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Selective inhibition of 17β-hydroxysteroid dehydrogenase type 1 (17βHSD1) reduces estrogen responsive cell growth of T47-D breast cancer cells

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ABSTRACT

The most potent estrogen estradiol (E2) plays a pivotal role in the initiation and progression of estrogen dependent diseases. 17β-Hydroxysteroid dehydrogenase type 1 (17βHSD1) catalyses the NADPH-dependent E2-formation from estrone (E1). It is often overexpressed in breast cancer and endometriosis. For this reason, inhibition of 17βHSD1 is a promising strategy for the treatment of these diseases. In the present paper, we investigate the estrogen responsive cell growth of T47-D breast cancer cells, the intracellular inhibitory activity of non-steroidal 17βHSD1-inhibitors and their effects on estrogen dependent cell growth *in vitro*. At equal concentrations the estrogens E1 and E2 induced the same extent of growth stimulation indicating fast intracellular conversion of E1 into E2. Application of inhibitors selectively prevented stimulation of proliferation evoked by E1-treatment whereas E2-mediated stimulation was not affected. Furthermore, intracellular E2-formation from E1 was significantly inhibited with IC_{50}-values in the nanomolar range. In conclusion, our findings strongly support suitability of non-steroidal 17βHSD1-inhibitors for the treatment of estrogen dependent diseases.

1. Introduction

17β-Hydroxysteroid dehydrogenase type 1 (17βHSD1) catalyses intracellularly the NADPH-dependent reduction of the weakly active estrone (E1) to the highly potent estradiol (E2). Besides its physiological effects in the development and differentiation of estrogen-sensitive tissues, E2 is involved in the initiation and progression of estrogen dependent diseases like breast cancer [1–3] and endometriosis [4,5].

In addition to the type 1 enzyme, until recently 17βHSD7 and 12 were supposed to primarily catalyse intracellular conversion of E1 into E2 [6,7]. But 17βHSD7 was found to be mainly involved in cholesterol synthesis [8,9], and 17βHSD12 was observed to be inefficient in intracellular E2-production compared to 17βHSD1 even at high expression levels as was demonstrated using T47-D cells [10].

In many breast cancer tissues [11–13] and endometriotic lesions [14] overexpression of 17βHSD1 could be detected. In contrast, expression of 17β-hydroxysteroid dehydrogenase type 2 (17βHSD2), which catalyses the deactivation of E2 and therefore plays a protective role [15–17], was not found to be increased, in several cases even decreased [5,18,19]. The resulting enhancement of 17βHSD1 to 17βHSD2 expression ratio [20] maintains the supply of tissue with E2 which is needed for further proliferation [21]. For this reason, the importance of 17βHSD1 in estrogen dependent diseases was recognised [22], and its inhibition is considered as a promising strategy for their treatment.

Until now different endocrine therapies have been administered in breast cancer [23]. Selective estrogen receptor modulators (SERMs) and pure antiestrogens like fulvestrant [24] block the estrogen action at the receptor level while aromatase inhibitors and GnRH-analogues restrain the formation of estrogens. This strong reduction of systemic estrogen action is a rather radical approach resulting in the well-known side effects of these strategies like osteoporosis, hot flushes, or depressive mood. A softer therapy could be the inhibition of 17βHSD1 catalysing the last step of the E2 biosynthesis. Compared to established endocrine breast cancer therapies, which systemically reduce E2-action [23], fewer side effects are expected, because mainly tissue overexpressing 17βHSD1 will be affected.

Abbreviations: 17βHSD1, 17β-hydroxysteroid dehydrogenase type 1; 17βHSD2, 17β-hydroxysteroid dehydrogenase type 2; DMEM, Dilbecco's modified eagle medium; E1, estrone; E2, estradiol; ER, estrogen receptor; FCS, fetal calf serum; HAP, hydroxyapatite; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; NADP(H), nicotinamide adenine dinucleotide phosphate; NAD(H), nicotinamide adenine dinucleotide; PBS, phosphate buffered saline; RBA, relative binding affinity; SDS, sodium dodecyl sulphate; SERM, selective estrogen receptor modulator; TE, Tris–EDTA.

