Chem Biol Drug Des 2016; **87:** 233–238

Research Letter

Synthesis and Biological Evaluation of Fluorinated 3-Phenylcoumarin-7-*O*-Sulfamate Derivatives as Steroid Sulfatase Inhibitors

Sebastian Demkowicz[1],*, Mateusz Daśko[1], Witold Kozak[1], Katarzyna Krawczyk[1], Dariusz Witt[1], Maciej Masłyk[2], Konrad Kubiński[2] and Janusz Rachon[1]

[1]Department of Organic Chemistry, Chemical Faculty, Gdansk University of Technology, Narutowicza 11/12, 80-233 Gdansk, Poland
[2]Department of Molecular Biology, Faculty of Biotechnology and Environment Sciences, The John Paul II Catholic University of Lublin, Konstantynów 1i, 20-708 Lublin, Poland
**Corresponding author: Sebastian Demkowicz, sebdemko@pg.gda.pl*

In the present work, we report the initial results of our study on a series of 3-phenylcoumarin sulfamate-based compounds containing C-F bonds as novel inhibitors of steroid sulfatase. The new compounds are potent steroid sulfatase inhibitors, possessing more than 10 times higher inhibitory potency than coumarin-7-*O*-sulfamate. In the course of our investigation, compounds 2b and 2c demonstrated the highest inhibitory effect on the enzymatic steroid sulfatase assay; both had IC_{50} values of 0.27 μM (the IC_{50} value of coumarin-7-*O*-sulfamate is 3.5 μM, used as a reference).

Key words: breast cancer, coumarin, steroid sulfatase, steroid sulfatase inhibitors, sulfamates

Received 12 May 2015, revised 20 July 2015 and accepted for publication 11 August 2015

Hormone-dependent breast cancer (HDBC) is a major cause of mortality, and there is a pressing need to develop novel treatment methods. One approach for treating HDBC involves the use of inhibitors for enzymes that are responsible for the biosynthesis of estrogens in peripheral tissues, for example, steroid sulfatase (STS) (1). STS is responsible for the hydrolysis of steroid sulfates to their active forms; therefore, it has a crucial role in regulating the formation of biologically active steroids. STS is widely distributed throughout the body, and its action is involved in physiological and pathological conditions (2).

In recent years, there has been intensive research toward finding novel steroidal and non-steroidal inhibitors of STS containing different functional groups, for example, phosphate, thiophosphate, or sulfamate moieties (3–8). The most promising compound was estrone-3-*O*-sulfamate (EMATE) (Figure 1)—a time- and concentration-dependent irreversible steroidal inhibitor, which exhibited very high activity in MCF-7 cells, with an IC_{50} value of 65 pM (9). Unfortunately, EMATE is not used for the treatment of breast cancer therapy due to its estrogenic properties. An important class of compounds that exhibits high activity against STS includes coumarin derivatives. 4-methylcoumarin-7-*O*-sulfamate (COUMATE) (IC_{50} value of 380 nM when evaluated against placental microsomes) and its tricyclic derivatives, for example, 667-COUMATE (IC_{50} value of 8 nM), have been shown to lack significant estrogenicity. Coumarin analogs are classified as irreversible inhibitors whose effects are time and concentration dependent (10,11).

With respect to the binding mode, our docking experiments revealed that sulfamated 3-phenylcoumarin analogs containing C-F bonds could, at least theoretically, bind STS. The new STS inhibitor candidates dock in a similar manner to the mode of the reported STS inhibitor (coumarin-7-*O*-sulfamate) (Figure 2). Sulfamate functional groups are directed to catalytic amino acid FGly75 coordinated to Ca^{2+}, and they are in the proximity of the proposed catalytic residues of Asp35, Asp36, Arg79, Lys134, His136, His290, Asp342, and Lys368. Furthermore, the fluorine-substituted phenyl rings of coumarin scaffolds are in close proximity to several lipophilic amino acids (Leu74, Arg98, Leu103, Leu167, Val177, Phe178, Thr484, His485, Val486, and Phe488), which are implicated in substrate recognition. Molecular modeling studies suggest that increasing the hydrophobic properties of coumarin frameworks (by introducing the substituted phenyl rings) could favor binding by the establishment of hydrophobic interactions, wherein the cavity is delimited by lipophilic amino acids in the enzyme pocket. All candidates expressed similar free docking energies (predicted free docking energies in the range of −6.2 to −6.9 kcal/mol). In contrast, the compounds lacking the hydrophobic substituents in the 3rd position of the coumarin scaffolds exhibited a poor ability to bind STS, resulting in an increase in the predicted

 doi: 10.1111/cbdd.12652