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**Anticancer effects of estrone derivatives and nonsteroidal 17 $\beta$ -  
hydroxysteroid dehydrogenase type 1 inhibitors**

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## INTRODUCTION

Cancer is a major health problem and leading disease in many countries worldwide. Accordance to the survey lung cancer is the leading cancer type of men's while breast cancer of women's. Growth control of the tumors is a very complex multifactorial process and characterized with dedifferentiation and uncontrolled cellular proliferation.

Natural steroid products and their synthetic analogs are intensively investigated in order to describe and exploit their anticancer capacity, thus it could be considered a basic source of innovative drugs. Therefore, steroidal-type structures with different function group may suggest starting structures for novel synthetic antiproliferative drugs. Diosgenin and many related steroidal alkaloids exhibit potent anticancer effects. The most intensively investigated estrane is an endogenous estradiol metabolite, 2-methoxyestradiol, which does not exhibit hormonal activity, but seems to be highly effective against a broad range of cancer cell lines.

The most important estrogen,  $17\beta$ -estradiol, is involved in many hormone-dependent proliferative disorders in humans, including cancers of gynecological origin and endometriosis. The suppression of estrogen exposure at the targeted tissue is therefore a part of the rational therapy and is nowadays successfully applied in patients using selective estrogen receptor modulators (SERMs) or aromatase inhibitors. An alternative approach, aiming at decreasing the level in active  $17\beta$ -estradiol, is inhibition of  $17\beta$ -hydroxysteroid dehydrogenase type 1 ( $17\beta$ -HSD1) which is responsible for the conversion of the much less potent estrogen estrone into  $17\beta$ -estradiol. Since most of the targeted hormone-dependent disorders share a proliferative character, it is conceivable that a direct antiproliferative action combined to the enzyme inhibition effect could be beneficial for the treatment of these diseases.

The G1-S transition is governed by an orchestrated interaction of a set of regulating factors, including Rb (retinoblastoma protein), CDK2, CDK4/6, p16, p21 and p53 determined crucial proteins. Entry into the S phase, and therefore cell proliferation, is inhibited as long as Rb remains unphosphorylated by a complex containing cyclin E-CDK2 and cyclin D-CDK4/6. Phosphorylated Rb dissociates from factor E2F allowing the transcription of S-phase-specific gene. The principal regulator of the cyclin E-CDK2 complex is tumor suppressor p21, which is transcriptionally activated by p53 encoded by the TP53 gene. The importance of this pathway is illustrated by the finding that nearly all

cancers have a mutation in the TP53 gene or in some components of its downstream event. The hypofunction of p16 protein (also referred to as CDK4 inhibitor) has been associated with several malignancies and its expression correlates with the chemotherapy response in patients with solid tumors. Therefore, inhibition of G1–S transition could be the target of innovative therapeutic modalities.

## AIMS

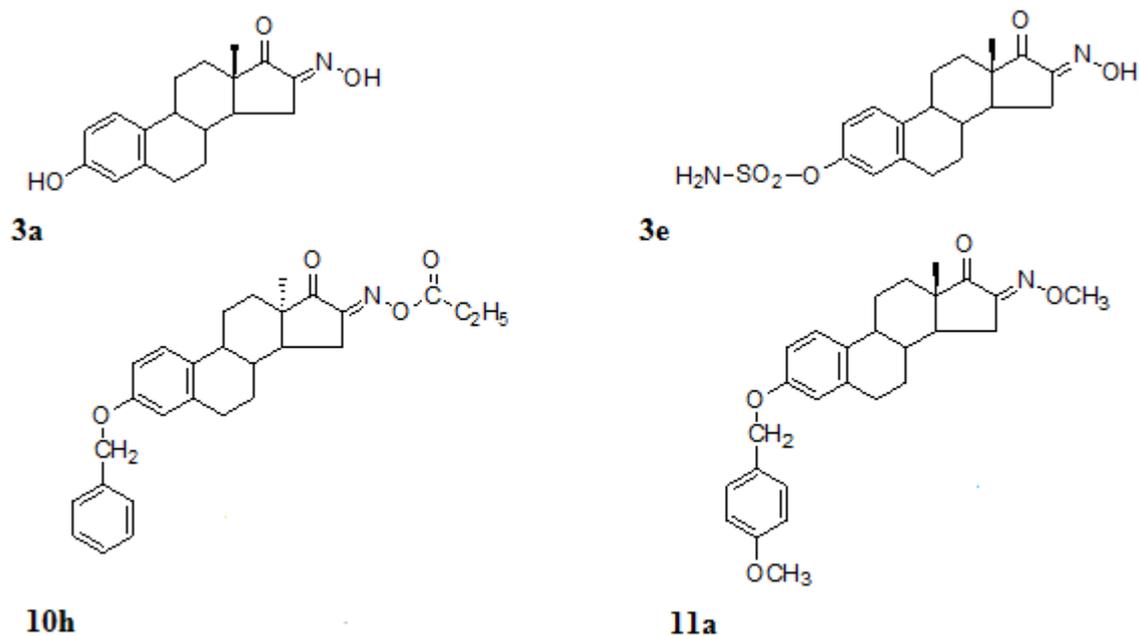
The aim of the present study was the investigation of the antiproliferative properties of synthetic compounds selected on the base of the previously described theoretical considerations:

- Investigation of antiproliferative action of newly synthesized estrone-16-oxime ethers *in vitro* using human adherent cancer cell lines. The most potent compounds were selected for a further set of experiments in order to describe the possible mechanism of the action.
- A set of estradiol derived steroid aglycones and glycosteroids was additionally screened for their antiproliferative effects.
- The determination of the antiproliferative capacity of a set of previously designed and synthesized nonsteroidal 17 $\beta$ -HSD1 inhibitors, *in vitro*. Further *in vitro* assays including cycle analysis, HOPI-double staining, BrdU incorporation assay, Caspase-3 activity assay, RT-PCR technique and Western blot studies are used to characterize the mechanism of the most potent agents.

