solid (91%). IR (film) *v* 3298 (OH), 1622 (C=O, amide); ^{1}H NMR (400 MHz, CD_3OD) δ 0.74 (s, 18′-CH_3), 0.93 and 0.97 (2t, J = 7.3 Hz, CH_2CH_3), 1.00–2.40 (m, 29H, CH and CH_2 of steroid skeleton and chain), 2.86 (m, 6′β-CH), 2.89 and 3.02 (2s, NCH_3), 3.32 (m, NCH_2), 3.65 (t, J = 8.6 Hz, 17′α-CH), 6.53 (dd, J_2 = 8.4 Hz, J_1 = 2.5 Hz, 2′-CH), 6.67 (d, J = 2.3 Hz, 4′-CH), 7.07 (d, J = 8.5 Hz, 1′-CH); ^{13}C NMR (75 MHz, CD_3OD) δ 11.6 (C18′), 14.2 (C4″), 20.9 and 21.0 (C3″), 24.1 (C15′), 26.3 and 26.7 (C3), 26.9 and 30.4 (2C) (C4–C6), 27.7 (C11′), 30.7 (C16′), 30.8 and 31.7 (C2″), 33.7 (C2), 33.9 and 36.0 (NCH_3), 34.4 (C2), 35.4 (C7′), 37.9 (C12′), 38.9 (C7), 39.1 (C6′), 40.1 (C8′), 44.1 (C13′), 45.0 (C9′), 48.6 and 51.0 (C1″), 51.5 (C14′), 82.5 (C17′), 113.5 (C2′), 115.0 (C4′), 126.9 (C1′), 133.4 (C10′), 142.8 (C5′), 156.1 (C3′), 175.5 (C1); LRMS calcd for $C_{30}H_{48}NO_3$ $[M+H]^+$ 470.3; HRMS calcd for $C_{30}H_{48}NO_3$ $[M+H]^+$ 470.36287, found 470.36383; HPLC purity of 89% (t_R = 31.06 min).

4.1.7.2. *N*-Butyl-*N*-methyl-7-(3′,17β′-dihydroxy-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (14). White amorphous solid (99%). IR (film) *v* 3272 (OH), 1618 (C=O, amide); ^{1}H NMR (400 MHz, CD_3OD) δ 0.78 (s, 18′-CH_3), 0.93 and 0.97 (2t, J = 7.1 Hz, CH_2CH_3), 1.10-2.45 (m, 29H, CH and CH_2 of steroid skeleton and chain), 2.69 (m, 6′α-CH), 2.90 and 3.03 (2s, NCH_3), 3.36 (m, NCH_2), 3.65 (t, J = 8.6 Hz, 17′α-CH), 6.54 (m, 2′-CH and 4′-CH), 7.06 (d, J = 9.0 Hz, 1′-CH); ^{13}C NMR (75 MHz, CD_3OD) δ 11.8 (C18′), 14.2 (C4″), 21.0 (C3″), 24.1 (C15′), 26.3 and 26.7 (C3), 27.4 (C11′), 28.9 and 30.4 (2C) (C4–C6), 30.6 (C2″), 30.7 (C16′), 31.7 (C7′), 33.7 (C2), 33.9 and 36.0 (NCH_3), 34.4 (C2), 35.3 (C8′), 38.0 (C12′), 39.1 (C6′), 39.4 (C7), 44.6 (C13′), 45.9 (C9′), 48.6 and 51.0 (C1″), 51.1 (C14′), 82.4 (C17′), 113.9 (C2′), 116.3 (C4′), 127.0 (C1′), 132.2 (C10′), 144.0 (C9′), 155.9 (C3′), 175.5 (C1); LRMS calcd for $C_{30}H_{48}NO_3$ $[M+H]^+$ 470.3; HRMS calcd for $C_{30}H_{48}NO_3$ $[M+H]^+$ 470.36287, found 470.36402; HPLC purity of 95% (t_R = 30.91 min).