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Over the last decade, several steroidal [25] and few non-steroidal [26–28] classes of 17βHSD1-inhibitors were discovered. Recently, we described two series of non-steroidal compound classes [29–32], which were evaluated by means of our screening system [33]. Since inhibitors show high selectivities towards 17βHSD2 and both subtypes (α and β) of the estrogen receptor (ER) as well as promising pharmacokinetic properties, they are appropriate for further investigations.

Before application in a suitable animal model can be envisaged, efficacy should be shown in cellular experiments. For this purpose, estrogen dependent breast cancer cell lines as T47-D or MCF-7 are available. T47-D cells were chosen, because they express 17βHSD1 and 17βHSD2 in sufficient amount in contrast to MCF-7 [34]. Additionally, they show a high 17βHSD1 to 17βHSD2 expression ratio [35], which is similar as in the diseased tissue. The main objective of this work is to examine whether selective 17βHSD1-inhibition is an appropriate way to reduce estrogen dependent cell proliferation.

2. Materials and Methods

2.1. Chemicals

E1, E2 and MTT were obtained from Sigma, Seelze. Radioactive labeled [2,4,6,7-^{3}H]-E1 (50–100 Ci/mmol) and [2,4,6,7-^{3}H]-E2 (70–115 Ci/mmol) were purchased from Perkin Elmer, Boston. Quickszint Flow 302 and Quickszint 212 scintillator fluids were bought from Zinsser Analytic, Frankfurt. Recombinant ERα and ERβ were purchased from Invitrogen, Carlsbad. Other chemicals were received from Sigma, Roth or Merck.

2.2. Cell culture

T47-D cells were obtained from ECACC, Salisbury. Stripped FCS and cell culture media were purchased from CCpro, Oberdorla. Stock culture of cells was routinely cultivated in RPMI 1640 medium supplemented with 10% FCS (Sigma), L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), insulin zinc salt (10 μg/mL) and sodium pyruvate (1 mM) at 37 °C under 5% CO_2 humidified atmosphere. Subculture was carried out every 2–3 days.

2.3. Cell free inhibition assays of 17βHSD1 and 17βHSD2

For the purification of 17βHSD1, the cytosolic fraction of human placenta was precipitated with ammonium sulphate following a well established procedure [36]. 17βHSD2 was obtained from the microsomal fraction.

Inhibitory activities were evaluated as described before [31,32]. Briefly, for determination of 17βHSD1-inhibition the enzyme preparation was incubated with NADH [500 μM] in presence of potential inhibitors at 37 °C in a phosphate buffer (50 mM) supplemented with 20% of glycerol and EDTA 1 mM. The enzymatic reaction was started by addition of [2,4,6,7-^{3}H]-E1 (500 nM, 0.15 μCi). After 10 min, the incubation was stopped with $HgCl_2$.

The 17βHSD2 inhibition assay was performed similarly to the 17βHSD1 procedure. The microsomal fraction was incubated with NAD^+ [1500 μM], test compound and [2,4,6,7-^{3}H]-E2 (500 nM, 0.11 μCi) for 20 min at 37 °C. Steroids were extracted into diethylether. Substrate and product were separated using acetonitrile/water (45:55) as mobile phase in a C18 reversed phase chromatography column (Nucleodur C18, 3 μm, Macherey-Nagel, Düren) connected to a HPLC-system (Agilent 1100 Series, Agilent Technologies, Waldbronn). Detection and quantification of the steroids were performed using a radioflow detector (Berthold Technologies, Bad Wildbad; Quickszint Flow 302, efficiency 50%).

