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ANTIPROLIFERATIVE AND ANTIMETASTATIC PROPERTIES OF D-RING MODIFIED ESTROGEN ANALOGS ON CERVICAL CANCER CELL LINES

Summary of Ph.D. Thesis

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1 INTRODUCTION

Besides cardiovascular diseases, cancer is the second leading cause of death worldwide. Epidemiological studies indicate that more than 600 million people are infected with human papillomavirus (HPV) worldwide, and around 5% of all cancer cases are associated with HPV including almost all cases of cervical, majority of anal, vaginal and increasing number of oral cavity and pharyngeal cancers. It has been proven that around 18 variants of HPV are associated with carcinogenesis, of which HPV-18 and -16 are responsible for approximately 50% and 20% of cervical cancerous cases, respectively. During the last few years vaccination and screening methods became widely available in developed countries, however women in less developed countries and over 25 years or influenced by antivaccination groups in developed countries are still compromised. Statistical data suggest that this special type of malignant cervical lesions even if they are diagnosed in early stage need rapid and aggressive treatment utilizing novel more potent anticancer agents with a more tolerable side-effect profile.

Carcinogenesis is a complex process including multiple changes in cell growth, differentiation and behavior, and characterized by dynamic changes in the gene expression of the affected cells. Generally, oncoviruses are not sufficient but necessary inducers of cancer development and characterized by a persistent infection and the onset of the malignant lesion occur years after the primary infection. Virus-induced oncogenesis is a multistep process: viruses disrupt the homeostasis of the infected cells and trigger cellular changes leading to malignity. Oncoviruses have some general mechanisms such as signaling mimicry (virus encoded proteins, which subvert the host-signaling mechanisms), changed DNA damage response (host cells acquire genetic instability and help viral replication) and chronic inflammatory response. More than 150 HPV genotype have been described already and some of them could be involved in oncogenesis depending on the immune state of the host and the type of the virus. The “high-risk” viruses compose of a small group, closely related to mucosal epithelial carcinomas which establish integrated HPV sequences in the host cells, encoding potent oncoproteins.

Estrogens are traditionally regarded as crucial factors of the reproductive systems, including the regulation of growth and differentiation, as well as it is well known that 17β-estradiol generally increases the proliferation of its target cells not only in the reproductive system but also in gynecological tumors. In recent years, an increasing body

of evidence indicated that structural modifications of 17β-estradiol may lead to estrone analogs with antiproliferative activity. The A-ring-modified 2-methoxyestradiol (2-ME) was one of the first discovered metabolite without any hormonal activity, but exerting antiproliferative actions against several cancerous cell lines owing to its apoptosis-activating, microtubule-disrupting, antimigratory and antiangiogenic effects. On the basis of these findings many new steroid derivatives with anticancer properties have been synthesized and investigated.

Several nonsteroidal triazole derivatives have antiproliferative and proapoptotic properties, therefore triazole ring can be regarded as a pharmacophore when built onto a steroid skeleton. Moreover, several lines of evidence suggest that triazolated steroids exert potent growth-inhibitory effects, including D-secooximes. Also some research has established that ring modifications, such as expansion or opening of D-ring may lead to homo- and secoestrones without any estrogenic activity. This kind of structural modifications can result in further promising estrone-based group of novel compounds which are potential candidates for anticancer investigations.

2 AIMS

The goal of the present study was to characterize the antiproliferative mechanism of action of recently synthesized D-ring modified (D-homo- or D-seco-) estrone analogues on cervical cancer cell lines. The specific aims of the performed investigations were the followings:

- Antiproliferative screening of the newly synthesized estran-based derivatives on human reproductive cancer cells, *in vitro*. Investigation of the tumor selectivity of the most potent compounds using non-cancerous cell lines.
- Comparing the activity of the most effective molecules on different HPV status cervical cell lines.
- Investigating the proapoptotic properties of the most effective compounds using fluorescent double staining, determination of caspase-3 activity and cell cycle analysis.
- Characterization of the alterations in cell cycle progression by flow cytometry, PCR technique, Western blot and tubulin polymerization analysis.
- Description of their effect on cell migration and invasion to reveal their influence on cancer cells motility.

3 MATERIALS AND METHODS

3.1 Chemicals

D-ring modified estrogen analogs, D-homo and D-secosteroids and their dimer derivatives were synthesized in the Institute of Organic Chemistry (SZTE). The chemical structure of the four most potent and widely investigated compounds of this study are presented in Figure 1.

