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Research paper

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

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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.

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0223-5234/

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].

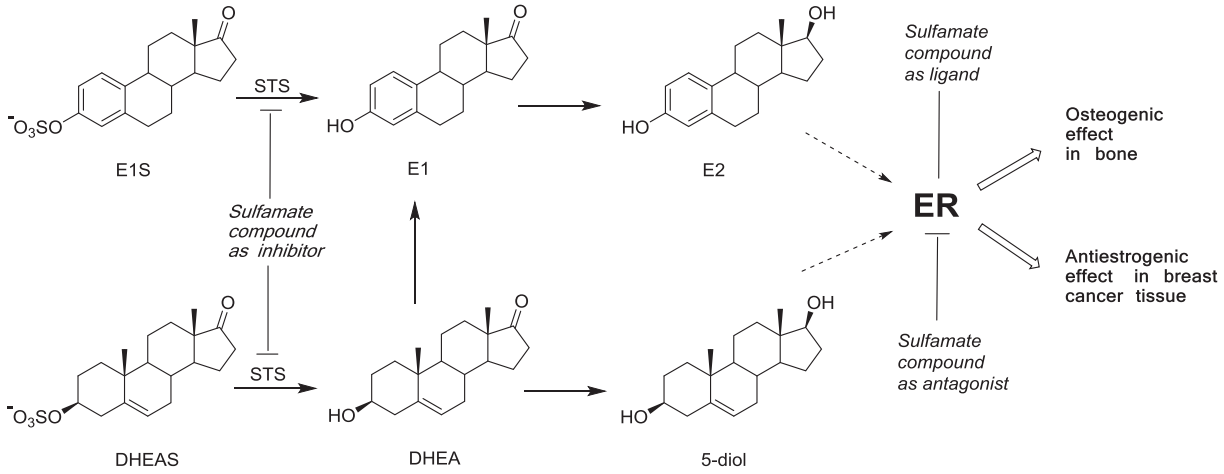



**Fig. 1.** Transformation of sulfated steroid E1S and DHEAS into estrogenic hormones (E2 and 5-diol) by steroid sulfatase (STS) and sites of dual-action sulfamate compounds. The sulfamate compound inhibits the STS and can act as agonist or antagonist of the estrogen receptor (ER) depending on the tissues.

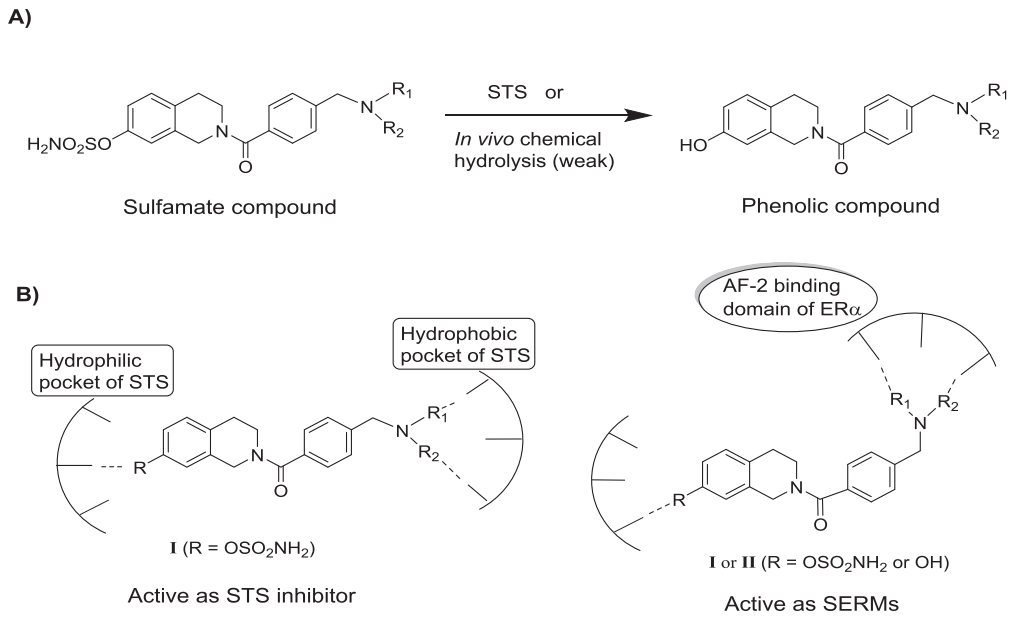



**Fig. 2.** Second generation of dual-action STS inhibitors and SERMs. A) Conversion of sulfamate compounds into phenolic compounds by STS or by chemical hydrolysis. B) The sulfamate compound (I) binds to the active site of STS, thus inactivating the enzyme. The sulfamate compound (I), by itself or via the released phenolic compound (II), binds to ERα and could act as a SERM.

### 2.2. Chemical synthesis of secondary amines as building blocks (compounds *1a-b, 2a-b, 3c, 4c, 5–11*)

Non commercially available secondary amines **1a**-**b**, **2a**-**b**, **3c**, and **4c** were synthesized under classic conditions of reductive amination, which consisted in reacting the aldehydes **1**–**4** with appropriate amines **a**-**c** followed by the reduction of the intermediate imine with sodium borohydride (Scheme 1) [41]. The aldehydes **1** and **2** were synthesized beforehand by reacting 2- or 4-hydroxybenzaldehyde, 1-(3-chloropropyl)piperidine, sodium carbonate and sodium iodide in refluxing acetone.

### 2.3. Chemical synthesis of compounds *19–34*

The general synthetic methodology for the preparation of all library members is outlined in Scheme 2. The starting 1,2,3,4-tetrahydroisoquinolin-7-ol (**12**) was selectively protected as the *N*-Fmoc derivative **13**, which after a sulfamoylation of the phenol group yielded **14**. This sulfamate derivative was then reacted with the trityl chloride resin to give the solid-phase bound compound **15**. Removal of the Fmoc protecting group by a treatment with 20% piperidine in DMF provided the resin **16** with a free NH, which was acylated with an activated ester of carboxybenzaldehyde to give the resin **17**. Diversification of **17** was obtained by performing a reductive amination with various secondary amines that yielded resins **18**. Finally, the phenolic derivatives **19**–**31** were obtained by a nucleophilic cleavage from resin **18**. The sulfamate compounds **32**–**34** were synthesized following the same solid-phase approach used for the synthesis of **19**–**31**. In that case, however, an acid cleavage using hexafluoroacetic acid (HFIP) was used at the end of the sequence of reactions to release **32**–**34**.

### 2.4. Screening of phenolic compounds *19–31* in T-47D ($ER^+$) cells

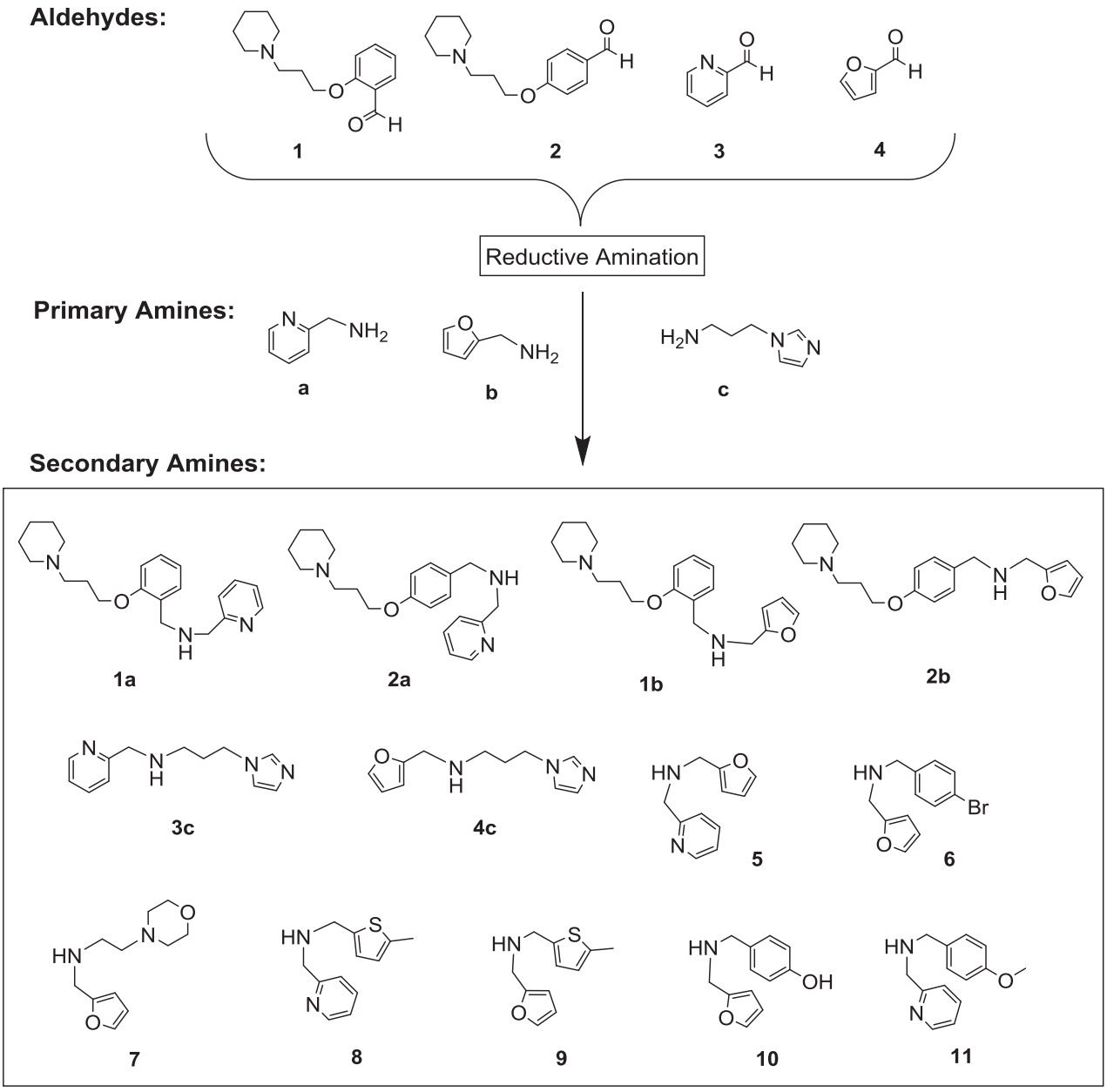
The library of phenolic compounds described above was tested on estrogen-sensitive breast cancer T-47D cells (Table 1). Compounds were discarded if they stimulated T-47D cell proliferation (estrogenic activity) or if they showed some cytotoxicity by reducing the cell number. Amongst the library of phenolic compounds, only compounds **25**, **29**, and **31** showed interesting results. In fact, they showed neither estrogenicity nor cytotoxicity on T-47D cells at the concentrations tested (0.01, 0.1 and 1 μM). Such results suggest that these three compounds do not possess the ability to activate ERα in breast cancer cells and consequently to induce unwanted cell proliferation.

