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RESEARCH ARTICLE

Comparative investigation of the *in vitro* inhibitory potencies of 13-epimeric estrones and D-secoestrones towards 17β-hydroxysteroid dehydrogenase type 1

Bianka Edina Herman[1], Johanna Szabó[2], Ildikó Bacsa[2], János Wölfling[2], Gyula Schneider[2], Mónika Bálint[3], Csaba Hetényi[4], Erzsébet Mernyák[2], and Mihály Szécsi[1]

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Abstract

The inhibitory effects of 13-epimeric estrones, D-secooxime and D-secoalcohol estrone compounds on human placental 17β-hydroxysteroid dehydrogenase type 1 isozyme (17β-HSD1) were investigated. The transformation of estrone to 17β-estradiol was studied by an *in vitro* radiosubstrate incubation method. 13α-Estrone inhibited the enzyme activity effectively with an IC_{50} value of 1.2 μM, which indicates that enzyme affinity is similar to that of the natural estrone substrate. The 13β derivatives and the compounds bearing a 3-hydroxy group generally exerted stronger inhibition than the 13α and 3-ether counterparts. The 3-hydroxy-13β-D-secoalcohol and the 3-hydroxy-13α-D-secooxime displayed an outstanding cofactor dependence, i.e. more efficient inhibition in the presence of NADH than NADPH. The 3-hydroxy-13β-D-secooxime has an IC_{50} value of 0.070 μM and is one of the most effective 17β-HSD1 inhibitors reported to date in the literature.

Keywords

13α-estrone, 17β-HSD1 inhibition, D-secoestrone, NADH, NADPH

History

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Introduction

The human 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1, EC 1.1.1.62) protein is comprised of 328 amino acids and exists as a cytosolic functional homodimer with a subunit molecular mass of 34 950 Da[1,2]. Amino acid sequence alignments and homology studies have revealed that it belongs to the short-chain dehydrogenase/reductase (SDR) superfamily. 17β-HSD1 is a pluripotent enzyme in terms of substrate, cofactor, and the oxidative and reductive direction of the 17β-hydroxy-17-oxo interconversion. This isozyme is capable of the 3β-hydroxy reduction of substrates bound in reverse mode[3].

Under *in vivo* conditions, as in living cells, however, the isoenzyme functions unidirectionally[4–6] and predominantly catalyze the NADPH-promoted stereospecific reduction of estrone (**1a**) to 17β-estradiol (E2) (Scheme 1), the final hormone-activating process in estrogen biosynthesis[5,7]. The highest expression and activity of the isozyme may be observed in the female steroidogenic reproductive tissues, such as the ovaries and the placenta[8]. This isozyme makes a major contribution to the general gonadal supply and to the circulating level of E2 in the blood. 17β-HSD1 is also expressed and active in peripheral tissues, where it regulates the intracellular accumulation of E2 and consequently the intracrine estrogen effect[3,9]. 17β-HSD1 has been reported to be responsible for the intracellular overproduction of E2 in various neoplasms. The pathophysiological accumulation of E2 then contributes to the development and progression of estrogen-dependent forms of endometriosis, breast cancer and ovarian cancer. The inhibition of 17β-HSD1 with suitable pharmacons may suppress both the systemic and the local or *in situ* synthesis of E2. The evoked pre-receptorial anti-hormonal effect offers a suitable option for the therapy of estrogen-dependent diseases. 17β-HSD1 inhibitors may serve as interesting drug targets of anti-estrogen therapy[2,10].

Numerous earlier studies have demonstrated that various estrone and 17β-estradiol derivatives inhibit 17β-HSD1 activity effectively[2,9,11,12]. Inhibitor design based on the estrane core is nonetheless limited, because they must be devoid of estrogenic activity[13–17].

Certain structural modifications of the estrane skeleton, such as the opening of ring D or inversion of the configuration at C-13, may lead to the complete loss of hormonal activity[18–20]. We recently described the synthesis and *in vitro* investigation of the 17β-HSD1-inhibitory activities of C-13 epimeric 17-(triazolylmethyl)carboxamido D-secoestrone derivatives bearing ether protecting groups on C-3[21]. The nature of the functional groups on C-3 and/or C-17 and the orientation of the angular methyl group influence the enzyme inhibitory potential substantially. Certain 3-methoxy-13β-D-secoestrones and one 3-benzyloxy-13α derivative displayed low micromolar 17β-HSD1 inhibitory

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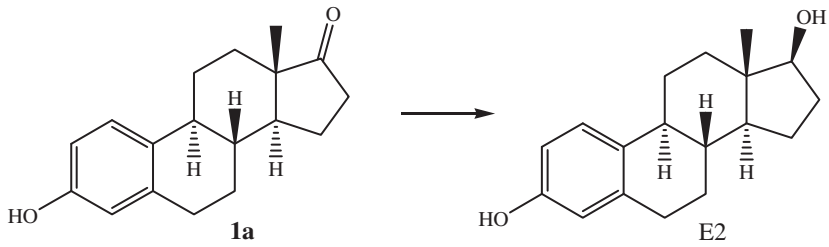
Scheme 1. Stereospecific reduction of estrone (**1a**) to 17β-estradiol (E2) by 17β-HSD1.



potentials. This biological activity of D-secoestrones was a novel finding and alludes to the design of hormonally inactive 17β-HSD1 inhibitors on these scaffolds in both the 13α- and 13β-estrone series.