2.4. ER-affinity assay

Binding affinity of inhibitors to ERα or ERβ was determined as described before [31] similarly to Zimmermann et al. [37]. Briefly, 0.25 pmol of ERα or ERβ, respectively, were incubated with [2,4,6,7-^{3}H]-E2 (10 nM, 0.07 μCi) and test compound for 1 h at room temperature. Non-specific-binding was performed with diethylstilbestrol (10 μM). After incubation, ligand-receptor complexes were bound to hydroxyapatite (HAP; 5 g/60 mL TE-buffer). After centrifugation the HAP-fraction was separated, washed and resuspended in ethanol. For radiodetection, scintillation cocktail (Quickszint 212) was added and samples were measured in a liquid scintillation counter (Rack Beta Primo 1209, Wallac, Turku). The relative binding affinity (RBA) was calculated after inhibitor and E2 concentrations required to displace 50% of the receptor bound labelled E2 were determined, using the following equation:

$$\mathrm{RBA}[\%] = \frac{\mathrm{IC}_{50}(\mathrm{E2})}{\mathrm{IC}_{50}(\mathrm{compound})} 100$$

The RBA value for E2 was arbitrarily set at 100%.

2.5. MTT-Cytotoxicity assay

The number of living cells was evaluated measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT). Experiments were performed in 96-well cell culture plates in DMEM supplemented with 10% FCS. Cells were incubated for 3 h with 2.5 μM of test compound at 37 °C in a humidified atmosphere at 5% CO_2. For cleavage reaction MTT-solution (5 mg/mL in PBS) was added and incubation was continued for another 3 h. Reaction stop and cell lysis were carried out by addition of sodium dodecyl sulphate (SDS) in 0.01 N HCl (10%). The produced blue formazan was quantified spectrophotometrically at 590 nm as described by Denizot and Lang [38] with minor modifications.

2.6. Cellular inhibition assay of 17βHSD1 and long-term incubation

Experiments were performed in 24-well plates in DMEM supplemented with 10% (v/v) FCS (Pan Biotech GmbH, Aidenbach), L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), insulin-zinc-salt (10 μg/mL) and sodium pyruvate (1 mM). After an adaption phase of 24 h the medium was changed for fresh serum free DMEM and a solution of test compound in DMSO was added. The final concentration of DMSO was adjusted to 1% in all samples. After a pre-incubation of 30 min, incubation was started by addition of [2,4,6,7-^{3}H]-E1 (50 nM, 0.15 μCi). After 30 min, the conversion reaction was stopped by removing the supernatant medium. The steroids contained in the aspirated medium were extracted into diethylether. Further treatment of the samples was carried out as described above.

2.7. Proliferation assay

RPMI 1640 (without phenol red) was used for the experiments and was supplemented with streptomycin (100 μg/mL), insulin zinc salt (10 μg/mL), sodium pyruvate (1 mM), L-glutamine (2 mM), penicillin (100 U/mL) and charcoal-stripped FCS 5% (v/v). Cells were grown for 2 days in phenol red-free medium before washing and addition of estrogens and/or compounds. Inhibitors, 4-hydroxytamoxifen, E1 and E2 were diluted in ethanol. The final ethanol concentration was adjusted to 1%. The medium was changed every 2–3 days and supplemented with the respective additive. After 8 days of culture without passage in the presence of the respective additives, the cell viability was evaluated measuring

the reduction of MTT as described above. Proliferation in presence of vehicle was set at 100%.

2.8. Statistics

Statistical significance was calculated by two-sided *t*-tests. Values are mean ± SEM. $p < 0.05$ was considered as significant, $p < 0.003$ as highly significant.

