

Steroid sulfatase inhibitors: promising new therapy for breast cancer

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Abstract

Manipulation of the hormone oestrogen has been used for decades to treat hormone-dependent breast cancer. Currently, aromatase inhibitors (AIs) are used as first-line therapy against early and metastatic breast cancer in post-menopausal women. Despite these advances, several patients eventually experience a relapse of breast cancer and declined clinical response to treatment. As per recent findings, steroid sulfatase (STS) has emerged as a novel therapy target. This review aims at summarising the emerging field of STS inhibitor development and highlighting current findings from pre-clinical and clinical trials. The recently-developed dual-targeting compounds, such as dual aromatase-sulfatase inhibitors (DASI), have shown encouraging preclinical results and represent important new treatments for hormone-dependent breast cancer.

Keywords: Oestrogen, Estrone (E1), Estrone sulfate (E1S), Estradiol (E2), Oestrogen receptor (ER), Aromatase (AROM), Aromatase inhibitors (AIs), Steroid sulfatase (STS), 667 COUMATE (STX64), Dual-aromatase-sulfatase inhibitors (DASI).

Introduction

According to the World Health Organisation (WHO), non-communicable diseases (NCDs) are the leading causes of death worldwide.¹ These NCDs comprise cardiovascular diseases, diabetes, chronic respiratory diseases and cancers.¹ Breast cancer is the most prevalent cancer in women globally, with an estimated 1.2 million new diagnoses each year.² Breast cancers caused nearly 400,000 deaths worldwide in 2008, and have a global economic impact of approximately \$88 billion.³

There are some prominent risk factors for breast cancer, such as advanced age, early menarche, nulliparity and late menopause.⁴ In addition to the aforementioned risk factors, certain hormones are also known to play a role in breast cancer etiology.⁵ Nearly two-thirds of all breast cancers exhibit the oestrogen receptor at the time of diagnosis, and require oestrogen to develop and propagate.⁵ Hence, manipulation of oestrogen synthesis

and action has been used to treat hormone-dependent breast cancer for decades. For several years, surgical ablation of oestrogen production, through oophorectomies or adrenalectomies, was utilised as a measure to control advanced breast cancer.^{5,6} In the past three decades, however, hormone-modulating drugs have become the primary endocrine treatments for hormone-dependent breast cancer.⁷ The anti-oestrogen tamoxifen was the gold standard of breast cancer treatment in the 1980s and the early 1990s, but in the late 1990s and in recent years, aromatase inhibitors (AIs) have been established as the dominant first-line therapy for early and metastatic, hormone-sensitive breast cancer in post-menopausal women.^{7,8}

Despite the observed benefits and widespread use of these drugs, clinical studies indicate that a majority of women given these therapies will eventually relapse and have progressing disease.⁹ The fact that relapsing tumours become resistant to existing hormone therapies, despite remaining ER-positive, suggests that the tumours are able to recruit alternative sources of oestrogens.^{9,10} The previous decade has seen considerable research in this field and the findings have shown that, in addition to aromatase (AROM), another enzyme, steroid sulfatase (STS), has a critical role in oestrogen biosynthesis in breast cancer.¹⁰ Several effective STS inhibitors have been developed as therapeutic agents for hormone-dependent breast cancer. The aim of this review is to summarise the recent and on-going development of STS inhibitors and findings from pre-clinical and early clinical trials of the major STS inhibitors being studied today. This paper thereby aims at demonstrating their potential value in treating hormone-dependent breast cancers as well as other conditions.^{9,10}

Oestrogen synthesis in breast cancers

It is known that tumours in hormone-dependent tissues, such as breast tissue, require oestrogens to mature.^{9,11} The exact sources of intra-tumoural oestrogen are still controversial and are the subject of intensive research.⁹ Either there is uptake of circulating oestrogens from plasma or there is in situ synthesis from oestrogen precursors. The fact that both hormone-sensitive and hormone-independent breast cancers display similar intra-tumoural oestrogen concentrations suggests that in situ