We recently described the halogenation of ring A of 13α-estrone with different protecting groups at position 3[22]. The halogen derivatives were designed on the basis of the literature analogy of similarly halogenated 13β-estrones as nanomolar inhibitors of 17β-HSD1. The inhibitory potential of the 13α-compounds depended markedly on the nature and the size of the protecting group on the phenolic OH function. The presence of H or methyl was advantageous relative to the more bulky benzyl group. Effective 13α-estrones such as these 17β-HSD1 inhibitors had not been published previously.

We now report an investigation of the inhibitory potentials of C-13 epimeric 3-OH and 3-ether estrone derivatives bearing an intact or seco ring D towards human placental 17β-HSD1. *In vitro* inhibition tests were performed with both the cofactors NADPH and NADH that are regularly applied in these assays.

Materials and methods

Chemistry

Compounds **1a** and **1c** were purchased from Sigma (St. Louis, MO) and **1b** from Steraloids (Newport, RI). 13α-Derivatives (**2b** and **2c**) were obtained by the epimerization of **1b** or **1c** using the literature methods[18],S3. 13α-Estrone (**1a**) was obtained by debenzylation of **1b**[S3]. The experimental details for the chemical synthesis and data on the compounds (**3–6**) are presented in the Supplemental Information.

Determination of 17β-HSD1 activity and its inhibition in the human placenta cytosol

Radioactive [6,7-3H(N)]estrone, S.A. = 50 Ci/mmol, was purchased from American Radiolabeled Chemicals (St. Louis, MO). Non-radioactive estrone (**1a**) and E2 standards, NADH and NADPH cofactors, other chemicals and solvents of analytical grade purity were purchased from Sigma (St. Louis, MO) or Fluka (Buchs, Switzerland). Kieselgel-G TLC layers (Si 254 F, 0.25 mm thick) were from Merck (Darmstadt, Germany). Human term placenta specimens were collected and used with the ethical approval of the Institutional Human Investigation Review Board.

The inhibitory effects of the newly synthesized compounds on the 17β-HSD1 activity were investigated via the conversion of **1a** to E2 *in vitro*. Human placental cytosol served as a source for the isozyme[21,23]. Human term placenta specimens were combined and homogenized with an Ultra-Turrax in 0.1 M HEPES buffer (pH = 7.3) containing 1 mM EDTA and 1 mM dithiotreitol and the cytosol was obtained by fractionated centrifugation. Substrate **1a** (1 μM) with its tritiated tracer (250 000 dpm) was added to the incubator in 10 μL of 25 v/v% propylene glycol in HEPES buffer solution, whereas the test compounds were applied in 10 μL of dimethyl sulfoxide solution. (These organic solvent contents in the 200 μL final volume of the HEPES buffer incubation medium did not reduce the enzyme activity substantially.) The cofactor, either NADH or NADPH, was used in an excess concentration of 100 μM. The enzymatic reaction was started by the addition of the cytosol aliquots. Incubation was carried out at 37 °C for 2.5 min and was then stopped by the addition of ethyl acetate and freezing. After extraction with ethyl acetate, unlabelled carriers of **1a** and the product E2 were added to the samples. The two steroids were separated by TLC with the solvent system dichloromethane/diisopropyl ether/ethyl acetate (70:15:15 v/v) and UV spots were used to trace the separated steroids. Spots were cut out and the radioactivity of the E2 formed and the **1a** remaining was measured by means of liquid scintillation counting. 17β-HSD1 activity was calculated from the radioactivity of the E2 with correction for the recovery.

The assays were performed in triplicate for determination of the percentages of relative inhibited conversions at a final inhibitor concentration of 10 μM, and the standard deviations (SDs) were also calculated. IC_{50} values (the inhibitor concentration that decreases the enzyme activity to 50%) were determined for the most effective and other selected test compounds. In these cases, conversions were measured at 10–15 different concentrations in the appropriate interval 0.001–50 μM. IC_{50} results were calculated by using unweighted iterative least squares logistic curve fitting by means of the ''absolute IC_{50} calculation'' function of the GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA). The IC_{50} of unlabelled estrone (**1a**) was measured as reference. The relative inhibitory potentials (RIPs) of the test compounds were calculated by using reference IC_{50} data measured with the corresponding cofactor: RIP = IC_{50} of test compound/IC_{50} of unlabelled estrone (**1a**).