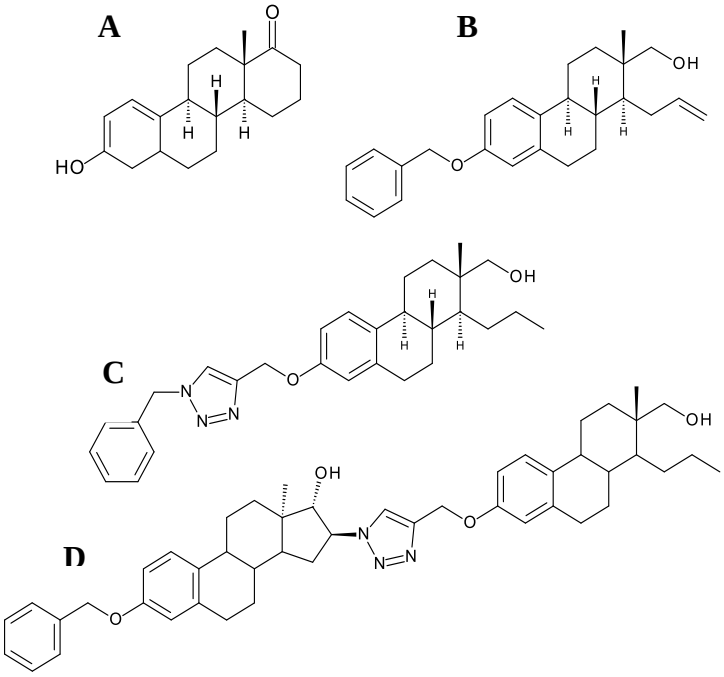

Figure 1. *Chemical structure of the selected compounds:* ***A:*** *D-homoestrone (D-HE)* ***B:*** *D-secoestrone, D-SE* ***C:*** *D-secoestrone-triazole, D-SET* ***D:*** *D-secoestrone and D-epiestrone linked dimer, DIM*

3.2 Cell lines and culturing conditions

For the antiproliferative screening, a panel of human adherent gynecological cancer cell lines was used: breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-361 and T47D), endometrial adenocarcinoma (Ishikawa), ovarian carcinoma (A2780), skin epidermoid carcinoma (A431) and three cervical carcinomas: HeLa (HPV 18+), SiHa (HPV 16+) and C-33 A (HPV –). Cells were maintain in Eagle's Minimum Essential Medium supplemented with 10% heat-inactivated fetal calf serum, 1% non-essential aminoacids, and 1% antibiotic-antimycotic mixture. The cells were maintained at 37 °C in humidified atmosphere containing 5% CO_2.

3.3 Antiproliferative MTT assay

The growth-inhibitory effects of the compounds were determined by standard MTT method. Cells were seeded onto 96-well, after overnight standing, new medium, containing the tested compounds at 10 and 30 μM, were added for the antiproliferative screening and increasing concentration of the test compounds (0.1-30.0 μM) were added for concentration-response curves. After incubation for 72 h MTT solution was added and the precipitated formazan crystals were solubilized in dimethyl sulfoxide and the absorbance was measured at 545 nm with an ELISA microplate reader.

3.4 Hoechst 33258 – propidium iodide (HOPI) double staining

HeLa, C-33 A and SiHa cells were seeded in 96-well plates, after incubation for 24 h with the test compounds Hoechst 33258 (HO) and propidium iodide (PI) were added directly to the cells. After 60 min incubation cells were examined under a Nikon ECLIPSE TS100 fluorescence microscope equipped with appropriate filters. This method allows a distinction between early apoptosis and late apoptosis (secondary necrosis) by the nuclear morphology and membrane integrity of the cells. To detect the form of cell death two separate pictures for HO and PI fluorescence from the same field were taken.

3.5 Cell cycle analysis by flow cytometry

To determine the cellular DNA content of the treated cells flow cytometric analysis was performed. HeLa, C-33 A and SiHa cells were seeded into 6-well plates. 24 or 48 h after treatment cells were harvested, fixed and stained with PI dye solution. Cells were analyzed by a Partec CyFlow instrument, in each analysis, 20,000 events were recorded, and the percentages of the cells in the different cell cycle phases (subG1, G1, S and G2/M) were determined by using ModFit LT 3.3.11 Software. The subG1 fraction was regarded as the apoptotic cell population.

3.6 Determination of in situ caspase activity

Caspases are the most crucial enzymes of the apoptotic machinery. To analyze the effect of the selected compounds on caspase-3 activity 10^7 cells were seeded in tissue culture flasks. After 24 or 48 hours incubation, cells were counted, and resuspended in lysis buffer. Equal portions of treated and untreated cell lysates were incubated with selective caspase-3 substrates. After an overnight incubation the absorbance of *p*-nitroaniline was measured at 405 nm with microplate reader. Comparison of the absorbance of *p*-nitroaniline from the treated and untreated samples.

3.7 Distinction of G2 and M phases by specific flow cytometry

To separate G2 and M populations M phase specific immunocytochemical flow cytometry was applied. The phosphorylation of histone H3 at Ser10 correlates with the G2 to M phase transition. HeLa cells were plated into 6-well plates and allowed to proliferate for 48 h. 24 hours after the treatment cells were washed and harvested and a commercially available FlowCellect^{TM} Bivariate Cell Cycle Kit for G2/M Analysis was used. Samples were analysed on the Partec CyFlow instrument. In each analysis, 20,000 events were recorded, and the data were evaluated using Flowing Software.

3.8 Reverse transcription PCR studies

The transition from the G2 to the M phase of the cell cycle is rigorously regulated by cyclin-dependent kinase 1 (Cdk1) and cyclins. The expressions of these regulating factors at mRNA level were determined by RT-PCR in HeLa cells. After a 48 h incubation with the appropriate test compound total RNA was isolated from the cells and cDNA was generated. PCR was carried out from cDNA, with sense and antisense primers of Cdk1, cyclin B1, cyclin B2, Cdc25B, Cdc 25C. As internal control human hGAPDH primers were used.

3.9 Western blotting studies

To investigate the downstream events of CDK1-cyclinB complex phosphorylated and total stathmin protein expression was determined by western blot. HeLa cells were treated with the test compound for 48 hours. Whole-cell extracts were prepared. 10 μg of protein per well was subjected to electrophoresis and proteins were transferred to nitrocellulose membranes, and blots were incubated with stathmin, phosphorylated stathmin and β-actin polyclonal rabbit antibody. Antibody binding was detected with the WesternBreeze Chemiluminescent Western blot immunodetection kit.