## MATERIALS AND METHODS

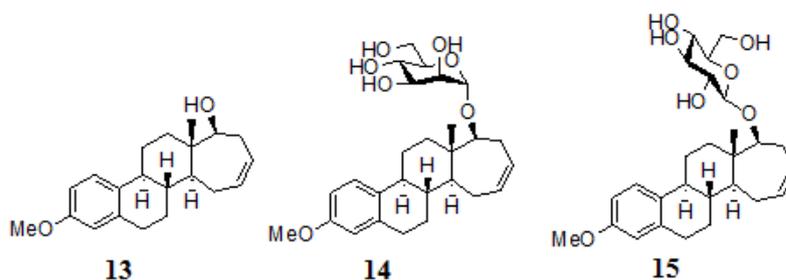
### Investigated compounds

63 investigated compounds of the estrone-16-oxime derivatives were substituted with different function groups at position 3 and 16 of ring A and D (Fig. 1). The most potent 16-oxime (**3a**, **3e**) included free phenolic hydroxyl-function or sulfamate groups at position C3. Both the propionate-ester (**10h**) from the group of oxim-esters, containing a 3-benzyl-protector group, and also the oxim-methylether (**11a**) from the group of oxim-ethers, containing p-methoxybenzyl-protector group, showed a significant cytostatic effect.



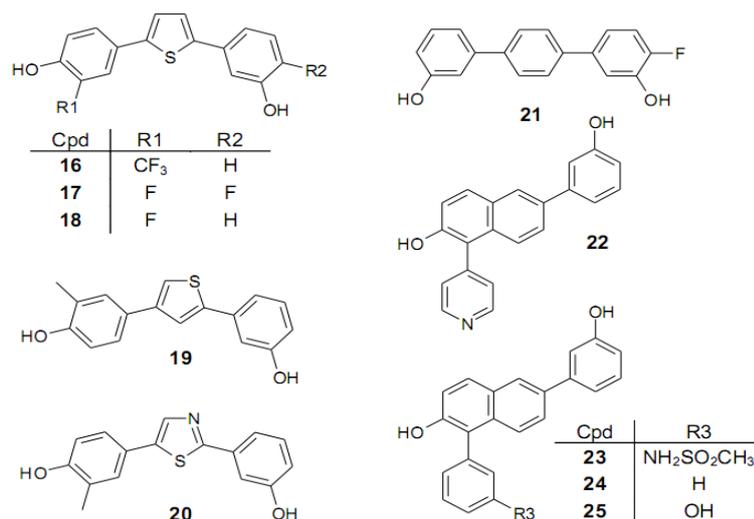
**Figure 1.** Chemical structures of four investigated steroidal oximes

In case of estradiol-derived steroids compound **13** belongs to the aglycones while compounds **14** and **15** possessed glyco steroid structure (Fig. 2).



**Figure 2.** Chemical structures of the estradiol derived steroid aglycones and glyco steroids

All the included  $17\beta$ -HSD1 inhibitors had an aromatic core with two phenolic substituents (Fig. 3). Five of the molecules contained thiophene (**16-19**) or thiazol (**20**) as central part. Compound **21** was a substituted para-terphenyl while agents **22-25** were diphenyl naphthols.



**Figure 3.** Chemical structures of the 17 $\beta$ -hydroxysteroid dehydrogenase type 1 inhibitors.

### Tumor cell lines and cell culture

Human cancer cell lines HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) as well as noncancerous MRC-5 human lung fibroblasts were maintained in minimal essential medium. A2780 cells (isolated from ovarian cancer) were maintained in RPMI medium. The cells were grown in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C.

### MTT assay

The effects on the viability of malignant cells were determined in vitro by using MTT assay. Cells were seeded onto 96-well plates and allowed to stand overnight, after which the medium containing the tested compound was added. 10 mM stock solutions of the tested compounds were prepared with dimethyl sulfoxide (DMSO). After a 72-hours incubation period, viability was determined by the addition of 20  $\mu$ L MTT solution (5 mg/mL). The precipitated formazan crystals were solubilized in DMSO and the absorbance was read at 545 nm with an ELISA reader. Two independent experiments were performed with five parallel wells and cisplatin, an agent clinically used in some gynecological malignancies, was used as positive controls. Sigmoidal dose-response curves were fitted to the measured points, and the IC<sub>50</sub> values were calculated by means of GraphPad Prism 4.0.

### **Hoechst 33258 - propidium iodide double-staining and flow cytometric analysis**

HeLa cells were seeded into a 96-well plate (5000 cells/well). After incubation for 24 hours with the test compound, Hoechst 33258 (HO) and propidium iodide (PI) were added to the culture medium to give final concentrations of 5 µg/mL and 2 µg/mL, respectively. The staining allowed the identification of live, early-apoptotic, late-apoptotic and necrotic cells. HO permeates all the cells and makes the nuclei appear blue. Apoptosis was revealed by nuclear changes such as chromatin condensation and nuclear fragmentation. The necrotic and the late-apoptotic cells were identified as cell with PI uptake, which indicates loss of membrane integrity, the cell nuclei being stained red.

