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Estrone Sulfatase Inhibitors as New Anticancer Agents

Svetlana N. Morozkina and Alexander G. Shavva

Abstract

Enzyme steroid sulfatase (STS) is considered as a promising therapeutic target for the treatment of hormone-dependent oncological diseases such as breast, endometrial, prostate cancers, and endometriosis. The discovery of potent and irreversible STS inhibitors stimulated huge efforts of preclinical and clinical work. Various STS inhibitors such as steroid sulfamate, steroid nonsulfamate, nonsteroidal sulfamate, and nonsteroidal nonsulfamate-based inhibitors have been developed. In the review known STS inhibitors from the point of view of their safety, side-effects and perspectives for clinical application are considered. Among STS inhibitors several dual (multitargeted) compounds have huge potential being nonestrogenic and acting in nanomolar levels on the targets. The dual aromatase-sulfatase inhibitors (DASI) approach has a great potential when a synergy between STS and aromatase inhibition is expected and, thus it could address acquired resistance mechanisms. Among STS inhibitors based on steroid skeleton 17α -benzyl-, 17β -arylsulfonamides, 17 -diisopropylcarbamoyl-3-O-sulfamates exhibit the best properties, especially as dual anticancer potential drugs. The same modifications result in the increased activity against STS in 2-OMe-3-O-sulfamates as well as 2-OMe-3, 17β -bissulfamates, which are also active against triple negative breast cancer. 8α -Steroid estrogen analogs without estrogenic properties also possess high STS-inhibitory activity and block breast cancer cells growth with the activity comparable to tamoxifen.

Keywords: steroid sulfatase (STS), inhibitors, breast cancer, hormone-dependent diseases

1. Introduction

Breast cancer (BC) is the most common malignant tumor in women (12%) worldwide and is the second leading cause of cancer mortality after lung cancer (26%) [1].

Approximately 95–97% of tumors are estrogen-dependent in the early stages of their development [2, 3] and more than 70% express very high levels of estrogen receptor alpha ($ER\alpha$) [4]. The fundamental difference of extragonadal estrogen synthesis is its autocrine nature—that an organ producing estrogens is a target organ at the same time. Thus, local concentration of estrogens in such organs may be markedly elevated. Peripheral estrogens formation is increased after menopause, and compensates estrogens deficiency in different organs and tissues [5]. Extragonadal estrogens' production may rise with the aging. Moreover, it was continually emphasized in the literature that the increased level of estrogens in the body is considered as a risk of the BC development [6, 7].

Biologically active hormones, in particular the most active estrogen estradiol (E2), play a critical role in the initiation and development of hormone-dependent breast cancer (HDBC). In premenopausal women, estrogens are mainly (75%) synthesized in the ovaries, and thus, a luteinizing hormone-releasing hormone (LH-RH) agonist [8, 9] is useful to suppress the function of pituitary hormone. In postmenopausal women estrogens are produced in peripheral tissues such as adipose tissues, skin, and mammary glands [10, 11].

Adrenal dehydroepiandrosterone sulfate (DHEAS), dehydroepiandrosterone (DHEA), and adrenal or ovarian androstenedione are also sources of E2 in peripheral tissues. In postmenopausal women, concentrations of DHEAS, DHEA, and androstenedione in plasma are relatively high; approximately 1.8, 6.6, and 1.9 nM, respectively. In contrast, plasma concentrations of estrone (E1) and (E2) are several-fold lower (70 and 30 pM, respectively) [12].

Another important steroid precursor for estrogen formation is E1-sulfate (E1S). It is the most important estrogen in the peripheral blood, with relatively high (0.6 nM) concentrations in postmenopausal women. E1S levels are associated with high body-mass index, which suggest that E1S originates from adipose tissue. Concentrations of E1S in plasma are 10–20 times higher than those of E1 and E2, as well as its half-life in the plasma is longer than the half-life of unconjugated estrogens.

Enzyme steroid sulfatase (STS) converts E1S to E1, followed by the reduction to the biologically active estrogen, E2, by 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1), which is overexpressed in many breast tumors.

