We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

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 (ERα) [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 17β-hydroxysteroid dehydrogenase type 1 (17β-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 17β-HSD1 leads to more active E2. Importantly, almost insoluble in aqueous media E1 is converted into water-soluble E1S under the action of

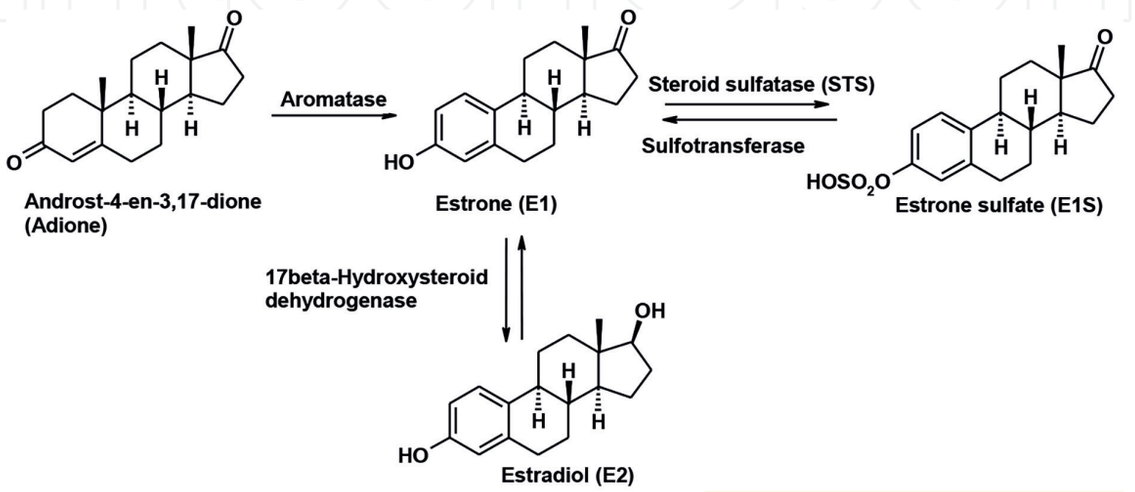


Figure 1.
Estrogens formation in human body.

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 ERα 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 HDBC. 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 ERα 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 thromboembolism [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 17β-HSD1 have been identified as essential parts in E2 production and subsequent promotion of cancer growth. Recently it was shown that 17β-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].

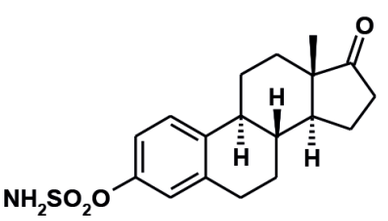
2.3 Steroid sulfatase enzyme (STS)

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 ERα-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 ($ArOSO_2NH_2$) 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 μM and has an IC_{50} value of 65 pM (IC_{50} = 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].



estrone-3-O-sulfamate (EMATE)

Despite the exceptional potency of the EMATE [67, 68], it is not used in clinical practice to treat hormone-dependent BC because metabolic conversion of EMATE by STS releases estrone, which act via estrogen receptors, and can directly promote tumor growth [69]. Nevertheless, EMATE is now the prototypical inhibitor, and used as standard during evaluation of other potential STS inhibitors [19].

2.4 Mechanisms of inactivation of steroid sulfatase

Several research groups made attempts to establish the mechanism(s) of sulfatase inactivation. However, the precise mechanism of inhibition is still uncertain. In 2010, Spillane and Malaubier have established that the hydrolysis of EMATE occurs by two different mechanisms: an SN2 mechanism below pH 9.5 and E1cB mechanisms involving N-sulfonylamines at higher pHs [70]. Detailed presumable mechanisms have been discussed in recent reviews [71–73].

Based on the mechanisms, the result of the hydrolysis is free estrone. Moreover, under per os administration, the activity of EMATE is several times higher than the activity of estrone, due slow metabolism of EMATE in liver [68]. EMATE is not subjected to metabolic inactivation in red blood cells. Thus, consideration of hormonal activity and side-effects of steroids with free phenolic group is important in the modeling of sulfatase inhibitors for therapeutic use [54, 74, 75].

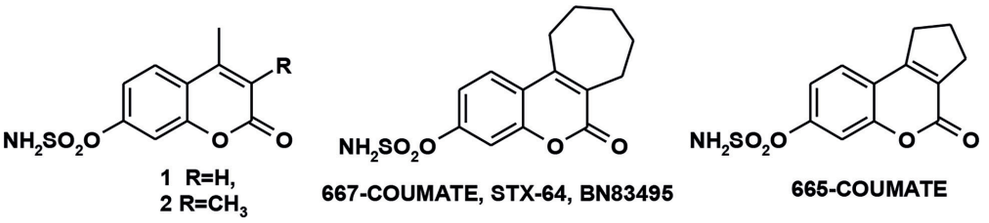
The knowledge of the crystal structure opens the rational drug design of molecules for the inactivation of steroid sulfatase.

2.5 Nonsteroidal STS inhibitors

Many investigations have been carried out to develop nonsteroidal STS inhibitors, because nonsteroidal drugs and their metabolites may have less undesirable effects.

4-Methylcoumarin-7-O-sulfamate (**1**, Coumate) was the first time- and concentration-dependent STS inhibitor (IC_{50} = 380 nM) in oral dose 10 mg/kg/day, and *in vivo* has no estrogenic activity. 3,4-Dimethylcoumarin-7-O-sulfamate (**2**) was a more potent inhibitor (IC_{50} = 30 nM) [76].

A search of an orally active, nonestrogenic, nonsteroidal STS inhibitors among tricyclic compounds based around the coumarin core resulted in the discovery of Irosustat (667-coumate, STX64, BN83495) [77], which is the first-in-class irreversible time- and concentration-dependent STS inhibitor for the treatment of hormone-dependent BC in postmenopausal women that has been clinically evaluated in breast, endometrial, and prostate cancers [77] and there is potential for innovative dual-targeting approaches [78, 79], with an IC_{50} value of 8 nM in placental microsomes. The inhibitor (**2**) does not possess any estrogenic activity in *in vitro* and *in vivo* assays [80].

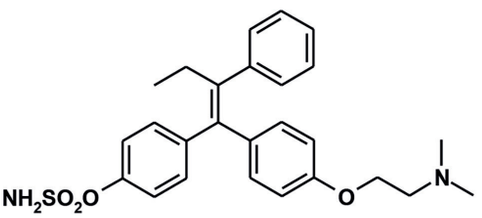


The optimum dose of 40 mg/day was estimated in phase I/II trials [81]. Efficiency of Irosustat has also been demonstrated in a phase II study in (ER+) endometrial cancer in women with advanced or recurrent disease [82]. The high bioavailability of Irosustat is explained by the prevention of degradation by sequestration

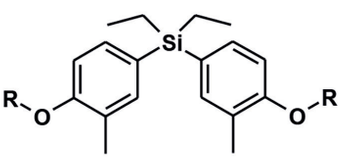
inside red blood cells where it, similarly to EMATE, binds to (and inhibits) carbonic anhydrase II (IC_{50} = 22 nM) [83]. The inactivation mechanism suggests that a sulfamate group is transferred to the gem-diol form of formylglycine 75 of steroid sulfatase due to a facile E1cB elimination of sulfamate 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 megesterol 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 Ki = 35.9 μM.



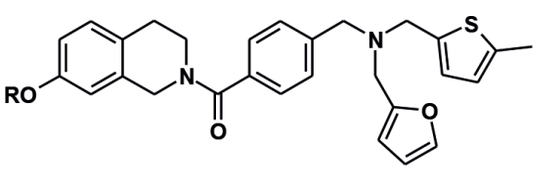
3



4 R = SO_2NH_2
5 R = H

Surprisingly, among silicon-containing derivatives compound **4** exhibits strong STS-inhibitory activity (IC_{50} = 0.17 μM). Furthermore, its metabolite **5** possesses potent ERα-antagonistic activity (IC_{50} = 29.7 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].



