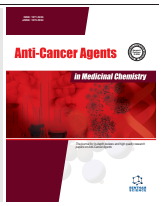
*Anti-Cancer Agents in Medicinal Chemistry*, 2018, *18*, 652-666

REVIEW ARTICLE



# Steroidal Anticancer Agents: An Overview of Estradiol-related Compounds

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ARTICLE HISTORY

Received: June 14, 2017
Revised: June 22, 2017
Accepted: July 04, 2017

*DOI:*
*10.2174/1871520617666171114111721*

**Abstract:** Research of steroidal compounds as anticancer agents started almost 50 years ago. During the past decades, several innovative new steroids, like cyproterone, finasteride, estramustin, exemestane and fulvestrant have successfully become a part of routine clinical practice. Meanwhile, a vast amount of new information have accumulated about the functions of the endogenous steroid system (including the characterization of enzymes, receptors, transcription pathways, *etc*.) and about the role of steroids in carcinogenesis. Therefore, it is regularly required to review the latest published results, focusing on a well-defined part within this research field that has definitely developed into a highly diversified speciality by now. Herein, we make an attempt to summarize the most recent results reported about anticancer agents of estrane backbone, focusing on their mechanisms of action and their structure-activity relationships. Due to the vast number and various accessibilities of scientific publications, neither other reviews nor this one can be considered as absolutely exhaustive. In spite of these restrictive factors, the current review makes a good opportunity to define the recent scientific trends in the field of estradiol-related anticancer agents.

**Keywords:** 2-Methoxyestradiol, cell cycle arrest, antitubulin effect, modified estradiol analogues, antiproliferative effect, apoptosis, autophagy, estrogenic activity.

## 1. INTRODUCTION

Despite decades of intensive and successful research in the field of anticancer agents, malignancies are still among the leading causes of death worldwide according to the latest report of the World Health Organisation [1]. Still in the 21st century, numerous types of tumours are characterized by a very poor survival rate due to the lack of a precise understanding of their mechanisms of proliferation and spreading (*e.g*. lung, liver, pancreas tumours) or due to their high resistance against diverse chemotherapeutic interventions [2]. If we also consider the continuous ageing of the world's population [3] and the well-known harms of environmental pollutants to the life cycle of human cells [4], it is not surprising that anticancer research is still challenging.

Chemotherapeutics with a steroidal structure form a unique group within the family of anticancer agents. They share a) their basic chemical skeleton of various types (*e.g*. estrane, pregnane, androstane, ergostane, *etc*.), b) distinct sources of origin or preparation like naturally occurring, semi-synthetic or synthetic compounds and c) extremely diverse mechanisms of action from receptor antagonism through enzyme inhibition to a direct antiproliferative activity. The first scientific publication about a therapeutically relevant steroid with antitumor effect, namely cyproterone acetate, was released in 1968 [5]. Since then, numerous drugs with a steroidal structure and tumour-inhibiting action have been discovered and developed, and introduced into the clinical practice. However, due to the adverse drug effects or the development of drug-resistance, not all of these pharmacological interventions can be regarded as optimal solutions for certain cancer cases. Thus, research and development of novel steroid compounds with an effective anticancer activity are still in the focus of undiminished scientific interest.

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Recently, several reviews have been published about steroids as anticancer agents. These papers summarize our current knowledge from different points of view. In 2013 Gupta *et al.* [6] gave a thorough overview of the most important steroidal lead molecules synthesized during the previous 25 years, including each type of mechanism of anticancer action and all forms of sterane framework. This paper provides the most detailed data about the antiproliferative effectiveness of the compounds discussed. In the same year, Frank and Schneider's publication [7] focused on the main routes of chemical synthesis of compounds with estrane or androstane skeletons, including N-containing steroidal heterocycles as well. Besides, the antiproliferative action of the compounds discussed, some details about their mechanisms of action are also summarized. The latest review on this research area has been published early this year in the journal Steroids. Kumar *et al.* [8] highlighted the chemical, pharmacological and clinical features of an anticancer drug candidate with estrane skeleton, 2-methoxyestradiol (2-ME2, an endogenous metabolite of estradiol) and its analogues.

Our current review focuses on novel anticancer agents with estrane skeleton published mainly during the last 5 years. We intend to highlight the differences in the mechanisms of action of various compounds classified within the same group based on their pharmacological targets. Moreover, we aim to draw attention to the current intensive research on the field of estradiol-related compounds exerting a direct cytostatic effect in a hormone-receptor independent manner. The currently available estrogen-related anticancer drugs are summarized in Table **1**.

## 2. ESTRADIOL-RELATED COMPOUNDS WITH A DIRECT ANTIPROLIFERATIVE EFFECT

In 1979 Stenkvist *et al.* [9] published a new observation about women chronically treated with cardiac glycosides: the volume of breast tumours developed among them was smaller compared to the non-treated population. Moreover, the morphology of cancer cells was also more uniform compared to those receiving no digitalis therapy. This was the first clinical evidence to suggest that a drug





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**Table 1. Currently available estrogen-related anticancer agents.**

| Drug Type | Mechanism of Action | Agent | Trade Name(s) | Indication | Route of Administration |
|---|---|---|---|---|---|
| Antiestrogens | Selective Estrogen Receptor Modulators | Tamoxifen | Nolvadex, Soltamox, Zitazonium | Breast cancer | Oral |
| | | Toremifene | Fareston | Breast cancer | Oral |
| | | Fulvestrant | Faslodex | Breast cancer | Intramuscular |
| | Aromatase inhibitors | Exemestane | Aromasin | Breast cancer | Oral |
| | | Anastrozole | Arimidex | Breast cancer | Oral |
| | | Letrozole | Femara | Breast cancer | Oral |
| Other | Mitotic inhibitor | Estramustine* | Emcyt | Prostate cancer | Oral |

*Chemically estramustine is a combination of estrogen and nitrogen mustard, so it can be regarded as an estrogen-related compound. It has a dual mechanism of action including the reduction of testosterone levels (by its estron and estradiol metabolites), as well as inhibiting mitosis in prostate cancer cell (*via* blocking microtubule formation and inducing microtubule degradation by its cytotoxic nitrogen mustard metabolites).

with a steroidal framework may exert some cell proliferation-inhibiting effect in a cancerous disease without influencing the signalling pathways of any steroid hormone receptors. However, some recent reports question these results, since increased breast cancer-specific mortality was found in digoxin users compared to non-users in a cohort study [10]. It has to be noted that this difference disappeared after adjustment for potential confounders. Moreover, for prostate cancer, no reduction in prostate cancer-specific mortality was observed in digoxin-users compared to non-users [11].

### 2.1. 2-methoxyestradiol

Although 17β-estradiol (E2) had been widely considered as a potent cell proliferation-inducing compound [12], at the beginning of '90s one of its endogenous metabolites, 2-ME2 (Fig. **1**) was discovered to inhibit the proliferation of MCF-7 cells [13]. This was the first antiproliferative agent with an estrane skeleton that was proved not to bind to estrogen receptor (ER) subtypes [14]. At this time point, a new trend in anticancer research evolved: the development of novel estradiol-related compounds with direct antiproliferative effect has become a new focus of research, keeping in mind that for these compounds, a low capability of steroid hormone receptor (especially ER) binding is desirable. On the other hand, extensive investigations started to describe the detailed mechanism of action of 2-ME2, which resulted in the characterization of a complex pharmacological action involving several different intracellular pathways [8].

In summary, 2-ME2 was tested and proved to be highly effective against at least 15 types of human cancer cell lines and five aspects of its anticancer mechanism of action (including antiproliferative, pro-apoptotic, antitubulin, antiangiogenic and antimetastatic properties) have been established.

Its antiproliferative activity is mediated by cell cycle arrest in the G2/M phase in most of the investigated cell lines [15-18]. In case of breast cancer, the cell cycle arrest may result from the up-regulation of cyclin B1 and cell division cycle protein 2 homolog (CDC2, also known as cyclin dependent kinase 1; CDK1), two regulatory factors of the G2/M phase transition [19]. The prolonged cell division process would lead to the activation of the intrinsic apoptotic pathway. Modulation of other intracellular pathways, including those that increase caspase-3 activity (*e.g.* p53, SAPJ/JNK, β-catenin, *etc*.) has also been demonstrated in several different cancer cell lines treated by 2-ME2 [20-22]. Moreover, in the PC3 prostate cancer cell line, the activation of the extrinsic apoptotic pathway has been detected after 2-ME2 exposure [23]. As the initial step of its pro-apoptotic effect various processes resulting