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biosynthesis makes significant contributions to tumour oestrogen levels.¹² The enzyme networks through which oestrogens are derived from precursor compounds in the breast are well characterised (Figure-1). ER-blocking therapy through selective oestrogen receptor modulators (SERMs), such as tamoxifen, constitutes the most common endocrine treatment strategy for hormone-dependent cancer in pre-menopausal women.¹³ It is pertinent to note that the incidence of breast cancer is found to increase in post-menopausal women when ovarian oestrogen production has ceased.¹⁴ However, oestrogens are

continually synthesised via peripheral conversion of androstenedione (Adione) to estrone (E1) through the action of aromatase, a cytochrome P450 (CYP450) enzyme.^{8,14} According to the 'St. Gallen' Panel, it is recommended for post-menopausal women to switch to AIs, such as anastrozole and letrozole, after 2-3 years of tamoxifen therapy, but AIs may also be used as first-line adjuvant therapy.¹⁵ Nevertheless, most patients eventually develop resistance to AI-based endocrine treatment and the cancer progresses, indicating that the tumour is able to recruit oestrogen through an alternative pathway.¹⁵

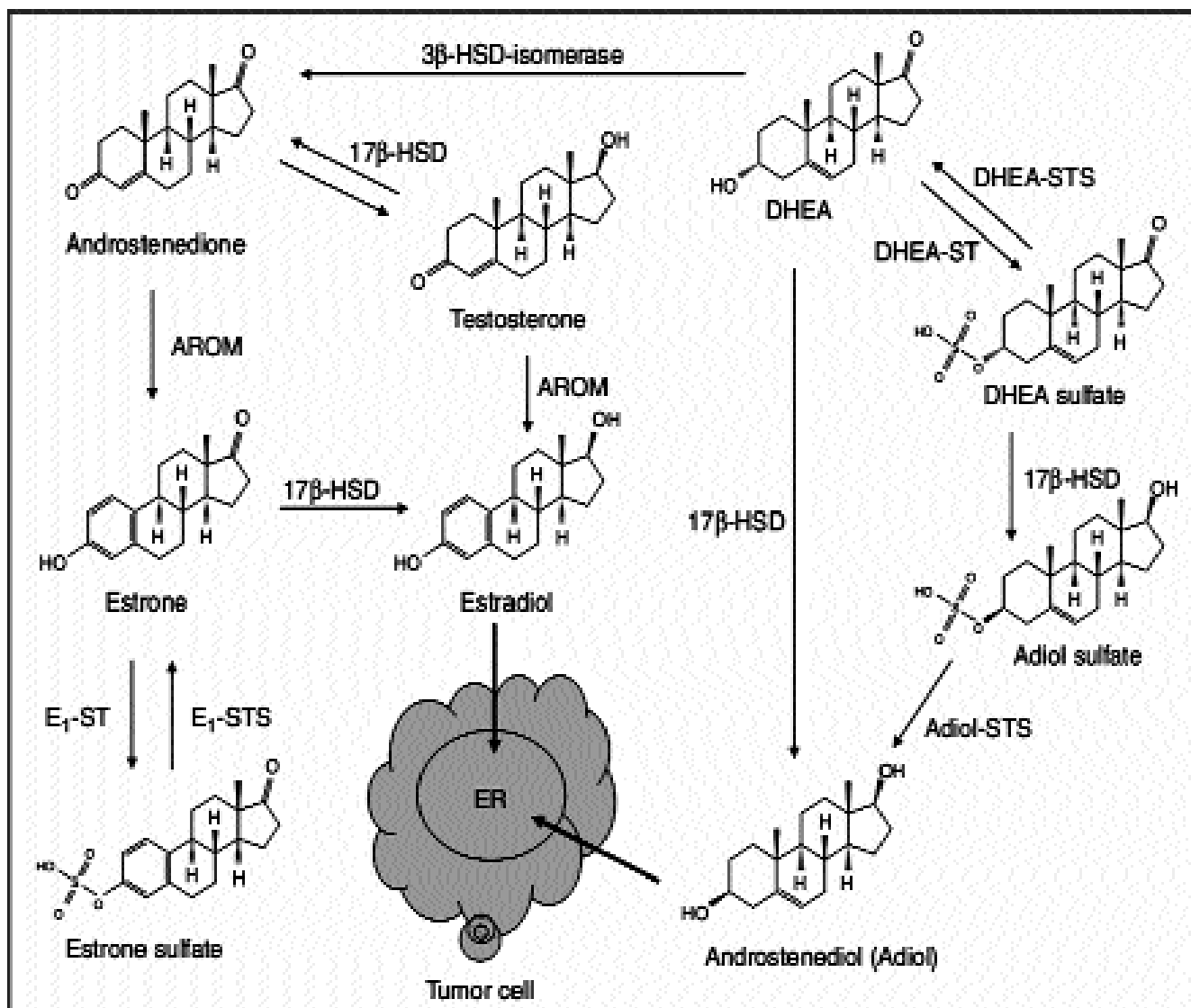


Figure-1: The origins of oestrogenic compounds. Estrone sulfate (E1S) is observed to be circulating at high concentrations. STS, elevated in many hormone-dependent cancers, causes peripheral tissue conversion of E1S to estrone (E1). E1 is then reduced to estradiol (E2) by 17 b-HSD type 1, binds to the oestrogen receptor (ER) and causes cell proliferation.

AROM: Aromatase; ST: Sulfotransferase; STS: Sulfatase; 17b-HSD, 17b-hydroxysteroid dehydrogenase; ER: Oestrogen receptor; DHEA: dehydroepiandrosterone; DHEA-ST: Dehydroepiandrosterone sulfotransferase.