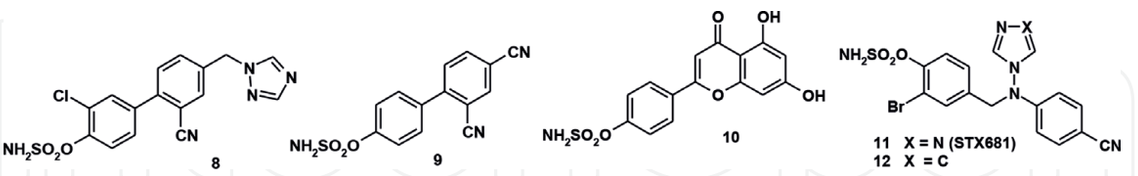
6 R = SO_2NH_2
7 R = H

2.5.2 Dual aromatase/STS inhibitors

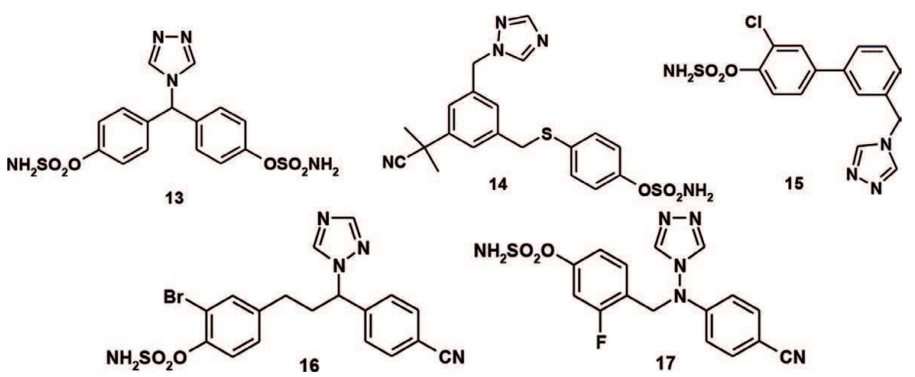
Another approach for the treatment of hormone-dependent BC is the development of DASIs, which may have an additive or synergistic antitumor effect. The potential advantages of a single chemical agent with the ability to interact with

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 μM [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 anastrazole 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 μM) [103]. When orally dosed, compound **15** reduces plasma estradiol levels and inhibits liver STS activity [103].

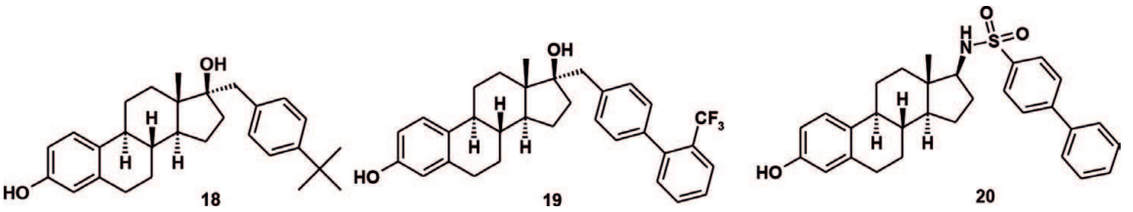
Potter with coauthors published the successful realization of the strategy when the core components of the two leading DASIs resulted in the hybrid structures that exhibit a very high level of dual inhibition against aromatase and STS *in vitro* (IC_{50} = 0.015–0.75 nM). Most active compound is analog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

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

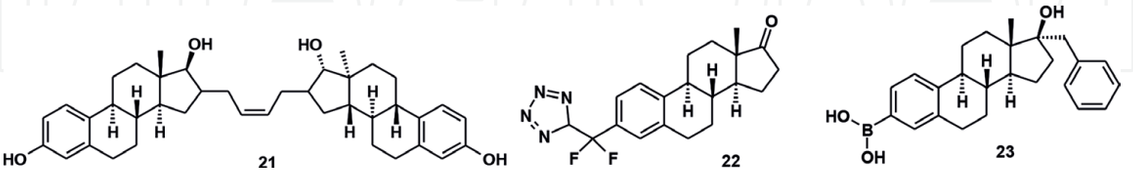
2.6 Steroidal STS inhibitors

2.6.1 Steroid-based STS inhibitors without sulfamate group

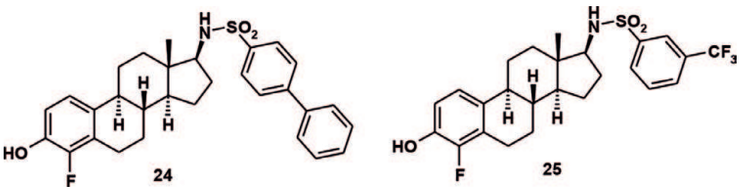
Nonsulfamated STS inhibitors based on estrogens are weaker than EMATE. Most active STS inhibitors without sulfamate group with highest activity are represented by compounds **18**, **19**, and **20** (IC_{50} = 12, 21, and 9, respectively) [107–109].



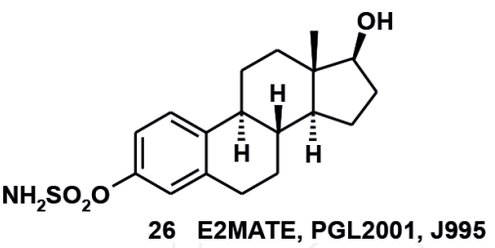
Estradiol dimer **21** also exhibits STS inhibitory activity in nanomolar range [110]. STS inhibitors are exemplified by tetrazole derivative **22** and boronic derivative **23** [111–113].



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 Ki app values of 1 and 2.5 nM [114].

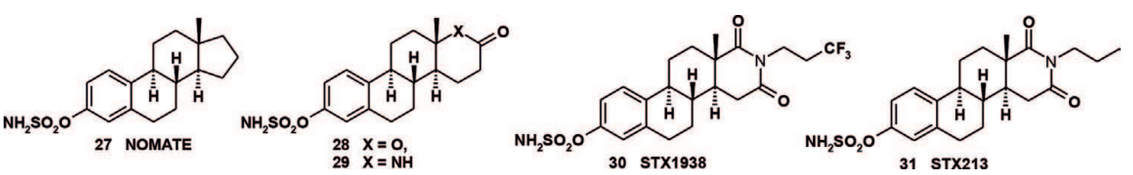


2.6.2 Steroid-based STS inhibitors with sulfamate group



26 E2MATE, PGL2001, J995

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].



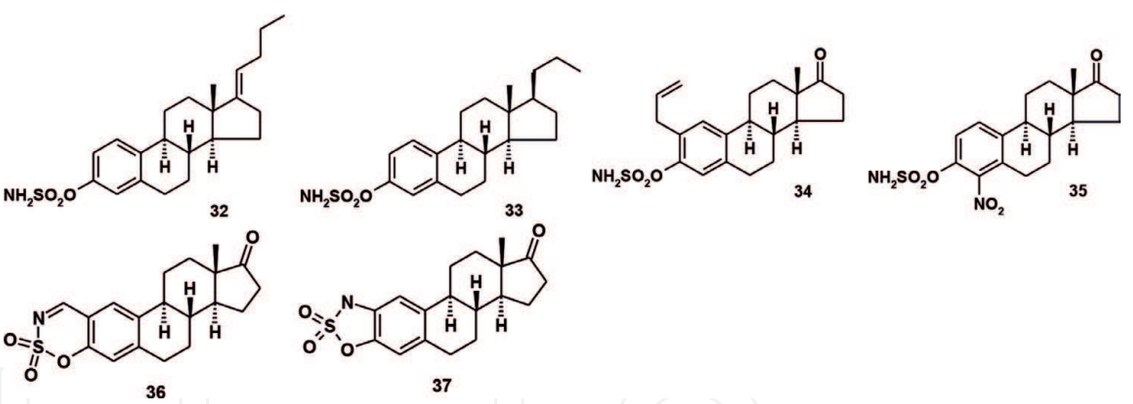
The sulfamate **27** (NOMATE) was evaluated as an STS inhibitor. This steroid without the 17-carbonyl group possesses ablated estrogenicity as well as reduced

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 μM, 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 lipophility 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].



