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Research paper

Discovery of a sulfamate-based steroid sulfatase inhibitor with intrinsic selective estrogen receptor modulator properties

Charles Ouellet [a], René Maltais [a], Étienne Ouellet [a], Xavier Barbeau [b], Patrick Lagüe [c], Donald Poirier [a, d, *]

[a] Laboratory of Medicinal Chemistry, CHU de Québec – Research Center (CHUL, T4), 2705 Laurier Boulevard, Québec, QC, G1V 4G2, Canada
[b] Département de chimie, Institut de biologie intégrative et des systèmes (IBIS), Centre de recherche sur la fonction, la structure et l'ingénierie des protéines (PROTEO), Université Laval, Québec City, QC, Canada
[c] Département de biochimie microbiologie et bio-informatique, Institut de biologie intégrative et des systèmes (IBIS), Centre de recherche sur la fonction, la structure et l'ingénierie des protéines (PROTEO), Université Laval, Québec City, QC, Canada
[d] Department of Molecular Medicine, Faculty of Medicine, Université Laval, Québec, QC, Canada

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ABSTRACT

Steroid sulfatase (STS), the enzyme which converts inactive sulfated steroid precursors into active hormones, is a promising therapeutic target for the treatment of estrogen-sensitive breast cancer. We report herein the synthesis and *in vitro* study of dual-action STS inhibitors with selective estrogen-receptor modulator (SERM) effects. A library of tetrahydroisoquinoline-*N*-substituted derivatives (phenolic compounds) was synthesized by solid-phase chemistry and tested on estrogen-sensitive breast cancer T-47D cells. Three phenolic compounds devoid of estrogenic activity and toxicity emerged from this screening. Their sulfamate analogs were then synthesized, tested in STS-transfected HEK-293 cells, and found to be potent inhibitors of the enzyme (IC_{50} of 3.9, 8.9, and 16.6 nM). When tested in T-47D cells they showed no estrogenic activity and produced a moderate antiestrogenic activity. The compounds were further tested on osteoblast-like Saos-2 cells and found to significantly stimulate their proliferation as well as their alkaline phosphatase activity, thus suggesting a SERM activity. These results are supported by molecular docking experiments.

1. Introduction

Hormonal therapy is commonly used for the treatment of estrogen-sensitive breast cancer. As the majority of breast cancers are initially estrogen-dependent, with approximately 55% in pre-menopausal women and 75% in post-menopausal women, this therapy efficiently blocks the stimulating effect of estrogens in breast cancer cells [1]. Selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene, are compounds that are presently used to treat breast cancer [2]. In breast tissues, SERMs effectively block the activation of estrogen receptor alpha (ERα) by endogenous ligands and prevent the transcription of genes mediated by estrogen response elements [3]. This class of compounds possesses the particularity of having tissue-specific effects on ERα, resulting in antagonist activity in breast and uterus tissues and agonist activity in bone. Although tamoxifen and raloxifene possess the desired SERM activity, they also increase the risk of venous thromboembolism [4,5]. As a result, the development of new SERMs is still active and needed in order to obtain SERMs with fewer side effects [6–9].

Inhibition of steroid sulfatase (STS) is a therapeutic approach for the treatment of estrogen-dependent breast cancer and different kinds of inhibitors were developed in this sense over the past years [10–22]. STS is an enzyme that converts inactive sulfated steroids, mainly pregnenolone sulfate (PREGS), estrone sulfate (E1S), and dehydroepiandrosterone sulfate (DHEAS), into corresponding

Abbreviations: ALP, alkaline phosphatase; DEA, diethylamine; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; 5-diol, 5-androstene-3β,17β-diol; DIPEA, diisopropylamine; E1, estrone; E1S, estrone sulfate; E2, estradiol; ERα, estrogen receptor alpha; HFIP, hexafluoroisopropanol; HOBt, hydroxybenzotriazole; IC_{50}, concentration inhibiting 50%; LRMS, low-resolution mass spectrometry; PREGS, pregnenolone sulfate; PyBOP, benzotriazol-1-yl-oxy-tripyrrolidinophosphonium hexafluorophosphate; SERM, selective estrogen receptor modulator; STS, steroid sulfatase; TEA, triethylamine.

* Corresponding author. Laboratory of Medicinal Chemistry, CHU de Québec – Research Center (CHUL, T4-42), 2705 Laurier Boulevard, Québec, QC, G1V 4G2, Canada.
E-mail address: donald.poirier@crchul.ulaval.ca (D. Poirier).

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unconjugated hormones (Fig. 1) [23,24]. E1S and DHEAS are particularly abundant in blood circulation and could act as a reservoir of steroid precursors [25]. It was also shown that STS activity in breast cancer tumors is much higher than aromatase activity and that *in situ* formation of estrone (E1) and estradiol (E2) is mainly done via the STS pathway rather than by the aromatase pathway [26–28]. Therefore, blocking STS could prevent estrogen-sensitive carcinomas from transforming sulfated steroids into potent estrogens, mainly E1, E2, and 5-androstene-3β,17β-diol (5-diol). Using a single compound inhibiting both STS and aromatase activities could be also a promising strategy [29,30].