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Once synthesised, E1 can be converted to estrone sulfate (E1S), primarily in the liver, by the action of oestrogen sulfotransferase (EST), and E1S can be converted to E1 via the action of the STS pathway. Circulating serum concentrations of E1S are higher than E1, and E1S is also found to be elevated in breast cancers.^{9,14} Once E1S is converted to E1 by the actions of STS, it can further be reduced to the more biologically potent compound, Estradiol (E2) (Figure-1), through the action of enzyme 17 β -hydroxysteroid dehydrogenase type 1 activity (17 β -HSD-1).¹⁶ Recently, a study, demonstrated that patients treated with neo-adjuvant AI therapy showed significant amplification of STS and 17 β -HSD-1 activity, signalling the potential importance of the STS pathway in mediating resistance to AI-based endocrine therapy.¹⁷

STS in Breast Cancer

Currently, there is a growing body of evidence that points to the importance of STS, as opposed to aromatase, as a therapeutic target in women with hormone-dependent breast cancer. STS activity was first demonstrated in mice liver in 1954, and has since been found to be nearly ubiquitously distributed in smaller quantities in human tissues.¹⁸ It has been shown that STS activity is elevated in human breast tumours and, thus, in human breast carcinomas, estrone (E1) formed through the STS pathway is detected to be ten-fold greater than that formed via aromatase.¹⁹ STS expression is found in 90% of breast tumours, whereas aromatase expression is detected in only 60-70%.^{20,21} Furthermore, a recent study indicated that STS and EST immunoreactivity is detected in a majority of breast carcinomas, and that STS might be an important prognostic factor for breast cancer.²² Using enzymatic assays, immunohistochemistry and reverse-transcription-polymerase chain reaction (RT-PCR), the researchers examined 113 cases of human breast invasive ductal carcinoma. EST and STS immunoreactivity was observed in 50 and 84 cases (44.2 and 74.3%), respectively and was significantly associated with their mRNA (ribonucleic acid) levels.²² STS immunoreactivity was also significantly correlated with tumour size ($p=0.003$), increased risk of recurrence ($p=0.0118$) and worse prognosis ($p=0.0325$).²² Therefore, ample evidence exists to warrant continued development of STS inhibitors and further research on STS as a therapeutic target in hormone-dependent breast cancers.