3. Results

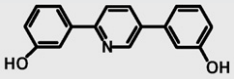
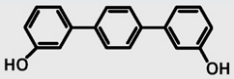
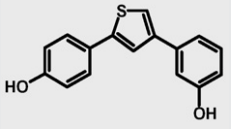
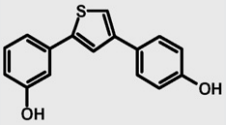
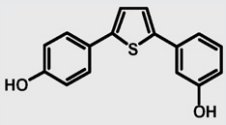
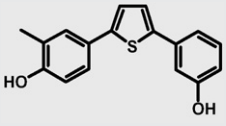
3.1. Selection of appropriate candidate compounds for extended investigation in T47-D cells

Following our screening procedure several compounds had been identified for further evaluation [31,32]. Table 1 shows data of the most promising 17βHSD1-inhibitors. They all are bis(hydroxyphenyl)substituted six or five membered aryls, the latter consisting of 2,5- and 2,4-disubstituted thiophenes.

For comparison of the compounds, their inhibitory activities at 17βHSD1 and 17βHSD2 were determined in cell free procedures. All compounds showed IC_{50}-values below 200 nM for inhibition of 17βHSD1 and selectivity factors of more than 10 towards 17βHSD2. Affinities to the ERs were marginal with RBA-values below 1.0% (E2: RBA = 100%) except for **5** with slightly enhanced ERβ-affinity. Nevertheless, binding affinities did never exceed the RBA of E1 (RBA(ERα) = 5.5%; RBA(ERβ) = 3.1%). Furthermore, survival rate of the cells after 3 h in presence of 2.5 μM of 17βHSD1-inhibitor was evaluated to ensure that intracellular inhibitory activities were not caused by unspecific cytotoxic effects. No significant reduction of cell number was observed indicating, that the compounds do not exert cytotoxic effects at the given concentration. Evaluation of the compounds in a cellular 17βHSD1-inhibition assay using T47-D cells showed inhibitory activities, which were less pronounced compared to the cell free assay. Nevertheless, all inhibitors showed IC_{50}-values below 500 nM in intact cells.

Based on the data presented in Table 1, candidates for the investigation of the influence of selective 17βHSD1-inhibitors on estrogen-responsive cell growth were chosen. For selection, different aspects were taken into account. Aside from inhibitory activity and selectivity towards 17βHSD2, affinities to the ERs were an important criterion. Since estrogenic or antiestrogenic effects would interfere with the investigation of estrogen dependent cell proliferation, inhibitors with lower RBA-values were preferred to those with higher binding affinity. Structural diversity was an additional aspect for selection. Thus, one representative of each core

Table 1
Inhibitory activity of compounds **1**–**6** at 17βHSD1 and 17βHSD2, binding affinities to ERα and ERβ and cell survival rate after compound application.

Compound	Structure	Cell-free assays; IC_{50} (nM)[a,b]			RBA (%)[a,b,f]		Survival rate[a,h] (%)	Cellular assay[a,i]
		17βHSD1[c]	17βHSD2[d]	Selectivity factor[e]	ERα[g]	ERβ[g]		IC_{50} (nM)
1		101	3399	34	<0.1	<0.01	90	382
2		173	2259	13	<0.001	<0.1	95	316
3		151	1690	11	<0.1	<1.0	105	404
4		77	1271	17	<0.1	<1.0	102	413
5		69	1953	28	<1.0	<10	99	469
6		46	1971	43	<0.01	<0.01	109	425

[a] Mean values of three determinations, standard deviation less than 16%.
[b] Data given for comparison.
[c] Human placenta, ammonium sulphate precipitate from cytosolic fraction, substrate [^{3}H]-E1 + E1 [500 nM], cofactor NADH [500 μM].
[d] Human placenta, microsomal fraction, substrate [^{3}H]-E2 + E2 [500 nM], cofactor NAD^+ [1500 μM].
[e] IC_{50}(17βHSD2)/IC_{50}(17βHSD1).
[f] Relative binding affinity (RBA), E2: 100%, < 0.01≙0.001 < RBA < 0.01, < 0.1≙0.01 < RBA < 0.1, < 1.0≙0.1 <RBA < 1.0, < 10≙1.0 <RBA < 10.
[g] Human recombinant protein, incubation with 10 nM [^{3}H]-E2 and inhibitor for 1 h.
[h] Survival rate of T47-D cells after incubation with compound at 2.5 μM for 3 h, amount of living cells determined by MTT-conversion, control: 100%.
[i] Intact T47-D cells substrate [^{3}H]-E1 + E1 [50 nM].