In BC tissues estrogens can be locally produced *de novo* by estrogen synthesis enzymes to promote tumor growth.

The level of estrogens in BC tissues of postmenopausal women can be 10–40 folds higher than in blood circulation and 5–10 times higher than in noncancerous breast tissues [13]. Furthermore, the intratumoral E2/E1 ratio is significantly higher in postmenopausal BC than in premenopausal BC. High concentrations of estrogen in breast tissue increase the risk of BC development [14, 15].

Thus, inhibition of enzymatic synthesis of estrogens is an effective therapeutic strategy for postmenopausal women with estrogen receptor-positive (ER+) tumors [16, 17]. *In situ* transformations of inactive steroids require activity of a series of enzymes that were found in hormone-sensitive cancers.

The scheme of estrogens formation in human body includes: (a) formation of E1 from androstenedione under the action of cytochrome P450 aromatase, (b) reduction of E1 by 17 β -HSD1 leads to more active E2. Importantly, almost insoluble in aqueous media E1 is converted into water-soluble E1S under the action of

Figure 1.
Estrogens formation in human body.

sulfotransferase (STS). E1S does not possess hormonal activity, however it may be transported into various targets (**Figure 1**) [18, 19]. Several reviews focus on aspects of human steroidogenesis [18, 20–29].

Free hormones are formed from sulfates of estrogen and androgens under action of steroid sulfatase. At high concentrations, androgens compete for binding with ERs. The activation of ER α under the action of androstenediol and DHEA in BC cells has been detected. It is confirmed by the inhibition of cell growth in the presence of antiestrogens. The evaluation of E1S level during diagnostic of various oncological diseases (for example, prostate cancer) is of high importance [30].

2. Approaches for the manipulation of estrogen level in tumors

2.1 Endocrine therapy

Hormonal (endocrine) therapy is effectively used for the treatment of HBC. Most types of BCs are estrogen-dependent, with approximately 55% in premenopausal women and 75% in postmenopausal women [31–34].

Selective estrogen receptor modulators (SERMs) or down-regulators (SERD), such as tamoxifen, raloxifene, ospemifine, and fulvestrant are compounds that are currently used in clinical practice to treat BC [9, 35]. In breast tissues, SERMs effectively block the activation of ER(α) by endogenous ligands, preventing the transcription of genes mediated by estrogen response elements [36, 37]. SERMs have tissue-specific effects on ER α that results in antagonist activity in breast and uterus tissues as well as agonist activity in bone. Although tamoxifen and raloxifene possess the desired SERM activity, they also increase the risk of venous thrombo-embolism [38] and exhibit toxicity [22]. Given that resistance (*de novo* or acquired resistance) is a major limiting factor in the use of endocrine therapy, additional endocrine therapies with other mechanisms of action are needed [39, 40].

2.2 Inhibitors of enzymes responsible for the estrogen formation in tumors

The aromatase enzyme is responsible for the conversion of testosterone and androstenedione to E2 and E1, respectively. Thus, inhibition of the aromatase enzyme is one of the approaches for the development of new drugs to treat BC [41–43].

Nonsteroidal third-generation aromatase inhibitors (AIs), such as anastrozole (Arimidex), letrozole (Femara), and exemestane (Aromasin), are often used for postmenopausal hormone-dependent BC treatment in clinical practice. Despite the success of AIs in the clinic, numerous BC patients still progress after AI therapy due to the development of resistance to AIs and side-effects such as osteoporosis caused by whole-body deprivation of estrogen [44, 45]. Mechanisms of AI resistance include ligand-independent activation of the ER and signaling via other growth factor receptors; new insights about resistance are published recently [45].

The overall response rates for AIs (40–50%) suggest the presence of alternative sources of estrogens. The production of E1, DHEA and androstenediol is an important mechanism of resistance to AI treatment [46].

It was demonstrated that AIs used sequentially with tamoxifen had higher efficacy compared to tamoxifen alone, with an improvement in overall survival [47].

