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16α-PROPYL DERIVATIVES OF ESTRADIOL AS INHIBITORS OF 17β-HYDROXYSTEROID DEHYDROGENASE TYPE 1

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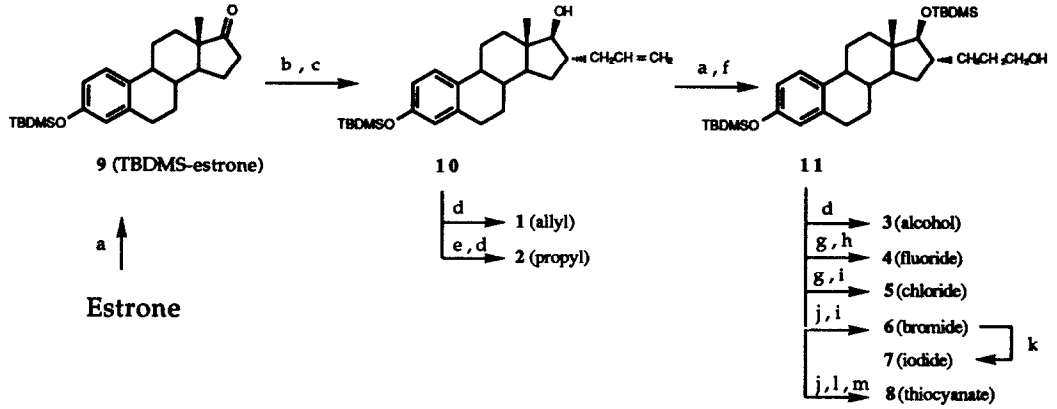
Abstract: The synthesis of eight 16α-propyl derivatives of estradiol is described, and structure-activity relationships are discussed. Potent inhibitors of cytosolic 17β-hydroxysteroid dehydrogenase of human placenta (type 1) can be obtained when a good leaving group is located at the end of a 16α-propyl side chain; the 16α-(iodopropyl)-estradiol (**7**) and 16α-(bromopropyl)-estradiol (**6**) gave the best irreversible inhibitions of 17β-HSD type 1, with IC_{50} values of 0.42 and 0.46 μM, respectively.

Human placental cytosolic 17β-hydroxysteroid dehydrogenase (17β-HSD) of type 1 [E.C.1.1.1.62]) was first described by Engel and co-workers.[1,2] This enzyme is responsible for the interconversion of estrone (E_1, the less active form) and estradiol (E_2, the most potent estrogen). In its native form, 17β-HSD is found as a dimer comprising two identical subunits and has a total molecular weight of *ca* 68 kDa.[3] Thus, according to the amino acid sequence predicted from the cDNA sequence, human 17β-HSD type 1 contains 327 amino acids with a calculated molecular weight of 34853 Da for each monomer.[4,5] Affinity-labelling studies with several steroidal bromoacetate derivatives have evidenced the presence of two histidines in the catalytic portion near the D-ring of steroids; these histidines might take part in the catalytic event.[6] Suicide inhibitors of 17β-HSD were developed by Thomas *et al.*[7] The 16-methylene-estradiol and the oxidated form, 16-methylene-estrone, represent this category of inhibitor. After enzymatic oxidation of 17β-OH group, these compounds become very reactive to basic amino acid residues, provoking the formation of a stable covalent bond responsible for 17β-HSD inhibition.

Estradiol is involved in the growth of a series of normal as well as tumoral tissues,[4,8,9] particularly in breast and endometrial cancers.[10] Thus, to better control estradiol formation, we are interested in developing inhibitors of 17β-HSD type 1 without estrogenic activity. First, we want to identify a side chain able to block 17β-HSD type 1. This appropriate side chain will be added thereafter on an antiestrogenic nucleus. Herein we report the synthesis of some 16α-estradiol derivatives (Table 1) bearing various chemical groups at the end of a three-carbon side chain. Using 17β-HSD type 1 activity, we evaluated the ability of the newly synthesized compounds to inhibit the enzymatic transformation of E_1 to E_2.