### 2.5. Steroid sulfatase inhibition

Following the results obtained in the initial screening with phenolic compounds **25**, **29**, and **31**, we synthesized the sulfamate analogs (compounds **32**, **33**, and **34**) and evaluated their capacity to inhibit the STS activity. For the enzymatic assay, we used homogenated HEK-293 cells overexpressing STS and evaluated the capacity of compounds to inhibit the conversion of [$^3$H]-E1S into [$^3$H]-E1 (Fig. 3). As reported previously in literature [42–46], phenolic compounds are less potent STS inhibitors than their sulfamoylated analogs. Our results confirm this affirmation, since only the sulfamate compounds inhibited STS. Effectively, the phenolic compounds showed no significant inhibition while the sulfamate compounds showed very good inhibition of STS with $IC_{50}$ values of 16.6 ± 2.7 nM for **32**, 8.9 ± 1.2 nM for **33**, and 3.9 ± 1.1 nM for **34**. Interestingly, these three sulfamate compounds demonstrated a higher STS inhibition potency than our previous generation of dual-action compounds, where one of the best STS inhibitors had an $IC_{50}$ of 300 nM [34]. Non-steroidal sulfamate compounds **32**–**34** were however less potent inhibitors than steroidal sulfamate derivative EM-1913 [42–44] used as reference compound ($IC_{50}$ ~ 0.05 nM). Therefore, only the sulfamate compounds **32**–**34** and corresponding phenolic compounds **25**, **29**, and **31** were tested for additional *in vitro* properties.

### 2.6. Effect on breast cancer T-47D ($ER^+$) cell proliferation of selected sulfamate and phenolic compounds (*25, 29, 31–34*)

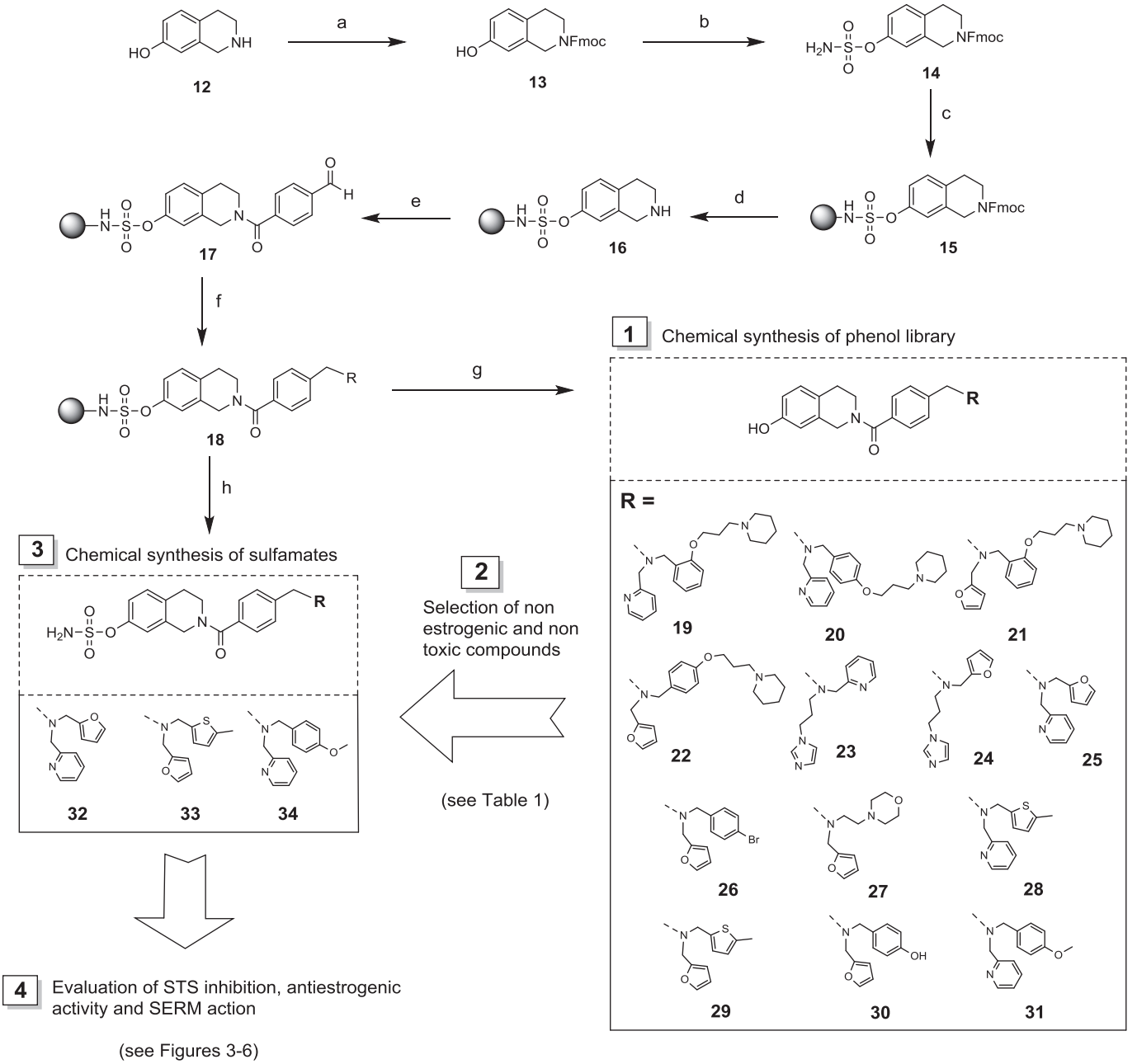
As the three sulfamate compounds **32**–**34** are potent inhibitors of STS, we wanted to know if they were devoid of estrogenic




**Scheme 1.** Preparation of building blocks (secondary amines **1a-b**, **2a-b**, **3c**, and **4c**) and structures of all commercially available secondary amines **5**–**11** used for the synthesis of targeted tetrahydroisoquinoline phenolic derivatives.

activity. Along with their three phenolic analogs **25**, **29**, and **31**, and to reconfirm the results of the initial screening, we tested the six compounds on breast cancer estrogen-sensitive T-47D cells (Figs. 4A and B). We used the estrogenic natural hormone E2 as a positive control of cell proliferation stimulation, and raloxifene, a SERM with no estrogenicity in breast tissue. E2, at a concentration of 0.1 nM, induced approximately 160% cell proliferation while raloxifene showed no estrogenic activity. Our six compounds showed interesting results because none of them stimulated the proliferation of estrogen-sensitive T-47D cells. On the other hand, we observed a reduction of cell proliferation at 5 μM, suggesting cytotoxic activity for raloxifene and compounds **31**–**34**. This should not be a problem as 5 μM is a high dose hardly achievable *in vivo* and that even raloxifene, a SERM used in clinic, induced some cytotoxicity at this concentration. As a result, both sulfamate and phenolic compounds can be used on breast cancer cells without stimulating their proliferation.

We next evaluated the capacity of compounds **25**, **29**, **31**–**34** to block the stimulation of T-47D cells proliferation induced by E2 (Fig. 5A and B). The cells were incubated with our six compounds and raloxifene at different concentrations in the presence of E2 (0.1 nM). Raloxifene was able to block the E2 (0.1 nM) stimulation at concentrations of 0.01–1 μM and still showed some cytotoxicity at 5 μM. For our six compounds, the results show that they all possess some antiestrogenic activity in T-47D cells. Because compounds **31**–**34** showed cytotoxicity at 5 μM in the previous test, their apparent antiestrogenicity at this concentration was not considered. The phenol derivative **29**, which did not reduce the cell proliferation at 5 μM, possesses the best antiestrogenic activity at this concentration since it blocked approximately 84% of the stimulation induced by E2. Even if compound **29** is not as good antiestrogenic as raloxifene, it still got the properties we were looking



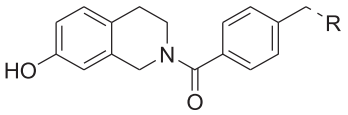

**Scheme 2.** Reagents and conditions for the chemical synthesis of phenolic derivatives (compounds **19**–**31**) and sulfamate derivatives (compounds **32**–**34**). Representation of the four steps (#1–4) behind the identification of dual-action compounds. (a) Fmoc-*O*-succinimide, $NaHCO_3$, $H_2O$; (b) $NH_2SO_2Cl$, 2,6-di-*tert*-butyl-4-methylpyridine, DCM; (c) trityl chloride resin, DIPEA, DMA, DCM; (d) 20% piperidine in DMF; (e) carboxybenzaldehyde, DIPEA, HOBt, PyBOP, DMF; (f) secondary amine ($R_1R_2NH$), $NaBH(OAc)_3$, 10% AcOH in NMP; (g) 30% DEA in THF, 60 °C; (h) 30% HFIP in DCM.

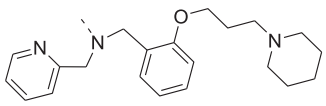
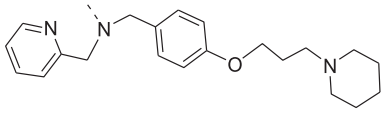
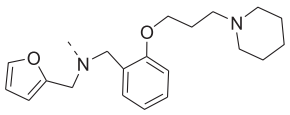
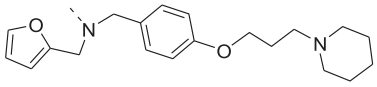
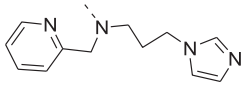
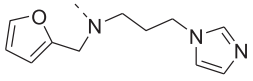
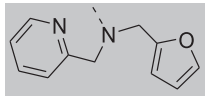
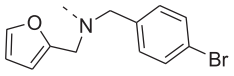
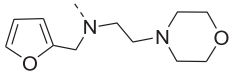
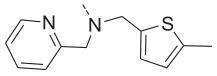
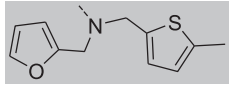
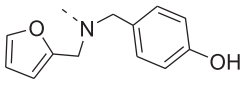
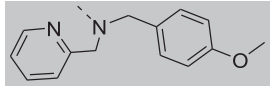
for in breast tissue.