The dual blocking of ERα and STS is an interesting therapeutic approach for the treatment of (ER^+) breast cancer by achieving maximum estrogen blockade. However, the maximum estrogen blockade obtained by such treatment should induce an estrogen depletion condition that could lead to undesirable side effects such as osteoporosis [31]. An approach to counter these unwanted effects resides in developing a sulfamoylated inhibitor of STS with SERM properties. This kind of compound should reduce the potential problem related to an estrogen depletion induced by STS inhibitor monotherapy. Indeed, this dual-action strategy was reported by Rasmussen et al. [32], who sulfamoylated the steroidal SERM SR 16137 to its corresponding sulfamate form (SR 16157) as STS inhibitor. However, this STS inhibitor was found to be active as SERM only in its phenolic form, which required the hydrolysis of the sulfamate group by STS in order to interact with ERα and to provide the desired SERM properties. Considering that a very small quantity of phenol should be released *in vivo* from the hydrolysis of the sulfamate group coming from irreversible STS inhibition, this approach has the disadvantage of not generating a sufficient physiological concentration of the phenol needed to exert a relevant SERM action.

An alternative approach, investigated in our laboratory [33], is to obtain a sulfamate-based STS inhibitor directly active as SERM, whose effect could be additive to the SERM action of the phenol counterpart released from sulfamate hydrolysis by STS. A first generation of compounds showed a good inhibition of STS but did not possess the SERM capacity we were looking for. Indeed, the compounds were found to be estrogenic on breast cancer cells [34]. Here we report the synthesis of second generation dual-action compounds designed to inhibit STS and to act as a SERM (Fig. 2). These non-steroidal compounds were built around a tetrahydroisoquinoline scaffold and the rational to use this scaffold was previously reported [33]. Phenol and sulfamate derivatives were both synthesized by parallel solid-phase chemistry using a multi-detachable sulfamate linker [35–37]. Three phenolic compounds showing good results and their corresponding sulfamate compounds were selected for further testing. The six selected compounds were tested in HEK-293 transfected cells as STS inhibitors, on T-47D cells to evaluate their non-estrogenic and antiestrogenic properties and on osteoblast-like Saos-2 cells to evaluate their capacity to stimulate the cell proliferation and alkaline phosphatase activity. Finally, molecular docking simulations were achieved to predict the binding modes of the compounds in the STS and ERα binding sites.

2. Results and discussion

2.1. Selection and chemical synthesis of secondary amines as building blocks

The choice of the secondary amines (compounds **1a**-**b**, **2a**-**b**, **3c**, **4c**, **5**–**11**) used as building blocks for the preparation of phenolic derivatives (compounds **19**–**31**) was guided by their potential capacity to interact either with STS enzyme (hydrophobic substituents) or with the estrogen receptor (H-bond acceptor groups). In the case of STS, it is well known that hydrophobic chains are well tolerated considering the presence of a large hydrophobic pocket in the active site of the enzyme [38]. We thus selected hydrophobic secondary amines with a 4-bromophenyl, furanyl, or thiophenyl group. Interestingly, the 4-bromophenyl and furanyl groups were previously found to be the most potent substituents from a series of tetrahydroisoquinoline derivatives synthesized as STS inhibitors in a first structure-activity relationship (SAR) study [33].

In addition to the use of a hydrophobic side-chain for STS inhibition, we were interested by amines that bear hydrogen bond acceptor group, such as pyridine, imidazole, morpholine, or piperidine. These chemical groups would favor interaction with a key amino acid of the ER. Indeed, it is known that a key amino acid like Asp351, which is involved in the stabilisation of ER-H12 helix, could be targeted to induce SERM activity [39]. For that purpose, we selected amines of different sizes, shapes and hydrogen bond acceptor capacity. Particularly, we synthesized the phenoxypropylpiperidine chains which have been frequently reported as an important pharmacophore in several SERM compounds [40].

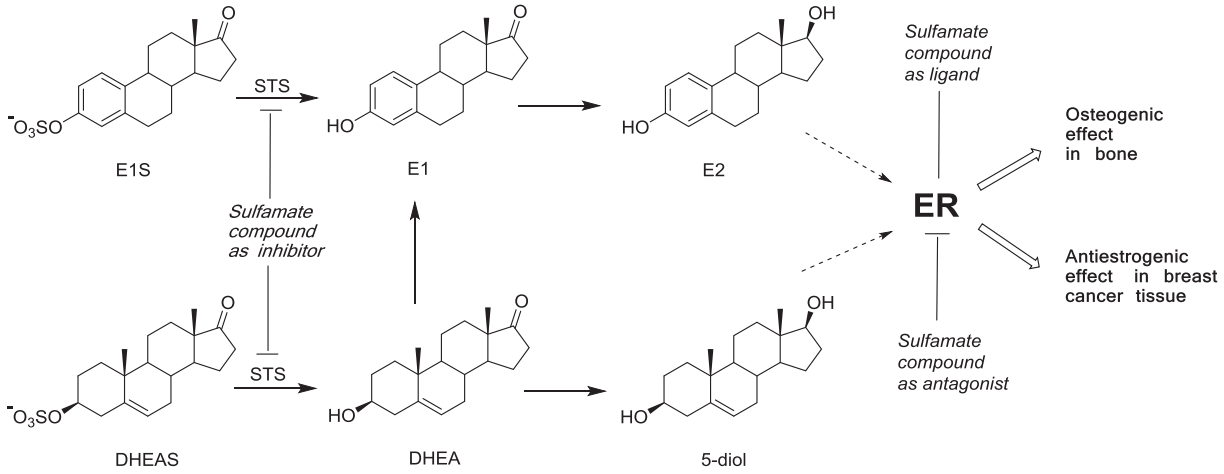

Fig. 1. Transformation of sulfated steroid E1S and DHEAS into estrogenic hormones (E2 and 5-diol) by steroid sulfatase (STS) and sites of dual-action sulfamate compounds. The sulfamate compound inhibits the STS and can act as agonist or antagonist of the estrogen receptor (ER) depending on the tissues.