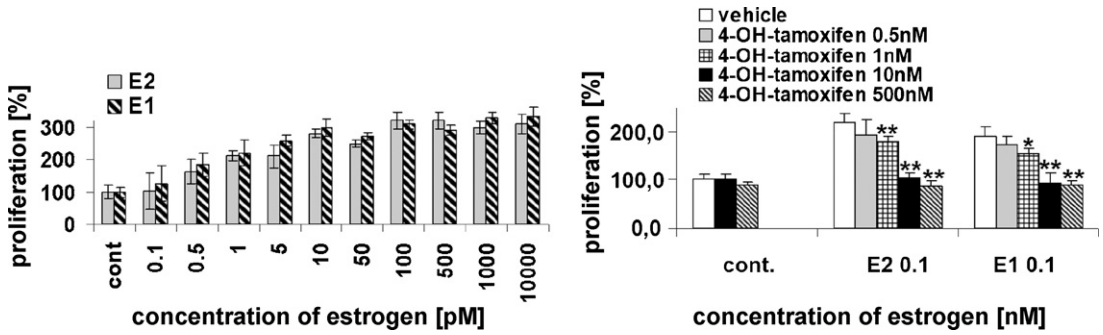


Fig. 1. (A) Concentration dependent stimulation of cell proliferation (T47-D). Grey bars represent E2-stimulation, striped bars E1-stimulation. The control was arbitrarily set at 100%. First significant stimulation was observed at 0.5 pM ($p < 0.05$). (B) Effect of 4-hydroxytamoxifen on estrogen-stimulated cell proliferation *in vitro*. T47-D cells were treated with 4-hydroxytamoxifen at 10 or 500 nM in presence (0.1 nM) or absence of E2 or E1, respectively and at 0.5 nM or 1 nM in presence (0.1 nM) of E2 or E2. $^{*}p < 0.05$, $^{**}p < 0.003$.

structure was chosen: **2** because of its very low ERα-affinity, **4** for its higher inhibitory activity and slightly better selectivity compared to **3**, and **6**, the most potent inhibitor in the cell free assay with promising data in the other tests (Table 1).

3.2. Investigation of estrogen stimulation of cell proliferation in T47-D cells

Extended evaluations of 17βHSD1-inhibitors were carried out in the estrogen dependent breast cancer cell line T47-D. For experiments, phenol red-free culture medium supplemented with 5% of stripped FCS was used. Prior to inhibitor application, estrogen responsiveness of the cells was confirmed. For this purpose, E1 or E2 was added to the culture medium at different concentrations ranging from 0.1 pM to 10 nM, and the number of living cells was determined after 8 days of incubation without passage (Fig. 1A). E1 stimulated cell proliferation to the same extent as E2, although E1 is known to be the weaker estrogen. A significant stimulation could be observed at a concentration as low as 0.5 pM. Maximum stimulation was reached at an estrogen concentration of 0.1 nM. Consequently, an estrogen-concentration of 0.1 nM was chosen for further experiments.

In order to confirm that estrogen stimulation is receptor-mediated, cells were treated with 4-hydroxytamoxifen, the active metabolite of tamoxifen, in presence or absence of 0.1 nM of E1 or E2, respectively (Fig. 1B). The number of living cells was determined after 8 days without passage. 4-Hydroxytamoxifen was added in four concentrations in presence of estrogen ranging from 0.5 to 500 nM and in two concentrations (10 and 500 nM) in absence of estrogen. Even at a concentration of 500 nM, 4-hydroxytamoxifen did not stimulate or reduce cell proliferation. The stimulation, which was evoked by 0.1 nM of E1 or E2, respectively, was dose dependent and fully prevented by simultaneous addition of 4-hydroxytamoxifen at 10 nM. This finding demonstrates, that E1 and E2 unfold their growth stimulatory effect via ER-activation.