### 2.6. Effect on osteoblast-like Saos-2 cells (proliferation)

As a SERM possesses the ability to block ERα in breast tissue and to activate ERα in other tissues, such as bone, we investigated the action of compounds **25**, **29**, **31**–**34** on the osteoblast-like Saos-2 cell proliferation. To do so, we incubated Saos-2 cells for 7 days with E2 or raloxifene, as a SERM reference compound, and our six compounds of interest at concentrations of 0.1 and 1 μM (Fig. 6A). All compounds, including E2 and raloxifene, significantly induced Saos-2 cell proliferation at 1 μM. However, the induced proliferation is quite low with the exception of the phenolic compound **29** and its sulfamate analog **33**, which both induced cell proliferation approximately by 160%. The slight cell proliferation observed is probably due to the osteoblast cells themselves. One of the main roles of osteoblasts in bone tissue is to construct a calcified extracellular matrix [31]. As such, bone formation is not principally due to osteoblast proliferation, but rather osteoblast maturation into mature osteocytes. To further investigate the high cell proliferation induced by compounds **29** and **33** and to confirm the first results, Saos-2 cells were incubated 7 days with **29** or **33** at a wider range of concentrations (Fig. 6B). We observed an apparent dose-dependent response from 1 nM to 1 μM for the cell proliferation induced by raloxifene, **29**, and **33**. At higher concentrations, however, all three compounds reduced Saos-2 cell proliferation, suggesting a

**Table 1**
Structure of phenolic compounds **19**–**31** and their capacity to modulate breast cancer T-47D ($ER^+$) cell prolifefation at three concentrations.




| ID | R | Proliferation of T-47D cells (%)[a] | | |
|---|---|---|---|---|
| | | 0.01 μM | 0.1 μM | 1 μM |
| **19** |  | 102.8 ± 6.9 | 99.8 ± 4.2 | 37.1 ± 4.2 |
| **20** |  | 105.9 ± 1.0 | 104.5 ± 4.8 | 60.7 ± 6.4 |
| **21** |  | 96.8 ± 1.2 | 105.9 ± 5.6 | 0 ± 9.1 |
| **22** |  | 95.6 ± 1.8 | 119.7 ± 4.8 | 133.7 ± 3.0 |
| **23** |  | 102.8 ± 2.2 | 112.9 ± 3.7 | 39.2 ± 2.9 |
| **24** |  | 94.9 ± 1.3 | 98.5 ± 4.5 | 114.6 ± 5.9 |
| **25** |  | 104.1 ± 2.3 | 103.2 ± 2.5 | 103.5 ± 6.5 |
| **26** |  | 106.3 ± 0.6 | 109.4 ± 1.2 | 59.2 ± 3.1 |
| **27** |  | 108.2 ± 5.5 | 105.9 ± 1.5 | 118.8 ± 9.6 |
| **28** |  | 100.6 ± 3.8 | 105.4 ± 4.4 | 78.9 ± 6.8 |
| **29** |  | 97.1 ± 2.6 | 103.2 ± 1.8 | 96.8 ± 2.2 |
| **30** |  | 108.2 ± 5.5 | 138.0 ± 1.2 | 200.3 ± 4.9 |
| **31** |  | 105.6 ± 0.6 | 104.6 ± 7.4 | 100.9 ± 7.9 |

The grey shades represent compounds showing interesting results.
[a] Control cell proliferation is set to 100%. Results are expressed as mean ± SD of triplicate measurements.

cytotoxic effect. Even if the mechanism by which **29** and **33** induced cell proliferation at concentrations lower than 1 μM remains unclear, all compounds tested clearly induced a desirable effect in osteoblasts.

### *2.7. Effect on osteoblast-like Saos-2 cells (alkaline phosphatase activity)*

Compounds **25**, **29**, **31** **34** had an apparently beneficial effect on

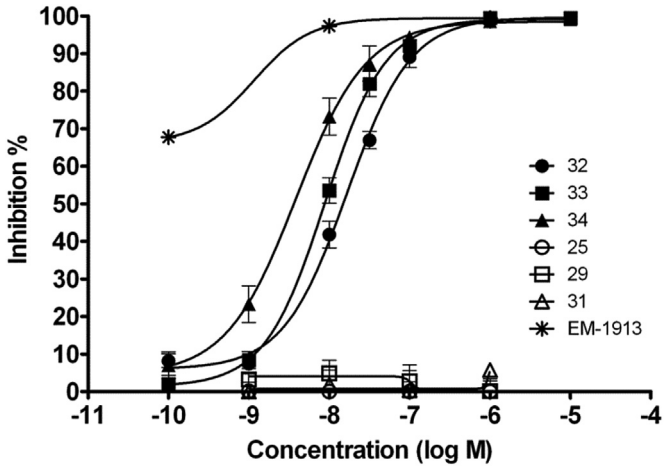



**Fig. 3.** Effect of sulfamate compounds **32**, **33**, and **34** and their phenolic analogs **25**, **29**, and **31**, respectively, on STS activity in homogenates of transfected HEK-293 cells. The three sulfamate derivatives inhibited STS ($IC_{50}$ = 16.6 ± 2.7 nM, 8.9 ± 1.2 nM, and 3.9 ± 1.1 nM for **32**, **33**, and **34**, respectively) while the three phenolic analogs showed no significant inhibition of STS. Results are expressed as % inhibition of [$^3$H]-E1S conversion into [$^3$H]-E1 by STS. Each point represents the mean ± SD of triplicate measurements.

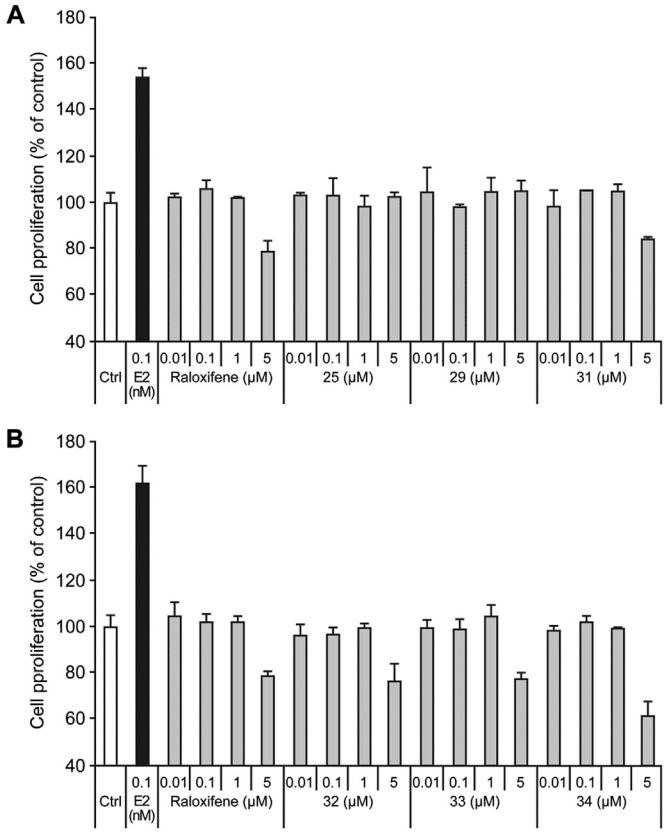



**Fig. 4.** Effect of phenolic (**A**) and sulfamate (**B**) compounds on estrogen-sensitive T-47D (ER$^+$) cell proliferation after 7 days of treatment. The estrogenicity of phenol derivatives **25**, **29**, and **31** as well as sulfamate derivatives **32**, **33**, and **34** was evaluated by incubating the cells with different concentrations (0.01, 0.1, 1, and 5 μM) of each compound. The proliferation of control cells is set to 100%. Results are expressed as means ± SD of triplicate measurements.

osteoblasts. Since they induced only a slight stimulation in Saos-2 cell proliferation, such as raloxifene, we decided to investigate the

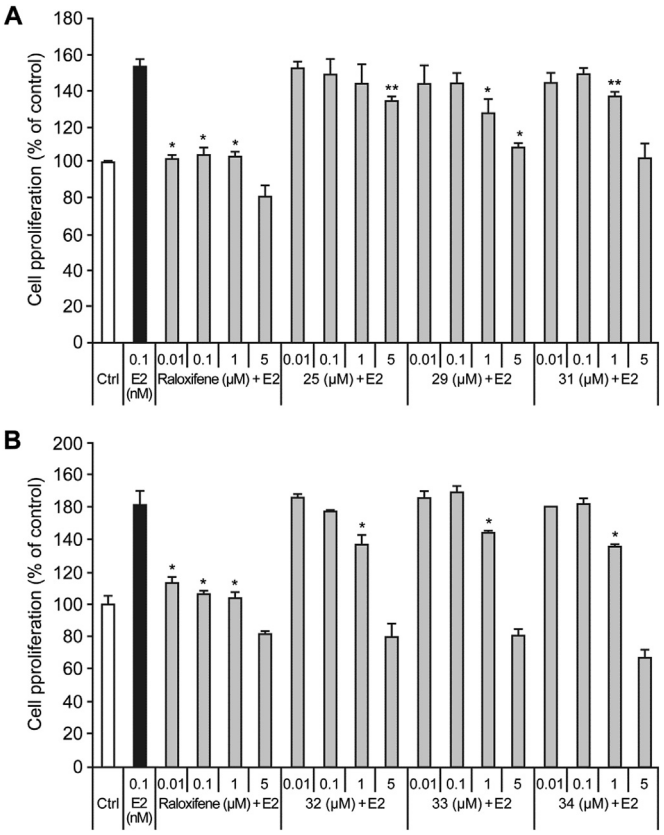



**Fig. 5.** Effect of phenolic (**A**) and sulfamate (**B**) compounds on estrogen-sensitive T-47D (ER$^+$) cell proliferation after 7 days of treatment. The antiestrogenicity of phenol derivatives **25**, **29**, and **31** as well as sulfamate derivatives **32**, **33**, and **34** was evaluated by incubating the cells with different concentrations (0.01, 0.1, 1, and 5 μM) of each compound and E2 (0.1 nM). The proliferation of control cells is set to 100%. Results are expressed as means ± SD of triplicate measurements. * $p \leq 0.01$ vs. E2, ** $p \leq 0.05$ vs. E2.

activity of a differentiation marker, the alkaline phosphatase (ALP). ALP is an enzyme that releases inorganic phosphate from different intracellular substrates such as pyrophosphate and pyridoxal 5'-phosphate [47]. The inorganic phosphate is used in the formation of hydroxyapatite crystals which are then inserted in the extracellular protein scaffold to form the solid part of bone tissue. In osteoblast cells, ALP is regulated by estrogens and is a good indicator of osteoblast differentiation [48]. We tested our compounds, raloxifene and E2, in Saos-2 cells and investigated their effect on ALP activity (Fig. 7). All compounds significantly increased ALP activity in Saos-2 cells, but it is interesting to see that **29** induced the highest ALP activity (138%).