3.10 Tubulin polymerization assay

The cell-independent direct effect of the test compound on tubulin polymerization was tested. 10 μl of test compounds, positive and negative controls were used and 50 min kinetic reaction was recorded in a cell free system to determine the absorbance of tubulin solution at 340 nm, per minute. Kinetic curves were recorded and their main parameters (Vmax, Δabsorbance/min) were statistically evaluated.

3.11 Analysis of cell migration using wound healing assay

To analyze the migratory capacity of HeLa cells wound healing assay was used. HeLa cells were seeded into each chambers of the specific wound healing inserts. After an overnight incubation culture inserts were removed, and cells were treated with test compounds in medium containing 1 % FBS for 24 hours. Migration of the cells into the wound site was visualized with phase-contrast inverted microscope.

3.12 Analysis of cell invasion using Boyden chamber assay

The invasion ability of HeLa cells was assessed using the BD BioCoat Matrigel Invasion Chamber. Cells were seeded into each top insert with or without test compounds in serum free medium. 10 % FBS containing medium was filled in the bottom well as chemoattractant. After 24 hours incubation the culture insert was removed the invading stained cells were counted under a phase-contrast inverted microscope.

4 RESULTS

4.1 Antiproliferative MTT assay

As a first phase of the work 46 estrane-based novel compounds were screened for antiproliferative effects. The four most potent compounds from these estrogen analogue library: D-homoestrone (D-HE). D-secoestrone (D-SE), D-secoestrone-triazol (D-SET) and the D-secoestrone and D-epiestrone linked dimer (DIM) were chosen for detailed investigation. We have found that the four estrogen analogs have highly different antiproliferative action against the cervical cell lines with various pathological backgrounds. The tumor selectivity of the compounds are better in the cases of D-HE, D-SE and D-SET or equal in the case of DIM as compared with cisplatin (Table 1).

Cell type	HPV status	IC_{50} values (μM)				
		D-HE	D-SE	D-SET	DIM	cisplatin
HeLa	18+	5.5	13.2	1.1	1.7	6.5
SiHa	16+	*>30*	13.6	>30	0.96	12.9
C33-A	–	*>30*	10.8	1.7	2.9	3.2
MRC-5		*>30*	*n.t.*	*>30*	*1.2*	*3.7*
skin fibroblast		*n.t.*	*>30*	*n.t.*	*3.0*	*21.6*

Table 1. *Calculated IC_{50} values of the test compounds and cisplatin, measured by MTT assay after incubation for 72 h on cervical cancer and intact human fibroblast cell lines. n.t. indicates not tested.*

4.2 HOPI double staining

In HeLa cells, after 24 h incubation D-HE, D-SET and DIM either could provoke the signs of early apoptosis with a bright chromatin condensation, and only higher concentration of D-SET presented the loss of membrane integrity. In C-33 A cells beside a huge chromatin condensation, increased rates of late-apoptotic (necrotic) cells could be observed after treatment with D-SET and DIM. Only DIM showed antiproliferative effect on HPV 16+ SiHa cells, but it does not reflected in HOPI results after 24 h (Figure 2).

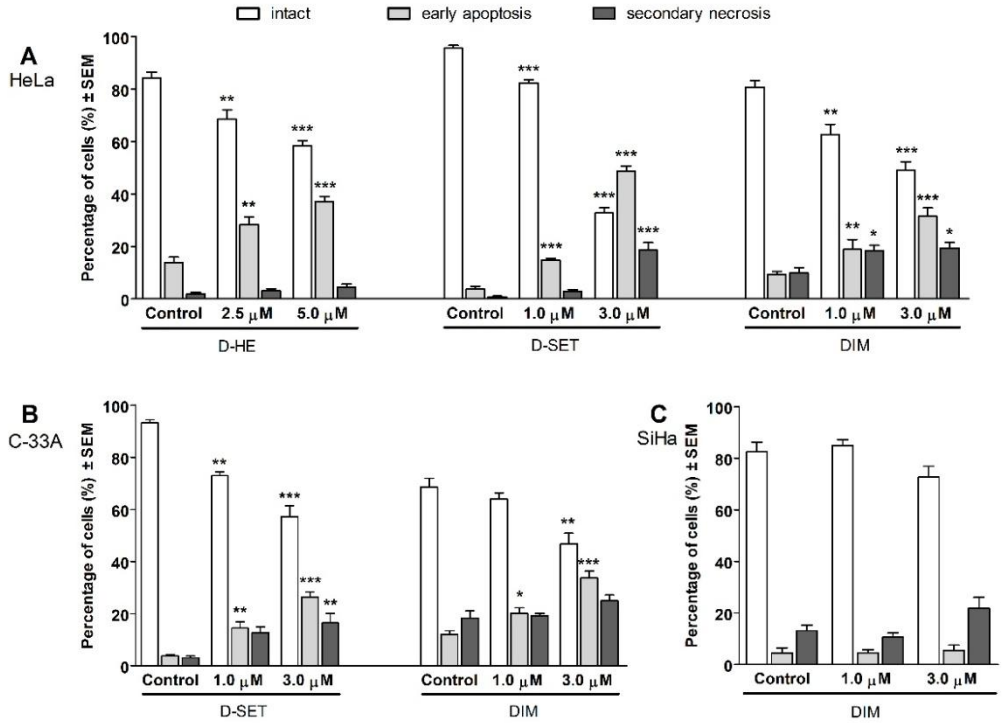

Figure 2. *Tested compounds induced apoptotic and necrotic cell death A: with D-HE, D-SET and DIM on HeLa; B: with D-SET and DIM on C-33 A; and C: with DIM on SiHa cells, after 24 h incubation. Results are means of percentage rates ± SEM. * indicates $p < 0.05$, ** indicates $p < 0.01$ and *** indicates $p < 0.001$ as compared with the control cells, non-significant changes are not indicated.*