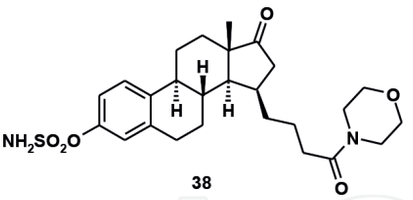
Among various 2- and 4-substituted and 2,4-disubstituted EMATE derivatives, most active compounds are 2-(2-prop-2-enyl)-EMATE (**34**, IC_{50} = 37 nM in MCF-7 cells) [126]; and 4-nitro-EMATE (**35**, IC_{50} = 0.01 nM in MCF-7 cells) (EMATE; IC_{50} = 0.83 nM in MCF-7 cells), and steroid **34** is nonestrogenic [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 17β-HSD1/STS inhibitors

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

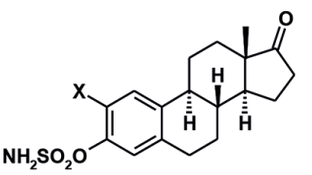
inhibitors of 17β-HSD and STS for the treatment of steroid hormone-dependent diseases are patented [135]. The example of such inhibitors is represented by the compound **38**.



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].



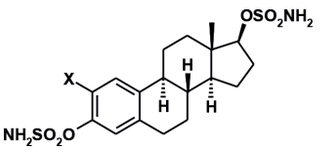
39 X = OMe (2-MeOEMATE)
40 X = Et

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]. 3,17β-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].

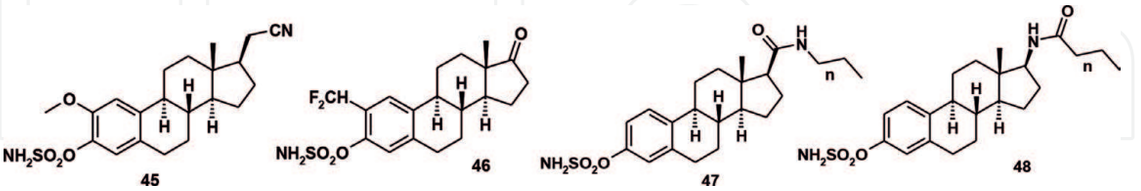


41 X = H
42 X = MeO (STX140)
43 X = MeS
44 X = Et (STX243)

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-3,17β-OO-bis(sulfamoyl)estradiol has been proposed as a new potential PET agent for imaging of steroid sulfatase in cancers [157].

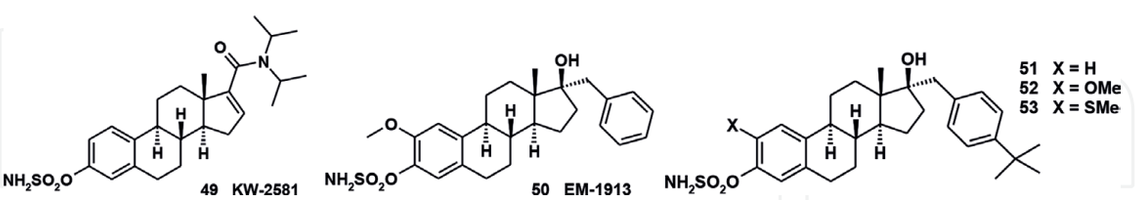
One more example of 2-MeO-derivatives as effective STS inhibitors is illustrated by compound **45** containing cyano group at position C-17 [158].



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

The level of STS inhibition for 17β-(N-alkylcarbamoyl)-estra-1,3,5(10)-trien-3-O-sulfamates (**47**) and 17β-(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 (IC_{50} = 4 nM) [161]. It was also demonstrated that the compound inhibits the ability of androstanediol-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 17α-alkylated estradiol sulfamates as STS inhibitors have been patented [163] and 17α-benzyl-derivatives have been investigated [164].

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

17α-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 IC_{50} = 0.04 nM. The compound without the tert-butyl group is nonestrogenic and effective STS inhibitor *in vivo* [167].

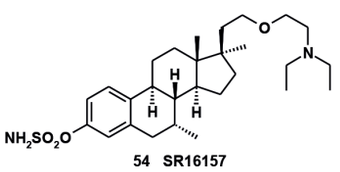
In the series of A-ring thioether-modified sulfamates, the steroid **53** is 50-fold more potent inhibitor of STS than steroid **52**; however, it possesses weak inhibitory activity against MCF-7 cells proliferation (IC_{50} = 10 μM) [168].

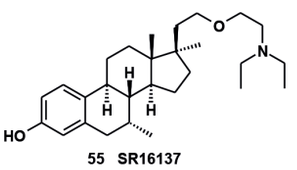
2.6.4 Dual STS/SERM inhibitors

Maximum estrogen blockade in the treatment of (ER+) BC may be achieved using dual ERα 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 μM) 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 ERα 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-alpha-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 dehydroepiandro-sterone-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 anastrazole, 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-diisopro-pylcarbomoyl-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.

Acknowledgements

The authors greatly appreciate Dr. Anna S. Chentsova for careful reading of the manuscript and her valuable comments. The reported study was funded by RFBR according to the Research Project No. 16-54-76024.

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
GPER, GPR30	G-protein-coupled estrogen receptor
HDBC	hormone-dependent breast cancer
17βHSD	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

Author details

Svetlana N. Morozkina[1,2*] and Alexander G. Shavva[1,2]

1 Laboratory of Medicinal Chemistry, School of Biomedicine, Far Eastern Federal University, Vladivostok, Russia

2 ITMO University, Russia

*Address all correspondence to: morozkina.svetlana@gmail.com

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;**68**(6):394-428

[2] Henderson BE, Ross R, Bernstein L. Estrogens as a cause of a human cancer: The Richard and Hinda Rosenthal Foundation award lecture. Cancer Research. 1988;**48**(2):246-253

[3] Lippman ME, Dickson RB, Bates S, Knabbe C, Huff K, Swain S, et al. Autocrine and paracrine growth regulation of human breast cancer. Breast Cancer Research and Treatment. 1986;**7**:59-70

[4] Fillmore CM, Gupta PB, Rudnick JA, Caballero S, Keller PJ, Lander ES, et al. Estrogen expands breast cancer stem-like cells through paracrine FGF/Tbx3. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(50):21737-21742

[5] Purohit A, Reed MJ. Regulation of estrogen synthesis in postmenopausal women. Steroids. 2002;**67**(12):979-983

[6] Epstein FH. Estrogens and the risk of breast cancer. The New England Journal of Medicine. 2001;**344**(4):276-285

[7] Liehr JG. Is estradiol a genotoxic mutagenic carcinogen? Endocrine Reviews. 2000;**21**(1):40-54

[8] Nishimura R, Anan K, Yamamoto Y, Higaki K, Tanaka M, Shibuta K, et al. Efficacy of goserelin plus anastrozole in premenopausal women with advanced or recurrent breast cancer refractory to an LH-RH analogue with tamoxifen: Results of the JMTO BC08-01 phase II trial. Oncology Reports. 2013;**29**(5):1707-1713

[9] Ghayee HK, Auchus RJ. Basic concepts and recent developments in human steroid hormone biosynthesis. Reviews in Endocrine & Metabolic Disorders. 2007;**8**(4):289-300

[10] Lin SX, Chen J, Mazumdar M, Poirier D, Wang C, Azzi A, et al. Molecular therapy of breast cancer: Progress and future directions. Nature Reviews. Endocrinology. 2010;**6**(9):485-493

[11] Labrie F. All sex steroids are made intracellularly in peripheral tissues by the mechanisms of intracrinology after menopause. The Journal of Steroid Biochemistry and Molecular Biology. 2015;**145**:133-138

[12] Lepine J, Audet-Walsh E, Gregoire J, Têtu B, Plante M, Ménard V, et al. Circulating estrogens in endometrial cancer cases and their relationship with tissular expression of key estrogen biosynthesis and metabolic pathways. The Journal of Clinical Endocrinology and Metabolism. 2010;**95**(6):2689-2698

[13] Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. The Journal of Steroid Biochemistry and Molecular Biology. 2000;**72**(1-2):23-27

[14] Sasano H, Miki Y, Nagasaki S, Suzuki T. In situ estrogen production and its regulation in human breast carcinoma: From endocrinology to intracrinology. Pathology International. 2009;**59**(11):777-789