With the selected incubation parameters, the enzyme reaction satisfied the conditions of the initial velocity measurements. The conversions in the non-inhibited control incubates reached similar rates (10–13%) with both cofactors, and the product formation was proportional to the enzyme concentration and the incubation duration. The 1 μM substrate was a saturation concentration in the presence of NADPH, whereas it was on the declining proportional phase with NADH (data not shown).

Results and discussion

17β-HSD1 inhibition

The steroid ligand binding site of 17β-HSD1[24,25] has been described as a hydrophobic tunnel with polar residues at each end. The surface of the tunnel is complementary to the C_{18} steroidal scaffold and ensures selectivity towards estrogenic substrates[26]. At the C-terminal recognition end, hydrophilic amino acids form hydrogen-bonds to the 3-hydroxy group of the substrate[27,28]. These interactions fix the substrate and its C-17 oxo into an appropriate orientation for the catalytic transformation[7,24,29], but they have been found not to be essential for the binding, and they may even establish a catalytically unfavourable position for non-cognate substrates[30]. Residues of the N-terminal catalytic end form triangular hydrogen-bond contacts with the C-17 carbonyl

oxygen and facilitate a charge-equalizing proton transfer following the hydride donation from the nicotinamide moiety of the cofactor[6,24,31].

NADPH and NADH bind in the same extended conformation to 17β-HSD1, pointing towards the active site with their nicotinamide ring[27,30], and both cofactors are able to promote the stereospecific reduction of the C-17 carbonyl of the substrate. Despite these resemblances, NADPH and NADH are not interchangeable as cofactors of 17β-HSD1. Their different interactions and the different ground and transition state structures[32] suggest that different modes of binding exist for the phosphorylated and the unphosphorylated cofactors. Binding differences induce different conformational changes in the cofactor binding cleft, which extends towards the catalytic cleft of the active centre in its close proximity[33].

Estrane-based inhibitors are assumed to occupy the substrate-binding site of 17β-HSD1, and are able to form other contacts to the enzyme than substrate molecules[2,9,12]. These interactions may improve the binding affinity and modulate the inhibitory potential[34]. The complexities of the interaction mechanisms of 17β-HSD1 have the result that relatively small changes in the shape of the steroid substrate or inhibitor ligand, and/or in the protein conformation induced by the cofactor or by other modulators can significantly affect the binding and catalytic arrangements, and consequently the binding affinity, the inhibitor potential and the selectivity.

Natural estrone possesses a tetracyclic steroidal framework with *trans* junctions of rings B/C and C/D. The other characteristics of this classical steroid are the typical conformations of rings C (chair) and D (strongly restricted). The rigid structure of estrone contains two oxygen functionalities with well-defined distances, which are crucial in the binding of estrone or estradiol to its nuclear hormone receptors. In contrast with the natural 13β compound, the 13 epimer has a quasi-equatorial angular methyl group, a *cis* junction of rings C/D and a ring D that is directed to the β side[18]. Poirier et al. reported the impact of inversion of the configuration at C-13 and/or C-17 of estradiols on their estrogenic activity[20]. They concluded that 13 epimers have low relative binding affinity for estrogen receptor alpha and have no significant uterotropic activity. Accordingly, inversion at C-13 in the estrane skeleton could be a correct strategy in the design of estrone-based anticancer agents lacking estrogenic activity.

In this work, we determined the *in vitro* inhibitory potencies on human placental 17β-HSD1 of the 3-hydroxy and the 3-ether derivatives of 13α- and 13β-estrones (**1**, **2**) and D-secoestrones (**3**–**6**) (Figure 1) in the presence of NADPH or NADH.

The reference IC_{50} data determined for unlabelled estrone (**1a**) were found to be 2.0 μM in the presence of NADH and 0.63 μM when NADPH was applied as cofactor (Table 1). These IC_{50} results are similar to those of the earlier published results by other authors (for placental 17β-HSD1)[5,23,35–37].

13α-Estrone (**2a**) proved here to be a potent inhibitor, displaying low micromolar IC_{50} values similar to those of the unlabelled reference estrone (**1a**). The 13α epimer (**2a**) of the natural estrogenic prehormone **1a** has long been known[38], but its inhibitory properties against the 17β-HSD1 activity have not been reported so far. Recently, 17β-HSD1 inhibition of 16-substituted derivatives of the 13α-estradiol has been investigated[39].

As concerns the inhibitory activities of the test compounds bearing an intact ring D (**1a–c** and **2a–c**), the nature of the substituent on C-3 was the determining factor. Similar to the earlier established relationships[22], the presence of the phenolic OH or the small methyl ether function was more advantageous than the bulky apolar benzyl group. The 13α methyl ether **2c** displayed a lower range activity than that of its 13β counterpart (**1c**). Our results tend to confirm the earlier observations[22] that the hydrogen-bonds of a phenolic OH function in this position might be beneficial, but not absolutely necessary for efficient inhibition.