Development of steroid sulfatase (STS) inhibitors

In the light of increasing evidence from earlier studies, the search for STS inhibitors began in the late 1980s and the early 1990s. The history of, and the drug development processes behind the production of STS inhibitors have

been comprehensively reviewed elsewhere.^{9,23} This section intends to provide an overview of the key STS inhibitors (Figure-2) that have been developed, and their performance in pre-clinical and clinical studies thus far. It is important to note that like AIs, STS inhibitors can only be employed in post-menopausal women. Any study of these compounds in pre-menopausal women would need to be accompanied by ovarian suppression, either surgically or medically.

Steroidal sulfamate-based inhibitors

Initial search for STS inhibitors centred around trying to modify the structure of E1S to create analogues, a justified approach as E1S is the substrate for STS.²³ The first breakthrough in the design process came when the sulfate group of E1S was replaced by a sulfamate moiety (-OSO₂NH₂), creating the compound estrone 3-O-sulfamate (EMATE) in 1998.²³ EMATE (Figure-2), was found to be an irreversible inhibitor, inhibiting STS in a time- and

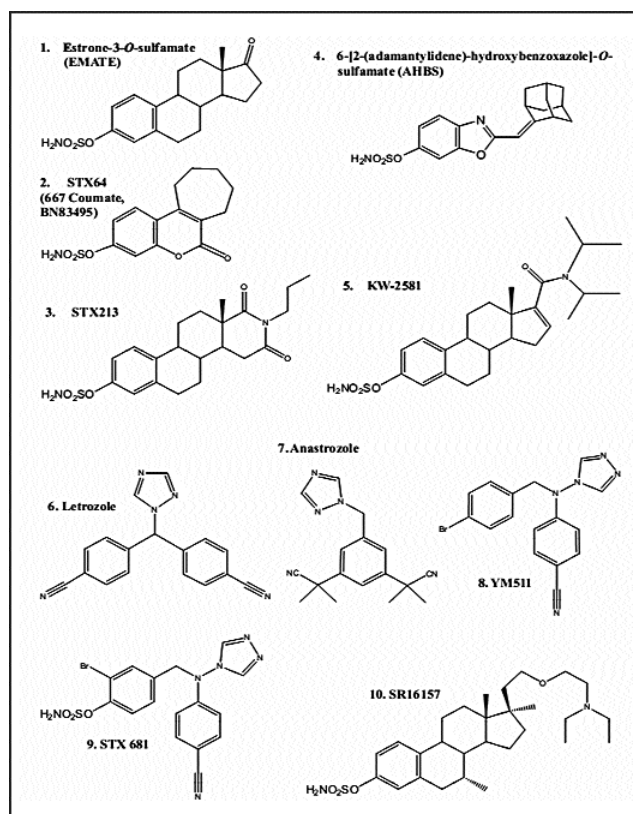


Figure-2: Relevant chemical structures: selected steroid sulfatase inhibitors (compounds 1-5), aromatase inhibitors (compounds 6-8), a dual aromatase-sulfatase inhibitor ("DASI"; compound 9), as well as a dual sulfatase-antioestrogen inhibitor (compound 10).

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concentration-dependent fashion. EMATE was found to be highly potent with an IC₅₀ of 18 nM in a human placental microsome formulation.²³ However, earlier studies had also found that EMATE has powerful oestrogenic activity when administered orally to rats.^{24,25} Even though oestrogen sulfamates were found to not have an oestrogenic activity in vitro and oestrogen sulfamates cannot bind the oestrogen receptor, EMATE remained unattractive for use against hormone-dependent breast cancer.²⁵