[15] Brueggemeier RW, Richards JA, Joomprabutra S, Bhat AS, Whetstone JL. Molecular pharmacology of aromatase and its regulation by endogenous and exogenous agents. The Journal of

Steroid Biochemistry and Molecular Biology. 2001;**79**(1-5):75-84

[16] Poirier D. Recent patents on new steroid agents targeting the steroidogenesis for endocrine cancer treatments. Recent Patents on Endocrine, Metabolic & Immune Drug Discovery. 2015;**9**(1):15-23

[17] Altundag K, Ibrahim NK. Aromatase inhibitors in breast cancer: An overview. The Oncologist. 2006;**11**(6):553-562

[18] Thomas MP, Potter BVL. The structure biology of estrogen metabolites. The Journal of Steroid Biochemistry and Molecular Biology. 2013;**137**:27-49

[19] Shah R, Singh J, Singh D, Jaggi AS, Singh N. Sulfatase inhibitors for recidivist breast cancer treatment: A chemical review. European Journal of Medicinal Chemistry. 2016;**114**:170-190

[20] Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocrine Reviews. 2011;**32**(1):81-151

[21] Thomas MP, Potter BVL. Estrogen O-sulfamates and their analogues: Clinical steroid sulfatase inhibitors with broad potential. The Journal of Steroid Biochemistry and Molecular Biology. 2015;**153**:160-169

[22] Avendano C, Menendez JC. Chapter 3: Anticancer drugs that modulate hormone action. Medicinal Chemistry of Anticancer Drugs. 2nd ed. Elsevier Science; 2015. pp. 82-129

[23] Piccinato CA, Neme RM, Torres N, Sanches LR, Derogis PBMC, Brudniewski HF, et al. Effects of steroid hormone on estrogen sulfotransferase and on steroid sulfatase expression in endometriosis tissue and stromal cells. The Journal of Steroid Biochemistry and Molecular Biology. 2016;**158**:117-126

[24] Rizner TL. Estrogen biosynthesis, phase I and phase II metabolism, and action in endometrial cancer. Molecular and Cellular Endocrinology. 2013;**381**(1-2):124-139

[25] Auchus RJ. Chapter 8: Human steroid biosynthesis. In: Knobil and Neill's Physiology of Reproduction. 4th ed. Academic Press; 2015. pp. 295-312

[26] Strauss JF III. Yen & Jaffe's Reproductive Endocrinology Physiology, Pathophysiology, and Clinical Management. 6th ed. Elsevier; 2009. p. 892

[27] Geyer J, Bakhaus K, Bernhardt R, Blaschka C, Dezhkam Y, Fietz D, et al. The role of sulfated steroid hormones in reproductive processes. The Journal of Steroid Biochemistry and Molecular Biology. 2017;**172**:207-221

[28] Hong Y, Chen S. Aromatase, estrone sulfatase, and 17β-HSD: Structure-function studies and inhibitor development. Molecular and Cellular Endocrinology. 2011;**340**(2):120-126

[29] Kulendran M, Salhab M, Mokbel K. Oestrogen-synthesising enzymes and breast cancer. Anticancer Research. 2009;**9**(4):1095-1109

[30] Giton F, de la Taille A, Allory Y, Galons H, Vacherot F, Soyeux P, et al. Estrone sulfate (E1S), a prognosis marker for tumor aggressiveness in prostate cancer. The Journal of Steroid Biochemistry and Molecular Biology. 2008;**109**(1-2):158-167

[31] International Breast Cancer Study Group. Toremifene and tamoxifen are equally effective for early-stage breast cancer: First results of International Breast Cancer Study Group Trials 12-93 and 14-93. Annals of Oncology. 2004;**15**(12):1749-1759

[32] Gennari L, Merlotti D, Paola VD, Nuti R. Raloxifene in breast cancer

prevention. Expert Opinion on Drug Safety. 2008;**7**(3):259-270

[33] Wardell SE, Nelson ER, Chao CA, McDonnell DP. Bazedoxifene exhibits antiestrogenic activity in animal models of tamoxifen-resistant breast cancer: Implications for treatment of advanced disease. Clinical Cancer Research. 2013;**19**(9):2420-2431

[34] LaCroix AZ, Powles T, Osborne CK, Wolter K, Thompson JR, Thompson DD, et al. Breast cancer incidence in the randomized PEARL trial of lasofoxifene in postmenopausal osteoporotic women. Journal of the National Cancer Institute. 2010;**102**(22):1706-1715

[35] Croxtall JD, McKeage K. Fulvestrant. Drugs. 2011;**71**(3):363-380

[36] McDonnell DP, Wardell SE. The molecular mechanisms underlying the pharmacological actions of ER modulators: Implications for new drug discovery in breast cancer. Current Opinion in Pharmacology. 2010;**10**:620-628

[37] Barrios C, Forbes JF, Jonat W, Conte P, Gradishar W, Buzdar A, et al. The sequential use of endocrine treatment for advanced breast cancer: Where are we? Annals of Oncology. 2012;**23**(6):1378-1386

[38] Duggan C, Marriott K, Edwards R, Cuzick J. Inherited and acquired risk factors for venous thromboembolic disease among women taking tamoxifen to prevent breast cancer. Journal of Clinical Oncology. 2003;**21**(19):3588-3593

[39] Obiorah I, Jordan VC. Progress in endocrine approaches to the treatment and prevention of breast cancer. Maturitas. 2011;**70**(4):315-321

[40] Rajapaksa G, Thomas C, Gustafsson J-A. Estrogen signaling and unfolded protein response in breast cancer. The Journal of Steroid Biochemistry and Molecular Biology. 2016;**163**:45-50

[41] Woo LWL. Enzyme inhibitors examples for the treatment of breast cancer. In: Smith HJ, Simons C, editors. Enzymes and Their Inhibition: Drug Development. Boca Raton: CRC Press LLC; 2005. pp. 221-241

[42] Johnson SRD, Martin L-A, Alex L, Heada J, Dowsett M. Clinical strategies for rational combinations of aromatase inhibitors with novel therapies for breast cancer. The Journal of Steroid Biochemistry and Molecular Biology. 2007;**106**(1-5):180-186

[43] Hong Y, Cho M, Yuan Y-C, Chen S. Molecular basis for the interaction of four different classes of substrates and inhibitors with human aromatase. Biochemical Pharmacology. 2008;**75**(5):1161-1169

[44] Dent SF, Gaspo R, Kissner M, Pritchard KI. Aromatase inhibitor therapy: Toxicities and management strategies in the treatment of postmenopausal women with hormone-sensitive early breast cancer. Breast Cancer Research and Treatment. 2011;**126**(2):295-310

[45] Higuchi T, Endo M, Hanamura T, Gohno T, Niwa T, Yamaguchi Y, et al. Contribution of estrone sulfate to cell proliferation in aromatase inhibitor (AI)-resistant, hormone receptor-positive breast cancer. PLoS One. 2016;**11**(5):e0155844

[46] Janicke F. Are all aromatase inhibitors the same? A review of the current evidence. Breast. 2004;**13**(1):S10-S18

[47] Coombes RC, Hall E, Gibson LJ, Paridaens R, Jassem J, Delozier T, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. New England Journal of Medicine. 2004;**350**(11):1081-1092

[48] Hanamura T, Niwa T, Gohno T, Kurosumi M, Takei H, Yamaguchi Y, et al. Possible role of the aromatase-independent steroid metabolism pathways in hormone responsive primary breast cancers. Breast Cancer Research and Treatment. 2014;**143**(1):69-80

[49] Haynes BP, Straume AH, Geisler J, A'Hern R, Helle H, Smith IE, et al. Intratumoral estrogen disposition in breast cancer. Clinical Cancer Research. 2010;**16**(6):1790-1801

[50] McNamara KM, Sasano H. The intracrinology of breast cancer. The Journal of Steroid Biochemistry and Molecular Biology. 2015;**145C**:172-178

[51] Stanway SJ, Delavault P, Purohit A, Woo LW, Thirieau C, Potter BVL, et al. Steroid sulfatase: A new target for the endocrine therapy of breast cancer. The Oncologist. 2007;**12**:370-374