### 2.8. Structural investigation

A molecular docking study was achieved to highlight the structural determinants that are essential for STS inhibition and SERM activity. As the same conclusions were drawn for all the compounds of interest, the docking results are presented only for phenolic compound **29** and sulfamate compound **33** (the results for compounds **25**, **31**, **32** and **34** are included in the Supporting Information).

Currently, only one experimental structure of human STS is available (PDB 1P49) [38]. Although there is no inhibitor present in this structure, a sulfate group is covalently bound to the catalytic residue hydroxyformylglycine, which is usually involved in a

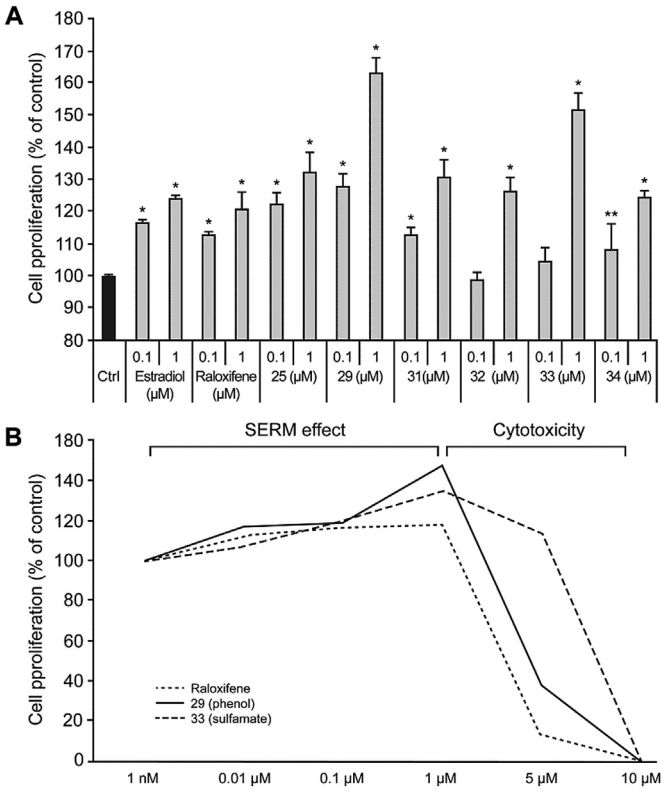



**Fig. 6.** Effect of phenolic and sulfamate compounds on Saos-2 cell proliferation after 7 days of treatment. **A)** Saos-2 cells were incubated with E2, raloxifene, **25**, **29**, **31** **34** at 0.1 and 1 μM. **B)** The cells were incubated with raloxifene, phenolic compound **29,** or sulfamate compound **33** at concentrations ranging from 1 nM to 10 μM. The proliferation of control cells is set to 100%. Results are expressed as means ± SD of triplicate measurements.

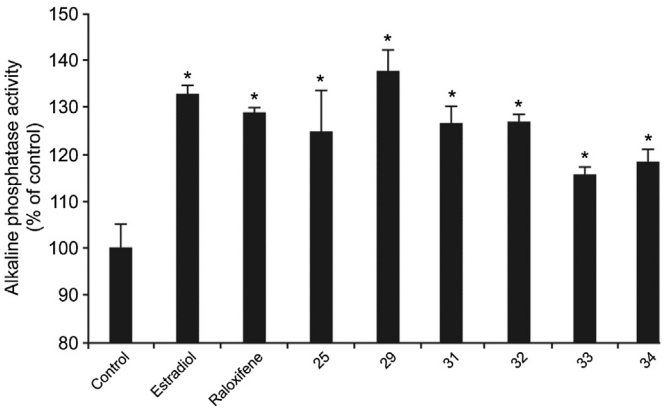



**Fig. 7.** Effect of phenolic and sulfamate compounds on alkaline phosphatase (ALP) activity in Saos-2 cells after 3 days of treatment. Saos-2 cells were incubated with E2, raloxifene, or a compound of interest at a concentration of 0.1 nM. The ALP activity of control cells is set to 100%. Results are expressed as means ± SD of triplicate measurements. * $p \leq 0.01$ vs. control.

covalent binding to the substrates [49,50]. As detailed in the *Experimental section*, we have used EM-1913, a highly potent STS inhibitor [42], to develop the docking procedure in STS. Shortly, the hydroxyformylglycine residue was replaced by a glycine and the corresponding covalently-bound sulfate group was removed. The coordinates of the EM-1913 molecule were initialized with its sulfamate moiety located at the position of the former crystallographic hydroxyformylglycine sulfate group. Using this procedure, the EM-1913 sulfamate moiety of the best docking pose is located within 0.6 Å of the crystallographic hydroxyformylglycine sulfate group (Fig. 8).

The STS binding site is composed of a long hydrophobic tunnel that accommodates the steroid scaffold. Fig. 8 presents the best docking results for sulfamate compound **33** superimposed on the best docking result of compound EM-1913 in the STS binding site. As for the sulfamate moiety of EM-1913, H-bond interactions are observed between the sulfamate moiety of compound **33** with the calcium ion and Lys134. These docking results clearly show that the hydrophobic tunnel of STS binding site comfortably accommodates the elongated structure of compound **33**, despite the absence of a steroidal scaffold. This docking result of compound **33** is in good agreement with its observed STS inhibition activity.

Many experimental structures of ERα are available for the APO protein or bound to either an agonist or an antagonist. In this study, molecular docking was carried out using the raloxifene-bound structure (PDB 1ERR) [51], and the docking results were compared to the agonist-bound structure (PDB 1ERE) [51]. These two structures differ in the conformation of the H12 α-helix: a conformational change involving this helix occurs in the presence of raloxifene and, as a result, the helix is no longer located in the binding pocket (Fig. 9, *black cartoon*). To induce this conformational change, the raloxifene core binds to the E2 binding site, interacting with Glu353, Arg394 on one side and His524 on the other side, and leaving the piperidine moiety at the entry of the pocket to interact with Asp351. The best docking conformations for compounds **29** and **33** in the ERα binding site are presented in Fig. 9. From these results, the furanyl and methylthiofuranyl groups of phenolic

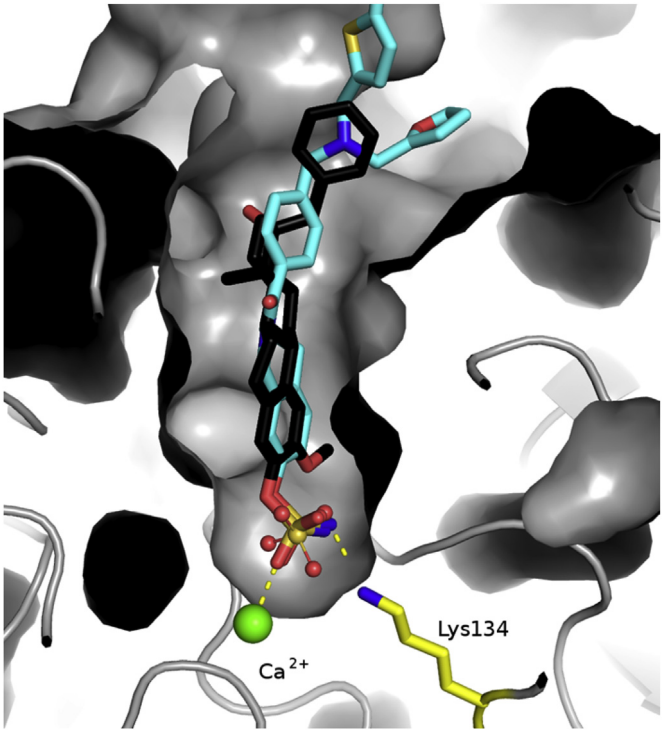



**Fig. 8.** The best docking results for the STS inhibitor EM-1913 and the sulfamate compound **33** in the STS binding site. EM-1913 and the sulfamate compound **33** are represented by black and cyan sticks, respectively. STS from PDB 1P49 is represented by gray cartoon, Lys134 by yellow sticks, and the calcium ion by a green sphere. The hydroxyformylglycine bound sulfate is shown as lines and spheres for reference. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