Non-steroidal sulfamate-based inhibitors

Due to the oestrogenic effects of EMATE, attempts were made to generate non-oestrogenic compounds, resulting in the production of non-steroidal sulfamates.²³ Research using tricyclic coumarin sulfamates identified the compound 667 COUMATE (also known as STX64 or BN83495).^{23,26} The compound 667 COUMATE (Figure-2) did not display any oestrogenic activity in vitro and in vivo, and was shown to be more potent at STS inhibition than EMATE, displaying an IC₅₀ of 8 nM in placental microsomes.²⁶ Furthermore, using the nitrosomethylurea (NMU)-induced mammary tumour model, it was observed that 667 COUMATE caused regression of estrone sulphate-stimulated NMU tumour growth in a dose-dependent manner. Thus, 667 COUMATE was highlighted as an ideal STS inhibitory compound and a valid candidate for clinical trials.⁹

667 COUMATE clinical trials

The 667 COUMATE (now given the generic name Irosustat) was the first STS inhibitor to be forwarded to a Phase I clinical trial in post-menopausal women with hormone-dependent breast cancer.^{9,27} The trial recruited 14 patients, all of whom had previously received hormone therapy for breast cancer, whereas some had also received chemo- and radiotherapy. Peripheral blood lymphocyte (PBL) STS activity was chosen as a biomarker permitting the duration and extent of STS inhibition to be measured during the trial.⁹ The primary endpoint of the study, therefore was to determine the dose of 667 COUMATE that inhibited STS activity by >90%. The ability of 667 COUMATE to inhibit STS in tumour samples obtained from some patients and its effect on serum androstenediol and oestrogen concentrations was also measured during the trial.^{9,27}

The 667 COUMATE was administered orally, with patients receiving an initial dose (cycle 0) followed by 3×2 weekly cycles (cycles 1–3). Each cycle consisted of daily dosing for 5 days followed by 9 days off treatment.⁹ The drug was well tolerated at 5 and 20mg. Peripheral blood lymphocytes (PBL) STS activity was inhibited by >90% with a dose of 5mg and was almost entirely eliminated at 20mg. Tumour tissue STS activity was also inhibited by

>95% in the 20 mg treatment group. Interestingly, the concentration of androstenedione, the main aromatase substrate in post-menopausal women, was found to be decreased by up to 86%.^{9,27} This indicates that androstenedione is derived from the peripheral conversion of dihydroepiandrosterone-sulphate (DHEAS) and not, as believed previously, by direct secretion from the adrenal cortex. As androstenedione can be converted to E2 via reactions involving aromatase and 17β-HSD type 1 (Figure-1), decreasing androstenedione concentrations signal a further potential advantage of STS inhibition. There were no serious toxicities associated with the 20mg dosage. The side effects included dry skin, hot flashes, taste disturbance, fatigue and headaches with none of these being higher than grade 2.²⁷

The final results from this trial were reassuring. Five out of 14 patients, all of whom had previously progressed on AI therapy, showed evidence of stable disease.²⁷ Unfortunately, Phase I/II clinical trials, performed by the pharmaceutical group Ipsen Ltd, did not repeat this success. The results from other Phase I/II trials, in metastatic breast and prostate cancer, involving Irosustat, have yet to be published.⁹ It has been suggested that there is a necessity of conducting further Phase II and III clinical trials using Irosustat in combination with AIs as a first-line dual-approach therapy for hormone-sensitive breast cancer.²⁷ Thus, further clinical studies with 667 COUMATE can be justified from current findings.

2nd and 3rd generation STS inhibitors

Parallel to the development of 667 COUMATE, a series of other compounds with STS inhibitory properties have also been developed and investigated. Two of these compounds have potent STS inhibitory qualities and show encouraging results from pre-clinical trials.^{9,23}