[52] Chanplakhorn N, Chanplakhorn P, Suzuki T, Ono K, Chan MSM, Miki Y, et al. Increased estrogen sulfatase (STS) and 17β-hydroxysteroid dehydrogenase type 1(17b-HSD1) following neoadjuvant aromatase inhibitor therapy in breast cancer patients. Breast Cancer Research and Treatment. 2010;**120**(3):639-648

[53] Woo LWL, Purohit A, Potter BVL. Development of steroid sulfatase inhibitors. Molecular and Cellular Endocrinology. 2011;**340**(2):175-185

[54] Reed MJ, Purohit A, Woo LWL, Newman SP, Potter BVL. Steroid sulfatase: Molecular biology, regulation, and inhibition. Endocrine Reviews. 2005;**26**(2):171-202

[55] Poulin R, Labrie F. Stimulation of cell proliferation and estrogenic response by adrenal C19-delta 5-steroids in the ZR-75-1 human breast cancer cell line. Cancer Research. 1986;**46**(10):4933-4937

[56] Lasley BL, Chen J, Stanczyk FZ, El Khodary SR, Gee NA, Crawford S, et al. Androstenediol complements estrogenic bioactivity during the menopausal transition. Menopause. 2012;**19**(6):650-657

[57] Rižner TL. The important roles of steroid sulfatase and sulfotransferases in gynecological diseases. Frontiers in Pharmacology. 2016;**7**(30):1-16

[58] Elger W, Laehteenmaeki P, Lehtinen M, Reddersen G, Zimmermann H, Oettel M, Schwarz S. Use of biogenic estrogen sulfamates for hormone replacement therapy. PCT Int. Appl. WO0006175; 2000

[59] Potter BVL, Reed MJ, Lebrond B, Leese MP. 17-Aryl linker derivatised estrogen 3-sulfamates as inhibitors of steroid sulfatase. U.S. Pat. Appl. US7067503; 2006

[60] Pautier P, Vergote I, Joly F, Melichar B, Kutarska E, Hall G, et al. A phase 2, randomized, open-label study of Irosustat versus megestrol acetate in advanced endometrial cancer. International Journal of Gynecological Cancer. 2017;**27**(2):258-266

[61] Secky L, Svoboda M, Klameth L, Bajna E, Hamilton G, Zeillinger R, et al. The sulfatase pathway for estrogen formation: Targets for the treatment and diagnosis of hormone-associated tumors. Journal of Drug Delivery. 2013;**957605**:1-13

[62] Pasqualini JR, Chetrite GS. Recent insight on the control of enzymes involved in estrogen formation and transformation in human breast cancer. The Journal of Steroid Biochemistry and Molecular Biology. 2005;**93**(2-5):221-236

[63] Suzuki T, Nakata T, Miki Y, Kaneko C, Moriya T, Ishida T, et al. Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. Cancer Research. 2003;**63**(11):2762-2770

[64] Geisler J, Sasano H, Chen S, Purohit A. Steroid sulfatase inhibitors: Promising new tools for breast cancer therapy? The Journal of Steroid Biochemistry and Molecular Biology. 2011;**125**(1-2):39-45

[65] Maltais R, Poirier D. Steroid sulfatase inhibitors: A review covering the promising 2000-2010 decade. Steroids. 2011;**76**(10-11):929-948

[66] Ghosh D. Human sulfatases: A structural perspective to catalysis. Cellular and Molecular Life Sciences. 2007;**64**(15):2013-2022

[67] Elger W, Barth A, Hedden A, Reddersen G, Ritter P, Schneider PB, et al. Estrogen sulfamates: A new approach to oral estrogen therapy. Reproduction, Fertility, and Development. 2001;**13**(4):297-305

[68] Hidalgo Aragones MI, Purohit A, Parish D, Sahm UG, Pouton CW, Potter BVL, et al. Pharmacokinetics of oestrone-3-O-sulphamate. The Journal of Steroid Biochemistry and Molecular Biology. 1996;**58**(5-6):611-617

[69] Woo LWL, Leblond B, Purohit A, Potter BVL. Synthesis and evaluation of analogues of estrone-3-O-sulfamate as potent sulfatase inhibitors. Bioorganic & Medicinal Chemistry. 2012;**20**(8):2506-2519

[70] Spillane WJ, Malaubier J-B. Mechanism of the hydrolysis of the sulfamate EMATE—An irreversible steroid sulfatase inhibitor. Tetrahedron Letters. 2010;**51**(15):2059-2062

[71] Mostafa YA, Taylor SD. Steroid derivatives as inhibitors of steroid sulfatase. The Journal of Steroid Biochemistry and Molecular Biology. 2013;**137**:183-198

[72] Williams SJ. Sulfatase inhibitors: A patent review. Expert Opinion on Therapeutic Patents. 2013;**23**(1):79-98

[73] Williams SJ, Denehy E, Krenske EH. Experimental and theoretical insights into the mechanisms of sulfate and sulfamate ester hydrolysis and the end products of type I sulfatase inactivation by aryl sulfamates. The Journal of Organic Chemistry. 2014;**79**(5):1995-2005

[74] Foster PA, Reed MJ, Purohit A. Recent developments of steroid sulfatase inhibitors as anti-cancer agents. Anti-Cancer Agents in Medicinal Chemistry. 2008;**8**(7):732-738

[75] Day JM, Purohit A, Tutill HJ, Foster PA, Woo LW, Potter BVL, et al. The development of steroid sulfatase inhibitors for hormone-dependent cancer therapy. Annals of the New York Academy of Sciences. 2009;**1155**:80-87

[76] Woo LWL, Fischer DS, Sharland CM, Trusselle M, Foster PA, Chander SK, et al. Anticancer steroid sulfatase inhibitors: Synthesis of a potent fluorinated second-generation agent, in vitro and in vivo activities, molecular modeling, and protein crystallography. Molecular Cancer Therapeutics. 2008;**7**(8):2435-2444

[77] Denmeade S, George D, Liu G, Peraire C, Geniaux A, Baton F, et al. A phase vertical bar pharmacodynamic dose escalation study of steroid sulphatase inhibitor Irosustat in patients with prostate cancer. European Journal of Cancer. 2011;**47**(Suppl. 1):S499

[78] Stanway SJ, Purohit A, Woo LWL, Sufi S, Vigushin D, Ward R, et al. Phase I study of STX 64 (667 Coumate) in breast cancer patients: The first study of a steroid sulfatase inhibitor. Clinical Cancer Research. 2006;**12**(5):1585-1592

[79] Lund MJ, Butler EN, Hair BY, Ward KC, Andrews JH, Oprea-Ilies G, et al. Age/race differences in HER2 testing and in incidence rates for breast cancer triple subtypes: A population-based study and first report. Cancer. 2010;**116**(11):2549-2559

[80] Coombes R, Schmid P, Isambert N, Soulie P, Cardoso F, Besse-Hammer T, et al. Phase I dose escalation study of steroid sulfatase inhibitor BN83495/STX64 in postmenopausal women with ER positive breast cancer. Cancer Research. 2009;**69**(24 Suppl):4097-4097

[81] Coombes RC, Cardoso F, Isambert N, Lesimple T, Soulié P, Peraire C, et al. A phase I dose escalation study to determine the optimal biological dose of irosustat, an oral sulfatase inhibitor, in postmenopausal women with estrogen receptor-positive breast cancer. Breast Cancer Research and Treatment. 2013;**140**(1):73-82

[82] Pautier P, Lobbedez FJ, Melichar B, Kutarska E, Hall G, Reed N. A phase II multicenter randomized open-label study of oral sulfatase (sulfatase) inhibitor Irosustat (BN83495) versus megestrol acetate (MA) in women with advanced/recurrent endometrial cancer (EC). Annals of Oncology. 2012;**23**(Suppl. 9):329

[83] Lloyd MD, Thiyagarajan N, Ho YT, Woo LWL, Sutcliffe OB, Purohit A, et al. First crystal structures of human carbonic anhydrase II in complex with dual aromatase-steroid sulfatase inhibitors. Biochemistry. 2005;**44**(18):6858-6866