Flow cytometric analysis was performed in order to characterize the cellular DNA content of treated HeLa cells. After 24 and 48 hours treatment the DNA of cells (200,000/condition) was stained with PI. The samples were analyzed by FACStar and 20,000 events were recorded, and the percentages of the cells in the different cell-cycle phases (subG1, G1, S and G2/M) were calculated by using winMDI2.9. The sub G1 fractions were regarded as the apoptotic cell population.

### **BrdU incorporation assay**

5-Bromo-2'-deoxyuridine (BrdU) incorporation into the cellular DNA was determined by BrdU Labeling and Detection Kit I and III on HeLa cells (5000 and 3000/well) treated with the test compound for 24 hours. The incorporation of BrdU in place of thymidine was monitored as a parameter for DNA synthesis. The cellular DNA was partially digested by nuclease treatment and peroxidase labeled antibody was added which bounded to BrdU or labeled with mouse monoclonal antibody. Finally, the peroxidase activity was determined colorimetrically by the addition of the substrate. In the case of Kit III the absorbance was measured with a microplate reader at 405 nm with a reference wavelength at 492 nm. Two independent experiments were performed with 4 parallel wells. Alternatively, the fluorescein-conjugated anti-mouse antibody was added and the wells were examined by fluorescent microscopy with the use of an appropriate optic block (ex: 465-495 nm, em: 515-555 nm, dichromatic mirror: 505 nm, Kit I). At least 400 cells were counted from four parallel wells for the expression of the BrdU-positive cells.

### **Caspase-3 assay**

The activity of caspase-3 from treated cells was determined by means of a commercially available colorimetric kit. Briefly, HeLa cells were exposed to the test item for 48 hours and then scraped, counted and resuspended in lysis buffer. The caspase-3 activity was measured by the addition of substrate (Ac-DEVD-pNA) and the amount of product (pNA) was measured at 405 nm after incubation for 17 hours. Results on treated cells are given as fold-increase by direct comparison with the untreated control results.

### **Reverse transcriptase-PCR studies**

The effects of the tested compounds on the mRNA expression pattern of retinoblastoma protein (Rb), cyclin-dependent kinase 2 (CDK2), cyclin-dependent kinase 4 (CDK4), cyclin-dependent kinase 6 (CDK6), p16, p21, p27 and p53 regulator factors were determined by RT-PCR in HeLa cells. After a 24 hours incubation period, total RNA was isolated from the cells ( $5 \times 10^5$ ) using TRIzol Reagent and cDNA was prepared in the presence of RT. Human glyceraldehydes-3-phosphate dehydrogenase (hGAPDH) primers were used as internal control in all samples. The sequences of the oligonucleotide primers for Rb, CDK2, CDK4/6, p16, p21, p27 and p53 were the same as previously reported.

### **Western blotting studies**

HeLa cells were treated with investigated compounds and whole-cell extracts were prepared by lysis buffer. 50  $\mu$ g of protein per well was subjected to electrophoresis on 4-12% NuPAGE Bis-Tris Gel in XCell SureLock Mini-Cell Units. Proteins were transferred from gels to nitrocellulose membranes, using the iBlot Gel Transfer System. Antibody binding was detected with the WesternBreeze Chromogenic Western blot immunodetection kit. The blots were incubated on a shaker with Rb, phosphorylated Rb (pRb) and  $\beta$ -actin polyclonal antibody 1:200 in the blocking buffer.

## **RESULTS**

### **MTT assay**

All of four estrone-16-oximes (**3a**, **3e**, **10h** and **11a**) affected the proliferation of HeLa cells comparably to the reference agent cisplatin, while MCF7, A413 and A2780 cells were less sensitive. The viability of noncancerous fibroblast cell line MRC-5 was affected only by **11a**, with a higher calculated IC<sub>50</sub> value than that of cisplatin. Agents with an

unsubstituted oxime function (**3a** and **3e**) could be regarded as selective for HeLa cells, with some modest action against ovarian cancer (A2780) cell line, while **10h** and **11a** displayed a broader spectrum of activities (Table 1).

Modified estrane-based steroid aglycone and glycosteroids (**13**, **14** and **15**) exerted moderate antiproliferative effect against three cancer cell lines HeLa, MCF7, A2780 and A431.

Compounds **16-22** exhibited antiproliferative activities comparable to that of the reference agent cisplatin on the HeLa cells, while the MCF7 and A2780 cells were generally less sensitive. None of compounds **16-22** exerted appreciable antiproliferative action against the noncancerous MRC-5 cells up to 30  $\mu\text{M}$ . Compound **21** proved to be the most potent antiproliferative agent, exhibiting a lower  $\text{IC}_{50}$  than that of the reference compound cisplatin. Test substances with a hydroxyphenylnaphthalene structure (compounds **23-25**) exhibited substantially weaker action against the cell lines used.

**Table 1.** Calculated  $\text{IC}_{50}$  values of the tested steroids (**3a-11a**, **13-15**) and  $17\beta$ -HSD1 inhibitors (**16-25**).