4.1.8. Synthesis of *N*-butyl-*N*-methyl-7-(3′-benzyloxy-17′-oxo-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (29). A mixture of **12** (41 mg, 0.087 mmol), cesium carbonate (88 mg, 0.27 mmol) and benzyl bromide (0.06 mL, 0.50 mmol) in acetonitrile (1.2 mL) was stirred for 2.5 h at reflux. The reaction mixture was cooled and diluted with EtOAc. Then the organic layer was washed with water, dried over $MgSO_4$, and evaporated. The crude residue was purified by chromatography (hexanes/EtOAc, 90:10 then 30:70) to afford **29** (50 mg, quantitative yield) as yellow oil. IR (film) *v* 1738 (C=O, ketone), 1644 (C=O, amide); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 18′-CH_3), 0.91 (m, CH_2CH_3), 1.20–2.40 (m, 28H, CH and CH_2 of steroid skeleton and chain), 2.51 (dd, J_2 = 18.2 Hz, J_1 = 8.1 Hz, 16′β-CH), 2.77 (m, 6′α-CH), 2.91 and 2.96 (2s, NCH_3), 3.26 and 3.36 (2m, NCH_2), 5.04 (s, OCH_2), 6.79 (m sharp, 2′-CH and 4′-CH), 7.19 (d, J = 9.2 Hz, 1′-CH), 7.38 (m, 5H-phenyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 13.9, 20.0, 21.6, 25.4, 25.6, 28.0, 29.5, 29.6, 29.8, 30.5, 31.6, 32.9, 33.4 (2C), 33.5, 35.4, 35.8 (2C), 37.8, 38.1, 44.4, 47.8, 48.2, 49.7, 50.2, 70.0, 112.0, 115.4, 125.9, 127.5 (2C), 127.9, 128.6 (2C), 132.1, 137.2, 143.0, 156.9, 173.0, 220.9; LRMS calcd for $C_{37}H_{52}NO_3$ $[M+H]^+$ 558.3.

4.1.9. Synthesis of *N*-butyl-*N*-methyl-7-(3′-benzyloxy-2′-iodo-17′-oxo-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (30). To a solution of **29** (58 mg, 0.104 mmol), $NaHCO_3$ (49 mg, 0.585 mmol) and silver trifluoroacetate (28 mg, 0.129 mmol) in dry CH_2Cl_2 (0.42 mL) was added iodine (33 mg, 0.129 mmol) dropwise at −40 °C, and the mixture was stirred for 4 h at −40 °C. The reaction was quenched by addition of Et_3N, then the mixture was poured into a column of silica gel and flash chromatography (hexanes/EtOAc, 90:10 then 30:70) gave the 4-iodo product (2 mg, 3% yield) and the desired 2-iodo product **30** (56 mg, 85 % yield) as yellow oil. IR (film) *v* 1738 (C=O, ketone), 1644 (C=O, amide); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 18′-CH_3), 0.91 (m, CH_2CH_3), 1.20–2.40 (m, 28 H, CH and CH_2 of steroid skeleton and chain), 2.52 (dd, J_2 = 19.0 Hz, J_1 = 8.0 Hz, 16′β-CH), 2.75 (m, 6′α-CH), 2.91 and 2.96 (2s, NCH_3), 3.25 and 3.35 (2m, NCH_2), 5.13 (s, OCH_2), 6.62 (s, 4′-CH), 7.32 (d, J = 7.1 Hz, 4″-CH of phenyl), 7.38 (t_{app}, J = 7.3 Hz, 2″- and 6″-CH of phenyl), 7.51 (d_{app}, J = 7.3 Hz, 3″- and 5″-CH of phenyl), 7.65 (s, 1′-CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9 (2C), 20.1, 21.5, 25.0, 25.4, 25.6, 27.9, 29.5, 29.7, 30.6, 31.5, 32.9, 33.2, 33.4, 33.5 (2C), 35.3, 35.8 (2C), 37.9, 38.2, 44.2, 47.4, 48.1, 49.7, 50.1, 71.0, 83.9, 113.6, 127.0 (2C), 127.8, 128.5 (2C), 134.4, 136.1, 136.8, 143.2, 155.2, 172.7, 220.6; LRMS calcd for $C_{37}H_{51}INO_3$ $[M+H]^+$ 684.2.

