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Design, synthesis, and biological evaluation of new arylamide derivatives possessing sulfonate or sulfamate moieties as steroid sulfatase enzyme inhibitors

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ABSTRACT

A series of new arylamide derivatives possessing terminal sulfonate or sulfamate moieties was designed and synthesized. The target compounds were tested for in vitro inhibitory effects against the steroid sulfatase (STS) enzyme in a cell-free assay system. The free sulfamate derivative **1j** was the most active. It inhibited the enzymatic activity by 72.0% and 55.7% at 20 μM and 10 μM, respectively. Compound **1j** was further tested for STS inhibition in JEG-3 placental carcinoma cells with high STS enzyme activity. It inhibited 93.9% of the enzyme activity in JEG-3 placental carcinoma cells at 20 μM with an efficacy near to that of the well-established drug STX64 as reference. At 10 μM, **1j** inhibited 86.1% of the STS activity of JEG-3. Its IC_{50} value against the STS enzyme in JEG-3 cells was 0.421 μM. Thus, **1j** represents an attractive new non-steroidal lead for further optimization.

1. Introduction

The steroid sulfatase (STS) enzyme catalyzes the hydrolysis of inactive sulfate metabolites such as estrone sulfate and dehydroepiandrosterone sulfate to the more active estrone and dehydroepiandrosterone, respectively. The production of 90% of androstenediol (Adiol) comes from dehydroepiandrosterone released through the STS pathway.[1] Despite the androgenic structure of Adiol, it still possesses some estrogenic properties. Adiol is about 100 times weaker than estradiol,[2–5] with lower affinity for the estrogen receptor.[6] However, the Adiol concentration in the circulation is 100-fold higher than estradiol. This led to speculation that it might be equipotent to estradiol.[7] In addition, the STS pathway produces a significant amount of estrogen besides that produced by aromatase, the enzyme which catalyzes the aromatization of androgen to estrogen. This has been supported by: (1) STS activity in liver, normal breast tissues, and breast cancer tissues is million fold higher than aromatase activity;[8] (2) estrone produced from estrone sulfate through the STS pathway is about 10-fold higher than that produced from androstenedione through aromatase action;[9] and (3) STS expression is a very essential prognostic factor in human breast carcinoma.[10,11] Thus, STS is an attractive target for the treatment of hormone-dependent breast,[12] endometrial,[13] prostate cancers, and endometriosis.[14]

Several articles have recently highlighted different steroidal and non-steroidal agents capable of inhibiting STS.[12,15–21] Estrone 3-*O*-sulfamate (EMATE, Fig. 1) is an example of a potent steroidal STS inhibitors, but when orally tested in vivo it exerted estrogenic side effects as demonstrated by its ability to increase the uterine weight in ovariectomized Wistar rats.[22] Attention was therefore switched to non-steroidal STS inhibitors to avoid such effects. The coumarin sulfamate derivative STX64 (Irosustat, 667 COUMATE, Fig. 1) has been the most potent and successful STS inhibitor to date. It is currently being investigated in clinical trials for treatment of estrogen-dependent breast cancer, and has been trialed in endometrial cancer and prostate cancer. STX64 is an irreversible STS inhibitor due to the presence of the sulfamate moiety that covalently binds to the enzyme.[16] On the other hand, some estrone sulfonate derivatives have been reported as reversible STS inhibitors because the sulfonate moiety is unable to make a covalent bond with the enzyme as the sulfamate analogues.[23,24]

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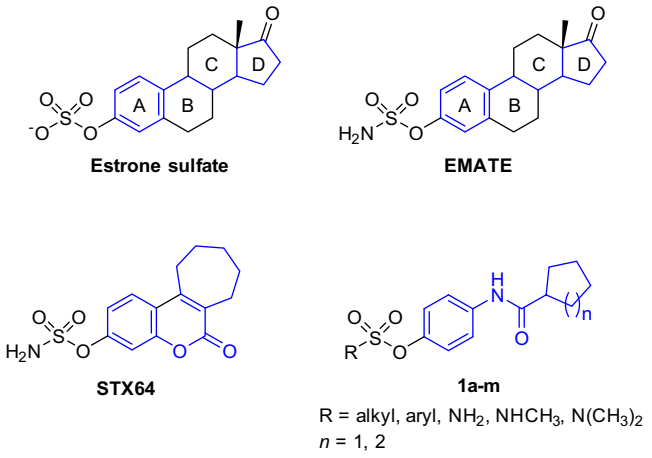


Figure 1. Structures of Estrone sulfate, EMATE, STX64, and the target compounds **1a**–**m**.

It is attractive to explore novel non-steroidal templates as potential sulfatase inhibitors. In the present study, a series of arylamide derivatives possessing sulfonate or sulfamate moieties was designed to mimic estrone sulfate and dehydroepiandrosterone sulfate, the substrates of STS. As illustrated in Figure 1, the two ring system of the target compounds mimic rings A and D of estrone sulfate and EMATE with a 2-atom spacer. In another orientation, it can also mimic the aromatic and the cycloheptane rings of STX64 with an amide linker as an isostere of the coumarin ester moiety. Thirteen target compounds were synthesized and evaluated for STS inhibitory effect in a cell-free enzymatic assay. The most promising compound was further tested for its STS inhibitory effect in whole JEG-3 placental carcinoma cells that have high STS enzyme activity. The results and experimental protocols are set out below.