4.3 Cell cycle analysis by flow cytometry

D-HE shows slight changes in the cell cycle after 24 . After incubation for 48 h the number of cells in the subG1 and G2/M phase increased significantly on the expense of G1 population. At 72 h a significant concentration-dependent increase of subG1 phase with concomitant decrease of G1 and G2/M phases was observed (Figure 3/A). These results indicate that D-HE may cause a blockade in G2/M phase of the cell cycle.

D-SET induces more robust changes of the cell cycle distribution after 24 h. Besides the increased apoptotic subG1 phase, a significant and concentration-dependent

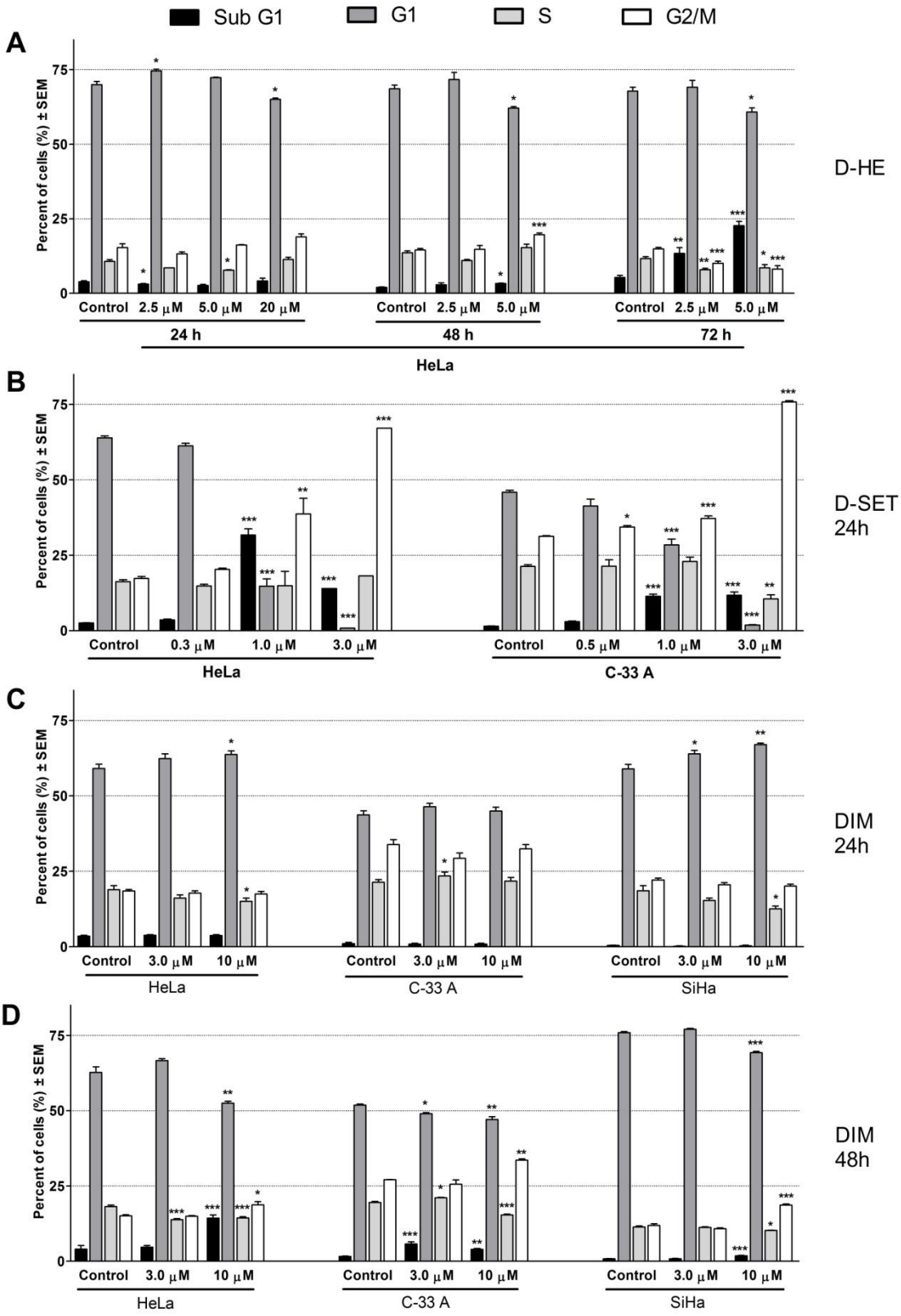

Figure 3. *Effects of tested compounds on cell cycle distribution after treatment with D-HE, for 24, 48 and 72 h on HeLa cells (**A**); D-SET for 24 h on HeLa and C-33 A cells (**B**); DIM for 24 h on HeLa, C-33 A and SiHa cells (**C**); and DIM for 48 h on HeLa, C-33 A and SiHa cells (**D**). Results are means ± SEM, in each analysis, 20,000 events were recorded. * indicates $p < 0.05$, ** indicates $p < 0.01$ and *** indicates $p < 0.001$ as compared with the control cells, non-significant changes are not indicated*

increase in the ratio of cells in the G2/M phase followed by a G1 phase reduction was established in both cell lines (Figure 3/B), indicating G2/M phase blockade.