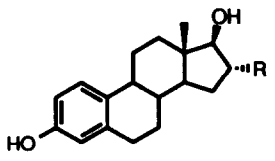


Scheme 1: Synthesis of proposed inhibitors of 17β-HSD type 1 (see Table 1). Reagents and conditions are: a) TBDMS-Cl, imidazole, DMF, rt, 17 h; b) LDA, allyl bromide, THF, -78°C to rt; c) $LiAlH_4$, -78°C, 2 h; d) Bu_4NF, THF, 1 h, 0°C to rt; e) H_2, Pd/C, EtOAc, 18 h; f) 1. BH_3, THF, 0°C, 3 h; 2. NaOH, H_2O_2, 1 h ; g) PPh_3, CCl_4, CH_2Cl_2, reflux, 18 h; h) Bu_4NF, THF, reflux, 20 h; i) MeOH-HCl (98:2), rt, 18 h ; j) PPh_3, CBr_4, CH_2Cl_2, rt, 18 h ; k) NaI, acetone, reflux, 18 h; l) KSCN, ethanol 95%, reflux, 27 h ; m) THF : H_2O : AcOH / 2:1:2, reflux, 21 h.

Chemistry (see note 12):

TBDMS-estrone (**9**) was synthesized from commercially available estrone (Scheme 1) after protection of the phenolic group at position 3 using *tert*-butyldimethylsilyl chloride (TBDMS-Cl) and imidazole in DMF. Transformation of compound **9** to the key intermediate **10** was performed according to the method of Fevig and Katzenellenbogen.[11] In brief, protected estrone (TBDMS-estrone) was treated with lithium diisopropylamine in THF at -78°C and alkylated in position 16 with allyl bromide. Reduction of the carbonyl group with lithium aluminium hydride at -78°C produced, after purification by chromatography, mainly the desired compound **10** (with 17β-hydroxyl and 16α-allyl group) and also a small amount of the 16β-allyl isomer. Deprotection of allyl derivative **10** by tetrabutyl ammonium fluoride (Bu_4NF) produced directly the 16α-allyl-estradiol (**1**), and 16α-propyl-estradiol (**2**) was obtained by catalytic hydrogenation of compound **10** (Pd/C, 10% w/w) followed by cleavage of the TBDMS group (Bu_4NF). After protection of the 17β-hydroxy group of compound **10** by TBDMS-Cl, the corresponding olefin was treated with borane followed by oxidation (NaOH, H_2O_2) to produce primary alcohol **11**. This intermediate was deprotected (Bu_4NF) to give triol **3**. In a standard procedure, chlorination (PPh_3, CCl_4) and bromination (PPh_3, CBr_4) of primary alcohol **11** was done, followed by cleavage of the TBDMS group (MeOH-HCl) to produce compounds **5** and **6** respectively. Fluoride analog **4** was obtained by treatment of chloride **5** with tetrabutyl ammonium fluoride in refluxing THF. From bromide **6**, iodination with sodium iodide in refluxing acetone gave compound **7**. The intermediate di-TBDMS-bromide (obtained from alcohol **11**) was used to synthesize the corresponding thiocyanate analogue (KSCN in refluxing ethanol 95%). The final TBDMS deprotection of the latter in a mixture of THF:H_2O:AcOH (2:1:2) afforded thiocyanate **8**.

Table 1. Inhibition of 17β-HSD type 1 by compounds **1** to **8**

General structure	R	No	Inhibition of 17β-HSD type 1 (E_1-->E_2) [a]		
			% at 1 μM [b]	% at 10 μM [b]	IC_{50} (μM) [c]
	$CH_2CH{=}CH_2$	**1**	26	63	-------
	$CH_2CH_2CH_3$	**2**	29	69	-------
	$CH_2CH_2CH_2OH$	**3**	17	57	-------
	$CH_2CH_2CH_2F$	**4**	49	81	4.30
	$CH_2CH_2CH_2Cl$	**5**	88	92	1.00
	$CH_2CH_2CH_2Br$	**6**	70	94	0.46
	$CH_2CH_2CH_2I$	**7**	68	92	0.42
	$CH_2CH_2CH_2SCN$	**8**	82	95	1.15

[a] Error = ± 5 %; [b] Concentration of inhibitor; [c] The concentration of inhibitor which causes 50% inhibition (obtained from inhibition curves, Figure 1A).

Inhibition of 17β-HSD type 1:

In vitro enzymatic assays were performed as described in note 13. As illustrated in Table 1, estradiol derivatives **4** to **8**, with a good leaving group on the 16α-side chain, resulted in the best inhibition (49 to 88% at 1 μM and 81 to 95% at 10 μM). Very weak inhibitions were observed with compounds **1** to **3**, which contain an allyl, propyl, or hydroxypropyl side chain (17 to 29% at 1 μM and 57 to 69% at 10 μM). For compounds **4** to **8**, the concentrations that cause 50% inhibition (IC_{50}) were determined from the inhibition curves (Figure 1A). According to IC_{50} values, among the halogen derivatives, the iodopropyl group exhibits a slightly better inhibition of 17β-HSD type 1 than bromopropyl, which is better than chloropropyl, and this latter is better than the fluoropropyl group (IC_{50} = 0.42, 0.46, 1.00, and 4.30 μM, respectively for **7**, **6**, **5**, and **4**). The thiocyanate group (IC_{50} = 1.15 μM) causes a better inhibition than fluoride but inferior to chloride. The lack of inhibition effect of compounds **1-4** suggests the need for a good leaving group, at the end of the three-carbon side chain, to provoke a good inhibition of 17β-HSD type 1. In addition, incubation of inhibitor **6** with 17β-HSD type 1 and the cofactor shows inactivation of the enzyme in a time- and concentration- dependent manner (Figure 1B). We also observed that the inactivation is independent of cofactor (data not shown). Inactivation of enzyme could be due to the formation of a covalent bond between the inhibitor and a reactive amino acid residue. Further experiments to determine the exact mechanism of inhibition, to optimize the effect of these inhibitors, and to decrease their estrogenic activity are now in progress and will be published later with a complete description of the experimental procedure (chemical synthesis and enzymatic test).

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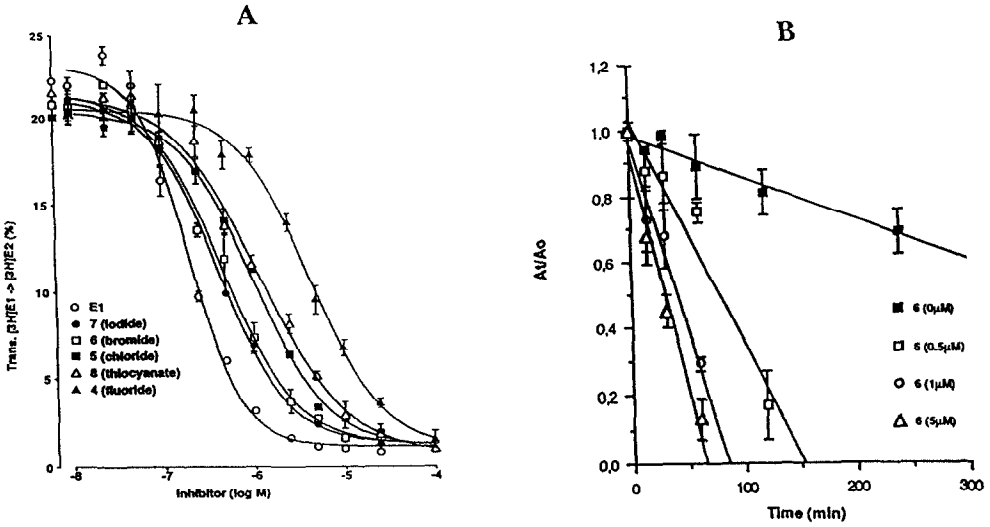

Figure 1: A. Inhibition curves of 17β-HSD type 1 by increasing concentrations of compounds **4-8**. B. Inactivation of 17β-HSD type 1 by bromide **6**, A_t: enzymatic activity at time t, and A_0: initial activity.

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12. All compounds were characterized by ^1H and ^{13}C NMR (300 MHz), FTIR and MS analysis; purity was checked by HPLC and elemental analysis.
13. Enzymatic assays (briefly): (A.) Assay for 17β-HSD type 1 activity was performed in a final volume of 1 ml of buffer (20% glycerol, 1 mM EDTA, 50 mM KH_2PO_4 and pH 7.4) containing 3-5 pmol of [^3H]estrone, 1 nmol NADH, 100 µl of partially purified human placental cytosolic 17β-HSD (type 1), and 10 µl of ethanolic solution of inhibitor (compounds **1** to **8**). Tubes in triplicate were incubated for 30 min at 37°C with shaking. After incubation, the reaction was stopped by cooling tubes in a mixture of ice and water and by adding immediately unlabelled estrone and estradiol as carriers. Steroids were then extracted, purified by TLC, and radioactivity associated to estrone (E_1) and estradiol (E_2) was measured to obtain the % of transformation. From these data, the % of inhibition was calculated at two concentrations of inhibitor (1 µM and 10 µM) and the IC_{50} value was determined from the inhibition curves. (**B.**) For inactivation assay, 100 µl of buffer solution containing 17β-HSD type 1, NADH and appropriate concentrations of compound **6** was incubated at 37°C. At intervals of time, sample tubes (triplicate) were diluted 20-fold with buffer solution of [^3H]estrone and enzymatic assay performed as above. The data were plotted according to Kitz and Wilson (J. Biol. Chem. **1962**, *237*, 3245).

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