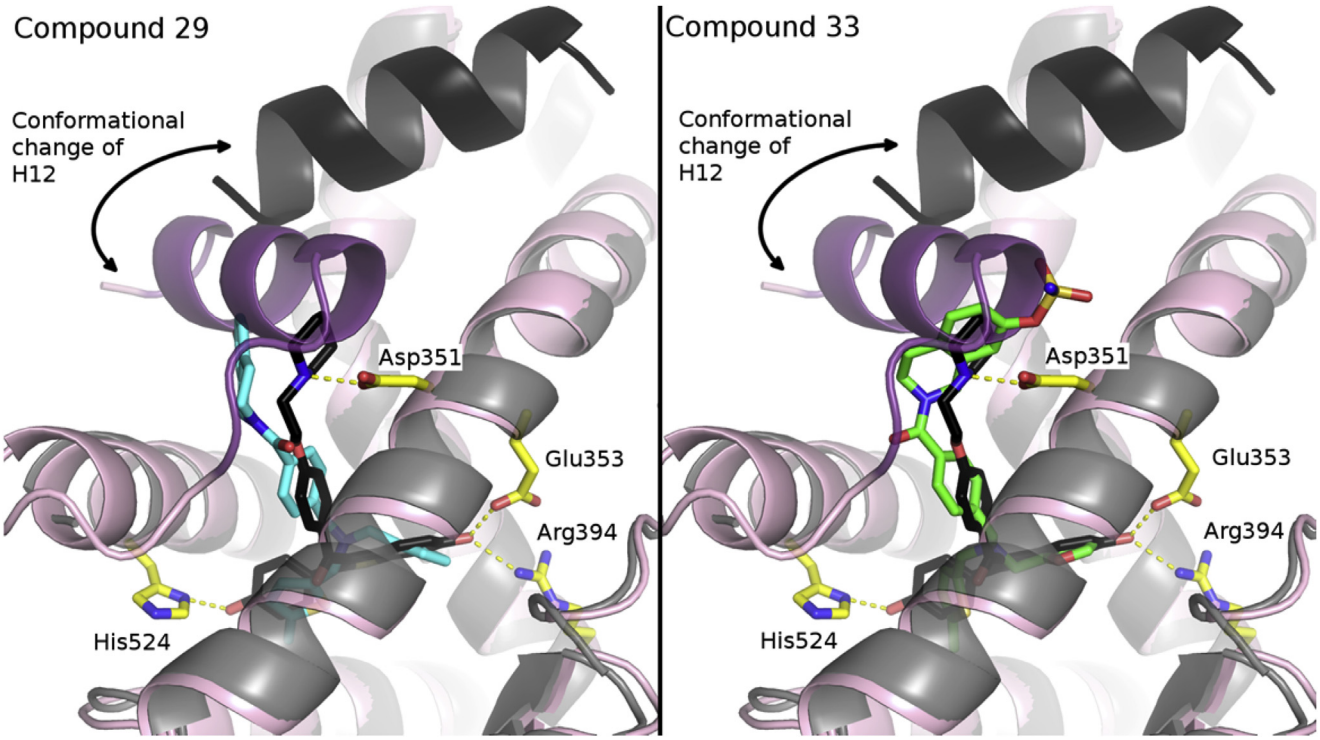



**Fig. 9.** Docking results for phenolic compound **29** (*left*, cyan sticks) and sulfamate compound **33** (*right*, green sticks) in the ERα binding site. ERα/raloxifene complex structure (PDB 1ERR) is represented by gray cartoon, H12 by black cartoon, and raloxifene, used as reference, by black sticks. H-bonds between ERα and raloxifene are identified by dashed yellow lines. ERα/E2 complex structure (PDB 1ERE) is represented by pink cartoon and H12 by purple cartoon. The black arrows emphasize the H12 conformational change between both PDB structures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compound **29** and sulfamate compound **33** are positioned in the estrogen pocket, orienting the phenol or sulfamate moiety toward the solvent. While compounds **29** and **33** realize no H-bond with ERα, this conformation is still in agreement with the hydrophobic character of the pocket and correlates with the geometry of raloxifene, suggesting a competitive binding mode with E2. In addition, the best binding conformation of both compounds occupy a similar location as raloxifene in reference with H12, suggesting that compounds **29** and **33** would also induce a H12 conformational change as does raloxifene, supporting the SERM activity observed in Figs. 4–6.

## 3. Conclusion

In summary, we described the chemical synthesis and *in vitro* biological evaluation of tetrahydroisoquinoline derivatives designed to inhibit STS and to act as SERMs. The phenolic compounds and three sulfamate analogs were all synthesized by parallel solid-phase chemistry using a multidetachable sulfamate linker. The library of phenols was tested on estrogen-sensitive breast cancer T-47D cells to discard compounds bearing estrogenicity or cytotoxicity at concentrations tested. In the library, phenolic derivatives **25**, **29**, and **31** showed no such undesirable activities and were selected for further testing. Their sulfamate analogs **32**, **33**, and **34** were tested on homogenated HEK-293 cells overexpressing STS and demonstrated to be strong STS inhibitors with $IC_{50}$ values of 16.6, 8.9, and 3.9 nM, respectively. All six compounds were tested on T-47D cells and showed no estrogenicity and even some antiestrogenic activity. Following this, we tested our compounds on osteoblast-like Saos-2 cells as SERMs must be active in tissues where estrogenic activity is beneficial. All compounds stimulated Saos-2 cell proliferation with phenolic compound **29** and sulfamate compound **33** being the best stimulator of each family. These molecules also increased the ALP activity. Results from molecular docking simulations suggest that the hydrophobic tunnel of the STS binding site accommodates the elongated structure of compound **33** despite the absence of a steroidal scaffold. This result is in agreement with its observed inhibition activity. Moreover, docking of phenolic compound **29** and sulfamate compound **33** led to a binding similar to that of raloxifene, *i.e.* the furanyl group of compound **29** and methylthiofuranyl group of compound **33** positioned in the estrogen pocket and the phenol or sulfamate moiety oriented toward the solvent. These results suggest that compounds **29** and **33** bind in a competitive manner with E2. The results obtained in this study are promising considering that compound **33** represents the first example of a sulfamate-based inhibitor with intrinsic SERM activity. This suggests that this compound could be used in future *in vivo* testing as a dual-action compound for the treatment of estrogen-dependent breast cancer.

## 4. Experimental section

### 4.1. Chemistry (general information)

Chemical reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and solvents were obtained from Fisher Scientific (Montréal, QC, Canada) and VWR (Ville Mont-Royal, QC, Canada). Trityl chloride resin was supplied by EMD Biosciences (Novabiochem, La Jolla, CA, USA). Flash chromatography was performed on Silicycle 60 230–400-mesh silica gel (Québec, QC, Canada). Thin-layer chromatography was performed on Whatman 0.25-mm silica gel 60 $F_{254}$ plates (Fisher Scientific, Nepean, ON, Canada) and compounds were visualized by exposure to UV light

(254 nm) or/and to a solution of ammonium molybdate/sulphuric acid/ethanol (plus heating). $^1$H NMR spectra were recorded at 400 MHz, using a Bruker AVANCE 400 spectrometer (Billerica, MA, USA). The chemical shifts ($\delta$) are expressed in ppm and referenced to chloroform (7.26 ppm), acetone (2.05 ppm), or methanol (3.31 ppm). Low-resolution mass spectra (LRMS) were recorded on a Shimadzu Prominence apparatus (Kyoto, Japan) equipped with an atmospheric pressure chemical ionization (APCI) source on positive mode.

### 4.2. Synthesis of aldehydes **1** and **2**

To a solution of 2- or 4-hydroxy-benzaldehyde (1.64 mmol) in anhydrous acetone (25 mL) was added cesium carbonate (4.92 mmol) and the solution was stirred at room temperature for 10 min under an argon atmosphere. Chloropropylpiperidine hydrochloride (2.46 mmol) and sodium iodide (0.82 mmol) was added to the solution and the mixture was heated at reflux overnight. The resulting solution was filtered and evaporated to dryness. The crude compound was diluted with EtOAc, the solution was washed successively with a saturated sodium carbonate solution and water, dried over $MgSO_4$, filtered, and evaporated under reduce pressure. Purification by flash chromatography (hexanes/acetone/TEA, 80:19:1 to 70:29:1) yielded the desired compound **1** (360 mg, 89%) or **2** (400 mg, 99%).

#### 4.2.1. 2-[3-(piperidin-1-yl)propoxy]benzaldehyde (**1**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.45 (q, $J$ = 6.0 Hz, 2H), 1.59 (p, $J$ = 5.6 Hz, 4H), 2.05 (m, 2H), 2.40 (broad s, 4H), 2.50 (t, $J$ = 7.4 Hz, 2H), 4.14 (t, $J$ = 6.3 Hz, 2H), 7.01 (t, J = 8.1 Hz, 2H), 7.53 (m, 1H), 7.83 (dd, $J$ = 1.8, 7.8 Hz, 1H), 10.51 (s, 1H). APCI-MS for $C_{15}H_{22}O_2N$ $[M+H]^+$: 248.3 *m/z*.

#### 4.2.2. 4-[3-(piperidin-1-yl)propoxy]benzaldehyde (**2**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.45 (m, 2H), 1.59 (q, $J$ = 5.6 Hz, 4H), 2.00 (m, 2H), 2.40 (broad s, 4H), 2.47 (t, $J$ = 7.4 Hz, 2H), 4.10 (t, $J$ = 6.4 Hz, 2H), 7.00 (d, $J$ = 8.7 Hz, 2H), 7.82 (d, $J$ = 8.7 Hz, 2H), 9.88 (s, 1H). APCI-MS for $C_{15}H_{22}O_2N$ $[M+H]^+$: 248.3 *m/z*.

### 4.3. Synthesis of secondary amines **1a**, **2a**, **1b**, **2b**, **3c** and **4c** (general procedure)

To a solution of aldehyde **1**, **2**, **3**, or **4** (1.21 mmol) in absolute ethanol (12 mL) was added the appropriate amine **a** (1-(pyridin-2-yl)methanamine), **b** (1-(furan-2-yl)methanamine), or **c** (3-(1*H* imidazol-1-yl)propan-1-amine) (1.45 mmol) and 4 Å molecular sieves. The solution was stirred at room temperature for 2.5 h and filtered to remove molecular sieves. Sodium borohydride (2.90 mmol) was then added in small portion to the resulting ethanol solution at 0 °C and the mixture allowed to return at room temperature and stirred overnight. Water (15 mL) was added and the solution stirred for 15 min before to be concentrated under reduced pressure. The aqueous layer was extracted with DCM (5 × 5 mL), and the combined organic phase was washed with brine, dried with $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography (DCM/MeOH/TEA, 98:1:1 to 90:9:1) yielded **1a** (370 mg, 90%), **2a** (318 mg, 78%), **1b** (320 mg, 70%), **2b** (347 mg, 76%), **3c** (400 mg, 99%), or **4c** (400 mg, 97%) according to the aldehyde and primary amine used.

#### 4.3.1. 1-{2-[3-(Piperidin-1-yl)propoxy]phenyl}-N-(pyridin-2-ylmethyl)methanamine (**1a**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.44 (m, 2H), 1.59 (p, $J$ = 5.6 Hz, 4H), 1.89 (broad s, NH), 2.01 (m, 2H), 2.38 (broad s, 4H), 2.48 (t, $J$ = 7.5 Hz, 2H), 3.87 (s, 2H), 3.92 (s, 2H), 4.03 (t, $J$ = 6.2 Hz, 2H), 6.85 (d, $J$ = 8.0 Hz, 1H), 6.90 (t, $J$ = 7.4 Hz, 1H), 7.14 (m, 1H), 7.21 (m, 1H), 7.27 (m, 1H), 7.37 (d, $J$ = 6.8 Hz, 1H), 7.63 (td, $J$ = 1.8, 7.7 Hz, 1H), 8.54 (ddd, $J$ = 0.9, 1.8, 4.9 Hz, 1H). APCI-MS for $C_{21}H_{30}ON_3$ $[M+H]^+$: 340.3 *m/z*.

#### 4.3.2. 1-{4-[3-(Piperidin-1-yl)propoxy]phenyl}-N-(pyridin-2-ylmethyl)methanamine (**2a**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.45 (m, 2H), 1.60 (p, $J$ = 5.6 Hz, 4H), 1.98 (m, 2H), 2.42 (broad s, 4H), 2.49 (t, $J$ = 7.5 Hz, 2H), 3.78 (s, 2H), 3.91 (s, 2H), 4.00 (t, $J$ = 6.4 Hz, 2H), 6.85 (d, $J$ = 8.6 Hz, 2H), 7.16 (ddd, $J$ = 1.2, 4.9, 7.5 Hz, 1H), 7.25 (d, J = 8.5 Hz, 2H), 7.31 (d, $J$ = 7.8 Hz, 1H), 7.64 (td, $J$ = 1.8, 7.7 Hz, 1H), 8.56 (ddd, $J$ = 1.0, 1.9, 4.9 Hz, 1H). APCI-MS for $C_{21}H_{30}ON_3$ $[M+H]^+$: 340.3 *m/z*.