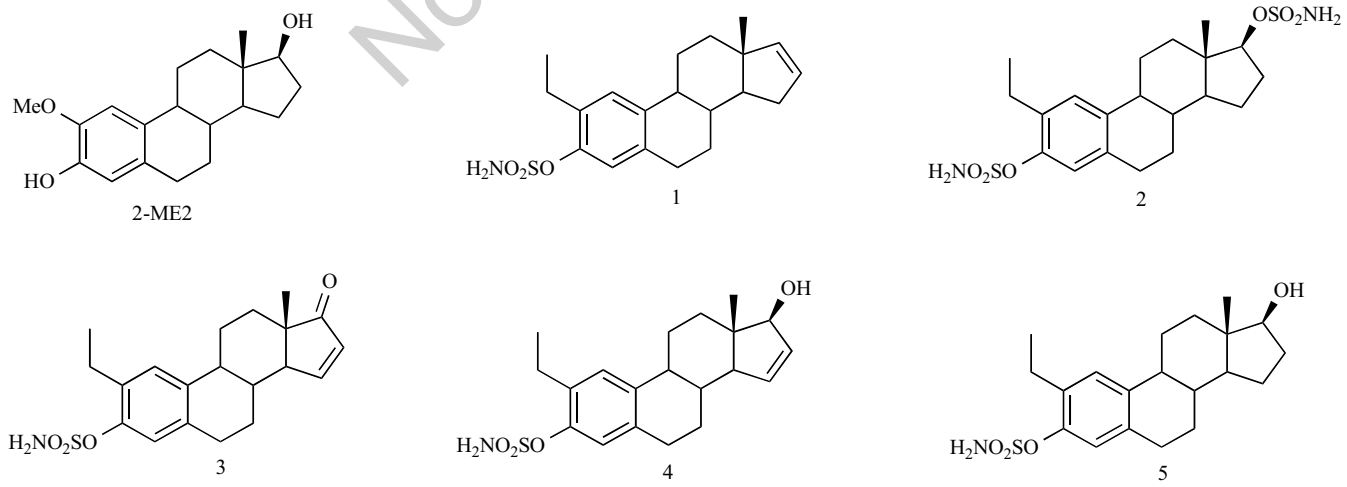


**Fig. (1).** 2-methoxyestradiol and its analogues. **2-ME2**: 2-Methoxyestra-1,3,5(10)-triene-3,17β-diol, **1**: 2-Ethyl-3-*O*-sulfamoyl-estra-1,3,5(10)16-tetraene, **2**: 2-Methoxyestra-1,3,5(10)-triene-3,17β-diyl bis(sulfamate), **3**: 2-Ethyl-3-*O*-sulphamoyl-estra-1,3,5(10),15-tetraen-17-one, **4**: 2-Ethyl-3-*O*-sulphamoyl-estra-1,3,5(10),15-tetraen-17β-ol, **5**: 2-Ethyl-3-*O*-sulphamoyl-estra-1,3,5(10)-triene-17β-ol.

in DNA damage have been identified, such as increasing the production of reactive oxygen species (ROS) accompanied by the disturbance of mitochondrial function in epithelial cancer cells [24] or increasing the activity of neuronal nitric oxide synthase (nNOS) in osteosarcoma cells [25]. The antitubulin effect of 2-ME2 is considered to play a substantial role in its cell proliferation-inhibiting effect. It is widely known that 2-ME2 effectively inhibits tubulin polymerization *via* interacting with the colchicine binding site [26] through its A-ring [27].

Angiogenesis blocking activity of an anticancer agent is also a beneficial property, as these agents interfere with the growth of new blood vessels which supply the continuously proliferating tumour. The antiangiogenic effect of 2-ME2 has been demonstrated in both *in vitro* and *in vivo* experiments [28]. This action was proved to be mediated by the down-regulation of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hypoxia-inducible factor-1α (HIF-1α) [29, 30]. The formation of tumour metastasis is a multistage process including changes in cell adhesion, migration and invasion [31]. Antimetastatic activity is another favourable aspect of an anticancer agent because the development of metastases is the most frequent cause of cancer death. First, an *in vivo* experiment established that 2-ME2 is able to inhibit the formation of lung colonies from pancreatic cancer [32]. Later, *in vitro* investigations verified the inhibition of cell motility, migration and adhesion after 2-ME2 exposure [33]. Moreover, it was also suggested that these effects would be associated with cytoskeletal changes resulting from the already known influence of 2-ME2 on the tubulin–microtubule system. Most recently, a new, convenient and robust *in vivo* assay using a zebrafish larvae model system has been reported to serve as a precise measure of the inhibition of cell migration capability at both the single cell and multicellular level initiated by 2-ME2 or other supposed antimetastatic compounds [34].

The latest reports about the mechanism of action of 2-ME2 offer further insight into the importance of its influence on HIF-1α function, supported by the observations that when 2-ME2 is combined with other conventional chemotherapeutics or radiation therapy, it can increase the anticancer activity of the concomitant agent(s) or that of the irradiation [35-37]. Moreover, based on its inhibitory effect on HIF-1α acute myeloid leukemia has also been identified as a possible novel therapeutic target for 2-ME2 [38].

Although 4-methoxyestradiol (4-ME2), another natural metabolite of estradiol is chemically closely related to 2-ME2, the experimental results concerning its action on the growth of cancer cells are sparse and contradictory. Chang and co-workers described the proapoptotic property of 4-ME2 against renal cancer cells, while other reports indicate its growth promoting activity on ovarian cancer cells [39-40]. Therefore, 4-ME2 is not regarded as a prototype of synthetic anticancer agents.

### 2.2. Analogues of 2-methoxyestradiol

2-ME2 has been revealed to be characterized by limited bioavailability and rapid metabolism *in vivo* [41]. Thus, numerous analogues have been synthesized and tested by several research groups to overcome this pharmacokinetic problem and to improve the antiproliferative activity of the parent compound. Kumar *et al.* [8] reviewed more than 100 derivatives of 2-ME2 and compared their antiproliferative activities to that of the original molecule. Many of these analogues inhibit cancer cell proliferation and tubulin polymerization more effectively than 2-ME2. Based on the structure-activity relationship analysis of 2-ME2 derivatives, it is now widely accepted that a) substitution with a relatively small functional group at position C2 on ring A has substantial importance in the antiproliferative activity, b) a free hydroxyl group at position C3 on ring A promotes metabolic degradation, c) modifications on ring B or C do not tend to be beneficial regarding the antiproliferative activity of the new compound and d) various alterations on ring D (*e.g.* sulfamoylation, unsaturation, homologation or substitution at positions C16 and 17, *etc.*) may be executed without the loss of the antiproliferative activity.

Recently the number of research publications about the synthesis and investigation of new analogues of 2-ME2 has dramatically decreased. Joubert and co-workers reported the detailed anticancer mechanism of action of some 2-ethyl-3-*O*-sulfamoyl-derivatives of 2-ME2 [42-46]. These five agents are sythetized by the *in silico* design and differ only in the structure of ring D (Fig. **1**). Since all the compounds are characterized by a 3-*O*-sulfamoyl substitution they are able to bind to carbonic anhydrase II in erythrocytes, resulting in a better metabolic feature compared to 2-ME2. On the other hand, carbonic anhydrase IX (CAIX) is known to be overexpressed in tumour cells assuring the development of the acidic environment that stimulates growth, survival and metastasis formation of tumours. Thus, the inhibition of CAIX would be an additional beneficial action of newly developed antiproliferative agents. Based on docking studies, three of the afore-mentioned five novel compounds, namely **1**, **3** and **4**, were selected as potent inhibitors [45, 47]. This gives another new insight into the anticancer mechanism of action of 2-ME2 analogues with a 3-*O*-sulfamoyl substitution. All these five compounds inhibit breast cancer cells' (MCF-7 and/or MDA-MB-231) proliferation within the concentration range of 0.05–1.0 μM which is comparable to the efficacy of 2-ME2 against the same cell lines [8]. They all share the main features of the antiproliferative mechanism of action of 2-ME2 such as cell cycle blocking, pro-apoptotic and antimitotic effects. However, two compounds, **2** and **5**, were reported to inhibit the proliferation of non-tumorigenic breast epithelial cells (MCF-12A) as well [43, 44], although it should be noted, that cancer cells were more sensitive to the **5** treatment compared to the MCF-12A cells.

On the other hand, this research group was the first one to report that 2-ME2 does not only activate the apoptotic growth inhibitory pathway, but also induces autophagy [48]. Their further investigations aimed to support this finding by testing the abovementioned 2-ME2 analogues for this specific mechanism of action. Thus, the presence of autophagy was demonstrated for both **1** and **3** by using transmission electron microscopy or the aggresome detection assay supplemented with LC3–II protein expression [46, 49]. Conversely, it was detected that **3** combined with a pyruvate dehydrogenase kinase inhibitor induced apoptosis accompanied by autophagy which had previously been suggested to prevent late apoptotic events. Both cell death pathways were activated by the formation of ROS and c-Jun terminal kinases [46]. Further investigations are needed to elucidate the interplay or counteraction between apoptosis and autophagy in cancer cells.

### 2.3. Other Estradiol-related Compounds

Besides 2-ME2 and its analogues, compounds with an estrane skeleton lacking substitution at position C2 were also demonstrated to exert an antiproliferative effect against several cancer cell lines. The mechanisms of their antiproliferative action seem to differ from that of 2-ME2 and its analogues several ways. By investigating these derivatives, new information can be gained about the intracellular events triggered by the test compounds in cancer cells, making this research area challenging too.

Most of the recently published results have been reported by Zupkó and co-workers. The investigated novel estradiol-related compounds can be classified into different sub-groups according to the chemical alteration (*e.g.* substitution, ring-opening, homologation, *etc.*) performed on the estrane skeleton. Their common feature is that all of these alterations affect the D-ring and/or the hydroxyl group at position C3.

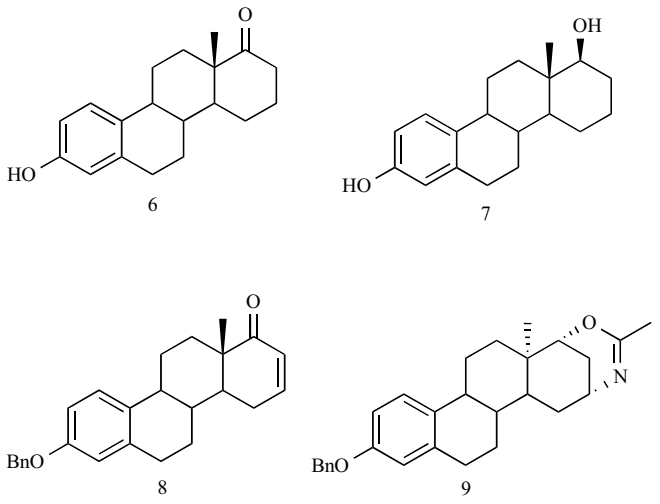


**Fig. (2).** D-homoestradiol and its analogues. **6**: 3-Hydroxy-D-homoestra-1,3,5(10)-trien-17a-one, **7**: 3,17α,β-Dihydroxy-D-homoestra-1,3,5(10)-triene, **8**: 3-Benzyloxy-17α,β-hydroxy-D-homoestra-1,3,5(10),16-tetraene, **9**: 16β-N-acetyl-3-benzyloxy-13α-D-homoestra-1,3,5(10)-trien-17aα-ol.