[84] Woo LWL, Ganeshapillai D, Thomas MP, Sutcliffe OB, Malini B, Mahon MF, et al. Structure-activity relationship for the first-in-class clinical sulfatase inhibitor Irosustat (STX64, BN83495). ChemMedChem. 2011;**6**:2019-2034

[85] Palmieri C, Stein RC, Liu X, Hudson E, Nicholas H, Sasano H, et al. IRIS study: A phase II study of the steroid sulfatase inhibitor Irosustat when added to an aromatase inhibitor in ER-positive breast cancer patients. Breast Cancer Research and Treatment. 2017;**165**(2):343-353

[86] Parra-Guillen ZP, Cendros Carreras JP, Peraire C, Obach R, Prunynosa J, Chetaille E, et al. Population pharmacokinetic modelling of Irosustat in postmenopausal women with oestrogen-receptor positive breast cancer incorporating nonlinear red blood cell uptake. Pharmaceutical Research. 2015;**32**(4):1493-1504

[87] Li P-K, Selcer KW. Compounds for the treatment of estrogen-dependent illnesses and methods for making and using the same. U.S. Pat. Appl. US6288107; 2001

[88] Komm BS, Mirkin S. An overview of current and emerging SERMs. The Journal of Steroid Biochemistry and Molecular Biology. 2014;**143**:207-222

[89] Kajita D, Nakamura M, Matsumoto Y, Makishima M, Hashimoto Y. Design and synthesis of silicon-containing steroid sulfatase inhibitors possessing pro-estrogen antagonistic character. Bioorganic & Medicinal Chemistry. 2014;**22**(7):2244-2252

[90] Ouellet C, Ouellet E, Poirier D. In vitro evaluation of a tetrahydroisoquinoline derivative as a steroid sulfatase inhibitor and a selective estrogen receptor modulator. Investigational New Drugs. 2014;**33**(1):95-103

[91] Ouellet C, Maltais R, Ouellet E, Barbeau X, Lagüe P, Poirier D. Discovery of a sulfamate-based steroid sulfatase inhibitor with intrinsic selective estrogen receptor modulator properties. European Journal of Medicinal Chemistry. 2016;**119**:169-182

[92] Zimmermann GR, Lehar J, Keith CT. Multi-target therapeutics: When the whole is greater than the sum of the parts. Drug Discovery Today. 2007;**12**(1/2):34-42

[93] Purohit A, Foster PA. Steroid sulfatase inhibitors for estrogen- and

androgen-dependent cancers. The Journal of Endocrinology. 2012;**212**(2):99-110

[94] Woo LWL, Jackson T, Purohit A, Reed MJ, Potter BVL, Reed G. Compound. U.S. Pat. Appl. US20100173963; 2010

[95] Ahmad I, Shagufta. Recent developments in steroidal and nonsteroidal aromatase inhibitors for the chemoprevention of estrogen-dependent breast cancer. European Journal of Medicinal Chemistry. 2015;**102**:375-386

[96] Saito T, Kinoshita S, Fujii T, Bandoh K, Fuse S, Yamauchi Y, et al. Development of novel steroid sulfatase inhibitors: II. TZS-8478 potently inhibits the growth of breast tumors in postmenopausal breast cancer model rats. The Journal of Steroid Biochemistry and Molecular Biology. 2004;**88**(2):167-173

[97] Reed MJ, Potter BVL. Compounds that inhibit oestrone sulphatase and/or aromatase and methods for making and using. U.S. Pat. Appl. US6506792; 2003

[98] Potter BVL, Reed MJ, Woo LWL, Purohit A, Burbert Ch, Wood PM, Sutcliffe OB. Sulfamic acid ester compounds useful in the inhibition of steroid sulphatase activity and aromatase activity. U.S. Pat. Appl. US20070213383; 2007

[99] Bubert C, Woo LWL, Sutcliffe OB, Mahon MF, Chander SK, Purohit A, et al. Synthesis of aromatase inhibitors and dual aromatase steroid sulfatase inhibitors by linking an arylsulfamate motif to 4-(4H-1,2,4-triazol-4-ylamino) benzonitrile: SAR, crystal structures, in vitro and in vivo activities. ChemMedChem. 2008;**3**(11):1708-1730

[100] Lafay J, Rondot B, Bonnet P, Clerc Th, Shields J, Duc I, Duranti E, Puccio F, Blot Ch, Maillos Ph. 1-N-phenyl-amino-1h-imidazole derivatives and pharmaceutical compositions containing them. U.S. Pat. Appl. US20070112009; 2007

[101] Wood PM, Woo LWL, Labrosse JR, Trusselle MN, Abbate S, Longhi G, et al. Chiral aromatase and dual aromatase-steroid sulfatase inhibitors from the letrozole template: Synthesis, absolute configuration, and in vitro activity. Journal of Medicinal Chemistry. 2008;**51**(14):4226-4238

[102] Lawrence LVL, Jackson T, Bubert Ch, Purohit A, Reed MJ, Potter BVL. Phenyl-sulfamates as aromatase inhibitors. PCT Int. Appl. WO2005118560; 2005

[103] Wood PM, Woo LWL, Thomas MP, Mahon MF, Purohit A, Potter BVL. Aromatase and dual aromatase-steroid sulfatase inhibitors from the letrozole and vorozole templates. ChemMedChem. 2011;**6**(8):1423-1438

[104] Woo LW, Bubert C, Purohit A, Potter BVL. Hybrid dual aromatase-steroid sulfatase inhibitors with exquisite picomolar inhibitory activity. ACS Medicinal Chemistry Letters. 2011;**2**(3):243-247

[105] Gobbi S, Rampa A, Belluti F, Bisi A. Nonsteroidal aromatase inhibitors for the treatment of breast cancer: An update. Anti-Cancer Agents in Medicinal Chemistry. 2014;**14**(1):54-65

[106] Kumler I, Knoop AS, Jessing CAR, Ejlertsen B, Nielsen DL. Review of hormone-based treatments in postmenopausal patients with advanced breast cancer focusing on aromatase inhibitors and fulvestrant. ESMO Open. 2016;**1**:e000062

[107] Maltais R, Fournier D, Poirier D. Quantitative structure-activity relationship (QSAR) study with a series of 17α-derivatives of estradiol: Model for the development of

reversible steroid sulfatase inhibitors. QSAR and Combinatorial Science. 2009;**28**(11-12):1284-1299

[108] Phan C-M, Liu Y, Kim B-M, Mostafa Y, Taylor SD. Inhibition of steroid sulfatase with 4-substituted estrone and estradiol derivatives. Bioorganic & Medicinal Chemistry. 2011;**19**(20):5999-6005

[109] Mostafa YA, Taylor SD. 17β-Arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol as highly potent inhibitors of steroid sulfatase. Bioorganic & Medicinal Chemistry. 2012;**20**(4):1535-1544

[110] Fournier D, Poirier D. Estradiol dimers as a new class of steroid sulfatase reversible inhibitors. Bioorganic & Medicinal Chemistry Letters. 2009;**19**(3):693-696

[111] Lapierre J, Ahmed V, Chen M-J, Ispahany M, Guillemette JG, Taylor SD. The difluoromethylene group as a replacement for the labile oxygen in steroid sulfates: A new approach to steroid sulfatase inhibitors. Bioorganic & Medicinal Chemistry Letters. 2004;**14**(1):151-155

[112] Ahmed V, Liu Y, Silvestro C, Taylor SD. Boronic acids as inhibitors of steroid sulfatase. Bioorganic & Medicinal Chemistry. 2006;**14**(24):8564-8573

[113] Ahmed V, Liu Y, Taylor SD. Multiple pathways for the irreversible inhibition of steroid sulfatase with quinone methide-generating suicide inhibitors. Chembiochem: A European Journal of Chemical Biology. 2009;**10**(9):1457-1461

[114] Mostafa YA, Kralt B, Rao PPN, Taylor SD. A-ring substituted 17β-arylsulfonamides of 17β-aminoestra-1,3,5(10)-trien-3-ol as highly potent reversible inhibitors of steroid sulfatase. Bioorganic & Medicinal Chemistry. 2015;**23**(17):5681-5692