2. Results and discussion

2.1. Chemistry

The target compounds **1a**–**m** were synthesized via the pathway illustrated in Scheme 1. 4-Aminophenol (**2**) was reacted with cyclohexanecarbonyl chloride (**3a**) or cyclopentanecarbonyl chloride (**3b**) in the presence of anhydrous potassium carbonate to afford the phenolic intermediates **4a**,**b**. Some precautions were taken into consideration in this reaction to avoid disubstitution, such as the order of addition, rate of addition, dilution with solvent, and stirring while adding the acid chlorides to 4-aminophenol. Interaction of the hydroxyl intermediates **4a**,**b** with the appropriate sulfonyl chloride derivatives in the presence of triethylamine produced the target sulfonate compounds **1a**–**i**. To obtain the target sulfamate analogues **1j**–**m**, compounds **4a**,**b** were reacted with the appropriate sulfamoyl chloride reagents in presence of anhydrous sodium hydride under N_2. The detailed structures of the target compounds are illustrated in Table 1.

2.2. Biological screening

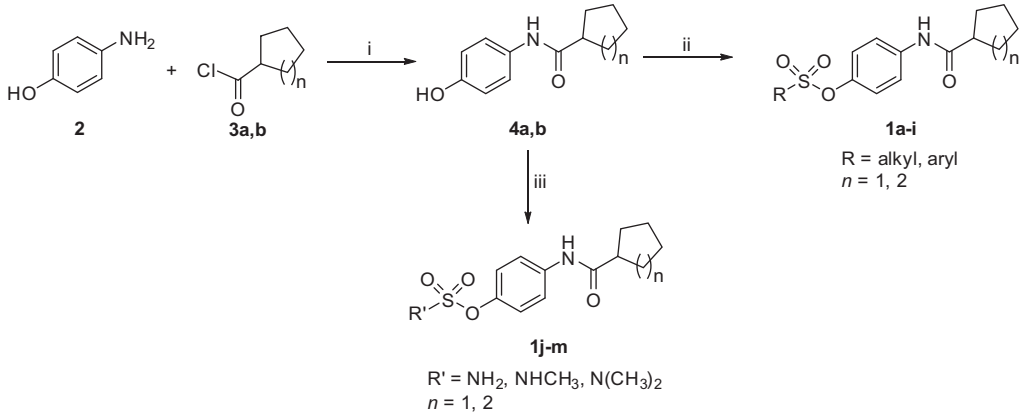
2.2.1. Cell-free enzyme inhibition testing

All the thirteen target compounds **1a**–**m** were tested at a single-dose concentration of 10 μM against the STS enzyme. The inhibitory effects are depicted in Figure 2. The results show that compound **1j** is the most active amongst this series of compounds. It possesses a free sulfamate 'warhead' moiety, similar to the lead compound STX64. Irosustat (STX64) has been reported as an irreversible inhibitor of STS. The irreversible inhibitors are usually stronger than the corresponding reversible inhibitory agents. This explains the stronger activity of the free sulfamate analogue **1j** that likely irreversibly also inhibits the enzyme similar to STX64, compared to the sulfonate derivatives that were less active.

The free sulfamate compound **1j** was significantly more active than the *N*-substituted sulfamate derivatives **1k** and **1l**. This finding complies with earlier data reported for STX64 and its steroidal counterparts compared with the corresponding substituted sulfamate analogues.[16] The substituted sulfamate moieties have been reported as reversible inhibitors and non-covalent binders relative to the free sulfamate.[25] This can rationalize the stronger activity of free sulfamate derivatives compared to the substituted sulfamates.

Among the aliphatic sulfonate analogues, the ethanesulfonate **1b** was the most active, and the *p*-tosylate derivative **1e** was more active than the other aromatic sulfonates. Upon investigating the effect of the cycloalkyl ring size on activity, the cyclohexyl derivatives **1e** and **1k** were more active than the corresponding cyclopentyl analogues **1i** and **1m**. So the bulkier cyclohexyl ring is more optimal for activity maybe due to stronger hydrophobic interactions and/or steric influence. Any or both of these effects might enhance the affinity to the enzyme and hence confer a stronger inhibitory effect.

The most promising compound **1j** was further studied in 5-dose testing mode at 20, 10, 5, 1, and 0.5 μM concentration in comparison with STX64. The results are illustrated in Figure 3. Compound **1j** inhibited the enzyme in a dose-dependent manner. It inhibited



Scheme 1. Reagents and conditions: (i) anhydrous K_2CO_3, acetone, 0 °C, rt, 4 h; (ii) appropriate sulfonyl chloride derivative, triethylamine, anhydrous THF, 0 °C, rt, 2 h, 80–88% (two steps); (iii) appropriate sulfamoyl chloride derivative, NaH, anhydrous DMF, 0 °C, rt, overnight, 83–90% (two steps).

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