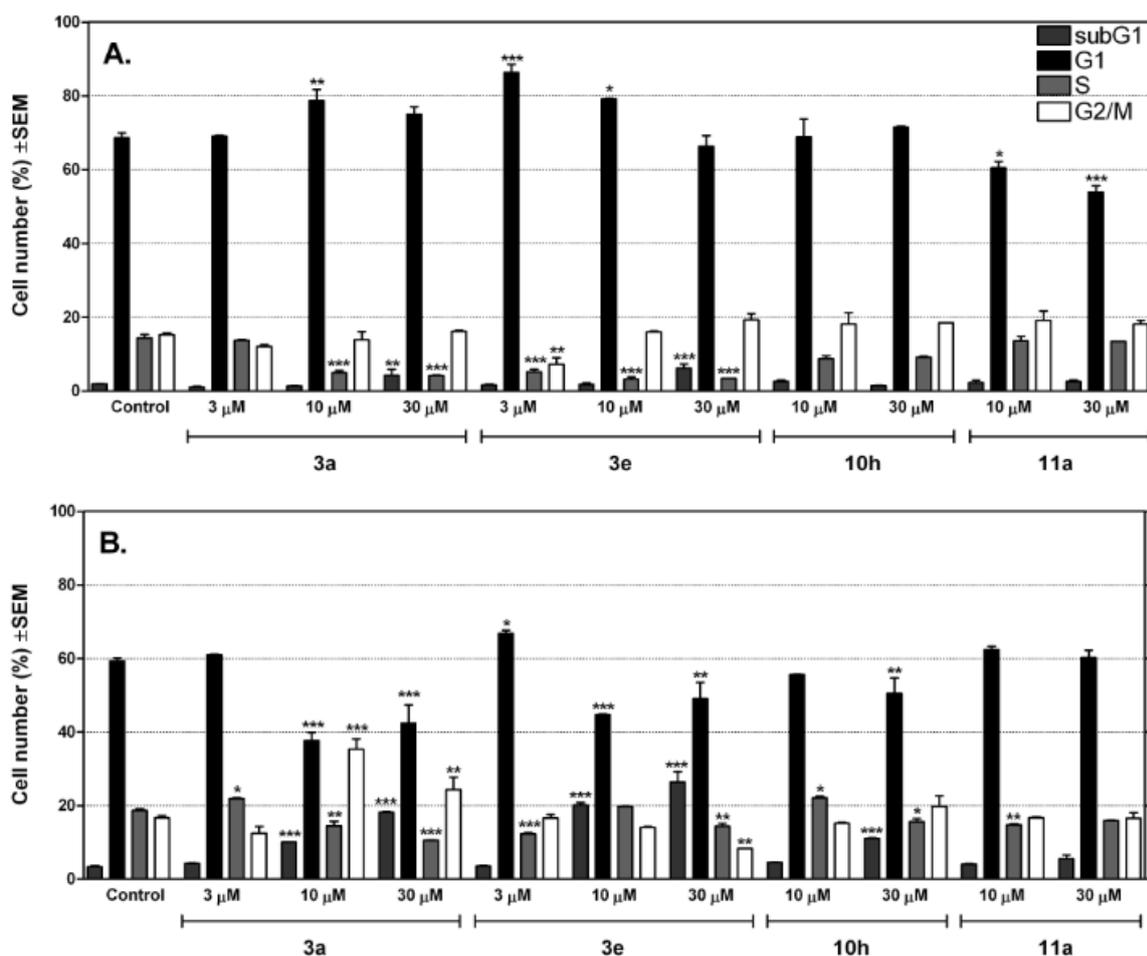
Compounds	$\text{IC}_{50}$ values ( $\mu\text{M}$ )				
	HeLa cells	MCF7 cells	A431 cells	A2780 cells	MRC-5 cells
<b>3a</b>	4.41	-	-	18.28	-
<b>3e</b>	4.04	-	-	11.96	-
<b>10h</b>	3.52	4.13	-	4.61	-
<b>11a</b>	5.63	-	13.25	12.62	6.94
<b>13</b>	15.68	19.91	15.20	10.45	-
<b>14</b>	10.82	12.40	10.39	-	-
<b>15</b>	-	27.45	-	19.88	-
<b>16</b>	5.29	25.23	-	-	-
<b>17</b>	6.84	13.88	-	-	-
<b>18</b>	3.81	16.11	-	-	-
<b>19</b>	14.53	-	-	-	-
<b>20</b>	5.66	-	-	-	-
<b>21</b>	1.41	-	-	-	-
<b>22</b>	5.30	25.51	-	9.71	-
<b>23</b>	-	-	-	25.87	n.d
<b>24</b>	-	-	-	-	n.d
<b>25</b>	-	-	-	-	n.d
<b>Cisplatin</b>	5.66	7.99	8.81	0.86	4.13