Studies were conducted on a series of steroidal sulfamates and the compound 17-diisopropylcarbonyl-1,3,5(10),16-estratetraen-3-yl sulfamate, also called KW-2581, was recognised as a potent steroidal-based selective STS inhibitor.²⁸ Using crude enzyme isolates from Chinese hamster ovary cells expressing human arylsulfatases, KW-2581 (Figure-2), was shown to exhibit high potency for STS inhibition with an IC₅₀ of 4.0 nM.²⁸ The compound also showed high selectivity, requiring 1000-fold greater concentrations to inhibit the other enzyme isolates. Moreover, it exhibited no oestrogenic activity in MCF-7 human breast cancer cells or in vivo, in ovariectomised rat models.²⁸ This group of researchers further tested the effects of KW-2581 in 2 hormone receptor-positive human breast cancer cell lines, ZR-75-1 and BT-474.²⁹ These cell lines express STS naturally and

display catalytic activity to produce E1 from E1S at levels comparable to human breast cancers.²⁹ Using ZR-75-1 cell xenografts in mice, KW-2581 was demonstrated to virtually abolish (>95%) STS activity after 24 hours, and cause tumour shrinkage after the 4-week dosing regimen.²⁹ These findings emphasise the possible therapeutic potential of this compound for hormone-dependent breast cancer and make it a viable candidate for Phase I clinical trials.²⁹ KW-2581 has yet to enter Phase I clinical trials.^{9,29}

In addition to the aforementioned compounds, there was also a concerted effort to design an EMATE-like compound retaining the steroidal scaffold but eliminating the undesirable oestrogenic effects. This resulted in the compound STX213 (Figure-2), where the natural steroid cyclopentanone D-ring is replaced by an N-substituted piperidine-2, 6-dione ring.^{30,31} This compound did not exhibit oestrogenic properties and was shown to have a far higher duration of STS inhibitory activity in rat livers, than 667 COUMATE (12 days versus 4 days respectively).³¹ A more recent study also demonstrated that STX213 had a far greater efficacy than 667 COUMATE at inhibiting tumours of Michigan Cancer Foundation (MCF)-7STS (MCF-7 breast cancer line overexpressing STS) xenografts in mice models.³² A similar compound, a third generation STS inhibitor called STX1938 was shown to have efficacy similar to STX213.³² Further studies using these novel compounds have yet to be conducted.^{9,32} Nevertheless, the results from initial pre-clinical studies are encouraging, highlighting the major breakthroughs in STS inhibitor design and demonstrating their excellent STS inhibitory potential. Several of these STS inhibitors are suggested to be employed in clinical trials where patients have already been treated and have progressed on AI therapy, or concurrently with AIs, as a dual-targeting therapy for hormone-dependent breast cancer.²⁷ There is, therefore, also an interest in creating compounds that can target both sulfatase and aromatase enzyme complexes and be used as a monotherapy.

Dual-targeting compounds

A combined therapy of STS inhibitors with AIs has been suggested as an enhanced strategy to maximise the intratumoural depletion of oestrogens.²⁷ Commonly, when such multi-mutargeted therapy is necessary, there are three possible pharmacological approaches.³³ The combination of drugs can be administered as a cocktail of two or more drugs (Figure-3A), or co-formulated into a multi-component tablet (Figure-3B) or, finally, a dual-targeting compound can be created that is able to modulate different targets concomitantly (Figure-3C). There are several pharmacologically justified motives

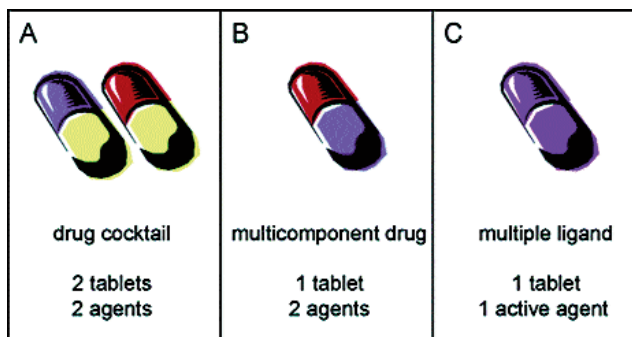


Figure-3: Three possible scenarios for polypharmacological multi-targeted therapy. Reprinted With Permission From (Morphy R, Rankovic Z. Designed Multiple Ligands. An Emerging Drug Discovery Paradigm. *J Med Chem* 2005; 48: 6523-6543). Copyright (2005) American Chemical Society.

