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Review

Steroid sulfatase inhibitors: Promising new tools for breast cancer therapy?

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ABSTRACT

Inhibition of aromatase is currently well-established as the major treatment option of hormone-dependent breast cancer in postmenopausal women. However, despite the effects of aromatase inhibitors in both early and metastatic breast cancer, endocrine resistance may cause relapses of the disease and progression of metastasis. Thus, driven by the success of manipulating the steroidogenic enzyme aromatase, several alternative enzymes involved in steroid synthesis and metabolism have recently been investigated as possible drug targets. One of the most promising targets is the steroid sulfatase (STS) which converts steroid sulfates like estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHEAS) to estrone (E1) and dehydroepiandrosterone (DHEA), respectively. Estrone and DHEA may thereafter be used for the synthesis of more potent estrogens and androgens that may eventually fuel hormone-sensitive breast cancer cells. The present review summarizes the biology behind steroid sulfatase and its inhibition, the currently available information derived from basic and early clinical trials in breast cancer patients, as well as ongoing research.

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Contents

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1. Introduction

Manipulation of estrogen synthesis and action has been used successfully for the treatment of hormone-dependent breast cancer for several decades [1]. While the antiestrogen tamoxifen was the gold standard of treatment during the 80s and early 90s, aromatase inhibitors (AIs) became 1st line therapy for metastatic, hormone-dependent breast cancer in postmenopausal women in the late 90s. Recently, aromatase inhibitors were also established as the 1st choice in estrogen receptor (ER) positive, postmenopausal early breast cancer [2]. Thus, estrogen suppression has turned out to be in general a more effective way to treat hormone-dependent breast cancer in selected patients compared to ER-blockade by selective estrogen receptor modulators (SERMs). However, in spite of all these advances, still many patients experience relapse of their breast cancer disease and all patients with metastatic, ER-positive breast cancer will have a progressive disease after a certain period of clinical benefit. Several studies have suggested conserved estrogen dependency of tumors following progression on first-line and even second-line endocrine therapies [1,3,4]. Thus, selective estrogen receptor downregulators (SERDs) like fulvestrant or steroidal aromatase inactivators like exemestane are currently used as (second-) third-line endocrine therapies in selected patients with MBC. However, other, non-cross-resistant therapies are urgently needed to give those patients an alternative treatment option prolonging the time-period without chemotherapy.

Inhibition of steroid sulfatase (STS) represents such a novel approach blocking the synthesis of a variety of steroids that have the potential to stimulate growth of human breast cancer (Fig. 1). Currently only one type of steroid sulfatase (also referred to as aryl sulfatase C) is known in humans, hydrolysing both aryl (estrone sulfate, E1S) as well as alkyl (DHEAS) steroid sulfates. Important STS crystallization and X-ray crystallographic studies carried out by Ghosh [5] have for the first time identified essential information about STS architecture and catalytic residues present at the active site. In particular, a catalytic cysteine residue, strictly conserved in all sulfatases, is posttranslationally modified into a formylglycine (FGS75). This is further hydrated to form hydroxyformylglycine. It is suggested that the mechanism of sulfate hydrolysis involves covalent attachment of the sulfate from the substrate to the hydroxyformylglycine. Similarly, irreversible inhibition of STS with compounds such as EMATE or Irosustat (i.e., arylsulfamates), involves mechanism-based irreversible inhibition of STS by suicide substrates such as EMATE [6].

As a consequence of steroid sulfatase inhibition, both estrogen and androgen synthesis will be reduced simultaneously. The relevance of steroid sulfatase in human breast cancer is underlined by findings of several studies suggesting steroid sulfatase mRNA in ER-positive breast cancer to be an independent prognostic indicator predicting relapse-free survival, with higher levels of expression being associated with a poor prognosis. Most interesting, Chanplakorn et al. recently showed increased steroid sulfatase and 17β-hydroxysteroid dehydrogenase type 1 (17βHSD1) immunoreactivity following neoadjuvant therapy with the aromatase inactivator exemestane, suggesting a role for steroid sulfatase in the adaptation processes during therapy with AIs [7].