The first extensively investigated structures were D-homoestrone (**6**) and D-homoestradiol (**7**), and their derivatives (Fig. **2**). **6** was reported to selectively inhibit the proliferation of HPV-18-positive HeLa cells at a low micromolar concentration compared to cervical cancer cell lines with various pathological backgrounds and other gynaecological cancer cell lines [50, 51]. **6** does not exert a significant proliferation-inhibiting effect on the non-cancerous human cell line, MRC-5, which has been further supported by the lack of any toxic effect on tissue morphology in the *in vivo* experiments. The most remarkable difference in its mechanism of action compared to that of 2-ME-2 is its impact on cell cycle progression: namely, it causes a cell cycle blockade at the G2 phase demonstrated by the loss of function of CDK1, the executioner regulating factor of the G2–M transition [51]. Consequently, it should not have any effect on tubulin polymerization and chromosome segregation, which has been proved by an *in vitro* cell-free direct tubulin polymerization assay and an immune-reaction-based flow cytometry analysis that demonstrates the lack of phosphorylated histone H3. **7** showed a highly similar efficacy and selectivity towards HeLa cells demonstrated on a four-member panel of gynaecological cancer cell lines [52]. Its 3-benzyl ether analogue with an unsaturated D-ring **8**, (Fig. **2**) proved to be another effective compound among the investigated D-homoestrone analogues (n=11) with an $IC_{50}$ value of 3.0 μM against the A2780 ovarian cancer cell line [52]. The analogues with an O-containing D-ring and a 16-halomethyl substituent were found to show a complete loss of the antiproliferative activity [52].

As a potent antiproliferative compound, a 13α-D-homoestrone analogue **9**, (Fig. **2**) was tested against 7 gynaecological malignant cell lines, and was found to be characterized by $IC_{50}$ values of 1.1–5.0 μM [53]. Additionally, it was observed to exert a limited inhibitory effect against the proliferation of MRC-5 cells. This was the first reported effective antiproliferative agent from the 13α-estrone series. Similarly to **6**, it elicits a G2/M phase arrest in cell cycle, but in this case it is supposed to result from a disturbed mitosis, as it was demonstrated that **9** enhances the rate of tubulin polymerization like paclitaxel, which is known to affect normal microtubule formation.

The second sub-group of estradiol-related antiproliferative agents includes D-secoestrones. Previously, no 2-ME2 analogues with an opened D-ring had been tested as antiproliferative agents, therefore the first publication concerning the anticancer activity of D-secoestrones was noteworthy [52]. Based on its potent antiproliferative activity against five malignant cell lines, a secoalcohol **10**, (Fig. **3**) was selected for further investigations to elucidate its precise mechanism of action. To reveal whether its antiproliferative activity is specific to tumour cells only or may appear in non-cancerous proliferating cell populations as well, it was tested against non-cancerous human foreskin fibroblast cells. Studies revealed a weaker proliferation-inhibiting activity in the concentration range applied for cancer cells compared to the positive control cisplatin. Moreover, an *in vitro* tubulin polymerization assay was also performed to determine an expected target of **10**. A concentration-dependent increase in the rate of tubulin polymerization was observed, similarly to paclitaxel and other steroidal anticancer agents. To confirm this result a molecular docking study was performed which revealed that similarly to several estrone analogues, **10** preferably binds to the colchicine binding site, rather, than to the vinblastine binding site. Due to the opening of ring D **10** is not expected to possess estrogenic activity, which would limit its utilization in the clinical practice. The following set of D-secoestrones share an oxime functional group [54] at position C17. One of the main aims of this study by Mernyak *et al.* was to compare the antiproliferative activity of 25 novel compounds belonging to the 13α- or 13β-estrone series. Eight effective compounds with a 13β-methyl functional group were identified, while their α -counterparts were found to be inactive. The two most potent agents were compound **11** and its ester analogue **12**, which were characterized by submicromolar $IC_{50}$ values against all four cancer cell lines investigated (Fig. **3**).

Since numerous triazole ring-containing compounds are biologically active, it is worth to consider the investigation of D-secoestrones substituted with a triazole group at position C3. Recently, 15 new D-secoestrone-triazole molecules from both the 13α- and the 13β-estrone series have been investigated to determine their anticancer potencies [55]. Similarly to D-secooximes, the highly potent agents within this set of compounds belong to the 13β-estrone series; however a 13α-methyl compound bearing an unsubstituted N-benzyl group **13**, (Fig. **4**) displayed a comparable proliferation-inhibiting activity too. This is the first published 13α-D-secoestrone with high antitumor potency.

All compounds proved to be inactive against 3 out of 4 breast cancer cell lines and against a HPV-16 positive cervical cancer cell line. However, the lowest $IC_{50}$ values (0.9–1.8 μM) were obtained for the 13β-counterpart of **13**, **14**, (Fig. **4**) [56]. It elicited a weak

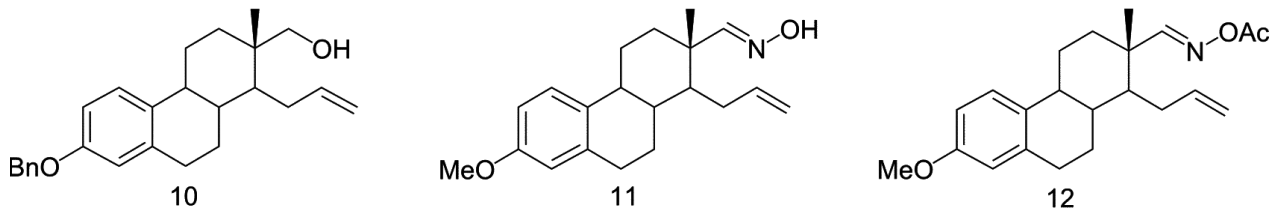


**Fig. (3).** D-secoestrones with a direct antiproliferative activity. **10**: 3-Benzyloxy-13α-hydroxymethyl-14β-(prop-2-en-yl)-des-D-estra-1,3,5(10)-triene, **11**: 3-Methoxy-14β-(prop-2-en-yl)-des-D-estra-1,3,5(10)-trien-13α-carbaldehyde oxime, **12**: 3-Methoxy-14β-(prop-2-en-yl)-des-D-estra-1,3,5(10)-trien-13α-carbaldehyde oxime acetate.

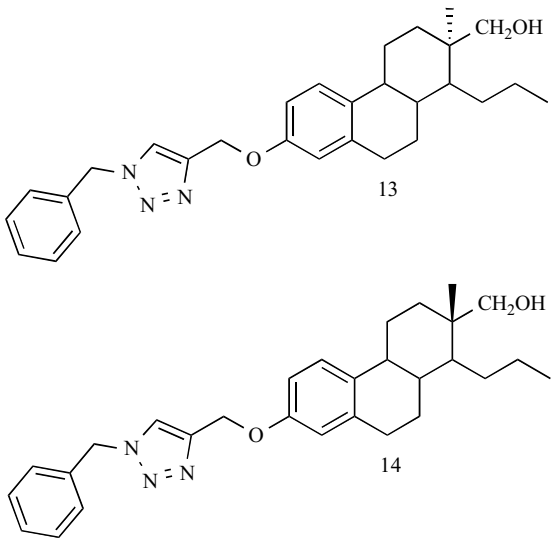


**Fig. (4).** D-secoestrone-triazoles with a direct antiproliferative activity. **13**: 3-[{1-Benzyl-1H-1,2,3-triazol-4-yl}methoxy]-13β-hydroxymethyl-14β-propyl-des-D-estra-1,3,5(10)-triene, **14**: 3-[{1-Benzyl-1H-1,2,3-triazol-4-yl}methoxy]-13α-hydroxymethyl-14β-propyl-des-D-estra-1,3,5(10)-triene.

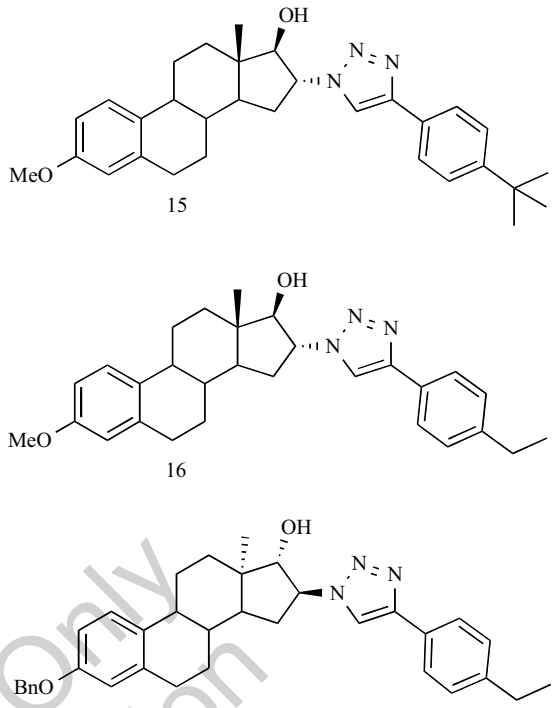


**Fig. (5).** 16-triazolylestrone derivatives with a direct antiproliferative activity. **15**: 16α-[4-(4-tert-Butylphenyl)-1H-1,2,3-triazol-1-yl]-3-methoxyestra-1,3,5(10)-trien-17β-ol, **16**: 16α-[4-(4-Ethylphenyl)-1H-1,2,3-triazol-1-yl]-3-methoxyestra-1,3,5(10)-trien-17β-ol, **17**: 3-Benzyloxy-16β-[4-(4-ethylphenyl)-1H-1,2,3-triazol-1-yl]-13α-estra-1,3,5(10)-trien-17α-ol.

inhibition against the proliferation of noncancerous fibroblast cells. Similarly to 2-ME2 and **6**, **14** activates apoptotic cell death preceded by a cell cycle blockade at the G2/M phase. However, like 2-ME2, but unlike **6**, it was revealed to trigger an M phase blockade resulting from the disturbed tubulin polymerization. Moreover, it also has a significant impact on metastasis formation *via* blocking cell migration and invasion, two key factors of cancer progression.