There are other factors involved in tumor growth [48]. The enzymes STS and 17 β -HSD1 have been identified as essential parts in E2 production and subsequent promotion of cancer growth. Recently it was shown that 17 β -HSD7 also plays a key role in increasing the E2/E1 ratio in BC tumors [49]. Very recently, some evaluations of the “sulfatase pathways” in tumor stroma have been carried out [50].

The STS is also responsible for the hydrolysis of DHEAS to DHEA, which is an immediate precursor of androstenediol, a potent estrogenic steroid [51], whose formation is not influenced by AIs. DHEAS stimulates proliferation of MCF-7 cells from BC, which could be blocked by an antiestrogen or STS inhibitor but by an AI. E1S and DHEAS are particularly abundant in blood circulation and could act as a reservoir of steroid precursors, specifically in BC [29, 52]. The formation of DHEA through the STS pathway accounts for the production of 90% of the androgen androstenediol [52], which possesses estrogenic properties, that are 100-times weaker than estradiol [13, 53]. Androstenediol is present at 100-fold higher concentrations than estradiol in the circulation, and may have estrogenic properties that are equal to estradiol [54]. Thus, inhibition of STS has the dual property of reducing local androstenediol biosynthesis [55, 56].

2.3 Steroid sulfatase enzyme (STS)

The STS enzyme (EC 3.1.6.2, aryl sulfatase C, steryl-sulfatase) is widely distributed throughout the body and plays critical role in steroidogenesis [54]. Publications in recent years indicate the role of STS activity in gynecological diseases [57], mentioning diminished endometriosis *in vivo* under the action of STS inhibitors [58, 59]. However, a phase II trial with STS inhibitors in endometrium cancer patients with advanced disease revealed no superior effects as compared to progestin megestrol acetate, and further studies are ongoing [60]. STS inhibitors are also useful for the treatment of ovary cancers and prostate cancer [16, 61].

According to the *in vitro* studies, STS is the main enzyme responsible for estrogen production in hormone-dependent breast tumors, and has several hundred times higher activity in liver and normal/malignant breast tissues than aromatase [13, 53, 62]. STS mRNA expression (74%) in ER α -positive breast tumors is an independent prognostic indicator in predicting relapse-free survival, with higher levels of expression being associated with a poor prognosis [63]. Like aromatase inhibitors, sulfatase inhibition can only be used in postmenopausal women. Probably, the greatest benefit with sulfatase inhibition is in those cases where DHEAS levels are high. To date, STS inhibitors are still in an early stage of development [53, 64, 65].

The human STS is a protein, integrated in microsomal membrane. Its three-dimensional structure has been determined (PDBcode 1P49) [66]. However, knowledge about regulation of its expression as well as activity is limited. The topology of the active site of the steroid sulfatase and the arylsulfatases A and B is similar [66].

Most of the STS inhibitors discovered to date, act as irreversible active-site-directed inhibitors. An aryl sulfamate group (ArOSO₂NH₂) is considered as the pharmacophore for irreversible inhibition of the enzyme. One of the first time-, pH-, and concentration-dependent irreversible active-site directed-steroidal inhibitor is estrone-3-O-sulfamate (EMATE), which inhibit STS in MCF-7 cells from BC by 99% at 0.1 μ M and has an IC₅₀ value of 65 pM (IC₅₀ = 80 nM in placental microsomes). EMATE was evaluated in clinical trials [67]. The highest effectiveness of EMATE has been demonstrated in rats (subcutaneous and oral administration). STS activity was also inhibited when EMATE was administered to humans in dose 0.5 mg/kg [68].

inside red blood cells where it, similarly to EMATE, binds to (and inhibits) carbonic anhydrase II ($IC_{50} = 22 \text{ nM}$) [83]. The inactivation mechanism suggests that a sulfamate group is transferred to the gem-diol form of formylglycine 75 of steroid sulfatase due to a facile E1cB elimination of sulfamate anion to give the corresponding coumarin, which has a long half-life in blood [84]. However, the further development of Irosustat in monotherapy was stopped in the phase I/II clinical studies, because Irosustat does not possess superior properties to the current standard of care megestrol acetate, and its relative bioavailability decreases with increasing dose. The study of its combination with other hormonal therapies (for example, with the aromatase inhibitor anastrozole) is underway [85]. Metabolism of Irosustat has been investigated [86]. Irosustat also inhibits skin and liver STS [86].