4.1.10. Synthesis of *N*-butyl-*N*-methyl-7-(3′-benzyloxy-2′-methoxy-17′-oxo-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (31). Na (88 mg, 3.8 mmol) was dissolved in MeOH (4 mL) under an inert atmosphere at rt. Then DMF (4 mL), **30** (121 mg, 0.190 mmol) in MeOH/DMF (1:1, 6 mL) and CuI (28 mg, 0.195 mmol) were added. The reaction mixture was warmed at 120 °C overnight, diluted with EtOAc, and quenched by addi-

tion of water. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The crude residue was purified by chromatography (hexanes/EtOAc, 60:40) to afford **31** (75 mg, 66% yield) as yellow oil. IR (film) ν 1738 (C=O, ketone), 1644 (C=O, amide); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 18′-CH_3), 0.93 (m, CH_2CH_3), 1.20–2.45 (m, 28H, CH and CH_2 of steroid skeleton and chain), 2.50 (dd, J_2 = 19.0 Hz, J_1 = 8.1 Hz, 16′β-CH), 2.67 (m, 6′α-CH), 2.91 and 2.96 (2s, NCH_3), 3.25 and 3.36 (2t, J = 7.4 Hz, NCH_2), 3.86 (s, OCH_3), 5.13 (d of AB system, J = 3.3 Hz, OCH_2), 6.66 (s, 4′-CH), 6.81 (s, 1′-CH), 7.29 (d, J = 7.3 Hz, 4″-CH of phenyl), 7.36 (t_{app}, J = 7.2 Hz, 2″- and 6″-CH of phenyl), 7.45 (d_{app}, J = 7.0 Hz, 3″- and 5″-CH of phenyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 14.0, 20.0, 21.5, 25.1, 25.5, 25.9, 28.0, 29.5, 29.7, 29.9, 30.6, 31.6, 32.9, 33.2, 33.4, 33.6, 35.3, 35.8 (2C), 37.3, 38.3, 44.8, 47.5, 48.2, 49.8, 50.2, 56.1, 71.3, 109.0, 115.4, 127.3 (2C), 127.7, 128.5 (2C), 132.1, 133.6, 137.5, 146.2, 147.7, 172.9, 220.9; LRMS calcd for $C_{38}H_{54}NO_4$ $[M+H]^+$ 588.3.

4.1.11. *N*-butyl-*N*-methyl-7-(3′-benzyloxy-17β′-hydroxy-2′-methoxy-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (32). The procedure reported above for the synthesis of **13** and **14** was used for the reduction of **31** into **32**. Yellow oil (99%). IR (film) ν 3419 (OH), 1633 (C=O, amide); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 18′-CH_3), 0.92 and 0.95 (2t, J = 7.3 Hz, CH_2CH_3), 1.10–2.35 (m, 29 H, CH and CH_2 of steroid skeleton and chain), 2.62 (m, 6′α-CH), 2.91 and 2.97 (2s, NCH_3), 3.26 and 3.36 (2t, J = 7.5 Hz, NCH_2), 3.74 (t, J = 8.5 Hz, 17′α-CH), 3.86 (s, OCH_3), 5.12 (d of AB system, J = 5.2 Hz, OCH_2), 6.65 (s, 4′-CH), 6.82 (s, 1′-CH), 7.29 (d, J = 7.2 Hz, 4″-CH of phenyl), 7.36 (t_{app}, J = 7.3 Hz, 2″- and 6″-CH of phenyl), 7.45 (d_{app}, J = 7.2 Hz, 3″- and 5″-CH of phenyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.2, 13.9, 20.0, 20.1, 23.1, 23.9, 25.1, 25.5, 26.2, 28.0, 29.4, 29.5, 29.6, 30.6 (2C), 33.0, 33.4, 33.5, 33.6, 35.3, 36.7, 37.4, 38.3, 43.4, 44.8, 47.4, 49.8 (2C), 56.1, 71.3, 81.8, 109.1, 115.4, 127.4, 127.7 (2C), 128.5 (2C), 132.7, 133.9, 137.5, 146.1, 147.6, 172.9; LRMS calcd for $C_{38}H_{56}NO_4$ $[M+H]^+$ 590.3.