In contrast, aromatase mRNA levels have not been associated with breast cancer prognosis so far. As high levels of steroid sulfatase activity have been detected in most breast cancers and with convincing evidence for active uptake of sulfates into breast cancer cells via a specific organic anion transporter (organic anion transporter polypeptide B, OATP-B), this pathway may be a major contributor to the well-known elevated estrogen levels in ER-positive human breast cancer tissue [8].

While 60–80% of all postmenopausal breast cancers are classified as ER-positive, the androgen receptor (AR) is co-expressed in up to 80% of the patients. In addition, the AR is still found in many patients with an ER/PGR-negative disease. These findings indicate that human breast cancer cells might be stimulated by androgens via the AR in the absence of ER/PGR.

The STS pathway is also responsible for the production of another steroid with estrogenic properties, namely 5-androstenediol (Adiol), from DHEAS and subsequent reduction of DHEA by 17β-HSD1. Adiol, although an androgen, can bind to the ER and has been shown to stimulate the proliferation of a number of ER-positive breast cancer cells in an ER-dependent manner. Despite its lower affinity for the ER, the 100-fold higher concentration of this hormone has led to the speculation that it may have equally efficacious estrogenic properties to estradiol. This might be the case particularly under clinical situations when patients are treated with aromatase inhibitors, estradiol synthesis has been suppressed by >99% to undetectable levels, but at the same time the tumors have become sensitised to very low estrogen concentrations [9]. Adiol has been shown to stimulate tumor growth even in the presence of an AI and Billich et al. [10] demonstrated that inhibition of steroid sulfatase blocked DHEAS-stimulated growth of MCF-7 breast cancer cells; the same effect was not achieved by the use of an aromatase inhibitor thus highlighting that the generation of Adiol from DHEAS occurred totally independent from the aromatase-pathway. This is of clinical significance because in postmenopausal breast cancer patients treated with AIs, unrestricted production of Adiol can occur via the steroid sulfatase pathway and may promote tumor progression.

Motivated by the findings presented here, several inhibitors of steroid sulfatase have been developed. These drugs have been shown to be very potent inhibitors of steroid sulfatase activity in vivo and are currently being tested in early clinical trials for the treatment of human breast cancer. The theoretical background, basic endocrine findings as well as clinical experience with these compounds available so far will be summarized in the following chapters.

2. Estrogen and androgen synthesis in breast cancer tissue (general introduction)

The origin and manipulation of estrogen levels in human breast cancer tissue has been the subject of intensive research [11–13]. It is currently believed that both uptake from the circulation as well as local synthesis in the tumor contribute to the local estrogen concentrations in a particular breast tumor [8]. Beside steroid sulfatase, a network of different enzymes is involved in human estrogen synthesis and metabolism (Fig. 1). Most of hormone-responsive breast tumors express three major enzyme systems [i.e., aromatase/CYP19, STS and 17β-HSD] that are responsible for the local formation of E2. Aromatase is a cytochrome P450 (CYP450). It interacts with NADPH-cytochrome P450 reductase and converts androgens (mainly androstenedione and minor testosterone) into estrogens (mainly E1 and minor E2). After E1 is synthesized by aromatase, it can be converted to E1S (mainly in liver) by the catalysis of estrogen sulfotransferase [14]. Through circulation, E1S can be then stored in tissues, including breast tumors. Steroid sulfatase catalyzes the hydrolysis of E1S to E1, which is subsequently reduced to E2 by 17β-HSD1. 17β-HSDs are a group of enzymes that catalyze dehydrogenation of 17-hydroxysteroids in steroidogenesis. 17β-HSD1 is the best studied isozyme and remains an important enzyme for E2 production because it can use E1 as a substrate from both aromatase and sulfatase pathways, and it principally synthesizes E2 using reduced nicotinamide adenine dinucleotide (NADPH) as a cofactor [15].