#### 4.3.3. 1-(Furan-2-yl)-N-{2-[3-(piperidin-1-yl)propoxy]benzyl} methanamine (**1b**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.45 (m, 2H), 1.59 (p, $J$ = 5.6 Hz, 4H), 1.99 (m, 2H), 2.40 (broad s, 4H), 2.48 (t, J = 7.5 Hz, 2H), 3.76 (s, 2H), 3.81 (s, 2H), 4.03 (t, $J$ = 6.2 Hz, 2H), 6.18 (dd, $J$ = 1.0, 3.2 Hz, 1H), 6.31 (dd, $J$ = 1.8, 3.1 Hz, 1H), 6.86 (d, $J$ = 8.0 Hz, 1H), 6.90 (t, $J$ = 7.4 Hz, 1H), 7.22 (m, 2H), 7.36 (dd, $J$ = 0.6, 1.6 Hz, 1H). APCI-MS for $C_{20}H_{29}O_2N_2$ $[M+H]^+$: 329.3 *m/z*.

#### 4.3.4. 1-(Furan-2-yl)-N-{4-[3-(piperidin-1-yl)propoxy]benzyl} methanamine (**2b**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.44 (m, 2H), 1.59 (p, $J$ = 5.6 Hz, 4H), 1.97 (m, 2H), 2.40 (broad s, 4H), 2.47 (t, $J$ = 7.5 Hz, 2H), 3.72 (s, 2H), 3.77 (s, 2H), 4.00 (t, $J$ = 6.4 Hz, 2H), 6.18 (dd, $J$ = 0.91, 3.2 Hz, 1H), 6.32 (dd, $J$ = 1.9, 3.2 Hz, 1H), 6.85 (d, $J$ = 8.6 Hz, 2H), 7.22 (d, $J$ = 8.6 Hz, 2H), 7.37 (dd, $J$ = 0.8, 1.9 Hz, 1H). APCI-MS for $C_{20}H_{29}O_2N_2$ $[M+H]^+$: 329.3 *m/z*.

#### 4.3.5. 3-(1H-imidazol-1-yl)-N-(pyridin-2-ylmethyl)propan-1-amine (**3c**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.97 (p, $J$ = 6.8 Hz, 2H), 2.64 (t, $J$ = 6.7 Hz, 2H), 3.88 (s, 2H), 4.06 (t, $J$ = 6.9 Hz, 2H), 6.91 (s, 1H), 7.04 (s, 1H), 7.18 (ddd, $J$ = 1.2, 4.9, 7.6 Hz, 1H), 7.26 (d, $J$ = 7.8 Hz, 1H), 7.47 (s, 1H), 7.65 (td, $J$ = 1.8, 7.7 Hz, 1H), 8.56 (m, 1H). APCI-MS for $C_{12}H_{17}N_4$ $[M+H]^+$: 217.3 *m/z*.

#### 4.3.6. N-(furan-2-ylmethyl)-3-(1H-imidazol-1-yl)propan-1-amine (**4c**)

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 1.92 (p, $J$ = 6.9 Hz, 2H), 2.59 (t, $J$ = 6.7 Hz, 2H), 3.75 (s, 2H), 4.04 (t, $J$ = 6.9 Hz, 2H), 6.15 (dd, $J$ = 0.6, 3.2 Hz, 1H), 6.32 (dd, $J$ = 1.9, 3.2 Hz, 1H), 6.89 (t, $J$ = 1.3 Hz, 1H), 7.05 (s, 1H), 7.37 (dd, $J$ = 0.8, 1.8 Hz, 1H), 7.45 (s, 1H). APCI-MS for $C_{11}H_{16}ON_3$ $[M+H]^+$: 206.3 *m/z*.

### 4.4. Synthesis of phenol derivatives **19**–**31**

The compounds **19**–**31** were synthesized in good quantity (28–39 mg) following the solid-phase strategy we previously developed and published for similar phenolic derivatives [33]. All these compounds were purified by reverse phase chromatography on LC-MS preparative system (Model Prominence, Shimadzu, Kyoto, Japan) equipped with a photodiode detector (SPD M 20A) and mass analyser (MS 2020) with atmospheric-pressure chemical positive ionisation (APCI) systems with a synergi Hydro-RP column (250 mm × 21.2 mm x 4 μm). Each compound was purified in a 50 min-run using a solvent gradient (70–100% MeOH, 30–0% water) at flow rate of 10 mL/min.

*4.4.1. (7-Hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)(4-{[{2-[3-(piperidin-1-yl)propoxy]benzyl}(pyridin-2-ylmethyl)amino] methyl}phenyl)methanone (**19**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 1.48 (broad s, 2H), 1.60 (q, *J* = 4.7, 5.3 Hz, 4H), 1.97 (m, 2H), 2.47 (broad s, 4H), 2.54 (t, *J* = 7.6 Hz, 2H), 2.76 (broad s, 1H), 2.85 (broad s, 1H), 3.58 (broad s, 1H), 3.70 (s, 2H), 3.71 (s, 2H), 3.76 (s, 2H), 3.91 (broad s, 1H), 4.02 (t, *J* = 6.1 Hz, 2H), 4.50 (s, 1H), 4.74 (s, 1H), 6.63 (d, *J* = 7.5 Hz, 2H), 6.94 (m, 2H), 6.97 (d, *J* = 7.8 Hz, 1H), 7.18 (td, *J* = 1.5, 7.7 Hz, 1H), 7.26 (m, 1H), 7.38 (d, *J* = 6.4 Hz, 2H), 7.46 (d, *J* = 6.3 Hz, 1H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.70 (dt, *J* = 1.2, 8.0 Hz, 1H), 7.79 (td, *J* = 1.80, 7.7 Hz, 1H), 8.40 (d, *J* = 4.4 Hz, 1H). APCI-MS for $C_{38}H_{45}O_3N_4$ $[M+H]^+$: 605.4 *m/z*. HPLC purity: 91.0%.

*4.4.2. (7-Hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)(4-{[{4-[3-(piperidin-1-yl)propoxy]benzyl}(pyridin-2-ylmethyl)amino] methyl}phenyl)methanone (**20**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 1.53 (d, *J* = 6.6 Hz, 2H), 1.67 (p, *J* = 5.8 Hz, 4H), 2.02 (p, *J* = 6.1 Hz, 2H), 2.68 (m, 6H), 2.76 (broad s, 2H), 2.85 (broad s, 1H), 3.56 (s, 2H), 3.59 (m, 1H), 3.63 (s, 2H), 3.71 (s, 2H), 3.91 (s, 1H), 4.05 (t, *J* = 6.0 Hz, 2H), 4.50 (s, 1H), 4.75 (s, 1H), 6.63 (d, *J* = 6.3 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 6.97 (d, *J* = 7.2 Hz, 1H), 7.29 (m, 3H), 7.40 (d, *J* = 7.3 Hz, 2H), 7.50 (d, *J* = 7.8 Hz, 2H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.82 (td, *J* = 1.8, 7.7 Hz, 1H), 8.41 (d, *J* = 4.4 Hz, 1H). APCI-MS for $C_{38}H_{45}O_3N_4$ $[M+H]^+$: 605.4 *m/z*. HPLC purity: 90.0%.

*4.4.3. 4-{[(Furan-2-ylmethyl){2-[3-(piperidin-1-yl)propoxy] benzyl}amino]methyl}phenyl)(7-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**21**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 1.67 (broad s, 2H), 1.85 (broad s, 4H), 2.11 (dq, *J* = 5.88, 11.4 Hz, 2H), 2.77 (broad s, 1H), 2.86 (broad s, 1H), 3.14 (m, 2H), 3.61 (broad s, 1H), 3.67 (s, 2H), 3.68 (s, 2H), 3.70 (s, 2H), 3.93 (broad s, 1H), 3.93 (t, *J* = 6.2 Hz, 1H), 4.05 (t, *J* = 5.8 Hz, 2H), 4.51 (s, 1H), 4.77 (s, 1H), 6.28 (d, *J* = 3.0 Hz, 2H), 6.38 (d, *J* = 3.1 Hz, 2H), 6.63 (d, *J* = 6.6 Hz, 2H), 6.95 (m, 4H), 7.23 (td, *J* = 1.6, 7.7 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 2H), 7.49 (m, 4H); APCI-MS for $C_{37}H_{44}O_4N_3$ $[M+H]^+$: 594.4 *m/z*. HPLC purity: 89.9%.

*4.4.4. (4-{[(Furan-2-ylmethyl){4-[3-(piperidin-1-yl)propoxy] benzyl}amino]methyl}phenyl)(7-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**22**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 1.51 (d, *J* = 5.9 Hz, 2H), 1.64 (p, *J* = 5.6 Hz, 4H), 2.00 (dq, *J* = 6.05, 11.9 Hz, 2H), 2.61 (m, 6H), 2.77 (broad s, 2H), 2.86 (broad s, 1H), 3.54 (s, 2H), 3.60 (s, 2H), 3.62 (m, 3H), 3.92 (s, 1H), 4.01 (t, *J* = 6.1 Hz, 2H), 4.52 (s, 1H), 4.76 (s, 1H), 6.24 (dd, *J* = 0.9, 3.3 Hz, 1H), 6.36 (dd, *J* = 1.9, 3.2 Hz, 1H), 6.63 (d, *J* = 6.2 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.6 Hz, 2H), 7.40 (d, *J* = 6.7 Hz, 2H), 7.49 (m, 3H). APCI-MS for $C_{37}H_{44}O_4N_3$ $[M+H]^+$: 594.5 *m/z*. HPLC purity: 92.0%.