As mentioned above, a triazole group is considered to be a pharmacologically beneficial functional group; therefore D-ring modified triazolylestrones are also in the focus of investigations. Molnár *et al*. [57] reported about 24 new 16α-triazolylestrones with low to moderate antiproliferative activity against three cancer cell lines. All compounds belong to the 13β-estrone series, but the orientation of the C17 hydroxyl group is different. Both of the most potent analogues **15** and **16**, (Fig. **5**) contain a 3-methoxyestra-1,3,5(10)-trien-17β-ol backbone.

Studies of their anticancer mechanism of action demonstrated pro-apoptotic and cell cycle blocking activities *via* the induction of the intrinsic apoptotic pathway and the accumulation of cells in the G2/M phase preceded by a down-regulation of factors governing the G2–M transition (*e.g*. CDK1, cyclin B1 and B2, CDC25B), respectively. The microtubule destabilizing protein stathmin is one of several targets of CDK1 that gets phosphorylated by CDK1 at the G2–M transition, resulting in the loss of stathmin's activity and consequently allowing microtubule formation and cell entry into mitosis [58]. Unexpectedly, an elevated level of phosphorylated stathmin expression was observed in **15** and **16** treated cells, indicating that besides CDK1 other kinases may also take part in the modulation of stathmin's function. Some members of a set of 16-triazolylestrones with 16α,17β functions belonging to the 13α-estrone series were reported to practically exhibit no activity against the four investigated cancer cell lines [59]. Some of their 17α-counterparts exerted an antiproliferative effect with low micromolar $IC_{50}$ values. The most potent molecule **17**, (Fig. **5**) displayed a limited cell proliferation-inhibiting effect against noncancerous fibroblast cells. Its antiproliferative mechanism of action is supposed to be identical with that of **15** and **16** (*i.e.* intrinsic apoptosis, G2/M blockade), although the orientations of their functional groups at positions C13, 16 and 17 are opposite. The estrogenic activity of **17** is presumed to be weak based on a previous observation about the low affinity of 13α-estradiols to ERs. Finally, Schneider *et al.* [60] described the antiproliferative activities of 28 D-ring substituted 17α and 17β-triazolyl-3-methoxyestranes. The introduction of a triazole moiety at position C17 produces novel compounds with low anticancer potency. Only three of the 28 analogues were found to display cell-growth inhibition similar to the positive control cisplatin.

Although the following two sets of estradiol derivatives differ in their chemical structures, their antiproliferative mechanism of action is highly comparable. The first set of compounds includes estrone-16-oxime analogues. Since no reports concerning the antiproliferative activity of 16-oxime estrone analogues had been published previously, a high number of oximes were investigated to determine the structure-activity relationship for this group of molecules [61]. Two potent unsubstituted oximes **18** and **19**, (Fig. **6**) with selective activity toward HeLa cells were chosen for further pharmacological investigations.

Both compounds were found to exert no significant inhibitory effect on the growth of noncancerous fibroblast cells. They initiated apoptotic cell death, as proved by morphological, enzymatic and flow cytomery analyses. However, unlike the above discussed estradiol-related compounds, these molecules induce cell cycle arrest at the G1 phase by reducing DNA synthesis and the expression of regulatory factors governing early G1–S transition (*e.g*. Rb, pRb and CDK4). Additionally, a significant increase in the expression of p16 (a well-known inhibitor of CDK4) was observed. The second set of compounds includes fused-ring oxadiazolidine analogues of estrone [62]. Seven out of the twelve investigated novel compounds proved to inhibit cancer cell proliferation at low micromolar concentrations ($IC_{50}$ < 10 μM). However, none of the compounds were able to influence the growth of human epidermoid cancer cells. The lowest $IC_{50}$ value was presented for the 16-iodo-*N*-phenyl-3-benzyloxy derivative **20**, (Fig. **6**) against ovarian carcinoma cells. Moreover, it was found to exert a weak inhibitory activity against

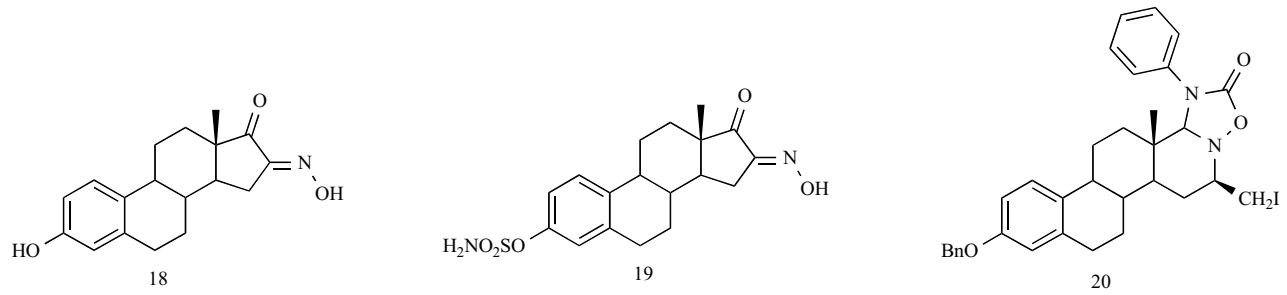


**Fig. (6).** Estrone-16-oxime ethers and oxadiazole derivative of estradiol. 18: Estra-1,3,5(10)-trien-16,17-dion-(Z)-16-monooxime, 19: Estra-1,3,5(10)-trien-16,17-dion-(Z)-16-monooxime-3-sulfamate, 20: 3-Benzyloxy-16β-iodomethyl-[(1',2',4'-N-phenyl)oxadiazolidin-5'-on:17,17aα]-D-homo-estra-1,3,5(10)-triene.

noncancerous fibroblast cells. Studies of its mechanism of action revealed that this compound also initiated the blockade of the G1–S transition like estrone-16-oximes. Further investigations are required to elucidate the exact mechanism and target of this type of cell cycle arrest.

Another research group reported that some estrone-17-hydrazones bearing an indole group **21** and **22**, (Fig. **7**) selectively inhibit the proliferation of HeLa cells [63]. They also synthesized hydrazones with a dehydroepiandrosterone skeleton exerting stronger antiproliferative activities.

Although the anticancer potencies of the estradiol-related compounds summarized above are somewhat lower compared to 2-ME2 and its analogues, they show some unique and interesting features which may serve as a good starting point for further research and development. Several molecules are able to selectively block the proliferation of certain cell lines (mostly HeLa cells), while other derivatives influence the function of exclusive targets or uncommonly disturb cell cycle at certain phases. There are still some highly potent compounds with submicromolar $IC_{50}$ values which are worthy of further detailed *in vivo* pharmacological and pharmacokinetic investigations and potentially suitable for clinical development. It is worth to note that due to the chemical modifications of ring D, these agents usually lose their estrogenic activities.

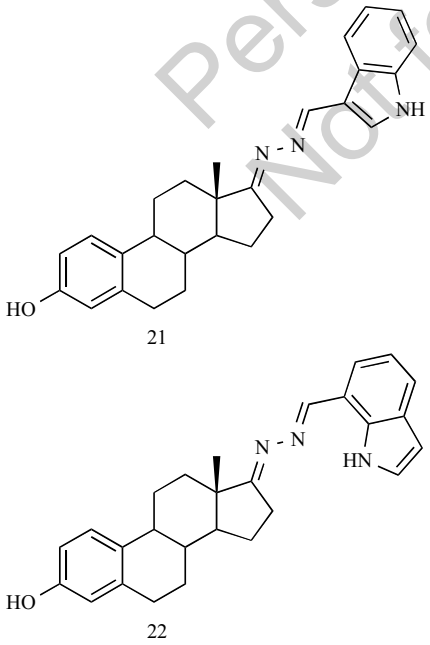


**Fig. (7).** Estrone-17-hydrazones with a direct antiproliferative activity. **21**: Estrone indol-3-methanylidenehydrazone, **22**: Estrone indol-7-methanylidenehydrazone.

Further estradiol-related compounds described in the literature exhibit not only a direct anticancer effect, but also influence other well-known molecular targets (*e.g.* enzymes, hormone receptors), therefore they are discussed in the following sections.

## 3. CONJUGATES OF ESTRADIOL-RELATED COMPOUNDS AND ANTICANCER AGENTS

The first report about the therapeutic application of a conjugate of an estrogen plus the anticancer agent estramustine (Estracyt) was published in 1972 [64]. Since then numerous further conjugates or complexes have been designed [6, 65]. Site-specific delivery of the anticancer agents is one of the leading reasons for the synthesis of these molecules. On the other hand, an enhanced anticancer activity can also be expected as the result of the chemical alteration of the original molecule.