In addition to estrogen uptake and synthesis, the expression of the ER has been suggested as a major factor influencing on estrogen disposition in human breast cancer [16].

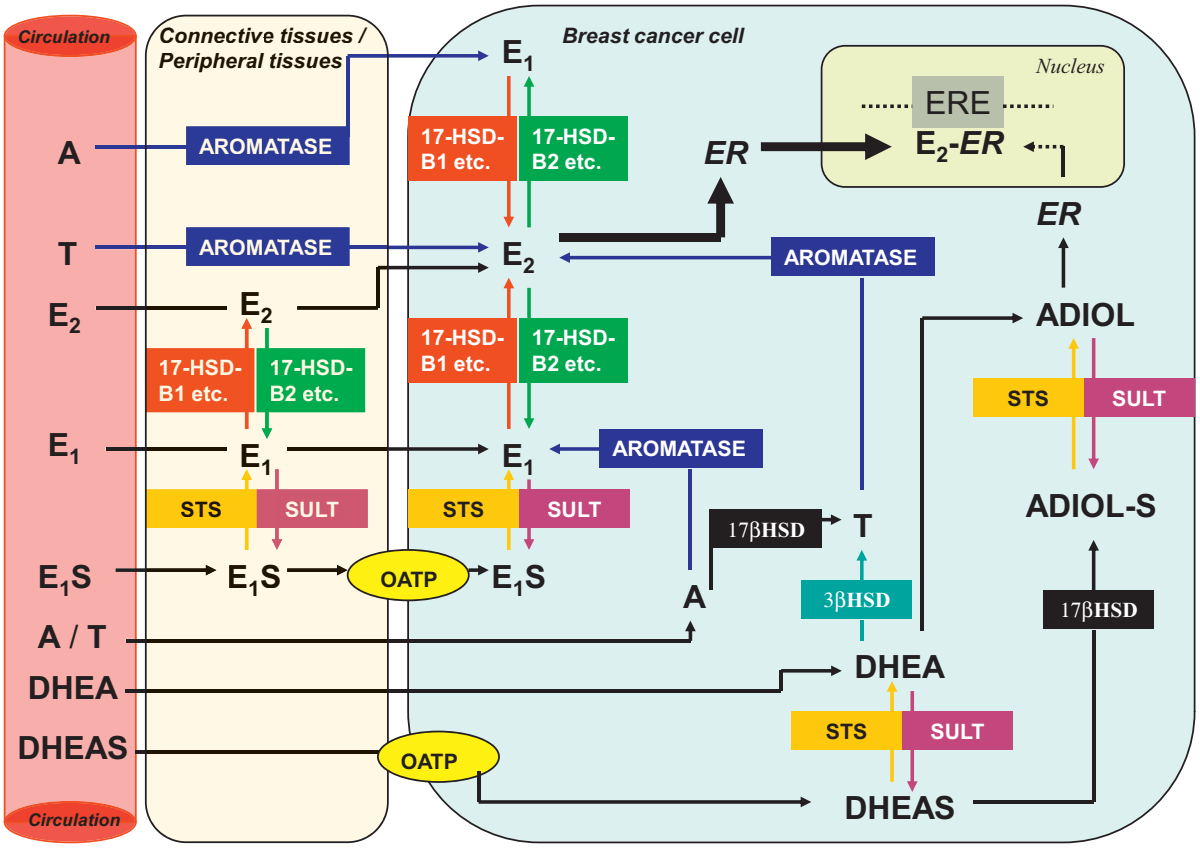

Fig. 1. Major pathways of estrogen and androgen synthesis in human breast cancer tissue. A, androstenedione; T, testosterone; E1, estrone; E2, estradiol; E1S, estrone sulfate; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; 17-β-HSD, 17β-hydroxysteroid dehydrogenase; STS, steroid sulfatase; SULT, steroid sulfotransferase; ER, estrogen receptor; ERE, estrogen receptor response element; ADIOL, androstenediol; ADIOL-S, androstenediol-sulfate.