2.5.1 Dual selective estrogen receptor modulators/STS inhibitors

One of the first examples of the dual SERM/STS inhibitor was published by Duquesne University [87]. 4-Hydroxytamoxifen is a metabolite of main drug tamoxifen used as endocrine therapy in (ER+) BCs [88]. This metabolite is a SERM and has antiestrogen effects in breast tissues, however, acts as an estrogen agonist in other tissues such as bone marrow. The sulfamate derivative **3** of 4-hydroxytamoxifen was shown to be an STS inhibitor with $K_i = 35.9 \text{ }\mu\text{M}$.

Surprisingly, among silicon-containing derivatives compound **4** exhibits strong STS-inhibitory activity ($IC_{50} = 0.17 \text{ }\mu\text{M}$). Furthermore, its metabolite **5** possesses potent ER α -antagonistic activity ($IC_{50} = 29.7 \text{ nM}$) [89].

Poirier with colleagues, among tetrahydroisoquinoline-N-substituted derivatives [90], found second-generation dual-action compounds that inhibit STS and act as a SERM. These compounds are devoid of estrogenic activity and toxicity. Their sulfamate derivatives possess high inhibitory activity toward STS (IC_{50} of 3.9, 8.9, and 16.6 nM). Both phenolic and their sulfamate derivatives show no estrogenic activity and moderate antiestrogenic properties. All compounds significantly stimulate osteoblast-like Saos-2 cell proliferation, thus suggesting a SERM activity. The results of molecular docking experiments suggest that the most active compounds **6** and **7** bind in a competitive manner with E2 [91].

2.5.2 Dual aromatase/STS inhibitors

Another approach for the treatment of hormone-dependent BC is the development of DASIs, which may have an additive or synergistic antitumor effect. The potential advantages of a single chemical agent with the ability to interact with

multiple biological targets were highlighted previously [92]. In the case of DASIs, this goal is being pursued by the introduction of the critical sulfamate unit in structures with known aromatase-inhibiting properties [93, 94]. All DASIs are still in preclinical investigations [95].

One of the best dual inhibitors is compound **8** with nonestrogenic properties. 2',4'-Di-cyanobiphenyl-4-O-sulfamate (TZS8478) (**9**) also shows the best STS inhibition [96].

One of the most potent dual inhibitor is compound **10** with 98 and 85% inhibition of STS and aromatase, respectively, at 10 μ M [97]. A series of DASIs have been investigated [98, 99]. Compound **11** (STX681, IC_{50} = 0.82 nM for aromatase and IC_{50} = 39 nM for STS) and similar analog **12** also exhibit an excellent profile against aromatase (IC_{50} = 0.13 nM) and STS (IC_{50} = 3.5 nM) and are not estrogenic [100]. Bissulfamate **13** at a single oral dose of 10 mg/kg inhibits aromatase and rat liver STS by 60 and 88%, respectively. The anastrozole inspired compound **12** is also potent dual inhibitor *in vivo* [101, 102].

Among compounds on letrozole and vorozole templates, the most potent inhibitors were compounds **15** (aromatase IC_{50} = 0.5 nM and STS IC_{50} = 5.5 nM) and **16** (IC_{50} = 0.0001 μ M) [103]. When orally dosed, compound **15** reduces plasma estradiol levels and inhibits liver STS activity [103].

Potter with coauthors published the successful realization of the strategy when the core components of the two leading DASIs resulted in the hybrid structures that exhibit a very high level of dual inhibition against aromatase and STS *in vitro* (IC_{50} = 0.015–0.75 nM). Most active compound is analog **17** (IC_{50} for aromatase = 0.0002 μ M, for STS = 0.0025 μ M) [104].

The latest achievements in the field of nonsteroidal AIs are presented in recent reviews [105, 106].