*4.4.5. (7-Hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)[4-({[3-(1H-imidazol-1-yl)propyl](pyridin-2-ylmethyl)amino} methyl)phenyl] methanone (**23**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 2.01 (p, *J* = 6.9 Hz, 2H), 2.51 (t, *J* = 6.8 Hz, 2H), 2.77 (broad s, 1H), 2.86 (broad s, 1H), 2.86 (t, *J* = 6.1 Hz, 1H), 3.60 (br t, *J* = 5.0 Hz, 1H), 3.68 (s, 2H), 3.74 (s, 2H), 3.92 (br t, *J* = 5.0 Hz, 1H), 4.02 (t, *J* = 6.9 Hz, 2H), 4.50 (s, 1H), 4.76 (s, 2H), 6.63 (d, *J* = 6.4 Hz, 2H), 6.88 (s, 1H), 6.98 (m, 2H), 7.30 (ddd, *J* = 1.3, 5.0, 7.5 Hz, 1H), 7.40 (d, J = 6.0 Hz, 2H), 7.49 (m, 2H), 7.59 (dt, *J* = 1.1, 7.9 Hz, 1H), 7.82 (td, *J* = 1.8, 7.7 Hz, 1H), 8.44 (ddd, *J* = 0.9, 1.8, 5.1 Hz, 1H). APCI-MS for $C_{29}H_{32}O_2N_5$ $[M+H]^+$: 482.3 *m/z*. HPLC purity: 99.0%.

*4.4.6. [4-({(Furan-2-ylmethyl)[3-(1H-imidazol-1-yl)propyl]amino} methyl)phenyl](7-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl) methanone (**24**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 1.99 (p, *J* = 6.8 Hz, 2H), 2.47 (t, *J* = 6.7 Hz, 2H), 2.77 (broad s, 1H), 2.86 (broad s, 1H), 3.65 (m, 5H), 3.93 (broad s, 1H), 4.07 (t, *J* = 6.8 Hz, 2H), 4.52 (s, 1H), 4.77 (s, 1H), 6.23 (d, *J* = 3.2 Hz, 1H), 6.35 (dd, *J* = 1.9, 3.2 Hz, 1H), 6.63 (d, J = 5.2 Hz, 2H), 6.93 (s, 1H), 7.00 (m, 1H), 7.03 (s, 1H), 7.46 (m, 5H), 7.59 (s, 1H). APCI-MS for $C_{28}H_{31}O_3N_4$ $[M+H]^+$: 471.3 *m/z*. HPLC purity: 90.0%.

*4.4.7. (4-{[(Furan-2-ylmethyl)(pyridin-2-ylmethyl)amino]methyl} phenyl)(7-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**25**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 2.77 (broad s, 1H), 2.86 (broad s, 1H), 3.60 (s, 1H), 3.70 (s, 2H), 3.71 (s, 2H), 3.80 (s, 2H), 3.91 (s, 1H), 4.51 (s, 1H), 4.75 (s, 1H), 6.26 (d, *J* = 3.1 Hz, 1H), 6.35 (dd, *J* = 1.8, 3.2 Hz, 1H), 6.63 (d, *J* = 4.6 Hz, 2H), 6.98 (d, *J* = 7.1 Hz, 1H), 7.28 (m, 1H), 7.40 (d, *J* = 6.1 Hz, 2H), 7.47 (s, 1H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.81 (td, *J* = 1.8, 7.7 Hz, 1H), 8.42 (d, *J* = 4.6 Hz, 1H). APCI-MS for $C_{28}H_{28}O_3N_3$ $[M+H]^+$: 454.2 *m/z*. HPLC purity: 94.7%.

*4.4.8. (4-{[(4-Bromobenzyl)(furan-2-ylmethyl)amino]methyl} phenyl)(7-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)methanone (**26**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 2.77 (t, *J* = 5.6 Hz, 1H), 2.86 (s, 1H), 3.55–3.95 (m, 8H), 4.51 (s, 1H), 4.76 (s, 1H), 6.33 (s, 1H), 6.40 (s, 1H), 6.63 (d, *J* = 4.4 Hz, 2H), 6.98 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.40–7.55 (m, 7H). APCI-MS for $C_{29}H_{28}BrO_3N_2$ $[M+H]^+$: 531.5 and 533.5 *m/z*. HPLC purity: 97.1%.

*4.4.9. [4-({(Furan-2-ylmethyl)[2-(morpholin-4-yl)ethyl]amino} methyl)phenyl](7-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl) methanone (**27**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 2.44 (s, 4H), 2.53 (dd, *J* = 5.7, 8.2 Hz, 2H), 2.66 (dd, *J* = 5.8, 8.4 Hz, 2H), 2.77 (broad s, 1H), 2.86 (s, 1H), 3.65 (m, 4H), 3.70 (s, 4H), 3.92 (broad s, 1H), 4.53 (s, 1H), 4.65 4.77 (s, 1H), 6.27 (d, *J* = 3.2 Hz, 1H), 6.36 (d, *J* = 2.9 Hz, 1H), 6.63 (d, *J* = 6.1 Hz, 2H), 6.98 (d, *J* = 7.1 Hz, 2H), 7.39–7.54 (m, 6H). APCI-MS for $C_{28}H_{34}O_4N_3$ $[M+H]^+$: 476.4 *m/z*. HPLC purity: 91.0%.

*4.4.10. (7-Hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)[4-({[(5-methylthiophen-2-yl)methyl](pyridin-2-ylmethyl)amino}methyl) phenyl]methanone (**28**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 2.44 (d, *J* = 1.2 Hz, 3H), 2.76 (t, *J* = 5.7 Hz, 1H), 2.85 (broad s, 1H), 3.60 (broad s, 1H), 3.69 (s, 2H), 3.75 (2s, 4H), 3.90 (broad s, 1H), 4.50 (s, 1H), 4.75 (s, 1H), 6.62 (m, 3H), 6.74 (d, *J* = 3.3 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 7.28 (ddd, *J* = 1.3, 5.0, 7.4 Hz, 1H), 7.40 (d, *J* = 6.5 Hz, 2H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.73 (dt, *J* = 1.1, 7.9 Hz, 1H), 7.83 (td, *J* = 1.8, 7.7 Hz, 1H), 8.42 (ddd, *J* = 0.89, 1.7, 5.1 Hz, 1H). APCI-MS for $C_{29}H_{30}O_2N_3S$ $[M+H]^+$: 484.2 *m/z*. HPLC purity: 99.4%.

*4.4.11. [4-({(Furan-2-ylmethyl)[(5-methylthiophen-2-yl)methyl] amino}methyl)phenyl](7-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl) methanone (**29**)*

$^1$H NMR (400 MHz, $CD_3OD$) δ: 2.44 (s, 3H), 2.78 (broad s, 1H), 2.86 (broad s, 1H), 3.65 (m, 5H), 3.74 (s, 2H), 3.92 (s, 1H), 4.53 (s, 1H), 4.76 (s, 1H), 6.26 (d, *J* = 3.1 Hz, 1H), 6.37 (dd, *J* = 1.9, 3.2 Hz, 1H), 6.60 (m, 3H), 6.73 (d, *J* = 3.4 Hz, 1H), 6.98 (d, *J* = 7.4 Hz, 1H), 7.44 (m, 3H), 7.53 (d, *J* = 7.7 Hz, 2H). APCI-MS for $C_{28}H_{29}O_3N_2S$ $[M+H]^+$: 473.3 *m/z*. HPLC purity: 98.1%.

*4.4.12. (4-{[(Furan-2-ylmethyl)(4-hydroxybenzyl)amino]methyl} phenyl)(7-hydroxy-3,4-dihydroiso quinolin-2(1H)-yl)methanone (**30**)*

$^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 2.77 (t, $J$ = 5.7 Hz, 2H), 2.86 (broad s, 1H), 3.49 (s, 2H), 3.62 (3s, 6H), 3.92 (broad s, 1H), 4.52 (s, 1H), 4.76 (s, 1H), 6.23 (d, $J$ = 3.0 Hz, 1H), 6.36 (dd, $J$ = 1.9, 3.1 Hz, 1H), 6.63 (m, 2H), 6.74 (d, $J$ = 8.0 Hz, 2H), 6.97 (d, $J$ = 7.1 Hz, 1H), 7.19 (d, $J$ = 8.3 Hz, 2H), 7.40 (d, $J$ = 6.9 Hz, 2H), 7.49 (t, $J$ = 8.1 Hz, 3H). APCI-MS for $C_{29}H_{29}O_4N_2$ $[M+H]^+$: 469.2 *m/z*. HPLC purity: 89.3%.

*4.4.13. (7-Hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)(4-{[(4-methoxybenzyl)(pyridin-2-ylmethyl)amino] methyl}phenyl) methanone (**31**)*

$^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 2.76 (broad s, 1H), 2.85 (broad s, 1H), 3.56 (s, 2H), 3.59 (broad s, 1H), 3.64 (s, 2H), 3.71 (s, 2H), 3.76 (s, 3H), 3.91 (broad s, 1H), 4.50 (s, 1H), 4.75 (s, 1H), 6.62 (broad s, 2H), 6.87 (d, $J$ = 8.6 Hz, 2H), 6.98 (d, $J$ = 8.1 Hz, 1H), 7.29 (m, 3H), 7.40 (m, 2H), 7.51 (d, $J$ = 7.8 Hz, 2H), 7.69 (d, $J$ = 7.9 Hz, 1H), 7.82 (td, $J$ = 1.78, 7.7 Hz, 1H), 8.40 (d, $J$ = 4.4 Hz, 1H). APCI-MS for $C_{31}H_{32}O_3N_3$ $[M+H]^+$: 494.3 *m/z*. HPLC purity: 98.3%.

### *4.5. Synthesis of sulfamate derivatives **32**–**34***

The compounds **32**–**34** were synthesized following a solid-phase strategy we previously developed and published for similar sulfamate derivatives [33]. These compounds were purified by flash chromatography (hexanes/EtOAc, 3:7).

*4.5.1. 2-[(4-{[(Furan-2-ylmethyl)(pyridin-2-ylmethyl)amino] methyl}phenyl)carbonyl]-1,2,3,4-tetrahydroisoquinolin-7-yl sulfamate (**32**)*

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.88 (m, 2H), 3.70 (m, 6H), 3.82 (s, 2H), 3.98 (broad s, 1H), 4.60 (broad s, 1H), 4.88 (broad s, 1H), 5.12 (broad s, 2H), 6.22 (dd, $J$ = 0.9, 3.2 Hz, 1H), 6.33 (dd, $J$ = 1.8, 3.2 Hz, 1H), 7.18 (m, 4H), 7.44 (m, 5H), 7.58 (d, $J$ = 7.8 Hz, 1H), 7.69 (td, $J$ = 1.8, 7.7 Hz, 1H), 8.53 (dd, $J$ = 1.5, 4.7 Hz, 1H). APCI-MS for $C_{28}H_{29}O_5N_4S$ $[M+H]^+$: 533.3 *m/z*. HPLC purity: 79.9%.