2.1. Steroid sulfatase in breast cancer tissue (detection, expression and regulation)

Steroid sulfatase activity has been reported to be higher in breast cancer tissues than that in normal breast tissues as has been stated above. In addition, the enzymatic activity of steroid sulfatase is detected in the great majority of human breast tumors [17,18], although Evans et al. [18] reported no significant association between steroid sulfatase activity and clinical parameters such as time to recurrence or overall survival time in breast cancer patients. Therefore, the analysis of steroid sulfatase enzymatic activity could be the gold standard in determining the status of steroid sulfatase in individual patients with breast cancer. However, rather laborious procedures of this enzymatic assays as well as requirement of frozen tissue specimens have made it difficult to be applied in a wide scale fashion for routine clinical practice. mRNA expression of steroid sulfatase could be evaluated in breast carcinoma tissues and results were usually correlated with those of enzymatic activities [13]. Utsumi et al. [19] reported that patients with high mRNA levels for steroid sulfatase were associated with an increased risk of recurrence after surgery. However, the analysis of mRNA in clinical specimens is usually associated with similar problems described above. Therefore, it then becomes important to apply more practical methods of evaluating the steroid sulfatase status in individual breast cancer patients.

Immunohistochemical evaluation using archival or 10% formalin-fixed and paraffin embedded tissue specimens have been in general considered ideal in this point. Various attempts have been made in immunohistochemical analysis of steroid sulfatase in clinical materials of breast cancer patients. Saeki et al. [20] reported the presence of steroid sulfatase immunoreactivity in carcinoma cells in 22 out of 25 cases (88.0%). Suzuki et al. further evaluated immunolocalization of steroid sulfatase in 113 cases of human breast invasive ductal carcinoma using immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR) [21]. Steroid sulfatase immunoreactivity was detected in carcinoma cells in 84 out of 113 carcinoma cases (74.3%), respectively, which was also associated with mRNA levels determined by RT-PCR analysis. This immunohistochemical detection kit is currently available for detection of steroid sulfatase immunoreactivity using the same primary antibody above [21].

Steroid sulfatase immunoreactivity was detected in cytoplasm of carcinoma cells as shown in Fig. 2. In addition, the combined analysis of micro-dissection/RT-PCR analyses demonstrated that both steroid sulfatase protein and mRNA were detected only in carcinoma or parenchymal cells, which is consistent with results of immunohistochemistry. In addition, steroid sulfatase immunoreactivity in these carcinoma cells was positively associated with tumor size of the patients.

2.2. Other steroidogenic enzymes in breast cancer tissue

2.2.1. Aromatase (CYP19)

The enzyme aromatase is encoded by the human CYP19 gene (P450 arom), a member of the cytochrome P_{450} superfamily, localized on the long arm of chromosome 15 (15q21) [22]. Aromatization of C_{19} steroid precursors is the rate-limiting step in estrogen synthesis in humans and is regulated by the use of 10 tissue-specific promoters [23,24]. Aromatase inhibition is currently the dominating treatment option for postmenopausal, hormone dependent breast cancer suitable for endocrine manoevres [25]. Three compounds, all belonging to the "third generation" of drugs are in use:

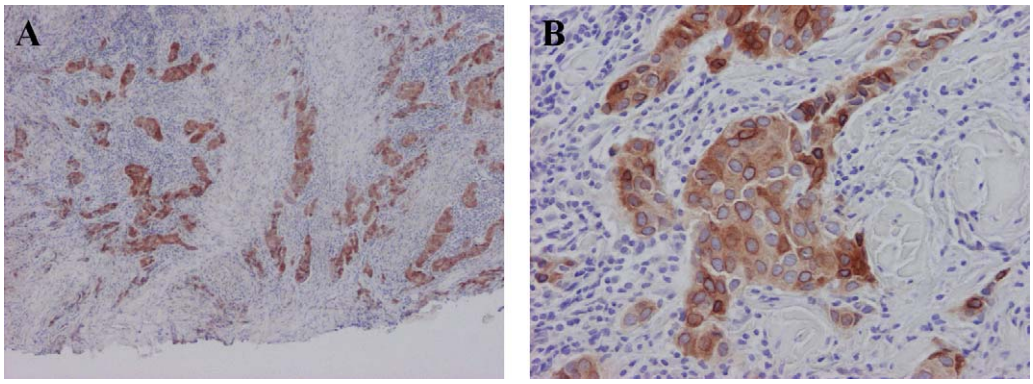

Fig. 2. Detection of steroid sulfatase in human breast cancer tissue. Steroid sulfatase immunoreactivity was detected in the cytoplasm of human breast cancer cells using low power (A) and high power (B) magnification.

the two nonsteroidal aromatase inhibitors anastrozole and letrozole as well as the steroidal aromatase inactivator exemestane. During therapy with these compounds, plasma and tissue estrogen levels have been shown to be suppressed significantly in breast cancer patients [26–28].

2.2.2. 17β-Hydroxysteroid dehydrogenases (17β-HSDs)

The 17β-hydroxysteroid dehydrogenases are pre-receptor activating/inactivating hormones in vivo [29]. At present, 15 isoforms of 17β-HSD have been identified [30–32]. One way to form the biologically most active estrogen estradiol (E2) is the reduction of estrone (E1) by the isoforms 17β-HSD1, 17β-HSD5, 17β-HSD7 and 17β-HSD12 (Fig. 1). While 17β-HSD1 has been suggested to play the major role in humans [33], recent data published by Haynes et al. showed a significant positive correlation of intratumor E2 levels with 17β-HSD7 only [16]. High mRNA levels of 17β-HSD5 have been shown to be related to a significantly higher risk of late relapse in ER-positive patients remaining recurrence-free later than 5 years after diagnosis [34]. Selective inhibitors of 17β-HSD isoforms have been synthesized with the goal to investigate their potential in breast cancer therapy [35,36]. STX 1040, a selective inhibitor of 17-HSD1, was recently reported to be efficacious in vivo in a breast cancer xenograft model [37]. In a separate study, Husen et al. reported the inhibitory activities of their inhibitors in a MCF-7 (17-HSD1) model in immunodeficient mice [38].

3. Sulfatase inhibitors

3.1. STX 64 (667 Coumate, BN83495, Irosustat)

The first specifically designed and synthesized steroid sulfatase inhibitor was estrone methylthiophosphonate (E1-MTP), an E1S-surrogate which possessed modest inhibitory properties. Extensive structure-activity relationship studies led to the identification of estrone 3-*O*-sulfamate (EMATE) as the first ever mechanism-based irreversible inhibitor of STS (Fig. 3, **1**). Unexpectedly, however, EMATE was found to have potent estrogenic properties, being 5 times more estrogenic than ethinylestradiol in rodents on oral application. This undesirable property stimulated the development of non-steroidal mimics which led to the discovery of STX 64, the only steroid sulfatase inhibitor that has entered into a phase I trial to date (Fig. 3, **2**). STX 64 is a non-steroidal agent with a tricyclic coumarin scaffold. It was shown to be devoid of estrogenic activity, as tested in an ovariectomised rat uterotrophic assay, and showed excellent efficacy in various in vivo tumor models [39].