[115] Loumaye E, Gotteland J-P. Treatment of oestrogen dependent conditions in pre-menopausal women. PCT Int. Appl. WO2009037539; 2009

[116] Pohl O, Bestel E, Gotteland J-P. Synergistic effects of E2MATE and norethindrone acetate on sulfatase inhibition: A randomized phase I proof-of-principle clinical study in women of reproductive age. Reproductive Sciences. 2014;**21**(10):1256-1265

[117] https://clinicaltrials.gov/ct2/show/NCT01631981

[118] Abbate F, Winum J-Y, Potter BVL, Casini A, Montero J-L, Scozzafava A, et al. Carbonic anhydrase inhibitors: X-ray crystallographic structure of the adduct of human isozyme II with EMATE, a dual inhibitor of carbonic anhydrases and steroid sulfatase. Bioorganic & Medicinal Chemistry Letters. 2004;**14**(1):231-234

[119] Hillisch A, Peters O, Gege Ch, Regenhardt W, Kosemund D, Siemeister G, et al. 2-Substituted estra-1,3,5(10)-triene-3-yl sulfamate with an anti-tumour action. U.S. Pat. Appl. US20090221841; 2009

[120] Reed MJ, Potter BVL. Steroid-3-O-sulphamate derivatives as inhibitors of oestrone sulphatase. PCT Int. Appl. WO9927935; 1999

[121] Potter BVL, Reed MJ. Use of compound in the manufacture of a pharmaceutical for in-hibiting steroid sulphatase and steroid dehydrogenase activity. WO0232409; 2002

[122] Potter BVL, Reed MJ, Woo LWL, Purohit A, Foster P. Steroidal compounds as steroid sulphatase inhibitors. U.S. Pat. Appl. US20090182000; 2009

[123] Fischer DS, Woo LWL, Mahon MF, Purohit A, Reed MJ, Potter BV. D-ring modified estrone derivatives as novel

potent inhibitors of steroid sulfatase. Bioorganic & Medicinal Chemistry. 2003;**11**(8):1685-1700

[124] Foster PA, Newman SP, Chander SK, Stengel C, Jhalli R, Woo LWL, et al. In vivo efficacy of STX213, a second-generation steroid sulfatase inhibitor, for hormone-dependent breast cancer therapy. Clinical Cancer Research. 2006;**12**(18):5543-5549

[125] Foster P, Chander S, Parsons M, Newman S, Woo L, Potter B. Efficacy of three potent steroid sulfatase inhibitors: Preclinical investigations for their use in the treatment of hormone-dependent breast cancer. Breast Cancer Research and Treatment. 2008;**111**(1):129-138

[126] Numazawa M, Tominaga T, Watari Y, Tada Y. Inhibition of estrone sulfatase by aromatase inhibitor-based estrogen 3-sulfamates. Steroids. 2006;**71**(5):371-379

[127] Tanabe M, Peters RH, Chao W-R, Shigeno K. Estrone sulfamate inhibitors of estrone sulfatase, and associated pharmaceutical compositions and methods of use. US6046186; 2000

[128] Tanabe M, Peters RH, Chao W-R, Shigeno K. Steroid inhibitors of estrone sulfatase and associated pharmaceutical compositions and methods of use. US5861388; 1999

[129] Li P-K, Murakata C, Akinaga Sh. Steroid sulfatase inhibitors and methods for making and using the same. U.S. Pat. Appl. US6376687; 2002

[130] Hanson SR, Whalen LJ, Wong C-H. Synthesis and evaluation of general mechanism-based inhibitors of sulfatases based on (difluoro) methyl phenyl sulfate and cyclic phenyl sulfamate motifs. Bioorganic & Medicinal Chemistry. 2006;**14**(24):8386-8395

[131] Peters RH, Chao WR, Sato B, Shigeno K, Zaveri NT, Tanabe M. Steroidal oxathiazine inhibitors of estrone sulfatase. Steroids. 2003;**68**(1):97-110

[132] Purohit A, Tutill HJ, Day JM, Chander SK, Lawrence HR, Allan GM, et al. The regulation and inhibition of 17β-hydroxysteroid dehydrogenase in breast cancer. Molecular and Cellular Biology. 2006;**248**(1-2):199-203

[133] Laplante Y, Cadot C, Fournier M-A, Poirier D. Estradiol and estrone C-16 derivatives as inhibitors of type 17β-hydroxysteroid dehydrogenase: blocking of ER+ breast cancer cell proliferation induced by estrone. Bioorganic & Medicinal Chemistry. 2008;**16**(7):1849-1860

[134] He W, Gauri M, Li T, Wang R, Lin S-X. Current knowledge of the multifunctional 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1). Gene. 2016;**588**(1):54-61

[135] Messinger J, Thole H-H, Husen B, Weske M, Koskimies P, Pirkkala L. 17SS-HSD1 and STS inhibitors. U.S. Pat. Appl. US20110021480; 2011

[136] Leese MP, Hejaz HAM, Mahon MF, Simone P, Newman SP, Purohit A, et al. A-ring-substituted estrogen-3-O-sulfamates: Potent multitargeted anticancer agents. Journal of Medicinal Chemistry. 2005;**48**(16):5243-5256

[137] Reed EJ, Woo LWL, Robinson JJ, Leblond B, Leese MP, Purohit A, et al. 2-Difluoromethyloestrone 3-O-sulphamate, a highly potent steroid sulphatase inhibitor. Biochemical and Biophysical Research Communications. 2004;**317**(1):169-175

[138] Reed MJ, Potter BVL. Compound. U.S. Pat. Appl. US20040127473; 2004

[139] Purohit A, Hejaz HAM, Walden L, MacCarthy-Morrogh L, Packham G, Potter BVL, et al. The effect of 2-methoxyoestrone-3-O-sulphamate

on the growth of breast cancer cells and induced mammary tumours. International Journal of Cancer. 2000;**85**(4):584-589

[140] Leese M, Purohit A, Reed M, Jourdan F, Potter BVL, Bubert Ch. Compound. U.S. Pat. Appl. US20070225256; 2007

[141] Potter BVL, Reed MJ, Packham GK, Leese MP. Thioether sulphamate steroids as steroid inhibitors and anti-cancer compounds. U.S. Pat. Appl. US20040009959; 2004

[142] Dahut WL, Lakhani NJ, Gulley JL, Arlen PM, Kohn EC, Kotz H, et al. Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. Cancer Biology & Therapy. 2006;**5**:22-27

[143] Kumar BS, Raghuvanshi DS, Hasanain M, Alam S, Sarkar J, Mitra K, et al. Recent advances in chemistry and pharmacology of 2-methoxyestradiol: An anticancer investigational drug. Steroids. 2016;**110**:9-34

[144] Machado-Linde F, Pelegrin P, Sanchez-Ferrer ML, Leon J, Cascales P, Parrilla JJ. 2-Methoxyestradiol in the pathophysiology of endometriosis: Focus on angiogenesis and therapeutic potential. Reproductive Sciences. 2012;**19**(10):1018-1029

[145] Jourdan F, Leese MP, Dohle W, Hamel E, Fernandis E, Newman SP, et al. Synthesis, antitubulin, and antiproliferative SAR of analogues of 2-methoxyestradiol-3,17-O,O-bis-sulfamate. Journal of Medicinal Chemistry. 2010;**53**(7):2942-2951

[146] Potter BVL, Reed MJ, Woo LWL, Hejaz H, Leblond B, Leese MP. Oestrogen-17-sulphamates as inhibitors of steroid sulphatase. U.S. Pat. Appl. US8030296; 2011

[147] Raobaikady B, Reed MJ, Leese MP, Potter BVL, Purohit A. Inhibition of MDA-MB-231 cell cycle progression and cell proliferation by C-2-substituted oestradiol mono- and bis-3-O-sulphamates. International Journal of Cancer. 2005;**117**(1):150-159

[148] Foster PA, Ho YT, Newman SP, Kasprzyk PG, Leese MP, Potter BVL, et al. 2-MeOE2bisMATE and 2-EtE2bisMATE induce cell cycle arrest and apoptosis in breast cancer xenografts as shown by a novel ex vivo technique. Breast Cancer Research and Treatment. 2008;**111**(2):251-260