In most cases, the anticancer agent of the conjugate is a compound already applied in therapy (*e.g.* cisplatin, nitrogen-mustards, doxorubicin, *etc.*), but conjugation makes its medicinal utilization more specific and/or more effective. Based on this theory, Kvasnica *et al.* have recently reported about new platinum–estradiol/estrone complexes [66]. Altogether sixteen complexes were synthesized and tested against seven cancer cell lines and a normal fibroblast cell line. Complexation was performed at the C3 hydroxyl group of the steroid molecules. Although a leukemia cell line was found to be the most sensitive, the proliferation of both ER-positive and -negative breast cancer cell lines were equally influenced by the test compounds. Its possible explanation may be the lack of the free phenolic hydroxyl group which is known to be essential for ER binding. Moreover, all the complexes displayed a more effective cytotoxic action than did the control cisplatin. The most potent complex **23**, (Fig. **8**) blocked cell proliferation at low micromolar concentrations. Chemical stability of the new complexes was regarded to be satisfactory which may be a beneficial feature during the further pharmacokinetic investigations.

In other conjugates, the anticancer agent is a natural product with at least moderate antiproliferative activity (*e.g.* artesunate [67]) or any special antitumor characteristic (*e.g.* inability to be transported by a multidrug resistance protein (MDR)). Prodigiosin is a natural compound with anticancer activity among others. It was conjugated to estrone at its C3 hydroxyl group by linkers of various lengths aiming to produce a localized effect on ER-positive breast cancer cells [68]. Although the shorter the linker was, the higher the anticancer efficacy proved to be, but the most effective conjugate **24**, (Fig. **8**) still displayed moderate inhibition on the proliferation of ER-positive MCF-7 cells. Surprisingly, it suppressed the proliferation of the ER-negative MDA-MB-132 cells even more effectively. Other breast cancer cell lines, independent of their ER status, showed a reduced sensitivity to the test compound. However, **24** inhibited the proliferation of some leukemia, lung, colon, prostate and melanoma cell lines at low micromolar concentrations.

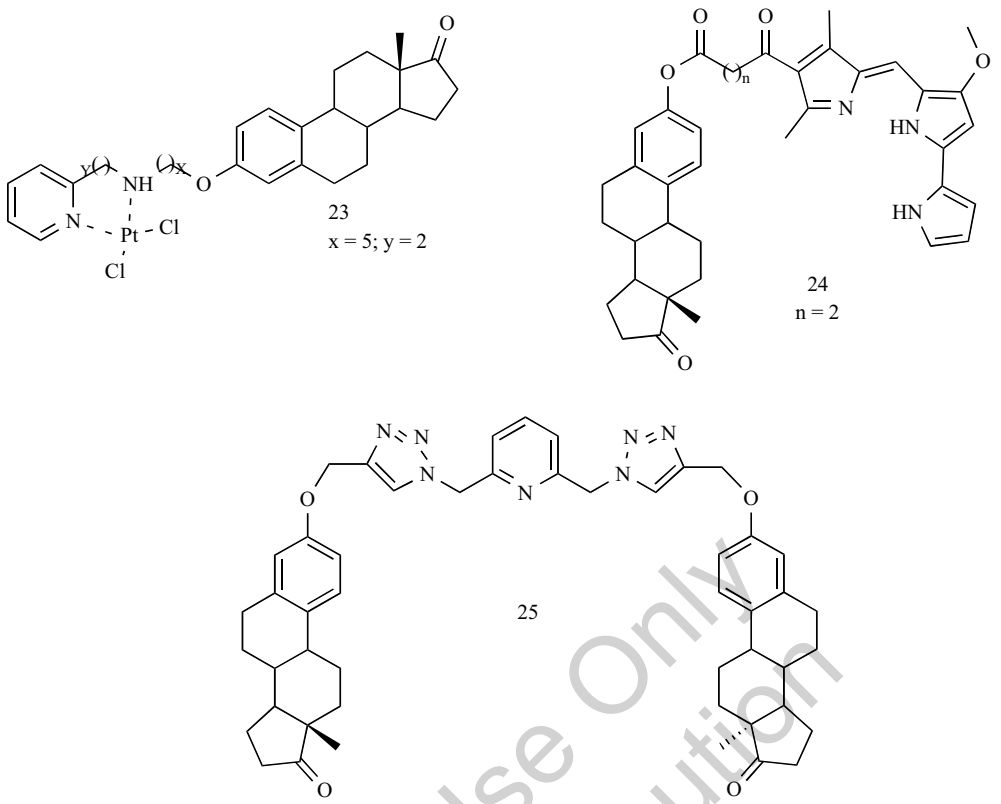


**Fig. (8).** Conjugates of estradiol-related compounds and anticancer agents. **23**: cis-Dichloro[3-(5-(2-(pyridin-2-yl-κN)ethylamino-κN)pentoxy)estra-1,3,5(10)-trien-17-one]platinum(II), **24**: (13S,14S)-13-Methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl 4-((Z)-2-((4-methoxy-1H,10H-[2,20-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrol-4-yl)-4-oxobutanoate, **25**: 3,30-[Pyridine-2,6-diylbis(methanediyl-1H-1,2,3-triazole-1,4-diylmethanediyloxy)]bisestra-1(10),2,4-trien-17-one.

Finally, Jurasek and co-workers investigated new compounds of interesting chemical structures [69]. These steroidal "ribbon-dimers" possess low to moderate estrogenic or antiestrogenic potency. However, the estrone-based dimer **25**, (Fig. **8**) was demonstrated to be active only against a daunorubicine-resistant cell line (CEM-DNR-BULK) which overexpresses MDR1 protein.

The detailed antiproliferative mechanism of action of the above mentioned conjugates is still unknown, but their unique chemical structures would give strong grounds for their detailed investigations to confirm their pharmacological effects *via* targeted experiments and also to clarify the recently reported unexpected data.

## 4. STEROID SULFATASE INHIBITORS

Endogenous steroidal biosynthesis and metabolism are governed by a group of chemical processes strictly regulated enzymatically. Thus, the pharmacological inhibition of certain enzymes which catalyze the formation of key molecules of cell proliferation results in beneficial therapeutic effects during the treatment of hormone-dependent carcinomas. One of these enzymes is steroid sulfatase (STS) which plays an important role not only in the formation of estrone from estrone sulphate, but also in the production of dehydroepiandrosterone (DHEA) and androstenediol from dehydroepiandrosterone sulphate (DHEAS) [70]. These androgens may contribute to excessive cell proliferation in hormone-dependent breast cancers, especially in menopausal women. Moreover, besides breast cancer, elevated STS activities were detected in endometrial, ovarian, prostate and colon carcinomas as well [6].

The first potent inhibitor of STS, estrone-3-*O*-sulfamate (EMATE) was reported in 1994 [71]. EMATE and its estradiol analogue (E2MATE) (Fig. **9**) became the basic molecules of the so called 1st generation (or irreversible) STS-inhibitors. They all share a common structural feature of an aryl-*O*-sulfamate moiety. In spite of their high effectiveness, neither EMATE, nor E2MATE have entered into the clinical practice, because they demonstrate estrogenic activity, which hindered their clinical development.

The 2nd generation STS-inhibitors are multi-targeted compounds, meaning that they block the enzyme activity on one hand, and exhibit a direct antiproliferative effect *via* the inhibition of tubulin polymerization on the other hand. This dual mechanism of action is thought to be beneficial in therapy. Usually, these molecules are 2-substituted derivatives of EMATE or E2MATE, occasionally accompanied by modification(s) in the D-ring of the steroidal skeleton [70]. From another point of view, some of them can be regarded as sulfamoylated derivatives of 2-ME2 and its analogues, blurring the frontier between these pharmacological groups [6].

Nowadays, the 3rd generation STS-inhibitors are also distinguished: molecules with a combined STS- and aromatase-inhibitory activity [6] are under pre-clinical development.

Most recently, few novel publications concerning the STS-inhibitors have been released. A pioneer work is focusing on the intracellular inhibition of the formation of active androgens using a previously described potent STS-inhibitor, **26** (Fig. **9**) [72]. Structurally this compound belongs to the sulfamoylated derivatives of 2-ME2 substituted with a 17α-benzyl functional group in the D-ring. In their *in vivo* experiments Roy *et al.* demonstrated that **26** prevents DHEAS-stimulation of androgen sensitive tissues without any androgen receptor mediated effects. The direct inhibition of STS was verified by the elevated plasma levels of DHEAS and the reduced plasma levels of DHEA and dihydrotestosterone.

Further novel EMATE analogues have been synthesized by Lawrence Woo and co-workers *via* the introduction of new sub-