2.6 Steroidal STS inhibitors

2.6.1 Steroid-based STS inhibitors without sulfamate group

Nonsulfamated STS inhibitors based on estrogens are weaker than EMATE. Most active STS inhibitors without sulfamate group with highest activity are represented by compounds **18**, **19**, and **20** (IC_{50} = 12, 21, and 9, respectively) [107–109].

Estradiol dimer **21** also exhibits STS inhibitory activity in nanomolar range [110]. STS inhibitors are exemplified by tetrazole derivative **22** and boronic derivative **23** [111–113].

In the series of 4-substituted 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol, compounds **24** and **25** are tight-binding inhibitors with K_i app values of 1 and 2.5 nM [114].

2.6.2 Steroid-based STS inhibitors with sulfamate group

The estrogenicity of EMATE and estradiol-3-O-sulfamate (**26**, E2MATE, PGL2001, J995) is the serious restriction for their development as anticancer agents. E2MATE effectively inhibits STS activity in endometrial tissue *in vitro* and *in vivo* (in doses 1.0 and 0.5 mg/kg) without affecting systemic E2 levels [58, 59, 115, 116], and is introduced into Phase IIa of clinical trials [117]. E2MATE has been also clinically investigated as a pro-drug for hormone-replacement therapy and some limited clinical data are available. EMATE and E2MATE are bound to carbonic anhydrase (for EMATE $IC_{50} = 23$ nM) within red blood cells, being dual inhibitors of carbonic anhydrases and STS [118].

The sulfamate **27** (NOMATE) was evaluated as an STS inhibitor. This steroid without the 17-carbonyl group possesses ablated estrogenicity as well as reduced

STS activity compared to EMATE. NOMATE was shown to exhibit antitumor activity against a range of tumor cell lines [119].

D-ring lactone **28** has been developed as an orally available STS inhibitor [114]. The latest together with related lactam **29** were independently developed by Imperial College and University of Bath [120]. These compounds are potent STS inhibitors (98 and 91% inhibition of STS activity in MCF-7 cells at 0.1 μ M, respectively; oral dose of 2 mg/kg/day) without estrogenic effects.

Simple modifications of the D-ring have led to dramatic variations in estrogenicity. Thus, the conversion of EMATE to the oxime results in a super-estrogen. From the other hand, D-ring heterocyclic derivatives exhibit reduced estrogenicity [121, 122].

The replacement of ring D with N-substituted piperidinedione moiety results in the loss of estrogenic properties and greater STS inhibitory activity *in vivo* compared to STX64, as it was shown by the compounds STX213 (**31**) [123] and STX1938 (**30**) [124]. The STX1938 (**30**) and STX213 (**31**) inhibit STS with IC_{50} of 1 nM and 35 pM correspondingly (90- and 18-fold more potent than EMATE, respectively) [125]. STX213 and STX1938 possess superior properties in comparison with STX64 *in vivo* models with once weekly oral dose 1 mg/kg [125, 126]. The docking studies explained the greater potency of STX1938 in comparison with STX213 by the increased lipophilicity of CF_3 group and the ability of the fluorine atoms to participate in C-F---H-O and C-F---H-N interactions in the STS binding site. STX213 (**31**) demonstrates a greater effect on tumor growth than Irosustat (oral dose 10 mg/kg/day) [21]. Most active among 17-modified EMATE derivatives as STS inhibitors was steroid **32** (IC_{50} = 11 pM) [126]. The saturated analog **33** possesses similar potency (IC_{50} = 34 pM), and is not estrogenic [126].

Among various 2- and 4-substituted and 2,4-disubstituted EMATE derivatives, most active compounds are 2-(2-prop-2-enyl)-EMATE (**34**, IC_{50} = 37 nM in MCF-7 cells) [126]; and 4-nitro-EMATE (**35**, IC_{50} = 0.01 nM in MCF-7 cells) (EMATE; IC_{50} = 0.83 nM in MCF-7 cells), and steroid **34** is nonestrogenic [127].