As concerns the secoestrones, the two epimeric D-secoalcohols (**3a** and **4a**) display IC_{50} values in the low or submicromolar range. Of the 3-methyl ethers of the secoalcohol (**3c** and **4c**), only the 13β counterpart (**3c**) was proved effective, but with higher IC_{50} value than that of its 3-OH derivative (**3a**). The epimeric 3-hydroxy-D-secooximes (**5a** and **6a**) displayed noteworthy inhibitory properties and C-13 chirality dependence. **5a** was found to be highly potent in the presence of either NADPH or NADH, with IC_{50} values of 0.070 μM and 0.077 μM, respectively. The 13α counterpart (**6a**) was effective only when NADH was used as a cofactor ($IC_{50} = 0.058$ μM). The oxime epimer pairs of **5a** and **6a** displayed a large difference, demonstrating inhibition around 400-fold stronger of the 13β than that of the 13α epimer in the presence of NADPH, whereas they exerted similar effect with NADH.

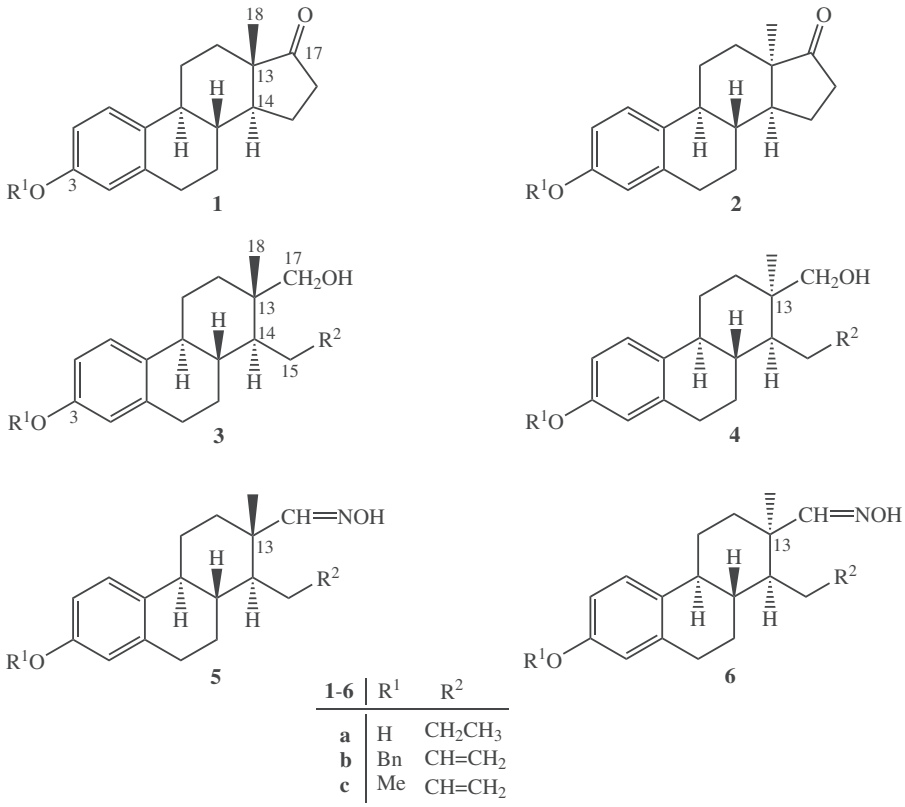
The inhibitory data of the D-seco compounds reveal that the nature of the 3 substituent has a crucial influence on the activities. Of the epimeric oxime ethers (**5b**, **c** and **6b**, **c**), only one 13β epimer (**5c**), bearing a small methyl group on the phenolic OH function, exerted substantial inhibitory effect, which was more pronounced than that observed for the 13β secoalcohol 3-methyl ether (**3c**).

Molecular mechanic and semi-empirical energy minimizations of the most potent 3-OH derivatives (**2a**–**6a**) were performed to demonstrate their structural features and differences (Figure 2). Figure 2 reveals that the epimerization of C-13 modifies the ring D region considerably. Functional groups in this region (carbonyl, hydroxymethyl or oxime) display alterations in position, direction and distance from those of 3-OH in the epimer pairs. Despite these structural differences, 13α-estrone (**2a**) binds to 17β-HSD1 with similar affinity as for the cognate substrate. As concerns the D-secoestrones (**3a**–**6a**), the 13β compounds (**3a** and **5a**) possess an axial angular methyl group and an equatorial functional group on C-13. In contrast with the 13β derivatives, the angular methyl group of 13α-D-secoestrones (**4a** and **6a**) has an equatorial orientation, and the oxime or primary alcoholic function is axial[40]. The oxime function has a double bond with *E* or *Z* orientation, but the primary alcoholic group can rotate freely. The difference in the inhibitory activities of the oximes and alcohols may therefore reflect the differences in the nature and the position of the C-17 functional groups. It may be postulated that in **5a** or **6a**, this oxime side-chain may take up an appropriate position to form strong hydrophilic interactions or hydrogen-bonds to certain amino acids of the enzyme, and these interactions may cause the high affinity and outstanding inhibitory potentials observed for the oximes. The noteworthy effectiveness of the oximes may be ascribed to the capability of the oxime function to form strong interactions with certain amino acid residues of the target proteins. Further investigations might identify the amino acid residues which are involved in these interactions.