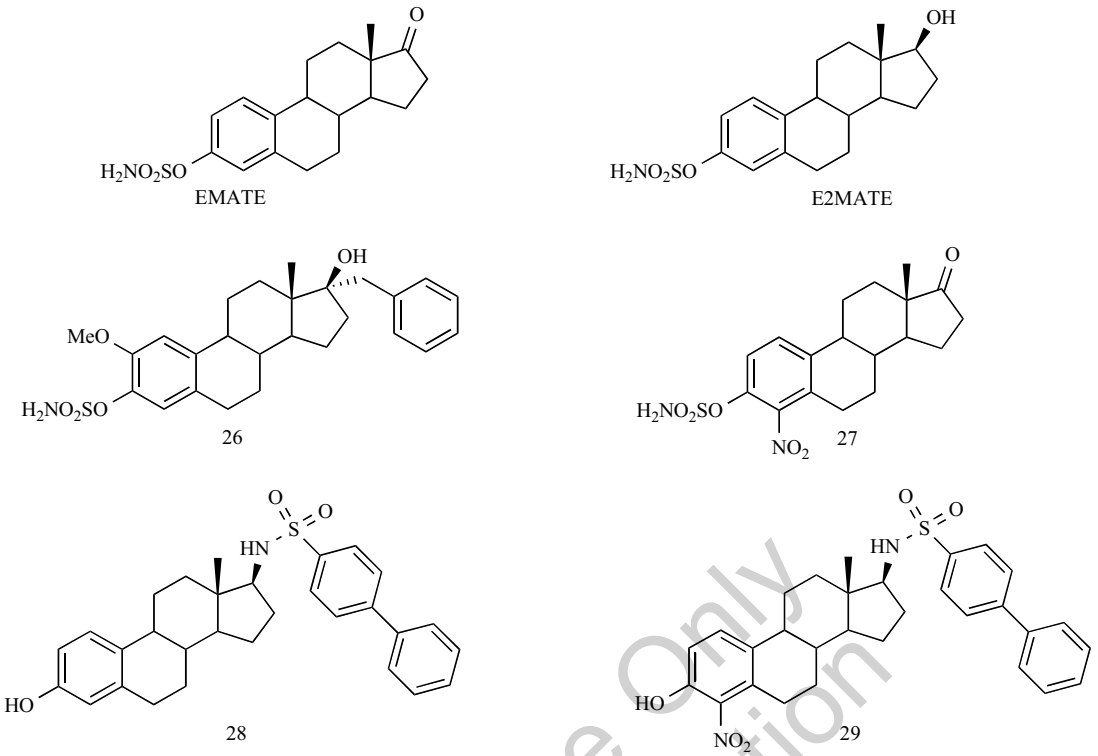


**Fig. (9).** Estradiol-related steroid sulfatase inhibitors. EMATE: Estra-1,3,5(10)-trien-17-one-3-sulfamate, E2MATE: 17β-Hydroxyestra-1,3,5(10)-trien-3-yl-sulfamate, **26**: 2-Methoxy-3-*O*-sulphamoyl-17α-benzyl-estra-1,3,5(10)-triene-3,17β-diol, **27**: 4-Nitro-estra-1,3,5(10)-trien-17-one-3-sulfamate, **28**: 17β-(4'-Phenylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol, **29**: 4-Nitro-17β-(4'-phenylbenzene)sulfonamide-1,3,5(10)-estratrien-3-ol.

stituents at positions C2 and/or C4, removal of the C17 carbonyl group and the formation of "cyclic sulfamates" [73]. Neither the 17-deoxy derivative of EMATE, nor the "cyclic sulfamates" demonstrated any inhibitory activity on STS. However, 4-nitro-EMATE (**27**, Fig. **9**) and the 2-halogenated compounds proved to be more potent inhibitors of STS compared to EMATE, based on the results of two different *in vitro* experimental methods. In fact, this investigation repeatedly supported a previous conclusion that a free or N-unsubstituted sulfamate group is essential for STS-inhibition and introduction of electron-withdrawing substituents enhance STS-inhibitory activity.

Reversible inhibition of an enzyme is more favourable in everyday practice than irreversible enzyme blocking, because it allows for the cessation of the therapeutic effect *via* the administration of a competitive antagonist agent. Thus, two recent publications about substituted 17β-aminoestradiol analogues by Mostafa *et al.* can be considered remarkable [74, 75]. The compounds they tested do not contain a sulfamoyl group at position C3, but they are 17β-arylsulfonamide derivatives. The 4'-biphenyl derivative **28**, (Fig. **9**) was found to be the most potent STS-inhibitor among the first set of new compounds, with an $IC_{50}$ value of one order of magnitude lower than that of EMATE, the former one determined in human placenta microsomes. Even this significant STS-inhibitory potency was exceeded by the 4-nitro derivative of **28**, **29**, (Fig. **9**). Moreover, some of these derivatives demonstrated a direct antiproliferative effect on the NCI 60 cell-line panel at low micromolar concentrations. Thus, it would be reasonable to further investigate the pharmacological and pharmacokinetic characteristics of these promising compounds, aiming the development of a therapeutically applicable STS-inhibitor.

## 5. 17β-HYDROXYSTEROID-DEHYDROGENASE INHIBITORS

The most potent cell proliferation-inducing steroidal compound is the well-known E2. The last step of its endogenous biosynthesis is the reduction of estrone (E1), mostly by type 1 17β-hydroxysteroid-dehydrogenase (17β-HSD1). It belongs to the large family of HSDs which catalyse either reductive (E1 to E2) or oxidative (E2 to E1 by 17β-HSD2) processes in different tissues [76].

Inhibition of 17β-HSD1 is considered as a medical approach of great importance, particularly in the therapy of hormone-dependent carcinomas (*e.g.* breast cancer). Although development of 17β-HSD1 inhibitors started in the '80s [77], still no agent is under clinical investigation. According to experts it might be explained by problems with their selectivity towards other members of the HSD family and also by the estrogenicity of the tested compounds.

Recently, Poirier and co-workers have published about several new compounds with a 17β-HSD1 inhibitory activity. They investigated various substituted E2 analogues to find a potent 17β-HSD1 inhibitor with no estrogenic activity and a good selectivity towards other HSDs, such as 17β-HSD2 or 17β-HSD12 (also take part in the transformation of E1 into E2). In 2008, a 16β-m-carbamoylbenzyl-estradiol derivative was reported to exert an extremely strong inhibitory activity on 17β-HSD1 **30**, (Fig. **10**) [78]. This compound acts as a reversible enzyme inhibitor, but it possesses residual estrogenic activity as demonstrated by both *in vitro* and *in vivo* experiments [79]. Thus, lactone derivatives of E2 were designed **31** and **32**, (Fig. **10**), expecting that these structural modification would reduce the estrogenicity of the compounds [80]. Unfortunately, these molecules were found to exert only a weak inhibitory activity on 17β-HSD1 as demonstrated by two different assays. However, they did not block the function of 17β-HSD2, which led the researchers to the conclusion that the estradiol-16β,17β-γ-lactone core can be considered as a promising structure for the development of new inhibitors. Later, modifying the structure of **30** by introducing a bromoethyl group at position C3, another strong, but irreversible 17β-HSD1 inhibitor was designed **33**, (Fig. **10**) and patented [81, 82]. This compound proved to exert no estrogenic activity neither *in vitro* nor *in vivo* [79].

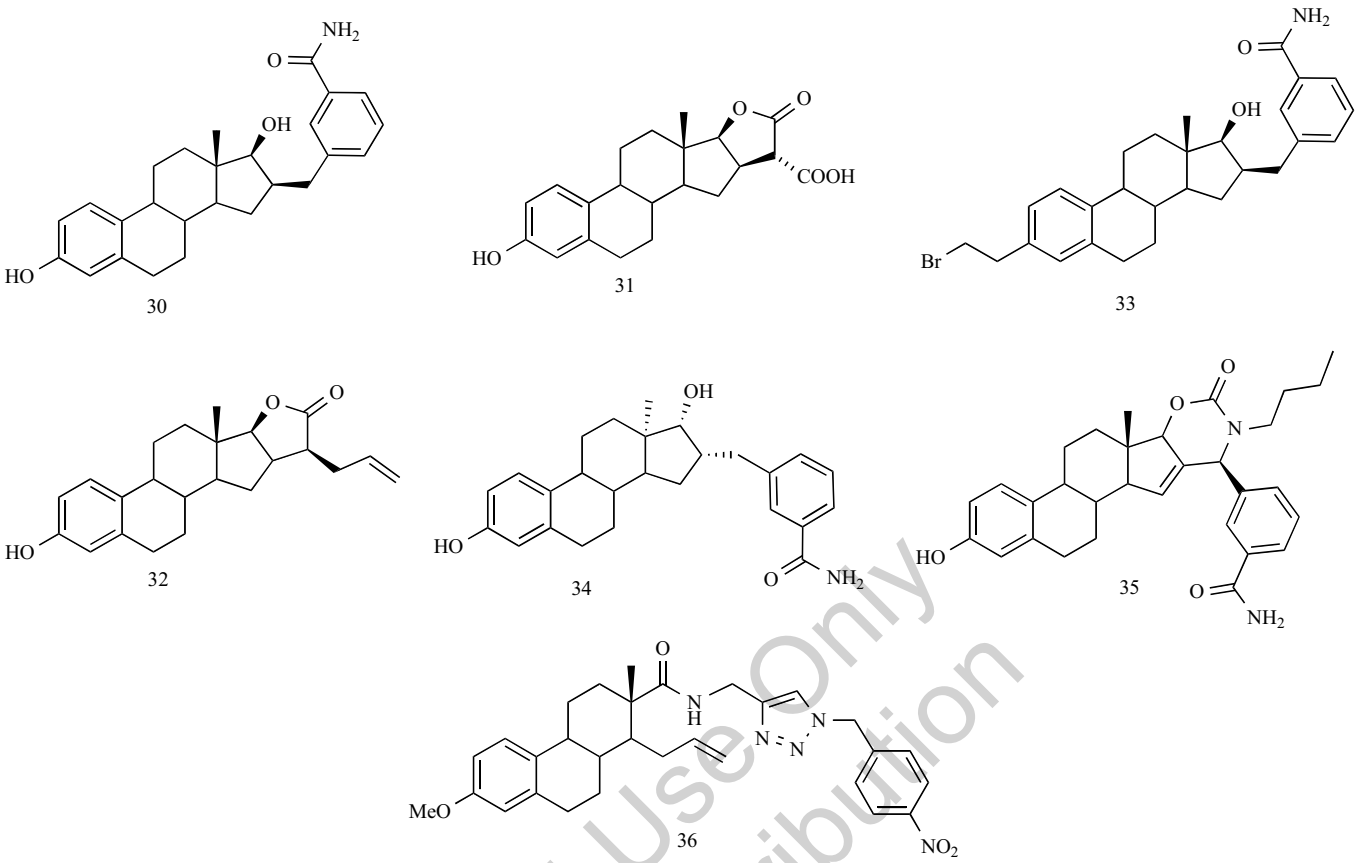


**Fig. (10).** Estradiol-related 17β-hydroxysteroid dehydrogenase inhibitors. **30**: 3-(3,17β-Dihydroxy-estra-1,3,5(10)-trien-16β-ylmethyl)-benzamide, **31**: (4bS,6aS,9aR,10aS,10bR)-2-hydroxy-6a-methyl-8-oxo-5,6,6a,6b,8,9,9a,10,10a,10b,11,12-dodecahydro-4bH-naphtho[2',1':4,5]indeno[1,2-b]furan-9-carboxylic acid, **32**: (4bS,6aS,6bS,9S,9aR,10aS,10bR)-2-hydroxy-6a-methyl-9-(prop-2-en-1-yl)-4b,5,6,6a,6b,9,9a,10,10a,10b,11,12-dodecahydro-8H-naphtho[2',1':4,5] indeno[1,2-b]furan-8-one, **33**: 3-(3-Ethylbromide-17β-hydroxy-estra-1,3,5(10)-trien-16β-ylmethyl)-benzamide, **34**: 3-{[(13α,16α,17α)-3,17-dihydroxyestra-1(10),2,4-trien-16-yl]methyl}benzamide, **35**: 3-[(4bS,6aS,6bR,10S,11aR,11bR)-9-butyl-2-hydroxy-6a-methyl-8-oxo-4b,5,6,6a,6b,8,9,10,11a,11b,12,13-dodecahydronaphtho[2',1':4,5]indeno[2,1-e][1,3]oxazin-10-yl]benzamide, **36**: 3-Methyloxy-N-{[1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl]methyl}-14β-(prop-2-en-yl)-des-D-estra-1,3,5(10)-trien-13α-carboxamide.