*4.5.2. 2-{[4-({(Furan-2-ylmethyl)[(5-methylthiophen-2-yl)methyl] amino}methyl)phenyl]carbonyl}-1,2,3,4-tetrahydro-isoquinolin-7-yl sulfamate (**33**)*

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.47 (s, 3H), 2.84 (m, 2H), 3.66 (s, 2H), 3.69 (s, 2H), 3.77 (s, 2H), 3.98 (broad s, 1H), 4.64 (broad s, 1H), 4.89 (broad s, 2H), 5.00 (m, 2H), 6.22 (dd, $J$ = 0.83, 3.1 Hz, 1H), 6.35 (dd, $J$ = 1.8, 3.2 Hz, 1H), 6.59 (dd, $J$ = 1.4, 3.3 Hz, 1H), 6.72 (d, $J$ = 3.4 Hz, 1H), 7.15 (m, 3H), 7.42 (d, J = 8.0 Hz, 3H), 7.50 (d, $J$ = 8.0 Hz, 2H). APCI-MS for $C_{28}H_{30}O_5N_3S_2$ $[M+H]^+$: 552.3 *m/z*. HPLC purity: 90.9%.

*4.5.3. 2-[(4-{[(4-Methoxybenzyl)(pyridin-2-ylmethyl)amino] methyl}phenyl)carbonyl]-1,2,3,4-tetrahydroisoquinolin-7-yl sulfamate (**34**)*

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.89 (m, 2H), 3.57 (s, 2H), 3.64 (m, 3H), 3.74 (s, 2H), 3.80 (s, 3H), 3.91 (broad s, 1H), 4.59 (s, 1H), 4.8–5.2 (m, 3H), 6.87 (d, $J$ = 8.6 Hz, 2H), 7.17 (m, 4H), 7.31 (d, $J$ = 8.6 Hz, 2H), 7.39 (m, 2H), 7.46 (m, 2H), 7.58 (d, $J$ = 7.9 Hz, 1H), 7.68 (td, $J$ = 1.8, 7.7 Hz, 1H), 8.51 (dt, $J$ = 1.3, 5.0 Hz, 1H). APCI-MS for $C_{31}H_{33}O_5N_4S$ $[M+H]^+$: 573.3 *m/z*. HPLC purity: 83.0%.

### *4.6. Biological assays (general information)*

17β-estradiol, Tris, EDTA, glycerol, insulin, and bovine serum albumin (BSA) were purchased from Sigma–Aldrich Ltd (Oakville, ON, Canada). 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt (MTS) was purchased from Promega (Madison, WI, USA). Radiolabeled [6,7-$^3$H] estrone sulfate (54.3 Ci/mmol) was purchased from Perkin Elmer (Woodbridge, ON, Canada). Raloxifene hydrochloride was bought from Cayman Chemical (Ann Harbor, MI, USA). Biodegradable Counting Scintillant was purchased from Amersham Biosciences (Waltham, MA, USA). Penicillin/streptomycin mix, L-glutamine, non-essential amino acids, sodium pyruvate, normal and charcoal-stripped Fetal Bovine Serum (FBS), and geneticin (G418 sulfate) were purchased from Wisent, Inc. (St-Bruno, QC, Canada). All cell culture media were purchased from Life Technologies (Grand Island, NY, USA) except for phenol-red free McCoy's 5A medium that was purchased from PromoCell (Heidelberg, Germany). STS inhibitor EM-1913 was synthesized in our Laboratory of Medicinal Chemistry using published procedures [42]. For the purpose of *in vitro* assays, all chemicals tested (inhibitors and reference compounds) were first dissolved in DMSO and subsequent dilutions were done in the proper buffer or cell culture media. The final concentration of DMSO in the culture medium was 0.1% or less.

*4.6.1. Steroid sulfatase inhibition assay*

An enzymatic assay previously described was used for the inhibition of the transformation of estrone sulfate (E1S) to estrone (E1) by STS [42]. Briefly, the transfected HEK-293 cells were homogenized by repeated (5 times) cycles of freezing (−80 °C) and thawing on ice (4 °C). The homogenates were then incubated for 2 h at 37 °C (shaking water bath) with or without inhibitors (0.01 μM–1 μM) in presence of [$^3$H]-E1S (9 nM), adjusted to 1 μM with E1S, in a Tris-acetate buffer (pH 7.4) containing 5 mM EDTA and 10% glycerol. After the incubation, 1 mL of xylene was added to each tube and the solutions were then centrifuged at 3000 rpm for 20 min to separate the organic ([$^3$H]-E1) and aqueous ([$^3$H]-E1S) phases. Once 500 μL of each phase was added to 10 mL of Biodegradable Counting Scintillant, the radioactivity of samples was recorded using a Wallac 1411 Liquid Scintillation Counter. The percentage of inhibition was determined by comparison with the control (buffer + homogenate + [$^3$H]-E1S) which was set to 0% of inhibition. $IC_{50}$ value was obtained using GraphPad Prism 5 software (La Jolla, CA, USA).

*4.6.2. Cell culture*

The $ER^+$ breast cancer T-47D cells and the osteoblast-like Saos-2 cells were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The HEK-293 cell line overexpressing STS was obtained from Dr. Van Luu-The (CHU de Québec - Research Center) [52]. All cell lines were maintained in culture flasks (175 $cm^2$ growth area, BD Falcon) at 37 °C in a 5% $CO_2$ humidified atmosphere. The T-47D cells were grown in phenol red free RPMI 1640 medium supplemented with 10% FBS, penicillin (100 IU/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), and 17β-estradiol (1 nM). The Saos-2 cells were grown in phenol red free McCoy's 5A medium supplemented with 10% FBS, penicillin (100 IU/mL), and streptomycin (100 μg/mL). The HEK-293 cells transfected with STS were maintained in Minimum Essential Medium supplemented with 10% FBS, penicillin (100 IU/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), non-essential amino acids (0.1 mM), sodium pyruvate (1 mM), and geneticin (G418 sulfate) (700 μg/mL).

*4.6.3. Cell proliferation*

CellTitter 96® Aqueous One Solution Cell Proliferation Assay was used as an indirect colorimetric measurement of cell proliferation according to the manufacturer's instructions. After the treatments, 20 μL of MTS solution was briefly added to each well (100 μL) of the plates and incubated at 37 °C for 2 h (Saos-2) or 4 h (T-47D). The absorbance at 490 nm was then measured with a Thermo max

microplate reader (Molecular Devices, Sunnyvale, CA, USA). The control (culture media + DMSO) is set to 100% of cell proliferation. T-47D cells were suspended in RPMI supplemented with insulin (50 ng/mL), instead of 17β-estradiol, and 5% charcoal-stripped FBS to deprive the media of estrogens. The cells were plated in 96-well plates at a density of 3000 cells/well and allowed to attach for 48 h. After this pre-incubation, the inhibitors and the reference compounds diluted in fresh culture media were added to the wells and replaced every 2 days for 7 days of treatment. Saos-2 cells were suspended in phenol-red free McCoy's 5A medium supplemented with 10% charcoal-stripped FBS, penicillin (100 IU/mL) and streptomycin (100 μg/mL). The cells were seeded in 96-well plates at a density of 3000 cells/well and allowed to attach. After 24 h, the inhibitors and the reference compounds diluted in fresh culture medium were added to the wells and replaced every 2 days for 7 days of treatment.

#### 4.6.4. Alkaline phosphatase activity

Saos-2 cells were treated similarly as reported in the cell proliferation assay. The cells were seeded at a density of 2000 cells/well and were treated with the inhibitors and the reference compounds for 3 days. The alkaline phosphatase (ALP) activity was measured using Sensolyte® pNPP Alkaline Phosphatase Assay Kit *Colorimetric* (AnaSpec, Fremont, CA, USA) following the manufacturer's protocol. Briefly, after the 3 days of treatment, the cells were washed twice with washing buffer (provided with the kit) and lysed with 0.2% Triton X-100. The cell lysates were centrifuged and the supernatants were used to determine the ALP activity. The supernatants were deposed in a 96-well plate and incubated 30 min with a *p*-nitrophenyl phosphate solution (provided with the kit). The absorbance at 405 nm was measured with a Thermo max microplate reader (Molecular Devices, Sunnyvale, CA, USA). The control (culture medium + DMSO) is set to 100% of alkaline phosphatase activity.

### 4.7. Molecular docking

Docking simulations for STS and ERα were performed using MOE 2014.09 (Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group Inc., Montréal, QC, Canada). Crystal structures, PDB 1P49 for STS and PDB 1ERR and 1ERE for ERα, were taken from RCSB PDB [53]. Phenolic compounds **25**, **29**, and **31**, and sulfamate compounds **32**, **33**, and **34**, and STS inhibitor EM-1913, were built and minimized in MOE. The same procedure was used for raloxifene, taken from PDB 1ERR.

#### 4.7.1. Steroid sulfatase docking

Structure of human placental estrone/DHEA sulfatase was obtained from PDB 1P49. STS inhibitors bearing an aryl sulfamate moiety are expected to form a covalent bond with the catalytic hydroxyformylglycine residue [49,50]. As covalent docking is not supported in MOE, the structure was prepared in two steps as follows: First, STS inhibitor EM-1913 [42–44] was manually oriented in the catalytic site, with its sulfamate moiety superposed to the hydroxyformylglycine-bound sulfate moiety. Then, this hydroxyformylglycine was mutated to Gly and the corresponding sulfate moiety deleted, creating enough space for non-covalent docking. One of the two water molecules originally present in the catalytic site was removed as it overlapped with the initial positioning. Hydrogens were added using the Protonate 3D in MOE and the binding site was minimized using the LigX procedure with MMFF94x and born solvation model. Docking simulations were realized using the rigid receptor protocol, the MMFF94x force field with the distance solvation model and default parameters. Docking of compound EM-1913 using this protocol confirmed the initial conformation as the best bound conformation, positioning the sulfamate moiety at a distance of 0.6 Å from the original sulfate (see Fig. 8). The best result from EM-1913 docking is used as the reference for the docking of sulfamate compounds **32**, **33**, and **34** using the same procedure.

#### 4.7.2. Estrogen receptor docking

Experimental structure of human ERα/raloxifene complex was obtained from PDB 1ERR. Hydrogens were added to the structure using Protonate 3D in MOE followed by a minimization process using the LigX procedure with force field MMFF94x, born solvation and default parameters. Docking was performed using the induced fit procedure with MMFF94x force field with the distance solvation model and default parameters. RMSD of 1.5 Å was obtained between the best docked conformation and the crystal structure of raloxifene, validating the procedure. The same procedure was used to perform docking of both phenolic as well as sulfamate compounds **25**, **29**, **31**, **32**, **33**, and **34**.

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## Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.04.044.

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