3.2. STX 213

Many efforts were also made in parallel to retain the steroidal scaffold but overcome the estrogenic drawbacks of EMATE. These strategies included modification of its ring system or the introduction of substituents at various positions of its steroidal scaffold to generate non-estrogenic derivatives which remained highly potent inhibitors of steroid sulfatase. STX 213 (Fig. 3) represents one such inhibitor, where the natural steroid cyclopentanone D-ring is replaced by a *N*-substituted piperidine-2,6-dione ring. STX 213 proved to be 8-fold and 18-fold more potent *in vitro* than STX 64 and EMATE respectively and was completely devoid of any estrogenic activity. It was thus chosen for preclinical development as a second-generation steroid sulfatase inhibitor. The most significant distinction of the second generation STS inhibitor was its prolonged duration of steroid sulfatase inhibition. The time to recover 50% of rat liver steroid sulfatase activity ($t½$) was around 3 days for STX 64 but 10 days for STX 213 when tested at a single oral dose of each inhibitor of 10 mg/kg [40,41].

3.3. Other steroid sulfatase inhibitors

In the past decade many other steroid sulfatase inhibitors have been identified including 6-[2-(adamantylidene)hydroxybenzoxazole]-*O*-sulfamate (AHBS, Fig. 3, **4**) [42] and KW-2581 (Fig. 3, **5**) [43]. Recently, a novel dual sulfatase–antiestrogen inhibitor, SR16157 (Fig. 3, **10**), has completed preclinical toxicity and PK evaluation in dogs and has excellent bioavailability and favourable safety profile [44]. However, to date, all highly active and irreversible steroid sulfatase inhibitors incorporate the phenol sulfamate ester pharmacophore required for potent steroid sulfatase inhibition, and first identified in EMATE.

4. Clinical experience with steroid sulfatase inhibitors in breast cancer patients

Clinical experience with sulfatase inhibitors is still limited. However, recently Stanway et al. published the results of a phase I, single-arm, open-label, study of the non-steroidal sulfatase inhibitor STX 64 (667 Coumate; BN83495, Irosustat) [45]. Briefly, 14 postmenopausal patients suffering from either metastatic or locally advanced breast cancer patients were enrolled in this study. The patients were heavily pretreated with antiestrogens, aromatase inhibitors, other endocrine options and with several lines of chemotherapy (median: 2). STX 64 was given in two different doses. A 5 mg daily dose was given to nine patients while five patients received a 20 mg daily dose. Steroid sulfatase activity in human tumor samples was inhibited at the 5 and 20 mg doses by