Moreover, for the first time it was demonstrated that a potent *in vitro* inhibitor of 17β-HSD1 was able to reduce the initial tumour size in an E1-stimulated xenograft model significantly [79]. Most recently, Maltais *et al.* have investigated derivatives of **30** with structures modified at positions C13, 16 and 17, aiming to reduce the estrogenic activity of the parent molecule [83]. The most promising chemical structure can be considered as the counterpart of **30**, as all of its functional groups are in the α position **34**, (Fig. **10**). It still has some estrogenic activity which is relatively weaker than that of **30**, but it does not activate the androgen-dependent cell proliferation. Based on molecular modeling experiments two significant interactions were identified between the ligand and the binding pocket of the enzyme: the interactions between C17-OH and the Ser142 residue, as well as between the benzamide moiety and the Asn152 and Leu95 residues, respectively. Another aim of the research group was to improve the 17β-HSD1 inhibitory activity of D-ring fused estradiol-lactone derivatives. Thus, saturated and unsaturated 16β,17β-oxazinone-estradiol derivatives were tested on intact T47D cells expressing 17β-HSD1. The most potent compound, **35** (Fig. **10**) was found to competitively inhibit the function of the enzyme at a low micromolar concentration [84].

It can be considered as a selective inhibitor, because it was shown to have no interaction with either 17β-HSD2 or17β-HSD12. The results of molecular modelling highlighted a novel and significant characteristics: namely, the presence of the C16-OH group (in case of saturated deivatives) was detected to worsen the binding ability of the ligand to the binding pocket of 17β-HSD1.

In 2015, the first report was published about the inhibitory effect of D-secoestrone derivatives on 17β-HSD1 [85]. The investigated molecules contain substituted triazole moieties on the D-seco-ring. The p-nitrophenyl-triazole derivative **36**, (Fig. **10**) displayed the highest inhibitory activity on 17β-HSD1 which was comparable to that of unsaturated E1. Additionally, the antiproliferative potency of all compounds was determined against four cancer cell lines. It was revealed that the compounds with good inhibitory activities on cell proliferation did not inhibit the function of 17β-HSD1 and *vice versa*. Based on previous structure-activity relationship analyses concerning ligand–ER binding, the authors supposed no estrogenic activity of these triazolyl D-secoestrone derivatives.

Finally, it is worthy of note that during the last decades endometriosis has been elucidated as an estrogen-dependent disease characterized by excessive cell proliferation induced by E2. As a consequence, one of its proposed therapeutic approaches is the reduction of E2 synthesis by the inhibition of 17β-HSD1 [86]. Several steroidal and non-steroidal 17β-HSD1 inhibitors are under pharmacological investigation targeting endometriosis [87-89], and an effort has been made to develop a reliable murine animal model of the disease as well [90].

## 6. AROMATASE INHIBITORS

By the formation of E1 and E2 from 4-androstene-3,17-dione and testosterone, respectively, endogenous steroidogenesis includes

the transformation of C19-steroids into C18-steroids, *ie.* estrogens. Besides STS and 17β-HSD1, the aromatase enzyme (CYP19) responsible for these transformations can also be considered as a key element of the biosynthesis of E2, a known promoter of cell proliferation [76] as mentioned before. Thus, effective inhibition of the function of aromatase is a promising pharmacological option in the therapy of estrogen hormone-dependent diseases.

Chemical development and even the clinical use of aromatase inhibitors (AIs) date back to over several decades. Based on the chemical structure of the agents, steroidal and non-steroidal AIs are distinguished from the very beginning of development. These compounds are able to inhibit the activity of aromatase either reversibly or irreversibly, but, as it is known, no clinical data can prove the superiority of steroidal AIs over non-steroidal AIs, or of reversible AIs over irreversible AIs [91]. As the exact crystal structure of aromatase was not clarified until 2009 [91], most of the investigated and clinically applied, up-to-date steroidal AIs are derivatives of androstendione, the endogenous substrate of the enzyme with an androgen backbone [92]. Thus, it is not surprising, that only few compounds with an estrane backbone as potential new AIs were reported during the last years. On the other hand, the number of publications about AIs with an androgen structure has also decreased significantly. Nevertheless, in 2013 a patent of 4-arylthia-4-androstene-3-one derivatives with potent aromatase-inhibiting effect was issued [93].

Based on promising molecular docking results, aromatase and lyase-inhibiting activities of a 17-oxa-D-homoestratriene derivative **37**, (Fig. **11**) was investigated [94]. Although it was found to have no estrogenic or antiestrogenic activity which is considered as a beneficial property, unfortunately the compound failed to justify the assumptions as it showed only a moderate effect towards both investigated enzymes.

A 16,17-secoestra-16,17a-dinitrile compound **38**, (Fig. **11**) was also predicted to be able to bind to more than one target among aromatase, androgen receptor, ERα and 17α-hydroxylase [95]. Nikolic and co-workers supposed that this could result from the opening and substitution of ring D which therefore becomes more flexible and its nitrile groups may interact directly with the Fe atom of heme, a common structural part of both investigated enzymes. Unfortunately, compound **38** was found to display a weaker antiproliferative activity than some D-secoandrosta dinitrile analogues, therefore its further investigations were rejected.

## 7. ANTIESTROGENS

It is well known that inhibiting the signal transduction pathway of ER, mainly ERα, blocks the cell proliferation inducing effect of E2. Therefore, efforts for the development of a potent ER-antagonist (an antiestrogen molecule), ideally selective for ERα, has always been a promising and challenging approach in pharmacological research targeting estrogen-dependent diseases. Since the discovery of the existence, tissue distribution and function of the second subtype of ER, ie. ERβ, this field of research has become more diverse [96]. All ER-antagonists can be divided into two groups based on their chemical structures, namely steroidal and non-steroidal antiestrogens.

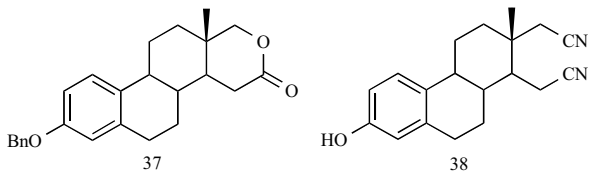


**Fig. (11).** Estradiol-related aromatase inhibitors. **37**: 3-Benzyloxy-17-oxa-D-homo-estra-1,3,5(10)-triene-16-on, **38**: 3-Hydroxy-16,17-secoestra-1,3,5(10)-triene-16,17a-dinitrile.

Based on the extensive research of steroidal antiestrogens it is well-established that modifications of the estrane skeleton at positions C7α or C11β are beneficial for the antiestrogenic potency of the new compounds [6], thus most of the recently reported novel antiestrogens possess one of these structural modifications. Moreover, special alterations at positions C15, C16 and C17 of E2 or E1 may produce direct cytotoxic molecules or dual acting antiestrogens [6].

Recently, Hanson and co-workers have published their results about the required nature of the substituents at position C11β. It was revealed that once the 11β-(4-substituted-phenyl) group has a substituent larger than a methoxy group, then the new compound behaves as an antiestrogen [97]. Moreover, incorporation of an azido group allows the incorporation of further diverse and bulky functional groups **39** and **40**, (Fig. **12**). Finally, they concluded that regarding the nature of the novel compound (agonist *vs.* antagonist) the sizes of the 11β-substituents are more important than the nature of the terminal group on the 4-substituted phenyl part of the molecule.

These findings established further investigations by the research group, focusing on molecules containing an antiestrogenic element connected to a traditional antitumor agent, like doxorubicin [98], mitomycin C [99] or geldanamycin [100], *via* a linker at position C11β. In fact, these compounds can be considered as conjugates or hybrid molecules. However, in all cases their steroidal part is a well-known antiestrogen, 11β-(4-(2-dimethylamino)ethoxy)phenyl)-estradiol, having a high binding affinity to ERα which is thought to play a significant role in their pharmacological action **41** and **42**, (Fig. **12**) and **43**, (Fig. **13**). By the development of these molecules the researchers aimed to implement multiple purposes, such as a) achieving a higher antiproliferative efficacy than that of the parent compounds, b) assuring a specific ER-mediated uptake and c) retain the antiestrogenic activity. Accordingly, the conjugation of doxorubicin with the 11β-substituted estradiol produced the most successful outcome with an enhanced and selective antiproliferative activity against the ER-positive MCF-7 cells followed by intracellular hydrolyzation, assuring a more targeted effect of doxorubicin. Although the mitomycin C–antiestrogen hybrid was also found to retain its high affinity to ERα and the antiestrogenic activity, neither an increase in its cell proliferation-inhibiting efficacy, nor selectivity towards the ER-positive cells was demonstrated. The conjugate containing geldanamycin was characterized by an antiproliferative activity lower than that of the parent antitumor agent, which was hypothesized to result from the lack of the dissociation of the molecule within cancer cells. Moreover, the conjugate inhibited the proliferation of ER-negative cells more effectively than that of ER-positive cells. The research group also drew some conclusions concerning the linker, based on previously described receptor–ligand interactions. Their results suggest that glycoloxy linkers may enhance the cellular uptake of certain molecules. However, once a linker contains two or more oxygen atoms, the length of the linker does not alter the ER-binding capacity of the novel agent, because the end of the molecule is outside of the ligand binding pocket of ER.