Cyclic sulfamate **36** is an effective STS inhibitor (IC_{50} = 9.3 nM) *in vivo* with dose regime 1 mg/mouse/day for 5 weeks [128]. The derivatives of oxathiazine **36** are claimed as estrogen-ablative agents; however, no data on their activity have been published [129]. Cyclic sulfamates with six-membered ring are time-dependent inactivators [130]. Acyclic mono-alkylated sulfamates are not time-dependent inactivators of sulfatases. Probably, imino compound **36** hydrolyzes to the ortho-formyl sulfamate *in situ* [53]. The five-membered ring compounds such as **37** are not time-dependent inactivators of STS [131].

2.6.3 Dual 17 β -HSD1/STS inhibitors

17 β HSD converts E1 to E2 and DHEA to androstanediol [132]. Several inhibitors based on steroidal skeleton have been successfully developed [133, 134]. Few dual

inhibitors of 17 β -HSD and STS for the treatment of steroid hormone-dependent diseases are patented [135]. The example of such inhibitors is represented by the compound **38**.

A-ring-modified steroidal sulfamates, for example, series of 2-OMe-estradiol sulfamates and analogs have been investigated as nonestrogenic STS inhibitors [136, 137]. 2-MeO-EMATE **39** demonstrates the excellent inhibitory properties in the relation to STS *in vitro* (IC_{50} = 30 nM) and *in vivo* and is not estrogenic [138]. It strongly shows the antiproliferative effects toward BC cells by inducing apoptosis and cell cycle arresting in the G2/M phase [139].

2-Ethyl-EMATE **40** was identified as a promising multitargeted anticancer agent with strong ability to arrest the cell cycle, inhibit angiogenesis, as well as inhibit tumor growth in a xenograft model [140]. It was found that 2-ethylestrone (desulfamoylated compound **40**) belongs to series of potent superoxide dismutase inhibitors [141].

It is known that 2-methoxyestradiol, a metabolite of E2, possesses antiangiogenic properties and prevents tumor growth through disrupting tubulin polymerization by binding at the colchicine-binding site [142, 143]. 2-Methoxyestradiol is considered as the perspective compound for the treatment of endometriosis [144].

The anticancer effects of the 2-substituted sulfamate estrogen derivatives arise from disruption of tubulin polymerization, and the compounds also binding at the colchicine site [145]. 3,17 β -Bissulfamates of estrogens are other representatives of multitargeted antitumor agents, acting as STS inhibitors with antiproliferative activity (IC_{50} = 18–250 nM) [146]. Such bissulfamates compete with colchicine for tubulin binding and disrupt microtubules resulting into cell cycle arrest just by apoptosis *in vitro* and *in vivo* [147, 148] and inhibit angiogenesis *in vitro* and *in vivo* [149]. The STS inhibitory activity of bissulfamate **41** is comparable to EMATE activity [150]. Bissulfamoylated derivatives with 2-MeO (**42**, STX140) and 2-Et (**44**, STX243) substituents in steroidal skeleton exhibit high STS inhibitory activity (IC_{50} = 39 and 1000 nM, respectively) [151].

STX140 (**42**) and STX243 (**44**) possess *in vivo* activity also against the MDA-MB-435 cell line (at 20 mg/kg oral) [152]. STX140 *in vivo* inhibits MDA-MB-231 breast tumors [152–154].

Coordination of the 17-sulfamate residue to the zinc in active site of the complex of STX140 with human carbonic anhydrase II is revealed [155].

STX140 depolarizes mitochondrial bioenergetics, activates caspase 3/7 causing apoptosis through the intrinsic mitochondrial pathway, and downregulates

the expression of caspase inhibitors [156]. The activity of such compounds is also explained by their ability to disrupt the tubulin-microtubule equilibrium in cells as being central to their antitumor activity. STX140 and STX243 bind with the colchicines binding site of tubulin. 2-(^{11}C)Methoxy-3,17 β -OO-bis(sulfamoyl)estradiol has been proposed as a new potential PET agent for imaging of steroid sulfatase in cancers [157].

One more example of 2-MeO-derivatives as effective STS inhibitors is illustrated by compound **45** containing cyano group at position C-17 [158].