3.3. Effects of selective 17βHSD1-inhibitors on estrogen stimulated cell proliferation

Compounds **2** and **6** were tested at higher concentrations. They were able to reduce E1-stimulation to control-level when added at a concentration of 1 μM. An example of the dose dependent reduction of stimulated cell growth is given in Fig. 2. First significant reduction could be observed at an inhibitor concentration of 0.1 nM. At 1 nM highly significant growth inhibition was seen. Therefore, a concentration of 1 nM was chosen for further experiments.

The ability of the inhibitors to reduce estrogen stimulated cell growth was evaluated in a proliferation assay using T47-D cells

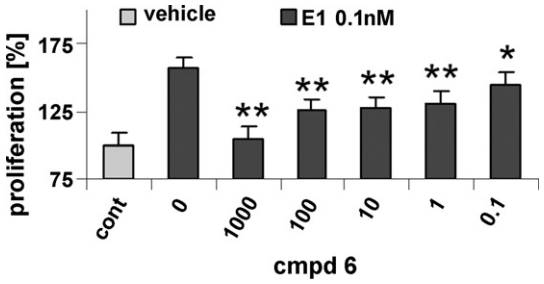


Fig. 2. Concentration dependent inhibition of E1-stimulated cell growth for compound **6** in T47-D cells. Light grey bar represents the vehicle treated control. Dark grey bars represent E1-treated cells. **6** was added at different concentrations ranging from 0.1 to 1000 nM. Cells were incubated with the respective additives for 8 days without passage. Medium was changed every 2–3 days. Vehicle = ethanol, $^{*}p < 0.05$, $^{**}p < 0.003$.

(Fig. 3). The compounds should not stimulate or reduce cell proliferation per se, because this finding would indicate estrogenic or toxic effects. In contrast, selective 17βHSD1-inhibitors should reduce stimulation when added simultaneously with E1, as they inhibit E1-conversion to E2. Consequently, the smaller amount of highly active E2 in the cells should result in a decrease of cell growth stimulation. In case of simultaneous addition of E2 and 17βHSD1-inhibitor, E2-stimulated cell proliferation should not be prevented, provided that the inhibitor does not exert antiestrogenic effects at the receptor level.

Compounds **2**, **4**, and **6** were evaluated in the proliferation assay as representatives of 1,4-disubstituted arenes (**2**), 2,4-disubstituted thiophenes (**4**), and 2,5-disubstituted thiophenes (**6**). Experiments were conducted in phenol red-free medium supplemented with 5% of stripped FCS. E1 or E2 was added in combination with 17βHSD1-inhibitor or alone. Inhibitors were added at a concentration of 1 nM in presence or absence of 0.1 nM of E1 or E2, respectively.

E1 and E2 stimulated cell proliferation to the same extent. None of the inhibitors caused any significant alteration of cell proliferation when added at 1 nM without estrogen. Even at concentrations of 100 nM, inhibitors did not show any significant reduction or stimulation of basal cell growth (data not shown). This result was expected regarding the determined RBA-values and shows that the inhibitors do not act as estrogens at the applied concentrations.

Simultaneous treatment of the cells with E1 and 17βHSD1-inhibitor led to significant reduction of cell proliferation compared to the treatment with E1 alone. The finding, that all inhibitors were effective at the same concentration is in accordance with the comparable IC_{50}-values determined in the cellular inhibition assay. In contrast, none of the three inhibitors influenced E2-stimulated cell growth. The finding that 17βHSD1-inhibitors are able to selectively