After incubation for 24 h DIM has no significant influence on the cell cycle in the cases of HeLa and C-33 A cells but it has induced a slight increase at G1 and decrease at S phase in the case of SiHa cells (Figure 3/C). After 48 h incubation regardless of the HPV status elevation of the apoptotic subG1 phase was observed; moreover, significant increase was detected in the number of cells in G2/M phase followed by a G1 phase reduction (Figure 3/D). Similar to the other two compounds DIM also can cause a cell cycle blockade at G2/M phase.

4.4 Determination of in situ caspase activity

Caspase-3 activity was determined to confirm the proapoptotic property of the tested compounds. Based on the subG1 phase elevation, HeLa cells were treated with D-HE for 48 h and with D-SET for 24 h. The results revealed that the enzyme activity was significantly increased after the appropriate incubation time with both of D-HE and D-SET at each applied concentration (Figure 4).

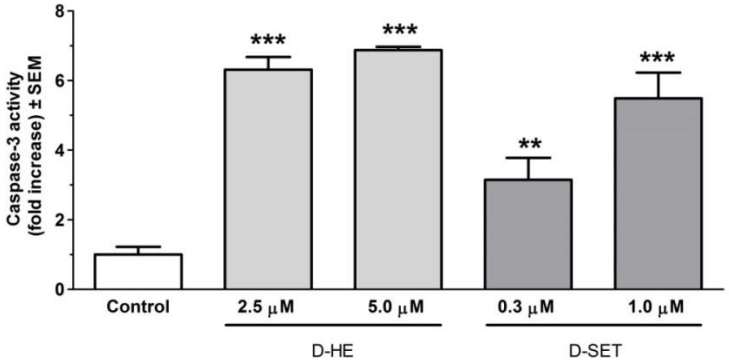

Figure 4. *Induction of in situ caspase-3 activity after 48 h treatment with D-HE and 24 hours treatment with D-SET in HeLa cells. Results show fold increase of enzyme activity of the treated samples compared to the untreated controls. Data are means ± SEM, n=3. * indicates $p < 0.05$ and *** indicates $p < 0.001$ as compared with the control cells.*

4.5 Distinction of G2 and M phases by specific flow cytometry

Immunocytochemical flow cytometric analysis was performed in order to distinguish the G2/M population on HeLa cells, based on their higher sensitivity. After 24 h incubation D-HE significantly decreased, while D-SET significantly increased the ratio of phosphorylated histone H3 protein. Paclitaxel was used as a positive control (Figure 5). These results support the theory that D-HE may cause cell accumulation and cell cycle blockade in the G2 phase while D-SET in the M phase.

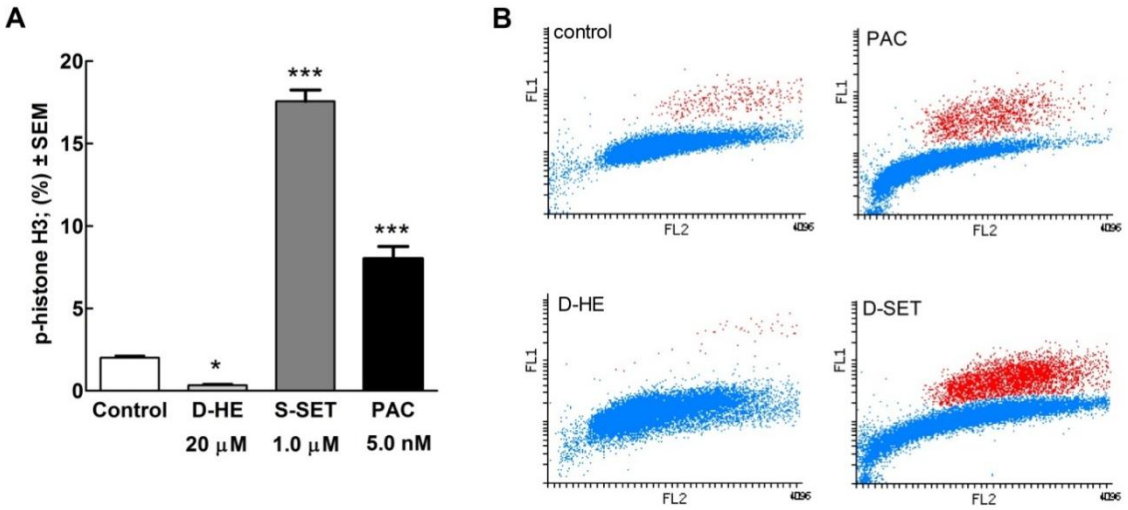

Figure 5. *Discrimination of M phase cell population by measuring the phosphorylation of Histone H3 on Ser10, after incubation for 24 hours and treatment with 20 μM D-HE, 1.0 μM D-SET and 5.0 nM PAC, on HeLa cells.* ***A:*** *Statistical analysis of p-Histone H3 positive cells* ***B:*** *Representative region analysis of flowing dot plot graph (cells in M phase are red labelled). Results are means ± SEM, in each analysis, 20,000 events were recorded. * indicates $p < 0.05$ and *** indicates $p < 0.001$ as compared with the control cells.*

4.6 Analysis of G2/M arrest

4.6.1 Reverse transcription PCR studies

Cdk1 is an executioner enzyme during the G2/M transition, cyclin B is responsible for its activation. However the mRNA expression of Cdk1 did not show significant alteration, the mRNA expression after incubation with D-HE for 48h, both of the activating isoforms of cyclin B (B1 and B2) decreased significantly. Cdc25B and Cdc25C are regulatory factors of Cdk1-cyclin B complex, and their mRNA expression were also significantly reduced as compared with the untreated control cells (Figure 6).