4.1.12. Synthesis of *N*-butyl-*N*-methyl-7-(3′,17β′-dihydroxy-2′-methoxy-estra-1′,3′,5′(10′)-trien-6′β-yl)-heptanamide (15). A mixture of **32** (24 mg, 0.041 mmol) and $Pd(OH)_2$ (10% in weight, 3 mg) in EtOAc (0.6 mL) under hydrogen atmosphere was stirred for 12 h at rt. The palladium catalysis was removed by filtration on Celite and washed with EtOAc. The filtrate was evaporated and the crude product was purified by chromatography (hexanes/EtOAc/MeOH, 67:28:5) to afford **15** (19.6 mg, 98% yield) as yellow amorphous solid. IR (film) ν 3350 (OH), 1620 (C=O, amide); ^{1}H NMR (400 MHz, CD_3OD) δ 0.79 (s, 18′-CH_3), 0.93 and 0.97 (2t, J = 7.1 Hz, CH_2CH_3), 1.10–2.40 (m, 29 H, CH and CH_2 of steroid skeleton and chain), 2.64 (m, 6′α-CH), 2.90 and 3.03 (2s, NCH_3), 3.35 (m, NCH_2), 3.66 (t, J = 8.6 Hz, 17′α-CH), 3.80 (s, OCH_3), 6.55 (s, 4′-CH), 6.79 (s, 1′-CH); ^{13}C NMR (75 MHz, CD_3OD) δ 11.8 (C18′), 14.2 (C4″), 20.9 and 21.0 (C3″), 24.1 (C15′), 26.3 and 26.6 (C3), 27.6 (C11′), 28.9 and 30.4 (2C) (C4–C6), 30.6 (C2″), 30.7 (C16′), 31.6 (C7′), 33.8 and 34.4 (C2), 33.9 and 36.0 (NCH_3), 35.1 (C8′), 38.1 (C12′), 38.5 (C6′), 39.5 (C7), 44.6 (C13′), 46.3 (C9′), 48.6 and 51.0 (C1″), 51.0 (C14′), 56.5 (OCH_3), 82.4 (C17′), 109.9 (C1′), 116.8 (C4′), 132.3 (C5′), 135.4 (C10′), 145.3 (C3′), 147.0 (C2′), 175.5 (C1); LRMS calcd for $C_{31}H_{50}NO_4$ $[M+H]^+$ 500.3; HRMS calcd for $C_{31}H_{50}NO_3$ $[M+H]^+$ 500.37344, found 500.37253; HPLC purity of 82% (t_R = 31.17 min).

4.2. Cell culture

4.2.1. HEK-293 intact cells (overexpressing type 1, 7 or 12 17β-HSD). Stable transfected HEK-293 cells overexpressing isoform 1, 7 or 12 were provided by Dr. Van Luu-The. Cells were maintained in 75 cm^2 culture flask at 37 °C under 5% CO_2 humidified atmosphere in minimum essential medium (MEM) containing non-essential amino acids (0.1 mM), L-glutamine (2 mM), sodium pyruvate (1 mM), 10% foetal bovine serum (FBS), penicillin (100 IU/mL), streptomycin (100 μg/mL) and geneticin (G418 sulfate) (Gibco, Burlington, On, Canada) (700 μg/mL).