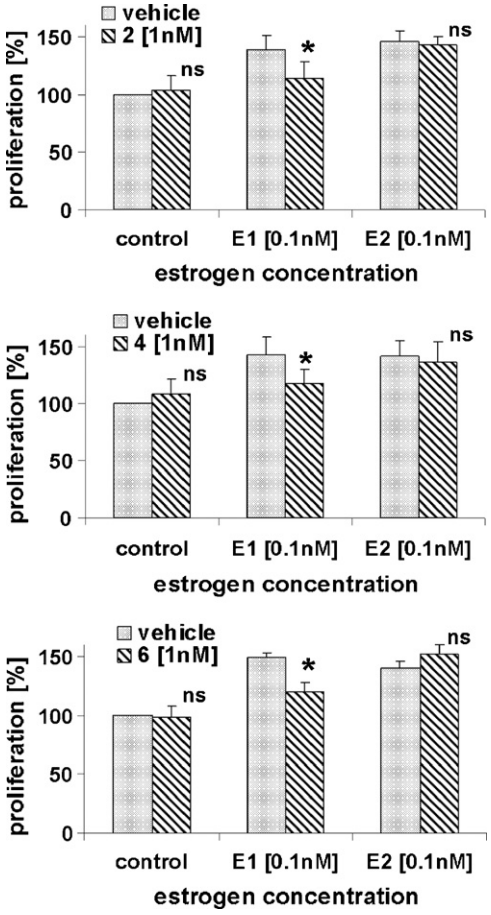


Fig. 3. Effects of compounds **2**, **4**, and **6** on estrogen-stimulated cell proliferation in T47-D. Cells were grown in phenol red-free RPMI 1640 medium supplemented with 5% stripped FCS before and during treatment. Proliferation was stimulated with E1 or E2 at a dose of 0.1 nM, respectively. Compounds **2**, **4**, and **6** were added at a concentration of 1 nM in presence or absence of E1 or E2. Cells were incubated with the respective additives for 8 days without passage. Medium was changed every 2–3 days. Vehicle = ethanol, $^{*}p < 0.05$, $^{ns}p > 0.05$. Each panel represents the mean of at least three independent experiments.

reduce E1-mediated cell proliferation without influencing E2-stimulation clearly demonstrates that this effect is caused by 17βHSD1-inhibiton and not by receptor blockade.

4. Discussion and Conclusion

The present paper shows that selective 17βHSD1-inhibitors are able to reduce the stimulation of proliferation induced by E1-addition to T47-D cells.

This cell line was chosen because intracellular enzyme concentrations especially 17βHSD1 and 17βHSD2 are similar to those found in estrogen dependent diseases [34]. T47-D cells express ERs and show estrogen dependent proliferation [39,40]. The enhanced ratio of 17βHSD1 to 17βHSD2 as it is observed in T47-D cells is very similar to that seen in diseased tissue. This elevated proportion leads to a pronounced intracellular E2-production from E1.

Estrogen responsive growth of the obtained cells was confirmed by addition of E1 or E2 at several concentrations. The stimulation of cell proliferation was dose dependent with both estrogens. The finding that the weaker estrogen E1 stimulates cell proliferation to the same extent as does E2 when given at the same concentration can be explained by a rapid intracellular conversion of E1 into E2. The high ratio of 17βHSD1/17βHSD2 leads to an estrogen balance in the cells which is characterised by an excess of E2. Hence, the formed E2 is responsible for ER-activation and consequently for enhanced cell growth in the E1-treated cells confirming the relevance of T47-D cells as a model for estrogen dependent diseases.

Since cell proliferation was not altered by application of 17βHSD1-inhibitors alone even at a concentration of 100 nM, we conclude that our compounds do neither activate the ER nor show unspecific reduction of proliferation by for example toxic effects.

As found in case of 4-hydroxytamoxifen, blockade of the ERs would reduce E2-mediated cell proliferation. When added in combination with E2, the inhibitors did not reduce hormone-mediated cell proliferation. Thus, it can be excluded that the compounds reduce cell proliferation via ER-blockade at the given concentration. Considering these findings, the tested 17βHSD1-inhibitors do not interfere with the ERs at the concentrations applied in the proliferation assay as was expected from the receptor assays.