Another research group reported about the pharmacology of two novel compounds **44** and **45**, (Fig. **13**) which were designed as antiestrogens [101]. Both molecules have a long side chain at position C7α with an altered terminal end. Although their binding affinities to ERα were shown to be similar to that of fulvestrant, a known complete ER-antagonist of this receptor type, the results of a transactivation assay and co-repressor recruitment assays revealed that **45** behaves like an agonist, and **44** can be considered as a selective ER-modulator (SERM), because they oppositely influence ERα function in HeLa and MCF-7 cells. Since these molecules only differ in the terminal ends (6-hydroxyhexanyl and 6-benzyloxyhexanyl group, respectively) of their side chains at position C7α, it was concluded that the length of the side chain at

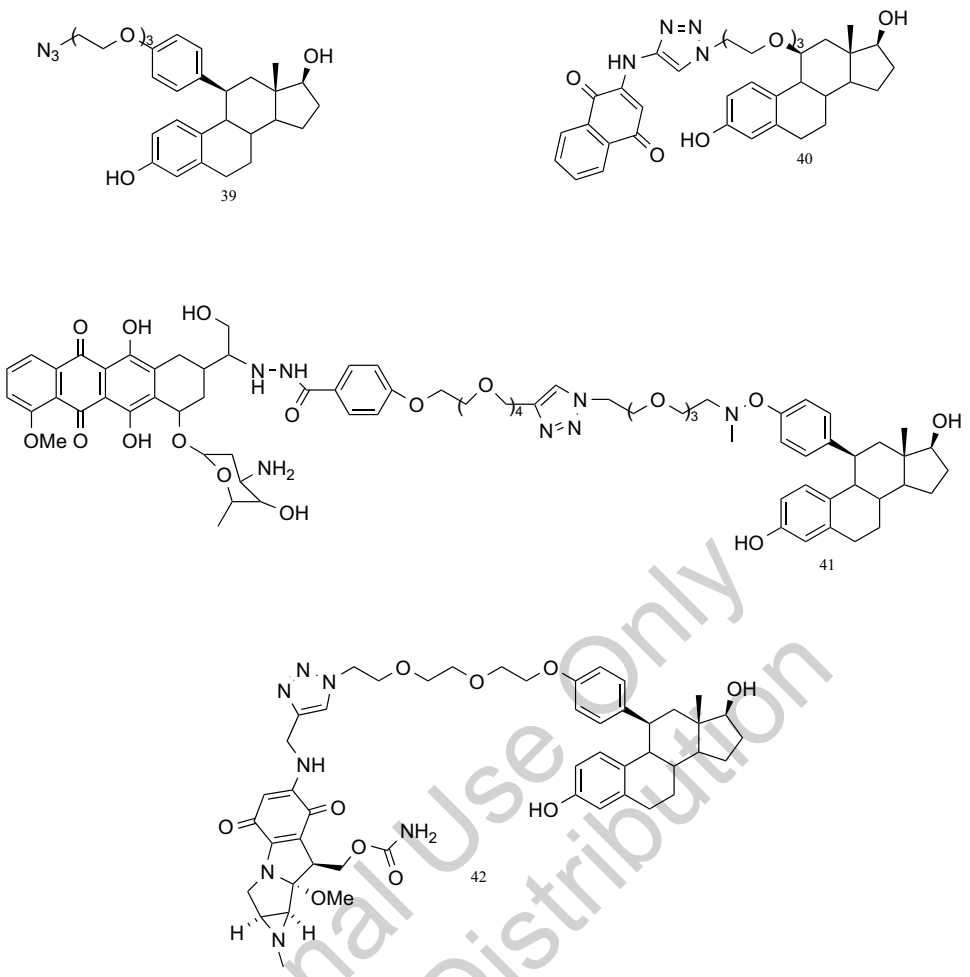

**Fig. (12).** Estradiol-related antiestrogens. **39**: 11β-(4-((2-(2-(2-Azidoethoxy)ethoxy)phenyl) estra-1,3,5(10)-trien-3,17β-diol, **40**: 2-(((1-(2(2(2-(4–3,17β-Dihydroxy-estra-1,3,5(10)-trien-11β-yl)phenoxy)ethoxy)ethoxy)ethyl-1H-1,2,3-triazol-4-yl)methyl)amino)naphthalenen-1,4-dione, **41**: 11β-(4-{2-[(2-{2-[2-(2-Azido-ethoxy)-ethoxy]-ethoxy}-ethyl)-methyl-amino]-ethoxy}-phenyl)-estra-1,3,5(10)-triene-3,17β-diol–doxorubicin conjugate, **42**: 11β-(4-((2-(2-(2-Azidoethoxy) ethoxy)ethoxy)phenyl)-estra-1,3,5(10)-triene-3,17β-diol conjugate with 7-*N*-propargyl-*N*-methylmitomycin C.

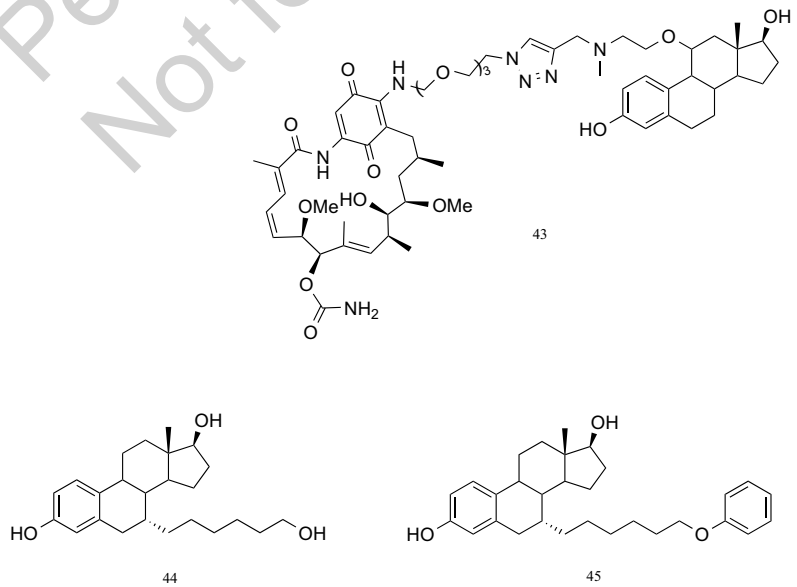

**Fig. (13).** Estradiol-related antiestrogens (continued). **43**: 11β-(4-N-propargyl-Nmethylaminoethoxyphenyl)-estra-1,3,5(10)-triene-3,17β-diol conjugate with 17-(azidopentaethylene glycolamino) geldanamycin, **44**: 7α-(6-hydroxyhexanyl)-estra-1,3,5(10)-triene-3,17β-diol, **45**: 7α-(6-benzyloxyhexanyl)-estra-1,3,5(10)-triene-3,17β-diol.

position C7α is less important for the pharmacological effect of these molecules than the exact nature of this functional group.

Although fulvestrant and tamoxifen are used with great success to treat hormone-dependent breast cancer, the development of novel antiestrogens is still in the focus of pharmacological research, fuelled by the increasing demand to develop more selective (to ERα or ERβ) and highly tissue specific molecules that may act as an agonist in the central nervous system and at the same time may behave as an antagonist in peripheral tissues. The results and conclusions concerning the compounds summarized here may compose the basis of further development of novel antiestrogens.

## CONCLUSION

Based on the overview of novel estradiol-related anticancer agents reported during the past five years, it can be established that the overall number of new publications has not declined substantially compared to the previous periods, which indicates an active developmental effort in this field of anticancer research. Obviously, certain topics achieved higher number of research papers than others which may partly result from the increasing efforts to find not only steroidal, but also novel non-steroidal anticancer agents. In some cases, the development of androstane or pregnane derivatives are preferred to that of estradiol analogues, based on the detailed structure-activity relationships elucidated recently.

To our best knowledge there are no ongoing or recently closed and published clinical trials concerning the innovative classes of agents described in the review. The reasons for that are complex, but these drugs typically exhibit some intrinsic estrogenic activity which limits their overall value as an endocrine disruptor.

However, for several families of the estradiol-related compounds, like for selective antiestrogens, estradiol-conjugates or estradiol derivatives with a direct antiproliferative effect (including 2-ME2 analogues), we may expect to witness a spectacular development in the near future. Hopefully, the most promising compounds of these pharmacological groups will finally reach clinical phase investigations or will even become new therapeutic agents against one or several types of cancer. To fulfil these aims, further persistent research is necessary on well-defined physico-chemical and pharmacological characteristics (*e.g*. tissue selectivity, receptor subtype selectivity, targeted uptake and accumulation, pharmacokinetic profile, *etc*.) of these compounds.



## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

RM collected the cited references from scientific databases and prepared the manuscript. IZ prepared the chemical structures and proof read the manuscript. This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Financial support from the Hungarian Scientific Research Fund (OTKA K-109293) is gratefully acknowledged.

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