-: Mean value above 30  $\mu\text{M}$ ; n.d: not determined

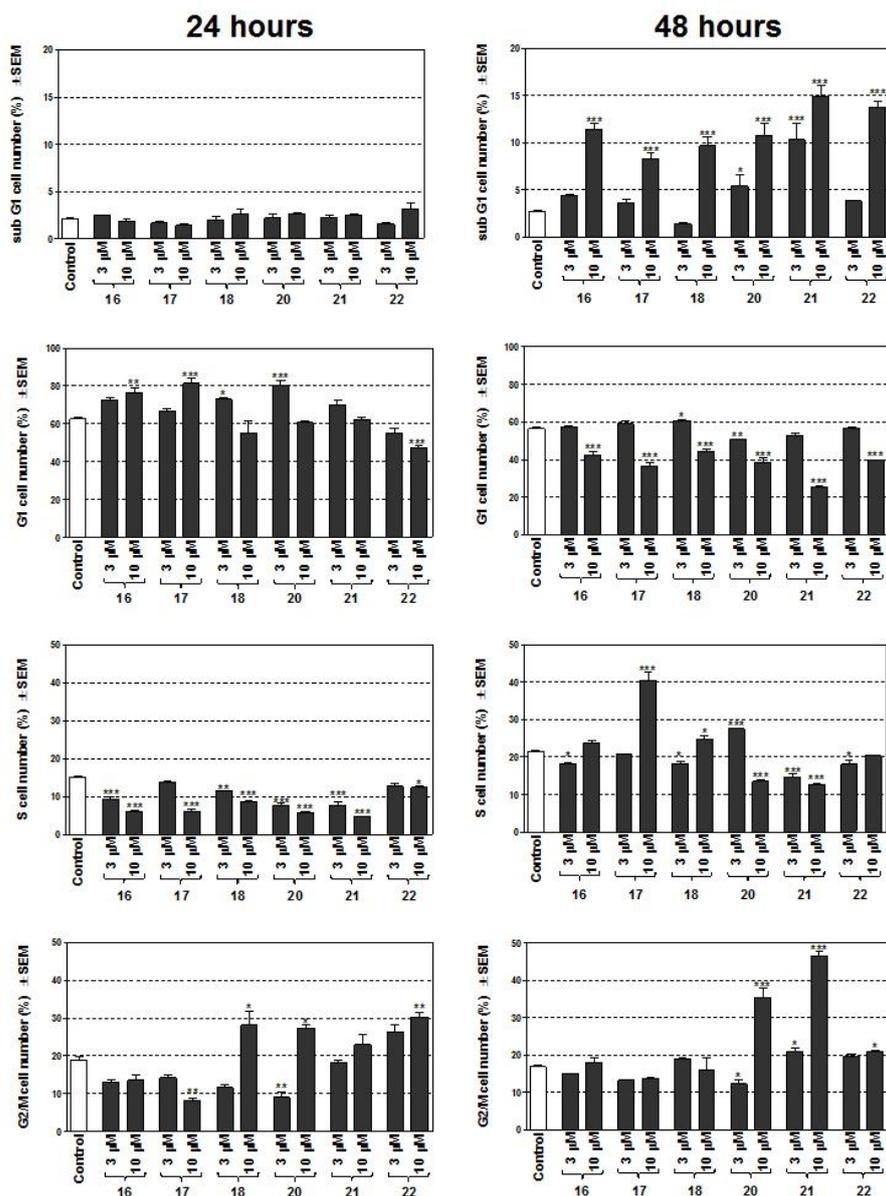
### Morphological studies and cell cycle distribution

The presence of apoptosis or necrosis was determined according to the cell morphology and membrane integrity. Concentration-dependent increases in nuclear condensation and fragmentation and in membrane permeability were generally observed by HOPI staining. The most markedly perturbed membrane integrity was seen in the case of **3e**, while

treatment with **10h** resulted in pronounced nuclear condensation with poor staining, even at 30  $\mu\text{M}$ , indicating apoptotic cell death. After 24 hours unsubstituted oximes (**3a** and **3e**) caused a pronounced decrease in the synthetic (S) phase of cell cycle and at the highest concentration applied (30  $\mu\text{M}$ ), an increase in the subdiploid (subG1) population. After a longer incubation (48 hours), **3a** and **3e** resulted in a concentration-dependent increase in the subG1 cells, but the S population was reduced (Fig. 4). At 17 $\beta$ -HSD 1 inhibitors the morphological evidence demonstrated that compound **18** was the most potent apoptosis inducer while compound **20** resulted in the most pronounced deterioration in membrane integrity, as evidenced by intensive staining with PI. After 24 hours, there was no essential change in the number of apoptotic cells. All the tested compounds decreased the population of the synthetic phase, resulting in an increase either of the G1 phase (compounds **16-18** and **20**) or the G2/M population (compounds **18**, **20** and **22**). After treatment for 48 hours, an unequivocal cumulation of hypodiploid (subG1) populations was detected for all items (Fig. 5).



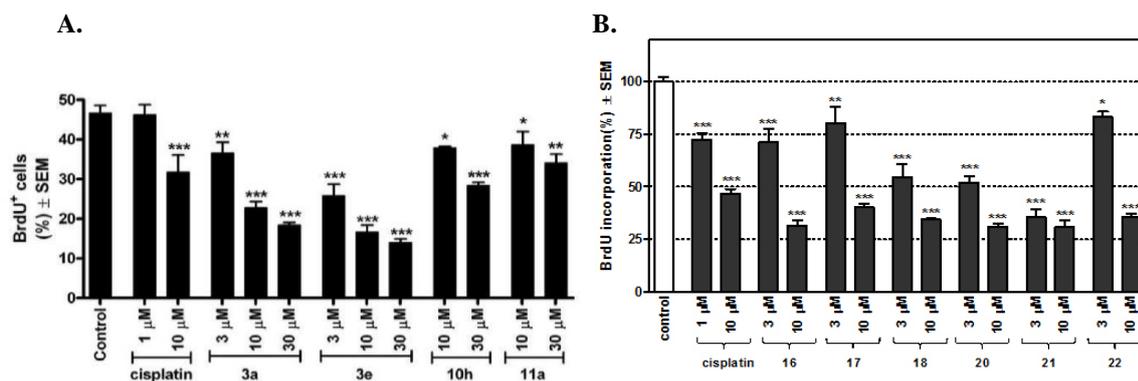
**Figure 4.** Effects of **3a**, **3e**, **10h** and **11a** on cell cycle distribution after incubation for 24 (panel A) or 48 hours (panel B). \*, \*\* and \*\*\* indicate  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively.