The stimulation achieved by E1-addition could be reduced by simultaneous application of different non-steroidal 17βHSD1-inhibitors. This observation is in agreement with experiments of Day et al. [10] and Laplante et al. [41], who describe significant reduction of E1-induced proliferation by steroidal 17βHSD1-inhibitors in a cell culture experiment. Both groups use higher inhibitor concentrations than 1 nM as used in our experiment: 500 and 100 nM, respectively. Moreover, in case of Laplante et al., the inhibitor caused a stimulation of cell proliferation when added at a concentration of 10 nM to the cells, suggesting estrogenic activity. In the experiment of Day et al., the used inhibitor reduced the E2-stimulated cell proliferation at the applied concentration. To our knowledge we are the first to show selective inhibition of E1-stimulated proliferation with non-steroidal inhibitors at very low concentrations without affecting the ER.

Inhibition of 17βHSD1 is an innovative, novel concept which might be superior or at least adequate to existing endocrine treatment modalities. One current therapy option is the application of SERMs like, e.g. tamoxifen, which block the ER. It is successfully administered in the majority of ER+ tumors. But SERMs have several drawbacks. At first they show estrogenic effects in the uterus and therefore can evoke endometrial carcinomas [42]. Secondly, they only antagonize estrogenic effects. The proliferation-stimulating agent E2 is still present in the diseased tissue. By antagonizing the estrogenic effects unselectively in the whole body, SERMs cause side effects [43]. Moreover, in long term treatment resistance development occurs often. Therefore, there is need for a therapy change after a certain time [44]. Consequently, SERMs are an effective but not an optimal treatment of breast cancer.

For the reduction of E2 in the diseased tissue there are different possibilities. One can use GnRH-analogues, which suppress estrogen production at ovarian level. Since they totally reduce the estrogens in the whole body, they cause strong side effects.

Another possibility is administration of aromatase inhibitors, which is normally applied in postmenopausal women. In reproductive age, usually the central feedback mechanism has to be suppressed by additional application of GnRH-analogues. The advantages of aromatase inhibitors are effectiveness, low risk of estrogenic effects and inhibition of the estrogen synthesis, which is a more causal approach than SERM-treatment. But the total blockage of estrogen synthesis is a reason for severe side effects [43,45]. As described for the SERMs there is also the risk of resistance development, which is an additional drawback.

We conclude that nowadays, we have effective medical treatments in hands but they are not the optimal therapy. Therefore, we think that new therapeutical approaches have to be considered. For this purpose, we propose 17βHSD1-inhibitors. In first animal studies, they show promising results in breast cancer treatment. 17βHSD1-inhibitors selectively suppress the biosynthesis of the proliferation-stimulating E2. Since the enzyme shows a tissue specific expression pattern and in case of breast cancer even an

overexpression, a better selectivity of E2-reduction will be reached. Therefore, 17βHSD1-inhibitors should show fewer side effects than current therapeutic agents and may be less problematic in treatment of premenopausal women. For these reasons, we consider 17βHSD1-inhibitors as potential further option in the treatment of breast cancer.

In the present paper, we focus on the indication of breast cancer but 17βHSD1-inhibitors may also have a benefit in endometriosis, which is also an estrogen dependent disease. In endometriosis 17βHSD1 was also found to be overexpressed [46] while expression of 17βHSD2 seems to be reduced [47]. Therefore, in endometriosis there is an environment present which favours E2-production over E2-inactivation. This enhanced ratio of 17βHSD1/17βHSD2 may be regulated by 17βHSD1-inhibitors. Therefore, 17βHSD1-inhibitors may also be valuable for the treatment of endometriosis.

In summary, we were able to show, that selective non-steroidal 17βHSD1-inhibitors are appropriate to reduce E1-mediated T47-D cell proliferation, while E2-induced stimulation was not affected. This finding validates 17βHSD1-inhibition as an effective strategy in inhibition of uncontrolled cell proliferation in estrogen dependent diseases. Consequently, 17βHSD1-inhibitors should be considered as an additional therapy option in breast cancer and endometriosis.

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