4.6.2 Western blotting studies

To investigate the function of CDK1-cyclin B complex protein expressions of phosphorylated and total stathmin were determined by western blot after treatment with D-HE for 48 h. Although the total protein expression of stathmin did not show remarkable alteration (Figure 7/A), the protein expression of phosphorilated (inactive) form (inactivated by CDK1-cyclin B complex) significantly decreased (Figure 7/B).

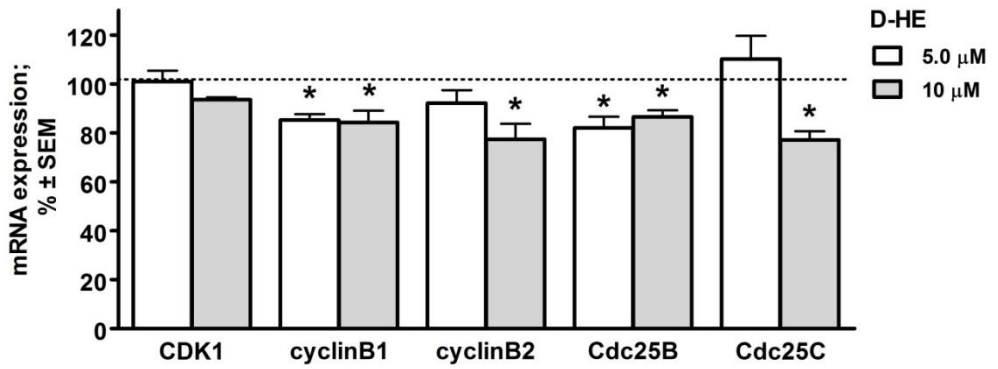

Figure 6. *The mRNA expression of G2/M checkpoint regulating factors after incubation with 5.0 and 10 μM D-HE for 48 h on HeLa cells, determined by reverse-transcription PCR. Results show rate of mRNA expression of the treated samples compared to the untreated controls (dashed line at 100%). Data are means ± SEM, of the data from two separate measurements and n=6. * indicates p < 0.05, as compared with the control cells, non-significant changes are not indicated.*

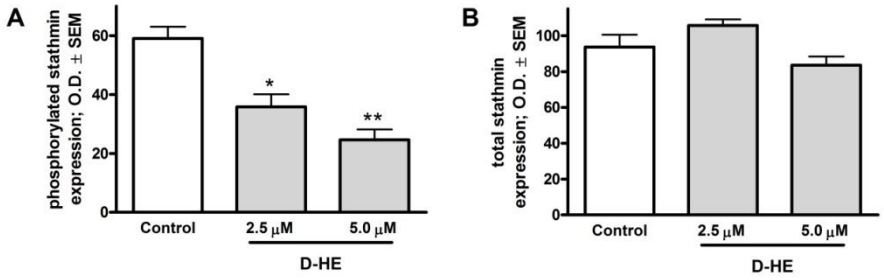

Figure 7. *Statistical analysis of protein expression of phosphorylated (**A**) and total stathmin (**B**) after treatment with 2.5 and 5.0 μM D-HE, for 48h, on HeLa cells.. Results are means ± SEM. * indicates p < 0.05 and ** indicates p < 0.01 as compared with the control cells, non-significant changes are not indicated.*

4.7 Tubulin polymerization assay

During the M phase tubulin can be a probable target, therefore the direct action of the test compounds on tubulin polymeriaztion has been determined by means of photometry. While D-HE did not show considerable effect on tubulin polymerization; both of the D-secoestrone derivatives (D-SE, D-SET) and their dimer analogue (DIM) significantly increased the calculated v_{max} value, similar to paclitaxel, a well-known microtubule stabilizer (Figure 8).

4.8 Analysis of cell migration using Wound healing assay

The migrating behavior of the cancer cells was observed after treatment with D-HE and D-SET by wound healing assay. D-HE did not show significant difference as compared with the untreated control cells while D-SET-treated cells failed to close the wound and concentration-dependently inhibited the migration of HeLa cells (Figure 9).

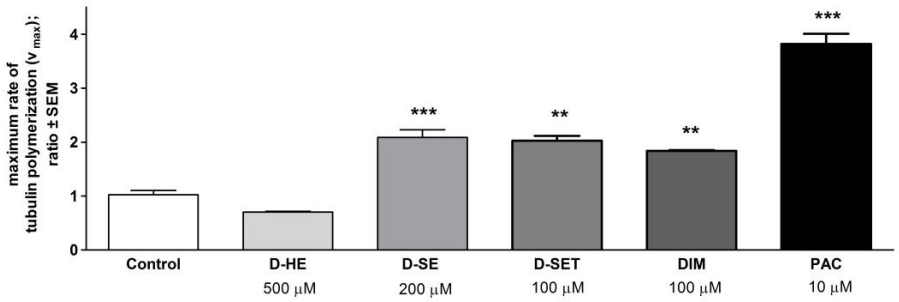

Figure 8. *The calculated maximum rate of tubulin polymerization of the test compounds in a cell independent experimental system, in vitro.. Results are means ± SEM of the data on two separate measurements with duplicates. ** indicates $p < 0.01$ and *** indicates $p < 0.001$ as compared with the negative control samples, non-significant changes are not indicated.*