**Figure 5.** Effects of compounds **16–18** and **20–22** on HeLa cell cycle distribution after incubation for 24 (left panels) and 48 (right panels) hours. \*, \*\* and \*\*\* indicate  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively.

### BrdU incorporation assay

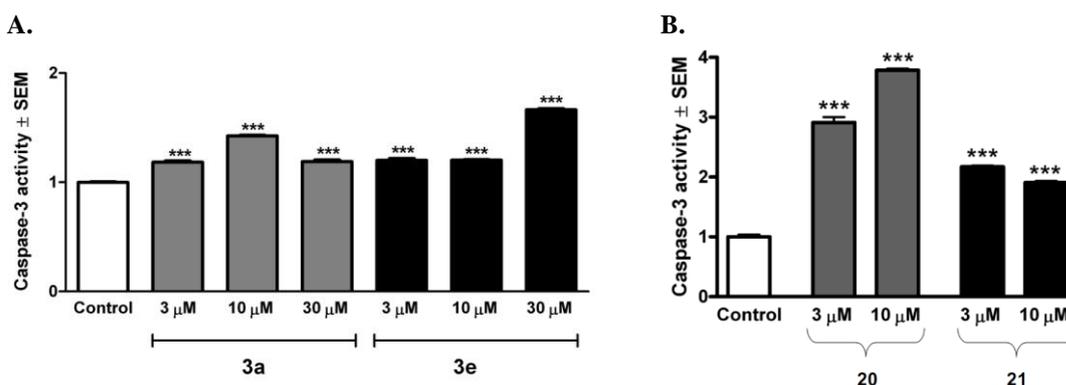
The incorporation of BrdU into the DNA was substantially inhibited by all of the selected agents. Compound **3e** was the most potent in inhibiting BrdU incorporation and, similarly to **3a**, exhibited a clear concentration dependence; their actions were comparable to that of cisplatin. In case of 17 $\beta$ -HSD1 inhibitors **21** was the most potent in this regard, exerting more than 50% inhibition even at 3  $\mu$ M (Fig. 6).



**Figure 6.** A. and B. Incorporation of BrdU into HeLa cells after incubation for 24 hours. \*, \*\* and \*\*\* indicate  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  as compared with the control cells, respectively.

### Caspase-3 activity assay

On the basis of the cell cycle results, compounds **3a**, **3e**, **20** and **21** were selected for additional experiments in order to determine their effects on the activity of caspase-3. All of these agents induced a substantial increase in the activity of this apoptosis-executing enzyme (Fig. 7).

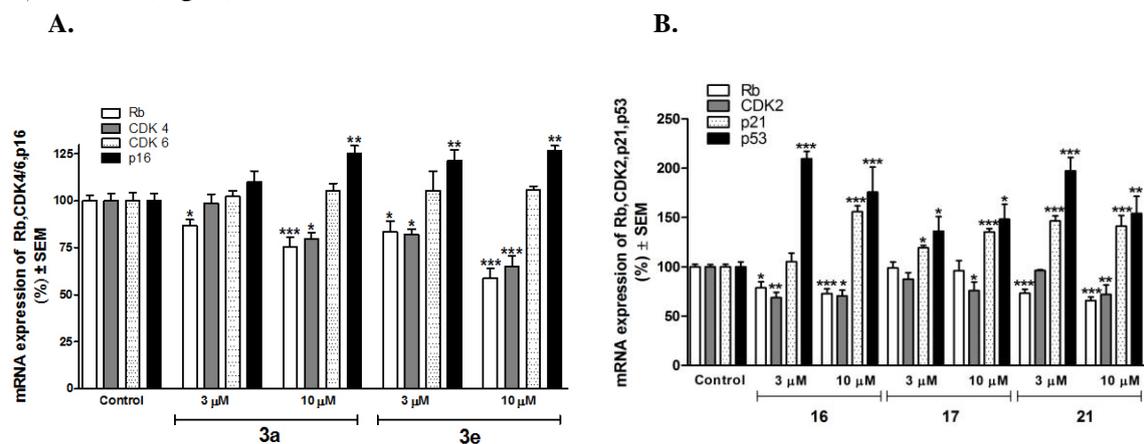


**Figure 7.** Induction of caspase-3 activity after incubation with compounds **3a**, **3e** (A) and with **20**, **21** (B) for 48 hours. The activity of untreated cells was taken as one unit. \*\*\* indicates  $p < 0.001$  as compared with the control cells.