Cofactor dependence

The 17β-HSD1 inhibition results of the test compounds demonstrate the influence of the cofactor partner. 13β-Estrone 3-methyl ether (**1c**) exerted a five-fold stronger inhibition in the presence of the phosphorylated cofactor. 13α-Estrone (**2a**) and its methyl ether (**2c**) displayed similar IC_{50} values with the two cofactors, but the RIP data demonstrated a 2–3-fold higher potential with NADH in comparison with the reference **1a**. The cofactor dependence was more pronounced among the D-seco compounds. The IC_{50} values were found to be 2–3-fold lower, indicating a 3–7-fold higher inhibition effect in terms of the RIP measured with NADH. The difference was further enhanced for the 13β epimer

Figure 1. Structural formulae of the test compounds (**1**–**6**).



1-6	R^1	R^2
a	H	CH_2CH_3
b	Bn	$CH{=}CH_2$
c	Me	$CH{=}CH_2$

of the D-secoalcohol (**3a**), which exerted an 8–9-fold more effective inhibition with NADH according to the IC_{50} data, and a 25-fold stronger effect in the sense of the RIP values.

The 13α epimer of the D-secooxime (**6a**) displayed an outstanding cofactor dependence. This compound exerted only weak inhibition with NADPH, but it was highly effective in the presence of NADH. The difference between the IC_{50} values was more than 500-fold, whereas the RIP ratio exceeded 1200.

Since the two secooxime epimers, **5a** and **6a**, differ only in the position of the angular methyl and the oxime function, the orientation of this part of the molecule seems to be favourable in the NADH complex of the enzyme for both the 13α and the 13β epimers (**6a** and **5a**), but only for **5a** in the NADPH complex. Effective binding of the 13α counterpart (**6a**) is possibly prevented by the increased specificity towards 13β compounds of the NADPH complex. The side chain at C-13 in **6a** may be directed into an unfavoured position, which cannot be modified because of the limited flexibility of the oxime function caused by its double bond. The related alcohols **3a** and **4a** display similar inhibitory potencies, irrespective of the orientation of the angular methyl group, as the shorter and more rotatable side chain may find its optimum position either in the NADH or in the NADPH complexed protein.

Other SDR enzymes feature NADH for the catalytic process and early studies annotated this cofactor to the reductive direction of 17β-HSD1[30,41,42]. Numerous *in vitro* inhibition tests have been performed with supplementation of the unphosphorylated cofactor too. Higher affinity for 17β-HSD1 of NADPH over NADH[5,29,43,44] and considerations of the abundance and metabolic roles of nicotinamide cofactors[4,45,46] have made it evident that NADPH might be the prevalent partner of 17β-HSD1 in its *in vivo* function, in the **1a**–E2 conversion[4,46].

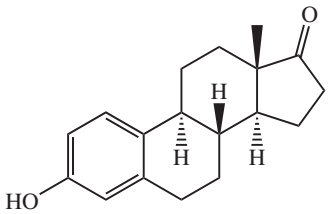
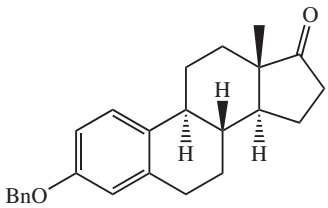
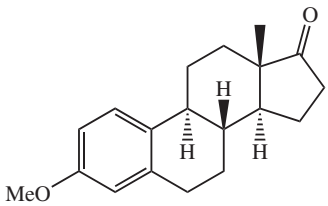
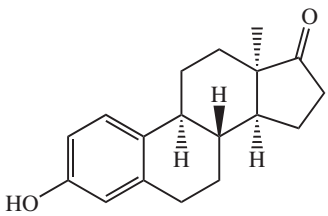
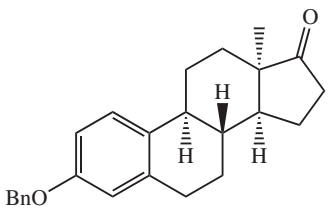
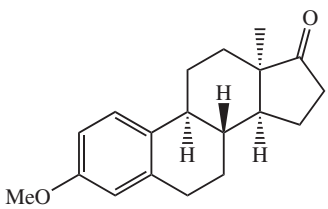
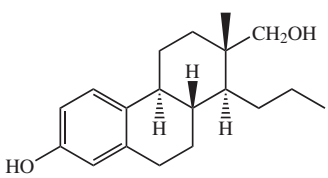
Only a few data are to be found in the literature as concerns the direct comparison of inhibitory potencies with NADPH versus NADH. The hybrid inhibitor EM-1745, in which an unphosphorylated cofactor-mimicking moiety was coupled to the estradiol core, and which was therefore planned to act on both the active centre and the cofactor binding site of the enzyme, proved to be a weaker inhibitor of 17β-HSD1 when NADPH was used as cofactor rather than NADH[47]. This difference, however, was explained specifically that the adenosine moiety of EM-1745 does not bind the cofactor-binding site of 17β-HSD1 as strongly as the phosphorylated adenosine moiety of NADPH, and thus the bisubstrate inhibitor EM-1745 (without a phosphate group) cannot compete efficient enough against the cofactor NADPH. Our D-seco compounds **3a** and **6a** do not possess a cofactor-mimicking moiety, but they display large differences in inhibitory potential measured in the presence of NADPH or NADH.