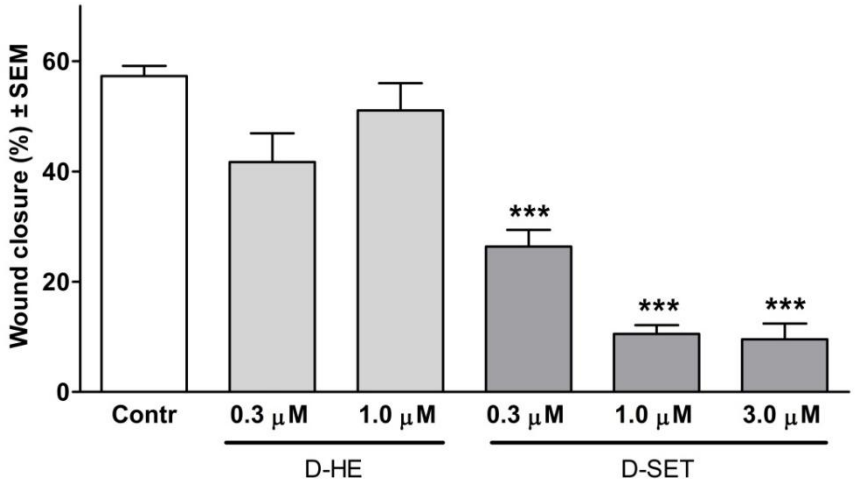

Figure 9. *Migratory capacity of HeLa cells after 24 hours D-HE and D-SET treatment, analysed by wound healing assay. Rate of migration was calculated as the ratio of wound closure in treated samples after 24 h and 0 h compared to the ratio of wound closure in untreated control samples after 24 h and 0 h. Results are mean values ± SEM of the data on three separate measurements with triplicates. *** indicates $p < 0.001$, non-significant changes are not indicated.*

4.9 Analysis of cell invasion using Boyden chamber assay

After the finding that D-SET significantly inhibit the migration of cancerous cells, the anti-invasive effect of the compound was additionally examined with the Matrigel-coated Boyden chamber. It was found that D-SET significantly inhibited the invasion of HeLa cells as compared with the untreated controls (Figure 10). This inhibition was present after the exposure of much lower than the effective antiproliferative concentration. Additionally, the inhibition became more pronounced as the concentration was raised.

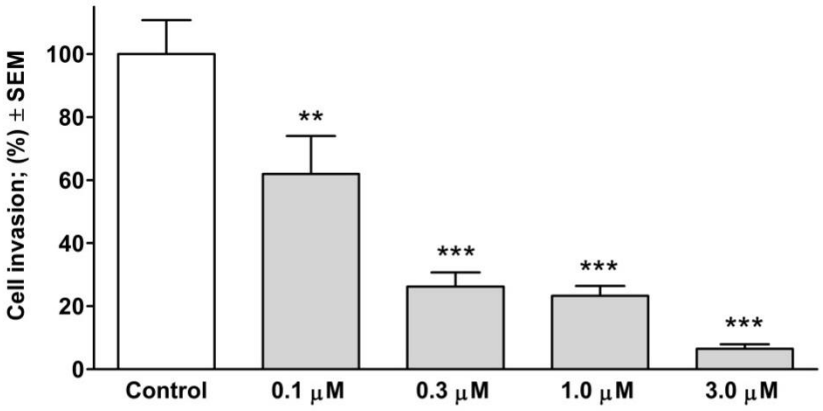

Figure 10. *Cell invasion capacity of HeLa cells through matrigel coated Boyden chambers. Statistical analysis of the percentage of invasive cells after 24 h D-SET treatment. Results are mean values ± SEM of the data on three separate measurements with duplicates. ** indicates $p < 0.01$ and *** indicates $p < 0.001$.*

5 DISCUSSION

The first use of compounds with steroid structure in anticancer therapy was from different plants. Besides these natural plant steroids there are some endogenous steroid metabolites inside the human body such as 2-ME or conjugated estrogen metabolites which have been described as potent antiproliferative agents. Active natural compounds and metabolites can be the basis for synthetic chemistry to design novel, more effective drug candidates. It has been described that D-homoestrone, does not exert estrogenic effect in an *in vivo* uterotrophic assay system. Additionally, *Jovanovic-Santa et al.* confirmed that D-secoestrogen derivatives undergo a total loss of estrogen activity due to the fragmentation of their D-ring.

In these studies, we have found that 3 D-homoestrogens, 13 D-secoestrogens and 1 dimer derivative from the investigated 46 novel estrogen analogs show potent antiproliferative effect on different gynecological cancer cell lines, *in vitro*. The 4 most active compounds were selected, and it was found that their efficacies were different based on the pathological bases of the cell lines. D-HE proved to be selective on the HPV 18+ cells; D SET inhibited the growth of both the HPV 18+ and HPV– cell lines. DIM shows the broadest spectrum with inhibition of all of the three tested cell lines (HPV 18+, HPV– and HPV 16+). These findings suggest that opening the D-ring may lead to a broader anitproliferative spectrum. The tumor selectivity of the four most potent compounds proved to be better than or equal to that of cisplatin, a routinely used drug in cancer therapy.