### RT-PCR

The expressions of cell cycle-regulating factors (CDK2, CDK4/6, p16, p21, p53 and Rb) that play key roles in the orchestration of the G1-S transition were additionally determined by means of a semiquantitative RT-PCR technique. The expression of tumor suppressor gene p16 was substantially and statistically significantly increased at the mRNA level under all tested conditions (**3a** and **3e**). Treatment with these selected agents resulted in a concentration-dependent repression of CDK4 and Rb, but not of CDK6. Tumor suppressor gene p53 and p21 was substantially and statistically significantly increased at an mRNA level under all the tested conditions (**16**, **17** and **21**). Treatment with the selected agents

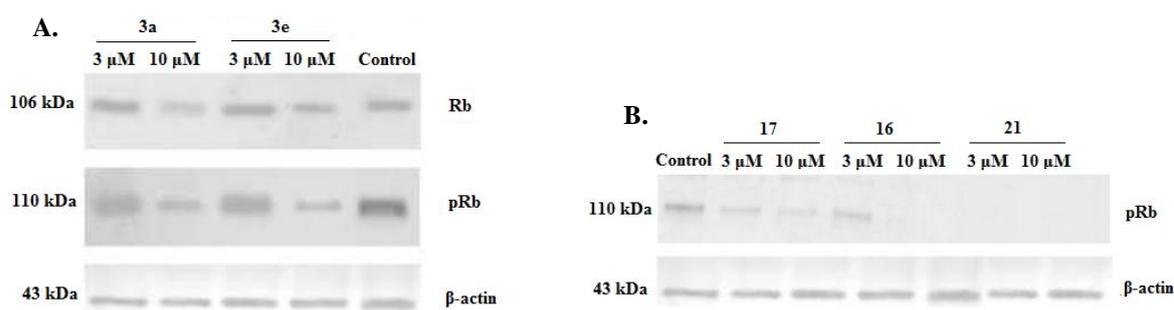
resulted in the concentration-dependent repression of CDK2 and Rb at the level of mRNA expression (Fig. 8).



**Figure 8.** (A.) Expression of Rb, CDK4, CDK6 and p16 at the mRNA level after incubation with compounds **3a**, and **3e** for 24 hours. (B.) Expression of p53, p21, CDK2 and Rb mRNA after incubation for 24 hours with compounds **16**, **17** and **21**. \*, \*\* and \*\*\* indicate  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively.

### Western blot analysis

Western blot analysis reduced the expression of Rb and postsynthetically phosphorylated Rb at a protein level. The relative expression of pRb, expressed as the density ratio Rb/pRb, was concentration-dependently decreased by **3a** and **3e**. This ratio in the control cells was 1.53, which was decreased to 0.59 and 0.46 by **3a**, and to 1.06 and 0.80 by **3e**, in concentrations of 3 and 10 μM, respectively. All of tested 17β-HSD1 inhibitors (compounds **16**, **17** and **21**) reduced the level of expression of phosphorylated Rb protein compared to the control HeLa cells. Compound **21** exerted the highest decrease in protein level in both 3 and 10 μM concentrations (Fig. 9).



**Figure 9.** (A.) Expression of Rb and phosphorylated Rb at the protein level after incubation with compounds **3a** and **3e** for 24 hours. (B.) Expression of phosphorylated Rb protein level after 24 hours treatment with compounds **16**, **17** and **21**.

## DISCUSSION

The oxime function has a widely accepted role in the design and synthesis of innovative anticancer drugs, including molecules with a steroidal skeleton. A set of novel estrone-16-oximes exerted antiproliferative property against human adherent cancer cells and the most effective agents were selected for additional experiments in order to elucidate the possible mechanism of the detected action. These members of the group induced cell cycle disturbances and programmed cell death as evidenced by flow cytometric analysis and increased the caspase-3 activity. Based on the decreased BrdU incorporation disturbed DNA synthesis can be suggested which could be responsible for the improperly orchestrated machinery of cell cycle regulating factors.

Similarly,  $17\beta$ -HSD1 inhibitors with natural polyphenol-related structures inhibited cancer cell growth, induced cell cycle disturbances and apoptosis. Our results indicate that the presented highly selective anticancer properties of the tested agents are not directly related to their originally described enzyme inhibitory action.

In conclusion, our current results provide the first evidence that substituted estrone oximes may selectively suppress cancer cell proliferation by promoting apoptotic cell death and modulate the cell cycle progression. Although relatively high concentrations are needed to exert substantial activity, their cancer selectivity seems to be more beneficial than that of the reference agent cisplatin. Accordingly, the estrone oxime skeleton is suggested as an appropriate scaffold for the design and development of novel antiproliferative agents.

In summary,  $17\beta$ -HSD1 blockade may be suggested as an attractive and selective point for intervention in estrogen-dependent proliferative disorders including gynecological cancers by inhibiting the excessive formation of potent  $17\beta$ -estradiol. Our currently investigated nonsteroidal  $17\beta$ -HSD1 inhibitor compounds chemically related to natural polyphenols exhibited considerable anticancer activity.

These results indicate that it is possible to combine direct antiproliferative activity with  $17\beta$ -HSD1 inhibition resulting in novel class of agents. Having in hand such compounds, it might be possible to further investigate whether these molecules with a dual mode of action are superior to pure  $17\beta$ -HSD1 inhibitors and might lead to the discovery of superior drugs for the treatment of estrogen-dependent proliferative disorders.

Consequently, our presented results concerning the antiproliferative action of chemically different agents with diverse pharmacological spectrum may contribute to design of novel anticancer agents.