These inhibition results indicate that the apparent *in vitro* potentials obtained with the two cofactors may differ substantially for certain compounds. Data on NADPH and NADH are not interchangeable and their direct comparison (e.g. in one table[48]) is not advised. The literature data must be reviewed with special attention to the cofactor supplementation, the screening systems[49] should be specified precisely, and NADPH should be preferred instead of NADH in cell-free *in vitro* inhibitor tests. The influence of cofactors might be an explanation for the altered, occasionally disappointingly decreased inhibition potentials obtained in cellular 17β-HSD1 inhibition assays performed following promising cell-free screening tests with NADH[50]. Data measured in the presence of NADH must be evaluated with caution in inhibitor optimization and in lead selection. NADH results are less relevant to the potential *in vivo* effect, but could be valuable in facilitating the understanding of the mechanism of catalysis and the inhibition of 17β-HSD1.

17β-HSD1 inhibition and antiproliferative effects

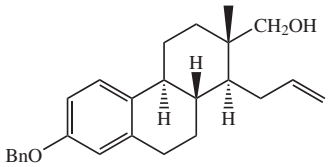
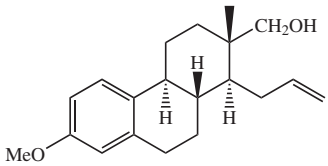
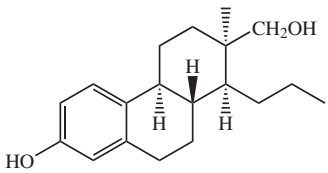
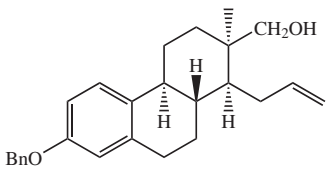
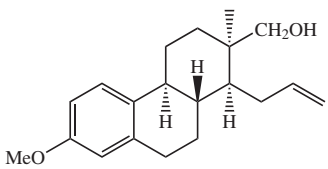
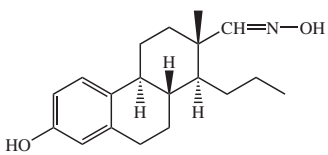
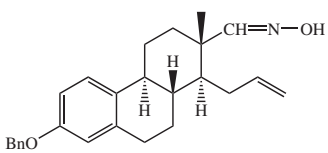
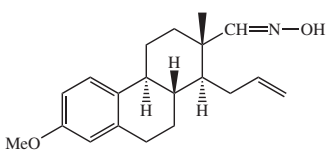
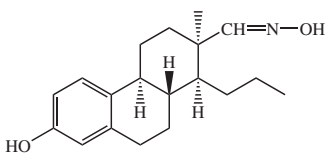
The synthesis and *in vitro* investigation of the antiproliferative potentials of the D-secoestrone derivatives tested here for their

Table 1. Inhibition results on 17β-HSD1.

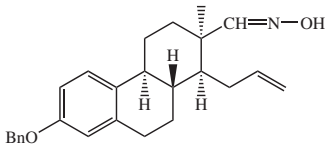
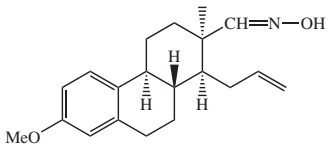
Compd.	Structure	IC_{50}±SD (μM) and RIP or relative conversion at 10 μM ± SD (%) NADPH	NADH
1a		$IC_{50} = 0.63 \pm 0.11$ RIP = 1.0	$IC_{50} = 2.0 \pm 0.18$ RIP = 1.0
1b		52 ± 2	52 ± 5
1c		$IC_{50} = 0.77 \pm 0.29$ RIP = 1.2	$IC_{50} = 4.2 \pm 1.6$ RIP = 2.1
2a		$IC_{50} = 1.2 \pm 0.2$ RIP = 1.7	$IC_{50} = 1.1 \pm 0.3$ RIP = 0.59
2b		55 ± 3	65 ± 10
2c		$IC_{50} = 5.5 \pm 1.5$ RIP = 8.8	$IC_{50} = 7.5 \pm 2.5$ RIP = 3.7
3a		$IC_{50} = 3.4 \pm 1.4$ RIP = 5.4	$IC_{50} = 0.41 \pm 0.24$ RIP = 0.21
3b		76 ± 7	70 ± 4