for the creation of dual-aromatase-sulfatase inhibitor (DASI) compounds for complex diseases such as breast cancer. These include the lack of adverse drug-drug interactions, decreased chances of developing resistance in tissue, and better compliance compared to drug cocktails or multi-component drugs.³³ Furthermore, the multiple ligand targeting approach can help in breakthroughs in designs for novel drugs for multifactorial diseases such as cancer.³³

The design strategy for DASI compounds focussed on engineering an STS inhibitory pharmacophore onto known aromatase inhibitors such as letrozole, anastrozole and YM511.^{8,9,34} An m-sulfamate derivative of YM-511, called STX681 (Figure-2) was shown to inhibit tumours of both MCF-7_{STS} (expressing STS cDNA) and MCF-7_{AROM} (expressing AROM cDNA), completely deterring the activity of STS and AROM. It was also found to greatly reduce plasma E2 levels.³⁴ Recently, studies have also been conducted on letrozole-derived and vorozole-based sulfamates and new compounds have been identified with potential DASI properties.³⁵ All these compounds, including STX681, show potent dual-action effects against STS and aromatase, and warrant further investigation in in vivo models and other pre-clinical pharmacological studies.

In ADDITION to the aforementioned compounds, research has also been conducted on creating a dual-targeting drug with anti-oestrogenic and STS inhibitory components.³⁶ A novel compound SR16157 (Figure-2), was designed as a sulfamate-containing STS inhibitor that also releases the compound SR16137, a powerful SERM.³⁶ This compound was found to be highly effective at inhibiting E1S-stimulated MCF-7 cells and displayed a 10-fold higher anti-oestrogenic potency than other SERMs such as tamoxifen and SR16137.³⁶ Derivatives of SR16157

have shown excellent in vivo potency and are currently being studied by the National Cancer Institute's Rapid Access to Intervention Development (RAID) programme. A pharmacokinetic study of SR16157 has shown very low toxicity in animal models and an excellent pharmacokinetic profile for the drug.³⁷ These findings are encouraging and pave the way for the development of additional multi-targeted therapies for possible uses in both pre-menopausal women and post-menopausal women with hormone-dependent breast cancer.

Other than breast cancer, STS has been implicated to play an important role in other pathologies. Recently, elevated sulfatase activity was detected in 97% of ovarian cancer specimens and was associated with worse progression-free survival in patients with advanced ovarian cancer.³⁸ Sulfatase activity is suggested to also play a role in disorders such as psoriasis and hirsutism, and, therefore, studies of STS inhibitors in these disorders are also imperative for a broader understanding of the biological role of STS in human health and disease.¹⁸

Finally, it can be argued that the advent of STS inhibitors and dual-acting compounds further demonstrates the intricate multifaceted etiology of cancer. It is important to adopt a pharmacogenetic approach to STS inhibiting drugs. Drugs such as tamoxifen are known to have pharmacogenetic differences in treatment outcomes.³⁹ Hence, conducting pharmacogenomic (genome-wide) investigations of STS inhibitor response, once validated for use in humans, may be highly beneficial. This drive towards personalised medicine, which seems inevitable and promises to be very favourable, echoes the thoughts of the illustrious Sir William Osler, who claimed, "If it were not for the great variability among individuals, medicine might as well be a science and not an art."⁴⁰

Conclusions

The review examined the current status of research into compounds with potent STS inhibitory activities as emerging drugs against hormone-dependent breast cancer. It can be concluded from current findings that STS plays a vital role in hormone-dependent breast cancers and that further investigation is necessary to validate the novel STS inhibitory compounds that are being developed. Though Phase I clinical trials of 667 COUMATE were extremely encouraging, further clinical studies are required before widespread use of these drugs can be mandated. The results from studies using dual-targeting compounds are extremely encouraging and possibly prognosticate the future direction of endocrine therapy of breast cancers. The fact that SR16157 was found to have a 10-fold higher anti-oestrogenic activity than tamoxifen

indicates that these novel compounds can target multiple ligands effectively.

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