2-Difluoromethyl-E1-3-O-sulfamate (**46**) is 91-fold more potent inhibitor compared to EMATE ($\text{IC}_{50} = 0.1$ and 9.1 nM, respectively) [159].

The level of STS inhibition for 17 β -(N-alkylcarbamoyl)-estra-1,3,5(10)-trien-3-O-sulfamates (**47**) and 17 β -(N-alkanoyl)-estra-1,3,5(10)-trien-3-O-sulfamates (**48**) is similar to or exceeded that of EMATE. Some of these compounds are nonestrogenic. 17-(N-alkylcarbamoyl)-estra-1,3,5(10)-triene-3-O-sulfamates and the inverse amides have been patented as good STS inhibitors [129].

Among a series of C17-ketone and amide-modified estrone-derived sulfamates, compound KW-2581 (**49**, 17-diisopropylcarbamoyl-1,3,5(10),16-estratetraen-3-yl-sulfamate) is the most promising, not estrogenic, orally active anticancer agent for the treatment of hormone-dependent BC and endometrial cancer [160]. KW-2581 as STS inhibitor is five times more potent compared to STX-64 ($\text{IC}_{50} = 4$ nM) [161]. It was also demonstrated that the compound inhibits the ability of androstenediol-S to stimulate the *in vivo* growth of MCF-7 cells from BC overexpressing STS. However, KW-2581 is practically insoluble in water (approx. 0.1 ng/mL). The attempts to increase its oral bioavailability showed that the milled powder exhibited poorer properties than the intact sample, including a lower level of crystallinity, higher water content, and increased decomposition rate [162].

Diverse 17 α -alkylated estradiol sulfamates as STS inhibitors have been patented [163] and 17 α -benzyl-derivatives have been investigated [164].

Compound EM-1913 (**50**) is nonestrogenic steroidal STS inhibitor with $\text{IC}_{50} = 0.05$ nM [165], which also inhibits dehydroepiandrosterone sulfate action in androgen-sensitive tissues, being therefore considered as a potential drug for the treatment of prostate cancer [166].

17 α -Benzyl substituent yields reversible STS inhibitors in the absence of a sulfamate group, and incorporation of an aryl sulfamate onto the A-ring results in a potent time-dependent irreversible inhibitor. The IC_{50} of the tert-butylbenzyl derivative **51** is low (8.3 nM); however, steroid **51** is estrogenic. A-ring substitution leads to the reduced estrogenicity. 2-Methoxyderivative **52** has an $\text{IC}_{50} = 0.04$ nM. The compound without the tert-butyl group is nonestrogenic and effective STS inhibitor *in vivo* [167].

In the series of A-ring thioether-modified sulfamates, the steroid **53** is 50-fold more potent inhibitor of STS than steroid **52**; however, it possesses weak inhibitory activity against MCF-7 cells proliferation ($IC_{50} = 10 \mu M$) [168].

2.6.4 Dual STS/SERM inhibitors

Maximum estrogen blockade in the treatment of (ER+) BC may be achieved using dual ER α antagonists and STS inhibitors, which might cause osteoporosis as a side effect [169]. In this case, the compound possessing SERM properties is needed.

Thus, a novel orally available irreversible dual STS/SERM inhibitor SR16157 (NSC 732011) (**60**) ($IC_{50} = 0.1 \mu M$) has been developed as a very promising inhibitor with excellent pharmacokinetics and acceptable toxicological profile [170].

Desulfamoylation of SR16157 (**54**) results in SR16137 (**55**), which is a tissue-selective antiestrogen with beneficial effects on bone and cardiovascular system [171]. SR16157 is 10 times more potent as a growth inhibitor of MCF-7 cells than either the antiestrogens tamoxifen or SR1613. Additionally, SR16137 has a 10-fold higher affinity for ER α as compared to tamoxifen. SR16157 was shown to possess minimal genotoxic activity [172]. SR16157 has been recommended in initial phase I of clinical trials with the starting dose of 1.3 mg/kg/day administered as a single dose in humans.