(*continued*)

Table 1. Continued

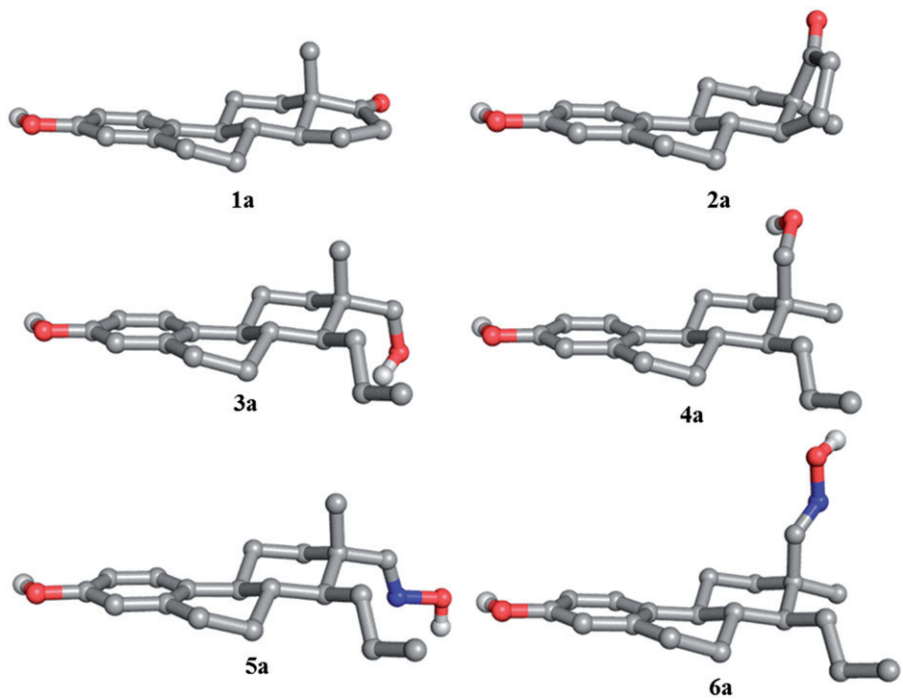
Compd.	Structure	IC_{50}±SD (μM) and RIP or relative conversion at 10 μM ± SD (%) NADPH	NADH
			
3c		$IC_{50} = 9.0 \pm 1.7$ RIP = 14.3	$IC_{50} = 5.5 \pm 1.9$ RIP = 2.7
4a		$IC_{50} = 3.7 \pm 1.3$ RIP = 5.8	$IC_{50} = 1.7 \pm 0.3$ RIP = 0.85
4b		84 ± 6	83 ± 7
4c		66 ± 10	67 ± 6
5a		$IC_{50} = 0.070 \pm 0.027$ RIP = 0.11	$IC_{50} = 0.077 \pm 0.036$ RIP = 0.039
5b		76 ± 10	82 ± 0.4
5c		$IC_{50} = 3.1 \pm 1.7$ RIP = 4.9	$IC_{50} = 1.9 \pm 0.8$ RIP = 0.93
6a		$IC_{50} = 30 \pm 7$ RIP = 48	$IC_{50} = 0.058 \pm 0.044$ RIP = 0.029

(*continued*)

Compd.	Structure	IC_{50}±SD (μM) and RIP or relative conversion at 10 μM ± SD (%) NADPH	NADH
6b		90 ± 17	77 ± 5
6c		IC_{50} = 21 ± 7 RIP = 34	IC_{50} = 24 ± 10 RIP = 12

Relative conversions (control incubation with no inhibition is 100%) measured in the presence of 10 μM of the compound tested. IC_{50}: The inhibitor concentration that decreases the enzyme activity to 50%. RIP: relative inhibition potency compared to reference E1. SD: standard deviation (for relative conversion $n = 3$).

Figure 2. Molecular structures of compounds **1a**–**6a**.