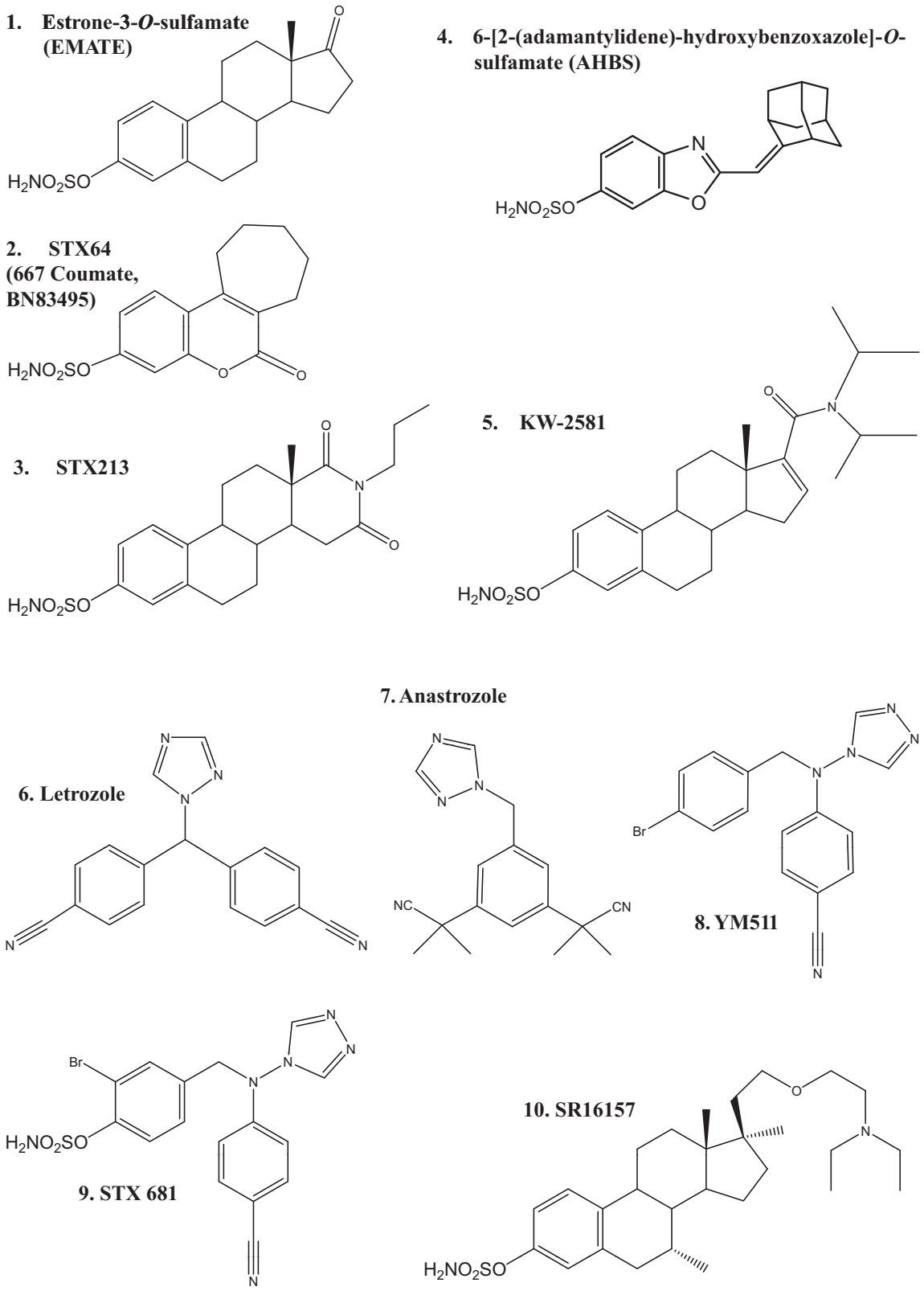

Fig. 3. Structures: selected steroid sulfatase inhibitors (compounds **1**–**5**), aromatase inhibitors (compounds **6**–**8**), a dual aromatase–sulfatase inhibitor ("DASI"; compound **9**), as well as a dual sulfatase–antiestrogen inhibitor (compound **10**).

99% (median) with both doses. In addition, plasma median concentrations of E1 were decreased at the 5 and 20 doses by 55% and 42%, respectively, with E2 plasma levels decreasing by 47% and 41%, respectively. Concerning plasma androgens, the levels of DHEA, androstenedione (A), and testosterone (T) were decreased by 52%, 63%, and 46%, respectively during therapy with STX 64. Although not a primary endpoint of this study, several patients experienced a stable disease for 2.75 to 7 months during therapy with STX 64 [45].

A further dose-escalation study in 29 patients has been completed recently [46]. The optimum dose was determined to be 40 mg of STX 64 per patient per day in tablet form.