[149] Ireson CR, Chander SK, Purohit A, Perera S, Newman SP, Parish D, et al. Pharmacokinetics and efficacy of 2-methoxyoestradiol and 2-methoxy-oestradiol-bis-sulphamate in vivo in rodents. British Journal of Cancer. 2004;**90**(4):932-937

[150] Leese MP, Leblond B, Smith A, Newman SP, Di Fiore A, De Simone G, et al. 2-substituted estradiol bis-sulfamates, multitargeted antitumor agents: Synthesis, in vitro SAR, protein crystallography, and in vivo activity. Journal of Medicinal Chemistry. 2006;**49**(26):7683-7696

[151] Peyrat J-F, Brion J-D, Alami M. Synthetic 2-methoxyestradiol derivatives: Structure-activity relationships. Current Medicinal Chemistry. 2012;**19**(24):4142-4156

[152] Newman SP, Foster PA, Stengel C, Day JM, Ho YT, Judde JG, et al. STX140 is efficacious in vitro and in vivo in taxane-resistant breast carcinoma cells. Clinical Cancer Research. 2008;**14**(2):597-606

[153] Tagg SLC, Foster PA, Leese MP, Potter BVL, Reed MJ, Purohit A, et al. 2-Methoxyoestradiol-3,17-O, O-bis-sulphamate and 2-deoxy-D-glucose in combination: A potential treatment for breast and prostate

cancer. British Journal of Cancer. 2008;**99**(11):1842-1848

[154] Meyer-Losic F, Newman SP, Day JM, Reed MJ, Kasprzyk PG, Purohit A, et al. STX140, but not paclitaxel, inhibits mammary tumor initiation and progression in C3(1)/SV40 T/t-antigen transgenic mice. PLoS One. 2013;**8**(12):e80305

[155] Lloyd MD, Pederick RL, Natesh R, Woo LWL, Purohit A, Reed MJ, et al. Crystal structure of human carbonic anhydrase II at 1.95 Å resolution in complex with 667-coumate, a novel anti-cancer agent. The Biochemical Journal. 2005;**385**:715-720

[156] Foster PA, Ho YT, Newman SP, Leese MP, Potter BVL, Reed MJ, et al. STX140 and STX641 cause apoptosis via the intrinsic mitochondrial pathway and down-regulate survivin and XIAP expression in ovarian and prostate cancer cells. Anticancer Research. 2009;**29**(10):3751-3757

[157] Wang M, Xu K, Gao M, Miller KD, Sledge GW, Zheng QH. Synthesis of 2-(11C)methoxy-3,17β-O,O-bis(sulfamoyl)estradiol as a new potential PET agent for imaging of steroid sulfatase (STS) in cancers. Steroids. 2012;**77**(8-9):864-870

[158] Jourdan F, Leese MP, Dohle W, Ferrandis E, Newman SP, Chander S, et al. Structure–activity relationships of C-17-substituted estratriene-3-O-sulfamates as anticancer agents. Journal of Medicinal Chemistry. 2011;**54**(13):4863-4879

[159] Reed JE, Woo LWL, Robinson JJ, Leblond B, Leese MP, Purohit A, et al. 2-Difluoromethyloestrone 3-O-sulfamate, a highly potent steroid sulphatase inhibitor. Biochemical and Biophysical Research Communications. 2004;**317**(1):196-275

[160] Ishida H, Nakata T, Suzuki M, Shiotsu Y, Tanaka H, Sato N, et al. A novel steroidal selective steroid sulfatase inhibitor KW-2581 inhibits sulfated-estrogen dependent growth of breast cancer cells in vitro and in animal models. Breast Cancer Research and Treatment. 2007;**106**(2):215-227

[161] Ishida H, Sato N, Hosogi J, Tanaka H, Kuwabara T. Inactivation of recombinant human steroid sulfatase by KW-2581. The Journal of Steroid Biochemistry and Molecular Biology. 2008;**108**(1-2):17-22

[162] Aoki M, Nishimura H, Mimura A, Kita S, Yasuzawa T, Terada K. Identification of the degradation products of the steroid. Sulfatase inhibitor KW-2581 in jet mill-micronized powder. Journal of Pharmaceutical Sciences. 2013;**102**(6):1760-1772

[163] Jinbo Y, Inoue Y. Novel estradiol derivatives. PCT Int. Appl. WO2000053620; 2000

[164] Ciobanu LC, Martel C, Labrie F, Poirier D. Inhibition of estrone sulfate-induced uterine growth by potent nonestrogenic steroidal inhibitors of steroid sulfatase. Cancer Research. 2003;**63**(19):6442-6446

[165] Poirier D, Roy J, Maltais R, Ayan D. A potent inhibitor of steroid sulfatase (EM-1913) blocks tumor growth in nude mice (MCF-7 xenograft). Current Enzyme Inhibition. 2015;**11**(1):65-73

[166] Roy J, Lefebvre J, Maltais R, Poirier D. Inhibition of dehydroepiandrosterone sulfate action in androgen-sensitive tissues by EM-1913, an inhibitor of steroid sulfatase. Molecular and Cellular Endocrinology. 2013;**376**(1-2):148-155

[167] Potter BVL, Reed MJ. 17-Alkyl-linker derivatized estrogen 3-sulphamates as inhibitors of steroid sulphatase. PCT Int. Appl. WO0216393; 2002

[168] Leese MP, Leblond B, Newman SP, Purohit A, Reed MJ, Potter BVL. Anti-cancer activities of novel D-ring modified 2-substituted estrogen-3-O-sulfamates. The Journal of Steroid Biochemistry and Molecular Biology. 2005;**94**(1-3):239-251

[169] Imai Y, Nakamura T, Matsumoto T, Takaoka K, Kato S. Molecular mechanisms underlying the effects of sex steroids on bone and mineral metabolism. Journal of Bone and Mineral Metabolism. 2009;**27**(2):127-130

[170] Rausch L, Green C, Steinmetz K, Le Valley S, Catz P, Zaveri N, et al. Preclinical pharmacokinetic, toxicological and biomarker evaluation of SR16157, a novel dual-acting steroid sulfatase inhibitor and selective estrogen receptor modulator. Cancer Chemotherapy and Pharmacology. 2011;**67**(6):1341-1352

[171] Rasmussen LM, Zaveri NT, Stenvang J, Peters RH, Lykkesfeldt AE. A novel dual-target steroid sulfatase inhibitor and antiestrogen: SR 16157, a promising agent for the therapy of breast cancer. Breast Cancer Research and Treatment. 2007;**106**(1 Suppl):191-203

[172] Doppalapudi RS, Riccio ES, Rausch LL, Shinon JA, Lee RS, Mortelmans KE, et al. Evaluation of chemopreventive agents for genotoxic activity. Mutation Research. 2007;**629**(2):148-160

[173] Pison U, Shavva AG, Morozkina SN. Preparation of 6-oxa-8-alpha-steroid estrogen analogues—A new group of unnatural estrogens and their use in medicine. JP Pat. Appl. JP2011503020; 2011

[174] Morozkina SN, Drozdov AS, Kovalev RA, Filatov MV, Shavva AG. Racemic 2,17β-disulfamoyloxy-3-methoxy-8α-estra-1,3,5(10)-triene as inhibitor of tumor cell proliferation of MCF-7. RU Pat. Appl. RU2562242; 2015

[175] Reddy DS. Neurosteroids: Endogenous role in the human brain and therapeutic potentials. Progress in Brain Research. 2010;**186**:113-137

[176] Meingassner JG. Combination of a steroid sulfatase inhibitor and ascomycin. PCT Pat. Appl. WO2006097293; 2006

[177] Loumaye E, Cayron-Elizondo V, Gotteland J-P. Use of steroid sulfatase inhibitors for the treatment of preterm labor. PCT Pat. Appl. WO2010013187; 2010

[178] Potter BVL. Steroid sulphatase inhibition via aryl sulphamates: Clinical progress, mechanism and future prospects. Journal of Molecular Endocrinology. 2018;**61**:T233-T252