## ANNEX

### Publications directly related to the subject of the dissertation:

- I. **Berényi Á**, Minorics R, Iványi Z, Ocsovszki I, Ducza E, Thole H, Messinger J, Wölfling J, Mótyán G, Mernyák E, Frank É, Schneider G, Zupkó I. Synthesis and investigation of the anticancer effects of estrone-16-oxime ethers in vitro. *Steroids* **78**: 69-78 (2013). **IF<sub>2011</sub>: 2.829**
- II. **Berényi Á**, Frotscher M, Marchais-Oberwinkler S, Hartmann RW, Minorics R, Ocsovszki I, Falkay G, Zupkó I. Direct antiproliferative effect of nonsteroidal 17 $\beta$ -hydroxysteroid dehydrogenase type 1 inhibitors in vitro. *J Enzyme Inhib Med Chem* **in press** (2012). **IF<sub>2011</sub>: 1.617**
- III. Ekholm FS, **Berényi Á**, Lagerquist L, Saloranta T, Zupkó I, Schneider G, Wölfling J, Leino R. Cytotoxic activity of some glycoconjugates including saponins and anthracyclines. *Carbohydr Res* **356**: 295-8 (2012). **IF<sub>2011</sub>: 2.332**

### Publications indirectly related to the subject of the dissertation:

- I. Fürst R, Zupkó I, **Berényi Á**, Ecker GF, Rinner U. Synthesis and antitumor-evaluation of cyclopropyl-containing combretastatin analogs. *Bioorg Med Chem Lett* **19**: 6948-51 (2009). **IF<sub>2009</sub>: 2.650**
- II. Csapi B, Hajdú Zs, Zupkó I, **Berényi Á**, Forgo P, Szabó P, Hohmann J. Bioactivity-guided isolation of antiproliferative compounds from *Centaurea arenaria*. *Phytother Res* **24**: 1664-69 (2010). **IF<sub>2010</sub>: 1.878**
- III. Vasas A, Sulyok E, Rédei D, Forgo P, Szabó P, Zupkó I, **Berényi Á**, Molnár J, Hohmann J. Jatrophone diterpenes from *Euphorbia esula* as antiproliferative agents and potent chemosensitizers to overcome multidrug resistance. *J Nat Prod* **74**: 1453-61 (2011). **IF<sub>2011</sub>: 3.128**

- IV. Ndonsta BL, Tatsimo JSN, Csupor D, Forgo P, Berkecz R, **Berényi Á**, Tene M, Molnár J, Zupkó I, Hohmann J, Tane P. Alkylbenzoquinones with antiproliferative effect against human cancer cell lines from stem of *Ardisia kivuensis*. *Phytochemistry Letters* **4**: 227–30 (2011). **IF<sub>2011</sub>: 1.222**
- V. Sarikaya BB, Zencir S, Somer NU, Kaya GI, Onur MA, Bastida J, **Berényi Á**, Zupkó I, Topcu Z. The effects of arolycoricidine and narciprimine on tumor cell killing and topoisomerase activity. *Rec Nat Prod* **4**: 381-5 (2012). **IF<sub>2011</sub>: 1.508**

#### Abstracts:

- I. Wölfling J, Bóna Á, Frank É, Schneider G, **Berényi Á**, Zupkó I. Synthesis and cytotoxic activity of 16-benzylidene estrone 3-methyl ethers. Hungarian-Austrian-Czech-German-Greek-Italian-Polish-Slovak-Slovenian Joint Meeting on Medicinal Chemistry, 24-27. June, 2009, Budapest, Hungary.
- II. Zupkó I, **Berényi Á**, Marchais-Oberwinkler S, Frotscher M. Direct antiproliferative effect of novel nonsteroidal 17beta-hydroxysteroid dehydrogenase type 1 inhibitors. 16<sup>th</sup> World Congress on Basic and Clinical Pharmacology, 17-23. July, 2010, Copenhagen, Denmark.
- III. Fürst R, Nowikow C, **Berényi Á**, Ecker GF, Schmid W, Zupko I, Rinner U. Synthesis and biological evaluation of novel carbocycle-containing combretastatin A-4 analogs. 240<sup>th</sup> National ACS Meeting and Exposition, 22-26. August, 2010, Boston, USA.
- IV. **Berényi Á**, Schneider G, Wölfling J, Ocsosvzki I, Zupkó I. Investigation of antiproliferative effect of estrone-16-oxime ethers in vitro. 3<sup>th</sup> PharmSciFair, 13-17. June, 2011, Prague, Czech Republic.
- V. Molnár J, Ocsosvzki I, **Berényi Á**, Hohmann J, Zupkó I: Investigation of antiproliferative effect of quinoline and acridone alkaloids isolated from Rutaceae plants in vitro. 3<sup>th</sup> PharmSciFair, 13-17. June, 2011, Prague, Czech Republic.
- VI. Zupkó I, Molnár J, **Berényi Á**, Kádár Z, Wölfling J: Antiproliferative action of novel triazol-containing estranes. Frontiers in Medicinal Chemistry. Joint German-Swiss Meeting on Medicinal Chemistry, 20-23. March, 2011, Saarbrücken, Germany.

- VII. Zupkó I, Molnár J, Ocsovszki I, **Berényi Á**, Hohmann J: Investigation of antiproliferative effect of kokusaginine and skimmianine in vitro. 4<sup>th</sup> International Conference on Drug Discovery & Therapy, 12-15. February, 2012, Dubai, UAE.
- VIII. Fürst R, Nowikow C, **Berényi Á**, Ecker GF, Schmid W, Zupkó I, Rinner U: Novel carbocycle-containing combretastatin analogs: Synthesis and biological evaluation. 3<sup>rd</sup> European Chemical Biology Symposium - 2<sup>nd</sup> Vienna Drug Action Conference, 1-3. July, 2012, Vienna, Austria.