5. Dual sulfatase/aromatase inhibitors

Since aromatase is needed for the synthesis of estrogens that are then converted into estrogen sulfates by estrogen sulfotransferase, hormone-dependent breast cancer may be more effectively treated by dual inhibition of aromatase and steroid sulfatase. A new design strategy was explored that involves introducing the aromatase inhibitory pharmacophore into a template that has been designed primarily for sulfatase inhibition [47]. A series of compounds that can inhibit both aromatase and sulfatase have been developed based on the structure of estrone 3-sulfamate, a typical estrone sulfatase inhibitor [48]. In contrast, a series of single agent dual aromatase-sulfatase inhibitors that are sulfamate derivatives of nonsteroidal AIs, including letrozole and anastrozole, have been successfully developed [49–51]. The design of these dual aromatase–sulfatase inhibitors shares a common strategy; that is, to engender the sulfatase inhibitory pharmacophore into an established aromatase inhibitor with minimal structural change incurred to the original scaffold in order to retain and maximize aromatase inhibition. At the same time, possible negative pharmacological interactions between several aromatase and sulfatase inhibitors given in concert could be avoided. It is also reasoned that resistance to drugs targeting two different enzymes is not likely to develop simultaneously. Thus, *D*ual *A*romatase-*S*ulfatase *I*nhibitors (DASIs) have been developed engendering the steroid sulfatase inhibitory pharmacophore into established aromatase inhibitors with minimal structural changes otherwise. At this stage, DASIs are available based on the triazoles letrozole (Fig. 3, **6** [52], anastrozole (Fig. 3, **7**) [53], and YM511 (Fig. 3, **8**) [51], in addition to alternative AIs characterized by their biphenyl templates [54].

STX 681 (Fig. 3, **9**) is a YM511-based DASI that has been shown to have in vivo activity. Using a xenograft nude mouse model, Foster et al. demonstrated that STX 681 completely inhibited the growth of MCF-7_{AROM} and MCF-7_{STS} tumors [55]. The authors conclude that targeting both the aromatase enzyme and the sulfatase enzyme at the same time has the potential to become a novel treatment strategy of hormone-dependent breast cancer (HDBC).

6. Sulfatase inhibitors: ongoing research and future aspects

Given the potency of this new class of sulfamate-based steroid sulfatase inhibitors, the large volume of preclinical data available on the use of steroidal and non-steroidal STS inhibitors in a variety of hormone-dependent cancer models and, given the encouraging results obtained in two phase I studies completed with BN83495 (STX 64) it will be important to carry out clinical trials to assess its efficacy in different clinical settings as well as in non-cancer disease indications. While clinical studies are planned to investigate the effect of BN83495 in women with ER-positive early breast cancer, the compound is currently in further clinical development for advanced endometrial cancer (phase II) as well as in phase I evaluation for castrate-resistant prostate cancer in North America. Additional trials will examine whether combining BN83495 with an AI or LHRH antagonist will improve response rates.

As the biological role of steroid sulfatase is also implicated in several disorders of the skin (acne, psoriasis, hirsuitism) and in memory function, BN83495 may find use in such non-cancer diseases [6].

7. Conclusions

Inhibition of steroid sulfatase is one promising new approach to develop alternative treatment strategies for hormone-sensitive breast cancer. In contrast to aromatase inhibition alone, suppressing plasma and tissue estrogen synthesis, sulfatase inhibition causes both estrogen and androgen depletion simultaneously. Early clinical findings suggest that breast cancer patients with progressive disease while on therapy with aromatase inhibitors, may experience a new response when treated with a steroid sulfatase inhibitor as monotherapy. Most interesting, upregulation of steroid sulfatase has recently been confirmed in breast cancer patients treated with an aromatase inhibitor, suggesting steroid sulfatase to be possibly involved in adaptation to estrogen deprivation and/or endocrine resistance. Phase I-II trials involving sulfatase inhibitors are now initiated to study the influence of these compounds on intra-tumor steroid levels and enzyme activity. Moreover, compounds inhibiting aromatase and sulfatase activity at the same time (DASIs) have been developed.

While sulfatase inhibition certainly is one of the most promising new treatment strategies for hormone-sensitive breast cancer, its role in daily praxis is currently unclear. Ongoing trials will investigate the potential of these drugs either as monotherapy or in combination with established drugs. Finally, the identification of biological relevant tumor markers that might serve as predictive factors (like steroid sulfatase activity in human cancer tissue, normal tissue, hair etc.) is urgently requested to allow the use of these drugs in groups of patients with a high chance for clinical responses.

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