We demonstrated that 8- α -analogs of steroid estrogens effectively inhibit the growth of BC cells, including triple negative BC [173, 174].

3. Conclusions

Manipulation of hormone biosynthesis in tumors by enzymes inhibitors is a very attractive approach for the treatment of hormone-dependent tumors such as breast, prostate cancer, and endometriosis.

The importance of STS in human body has been underlined by many investigations. Thus, STS-catalyzed hydrolysis of pregnolone-3-sulfate and dehydroepiandrosterone-3-sulfate in the brain regulates neurosteroid synthesis and influences memory. STS inhibition for the potentiation of memory in sufferers of neurological diseases such as Alzheimer's disease and dementia has been postulated [175]. The role of STS inhibitors as agents to reveal beneficial endogenous glucocorticoid effects was also claimed. The use of STS inhibitors in combination with the immunosuppressive ascomycin for the treatment of acne, seborrhoea, androgenetic alopecia, and hirsutism is patented. The administration of an estrogen (including norgestimate and norelgestromin), in combination with a progestogen in hormone-replacement therapy act by inhibiting STS, thus reduce estrogen production and protect the endometrium and breast from hormone-dependent cancers [176]. STS inhibitors prevent ovarian cycle disturbance, prolonged unopposed secretion of estrogens, and ovarian follicular cyst formation in premenopausal women, as well as prevent premature uterine contractions, particularly for preterm labor [177].

The importance of STS inhibition in endometriosis, prostate cancer, as well as latest discussions about mechanism of inhibition is well considered in the review of Prof. Potter [178]. The significance of steroid sulfatase and sulfotransferases in gynecological diseases are summarized in the review [57].

As far as estrogenic compounds may stimulate tumor cells growth, the main requirement for STS inhibitors and their metabolites is the absence of estrogenicity. Among nonsteroidal STS inhibitors only one nonestrogenic compound-Irosustate was evaluated in clinical trials with excellent properties, however its further development was stopped. Currently, the action of Irosustate in the combination with AIs is investigated.

The discovery of dual (multitargeted) inhibitors is the most promising nowadays. For example, several DASIs based on anastrozole, letrozole, and vorozole templates inhibit both STS and aromatase in nanomolar concentrations, being nonestrogenic; and have a chance to be introduced in clinical trials.

Among STS inhibitors based on steroid skeleton 17α -benzyl-derivatives, 17β -arylsulfonamides, and 17-diisopropylcarbomoyl-3-O-sulfamates exhibit the best properties, especially as multitargeted (dual) anticancer potential drugs. The same modifications result in the increased activity against STS in the case of 2-OMe-3-O-sulfamates as well as 2-OMe-3, 17β -bissulfamates. The latter also possess activity against most aggressive form—triple negative BC.

Additionally, 8α -steroid estrogen analogs without estrogenic properties possess high STS activity and block BC cells growth with the activity comparable to standard of care for BC treatment tamoxifen.

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Conflict of interest

The authors confirm that this article content has no conflicts of interest.

Abbreviations

AIs	aromatase inhibitors
BC	breast cancer
COMT	catechol-O-methyltransferase
Coumate	4-methylcoumarin-7-O-sulfamate
DASI	dual aromatase-sulfatase inhibitor
DHEAS	dehydroepiandrosterone sulfate
FGly	for-mylglycine
GP _{ER} , GPR30	G-protein-coupled estrogen receptor
HDBC	hormone-dependent breast cancer
17β HSD	17β -hydroxysteroid dehydrogenase
E1	estrone
E2	estradiol
E2MATE	estradiol-3-O-sulfamate
EMATE	estrone-3-O-sulfamate
ER	estrogen receptor
LH-RH	luteinizing hormone-releasing hormone
2-OHE1	2-hydroxyestrone
4-OHE1	4-hydroxyestrone

2-OHE2	2-hydroxyestradiol
4-OHE2	4-hydroxyestradiol
STS	steroid sulfatase
SERD	selective estrogen receptor down-regulators
SERM	selective estrogen receptors modulator
UGT	UDP-glucuronosyltransferase

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