To investigate the mechanism of antiproliferative action, the apoptosis-inducing effects of the compounds were proven by three independent methods. Morphological

changes confirm the concentration-dependent proapoptotic effect of the compounds on the concerned cell lines. Cell cycle analysis revealed that all of the tested compounds, on all of the affected cell lines, may increase the cell number of subG1 population, indicating progressive DNA elimination as a marker of apoptosis. Additionally, both D-HE and D-SET increased the activity of caspase-3 enzyme. According to these data we can infer that all of the investigated compounds have potent proapoptotic activity resulting in apoptotic attributes: specific morphological changes, DNA fragmentation and caspase enzyme activation.

Even the regular flow cytometric analysis revealed that all of D-HE, D-SET and DIM cause G2/M arrest in cell cycle progression, such as the widely investigated 2-ME; the detailed investigations show, that D-HE exerts its effect in G2 phase resulting functional failure of CDK1/cyclinB complex at G2/M checkpoint. In contrast D-SET and DIM provoke M phase blockade as a consequence of their influence on tubulin polymerization. The opened ring secoestrogen derivatives shift the dynamic equilibrium between tubulin dimers and microtubules towards microtubule assembly and stabilization, resulting in an aberrant mitosis. As a consequence, secoestrogens treated cells cannot continue the cell cycle progression and accumulate at the M-phase; triggering apoptotic cell death. These results lead to the conclusion, that different modifications of the D-ring determine different mechanisms of action during the cell cycle.

Besides the antiproliferative and proapoptotic capacities, antimetastatic effect is another important characteristic in question during the investigation of the anticancer mechanism of new drug candidate. The presence of metastasis is highly associated with poor prognosis and the dissemination of cancer cells from the primary tumor to a distant organ can lead to severe organ failure, and that is the most frequent cause of patient's death

17β-estradiol has been described as an invasion inducing agent in cervical, endometrial and ovarian cancer cell lines. Previous studies also revealed that A-ring modified estrogens have antimetastatic effects on several cancer cell lines. D-HE did not able to inhibit cell migration in the used subantiproliferative concentrations, while D-SET, similar to the widely investigated 2-ME had a potent anti-migratory and anti-invasion capacity below its antiproliferative concentration. The used subantiproliferative concentration is another major highlight of tumor-specificity, since at the used low concentrations compounds have limited toxic effect on intact cells (cells without motility changes) and therefore this approach can reduce the incidence of side effects. According

to these findings opening the D-ring may lead to potent anti-metastatic compounds, with a significant inhibitory activity on cell motility.

In conclusion, even in recent years there has been an increasing interest in antiproliferative estogens, before our study D-ring modified analogues have not been investigated. Our findings suggest that D-homo- and D-secoestrogens have different selectivity with a different mechanism of action. D-HE causes a functional loss of CDK/cylinB complex and provokes cell cycle arrest before cervical cancer cells start to enter mitosis at G2 phase, followed by the induction of apoptosis. In contrast, molecules with opened D-ring cause M phase blockade by stabilizing tubulin polymers, leading to programmed cell death. Results revealed anti-migrating and anti-invading potential of D-SET leads to the conclusion that D-SET shows antimetastatic potential by preventing cancer cell motility.

As regards all of the presented findings, D ring modified estrogen derivatives can be considered as drug candidates with a promising new mechanism of action among the antiproliferative steroids and potentially allows the design of further anticancer drugs.

SCIENTIFIC PUBLICATION RELATED TO THE SUBJECTS OF THE THESIS

1. *Bózsity N*, Minorics R, Szabó J, Mernyák E, Schneider G, Wölfling J, Wang HC, Wu CC, Ocsovszki I, Zupkó I. Mechanism of antiproliferative action of a new d-secoestrone-triazole derivative in cervical cancer cells and its effect on cancer cell motility. *The Journal of Steroid Biochemistry and Molecular Biology*. 2017; 165:247-257 (IF_{2016}: 4.561)
2. Szabó J, Jerkovics N, Schneider G, Wölfling J, *Bózsity N*, Minorics R, Zupkó I, Mernyák E. Synthesis and *in vitro* antiproliferative evaluation of C-13 epimers of triazolyl-D-secoestrone alcohols: The first potent 13α-D-secoestrone derivative. *Molecules*. 2016; 21:611 (IF_{2016}: 2.861)
3. Minorics R, *Bózsity N*, Molnár J, Wölfling J, Mernyák E, Schneider G, Ocsovszki I, Zupkó I. A molecular understanding of D-homoestrone-induced G2/M cell cycle arrest in HeLa human cervical carcinoma cells. *Journal of Cellular and Molecular Medicine*. 2015; 19:2365-2374 (IF_{2015}: 4.938)
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Additional Publications

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3. Schneider G, Kiss A, Mernyák E, Benke Z, Wölfling J, Frank É, Bózsity N, Gyovai A, Minorics R, Zupkó I. Stereocontrolled synthesis of the four 16-hydroxymethyl-19-nortestosterone isomers and their antiproliferative activities. *Steroids*. 2016; 105:113-120 (IF_{2016}: 2.282)
4. Ványolós A, Kovács B, Bózsity N, Zupkó I, Hohmann J. Antiproliferative activity of some higher mushrooms from Hungary against human cancer cell lines. *International Journal of Medicinal Mushrooms*. 2015; 17:1145-1149 (IF_{2015}: 1.357)