4.2.2. Breast cancer cell lines. Two ER-positive breast cancer cell lines, T-47D and MCF-7, were obtained from the American Type Culture Collection (ATCC) and maintained in 75 cm^2 culture flask at 37 °C under 5% CO_2 humidified atmosphere. The T-47D cells were grown in RPMI medium supplemented with 10% FBS, L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL) and estradiol (1 nM). The MCF-7 cells were propagated in DME-F12 medium supplemented with 5% FBS, glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL) and estradiol (1 nM).

4.3. Cell proliferation assay

Cell growth was measured using CellTitter 96® AQ_{ueous} Solution Cell Proliferation Assay (Promega, Nepean, ON, Canada) following the manufacturer's instructions. T-47D and MCF-7 cells were resuspended in their respective medium supplemented with insulin (50 ng/mL) and 5% dextran-coated charcoal treated FBS to remove the remaining estrogen present in the serum and medium. Aliquots (100 μL) of the cell suspension were seeded in 96-well plates (3000 cells/well) in triplicate. After 48 h, the medium was changed with an appropriate dilution of the inhibitor in growth medium and was replaced every 2 days. Cells were grown in absence or presence of inhibitors for 10 days in triplicate.

4.4. Assay of inhibition of isoforms 1, 7 or 12 of 17β-HSD

4.4.1. Type 1 17β-HSD (homogenated HEK-293 cells). Compounds were evaluated for their ability to inhibit the reductive transformation of E_1 (100 nM) into E_2 by type 1 17β-HSD (an homogenate of HEK-293 cells) in the presence of cofactor NADH according to an established procedure.[18]

4.4.2. Type 1 17β-HSD (intact HEK-293 cells). Compounds were evaluated for their ability to inhibit the reductive transformation of E_1 (60 nM) into E_2 by type 1 17β-HSD (transfected HEK-293 intact cells). Transfected cells were plated at 5000 cells/well in a 24-well plate. For the inhibitory activity assay, ethanolic solutions of inhibitors were immediately added to each well and incubated for 24 h. After incubation, [4-^{14}C]-E_1 (American Radiolabeled Chemicals Inc., St. Louis, MO, USA) was added and incubated for 15 min. After incubation, culture medium was removed and labelled steroids (E_1 and E_2) were extracted and quantified according to an established procedure.[42]

4.4.3. Type 1 17β-HSD (intact T-47 D cells). T-47D cells were seeded in 24-well plate at 3×10^4 cells/well in proliferation assay medium. After 48 h, 60 nM of [4-^{14}C]-E_1 and an ethanolic solution of inhibitor (0.5 %, v/v) at concentrations of 0.1, 1 and 10 μM were added to freshly changed culture medium and the cells were incubated for 24 h. After incubation, labelled steroids (E_1 and E_2) were extracted and quantified according to an established procedure.[42]

4.4.4. Type 7 17β-HSD (intact cells). Compounds were evaluated for their ability to inhibit the reductive transformation of E_1 (60 nM) into E_2 by type 7 17β-HSD (transfected HEK-293 intact cells) according to an established procedure.[43]

4.4.5. Type 12 17β-HSD (intact cells). Compounds were evaluated for their ability to inhibit the reductive transformation of E_1 (60 nM) into E_2 by type 12 17β-HSD (transfected HEK-293 intact cells). Transfected cells were plated at 1.5×10^5 cells/well in a 24-well plate. For the inhibitory activity assay, ethanolic solutions of inhibitor and [4-^{14}C]-E_1 were added to freshly changed culture medium and incubated for 24 h. After incubation, culture medium was removed and labelled steroids (E_1 and E_2) were extracted and quantified according to an established procedure.[42]

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