17β-HSD1 inhibitory properties have recently been reported[51,52]. Our potential anticancer agents were designed on the hormonally inactive D-seco- and/or 13α-estrone core. 3-Benzyloxy-D-secoestrone alcohol (**3b**), the first D-secoestrone in the literature, displays substantial *in vitro* antiproliferative effects against a number of human reproductive cancer cell lines with good tumour selectivity[51]. The debenzylated secoalcohol (**3a**) containing a 3-phenolic group did not inhibit tumour cell growth markedly. These results led to further D-secoestrone derivatives as potential antitumour agents. 17-Oxime derivatives of the potent secoalcohol (**3b**) were synthesized and diversified at several sites of the molecule: 3-ethers (**5b**,**c** and **6b**,**c**) or 3-hydroxy derivatives (**5a** and **6a**) were investigated in both the 13β- and the 13α-estrone series[52]. None of the 13α epimers (**6**) or the 3-hydroxy derivatives (**5a**) exerted substantial antiproliferative activities, but the 13β-D-secoestrone-3-ethers (**5b**,**c**) proved to be effective against various cell lines (HeLa, A2780, A431 and MCF-7) with IC_{50} values in the low μM range. Tests were performed on cell lines with diverse steroidogenic and steroid responsive properties, and the results suggested that the cytotoxic effect is most probably independent of the estrogen hormonal mechanisms, and the 17β-HSD1 inhibition among them.

The literature reveals that it is possible to combine direct cytostatic activity with 17β-HSD1-inhibitory potential, resulting in dual action against estrogen-dependent tumours. It is therefore reasonable to evaluate our present results in this sense too (Figure 3). Depending on the nature of the substituent at C-3, 13β-methyl-D-secoestrone oxime (**5**) is able to exert different

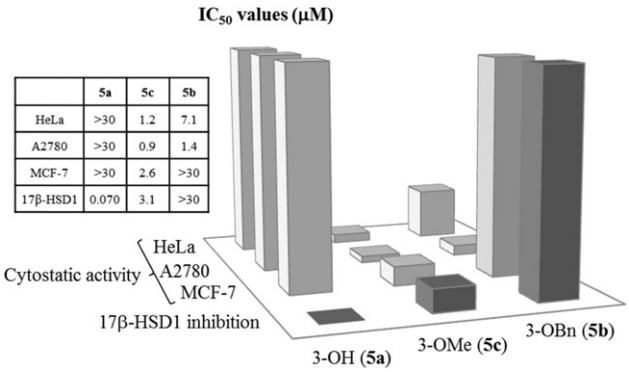


Figure 3. 17β-HSD1 inhibition (in the presence of NADPH) and cytostatic potentials[51,52] of the tested D-secooximes (IC_{50} values on a relative scale).

important biological activities: bearing an unsubstituted 3-OH (**5a**), which belongs to the highly potent 17β-HSD1 inhibitors with unmarked antiproliferative action on the examined cell lines. Compound **5b**, possessing a bulky apolar benzyl protecting group, substantially inhibits the growth of certain cell lines, but does not influence the estrone–estradiol conversion catalysed by 17β-HSD1. The methyl ether (**5c**) behaves dually by inhibiting both the cell growth and 17β-HSD1 as enzyme. The same tendency appears in the results of the antiproliferative and 17β-HSD1 inhibitory measurements as concerns 13β-methyl-D-secoestrone alcohol (**3**), but it can be stated that the biological activities of the secooximes are more pronounced than those of their alcoholic counterparts. Compound **5a** or **5b**, however, is a selective 17β-HSD1 inhibitor or an antiproliferative agent respectively, in this comparison.

The 3-methyl ether of D-secoestrone oxime (**5c**) may be considered as a compound with a dual mode of action, as it displays a noteworthy direct antiproliferative effect against a number of human reproductive cancer cell lines (independently of their 17β-HSD1 or ER status), and exerts substantial inhibitory potential against 17β-HSD1. Since 17β-HSD1 inhibition is a promising approach for the treatment of estrogen-dependent tumours, it decreases the level of estradiol in the tumour cells, a compound with a dual mode of action may be superior to simple 17β-HSD1 inhibitors.

Conclusions

17β-HSD1 has been studied for more than half a century, but none of its inhibitor candidates have yet reached clinical trials for the treatment of estrogen-dependent diseases. Since breast cancer is the most common cancer among women in the Western world, further intensive research efforts are demanded. In order to develop potent and selective 17β-HSD1 inhibitors, a profound understanding of the enzymatic mechanisms and the structure–function relationships is essential.

The present study has revealed that 13α-estrone (**2a**) and some D-secoestrone derivatives (**3a**–**6a**, **3c** and **5c**), might be promising inhibitors. The very low *in vitro* IC_{50} of **5a** indicates that this compound is one of the most effective 17β-HSD1 inhibitors ever reported. Its 3-methyl ether (**5c**) may be regarded as the first published D-secoestrone that exerts dual independent pre-receptorial antihormonal and antiproliferative effects. Further derivatization of the promising 13α-estrone and D-secoestrone oxime scaffold may lead to drug candidates that possess a beneficial combination of direct cytostatic and endocrine disruptor behaviour. The different *in vitro* inhibitory potentials observed for the C-13 epimer pairs, with the cofactor NADH instead of NADPH, are interesting findings. Additional investigations with the aim of elucidating the binding mechanisms may provide new data clarifying the structure–function relationships of 17β-HSD1.

Declaration of interest

The authors report no declarations of interest. The authors are grateful for the financial support from the Hungarian Scientific Research Fund [OTKA K113150].

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